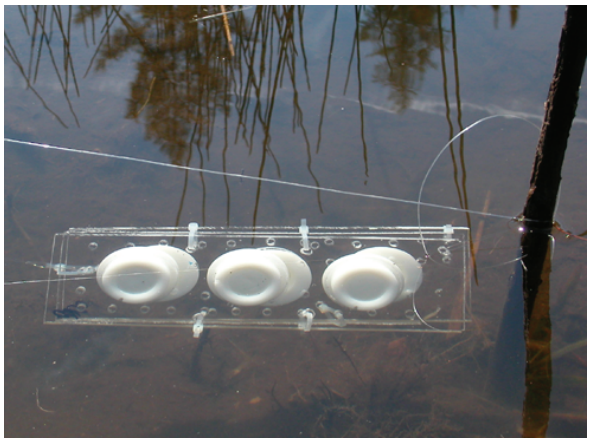
ERDC/EL TR-09-22

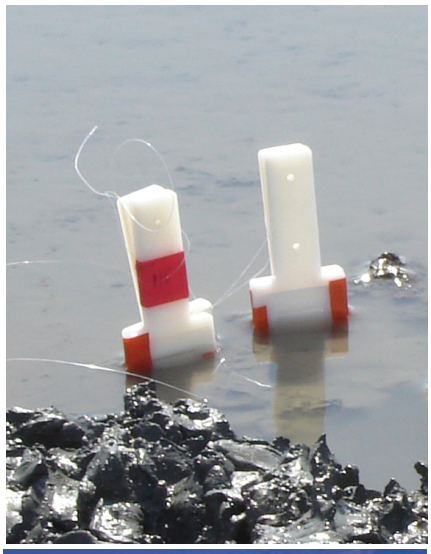
US Army Corps
of Engineers®
Engineer Research and
Development Center

Comparison of DGT Sentinels and Bioassays for Long-term Mercury TMDL Monitoring under San Francisco Bay Field Conditions

E. P. H. Best, H. Hintelmann, O. Clarisse, J. S. Furey,
B. Greenfield, and B. Dimock

December 2009





Environmental Laboratory

ERDC/EL TR-09-22
December 2009

Comparison of DGT Sentinels and Bioassays for Long-term Mercury TMDL Monitoring under San Francisco Bay Field Conditions

E. P. H. Best and J. S. Furey

Environmental Laboratory
U.S. Army Engineer Research and Development Center
3909 Halls Ferry Road
Vicksburg, MS 39180-6199

H. Hintelmann, O. Clarisse, and B. Dimock

Trent University
1600 West Bank Drive
Peterborough, ON K9J 7B8, Canada

B. Greenfield

San Francisco Estuary Institute
7770 Pardee Lane
Oakland, CA 94621-1424

Final report

Prepared for U.S. Army Engineer District, San Francisco
1455 Market Street, San Francisco, CA 94103

Abstract: Compliance with the mercury total mean daily load (TMDL) for San Francisco Bay adopted in 2004 requires long-term monitoring of mercury loadings from a range of sources, including extant and restored wetlands, into the Bay. This study explores the use of DGTs for long-term monitoring of MeHg in the field, by determining the performance of DGTs as indicators for potential MeHg accumulation at relevant field sites over a range of time periods (up to 28 days); comparing/ correlating the DGT results with MeHg bioaccumulation over the same time periods in originally 'clean' bioassay organisms (two clam species); and comparing/ correlating the DGT results with the MeHg levels in site-inhabiting clams and small fish.

The following conclusions can be reached based on study results:

- DGT-labile MeHg concentrations of the water-DGTs were usually less than the unfiltered water concentrations.

- The MeHg-time relationships of the water-DGTs differed from those of the +1.5-cm-sediment-DGTs.

- The MeHg concentrations of the water-DGTs were not significantly related to those of *M. nasuta* test clams.

- The MeHg concentrations of the water DGTs ranked similar to site as those of *T. japonica* test clams and, therefore, appeared to respond to the same processes.

- The MeHg concentrations of the 14-day incubated water-DGTs were significantly related to those of the site-inhabiting clam *Mya arenaria* and weakly related to those of the site-inhabiting fish *M. audens*, and, therefore, water-DGTs appeared to respond to the same processes as these organisms.

Contents

Figures and Tables

Figures

Tables

Preface

The work reported herein was conducted by the Environmental Laboratory (EL) of the U.S. Army Engineer Research and Development Center (ERDC), Vicksburg, MS. Funding was provided by the Long Term Management Strategy (LTMS), administered by the U.S. Army Engineer District, San Francisco (CESPN).

In March 2003, the CESPN requested an expansion of pre-construction monitoring of total mercury (THg) and methylmercury (MeHg) concentrations in sediments and soils of existing wetlands bordering the Hamilton Army Airfield (HAAF) Wetlands Restoration Site on San Pablo Bay, California. The purpose of the expanded activities was to gain site-specific knowledge of the geochemical/geophysical, microbial, predominant plant- and animal-related interactions that affect the stabilization and mobilization of Hg and MeHg in the sediments/soils of the area. Exploratory research data from 2003 formed the basis for a site-specific screening-level model to estimate Hg species mobility during wetlands reconstruction. Follow-up research in 2004-05 described (1) site-specific (de)methylation and sedimentary microbial community characterization; (2) Hg dynamics in decomposing plant litter; (3) Hg dynamics in food webs; and (4) bioavailability of sediment-associated Hg to macrobenthos. Subsequent research in 2006 focused on (1) site-specific (de)methylation and Hg cycle parameters measured by established and gel-based techniques, e.g., diffusive gradient in thin film (DGT); (2) accumulation of water- and sediment-associated Hg in clams, fish, and DGT; (3) exploring food web sources and pathways using multi-source mixing models; and (4) recalibration of the screening-level model. The current project conducted in 2007-08 further explores the performance of DGTs for long-term Hg total mean daily load (TMDL) monitoring in the field.

The project leader of this work was Dr. Elly P. H. Best, Environmental Risk Assessment Branch (ERAB), of the Environmental Processes and Engineering Division (EPED), Environmental Laboratory (EL), U.S. Army Engineer Research and Development Center (ERDC). The multidisciplinary team was composed of the following investigators: J. S. Furey, Environmental Processes Branch (EPB), EL; Dr. H. Hintelmann, Dr. O. Clarisse, and B. Dimock (Trent University, Department of

Chemistry, Peterborough, Ontario, Canada) for the work on DGTs, water, and clams; and Dr. B. Greenfield (San Francisco Estuary Institute, Oakland, California) for the work on fish.

Dr. A. J. Bednar and Dr. Charles H. Lutz, ERDC, are gratefully acknowledged for their reviews of two earlier drafts of this report. Dr. D. Yee of the San Francisco Estuary Institute is gratefully acknowledged for serving as external reviewer.

The study was conducted under the general supervision of Warren Lorentz, Chief, ERAB; Richard E. Price, Chief, EPED; Dr. Mike Passmore, Deputy Director, and Dr. Beth Fleming, Director of EL.

COL Gary E. Johnston was Commander and Executive Director of ERDC. Dr. James R. Houston was Director.

1 Introduction

Compliance with the mercury TMDL for San Francisco Bay adopted in 2004 requires long-term monitoring of mercury loadings from a range of sources, including extant and restored wetlands, into the Bay. Although the TMDL has as a goal to attain levels of total mercury (THg) that are protective of wildlife and human health, the toxicity of mercury species is largely associated with monomethylmercury (MeHg), which accumulates up food chains. It is clear from the large databases of directly measured THg and MeHg that THg is found almost everywhere in sediments at about 0.3 μg/g dry weight, and that the levels of MeHg in water and sediment are extremely variable in both time and space (Best et al. 2007). Recent studies indicate that the extent to which MeHg bioaccumulates in organisms of food chains associated with tidal wetlands, such as China Camp and potentially with a restored Hamilton Army Airfield (HAAF) marsh, depends largely on feeding behavior and diet (Best et al. 2007). Fish sentinels are being developed for use in the Sacramento River Delta for integrating potential exposures (Slotton et al. 2004; Greenfield et al. 2006). Future monitoring activities would greatly benefit from sentinels that would serve as direct indicators of potential impacts of MeHg on threatened and endangered species and the Bay fishery, and would provide feedback to environmental regulators and enable them to adaptively manage the progress of wetland restorations.

In previous studies on mercury biogeochemistry, new gel techniques (DGT and DET) were used to sample key parameters in sediment pore water. The results of these techniques correlated well with those of conventional pore water sampling (Best et al. 2005). DGT-based measurement methods have been developed by Davison and Zhang (1994). The DGT device is comprised of an ion-exchange resin immobilized in a gel (resin gel), which is separated from the test solution by an ion-permeable gel (diffusive gel). Concentration gradients develop across the diffusive gel and the contaminants are transported to the resin gel where they are fixed (in the case of MeHg by an ion-exchange reaction) and accumulate during the deployment time. The DGT approach has several advantages over other techniques proposed for measuring trace metals in natural waters:

1. The device can be mass produced and is easy to use.
2. The device can provide information about the actual MeHg species present in the water by varying the thickness and pore size of the diffusion gel layer.
3. The device concentrates MeHg in situ.
4. The device yields time-averaged concentrations over the length of the deployment period.
5. Analysis of the devices can be optimized for high-throughput analyses.

DGT devices accumulate only certain forms of a metal, i.e., mainly the labile metal species able to pass through the diffusion layer and bind with the resin layer. After the DGT device is removed from the sampling site, the mass of metal in the resin layer is determined analytically. The well-defined geometry of the DGT device enables quantitative interpretation of the mass accumulated, either in terms of dissolved concentrations, or remobilization fluxes from sediments to pore waters. In waters that are reasonably well mixed, the interpretation of DGT measured fluxes as labile metal concentrations in solution external to the DGT device is relatively straightforward (Zhang and Davison 1995). In sediments and saturated soils, interpretation is more complicated, due to the interaction of metal in solution with metal associated with the solid phase. Simple interpretations can provide estimates of a time-averaged remobilization flux from solid phase to solution and estimates of pore water concentrations (Zhang et al. 1995). A numerical modeling approach (Harper et al. 1998) is used to provide more quantitative interpretations in terms of the rate of supply from sediment to solution (i.e. the exchangeable metal fractions associated with sediment particles). The model enables the determination of the characteristic sorption-desorption reactions, together with information on the size of the exchangeable fraction associated with the solid phase. In the case of MeHg, methylation of proximate Hg as a source of MeHg also affects DGT measurements. Interpretation of such measurements is therefore not simple but, if done correctly, it may provide invaluable data for the understanding of mercury biogeochemical cycles in sediments. MeHg concentrations calculated from the DGT measurements are supposed to correspond to the concentration of the aqueous $MeHg^{+}$ ion and small inorganic MeHg complexes with comparable diffusion coefficients such as MeHgCl.

Initial studies associated with the HAAF Wetland Restoration Project in San Pablo Bay have shown that DGT devices are useful to integrate

exposures to MeHg. Short-term field incubations indicated that MeHg concentrations in DGTs (ng/L) were strongly correlated with MeHg concentrations in the interstitial water of sediments (ng/L) determined by conventional techniques over a range of salinities varying from saline to brackish (Best et al., in preparation). Subsequent short-term field incubations formed the basis for strong correlations between MeHg concentrations in DGTs (ng L^{-1}) and net methylation rates (ng g^{-1} DW day^{-1}) in sediments over the same range of salinities (Best et al. 2009). In addition, results of laboratory incubations in which DGTs, clams, and fish were exposed to aqueous MeHg and MeHg-spiked food also showed correlations between the MeHg mass contained in the DGTs (pg per DGT device) and the MeHg concentrations (ng g^{-1} DW) in the organisms, but correlations were stronger when exposure originated only from aqueous MeHg than when exposure originated from aqueous MeHg and from MeHg-spiked food (Best et al., in preparation).

The objectives of the current study were to further explore the DGT sentinels for long-term monitoring of MeHg in the field, by:

- Determining the performance of DGTs as indicators for potential MeHg accumulation at relevant field sites over a range of time periods (up to 28 days).
- Comparing/correlating the DGT results with MeHg bioaccumulation over the same time periods in originally 'clean' bioassay organisms (two clam species).
- Comparing/correlating the DGT results with the MeHg levels in site-inhabiting clams and small fish.

