HEAVY METAL ASSESSMENT OF SEDIMENTS AND SELECTED BIOTA FROM AMERICAN MEMORIAL PARK NEARSHORE WATERS, SAIPAN, COMMONWEALTH OF THE NORTHERN MARIANA ISLANDS (CNMI)

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(*Photo credit: U.S. National Park Service*).

Plate 1: One of several public walking pathways that meander through the beautifully landscaped American Memorial Park in Saipan

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ABSTRACT

The American Memorial Park (AMME) is located on the western side of central Saipan and occupies an area of 133 acres (54 ha). It was established in 1978 to commemorate those who sacrificed their lives during the Marianas Campaign of WWII and has been under the administrative control of the U.S. National Park Service since 1979. The seaward boundary of AMME extends along the shoreline of Saipan Lagoon and provides easy access to the ocean for park visitors. The nearshore waters in this region are popularly used for fishing, water recreation, and aesthetic enjoyment by tourists and local residents alike.

The land upon which the park was built was occupied by the U.S. Navy immediately after WWII and served primarily as a vehicle pool with onsite maintenance, repair and refueling facilities (Ogden 1998). Allotments were also set aside on the property for garbage disposal and the stockpiling, detonation and burial of residual munitions (AMPRO 2005). These previous land-use practices have left behind a significant heavy metal footprint throughout the park. The NE boundary of AMME abuts a municipal dump that dates back to the end of WWII. This facility was the island's primary solid waste disposal site until it was closed in 2003. Previous marine monitoring and assessment studies near the dump have identified significant heavy metal enrichment in sediments and dominant ecological representatives (Denton *et al*. 2001, 2006a, 2008, 2009, 2010). The study reported herein continued this research emphasis along the AMME shoreline, and was seen as a logical extension of these earlier investigations.

Shoreline sediments were collected from 37 sites along the entire seaward boundary of AMME. Biological samples were also taken from 11 sites along this stretch of coastline. Emphasis was placed on marine plants with known or suspected biomonitoring capability, and shellfish traditionally harvested for food. The final selection of biota consisted of one species of seaweed, two species of seagrass, and four species of bivalve mollusk. All samples were analyzed for eight heavy metals, viz., cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), nickel (Ni), silver (Ag) and zinc (Zn), and the metalloid, selenium (Se). These elements were selected based on their wide range of industrial uses, their toxicity, and their ubiquity as environmental contaminants.

The analytical findings were weighed against previously reported metal values in sediments and marine organisms from elsewhere. It was concluded that the heavy metal status of the AMME shoreline was generally low by world standards; however localized areas of metal enrichment were identified near obvious sources of contamination. Shoreline sediments in the central region of the property, for example, were classified as significantly enriched with Cu, Pb, Hg, and Zn in the *Smiling Cove Marina* area and at various locations east of the marina causeway towards the dump. High Hg enrichment was revealed in sediments down gradient of a stormdrain that serviced the AMME natural forest protected area and at an adjacent site where an old military field hospital once stood. Interestingly, levels of all detectable metals in surface deposits near the dump were substantially lower than values reported back in the 1980s and 1990s (DEQ 1987, Denton *et al.* 2001) and none exceeded existing sediment quality guidelines. This apparent attenuation was attributed to sediment accretion processes involving cleaner materials since the dump closed its gates in 2003.

Biotic representatives generally mirrored metal profiles in sediments throughout the study area, although some organisms were clearly more effective biomonitors than others, at least for some elements. The bivalve, *Quidnipagus palatum*, for example, demonstrated an extraordinary capacity to accumulate Cu and Zn and was a sensitive biomonitor for both elements in addition to Cr, Hg and Pb. All of these metals were accumulated to levels well above baseline in specimens collected along the muddy shoreline east of the causeway. The seagrass, *Enhalus acoroides*, also proved to be a highly sensitive indicator of Cu and clearly identified Cu amplification in the *Smiling Cove Marina* area. Other pertinent biomonitoring issues are discussed throughout the text; additional information on candidate species prerequisites and sampling considerations are provided in Appendix D.

The Se data reported here are the first of their kind reported for Saipan coastal waters and are of considerable interest given the toxicological significance of this element in ameliorating Hg toxicity. Levels of both elements are generally higher in seafood than most other foods. Selenium concentrations recorded in AMME sediments and biota were generally low compared with reported values in similar matrices from elsewhere. Levels in AMME bivalves exceeded those in sediments and plants and were consistently in molar excess of Hg. The dietary protective effect of Se on Hg toxicity is widely believed to occur providing Se:Hg molar ratios in consumed foods are greater than 1. Average ratios determined in bivalves during the present study ranged from 16-118 and were lowest in *Q. palatum* east of the causeway. The relatively low Se:Hg ratios in *Q. palatum* from this location were attributed to Hg enrichment rather than diminished Se levels.

Human health risks associated with AMME bivalve consumptions were determined by comparing elemental levels in each species with international food standards. Exceedances of permissible limits were noted only for Pb and Cu and only in *Q. palatum* east of the causeway. Tolerable consumption limits for each species were computed using USEPA reference dose (Rfd) values and provisional tolerable daily intake (PTDI) benchmarks established by the FAO/WHO Joint Expert Committee on Food Additives (JECFA). By this means, Hg and Pb were identified as the main elements of toxicological concern, limiting safe consumption of *Q. palatum* to no more than 63 g (flesh weight) per person per day for Hg, and 89 g per person per day for Pb. Bivalve consumption rates in Saipan are currently unknown, although top consumer countries of the world rarely exceed 70 g per person per day. All other metals in *Q. palatum* occurred at levels well below those necessary to exceed tolerable daily intake limits at realistic consumption rates.

The principal findings of the aforementioned study are brought together in the final chapter of this report, together with more expansive notes on toxicological issues of importance from an ecological and human health standpoint. Thoughts on future research directives are also provided.

INTRODUCTION

The American Memorial Park (AMME) in Saipan is a 133-acre (54 ha) parcel of land that borders the central region of a large lagoon on the western side of the island (Fig. 1). It was constructed in 1978 under the administrative control of the US National Park Service (NPS) to commemorate the sacrifices of US soldiers and local residents killed during World War II in the Saipan invasion. Located in the village of Garapan, the land upon which the park now sits was occupied by the US Navy immediately after WWII, and aerial photographs taken in 1948 reveal military buildings scattered over much of the property. The area served primarily as a motor pool and maintenance and repair facility as well as a refueling station for military and civilian personnel (Ogden 1998). Allotments were also set aside for the stockpiling and disposal of residual munitions and other hazardous materials (AMPRO 2005). The indiscriminate dumping of garbage on the property was commonplace and continued until well into the 1970s (Raulerson and Rinehart 1989).

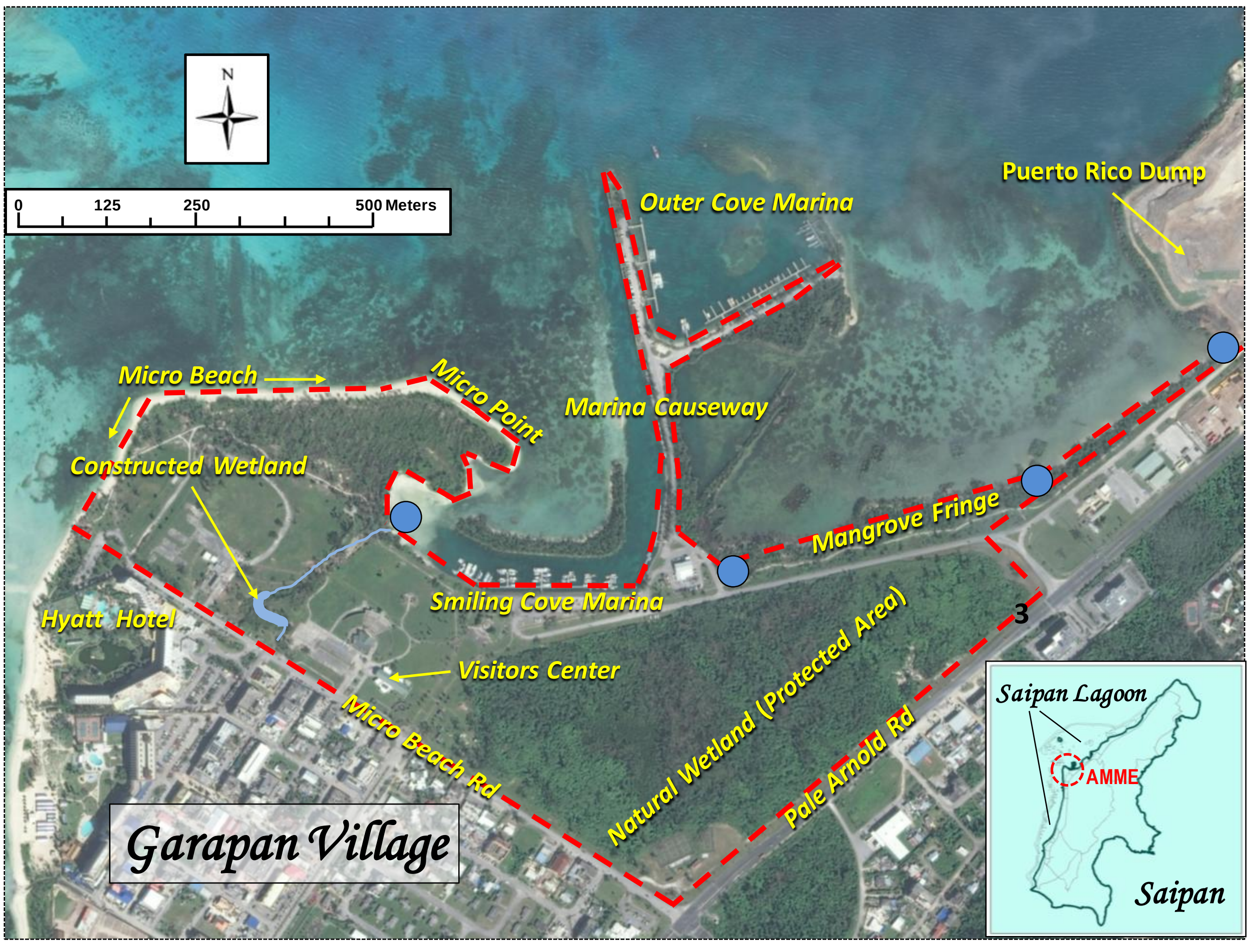

Figure 1: Image of American Memorial Park (AMME) project location and adjacent areas with inset of Saipan Island. Dashed red line on main picture indicates AMME boundary. Blue circles are stormwater discharge points that drain runoff and seepage from adjacent lands into the ocean.

Unexploded ordnance (UXO) from WWII are scattered all over Saipan and continue to contribute to the pollution load entering the coastal belt. Mercury fulminate was the primary explosive in primers and detonators of artillery shells and percussion caps of bullets manufactured back then (US Navy 1947). Mercury switches were also commonplace in certain types of rockets and projectiles used at the time (US Navy 1946). Given the sheer numbers of UXO remaining on Saipan, even modest releases of mercury from such devices could potentially have far reaching effects on environmental and human health over time. Elevated mercury levels have certainly been identified in soils and sediments from ordnance detonation sites around the island (Denton *et al* 2014, 2016), and frequent detections of this element have also been recorded in stormwater discharges entering the southern half of Saipan Lagoon – an area that was heavily bombarded during WWII (Environet Inc. 2007).

Efforts to remediate lands contaminated by past military activities on Saipan are ongoing and fall under the *Department of Defense Formerly Used Defense Site*s (FUDS) cleanup program. Of the twenty-one FUDS slated for restoration by the Army Core of Engineers in 2012 (USACE 2012), nine remain to be dealt with (Eugenio 2014). The AMME site is one of them and for good reason. In 2004, a 'hot spot' of unexploded ordnance was unearthed on the western side of the property during construction of the visitor center parking lot. Hundreds of high explosive projectiles were recovered from the site, and a magnetometer sweep suggested several hundred more projectiles, or parts thereof, lay buried outside the construction area (AMPRO 2005).

The Water and Environmental Research Institute (WERI) at the University of Guam examined four soil samples from the eastern side of AMME and detected mercury, lead, cadmium, copper and zinc concentrations that exceeded ecological soil screening levels developed by the USEPA (2005). These benchmarks are conservatively protective of ecological receptors that either commonly come into contact with soil or ingest other biotic representatives that live in or on the soil (Denton and Gawel 2012 unpublished data). Concentrations of all five elements were also appreciably higher than those recently reported for uncontaminated soils on Saipan (Denton *et al.* 2016). Whether or not these metals are reaching the coastal zone and entering the food web, including species harvested by humans for food, remains to be determined and is the subject of the current study.

In accordance with the National Park Service Task Agreement (Appendix 1) and subsequent modification (Appendix 2), surface sediment samples and representative biota were collected from a number of sites along the entire shoreline perimeter of AMME then analyzed for a suite of heavy metals commonly associated with past and present land-use activities in the park and adjacent areas. The primary elements of interest to NPS are lead, mercury and selenium, while those of secondary interest, added later by WERI, are cadmium, copper, chromium, nickel, silver and zinc. All nine elements have a diversity of uses in industry and, as a consequence, are common global pollutants found in all environmental compartments. While the majority of these elements (Cu, Zn, Se, Cr, Ni) are essential micronutrients or are used therapeutically (Ag), several (Cd, Hg, Pb) have no known biological function and rank among the most toxic heavy metals known to man.

The primary goal of the study was to determine if any of the above elements are being mobilized from AMME into the coastal belt and accumulating in various components at concentrations that approach or exceed sediment quality guidelines and human health criteria. To achieve this goal, two objectives were pursued. The first was to determine the spatial distribution of heavy metals in surface sediments and biota (seaweed, seagrass and bivalve mollusks) along the AMME shoreline. Using a comparative assessment of both biotic and abiotic samples, the second objective was to differentiate between total and biologically available heavy metal concentrations, and note any abnormal levels in edible species that present possible human health concerns.

Table 1: Past and Present Industrial Uses of the Heavy Metals Examined[a]

Metal (Chemical Symbol)	Uses of Metals and Metal Compounds[b]
Cadmium (Cd):	Electroplating (anticorrosion coatings); thermoplastic stabilizers, e.g. in PVC; Ni-Cd batteries; alloys; solders; catalysts; engraving; semi-conductors; TV tube phosphors; pigments in paints and plastics; glass ceramics; biocides.
Chromium (Cr):	Metallurgy—ferrochromium alloys; refractory bricks; electroplating; industrial dyes; ink; tanning; paint; wood preservative; glass making; cement production.
Copper (Cu):	Electrical industry; alloys; e.g. brass; chemical catalyst; anti-fouling paint; algaecide; wood preservative.
Lead (Pb):	Storage batteries; leaded gasoline; pigments; red lead paint; ammunition; solder; cable covering; anti-fouling paint; glazing; PVC stabilizers.
Mercury (Hg):	Chlorine production; electrical apparatus; anti-mildew paint; instruments; catalyst e.g. for PVC and acetaldehyde production; pesticides; preservatives; pharmaceuticals; dentistry; anti-fouling paint.
Nickel (Ni):	Metallurgy—steel and other alloys; electroplating; catalyst; rechargeable Ni-Cd batteries.
Selenium (Se)	Glass industry; electrical, e.g. rectifiers, photocells, solar cells; metallurgy, e.g. degassifier, improves machinability of steel and copper alloys (e.g. lead replacement); dietary supplements; paints; rubber; insecticide (sodium selenite); shampoo (selenium sulfide).
Silver (Ag)	Photography; electric conductors; sterling ware; solders; coinage; electroplating; catalyst; batteries; food and beverage processing.
Zinc (Zn)	Zinc based alloys; brass and bronze; galvanizing; rolled zinc; paints; batteries; rubber; sacrificial anodes on marine water-craft.

[a] from Bryan (1976), Förstner and Wittmann (1983), Moore (1991), Bryan and Langston (1992)
[b] importance generally decreasing from left to right.

MATERIALS AND METHODS

General Description of Study Area

All samples analyzed during the present study were taken from multiple sites along the entire seaward boundary of AMME. The western limit of the boundary aligns with Micro Beach Road approximately 125 m NE of the Hyatt Regency Hotel property line. The eastern limit abuts the SE corner of the now closed Puerto Rico Dump. The shoreline distance of the boundary is a little over 2 km. It is bisected by a narrow causeway that extends perpendicular to the general coastline between *Smiling Cove Marina* and *Outer Cove Marina* (Fig 1.).

Sediments taken throughout the study area were composed largely of bioclastic carbonates derived from degraded corals, coralline algae, mollusk shells and foraminifera. Inter-site grain size disparities were often appreciable reflecting energetic and circulatory differences between the water masses bathing these shores. Such disparities were especially noticeable between samples collected from either side of the causeway. The foreshore to the east of the marina causeway, for example, is essentially a mudflat backed by a narrow strip of mangrove and strand forest. In sharp contrast, intertidal sediments to the west range from medium coarse, muddy sand along the inner *Micro Point* area next to the marina to cleaner deposits NW of the point towards *Micro Beach*. Subtidal deposits in this general area are dominated by poorly sorted muddy sand, shell gravel and calcareous algal remnants. Beyond this region, shoreline sediments and subtidal deposits give way to cleaner, coarser material that gradually transitions to fine, white coral sand within 100 m of the Hyatt boundary.

There are four freshwater sources that discharge into the coastal belt within the bounds of the AMME shoreline. The first of these sources is an artificial wetland that was constructed in the park in 1999 as part of a flood mitigation plan for northern Garapan. The wetland is essentially a crescent-shaped pond that receives runoff from the Garapan commercial center and discharges it into the ocean beside *Smiling Cove Marina* (Fig. 1). The second source is the AMME natural wetland on the eastern side of the property. The wetland is a 30-acre (12 ha) triangular mosaic of secondary forest interspersed with emergent wetland. It provides critical habitat for many avian species and other indigenous wildlife and is now a designated protected area (Williams 2007). Drainage from the natural wetland exits the property through a stormdrain near the eastern wall of the causeway (Fig 1). The third freshwater source is runoff from a small industrial complex on the landward side of a narrow road that runs parallel to the shoreline. The runoff is channeled into the ocean via two storm drains, the more easterly one of which abuts the SE corner of the Puerto Rico Dump (Fig. 1). The dump is an unlined facility that occupies an area of approximately 20 acres (8 ha). It was started by the military shortly after WWII and served as the island's primary solid waste disposal site for over 50 years before closing in February 2003. Stormwater runoff and seepage from this facility constitute the fourth freshwater source that discharges into the study area and it has long contaminated the adjacent shoreline with a variety of organic and inorganic wastes (DEQ 1987, Ogden 1994, Denton 2001, 2006a, 2009). Federal funds for capping the dump were secured by the Saipan government in June 2015 (Chan 2015). The remediation process was well underway by the time the current study had finished and is expected to greatly reduce the adverse impacts of the dump on surrounding ocean ecosystems (Deposa 2014).

Sample Collection and Preparation

Surface sediments (top 5 cm) were collected from 37 shoreline sites along the seaward perimeter of AMME in March 2016. Western sites between the *Hyatt Regency Hotel* and *Smiling Cove Marina* were located 50 m apart with the exception of site 26 (see Fig. 2 legend). Sites to the east of the causeway were set at 100-m intervals along the shoreline. The distance between sites was measured with a standard surveyor's tape. Three separate sediment samples (~100 g each) were taken within a 3-m diameter circle at each site and pooled for single analysis. Each replicate was scooped up in a hand-held, pre-cleaned, plastic vial (70 ml). In the laboratory, the samples were oven dried in their vials to constant weight at ~30^{o}C and disaggregated in clean Ziploc bags between finger and thumb. Site replicates were then combined and dry sieved through a 1-mm Teflon screen in preparation for analysis. Sediment fractions >1 mm were discarded.

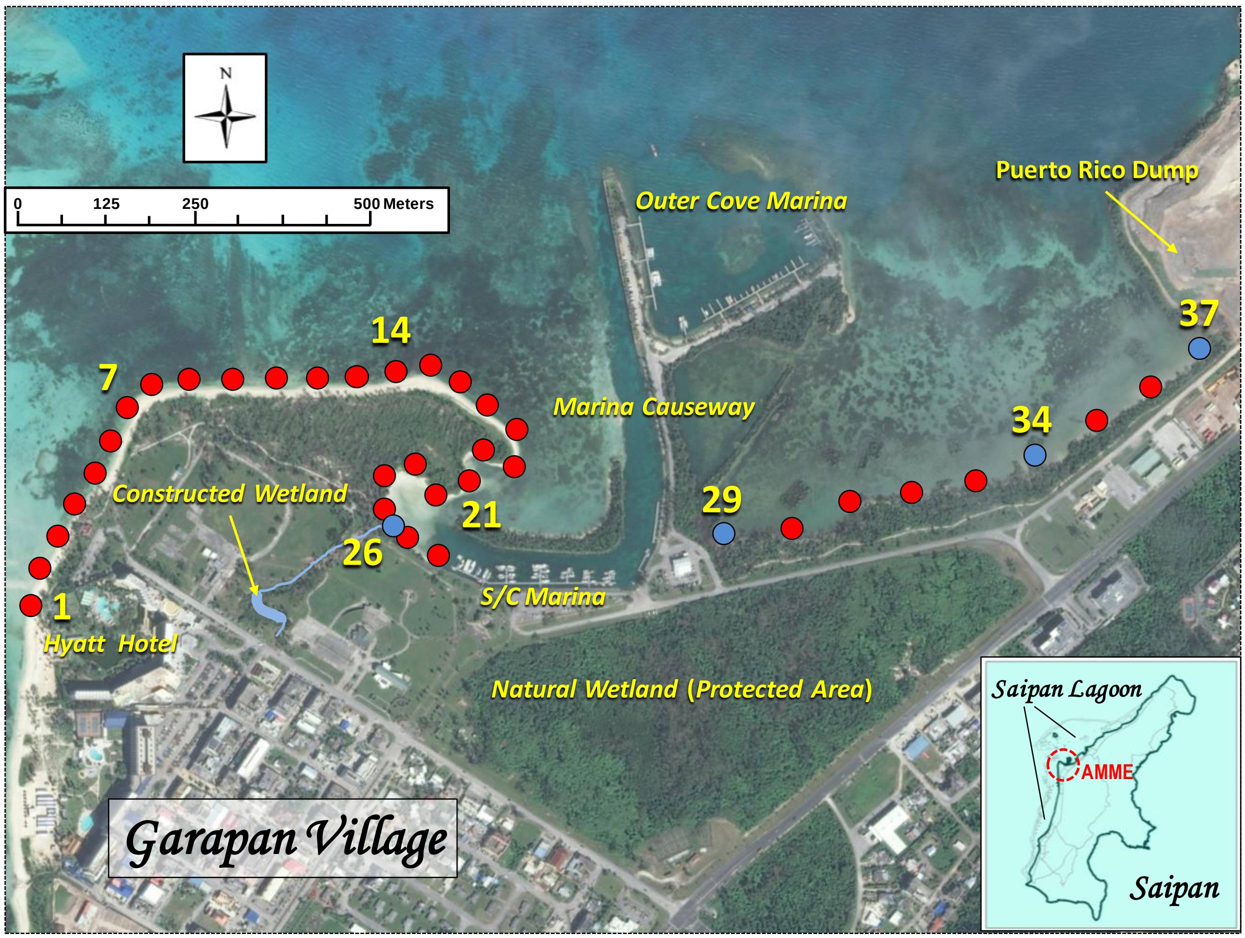

Figure 2: Sediment sampling sites (red and blue circles) along seaward perimeter of AMME. Sites west of causeway were set 50 m apart except for site 26 (blue circle) at the mouth of the small stream that drains the constructed wetland. Sites 29-37 east of causeway were set 100 m apart. Sites 29, 34 and 37 (blue circles) were located down gradient of stormdrains.

