

# INTRODUCED AQUATIC SPECIES IN THE MARINE AND ESTUARINE WATERS OF CALIFORNIA

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## 1.0 EXECUTIVE SUMMARY

In response to the potential threat from the introduction of non-native species from the ballast of ships into the marine waters of the state, the Legislature passed Assembly Bill 703 (Lempert) Chapter 849, Statutes of 1999. Under this bill and subsequent extensions of its provisions, the California Department of Fish and Game (DFG) is required to conduct a study of California coastal waters for new introductions of Non-native Aquatic Species (NAS) that could have been transported into state waters in ballast or through hull-fouling and assess results of the effectiveness of the Marine Invasive Species Program (MISP) in controlling NAS introductions from ship-related vectors.

There is a substantial number of introduced species in California's coastal waters. Statewide, 307 NAS have been identified from the literature and field investigations. Some of the NAS originally listed in the DFG 2002 report were subsequently re-identified or reclassified as native, cryptogenic (not demonstrably introduced or native), or unresolved, or are considered part of species complexes, thereby creating an artificial apparent reduction in NAS from 2002 to 2008. An additional 453 species are currently classified as cryptogenic. Many of these species are likely introduced, but require further analysis before they can be confidently categorized as introduced or native. In addition to the combined 760 species classified as introduced or cryptogenic, another 1,362 cannot be identified to species level with any degree of certainty. Reasons for non-identification include presence of juveniles, unrecognized or undescribed specimens, and damaged specimens.

DFG conducted numerous large-scale field surveys of California coastal waters that indicate all major harbor areas in California have received significant introductions. Each major commercial harbor area of the state has between 40 and 190 NAS and another 15 to 138 species that are possibly introduced (cryptogenic). San Francisco Bay had more NAS (190) than any other estuary or harbor. Substantial numbers of introduced species were also found in the smaller ports and bays. Two broad-scale field surveys of the outer coast revealed only nine introduced species, far fewer than in estuaries. The majority of the species introduced to California appear to be native to the northwest Atlantic, the northwest Pacific and the northeast Atlantic. The number of species with unknown origins is substantial, however, indicating a need for further research.

The fouling vector was most often attributed to the introduction of species to California. In major ports and waters adjacent to ports, it was impossible to distinguish between fouling due to recreational boats and fouling due to commercial ships. Statewide, 165 species were probably introduced via fouling. Among those 165 species, 153 (93%) had both recreational boats and commercial ships as possible sources of introduction. Further assessment of the role of recreational boat fouling as an NAS vector is needed. Ballast water, however, is still a major vector of introductions. Ship ballast water discharge was

the second largest category of potential vectors and, together with fouling from ship's hulls, indicates that shipping plays a substantial role in dispersal of species.

The rate of introductions into California has accelerated more or less exponentially over time since 1850. Yet the discovery rate has actually dropped below the exponential curve since the late 1970s. However, observations of the rate of discoveries cannot be relied upon as an indicator of introduction rate, due to such factors as sampling bias, occurrence of serendipitous discoveries, lag time between introduction and discovery, and high variability in sampling effort. Although as many as 14 new discoveries of NAS in California have been made in one year (in 1993, which is mainly attributable to increased sampling), no new discoveries are known since 2006.

***Recommendation 1: Increase the role of genetic studies and DNA sequencing in species identification.***

The detection and monitoring of marine invasions is complicated by the presence of cryptic species. When native and introduced species belong to the same cryptic species complex, the arrival of the introduced species will not be noticed until genetic analyses are performed; the replacement of the native by the introduced species may occur without notice. Recent research funded by DFG has shown that genetic analysis is effective in discovering these introductions.

***Recommendation 2: Fund special studies to resolve gaps in taxonomic identification.***

In order to determine if new species are being introduced to California, species need to be clearly identified and named. However, certain groups of marine organisms need taxonomic clarification. Special studies should be funded that are designed to revise or refine the taxonomy of various groups of organisms, determine and describe the geographic ranges of origin of species currently classified as cryptogenic, or facilitate calibration of taxonomic knowledge amongst taxonomists.

***Recommendation 3: Modify MISP sampling design to accomplish NAS monitoring.***

Potential changes to the study plan include: (1) more frequent smaller-scale sampling, to avoid missing seasonal dynamics, (2) combine short-term, focused surveys with large-scale surveys to provide the best opportunity to detect new species while minimizing the impact of lag-time in specimen identification before species are discovered, (3) scale back the number of sites sampled in the broad statewide surveys, particularly on the outer coast.

***Recommendation 4: Analyze shipping and ballast water dumping patterns and their relationship to NAS.***

California dates of discovery for species introduced or dispersed by ballast water can be compared to volume of discharged or exchanged ballast water in nearshore locations. Examine relationships between shipping traffic patterns along the California coast and occurrence of NAS likely introduced via shipping.

***Recommendation 5: Further research the role of vectors in introducing and dispersing NAS.***

There is little knowledge of the roles of many non-shipping vectors in introducing or spreading NAS, especially recreational boating. Many species are thought to have arrived via multiple pathways. Further research will make it possible to more effectively target prevention measures on the most problematic vectors. Implementation of this recommendation is underway through a risk assessment study of six non-shipping vectors funded by the Ocean Protection Council and directed by the Ocean Science Trust.

***Recommendation 6: Continue to provide opportunities for peer input to sampling methodology and review of reports.***

Develop a standardized review program to continue to improve the ongoing sampling program and ensure data quality. The review program should include an adequate timeline for identifying and contacting appropriate peer reviewers, adequate time for review, and an honorarium for academic peers.

## **DEFINITIONS OF TERMS USED IN THIS REPORT**

Algae: A class of microscopic plants which contain chlorophyll and live suspended in water or attached to structures like rocks or other submerged surfaces.

Alien species: Species occurring in an area outside of its historically known natural range as a result of intentional or accidental dispersal by human activities.

Antifouling: A particular type of paint used on the bottom of vessels in order to reduce the growth of marine organisms.

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

Benthic: Organisms that live on or in the ocean bed.

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.

Cryptogenic: Of unknown origin.

Epifaunal: Organisms that live on the ocean floor or upon bottom objects such as sea anemones and barnacles.

Exotic Species: Synonym for “introduced species” (species occurring in an area outside of its historically known natural range as a result of intentional or accidental dispersal by human activities).

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.

Haplotype: A way of denoting the collective genotype of a number of closely linked loci on a chromosome.

Infaunal: Organisms that live within the surface sediments such as clams and worms.

Intertidal: Coastal area between low and high tide.



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

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

Nonindigenous: Organisms living outside their natural geographical boundaries.

Phytoplankton: Microscopic aquatic plants suspended in water

Plankton: A diverse group of small, usually microscopic animals (zooplankton) and plants (phytoplankton) that freely drift in the water.

Rip-Rap: A combination of large stone, cobbles and boulders used to prevent coastal erosion.

Soft Bottom Benthos: Organisms that live in unconsolidated soft sediment (sand, silt, and clay).

Species Complex: A group of species that are not distinguishable (at least are not reliably) based on form and structure.

Substrate: Surface on which an organism lives.

Subtidal: A marine or estuarine environment that lies below the low tide usually flooded near edge of tidal waters.

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

Vector (Introduction Vector): A carrier (such as ballast water) that transfers or introduces aquatic species from one geographical location to another.

Water Column: A hypothetical cylinder of water from the surface of a water body to the bottom.

Zooplankton: Microscopic animals that are free-floating or swim that live in aquatic environments.

## **ACRONYMS AND ABBREVIATIONS USED IN THIS REPORT**

<u>AIS Plan</u> :	California Aquatic Invasive Species Management Plan
<u>BWM</u> :	Ballast Water Management
<u>BWT</u> :	Ballast Water Treatment
<u>CANOD</u> :	California Aquatic Non-native Organism Database
<u>DFG</u> :	California Department of Fish and Game
<u>EPA</u> :	Environmental Protection Agency
<u>MISP</u> :	Marine Invasive Species Program
<u>MLML</u> :	Moss Landing Marine Laboratories
<u>NAISA</u> :	National Aquatic Invasive Species Act
<u>NAS</u> :	Non-native Aquatic Species
<u>NEMESIS</u> :	National Exotic Marine and Estuarine Species Information System
<u>NIS</u> :	Nonindigenous Species
<u>NISA</u> :	National Invasive Species Act (1996)
<u>NRC</u> :	National Research Council
<u>OSPR</u> :	Office of Oil Spill Prevention and Response
<u>SCUBA</u> :	Self-Contained Underwater Breathing Apparatus
<u>SERC</u> :	Smithsonian Environmental Research Center
<u>SFSU/RTC</u> :	San Francisco State University/ Romberg Tiburon Center
<u>SMURF</u> :	Standard Monitoring Units for the Recruitment of Fish

US EPA: United States Environmental Protection Agency

USCG: United States Coast Guard

USDS: United States Department of the State

USFWS: United States Fish and Wildlife Service

USGS: United States Geological Survey

## 2.0 INTRODUCTION

Introduced aquatic animals and plants have had a profound impact on the ecology of the marine and freshwater regions of California. Several transport vectors have been implicated in the spread of NAS; however, the ballast water of ocean-going ships is a primary mechanism of marine and estuarine introductions. A modern day cargo ship can take on enormous quantities of water (in excess of 200,000 m<sup>3</sup>) in ballast tanks to achieve proper buoyancy and trim (NRC 1996). This water may later be discharged in another port, perhaps thousands of miles from its source, before the vessel takes on additional cargo. Ballast water can contain numerous species in great abundance, including phytoplankton, zooplankton, and the eggs, larvae or adults of clams, crabs, shrimp, worms, and other marine species. Within a few hours, tens of millions of living introduced organisms may be de-ballasted from a single ship.

It is estimated that more than 10,000 marine species are transported each day around the globe in the ballast water of cargo ships (Carlton 1999). The volume of water is so enormous, and the transit time that organisms spend in the ballast water tank is so short, that the number of species successfully invading new habitats via shipping pathways is increasing at an ever higher rate (Cohen and Carlton 1998). In a study of the introduction of aquatic species in the San Francisco Bay, Cohen and Carlton (1998) found that from 1851 to 1960, new introductions occurred at the rate of one species every 55 weeks. From 1961 to 1995, the rate of introduction increased to an estimated one new species every 14 weeks.

### 2.1 Effects of Introduced Species

The problem of ballast introductions has become all the more urgent as international commerce increases, resulting in a corresponding increase in the rate at which NAS are transported and introduced. The introduction of NAS has created serious ecological, operational, and engineering problems in many areas of the United States, including many of the harbors and bays in California.

#### 2.1.1 Economic Effects

The economic impacts of NAS introductions can be severe. One recent study placed U.S. economic damages associated with alien invasive species effects and their control amount to approximately \$120 billion/year (Table 1, Pimentel et al. 2005). This table does not include impacts of some harmful introduced species for which data were unavailable.

**Table 1:** Estimated annual costs associated with some alien species introduction in the United States (Pimentel et al. 2005).

Category	Cumulative loss estimates (millions of dollars)
Plants	\$ 34,661
Terrestrial vertebrates	\$ 39,387
Fish	\$ 5,400
Aquatic invertebrates	\$ 2,205
Arthropods	\$ 16,455
Plant pathogens	\$ 25,700

#### 2.1.1.1 Examples of Economic Effects of NAS

- Zebra and quagga mussels are a notorious example of the economic impact that can result from the introduction of NAS. Transported from Europe in the ballast water of transoceanic ships, zebra mussels (*Dreissena polymorpha*) and quagga mussels (*D. bugensis*) were first discovered in North America in the late 1980's. The first account of an established population came from the Canadian waters of Lake St. Clair, a small water body connecting Lake Huron and Lake Erie. By 1990, zebra mussels had been found in all the Great Lakes. The following year, zebra mussels escaped the Great Lakes basin and found their way into the Illinois and Hudson rivers (NRCS 2007). By 2000, the mussels inhabited the waters of at least 20 states and the Canadian provinces of Ontario and Quebec. In 2007 quagga mussels were discovered in Lake Mead, Nevada, and later throughout Lake Mead's lower basin, the Colorado River, and the Colorado River Aqueduct System that serves Southern California. This was the first discovery of dreissenid mussels west of the Continental Divide, and can be assumed to be the result of the overland transport of mussels. In January of 2008 zebra mussels were discovered at San Justo Reservoir, California, another example of the overland spread.

The mussels' penchant for adhering to hard surfaces has caused technical problems for the American power industry. Water intake pipes are often encrusted with thousands of mussels which increase sedimentation and corrosion of the pipes, as well as restricting or even stopping water flow. Maintenance of pipes clogged with mussels costs the power industry up to \$60 million per year and temporary shutdowns due to insufficient water flow can cost over

\$5,000 per hour. According to the U.S. Department of State, the estimated cost of the zebra mussel invasion to the United States will total \$3.1 billion over the next ten years (USDS 2008). The California Department of Water Resources estimates a mussel infestation will add millions of dollars in maintenance costs to the operation of the State Water Project. Zebra and quagga mussels also attach to boat hulls, docks, locks, breakwater and navigation aids, increasing maintenance cost and impeding waterborne transport (SeaGrant 2008).

- Chinese mitten crabs (*Eriocheir sinensis*) were first collected in San Pablo Bay in fall 1994. By 1998, they were found in the Sacramento River as far north as Colusa, and in the San Joaquin River as far south as the San Luis National Wildlife Refuge. The most probable mechanism of introduction to the estuary was either deliberate release to establish a fishery or accidental release via ballast water (DFG 1998). Based on the impacts of mitten crabs in their native range and in Europe, they pose several possible threats: burrowing that may accelerate the erosion of banks and levees; damaging rice crops by consuming young rice shoots; and damaging commercial fishing nets and reducing the size of the catch when the crabs are caught in high numbers (it is very costly and time consuming for the fishermen to remove the crabs from the nets) (Hieb and Veldhuizen 1998). It is considered one of the world's worst 100 invasive species (ISSG 2008).

Mitten crabs pose several economic impacts. They steal fish bait, causing a substantial economic impact on anglers, commercial fishermen, and sports fishing in the San Francisco Estuary. In San Francisco Bay, they interfere with fisheries by competition for food and shelter (NEMESIS 2008) and commercial shrimpers claimed that as much as 40% of their catch was damaged or killed by mitten crabs (Rudnick and Resh 2002). In California, they caused serious mortality in fish-salvage facilities, which were designed to divert fish from irrigation facilities in the Sacramento-San Joaquin Delta. In 1998, at peak migration times, mortality of migrating fish was 98-99%. The economic costs to prevent the crabs' entry into federal and state water diversion facilities are high (ISSG 2008). Retrofitting of the facilities to prevent mitten crab entrapment was expensive: costs for control and research were \$1 million in 2000-2001 (Carlton 2001).

### 2.1.2 Ecological Effects

Introduced species may out-compete or alter local habitats to such an extent that they make it impossible for native species to survive. Introduced species are often predators, competitors or parasites and many introduced species can

cause or carry disease. Regardless of the direct or indirect nature of the effect, NAS can significantly impact human health, devastate fishery and aquaculture resources and severely disrupt habitat and ecosystem stability.

One of the most serious ecological costs of introduced species is the extinction of native species. Approximately 42% of the species on the Threatened or Endangered species lists are at risk primarily because of predation, parasitism, and competition from alien-invasive species (Pimentel et al. 2004). About 40% of the species forced to extinction in aquatic ecosystems are due to biological invaders (Pimentel 2003).

#### 2.1.2.1 Examples of Ecological Effects

- Plankton that have caused harmful toxic blooms or 'red tides' are among the better known and documented instances of successful invaders causing great harm at a considerable cost. Red tides occur when toxic, microscopic plankton in seawater proliferate to higher-than-normal concentrations (bloom), often discoloring the water red, brown, green, or yellow. More than 40 species of toxic plankton live in the Gulf of Mexico. The most common is the dinoflagellate *Karenia brevis*, the Florida red tide organism. Florida red tides occur in the Gulf of Mexico almost every year, generally in the late summer or early fall. Most blooms last three to five months and may affect hundreds of square miles. Occasionally, however, blooms continue sporadically for as long as 18 months and may affect thousands of square miles. Red tides can kill fish, birds, and marine mammals; cause health problems for humans; and adversely affect local economies (FFWCC 2008).
- *Caulerpa taxifolia*, an introduced seaweed, was first discovered in June 2000 in a coastal lagoon in Carlsbad, California, within San Diego County. It is a green alga native to tropical waters where it typically grows to small size and in limited patches. In the late 1970's, this species attracted attention as a fast-growing and decorative aquarium plant that became popular in the saltwater aquarium trade. In the early 1980's, it escaped or was released from an aquarium in Germany into Mediterranean waters and by 1989 had spread from an initial patch of about one square yard to over two acres. By 1997, it blanketed more than 11,000 acres of the northern Mediterranean coastline and has recently been reported off northern Africa. It is considered one of the world's worst 100 invasive species (ISSG 2008).