2 Materials and Methods

Approach

Water DGTs, sediment DGTs, and two clam species (further referred to as 'test-clams') were deployed in the field on 22 October 2007, and sampled after 3, 14, and 28 days of on-site exposure. Additional characteristic site parameters collected included water temperature, salinity, and MeHg in surface water (each sampling date), and MeHg in site-inhabiting clams and small fish (the last sampling date only). MeHg concentrations in DGTs and test clams were determined, compared, and correlated to evaluate tentative relationships between MeHg concentrations in these monitoring devices, exposure period, and site characteristics. In addition, MeHg concentrations in DGTs, test clams, and site-characteristic clams and fish were determined, compared, and correlated to explore relationships between monitoring devices and site-inhabiting clams and fishes.

Study sites

Site selection was based on three criteria. The first criterion was relevance for HAAF wetland MeHg studies (Best et al. 2005, 2007, in preparation), the second criterium relevance for a small fish mercury biosential project (Greenfield et al. 2006), and the third criterion elevated MeHg levels in water. Based on the first criterion, three sites adjacent to wetlands were selected: China Camp as a reference wetland, the HAAF wetlands restoration site, and a brackish Petaluma River marginal wetland site where elevated MeHg net production in the sediment was found in previous studies (Best et al., in preparation). Based on the second and third criteria, two additional sites adjacent to wetlands were selected: Alviso Slough, a brackish existing wetland with elevated MeHg levels in the south Bay, and Point Isabel, a marine existing, interior wetland with elevated MeHg levels in the mid Bay. Characteristics of the study sites are provided in Figure 1 and Table 1.

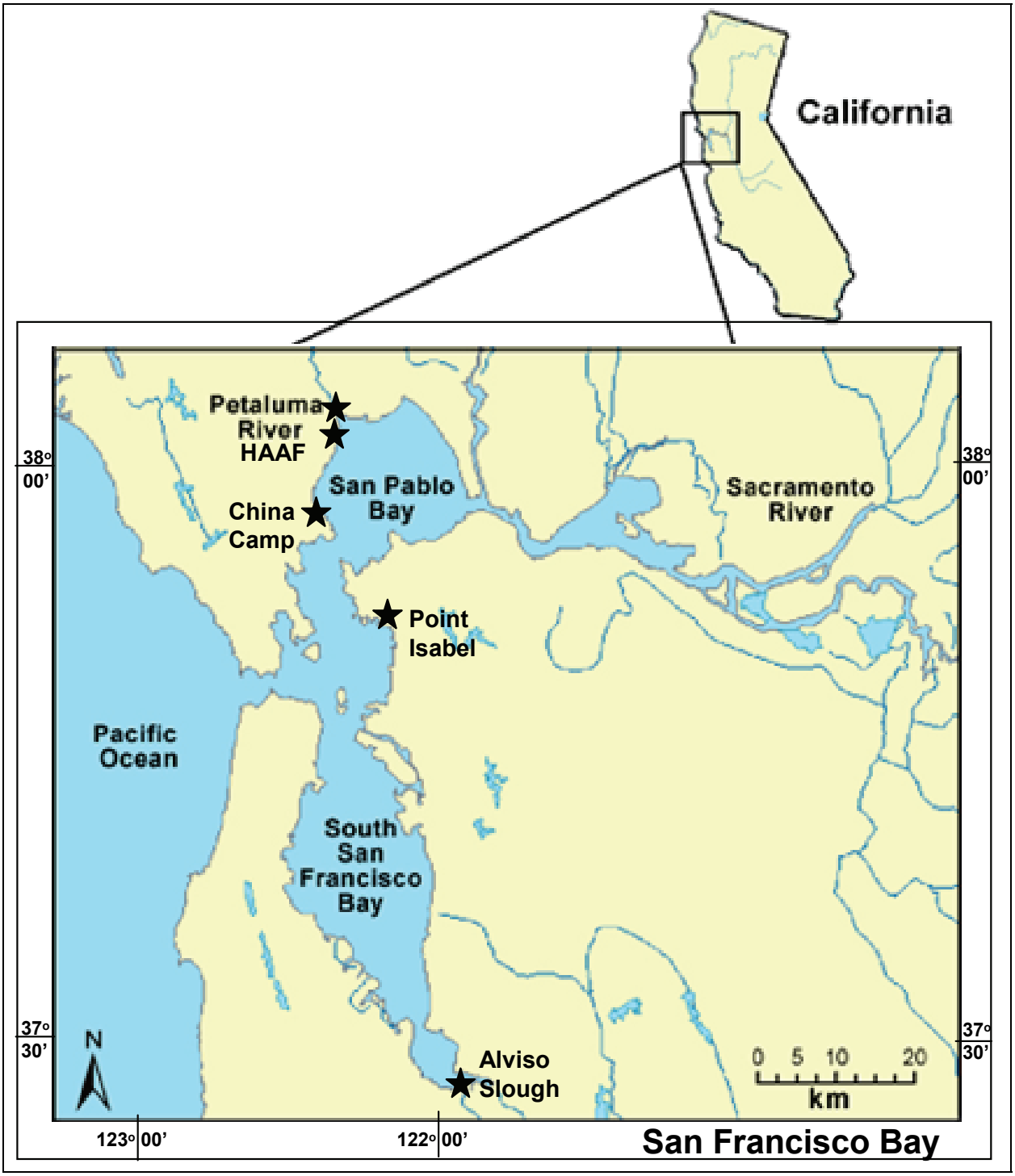


Figure 1. Location of sites.

Table 1. Site situation in the San Francisco Bay area.

Site name	Latitude	Longitude	Situation in landscape
China Camp	38° 00.533 N	122° 28.967 W	San Pablo Bay
HAAF	38° 03.111N	122° 29.55 W	San Pablo Bay (close to site SM-10)
Petaluma River	38° 06.964 N	122° 30.249 W	Petaluma River/point of inflow San Pablo Bay
Alviso Slough	37° 27.504 N	122° 01.183 W	South San Francisco Bay
Point Isabel	37° 54.235 N	122° 19.170 W	Mid San Francisco Bay

Monitoring devices

DGTs

DGT probes for sampling Hg species in porewater of marine sediments have previously been deployed for the measurement of Hg^{2+} (Divis et al. 2005). The same gels were contained in the DGT probes used to sample MeHg in water and in sediments. In the water DGTs, the gels were mounted on perspex plates designed to float in the water or lie flat on top of the sediment, in triplicate. In the sediment DGTs, the gels were mounted in elongated Perspex holders designed to be inserted into the sediment so as to expose the gel to water just above the sediment and a sediment depth range of 0 to 10 cm (Figure 2). DGT probes for accumulating MeHg were constructed with standard filter membranes (cellulose nitrate), diffusive gels ($\Delta d = 0.053$), and a binding resin consisting of mercapto-propyl functionalized silica gel embedded in a 0.05-cm thick polyacrylamide gel (Clarisse and Hintelmann 2006). The probes were deployed after de-aeration and retrieved in the standard manner for DGT-sediment probes. The diffusive gel was removed from the binding resin, which was cut into 1-cm sections and placed in clean glass vials. The resin sections were preserved by refrigeration. In the laboratory, the resins were leached using a thiourea/HCl solution (0.005% in 0.1 M HCl) and analyzed by gas chromatography coupled to inductively coupled plasma/mass spectrometry (GC-ICP/MS). Pore water MeHg concentrations were computed using the recorded incubation times in hours. Calculated concentrations correspond to the interval from 5 mm above to 5 mm below the indicated nominal depth in the cores.

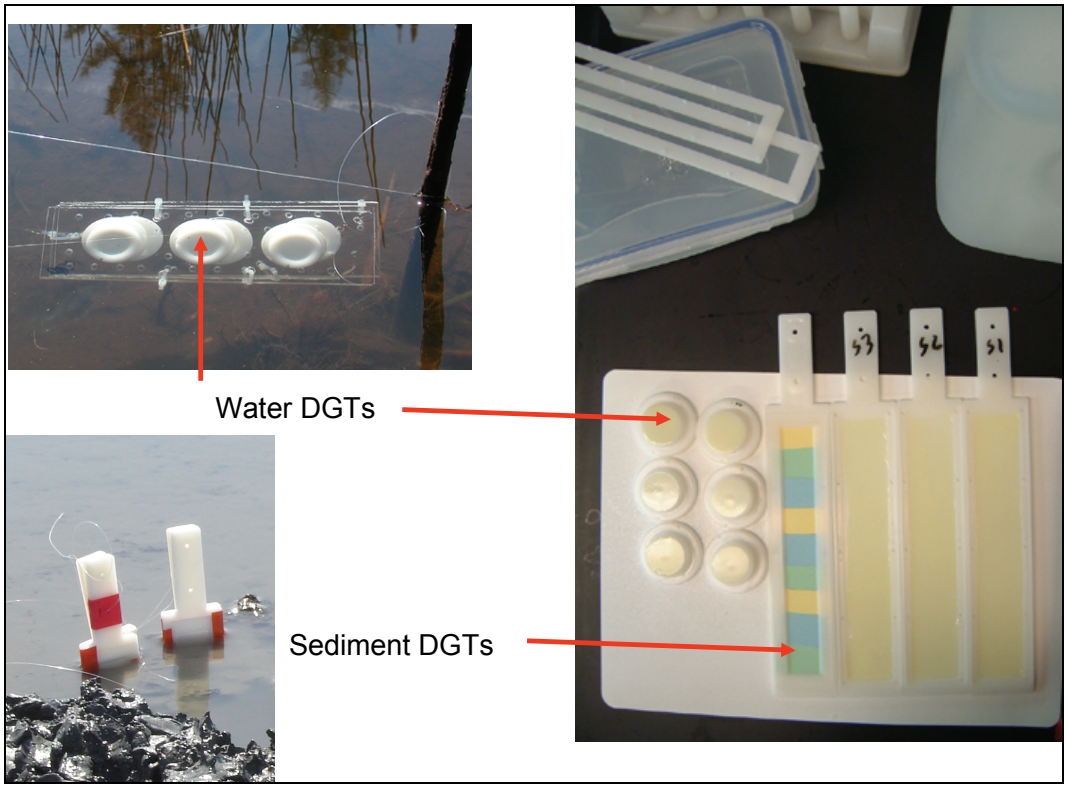


Figure 2. Water and sediment DGTs were deployed on the sites located in San Pablo Bay and South San Francisco Bay (CA) and sampled after 3, 14, and 28 days of exposure to site conditions.

Test clams

Macoma nasuta (bent-nosed clam; Figure 3) is a native estuarine bivalve common in intertidal and subtidal zones, where it inhabits shallow mud to muddy-sand substrates. It occurs from Alaska to Southern California (Hylleberg and Gallucci 1975). The species has been recorded from the San Francisco Bay sediments, and fills a niche similar to that of *Macoma balthica*, a clam common in the intertidal sediments in San Pablo Bay. *M. nasuta* is a facultative deposit feeder, capable of suspension filter feeding and selective deposit feeding, and typically burrows down to a depth of 15 cm. Its siphons are separated: the inhalant siphon takes up detritus and organic matter directly from either the overlying water or from the substrate, while the exhalant siphon deposits the indigestible particles and sediment on the sediment surface. Predators of all clams include snails, crabs, starfish, soles, flounder, perch, and shorebirds (Table 2).

Figure 3. Test clams, *Macoma balthica* (left) and *Tapes japonica* (right) collected from Tomales Bay (CA), were deployed on the sites located in San Pablo Bay and South San Francisco Bay (CA) and sampled after 3, 14, and 28 days of exposure to site conditions.