Biotic samples were collected from 11 sites along the AMME boundary from June-August, 2016 (Fig. 3; see Appendix 3 for site coordinates). Species selected for study included those traditionally harvested for food as well as those with established or potential biomonitoring capability. Representatives of each were collected from either intertidal or subtidal locations along the shoreline depending upon their availability. All subtidal collections were made within 10 m of mean low tide. Table 2 lists the species that were collected and their respective collection sites (Fig. 3). As shown, not all species of interest were available at all collection sites. Of note, the beach and adjacent subtidal zone approximately 150 m either side of the Hyatt property line were completely devoid of all visible macroflora and fauna.

All algal specimens were hand-plucked from their anchorage points and vigorously shaken in clean seawater to remove adhering particulates. Holdfasts and epiphytically encrusted plant parts were discarded. Seagrass blades were removed as close to their respective growing tips as possible. With the larger seagrass (*Enhalus acoroides*), the proximal 20 cm of each blade was relatively free of encrusting organisms and the only portion analyzed. Bivalves were scrubbed clean of sedimentary material and purged of their gut contents in clear seawater for 48 h prior to storage at -20^{o}C. Subsequently, the entire soft parts of thawed specimens were taken for analysis.

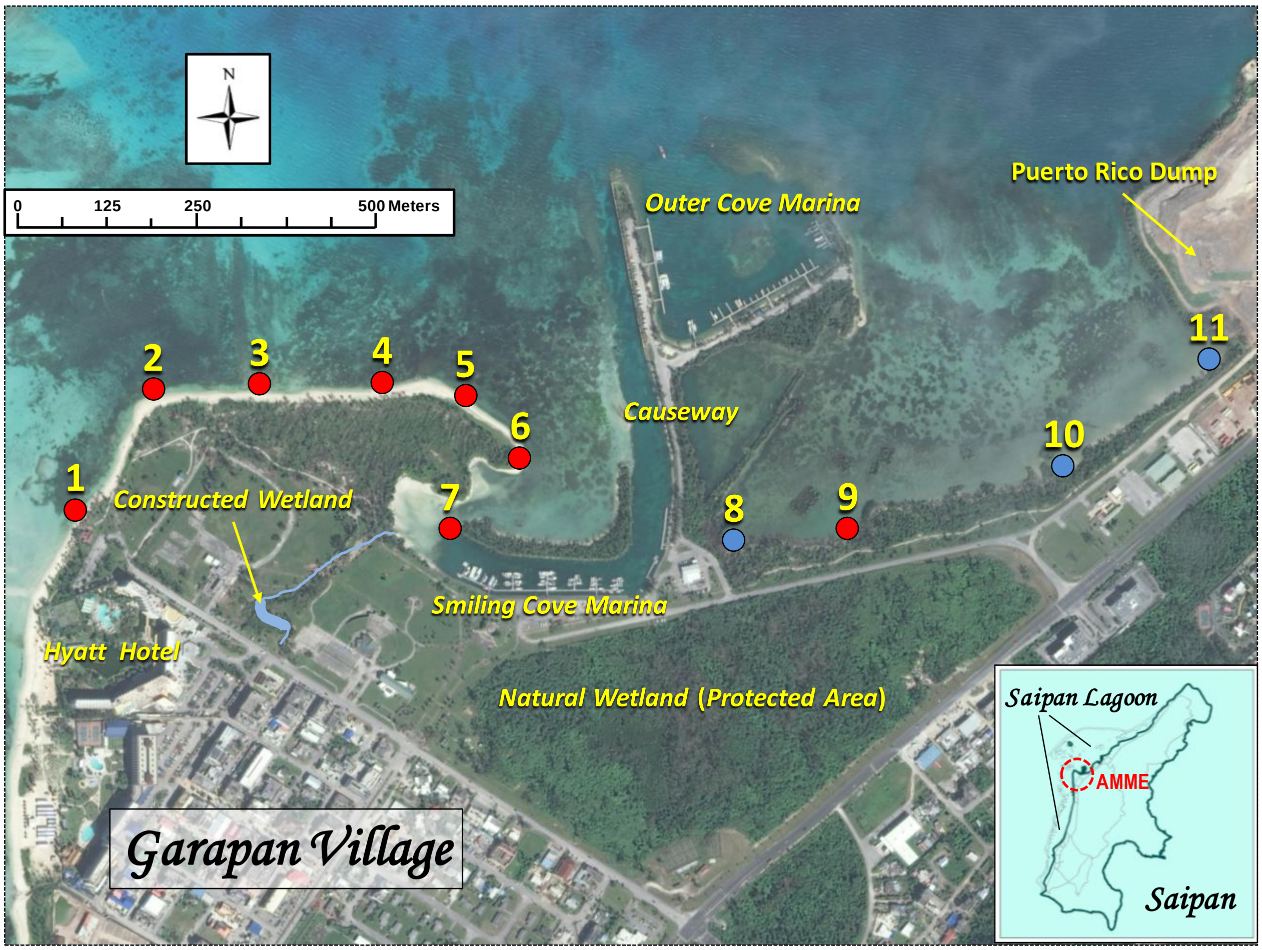

Figure 3: Biota sampling sites along the seaward perimeter of AMME. Sites 8, 10 and 11 (blue circles) located down gradient of stormdrain discharge points.

All cleaned and processed biotic samples were stored in acid-washed, polypropylene vials (80 ml) at -20^{o}C until required. Chemical determinations were performed on samples dried to constant weight at 60^{o}C for all elements except mercury and selenium. The relatively high volatility of the latter elements required they be analyzed on wet rather than dried tissues.

Table 2: Flora and Fauna Sampled During the Present Survey

Biotic Representative[a]	Site[b]										
	1	2	3	4	5	6	7	8	9	10	11
ALGA (Rhodophyta)											
Acanthophora spicifera	✓	✓	✓	✓	✓	✓	✓	✓	-	-	-
SEAGRASSES											
Enhalus acoroides	-	-	✓	✓	✓	✓	✓	✓	✓		
Halodule uninervis	✓	✓	✓	✓	-	-	-	-	-	-	-
BIVALVES											
Atactodea striata	-	✓	✓	✓	✓	✓	-	-	-	-	-
Ctena bella	-	-	✓	✓	✓	✓	-	-	-	-	-
Gafrarium pectinatum	-	-	-	✓	✓	✓	-	✓	-	-	✓
Quidnipagus palatum	-	-	-	✓	-	-	-	✓	✓	✓	✓

[a]Color photographs of all flora and fauna examined are shown in Plates 2-5 on pages 10-11.

[b]Visual characterstics of sediment:

Site 1: clean fine sand intertidally and subtidally
Sites 2-4: clean medium coarse sand intertidally and subtidally
Sites 5 & 6: clean medium coarse sand intertidally; silty medium coarse sand subtidally
Site 7: gravelly, muddy sand intertidally; silty mud subtidally
Sites 8-10: fine, flocculent, sticky mud intertidally; silty mud subtidally
Site 11: gravelly mud intertidally; silty mud subtidally

SEDIMENT ANALYSES:

All sediment samples were analyzed for heavy metals by atomic absorption spectroscopy (AAS) following conventional wet oxidation in hot mineral acids. This digestion procedure followed EPA method 3050A, SW-846 (USEPA 1995) with minor modifications. It was designed to release weakly-to-strongly bound metals in the sample without completely destroying the non-carbonate, mineral matrix of the sample. The procedure is briefly described below and is a useful means of identifying metal enrichment in sediments associated with anthropogenic activities.

Mercury and Selenium:

Approximately 2 g of sieved, sediment samples were weighed into 80-ml polypropylene digestion tubes specifically made for a MOD BLOCK digestion block (CPI International). The tubes were loosely capped with Teflon stoppers and refluxed with ~20 ml of concentrated nitric acid at 100°C for 3 hours. Upon cooling, the digests were reheated to 100°C for 15 minutes following the addition of 5 ml of concentrated hydrochloric acid to reduce Se^{6+} to Se^{4+} (Plessi *et al.* 2001). Each digest was then topped up to a final 50-ml volume with 10% hydrochloric acid.

Mercury was analyzed by flameless (cold vapor) atomic absorption spectrophotometry (AAS) and involved the generation of metallic mercury vapor (Hg^{o}) following reduction with 2% stannous chloride (Hatch and Ott 1968). The process was facilitated using the syringe technique described by Stainton (1971). All calibration standards (5-20 ng/l) for mercury were made up in 10% nitric acid containing 0.05% potassium dichromate as a preservative (Feldman 1974). Selenium analysis was accomplished by flow-injection hydride generation AAS, where Se^{4+} was converted to the volatile hydride (SeH_2) by reduction with 0.4% sodium borohydride (in 0.5% sodium hydroxide) and 5M hydrochloric acid. Same-day calibration standards (1-10 μg/L) for this element were made up in 10% hydrochloric acid. Matrix interferences were accounted for using the standard addition method, whereby 10 ml aliquots of sample were spiked with 50 µl of 1 μg/L standard to determine selenium recovery percentages (Plessi *et al.* 2001).

Other Metals:

Approximately 1 g of each dried sediment sample was weighed into an 80-ml glass MOD BLOCK tube and digested with ~10 ml of concentrated nitric acid at 100°C for 3 hours. The digests were then evaporated to near dryness at 135°C and allowed to cool before re-dissolving in 10 ml of 10% nitric acid with gentle warming. The contents of each tube was thoroughly mixed and allowed to stand for several hours at room temperature to permit residual particulates to settle out. Clear aliquots of each sample were then decanted into clean polypropylene vials ready for analysis by flame AAS. Simultaneous corrections for non-atomic absorption were made by the instrument (deuterium lamp). All calibration standards (0.2-10 mg/L) were prepared from a commercial mixed stock solution (100 mg/L of each metal) and were made up in 10% nitric acid. In this form they are stable for several weeks.

BIOTA ANALYSES:

The procedures for biota analyses were essentially the same as those described for sediments with three notable exceptions. First, all samples were cold digested overnight to minimize frothing during the initial warming phase. Second, samples for mercury and selenium analyses were digested in 2:1 nitric and sulfuric acids rather than nitric acid alone. The more powerful oxidizing mixture was required for the complete destruction of organic matter in the wet tissues. Finally, samples for all other metals were subjected to two 3-hour digestion/drying cycles with hot nitric acid prior to topping up to final volume with 10% nitric acid.

QUALITY ASSURANCE AND QUALITY CONTROL (QA/QC):

All reagents used were analytical grade and all plastic and glassware were acid-washed and rinsed with deionized water prior to use. Standard stock solutions were purchased from a commercial supplier (*AccuStandards*). Approximately 10% of samples were run in duplicate and were accompanied by appropriate method blanks and matrix spikes. Accuracy and precision estimates were based on heavy metal recoveries from certified standard reference materials and were within acceptable limits for all elements examined (Tables 3 and 4).

Table 3: Heavy Metal Recoveries (µg/g dry wt.) from Soil Standard Reference Material

Metal	This Study		Certified Values	
	Mean	Range	Mean	Range
	***PriorityPollutnT™/CLP Inorganic Soils* [Cat N° PPS-46; Lot N° 232][a]**			
Cadmium	91.2	86.9-101	88.8	66.9-111
Chromium	109	97.8-117	133	104-163
Copper	80.3	71.6-92.1	85.0	68.0-102
Lead	83.3	69.0-94.5	86.4	58.0-115
Mercury	2.86	2.46-3.15	2.86	1.78-3.94
Nickel	95.5	78.0-108	95.5	72.1-119
Selenium	124	110-139	129	93.9-165
Silver	120	112-133	117	84.5-150
Zinc	64.9	59.0-75.1	71.8	53.4-90.2

[a] *ERA-CRM Trace Metals PriorityPollutnT™* [Cat N° 540]

Table 4: Heavy Metal Recoveries (µg/g dry wt.) from Biotic Standard Reference Materials

Metal	Mean ± 95% Confidence Limits		Mean ± 95% Confidence Limits	
	This Sudy	Certified Value	This Sudy	Certified Value
	Apple Leaves (SRM 1515) [a]		*Bovine Liver (SRM 1577b)* [b]	
Cadmium	0.03 ± 0.01	0.013 ± 0.002	0.48 ± 0.01	0.50 ± 0.03
Chromium	0.36 ± 0.10	0.3[c]	0.92 ± 0.13	-
Copper	5.30 ± 0.25	5.64 ± 0.24	145 ± 2.94	160 ± 8
Lead	0.39 ± 0.01	0.47 ± 0.02	0.14 ± 0.01	0.129 ± 0.004
Nickel	0.99 ± 0.08	0.91 ± 0.12	0.63 ± 0.15	-
Silver	0.04 ± 0.01	-	0.05 ± 0.01	0.039 ± 0.007
Zinc	12.2 ± 0.55	12.5 ± 0.03	118 ± 3.17	127 ± 16
	Fish Protein (Dorm-4) [d]		*Albacore Tuna (RM 50)* [b]	
Mercury	0.397 ± 0.033	0.410 ± 0.055	0.98 ± 0.03	0.95 ± 0.01
Selenium	3.31 ± 0.22	3.56 ± 0.34	-	-

[a] *National Bureau of Standards;* [b] *National Institute of Standards and Technology;* [c] Unconfirmed reference value only
[d] *National Research Council Canada;* dashes indicate no data

<u>COMPENSATORY PROCEDURE FOR SEDIMENT HETEROGENEITY:</u>

Sediment particle size heterogeneity between sites was accounted for by normalizing the data against measured chromium concentrations. Chromium was chosen as the normalizing metal because it occurred at low concentrations and showed the least variability with and between sites. These characteristics suggested zero to minimal input of this element from anthropogenic sources and qualified it as a reasonable proxy for the finer sediment fraction (<63 µm) in all samples. Anthropogenic metal inputs within the study area were identified by comparing the Cr normalized data of the samples with that obtained from the reference materials (from sites 1-3 in current study) to obtain enrichment factors (EFs). The equation used to derive EF is as follows:

$$EF = \frac{[C_{metal}/C_{Cr}]_{\text{sample}}}{[C_{metal}/C_{Cr}]_{\text{reference material}}}$$

where C_{metal} and C_{Cr} are the geometric mean concentrations of the metal of interest and Cr in the sample and reference material respectively (Salomons and Förstner 1984). All EF values were ranked using a scaling system developed by Sutherland (2000) whereby: EF <2 = no enrichment; 2 <EF <5 = light to moderate enrichment; 5 <EF <20 = significant enrichment; 20 <EF <40 = high enrichment and EF >40 = very high enrichment.

<u>DATA INTERPRETIVE ANALYSIS:</u>

All analytical data were weighed against values reported for sediments and similar biotic groups from other parts of the world with emphasis on identical species collected from Guam, northern Australia, and elsewhere around Saipan. Baseline values were derived from these regional databases and used in the following section as benchmarks for comparative assessment purposes. Ecological and human health aspects of the study are addressed in the final chapter of this report.

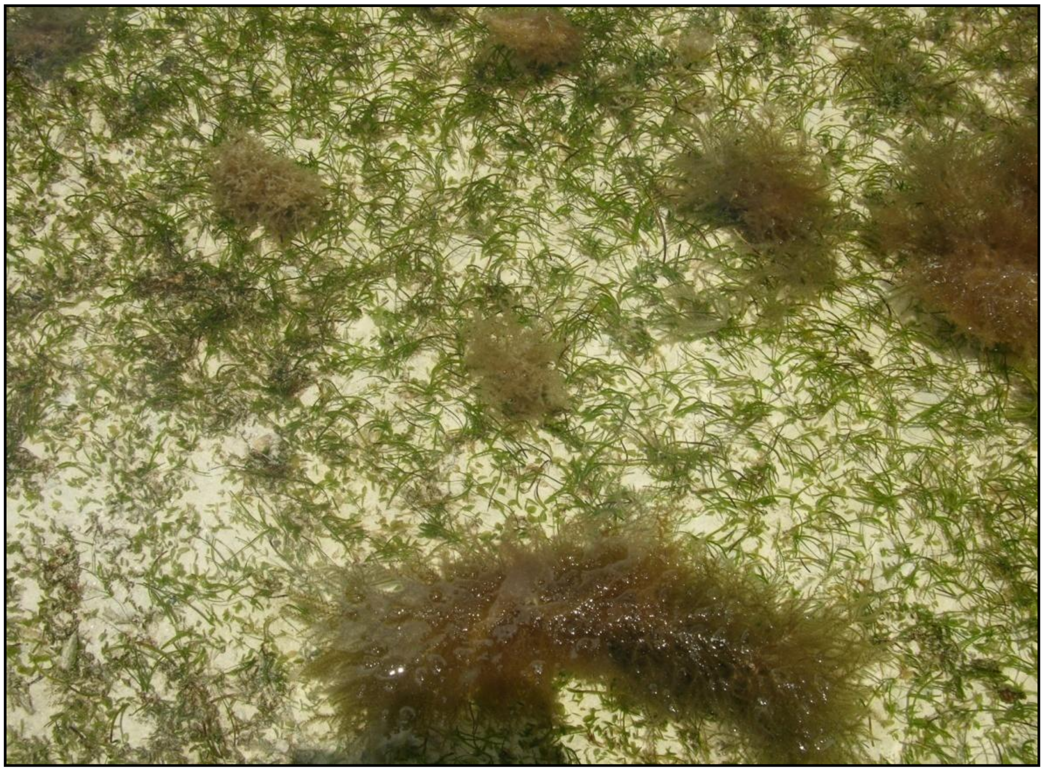

Plate 2: Biomonitors: Narrow blade seagrass, *Halodule uninervis*, and red alga, *Acanthophora spicifera*

Plate 3: Biomonitors: Broad blade seagrass, *Enhalus acoroides,* and three common bivalves (inset) found in bed sediments: (a) *Ctena bella,* (b) *Gafrarium pectinatum*, (c) *Quidnipagus palatum*

Plate 4: Biomonitor: *Atactodea striata,* (inset), mid- to low-tide resident of clean, sandy beaches

Plate 5: Close-up of *Atactodea striata* showing preferred sediment composition and texture

RESULTS AND DISCUSSION

Tabulated data summaries for all elements considered in sediments (Tables 5 and 6) and biota (Tables 7-10) are located at the end of this section. Numerical sediment quality guidelines commonly used to predict potential ecological impacts of sediment associated metals are listed in Table 11, and a compilation of national and international regulatory limits and guidelines for heavy metal in seafood are shown in Tables 12 and 13. The tables are preceded by notes that compare and contrast the current findings with the work of others on a metal-by-metal basis. All referenced data are expressed on a dry weight basis unless stated otherwise.

SILVER (Ag):

Silver ranks among the most toxic of heavy metals to marine organisms (Moore 1991). Levels in abiotic components of the marine environment are usually low. Dissolved levels in seawater, for example, are generally less than 0.001 µg/L (Shafer 1995) while levels in uncontaminated sediments are in the order of 0.1 µg/g (Bryan and Langston 1992). Sedimentary silver concentrations in highly polluted environments can exceed 100 µg/g (Skei *et al.* 1972).

AMME Shoreline Sediments:

Silver concentrations in beach sediments examined during the present study were consistently below an analytical detection limit of ~0.2 µg/g (Table 5). Similar findings were recently reported for intertidal deposits collected down gradient of 22 stormdrains further south in Saipan Lagoon (Denton *et al.* 2014). An earlier investigation revealed similar concentrations in beach sediments from 11 locations further north (Denton *et al.* 2009). The only shoreline sediments in the lagoon to have ever shown silver enrichment have been those at the foot of the Puerto Rico Dump. In the mid 1980s, for example, the Saipan Division of Environmental Quality (DEQ), in collaboration with USEPA (Region 9) scientists, noted a high of 2.3 µg/g for silver in sediments taken around the SW perimeter of the dump (DEQ 1987). In the late 90s, WERI re-sampled some of these sites and failed to detect silver in any of them (Denton *at el.* 2001). However, in 2003 WERI conducted another study and found 0.75 µg/g of silver in deposits from the SE corner of the property where the current site 37 is now located (Denton *et al.* 2009). The failure to detect silver in any samples from this location during the current study, likely reflects burial of the older sediments under cleaner material deposited since the dump was closed in 2003.

Marine Plants:

Marine algae and macrophytes generally do not concentrate silver to levels above 0.4 µg/g in clean waters (Preston *et al.* 1972, Bryan and Uysal 1978, Burdon-Jones *et al.* 1975, Denton *et al.* 1980). In metal enriched environments, however, algae and macrophyte levels may be somewhat higher. For example, Bryan and Hummerstone (1977) reported a maximum value of 2.42 µg/g for *Fucus* spp. (brown algae) collected from the metal enriched Looe River estuary in Cornwall, UK. In the current study, silver concentrations did not exceeded 0.3 µg/g in any algal sample analyzed (Table 7). The element was also undetectable (<0.14 µg/g) in all seagrass replicates tested (Tables 8).