The first confirmed occurrence of this invasive species in California caused considerable alarm. If established, it could cause considerable ecological and economic devastation by overgrowing

and eliminating native seaweeds, sea grasses, reefs, and other communities. In the Mediterranean, it is reported to have harmed tourism and pleasure boating, devastated recreational diving, and had a costly impact on commercial fishing both by altering the distribution of fish as well as creating a considerable impediment to net fisheries. In Southern California, this alga posed a substantial threat to marine ecosystems, particularly to the extensive eelgrass beds and other benthic environments that make our coastal water such a rich and productive environment for fish and birds (Woodfield 2002). *Caulerpa taxifolia* was successfully eradicated from California in 2006 at a cost of approximately six million dollars (Williams and Grosholz 2008).

- In San Francisco Bay, the overbite clam (*Corbula amurensis*) spread throughout the region's waterways within two years of first being detected in 1986. The clam accounts for up to 95% of the living biomass in some shallow portions of the bay floor (Nichols et al. 1990). It has contributed to a persistent decline in the availability of plankton in the Sacramento-San Joaquin River Delta (Jassby et al. 2002) which, in turn, may be a cause of declines in fish populations (Feyrer et al. 2003).
- The European green crab (*Carcinus maenas*) has been introduced to the east and west coasts of the US, as well as to the waters of Australia, Brazil, Panama and South Africa (Grosholz and Ruiz 1996). Introductions to the United States are likely the result of ballast water or from crabs clinging to heavily fouled ship hulls. They were first detected on the West Coast in San Francisco Bay in the late 1980's, and found in Humboldt Bay in 1995. By 1998, large numbers were found in the intertidal areas of Humboldt Bay where their habitat and feeding preferences overlap many of the indigenous species, primarily those of the Dungeness crab. Documented destruction of shellfish resources on the Eastern Atlantic Coast by green crabs caused concern among Humboldt resource managers and fishermen. Green crabs may impact juvenile Dungeness crabs that settle by the thousands in Humboldt Bay and may also prey upon juvenile cultured oysters, clams and mussels (SeaGrant California 2001).

The green crab is a voracious predator and has caused a substantial decline in the abundance of native crabs and clams in Bodega Harbor. The native symbiotic nemertean egg predator, *Carcinonemertes epialti*, now also infests green crabs and threatens fisheries and aquaculture (Carlton and Cohen 2007). The green crab is considered one of the world's worst 100 invasive species (ISSG 2008). There are fears that it will compete for food with the valuable



Dungeness crab (*Cancer magister*) threatening the west coast fishery (McDonald et al. 2001).

- NAS impact on biodiversity can also be enormous. Fully half of all threatened or endangered species are imperiled by introduced species, making introduced species second only to habitat loss as the greatest threat to endangered species (Wilcove, et al. 1998). The environmental damage attributable to introduced species in the San Francisco Estuary includes: reduction or local extinction of native species (some bay waters now contain virtually no native species); disruption of the aquatic food chain through elimination of phytoplankton by highly efficient introduced filter feeders; erosion of shorelines by introduced burrowers; and other ecosystem alterations which extend to bird and wildlife population (Cohen and Carlton 1995).

Species extinctions do not have to occur for biological communities to be radically and permanently altered. Nor are extinctions necessary for California to experience a significant decline in the abundance, diversity, and aesthetic value of its biological resources as populations of indigenous species shrink and numbers of NAS increase.

## 2.2 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. This program was an outgrowth of the initial effort at the federal level to combat the problem in the Great Lakes and elsewhere. The following summarizes the origins and evolution of the federal effort and the subsequent California initiative.

### 2.2.1 Federal Ballast Water Management Initiatives

In 1996, Congress re-authorized the Non-native Aquatic Nuisance Species Prevention and Control Act of 1990 (NANPCA), re-titled as the National Invasive Species Act of 1996 (NISA). The new law established a national ballast management program that included provisions for mandatory ballast water control procedures for vessels traveling in the Great Lakes, but voluntary procedures for vessels entering other ports in the U.S. The law required that the Secretary of Transportation report to Congress by the end of 2001 regarding the level of compliance with the voluntary ballast management guidelines. If the rate of compliance was not adequate, NISA provided the U.S. Coast Guard (USCG) the authority to make the voluntary guidelines mandatory.

In May 2002, the USCG issued a report to Congress as required under NISA. The report assessed the effectiveness and rate of compliance with the ballast water management requirements since the passage of the law in 1996. The report found that only about 40% of vessels complied with mandatory ballast reporting requirements, and of those only about half (51.2%) conducted a mid-ocean exchange of ballast water. Based on these results, the USCG determined that voluntary guidelines should be made mandatory and should be enforced. The USCG decided to implement a national ballast water management (BWM) program, maximizing existing BWM techniques and fostering the development of new ballast water treatment (BWT) technologies (USCG 2001).

In 2004, the USCG established regulations for a national mandatory BWM program for all vessels equipped with ballast water tanks that enter or operate within U.S. waters. These regulations also require vessels to maintain a BWM plan that is specific for that vessel and assigns responsibility to the master or appropriate official to understand and execute the BWM strategy for that vessel. Congress reauthorized the National Aquatic Invasive Species Act (NAISA) in 2007. This statute, among other provisions, requires all ships (transoceanic and coastal) to: (a) prepare Ship Invasive Species Management Plans outlining procedures to prevent introductions of invasive organisms, (b) report all ballast operations, treatment and management practices, (c) carry out Best Management Practices to reduce the movement of species by ships, and (d) until the end of 2011, all existing ships entering a U.S. port must conduct ballast water exchange and any other management practices included in regulations unless the safety of the vessel is at stake. Beginning in 2012, all vessels entering a US port shall conduct ballast water treatment so that ballast water discharged contains less than one living organism that is larger than 50 micrometers in dimension per ten cubic meters of water and less than one living organism that is smaller than 50 micrometers per ten milliliters of water.

### 2.2.2 California Ballast Water Management Act

In California, concern was raised over the adequacy of the federal program. The voluntary nature of the federal provisions, and the lack of funding for research on more effective methods to prevent species introductions, convinced the California legislature to enact a program to better protect the marine resources of the state.

In response to the potential threat by the introduction of NAS from the ballast of ships into the marine waters of the state, the Legislature passed the Ballast Water Management Act of 1999. The Act was due to sunset in January 2004. Three agencies were responsible for implementing the various provisions of the Act: the DFG, the State Water Resources Control Board, and the State Lands Commission. The DFG, 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. 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 new law came into effect January 1, 2004.

### 2.2.3 Marine Invasive Species Act

The MISA of 2003 repealed the sunset provision of the Ballast Water Management Act of 1999 and reauthorized the statewide multi-agency program, the MISP, to control the introduction and spread of NAS in marine and estuarine waters. The Act expanded the MISP to include coastwise traffic and DFG'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 DFG to conduct a monitoring program to determine whether new introductions have occurred since the original baseline was established. The MISA was authorized to sunset in December 2009.

### 2.2.4 Coastal Ecosystem Protection Act

In 2006 the legislature passed Senate Bill 497, which repealed the sunset provision of December 2009 that would have ended the MISP. The program is now ongoing, and the DFG has been given several new research and reporting responsibilities, as follows:

- The Department must monitor coastal and estuarine waters for new introductions of NAS that could have been transported into state waters in ballast or as hull-fouling.
- Data from the monitoring effort must be posted to the internet and updated on an annual basis. The data from the monitoring efforts can be viewed at <http://www.dfg.ca.gov/ospr/about/science/misp.html>.
- A report must be drafted and submitted 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 is due December 31, 2008, and must be updated every three years thereafter.

### 2.2.5 California Ballast Water Management

In 1999, Assembly Bill 703 required that vessels originating from outside the 200 nautical mile Exclusive Economic Zone (EEZ) of the United States carry out mid-ocean ballast water exchange, or use an approved ballast water treatment method, before discharging ballast in California state waters. Beginning in 2006,

vessels operating within the Pacific Coast Region (i.e. coastal voyages) were required to manage their ballast water, either through retention or by conducting an exchange in near-coastal waters. The California State Lands Commission (CSLC) was required to adopt regulations on performance standards for the discharge of ballast water by January 2008.

CSLC regulations require vessels, upon departure from each port or place in California, to submit a Ballast Water Reporting Form which details their ballast management practices. According to the CSLC, over 25,000 reporting forms have been submitted since 2004 and ballast water management compliance in California is extremely high. In 2006, 99% of all vessel-reported ballast water carried into State waters complied with management requirements; either through complete retention of ballast onboard or undergoing a legal exchange prior to discharge. The largest category (35% on average) of non-compliant ballast water discharged between January 2004 and June 2006 originated from Mexican coastal waters, with tank vessels and bulk carriers responsible for almost all (approximately 89%) of these discharges. The second largest proportion (28% on average) of noncompliant ballast water discharges originated within the U.S. West Coast Exclusive Economic Zone (California State Lands Commission 2007).

Regulations allow two methods of ballast water exchange. The empty-refill method requires that the ballast tank be emptied once, and subsequently refilled with mid-ocean water. Vessels utilizing the flow-through exchange method must pump three full volumes of mid-ocean water through the ballast tank, but tanks are not emptied (California State Lands Commission 2007). Both types of exchange are highly effective when performed according to guidelines and regulations. When replacing coastal ballast water with mid-ocean water there is 88-99% replacement of original water and 80-95% reduction in concentration of coastal planktonic organisms across ship types (Ruiz et al. 2007). A study that examined ballast water movement from Sacramento to the Columbia River found that coastal exchange was highly effective in removing potentially high-risk freshwater species from ballast tanks (Noble et al. 2006).

## 2.2.6 California Aquatic Invasive Species Management Plan

In 2008, Governor Schwarzenegger signed the California Aquatic Invasive Species Management Plan (AIS Plan), which provides a framework for agency coordination and 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.

### 3.0 STUDY PLAN AND FIELD SURVEYS

To detect marine invasions, we utilized a 4-step process, outlined by Wasson et al. (2000): (1) thorough field surveys, (2) systematic analyses to identify all species found, (3) literature searches for the worldwide distribution of these species and for previous records of these species in this region, and (4) application of rigorous criteria to assess whether each species found is introduced or native. The remainder of this section of the report elaborates on the details of this process.

The initial NAS baseline for California harbors and bays was developed from 2000 to 2001 from a combination of surveys and literature searches. The study plan for the 2000-2001 surveys focused on those areas of the coast that had not been surveyed specifically for NAS in past investigations and then, within those areas, concentrated on the regions most likely to be impacted by ballast introductions. The sampling data from these sites were supplemented by information generated from an extensive literature review, as well as data from comparable studies being conducted independently during the same period by other organizations. The initial study targeted the seven major ports and many of the smaller ports and bays along the California coast. The seven port areas are: San Diego, Los Angeles/Long Beach, Port Hueneme, Stockton, Sacramento, San Francisco Bay and Humboldt Bay. Sampling and identification of NAS was done in all these port areas except San Francisco Bay which had already been extensively studied in recent years, most notably by Cohen and Carlton in 1995.

The MISA of 2003 stipulated that the DFG will conduct several studies, including a supplemental survey of the open coast, to augment the baseline data from the harbors and bays that was previously compiled. Table 2 lists the different field surveys and the years that they were conducted. Multiple habitats were surveyed during each survey (Table 3).

The methods for these surveys were previously detailed in DFG's 2002 report to the State Legislature (DFG 2002) and in Foss et al. (2007). The 2002 Legislative report included a comprehensive species list that was compiled from the literature review and the results of DFG's sampling program.

**Table 2.** DFG/MLML field survey per year.

Survey	Year				
	2004	2005	2006	2007	2008
Bays and Harbors			X		
Outer Coast	X			X	
San Francisco Bay		X			
Plankton			X	X	
Fish					X

**Table 3.** DFG/MLML habitats sampled during each survey.

Habitats	Survey		
	Bays and Harbors	Outer Coast	San Francisco Bay
Water Column	X		X
Subtidal fouling	X		X
Intertidal Rocky		X	
Intertidal Sandy		X	
Subtidal Rocky		X	
Subtidal Benthic	X	X	X

### 3.1 San Francisco Bay Survey

The survey of San Francisco Bay was conducted during the summer and fall of 2005 and is a part of a long-term monitoring effort that includes surveys in ports, harbors, estuaries, and outer coast. Previous studies indicated that San Francisco Bay was the most invaded estuary in California, with other ports and harbors not far behind in terms of numbers of NAS. DFG contracted with San Jose State University Foundation's Moss Landing Marine Laboratories (MLML) as the principal investigator for the biological survey.

Literature and data reviews were complimented by field and laboratory studies jointly conducted by OSPR and MLML. Additional universities and specialized laboratories provided taxonomic expertise in identification of marine species.

The sampling design was adapted from the design used in previous DFG/MLML NAS surveys conducted in California bays and harbors and outer coast habitats, and focused on whole community structure rather than singling out any one species or habitat. Multiple habitats were surveyed at each of 70 San Francisco Bay sites. For the purpose of examining trends of introduced species distribution within the Bay, San Francisco Bay was divided into 4 sub-regions: South San Francisco Bay, Central San Francisco Bay, San Pablo Bay, and Suisun Bay (Figures 1-3 show the sampling sites in each region).

# San Pablo & Suisun Bay Sites

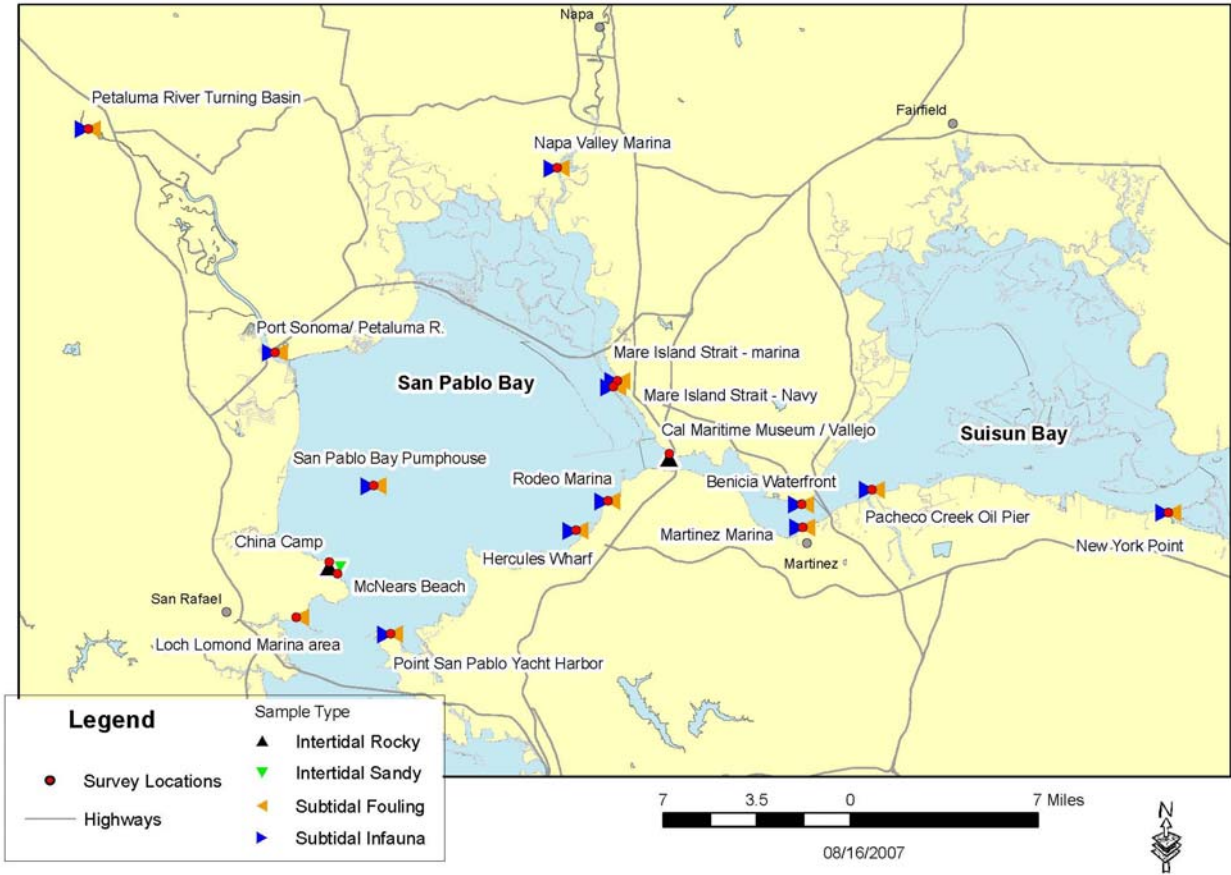


Figure 1. Sub-regions San Pablo Bay and Suisun Bay.