Table 2. Information relating to sediment/water access of DGTs, and of water layer access, feeding mode, food items, predators, and continent of origin (endemic or exotic) of organisms employed to monitor exposure to MeHg in nearshore areas of San Francisco Bay. DGTs, *M. nasuta* and *T. japonica* were deployed as MeHg monitoring devices with known exposure time. The other clams and fish inhabited the field sites; their MeHg levels were expected to be site-specific, and were used for comparison.[1]

DGT/Organism	Sediment/ Water Layer Access	Feeding Mode	Food Items	Predators	EN/EX	Reference (see notes at end of table)
DGT						
Water-DGT	Water-sediment interface; water column					
Sediment DGT	Sediment 0 to -10-cm; water-sediment interface					
Clams						
Macoma nasuta (bent-nosed clam)	Sediment -15-cm; water-sediment interface	Suspension feeder/selective deposit feeder	Detritus, phytoplankton	Snails; crabs; siphon-eating fish; shore birds	EN	1
Tapes japonica (Manila clam)	Sediment -10-cm; water-sediment interface	Non-selective suspension feeder	Phytoplankton, detritus	Snails; crabs, shrimp; siphon-eating fish; ducks, shore birds, gulls	EX	2, 3, 4
Macoma balthica (Baltic clam)	Sediment -15-cm; water-sediment interface	Deposit/ suspension feeder; herbivore	Detritus, phytoplankton	Snails; crabs, shrimp; siphon-eating fish; ducks, shore birds, gulls	EX	2, 5, 6, 7

DGT/Organism	Sediment/ Water Layer Access	Feeding Mode	Food Items	Predators	EN/EX	Reference (see notes at end of table)
Clams (cont.)						
Mya arenaria (soft-shell clam)	Sediment -15-cm; water-sediment interface	Non-selective suspension feeder	Plankton, detritus	Snails; crabs; various benthi-vorous fish; ducks, cormorants, gulls, shore birds; otters, raccoons	EX	2, 8
Potamocorbula amurensis (Asian clam)	Sediment just under surface; water-sediment interface	Suspension feeder; omnivore	Phytoplankton, zooplankton, bacteria, larvae, detritus	Diving ducks (scaup); starry flounder	EX	2, 7, 9, 10, 11
Fish						
Atherinops affinis (topsmelt)	Water column	Predator; carnivore adults; omnivore juveniles	Zooplankton; epi-benthic inverte-brates; algae	Gobies, California flounder, rays, sharks, seals	EN	3, 12 13,14
Clevelandia ios (arrow goby)	Water-sediment interface	Predator; benthivore	Small invertebrates (copepods, amphi-pods, nematodes, worms); siphon tips; potential cannibalism	Sculpin, halibut, turbot; shore birds	EN	15, 16, 17, 18
Menidia audens (Mississippi silverside)	Water column	Predator; carnivore	Zooplankton; epibenthic invertebrates	Various bass species, gar	EX	19, 20, 21, 22, 23

Notes: 1. Hylleberg and Galluci 1975. 2. Cohen and Carlton 1995. 3. Emmett et al. 1991. 4. Morris et al. 1980. 5. Black 1980. 6. Olafsson 1986. 7. Richman and Lovvorn 2004. 8. Newell and Hidu 1986. 9. NIMPIS 2002. 10. Peterson 1997. 11. Poulton et al. 2002. 12. Fish Base 2009. 13. Fitch and Lavenberg 1975. 14. Visintainer et al. 2006. 15. Hieb 2000. 16. Maginnis 2006. 17. Svensson et al. 1998. 18. Svensson and Karnemo 2007. 19. Matthews et al. 1992. 20. Moyle 2002. 21. Page and Burr 1991. 22. Robins and Ray 1986. 23. Ross 2002.

[1]EN = Endemic, EX = Exotic

Tapes japonica (Manila clam or Japanese littleneck clam; Figure 3) is an exotic estuarine bivalve common in intertidal and subtidal zones where it inhabits shallow fine gravel, sand, mud, and shell substrates (Emmett et al. 1991). *T. japonica* was introduced from Asia (China, Japan, Korea) in San Francisco Bay in 1946, and is currently common in San Francisco Bay sediments (http://www.exoticsguide.org/species_pages/v_philippinarum.html). The species has a spatial distribution from Washington to Southern California, and is the second-most important commercial clam species on the Pacific coast of North America. It occurs in a temperate climate, prefers a salinity range of 24 to 31 ppt with prolonged salinities below 10 ppt being lethal, is

tolerant towards pollution, and may accumulate pollutants harmful to humans. *T. japonica* is a non-selective suspension filter feeder (phytoplankton and detritus), and typically resides just under the mud surface (Table 2; Emmett et al. 1991).

The test clams were purchased from a commercial vendor (John Brezina & Associates, Dillon Beach, CA), who collected the organisms from Tomales Bay, CA, and kept them refrigerated for 1-3 days in bay water until deployment in the field.

Deployment and sampling of DGTs and test clams

All field sites were marked by stakes (Figure 4). The water DGTs were tied to floats manufactured from plastic tubing, which in turn were tied to crab cages fastened to the stake (Figure 4). This arrangement enabled the DGTs to stay submerged most of the time, follow the tidal movements of the water, and be subjected to the same exposure as the test clams. The sediment DGTs were inserted into the sediment next to the stake, enabling contact with water just above the sediment surface and a sediment depth range of 0 to 10 cm. The test clams were placed in crab cages tied to the stake. DGTs and test clams were sampled after 3, 14, and 28 days of exposure to site conditions.

At the initiation of the experiment, each field site was marked by three stakes (one for each harvest after 3, 14, and 28 days of incubation). Each stake served as a one-time marker/connector of three water DGTs, one sediment DGT, and three crab-cages each containing four *M. nasuta* and four *T. japonica* clams. At the initiation of the experiment and each sampling time, surface water was sampled and salinity and temperature were measured. At each sampling time, the DGTs in their mounts and the clams were removed from water and sediment, lightly rinsed on site with demineralized water, kept moist in Ziploc bags and refrigerated until further processing and analysis in the laboratory. In the laboratory, the clams were removed from their shells and freeze-dried prior to MeHg analysis.

Figure 4. Deployment and sampling of the DGTs and test clams in the field. All field sites were marked by stakes (upper left). The water DGTs were tied to floats manufactured from plastic tubing, which in turn were tied to crab-cages fastened to the stake (upper right). This arrangement enabled the DGTs to stay submerged most of the time, follow the tidal movements of the water, and be subjected to the same exposure as the test clams. The sediment DGTs were inserted into the sediment next to the stake, enabling contact with surface water just above the sediment, and a sediment depth range of 0 to 10 cm. The test clams were placed in crab cages tied to the stake (lower left). DGTs and test clams were sampled after 3, 14, and 28 days of exposure to site conditions (lower right).

Clams and fishes inhabiting the field sites

Macoma balthica (Balthic clam; Figure 5) is an exotic estuarine bivalve common in intertidal and subtidal zones where it inhabits shallow mud to muddy-sand substrates. The species is common in most estuaries of northern Europe and in North America at least as far south as San Francisco and Chesapeake Bays. It has a spatial distribution, fills a niche, has a feeding behavior and predators similar to *M. nasuta* (Waugh 1960; Nichols and Thompson 1982; Table 2).

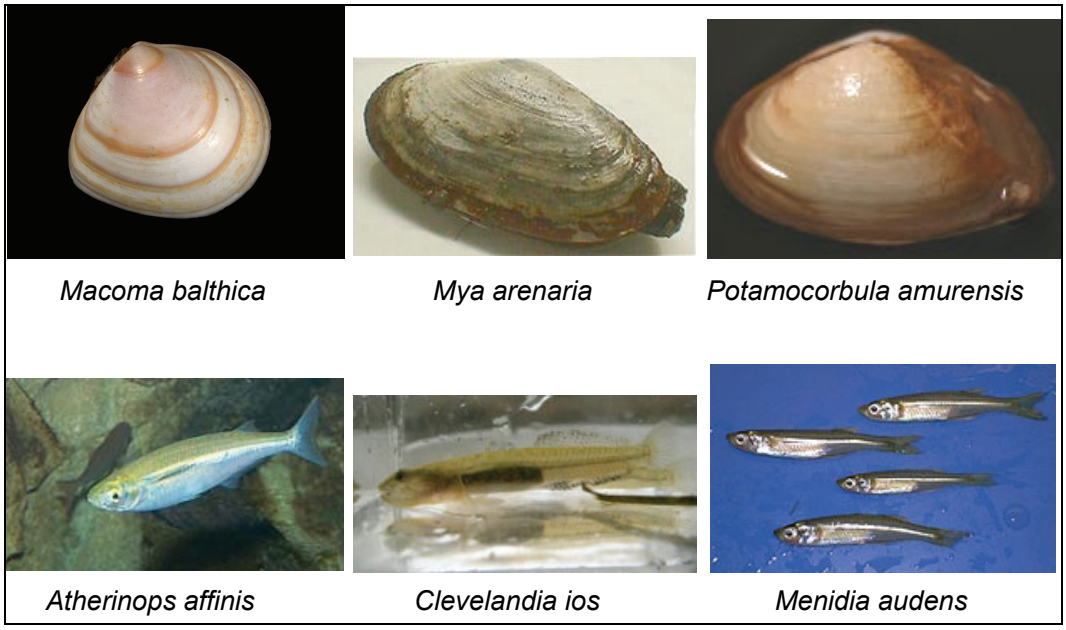


Figure 5. Site-characteristic clams (*Macoma balthica*, *Mya arenaria, Potamocorbula amurensis*, *and Tapes japonica*-the latter shown in Figure 3) and fish (*Atherinops affinis*, *Clevelandia ios,* and *Menidia audens*) were sampled on the sites located in San Pablo Bay and South San Francisco Bay (CA) at the end of the exposure period of the DGTs and test clams.

Mya arenaria (soft-shell clam; Figure 5) is an exotic, estuarine bivalve common in upper intertidal zones, but also found in low intertidal and shallow subtidal zones where it inhabits sand, mud and clays, often in mixtures with coarse gravel (Abraham and Dillon 1986). It occurs in eastern North America from Labrador to Cape Hatteras in North Carolina, in Alaska north of the Aleutian peninsula, and in Korea, the Kurile Islands and northern Japan. On the North American Pacific coast, introductions occurred since the 1880's when *M. arenaria* entered San Francisco Bay, where it is presently common (Stearns 1881; Cohen and Carlton 1995). It can tolerate salinities down to 5 ppt. *M. arenaria* feeds on plankton and detritus from the water column. It serves as prey for snails, crabs, rays, sharks, flounder, sculpin, ducks, cormorants, gulls, shorebirds, sea otters and raccoons (Table 2).

Potamocorbula amurensis (Asian or Chinese clam; Figure 5) is an exotic bivalve common mostly in subtidal zones, but also occurring intertidally, where it inhabits mud, peat, clay, sand and is most abundant on muddy-sand substrates. It occurs in cold temperate to tropical waters, can tolerate salinities from 5 to 28 ppt, and is tolerant of pollution. The species is native in Japan, China, and Korea and was introduced into San Francisco Bay in 1986, where it is a dominant species and has caused dramatic

changes in the soft-sediment communities in the area. *P. amurensis* is a suspension feeder that can consume large amounts of phyto- and zooplankton larvae (NIMPIS 2002). It typically exposes one half to three quarters of its shell above the sediment-water interface. It serves as prey for starry flounder, and diving ducks, particularly scaup (Table 2; Richman and Lovvorn 2004).

Atherinops affinis (topsmelt; Figure 5) is native to the eastern Pacific Ocean, and is found along the west coast of North America from British Columbia to Baja, California. This species is common in San Francisco Bay and the Sacramento-San Joaquin River Delta in California. It is a marine species and often schools in shallow water such as estuaries, bays, intertidal zones, and kelp forests, where it feeds on zooplankton and epibenthic invertebrates. It serves as prey for gobies, California flounder, rays, sharks, and seals (Table 2; Emmett et al. 1991; Visintainer et al. 2006).

Clevelandia ios (arrow goby; Figure 5) is the most abundant native goby in San Francisco Bay. It is common to intertidal mudflats and shallow subtidal areas of bays, estuaries, and coastal lagoons, where it feeds on small invertebrates such as harpactoid copepods, nematodes, oligochaetes, ostracods, and cyclopoid copepods. This small fish is an important component of the intertidal food web, and predators include sculpin, halibut, turbot, and shorebirds (Table 2; Hieb 2000; Maginnis 2006; Svensson et al. 1998; Svensson and Karnemo 2007).

Menidia audens (Mississippi silverside; Figure 5) is not native to the west coast; it was introduced from Oklahoma to several California water bodies in the late 1960s, and has spread widely throughout the Bay-Delta region (Moyle 2002; Suttkus et al. 2005). It is a species that may occur in the pelagic zones of freshwater, brackish, and marine waters, where it feeds on zooplankton and epibenthic invertebrates. It serves as prey for various bass species and gar (Table 2; Robins and Ray 1986; Page and Burr 1991; Matthews et al. 1992; Ross 2002; Moyle 2002; Visintainer et al. 2006).

Sampling of clams and fish inhabiting the field sites

Four Birge-Ekman samples were taken in the direct vicinity of the stakes at each field site at the end of the experiment. The contents of the samples were sieved, and the clams inhabiting the site were collected. These field clams were separated into species, and processed and analyzed in the same manner as the test clams.

