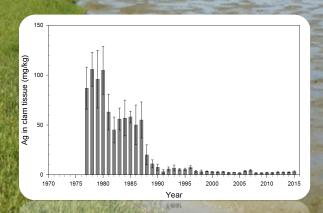


Prepared in Cooperation with the City of Palo Alto, California

Near-Field Receiving Water Monitoring of Trace Metals and a Benthic Community Near the Palo Alto Regional Water Quality Control Plant in South San Francisco Bay, California: 2015



Open File Report 2016–1118

U.S. Department of the Interior U.S. Geological Survey

Cover Image: Marsh and mudflat at Sand Point, Baylands Nature Preserve, Palo Alto, Calif. Photo courtesy of Dan Cain, USGS, 2014.



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By Daniel J. Cain, Janet K. Thompson, Jeff Crauder, Francis Parchaso, Robin Stewart, Mathew Turner, Michelle I. Hornberger, and Samuel N. Luoma

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U.S. Department of the Interior U.S. Geological Survey

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## **Conversion Factors**

Multiply	Ву	To obtain
foot (ft)	0.3048	meter (m)
gallon (gal)	3.785	liter (L)
inch (in)	2.54	centimeter (cm)
inch (in)	25,400	micrometer (µm)
meter (m)	1,000,000	micrometer (µm)
mile (mi)	1.609	kilometer (km)
ounce (oz)	28.35	gram (g)
part per million (ppm)	1	microgram per gram (µg/g)
milligram per kilogram (mg/kg)	1	microgram per gram (µg/g)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:

°F=(1.8×°C)+32

Temperature in degrees Fahrenheit (°F) may be converted to degrees Celsius (°C) as follows:

°C=(°F-32)/1.8

Concentrations of chemical constituents in water are given either in milligrams per liter (mg/L) or micrograms per liter (µg/L).

NOTE TO USGS USERS: Use of liter (L) as a special name for cubic decimeter (dm3) is restricted to the measurement of liquids and gases. No prefix other than milli should be used with liter.

# Abbreviations and Acronyms

mL	milliliter
μΏ-cm	microohm per cm
µg∕g	microgram per gram
mg/kg	milligram per kilogram
μm	micrometer
CI	condition index
ERL	effects range-low
ERM	effects range-median
ICP-OES	inductively coupled plasma-optical emission spectrophotometry
IRMS	isotopic ratio mass spectrophotometry
MDL	method detection limit
MLLW	mean low low water
MRL	method reporting level
NIST	National Institute of Standards and Technology
NPDES	National Pollutant Discharge Elimination System
PARWQCP	Palo Alto Regional Water Quality Control Plant
RWQCB	California Regional Water Quality Control Board
SFEI	San Francisco Estuary Institute
USEPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey

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## **Executive Summary of Past Findings**

U.S. Geological Survey (USGS) personnel have assessed trace-metal concentrations in sediments and sediment-dwelling species since 1977 at an intertidal site in the vicinity of the discharge of the Palo Alto Regional Water Quality Control Plant (PARWQCP). They have also profiled that area's benthic community structure since 1974. Ancillary biotic and abiotic factors that could affect metal concentrations and benthic community structure—exotic species invasions, pelagic food availability, and weather anomalies—have also been measured during this time. Collectively, this dataset describes a long-term, detailed history of metal concentrations and benthic community dynamics at this site.

Initially, these studies found exceptionally high concentrations of copper (Cu) and silver (Ag) in mud-dwelling animals in this area, with strong seasonal variability. Additional studies identified the PARWQCP as a point source for Cu and Ag and established the clam *Macoma petalum* as a biological indicator of metal exposure. The annual mean concentrations of Cu and Ag in *Macoma petalum* were 287 milligrams per kilogram (mg/kg) and 105 mg/kg, respectively, in 1980. These levels exceeded tissue concentrations reported in the literature for this species and were much greater than seen elsewhere in San Francisco Bay. Elevated metal concentrations coincided with reduced reproductive activity in *M. petalum*. Related studies supported the theory that elevated Ag concentrations inhibited the development of reproductive tissue. The benthic community also showed signs of environmental stress during this time. Opportunistic organisms that are capable of fast invasion and propagation in disturbed environments, such as the amphipod *Ampelisca abdita*, dominated the community. These organisms thrived on the surface of the mud in tubes or as shelled animals, brooded their young, and fed on waterborne particles.

Concentrations of Cu and Ag in both sediments and clams declined significantly during the 1980s as the PARWQCP implemented more advanced wastewater treatment and source control programs. The downward trends in Cu in sediments and in the tissues of *M. petalum* correlated with reduced Cu discharge from the PARWQCP. Coincident with the decline in Cu and Ag in the sediment and clams, the reproductive activity of the clam greatly increased. The composition of the benthic community also shifted during this period. Opportunistic species became less dominant, and other species became more persistent. Other environmental factors that vary seasonally and annually (for example, sediment composition, grain-size distribution, organic content, and ambient water salinity) were not associated with the observed temporal trends in metal concentrations, inferred metal effects on species, and benthic community changes. The only unidirectional change in an environmental factor

during this period (1980–1990) was the decline in metal concentrations in discharge from the waste treatment plant.

Following the significant reductions in the 1980s, concentrations of Cu and Ag in sediments and clams have remained relatively low and stable. Concentrations have fluctuated modestly and without a sustained temporal trend. However, Ag in sediments remains greater than what may be considered the regional background (0.09 mg/kg). This persistent, low level of contamination likely derives from Ag introduced to the site before the 1990s. The concentrations of Cu and Ag in *M. petalum* have fluctuated as much as four-fold. Concentration minima for Cu observed during this period (1991, 2000–2005, and 2008–2012) were comparable to what can be considered baseline concentrations for this species in San Francisco Bay (20–30 mg/kg). Thus, metal concentrations in sediments and tissue of *M. petalum* are more likely a combination of inputs from the PARWQP and other regional sources, cycling of contaminants stored within sediments, and regionally-scaled physical and biogeochemical processes controlling the distribution and bioavailability of metals.

As concentrations of Ag and Cu in *M. petalum* declined, reproductive activity increased both in terms of the percentage of individuals that were in a reproductively active stage and the frequency of reproductive activity during the year. Overall, the reproductive status of the population has improved and stabilized over the 20 years of reduced exposure to Ag and Cu at the site.

Over the same period, the composition of the infaunal community shifted from a dominance of surface-dwelling, brooding species to species with various life-history characteristics. In particular, species that lay their eggs in the mud and feed by burrowing through and consuming the mud, which were rare in the community in the 1970s and 1980s, have increased in abundance. This pattern continued through 2007, with the less opportunistic species becoming more dominant in abundance. A disturbance occurred on the mudflat in early 2008 (possible causes include sediment accretion or freshwater inundation) that resulted in the loss of the benthic animals, except for those deep-dwelling animals like M. petalum. Animals returned to the mudflat within 2 months of the event, however, which suggested that the disturbance was not due to a persistent toxin or to anoxia. Benthic community data in 2009 showed that the animals that returned to the mudflat were those that can respond successfully to a physical, nontoxic disturbance. The most recent community surveys showed a mix of animals that consume the sediment, filter feed, brood their young, and have pelagic larvae that must survive life on the sediment at a young age. The 2008 defaunation event allowed for an examination of the response of the community to a natural disturbance and a comparison of this recovery to the long-term recovery observed in the 1970s, when the decline in sediment pollutants was the dominating factor. Today, the community at this site is very similar to the benthic community observed by Thompson and Parchaso (2012) throughout South San Francisco Bay: although small filter feeding species are numerically dominant, there is a significant proportion of the community that feeds on surface and subsurface sediment particles, a feature of community structure that is not present where sediments have high concentrations of toxicants.

When this study started in the late 1970s, the site was already heavily contaminated with metals. Although the authors assume that the biological conditions reflected the consequences of elevated metal exposures, there is a scarcity of preexisting data to evaluate impacts due to elevated metals. However, the long-term record contained in this study provides a unique opportunity to document biological response when the stress of metal exposure is relaxed. The data make a compelling case that the mitigation of Ag and Cu in wastewater effluent during the 1980s allowed for biological recovery and the establishment of a more diverse and stable infaunal community.

## Abstract

Trace-metal concentrations in sediment and in the clam *Macoma petalum* (formerly reported as *Macoma balthica*), clam reproductive activity, and benthic macroinvertebrate community structure were investigated in a mudflat 1 kilometer south of the discharge of the Palo Alto Regional Water Quality Control Plant (PARWQCP) in South San Francisco Bay, California. This report includes data collected by U.S. Geological Survey (USGS) scientists for the period from January 2015 to December 2015. These data are appended to long-term datasets extending back to 1974, and serve as the basis for the City of Palo Alto's Near-Field Receiving Water Monitoring Program, initiated in 1994.

Following significant reductions in the late 1980s, silver (Ag) and copper (Cu) concentrations in sediment and *M. petalum* appear to have stabilized. Data for other metals, including chromium (Cr), mercury (Hg), nickel (Ni), selenium (Se), and zinc (Zn), have been collected since 1994. Over this period, concentrations of these elements have remained relatively constant, aside from seasonal variation that is common to all elements. In 2015, concentrations of Ag and Cu in *M. petalum* varied seasonally in response to a combination of site-specific metal exposures and annual growth and reproduction, as reported previously. Seasonal patterns for other elements, including Cr, Ni, Zn, Hg, and Se, were generally similar in timing and magnitude as those for Ag and Cu. In *M. petalum*, all observed elements showed annual maxima in January–February and minima in April, except for Zn, which was lowest in December. In sediments, annual maxima also occurred in January–February, and minima were measured in June and September. In 2015, metal concentrations in both sediments and clam tissue were among the lowest on record. This record suggests that regional-scale factors now largely control sedimentary and bioavailable concentrations of Ag and Cu, as well as other elements of regulatory interest, at the Palo Alto site.

Analyses of the benthic community structure at the same mudflat over a 40-year period show that changes in the community have occurred concurrent with reduced concentrations of metals in the sediment and in the tissues of the biosentinel clam, M. petalum, from the same area. Analysis of M. petalum shows increases in reproductive activity concurrent with the decline in metal concentrations in the tissues of this organism. Reproductive activity is presently stable (2015), with almost all animals initiating reproduction in the fall and spawning the following spring. The entire infaunal community has shifted from being dominated by several opportunistic species to a community where the species are more similar in abundance, a pattern that indicates a more stable community that is subjected to fewer stressors. In addition, two of the opportunistic species (Ampelisca abdita and Streblospio benedicti) that brood their young and live on the surface of the sediment in tubes have shown a continual decline in dominance coincident with the decline in metals; both species had short-lived rebounds in abundance in 2008, 2009, and 2010 and showed signs of increasing abundance in 2015. Heteromastus filiformis (a subsurface polychaete worm that lives in the sediment, consumes sediment and organic particles residing in the sediment, and reproduces by laying its eggs on or in the sediment) showed an increase in dominance, concurrent with the decrease in Ag and Cu concentrations, and in the last several years before 2008, showed a stable population. H. filiformis abundance increased slightly in 2011–2012 and returned to pre-2011 abundance in 2015. An unidentified disturbance occurred on the mudflat in early 2008 that resulted in the loss of the benthic animals, except for deep-dwelling animals like *M. petalum*. However, within two months of this event animals returned to the mudflat. The resilience of the community suggested that the disturbance was not due to a persistent toxin or to anoxia. The reproductive mode of most species present in 2015 is reflective of species that were available either as pelagic larvae or as mobile adults. Although oviparous (live-birth) species were lower in number in this group, the authors hypothesize that these species will return slowly as more species move back into the area. The use of functional ecology was highlighted in the 2015 benthic community data, which showed that the animals that have now returned to the mudflat are those that can respond successfully to a physical, nontoxic disturbance. Today, community data show a mix of species that consume the sediment, or filter feed, have pelagic larvae that must survive landing on the sediment, and those that brood their young. USGS scientists view the 2008 disturbance event as a response by the infaunal community to an episodic natural stressor (possibly sediment accretion or a pulse of freshwater), in contrast to the long-term recovery from metal contamination. We will compare this recovery to the long-term recovery observed after the 1970s when the decline in sediment pollutants was the dominating factor.

## Introduction

Determining spatial distributions and temporal trends in trace metals in sediments and benthic organisms is common practice for monitoring environmental contamination. These data can be the basis for assessing metal exposure and the potential for adverse biological effects. Another common method of environmental monitoring is to examine the community structure of sediment-dwelling benthic organisms (Simon, 2002). Spatial and temporal changes in community structure reflect the integrated response of resident species to environmental conditions, although the underlying cause(s) for the response may be difficult to identify and quantify. Together, measurements of metal exposure and biological response can provide a more complete view of anthropogenic disturbances and the associated effects on ecosystem health.

Sediment particles can strongly bind metals, effectively removing them from solution. As a result, sediments may accumulate and retain metals released to the environment. Contaminated sediments may become a chronic source of metals to the environment. Thus, concentrations of metals in sediments serve as a record of metal contamination in an estuary, with some integration over time. Fluctuations in the record may be indicative of changes in anthropogenic releases of metals into the environment (Förstner and Wittmann, 1979).

Metals in sediments are also indicative of the level of exposure of benthic animals to metals through contact with, and ingestion of, bottom sediments and suspended particulate materials. However, geochemical conditions of the sediment affect the biological availability of the bound metals. Assimilation of bioavailable sediment-bound metal by digestive processes and the contribution of this source of metals relative to metals in the aqueous phase are not well understood. Thus, in order to better estimate bioavailable metal exposures, the tissues of the organisms themselves may be analyzed for trace metals. Benthic organisms concentrate most metals to levels higher than those that occur in solution (Phillips and Rainbow, 1993). Therefore, the record of metal concentrations in clam tissue can be a more sensitive indicator of anthropogenic metal inputs than the sediment record. Different species concentrate metals to different degrees. However, if one species is analyzed consistently, the results can be used to indicate trace-element exposures to the local food web.

Contaminants can adversely affect benthic organisms at several organizational levels. For example, responses to a pollutant at the cellular or physiological level of an individual can result in changes at the population level, such as reductions in growth, survival, and reproductive success (Newman, 1998). Community level responses to population level impairment can include overall shifts in species abundance, favoring metal-tolerant species, which can result in changes in predator/prey interactions and competition for available resources. Changes in the benthic community can ultimately result in changes at the ecosystem level due to that community's importance as primary consumers in the cycling of carbon in aquatic environments (Alpine and Cloern [1992] provides a local example).

In all aquatic environments, benthic organisms may be exposed to contaminants at all life stages through a variety of routes—sediment, water, and food (Wang and Fisher [1999] provides a summary of

the potential transport of trace elements through food). Toxicant exposure is related to contaminant concentration as well as duration. Even at relatively low contaminant levels, long-term exposure can affect benthic organisms (Long and others, 1995). The added complexity of synergistic or antagonistic effects between different contaminants, and between contaminants and natural stressors, makes causal relationships difficult to identify and quantify, even on a site-specific basis. However, a time-integrated picture of ecosystem response to contaminant loading can be provided by field studies that link changes in exposure at multiple time scales (in this case seasonal to decadal) to changes at individual, population, and community levels.

The California Regional Water Quality Control Board (RWQCB) has prescribed a selfmonitoring program with its reissuance of the National Pollutant Discharge Elimination System (NPDES) permits for South San Francisco Bay dischargers. The recommendation includes specific receiving-water monitoring requirements.

Since 1994, the Palo Alto Regional Water Quality Control Plant (PARWQCP) (fig. 1) has been required to monitor metals and other specified parameters in sediments and the clam *Macoma petalum* at an inshore location in South San Francisco Bay, Calif. In addition to the required monitoring, PARWQCP has undertaken monitoring of the benthic community as a whole. The monitoring protocols have been designed to be compatible with or complementary to the RWQCB's Regional Monitoring Program. In this report, the USGS monitoring study is described, and data collected during 2015 are presented within the context of more than 35 years of previous data collections and investigations by the USGS at this inshore location.

The data collected during this study include trace-metal concentrations in sediments and clams, clam reproductive activity, and benthic community structure. These data and those reported earlier (for example, Hornberger and others, 2000a; Luoma and others, 1991, 1995a, 1996; Moon and others, 2005; Shouse and others, 2003, 2004; Thompson and others, 2002; Cain and others, 2006; Dyke and others, 2011; 2012, 2014) were used to meet the following objectives:

- Provide data to assess seasonal and annual trends in trace-element concentrations in sediments and clams, reproductive activity of clams, and benthic community structure at a site designated in the RWQCB's self-monitoring program guidelines for PARWQCP.
- Present the data within the context of historical changes in South San Francisco Bay and within the context of other locations in the Bay published in the international literature.
- Coordinate inshore receiving water monitoring program for PARWQCP and provide data compatible with relevant aspects of the regional monitoring program. The near-field data will augment the regional monitoring program as suggested by the RWQCB.
- Provide data that could support other South San Francisco Bay issues or programs, such as development of sediment quality standards.

Despite the complexities inherent in monitoring natural systems, the adopted approach has been effective in relating changes in near-field contamination to changes in reproductive activity of a clam (Hornberger and others, 2000b) and in benthic community structure (Kennish, 1998). This study, with its basis in historical data, provides a context within which future environmental changes can be assessed.

Metal concentrations were monitored in sediments and a resident clam species, *Macoma petalum*. Analysis of trace-metal concentrations in the sediments over time provides a record of metal contamination of the site. The concentration and bioavailability of sediment-bound metals are affected by hydrology and geochemical factors (Thomson-Becker and Luoma, 1985; Luoma and others, 1995b). Thus, ancillary data, including grain-size distribution, organic carbon, aluminum and iron content of the sediment, regional rainfall, and surface salinity were collected to interpret seasonal, annual, and inter-

annual variation in metal concentrations. The tissue of *M. petalum* provides a direct measure of exposure to bioavailable metals.

Biological response of the benthic community to metal exposure was examined at three levels of organization: individual, population, and community. At the individual level, concentrations of metals in the tissues of *M. petalum* were compared with physiological indicators. Two common animal responses to environmental stress are reduced growth and reproductive activity. Growth and reproduction in *M. petalum* occur on fairly regular seasonal cycles. Seasonally, a clam of a given shell length will increase somatic tissue weight as it grows during the late winter and spring (Cain and Luoma, 1990). Reproductive tissue increases during the early stages of reproduction and declines during and after spawning. These cycles can be followed with the condition index (CI), which is an indicator of the physiological condition of the animal. The CI is the total soft-tissue weight of a clam standardized to shell length (Cain and Luoma, 1990). Inter-annual differences in growth and reproduction, expressed in the CI, are affected by the availability and quality of food, as well as other stressors, such as pollutant exposure and salinity extremes. An earlier study (Hornberger and others, 2000b) has shown that reproductive activity of *M. petalum* has increased with declining metal concentrations in animals from this location. Therefore, CI and reproductive activity of *M. petalum* appear to be useful indicators of physiological stress by pollutants at this location and continue to be monitored in this study.