# Central San Francisco Bay

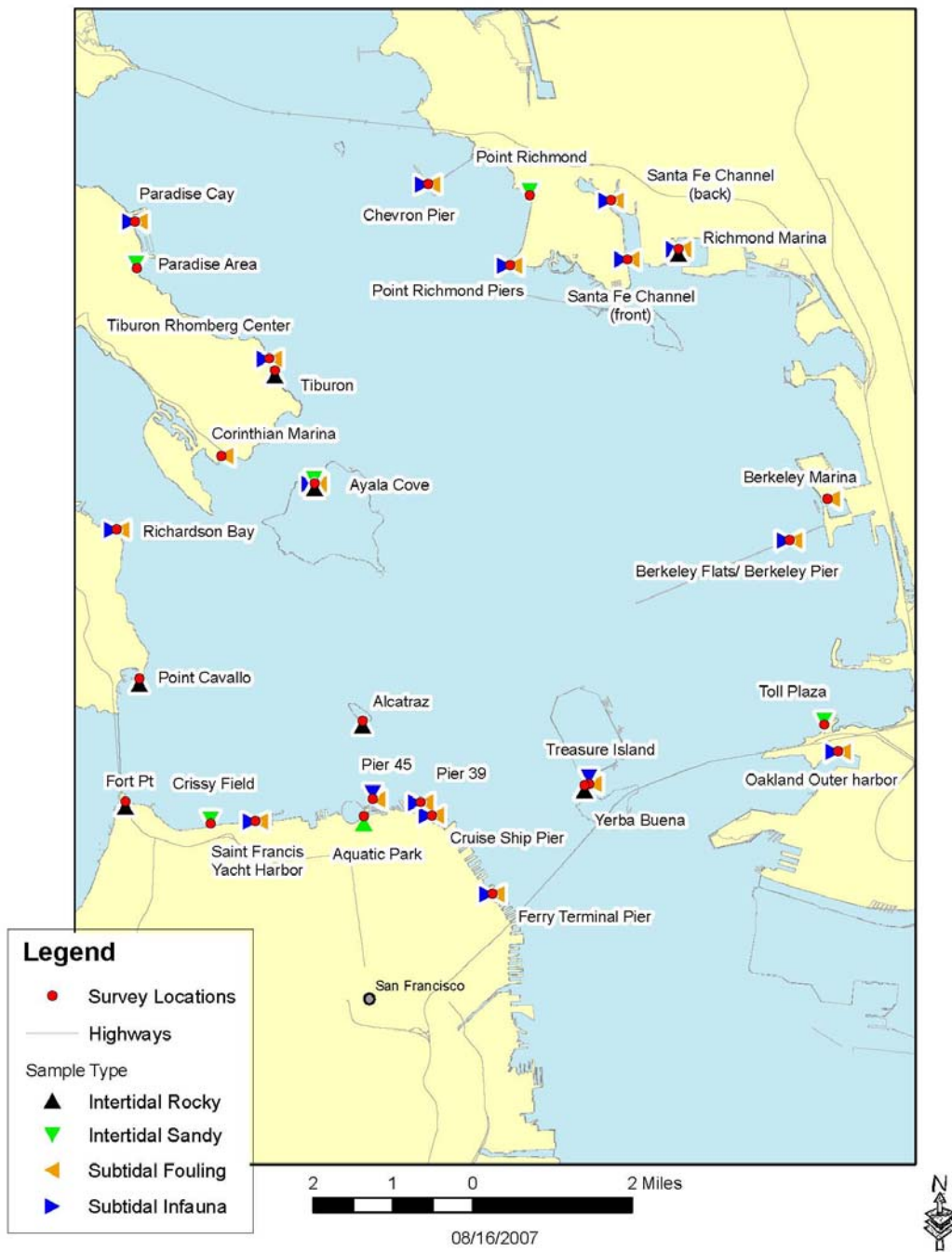
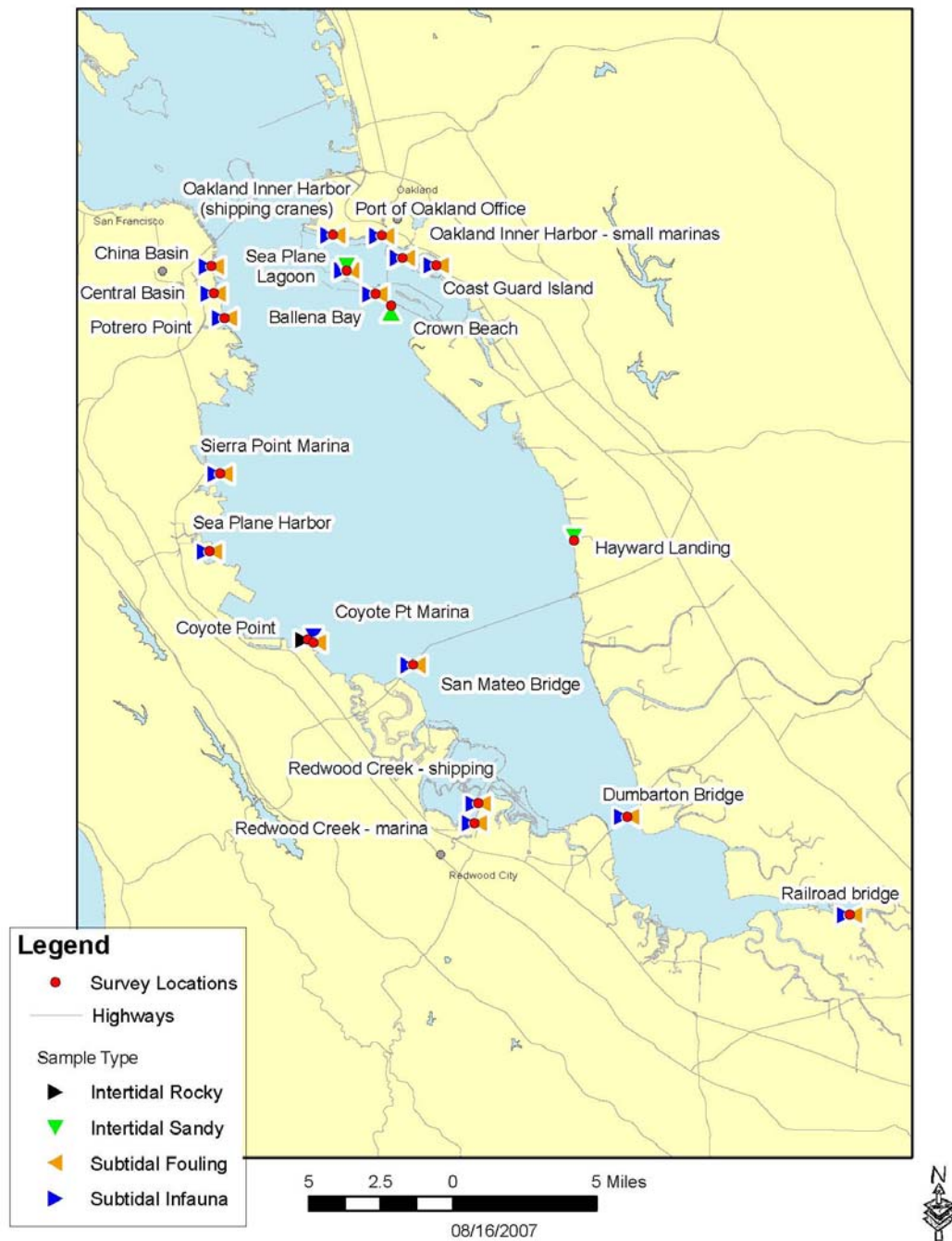


Figure 2. Sub-region Central San Francisco Bay.



## South San Francisco Bay



**Figure 3. Sub-region South San Francisco Bay.**

Four types of habitats were surveyed: rocky intertidal, sandy intertidal, subtidal fouling and subtidal infauna. Criteria used during site selection included (1) obtain good geographic distribution over sample regions, (2) target areas likely to

be impacted by anthropogenic activities, (3) locate and sample sites harboring a variety of hard substrates with fouling communities (for subtidal surveys), (4) locate and sample sites with available intertidal natural rocky reef if possible (for rocky intertidal surveys), and (5) overlap with historical and/or current survey sites if possible. Natural rocky and sandy intertidal habitat is limited within the Bay so geographic distribution for those sample sites was limited.

Methods included the use of sediment cores and grabs, quadrat clearings, and qualitative taxonomic surveys. In addition, qualitative samples were collected during the visual scans. Samples were then preserved and transported to the appropriate laboratories and taxonomists for identification and enumeration. Taxonomists also occasionally provided information about historical or ongoing ecological or monitoring research conducted at or near survey sites.

### 3.2 Bays and Harbors Survey

In California's bays and harbors, two main habitat types were targeted: subtidal fouling (also called epifaunal in this report), and subtidal infaunal communities. Sampling included the use of qualitative and quantitative sampling protocols to survey representative communities for the presence of NAS. The survey focused on whole community structure rather than singling out any one "invasive" species or habitat. Methods employed included the use of sediment cores and grabs, quadrat clearings, qualitative taxonomic surveys and plankton tows. Sampling focused on macroinvertebrates, algae, plankton, fish, and some aquatic vascular plants; each were identified and counted by specialized taxonomists.

The majority of the field sampling for this survey was done by MLML. The field investigation for the bays and harbors was done in 2006 and 2007, and taxonomic identification work extended through much of 2007. MLML sampling protocols were designed to maximize the probability that NAS would be detected by directing effort to locations and habitats most likely to have been colonized by these organisms. Sampling focused on (1) areas within harbors and bays that had a high potential for ballast water release, (2) calm backwaters where species could collect and flourish, (3) recently established docks which could provide a comparison to growth on older docks, and (4) habitats at harbor entrances. Within these general areas, priority was given to active and inactive shipping berths, fishing vessel docks, recreational marinas, aquaculture facilities, and newly constructed structures. Sample sites were spread throughout each port, harbor, or bay to give spatial representation and to accommodate differences in tidal flushing and mixing.

The NAS baseline for bays, ports and harbors was established through surveys conducted in 2000/2001 (Foss et al. 2007). In the present survey, sites in the harbors and bays of northern, central and southern California were revisited, except for the site at Fort Bragg, because earlier sampling revealed it to be a freshwater environment. These areas include: the ports of San Diego, Los

Angeles/Long Beach, Hueneme, Stockton, Sacramento, San Francisco Bay and adjacent waters, Humboldt Bay, Tomales Bay and numerous small harbors encompassing the entire California coast (Figure 4). Samples were collected at over 450 stations in 21 harbors, marinas, and bays; epifaunal samples were taken in all locations, infaunal communities were sampled in 4 harbors, zooplankton were identified from samples taken in Humboldt Bay, Port Hueneme, LA/LB Harbor and San Diego Bay, and fish surveys were conducted in the ports of Sacramento and Stockton (Foss et al. 2007). Benthic infaunal samples (sediment grabs) were collected at 77 stations in 4 harbors. Voucher specimens of all identified NAS and cryptogenic taxa were stored and maintained by MLML.

Due to habitat differences that could influence larval recruitment and subsequent colonization, the sampling strategy encompassed multiple depths, substrates, orientations, and light exposure conditions. Although subtidal sampling focused on average depths up to 30 feet, epifaunal subtidal sampling substrates were at or near the surface.

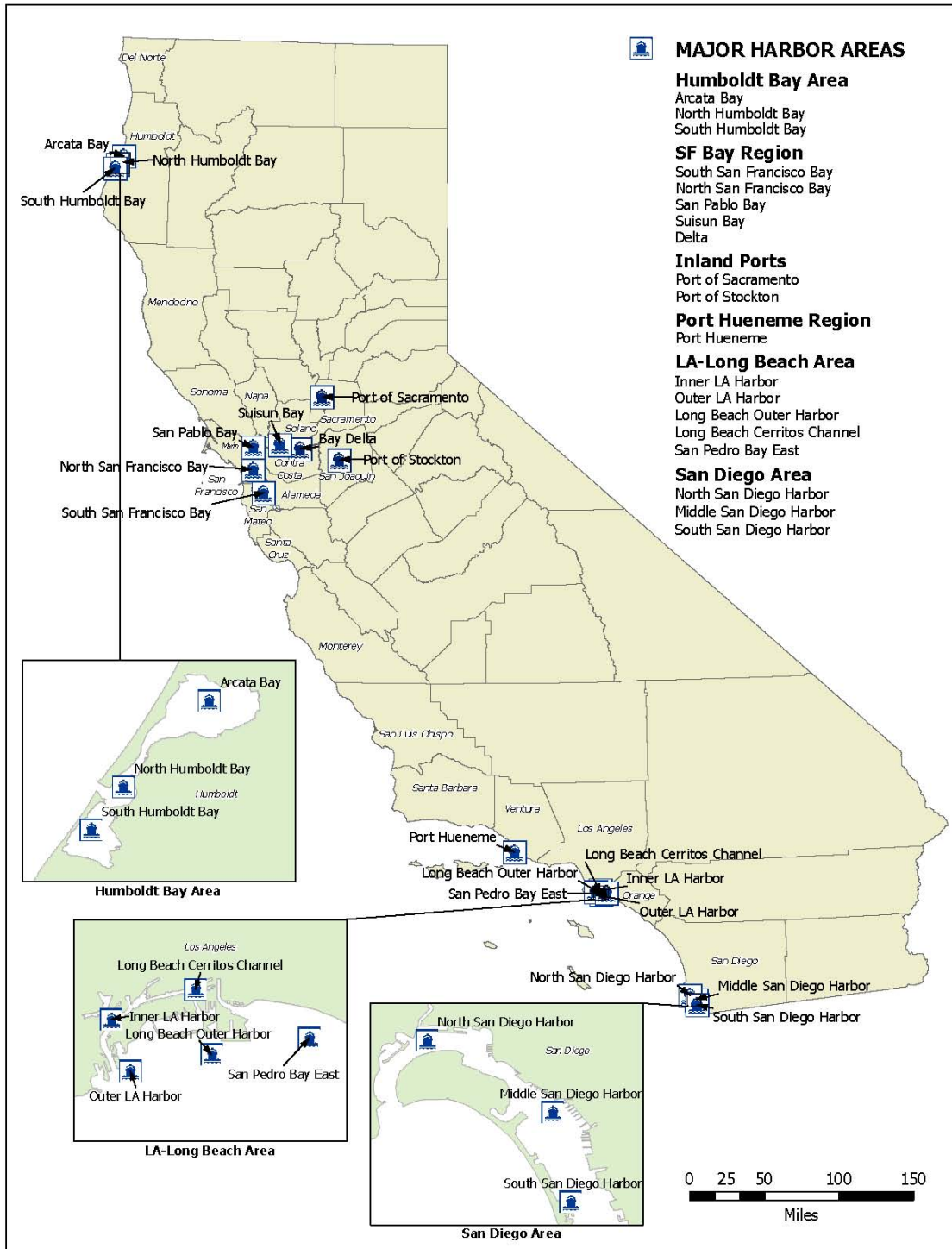


Figure 4. Harbors and bays sampled during MISP surveys.

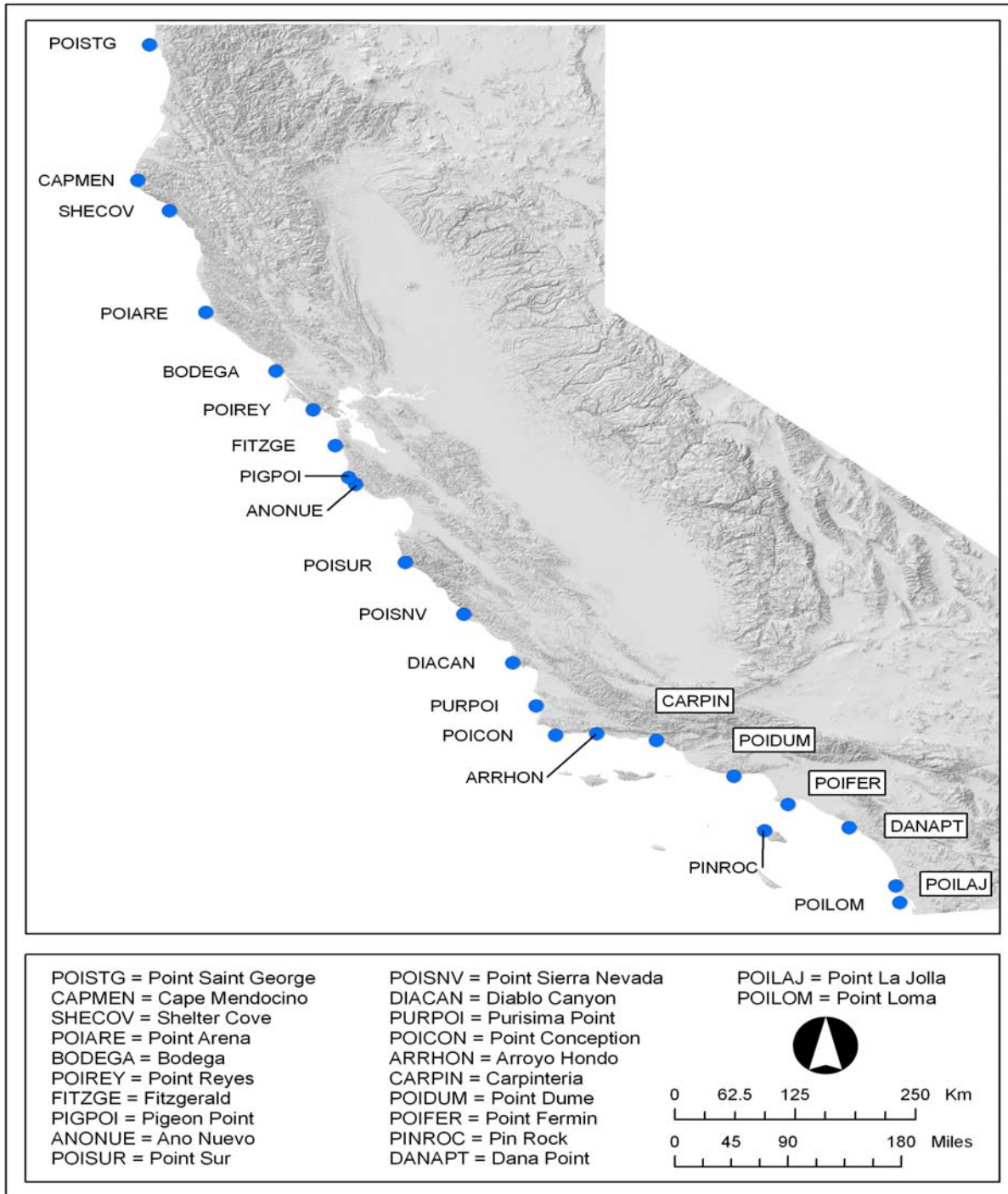
### 3.3 Outer Coast Surveys

DFG contracted with MLML to conduct the outer coast surveys. The initial field investigation for the outer coast was done in 2004 and 2005 with taxonomic identification work extending through much of 2006. A second survey was conducted in 2007 and 2008 (Table 2) to help address whether or not ballast water exchange initiatives have been successful in slowing the rate of species invasions. Literature and data reviews were complimented by field and laboratory studies jointly conducted by DFG/OSPR and San Jose State University Foundation's, MLML. Additional universities and specialized laboratories provided taxonomic expertise in identification of marine species.