Four composites of whole fish from each site were targeted for mercury species analysis. Five to ten individual fish were targeted for inclusion in each composite. Fish were collected by beach seine in the direct vicinity of each field site in the same manner as the fish were collected for the Hg in small fish biosentinel project (Greenfield et al. 2006). The fish were separated by species, freeze-dried, and analyzed for MeHg and total-Hg.

Methylmercury determination

Fish and clam samples were subjected to an alkaline digestion in order to isolate MeHg from biological matrices (Hintelmann and Nguyen 2005). The whole sample (0-0.2 g wet weight) was dissolved in 8 mL of 20% KOH in methanol at 47 °C for 24 hr. Prior to the alkaline digestion, an internal standard $Me^{201}Hg$ (500 pg) was spiked on the biological tissue. A 50-µL aliquot of the alkaline digest was processed for aqueous ethylation and MeHg was determined by GC-ICP-MS.

Sea water samples were distilled in the laboratory. Approximately 50 mL of sample was transferred into a 50-mL glass vial. An internal standard $Me^{201}Hg$ (25 pg), 200 µL of H_2SO_4 (9M), and 500 µL of KCl (20%) were added. The distillation vials were placed in a heating block at 50 °C. Methylmercury was distilled from the sample under a supporting nitrogen stream (80 mL/min). Distillation time was approximately 3-4 hr per sample.

A reaction vessel was filled with 100 ml Milli-Q water, and the distillate was added for measurement of MeHg. Then 0.2 ml of acetate buffer (2 M) was added to adjust the pH to 4.9. Sodium tetraethylborate (100 µL, 1 % w/v) was added and the solution was left sitting at room temperature for 20 min for the tetraethylborate to react. Tenax adsorber traps were connected to the reaction vessel and the generated MeHg was purged from the solution using nitrogen (200 mL min^{-1}) and collected on the Tenax trap. Finally, mercury species were thermally desorbed from the trap (250 °C), separated by gas chromatography, and quantified by ICP/MS (Micromass Platform). The following isotopes of Hg were measured: ^{201}Hg (internal standard) and ^{202}Hg (to calculate ambient MeHg). Peak areas were used for quantification, and ambient MeHg concentrations were calculated by isotope dilution.

Hg analysis QA/QC

QA/QC was performed on a regular basis by analyzing MeHg in bubbler blanks, thiourea blanks (DGT), KOH-methanol blanks (fish and clams), and distillation blanks (seawater). MeHg was also analyzed in certified reference materials after alkaline digestion: dogfish muscle tissue (DORM-2; NRCC, Ottawa, ON, Canada), lobster hepatopancreas (TORT-2; NRCC, Ottawa, ON, Canada), and oyster tissue (NIST 1566b; NIST, Gaithersburg, MD, USA). Individual elution or digestion yields were determined by adding the internal ^{201}Hg isotope standard.

Data analysis

The mass of MeHg accumulated in the DGT gels was used to calculate the solution concentration of MeHg (in sea and pore-water). Based on Fick's first law of diffusion, the mass of MeHg accumulated by the resin inside the DGT unit depends on its concentration in solution (C), diffusive coefficient (D) in the polyacrylamide gel, the thickness (Δd = 0.053 cm) and surface area (A = 3.14 cm^2) of the diffusive gel layer, and the deployment time (t) of the DGT device (Clarisse and Hintelmann 2006):

$$M = \frac{D \times A \times C \times t}{\Delta d} \quad (1)$$

Hence, the concentration is obtained by

$$C = \frac{M \times \Delta d}{D \times A \times t} \quad (2)$$

A diffusive coefficient of 5.75 x 10^{-6} cm^2 sec^{-1} at 25 °C (as reported by Best et al. 2007) was used and the actual value was corrected for the respective deployment temperature.

Statistical analyses were conducted with the software STATGRAPHICS Plus for Windows version 32S package (Manugistics, Rockville, MD). Normal data distribution was tested using the Shapiro-Wilk's test. Analysis of variance (ANOVA) was expanded with a multiple range test using the Fisher's least significant difference procedure. The p-value in the ANOVA is a measure of the significance of the analysis; it was set at a 95-percent confidence level (p value of $\leq$0.05). Regression analyses were conducted using the least squares method. The p-value in the regression

model was set at a 95-percent confidence level (p value of $\leq$0.05) unless stated otherwise. The R^2-value of the regression model indicates the proportion of the variance explained by the model.

3 Results and Discussion

Performance of DGTs: Comparison of MeHg levels in water, DGTs, and test clams

The MeHg concentrations in surface water, DGT-labile MeHg calculated from MeHg accumulated by water-DGTs, selected layers of sediment-DGTs, and the test clams *M. nasuta* and *T. japonica* are presented in Table 3.

Table 3. Concentrations of DGT-labile MeHg calculated from the mass of MeHg accumulated in water-DGTs, selected sediment-DGTs, and MeHg measured directly in test clams exposed for 3, 14, and 28 days to site conditions. MeHg concentrations in surface water, salinity, and temperature initially and at each sampling date provided for reference. Mean values and standard deviations (N=1 to 5). NA = not applicable.

Site/Time	Water-DGT MeHg (ng/L)	Sed-DGT MeHg (ng/L) +1.5-cm	Sed-DGT MeHg (ng/L) -0.5-cm	Sed-DGT MeHg (ng/L) -1.5-cm	*M. nasuta* MeHg (ng/g DW)	*T. japonica* MeHg (ng/g DW)	Water MeHg (ng/L)	Salinity (‰)	Temperature (oC)
China Camp									
0 days	NA	NA	NA	NA	10.1+3.5	239.5+18.9	0.082	24.5	14
3 days	0.267+0.134	0.53	0.99	2.03	11.8+1.4	242.5+19.7	0.280	25	11
14 days	0.031+0.018	0.21	0.42	0.22	18.6+8.3	174.8+20.3	0.095	30	14
28 days	0.019+0.007	0.03	0.04	0.04	17.3+7.2	162.1+10.6	0.090	26	13
HAAF									
0 days	NA	NA	NA	NA	10.8+3.0	230.7+19.3	0.275	25	20
3 days	0.081+0.021	0.11	0.12	0.30	17.8+5.1	196.5+20.6	0.030	25	11
14 days	0.035+0.010	0.16	0.07	0.08	11.4+4.1	159.0+9.4	0.229	28	15
28 days	0.014+0.004	0.03	0.03	0.03	19.6+7.6	137.0+15.6	0.178	24	15
Petaluma River									
0 days	NA	NA	NA	NA	12.0+2.3	235.6+15.1	0.128	30	17
3 days	0.046+0.032	0.21	0.33	0.34	15.5+5.2	210.7+7.8	0.165	25	15
14 days	0.030+0.018	0.07	0.08	0.07	24.1+6.8	205.8+15.6	0.382	26	16
28 days	0.013+0	0.03	0.07	0.05	11.5+2.6	177.0+10.0	1.270	24	15
Alviso Slough									
0 days	NA	NA	NA	NA	10.8+3.0	230.7+19.3	ND	22	20
3 days	0.326+0.062	0.40	0.35	0.42	21.2+3.5	184.0+16.3	1.526	30	14
14 days	0.076+0.012	0.09	0.25	0.57	16.3+2.6	213.5+18.7	0.675	21	18
28 days	0.063+0.009	0.58	0.39	0.39	27.1+5.7	208.4+18.8	0.706	20	16
Point Isabel									
0 days	NA	NA	NA	NA	10.8+3.0	230.7+19.3	ND	22	19
3 days	0.296+0.037	0.47	0.82	1.10	16.1+5.1	207.5+13.5	0.425	30	14
14 days	0.310+0.214	0.09	0.15	0.27	14.5+2.4	217.4+17.0	0.286	30.5	16
28 days	0.073+0.011	0.14	0.12	0.14	18.2+5.5	223.4+24.8	0.359	30	15

The mean unfiltered MeHg concentrations in surface water increased in the order China Camp (0.137 ng/L)<HAAF (0.178 ng/L)<Point Isabel (0.357 ng/L)<Petaluma River (0.486 ng/L<Alviso Slough (0.969 ng/L; Table 3).

DGTs do not directly measure concentrations of MeHg in water. Instead, the means of MeHg accumulated on gels is determined. The estimated concentration of dissolved MeHg then depends on the accumulated mass and the deployment time over which this mass was taken up. Hence, if the rate of accumulation changes over time (particularly, slows down towards the end of long exposure periods) it leads to erroneous low estimates of aqueous concentrations. The (dissolved) MeHg concentrations of the water-DGTs increased in the order of Petaluma River $\leq$HAAF<China Camp<Point Isabel$\leq$Alviso Slough. The MeHg mass quantities accumulated in the water and sediment DGTs, from which the concentrations in the DGT gels were calculated, are provided in Table A1. The concentrations ranged from 0.013 to 0.046 ng/L in the Petaluma River, 0.014 to 0.081 ng/L at HAAF, 0.019 to 0.267 ng/L at China Camp, 0.063 to 0.326 ng/L in Alviso Slough, and 0.073 to 0.310 ng/L at Point Isabel (Table 3). The MeHg concentrations of the water-DGTs decreased with incubation time (Figure 6). Since the gels exhibited substantial fouling after 28 days, limiting diffusion and resulting in an apparently low concentration, an incubation period between 3 and 14 days was considered long enough to adsorb a MeHg quantity in equilibrium with the mean MeHg concentration in the surface water. The MeHg concentrations of the water DGTs were usually less than of the surface water samples. It is likely that the water DGT MeHg concentrations correspond to the concentrations of the aqueous $MeHg^+$ ion + small inorganic MeHg complexes, and, therefore, did not encompass all MeHg present in the water column. Another possibility would be that the DGT-gel became saturated at all sites except when incubated for 3 days at HAAF.

MeHg burdens in clams and fish are thought to accumulate largely from dietary sources, but direct accumulation from water can also contribute. For instance, contributions of 97% from food and 3% from water were quantified in sheephead minnows (*Cyprinodon variegatus*) in an earlier study (Best et al., in preparation). Both test clams are in contact with water

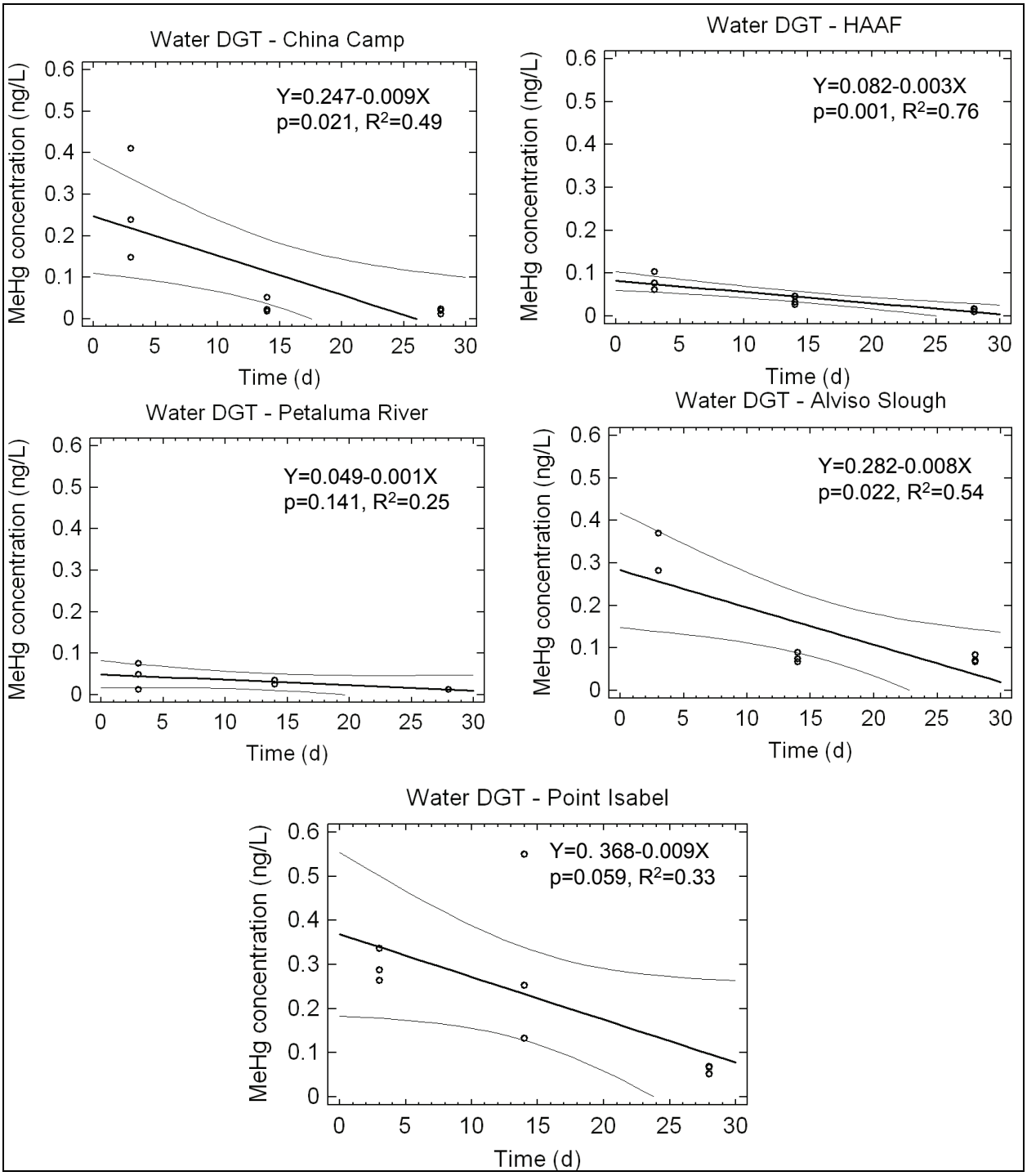


Figure 6. Relationships between MeHg concentration and length of incubation period of water DGTs exposed to field conditions. Regression lines and 95% confidence limits indicated; Y = DGT response, X = incubation period.