At the population level, trends of the dominant benthic species were examined to see if certain species have been more affected than others by environmental change. It has been shown that most taxonomic groups have species that are sensitive to elevated Ag (Luoma and others, 1995b) and that some crustacean and polychaete species are particularly sensitive to elevated sedimentary Cu (Morrisey and others, 1996; Rygg, 1985). In addition, the benthic community was examined for changes in structure: that is, shifts in the species composition of the macroinvertebrate community resulting in a change in the function of the community. The authors hypothesized that a shift in community composition and potentially in the function of the benthic community in the ecosystem would result from changes in the concentrations of either specific metals or a combination of contaminants for several reasons. First, prior studies have shown that South San Francisco Bay benthic communities were dominated by opportunistic species in the 1980s (Nichols and Thompson, 1985a). These opportunistic species might become less dominant as environmental stressors decrease. Second, environmental pollutants may differentially affect benthic species that use different feeding and reproductive modes. An intertidal mudflat community, such as this study site, should include a combination of species that feed on particles in the water column, on settled and buried food particles in the mud, and on other organisms. The absence of any one of these feeding groups may show limitations on species as a result of environmental stressors that target specific feeding groups. For example, pollutants attached to sediment particles are likely to affect species that consume the sediment as part of their feeding mode and (or) those species that lay their eggs in the sediment (Newman and Jagoe, 1996).

Previous analysis of this community has shown no correlation between changes in the community and measured environmental parameters (salinity, air and water temperature, delta outflow, precipitation, chlorophyll *a*, sediment total organic carbon, and biological oxygen demand; Shouse, 2002). The community data are compared only to trace-metal data in this report.

The Palo Alto site (PA) includes the benthic community sampling site and the *M. petalum* and sediment sampling site, both adjacent to Sand Point in Palo Alto Baylands Nature Preserve on a mudflat on the western shore of San Francisco Bay (fig. 1). The site is 1 kilometer southeast of the intertidal discharge point of the PARWQCP. The sampling locations are approximately 12 meters (m) from the edge of the marsh and 110 centimeters (cm) above mean low low water (MLLW).

The sediment and biological samples from this location reflect a response of the receiving waters to the effluent just beyond the location of discharge. Earlier studies (Thomson and others, 1984) have shown that dyes, natural organic materials in San Francisquito Creek, and waters in the PARWQCP discharge move predominantly south toward Sand Point, thereby affecting the mudflats in the vicinity of Sand Point. Thomson and others (1984) showed that San Francisquito Creek and the Palo Alto Yacht Harbor were minor sources of most trace elements compared to the PARWQCP. Based on spatial and temporal trends of Cu, Ag, and Zn in clams and sediments, the PARWQCP appeared to be the primary source of elevated metal concentrations at the PA site in the spring of 1980 (Thomson and others, 1984; Cain and Luoma, 1990). Metal concentrations in sediments and clams (*M. petalum*), especially Cu and Ag, have declined substantially since the original studies as more efficient treatment processes and source controls were employed (Hornberger and others, 2000b). Frequent sampling each year was necessary to characterize those trends because there was significant seasonal variability (Cain and Luoma, 1990; Luoma and others, 1985). This report characterizes data for the year 2015, thereby extending the long-term record at this site.

## Methods

### Sampling Frequency and Duration

In dynamic ecosystems such as San Francisco Bay, the environmental effects of anthropogenic stressors are difficult to distinguish from natural seasonal changes. Sustained sampling at frequent intervals can characterize seasonal patterns, capture episodic events, and identify longer-term trends, thereby increasing the probability that anthropogenic effects can be identified. Analyses of early community data (1974–1983; Nichols and Thompson, 1985a,b) showed that benthic samples need to be collected at monthly to bimonthly intervals to distinguish between natural and anthropogenic effects. Therefore, data reported herein are based on samples collected on a bimonthly basis from the exposed mudflat at low tide between January and December. Samples collected in the field include surface sediment, the deposit-feeding clam *M. petalum*, surface water, and sediment cores for community analysis. Surface water, surface sediment, and *M. petalum* were not collected during the months May and July. Data on sediments, *M. petalum*, and surface water have been collected continuously since 1977, while community data were collected during 1974–1990 and 1998 to the present (2015).

#### Measurements of Metal Exposure

Metal concentrations in surficial sediments and in the soft tissues of *M. petalum* were interpreted as indicative of metal exposure to the broader benthic community. The collection and preparation of sediment and *M. petalum* samples have previously been described in detail (see, for example, Dyke and others, 2014). The following is an overview of those procedures.

#### Sediment

Sediment samples were scraped from the visibly oxidized (brownish) surface layer (top 1–2 cm) of mud and later that day sieved through a 100-micrometer ( $\mu$ m) mesh polyethylene screen with distilled water to remove large grains that might bias interpretation of concentrations. The mesh size was chosen to match the largest grains typically found in the digestive tract of *M. petalum*. All chemical data reported herein were determined from the fraction that passed through the sieve (<100 µm), termed the "silt/clay fraction."

The percent silt/clay fraction of sediment samples was determined to provide some comparability with bulk sediment determinations such as that employed in the Regional Monitoring Program (San Francisco Estuary Institute, 1997). The sediment that did not pass through the sieve (>100  $\mu$ m)—termed the sand fraction—was collected, as was the silt/clay fraction (<100  $\mu$ m) (see above). Each fraction was dried to constant weight, and their percent contributions to the bulk sample were determined gravimetrically.

The silt/clay fraction was subsampled to provide replicates weighing 0.4–0.6 grams (g). These were redried (70 °C), reweighed, and then digested by hot acid reflux (10 milliliters [mL] of 16 normal [N] nitric acid). This method provides a "near-total" extraction of metals from the sediment and is comparable to the recommended procedures of the U.S. Environmental Protection Agency (USEPA) and to the procedures employed in the regional monitoring program. Another set of replicate subsamples from the silt/clay fraction were directly extracted with 12 mL of 0.6 N hydrochloric acid (HCl) for 2 hours at room temperature. This partial extraction method extracts metals bound to sediment surfaces and is operationally designed to obtain a crude chemical estimate of bioavailable metal.

Total organic carbon (TOC) concentrations were determined using a continuous flow isotope ratio mass spectrophotometer (IRMS) (table 1). Before the analysis, sediment samples were acidified with 12 N HCl vapor to remove inorganic carbon (Harris and others, 2001).

#### **Clam Tissue**

Specimens of *M. petalum* were collected by hand on each sampling occasion. Typically, 60–120 individuals were collected, representing a range of sizes (shell length).

In the laboratory, the clams were removed from the containers and gently rinsed with deionized water to remove sediment. Clams were immersed in seawater diluted with deionized water as necessary to the equivalent of ambient salinity, and moved to a constant temperature room (12 °C) for 48 hours to allow for the egestion of sediment and undigested material from their digestive tracts.

#### Sample Preparation and Analysis for Metals, Excluding Mercury and Selenium

The shell length of each clam was measured with electronic calipers and recorded digitally. Clams were separated into 1- or 2-millimeter (mm) size classes (for example, 10.00–10.99 mm or 10.00–11.99 mm). The soft tissues from all of the individuals within a given size class were dissected from the shell and collected in preweighed 20-mL screw-top borosilicate glass vials to form a single composite sample for elemental analysis. The vials were transferred to a convection oven (70 °C). After the tissues were dried to constant weight, they were digested by reflux in subboiling 16 N nitric acid. The tissue digests were then dried and metals resolubilzed in 0.6 N hydrochloric acid for trace-metal analysis.

Sediment and tissue concentrations of aluminum (Al), chromium (Cr), copper (Cu), iron (Fe), nickel (Ni), silver (Ag), and zinc (Zn) were determined using inductively coupled plasma optical emission spectrophotometry (ICP-OES). Samples and standard reference materials (see below) were filtered (0.45  $\mu$ m) prior to analysis. Analytical ICP-OES results are available upon request.

#### Sample Preparation and Analysis for Mercury and Selenium

Approximately 40 clams were selected from each collection. The only criterion for selection was that the range of sizes (shell length) within this group was representative of the larger collection. Otherwise, the selection of individuals was random. Selected individuals were grouped according to size to form three composites, each containing a minimum of ~1.25 grams wet weight. To meet this

requirement, especially for the smaller clams, the 1-mm size classes were usually combined to form broader size classes (within 3–4 mm of each other, as appropriate). Once the composites were formed, the clams were dissected as described above, and the soft tissue was placed into preweighed 30-mL screw-top polycarbonate vials. These vials were closed and temporarily transferred to a freezer (–20 °C). The tissue sample was partly thawed and homogenized with a high-speed tissue homogenizer. Once homogenized the samples were refrozen (–80 °C), and then freeze-dried.

Tissue and sediment samples were subsampled and analyzed for total mercury by acid-digestion, BrCl oxidation, purge and trap, and cold vapor atomic fluorescence spectrometry according to the U.S. Environmental Protection Agency Method 1631, Revision E (2002), and for selenium by acid digestion, hydrogen peroxide oxidation, hydride generation inductively coupled plasma mass spectrometry (HG-ICP-MS) according to a method modified from Liber (2011) and Elrick and Horowitz (1985).

#### **Quality Assurance**

All glass and plastic materials used in the collection, preparation, and storage of samples were first cleaned thoroughly to remove metal contamination. Cleaning consisted of a detergent wash and rinse in deionized water, followed with a 10-percent hydrochloric-acid wash and thorough rinse in double-deionized water (approximately 18 mega-ohm [M $\Omega$ -cm] resistivity). Materials were dried in a dust-free positive-pressure environment, sealed, and stored in a dust-free cabinet.

Samples prepared for ICP-OES analysis (that is, all elements except Se and Hg) were accompanied by procedural blanks and standard reference materials (SRMs) issued by the National Institute of Standards and Technology (NIST). Analysis was preceded by instrument calibration, followed by quality-control checks with prepared quality-control standards before, during (approximately every 10 samples), and after each analytical run. Metal recoveries of sediment digests were evaluated with NIST 2709a San Joaquin soils and NIST 2711a Montana II Soil (appendix 1). Metal recoveries for soft tissue digests were evaluated with NIST 2976 Mussel Tissue and NIST 1566b Oyster Tissue (appendix 2). Results were consistent within methods, and most elements were within the ranges of certified values, with the following exceptions. The near-total extraction method recovered on average 43 and 38 percent of the Al and 58 and 55 percent of Cr in NIST 2709a and 2711a, respectively. Recovery of Pb averaged 60 percent in NIST 2709a. Recoveries for the near-total extraction method compared favorably to those reported by NIST for USEPA methods 200.7 and 3050B. Recoveries of elements in biological SRMs were consistently greater than 80 percent with a few exceptions. Nickel recovery ranged from 69 to 77 percent among the three SRMs (2976, 1566b, and Tort2). Lead averaged 77 percent in SRM 2976. Zinc was not quantified in SRM 1566b due to the high concentrations. The lower recovery in SRM 1566b likely reflects poor quantitation of the high Zn concentrations present in the samples.

Quality assurance for Hg and Se methods comprised analyses of sample splits (appendix 3) and a variety of standard reference materials (appendix 4). Observed concentrations of reference material fell within the range of their certified values (appendix 4).

Method detection limits (MDL) and reporting levels (MRL) for ICP-OES methods were determined using the procedures outlined by Glaser and others (1981), Childress and others (1999), and U.S. Environmental Protection Agency (2004) (appendix 5). A full quality-assurance/quality-control plan is available upon request.

#### Salinity

A small volume (approximately 25 mL) of water that had pooled on the surface of the mudflat was collected in a polypropylene bottle and returned to the laboratory, where it was measured for

salinity with a handheld refractometer. Additionally, salinity is determined in a small volume (approximately 1-2 mL) of bay water that is collected from the body cavity of *M. petalum*. Salinity of the surface water sample and the body water sample consistently agreed within  $\pm 2$  parts per thousand (ppt).

#### **Other Data Sources**

Precipitation data for San Francisco Bay are reported from a station at San Francisco International Airport (station identification SFF) and were obtained from the California Data Exchange Center (http://cdec.water.ca.gov/).

#### **Biological Response**

#### Condition Index

The condition index (CI) is a measure of the clam's physiological state derived from the relation between soft tissue weight and shell length and reported as the soft tissue dry weight (in grams) for a clam of a particular shell length (in mm). Specifically, for each collection, the relation between the average shell length and tissue dry weight of the composites was fit with a linear regression, and from that regression, the tissue dry weight was predicted for a normalized shell length of 25 mm.

#### **Reproductive Activity**

A minimum of 10 clams of varying sizes ( $\geq 10$  mm shell length) were processed for reproductive activity concurrent with samples for metal analyses. Clams were immediately preserved in 10-percent formalin at the time of collection. The visceral mass of each clam was removed in the laboratory, stored in 70-percent ethyl alcohol, and then prepared using standard histological techniques. Tissues were dehydrated in a graded series of alcohol, cleared in toluene (twice for 1 hour each), and infiltrated in a saturated solution of toluene and Paraplast® for 1 hour, and two changes of melted Tissuemat® for 1 hour each. Samples were embedded in Paraplast® in a vacuum chamber and then thin sectioned (10 µm) using a microtome (Weesner, 1960). Sections were stained with Harris' hematoxylin and eosin, and examined with a light microscope. Each individual was characterized by size (length in mm), sex, developmental stage, and condition of gonads, thus allowing each specimen to be placed in one of five qualitative classes of gonadal development (previously described by Parchaso, 1993).

#### **Community Analysis**

Samples for benthic community analysis were collected with hand-held core 8.5-cm in diameter and 20-cm deep. Three replicate samples were taken arbitrarily, within a square-meter area, during each sampling date.

Benthic community samples were washed on a 500-µm screen, fixed in 10-percent formalin, and then later preserved in 70-percent ethanol. Samples were stained with rose bengal solution. All animals in all samples were sorted to species level where possible (some groups, such as the oligochaetes, are still not well defined in the Bay), and individuals for each species were enumerated. Taxonomic work was performed in conjunction with a private contractor familiar with the taxonomy of San Francisco Bay invertebrates (Susan McCormick, Colfax, California) (appendix 8). McCormick also compared and verified her identifications with previously identified samples.

### Results

#### Salinity

Surface-water salinity in the Bay is related to and roughly the inverse of the seasonal weather pattern in Northern California, which is characterized by a winter rainy season, defined as months with rainfall amounts greater than 0.25 inches (November through April), and a summer dry season (May through October). Total precipitation measured at station SFF in 2015 was 9.7 inches with slightly more than half of that occurring in December (http://cdec.water.ca.gov/) (fig. 2). Cumulative precipitation in 2015 was less than half the average annual rainfall of 20 inches during the period of record (1994–2015) (fig. 2). Salinity varied between 23 and 30 ppt during 2015 (fig. 3, table 1). Mean annual salinity correlated with annual cumulative rainfall (Pearson r = -0.53; p = 0.01; n = 22), and appears to have increased over the period of record (fig. 3).

#### Sediments

Metal concentrations in surface sediments from Palo Alto typically display an annual periodicity that generally corresponds to seasonal fluctuations in the relative abundance of fine particles (figs. 4–8, appendix 1). Thomson-Becker and Luoma (1985) suggested that this intra-annual variation is related to changes in the size distribution of sediment particles caused by allocthonous inputs and deposition of fine-grained particles in the winter and their subsequent wind-driven resuspension in the summer and fall. Because metal concentrations vary as a function of the ratio of surface area to volume of a particle, metal concentrations tend to track the percent of fine particles. The seasonal variation in metal concentrations in surface sediment suggests that the particle-size distribution within the <100  $\mu$ m sediment fraction varies seasonally. In particular, the concentrations of Al and Fe correlate with the abundance of fine particles, reflecting the contribution of clays rich in Al and Fe (fig. 4C).

Surface sediments from Palo Alto in 2015 contained on average 1.28 percent (by weight) total organic carbon (TOC) (table 1). As with metals, TOC varied with the percentage of fine-grained (<100  $\mu$ m) sediment particles throughout the year.

Chromium (Cr) and nickel (Ni) are highly enriched in some geologic formations within the watershed. Studies of sediment cores from North San Francisco Bay indicated that concentrations of these elements similar to those reported here were derived from natural geologic inputs (Hornberger and others, 1999; Topping and Kuwabara, 2003). At Palo Alto, seasonally varying concentrations of Cr and Ni in surface sediments may indicate inputs of Cr and Ni bearing minerals from the surrounding watershed. In 2015, as in previous years, maximum concentrations coincided with winter/spring maxima in fine sediments, consistent with inputs from the surrounding watershed, whereas minimum concentrations occurred during the late summer/fall (fig. 5, table 1). However, concentrations of both elements typically vary by less than 20 percent. Moreover, identification of inputs is confounded by seasonal changes in the particle size distribution of sediments described above. Chromium and Ni concentrations have differed between years during the record (p<0.05; ANOVA). For example, Cr was relatively high in 2003 and during 2009–2010. In 2015, the respective concentrations of Cr and Ni were 118±4 (mean ±1 standard error of the mean (SEM)) and 102±6.

Concentrations of Cu and Zn in sediments are shown with sediment guidelines set by the National Oceanic and Atmospheric Administration (Long and others, 1995) in figures 6 and 7 and table 1. Long and others (1995) defined values between ERL (effects range-low) and ERM (effects range-median) as concentrations that are occasionally associated with adverse effects on sensitive species (21–47 percent of the time for different metals). Values greater than the ERM were frequently associated

with adverse effects on sensitive species (42–93 percent of the time for different metals). It is important to note, however, that these effects levels were derived mostly from bioassay data and are not accurate estimates of site-specific sediment toxicity. For most of the record, Cu concentrations have exceeded the ERL (34 mg/kg). In 2015, the average annual copper was  $34\pm2$  (SEM) mg/kg, practically equivalent to the ERL, as a result of the relatively higher concentrations during the middle part of the year. The typical annual pattern was evident: Cu increased during the first half of the year, then declined in the fall (fig. 6, table 1). In general, the partial-extractable Cu concentrations correlate with the near-total Cu (p<0.001 for the 1994–2015 dataset). The partial extractions represent approximately 42 percent of the Cu recovered in the near-total digest. Near-total Zn concentrations were below the Zn ERL (150 mg/kg) for much of the record (fig. 7, table 1). Exceedances typically occurred during the winter season. In 2015, the average Zn concentration was 139 $\pm$ 6 mg/kg with a maximum concentration of 161 mg/kg in March.

The annual average of silver extracted from sediments using the partial-extraction method has varied from a high of 1.62 mg/kg in 1979 to a low of 0.20 mg/kg in 2008–2009 (appendix 2). In 2015, Ag concentrations averaged  $0.33\pm0.04$  mg/kg. Like other trace elements, Ag concentrations have displayed a seasonal pattern over the record (fig. 8, table 1). In 2015, annual maxima were observed during February and March (0.44–0.49 mg/kg, and annual minima occurred from September to December (0.10–0.26 mg/kg).

Mercury concentrations in sediment from Palo Alto during 2015 averaged 0.44 mg/kg (table 1), which was slightly elevated compared to the previous four years (fig. 9). Concentrations in February (0.61 mg/kg) and in December (0.67 mg/kg) were notable for being among the highest concentrations recorded at the site. These concentrations appear to be at the upper range of concentrations typical for South Bay sediment (Davis and others, 2012).