Bivalves:

Mollusks show considerable inter- and intra-specific variations in silver concentrations with levels reported in the literature ranging from <0.1-185 µg/g. In most cases, the highest values are found in bivalves taken from polluted environments (Eisler 1981). While generally low, silver concentrations in specimens examined during the present work (Table 9) were notably higher than those found in similar species from Pago Bay, a relatively clean coastal site in Guam. *Quidnipagus palatum* in particular, appears to have a relatively high affinity for silver and is thought to possess some biomonitoring capability for this element (Denton *et al.* 2009). Also noteworthy in this regard is the small beach bivalve, *Atactodea striata*. This species is prolific on sandy beaches throughout the region with population densities well suited for trend monitoring purposes. As a consequence, WERI has compiled a relatively large heavy metal database for this species over the years, with representatives having been examined from numerous sites along the entire length of Saipan Lagoon. Clear spatial differences have subsequently been identified for several elements, including silver, giving credence to the biomonitoring capability of this organism. As such, there is no evidence to suggest that silver is a problem element anywhere in Saipan Lagoon, including sites examined during the present investigation.

Concluding Remarks:
The absence of detectable silver levels in any of the AMME shoreline sediments or marine plants analyzed, coupled with low concentrations of less than 1.0 µg/g in the great majority of bivalves examined, support earlier conclusions of low-level silver abundance throughout Saipan Lagoon (Denton *et al*. 2001, 2006a, 2009).

CADMIUM (Cd):
Cadmium, particularly as the free cadmium ion, is highly toxic to most plant and animal species. Normally, soluble levels of cadmium in uncontaminated aquatic environments are well below 1 µg/L (Moore 1991). Concentrations in remote open ocean waters may be as low as 0.002 µg/L and rarely exceeds 0.5 µg/L in nearshore waters, even in heavily industrialized areas (Yeates and Bewers 1987). Non-polluted sediments typically contain around 0.2 µg/g cadmium or less while levels may exceed 100 µg/g at heavily contaminated sites (Naidu and Morrison 1994).

AMME Shoreline Sediments:
Cadmium levels in sediments examined during the present study (Table 5) were consistently below the limits of analytical detection (~0.20 µg/g) and support previous sediment studies conducted elsewhere in Saipan Lagoon (Denton *et al.* 2009, 2014). However, back in the mid-1980s, DEQ detected 1.0-2.7 µg/g of cadmium in samples taken along the SW perimeter of the dump (DEQ 1987). Such values exceed threshold sediment quality guidelines for the protection of sensitive, sediment-dwelling species (Table 11). A little over a decade later, Denton and coworkers reexamined three of these sites and reported mean cadmium levels of 0.24 µg/g and 0.58 µg/g in two of them (Denton *et al.* 2001). Shortly after, a single sediment sample from the SW corner of the property where the current site 37 is located, contained 1.69 µg/g of cadmium (Denton *et al.* 2009). The subsequent decline in sedimentary cadmium levels at site 37 certainly implies an overall attenuation of metal contamination in this area in recent years.

Marine Plants:
The ability of algae to accumulate cadmium from seawater is well documented, and levels as high as 220 µg/g have been recorded in brown algae (*Fucus vesiculosus*) from the metal enriched waters of the Severn Estuary in the UK. (Butterworth *et al* 1972). Levels recorded in *Acanthophora spicifera* during the present study ranged from <0.12-0.87 µg/g with highest

levels recorded in the sample collected closest to the dump at site 8 (Table 7). Values reported for all other sites compare well with levels found in the same species from the Australian Great Barrier Reef (Denton and Burdon-Jones 1986a).

Cadmium in uncontaminated seagrass typically occurs at the sub parts per million level and is normally undetectable by conventional AAS analysis. Such was the case for plants taken west of the causeway (Fig. 3) with just a hint of enrichment observed in *Enhalus acoroides* from *Smiling Cove Marina* (Table 8). Values determined in *E. acoroides* east of the causeway, however, provide evidence of light to moderate cadmium enrichment in nearshore waters despite the absence of supporting evidence from the accompanying sediment analysis. The reason for this discrepancy is that seagrasses are rooted macrophytes and accumulate heavy metals from sub-surface sediments within their root zone as well by direct foliar partitioning from the water column. The data thus lend further weight to the suggestion that surface sediments previously shown to be heavy metal contaminated in this region now lay buried under cleaner deposits.

As an addendum here, it should be noted that while algae are generally considered to be useful biological indicators of dissolved cadmium, the presence of elevated levels of iron and/or manganese in the water can significantly reduce cadmium uptake (Moore 1991). This is thought to occur as a result of competition between the metals for cellular binding sites. Since harbors, marinas, and dump sites are typically enriched with both metals, some caution is required in interpreting cadmium contamination profiles in such areas from the analysis of algae alone. Whether seagrass is similarly affected is currently unknown.

Bivalves:
Bivalve mollusks have been widely used to monitor cadmium pollution in aquatic environments. The group as a whole generally demonstrates a high affinity for cadmium and other metals of environmental concern, with little to no metabolic control over levels accumulated. While such characteristics certainly make these organisms ideal candidates for monitoring the heavy metal status of coastal environments, they can place severe constraints on the usefulness of bivalves harvested for food from metal contaminated waters.

While there is considerable data for cadmium and other heavy metals in bivalves from temperate waters, not much in the way of comparable data exists for the species analyzed during the present investigation. What little data there is largely reflects previous works conducted elsewhere in Saipan (Denton *et al.* 2009) and on Guam (Denton and Morrison 2009). During the present study, cadmium levels in all bivalves examined (Table 9) were generally lower than those determined in similar species elsewhere in Saipan Lagoon and on a par with values previously reported for a relatively clean environment in Guam. The US Food and Drug Administration's guideline for maximum cadmium levels in shellfish harvested for food currently stands at 4 µg/g wet weight (USFDA 2001) and is well above values encountered in bivalves during the current study. International food standards for cadmium vary considerably with maximum permissible levels in seafood typically ranging between 0.1 and 2.0 µg/g wet weight (Nauen 1983).

Concluding Remarks:
Based on the foregoing data and discussions, there is no indication that cadmium poses a threat to the health of ecosystems, or integrity of potential food resources within the AMME study area.

CHROMIUM (Cr):
Chromium is only moderately toxic to aquatic organisms (Moore 1991). Total dissolved chromium levels in seawater show little variability and range from around 0.6 µg/L in offshore areas to 1-2 µg/L in highly polluted areas (Riley and Chester 1971, Beukema *et al.* 1986). Nakayama *et al.* (1981) showed that dissolved chromium in the Pacific Ocean and Sea of Japan existed as 10-20% inorganic-Cr^{3+}, 25-40% inorganic-Cr^{6+}, and 45-65% organic-Cr. Levels in particulate form were also found to outweigh dissolved concentrations by a factor of 6 and 5.25 in each location respectively. These findings imply that sediments may rapidly accumulate chromium in waters receiving elevated concentrations of this element.

AMME Shoreline Sediments:
Chromium levels in uncontaminated sediments vary according to their mineralogical characteristics and range between 10-100 µg/g (Turekian and Wedepole 1961). Calcareous sediments of biogenic origin, like those found on Saipan and Guam seldom exceed 10 µg/g of chromium and are generally less 5 µg/g in non polluted waters (Denton and Morrison 2009, Denton *et al.* 2006b, 2009, 2014). In severely contaminated areas, however, sedimentary chromium concentrations have exceeded 2,000 µg/g (Young and Means 1987). Chromium levels present in shoreline sediment during the current work ranged from 3.47-8.25 µg/g with the highest level occurring close to the dump (Table 5). These values are well below sediment quality guideline threshold values for adverse biological effects (Table 11). A single sample collected from the latter site in 2003 contained 17.5 µg/g of this element demonstrating an approximate 50% decrease in surface chromium levels in a little over a decade. Levels currently encountered in this general area are indicative of only light chromium enrichment.

Marine Plants:
Chromium levels in algae and seagrass normally range from <1-3 µg/g. Rarely do values exceed 5.0 µg/g in plants from clean coastal waters (Eisler 1981). Chromium concentrations found in specimens analyzed during the present work were generally well below 5.0 µg/g (Tables 7-8). In chromium-contaminated environments, levels in algae may be appreciably higher. For example, Burdon-Jones *et al.* (1975, 1982) reported a high of 31.5 µg/g in *Padina* sp. from the upper reaches of Townsville Harbor in north Queensland, Australia, while Gryzhanková *et al.* (1973) recorded a high of 140 µg/g in algae from polluted coastal waters in Japan. Whether seagrasses are as responsive as algae to changes in ambient chromium availability is currently unknown.

Bivalves:
Chromium concentrations in the edible tissues of uncontaminated marine mollusks usually lie between 0.5-3.0 µg/g (Eisler 1981). Levels recorded here ranged from 1.00 µg/g or less in *Ctena bella* and *Gafrarium pectinatum* to over 10.0 µg/g in *Quidnipagus palatum* taken near the dump. (Table 9). All three species were significantly higher in chromium than their counterparts taken from Pago Bay, a clean coastal environment in Guam (Denton and Morrison 2009). The findings demonstrate the sensitivity of these bivalves to changes in ambient chromium concentrations and provide additional evidence of light chromium enrichment around the Puerto Rico Dump. Of

additional note, chromium levels in all bivalves examined were well below the USFDA guideline value of 13 µg/g wet weight (USFDA 2001). Most other countries do not have regulatory or guideline limits for chromium in seafood because it is not an element that is readily accumulated. China is one exception to this general rule and currently enforces a maximum chromium level in aquatic animals and products of 2 µg/g wet weight (NFSSC 2014).

Concluding Remarks:
Although modest localized chromium enrichment was apparent in sediments and some biotic components from within the study area, the levels encountered were generally within the bounds of those found elsewhere in the lagoon in earlier studies and are of no concern from an environmental or human health standpoint (Denton *et al.* 2006a, 2009, 2014).

COPPER (Cu):
Copper is particularly noxious to plants and invertebrates (Brown and Ahsanulla 1971, Denton and Burdon-Jones 1982a), and ranks among the more toxic heavy metals to fish (Denton and Burdon-Jones 1986b, Moore 1991). Dissolved copper levels in open ocean surface waters are low and generally in the order of 0.2 µg/L, or less. In uncontaminated nearshore surface waters, levels are appreciably higher, often approaching 1 µg/L, while in highly polluted waters they may exceed 10 µg/L (Denton and Burdon-Jones 1986c). Copper levels in clean, non-geochemically enriched coastal sediments rarely exceed 10 µg/g whereas values in excess of 2,000 µg/g can occur in severely polluted environments (Legoburu and Canton 1991, Bryan and Langston 1992).

AMME Shoreline Sediments:
Clean coral reef sediments typically contain 0.5-1.5 µg/g copper nearshore (Denton and Morrison 2009), whereas levels of 0.1 µg/g or less are frequently encountered further offshore away from terrestrial influences and contributing anthropogenic sources (Denton *et al.* 2014). In the present study, sedimentary copper levels (Table 5) ranged from 4.93 µg/g in the fine coral sand adjacent to the AMME pavilion (site 4) to a high of 15.6 µg/g at site 29 immediately east of the causeway and down gradient of the stormdrain that receives drainage derived exclusively from the AMME protected wetland area (Fig. 2). The latter value is not too far removed from the sediment quality guideline threshold effects level adopted by the Florida Department of Environmental Protection for the protection of sensitive species in calcareous sediments (Table 11). Relatively high levels (11.1 µg/g) were also encountered in sediments taken from the mouth of the small stream that drains the AMME constructed wetland (site 26) and down gradient of the stormdrain (site 37) that discharges close to the dump (12.2 µg/g). In 2003, the copper concentration in a single sediment sample taken from the latter site was 102 µg/g (Denton *et al.* 2009). The lower level noted in the present investigation lends weight to the suggestion that cleaner deposits have contributed to sediment accretion processes next to the dump in recent years. Copper enrichment factor (EF) analysis placed sediments from all but one site in the 'significant' enrichment category (Table 6). The copper contamination likely reflects a combination of past and present land-use activities dating back to WWII and the US invasion, superimposed upon more recent industrial, shipping, and other activities in the area.

Marine Plants:
According to Moore (1991), total copper levels in marine plants are normally less than 10 µg/g, except near polluting sources where values upwards of 50 µg/g are not uncommon (Bryan and Hummerstone 1973, Burdon-Jones *et al.* 1975). Copper concentrations recorded in seaweed and seagrass during the present investigation were mostly well below 10 µg/g. The notable exceptions in both biotic groups were at site 7, near *Smiling Cove Marina*, where copper concentrations in *A. spicifera* and *E. acoroides* averaged 11.4 µg/g and 29.8 µg/g respectively (Tables 7-8). Interestingly, copper values in sediment from site 7 (13.3 µg/g) were only modestly higher than baseline. In an earlier study, WERI examined copper in *A. spicifera* and *E. acoroides* growing near a dry dock facility in Saipan Lagoon and found even higher levels of 30.5 µg/g and 47.9 µg/g in each species, respectively. The sedimentary copper value measured at the time was 39.8 µg/g (Denton *et al.* 2009). Antifouling paints are suspected of being the most likely source of copper at both locations.

From the foregoing, it would appear that *E. acoroides* is a more sensitive indicator of copper contamination than *A. spicifera.* Further evidence for this emerged during the current study with *E. acoroides* demonstrating a gradual increase in copper concentrations from 3.70-12.4 µg/g between sites 3 and 6 before rapidly increasing to 29.8 µg/g at site 7. By comparison, inter-site copper increases shown by *A. spicifera* were not clearly discernible until site 6 (Tables 7-8).

As far as we know, these are the first reports highlighting the exceptional biomonitoring capacity of *E. acoroides* for copper. Companion studies also suggest other seagrasses are similarly sensitive to copper. For example, *Halodule* from a former dump site at the southern end of Saipan Lagoon accumulated in excess of 50 µg/g of this element (Denton *et al*. 2009). Copper levels in sediments at this site ranged from 12.1-16.7 µg/g compared with baseline levels of <1.00 µg/g in adjacent sites further north (Denton *et al.* 2014). The copper sources in this instance was metallic debris, including copper wire (discarded power lines), and discarded WWII munitions. More recently, Díaz et al. (2018) examined heavy metals in the seagrass, *Syringodium filiforme* from a former US Navy bombing range in Puerto Rico. Copper levels in samples from the impacted site ranged from 12.7-30.5 µg/g compared with 12.2-16.9 µg/g at a reference site located several kilometers away. Unfortunately, no sediment copper data were provided.

Bivalves:
Bivalve mollusks have been used extensively to monitor copper in the marine environment, although some species are far more sensitive to ambient changes in the biological availability of this element than others. Not much is known about the biomonitoring potential of the species examined here although what little information there is suggests *Quidnipagus palatum* is an extremely promising candidate. Specimens from Pago Bay in Guam, for example, yielded a maximum copper concentration of 68.5 µg/g (Denton and Morrison 2009) compared with an impressive 1,876 µg/g in specimens collected near the Puerto Rico Dump in Saipan back in 2003 (Denton *et al.* 2009). With copper values in excess of 1,000 µg/g in specimens analyzed during the present study (Table 9), it is clear that a respectable degree of copper contamination still exists in sub-surface sediments within the dump-adjacent embayment. Copper levels determined in this species from comparatively clean sites elsewhere in Saipan Lagoon generally did not exceed 100 µg/g and were mostly below 50 µg/g (Denton *et al.* 2009, Denton 2008 unpublished data).

Copper values derived from all other bivalve species examined during this study were generally low. Mean values in *Atactodea striata* were remarkably similar between sites spanning a narrow range of 10.3-12.4 µg/g. While such stability suggests copper may be regulated in this species, a high of 82.2 µg/g was noted earlier in representatives collected near an old dumpsite at the southern end of the lagoon (Denton 2009 unpublished data). Copper regulation in *A. striata* therefore seems unlikely. *Gafrarium pectinatum,* might also be suspected of exerting some metabolic control over copper given the similarly low values emerging in samples taken either side of the causeway (Table 9). Again, however, previous studies have proved otherwise with levels in excess of 50 µg/g recorded in samples from copper enriched areas further south in the lagoon (Denton 2008 and 2009 unpublished data).

Ctena bella were only available for copper analysis from two sites during the current study and the range of values found was similar in specimens from both populations. Copper levels encountered in this species were consistently higher than those found in *A. striata* and *G. pectinatum* from within the study area (Table 9). Previous studies on Guam suggest baseline copper levels in *C. bella* are generally less than 10 µg/g (Denton and Morrison 2009). Much of WERI's unpublished heavy metal data for *C. bella* from elsewhere in Saipan Lagoon also support a less than 10 µg/g copper baseline. Moreover, copper levels approaching 50 µg/g have been recorded in this species from mildly contaminated waters in the lagoon, thus highlighting its biomonitoring potential for this particular element. The collective implications of these findings therefore suggest that *C. bella* is a more sensitive indicator of copper than *A. striata* and *G. pectinatum*. The relatively high levels determined in *C. bella* during the current study also imply it was responding to the moderate copper enrichment identified in sediments and seagrass from the same general area.

Concluding Remarks:

Despite light to significant copper enrichment in shoreline sediments throughout the study area and clear indications of this element's migration into biological systems, there is, as yet, no compelling evidence to suggest the edible quality of marine food resources has been compromised, or that adverse biological effects have occurred in sensitive species when weighed against existing food standards and sediment quality criteria. Many bivalves rely on copper based hemocyanins as their respiratory blood pigment and are thus naturally high copper. The greenish grey color of *Q. palatum* flesh certainly suggests the presence of hemocyanin.

While US food standards do not include a maximum permissible level for copper in bivalves sold for human consumption, those adopted by other countries have enforceable copper limits ranging from 10-100 µg/g wet weight to accommodate species naturally high in this element (Nauen 1983, Tables 12 and 13). Mean copper values recorded in *Q. palatum* ranged from 44-140 µg/g when expressed on a wet weight basis, thus exceeding the maximum allowable concentration in some individuals from site 11 beside the dump (Table 9).

MERCURY (Hg):

Mercury is highly toxic to aquatic organisms, particularly in the organic form (Moore 1991). The number one anthropogenic source of mercury to the environment is discharge from coal-burning power plants followed by atmospheric fallout from other sources (e.g. incineration of municipal refuse), chemical manufacturing processes, and the discharge of domestic wastes

(Moore 1991). Mercury levels in urban runoff are generally low (Marsalek and Schroeter 1988), although relatively high concentrations have been found in oil and other petroleum products (Patterson *et al.* 1987), and some enrichment can be expected in harbor areas. Substantial amounts of mercury were once used in anti-fouling paints to prevent the growth of marine organisms on the hulls of ships and other watercraft. In 1969, for example, 12% of all mercury used in the U.S. went into such paints (Gerlach 1981).

Dissolved mercury concentrations in the open ocean typically range from <0.001-0.003 µg/L (Miyake and Suzuki 1983). Slightly higher values of 0.003-0.02 µg/L are found closer to shore, and polluted estuarine waters may contain up to 0.06 µg/L (Baker 1977). Sediment concentrations of mercury in unpolluted, non-geochemically enriched areas, usually do not exceed 30 ng/g (Bryan and Langston 1992, Benoit *et al.* 1994) and may be as low as 1 ng/g or less in clean bioclastic sediments (Denton and Morrison 2009). Estuarine sediments adjacent to heavily industrialized areas or mercury mining activities can be three to five orders of magnitude higher than these baseline values (Langston 1985, Benoit *et al.* 1994). Concentrations in excess of 2000 µg/g were found in sediments from the grossly contaminated Minimata Bay area in Japan following the mass mercury-poisoning episode of the late 1950s, and probably rank among the highest levels ever reported for this element (Tokuomi 1969).

AMME Shoreline Sediments:
Mercury levels found in shoreline sediments west of the causeway during the current investigation ranged from a low of 0.42 ng/g in the fine sandy deposits near the AMME pavilion (site 1), to a high of 8.9 ng/g in the muddy sand at the constructed wetland discharge point (site 26). The latter value is indicative of significant mercury enrichment according to the classification scheme described earlier. All other sites west of the causeway showed either light to moderate mercury enrichment or no enrichment at all (Table 6). Sediment taken from site 29 on the other side of causeway contained the highest level of mercury at 37.5 ng/g and was considered to be very highly enriched. Nevertheless, this maximum was well below that necessary to promote adverse biological effects in sensitive sediment dwelling species (Table 11). All other sites along the latter coastline were either significantly or highly enriched with mercury. Interestingly, mercury in sediment from site 37 near the dump, appears to have attenuated in recent years dropping from ~75 ng/g in 2003 (Denton *et al.* 2009) to 27.3 ng/g in the current study (Table 5).

Marine Plants:
Marine algae are noted accumulators of heavy metals and have a relatively high affinity for mercury. Algae also represent the soluble metal load in their immediate environment rather than that associated with sediments and particulate material. Thus, the need for thoroughly cleaning samples prior to analysis is emphasized.

Algae from clean environments seldom contain more than 20 ng/g (ppb) mercury in their tissues on a wet weight basis (Denton and Burdon-Jones 1986a). In polluted waters, however, levels may be several orders of magnitude higher. In Hardangerfjord, Norway, for example, up to 20 µg/g (ppm) of mercury was reported for the brown alga, *Ascophyllum nodosum* (Haug *et al.* 1974). Apparently, contaminated wastewaters from a nearby metal smelter were the primary source of mercury pollution in this instance (Myklestad *et al.* 1978). In an earlier study, Jones *et* *al.* (1972) measured mercury in 10 species of algae from the polluted Tay estuary in the UK and reported a maximum of 6.3 µg/g wet weight in the green alga, *Ulva lactuca*. This extraordinary finding still stands as one of the highest levels ever recorded for marine algae. Among the lowest values in the literature are those given by Denton and Burdon-Jones (1986a) for 48 species of algae from the Great Barrier Reef. Measured mercury concentrations in the latter study ranged from <1-24 ng/g wet weight and were comparable with the values of 2-52 ng/g in 17 algal species from Korean waters (Kim 1972).

Very low mercury concentrations were detected in the red algal representative, *Acanthophora spicifera*, during the current work. Mean levels ranged from 1.2-1.9 ng/g wet weight (Table 7) in plants collected from all sites west of the causeway (Fig. 3). A slightly higher average of 3.20 ng/g was determined in the only representative sample collected from site 8 to the east of this structure. While hardly indicative of polluted conditions, these findings do suggest that soluble mercury is slightly enriched east of the causeway, and is to be expected given the mercury status of sediments here.

Comparable mercury data exists for *A. spicifera* from the pristine Great Barrier Reef waters in Northern Australia (Denton and Burdon Jones 1986a) and from a clean coastal environment (Pago Bay) on Guam (Denton and Morrison 2009). In both instances, levels determined were similar to those reported in the current work and collectively rank among the lowest mercury values recorded in the literature for this species.