most of the time, depending on the tidal movements. Based on their feeding habits, with *M. nasuta* being a facultative deposit feeder capable of suspension filter feeding and selective deposit feeding, and *T. japonica* being a suspension feeder, it was expected that both clams would obtain portions of their diet from the water column, the water layer just above the sediment, the sediment surface, and the upper sediment layer. Therefore, surface water and sediment layers considered as relevant for the test clams were +1.5 cm, -0.5 cm, -1.5 cm.

The MeHg concentrations of the sediment-DGTs are presented in Table 4 and Figure 7. The MeHg concentrations were at least four times less in the sediment of HAAF and the Petaluma River than in the sediment of China Camp, Alviso Slough, and Point Isabel. MeHg concentrations fluctuated somewhat with depth, and decreased with length of incubation period. Because the incubations started in the last week of October 2007, decreasing effects of the decreasing temperatures on the net MeHg production may have affected the MeHg concentrations in the sediment DGTs also, besides length of incubation period. The greatest MeHg concentration of 3.22 ng/L was found in China Camp sediment at a 2.5-cm depth in DGTs exposed for 3 days.

Multifactor ANOVA was used to evaluate the effect of length of incubation period and site on the MeHg concentrations in the DGTs and test clams. The MeHg concentrations of the water DGTs decreased significantly with length of incubation period, and were also significantly affected by site (Table 5). The MeHg concentrations of the sediment DGTs were not significantly affected by length of incubation period, but the MeHg concentration at 1.5 cm above the sediment surface at China Camp was significantly greater (mean 1.183 ng/L) than those at the other sites (means ranging from 0.100 to 0.357 ng/L; Table 5). The MeHg concentrations of *M. nasuta* were approximately 20 times less than those of *T. japonica* (Table 3). Since both organisms originated from the same site in Tomales Bay, with considerable Hg contamination from the Gambonini Mine and Walker Creek, the differences in initial MeHg levels were attributed to differences in feeding mode.

Table 4. DGT-labile MeHg concentrations calculated from the mass of MeHg accumulated in sediment DGTs exposed for 3, 14, and 28 days to site conditions, in ng MeHg/L. One-replicate values.

Site/Time	MeHg concentration (ng/L)							
	+1.5-cm	-0.5-cm	-1.5-cm	-2.5-cm	-5.5-cm	-6.5-cm	-8.5-cm	-9.5-cm
China Camp								
3 days	0.53	0.99	2.03	3.22	1.21	1.33	0.56	0.53
14 days	0.21	0.42	0.22	0.29	0.58	0.20	0.07	0.07
28 days	0.03	0.04	0.04	0.06	0.35	0.43	0.15	0.14
HAAF								
3 days	0.11	0.12	0.30	0.18	0.23	0.28	0.39	0.40
14 days	0.16	0.07	0.08	0.08	0.08	0.08	0.08	0.08
28 days	0.03	0.03	0.03	0.02	0.02	0.02	0.03	0.03
Petaluma River								
3 days	0.21	0.33	0.34	0.33	0.27	0.28	0.25	0.21
14 days	0.07	0.08	0.07	0.09	0.08	0.18	0.20	0.09
28 days	0.03	0.07	0.05	0.06	0.05	0.06	0.08	0.11
Alviso Slough								
3 days	0.40	0.35	0.42	0.42	1.25	1.07	1.09	1.18
14 days	0.09	0.25	0.57	0.63	0.65	0.69	0.41	0.35
28 days	0.58	0.39	0.39	0.35	0.18	0.17	0.13	0.13
Point Isabel								
3 days	0.47	0.82	1.10	1.18	0.88	0.57	0.39	0.32
14 days	0.09	0.15	0.27	0.35	0.39	0.43	0.60	0.71
28 days	0.14	0.12	0.14	0.15	0.21	0.24	0.21	0.17

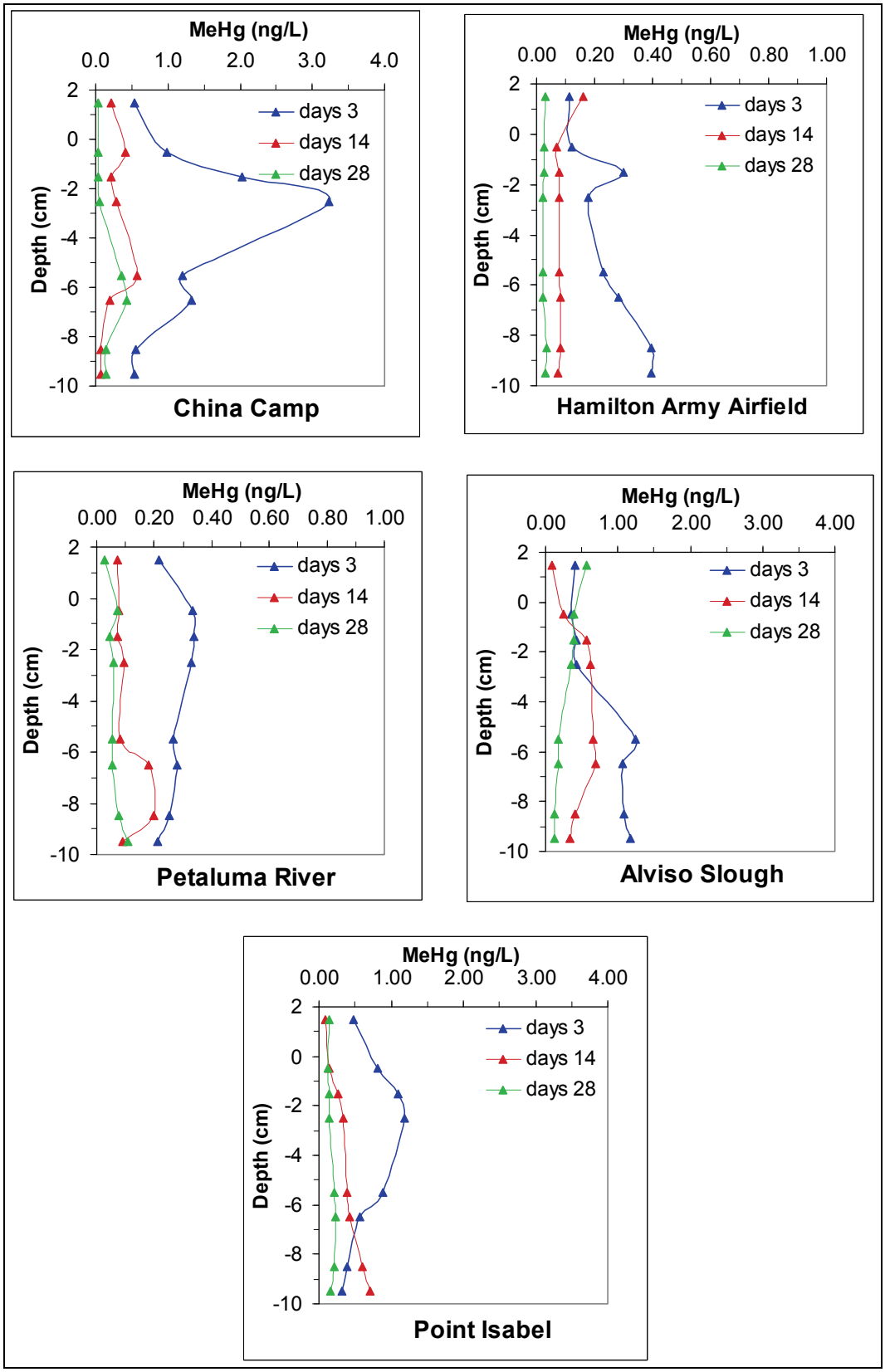


Figure 7. MeHg concentrations over time derived from MeHg accumulation in sediment-DGTs.

Table 5. Analysis of variance of the effects of length of incubation period (time) and site (site No.) on the MeHg concentrations obtained from the DGTs and determined in test clams. Statistically significant relationships at the 95% confidence level underlined.

Time/Site	MeHg concentration (mean ± standard error of mean)							
	Water-DGT (ng/L)	Sed-DGT (ng/L)	Sed-DGT (ng/L)	Sed-DGT (ng/L)	*M. nasuta* (ng/g DW)	*T. japonica* (ng/g DW)	Water-DGT (ng/L)	Sed-DGT (ng/L)
		+1.5-cm	-0.5-cm	-1.5-cm				
Factor								
Time								
3 days	0.197+0.024 b	0.344+0.172 a	0.366+0.083 a	0.438+0.099 a	16.42+1.39 a	208.13+5.79 b		
14 days	0.095+0.024 a	0.280+0.172 a	0.194+0.083 a	0.206+0.099 a	16.57+1.39 a	193.63+5.79 ab		
28 days	0.031+0.024 a	0.562+0.172 a	0.166+0.083 a	0.130+0.099 a	18.62+1.43 a	181.66+5.63 a		
ANOVA								
p-value	<0.001	0.511	0.248	0.112	0.476	0.007		
MS	0.096	0.109	0.058	0.131	27.561	3412.21		
F-ratio	11.90	0.73	1.66	2.65	0.75	5.37		
Factor								
Site								
China Camp (1)	0.105+0.029 ab	1.183+0.223 b	0.283+0.108 a	0.036+0.128 a	15.90+1.82 a	193.14+7.27 b		
HAAF (2)	0.043±0.029 a	0.100±0.223 a	0.073±0.108 a	0.136±0.128 a	16.08±1.82 a	164.17±7.27 a		
Petaluma River (3)	0.019±0.034 a	0.103±0.223 a	0.160±0.108 a	0.153±0.128 a	16.31±1.82 a	197.04±7.98 bc		
Alviso Slough (4)	0.149±0.031 bc	0.356±0.223 a	0.330±0.108 a	0.460±0.128 a	21.51±1.74 b	201.99±7.27 bc		
Point Isabel (5)	0.223±0.029 c	0.233±0.223 a	0.363±0.108 a	0.503±0.128 a	16.21±1.82 a	216.03±7.27 c		
ANOVA								
p-value	<0.001	0.042	0.359	0.135	0.130	<0.001		
MS	0.056	0.615	0.044	0.128	68.639	4339.68		
F-ratio	6.98	4.12	1.26	2.59	1.87	6.83		

Note: Values that are followed by the same letter are not significantly different according to Fisher's least significant difference procedure.

The MeHg concentrations of the *M. nasuta* samples were not affected by length of incubation period, but the mean MeHg concentration at Alviso Slough (21.5 ng/g) was significantly greater than those at the other sites (means ranging from 15.9 to 16.3 ng/g; Table 5; Figure 8). In contrast, the MeHg concentrations of the *T. japonica* samples were significantly affected by both length of incubation period and site: they decreased with increasing incubation period by 15%, and the mean MeHg concentration increased significantly in the order of HAAF<China Camp<Petaluma River≤Alviso Slough<Point Isabel (Table 5; Figure 9).