Selenium concentrations in 2015 ranged modestly from 0.30 mg/kg (December) to 0.44 mg/kg (September), and averaged 0.36 mg/kg (fig. 9, table 1). Concentrations in 2015 were comparable to those of the previous year. Sedimentary Se hasn't exhibited a sustained temporal trend over the period of the record. During this time, Se concentrations have alternated from relatively high (2004, 2006, 2008, and 2011) to relatively low (2005, 2007, 2009–2010, 2012, and 2014–2015).

#### Clam Tissue

Metal concentrations in the soft tissues of *Macoma petalum* reflect a combination of metal exposures from water and food, and the diluting and concentrating effects of gaining and losing tissue mass associated with annual growth and reproductive cycles. Exposures to Cu and Ag at Palo Alto are of special interest because of the high tissue concentrations observed at this site in the past (figs. 10 and 11, appendix 7). During 1977–1990, the ranges in annual concentrations of Cu and Ag were 35–287 mg/kg and 7.7–106 mg/kg, respectively. Since 1990, concentrations have been considerably lower, 24–71 mg/kg for Cu and 1.8–7.5 mg/kg for Ag. Concentrations have been particularly low and stable since 1997, except for a 2-year period in 2006–2007 when concentrations increased modestly. Annual mean concentrations of Cu and Ag for 2015 were 43±7 mg/kg and 3.3±0.6 mg/kg, respectively (table 2).

Intra-annual variations in Ag and Cu concentrations in clam soft tissues display a consistent seasonal signal characterized by fall/winter maxima and spring/summer minima. The amplitude of this seasonal cycle varies from year to year. For example, the winter maxima and the magnitude of seasonal Ag and Cu concentrations during 1994–1997 and in 2007 were large relative to other years (figs. 12 and 13). In 2015, both Cu and Ag concentrations followed a typical seasonal pattern that was of similar magnitude as that observed in 2007. These patterns most likely reflect the interaction between the exposure regime at the site and the annual growth and reproductive cycles of *M. petalum* (Cain and

Luoma, 1990). The latter can largely account for intra-annual variability in tissue metal concentrations during periods of relatively stable metal exposures. For example, over the period of 1994 to 2015, the seasonal variations of Cu and the CI varied inversely of one another and were of similar magnitude, suggesting that variations in tissue weight driven by growth and reproduction highly influenced the tissue Cu concentrations (fig. 12*C*).

As with Cu and Ag, tissue concentrations of Cr (fig. 14, table 2), Ni (fig. 15, table 2), and Zn (fig. 16, table 2) also exhibited annual cycles of variable magnitude throughout the record. None of these elements exhibit a clear temporal trend (either downward or upward) in concentration. In 2015, the annual average Cr, Ni, and Zn concentrations were 2.6, 5.6, and 195 mg/kg, respectively. Wellise and others (1999) observed that intra-annual and inter-annual patterns of Cr, Ni, and Zn in *M. petalum* at Palo Alto were generally similar to those observed near the San Jose/Santa Clara Water Pollution Control Plant, indicating that regional-scale processes may be more important than treatment plant inputs in controlling the bioavailability of these elements.

The average Hg concentration in *M. petalum* in 2015 was 0.43 mg/kg (fig.17, table 2). Intraannual variation of Hg in 2015 displayed the typical pattern of fall/winter maxima and spring/summer minima. The maximum concentrations of Hg in *M. petalum* (January and September) did not coincide with maximum total sedimentary Hg concentrations (February and December). Although Hg concentrations increased in 2015 relative to the previous four years, a long-term trend in Hg concentration is not evident (fig. 17*A*).

Selenium concentrations in *M. petalum* averaged 4.49 mg/kg in 2015 (table 2). An annual cycle of moderate magnitude was displayed with the highest concentrations in January (5.07 mg/kg) and the following September and December (4.93 and 4.63 mg/kg, respectively; fig. 18, table 2). Although peak concentrations of Se have been lower during 2012 to 2015 than most previous years, a long-term trend is not evident.

Data on the condition index for *M. petalum* at Palo Alto extends back to 1988 (fig. 19, table 2). As previously discussed, the data fluctuate seasonally in relation to growth and reproductive cycles, and annual cycles differ in magnitude. Condition index values typically increase to a maximum in the spring (pre-spawning period) then taper off throughout the year (fig. 19*C*). The seasonal variation of CI in 2015 was typical for the record, and the annual average CI (134 mg) was consistent with values observed since 2007. The maximum CI in May (228 mg) coincided with the minima for most of the metals concentrations in *M. petalum* in 2015, and the minimum CI (65 mg in February) coincided with the maxima for most of the metal concentrations in *M. petalum*.

#### Reproduction of Macoma petalum

Previous studies suggested that reproduction in *M. petalum* is affected by Ag (Hornberger and others, 1999; Shouse and others, 2003). The time series of reproductive activity (fig. 20) shows that reproductive activity was lowest with periods of reproductive inactively during 1974–1983, coincident with the period of highest Ag and Cu exposure. As metal exposures declined during the 1980s and into the early 1990s, reproductive activity increased. Data from 2015 show that *M. petalum* continues to be highly reproductive relative to the 1970s with a relatively high percentage of the animals being reproductively active in any given sample. A closer look at the last five years of data (2011–2015) demonstrates the seasonality of reproduction in *M. petalum* (fig. 21, table 3). Reproductive activity commences in the fall and concludes the following spring.

#### **Benthic Community**

Estimates of species diversity and total animal abundance are simple metrics that are used in assessing environmental stress on biological communities. Species diversity at the Palo Alto site, as estimated by a time series of number of species was at a minimum in 1998, trended upward through 2012, and has shown a slight decline since its peak in 2012 (fig. 22). Total animal abundance has varied significantly during the sampling period (fig. 23). The difficulty with these types of metrics is that they do not consider the possibility that one species can take the place of another or that high abundance is based on one species. Depending on the characteristics of a species new to the community or newly dominant in the community, the community structure and function may change as a result of this change in species composition or dominance. The details of changes in species composition are important because they may reflect the relative ability of species to accommodate environmental stress and redistribute site resources. In general, the species composition at the study site has changed little since 1998, although there have been seasonal eruptions of several species in some years.

Three common bivalves (*Macoma petalum*, *Mya arenaria*, and *Gemma gemma*) have not shown any consistent trend over the 42-year period from 1974 to 2015 (figs. 24–26). Significant seasonal and inter-annual variability has been displayed in species abundances for all species found at the Palo Alto site. The three common bivalves illustrate this variability well. *Gemma gemma* has been particularly volatile since 2005. *Gemma gemma* abundance dropped to near zero in late fall 2007, took three years to recover to previous average densities, and then continued this abundance through 2015.

Six species have shown trends in their abundance since the 1970s, and these trends continued through 2015. The first species, Ampelisca abdita, is a small crustacean that lives above the surface of the mudflat in a tube built from selected sediment particles. A. abdita showed a general decline in abundance (fig. 27) after 1998 and mostly low abundances persisted through 2014. In 2015, A. abdita showed an order of magnitude increase in abundance. The second species to show a significant trend is the small polychaete worm Streblospio benedicti, which also builds a tube above the surface of the mudflat. S. benedicti abundance has declined through the study years and, over the past 7 years, the species has maintained a seasonal pattern of increasing spring abundance followed by a fall/ winter decline throughout the study (fig. 28). The abundance of the small burrowing crustacean Grandiderella japonica, a deposit feeder, became more seasonally consistent after 2000 (fig. 29), with particularly low abundances in 2006, 2007, and 2011. This species has shown a consistent seasonal peak in abundance in the fall since 1999 with the exception of 2011. Neanthes succinea, a burrowing polychaete that feeds on surface deposits and scavenges for detrital food, showed large seasonal fluctuations in abundance throughout the study (fig. 30) and like other species its abundance declined in 2011 and remains in low numbers in 2015. Two species showed an increase in abundance within the time series. The first was the polychaete worm Heteromastus filiformis (fig. 31), a burrowing, subsurface-deposit-feeding species that lives deep in the sediment (usually 5–20 cm below the surface of the mudflat). Abundance increased sharply in 1985 and then partly receded in the late 1980s. Abundance remained higher than in the late 1970s until 2008, when there was a large decline in H. filiformis abundance (fig. 31). H. filiformis abundance increased slightly after 2010. Abundance data from the 1980s indicate that this is a species that increases slowly, possibly because of their egg-laying mode of recruitment. The second species showing an increase was Nippoleucon hinumensis, a small, burrowing, surface-deposit-feeding crustacean, which appeared in the dataset in 1988 (fig. 32) following its introduction into the bay in 1986 (Cohen and Carlton, 1995). A complete list of the benthic species found at the Palo Alto site in the year 2015 is shown in appendix 8. The benthic species name changes (as of 2015) for the species listed in appendix 8 are shown in appendix 9.

A sudden drop in animal abundance was observed in February 2008. Very few animals were found at the site, and the mudflat community was evidently stressed by some event between the January and February sampling. Possible causes of the stress include sedimentation or freshwater inundation. There was a large storm on January 25, 2008, with rainfall rates exceeding 0.5 cm/hr for more than half the day, including during the low-tide period. No obvious changes in the sediment surface were observed, but sediment changes can occur and be incorporated quickly in this tidal environment. Other possible causes of benthic community death or exodus include a toxic event or anoxia. It is unlikely that either of these occurred, because M. petalum were present in the deep sediment in February 2008, and animals were found again at the site in March 2008. This would not happen with toxicity or anoxia. The timeline for recovery from anoxia can be estimated on the basis of observations following an anoxic event at this site in 1975. Macroalgae were deposited on the mudflat surface and began to decay, and the resulting bacterial consumption of oxygen led to anoxia. The benthic community took many months to recover from this anoxic event. Animals that returned after the disturbance in 2008 include those species with pelagic larvae and mobile adults, as would be expected. Nonmobile brooders returned to the site in 2009, concurrent with an increase in abundance of the brooding clam G. gemma and the brooding polychaete Streblospio benedicti. This trend continued into 2015, when brooder species were more than half of the top 10 most abundant species, while oviparous and spawner species made up the rest.

Multivariate analyses of population data of the dominant species with environmental parameters did not reveal any relations, except with the concentration of Ag and Cu in the sediment and in the tissue of *M. petalum* (using data reported by David and others, 2002). Therefore, this update will consider only those metals. Metal concentration and abundance of species with the most susceptible mode of feeding and reproduction will be compared over the period of the study. One such susceptible species, the worm *H. filiformis*, increased in abundance with the decrease in Ag and Cu until 2008 (fig. 31). This was of interest because H. filiformis has continual tissue contact with the sediment at the exterior of its body, as well as within its body, as a result of its lifestyle of burrowing through the sediment and consuming a diet of mud and organic particles. In addition, this is one of the few species in the present community that reproduces exclusively by laying its eggs in the sediment. The larvae hatch after 2 or 3 days and spend a very short period (2–3 days) in the plankton before settling back to the mud as juvenile worms (Rasmussen, 1956). The short planktonic period limits the species' speed of expansion into new areas. The authors hypothesize that once a few individuals successfully arrived at the study site, *H. filiformis* increased in abundance because either the adult worms or the eggs found the environment agreeable. This species is not likely to move into an area quickly after an environmental stressor because of its mode of reproduction and short planktonic larval period. A large spike in H. filiformis abundance was observed in January 2008 because of the settling of larvae, but these larvae did not survive the event that occurred before the February sampling. So far, the species has not returned in high numbers to the study site. The dynamics of recovery for this species will continue to be monitored closely.

Two species have shown the opposite trend of *H. filiformis*, the crustacean *A. abdita* (fig. 27) and the worm *S. benedicti* (fig. 28) have declined in abundance coincident with the decline in metals. These species have very similar life-history characteristics that make them less susceptible to high Ag and Cu concentrations in sediment. Both species live on the surface of the sediment in tubes that are built from sediment particles. They feed on particles in the water column or on particles that have settled to the sediment surface, brood their young, and produce young that are capable of either swimming or settling upon hatching. These opportunistic characteristics make these species ideal for invading a disturbed or stressed environment; thus, they are capable of rapid increase in population size and distribution. It is not surprising that both species immediately responded to the near-empty community

in February 2008 and have subsequently declined (figs. 27 and 28). This abundance pattern is consistent with what is expected of an opportunistic species, confirming both their tolerance to elevated metals as well as an inability to out-compete less opportunistic diverse communities in non-stressed conditions.

Other species share the characteristics highlighted in our discussion of H. filiformis, S. benedicti, and A. abdita; the species with similar characteristics have been combined into plots that examine the percent of abundance represented by each feeding and reproductive mode (figs. 33–36). Because the natural spatial variability (that is, the large standard deviations around the monthly means) and seasonal variability of invertebrate abundance can be quite large, the average percentages for the month of August of each characteristic reproductive and feeding mode are shown in figures 33–36. To interpret these plots, the life-history characteristics must first be examined to determine if there is some mechanism by which this organism could be responding to a decrease in Ag or Cu in the environment. It is likely that Ag, but probably not Cu, adversely affected reproduction of all animals (Hornberger and others, 2000b). If species with pelagic larvae were transported into the area they did not survive to dominant the community. Species having oviparous and mixed (species capable of oviparity and brooding) reproductive modes (Ahn and others, 1995; Hornberger and others, 2000b) are worth examining in more detail. The gradual increase in abundance of this group through 1983 occurred concurrently with the gradual reduction of metals in the environment during that time. In the present environment, with much lower metal concentrations, these species respond to a different variety of stresses, and the percentage of brooding and oviparous individuals in the community reflects those stresses (figs. 33 and 34). Although the percentage is variable, the brooders plus oviparous individuals has never been as high as it was in the early 1970s. The authors interpret this as being a reflection of the general health of the benthic environment. In a similar manner, we can examine the feeding modes of the majority of the individuals (figs. 35 and 36). High concentrations of either Cu or Ag in the sediment are unlikely to be healthy for species that ingest the sediment in order to consume the interstitial and attached carbon; thus, it is reasonable to expect species that consume particles from the water column to be more protected from the contaminants in the sediment. Filter feeding species are usually the dominant group throughout the data set, and the subsurface deposit feeders are the group that shows the largest increase in dominance after the 1970s. This is consistent with the conceptual model posed here.

The change in function of the benthic community over time can be examined by ranking the top 10 species by abundance and plotting the  $\log (n + 1)$  of mean abundance against the rank of each species. The plot for 2015 (fig. 37) is indicative of a healthy benthic community with species dominance, as revealed by abundance, not showing large differences among the top 10 species. An examination of similar plots for August of three hydrologically dry years during this study (1977, 1989, and 2002) shows that the shape of the curve has changed greatly and that the 2015 curve is similar to that seen in 1989. The figure shows a community that was, with the rest of the species having similar abundances, heavily dominated by three species in 1977, 1989, and 2015, versus a community with one dominant species in 2002. Although G. gemma is the most dominant species in all years except 1989; in 2015, the graph shows a smooth downward slope showing a more homogenized decrease in species abundance. The 1977 community plot is the most extreme, with three species dominating the community and the remainder having similar but relatively low abundances. It is informative to examine the rank-abundance plots within the context of the life-history characteristics of each species to determine if shifts in plot shape coincide with a shift in community structure and function that might be indicative of a healthier environment. Two critical life history characteristics are shown: feeding mode in figure 38 and reproductive mode in figure 39. The 1977 community was dominated by filter feeding species (species that consume particles in the water column), mixed feeders (species that have the option of either filter feeding or feeding on the sediment surface), and two species that feed on food particles

on the sediment surface. In 1989, the species composition had shifted such that filter feeding species, subsurface deposit and surface deposit feeding species (those that ingest sediment and strip the food off of the sediment in their gut) dominated the community. In 2002, a shift was observed towards species that could either filter feed or deposit feed (mixed feeders) and those species that feed on subsurface sediment. The most recent data (2015) show the community to be similar to that of 1989, with the composition having shifted to that of filter feeding species, subsurface deposit and surface deposit feeding species. Over the period of this study, a shift has occurred from a community dominated by species that feed either in the water column or on recently settled food particles on the sediment surface to a mixed community of species that feed directly on the subsurface sediment, those capable of feeding in the water column, and those feeding on the sediment surface. The species that returned following the defaunation event in January/February 2008 have maintained this pattern. Thus, it is unlikely that any sediment-borne pollutant caused the collapse of the community in early 2008.

An examination of these rank-abundance plots using reproductive mode as the descriptor for each point is equally informative (fig. 39). The dominant species in 1977 were species that brood their young and release fully functional juveniles into the environment. In 1989, there were still several brooders, but there were also two species that lay their eggs in the sediment. Although brooding species remain the most abundant species for all years, species that spawn their gametes into the water column in combination with those that lay eggs in the sediment (oviparous) have the highest presence. It is possible that some of the metal contaminants found in the sediment in the 1970s at this location limited the success of species that consumed the sediment for food, laid eggs in the sediment, or depended on water-borne larvae to repopulate the community. Interestingly, the reproductive mode of most species present in 2015 is brooding with some egg laying (oviparous) and spawning species

## Summary

#### Long-Term Observations

Since 1974, USGS personnel have monitored and conducted basic research on the benthic sediments and biological community in the vicinity of the discharge of the Palo Alto Regional Water Quality Control Plant (PARWQCP). The time series presented here update previous findings (for example, Hornberger and others, 2000a; Luoma and others, 1991, 1995a, 1996; Moon and others, 2005; Shouse and others, 2003, 2004; Thompson and others, 2002; Cain and others, 2006; Dyke and others, 2011, 2012) with additional data from January 2015 through December 2015 to create a record spanning 41 years. This long-term dataset includes sediment chemistry and tissue concentrations of metals (1977–2015 for Cu and Ag, 1994–2015 for other metals), condition index (1988–2015) and reproductive activity in *M. petalum*, and population dynamics of benthic invertebrate species (1974–2015). This time series encompasses the period when exceptionally high concentrations of Cu and Ag were found in *M. petalum* (1970s and early 1980s) and the subsequent period when those concentrations declined. The sustained record of biogeochemical data at this site provides a rare opportunity to examine the biological response to metal contamination within this ecosystem.

Studies during the 1970s showed that sediments and *M. petalum* at the Palo Alto site contained highly elevated levels of metals, especially Ag and Cu, as a result of metal-containing effluent being discharged from the Palo Alto Regional Water Quality Control Plant (PARWQCP) to South San Francisco Bay. In the early 1980s, the point-source metal loading from the nearby PARWQCP was significantly reduced as a result of advanced treatment of influent and source mitigation. Coincident with declines in metal loadings, concentrations of metals in the sediment and in the clam *M. petalum* (serving as a biomonitor of metal exposures) also declined, as previously described by Hornberger and

others (2000b). Inter-annual trends in clams and sediments were highly correlated with Cu loadings from PARWQCP (concurrent loading data for Ag were not available). Metal levels in sediments and clams responded relatively quickly to changes in metal loading; the reduction in metal loadings by the PARWQCP resulted in a reduction in metal concentrations in both the sediment and *M. petalum* within a year (Hornberger and others, 2000b).

Biological responses to metal inputs to South San Francisco Bay were assessed at different levels of organization. These responses are interpreted within the appropriate temporal context. Because metal exposures were already high when the study began, interpretations are based on observed changes in biological attributes as metal inputs declined. In general, discernable responses at the organism level (that is, reproductive activity, a manifestation of a cellular or physiological change) to metal exposure may occur within a relatively short time (for example, at intra- and inter-annual time scales as previously exposed individuals recover and successive cohorts become established), whereas population and community level responses take longer to develop. Stable changes in the benthic community may take a relatively long period of time to be expressed because of the normally high degree of intra-annual variability of benthic community dynamics, which reflects the cumulative response to natural and anthropogenic disturbances. It is therefore critical that sampling frequency and duration be conducted at temporal scales appropriate to characterize the different biological responses.