What available evidence there is in the literature suggests that *E. acoroides* possesses some indicator capability for mercury. In an earlier WERI study, for example, Denton *et al.* (2009) examined representatives from four relatively clean and four relatively polluted sites in the central region of Saipan Lagoon. Samples from the clean locations contained 0.9-1.7 ng/g mercury (wet weight) while their counterparts from the more contaminated sites yielded 3.8-9.0 ng/g. Mean levels in both sets of plants were significantly different from one another at 1.1 ng/g and 4.6 ng/g respectively. Further evidence supporting the biomonitoring potential of this species was provided in the current study with plants from sites 8 and 9 containing higher mercury levels (3.5-4.9 ng/g) than specimens taken elsewhere in the study area (1.3-2.5 ng/g) (Table 8). Whether *Halodule uninervis* possesses the same sentinel capability for this element remains to be established.

Bivalves:

Although bivalve mollusks are excellent accumulators of mercury, tissue concentrations in specimens from clean environments rarely exceed 100 ng/g wet weight and are usually less than 50 ng/g. In polluted environments, however, levels can climb considerably higher. In the 1960s for example, levels in bivalves from the mercury contaminated Minimata Bay area of Japan frequently ranged between 10-100 µg/g. These levels are without question the highest values ever recorded in field specimens (Irukayama *et al.* 1961, 1967, Matida and Kumada 1969).

Mercury levels in bivalves during the present work were unremarkable in samples collected west of the causeway. Despite clear differences between species, levels determined in all specimens were under 30 ng/g wet weight. To the east of the causeway, however, the picture was rather different with levels in *Quidnipagus palatum* exceeding 200 ng/g wet weight in some instances.

The single *Q. palatum* taken west of the causeway (site 4) contained only 25 ng/g by comparison (Table 9). Baseline levels for this species typically range between 20-60 ng/g (Denton and Morrison 2009, Denton *et al.* 2009). International food standards for mercury in seafood range from 0.1-1.0 µg/g wet weight and vary between countries and seafood types in question (Tables 12 and 13). In the US, the current maximum permissible mercury levels in all seafood sold for human consumption is 1.0 µg/g. This standard specifically applies to methyl mercury, which typically accounts for 80-100% of total mercury levels in most species (Holden 1973). No mercury values recorded during the present study exceeded this benchmark.

Mercury levels of 17-19 ng/g wet weight were found in *Gafrarium pectinatum* from site 11 near the dump. Since these values approximate baseline levels for this species, they were naturally surprising. Even more remarkable was the fact that *Q. palatum* from the same site yielded much higher values of 149-232 ng/g. From previous studies we know that both species are highly responsive to changes in ambient mercury concentrations, and specimens retrieved from moderately contaminated environments elsewhere in the lagoon have been shown to accumulate levels well in excess of 100 ng/g (Denton *et al.* 2009).

The discrepancy noted between the above species is thought to be related to the depths that they occupy in their preferred sediment types. *G. pectinatum*, for example, is a short-siphoned, suspension feeding bivalve that normally resides within 2-5 cm of the sediment surface. Its preferred substrate ranges from medium to coarse muddy sand and gravel to clean medium to coarse sands. In marked contrast, *Q. palatum* is a long-siphoned deposit feeder and typically occupies a zone 12-15 cm below the surface of soft clayey substrates where redox conditions are often highly reducing. The pore waters in such sediments are typically enriched with soluble metals that are sequestered and immobilized by insoluble iron and manganese oxyhydroxides at shallower depths (Förstner and Wittman 1983). Thus, the two bivalves are exposed to very different metal regimens and depth-related difference in metal bioavailability. Further, surface sediment accretion processes east of the causeway appear to have commandeered less contaminated materials over the last decade or so. In all probability then, *G. pectinatum* taken from site 11 derived their metal load predominantly from younger, cleaner surface deposits, whereas *Q. palatum* were exposed primarily to the older, more contaminated sediments that prevail at greater depths.

Concluding Remarks:

The findings presented here confirm earlier evidence of increased mercury availability to the biota in shoreline communities adjacent to the *Puerto Rico Dump.* While levels in surface sediments along this stretch of coastline are clearly enriched, they are no longer excessively so due to the deposition of cleaner materials in recent years. Nevertheless, the impact of existing levels of contamination on fisheries resources in the area is significant and should be evaluated in greater detail with emphasis on popular table fish with restricted foraging ranges.

NICKEL (Ni):

Nickel is only moderately toxic to most species of aquatic plants and is one of the least toxic heavy metals to invertebrates and fish (Denton and Burdon-Jones 1982a, 1986b, Moore 1991). Open ocean concentrations of dissolved nickel normally lie between 0.1-0.3 µg/L (Boyle *et al.* 1981, Bruland 1980, Denton and Burdon-Jones 1986c). In polluted nearshore and estuarine waters, levels of between 5 and 30 µg/L have been reported (Halcrow *et al.* 1973, Abdulla and Royle 1974, Boyden 1975). Total nickel residues in lithogenic sediments normally range between 10-20 µg/g (Bryan and Langston 1992) but can exceed 200 µg/g (Fowler *et al.*1993) in contaminated regions. Clean bioclastic sediments from Guam and Saipan coastal waters typically contain concentrations of less than 1 µg/g (Denton *et al.* 1997, 2001, 2014).

AMME Shoreline Sediments:
Nickel was undetectable in sediments from 23 of the 37 sites sampled during the current study. Detectable levels ranged from 0.38-0.59 µg/g over only five sites west of the causeway and from 0.38-1.69 over all sites to the east (Table 5). Overall, only six sites showed any nickel enrichment and all were considered to be only lightly contaminated (Table 6). As expected, site 37 near the dump was one of the higher concentration locations, although the level recorded was extremely modest (0.93 µg/g) compared with 11.9 µg/g reported by Denton *et al.* (2009) for a single sediment sample from the same place in 2003. The highest nickel value found in surface sediments from the SW perimeter of the dump back in the mid 1980s was 16.1 µg/g (DEQ 1987) and exceeded threshold levels for adverse biological effects (Table 11). Denton *et al.* (2001) revisited some of these sites in 1999 and found lower levels ranging from 1.11-5.23 µg/g. Clearly, nickel concentrations in surface sediments have decreased over time along the eastern shoreline of AMME.

Marine Plants:
Algae rarely concentrate nickel above 3 µg/g in uncontaminated environments (Denton and Burdon Jones 1986a, Denton and Morrison 2009), whereas levels in excess of 30 µg/g have been recorded in specimens from nickel-enriched waters (Stevenson and Ufret 1966). The great majority of algal samples analyzed during the present study yielded values below 3 µg/g while levels in seagrass samples were all less than 2 µg/g. Similarly low nickel concentrations have previously been reported in both groups of plants from several sites to the north of the Puerto Rico dump in Saipan (Denton *et al.* 2009) and from Pago Bay in Guam (Denton and Morrison 2009).

Bivalves:
Bivalves are generally more effective accumulators of nickel than marine plants, although their biomonitoring capacity for this element remains in question. Certainly, the similarity between earlier Saipan and Guam data sets for *Ctena bella*, *Gafrarium pectinatum* and *Quidnipagus palatum* suggest that all three species exert some metabolic control over this element. This is also true of *Atactodea striata* whose total body nickel concentrations consistently fell within 2-5 µg/g during the current study as did 90% of all other representatives taken earlier from 25 additional sites along the entire length of Saipan Lagoon (Denton 2008 and 2009 unpublished data).

Concluding Comments:
In light of the data presented, nickel does not appear to be a metal of environmental concern within the AMME study area. The USFDA (2001) guideline for nickel in seafood currently stands 80 µg/g wet weight (Table 13) and is well above any value recorded in bivalves during the present work.

LEAD (Pb):

Although inorganic lead is only moderately toxic to aquatic plants and animals, organolead compounds, particularly those used as antiknock agents in gasoline, are highly toxic to all forms of life (Moore 1991). Inorganic lead is barely soluble in seawater and levels in open ocean waters typically range from 0.005-0.015 µg/L. Even in highly polluted waters, levels are unlikely to rise much above 0.050 µg/L (Burnett *et al*. 1977). Thus, particulate lead accounts for >75% of total lead in most waters (Moore 1991).

Lead concentrations in clean, non-geochemically enriched, lithogenic sediments generally do not exceed 25 µg/g (Bryan and Langston 1992), while levels in clean bioclastic deposits rarely exceed 1.0 µg/g (Denton *et al.* 1997, 2001, 2006a). In severely polluted locations, near mining activities, or industrial processes that utilize lead, sedimentary lead concentrations may exceed 2,000 µg/g (Jones 1986, Bryan and Langston 1992). The highest level reported to date is 266,000 µg/g in sediments adjacent to a battery factory in Suva Harbor, Fiji (Naidu and Morrison 1994).

AMME Shoreline Sediments:

Lead levels recorded in shoreline sediments west of the causeway were mostly undetectable with positive hits occurring only in areas impacted by outflows from the constructed wetland and boating activities in the *Smiling Cove Marina* (sites 26-28; Table 5). Degrees of lead enrichment in sediments from the marina area varied between light to moderate and significant (Table 6). The majority of sediments taken east of the causeway revealed similar enrichment categories with lead levels ranging from 1.84-13.8 µg/g. The highest lead concentrations were confined to sediments at either end of this muddy embayment with the maximum value (13.8 µg/g) occurring in deposits collected next to the dump (site 37).

Compared with earlier lead data reported for this site (Denton *et al.* 2009), levels have attenuated markedly since the closure of the dump: down from a high 158 µg/g in 2003 to the current 13.8 µg/g. Evidence of this having occurred elsewhere around the dump is also available. In the mid 1980s, for example, DEQ monitored lead in sediments from several sites around the SW perimeter of the property and reported levels of up to 201 µg/g. Likewise, in 1999, WERI retrieved sediments from the same area and reported highly variable levels ranging from 3.56-43.4 µg/g. The researchers concluded that the distribution of lead in the surrounding sediments was highly heterogeneous, with pockets of contamination continuing to exceed the sediment quality guidelines proposed by MacDonald *et al.* (1996) for the protection of organisms inhabiting carbonate rich sediments (Table 11).

In early 1997, the Saipan Department of Public Works started covering trash at the dump with sediments dredged from a deep water shipping lane located some distance offshore. Over 500,000 cubic yards of dredged material were placed on the dump up until late 1998 when the practice ceased. Most of the material was placed on the northeast and southeast sides of the dump resulting in side slopes that were very steep (>1:1) and unstable. As a consequence, they were heavily eroded during the wet season. Landslides were also common and severe wave and storm damage was evident all along the seaward facing side of the dump after four typhoons passed the island in 1997 (Brian Bearden, former DEQ employee, pers. com.). Hence, the attenuating elemental composition of sediments near the dump over the past few years almost certainly reflects burial of the earlier metal enriched deposits under cleaner offshore material originally intended for the daily coverage of trash at this facility.

Marine Plants:
While algae generally have a high affinity for lead and may concentrate it to well over 100 µg/g in polluted waters, levels in specimens from clean environments rarely exceed 1 µg/g (Denton and Burdon-Jones 1986a). In the present study, lead concentrations in the red alga, *Acanthophora spicifera* ranged from 0.83-2.89 µg/g (Table 7) and were within the range reported earlier for this species from pristine Great Barrier Reef waters in Australia (Denton and Burdon Jones 1986a). In a more recent study, Denton *et al.* (2009) examined metal levels in this species from several sites to the north of the current study area. A high of 8.3 µg/g was reported for a composite sample collected from *Seaplane Ramps.* At all other sites, levels in this species were all less than 1 µg/g. Similarly low values were also reported by Denton and Morrison (2009) for *A. spicifera* from Pago Bay, a relatively clean coastal environment in Guam.

Lead was not detected in the great majority of seagrass samples and barely detectable in the rest. *Enhalus acoroides* consistently yielded data below the limits of analytical detection (Table 8). These data notwithstanding, it seems fairly safe to say here that seagrasses do possess some biomonitoring capability for lead, based on earlier studies. For example, Denton *et al.* (2009) recorded 2 µg/g in *E. acoroides* from *Seaplane Ramps* and more recently noted values of 25.1-56.3 µg/g (mean 33 µg/g) in *Halodule uninervis* from a known lead contaminated site at the southern end of Saipan Lagoon (Denton *et al.* 2014, Denton 2008 unpublished data).

Bivalves:
Bivalves are generally considered to be excellent indicators of heavy metal pollution (Phillips 1980) although some species are clearly better suited than others for such purposes (Eisler 1981, Klumpp and Burdon Jones 1982). The bivalves examined in this study provide examples of such lead sensitivity disparities between species. For example, *Atactodea striata* and *Gafrarium pectinatum* from the lead contaminated site referred to above contained very different lead levels of 9.8 µg/g and 74.4 µg/g respectively (Denton 2009 unpublished data). Likewise, *Quidnipagus palatum* taken near the *Puerto Rico Dump* in 2003 yielded lead values of 163-184 µg/g compared with only 31-45 µg/g in *G. pectinatum* from the same location (Denton *et al.* 2009). Such disparities may also be due, at least in part, to depth related differences in habitat preference as previously discussed for mercury.

Baseline lead levels for all bivalve species examined during the present study hover around 1 µg/g (Denton and Morrison 2009, Denton 2008 unpublished data). Values encountered in *A. striata* and *G. pectinatum* west of the causeway during the present study were, therefore unremarkable (Table 9), while those noted for *Ctena bella* from subtidal sites most likely reflect mild contributions from leaded gasolines that once powered boating activities in the area. As expected, greater lead enrichment was identified at all sites east of the causeway with significantly higher biologically available levels indicated at site 11 next to the dump. Interestingly, mean levels in *Q. palatum* from this site were ~50% lower than they were back in 2003, thus mirroring the decline in sedimentary lead levels noted over the same time frame.

The USFDA (2001) guideline for maximum lead levels in bivalves sold for human consumption is 1.7 µg/g. International standards are of the same order and typically lie between 1.0 and 2.0 µg/g wet weight (Tables 12 and 13). Clearly then, lead levels in *Q. palatum* east of the causeway are cause for concern especially at site 11 beside the dump where average concentrations are an order of magnitude above the lower regulatory limit.

Concluding Remarks:
Despite evidence of attenuating lead concentrations in shoreline sediments from the NE boundary of AMME, moderate to significant lead enrichment still exists in surface deposits between *Smiling Cove Marina* and the dump. While levels determined in this region were below the threshold level (30.2 µg/g) necessary to induce adverse biological effects in sensitive species (McDonald *et al.* 1996), they were sufficient to elevate lead in resident bivalves east of the causeway to levels beyond that considered suitable for human consumption.

SELENIUM (Se):
Selenium is geochemically similar to sulfur and the two elements often coexist in nature. The average crustal abundance of selenium in igneous and sedimentary rocks is 50 ng/g and 80 ng/g respectively with up to 600 ng/g in certain shales (Turekian and Wedepohl 1961). Selenium in soils depends on the nature of the underlying bedrock, and ranges from 10 ng/g in selenium deficient areas, to well over 1000 µg/g in naturally enriched soils (Ralston *et al.* 2008a). Background levels of selenium in marine waters normally range between 20-40 ng/L. Near polluting sources, levels of 1-10 µg/L are not unusual and may even exceed 100 µg/L in rare instances (Ralston *et al.* 2008a). Fossil fuel combustion is without doubt the leading cause of selenium releases to the environment. Nonferrous metal mining and smelting also add significantly to the environmental load. Localized areas may additionally be impacted by selenium released from municipal landfills, industrial and domestic wastes and sludges, agricultural irrigation, feedlot wastes, and urban runoff (Moore 1991, Lemly 1999, 2002).

Selenium is an essential dietary trace element to humans and plays an important role in antioxidant defense systems, thyroid activity, immune responses, brain function and cardiac health (Raymond and Ralston 2004, Mozaffarian 2009). Of all essential elements, however selenium has one of the narrowest ranges between dietary deficiency (<40 µg/day) and toxic levels (>400 µg/day) (WHO 1996). The U.S. *Recommended Daily Allowance* (RDA) for adults is 55 µg/day (Raymond and Ralston 2004). The fact that selenium is known to have a protective effect against mercury toxicity has captured public attention in recent years, especial among voracious consumers of seafood. Fish have a high propensity for mercury, and current USEPA doctrine dictates that specimens with more than ~0.10 µg/g (wet weight) in their tissues should not be eaten on an unrestricted basis (USEPA 2000). Recent research by Ralston and coworkers suggests otherwise, providing selenium-mercury molar ratios in the fish consumed are equivalent (1:1) or in molar excess for selenium (Ralston *et al.* 2008b).

Mercury has an exceptionally strong affinity for selenium and, at the cellular level, sequesters selenium from functional biomolecules (e.g. selenoenzymes) to form mercury selenide. The mercury selenide is biologically inactive and eventually excreted from the body. Impaired selenoproteins are replaced by the cell and draw upon the body's selenium reserves in the process. Insufficient selenium reserves compromise further selenoprotein production and selenium-dependant enzyme activity (Raymond and Ralston 2004, Kanko 2010). Fish and shellfish consumption of specimens with equimolar selenium-mercury concentrations in their tissues, or proportionately greater amounts of selenium, minimizes depletion of the body's selenium reserves and maximizes the protective effect selenium affords against mercury toxicity (Burger and Gochfeld 2012, 2013).

AMME Shoreline Sediments

Total selenium levels determined in shoreline sediments during the current study ranged from 16-105 ng/g west of the causeway and 43-407 ng/g to the east (Table 5). Geometric mean concentrations within each of these regions were 57 ng/g and 94 ng/g respectively. Little comparative data exist in the literature for selenium in carbonate-rich sediments, although values of <10-80 ng/g have previously been reported for limestone rock of marine origin (Koljonin 1973). One study in American Samoa retrieved coralline sediments for chemical analysis from around Tutuila Island and failed to detect selenium in any sample analyzed (EnviroSearch Int. 1994). The analytical detection limit reported in the latter study was 180 ng/g. More recently, NOAA scientists published selenium levels found in coral reef sediments from four different locations in Puerto Rico. Their reported means ranged from 150-330 ng/g with maximum values of 430-1,560 ng/g (Whitall *et al.* 2014). The NOAA *National Status and Trends* (NS&T) program has been compiling sediment contaminant data from sites throughout the U.S since 1984. The program's national median for selenium in marine sediments currently stands at 330 ng/g (Apeti *et al.* 2012). Individual site means reported earlier in the program's 1991 data summary ranged from <10-9,400 ng/g and were log-normally distributed (NOAA 1991).

Selenium concentrations measured in AMME shoreline deposits during the present investigation generally rank among the lower sediment values reported above for US waters and were therefore considered to represent background with only light to moderate selenium enrichment in localized areas along the property boundary (Table 6).

Marine Plants:

Marine algae accumulate selenium directly from seawater and essentiality has been demonstrated in several unicellular species (Araie and Shiraiwa 2009). Reported levels in macroalgae are highly variable and range from 10 ng/g to over 760 ng/g (Horiguchi *et al.* 1971, Pak *et al.* 1977, Maher 1985, Maher *et al.* 1992, Smith *et al.* 2010, Duinker *et al.* 2016). From the literature it would appear that algae possess some indicator capability for selenium. For example, seaweeds from selenium depleted waters in New Zealand were reported to contain mean values of 160 ng/g, 170 ng/g and 150 ng/g in red, green and brown algae respectively (Smith *et al.* 2010). Mean levels recorded in the same three phyla from more enriched coastal waters in Japan were 610 ng/g, 310 ng/g and 300 ng/g (Horguchi *et al.* 1971). Selenium values derived from the red alga, *Acanthophora spicifera*, during the current study ranged from <2-18 ng/g on a wet weight basis (Table 7). The dry weight equivalent values were <17-150 ng/g. Such low values are therefore considered to be representative of selenium impoverished waters when weighed against the aforementioned published data.

Higher plants appear to have lost Se essentiality (Araie and Shiraiwa 2009, Pilon-Smit 2015) and many land species are either poor accumulators or non accumulators of this element. Grasses in particular show very little propensity for selenium with reported levels typically ranging from

10-40 ng/g on a dry weight basis (Fordyce 2012). Interestingly, plants that have returned to the ocean do not regain their selenium dependence. Little wonder, then, that seagrasses analyzed during the present study almost always tested negative (<2 ng/g wet weight or approximately <20 ng/g on a dry weight basis) for this element (Table 8). Low selenium levels occasionally recorded in *Halodule uninervis* were attributed to residual sediment contamination.

Bivalves:
Bivalves typically biomagnify selenium to levels higher than those found in surrounding sediments, and is evidenced in the current work (Table 9). They also accumulate this element largely via the ingestion of particulate matter (planktonic and mineral) rather than by direct uptake from water alone (Stewart *et al.* 2004). Since bivalves are a significant source of selenium to major predators that feed upon them, including humans, existing levels in some species may be a source of risk to unwary consumers (Fan *et al.* 1988).

Several studies have exploited the biomonitoring potential of bivalves for monitoring selenium abundance in aquatic environments, and numerous papers on this subject exist in the literature. It is interesting to note that the selenium data for bivalves from selenium enriched and non enriched water rarely differs by more than an order of magnitude. This strongly suggests that the group as a whole possesses some degree of metabolic control over selenium levels in their tissues, which in turn implies that bivalves are not particularly sensitive biomonitors for this element. Examples of selenium levels reported in the literature for bivalves are provided below.

Maher *et al.* (1992) reviewed the selenium data for a number of Australian mollusks and noted that 95% of reported levels were less than 3.00 µg/g wet weight, with approximately half of these coming in under 0.50 µg/g. Benthic clams from the selenium enriched waters of North San Francisco Bay accumulated 5-20 µg/g of selenium on a dry weight basis (Linville *et al.* 2002, Stewart *et al.* 2004). These values are equivalent to ~0.60-2.4 µg/g wet weight and hence within the range reported above by Maher *et al.* (1992). The 1986 summary report of the NS&T Mussel Watch program (145 sites) provided a national grand median value for selenium in shellfish of 0.30 µg/g wet weight, with a range of 0.11-0.98 µg/g (IOM 1991). From these data it seems likely that baseline selenium levels in bivalves from clean coastal environments normally lie somewhere between 0.10 µg/g and 0.30 µg/g, and rarely exceed 3.0 µg/g even in highly enriched waters.