The data set pertaining to the monitoring devices was further interpreted per site, because length of incubation time and site significantly affected the MeHg accumulation of selected monitoring devices (water-DGTs, *T. japonica*).

Regression analysis was used to evaluate tentative relationships between MeHg concentrations in selected monitoring devices (water DGTs and test clams) and length of incubation period. Trends rather than statistics on changes in MeHg concentration with sediment layer and length of incubation period were described, because insufficient data (only one value per sampling time) were available for the sediment DGTs, preventing the use of regression analysis. The MeHg concentrations of the water DGTs decreased linearly with length of incubation period, significantly at China Camp, HAAF, and Alviso Slough (Table 6, Figure 6). The MeHg concentrations of the sediment DGTs exposed to water at +1.5 cm above the sediment surface increased also with length of incubation period at two sites (China Camp, Alviso Slough), but decreased at the remaining sites (HAAF, Petaluma River, Alviso Slough; Table 6). The MeHg-time relationships of the sediment DGTs exposed to -0.5-cm sediment and those exposed to -1.5-cm sediment differed from those of the sediment-DGTs exposed to +1.5-cm. The MeHg concentrations were greatest at the -1.5-cm depth within the sediment and after 3 days incubation. In summary, the MeHg-time relationships of water DGTs differed from those in the +1.5-cm-sediment DGTs, with both being exposed to surface water but the water DGT accumulating from the whole water column and the +1.5-cm-sediment DGT accumulating from the water layer just above the sediment.

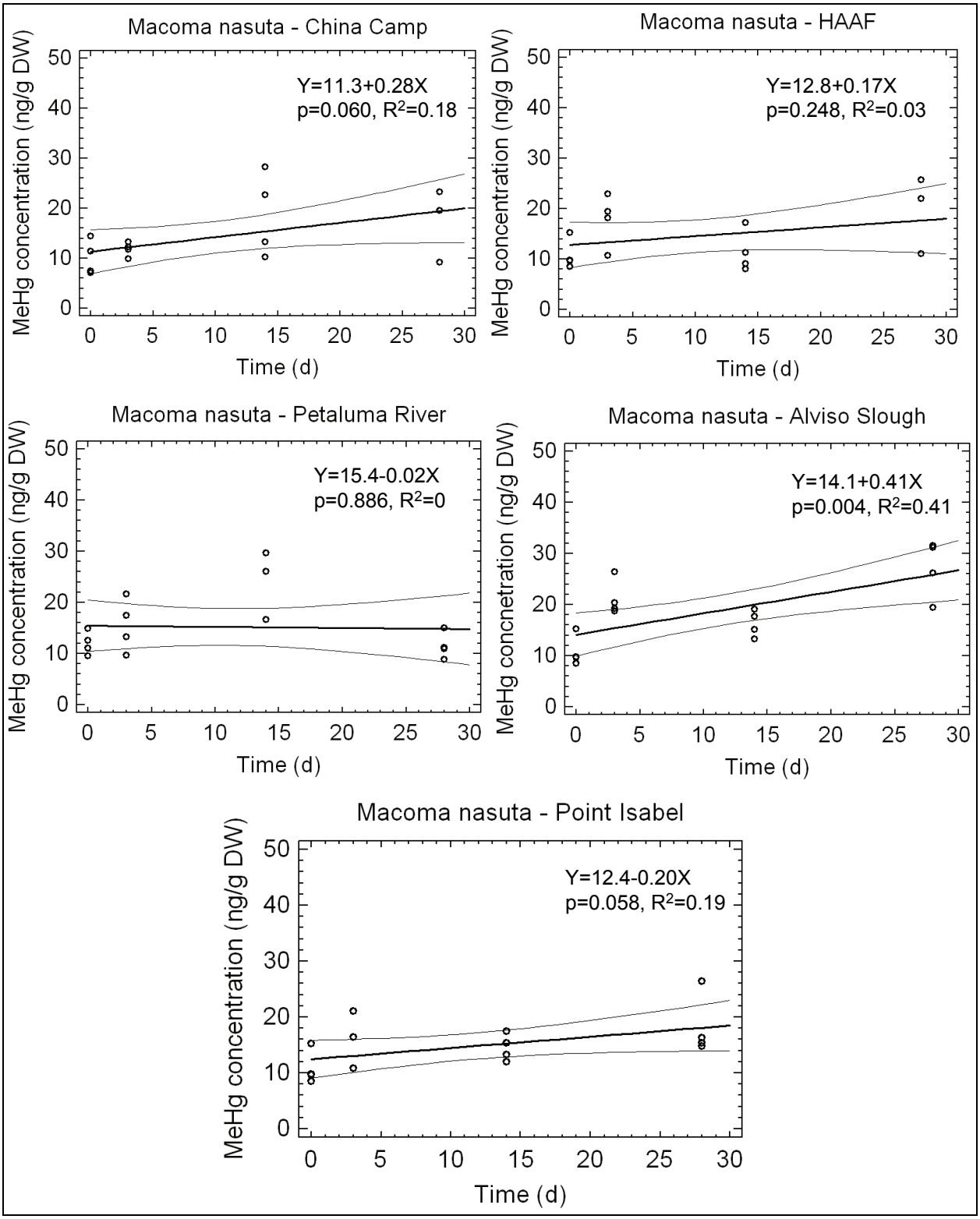


Figure 8. Relationships between MeHg concentration and length of incubation period of *Macoma nasuta* test clams exposed to field conditions. Regression lines and 95% confidence limits indicated; Y = clam response, X = incubation period.

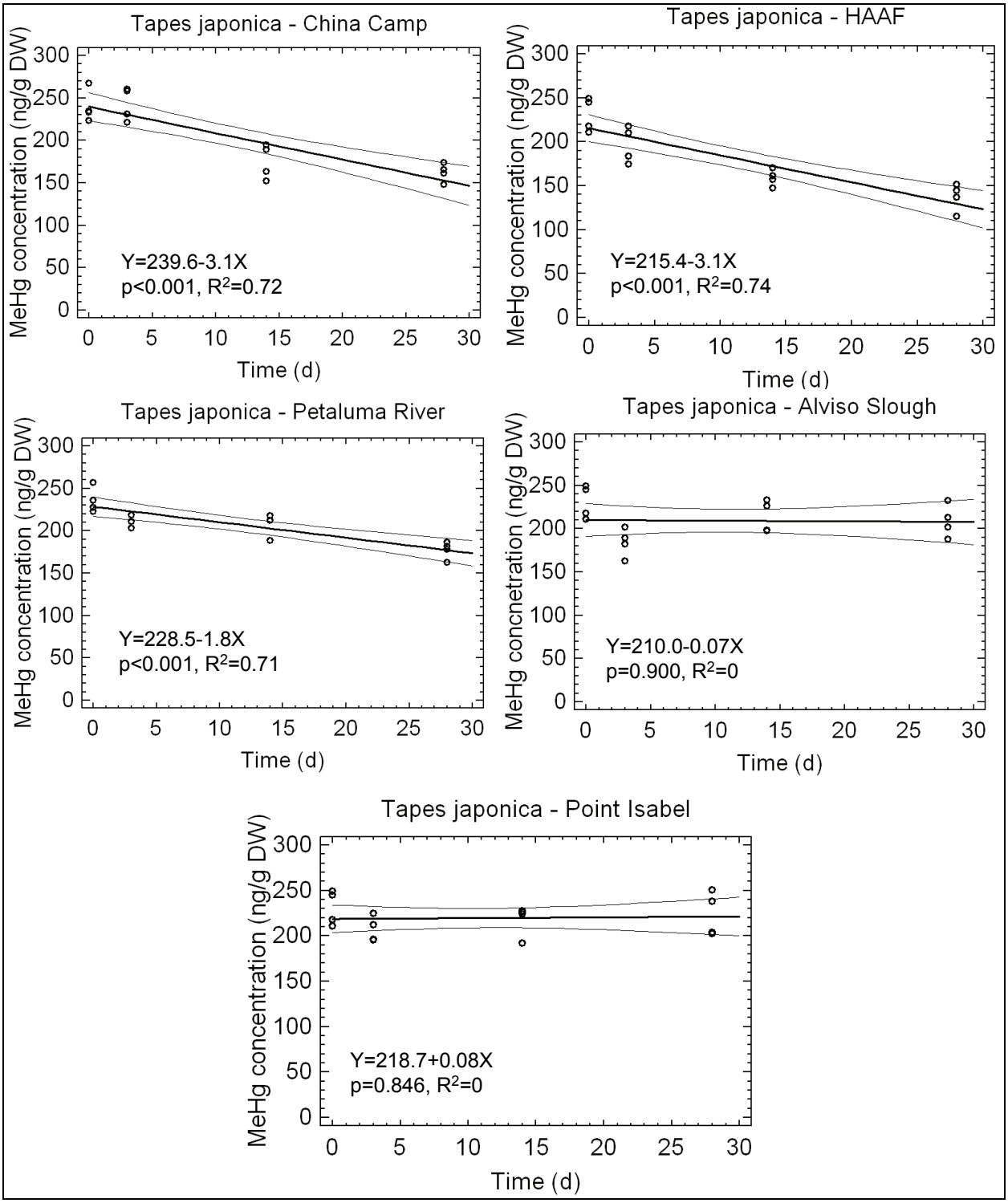


Figure 9. Relationships between MeHg concentration and length of incubation period of *Tapes japonica* test clams exposed to field conditions. Regression lines and 95% confidence limits indicated; Y = clam response, X = incubation period.

Table 6. Trends in relationships between MeHg concentrations in monitoring devices and length of incubation period.

Monitoring device	Increasing	Decreasing	No trend
Water-DGT[1]		All sites	
Sediment-DGT +1.5-cm	China Camp, Alviso Slough	HAAF, Petaluma River, Point Isabel	
Sediment-DGT -0.5-cm		HAAF, Petaluma River, Point Isabel	China Camp, Alviso Slough
Sediment-DGT -1.5-cm	China Camp	HAAF, Petaluma River, Point Isabel	Alviso Slough
M. nasuta[1]	China Camp, Alviso Slough, Point Isabel		HAAF, Petaluma River
T. japonica[1]		China Camp, HAAF, Petaluma River	Alviso Slough, Point Isabel

[1]Relationships supported by regression analysis.

This suggested that the dissolved MeHg at the sediment-water interface was consistently greater than the water column average, originating from MeHg diffusion/export from the sediment and/or MeHg loss from the water column. In addition, the MeHg-time relationships of the sediment DGTs differed between depth in the sediment and sites, all being exposed to sediment with different characteristics and net MeHg production, but with greatest MeHg levels at the -1.5-cm depth.

Regression analysis was also used to explore relationships between MeHg concentrations of the water DGTs and clams. The MeHg concentrations of water DGTs were not significantly related to those of *M. nasuta* (Table 7). This was attributed to either duration of the exposure period up to 28 days that may have been too short for *M. nasuta* to accumulate measurable amounts of MeHg, or to high variability in the data set caused by analyzing whole animals, including gut contents, that may have contained particulate materials. Therefore, it was not possible to decide if water DGTs respond to the same processes as *M. nasuta*. The MeHg concentrations of water DGTs ranked similar to site as those of *T. japonica* test clams, and, therefore, appeared to respond to the same processes. The MeHg concentrations of *T. japonica* were far greater than those of *M. nasuta* to begin with and decreased significantly with exposure time at low MeHg sites, while remaining unchanged at perceived MeHg hot spots (Table 7).

Table 7. Relationships between MeHg concentrations obtained from water DGTs (ng/g DW) and test clams established by regression analysis, Y = A + BX (Y = MeHg concentration in water-DGT; X = MeHg concentration in test clam). Statistically significant relationships at the 95% confidence level underlined.

Site	Water-DGT MeHg range	*M. nasuta*				*T. japonica*			
	(ng/L)	Statistic fitted model				Statistic fitted model			
		p-value	R^2	A	B	p-value	R^2	A	B
China Camp	0.019-0.267	0.302	0.03	16.4	-15.5	<u>0.006</u>	0.64	164.6	270.0
HAAF	0.014-0.081	0.955	0	15.9	-4.3	<u>0.029</u>	0.45	133.5	630.4
Petaluma River	0.013-0.046	0.413	0	14.0	116.2	0.065	0.42	176.3	646.1
Alviso Slough	0.063-0.326	0.869	0	20.5	3.7	<u>0.036</u>	0.46	222.8	-147.7
Point Isabel	0.073-0.310	0.684	0	17.6	-5.14	0.788	0	212.5	-10.5

DGTs were exposed to surface water, whereas clams may have been feeding largely on deposits (with deposits being low at non-hotspot sites) rather than plankton and detritus from the water column in San Francisco Bay. This clam behavior is feasible because phytoplankton production is very low in San Francisco bay (Cloern 1987; Cole and Cloern 1987; Kimmerer and Orsi 1996). Thus, water DGTs may be suitable surrogates for *T. japonica*.