During the first 10 years of this study, when the metal concentrations were high and declining, the benthic community was largely composed of nonindigenous, opportunistic species that dominated because of their ability to survive the many physical disturbances on the mudflat (Nichols and Thompson, 1985a,b). These disturbances included sediment erosion and deposition and aerial exposure at extreme low tides, as well as less well defined stresses. The possible effects of metal exposure as a disturbance factor were not considered in the analyses by Nichols and Thompson because the decline in metal concentrations in *M. petalum* and sediment had just begun.

However, data collected throughout the period of declining metal exposure have revealed biological responses to this metal decline. Reproductive activity improved within a year or two of reduced metal exposure, and responses at the population and community levels were observed afterward. Identification of these responses was possible because the frequency of sampling allowed long-term trends related to metal contamination to be identified within the context of repeating seasonal cycles and unrelated intra-annual variation. Decreasing particulate concentrations of trace metals in the local environment have improved the condition of resident populations of invertebrates.

The dramatic decline in the benthic community in early 2008 did not appear to be related to metals, but instead may have been the result of a natural stressor, such as a sedimentation or freshwater event. The composition of the benthic community supports that supposition. Mobile animals such as *M. petalum* that were capable of burrowing down to avoid the stressor probably did so, but many other species either relocated or were killed. This natural disturbance gives scientists the opportunity to observe mudflat community recovery from a natural stressor and to compare this recovery to that observed during the long-term decline in metals. Long-term shifts in species abundance at Palo Alto have been interpreted to be a response to decreasing sediment contaminants. These community changes have included a shift from species that live on the surface, filter food out of the water column or consume particles on the sediment surface, consume the sediment directly to harvest food particles, and spawn and lay eggs in the sediment. The 2008–2015 data reveal a community that had a short-term physical stressor but not one that was subject to unhealthy sediment. This "natural experiment" has given USGS scientists a great opportunity to test various hypotheses on the benthic community response

to different stressors. Future data will further refine the understanding of the response of this benthic community to natural and anthropogenic.

The ecology of the Palo Alto mudflats is part of the larger South San Francisco Bay, which has been undergoing some changes in recent years. During 1999–2005, USGS scientists noticed an increase in phytoplankton biomass in the southern bay. Sampling in the deeper water of the southern bay showed that the bivalves were mostly absent from the system during this increase in primary production. Cloern and others (2007) indicate that the cause of the decline in bivalves was an increase in fish predators resulting from increased offshore upwelling activity. The higher reproductive success of demersal fish, crabs, and shrimp during this period resulted in a higher number of juveniles moving into the South San Francisco Bay to grow. Since 2005, scientists have seen the large bivalve populations fluctuate more than in previous years, and these fluctuations have been reflected in changes in phytoplankton biomass in the system (primarily through an increase in phytoplankton biomass in late summer and fall). The value of these findings in greater South San Francisco Bay to this study is twofold. First, it reinforces the importance of the benthic community's impact on ecosystem function. Second, it shows that the high intertidal community at the Palo Alto site has not been demonstrably affected by these greater South San Francisco Bay influences during these years. This finding solidifies the authors' confidence that the changes observed in the benthic community are in large part due to local factors.

#### 2015 Observations

Throughout 2015, Cu and Ag concentrations in sediments and soft tissues of the clam, M. petalum, remained representative of the concentrations observed since 1991, following the significant reductions in concentrations during the 1980s that coincided with reductions in the discharge of these elements from PARWQCP. Since 1991, annual mean Cu and Ag concentrations have fluctuated modestly and without any extended trends. This is also true for other elements. For example, sedimentary selenium (Se) concentrations have been variable from year to year and showed no sustained temporal trend. Selenium concentrations in surface sediment declined in 2009–2010 from the record high concentrations observed in 2008, increased in 2011, and subsequently (2012-2015) decreased to about half the annual average observed in 2008. In another example, annual average concentrations of Cu and Ag in M. petalum were relatively low from 1997 to 2005, increased notably in 2006–2007, returned to 1997–2005 levels during 2008–2014, and in 2015 increased again to levels comparable to 2006–2007. Silver annual averages in *M. petalum* also were slightly greater in 2015 compared to the previous seven years. However, the most recent results (2015) show that Ag and Cu in *M. petalum* are only 3 percent and 15 percent, respectively, of the maximum values observed during 1978–1980. Since 1994, the magnitude of the annual growth and reproductive cycle of *M. petalum* appears to influence the modest inter-annual variation in elemental tissue concentrations. Concentrations of Ag and Cu in sediments in 2015 were 20 percent and 40 percent, respectively, of the record high concentrations observed in 1979. Inter-annual variation in Cu and Ag in M. petalum from 1991 to 2006 did not correlate with discharge of Cu and Ag from PARWQCP (Lorenzi and others, 2007), indicating that, as with other elements of regulatory interest, including Cr, Ni, and Zn, regional-scale factors now largely influence sedimentary and bioavailable concentrations (see, for example, Luoma and others, 1998). Abiotic factors that affect the seasonal and year-to-year patterns in sedimentary and tissue concentrations may include precipitation, nonpoint-source runoff, cycling of legacy contamination, accelerated erosion of salt marsh banks in recent years, and periods of accretion and erosion of sediment on the mudflat.

In 2015, individual and community-level metrics did not exhibit any obvious signs of metal stress. Recovery of reproductive activity in *M. petalum* following the decline in metal exposures has

been sustained. The species abundance data and the constancy of functional groups within the benthic community continues to show signs of a community that has recovered from metal stress and has stabilized.

#### Value of Long-Term Monitoring

This study highlights the importance of long-term ecosystem monitoring. Often, antecedent data are not available to evaluate ecological responses to contaminants. However, long-term studies may be able to separate contaminant-influenced biological responses from those related to natural processes and other potential stressors. Such was the case in this study wherein the linkage between metal exposure and biological effects was established by examining long-term biological responses at different levels of organization (individual, population, community) relative to the mitigation of metal loadings from a point source. The decadal time series produced during the course of sustained efforts at the Palo Alto site have made it possible to describe trends, identify previously undocumented phenomena, and pose hypotheses that have guided past detailed explanatory studies and can guide future studies. Monitoring studies cannot always unambiguously determine the causes of trends in metal concentrations or benthic community structure. The strength and uniqueness of this study is the integrated analysis of metal exposure and biological response at intra- and inter-annual time scales over multiple decades. Changes and trends in community structure that may be related to anthropogenic stressors, as was seen in this study, can be established only with a concerted and committed effort of sufficient duration and frequency of sampling. Such rare field designs allow biological responses to natural stressors to be characterized and separated from those stressors introduced by humans. Through interpreting the timeseries data, it has been possible to separate anthropogenic effects from natural annual and inter-annual variability. The data from the recent record (that is, within the past decade) with indicators of metal contamination, physiological status of an indicator species (*M. petalum*), and benthic community structure increasingly appear to be indicative of an integrated regional ecological baseline. Changes are occurring in the South San Francisco Bay watershed. For example, the South Bay Salt Ponds Restoration Program has been implemented with unknown implications (positive or negative) for all of South San Francisco Bay. Nanomaterials, many of which include metal-based products in forms for which environmental scientists and regulators have little or no experience, are being used in an increasing number of common consumer products. The long-term, detailed, integrated ecological baseline that has been established at this sampling site will be uniquely valuable in assessing the response of the environment as human activities in the watershed continue to change.

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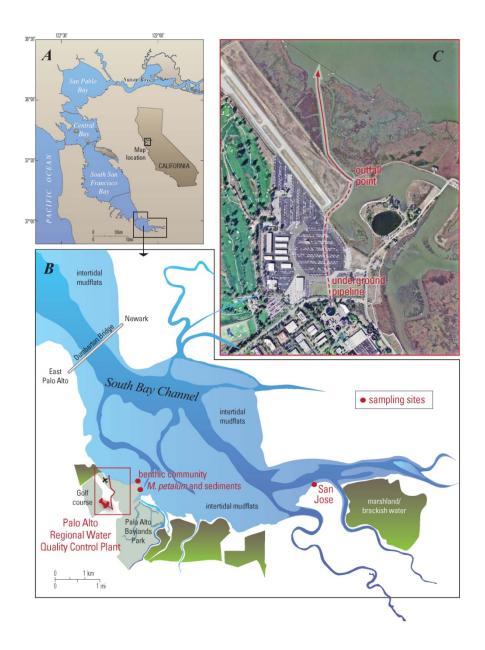
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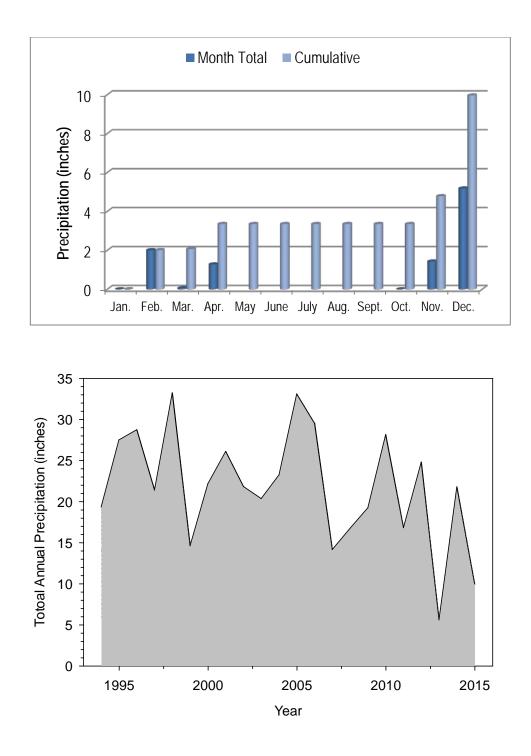
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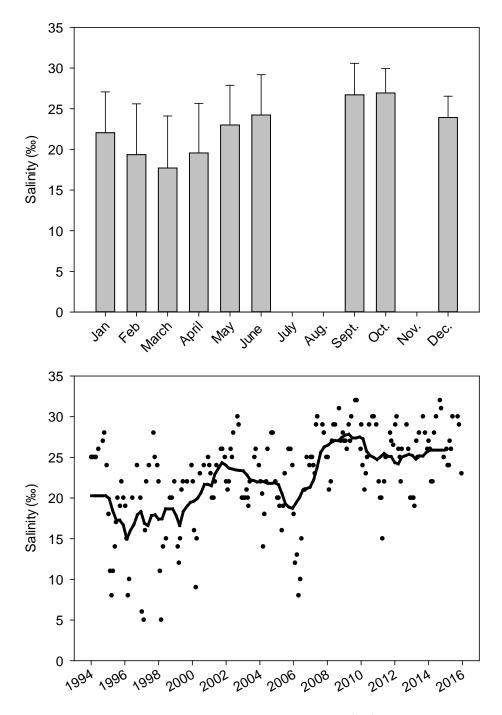
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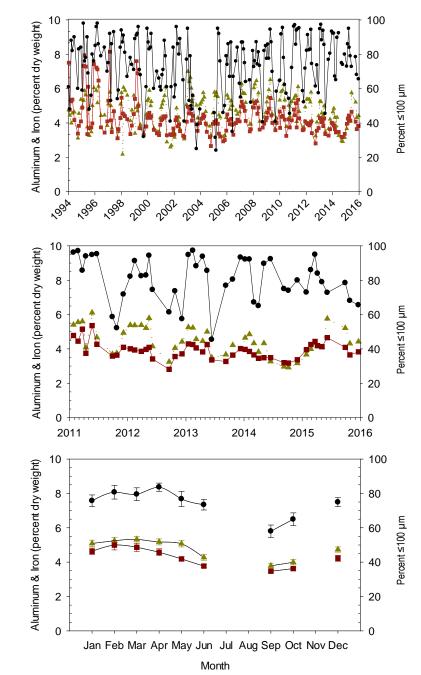
**Figure 1.** Location of the Palo Alto sampling site in South San Francisco Bay, Calif. (*A*) Sampling area within the greater San Francisco Bay region. (*B*) The intertidal mudflats are shaded light blue, subtidal in dark blue, and marshland/brackish water in green/brown. The benthic community and *M. petalum* and sediments points make up the Palo Alto sampling site. The San Jose sampling site (inactive) is also shown for reference. (*C*) Effluent from the Palo Alto Regional Water Quality Control Plant (red thumbtack, insert *B*) is discharged by way of underground pipe (dashed red line) until it reaches the mouth of a small channel that connects to the intertidal mudflat approximately 1 kilometer northwest of the sampling sites.



**Figure 2.** Precipitation recorded at San Francisco WB AP in San Mateo County, CA. Top panel shows the monthly and cumulative precipitation in 2015. The bottom panel shows the total annual precipitation for the period 1994–2015. The station (identification SFF) is operated by the National Weather Service.



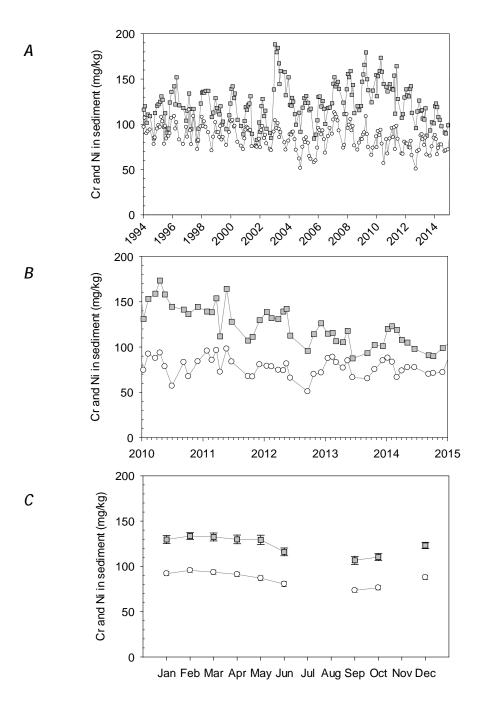
**Figure 3.** Surface-water salinity in parts per thousand (ppt) at the Palo Alto site, Calif., 1994–2015. The top panel illustrates seasonal variation as the monthly averages ( $\pm 1$  std, n = 21), and the bottom panel shows individual monthly data (dots) and the running average (solid line) indicating the long-term pattern.



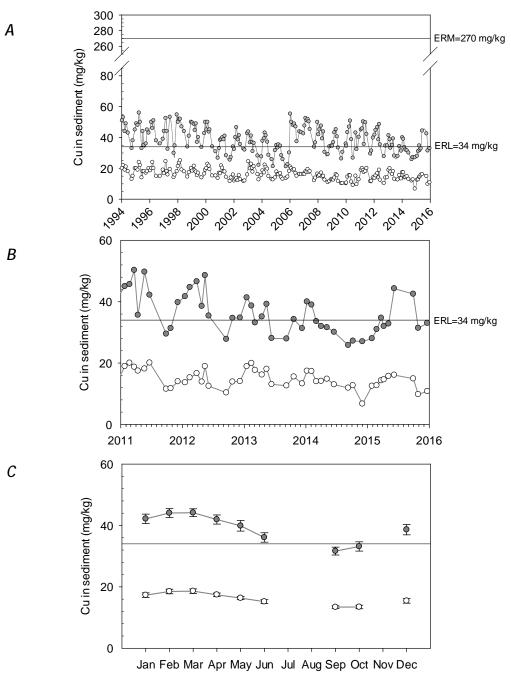
**Figure 4.** Aluminum, iron, and silt/clay in sediments, Palo Alto, Calif., 1994–2015. (*A*) Percent aluminum ( $\blacktriangle$ ), iron ( $\blacksquare$ ) (extracted by near-total digest), and percent of sediments as silt/clay  $\leq 100 \mu m$  ( $\bigcirc$ ). Data on percent of sediment as silt/clay for 2004 contain unquantifiable biases due to errors in sample processing and, therefore, have been censored. (*B*) Data for the past 5 years (2011–2015). (*C*) The monthly mean of all samples collected from 1994–2015, illustrating the general seasonal variation in Al, Fe, and percent fine sediments. Collections are not made in July, August, and November. The error bar is the standard error of the mean.

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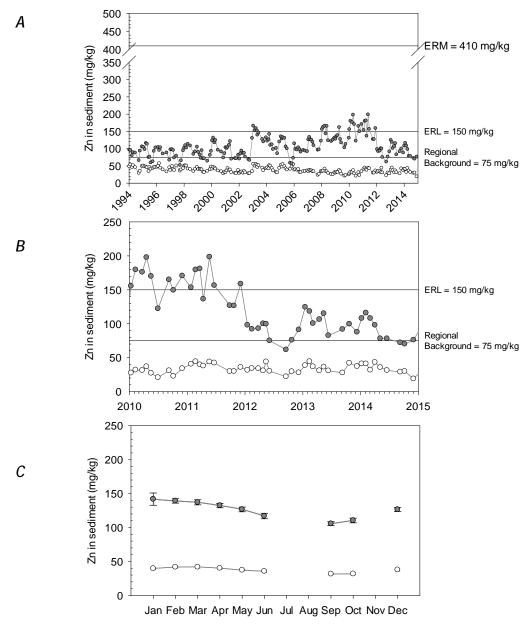
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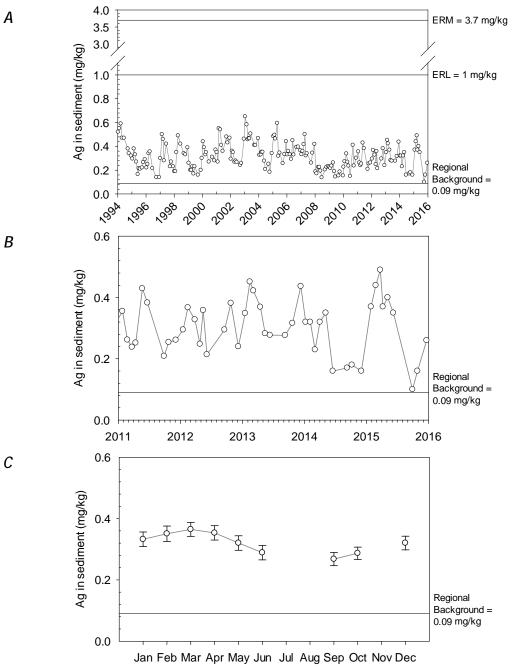
**Figure 5.** Chromium and nickel in sediments, Palo Alto, Calif., 1994–2015. (*A*) Concentrations of chromium (Cr) ( $\square$ ) and nickel (Ni) ( $\bigcirc$ ) extracted by near-total digest in milligrams per kilogram (mg/kg). (*B*) Data for the past 5 years (2011–2015). (*C*) The monthly mean of all samples collected from 1994–2015, illustrating the general seasonal variation in Cr and Ni. Collections are not made in July, August, and November. The error bar is the standard error of the mean.