The surveys covered 22 sites from Oregon to the Mexican border, focusing primarily on areas around the prominent coastal headlands (Figure 5). Given the vast expanse of the California coastline it was not possible to collect specimens from all locations. In an effort to limit the field work to a manageable number of sites, while still including a representative array of habitats and covering the 1100 miles of coastline, emphasis was placed on choosing sites most likely impacted by a discharge from ocean-going vessels traveling in near-shore waters. The coastal headlands seemed to be areas that best fit these criteria, since these are areas where species from a ballast discharge would likely wash ashore and become established. This study plan may be modified in the future.

Four habitats (rocky intertidal, sandy intertidal, rocky subtidal and sandy subtidal) were surveyed at 22 open coastal sites. While the basic sampling regime used in the previous surveys was retained, protocol details were adjusted for the current survey by MLML to accommodate for the more natural substrates found at outer coast habitats. The sampling design focused on whole community structure rather than singling out any one invasive species or habitat. Taxonomic experts and literature review for each phylum were often relied upon to help determine the status of species as introduced, cryptogenic, or native.

Outer coast rocky subtidal sampling focused on kelp forest habitat whenever possible to target high diversity communities. The sampling strategy encompassed multiple depths, intertidal zones, substrates and light exposure conditions due to habitat differences that could influence larval recruitment and colonization. At each of the 22 outer coast sites, epifaunal samples were collected quantitatively from rocky intertidal and subtidal substrate. When collecting infaunal samples, five quantitative benthic infaunal cores were collected for community analyses from the low intertidal, the high intertidal and subtidal sand in order to target as many habitats as possible at each site. Subtidal surveys were conducted via self-contained underwater breathing apparatus (SCUBA) at all sites unless white shark presence prohibited diving.



**Figure 5.** Sites sampled during Outer Coast Survey. Source: Maloney et al. 2007.

### 3.4 Zooplankton Sampling

To augment the sampling methods described above, we collected zooplankton in seven bays and harbors including Humboldt Bay, San Francisco Bay, the Port of Oakland, Port Hueneme, Los Angeles Harbor, Long Beach Harbor and San Diego Bay. Each harbor was sampled for zooplankton three times between March 2006 and September 2007 (with the exception of San Francisco Bay, which was sampled four times within that time period). Field sampling was conducted by DFG staff at all bays and harbors except San Francisco, where sampling was conducted by San Francisco State University/Romberg Tiburon Center (SFSU/RTC) staff.

Sampling in San Francisco Bay was conducted on the *RV Questuary* at twelve predetermined stations in South San Francisco Bay, Central San Francisco Bay, and San Pablo Bay. Six stations were located in the channel and six stations were located near the shoal. A 150 µm mesh net with a 50 cm diameter mouth was towed obliquely for three minutes by wire cable and winch at the six channel stations. The net was slowly dropped to one meter off the bottom, allowed to tow for two minutes at depth and then slowly returned to the surface. At the six shoal stations the zooplankton net was towed for three minutes at the surface. Vertical tows were made by hand at four sites at both shoal and channel locations within the Port of Oakland.

## 4.0 SPECIAL STUDIES

DFG funded special studies designed to detect NAS or improve our knowledge about geographic ranges of cryptic or poorly understood NAS.

### 4.1 Rip-rap Fish Sampling

The DFG 2002 report to the State Legislature identified one habitat as being undersampled: the crevices between the rip-rap of breakwaters. Rip-rap consists of boulders that are often used in harbors and jetties to reduce water erosion by dissipating the energy of flowing water or waves. The spaces between the rocks create habitat for fish and macroinvertebrates, but are difficult to sample by conventional methods. To investigate efficient sampling methods and which NAS are utilizing the habitat, DFG/OSPR funded a California State University-Northridge pilot study in 2008 to examine NAS near the southern breakwater of the Cabrillo Marina within Los Angeles Harbor in the city of San Pedro, California. At least two introduced species of gobies (*Tridentiger trigonocephalus* and *Acanthogobius flavimanus*) are known to already occur in this location.

The fish study was designed to compare the cost effectiveness of three sampling methods in detecting the presence and abundance of introduced species. The first is a quantitative method that has been used in previous nearshore fish

studies, which uses a specially designed enclosure net lifted by divers. Bleach then will be injected by syringe into the area through the netting. Because this method uses SCUBA, it is labor intensive. In addition, two types of passive collectors were used to collect cryptic fish and invertebrates: (1) Standard Monitoring Units for the Recruitment of Fish (SMURFs), and (2) standard cages modeled after “Crab Condos”, which were originally designed to catch mitten crabs. SMURFs are portable collection units made of an artificial substrate that are placed in the water column, and act as a safe haven for new settlers while protecting them from predators. The use of artificial substrate for collection units has been used widely by ecologists studying intertidal invertebrates and only recently been used to monitor settlement patterns in fishes. The cages consist of at least twelve vertically-oriented PVC pipes clustered in a honey-comb pattern to provide shelter space for cryptic fish and invertebrates.

#### 4.2 Watersipora Genetics Study

*Watersipora*, a type of colonial animal called a bryozoan, has a potential for rapid population growth and can be found on a variety of hard substrates including docks, pilings, rocks, vessel hulls, debris, and kelp holdfasts. *Watersipora* can grow rapidly by lateral growth of established colonies and settlement of short-lived larvae that may be retained near parents owing to short dispersal durations and has become a major space occupier in the protected bays of California. They facilitate other introduced species because they are resistant to anti-fouling paints and provide secondary substrata for other introduced species. When settled on ships’ hulls, they help carry other introduced species to harbors statewide (Geller et al. 2008).

The goal of this project was to survey variation in the genetics in *Watersipora* spp. collected along the California coast to determine the number of distinct species and their distribution. The study was conducted by Dr. Jonathan Geller of Moss Landing Marine Laboratories.

*Watersipora* present taxonomic difficulties that are typical of many invasive marine plants and animals, in that morphology alone is often insufficient for species identification. Multiple species of *Watersipora* are known to be widespread in California waters, but distinguishing among the different species based on morphological characters is difficult (Soule and Soule, 1976; Seo, 1999).

Sample colonies were collected on settlement panels or marina installations. After samples were collected, intact colonies were sorted, then DNA was extracted and sequenced. Sequence data was included for populations that were analyzed previously for phylogenetic and population analyses. A sample of colonies of ‘subovoid’ (or keyhole-shaped aperture) species were also analyzed morphometrically to determine whether a conventional series of measurements allowed resolution of divergent lineages distinguished by genetic sequence.



Colonies were photographed at 40x magnification under a dissecting light microscope and the scanned images were measured (Geller et al. 2008).

## **5.0 DATA ANALYSIS AND QUALITY ASSURANCE**

### **5.1 Analysis of Probable Vectors of Introduction**

In an effort to increase the number of taxa for which we can identify a probable pathway (vector) of introduction, DFG contracted with the SERC to research probable vectors for California NAS. DFG previously assigned vectors to the extent that this information was available in the literature, but there were widespread gaps in the published information on many of the taxa in our database. Since this information is critical to summarizing the extent of the introduced species introductions, these data gaps impair our ability to address the key questions well. The methods used in this vector analysis are described in Appendix A of this report. Results of the study are the basis for the analysis of introduction vectors in the Results and Discussion section of this report.

### **5.2 Collaboration and Data Sharing**

DFG collaborates with other agencies and organizations conducting similar surveys for NAS in coastal waters and shares data generated by these studies to maximize financial and personnel resources. One such collaboration has been with the U.S. Fish and Wildlife Service (USFWS). In 2005, USFWS conducted a survey for NAS in San Diego Bay and in the fresh water areas of the Sacramento/San Joaquin Delta. MLML was the principal investigator for USFWS. The data generated for those areas was incorporated into California Aquatic Non-native Organism Database (CANOD) and the data generated by the DFG surveys of the remaining areas of the coast will be shared with USFWS for use in their programs.

The database for this report (CANOD) also includes information obtained from the U.S. Environmental Protection Agency's Western Environmental Monitoring and Assessment Program (WEMAP). WEMAP is a regional program designed to collect coastal and estuarine samples from the states of California, Oregon, and Washington. In California, infaunal samples were collected along the length of the State and at various depths.

Information collected by DFG field surveys is shared with other agencies that have regional or national NAS databases. One example is the Pacific Coast Ecosystem Information System (PCEIS), a georeferenced database of the native and nonindigenous marine/estuarine species and coastal landscape characteristics for Washington, Oregon, and California, administered jointly by the United States Environmental Protection Agency (U.S. EPA) and United States Geological Survey (USGS). The SERC has developed and maintains a national database of marine and estuarine invasions of the continental U.S. and

Alaska called National Exotic Marine Estuarine Species Information System (NEMESIS). Data are shared between DFG and SERC and substantial efforts have been made to standardize lists of California introduced species.

### 5.3 Quality Assurance

DFG's list of introduced species underwent an extensive review by the SERC during 2007 and 2008, which involved an intensive comparison of taxonomic records and invasion status to identify and distinguish introduced from cryptogenic and native species. SERC's review of the CANOD species list included literature-based research, discussion with taxonomic experts, and discussion with aquatic invasion experts. Additional partial review was done by Dr. James T. Carlton.

### 5.4 Terminology

We categorized the introduction status of taxa as "introduced," "cryptogenic," or "unresolved." Introduced species are those plants and animals that are living outside their natural geographic boundaries. A variety of terms have been used to describe plants and animals that are living outside their natural geographic boundaries including, aliens, exotics, non-natives, introduced species, and invasive species. The term "invasive" is generally reserved for species that are likely to cause economic or environmental harm or harm to human health. Although the term "invasive" does apply to many species in California, we limit its use in this report since impacts of many introduced species are unknown or speculative.

Cryptogenic species are those that are neither demonstrably native nor introduced (Cohen and Carlton 1995, Carlton 1996). These species have been identified but their native range or region is unknown. In some cases, specimens could not be identified to a sufficiently low taxonomic level (usually species) to classify as native or nonindigenous, so were conservatively assigned "cryptogenic" or "unresolved" status. For these unresolved taxa, introduction status was determined on a case-by-case basis. In some instances, if we were confident that all the species from a particular genus were introduced to California, we assumed that any species from that genus found in California was introduced. The introduction status of each organism was based on documented research, personal communication with taxonomic experts, and communication with introduced species experts.

We labeled specimens that could not be identified unambiguously to species level as "unresolved." Many genera identified in the study have at least one species that is indigenous to California. Thus, it was often unclear whether an organism identified as "*Genus sp.*" represents a unique (distinct) species and/or whether that species is native or introduced. Unless otherwise noted, summary figures reflect only those organisms that have been identified to species level,

which results in a somewhat conservative listing of introduced and cryptogenic species.

## **6.0 RESULTS AND DISCUSSION**

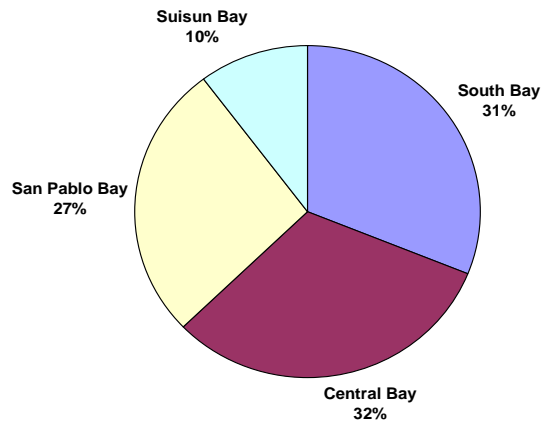
### **6.1 Summaries of Field Surveys**

#### **6.1.1 San Francisco Bay Survey - 2005**

From the samples collected during the 2005 field survey, 513 species were identified, of which 88 (19% of all species identified) were classified as introduced, 98 were classified as cryptogenic, and 327 were classified as native to California. In addition, another 316 taxa were collected which could not be identified to species level and were classified as unresolved. The majority of unresolved identifications were annelids (marine worms). Juvenile or non-reproductive specimens represented about half of the unresolved identifications. Unrecognized species and damaged specimens were other factors responsible for unresolved identifications.

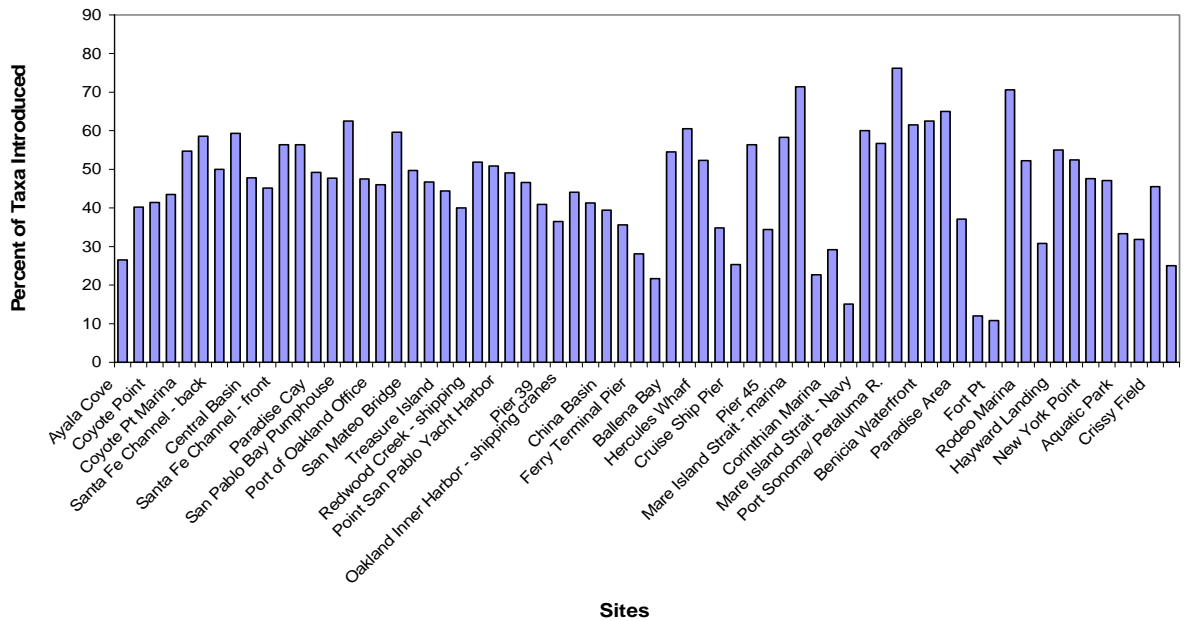
The NAS list from the current survey was compared to a list of NAS in San Francisco Bay from the Department of Fish and Game's statewide database (CANOD). Many of the species found historically in the Bay were not identified in the current study in part because the sampling methods employed in this survey tend to miss pelagic organisms, such as hydromedusae and mysids. Another group that was poorly sampled includes mobile or migratory species, such as the mitten crab, *Eriocheir sinensis*, and gastropods, including nudibranchs. Excluding strictly freshwater species, fish, vascular plants, and phytoplankton, 70 of the species found in the database were identified in the current survey, but 52 were not. An additional 44 NAS that were not previously listed in CANOD were identified during the survey. No new invaders to San Francisco Bay were found during the survey.