Comparison of MeHg levels in DGTs and site-inhabiting clams and small fish

The MeHg concentrations in the site-inhabiting clams (*Macoma balthica*, *Mya arenari* , *Potamocorbula amurensis*, *Tapes japonica*) and small fish (*Atheropsis affinis*, *Clevelandia ios*, *Menidia audens*) are presented in Table 8.

Based on the clam feeding habits, with *M. balthica* being a facultative deposit feeder capable of suspension filter feeding and selective deposit feeding, and the other clams being suspension feeders (Table 2), it was expected that the clams would obtain portions of their diet from the water column, the water layer just above the sediment, the sediment surface, and the upper sediment layer. Therefore, surface water and sediment layers considered as relevant for the clams were +1.5 cm, -0.5 cm, and -1.5 cm.

Table 8. Concentrations of MeHg in site-inhabiting clams and fish sampled at the end of the exposure period of the DGTs and test clams. Mean values ± standard deviations (N).

	Clams				Fishes		
	M. balthica	*M. arenaria*	*P. amurensis*	*T. japonica*	*A. affinis*	*C. ios*	*M. audens*
Site	MeHg (ng/g DW)	MeHg (ng/g DW)	MeHg (ng/g DW)	MeHg (ng/g DW)	MeHg (ng/g DW)	MeHg (ng/g DW)	MeHg (ng/g DW)
China Camp	37.2±2.0 (2)	31.8 (1)			146.5±49.4 (5)		431.0±46.1 (3)
HAAF	40.2±6.5 (2)	19.7 (1)			134.0±19.0 (3)		302.6±60.2 (5)
Petaluma River	73.5±1.6 (2)	26.0 (1)	57.4 (1)				361.7±90.3 (5)
Alviso Slough					227.2±42.0 (4)	286.6±142.7 (2)	728.9±181.2 (4)
Point Isabel	66.6 (1)	257.4±10.8(1)		270.3 (1)	212.1±31.7 (4)		793.3±162.9 (4)

Based on the fish feeding habits, with *A. affinis* and *M. audens* feeding on zooplankton in the water column and *C. ios* largely on sediment-associated invertebrates, surface water and the +1.5-cm, -0.5-cm, -1.5-cm sediment layers were also considered as relevant for the fish.

The MeHg levels in the three clam species, *M. balthica*, *P. amurensis,* and *M. arenaria*, were usually less than the levels in the two fish species, *A. affinis* and *M. audens*. However, MeHg accumulated to an elevated level in *M. arenaria* and *T. japonica* at one site only, Point Isabel. The latter MeHg level of 257 to 270 ng/g DW was in the MeHg range exhibited by two fish species, *A. affinis* and *C. ios*, but a factor of 2 to 3 less than the MeHg level in *M. audens*.

One-factor ANOVA was used to evaluate the effect of site on the MeHg concentrations in the site-inhabiting clams and fish. The MeHg concentrations in clams and fish, for which enough data were available for analysis, were significantly affected by site (Table 9). The MeHg concentration was significantly elevated in all clams and fish at Point Isabel, and in fish also at Alviso Slough. It was also significantly elevated in *M. balthica* in the Petaluma River, but not in the other organisms.

Regression analysis was used to evaluate tentative relationships between mean MeHg concentrations of the water-DGTs incubated for 14 days, assuming that MeHg levels were at equilibrium at that time, and clams and fish, for which enough data were available for analysis. Regression analysis of the 3 days incubated water DGT MeHg concentrations was also

Table 9. Analysis of variance of the effects of site (site No.) on the MeHg concentrations of site-inhabiting clams and fishes. Statistically significant relationships at the 95% confidence level underlined.

Site/Time	MeHg Concentration (mean + standard error of mean)			
	Clams		Fishes	
	M. balthica (ng/g DW)	M. arenaria (ng/g DW)	A. affinis (ng/g DW)	M. audens (ng/g DW)
Factor				
Site				
China Camp (1)	37.2+2.9 a	31.8+10.6 a	146.5+17.7 a	431.0+69.1 a
HAAF (2)	40.2+2.9 a	19.7+ 10.6 a	134.0+22.8 a	302.6+53.5 a
Petaluma River (3)	73.5+2.9 b	26.0+10.6 a		361.7+53.5 a
Alviso Slough (4)			227.2+19.7 b	728.9+59.8 b
Point Isabel (5)	66.6+2.9 b	257.4+6.1 b	212.1+19.7 b	793.3+59.8 b
ANOVA				
p-value	0.007	0.004	0.014	<0.001
MS	615.76	27841.0	8336.57	214105
F-ratio	16.30	245.88	5.33	14.93

Note: Values that are followed by the same letter are not significantly different according to Fisher's least significant difference procedure.

explored, but did not generate significant results. The values of the water DGTs incubated for 14 days were selected for this analysis since these were considered most indicative of accumulation from surface water. The MeHg level of the clam *M. arenaria* increased significantly with water DGT MeHg, and the fitted linear regression equation explained 99% of the variability in the data set (Figure 10). No significant relationships between the MeHg level of the clam *M. balthica*, fish *A. affinis*, and the water-DGT MeHg were found ($p>0.05$ and R^2 close to zero; Figure 10). A weak linear relationship between the MeHg level of the fish *M. audens* and water-DGT MeHg was identified, with the fit of the regression being not significant ($p>0.05$), but explaining half of the variability in the data set (Figure 10).

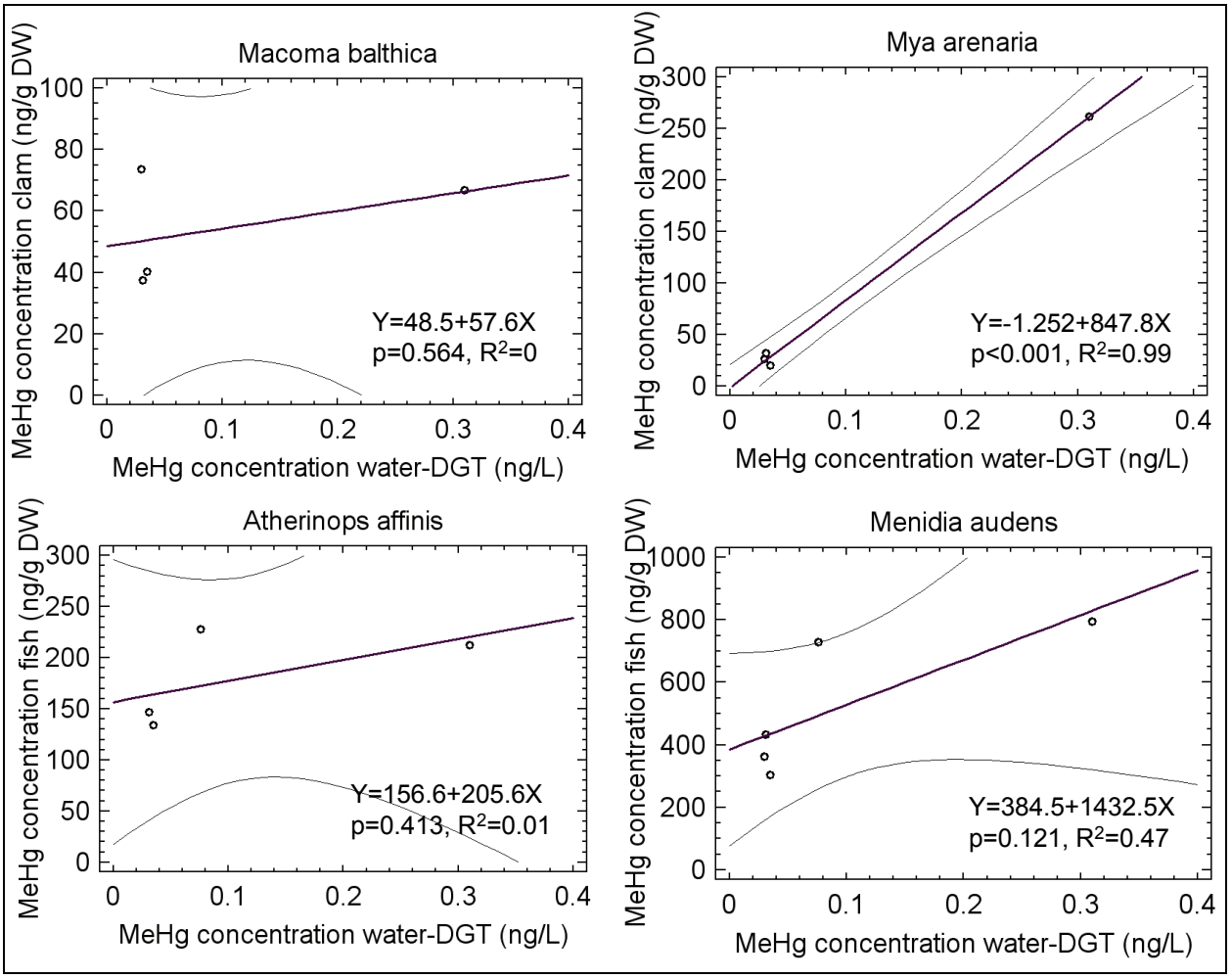


Figure 10. Relationships between mean MeHg concentrations in site-inhabiting clams and fish, and in water DGTs incubated for 14 days in the field. MeHg concentrations in species occurring only at one site were: in clams 57.4 mg/g DW in *Potamocorbula amurensis*, 270.3 ng/g DW in *Tapes japonica*, and in fish 286.6 ng/g DW in *Clevelandia ios*.

4 Conclusions

The study described herein resulted in the following conclusions:

1. DGT-labile MeHg concentrations of the water DGTs were usually less than the unfiltered water concentrations. The MeHg concentrations of the water DGTs decreased with increasing length of incubation period. It is likely that the water DGT MeHg concentrations correspond to the concentrations of the aqueous $MeHg^{+}$ ion + small inorganic MeHg complexes, and, therefore, did not encompass all MeHg present in the water column. Another possibility would be that the DGT gel became saturated at all sites except when incubated for 3 days at HAAF.

2. The MeHg-time relationships of the water DGTs differed from those of the +1.5-cm-sediment DGTs. This suggested that the dissolved MeHg concentration at the sediment-water interface was consistently greater than the water column average, originating from MeHg diffusion/export from the sediment and/or MeHg loss from the water column. In addition, the MeHg-time relationships of the sediment DGTs differed between depth in the sediment and sites, all being exposed to sediment with different characteristics and net MeHg production but with greatest MeHg levels at the -1.5-cm depth.

3. The MeHg concentrations of the water DGTs were not significantly related to those of *M. nasuta* test clams. This was attributed to either duration of the exposure period up to 28 days that may have been too short for *M. nasuta* to accumulate measurable amounts of MeHg, or variability in the data set that was too high because of analyzing whole animals, including gut contents, that may have contained particulate materials. Therefore, it was not possible to decide if water DGTs respond to the same processes as *M. nasuta*.

4. The MeHg concentrations of the water DGTs ranked similar to site as those of *T. japonica* test clams and, therefore, appeared to respond to the same processes. The MeHg concentrations of *T. japonica* were far greater than those of *M. nasuta* to begin with and decreased significantly with exposure time at low MeHg sites, while remaining unchanged at perceived MeHg hot spots. Thus, water DGTs may be suitable surrogates for *T. japonica*.

5. The MeHg concentrations of the 14-day incubated water DGTs were significantly related to those of the site-inhabiting clam *Mya arenaria* and weakly related to those of the site-inhabiting fish *M. audens*, and, therefore, water DGTs appeared to respond to the same processes as these organisms.

References

Abraham, B. J., and P. L. Dillon. 1986. Species profiles: Life histories and environmental requirements of coastal fishes and invertebrates (Mid-Atlantic) – softshell clam. Biological Report 82 (11.68). Washington, DC: U.S. Fish and Wildlife Service. Technical Report EL-82-4. Vicksburg, MS: U.S. Army Corps of Engineers.