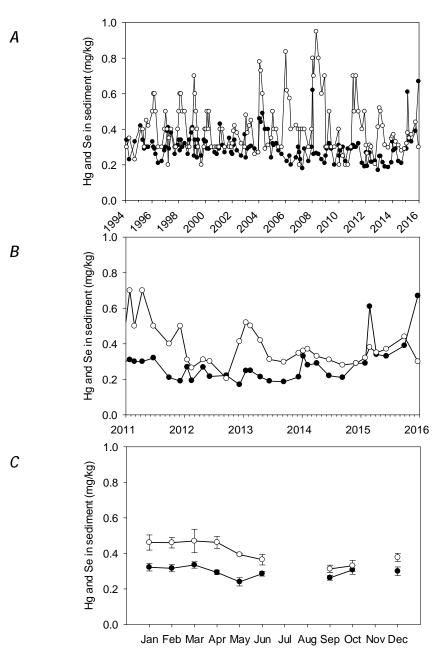
**Figure 6.** Copper in sediments, Palo Alto, Calif., 1994–2015. (*A*) Near-total ( $\bigcirc$ ) and partial-extractable ( $\bigcirc$ ) copper in milligrams per kilogram (mg/kg). (*B*) Data for the past 5 years (2011–2015). (*C*) The monthly mean of all samples collected from 1994–2015, illustrating the general seasonal variation in Cu. Collections are not made in July, August, and November. The error bar is the standard error of the mean. The effects range-low (ERL) is the concentration below which the expected incidence of adverse effects is low (9 percent). The effects range-median (ERM) is the concentration above which the expected incidence of adverse effects is high (84 percent).



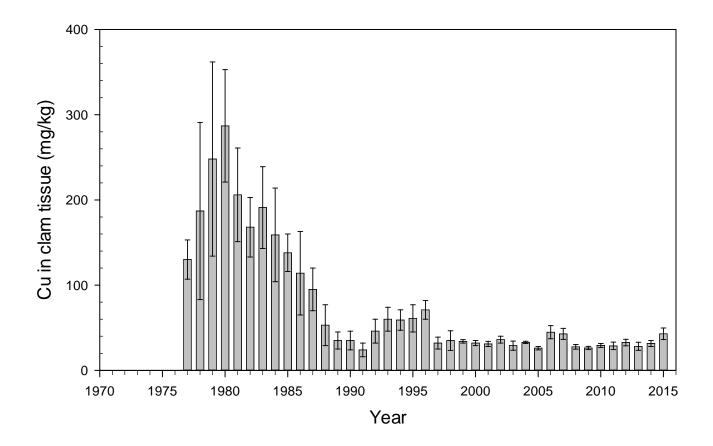
**Figure 7.** Zinc in sediments, Palo Alto, Calif., 1994–2015. (*A*) Near-total ( $\bigcirc$ ) and partial-extractable ( $\bigcirc$ ) zinc in milligrams per kilogram (mg/kg). (*B*) Data for the past 5 years (2011–2015). (*C*) The monthly mean of all samples collected from 1994–2015, illustrating the general seasonal variation in Zn. Collections are not made in July, August, and November. The error bar is the standard error of the mean. The effects range-low (ERL) is the concentration below which the expected incidence of adverse effects is low (6 percent). The effects range-median (ERM) is the concentration above which the expected incidence of adverse effects is high (70 percent).



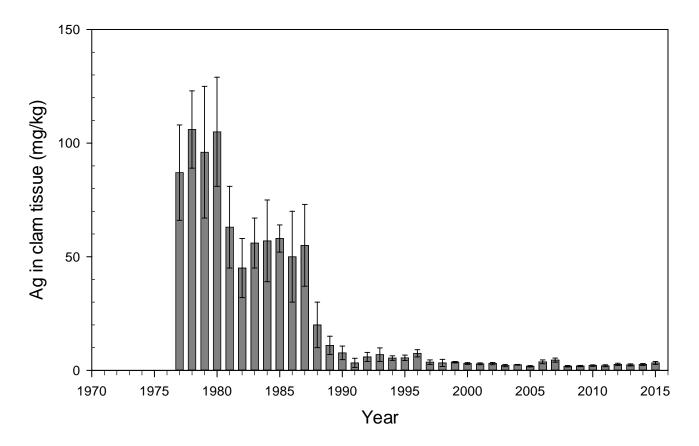
**Figure 8.** Silver in sediments, Palo Alto, Calif., 1994–2015. (*A*) Data represent partial-extractable silver (treatment with 0.6 N hydrochloric acid) in milligrams per kilogram (mg/kg). (*B*) Data for the past 5 years (2011–2015). (*C*) The monthly mean of all samples collected from 1994–2015, illustrating the general seasonal variation in Ag. Collections are not made in July, August, and November. The error bar is the standard error of the mean. The effects range-low (ERL) is the concentration below which the expected incidence of adverse effects is low (3 percent). The effects range-median (ERM) is the concentration above which the expected incidence of adverse effects is high (93 percent).



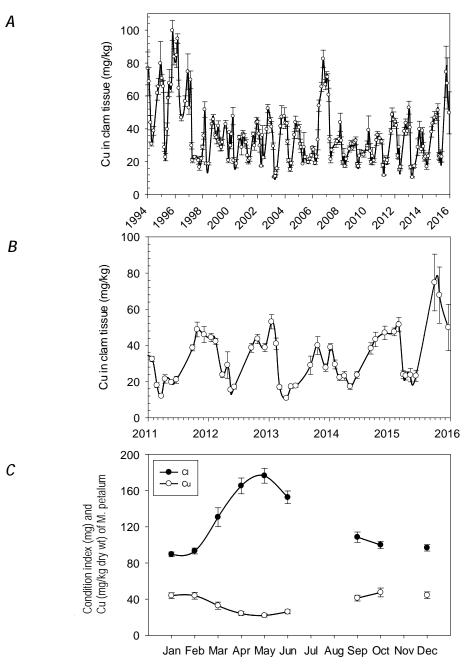
**Figure 9.** Mercury and selenium in sediments, Palo Alto, Calif., 1994–2015. (*A*) Mercury ( $\bigcirc$ ); selenium ( $\bigcirc$ ) in milligrams per kilogram (mg/kg). (*B*) Data for the past 5 years (2011–2015). (*C*) The monthly mean of all samples collected from 1994–2015, illustrating the general seasonal variation in Se and Hg. Collections are not made in July, August, and November. The error bar is the standard error of the mean.



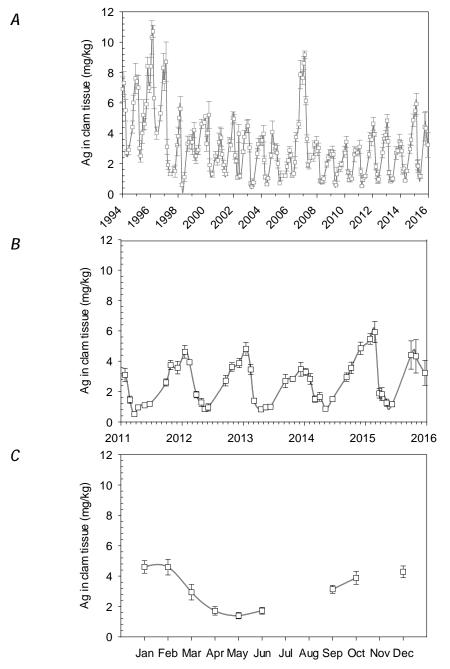
**Figure 10.** Annual mean copper concentrations in milligrams per kilogram (mg/kg) in the clam *Macoma petalum*, Palo Alto, Calif., 1977–2015. Values are the annual (grand) means for 7 to 12 separate samples per year and error bars are standard errors of those means.



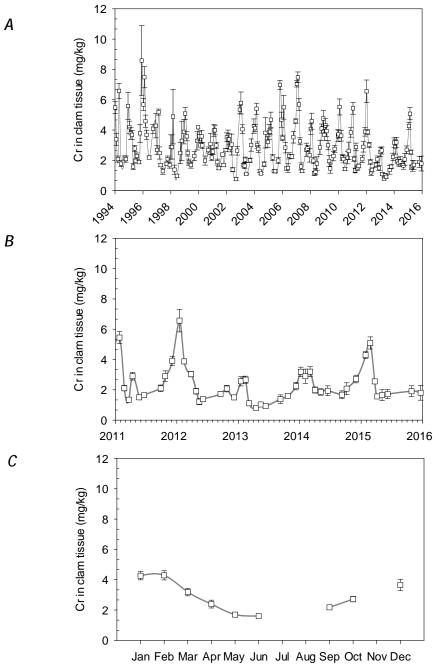
**Figure 11.** Annual mean silver concentrations in milligrams per kilogram (mg/kg) in the clam *Macoma petalum*, Palo Alto, Calif., 1977–2015. Values are the annual (grand) means for 7 to 12 separate samples per year and error bars are standard errors of those means.



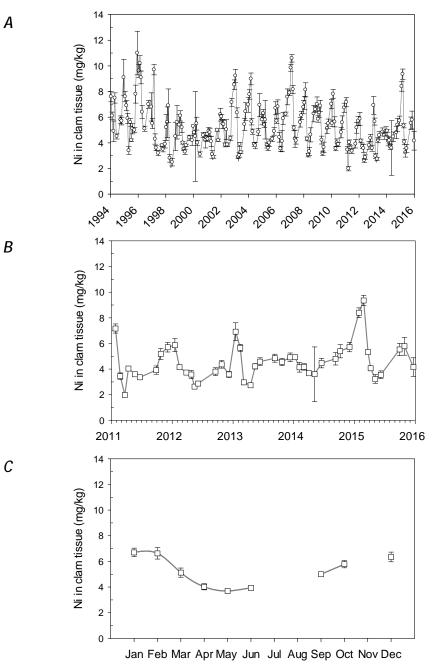
**Figure 12.** Copper concentrations in the clam *Macoma petalum*, Palo Alto, Calif., 1994–2015. (*A*) Each value is the mean concentration in milligrams per kilogram (mg/kg) for the sample collected on a given date. The error bar is the standard error of the mean. (*B*) Data for the past 5 years (2011–2015). (*C*) The monthly mean of all samples collected from 1994–2015, illustrating the general seasonal variation in Cu and the condition index. Collections are not made in July, August, and November. The error bar is the standard error of the mean.



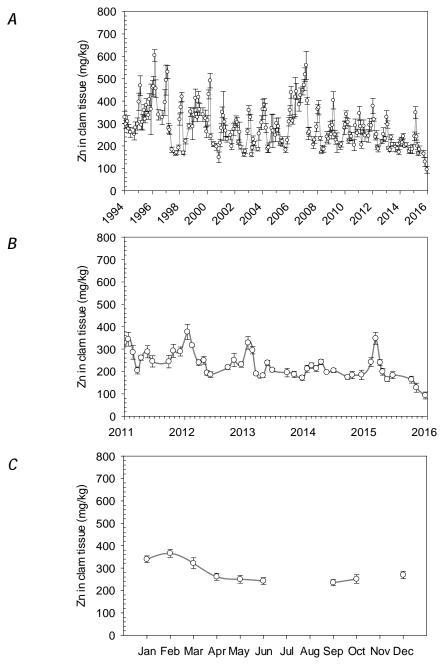
**Figure 13.** Silver concentrations in the clam Macoma petalum, Palo Alto, Calif., 1994–2015. (*A*) Each value is the mean concentration in milligrams per kilogram (mg/kg) for the sample collected on a given date. The error bar is the standard error of the mean. (*B*) Data for the past 5 years (2011–2015). (*C*) The monthly mean of all samples collected from 1994–2015, illustrating the general seasonal variation in Ag. Collections are not made in July, August, and November. The error bar is the standard error of the mean.



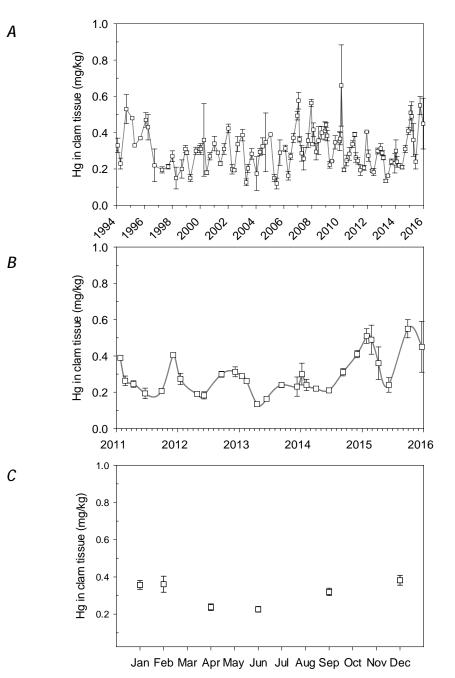
**Figure 14.** Chromium concentrations in the clam *Macoma petalum*, Palo Alto, Calif., 1994–2015. (*A*) Each value is the mean concentration in milligrams per kilogram (mg/kg) for the sample collected on a given date. The error bar is the standard error of the mean. (*B*) Data for the past 5 years (2011–2015). (*C*) The monthly mean of all samples collected from 1994–2015, illustrating the general seasonal variation in Cr. Collections are not made in July, August, and November. The error bar is the standard error of the mean.



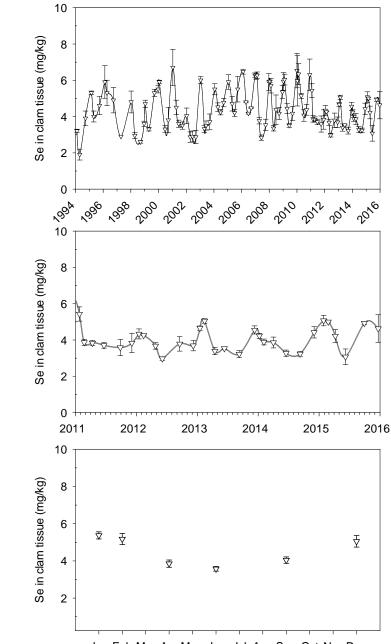
**Figure 15.** Nickel concentrations in the clam *Macoma petalum*, Palo Alto, Calif., 1994–2015. (*A*) Each value is the mean concentration in milligrams per kilogram (mg/kg) for the sample collected on a given date. The error bar is the standard error of the mean. (*B*) Data for the past 5 years (2011–2015). (*C*) The monthly mean of all samples collected from 1994–2015, illustrating the general seasonal variation in Ni. Collections are not made in July, August, and November. The error bar is the standard error of the mean.



**Figure 16.** Zinc concentrations in the clam *Macoma petalum*, Palo Alto, Calif., 1994–2015. (*A*) Each value is the mean concentration in milligrams per kilogram (mg/kg) for the sample collected on a given date. The error bar is the standard error of the mean. (*B*) Data for the past 5 years (2011–2015). (*C*) The monthly mean of all samples collected from 1994–2015, illustrating the general seasonal variation in Zn. Collections are not made in July, August, and November. The error bar is the standard error of the mean.



**Figure 17.** Mercury concentrations in the clam *Macoma petalum*, Palo Alto, Calif., 1994–2015. (*A*) Each value is the mean concentration in milligrams per kilogram (mg/kg) for the sample collected on a given date. The error bar is the standard error of the mean. (*B*) Data for the past 5 years (2011–2015). (*C*) The monthly mean of all samples collected from 1994–2015, illustrating the general seasonal variation in Hg. Collections are not made in March, May, July, August, October, and November. The error bar is the standard error of the mean.



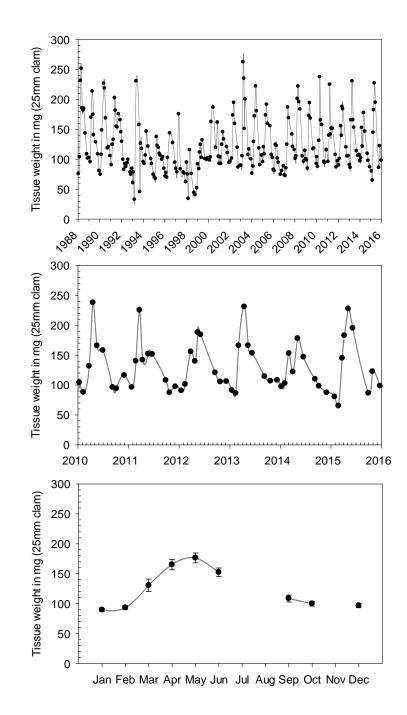
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec

**Figure 18.** Selenium concentrations in the clam *Macoma petalum*, Palo Alto, Calif., 1994–2015. (*A*) Each value is the mean concentration in milligrams per kilogram (mg/kg) for the sample collected on a given date. The error bar is the standard error of the mean. (*B*) Data for the past 5 years (2011–2015). (*C*) The monthly mean of all samples collected from 1994–2015, illustrating the general seasonal variation in Se. Collections are not made in March, May, July, August, October, and November. The error bar is the standard error of the mean.

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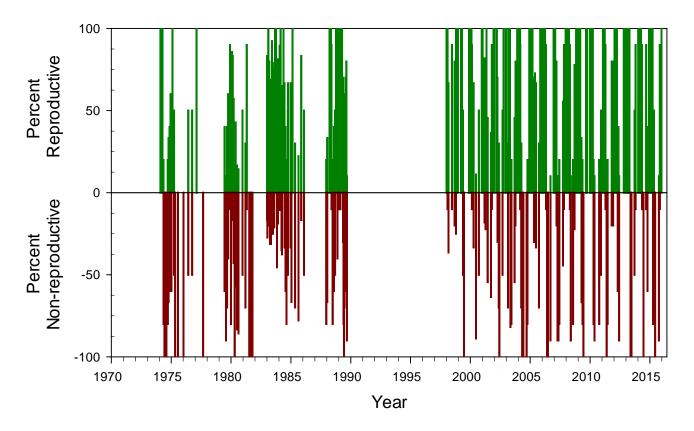


**Figure 19.** Condition index of the clam *Macoma petalum*, Palo Alto, Calif., 1988–2015. (*A*) The condition index is defined as the weight in milligrams (mg) of the soft tissues for an individual clam having a shell length of 25 millimeters (mm). (*B*) Condition index over the past 5 years (2011–2015). (*C*) The monthly mean of samples collected from 1988–2015, illustrating the general seasonal variation in condition index. The error bar is the standard error of the mean. Samples were not collected in July, August, and November after 1994, and any samples collected in those months prior to 1994 were not included for the purposes of the illustration.

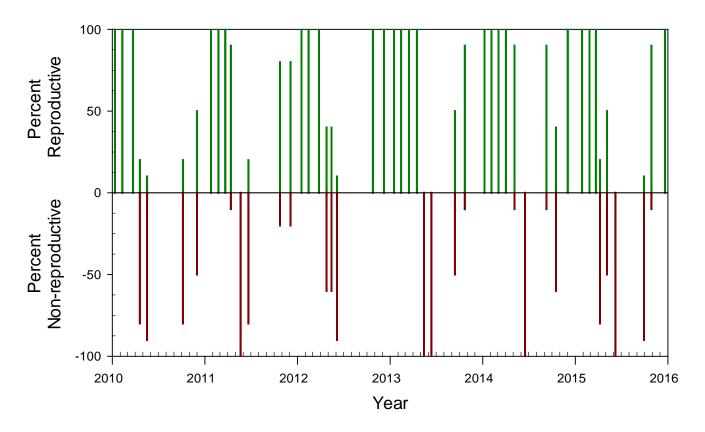
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**Figure 20.** Reproductive activity of the clam *Macoma petalum*, Palo Alto, Calif., 1974–2015. Values are the percent of individuals that were either reproductively inactive (non-reproductive; shown in red) or in various stages of reproduction (reproductive; shown in green). The percent of non-reproductive individuals is reported as a negative value.