More introduced species were found in the South and Central bays than in San Pablo and Suisun bays, even after standardizing for the number of samples taken in each (sub)bay (Figure 6). This phenomenon appears to be due at least in part to a bias in sample design. The Central Bay had seven of the survey's ten subtidal rocky samples, which tended to have the highest number of introduced (and native) species. Intertidal rocky samples yielded 6.9 introduced species per sample (69 species in ten samples) compared to an average of only 2.7 introduced species per intertidal sandy sample and about 1.0 introduced species per sample for subtidal samples. Therefore, it is likely that the relatively high number of rocky intertidal samples in the Central Bay contributed the higher numbers of introduced species found in this sub-region. This does not explain the relatively high numbers of introduced species in South San Francisco Bay, however.



**Figure 6.** Percent of introduced species per San Francisco Bay Sub-region.

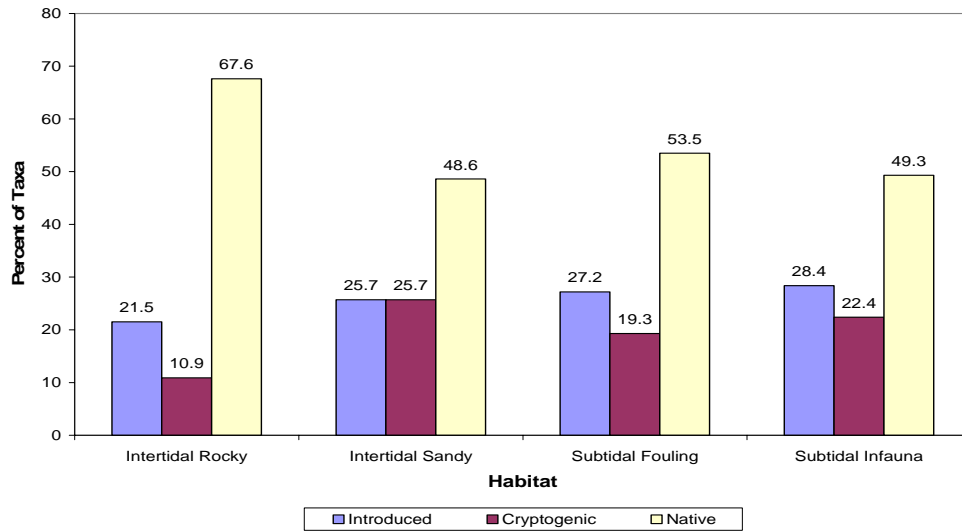
The site at Port Sonoma on the Petaluma River had the highest percentage of introduced species (76.2%) (Figure 6). The two sites at Mare Island also had very high percentages of introduced species (71.4% and 63.0%).



**Figure 7.** Percent of total taxa introduced at San Francisco Estuary sites.

Subtidal habitats tended to have a higher percent of introduced species (Figure 8). Epifaunal habitats (intertidal rocky and subtidal fouling) had the highest

numbers of introduced species (69). Intertidal rocky habitat samples produced more total taxa than other habitats and had the highest percent of native species.

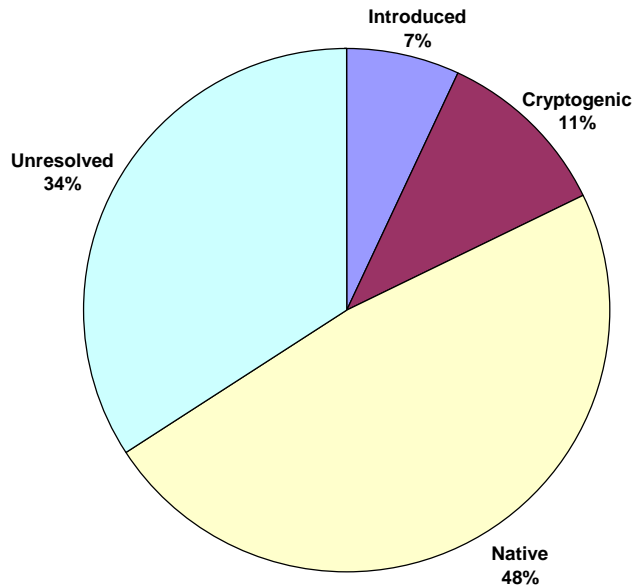


**Figure 8.** Percentage of total taxa within each classification for each habitat type sampled.

The higher number of introduced species found in epifaunal habitat may be due, in part, to greater sampling effort in epifaunal habitat than in infaunal habitat. Additionally, an on-site, qualitative visual search conducted via SCUBA accompanied surveys in epifaunal habitat but not in infaunal habitat. Several species were identified from the visual searches in epifaunal habitat which were not detected in the quantitative samples collected from the same sites and habitats. Investigation into possible habitat type preferences for introduced species may help explain the trends observed in the current survey. Although challenging, a quantification of the total available area of each type of habitat might provide insight into differences between numbers of species found among habitat types

### 6.1.2 Survey of California Bays and Harbors - 2006

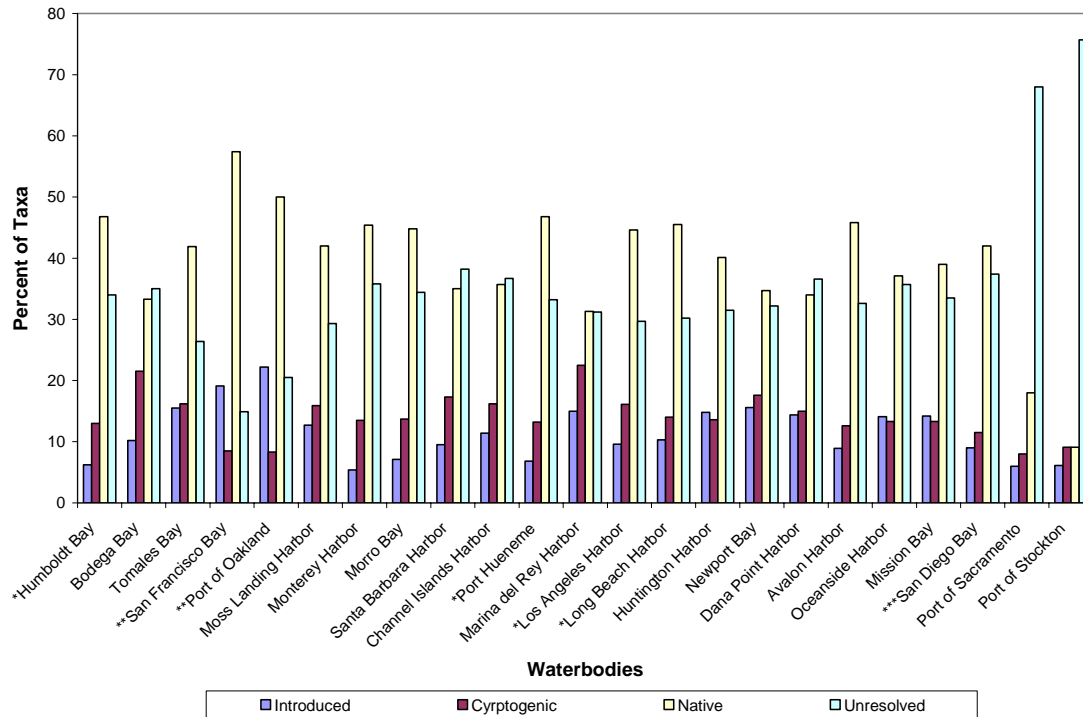
From the samples collected during the 2006 field survey, a total of 775 species were identified, of which 82 (7%) were classified as introduced, 126 (11%) as cryptogenic and 567 (48%) as native to California. In addition, 402 (34%) different taxa were not resolved to the species level (for reasons discussed below), and have been classified as unresolved (Figure 9). Several of the unresolved taxa are identified to the genus level and are listed with a provisional species name. No new introduced species were discovered during this survey.



**Figure 9.** Percentage of total taxa identified for each classification.

Figure 10 lists all the bays and harbors surveyed and the percentage of taxa identified within each introduction status classification. It is important to note that different combinations of habitats were sampled at the different harbors, as indicated by the asterisks in the figure, so direct comparisons between bays based on this table should be made cautiously. Figure 10 also includes results from a survey of San Diego Bay conducted in 2005 by MLML/USFWS (Maloney et al., 2007). For the 2005 survey of San Diego Bay, epifaunal and infaunal habitats were sampled using the same sampling protocol used in the current survey.

In the marine bays and harbors where infaunal and epifaunal habitats were sampled (and for some harbors, water column for zooplankton as well), introduced species ranged from a low of 14 species (at two different harbors) to a high of 44 species at San Diego Bay, and represented 5.4% to 15.6% of the total taxa collected from each harbor. Also in the marine harbors, cryptogenic species ranged from 19 species collected in Oceanside Harbor to 57 species collected in Los Angeles Harbor, representing 12.6% to 22.5% of total taxa at each site. Native species in marine harbors ranged from 50 to 204 species collected, representing 31.3% to 57.4% of total taxa collected from each harbor, while up to three taxa were classified as unresolved complex from each marine harbor, representing 0.3% to 1.3% of the total taxa in each harbor.



**Figure 10.** Percentage of taxa identified from samples for each classification in each harbor where infaunal, epifaunal and water column samples were collected.

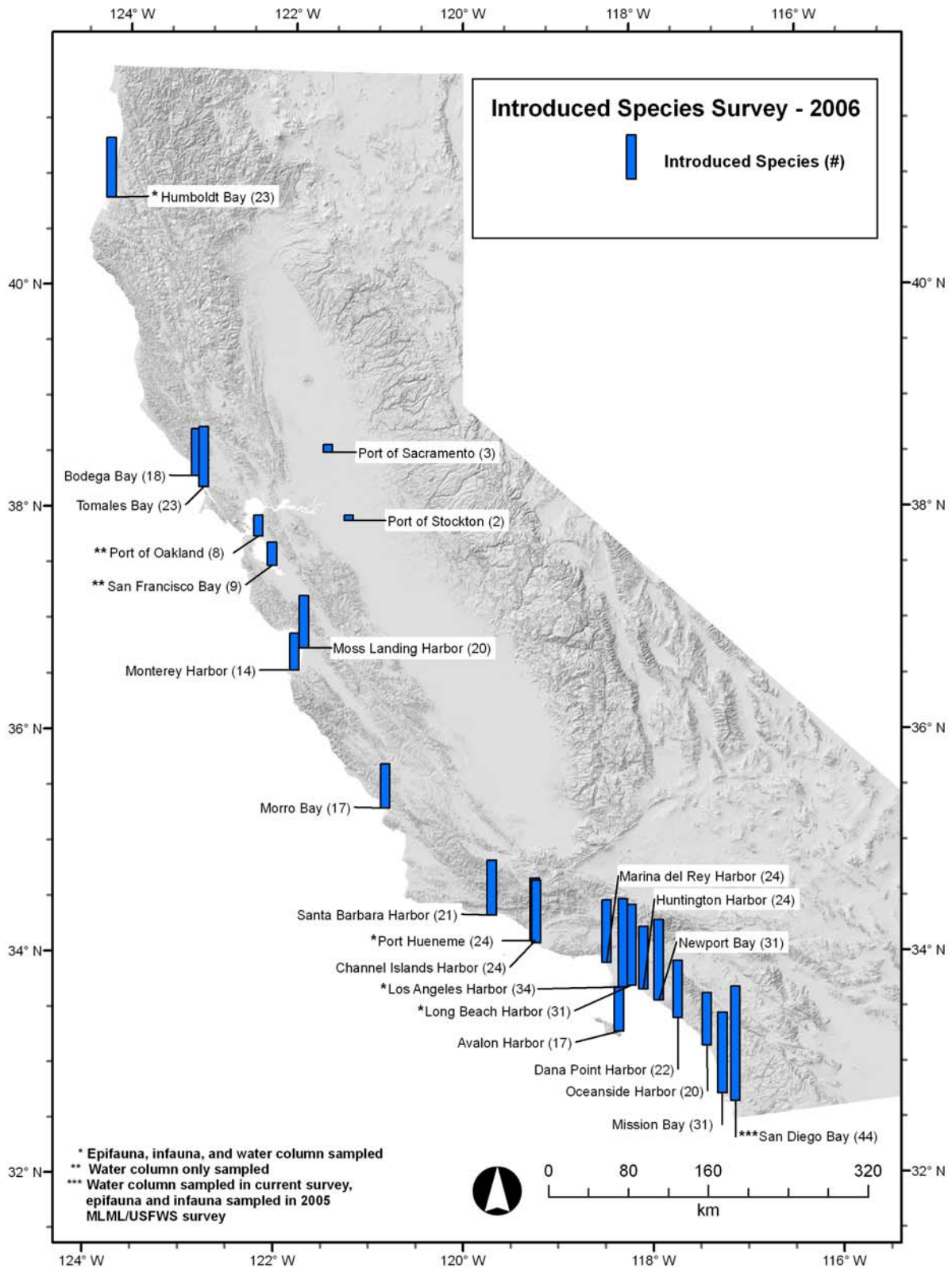
\* includes sites where the water column was sampled for zooplankton

\*\* only includes sites where the water column sampled for zooplankton

\*\*\*includes water column results from current survey as well as epifaunal and infaunal results from 2005 MLML/USFWS survey of San Diego Bay.

No strong trends were observed between the bays and harbors, although southern California had a higher average number of introduced species than northern and central California bays and harbors. The two phyla with the highest number of introduced species from the epifaunal and infaunal samples were arthropoda (25 introduced species) and chordata (18 introduced species). The only phylum in which introduced species were identified from the water column surveys was arthropoda, which had 11 introduced zooplankton species.

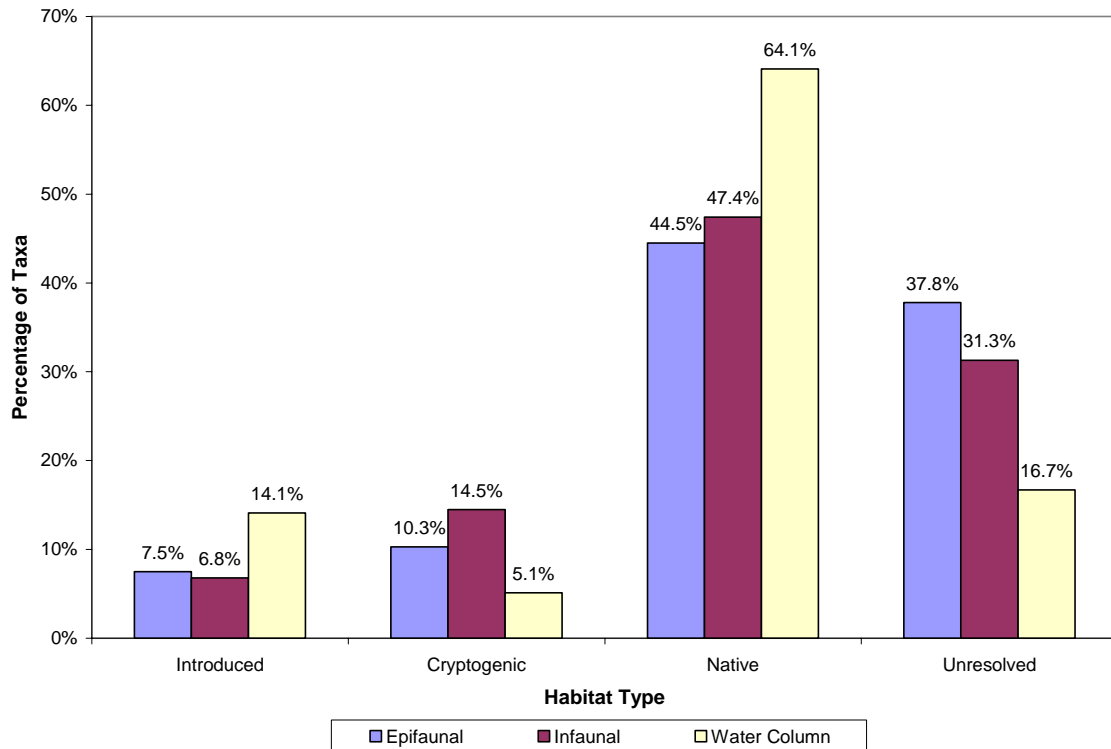
The number of introduced species found at each of the 2006 Bays and Harbors survey sites is shown in Figure 11. Freshwater ports had far fewer introduced species than did the marine bays and harbors. Sites with the highest numbers of introduced species tended to be in southern California, although direct comparison of sites is difficult, due to differences in habitats sampled and number of samples per site. The most species per site in northern and central California was 24, whereas five different harbors in southern California had over 30 introduced species. Since only water column sampling was done in San Francisco and Port of Oakland, it is possible that the percentage of taxa in each category reported for these sites is biased, since most other sites include data from multiple habitats.



**Figure 11.** Number of introduced species identified from each of bays and harbors surveyed. Source: Maloney et al. 2007.