In the current study, selenium concentrations in all bivalves examined ranged from 0.32-0.70 µg/g wet weight (Table 9). Overall mean values in *Atactodea striata, Ctena bella, Gafrarium pectinatum* and *Quidnipagus palatum* were of 0.57, 0.37, 0.47 and 0.49 µg/g respectively. These findings are generally considered to be indicative of light to moderate selenium enrichment along the AMME shoreline when weighed against the preceding data.

Concluding Remarks:
Some degree of selenium enrichment along the AMME shoreline is to be expected given prevalence of fossil fuel burning facilities in the general area. The Port of Saipan lies just 0.5 km to the NE, for example, and a major power station operates just 1 km beyond that. Exhaust plumes from shipping and boating activities are also contributing sources of selenium to the local environment, as were past land-use activities associated with the military occupation during and

immediately after WWII. This diversity of potential sources notwithstanding, the AMME shoreline appears to be only mildly impacted by selenium with sediments serving as the primary repository for this element. Data from the seaweed analysis suggests that dissolved selenium levels in adjacent nearshore waters are unremarkable from an environmental standpoint. Selenium-mercury molar ratios in the bivalves examined were well in excess of 1 (Table 10) and thus afford more than adequate protection against mercury toxicity to anyone who cares to eat them. The risks of selenium toxicity to avid consumers of shellfish along the AMME shoreline is also low, given that the safe and adequate range of dietary selenium intake is 50-200 µg/day (US Food and Nutrition Board 1980, NRC 1980). An individual would need to consume around 40 *Quidnipagus palatum* on a daily basis just to match the upper dietary limit of 200 µg.

ZINC (Zn):

Although zinc is not appreciably toxic, it is a ubiquitous contaminant and is sometimes released into the aquatic environment in substantial quantities (Bryan and Langston 1992). Surface water concentrations in the open ocean are around 0.01 µg/L (Bruland *et al.* 1978, Bruland 1980) while closer to shore they are generally higher and show greater variability. A mean value of 0.161 µg/L was reported by Bruland and Franks (1981) for uncontaminated coastal waters of the NW Atlantic, and Denton and Burdon-Jones (1986c) recorded levels of 0.06-0.44 µg/L in waters from the Australian Great Barrier Reef.

In harbor environments and polluted estuaries dissolved zinc levels are considerably higher and typically range from 10-50 µg/L (Preston *et al.* 1972, Abdullah and Royle 1974, Zingde *et al.* 1976, Burdon-Jones *et al.* 1982, Scoullos and Dassenakis 1983). One of the highest levels recorded is 305 µg/L from Restronguet Creek, a tidal arm of a large Cornish estuary in the UK that drains an area of heavily mineralized Devonian rocks and ancient mine workings (Klumpp and Peterson 1979).

Lithogenic sediments from uncontaminated waters typically contain zinc levels of 5-50 µg/g depending upon local geology (Moore 1991). Residues exceeding 3,000 µg/g are frequently found in the vicinity of mines and smelters (Bryan *et al.* 1985) and in contaminated harbor environments (Poulton 1987, Legorburu and Canton 1991). Levels in nearshore bioclastic deposits are normally within 3-5 µg/g, and may drop below 1 µg/g in clean coral sands (Denton *et al.* 1997, 2001, 2014).

AMME Shoreline Sediments:

Sedimentary zinc levels reported here for AMME shoreline sediments were generally low with over 80% of all sites showing either light to moderate enrichment or no enrichment at all (Tables 5-6). As with copper, mercury and lead, the highest levels west of the causeway were confined to sites 25-28 near the *Smiling Cove Marina* and constructed wetland discharge point. Generally speaking, higher zinc concentrations occurred east of the causeway with maximum levels confined to shoreline sediments at either end of the embayment, i.e., at sites 29, 30, 37. These sites were classified as significantly enriched, whereas all other sites along this stretch of coastline fell into the light to moderately enrichment category. Zinc levels in surface sediment from site 37 were just 42.2 µg/g compared with 358 µg/g 13 years ago (Denton *et al.* 2009). This is a remarkable decline that can only be explained by cleaner material contributing to sediment accretion processes in recent years.

Marine Plants:
Marine algae readily concentrate zinc. Among the brown algae, which are most commonly used as biomonitors of heavy metal pollution, levels ranging from several hundred to several thousand µg/g have been recorded in species from severely polluted environments (Bryan and Hummerstone 1973, Fuge and James 1973, Haug *et al.* 1974, Stenner and Nickless 1974, Melhuus *et al.* 1978). In clean environments, levels are usually less than 10 µg/g. Denton and Burdon-Jones examined zinc levels in 48 species of algae from the Australian Great Barrier Reef and reported overall mean zinc concentrations of 2.0, 2.7, and 2.2 µg/g for brown, red, and green representatives respectively (Denton and Burdon-Jones 1986a).

Baseline levels for *Acanthophora spicifera* from a relatively clean coastal environment in Guam ranged from 3.14-8.14 µg/g (Denton and Morrison 2009). These values compare well with values of 8.0-13.0 µg/g reported for this species from the Australian Great Barrier Reef (Denton and Burdon-Jones 1986a). Mean zinc levels found in *A. spicifera* from Saipan Lagoon back in 2003 ranged from 17.6-22.6 µg/g, in samples from more remotely located sites in the northern part of the lagoon to 130 µg/g at *Seaplane Ramps* near the port (Denton *et al.* 2009). A composite sample taken from Micro Beach at the time yielded a zinc value of 57.4 µg/g. The latter value falls within the concentration range reported here for *A. spicifera* (Table 7) and implies the soluble zinc loading of AMME nearshore waters has remained reasonably constant over this time frame.

Harbor waters and marinas are typically enriched with soluble zinc leached predominantly from boat paints, galvanized structures and the sacrificial anodes of watercraft. Highway runoff also contains relatively high levels of zinc from vehicular sources (Makepeace *et al.* 1995). Not surprisingly then, levels determined in *A. spicifera* during the present work showed a progressive increase in zinc levels towards *Micro Point* and *Smiling Cove Marina* (sites 5 and 7).

The ability of seagrasses to reflect changes in ambient zinc availability remains in question. Baseline value for this element in *Enhalus acoroides* from clean waters are in the order of 5-20 µg/g (Denton and Morrison 2009), and comparatively high levels have certainly been measured in this species from zinc enriched waters. At *Fishing Base* in Saipan, for example, levels of 74.5-87.5 µg/g were found in specimens taken adjacent to the public boat ramp. Likewise, specimens impacted by runoff from a car dealership further south in the lagoon contained 77.1-102 µg/g zinc in blade tissue. Sedimentary zinc levels at both locations were 46 µg/g and 56 µg/g respectively and fell within the high to very high enrichment category for this element (Denton *et al.* 2014). Conflicting results were found earlier by Denton *et al.* (2009) who reported near baseline zinc levels of 29 µg/g and 33 µg/g in *E. acoroides* from polluted shoreline waters beside *Seaplane Ramps* and the *Puerto Rico Dump* respectively. Corresponding zinc concentrations in sediments at each of these locations were 84 µg/g and 358 µg/g.

In the current study, mean zinc levels in *E. acoroides* ranged from 18-25 µg/g. Once again, it can be seen that levels sequestered by these plants hover around baseline values (Table 7) despite obvious zinc enrichment in sediments at biota site 7 alongside *Smiling Cove Marina,* and biota site 8 on the opposite side of the causeway (Table 5, Figs 2-3).

Clearly metal kinetics in seagrasses are very different than in algae and have yet to be fully elucidated. As land plants that have essentially returned to the ocean, seagrasses have had to overcome many challenges, including those associated with nutrient uptake and metal transport, and have developed intriguing strategies in order to survive sediments high in sulfur and other toxic elements. Comparatively little is known about their physiology in this regard, although it would appear that metal binding proteins and other chelating compounds are involved (Papenbrock 2012).

Bivalves:
Bivalves are frequently used to monitor zinc levels in the marine environment although comparatively little data exists for the species examined during the present study. Based on dataset comparisons with specimens taken from Pago Bay in Guam, *Quidnipagus palatum* obviously possesses some biomonitoring potential for zinc, whereas *Gafrarium pectinatum* clearly does not (Denton and Morrison 2009). Levels recorded in the former species from AMME in the current study ranged from 303-1,142 µg/g (overall mean: 692 µg/g) compared with 94-341 µg/g (mean 222 µg/g) in specimens from Pago Bay.

Ctena bella also appears to have biomonitoring potential for this element with mean levels approaching 300 µg/g in the current study compared with a little under 200 µg/g in representatives from Pago Bay (Denton and Morrison 2009). Interestingly, Denton and coworkers examined the latter species from *Micro Point* beach in 2003 and reported zinc levels of 384-430 µg/g (Denton *et al.* 2009). The somewhat higher levels emerging from their study compared with current investigation is thought to represent the closer proximity of their sampling site to *Smiling Cove Marina.*

Whether *Atactodea striata* is suitable as a biomonitor for zinc now seems unlikely in light of recent data acquired for this species during the current study. An earlier investigation by Denton *et. al.* (2009) certainly cast doubts on this bivalve's ability to perform as a zinc sentinel after levels in representatives from *Micro Point* (112-129 µg/g) were found not to differ significantly from their counterparts at several more remotely located sites further north in the lagoon (71.8-147 µg/g). Comparable zinc concentrations of 81.3-134 µg/g were determined during this work providing further evidence of zinc regulation in this species.

Concluding Remarks:
Zinc enrichment was fairly widespread throughout the study area reflecting past and present land-use activities in the park and surrounding properties as well the ubiquity of this element as an environmental contaminant. In fact, of all the elements considered here, zinc is perhaps the only metal in developed coastal waters that consistently occurs at levels well beyond those typically released from the lithosphere by volcanism and natural weathering processes (Phillips 1980). But be that as it may, levels encountered in sediments examined in AMME shoreline sediments were, for the most part, comparatively low by world standards with levels well below sediment quality guidelines formulated for the protection of aquatic species (Table 11). Levels in the biota examined generally convey the same message despite significant interspecific differences in each organism affinity for this element.

Table 5: Heavy Metals in Shoreline Sediments from AMME Seaward Boundary

Site	Shoreline Distance (m)	Metal Levels (µg/g dry weight)								
		Ag	Cd	Cr	Cu	Hg[a]	Ni	Pb	Se[a]	Zn
***Micro Beach* (from Hyatt Boundary)**										
1	0	<0.14	<0.14	4.15	5.41	0.42	<0.18	<0.68	88.0	1.35
2	50	<0.15	<0.15	3.71	5.22	0.94	<0.20	<0.75	105	1.34
3	100	<0.15	<0.15	3.61	5.55	1.42	<0.20	<0.75	96.1	1.95
4	150	<0.14	<0.14	3.84	4.93	1.83	<0.19	<0.72	-	2.32
5	200	<0.14	<0.14	4.09	5.36	1.38	<0.19	<0.72	-	2.90
6	250	<0.15	<0.15	3.77	5.85	1.38	<0.20	<0.73	-	2.78
7	300	<0.15	<0.15	3.47	5.40	2.90	<0.20	<0.75	-	2.70
8	350	<0.15	<0.15	3.57	5.43	1.95	<0.20	<0.75	-	3.17
9	400	<0.15	<0.15	3.67	6.78	1.95	<0.20	<0.75	-	3.00
10	450	<0.14	<0.14	4.89	6.09	1.87	<0.19	<0.72	-	2.75
11	500	<0.15	<0.15	6.51	6.24	3.35	<0.20	<0.74	64.0	3.12
12	550	<0.15	<0.15	5.33	5.70	3.39	<0.20	<0.75	-	2.85
13	600	<0.14	<0.14	4.36	5.14	2.24	0.38	<0.69	-	2.64
14	650	<0.15	<0.15	3.95	5.55	1.94	0.41	<0.75	-	2.40
15	700	<0.15	<0.15	3.58	5.71	1.42	0.59	<0.73	-	2.63
16	750	<0.15	<0.15	3.69	5.97	3.12	<0.20	<0.73	-	3.06
17	800	<0.15	<0.15	3.80	5.33	1.63	<0.20	<0.76	-	3.65
18	850	<0.15	<0.15	4.03	5.76	1.08	<0.20	<0.76	-	3.18
19	900	<0.15	<0.15	4.29	5.91	1.08	<0.20	<0.76	-	3.33
20	950	<0.15	<0.15	4.20	5.76	1.06	<0.20	<0.74	-	3.25
21	1000	<0.15	<0.15	4.11	6.65	1.58	<0.20	<0.74	-	4.14
22	1050	<0.15	<0.15	3.92	6.60	2.14	<0.20	<0.75	-	3.75
23	1100	<0.15	<0.15	3.74	6.67	2.07	<0.19	<0.72	-	4.20
24	1150	<0.15	<0.15	5.15	6.21	2.11	<0.20	<0.74	-	4.58
25	1200	<0.15	<0.15	7.10	8.93	5.75	<0.20	2.93	37.0	9.66
26[b]	1220	<0.15	<0.15	6.38	11.1	8.89	0.20	6.95	16.0	23.4
27	1250	<0.15	<0.15	5.07	7.98	3.17	0.39	2.59	-	7.98
28	1170	<0.15	<0.15	4.03	13.3	6.24	0.58	4.00	-	10.5
***Puerto Rico Dump Bay* (from causeway)**										
29[c]	0	<0.15	<0.15	5.47	15.6	37.5	1.69	12.5	75.0	52.9
30	100	<0.15	<0.15	7.03	9.90	18.9	0.58	5.91	181	22.2
31	200	<0.15	<0.15	6.89	9.80	31.1	1.52	8.17	470	13.2
32	300	<0.15	<0.15	5.88	6.52	16.8	0.56	2.54	159	8.41
33[c]	400	<0.15	<0.15	5.88	6.47	14.2	0.38	1.84	63.0	7.94
34	500	<0.15	<0.15	6.75	7.09	11.8	0.39	3.77	55.0	11.3
35	600	<0.15	<0.15	6.12	6.87	9.79	0.77	2.99	44.0	8.66
36	700	<0.15	<0.15	5.30	6.83	8.35	0.57	4.08	43.0	7.43
37[c]	800	<0.15	<0.15	8.25	12.2	27.3	0.93	13.8	88.0	42.2

[a]Mercury and selenium as ng/g dry weight; [b]AMME constructed wetland disharge point; [c]Stormdrain discharge point; Dashes indicate no data

Table 6: Heavy Metal Enrichment in Shoreline Sediments from AMME Seaward Boundary

Enrichment Category	Sites Showing some Degree of Enrichment above Baseline				
	Cu	Hg	Ni	Pb	Zn
Very high	-	-	-	-	-
High	-	29, 31	-	-	29
Significant	all other sites	26, 28, 30, 32-37	29	26, 28, 29, 31, 37	26, 28, 30, 37
Light to moderate	11	4, 7-9, 11-14, 16,17, 22, 23, 25, 27	14, 15, 28, 31, 35-37	25, 27, 30, 32, 34-36	8, 9, 16-25, 27, 31-36
None	-	all other sites	all other sites	all other sites	all other sites

Metal values from all sites were normalized against those obtained from sites 1-3 (reference sites) using Cr as the normalizing element (Salomons and Förstner 1984). Overall geometric mean values for Cr, Cu, Hg, Ni, Pb, and Zn from the reference sites were: 3.92, 0.81, 0.00083, 0.19, 0.72, and 1.52 µg/g respectively. Since Cu was clearly enriched at all AMME sites, the reference value adopted for this element (0.81 µg/g) was derived from bioclastic deposits taken from a clean coastal environment on Guam (Denton and Morrison 2009). No suitable reference value was found for Se. Cadmium and Ag were consistently below analytical detection limits. Dashes indicate no site enrichment at category indicated.

Table 7: Heavy Metals in Seaweed from AMME Seaward Boundary

Species/Site	Collection Date	Replicates/Metal Hg/Se	Replicates/Metal All Others	Statistic[a]	Ag	Cd	Cr	Cu	Hg[b]	Ni	Pb	Se[b]	Zn
					Metal Levels (µg/g dry weight)								
***Acanthophora spicifera* (red alga)**													
1	7/1/2016	2	3	Mean:	nc	0.24	3.16	2.70	1.69	3.19	1.53	2.22	43.4
				Range:	all <0.10	0.18-0.30	2.99-3.25	2.56-2.78	1.29-2.20	2.90-3.51	1.40-1.68	1.98-2.48	42.0-44.6
2	7/1/2016	2	3	Mean:	nc	0.24	3.98	4.02	1.86	3.18	1.76	14.1	49.4
				Range:	all <0.10	0.19-0.28	3.92-4.06	3.97-4.06	1.74-1.99	3.01-3.37	1.74-1.78	11.8-16.8	48.6-50.7
3	7/1/2016	2	3	Mean:	nc	nc	3.06	2.30	1.67	3.31	0.98	12.5	35.6
				Range:	all <0.12	all <0.12	3.03-3.08	2.14-2.43	1.64-1.70	3.16-3.54	0.81-1.16	9.49-16.4	34.5-36.8
4	7/2/2016	2	3	Mean:	nc	0.17	4.55	4.35	1.39	2.58	2.39	17.6	47.0
				Range:	all <0.09	0.16-0.17	4.42-4.66	4.30-4.46	1.61-1.20	2.51-2.67	2.22-2.60	17.3-17.9	45.6-48.5
5	7/2/2016	2	3	Mean:	nc	0.18	2.80	4.80	1.57	2.40	0.91	4.75	55.8
				Range:	all <0.09	0.16-0.19	2.78-2.82	4.63-4.95	1.39-1.78	1.56-3.69	0.77-1.15	4.12-5.48	53.5-57.3
6	7/3/2016	2	3	Mean:	nc	0.25	3.13	6.47	1.58	1.96	1.13	1.43	67.2
				Range:	all <0.12	0.24-0.26	3.08-3.23	5.91-6.85	1.50-1.67	1.89-2.07	1.08-1.24	1.29-1.59	64.0-69.5
7	7/3/2016	2	3	Mean:	nc	0.13	2.40	11.4	1.20	2.06	0.83	nc	74.4
				Range:	all <0.13	0.14-0.13	2.35-2.42	10.8-11.8	1.14-1.25	1.92-2.21	0.81-0.87	all <2.00	73.8-75.1
8	7/20/2016	2	3	Mean:	0.21	0.84	2.62	5.44	3.20	2.86	1.01	nc	60.8
				Range:	<0.14-0.29	0.81-0.87	2.56-2.70	5.35-5.58	3.01-3.40	2.77-2.96	0.87-1.21	all <2.00	58.4-63.3

[a]Mean = geometric mean; [b]Mercury and selenium as ng/g wet weight; nc = not calculable

Table 8: Heavy Metals in Seagrass from AMME Seaward Boundary

Species/Site	Collection Date	Replicates/Metal		Statistic[a]	Metal Levels (µg/g dry weight)								
		Hg/Se	All Others		Ag	Cd	Cr	Cu	Hg[b]	Ni	Pb	Se[b]	Zn
Enhalus acoroides													
3	7/1/2016	2	3	Mean:	nc	nc	0.72	3.70	1.85	1.02	nc	nc	17.7
				Range:	all <0.16	all <0.16	0.67-0.77	3.57-3.82	1.38-2.49	0.91-1.12	all <0.33	all <2.00	17.7-17.7
4	7/2/2016	2	3	Mean:	nc	nc	0.77	5.49	1.61	0.78	nc	nc	22.3
				Range:	all <0.16	all <0.16	0.57-0.98	4.82-6.03	1.44-1.80	0.60-0.88	all <0.33	all <2.00	21.0-24.0
5	7/2/2016	2	3	Mean:	nc	nc	0.65	7.76	1.41	0.93	nc	nc	24.6
				Range:	all <0.20	all <0.20	0.59-0.73	7.10-8.27	1.29-1.53	0.84-1.04	all <0.16	all <2.00	23.9-26.1
6	7/3/2016	2	2	Mean:	nc	nc	0.70	12.4	1.86	1.02	nc	nc	24.5
				Range:	all <0.19	all <0.19	0.61-0.81	12.4-12.4	1.58-2.20	1.96-1.08	all <0.39	all <2.00	22.6-26.5
7	7/3/2016	2	3	Mean:	nc	0.29	0.75	29.8	1.99	0.92	nc	nc	24.0
				Range:	all <0.14	0.22-0.41	0.65-0.90	29.2-30.6	1.86-2.14	0.80-1.09	all <0.29	all <2.00	23.3-24.6
8	7/20/2016	2	3	Mean:	nc	0.47	0.48	5.71	3.56	0.99	nc	nc	19.9
				Range:	all <0.49	0.43-0.50	0.39-0.70	5.49-5.88	3.47-3.65	0.86-1.12	all <0.52	all <2.00	17.1-21.6
9	7/20/2016	2	3	Mean:	nc	0.84	0.88	7.96	3.50	1.27	nc	nc	20.4
				Range:	all <0.27	0.67-1.11	0.72-1.08	7.67-8.56	2.51-4.89	1.24-1.28	all <0.56	all <2.00	19.7-21.0
Halodule uninervis													
1	7/1/2016	2	3	Mean:	nc	nc	1.18	4.15	2.90	0.94	0.77	nc	44.2
				Range:	all <0.13	all <0.13	1.00-1.51	3.99-4.26	2.90-2.90	0.85-1.07	0.70-0.83	all <2.00	43.5-45.1
2	7/1/2016	2	3	Mean:	nc	nc	1.47	3.90	3.20	1.00	0.82	17.2	39.3
				Range:	all <0.13	all <0.13	1.29-1.70	3.67-4.14	3.12-3.28	0.92-1.06	0.80-0.83	16.8-17.5	38.5-40.5
3	7/1/2016	2	3	Mean:	nc	nc	1.09	3.68	2.48	1.26	0.92	2.56	47.3
				Range:	all <0.12	all <0.12	0.78-1.38	3.55-3.77	2.19-2.81	1.11-1.40	0.75-1.03	<2.00-3.50	43.9-50.3
4	7/2/2016	2	3	Mean:	nc	nc	1.43	4.23	2.40	1.58	0.79	7.64	57.8
				Range:	all <0.14	all <0.14	1.34-1.53	3.99-4.62	2.18-2.64	1.09-2.91	0.74-0.84	4.82-12.1	56.5-58.9

[a]Mean = geometric mean; [b]Mercury and selenium as ng/g wet weight; nc = not calculable