**Figure 21.** Reproductive activity of the clam *Macoma petalum*, Palo Alto, Calif., 2011–2015. Values are the percent of individuals that were either reproductively inactive (non-reproductive; shown in red) or in various stages of reproduction (reproductive; shown in green). The percent of non-reproductive individuals is reported as a negative value.

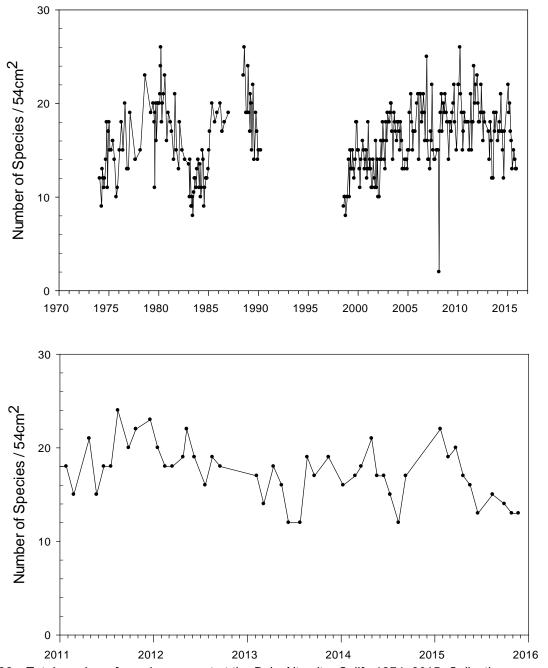


Figure 22. Total number of species present at the Palo Alto site, Calif., 1974–2015. Collections were not made between 1991 and 1998. Cm, centimeters.

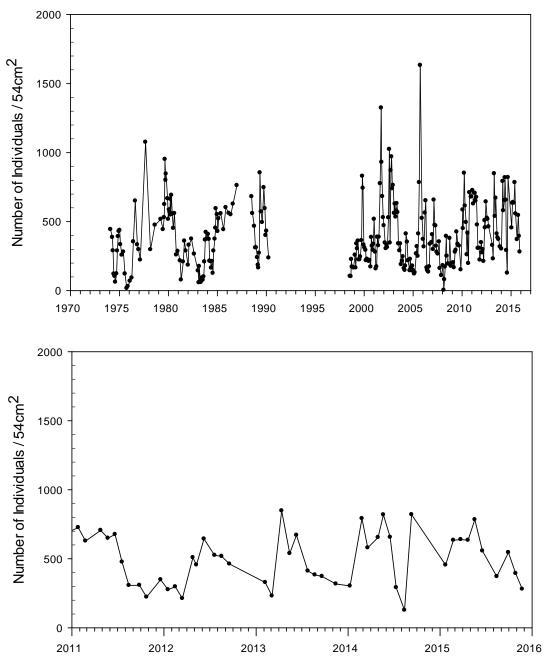
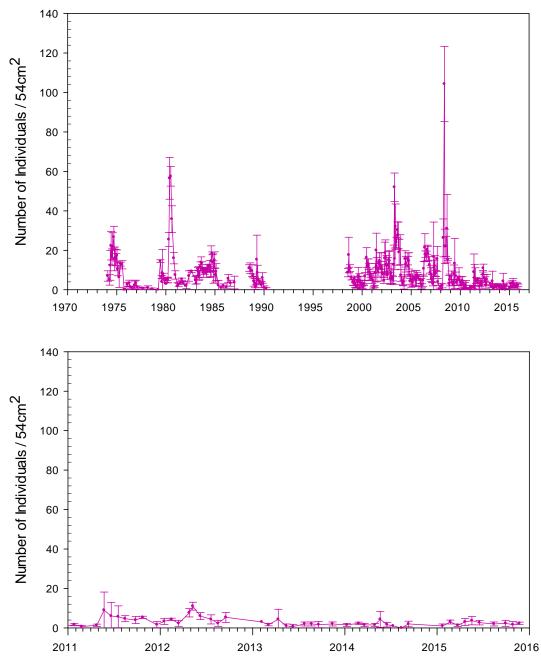
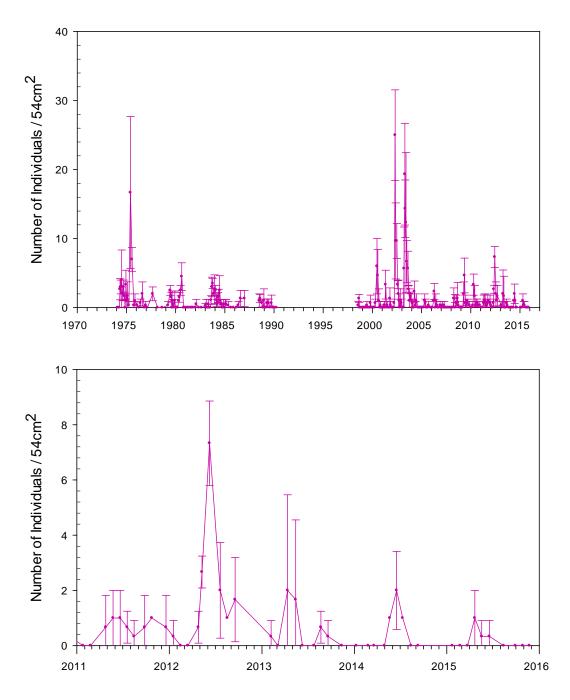


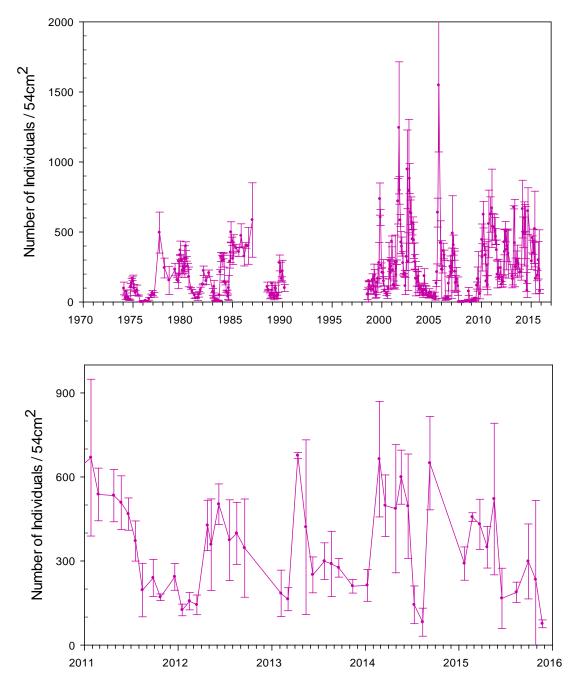
Figure 23. Total average number of individuals present at the Palo Alto site, Calif., 1974–2015. Collections were not made between 1991 and 1998. Cm, centimeters.



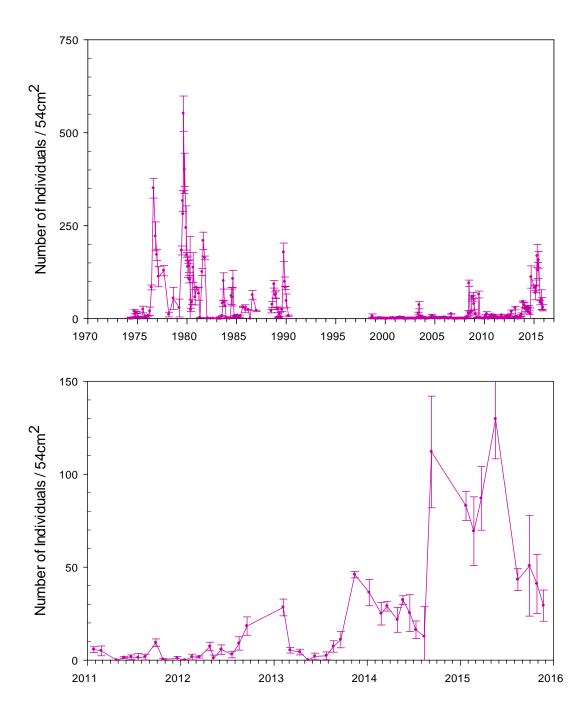
**Figure 24.** Monthly average abundance of *Macoma petalum*, Palo Alto, Calif., 1974–2015. Error bars represent standard deviation from three replicate samplings. Collections were not made between 1991 and 1998. Cm, centimeters.



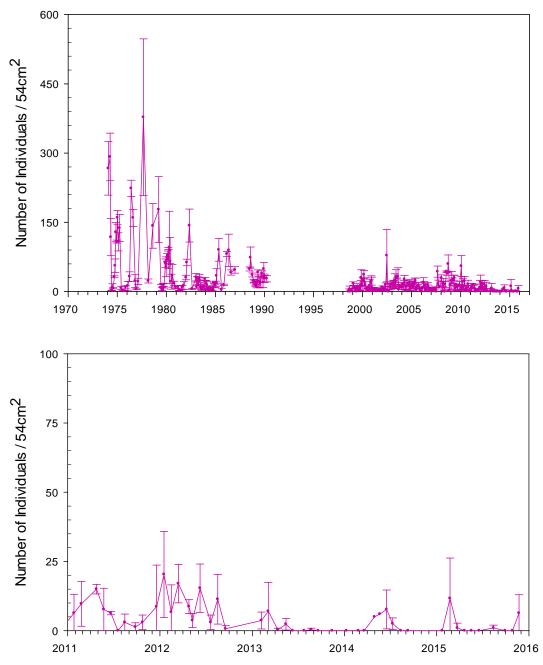
**Figure 25.** Monthly average abundance of *Mya arenaria*, Palo Alto, Calif., 1974–2015. Error bars represent standard deviation from three replicate samplings. Collections were not made between 1991 and 1998. Cm, centimeters.



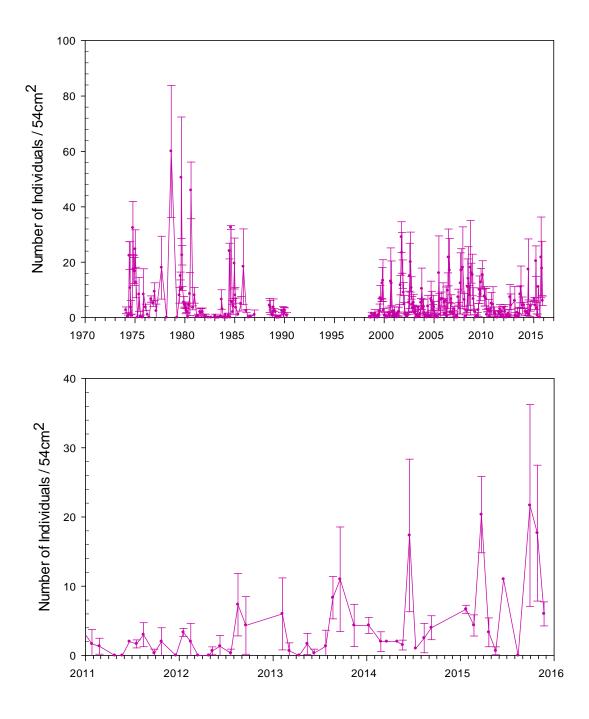
**Figure 26.** Monthly average abundance of *Gemma gemma*, Palo Alto, Calif., 1974–2015. Error bars represent standard deviation from three replicate samplings. Collections were not made between 1991 and 1998. Cm, centimeters.



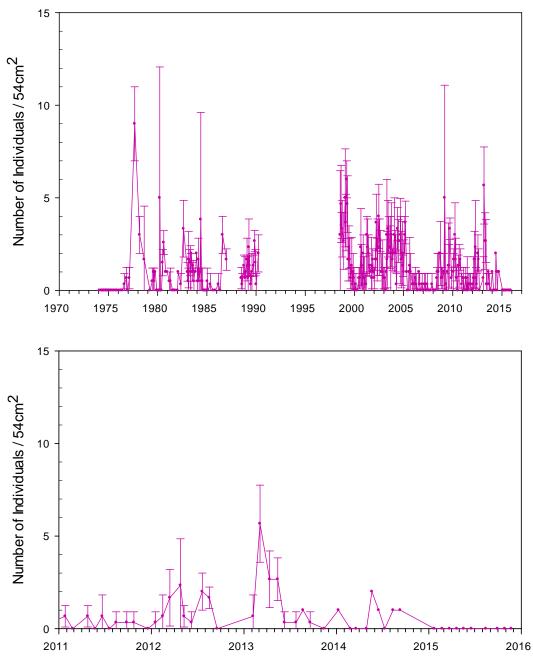
**Figure 27.** Monthly average abundance of *Ampelisca abdita*, Palo Alto, Calif., 1974–2015. Error bars represent standard deviation from three replicate samplings. Collections were not made between 1991 and 1998. Cm, centimeters.



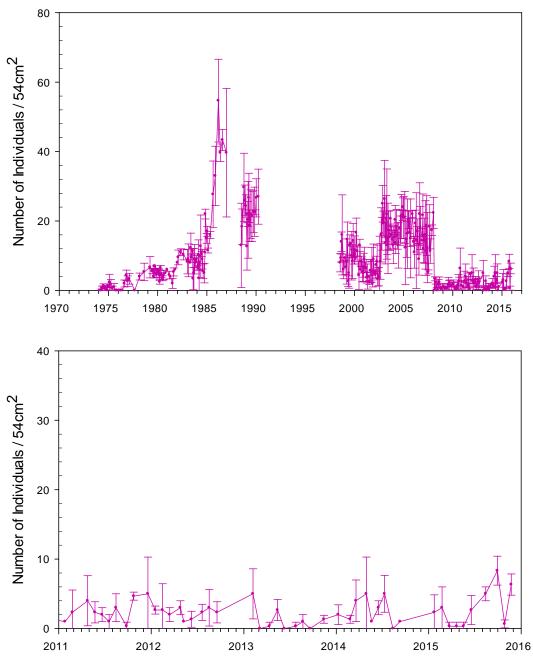
**Figure 28.** Monthly average abundance of *Streblospio benedicti*, Palo Alto, Calif., 1974–2015. Error bars represent standard deviation from three replicate samplings. Collections were not made between 1991 and 1998. Cm, centimeters.



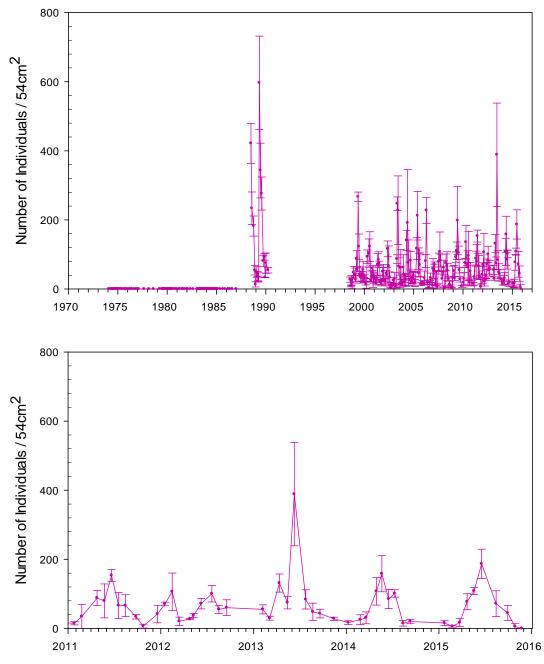
**Figure 29.** Monthly average abundance of *Grandiderella japonica*, Palo Alto, Calif., 1974–2015. Error bars represent standard deviation from three replicate samplings. Collections were not made between 1991 and 1998. Cm, centimeters.



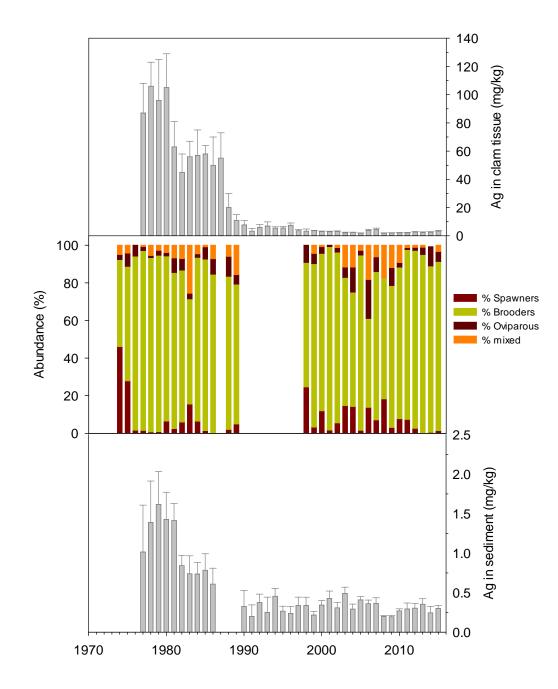
**Figure 30.** Monthly average abundance of *Neanthes succinea*, Palo Alto, Calif., 1974–2015. Error bars represent standard deviation from three replicate samplings. Collections were not made between 1991 and 1998. Cm, centimeters.



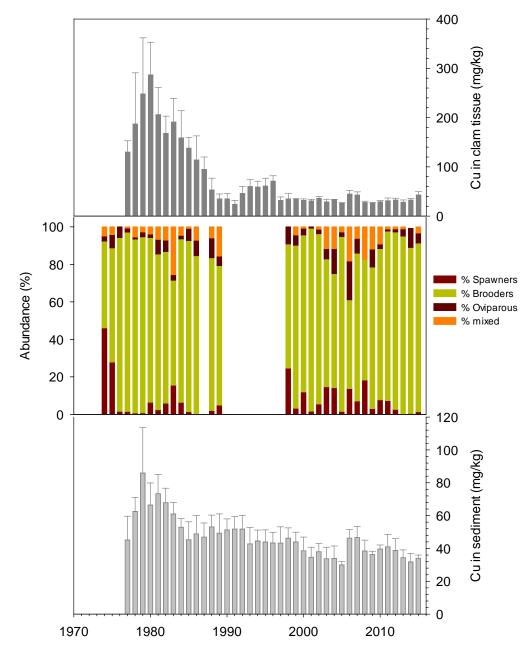
**Figure 31.** Monthly average abundance of *Heteromastus filiformis*, Palo Alto, Calif., 1974–2015. Error bars represent standard deviation from three replicate samplings. Collections were not made between 1991 and 1998. Cm, centimeters.