Figure 12 shows the percentage of total taxa within each classification (introduced, cryptogenic, native and unresolved) for the three different habitats (epifaunal, infaunal, and water column). The highest number of overall species collected were from the epifaunal habitat (884), followed by infaunal (456) and water column habitat (78). Likewise, more introduced and cryptogenic species were identified from epifaunal habitat (66 introduced, 91 cryptogenic), followed by infaunal (31 introduced, 66 cryptogenic) and water column habitats (11 introduced, 4 cryptogenic). Although the number of introduced species identified from epifaunal samples was over twice the number of introduced species identified from infaunal samples, the percent of total taxa represented by introduced species was relatively similar for the two habitats. The percentages of introduced and native zooplankton taxa were higher than the percentages from epifaunal and infaunal habitats for those classifications. In contrast, the percentages of cryptogenic and unresolved zooplankton taxa were lower than what was seen in the epifaunal and infaunal habitats.



**Figure 12.** Percentage of total taxa within each classification (introduced, cryptogenic, native, and unresolved) found in three different habitats (epifaunal, infaunal, and water column).

Introduced zooplankton species ranged from a low of one in Port Hueneme to a high of nine species in San Francisco Bay, and represented 2.6% to 22.2% of the total zooplankton taxa collected from each harbor. Cryptogenic species ranged from a low of two species to a high of four species, and represented 5.4% to 8.6% of the total zooplankton taxa collected from each harbor. The number of

native zooplankton species collected from the water column was higher than any other introduction classification in each harbor sampled. Native species ranged from 18 to 28 species per harbor, and represented 50% to 74.3% of the total zooplankton taxa collected from each harbor. Unresolved taxa were collected from each harbor sampled, while taxa classified as unresolved complexes (not morphologically distinguishable) were collected from only two harbors sampled.

For a variety of reasons, some specimens collected in the survey were unable to be identified to species level. Of these unresolved identifications, approximately 53% were due to juvenile specimens or specimens without reproductive characters that are necessary to differentiate species. Approximately 21% were due to undescribed or unrecognized species, 6% were due to damaged specimens (presumably damaged during the collection process), 15% were both juvenile and damaged, and 5% were due to other reasons which were not specified by the taxonomists. This information was used to modify sampling procedures in subsequent surveys, particularly to attempt to reduce the number of damaged specimens in certain groups of organisms.

### 6.1.3 Outer Coast Surveys - 2004 and 2007

During the 2004 survey, 166 epifaunal samples (rocky substrate scrapings) and 321 infaunal samples (sandy cores) were collected along California's outer coast from 22 sites. In addition, a total of 208 qualitative samples were collected during the visual scans at the 22 outer coast sites. There were 63 sediment samples also collected for grain-size analysis.

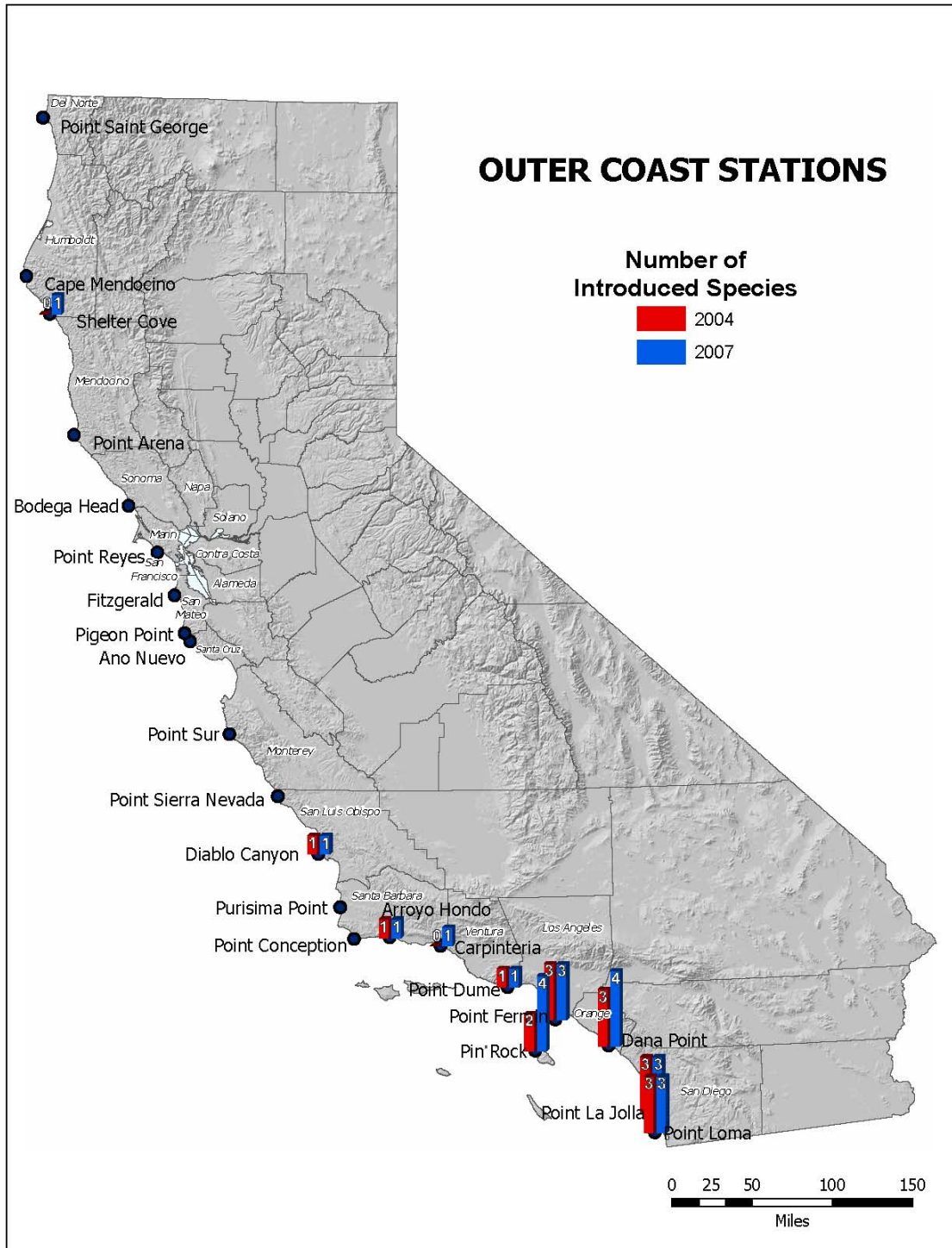
Of the 1,222 species identified from the 2004 field collections, four species were classified as introduced, 147 as cryptogenic, and 1,069 as native. There were 618 additional unique taxa not identified to species level, which were classified as unresolved. The group with the highest number of taxa not identified to species level (and therefore classified as unresolved) was annelids; the presence of juvenile and/or non-reproductive specimens was the main factor that limited their identification. Additionally, there were eight taxa that were classified as unresolved species complexes.

From the samples collected during the 2007 field surveys, a total of 956 species were identified, of which eight were classified as introduced, 144 were classified as cryptogenic and 802 were classified as native to California. There were also 474 taxa which were classified as unresolved and five taxa were classified as unresolved complexes. Introduced species were found at only ten of the 22 sites and up to four introduced species were found at one site. On average, only slightly higher numbers and percentages of introduced species were identified from sites in southern California.

Only 2 species, *Sargassum muticum* and *Caulacanthus ustulatus*, were collected at the same sites in both 2004 and 2007. The combined results of the two

surveys indicate that most introduced species were found at southern California sites (Figure 13).

A comparison of the outer coast survey results to those from the Bays and Harbors 2000-2001 survey revealed that there were more cryptogenic and native species and fewer introduced species on the outer coast. Also, introduced species comprised a lower percentage of the total species identifications in the open coast relative to bays and harbors. Overall, 1,222 species were identified in the 2004 outer coast survey as compared to 818 in the Bays and Harbor survey. There was very little overlap of introduced species at individual sites between the two surveys; only six of the 26 outer coast introduced species were identified in the Bays and Harbor survey (Table 4).



**Figure 13.** Number of introduced species found at each station in 2004 and 2007 Outer Coast Surveys. All stations w/o bars had no introduced species during either survey.

**Table 4.** Occurrence of introduced species at Outer Coast Survey sites, 2004 and 2007.  
P = present.

Station Name	Year / Taxon											Year	Number of Species at Station																							
	2004	2007	2004	2007	2004	2007	2004	2007	2004	2007	2004			2007																						
	Heterokontophyta		Sargassum muticum		Sargassum filicinum		Rhodophyta		Caulacanthus		Lomentaria ustulatus		Amelita hakodatensis		Hydroides elegans		Mollusca: Polychaeta		Mollusca: Bivalvia		Annelida		Moluscopoda: Cerithiidae		Moluscopoda: Amphipoda		Ectopoda		Watersipora sp A Schroeder		Chordata: Ascidiacea		Bohrillus schlosseri			
Point Saint George	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				
Cape Mendocino	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
Shelter Cove	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
Point Arena	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
Bodega	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
Point Reyes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
Fitzgerald	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Pigeon Point	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Ano Nuevo	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Point Sur	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Point Sierra Nevada	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Diablo Canyon	P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Purisima Point	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Point Conception	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Arroyo Hondo	P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Carpinteria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Point Dume	P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Point Fermin	P	0	P	P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dana Point	P	0	P	P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pin Rock (Santa Catalina Island)	P	P	P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Point La Jolla	P	0	P	P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Point Loma	P	0	P	P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Number of Sites Where Present	8	0	4	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	7	1	5	1	1	1	2	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

#### 6.1.4 Estuarine vs. Outer Coast Level of Invasion

A comparison of the outer coast survey results to those from the Bays and Harbors 2000-2001 survey revealed that there were far fewer introduced species on the outer coast than in estuaries. Also, introduced species comprised a lower percentage of the total species identifications in the open coast relative to bays and harbors. Overall, 1,265 species (including introduced, native, and cryptogenic) were identified in the outer coast survey as compared to 818 in the Bays and Harbor survey.

Wasson et al. (2005) outlined a number of factors that contribute to the higher invasion rate seen in estuaries: (1) many estuaries are subject to intensive shipping and thus a high potential infection rate, (2) brackish, estuarine species have a better chance of being transported alive than other species due to their physiological tolerances, (3) estuaries have lower natural species diversity and abundance compared the outer coast, therefore more invasive species are able to establish in estuaries, and (4) estuaries receive invasion pressure from both ocean and inland aquatic species (Nehring 2006). California estuaries are also geologically young, heavily modified by humans, and are exposed to several introduction vectors.

### 6.2 Special Study Results

#### 6.2.1 Rip-rap Fish Sampling

Preliminary results from sampling with SMURFs and cages indicate that one introduced fish was captured, the chameleon goby, *Tridentiger trigonocephalus*. Many native fish and invertebrates were captured by the traps, but fish may have been displaced by small octopuses, which were found in relatively high numbers in the cages. For this reason, the cages may have greater utility in areas with lower salinity.

#### 6.2.2 Watersipora Genetics Study

Whereas traditional taxonomic methods could only detect two species of *Watersipora*, the genetics study results confirmed there are three species of *Watersipora* in California (*W. arcuata*, *W. subtorquata*, and *Watersipora* “new sp.”) which are almost certainly introduced to California. Results also show evidence that *W. arcuata* and *W. subtorquata* have been transported widely within California.

The genetics study also revealed patterns of abundance and geographical distribution. *W. subtorquata* was the most abundant of the three species; it was found at 12 of 18 sites examined throughout the State. A single *W. arcuata* colony occurred in the Oceanside sample, while the species was not found in samples between Oceanside and Mission Bay. The new species of *Watersipora*

occurred in samples from Bodega Bay to Marina Del Ray and was the only species collected at Bodega Bay and Morro Bay. The new species was apparently not abundant prior to the last decade given the absence of historical records of *Watersipora* in sites where it is now common, suggesting it is a recent cryptic invasion (Geller et al. 2008).

*Watersipora* specimens have been reported from many parts of the world. The native region of *W. subtorquata* is unknown, but its distribution and spread suggests the northwest Pacific as the most probable origin (Geller et al. 2008). Transport as fouling on ship hulls is the most likely vector of introduction, as *Watersipora* has been frequently found both in fouling and on ship bottoms and is highly tolerant of copper-based anti-fouling compounds. *Watersipora* species have larvae which remain in the plankton stage for less than a day before settling, so they could not have been transported long distances as larvae in currents or in ballast water. The scenario of introduction for introduced species can sometimes be reconstructed from genetic studies. Genotyping of colony populations on a sample of ship hulls, and delineation of haplotype diversity and spatial segregations of haplotypes in native ranges, represent two projects necessary for reconstructing links between populations, ports, and the global dispersal by ships and other vectors (Geller et al. 2008).

Further sampling at multiple locations and habitats is necessary to better understand potential patchiness in distribution. Ten of 19 sites had only one species - these sites should be sampled more exhaustively to verify the absence or scarcity of other species. A geographically patchy distribution of *Watersipora* "new sp." suggests potential saltatory dispersal (jumps in distribution), perhaps through small boat movement. Continued sampling is necessary to reveal temporal patterns of distribution as affected by anthropogenic and natural processes. Species distribution appears to be strongly influenced by latitudinal environmental gradients, perhaps most importantly by temperature, implying that *Watersipora* may be a good model for studies of distributional changes (and underlying causal mechanisms) driven by climate change (Geller et al. 2008).

## 6.3 Occurrence of NAS

### 6.3.1 State-Wide Totals

The state-wide totals summarized in this section show the number of individual taxa recorded during the sampling effort or identified in the literature. Although we attempted to sample or record information for a broad range of habitats, it was not possible to sample in all subtidal and intertidal habitats or include all communities in the study design. As a result, the numbers presented here may, to a certain extent, underestimate the true populations of NAS.

A total of 307 NAS have been identified from the literature and field investigations. Arthropods (crabs, shrimp, etc.) were the dominant phylum, comprising 27% of the species identified. A total of 83 introduced arthropods have been identified from the marine and estuarine waters of the State. Amphipods were the most common group of arthropods identified during this study. Chordates (fish, sea squirts, etc.) were the second most numerous phylum identified, comprising 22% of the species. Many of the fish species were identified from freshwater habitats, including the Sacramento-San Joaquin Delta and the location of two primary study sites, the Ports of Sacramento and Stockton. About 17% of California NAS are annelids (aquatic worms), primarily polychaete worms.

Although the previous DFG report (2002) listed 360 NAS along the California coast, some of those species were subsequently re-identified or reclassified as native, cryptogenic, or unresolved, or are considered part of species complexes. Still others were found to be synonyms of other species on the NAS list. These changes were made after extensive review and research of each species in the NAS database. Another factor explaining the discrepancy between this report and the 2002 report is the types of organisms that were included in each report's list; this report excluded some species that were previously included, such as most vascular plants (e.g. *Spartina*). Therefore, the apparent reduction in NAS between 2002 and 2008 is entirely an artifact caused by the species list changes described above.

Field surveys and literature sources indicate that there are 453 cryptogenic species. These are specimens that were identified to the species level but not enough is known about them to unambiguously determine if they are introduced or native. 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. Sixty percent of cryptogenic species (273 species) were annelids. A total of 82 cryptogenic arthropods (18%) were identified.

In addition to the combined 760 species classified as introduced or cryptogenic, another 1,362 taxa were identified as unresolved. For reasons described earlier, these taxa could not be identified to the species level with any degree of certainty. Additionally, though not a focus of our field surveys or research, our database (CANOD) contains location information on 1,884 native species sampled in our field surveys.

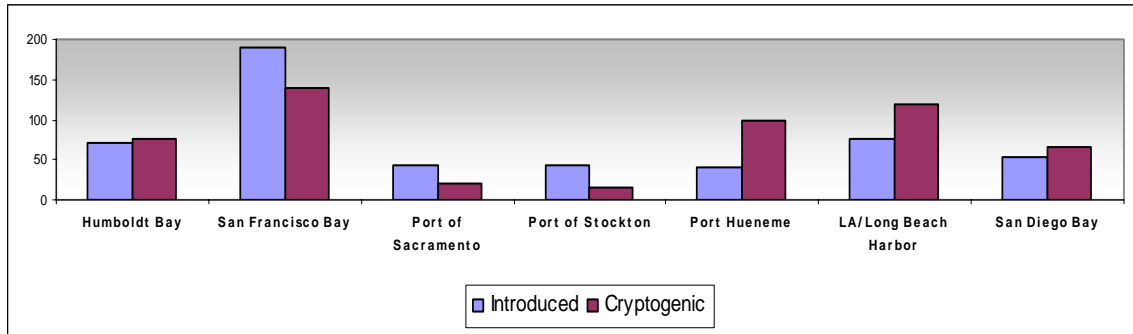
There are a substantial number of species in California's coastal waters that are clearly introduced to the habitats where they were found. There are also, however, a large number of species that are possibly introduced, but must be analyzed further. For a detailed list of all the species included in this summary and their status of introduction, see Appendix C.



### 6.3.2 Major Harbor Areas

Based on the entire compilation of NAS data, all the major harbor areas in California have received significant NAS introductions (Figure 14). Each major commercial harbor area of the state had between 40 and 190 species that are classified as introduced and another 15 to 138 species that are possibly introduced (cryptogenic). The entire list of introduced and cryptogenic species is presented in Appendix C of this report.