Best, E. P. H., H. L. Fredrickson, V. A. McFarland, H. Hintelmann, R. P. Jones, C. H. Lutz, G. A. Kiker, A. J. Bednar, R. N. Millward, R. A. Price, G. R. Lotufo, and G. L. Ray. 2005. *Pre-construction biogeochemical analysis of mercury in wetlands bordering the Hamilton Army Airfield Wetlands Restoration Site.* ERDC/EL TR-05-15. Vicksburg, MS: U.S. Army Engineer Research and Development Center.

Best, E. P. H., H. L. Fredrickson, H. Hintelmann, O. Clarisse, B. Dimock, C. H. Lutz, G. R. Lotufo, R. N. Millward, A. J. Bednar, and J. S. Furey. 2007. *Pre-construction biogeochemical analysis of mercury in wetlands bordering the Hamilton Army Airfield (HAAF) wetlands restoration site. Part 2.* ERDC/EL TR-07-21. Vicksburg, MS: U.S. Army Engineer Research and Development Center.

Best, E. P. H., H. Hintelmann, O. Clarisse, B. Dimock, G. R. Lotufo, and J. S. Furey. 2009. Pre-construction biogeochemical analysis of mercury in wetlands bordering the Hamilton Army Airfield (HAAF) wetlands restoration site. Part 3. ER/EL TR-09-21. Vicksburg, MS: U.S. Army Engineer Research and Development Center.

Black, L. F. 1980. The biodepositional cycle of a surface deposit-feeding bivalve, *Macoma balthica* (L.).In *Estuarine Perspectives*, ed. V.S. Kennedy, 389-402. New York: Academic Press.

Clarisse, O., and H. Hintelmann. 2006. Measurements of dissolved methylmercury in natural waters using diffusive gradients in thin film. *Journal of Environmental Monitoring* 8: 1242-1247.

Cloern, J. E. 1987. Turbidity as a control on phytoplankton biomass and productivity in estuaries. *Continental Shelf Research* 7: 1367-1381.

Cohen, A. N., and J. T. Carlton. 1995. Nonindigenous aquatic species in a United States Estuary: A case study of the biological invasions of the San Francisco Bay and Delta. Washington, DC: U.S. Fish and Wildlife Service, 65-66.

Cole, B. E., and J. E. Cloern. 1987. An empirical model for estimating phytoplankton productivity in estuaries. *Marine Ecology Progress Series* 36: 299-305.

Davison, W., and H. Zhang. 1994. In situ speciation measurements of trace components in natural waters using thins film gels. *Nature* 367:546-548.

Divis, P., M. Leermakers, H. Docekalova, and Y. Gao. 2005. Mercury depth profiles in river and marine sediments measured by the diffusive gradients in thin films technique with two different specific resins. *Analytical and Bioanalytical Chemistry* 382: 1715-1719.

Emmett, R. L., S. L. Stone, S. A. Hinton, and M. E. Monaco. 1991. *Distribution and abundance of fishes and invertebrates in west coast estuaries. Volume II: species life history summaries*. ELMR Report No 8. Rockville, MD: National Oceanic and Atmospheric Administration/National Ocean Service, 44-48.

Fish Base. 2009. http://www.fishbase.org; Date of access: 22 July 2009.

Fitch, J. E., and R. J. Lavenberg. 1975. *Tidepool and nearshore fishes of California*. California Natural History Guides: 38. Berkeley and Los Angeles, CA: University of California Press.

Goals Project. 2000. Baylands Ecosystem Species and Community Profiles: Life histories and environmental requirements of key plants, fish and wildlife. Prepared by the San Francisco Bay Area Wetlands Ecosystem Goals Project. P. R. Olofson (ed.). San Francisco Bay Regional Water Quality Control Board, Oakland, CA: 136-141.

Greenfield, B. K., A. Jahn, J. L. Grenier, S. Shonkoff, and M. Sandheinrich. 2006. *Mercury in biosentinel fish in San Francisco Bay: First-year project report*. San Francisco Estuary Institute, Oakland, CA.

Harper, M., W. Davison, H. Zhang, and W. Tych. 1998. Solid phase to solution kinetics in sediments and soils interpreted from DGT measured fluxes. *Geochimica and Cosmochimica Acta* 62: 2757-2770.

Hieb, K. A. 2000. Arrow goby. *Clevelandia ios*. In: *Goals project. Baylands Ecosystem Species and Community Profiles. Life histories and environmental requirements of key plants, fish and wildlife*. Prepared by the San Francisco Bay Area Wetlands Ecosystem Goals Project. ed. P. R. Olofson, 136-138. Oakland, CA: San Francisco Bay Regional Water Quality Control Board.

Hintelmann, H., and H. T. Nguyen. 2005. Extraction of methylmercury from tissue and plant samples using acid leaching. *Analytical and Bioanalytical Chemistry* 381: 360-365.

Hylleberg, J., and V. F. Gallucci. 1975. Selectivity in feeding by the deposit-feeding bivalve *Macoma nasuta*. *Journal of Marine Biology* 32: 167-178.

Kimmerer, W. J., and J. J. Orsi. 1996. Changes in the zooplankton of the San Francisco Bay estuary since the introduction of the clam *Potamocorbula amurensis*. In *San Francisco Bay: The Ecosystem,* ed. J. T. Hollibaugh, 403-425. San Francisco, CA: American Association for the Advancement of Science.

Maginnis, T. L. 2006. The costs of autonomy and regeneration in animals: A review and framework for future research. Review. *Behavioral Ecology* 17: 857-872.

Matthews, W. J., F. P. Gelwick, and J. J. Hoover. 1992. Food of and habitat use by juveniles of species of *Micropterus* and *Morone* in a southwestern reservoir. *Transactions of the American Fisheries Society* 121: 54-66.

Moyle, P. B. 2002. *Inland Fishes of California*. Berkley, CA: University of California.

Morris, R. H., D. P. Abbott, and E. C. Haderlie. 1980. *Intertidal Invertebrates of California*. Stanford, CA: Stanford University Press: 375.

National Introduced Marine Pest Information System (NIMPIS). 2002. *Potamocorbula amurensis* species summary. C. L. Hewitt, R. B. Martin, C. Sliwa, F. R. McEnnulty, N. E. Murphy, T. Jones, and S. Cooper, editors. Web publication http://crimp.marine.csiro.au/nimpis, Date of access: 06/12/2008.

Newell, C. R., and H. Hidu. 1986. *Species profiles: Life histories and environmental requirements of coastal fishes and invertebrates (North Atlantic) Softshell Clam*. Biological Report 82(11.53). Washington DC: U.S. Fish and Wildlife Service.

Nichols, F. H., and J. K. Thompson. 1982. Seasonal growth of the bivalve *Macoma balthica* near the southern limit of its range. *Estuaries* 5: 110-120.

Olafsson, E. B. 1986. Density dependence in suspension-feeding and deposit-feeding populations of the bivalve *Macoma balthica*: a field experiment. *Journal of Animal Ecology* 55: 517-526.

Page, L. M., and B. M. Burr. 1991. *A field guide to freshwater fishes of North America north of Mexico*. The Peterson Field Guide Series, Volume 42. Boston, MA: Houghton Mifflin.

Peterson, H.1997. Clam stuffed sturgeon. *IEP (Interagency Ecological Program for the San Francisco Estuary) Newsletter* 9: 19.

Poulton, V. K., J. R. Lovvorn, and J. Y. Takekawa. 2002. Clam density and scaup feeding behavior in San Pablo Bay, California. *The Condor* 104: 518-527.

Richman, S. E., and J. R. Lovvorn. 2004. Relative foraging value to lesser scaup ducks of native and exotic clams from San Francisco Bay. *Ecological Applications* 14: 1217-1231.

Robins, C. R., and G. C. Ray. 1986. *A field guide to Atlantic coast fishes of North America*. Boston, MA: Houghton Mifflin.

Ross, S. T. 2002. *The Inland Fishes of Mississippi*. Jackson, MS: University Press of Mississippi.

Slotton, D. G., S. M. Ayers, T. H. Suchanek, R. D. Weyland, and A. M. Liston. 2004. Mercury bioaccumulation and trophic transfer in the Cache Creek watershed of California., in relation to diverse aqueous exposure conditions. Final Report to CALFED Bay Delta Program Project, Component 5B of Assessment of Ecological and Human Health Impacts of Mercury in the San Francisco Bay-Delta Watershed. Sacramento, California, 25 January 2004. http://loer.tamug.edu/calfed/Report/Final/UCDavis_Cache_Bio_Final.pdf.

Stearns, R. E. C. 1881. *Mya arenaria* in San Francisco Bay. *American Naturalist* 15: 142-146.

Suttkus, R. B., B. A. Thompson, and J. K. Blackburn. 2005. An analysis of the *Menidia* complex in the Mississippi River Valley and in two nearby minor drainages. *Southwest Fishes Council Proceedings* 48: 1-9.

Svensson, O., and C. Karnemo. 2007. Parasitic spawning in sand gobies: An experimental assessment of nest-opening size, sneaker male cues, paternity, and filial cannibalism. *Behavioral Ecology* 18: 410-419.

Svensson, O., C. Magnhagen, E. Forsgren, and C. Karnemo. 1998. Parental behaviour in relation to the occurrence of sneaking in the common goby. *Animal Behaviour* 56: 175-179.

Visintainer, T. A., S. M. Bollens, and C. Simenstad. 2006. Community composition and diet of fishes as a function of tidal channel geomorphology. *Marine Ecology Progress Series* 321: 227-243.

Waugh, J. 1960. The ecology and mode of life of the edible mollusk. *Journal of Molluscan Studies* 34: 113-122.

Zhang, H., and W. Davison. 1995. Performance characteristics of diffusion gradients in thin films for the in situ measurement of trace metals in aqueous solution. *Analytical Chem*istry 67: 3391-3400.

Zhang, H., W. Davison, S. Miller, and W. Tych. 1995. In situ high resolution measurements of fluxes of Ni, Cu, Fe, and Mn and concentrations of Zn and Cd in porewaters by DGT. *Geochimicha Cosmochimica Acta* 59: 4181-4192.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
December 2009	Final report	

4. TITLE AND SUBTITLE

Comparison of DGT Sentinels and Bioassays for Long-term Mercury TMDL Monitoring under San Francisco Bay Field Conditions

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

E. P. H. Best, H. Hintelmann, O. Clarisse, J. S. Furey, B. Greenfield and B. Dimock

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

See reverse.

8. PERFORMING ORGANIZATION REPORT NUMBER

ERDC/EL TR-09-22

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Engineer District, San Francisco
1455 Market Street
San Francisco, CA 94103

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution is unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Compliance with the mercury total mean daily load (TMDL) for San Francisco Bay adopted in 2004 requires long-term monitoring of mercury loadings from a range of sources, including extant and restored wetlands, into the Bay. This study explores the use of DGTs for long-term monitoring of MeHg in the field, by determining the performance of DGTs as indicators for potential MeHg accumulation at relevant field sites over a range of time periods (up to 28 days); comparing/ correlating the DGT results with MeHg bioaccumulation over the same time periods in originally 'clean' bioassay organisms (two clam species); and comparing/correlating the DGT results with the MeHg levels in site-inhabiting clams and small fish.

The following conclusions can be reached based on study results:

- DGT-labile MeHg concentrations of the water-DGTs were usually less than the unfiltered water concentrations.
- The MeHg-time relationships of the water-DGTs differed from those of the +1.5-cm-sediment-DGTs.
- The MeHg concentrations of the water-DGTs were not significantly related to those of *M. nasuta* test clams.
- The MeHg concentrations of the water DGTs ranked similar to site as those of *T. japonica* test clams and, therefore, appeared to respond to the same processes.
- The MeHg concentrations of the 14-day incubated water-DGTs were significantly related to those of the site-inhabiting clam *Mya arenaria* and weakly related to those of the site-inhabiting fish *M. audens*, and, therefore, water-DGTs appeared to re-spond to the same processes as these organisms.

15. SUBJECT TERMS

Clams
Coastal wetlands
DGT
Fish
Management
Methylmercury
Monitoring
San Francisco Bay
Sediment

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED		46	

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) (Concluded)

U.S. Army Engineer Research and Development Center
Environmental Laboratory
3909 Halls Ferry Road
Vicksburg, MS 39180-6199;
Trent University
1600 West Bank Drive
Peterborough, ON K9J 7B8, Canada;
San Francisco Estuary Institute
7770 Pardee Lane
Oakland, CA 94621-1424