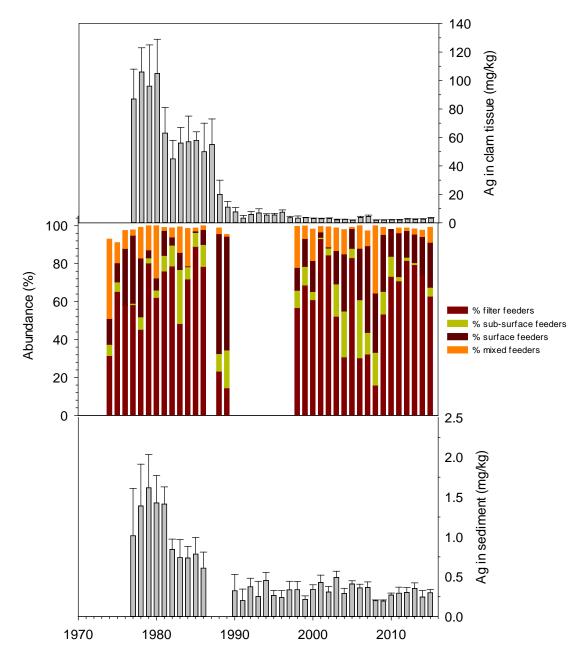
**Figure 32.** Monthly average abundance of *Nippoleucon hinumensis*, Palo Alto, Calif., 1974–2015. Error bars represent standard deviation from three replicate samplings. Collections were not made between 1991 and 1998. Cm, centimeters.



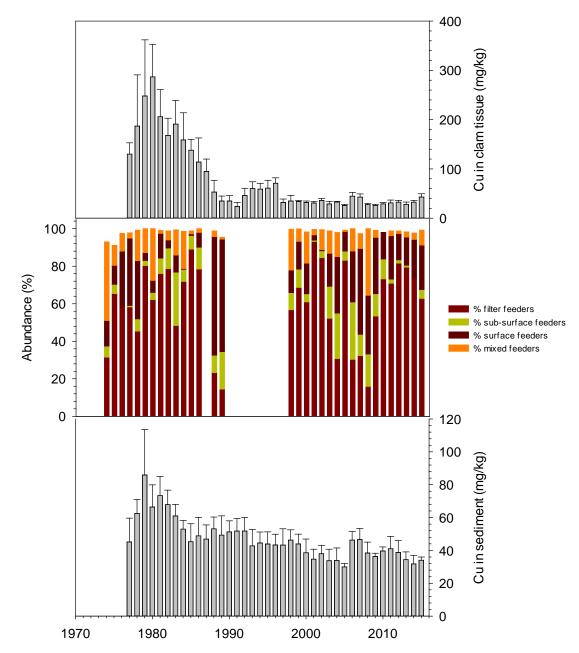
**Figure 33.** Reproductive mode annual abundance with silver concentrations in the clam *Macoma petalum* and in sediment, Palo Alto, Calif., 1974–2015. Annual abundance data is from August of each year. The reproductive mode of the top ten ranked species for each year is shown. Brooder: broods young and release juveniles as fully functional "miniature adults"; Oviparous: lays eggs in or on sediment; Spawner: releases gametes into water column and juveniles settle out of plankton onto sediment surface after growth in the plankton. %, percent; mg/kg, milligrams per kilogram.



**Figure 34.** Reproductive mode annual abundance with copper concentrations in the clam *Macoma petalum* and in sediment, Palo Alto, Calif., 1974–2015. Annual abundance data is from August of each year. The reproductive mode of the top ten ranked species for each year is shown. Brooder: broods young and release juveniles as fully functional "miniature adults"; Oviparous: lays eggs in or on sediment; Spawner: releases gametes into water column and juveniles settle out of plankton onto sediment surface after growth in the plankton. %, percent; mg/kg, milligrams per kilogram.



**Figure 35.** Feeding mode annual abundance with silver concentrations in the clam *Macoma petalum* and in sediment, Palo Alto, Calif., 1974–2015. Annual abundance data is from August of each year. The feeding mode of the top ten ranked species for each year is shown. Filter: filters food particles from water column; Subsurface Deposit: ingests subsurface sediment and removes food from sediment in gut; Surface Deposit: ingests food particles on surface sediment; Mixed: capable of filter feeding and surface deposit feeding. %, percent; mg/kg, milligrams per kilogram.



**Figure 36.** Feeding mode annual abundance with copper concentrations in the clam *Macoma petalum* and in sediment, Palo Alto, Calif., 1974–2015. Annual abundance data is from August of each year. The feeding mode of the top ten ranked species for each year is shown. Filter: filters food particles from water column; Subsurface Deposit: ingests subsurface sediment and removes food from sediment in gut; Surface Deposit: ingests food particles on surface sediment; Mixed: capable of filter feeding and surface deposit feeding. %, percent; mg/kg, milligrams per kilogram.

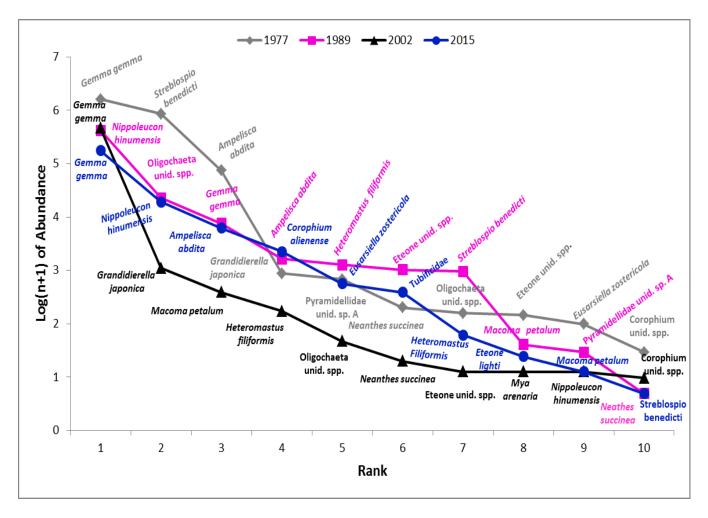
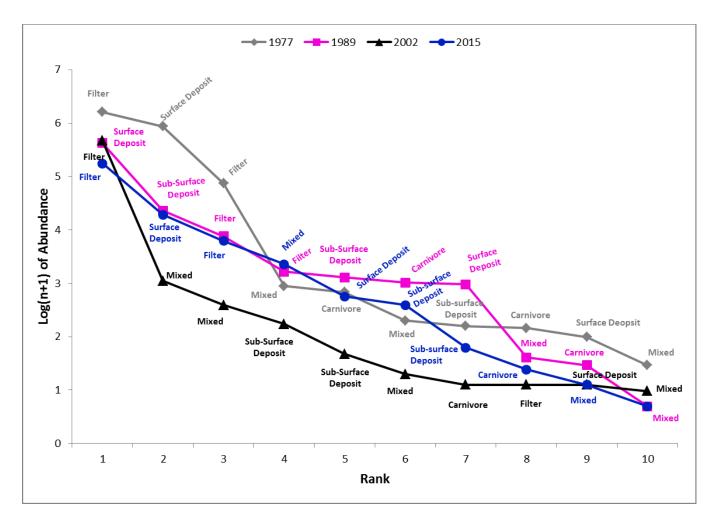
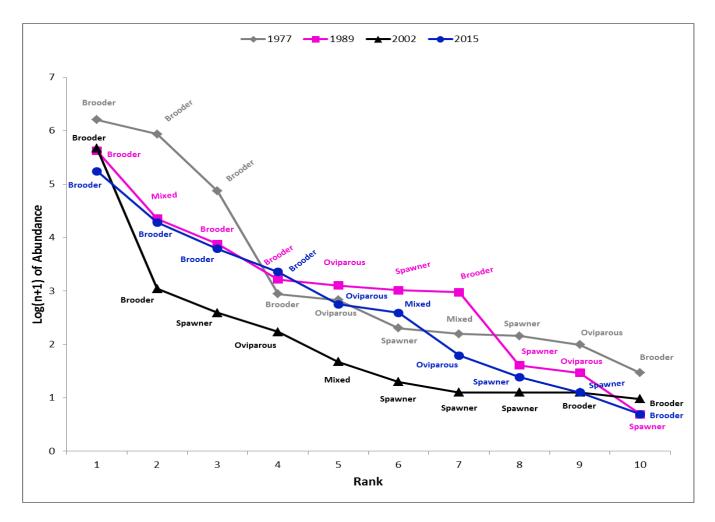


Figure 37. Species rank-abundance for the benthic community, Palo Alto, Calif., for 1977, 1989, 2002, and 2015.



**Figure 38.** Species rank-abundance identified by feeding mode, Palo Alto, Calif., for 1977, 1989, 2002, and 2015. The feeding mode for each species at each rank shown. Filter: filter food particles from water column; Subsurface Deposit: ingests subsurface sediment and removes food from sediment in gut; Surface Deposit: ingests food particles on surface sediment; Mixed: capable of filter feeding and surface deposit feeding; Carnivore: predator on other fauna.



**Figure 39.** Species rank-abundance data identified by reproductive mode, Palo Alto, Calif., for 1977, 1989, 2002, and 2015. Reproductive mode for each species at each rank is shown. Brooder: broods young and release juveniles as fully functional "miniature adults"; Oviparous: lays eggs in or on sediment; Spawner: releases gametes into water column and juveniles settle out of plankton onto sediment surface after growth in the plankton.

**Table 1.** Concentrations of major and minor inorganic elements, total organic carbon, percent of fine particles in surface sediments, and salinity of water pooled on the surface of sediments for samples collected from Palo Alto, Calif., 2015.

[Units for Al, Fe, total organic carbon (TOC), and silt/clay are percent (%) of dry weight. Silt/clay is operationally determined as  $\leq 100$  micrometer grain size. Salinity is reported in units of parts per thousand (ppt) for water pooled at the sediment surface during low tide. Elemental concentrations for the monthly samples are reported as the mean  $\pm 1$  standard deviation (std) for replicate subsamples (n=2). Units are milligram per kilogram dry weight. Results for TOC, silt/clay, and salinity are for a single (n=1) measurement. Means for monthly samples were summarized and reported as the annual mean  $\pm$  the standard error of the mean (SEM) (n=6 or 9). All concentrations are based on near-total extracts, except for silver (Ag), which is based on partial extraction (see text section in Methods). ND, no data]

											Silt/Clay	Salinity
Date	AI (%)	Ag	Cr	Cu	Fe (%)	Hg	Ni	Se	Zn	TOC (%)	(%)	(ppt)
1/28/2015	$3.7~\pm~0.2$	$0.37~\pm~0.00$	$107 \pm 6$	$28 \pm 1$	$3.9~\pm~0.1$	0.29	$99 \pm 1.9$	0.32	$140 \pm 2$	$1.23~\pm~0.03$	73	26
2/25/2015	$4.0~\pm~0.1$	$0.44~\pm~0.01$	$113 \pm 0$	$31 \pm 0$	$4.3~\pm~0.02$	0.61	$104 \pm 1.0$	0.38	$151 \pm 1$	$1.27~\pm~0.00$	86	24
3/23/2015	$4.3~\pm~0.0$	$0.49~\pm~0.01$	$120 \pm 3$	$35 \pm 0$	$4.4~\pm~0.2$	ND	$111 \pm 5.8$	ND	$161 \pm 7$	$1.41~\pm~0.00$	95	24
4/8/2015	$4.2~\pm~0.5$	$0.37~\pm~0.01$	$112 \pm 13$	$32 \pm 2$	$4.2~\pm~0.2$	0.34	$143 \pm 5.4$	0.35	$143 \pm 5$	$1.42~\pm~0.01$	84	27
5/6/2015	$4.1~\pm~0.2$	$0.40~\pm~0.01$	$108 \pm 2$	$33 \pm 0$	$4.1~\pm~0.0$	ND	$99 \pm 0.6$	ND	$137 \pm 1$	$1.52~\pm~0.03$	79	26
6/8/2015	$5.8 \pm 0.0$	0.35	$144 \pm 1$	$44 \pm 0$	$4.7~\pm~0.0$	0.33	$107 \pm 0.4$	0.37	$157 \pm 0$	$1.58 \pm 0.01$	73	30
9/29/2015	$5.2 \pm 0.3$	$0.10~\pm~0.00$	$130 \pm 7$	$42 \pm 2$	$4.1~\pm~0.2$	0.39	$96 \pm 3.8$	0.44	$142 \pm 5$	$1.22 \pm 0.01$	79	30
10/28/2015	$4.3~\pm~0.0$	$0.16 \pm 0.00$	$112 \pm 4$	$31 \pm 0$	$3.7~\pm~0.0$	ND	$85 \pm 0.2$	ND	$114 \pm 0$	$0.91 \pm 0.14$	68	29
12/21/2015	$4.4~\pm~0.2$	$0.26~\pm~0.00$	$116~\pm~8$	$33 \pm 2$	$3.8~\pm~0.2$	0.67	$77 \pm 3.2$	0.30	$109~\pm~6$	$0.98~\pm~0.01$	66	23
Annual Mean:	4.4	0.33	118	34	4.1	0.44	102	0.36	139	1.28	78	27
SEM:	0.2	0.04	4	2	0.1	0.07	6	0.02	6	0.08	3	1

 Table 2.
 Concentrations of trace metals in and the condition index for the clam Macoma petalum, Palo Alto, Calif., 2015.

[Monthly data are the mean and standard deviation for replicate composites (n=11-13, n=3 for Se and Hg). Means for monthly samples were summarized and reported as the annual mean  $\pm$  the standard error (SEM) (n=6 or 9). All concentrations are based on near-total extracts. Elemental concentrations are milligram per kilogram soft tissue dry weight. The condition index is the soft tissue weight in milligrams of a clam of 25-millimeter shell length. <MDL, less than the method detection limit; ND, no data]

Date	Ag	Cr	Cu	Hg	Ni	Se	Zn	Condition Index
1/28/2015	55 1 2	42 08	40 0	0.51 0.00	Q 4 1 1 4	$5.07 \pm 0.50$	242 + 60	80
1/28/2015	$5.5 \pm 1.3$	$4.3 \pm 0.8$	48 ± 8	$0.51 \pm 0.08$	$8.4 \pm 1.4$		242 ± 69	
2/25/2015	$5.9 \pm 2.3$	$5.1 \pm 1.3$	$52 \pm 13$	$0.49 \pm 0.14$	$9.4 \pm 1.3$	$5.00 \pm 0.10$	$348 \pm 87$	65
3/23/2015	$1.9 \pm 1.0$	$2.6 \pm 0.5$	$24 \pm 7$	ND	$5.3 \pm 0.5$	ND	$239 ~\pm~ 45$	145
4/8/2015	$1.8 \pm 1.3$	$1.6 \pm 0.4$	$23 \pm 7$	$0.36 \pm 0.16$	$4.1 \pm 0.6$	$4.22 ~\pm~ 0.61$	$199 \pm 45$	183
5/6/2015	$1.3 \pm 0.8$	$1.7 \pm 1.2$	$24 \pm 14$	ND	$3.2 \pm 1.2$	ND	$165 \pm 33$	228
6/8/2015	$1.2 \pm 0.4$	$1.7 \pm 0.7$	$23 \pm 9$	$0.24 \pm 0.08$	$3.6 \pm 0.9$	$3.07 \pm 0.74$	$183 \pm 47$	195
9/29/2015	4.4 ± 2.6	<mdl< td=""><td><math>75 \pm 44</math></td><td><math>0.55 \pm 0.07</math></td><td><math>5.5 \pm 1.4</math></td><td><math display="block">4.93 ~\pm~ 0.13</math></td><td><math>163 \pm 43</math></td><td>87</td></mdl<>	$75 \pm 44$	$0.55 \pm 0.07$	$5.5 \pm 1.4$	$4.93 ~\pm~ 0.13$	$163 \pm 43$	87
10/25/2015	4.3 ± 3.5	$1.9 \pm 1.0$	$68 \pm 49$	ND	$5.8 \pm 2.2$	ND	$128 \pm 64$	123
12/21/2015	$3.2 \pm 2.3$	$1.8 \pm 1.1$	$50 \pm 36$	$0.45 ~\pm~ 0.24$	$4.2 ~\pm~ 2.0$	$4.63 ~\pm~ 1.32$	$93 \pm 43$	99
Annual Mean:	3.3	2.6	43	0.43	5.5	4.49	195	134
SEM:	0.6	0.5	7	0.05	0.7	0.31	25	19

Table 3.	Reproduction da	ata for <i>Macoma</i>	petalum, Palo	Alto, Calif., 2015
Table J.	INCPRODUCTION UC		$p \in a u u u$	$A_{IIO}$ , $Ca_{III}$ , $ZOIJ$

[Data are percentage of clams in each stage of reproduction. Reproductive represents the percentage of clams in Active, Ripe, and Spawning stages. Non-reproductive represents the percentage of clams in Inactive and Spent stages. Spent means the clams have released all their gametes. ND, no data; n, number of clams analyzed]

Date	Inactive	Active	Ripe	Spawning	Spent	n	Reproductive	Non- reproductive
28-Jan-15	0.0	0.0	90.0	10.0	0.0	10	100.0	0
26-Feb-15	0.0	0.0	40.0	60.0	0.0	10	100.0	0
23-Mar-15	0.0	0.0	40.0	60.0	0.0	10	100.0	0
8-Apr-15	0.0	0.0	10.0	10.0	80.0	10	20.0	-80
6-May-15	0.0	0.0	0.0	50.0	50.0	10	50.0	-50
8-Jun-15	0.0	0.0	0.0	0.0	100.0	10	0.0	-100
1-Jul-15	ND	ND	ND	ND	ND	ND	ND	ND
1-Aug-15	ND	ND	ND	ND	ND	ND	ND	ND
29-Sep-15	90.0	10.0	0.0	0.0	0.0	10	10.0	-90
1-Nov-15	ND	ND	ND	ND	ND	ND	ND	ND
28-Oct-15	10.0	70.0	20.0	0.0	0.0	10	90.0	-10
21-Dec-15	0.0	0.0	100.0	0.0	0.0	10	100.0	0

# Appendix 1. Certified concentrations and the percent recoveries of inorganic elements in National Institute of Science and Technology Standard Reference Material 2709a (San Joaquin Soil) and 2711a (Montana Soil) prepared in 2015.

Constituent	Number of analyses	Dilution ratio	Certified concentration, in µg/g	Mean SRM recovery, in percent	95-percent confidence interv for SRM recover in percent				
			SRM 2709a - Near-total metal	extraction					
Aluminum	11	1:10	73,700	49	46	_	51		
Chromium	11	1:10	130	68	64	_	71		
Copper	10	1:10	33.9	76	73	_	80		
Iron	11	1:10	33,600	86	83	_	88		
Lead	11	1:10	17.3	56	51	_	61		
Manganese	11	1:10	529	85	82	_	88		
Nickel	11	1:10	85	87	86	_	89		
Silver			unknown						
Vanadium	11	1:10	110	89	83	_	95		
Zinc	11	1:10	103	94	91	_	96		
			SRM 2711a - Near-total metal e	xtraction					
Aluminum	17	1:10	67,200	40	38	_	42		
Chromium	17	1:10	52.3	58	54	_	62		
Copper	17	1:10	140	89	87	_	92		
Iron	17	1:10	28,200	81	79	_	83		
Lead	17	1:10	1,410	95	93	_	97		
Manganese	17	1:10	675	77	74	_	79		
Nickel	17	1:10	21.7	88	86	_	90		
Silver	17	1:1	6	90	88	_	93		
Vanadium	17	1:10	81	87	81	_	93		
Zinc	17	1:10	414	98	95	_	101		

[µg/g, micrograms per gram; SRM, standard reference material]

## Appendix 2. Certified concentrations and the percent recoveries of inorganic elements in National Institute of Science and Technology Standard Reference Material 2976 (Mussel Tissue) and 1566b (Oyster Tissue) prepared in 2015.