San Francisco Bay had the greatest number of NAS. The major harbor areas had a number of NAS in common. Both Humboldt Bay and LA/LB Harbor had 55 NAS in common with San Francisco Bay; 43 of the NAS found in LA/LB Harbor were also found in San Diego Bay. The Ports of Sacramento and Stockton had 36 of their 43 NAS in common. However, quantitative comparisons among ports or bays are problematical because sampling methods, seasons, and effort varied considerably among the different studies.



**Figure 14.** Introduced and cryptogenic species in major port regions. Includes literature-based records and non-DFG field surveys.

### 6.3.3 Minor Harbors

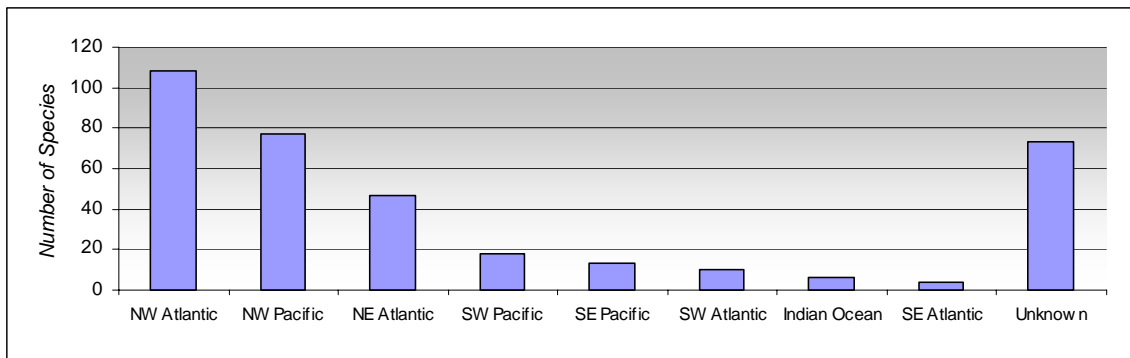
Substantial numbers of introduced taxa were found in the smaller ports and bays. Over 101 introduced species were found in the Sacramento-San Joaquin Delta (the brackish upstream portion of the San Francisco Estuary) and over 50 NAS were identified in Elkhorn Slough (Table 5). Tomales Bay, Mission Bay, Newport Bay, and Channel Island Harbor also contain high numbers of NAS.

**Table 5.** Numbers of introduced and cryptogenic species in minor harbors.

Bay	Introduced	Cryptogenic
Bodega Bay	33	51
Tomales Bay	46	47
Delta	101	62
Moss Landing Harbor	23	31
Elkhorn Slough	52	9
Monterey Harbor	27	40
Morro Bay	32	49
Marina del Rey Harbor	29	45
Santa Barbara Harbor	22	46
Channel Islands Harbor	35	43
King Harbor	11	3
Alamitos Bay	22	4
Huntington Harbor	28	35
Anaheim Bay	12	5
Newport Bay	37	45
Dana Point	23	27
Oceanside Harbor	28	29
Agua Hedionda Lagoon	10	6
Mission Bay	38	35
Avalon Harbor	18	36

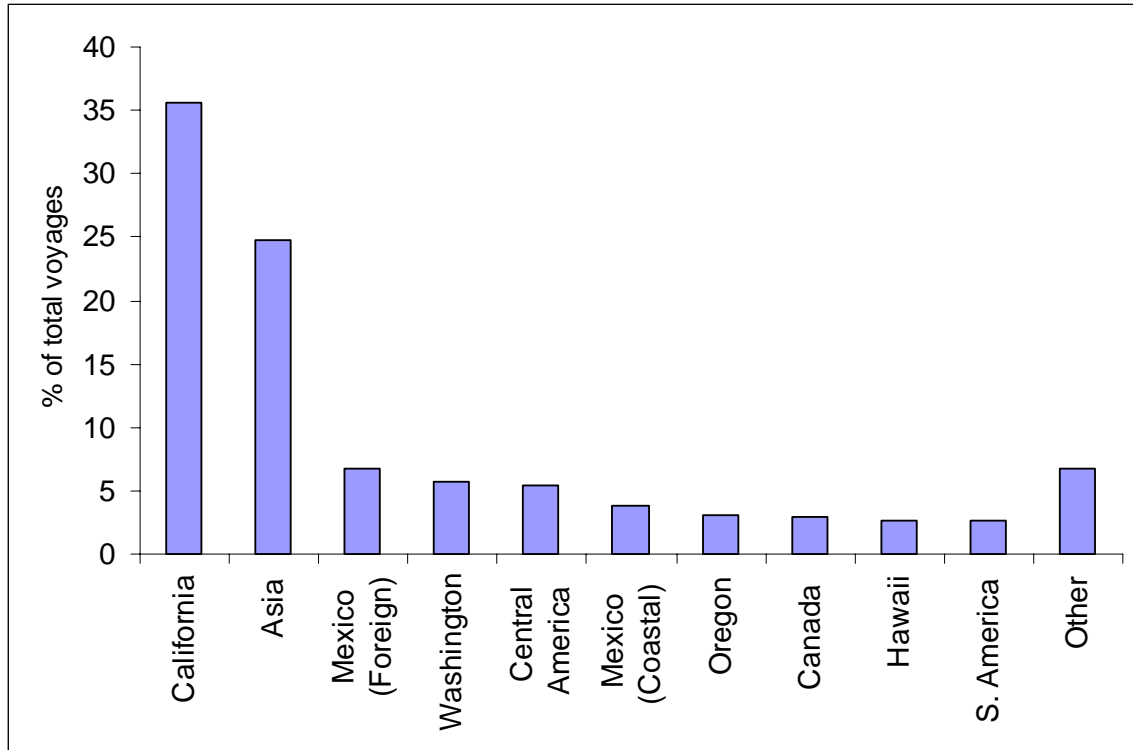
#### 6.4 Regions of Origin of Introduced Species

Figure 15 summarizes the number of introduced species that originate from various regions of the world organized by major oceanic quadrants. The majority of the species introduced to California appear to be native to the northwest Atlantic, the northwest Pacific, and the northeast Atlantic. The number of species with unknown origins is substantial, however, and data regarding the region of origin for many species was non-specific or speculative. Some species are included in each of the regions of possible origin identified in the literature. This approach has limitations, but provides a general sense of the potential regions from which the introduced species do or at least can originate. This area requires substantial additional research before confident conclusions can be made about regions of origin and their relationship to vectors of introduction.



**Figure 15.** Native regions of California introduced species.

The major regions of origin are areas of the world from which California receives a considerable amount of ship traffic as well as the source materials for much of our aquaculture. Figure 16 shows the percent of voyage arrivals in California by last port of call region. About 25% of arrivals are from Asian ports. Ruiz and others (2000) found that most marine invasions to the West Coast originated from the Indo-West Pacific (including Western Pacific) and Western Atlantic and that introduction routes correlate to the principal trade corridors. Oysters culture from the Atlantic is responsible for many of the introductions from that region.



**Figure 16.** Percent of voyage arrivals in California ports by last port of call region from July 1, 2006, through June 30 2008. Source: California State Lands Commission.

Although native range information can indicate where species originate, it can't tell us if they were introduced to California directly from their native region or from some intermediate location. To make this determination, information on source region (the probable area from which an introduction occurred) is needed and should be researched.

## 6.5 Vectors of Introduction

At least 13 different vectors have been implicated in the introduction of new species to California. These vectors can be grouped into broad vector classes (e.g. Shipping). Appendix A lists those vector classes and briefly describes each vector.

Introduced species were assigned probable vectors of introduction for each bay in which they were found and those vectors were summed to give statewide totals for each vector category (Table 6). However, determining vectors for any unintentional introduction with absolute certainty is impossible, and so vectors are determined on the basis of probability. Discussion of the role of various introduction vectors is complicated by the fact that many taxa are polyvectic, a term used by Carlton and Ruiz (2005) to describe species introduced by more than one vector. Some species have been introduced to California waters more than once and by more than one mechanism, so multiple vectors were assigned to those species.

Fouling was the vector that was most often attributed to the introduction of species to California. In most cases, it was impossible to distinguish between fouling due to recreational boats and fouling due to commercial ships. In major ports and waters adjacent to ports either vector is possible, so both vectors were listed for species found in these areas. Statewide, 165 species have fouling as a probable vector. Among those 165 species, 153 (93%) had both recreational boats and commercial ships as possible sources of introduction.

The previous DFG NAS report (DFG 2002) also assigned vectors to introduced species found in California. In that analysis, the largest vector category was “unknown.” The recent SERC vector study for DFG (unpublished data) has greatly reduced the number of species with unknown vectors. In contrast with the 2002 results which identified ballast water as the leading vector of introductions, we now see the fouling vector as the one most responsible for introductions into California coastal waters. Ballast water, however, is still a leading vector of introductions. Ship ballast water discharge was the second largest category of potential vectors and, together with fouling from ship’s hulls, indicates that shipping plays a substantial role in dispersal of species.

The 2001-2002 DFG study also found that shipping was the main probable vector responsible for introductions of coastal aquatic species in California before 2002. Similarly, Ruiz and et al. (2000) found that shipping was the sole vector for 51% of initial North American invasions and 59% of the repeat invasions. However, the relative contribution of the ballast water and hull fouling subvectors is extremely difficult, if not impossible to distinguish (Fofonoff et al. 2003). It is obvious that shipping traffic continues to play a significant role in dispersal of new species into California waters through a combination of ballast discharges and hull fouling.

Hull fouling, which is a dominant source of introductions in many harbors, appears to have had less of an impact in the Sacramento/San Joaquin Delta and Inland Ports. It is likely that low salinity is a limiting factor for marine fouling organisms, acting as a barrier to survival. Freshwater exposure has been used as an effective means of eliminating marine fouling organisms from ship’s hulls (Brock et al. 1999).

Oyster and fisheries introductions have been important historically in bringing new species into the State. In Elkhorn Slough (including Moss Landing Harbor), oyster culture is a probable vector for 44 of 69 NAS.

**Table 6.** Statewide total number of introduced species potentially introduced by each vector category. Totals include all possible vectors for each species, statewide.

<u>Vector</u>	<u>Total Probable Vectors</u>
Fouling (Recreational Boats)	159
Fouling (Commercial shipping)	155
Ballast Water	148
Oyster -All	91
Fisheries -All	64
Natural Dispersal	24
Unknown Vector	20
Aquatic Plant Shipments	14
Discarded Seafood	12
Dry Ballast	11
Pet Release	9
Discarded Bait	8
Aquaculture Escape	5
Biocontrol	4
Cargo	3
Scientific Escape	3
Habitat Restoration	3

### 6.5.1 Secondary Introductions

After a coastal region becomes invaded, it then serves as a potential donor region for other regions and introduced species spread along the coast. The vectors responsible for the post-introduction spread of aquatic organisms along the California coast are poorly understood, but certainly shipping vectors are responsible for much of the coastwise dispersal of species. West Coast containership traffic fits a pendulum model, where ships make transoceanic voyages followed by several shorter trips to coastwise ports before returning. Coastwise arrivals account for about one-third of ship arrivals in California (CSLC unpublished data). It is likely that many introductions attributed to recreational boat fouling are secondary introductions.

### 6.6 Rate of Introduction

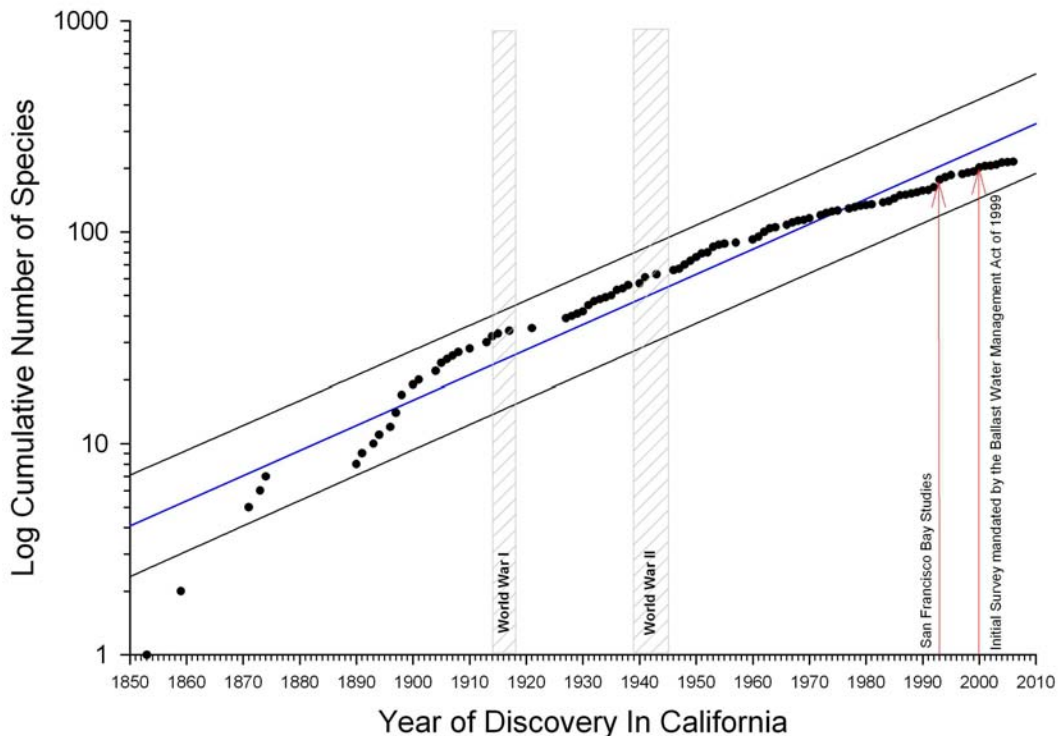
The rate of introductions into California waters is difficult to quantify because baseline biotic inventories of native species over appropriate temporal and spatial scales have not been conducted prior to the arrival of non-natives. Without such a baseline, it is impossible to distinguish a native species from an introduced one that had become well established in the past. The relative incompleteness of baseline inventories may be attributed to logistical challenges presented by a water-dominated ecosystem that is structurally, functionally, and

ecologically diverse. SCUBA gear only became widely available in the 1950s. Prior to that time, most marine organisms were collected through the use of specialized, remotely-operated sampling equipment suitable for a limited range of habitats.

Transoceanic voyages to California are known to have occurred during pre-Columbian times (Heizer 1971; Olson 1971; and Rolle 1998), thus the potential for incidental ship-borne non-native organism introductions has existed for at least two millennia. However, early forays across the Pacific Ocean by small, primitive watercraft into California waters were extremely rare occurrences. Maritime activity in California began in earnest with the arrival of European explorers, settlers, and traders during the sixteenth century (Rolle 1998; Wonham and Carlton 2005). Accounts of incidental non-native species introductions in California from as early as the sixteenth century do exist, but prior to the mid-nineteenth century, most observations were anecdotal in nature (Carlton 1979). Prior to the late 19<sup>th</sup> century, it can be assumed that hull fouling was the primary source of the introductions, and these introductions were likely to have increased in proportion to the volume of ship traffic arriving from other ports (Carlton 1979). Since then, matters have been further complicated by the addition of new vectors (e.g., through the advent of metal-hulled ships equipped with ballast tank systems, shellfish culture [especially oysters], fisheries development, recreational boating, aquarium releases, and aquatic vegetation introductions) and the change in the relative contributions of each over time (Carlton 1979, Wonham and Carlton 2005).

In lieu of hard data from early baseline and subsequent time-series surveys, the rate of NAS introductions might be inferred from discovery dates reported from a combination of existing literature and recent field surveys. Such analyses should be viewed with caution, however, because survey efforts and publications generated from them are primarily driven by a fortuitous combination of interest, technical expertise, opportunity, and access. Factors that may contribute to an inaccurate introduction rate estimation include: (1) a bias toward detection of larger and more conspicuous organisms over smaller (not readily visible without magnification) or obscure ones, (2) substantial time lapse (often many years) between actual introduction and discovery (Carlton 1979; Cohen and Carlton 1995; Dames and Moore 1999; Ruiz et al., 2000; Ruiz and Reid 2007), (3) sampling methodologies which overlook rare, elusive, or spatially-isolated organisms (Ruiz et al. 2000; Thompson 2004), (4) lack of sufficient taxonomic information to identify suspect organisms (includes those for juvenile stages), (5) lack of sufficient information to determine the origin and natural distribution of a given species, and (6) limited availability of information about new introduced species discoveries due to publication delays or failure to publish. James Carlton stated in 2007 that "...numerous and growing lines of evidence, enhanced by molecular techniques for the detection of cryptic species, suggest that the number of invasions occurring in the past 500 years has been severely underestimated."

Ruiz et al. (2000) reported that marine species introduction rates in North America increased exponentially between 1790 and 1990. Similar rates were observed by Wonham and Carlton (2005) for the Puget Sound area, Willapa Bay, Coos Bay, and Humboldt Bay, from 1900 to 2000. In California, the cumulative number of introduced marine and estuarine species has also accelerated more or less exponentially over time (Figure 17), based on records of 212 determinate, established introduced taxa first discovered from 1853 to 2006. Data resolution was poor prior to the last decade of the 19<sup>th</sup> century due to paucity of records. During 2007, no new taxa were found through fall of that year.