Table 9: Heavy Metals in Bivalves from AMME Seaward Boundary

Species/Site	Collection Date	Shell Length[a] (replicates)		Statistic[b]	Metal Levels (µg/g dry weight)									Wet:Dry wt. Ratio
		Hg/Se	Other Metals		Ag	Cd	Cr	Cu	Hg[c]	Ni	Pb	Se[d]	Zn	
Atactodea striata														
2	7/1/2016	2.5-2.6 (5)	2.7-3.0 (5)	Mean:	0.35	0.42	1.14	10.3	18.0	3.97	nc	0.59	102	6.2
				Range:	0.29-0.62	0.29-0.62	0.91-1.60	9.01-12.0	16.3-20.6	3.36-4.24	<0.38-0.72	0.48-0.69	81.3-130	5.6-7.0
3	7/1/2016	2.4-2.6 (5)	2..4-2.9 (5)	Mean:	0.30	0.35	1.00	10.7	19.3	3.51	nc	0.62	97.7	6.2
				Range:	<0.17-0.63	0.19-0.47	0.84-1.13	9.83-11.5	18.2-20.5	3.20-3.83	<0.34-0.83	0.59-0.66	84.5-114	6.1-6.5
4	7/2/2016	2.3-2.8 (5)	2.4-3.0 (4)	Mean:	nc	0.26	1.06	12.4	22.0	3.69	0.54	0.60	99.3	6.1
				Range:	all <0.14	0.14-0.38	0.94-1.25	11.5-14.5	19.1-24.2	3.47-4.20	0.36-0.70	0.56-0.66	86.8-112	5.9-6.4
5	7/2/2016	2.8-3.0 (5)	2.5-3.3 (5)	Mean:	nc	0.32	0.97	12.2	11.6	3.09	0.68	0.58	109	5.9
				Range:	all <0.12	0.26-0.37	0.82-1.12	11.6-13.1	11.2-11.8	2.86-3.34	0.51-0.93	0.56-0.64	94.6-117	5.5-6.4
6	7/3/2016	2.2-2.6 (5)	2.4-2.9 (5)	Mean:	nc	0.34	1.21	11.8	10.8	2.24	0.67	0.43	108	6.60
				Range:	all <0.18	0.31-0.36	1.01-1.82	11.3-12.5	8.76-12.8	2.11-2.38	<0.39-0.91	0.32-0.55	97.2-134	6.1-7.2
Ctenna bella														
3	7/1/2016	2.1 (1)	-	Mean:	-	-	-	-	11.6	-	-	0.39	-	-
				Range:	-	-	-	-	-	-	-	-	-	-
4	7/2/2016	2.7- 2.8 (2)	2.4-3.2 (3)	Mean:	0.85	0.65	1.07	38.7	15.3	6.28	8.06	0.44	326	5.3
				Range:	0.71-1.15	0.38-1.04	0.61-1.51	28.1-49.4	14.0-16.7	4.77-8.50	6.68-10.9	0.30-0.65	227-463	5.2-5.4
5	7/2/2016	2.9-3.6 (4)	2.5-3.1 (4)	Mean:	0.44	1.13	0.95	33.4	5.91	3.30	6.94	0.36	277	4.20
				Range:	0.35-0.57	0.74-1.70	0.84-1.07	28.9-37.5	5.10-6.85	2.72-4.77	5.64-11.4	0.34-0.39	245-314	4.0-4.6
6	7/3/2016	3.1 (1)	-	Mean:	-	-	-	-	9.21	-	-	0.30	-	-
				Range:	-	-	-	-	-	-	-	-	-	-

[a]Shell length in cm; [b]Mean as geometric mean; [c]Mercury as ng/g wet weight; [d]Selenium as µg/g wet weight; nc = not calculable; dashes indicate no data

Table 9 (cont.): Heavy Metals in Bivalves from AMME Seaward Boundary

Species/Site	Collection Date	Shell Length[a] (replicates)		Statistic[b]	Metal Levels (µg/g dry weight)									Wet:Dry wt. Ratio
		Hg/Se	Other Metals		Ag	Cd	Cr	Cu	Hg[c]	Ni	Pb	Se[d]	Zn	
Gafrarium pectinatum														
4	7/2/2016	3.4-3.7 (2)	2.9-3.3 (3)	Mean:	0.19	0.19	0.62	11.0	16.0	10.9	1.00	0.46	62.4	6.1
				Range:	0.17-0.24	0.17-0.24	0.39-1.08	8.78-16.6	15.4-16.8	9.51-12.6	0.72-1.39	0.41-0.51	50.1-88.8	5.6-6.6
5	7/2/2016	3.4-3.6 (2)	-	Mean:	-	-	-	-	22.9	-	-	0.51	-	-
				Range:	-	-	-	-	20.2-26.0	-	-	0.46-0.56	-	-
6	7/3/2016	2.9 (1)	-	Mean:	-	-	-	-	17.1	-	-	0.42	-	-
				Range:	-	-	-	-	-	-	-	-	-	-
8	7/20/2016	-	3.3 (1)	Mean:	0.97	0.65	0.78	20.2	-	8.53	10.1	-	65.7	6.3
				Range:	-	-	-	-	-	-	-	-	-	
11	7/19/2016	3.1 (2)	3.7 (1)	Mean:	0.31	0.47	1.01	11.4	17.8	9.70	27.7	0.50	69.9	6.4
				Range:	-	-	-	-	16.7-19.1	-	-	0.50-0.50	-	
Quidnipagus palatum														
4	7/2/2016	5.4 (1)	-	Mean:	-	-	-	-	26.0	-	-	0.67	-	-
				Range:	-	-	-	-	-	-	-	-	-	-
8	7/20/2016	3.9-4.1 (5)	3.7-5.3 (7)	Mean:	0.38	nc	4.14	472	210	6.08	16.3	0.43	610	5.8
				Range:	0.17-0.67	all <0.16	2.85-4.77	173-1179	168-250	3.84-7.78	8.97-24.6	0.39-0.55	405-1018	5.4-6.2
9	7/20/2016	3.6-4.3 (5)	3.4-5.0 (5)	Mean:	1.22	nc	5.87	399	88.0	7.67	12.5	0.58	615	5.7
				Range:	0.33-4.90	all <0.16	3.66-8.16	95.6-892	44.8-185	4.30-10.6	6.59-20.7	0.45-0.70	303-964	5.5-8.3
10	7/19/2016	3.4-4.0 (5)	4.0-4.7 (5)	Mean:	0.34	nc	6.53	301	44.4	11.1	12.1	0.43	935	6.8
				Range:	0.24-0.59	all <0.16	5.88-7.01	181-501	40.2-44.4	9.83-13.1	10.7-14.4	0.38-0.46	780-1142	6.1-7.4
11	7/19/2016	3.0- 3.7 (4)	3.0-3.9 (4)	Mean:	0.27	nc	8.24	908	186	14.4	83.9	0.49	685	6.5
				Range:	0.18-0.42	all <0.12	6.76.11.2	646-1219	149-232	11.4-20.1	55.0-134	0.38-0.60	516-891	5.9-7.2

[a]Shell length in cm; [b]Mean as geometric mean; [c]Mercury as ng/g wet weight; [d]Selenium as µg/g wet weight; nc = not calculable; dashes indicate no data

Table 10: Selenium:Mercury Molar Ratios in Bivalves from AMME Seaward Boundary

Species/Site	Shell Length (cm)	Replicate	ng/g wet wt.		nM/g		Se:Hg Molar Ratio
			Se	Hg	Se	Hg	
Atactodea striata							
2	2.5-2.6	1	479	16.3	6.07	0.08	75
		2	609	20.6	7.72	0.10	75
		3	522	18.1	6.61	0.09	73
		4	676	17.0	8.56	0.08	101
		5	690	18.6	8.74	0.09	94
3	2.4-2.6	1	651	20.5	8.24	0.10	81
		2	599	18.7	7.58	0.09	82
		3	588	19.7	7.45	0.10	76
		4	597	19.8	7.57	0.10	77
		5	657	18.2	8.32	0.09	92
4	2.3-2.8	1	643	24.2	8.14	0.12	68
		2	604	19.1	7.65	0.10	80
		3	657	20.6	8.32	0.10	81
		4	565	23.2	7.16	0.12	62
		5	558	23.2	7.06	0.12	61
5	2.8-3.0	1	570	11.7	7.21	0.06	124
		2	562	11.2	7.11	0.06	127
		3	644	11.8	8.15	0.06	139
		4	562	11.5	7.12	0.06	124
		5	590	11.8	7.48	0.06	127
6	2.2-2.6	1	547	12.8	6.92	0.06	109
		2	487	10.3	6.17	0.05	120
		3	523	11.7	6.62	0.06	114
		4	325	10.8	4.11	0.05	76
		5	332	8.76	4.21	0.04	96
Ctena bella							
3	2.1	1	388	11.6	4.92	0.06	85
4	2.7- 2.8	1	304	14.0	3.85	0.07	55
		2	648	16.7	8.20	0.08	98
5	2.9-3.6	1	357	5.53	4.52	0.03	164
		2	341	5.10	4.32	0.03	170
		3	358	6.85	4.53	0.03	133
		4	386	6.31	4.89	0.03	155
6	3.1	1	299	9.21	3.79	0.05	82

Table 10 (cont.): Selenium:Mercury Molar Ratios in Bivalves from AMME Seaward Boundary

Species/Site	Shell Length (cm)	Replicate	ng/g wet wt.		nM/g		Se:Hg Molar Ratio
			Se	Hg	Se	Hg	
Gafrarium pectinatum							
4	3.4-3.7	1	408	16.7	5.17	0.08	62
		2	510	15.4	6.46	0.08	84
5	3.4-3.6	1	564	26.0	7.14	0.13	55
		2	462	20.2	5.85	0.10	58
6	2.9	1	420	17.1	5.32	0.09	62
11	3.1	1	500	16.7	6.34	0.08	76
		2	504	19.1	6.38	0.09	67
Quidnipagus palatum							
11	3.0-3.7	1	529	232	6.70	1.16	6
		2	378	215	4.79	1.07	4
		3	597	160	7.56	0.80	10
		4	501	149	6.35	0.74	9
10	3.4-4.0	1	385	42.5	4.87	0.21	23
		2	461	45.3	5.84	0.23	26
		3	447	40.2	5.66	0.20	28
		4	436	40.6	5.52	0.20	27
		5	404	55.1	5.12	0.27	19
9	3.6-4.3	1	697	138	8.82	0.69	13
		2	634	185	8.03	0.92	9
		3	669	44.8	8.47	0.22	38
		4	454	64.5	5.75	0.32	18
		5	469	71.6	5.94	0.36	17
8	3.9-4.1	1	388	168	4.91	0.84	6
		2	506	210	6.40	1.04	6
		3	420	225	5.32	1.12	5
		4	457	229	5.79	1.14	5
		5	502	189	6.36	0.94	7
		6	545	250	6.90	1.25	6
4	5.4	1	669	26	8.47	0.13	65

Molar concentrations were calculated by dividing Hg and Se concentrations by their respective atomic weights.
Thus Se and Hg tissue concentrations presented above were divided by 78.96 and 200.59 respectively.

Table 11: Numerical Sediment Quality Guidelines for US Waters

Element	NOAA National Status & Trends Program[a]		Florida Department of Environmental Protection[b]	
	Effects Range-Low (ERL)[c]	Effects Range-Median (ERM)[c]	Threshod Effects Level (TEL)[c]	Probable Effects Level (PEL)[c]
Arsenic	8.2	70	7.24	41.6
Cadmium	1.2	9.6	0.68	4.21
Chromium	81	370	52.3	160
Copper	34	270	18.7	108
Lead	46.7	218	30.2	112
Mercury	0.15	0.71	0.13	0.7
Nickel	20.9	51.6	15.9	42.8
Silver	1	3.7	0.73	1.77
Zinc	150	410	124	271

[a]Long *et al*. 1995; [b]MacDonald *et al*. 1996; [c]All values as µg/g dry weight; No SQG currently available for selenium

Note: Below the ERL and TEL values adverse effects are rarely reported; between the ERL/ERM guidelines and TEL/PEL guidelines negative biological effects are occasionally observed, and above the ERM/PEL guidelines adverse effects frequently occur.

Table 12: Compilation of International Regulatory Limits for Heavy Metals in Seafood. (All values listed as μg/g wet wt.)

Country	Cd	Cr	Cu	Hg	Ni	Pb	Se	Zn
Australia	2.0 5.5	-	30 70[a]	0.5[b,c]	-	2.5 5.5	1.0 2.0[b]	40 1000[a]
Brazil	-	-	-	0.5	-	-	-	-
Canada	-	-	-	0.5	-	0.5[b]	-	-
Chile	0.05[c]	-	10[c]	-	-	2.0[c]	0.3[c]	100[c]
Finland	-	-	-	1.0[b]	-	2.0	-	-
France	-	-	-	0.5	-	-	-	-
Germany	0.5[b]	-	-	1.0[b]	-	0.5[b]	-	-
Greece	-	-	-	0.7[b,d]	-	-	-	-
Hong Kong	2.0	1.0	-	0.5	-	6.0	-	-
India	-	-	10[b]	0.5[b]	-	5.0[b]	-	50[b]
Israel	-	-	-	0.5[b]	-	-	-	-
Italy	-	-	-	0.7[b]	-	2.0	-	-
Japan	-	-	-	0.3[c,d] 0.4[c]	-	-	-	-
Korea	-	-	-	0.5[c]	-	-	-	-
Netherlands	1.0	-	-	1.0[c]	-	2.0	-	-
New Zealand	1.0	-	30	0.5[b]	-	2.0	-	40
Philippines	-	-	-	0.5[c,d]	-	0.5	-	-
Poland	-	-	10 30[b]	-	-	1.0 2.0[b]	-	30 50[b]
Spain	-	-	-	0.5[c]	-	-	-	-
Sweden	-	-	-	1.0[b]	-	1.0[b]	-	-
Switzerland	0.1[c]	-	-	0.5[c]	-	1.0[c]	-	-
Thailand	-	-	20[c]	0.5[c]	-	1.0[c]	-	-
United Kingdom	-	-	20	-	-	2.0[b] 10	-	50
United States	-	-	-	1.0[c]	-	-	-	-
U.S.S.R	-	-	-	0.5[b]	-	-	-	-
Venezuela	0.1	-	10[c]	0.1	-	2.0[c]	-	-
Zambia	-	-	100[c]	0.2 0.3[e,d]	-	0.5 10[b]	-	100[c]

After Nauen (1983). Note: food standards are periodically updated and some of those listed may have been changed or withdrawn.
[a]Oysters. [b]Fish and fish products. [c]Seafood and seafood products including bivalves. All undenoted values are for bivalves only.
[d]Methyl Hg; all other values represent Total Hg; [e]Tuna. Smaller Hg value for Zambia for all other fish.
Dashes indicate no data. No maximum limits avialable for silver (Ag).

Table 13: Current Regulations and Guidelines for Heavy Metal Limits in Seafood (all values listed as µg/g wet weight)

Organization/Country	Metal (µg/g wet weight)								Reference
	Cd	Cr	Cu	Hg	Ni	Pb	Se	Zn	
Australia New Zealand Food Standards Code	2.0	-	-	0.5, 1.0[a]	-	2.0	-	-	FSANZ 2017
FAO/WHO Food Standards Program (CODEX)	2.0	-	-	0.5, 1.0[b,c]	-	0.3[d]	-	-	CODEX 2016
European Union (EU) Commission (EC)	1.0	-	-	0.5 1.0[e]	-	1.5[d]	-	-	EC 2006
US Food and Drug Administration (USFDA)	4[g]	13[g]	-	1.0[cf]	80[g]	1.7[g]	-	-	USFDA 2000, 2001
Health Canada Bureau of Chemical Safety	-	-	-	0.5 1.0[h]	-	0.5	-	-	Health Canada 2016
Food Safety and Standards Authority of India (FSSAI)	2.0	12[i]	30	0.5 1.0[a]	-	1.5	-	50	FSSAI 2011, 2015
Korean Ministry of Food and Drug Safety (KMFDS)	2.0	-	-	0.5	-	2.0	-	-	KMFDC 2014, cited in Mok *et al.* 2014
National Food Safety Standard of China (NFSSC)	2.0	2.0	50	0.5 1.0[j]	-	1.5	1.0[d]	100	NFSSC 2005, 2014
Malaysian Food Regulations (MFR)	1.0	-	30	0.5	-	2.0	-	100	MFR 1983, cited in Hossen *et al.* 2015
Singapore Food Regulations (SFR)	1.0	-	20	0.5 1.0[k]	-	2.0	-	-	SFR 2005

All undenoted values are for bivalve molluscs. All denoted values are defined as follows:

[a]Low Hg value for non predatory fish, crustaceans and molluscs; high value for certain predatory fish species

[b]Low and high Hg values for non predatory and predatory fish species respectively. No Hg value provided for shellfish

[c]Methyl Hg; all other reported values as Total Hg.

[d]Fish only. No Maximum Pb Level provided for other seafood

[e]High mercury value for cetratain predatory fish species; low value for all other fish, fisheries products and crustaceans

[f]Fish, shellfish crustaceans and other aquatic organisms

[g]Non-enforceable guideline value

[h]Low Hg value for all retail fish species with six exceptions (high value)

[i]All fisheries products

[j]High value for predatory fish and their products; low value for all other aquatic animals and products.

[k]High value for predatory fish and their products; low value for all other fish and fish products.

Dashes indicate no data

CONCLUSIONS AND RECOMMENDATIONS

This preliminary investigation adds significantly to the contaminant database for Saipan's coastal waters and identifies areas of heavy metal enrichment within sediments and selected biota from AMME nearshore waters. The results provide a useful database with which future findings can be compared and evaluated. Highlights of the study are summarized below.

HEAVY METAL POLLUTION EAST OF THE MARINA CAUSEWAY:
The study confirmed previous findings of trace metal enrichment in shoreline sediments bordering the Puerto Rico Dump, and has identified other hitherto unknown areas of contamination further west towards the marina causeway. Of particular interest in this regard are the declining metal gradients shown between our sediment collection sites 29 and 31 at the causeway end of this stretch of coastline. The aerial photograph shown below (Fig. 4) clearly identifies buildings and various structures in this section of the park in 1948 with significant human intrusion into this sensitive area during that post-war period. The residual copper, lead and zinc levels noted here may very well have been associated with these constructions and the activities that took place around and since then.

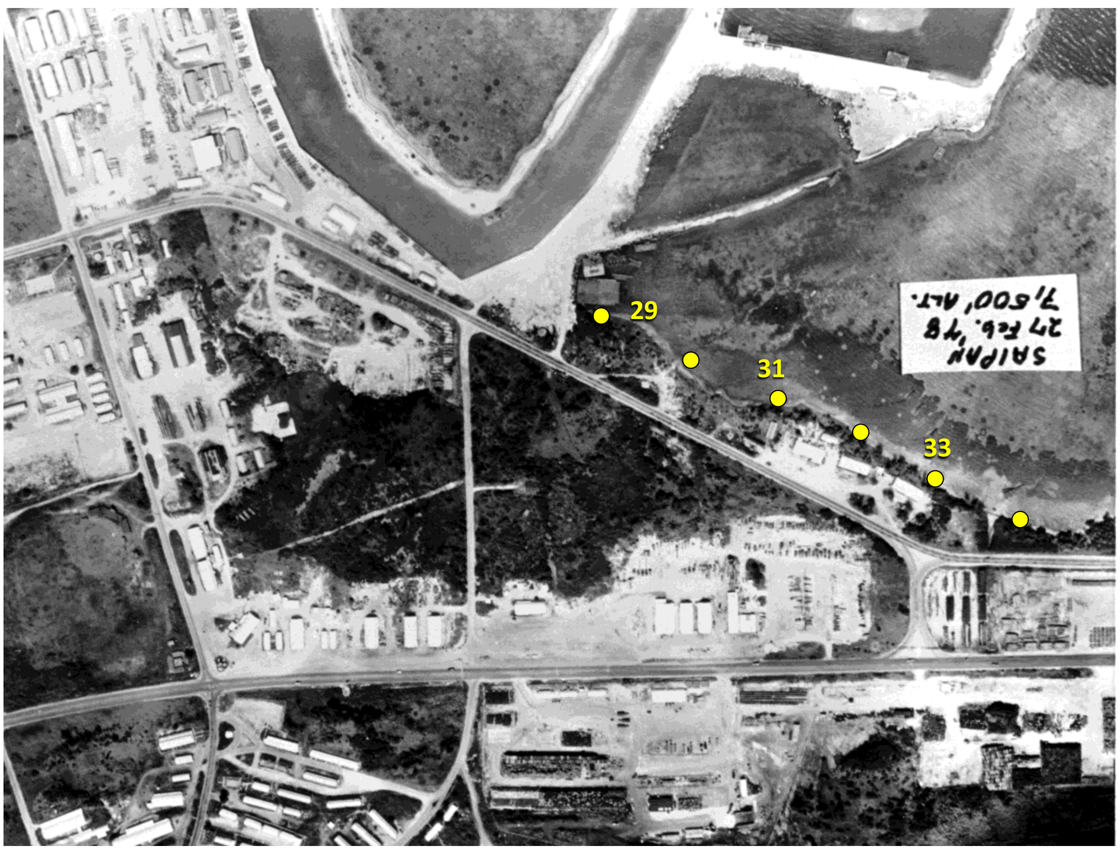

Figure 4: 1948 Aerial photograph of SE section of AMME showing military buildings and other structures in the area. Yellow circles approximate the locations of sediment sampling sites 29-34

The unfolding story for mercury in shoreline sediments bordering this embayment is somewhat different insofar as displaying a distinct gradient that starts at the causeway and declines steadily along the coast towards the dump. This pattern suggests that mercury at the causeway emanates from a source or sources that remain active to this day and have plumed eastwards along the shoreline. Anecdotal evidence suggests a field hospital was among the buildings shown between sites 31 and 33. Since mercury was commonly used in medicinal preparations and dentistry back then, wastes from this building could well account for the notable spike in sediment concentrations of this element at site 31. However, the old field hospital is unlikely to have caused the higher mercury levels found at site 29, owing to the low energy tidal movements and circulatory characteristics in this part of the bay (personal observation).

At this point in time, the elevated mercury levels at site 29 are believed to be associated with stormwater discharges directed into the bay via the stormdrain located immediately above it. As mentioned earlier, this stormdrain receives drainage exclusively from the protected wetland area. Previously tested soil from four sites within this area contained relatively high levels of mercury (Denton and Gawel 2012 unpublished data). Assuming these data are reflective of soil mercury levels throughout the wetland, it is not difficult to imagine how drainage from the property might be enriched with this element as it partitions back and forth between soil and soil pore waters and slowly migrates towards the coast. An examination of stormwater and stormdrain sediments up gradient of the discharge point would therefore seem prudent and a necessary preliminary to a more expansive assessment.