Constituent	Number of analyses	Certified concentration, in µg/g	Mean SRM recovery, in percent	interval for S	95-percent confidence interval for SRM recovery, i percent			
		SRM 297	6					
Cadmium	18	0.82	127	126	_	128		
Chromium	18	0.5	109	105	_	113		
Copper	18	4.02	90.7	89.7	_	91.6		
Lead	18	1.19	77.1	76.0	_	78.2		
Nickel	18	0.93	68.9	68.2	_	69.7		
Silver	18	0.011	<mrl< td=""><td></td><td></td><td></td></mrl<>					
Vanadium	NA	unknown	unknown	unknown				
Zinc	18	137	95.7	94.6		96.9		
		SRM 1566	bb					
Cadmium	15	2.48	87	71	_	102		
Chromium	NA	unknown	unknown	unknown				
Copper	15	71.6	84	71.9	_	95.3		
Lead	15	0.308	95	79	_	111		
Nickel	15	1.04	76	62.7	_	89.9		
Silver	2	0.666	92	91.5	_	93.7		
Vanadium	15	0.577	84	72.4	_	96.3		
Zinc	15	1,424	43	31.4	_	53.7		
		SRM Tort	2					
Cadmium	6	26.7	97	94	_	100		
Chromium	NA	unknown						
Copper	6	106	87	82	_	91		
Lead	6	0.35	104	93	_	115		
Nickel	6	2.5	77	70	_	83		
Silver	6	unknown						
Vanadium	6	1.64	66	46	-	87		
Zinc	6	180	98	92	-	103		

[Samples were not diluted prior to analysis; µg/g, micrograms per gram; SRM, standard reference material]

## Appendix 3. Mercury and selenium concentrations determined in sample splits of surface sediments and *Macoma petalum* collected at Palo Alto, Calif., 2015.

[One sediment sample and one clam tissue sample were split and analyzed for Se and Hg. The split results are shown here. Units are milligrams per kilogram dry weight]

Date	Sample ID	Sedi	iment	M. petalum				
		Mercury	Selenium	Mercury	Selenium			
Jan. 2015	R3				0.42			
	R3- duplicate				0.44			
June 2015	R1		0.33					
	R1- duplicate		0.33					

#### Appendix 4. Certified and measured concentrations of mercury and selenium in standard reference materials analyzed in 2015.

		Mercury	Selenium						
SRM	Observed			Observed	Certified	Percent recovery			
PACS-3	$2.83\pm0.01$	$3.04\pm0.2$	94	1.03					
NIST-2976	$0.059 \pm 0.002$	$0.061\pm0.004$	98	1.81	$1.80\pm0.15$	100			
TORT-3	$0.27\pm0.03$	$0.292\pm0.022$	93	10.5	$10.9\pm1.0$	96			

[Concentration is reported as milligram per kilogram. SRM, standard reference material]

## Appendix 5. Method detection limits and reporting levels for inductively coupled plasma-optical emission spectrophotometry methods.

Method	Marker	Ag	AI	Cd	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn
Sediment	MDL	0.0003	0.0255	0.0001	0.0100	0.0024	0.0327	0.0034	0.0005	0.0026	0.0005	0.0072
	MRL	0.0006	0.0509	0.0002	0.0199	0.0048	0.0654	0.0067	0.0010	0.0051	0.0010	0.0143
Tissue	MDL	0.0007	0.0092	0.0002	0.0120	0.0020	0.0094	0.0010	0.0002	0.0030	0.0002	0.0022
	MRL	0.0013	0.0184	0.0003	0.0240	0.0039	0.0189	0.0020	0.0005	0.0059	0.0004	0.0043

[Concentration is reported as milligrams per liter; MDL, method detection limit; MRL, method reporting limit]

#### Appendix 6. Statistical summary of percentage of sediment in samples composed of clay- and silt-sized particles, collected from Palo Alto, Calif., 1994–2015.

[Statistical results are for percent fine-grained particles (silt and clay,  $\leq 100$  micrometer) observed each month for N collections made during the period 1994–2015. Data for percent fines for 2004, which contain unquantifiable biases due to errors in sample processing, are not included in these statistical calculations]

Month	Ν	Maximum	Minimum	Mean	Median
January	20	96	46	76	76
February	20	98	32	83	87
March	20	98	24	80	85
April	20	96	50	84	86
May	15	95	35	77	80
June	20	95	46	73	71
July	1	69	69	69	69
August	1	48	48	48	48
September	20	84	25	59	60
October	16	84	39	64	65
November	3	66	50	59	61
December	20	95	48	74	74

#### Appendix 7. Statistical summary of silver and copper concentrations in sediment and the clam *Macoma petalum*, Palo Alto, Calif., for 2015 and 1977–2015.

[The 2015 column presents the mean and standard error of the monthly samples. Mean, median, minimum, and maximum are calculated from the annual means during the period from 1977–2015. Units are milligrams per kilogram dry weight of soft tissue for the clam (*Macoma petalum*) and milligram per kilogram dry weight for sediment]

Sample type	Element	Method		201	5	Mean	Median	Minimum	Maximum
Sediment	Ag	Partial extraction	0.33	±	0.04	0.50	0.36	0.20	1.62
	Cu	Partial extraction	13	±	1	23	19	13	55
	Cu	Near total	34	±	2	44	43	30	86
M. petalum	Ag	Tissue digest	3.3	±	0.6	23	5	1.8	106
	Cu	Tissue digest	43	±	7	77	43	24	287

#### Appendix 8. Complete list of benthic species found at Palo Alto in the year 2015.

[Three samples are taken at each sampling event. The mean and standard deviation (std dev) of the three samples are shown]

TAXON	1/23	/2015	2/23	/2015	3/24	/2015	4/22	/2015	5/19	/2015	6/18	/2015	8/14	/2015	9/29	/2015	10/28	3/2015	11/23	3/2015
	Mean	std dev	Mean	std dev	Mean	std dev														
PHYLUM ANNELIDA																				
Class Oligochaeta																				
Naididae unid. spp.	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	0.0	0.0
Oligochaeta unid. spp.	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	0.0	0.0
Tubificidae unid. spp.	0.7	0.6	23.7	31.0	1.7	2.9	4.7	3.8	0.0	0.0	9.0	15.6	12.3	8.1	29.0	14.5	1.0	1.7	63.3	56.8
Class Polychaeta																				
Capitella capitata complex	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	0.0	0.0
Cirratulidae unid. spp.	0.3	0.6	0.0	0.0	0.3	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.6	0.0	0.0	0.3	0.6	0.3	0.6
Eteone lighti	4.0	2.0	3.0	1.0	2.0	1.0	5.0	2.6	1.3	1.2	4.7	3.2	3.0	3.6	2.3	2.1	1.3	0.6	2.3	3.2
Eteone unid. spp.	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	0.0	0.0
Euchone limnicola	1.3	1.5	1.0	1.0	1.0	1.0	0.0	0.0	0.3	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Euchone unid. spp.	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	0.0	0.0
Glycera unid. spp.	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	0.0	0.0
Glycinde armigera	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	0.0	0.0
Glycinde picta	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	0.0	0.0
Glycinde unid. sp. SF1	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	0.0	0.0
Glycinde unid. spp.	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	0.0	0.0
Harmothoe imbricata	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	0.0	0.0
Heteromastus filiformis	2.3	2.5	3.0	3.0	0.3	0.6	0.3	0.6	0.3	0.6	2.7	2.1	5.0	1.0	8.3	2.1	0.7	0.6	6.3	1.5
Maldanidae unid. spp.	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	0.0	0.0
Marphysa sanguinea	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	0.0	0.0
Neanthes succinea	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	0.0	0.0
Polychaeta unid. spp.	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	0.0	0.0
Polydora cornuta	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	0.3	0.6
Polydora unid. spp.	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	0.0	0.0
Pseudopolydora kempi	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	0.0	0.0
Pseudopolydora paucibranchiata	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	0.0	0.0
Sabaco elongatus	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	0.0	0.0
Sphaerosyllis californiensis	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	0.0	0.0
Sphaerosyllis erinaceus	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	0.0	0.0
Sphaerosyllis unid. sp. A	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	0.0	0.0
Streblospio benedicti	11.7	14.6	0.0	0.0	1.0	1.7	0.0	0.0	0.0	0.0	0.0	0.0	1.0	1.0	6.3	6.7	0.0	0.0	0.0	0.0
Spionidae unid. spp.	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	0.0	0.0

TAXON	1/23/2015		2/23/2015		3/24/2015		4/22/2015		5/19/2015		6/18/2015		8/14/2015		9/29/2015		10/28/2015		11/23/2015	
	Mean	std dev	Mean	std dev	Mean	std dev														
PHYLUM ARTHROPODA																				
Class Arachnida																				
Acari	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	0.0	0.0
Class Copepoda																				
Calanoida unid. spp.	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	0.0	0.0
Harpacticoida unid. spp.	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	0.0	0.0
Class Insecta																				
Chironomidae unid. spp.	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	0.0	0.0
Class Malacostraca																				
Americhelidium unid. spp.	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	0.0	0.0
Ampelisca abdita	83.0	7.8	69.3	18.5	87.0	17.1	168.7	31.5	129.7	21.4	157.3	23.4	43.3	5.9	50.7	27.0	41.0	15.9	29.3	8.4
Ampithoe unid. spp.	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	0.0	0.0
Callianassidae unid. spp.	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	0.0	0.0
Caprella californica	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	0.0	0.0
Corophium alienense	2.3	0.6	4.0	1.7	33.0	31.7	6.3	5.5	6.7	3.8	5.7	0.6	27.7	2.1	38.3	37.6	23.0	4.4	52.7	29.1
Corophium heteroceratum	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	0.0	0.0
Corophium spinicorne	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	0.0	0.0
Corophium unid. spp.	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	0.0	0.0
Corophiidae unid. spp.	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	0.0	0.0
Crangon nigricauda	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	0.3	0.6	0.0	0.0	0.0	0.0
Cumella vulgaris	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	0.0	0.0
Eochelidium cf. miraculum	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	0.0	0.0
Eogammarus confervicolus	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	0.0	0.0
Gnorisphaeroma oregonensis	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	0.0	0.0
Grandidierella japonica	6.7	0.6	4.3	1.5	20.3	5.5	3.3	2.1	0.7	0.6	11.0	0.0	0.0	0.0	21.7	14.6	17.7	9.8	6.0	1.7
Hemigrapsus oregonensis	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	0.0	0.0
Melita nitida	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	0.0	0.0
Melita unid. spp.	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	0.0	0.0
Monocorophium acherusicum	1.3	1.2	0.7	0.6	3.7	3.5	0.0	0.0	0.7	1.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Monocorophium insidiosum	0.3	0.6	3.0	1.7	5.0	3.6	2.0	2.0	1.3	1.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Monocorophium unid. spp.	2.3	2.5	2.0	1.0	8.3	4.9	2.0	2.6	1.3	0.6	1.3	1.5	0.0	0.0	45.0	26.1	0.0	0.0	0.0	0.0
Mysidacea unid. spp.	0.7	0.6	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
Nippoleucon hinumensis	15.7	6.7	6.0	2.6	17.0	11.5	77.3	23.1	108.3	10.2	187.0	42.2	71.7	37.2	44.7	22.2	5.3	8.4	0.0	0.0
Sinelobus unid. spp.	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	2.3	0.6	0.0	0.0	0.0	0.0
Sphaeromatidae unid. spp.	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	0.0	0.0
Synidotea laevidorsalis	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	0.0	0.0
Synidotea unid. spp.	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	0.0	0.0
Class Ostacoda																				
Eusarsiella zostericola	33.3	7.6	34.3	3.8	22.3	6.7	8.3	5.1	7.0	3.0	7.3	3.2	14.7	4.0	0.0	0.0	0.0	0.0	0.0	0.0
Cyprideis unid. spp.	0.3	0.6	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
Class Thecostraca																				
Amphibalanus improvisus	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	0.0	0.0
Balanomorpha unid. spp.	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	0.0	0.0
Cirripedia unid. spp.	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	0.0	0.0

TAXON	1/23/2015		2/23/2015		3/24/2015		4/22/2015		5/19/2015		6/18/2015		8/14/2015		9/29/2015		10/28/2015		11/23/2015	
TAXON	Mean	std dev	Mean	std dev	Mean	std dev														
PHYLUM CNIDARIA																				
Class Anthozoa																				
Actiniaria - attached	1.7	0.6	2.0	2.0	0.0	0.0	0.7	0.6	0.0	0.0	0.0	0.0	0.3	0.6	1.7	1.5	4.3	3.5	6.3	6.7
Actiniaria - burrowing	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	0.0	0.0
Actiniaria unid. spp.	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	0.0	0.0
PHYLUM MOLLUS CA																				
Class Bivalvia																				
Bivalvia unid. spp.	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	0.0	0.0
Gemma gemma	291.0	59.6	457.0	15.4	431.0	89.6	349.3	74.4	521.3	270.6	166.7	107.8	188.3	36.3	298.7	133.8	233.7	282.2	76.0	14.0
Macoma petalum	1.0	1.0	3.0	1.0	1.3	0.6	3.0	1.7	3.7	2.1	2.7	1.2	2.0	1.0	2.3	1.5	1.7	1.5	2.3	0.6
Macoma unid. spp.	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	0.0	0.0
Musculista senhousia	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	0.0	0.0
Mya arenaria	0.0	0.0	0.0	0.0	0.0	0.0	1.0	1.0	0.3	0.6	0.3	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Potamocorbula amurensis	1.0	1.0	1.3	0.6	0.7	0.6	0.0	0.0	0.0	0.0	1.3	1.2	0.3	0.6	0.3	0.6	0.3	0.6	0.0	0.0
Rochefortia grippi	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	0.0	0.0
Rochefortia unid. spp.	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	0.0	0.0
Tellinidae unid. spp.	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	0.0	0.0
Class Gastropoda																				
Gastropoda unid. sp. B	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	0.0	0.0
Ilyanassa obsoleta	0.0	0.0	0.0	0.0	0.0	0.0	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	0.0
Odetta bisuturalis	4.3	3.5	3.0	1.7	2.0	3.5	0.3	0.6	0.3	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Philine unid. spp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.6	0.0	0.0	0.0	0.0	0.0	0.0
Pyramidellidae unid. sp. A	0.7	0.6	1.7	1.5	0.7	1.2	0.0	0.0	0.3	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Urosalpinx cinerea	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	0.0	0.0
PHYLUM NEMATODA																				
Nematoda unid. spp.	0.3	0.6	0.0	0.0	0.0	0.0	0.7	0.6	0.0	0.0	0.0	0.0	0.7	1.2	0.0	0.0	0.0	0.0	0.0	0.0
PHYLUM PLATYHELMINTHES																				
Class Turbellaria																				
Turbellaria unid. sp. A	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	0.0	0.0
Turbellaria unid. spp.	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	0.0	0.0

Current species name:	Formerly documented as							
PHYLUM ANNELIDA								
Class Oligochaeta								
Naididae unid. spp.	Naididae							
Oligochaeta unid. spp.	Oligochaeta, Unid. Oligochaeta family							
Tubificidae unid. spp.	Tubificidae							
Class Polychaeta								
Capitella capitata complex	Capitella "capitata"							
Cirratulidae unid. spp.	Tharyx spp. ?, Cirratulidae							
Eteone unid. spp.	Eteone spp., Eteone ?californica							
Euchone unid. spp.	Euchone spp.							
Glycera unid. spp.	Glycera spp.							
Glycinde picta	Glycinde polygnatha							
Glycinde unid. sp. SF1	Glycinde sp. SF1							
Glycinde unid. spp.	Glycinde spp.							
Maldanidae unid. spp.	Unid. Malanidae							
Polychaeta unid. spp.	Unid. Polychaeta, Polychaeta							
Polydora cornuta	Polydora lighti, Polydora ligni							
Polydora unid. spp.	Polydora spp.							
Sphaerosyllis unid. sp. A	Sphaerosyllis spp.							
Spionidae unid. spp.	Unid. Spionidae, Spionidae Unidentified							
PHYLUM ARTHROPODA								
Class Copepoda								
Calanoida unid. spp.	Calinoida							
Harpacticoida unid. spp.	Harpacticoida							
Class Insecta								
Chironomidae unid. spp.	Chironomidae							
Class Malacostraca								
Americhelidium unid. spp.	Americhelidium spp., Synchelidium spp.							
Ampithoe unid. spp.	Ampithoe spp.							
Callianassidae unid. spp.	Callianassidae, Callianassidae unidentified							
Corophium unid. spp.	Corophiidae - unidentified							
Corophiidae unid. spp.	Corophium spp.							
Eogammarus confervicolus	Anisogammarus confervicolus							
Gnorisphaeroma oregonensis								
	Gnorisphaeroma oregonensis, Gnorimosphaeroma oregonensis							
Melita unid. spp.	Melita spp.							
Monocorophium acherusicum	Corophium acherusicum							
Monocorophium insidiosum	Corophium insidiosum							

#### Appendix 9. Benthic species name changes as of 2015.

Current species name:	Formerly documented as
Monocorophium unid. spp.	Corophium ?insidiosum, Corophium spp. (female and juvenile), Corophium spp. (male), Monocorophium spp.
Mysidacea unid. spp.	Mysidacea
Nippoleucon hinumensis	Hemileucon hinumensis
Sinelobus unid. spp.	Sinelobus stanfordi, Sinolobus spp., Sinolobus stanfordi, Tanais spp.
Sphaeromatidae unid. spp.	Sphaeromatidae (juv.), Sphaeromatidae unid., Dynamella spp.
Synidotea unid. spp.	Synidotea spp.
Class Ostacoda	
Eusarsiella zostericola	Sarsiella zostericola
Cyprideis unid. spp.	Cyprideis spp.
Class Thecostraca	
Amphibalanus improvisus	Balanus improvises
Balanomorpha unid. spp.	Balanus ?aquila, Balanus spp., Unid. Balanomorpha, Balanomorpha – unidentified
Cirripedia unid. spp.	Cirripedia
PHYLUM CNIDARIA	
Class Anthozoa	
Actiniaria unid. spp.	Anthozoa, Unid. Actiniaria
PHYLUM MOLLUSCA	
Class Bivalvia	
Bivalvia unid. spp.	Unid. Bivalvia
Macoma petalum	Macoma balthica
Macoma unid. spp.	Macoma spp.
Potamocorbula amurensis	Corbula amurensis
Rochefortia unid. spp.	Rochefortia spp.
Tellinidae unid. spp.	Tellinidae
Class Gastropoda	
Gastropoda unid. sp. B	Unidentified Gastropoda B
Ilyanassa obsoleta	Nassarius obsoletus
Odetta bisuturalis	Boonea bisuturalis, Odostomia fetella
Philine unid. spp.	Philine spp.
Pyramidellidae unid. sp. A	Odostomia spp., Pyramidellidae, Unidentified Gastropod A
PHYLUM NEMATODA	
Nematoda unid. spp.	Nematoda
PHYLUM PLATYHELMINTHES	
Class Turbellaria	
Turbellaria unid. sp. A	Planariidae A
Turbellaria unid. spp.	Turbellaria