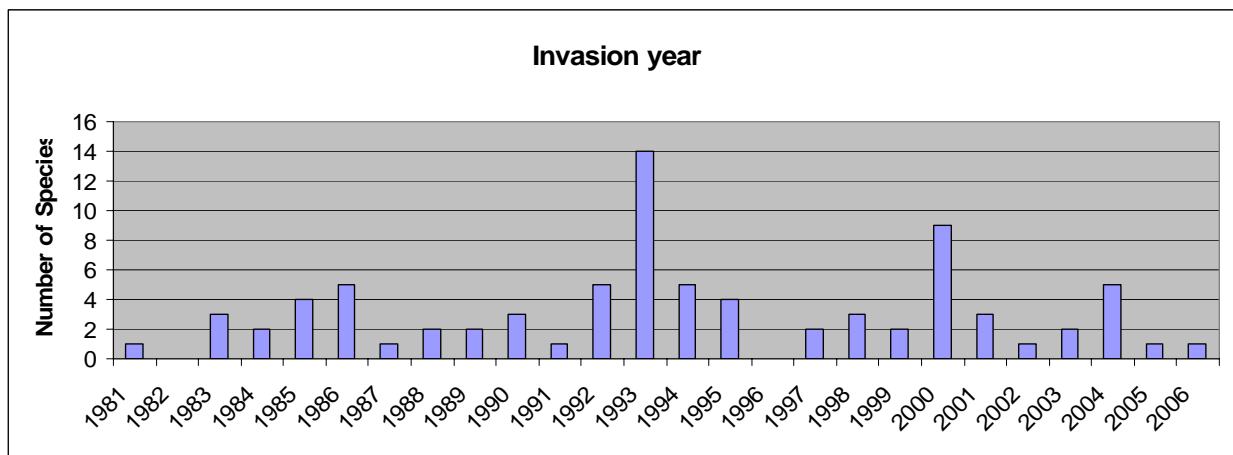


**Figure 17.** Cumulative number of established, determinate introduced marine and estuarine taxa, [marine and estuarine spp., excludes vascular plants] by year of first discovery in California. (Based, in part, on data provided by SERC.)

Yet despite growing awareness of the introduced species problem and greater effort placed on monitoring and research (Grosholz 2002; Ruiz and Reid 2007), the discovery rate had actually dropped below the exponential curve since the late 1970s. Sampling effort, sampling bias, occurrence of serendipitous discoveries, and various other factors may account for discrepancies between the actual introduction rate and that derived from discovery-dependent datasets. The apparent rate of introduction in California's coastal and estuarine waters might therefore be expected to change if, in the future, the status of the following categories of taxa were to be clarified: (1) introduced taxa for which

establishment is yet to be confirmed (N = 15), (2) cryptogenic species (N = 453), (3) and unresolved taxa (N = 1362). In addition, given the relatively incomplete state of marine biotic inventories worldwide, and expansion of mandated introduced organism surveys, the probability that new taxa will continue to be discovered in the future remains high, despite precautions to reduce or eliminate their sources. Indeed, Costello and Solow (2003) have provided mathematical evidence that the discovery curve can continue to increase despite no increase in either the introduction or sampling rate. Therefore, observations of the rate of discoveries should definitely not be relied upon as an indicator of introduction rate.

The effect of increased sampling effort for NAS is shown in Figure 18. Since 1981, annual new discoveries of established introduced taxa ranged between 0 and 14, and averaged 5.06. The relatively large number of discoveries made in 1993 (N = 14 taxa) and 2000 (N = 9 taxa) are mainly attributable to accelerated effort. In 1993, Cohen and Carlton (1995) accounted for six of the discoveries, and two were made by Orsi and his colleagues (Orsi and Ohtsuka 1999; Modlin and Orsi 1997). Six of the nine new introduced taxa discovered in 2002 were the result of surveys mandated by the Ballast Water Management Act of 1999 (DFG 2002 [Appendix B], Cohen et al. 2005).



**Figure 18.** Number of established, determinate introduced marine and estuarine taxa, per year of first discovery in California, 1981 through 2006. No new discoveries were made through fall of 2007.

Although no new discoveries are known since 2006, it is expected that more non-native marine taxa will continue to be found in California waters with continued sampling efforts. Despite expected reduced introductions through ballast water exchange and treatment policies and regulations, other anthropogenic sources of non-native organisms remain unregulated. Thus the spread of polyvectic organisms could continue by these means.

However, despite the perception that California’s marine and estuarine waters are constantly beset by non-native organisms, not all survive and establish in the



long term. Even though some populations may flourish for several years, environmental conditions may become unfavorable due to interannual variation and localized disturbances. Therefore, rather than merely tracking the number of non-native organism discoveries, future research and monitoring should consider the following: 1) increased frequency of monitoring, especially capturing seasonal variations; 2) evaluating potential displacement of native organisms by spread of non-native organisms; and 3) correlation of physical and other environmental factors with establishment and spread of non-native organisms.

## 7.0 RECOMMENDATIONS

### ***Recommendation 1: Increase the role of genetic studies and DNA sequencing in species identification and determination of introduction status.***

The detection and monitoring of marine invasions is complicated by the presence of cryptic species, as noted by Geller (1996, 1999). When native and introduced species belong to the same cryptic species complex, the arrival of the introduced species will not be noticed until genetic analyses are performed; the replacement of the native by the invader may even occur without notice (Geller, 1999). At least one author has claimed that cryptic species complexes are highly prevalent in the marine environment (Knowlton 1993). Multiple invasions by members of the same species complex will be overlooked, and simultaneous or sequential invasions will be regarded as one. The co-occurrence of the introduced crabs *Carcinus maenas* and *C. aestuarius* went undetected in Japan and South Africa for this reason (Geller et al. 1997).

It is likely that there are many other cases of cryptic species complexes falling into these two categories in California: multiple invasions by an entirely introduced complex, and introduced species belonging to a complex that include California natives. The presence of a cryptic species often is not recognizable, even by expert taxonomists. However, Geller's research has shown that genetic analysis is effective in discovering these invasions. Accurately cataloging and mapping introduced organisms and assessment of the efficacy of preventative measures will require routine genetic analysis.

Another taxonomic issue that genetic studies may help resolve is that of cosmopolitan species. Many species which can be identified cannot be designated native or introduced, so are categorized as cryptogenic. Some cryptogenic species are placed in this category because they are cosmopolitan (globally distributed). Knowing which cosmopolitan species are naturally distributed globally or are introduced is essential to an accurate accounting of invasive species. Genetics can test whether a truly cosmopolitan species has a genetic structure consistent with isolation by distance. Introduced species would show apparent gene flow across obvious barriers (e.g., continents or ocean basins) and would show incongruent patterns of population similarity (i.e.,

populations from California and Italy might appear more closely related than from Italy and France - as is the case for introduced mussels) (J. Geller pers. comm.).

Still another taxonomic problem is the difficult identification of several taxa that are prominent among introduced species, such as sponges, tunicates, and polychaete worms. Reliance on expert taxonomists is labor-intensive, expensive, somewhat risky (taxonomists may retire or decline to take new work), and essentially unverifiable. Genetics analysis could address this problem in this way: once experts have identified specimens, DNA sequences derived from those specimens can serve to identify newly collected material. Then, new specimens that have unknown sequences can be referred back to the taxonomic expert, with the DNA sequence itself serving as a temporary taxonomic label. Fortunately, methods for genetic analysis are becoming continually easier and more streamlined, and the cost is also decreasing.

***Recommendation 2: Fund special studies to resolve gaps in taxonomic identification.***

In order to determine if new species are being introduced to California, species need to be clearly identified and named. Certain groups of marine organisms (especially polychaete worms and sponges) found during our field surveys need taxonomic clarification because: (1) they are new species, (2) they are part of species complexes which can include both introduced or native species, (3) they are incompletely described either because of incomplete original descriptions or because new populations exhibit variant characteristics which need to be verified before they can be properly categorized as introduced or native, or (4) they are unknown species found out of their range and have been displaced by man-caused translocation. Still others have been previously misidentified. All these factors add to the uncertainty of classifications and many of these species have been designated as cryptogenic, due to lack of information about their ecology and origin.

DFG should look for opportunities to secure funding for special studies designed to revise or refine the taxonomy of various groups of organisms, determine and describe the geographic ranges of origin of species currently classified as cryptogenic, or facilitate calibration of taxonomic knowledge amongst taxonomists involved with identification of MISP specimens. The aim of such studies is to gain an accurate accounting of introduced species by determining which species presently categorized as cryptogenic can be designated native or introduced.

***Recommendation 3: Modify current MISP sampling design to accomplish future NAS monitoring.***

In March 2008, DFG held a meeting to obtain recommendations for modification and improvement of the DFG long-term NAS monitoring program. Meeting

participants included representatives of SERC, USEPA, USFWS, MLML, CSLC, and DFG. Some of the recommendations resulting from discussions were:

- To avoid missing seasonal dynamics, some short-term tracking of fouling organisms should be done, at least in one bay system, perhaps San Francisco Bay. Sampling could be done quarterly using settling plates.
- A combination of short-term, focused surveys and larger, statewide surveys may provide the best opportunity to detect new species while minimizing the impact of lag-time in specimen identification before species are discovered.
- The number of sites sampled in the broad statewide surveys could be scaled back, particularly on the outer coast, which tends to have fewer NAS.
- Genetic/molecular methods could be inserted into the specimen identification process, which requires building a genetics database of California coastal aquatic species.

If eradication is to be successful, it is essential to detect invaders as early in the colonization phase as possible, to limit the number of individuals that need to be removed and the area that must be treated. Therefore, monitoring with a rapid turn-around time is desirable.

***Recommendation 4: Analyze shipping and ballast water dumping patterns and their relationship to NAS***

California dates of first introduction/discovery for species introduced or dispersed by ballast water can be compared to volume of discharged or exchanged ballast water in nearshore locations. Examine relationships between shipping traffic patterns along the California coast and occurrence of NAS likely introduced via shipping vectors.

***Recommendation 5: Further research the role of vectors in introducing and dispersing NAS.***

The research by SERC on introduction vectors has provided a more accurate picture of the mechanisms of introduction for species introduced into California. However, there is little knowledge of the roles of non-shipping vectors in introducing or spreading NAS, especially recreational boating. Basic information about boat movement patterns within California coastal areas is lacking. Many species are thought to have arrived via multiple pathways. Further research will make it possible to more effectively target prevention measures on the most problematic vectors.

In the Executive Summary of the California AIS Plan, five top priorities were identified from the 163 actions listed in the plan. One of these priorities is to conduct a statewide risk assessment of NAS introduction pathways that are believed to be important but have not been investigated in a systematic manner. The introduction of NAS from ballast water discharge by commercial ships and from hull fouling of commercial ships has been studied and reported on in California. These are considered to be the largest contributors of NAS, but additional vectors exist that experts believe contribute significantly to NAS introductions. These six vectors are:

- commercial fishing;
- recreational boating;
- live bait;
- live imported seafood;
- aquariums and aquascaping; and
- aquaculture.

In 2008, the Ocean Protection Council approved \$1M for the purpose of performing the risk assessments, which will result in comprehensive reports on these vectors for introductions of NAS in California's ocean and coastal ecosystems. The data generated by these assessments will be incorporated into CANOD and will no doubt add to our understanding of the vectors responsible for introduction of new species into California.

***Recommendation 6: Continue to provide opportunities for peer input to sampling methodology and review of reports.***

In 2008, the MISP invited researchers and agency representatives to a workshop to discuss possible improvements and modifications to the existing sampling program. This type of coordination and review should continue, so we propose to develop a standardized review program to continue to make improvements to the ongoing sampling program. In addition, it is valuable to have access to experts to assist with data review. It is critical that any peer input includes an adequate timeline for identifying and contacting appropriate peer reviewers, adequate time for review, and an honorarium for academic peers.

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## **Appendix A. Vectors of Introduction (based on National Introduced Marine Pest Information System 2008)**

### **Shipping (Ballast water, Dry ballast, Fouling)**

This class includes vectors associated with maritime transport and shipping activities. Types of vessels are: commercial ships (e.g. tankers, container ships, ferries, barges), domestic ships, passenger vessels, drilling platforms and research vessels.

#### Ballast water

Various types and life stages of species can be transported in ballast water, including plankton, crustaceans, fish, larvae, eggs or cysts. Ballast water is used in commercial vessels to stabilize the vessel and is uploaded or discharged depending on the amount of cargo onboard. The ballast water vector also includes sediments that accumulate in the bottom of ballast tanks. Species that are able to survive within these sediments include those that have a resistant stage or resting cyst (e.g. dinoflagellates) as well as adult stages of benthic organisms.

#### Dry ballast

Dry ballast included rocks and sand, which historically was used in vessels for ship stability during transit, but has been replaced by ballast water in modern ships. When no longer necessary, the ballast was usually thrown overboard, resulting in release of organisms to a new environment.

#### Fouling

Fouling communities are typically composed of encrusting or sessile species, but they can include mobile species. This vector can introduce species through a variety of means. The most obvious means is through dislodgement of fouling species from a vessel in port through abrasion with wharf structures, through in-water vessel hull cleaning, or in transit through high vessel speeds. Another method of introduction of fouling organisms is through spawning of a fouling species on a vessel in port and its successful settlement and establishment of a reproductive population.

For hull fouling as a vector, it is not currently possible to distinguish the potential role of commercial and recreational vessels.

**Aquaculture** (Discarded bait, Fisheries intentional [not oyster], Fisheries accidental [not oyster], Oyster accidental, Oyster intentional, Packing material, Scientific escape)

This class includes vectors associated with fisheries and aquaculture activities and trade. An example of a vector from this class is Fisheries intentional, which

would incorporate, for example, the introduction of the Pacific oyster *Crassostrea gigas* for aquaculture purposes. Other vectors included in this class are:

#### Oyster accidental

The introduction of organisms associated with oysters and their deliberate translocation. Oysters are a highly valued food species and have been translocated across the globe for farming. Many species live cryptically on oyster shells and have subsequently been introduced along with oysters into new localities. This was more common historically when shells were not cleaned of other species.

#### Oyster intentional

This vector describes the deliberate introduction of oysters. Oysters are a highly valued aquaculture species and have been translocated to many locations around the world to establish aquaculture industries and also to restock areas where native oysters have been lost through either disease or overfishing.

#### Fisheries intentional (not oyster)

The deliberate translocation of fish, crustaceans or molluscs (not oysters) to establish or support a new fishery. For example many aquaculture operations use species that are not native, which involves introducing species from elsewhere in the world.

#### Fisheries accidental (not oyster)

The accidental translocation of species through aquaculture and fisheries activities. This vector includes the accidental release of live fish, crustaceans and molluscs (other than oysters) imported for human consumption, as well as the accidental translocation of species attached to aquaculture gear (floats, cages, etc).

#### Discarded bait

The release of bait species (and associated organisms) from commercial fishing operations, sport and recreational fishing activities. The release from vessels or from shore of organisms originally imported for sale as bait or human food may have led to the establishment of two species of shrimp into San Francisco Bay.

#### Packing material

The accidental release of species associated with seaweed (and other packing materials) for bait and fishery products. These packaging materials are often disposed of at sea by fishers, which can release organisms into the marine environment.

### Scientific escape

The accidental introduction of species during research activities conducted by educational, scientific, and private institutions.

### **Ornamental**

This class includes vectors associated with ornamental species in the aquarium trade or horticulture and landscaping industries. Many aquatic plants have been introduced for decorative purposes in aquaria ponds via these shipments.

#### Aquatic plant shipments

The accidental or deliberate release of aquatic plants (and associated organisms) from aquatic plant shipments. Many species of aquatic plants (both marine and freshwater), including known pest species, can be purchased over the Internet (e.g. the marine alga *Caulerpa* spp.).

#### Pet release

The accidental and deliberate release of domestic animals kept as pets by individuals. This often involves releasing introduced species directly into natural waterways or indirectly via drainage and sewer systems.

### **Natural Dispersal**

Natural dispersal is a mechanism for the range expansion of a species through natural processes such as the movement of larvae or adults to a new location, for example, through passive movement in water currents; or active movement (migration) in response to changes in environmental conditions such as salinity changes or water flow dynamics. Natural dispersal also allows for the successful settlement of recruits in a new location.

Characteristics of a species that may be translocated via this vector include having a planktonic dispersal phase; and readily fouling floating objects. Natural dispersal is a mechanism for the range expansion of a species through the movement of larvae or adults to a new location, and the successful settlement of recruits in this new location.

**Appendix B. Methods for NAS Vector Analysis by Smithsonian Environmental Research Center**

*[Temporarily removed pending article publication]*

**Appendix C. Table: Introduced And Cryptogenic Species In California By Location**

Source: California Aquatic Non-native Organism Database (CANOD), updated on September 18, 2008.

Format: The location information on the even numbered pages is a continuation of the data for each species that begins on the odd numbered pages.