Copper, lead and zinc often correlate strongly with human activities and traffic densities, and are by far the most prevalent priority metal pollutants found in urban runoff (USEPA 1983). Mercury on the other hand, has no vehicular source and is rarely encountered in highway runoff unless there is a nearby source (Fulkerson *et al.* 2007). In USEPA's 5-year National Urban Runoff Program, for example, mercury was found in only 9% of several thousand samples analyzed (USEPA 1983). This contrasts sharply with a more recent study in Saipan where it was detected in 70% of all samples collected from stormdrains along the southern border of Saipan Lagoon (Environet Inc. 2007). Levels reported in the latter study ranged from 8-150 ng/L and were generally well in excess of those found in local groundwater (2-5 ng/L). The high detection frequency and levels detected were believed to be associated with mercury releases from corroding ordnance that remain buried on land up gradient of the stormwater discharge points (Denton *et al.* 2014). Large numbers of unexploded ordnance have previously been removed from AMME and all indications are that numerous others remain buried throughout the property (AMPRO 2005). A connection between these discarded munitions and the elevated mercury status of the park's wetland soils and drainage waters therefore seems likely.

Recent sediment accretion processes involving cleaner materials almost certainly account for the marked attenuation of heavy metals in surface deposits alongside the dump. However, relatively high concentrations of these contaminants still exist in deeper layers of the bioturbation zone, as evidenced by the unwavering metal profiles in *Quidnipagus palatum* over the past decade or so. Any future thoughts of dredging this area will need to take this fact into account when considering appropriate sediment disposal options. Core sampling could help define the vertical distribution of heavy metals in this region and provide greater insight into the long-term impact of any residual metal levels on the edible status of resident *Q. palatum* populations. The role of iron and manganese in regulating metal recycling processes in aquatic environments is well known (Förstner and Wittmann 1983), and undoubtedly of primary importance here considering that both elements are typically high in leachate streams emerging from municipal dumps (Denton *et al.* 2005). Likewise, the complexation of free metal ions with dissolved organic ligands released from decomposing organic wastes in the dump, coupled with dilution and dispersion processes associated with the continual tidal flushing in and out of the area, all serve to keep the biologically available metal fraction in the water column close to baseline. Low water column metal concentrations are consistent with metal profiles identified in the algal representative, *Acanthophora spicifera,* and short-siphoned surface dwelling bivalve, *Gafrarium pectinatum,* near the dump.

HEAVY METAL POLLUTION WEST OF THE MARINA CAUSEWAY:

The discovery of widespread copper contamination in shoreline sediments at all sites between the *Hyatt Hotel* boundary and *Smiling Cove Marina* was undoubtedly the biggest surprise here. Levels encountered were at least an order of magnitude above those typically found in clean bioclastic deposits examined further south in the lagoon (Denton *et al.* 2014). Oddly enough levels diminish quite rapidly sub-tidally with samples taken 10 m offshore from *Micro Beach* in 1999 yielding baseline values of 0.48-0.75 µg/g (Denton *et al.* 2001). One therefore has to wonder at the possibility of dredged material being used for beach augmentation during the economic boom of the late 1980s to early 1990s. A report from one who witnessed the deep water dredging and dock expansion activities back in 1998, suggests the latter scenario was most unlikely. They do admit, however, that the dredging created a silt plume that would have undoubtedly contributed to the composition of beach sands in adjacent areas (Brian Bearden, former DEQ employee, pers. com.).

Shipping lane deposits would almost certainly be copper enriched from copper based antifouling paints. Copper has been used for such purpose since historic times, first as thin copper sheet nailed to the hulls of ancient wooden sailing vessels to the more modern copper formulated paints that have their beginning back in the mid-nineteenth century and remain in use to this day (Franz 2009). It therefore seems likely that these biocides have indeed played a major role in elevating copper levels in AMME shoreline sediments, in one way or another, along with contributions from various past and present land-use activities.

Copper levels encountered in AMME sediments were well below acute toxicological thresholds reported for sensitive marine species (Denton and Burdon-Jones 1982a, 1986b). However, the long-term, sub-lethal effects of copper on such species can manifest themselves at concentrations several orders of magnitude below those that cause death. In a recent communication, for example, Prato *et al.* (2013) reported negative effects on survival, growth and fecundity of the amphipod, *Gammarus aequicauda*, at environmentally realistic concentrations as low as 50 µg/L. Sediment pore water copper concentrations in AMME beach sediments and their impact on meiofauna communities in the area is currently unknown, and would be one of several appropriate follow-on studies.

Mercury was the only other element that showed widespread enrichment in beach sediments along this stretch of coastline, despite levels being relatively light for the most part. In fact, greater enrichment was only identified at the mouth of the small stream that drains the AMME constructed wetland (site 26), and the western edge of the *Smiling Cove Marina* (site 28). Denton and coworkers previously examined mercury in sediment from two centrally located sites within the marina, and reported levels of 30-37 ng/g at one of them and 41-53 ng/g at the other (Denton *et al.* 2001). Unpublished data by these researchers for mercury in the wetland deposits range from 12-49 ng/g at the head of the stream to 10-12 ng/g at the mouth. The lower values at the mouth likely reflect losses associated with tidal flushing.

Sources of mercury at the marina undoubtedly include antifouling paints, as formulations were once available that contained up to 5% of this element (Johnsen and Engøy 1999). Contributions channeled into the area via drainage from the constructed wetland have likely come from at least two other sources over the years. Perhaps the most important of these was the medical incinerator at the *Commonwealth Health Center*, in Middle Road. This facility was operated for over 20 years before it was shut down in 2006 for violations of the Clean Air Act. Smoke stack emissions and residual ash from the incinerator caused widespread mercury contamination over much of northern Garapan and significantly raised mercury levels in nearshore fisheries (Denton *et al.* 2011a & b). Mercury fallout in the catchment of the constructed wetland would ultimately have been flushed into the park in runoff during storm events. The second mercury source is, of course, any buried munitions that remain in and near the park, and continue to provide low-level contributions to the wetland and the receiving waters into which it drains.

SEDIMENT TOXICITY ASSESSMENT:

The full impact of metal contaminated sediments on biotic communities cannot be determined from chemical analysis alone (Burton 2002). Other interpretive tools are necessary to predict potential adverse biological effects, including toxicity tests, bioaccumulation tests, equilibrium partitioning studies, and acid volatile sulfide determinations (Hansen *et al.* 1996, Burton 2002, Hansen *et al.* 2005). The recent development of numerical sediment quality guidelines (SQGs) also greatly assists with sediment toxicity evaluations. While SQGs cannot be expected to apply equally to all types of sediments and environmental conditions, they do provide a useful point of reference for the identification of contaminants and sites of potential concern, and are of value from the standpoint of prioritizing actions and management decisions.

The most widely accepted SQGs are those developed for the NOAA National Status and Trends Program (Long *et al.* 1995) for sediments from northeastern and western shelf water of the US. The guidelines were empirically derived from the systematic analysis of numerous field, laboratory, and modeling studies that linked sediment contaminant data with biological effects information in the U.S. The statistical approach involved ranking the effects data in order of concentration for each contaminant considered and calculating the 10^{th} and 50^{th} percentile of concentrations associated with adverse biological effects. These percentiles respectively equate to *Effects Range Low* (ERL) and *Effects Range Median* (ERM) benchmarks and conveniently separate out three contaminant concentration categories, namely those that are 'rarely,' (≤ERL), 'occasionally' (>ERL to <ERM), and 'frequently' (≥ERM) associated with biological effects.

The database was later expanded and the statistical approach refined to accommodate Florida sediment monitoring sites in the southeastern part of the country (MacDonald *et al.* 1996). Both 'effects' and 'no effects' datasets were used to derive *Threshold Effects Level* (TEL) and the *Probable Effects Level* (PEL) benchmarks for each contaminant considered. The concentration categories derived in this instance were referred to as the ‘minimal effects range’ (≤TEL), the ‘possible effects range’ (>TEL to <PEL), and the ‘probable effects range’ (≥PEL). Both sets of SQGs are listed in Table 13 below and are currently available for nine elements. The Florida SQGs are more conservative and designed specifically for calcium carbonate-rich sediments. Thus, they are more appropriate as an evaluative tool for the current findings. The absence of any TEL or PEL exceedances in all AMME sediments examined is therefore encouraging despite indications of copper enrichment in sediments along much of the AMME shoreline boundary (Tables 5 and 6).

PUBLIC HEALTH CONCERNS:

In the 1970s, following the realization that many aquatic organisms naturally contain relatively high levels of heavy metals and are capable of accumulating even more under polluted conditions, government agencies around the world began establishing enforceable heavy metal standards used in controlling the harvesting, transportation and sale of fish and shellfish sold for human consumption (Nauen 1983). Standards were only set for those elements that presented both a significant risk to public health and a known or expected problem in intestate/international trade. As a general rule the regulatory limits were set at a level slightly above the normal range of variation so as not to disrupt food production or trade. From Tables 11 and 12 it is clear that mercury, followed by lead and cadmium continue to rank among the most important elements of concern. In comparing both tables, it is also evident that maximum acceptable levels of most metals listed have changed very little over the past 40 years or so.

Food standards in the U.S. are under the jurisdiction of the U.S. Food and Drug Administration (USFDA) with non-regulatory technical guidance provided by the U.S. Environmental Protection Agency. The only enforceable metal standard currently in existence is for mercury. The ‘action level’ for this element presently stands at 1.0 µg/g wet weight for organic (methyl) mercury rather than total mercury. None of the bivalves examined here exceeded this value although maximum levels encountered in *Quidnipagus palatum,* east of the causeway, approached the mercury standard for Japan and even exceed those for Venezuela and Zambia (Table 11).

A series of non-enforceable guidelines also exist in the U.S. for cadmium, chromium, nickel and lead in shellfish. These are also expressed on a wet weight basis and the applicable guideline maxima for bivalves are 4, 13, 80 and 1.7 µg/g for each metal respectively (USFDA 2001). Based on these standards, lead clearly stands out with exceedances noted in *Q. palatum* from all sites between the dump and the causeway. Average wet weight values for lead in this bivalve from sites 8-11 were 2.8 µg/g, 2.2 µg/g, 1.8 µg/g, and 12.9 µg/g respectively. It is noteworthy that individual lead concentrations in specimens closest to the dump (site 11) were 5-12 times higher than the 1.7 µg/g wet weight consumption guideline noted above. The only other metal of elevated status in *Q. palatum* was copper and was detected at concentrations ranging from 104-180 µg/g wet weight in site 11 samples near the dump. Levels encountered elsewhere were generally lower although one outlier value topped 200 µg/g wet weight at site 8 near the causeway. While there are no current U.S. standards for copper in seafood, maximum permissible concentrations in other parts of the world range from 10-100 µg/g wet weight (Tables 12 and 13). Copper concentrations in *Q. palatum* populations along this stretch of coastline emerge as potentially problematic if evaluated solely on comparisons with regulatory limits. Other considerations of importance in evaluating human health risks are addressed below.

DIETARY RISK ASSESSMENT OF AMME BIVALVES

Regulatory limits and guidelines for contaminants in seafood serve an effective risk management function in trade and commerce. Such standards are typically based upon per capita consumption data and thus afford protection to those who consume average amounts of seafood or less. They are not intended to protect individuals whose consumption of fish and shellfish exceed national averages. Hence, avid consumers of seafood, as well as recreational fishers and those living in coastal regions and island communities may be under-protected (USEPA 1989). Tolerable intake (TI) risk assessment models offer a more flexible approach that can be tailored to specific consumption rates and contaminant levels in foods consumed. These are weighed against precise toxicological benchmarks to determine if amounts ingested are safe to eat on a regular basis over extended periods of time (Figueira *et al.* 2011, Mok *et al.* 2014, Yap *et al.* 2016, Liu *et al*. 2017).

USEPA oral reference dose (RfD) values were employed during the current investigation to determine safe consumption limits of AMME bivalves for all elements except copper and zinc. The RfD represents the maximum (tolerable) amount of a substance to which a person can be exposed each day over a lifetime without an unacceptable risk of adverse health effects (Bhupander and Mukherjee 2011). RfD values are available from the USEPA *Integrated Risk Information System* (IRIS) *Chemical Assessment Summary* website (https://www.epa.gov/iris) and are typically expressed on a µg/kg body weight/day basis. They are analogous to the World Health Organization (WHO) *Provisional Tolerable Daily Intake* (PTDI) benchmarks formulated by the FAO/WHO Joint Expert Committee on Food Additives (JECFA). PTDI values for copper and zinc were obtained from the JECFA database (http://apps.who.int/food-additives-contaminants-jecfa-database) for use in this study.

Bivalve consumption rates in Saipan are currently unknown. For this reason, flesh weights that would need to be consumed to equal the adopted RfD and PTWI benchmarks were computed for each element on the basis of existing mean metal concentrations in each species as follows:

$$\text{Tolerable Weekly Consumption Limit (kg)} = \frac{\text{RfD or PTDI } (\mu\text{g/kg b.w./day}) \times 7 \text{ days} \times 70 \text{ kg adult b.w.}) \times 10^{-3}}{\text{Mean Metal Concentration in Bivalve (mg/kg wet wt.)}}$$

Table 14 summarizes the risk assessment findings and clearly identifies mercury and lead as the main elements limiting safe consumption of *Q. palatum* harvested east of the causeway. Maximum safe consumption limits for specimens from this location were estimated to be 440 g/person/week or a little over 60 g/day (approximately 6 medium sized clams), which is certainly conceivable for avid consumers of bivalve mollusks (Nguyen *et al.* 2009). The consumption of 100 g of clam meat per day (10 medium sized clams) carries an additional threat of lead toxicity. Chromium was the only other element that posed some measurable risk associated with *Q. palatum* consumption from this location but only if intake regularly exceeds 227 g/day. Such a high rate of consumption seems unlikely given that top consumer countries of the world rarely surpass 70 g/person/day (Cauwenberghe and Janssen 2014). Mercury was also the main element limiting consumption of all other bivalve species examined, but only if consumption rates consistently exceed 380, 450 and 810 g/day for *G. pectinatum*, *A. striata* and *C. bella*, respectively. Once again, such high consumption rates seem unlikely to occur long-term. All other metals in these bivalves, including copper, were found to be well below levels sufficient to exceed tolerable daily intake limits at realistic and sustainable consumption rates.

Table 14: Risk Assessment of Heavy Metal Levels in AMME Bivalves: Tolerable Weekly Consumption Limits

Metal	Mean Metal Concentration[a] (mg/kg wet wt.)				USEPA RfD[b] (µg/kg b.w./day)	Tolerable Intake (TI) (mg/person/week)[c]	Tolerable Weekly Consumption (kg)[d]			
	A. striata	*C. bella*	*G. pectinatum*	*Q. palatum*			*A. striata*	*C. bella*	*G. pectinatum*	*Q. palatum*
Ag	0.03	0.12	0.05	0.07	5	2.45	81.7	20.1	52.9	33.4
Cd	0.05	0.19	0.05	0.02	1	0.49	9.07	2.62	10.6	25.5
Cr	0.17	0.21	0.11	0.92	3	1.47	8.50	7.08	13.0	1.59
Cu	1.84	7.41	1.99	74.5	500	245	133	33.1	123	3.29
Hg	0.016	0.009	0.018	0.112	0.1	0.049	3.12	5.68	2.65	0.44
Ni	0.52	0.91	1.61	1.41	20	9.8	18.9	10.8	6.10	6.95
Pb	0.09	0.12	0.49	3.14	4	1.96	21.78	16.9	3.99	0.62
Se	0.56	0.37	0.48	0.49	5	2.45	4.37	6.55	5.12	5.04
Zn	16.6	61.9	10.2	112	1000	490	29.4	7.92	47.9	4.39

[a]All sites; [b]RfD values derived from USEPA IRIS database for all elements except copper (not evaluated by USEPA). As a consequence, the WHO Provisional Tolerable Daily Intake (PTDI) for copper was adopted as the appropriate copper RfD (JECFA 1982). USEPA RfDs for chromium, mercury and nickel were for Cr^{6+}, methyl-Hg and Ni-soluble salts respectively.
The JECFA (1982) PTDI for zinc (1000 µg/kg b.w./day) was preferred to USEPA's RfD for this metal (300 µg/kg b.w./day) because bivalves naturally accumulate zinc in their soft tissues.
[c]Based on 70 kg adult body weight; [d]Wet flesh weight. **Note:** The above risk assessement assumes that all metals of interest in bivalves are 100% biologically available and in a potentially toxic form.
Calculations: TI (mg/person/day) = (RfD x 7 days x 70 kg person) x 10^{-3}(unit conversion factor); Tolerable Weekly Consumption (kg) = TI/Mean Metal Concentration in bivalve (mg/kg wet wt.).

SELENIUM:MERCURY MOLAR RATIOS:

It has long been recognized that selenium affords some protection to animals and plants against the toxic effects of mercury (Parizek and Ostadelova 1967). Recent, attention has focused on selenium values in seafood because fish and shellfish are typically high in mercury compared with most other kinds of food (Holden 1973). It is now widely believed that selenium's protective effect only occurs if the Se:Hg molar ratio in the food consumed is 1:1 or greater for selenium (Ralston 2008, Ralston *et al.* 2008b) although there is some contradictory evidence (Lémire *et al.* 2010). Selenium levels in all bivalves analyzed during the present study were consistently in molar excess of mercury with Se:Hg ratios varying from 5-170. Average molar ratios for each species examined were 16:1, 66:1, 93:1, 118:1 for *Q. palatum, Gafrarium pectinatum, Atactodea striata,* and *Ctena bella* respectively (Table 11). The relatively low molar ratios noted in *Q. palatum* east of the marina causeway were attributed to higher levels of mercury in these specimens rather than a depletion of their selenium reserves (Table 10).

Although selenium levels separating deficiency from toxicity in humans barely differ by more than an order of magnitude, anthropogenic sources of this element rarely, if ever, elevate environmental concentrations to levels that pose a significant health problem. In fact, selenium deficient environments are far more widespread than seleniferous ones (Fordyce 2012). As a consequence more than one billion people suffer from selenium deficiency symptoms worldwide (Gupta and Gupta 2016). Selenium toxicity (selenosis) on the other hand is far less common and largely restricted to seleniferous regions where locally grown crops are excessively enriched by this element (Fordyce 2012).

The USEPA risk based consumption limit guidelines for selenium in fish (USEPA 2000) provides the number of 8-oz (227 g) fish meals that may be consumed each month for any given level of selenium in edible tissues. Accordingly, fish containing selenium concentrations of 1.5 µg/g wet weight or less, may be eaten on an unrestricted basis. Above this value, restrictions come into play. For example, 8-oz fish portions containing selenium levels of 6.0-12 µg/g should not be consumed more than four times per month.

While all AMME bivalves examined thus far contained selenium levels well below the unrestricted consumption limit, no selenium data currently exists for fish from Saipan waters. Fish typically contain lower levels of selenium than shellfish (Maher *et al.* 1992), while the opposite trend generally applies for mercury. Moreover fish appear to regulate selenium levels in their tissues, whereas mercury levels tend to increase with age (Eisler 2000). For any given species, then, Se:Hg ratios generally tend to be lower in larger (older) fish. This is especially apparent in higher trophic level representatives like shark, marlin, swordfish and tuna (Burger and Gochfeld 2013). Consumers who prefer larger, predatory fish species may therefore be losing the protection that selenium affords them against mercury toxicity. Determining the relationships between mercury, selenium and size in popular table fish from AMME nearshore waters and elsewhere in the lagoon therefore have important public health implications that have yet to be addressed.

BIOMONITOR PERFORMANCE:

Notes on biomonitor prerequisites and the influence of various biological and environmental factors on the utility of candidate species as sentinels of heavy metal pollution are provided in

Appendix D. By now it should be realized that not all aquatic species are suited for such purposes, but those that are have one thing in common, namely an inability to metabolically regulate heavy metals of environmental interest. It is also evident that different biomonitors can respond quite differently to different metal fractions in the hydrosphere. Algae, for example, respond to soluble metals in the water column and do not respond to fractions associated with sediments or suspended particulates, in the same way as bivalves might. Rooted macrophytes like seagrasses have the added advantage of reflecting metal contributions from sediment pore waters and together with algae and bivalves, provide a triad of sentinels that facilitates a greater understanding of the movement and partitioning of heavy metals within marine and estuarine ecosystems than any single biomonitor alone.

All of the species selected for study here have been used in heavy metal monitoring programs elsewhere (Burdon Jones *et al.* 1975, Denton *et al.* 1980, Denton and Burdon Jones 1986a, Denton and Morrison 2009, Denton *et al* 2009). As a consequence, a fairly extensive heavy metal database now exists for representatives of each species from clean and polluted waters within the region. Much of these data are referred to in the preceding discussions and are consolidated in Table 15 below for convenience and ease of access. Comparative assessments between these regional datasets provide insight into each organism's ability to respond to changes in ambient metal availability in their immediate environment. Moreover they also permit the formulation of reliable baseline benchmarks, which are of fundamental importance in site assessment studies such as the one conducted here.

Table 16 summarizes the biomonitoring capability of each species as currently understood. Further refinement of this table is expected as additional data come to light. Follow-up field and lab investigations are highly recommended to determine metal uptake and depuration kinetics in these organisms. Understanding just how quickly they respond to changes in ambient metal availability is a necessary prerequisite for determining their value as long- or short-term indicators for any particular metal of interest. Growth/age dependant variability in heavy metal levels also needs to be examined, especially in the bivalves. Weight gains and losses associated with sexual development and spawning are additional factors that need to be evaluated ahead of time in order to minimize their diluting/concentrating effects on an organism's total metal loading (Phillips 19980).

A common problem with biomonitoring, especially in tropical waters, is the often patchy distributions of suitable sentinel species coupled with their relatively low abundances. These failings are partially overcome by adopting a multi-species approach although data comparisons between representatives from different habitat types is usually not possible. Such habitat related disparities were certainly evident during the current study. The selected seaweed and seagrass representatives, for example, were far more abundant in sandy, subtidal regions than they were in muddy areas. Among the bivalves, *Atactodea striata* was exclusively confined to relatively clean, coarse sands in the mid tidal region of the AMME shoreline, whereas *Quidnipagus palatum* occupied muddy substrates in the lower intertidal and immediate subtidal zones. The habitat preferences of *Gafrarium pectinatum* and *Ctena bella* were relatively broad by comparison, permitting their coexistence with both *A. striata* and *Q. palatum* on sandy and muddy shores respectively. They were, however, generally less abundant and more patchily distributed throughout the study area than the latter two bivalves.

Table 15: Heavy Metals in Sediments and Biota from AMME and Other Regional Sites

Species	Location	Metal (µg/g dry wt.)									Reference
		Ag	Cd	Cr	Cu	Hg[a]	Ni	Pb	Se[b]	Zn	
SURFACE SEDIMENTS:											
Lower shore	American Memorial Park nearshore waters, Saipan (37 sites)	all <0.20	all <0.20	3.47-8.25	4.93-15.6	0.42-37.5	0.18-1.69	<0.68-13.8	16-470	1.34-52.9	This Study
Lower shore	Tanapag Lagoon, Saipan (12 sites)	<0.20-0.75	<0.20-1.69	1.42-17.5	0.50-102	2.38-74.7	<0.20-11.9	0.65-158	-	2.42-358	Denton *et al.* 2009
Lower shore	Garapan Lagoon, Saipan (13 sites)	all <0.20	all <0.20	1.72-8.28	0.60-50.7	3.57-80.5	<0.39-6.23	0.80-22.5	-	3.00-98.5	Denton *et al.* 2014
1000 m offshore	Garapan Lagoon, Saipan (7 sites)	all <0.20	all <0.20	1.48-3.4	all <0.20	0.56-13.4	all <0.40	all <0.40	-	<0.19-0.4	Denton *et al.* 2014
Intertidal: lower shore	Chalan Kanoa Lagoon, Saipan (3 sites)	all <0.20	all <0.20	2.90-3.13	0.59-12.1	4.85-18.20	all <0.40	<0.39-31.0	-	2.75-26.6	Denton *et al.* 2014
Lower shore	Pago Bay, Guam (7 sites)	all <0.15	all <0.15	1.96-4.03	0.68-1.34	2.98-8.52	0.20-1.23	0.25-3.19	-	0.77-6.89	Denton and Morrison 2009
100-500 m offshore	Pago Bay, Guam (5 sites)	all <0.15	all <0.15	3.76-5.94	0.60-1.61	1.55-3.67	1.65-2.41	0.25-0.47	-	0.76-1.64	Denton and Morrison 2009
Lower shore	Townsville coastal waters, northern Australia (24 sites)	all <0.20	all <0.20	1.9-9.6	0.6-3.6	-	0.6-3.6	<0.4-5.3	-	4.2-26.3	Denton 1977 (unpublished data)
5-20 km offshore	Townsville coastal waters, northern Australia (24 sites)	-	-	-	6.0-6.9	10.9-11.7	9.3-11	16-16	-	30-32	Knauer 1976, 1977
Offshore	Great Barrier Reef, Australia (3 sites)	-	all <0.10	-	3.00-3.38	-	all <0.40	all <0.60	-	-	Denton 1981 (unpublished data)
ALGAE:											
Acanthophora spicifera	American Memorial Park nearshore waters, Saipan (8 sites)	<0.08-0.29	<0.12-0.87	2.35-4.66	2.14-11.8	1.14-3.40	1.56-3.69	0.77-2.60	<2.00-17.9	24.5-75.1	This Study
Acanthophora spicifera	Tanapag Lagoon, Saipan (6 sites)	<0.08-0.51	0.13-0.70	<0.26-1.54	2.88-30.5	1.86-10.2	1.78-2.52	0.49-8.14	-	17.6-130	Denton *et al.* 2009
Acanthophora spicifera	Pago Bay, Guam (5 sites)	all <0.16	<0.16-0.47	<0.21-1.88	1.22-3.03	1.09-2.83	3.05-5.20	0.31-1.36	-	3.36-8.04	Denton and Morrison 2009
Acanthophora spicifera	Great Barrier Reef, Australia (3 sites)	-	0.21-0.32	-	2.6-3.5	2.03-3.90	3.0-5.1	<0.69-2.9	-	8.0-13.0	Denton and Burdon-Jones 1986
SEAGRASSES:											
Enhalus acoroides	American Memorial Park nearshore waters, Saipan (7 sites)	all <0.14	<0.14-1.10	<0.39-1.08	3.57-30.6	0.99-4.89	0.60-1.28	all <0.50	all <2.00	17.1-26.5	This Study
Enhalus acoroides	Tanapag Lagoon, Saipan (8 sites)	all <0.20	0.15-0.60	<0.30-0.87	2.03-47.9	0.85-9.01	0.60-2.34	<0.22-2.05	-	20.0-33.0	Denton *et al.* 2009
Enhalus acoroides	Garapan Lagoon, Saipan (15 sites)	<0.10-2.00	<0.10-1.36	all <0.40	1.58-23.7	1.24-3.69	0.67-14.1	<0.43-1.35	-	12.4-102	Denton 2008 (unpublished data)
Enhalus acoroides	Pago Bay, Guam (11 sites)	all <0.16	all <0.16	<0.15-0.64	0.74-5.73	1.13-3.56	1.26-4.26	<0.30-1.07	-	4.96-16.6	Denton and Morrison 2009
Halodule uninervis	American Memorial Park nearshore waters, Saipan (4 sites)	all <0.14	all <0.14	0.78-1.70	3.55-4.62	2.18-3.28	0.85-2.91	0.70-1.03	<2.00-17.5	38.5-58.9	This Study
Halodule uninervis	Tanapag Lagoon, Saipan (4 sites)	all <0.20	0.29-0.66	0.42-0.69	2.45-6.46	1.80-3.53	0.70-1.25	<0.32-1.09	-	21.1-35.8	Denton *et al.* 2009
Halodule uninervis	Garapan Lagoon, Saipan (13 sites)	<0.20-1.70	<0.10-1.00	<0.11-3.94	1.20-15.6	<0.24-3.85	0.60-9.83	<0.34-7.06	-	5.70-75.1	Denton 2008 (unpublished data)
Halodule uninervis	Chalan Kanoa Lagoon, Saipan (2 sites)	0.52-1.10	all <0.30	1.05-5.58	6.72-52.6	1.83-3.96	6.17-9.67	3.21-56.3	-	78.8-187	Denton 2008 (unpublished data)
Halodule uninervis	Townsville coastal waters, Australia (2 sites)	<0.3	0.5	1.6	2.7	-	0.7	7.0	-	11.0	Denton *et al.* 1980
BIVALVES (whole flesh):											
Atactodea striata	American Memorial Park nearshore waters, Saipan (5 sites)	<0.09-0.63	0.14-0.62	0.82-1.82	9.01-14.5	8.76-24.2	2.11-4.24	<0.34-0.93	0.32-0.69	81.3-134	This Study
Atactodea striata	Tanapag Lagoon, Saipan (6 sites)	<0.23-5.08	<0.08-5.45	0.42-6.56	7.35-20.2	8.22-23.8	1.81-4.76	0.34-3.14	-	71.8-147	Denton *et al.* 2009
Atactodea striata	Garapan Lagoon, Saipan (17 sites)	<0.09-2.86	<0.26-3.47	<0.12-3.27	6.57-15.7	10.2-53.3	1.54-6.53	<0.20-4.40	-	54.1-138	Denton 2008 (unpublished data)
Atactodea striata	Chalan Kanoa Lagoon, Saipan (2 sites)	0.12-0.67	<0.15-1.33	<0.10-2.27	6.74-82.2	11.5-41.1	1.57-4.26	0.34-9.80	-	63.0-129	Denton 2009 (unpublished data)
Atactodea striata	Townsville coastal waters, Australia (1 site)	0.8	1.1	1.8	13	-	2.4	2.0	-	138	Burdon-Jones *et al.* 1975
Ctena bella	American Memorial Park nearshore waters, Saipan (4 sites)	0.35-1.15	0.38-1.70	0.61-1.51	28.1-49.4	5.10-16.7	2.72-8.50	5.64-11.4	0.39-0.65	227-463	This Study
Ctena bella	Tanapag Lagoon, Saipan (1 site)	0.33-0.81	1.16-2.71	0.82-0.92	5.31-14.1	22.0	4.40-5.57	5.94-6.38	-	384-430	Denton *et al.* 2009
Ctena bella	Garapan Lagoon, Saipan (12 sites)	<0.04-1.05	0.20-8.79	0.10-1.35	4.39-41.3	3.25-19.8	1.38-5.97	0.61-19.5	-	86.9-395	Denton 2008 (unpublished data)
Ctena bella	Pago Bay, Guam (4 sites)	0.09-0.12	0.66-2.51	0.14-0.18	5.79-20.9	5.63-17.4	7.83-21.2	<0.20-100	-	112-289	Denton and Morrison, 2009
Gafrarium pectinatum	American Memorial Park nearshore waters, Saipan (4 sites)	0.17-0.97	0.17-0.65	0.39-1.08	8.78-20.2	15.4-26.0	8.53-12.6	0.72-27.7	0.41-0.56	50.1-88.8	This Study
Gafrarium pectinatum	Tanapag Lagoon, Saipan (4 sites)	<0.14-0.62	0.78-1.79	0.58-1.31	6.69-35.3	9.91-23.3	10.6-14.1	7.97-46.9	-	42.3-63.2	Denton *et al.* 2009
Gafrarium pectinatum	Garpan Lagoon, Saipan (14 sites)	<0.06-2.19	<0.12-1.40	<0.07-1.53	6.76-65.5	9.01-118	4.94-23.8	0.48-10.6	-	27.6-76.3	Denton 2008 (unpublished data)
Gafrarium pectinatum	Chalan Kanoa Lagoon, Saipan (1 site)	<0.06-1.18	<0.31-0.59	<0.69-1.34	16.3-71.8	-	3.46-19.0	5.74-74.4	-	29.7-75.0	Denton 2009 (unpublished data)
Gafrarium pectinatum	Pago Bay, Guam (1 site)	0.14	1.14	0.21	17.0	-	16.4	0.27	-	59.6	Denton and Morrison 2009
Gafrarium tumidum	Townsville coastal waters, Australia (2 sites)	5.3-5.7	0.3-0.3	0.6-1.6	7.1-7.7	-	64.5-145	3.1-5.1	-	26.3-68.8	Burdon-Jones *et al.* 1975
Quidnipagus palatum	American Memorial Park nearshore waters, Saipan (5 sites)	0.17-4.90	all <0.09	2.85-11.2	95.6-1219	26.0-250	3.84-20.1	6.59-134	0.38-0.70	303-1142	This Study
Quidnipagus palatum	Tanapag Lagoon, Saipan (4 sites)	0.32-24.1	0.19-1.40	4.86-10.6	14.7-1876	33.6-111	8.32-13.1	9.01-184	-	305-1027	Denton *et al.* 2009
Quidnipagus palatum	Garpan Lagoon, Saipan (10 sites)	<0.05-0.94	<0.05-0.11	0.69-12.6	10.4-60.9	7.18-96.9	3.92-21.0	1.96-48.1	-	177-1518	Denton 2008 (unpublished data)
Quidnipagus palatum	Pago Bay, Guam (4 sites)	<0.08-0.13	<0.08-0.10	<0.13-0.46	4.26-68.5	21.9-62.4	10.4-24.7	0.20-0.89	-	93.6-341	Denton and Morrison 2009

[a]Mercury in sediments and biota as ng/g dry weight and ng/g wet weight respectively; [b]Selenium in sediments as ng/g dry weight, in seaweed and seagreass as ng/g wet weight, and in bivalves as µg/g wet weight; dashes indicate no data

Table 16: Preliminary Evaluation of Selected AMME Biota to Act as Biomonitors[a]

Species	Metal Fraction Reflected	Species Sensitivity[a]								
		Ag	Cd	Cr	Cu	Hg	Ni	Pb	Se	Zn
Algae										
Acanthophora spicifera	Dissolved metals in water column	✓	✓✓	✓✓	✓✓✓	✓✓	✓✓	✓✓✓	✓✓	✓✓✓
Seagrass										
Enhalus acoroides	Dissolved metals in water column and sediment	✓	✓✓	?	✓✓✓	✓✓	✓	✓	x	?
Halodule uninervis	pore waters	✓	?	✓	✓✓✓	?	✓	✓✓✓	x	?
Short Siphoned Bivalves										
Atactodea striata	Dissolved/particulate bound metals in surface water and beach sediment pore waters	✓✓	✓✓	✓	✓✓✓	✓	x	✓	✓	x
Ctenna bella	Dissolved/particulate bound metals in surface	✓	✓✓	✓	✓✓✓	?	x	✓✓✓	✓	✓✓
Gafrarium pectinatum	water and shallow sediment pore waters	✓	✓✓	✓	✓✓✓	✓✓✓	x	✓✓✓	✓	x
Long Siphoned Bivalve										
Quidnipagus palatum	Dissolved/particulate bound metals in surface water and deep sediment pore waters	✓✓✓	✓✓	✓✓✓	✓✓✓	✓✓✓	x	✓✓✓	✓	✓✓✓

[a]Sensitivity to changes in ambient heavy metal availability: ✓✓✓= high sensitivity; ✓✓ = moderate sensitivity; ✓ = low sesitivity; x = regulates element; ? = unknown

FUTURE DIRECTIVES:

On the basis of the work already completed within the study area and beyond, the following recommendations are made for future investigations within AMME and the adjacent waters of Saipan Lagoon.

- ***Identify heavy metal profiles in surface soils throughout AMME natural wetland area:***
  Past land-use activities within the park have left behind heavy metal signatures in certain areas. A preliminary analysis of heavy metals in surface soil from the central and southeastern perimeter of the property was undertaken in 2012 and revealed elevated mercury, cadmium, lead, copper and zinc that exceeded ecological benchmarks developed by the USEPA (2005). As a first step towards evaluating the full impact of this contamination on sensitive species within the area, the distribution and abundance of heavy metals in the area need to be determined. It is therefore recommended that surface and subsurface soil samples be taken for analysis from around the perimeter of the protected area and along at least five NW-SE bearing transects running through it.

- ***Determine origin and extent of heavy metal enrichment immediately east of the causeway:***
  Excess waters from the natural wetland are channeled into the ocean via a storm drain located immediately east of the causeway (Fig. 1). Shoreline sediments and biotic representatives collected down gradient of the storm drain discharge point were shown to be relatively enriched with mercury, lead, copper and zinc during the present study. Whether such contamination emanates from the wetland itself or from shoreline structures that once existed along this stretch of coastline, remains to be identified. Soil sampling for heavy metal analysis within the protected area immediately up gradient of the storm drain discharge point will provide a useful starting point for such investigations. Further seaward sampling of sediments and biotic representatives from the storm drain to *Outer Cover Marina* is also recommended, to determine the extent and intensity of heavy metal contamination along the eastern side of the causeway.

- ***Evaluate impact of heavy metal enrichment east of the causeway on popular food fish:***
  When WERI released their 2003 study identifying elevated heavy metal levels in bivalves taken beside the dump, the opinion of local officials was that few people, if any, harvest seafood from this area. Observations made after the dump closed and during the current study indicate otherwise and suggest even more people will frequent these waters once the capping and restoration of the dump is complete. Mercury and lead remain primary elements of concern in these waters, with contributions emanating from the dump and an as yet unidentified secondary source or sources on the opposite side of the embayment. Heavy metal levels in nearshore fisheries (particularly Hg, Pb and Se) within the embayment have yet to be determined and are important, given the growing popularity of this area among local fishing enthusiasts. Such a survey should focus on representatives with restricted foraging ranges. Emperor fish are ideal candidates for such purposes, particularly *Lethrinus atkinsoni* and *L. harak*, which are highly targeted species (Graham 1994, Taylor & McIlwain 2010).

- ***Evaluate impact of AMME constructed wetland drainage on nearshore fisheries:***
  Waters feeding this artificial wetland are, in fact, contaminated storm and wastewater discharges that are redirected from the streets of Garapan into the ocean at the western end of

Smiling Cove Marina (Fig. 1). Such discharges are typically enriched with heavy metals associated with highway traffic (Makepeace *et al.* 1995). While Hg has no known vehicular source and is not normally encountered in urban runoff (USEPA 1983), mercury emissions from a nearby medical incinerator contaminated much of the Garapan landscape from the mid 1980s until early 2006 (Denton *et al.* 2011a) and undoubtedly raised mercury levels in runoff discharged into the constructed wetland over this time frame.

The receiving coastal waters seaward of the marina are largely contained within a shallow embayment bounded by *Micro Point* and the causeway to the east and west and a protective earthen rampart shielding *Smiling Cove Marina* to the south and southeast (Fig. 1). Water circulation within this embayment is highly restricted rendering it more or less a permanent sink for recalcitrant contaminants flushed into it. The area is easily commonly accessed by local fisherman.

Two *L. harak* specimens recently retrieved from this area yielded mercury concentrations substantially above those found in similar sized fish elsewhere in the lagoon (Fig. 5). Indeed, the level encountered in the larger specimen (1.18 µg/g wet wt.) is the highest value recorded in close to 200 specimens so far examined by WERI. According to USEPA guidelines, an 8oz portion of fish with this amount of mercury in its tissues should not be eaten more than twice a month and preferably not at all by nursing mothers, women of child bearing age and sensitive individuals (USEPA 2000). Further analysis of mercury in fish from these waters together with and an assessment of any health risks associated with their consumption, therefore, seem appropriate under the circumstances.

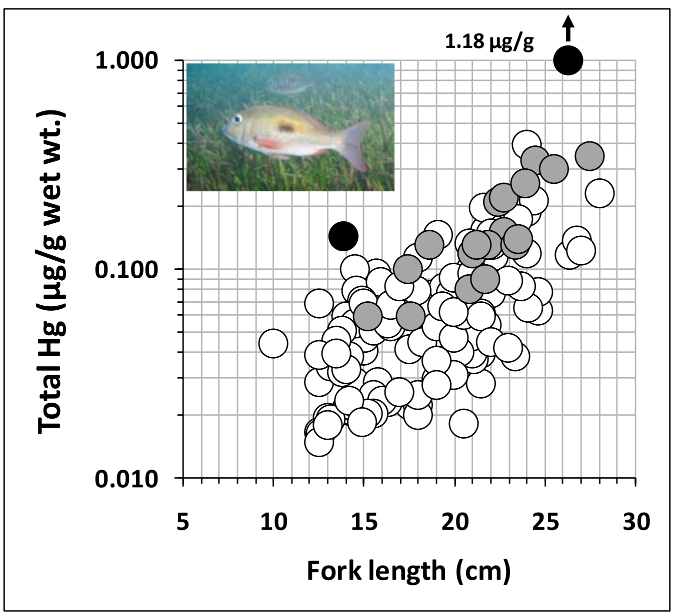

Figure 5: Scatterplot of mercury levels in *Lethrinus harak* (inset) from Saipan Lagoon. Black circles are values from two recently caught fish from the outer embayment adjacent to *Micro Point* and *Smiling Cove Marina*; grey circles represent fish previously caught off *Micro Beach* ~500 m to the west. All other plots (white circles) are from fish taken from relatively clean and contaminated sites elsewhere in the lagoon. Mercury levels in fish from uncontaminated waters typically range between 0.001 and 0.10 µg/g wet wt. (Holden 1973).

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APPENDIX A.

(Task Agreement P14ACO1579, Cooperative Agreement P14AC00637)

Hawai'i-Pacific Islands Cooperative Ecosystem Studies Unit
Task Agreement
National Park Service

TASK AGREEMENT NO.: P14AC01579

COOPERATIVE AGREEMENT NO.: P14AC00637

TERM OF AGREEMENT: 8/18/2014 – 2/17/2017

RECIPIENT: University of Guam

PROJECT TITLE: American Memorial Park Shoreline Infauna and Heavy Metals Assessment

FISCAL YEAR FUNDING	**COST STRUCTURE**	**AMOUNT:**
2014	PPWONRADW0 PPMRSNR1Y.NM0000	$ 21,100

PROJECT ABSTRACT: American Memorial Park, the Water Resources Division of the National Park Service Natural Resources Stewardship and Science Directorate, and the University of Guam are collaborating through this task agreement to determine the types of common macro-invertebrates along the park shoreline and the concentrations of heavy metals within invertebrate representatives. The project outcomes include: informing the National Park Service about the types of, abundance of and sampling methods for monitoring macro-invertebrates inhabiting the park shoreline; documenting the amount and distribution of heavy metals in park shoreline invertebrates popularly harvested for food; providing scientific information to National Park Service managers that can be utilized to inform management alternative formulations and decisions; and providing information that can be distributed to the public and students about invertebrates and heavy metal concentrations in selected representatives.

PRINCIPAL INVESTIGATOR(S): Gary Denton and Maria Celia Defrance Malay

CFDA #: 15.945, Cooperative Research and Training Programs – Resources of the National Park System (CESU)

Unless otherwise provided herein, the terms and conditions of P14AC00637 apply to this Task Agreement.

University of Guam

Robert A Underwood

Dr. Robert Underwood
President, University of Guam

SEP 23 2014

Date

Victorina My Renacia

Victorina M.Y. Renacia
Legal Counsel, University of Guam

9/4/14

Date

NATIONAL PARK SERVICE
PACIFIC WEST REGIONAL OFFICE

Lilette Baltodano

Lilette J. Baltodano
Financial Agreements Officer

9/23/14

Date

TASK AGREEMENT NO. P14AC01579

"American Memorial Park Shoreline Infauna and Heavy Metals Assessment"

This Task Agreement by and between the National Park Service (NPS) and University of Guam (UOG) is issued against the Hawai'i-Pacific Islands Cooperative Ecosystem Studies Unit Cooperative and Joint Venture Agreement, P14AC00637, for the purpose of mutual assistance in conducting a project entitled "American Memorial Park Shoreline Infauna and Heavy Metals Assessment ." Unless otherwise provided herein, the terms and conditions of P14AC00637 apply to this Task Agreement.

ARTICLE I – BACKGROUND AND OBJECTIVES

A. Background

The American Memorial Park (AMME) is a 133 acre parcel of land that borders the central region of Saipan Lagoon. The park has been under the administrative jurisdiction of the NPS since 1979. The AMME shoreline and adjacent waters, like the rest of the lagoon, are used for fishing and aesthetic enjoyment and are of great cultural significance to the people of Saipan. The AMME park shoreline is adjacent to the Puerto Rico dump site, and Denton et. al. (2008) documented enrichment of heavy metals in biota in the area of the dump. However, biota from the dump-adjacent AMME shoreline were not part of the study.

Like much of Saipan, the AMME area was occupied by the US military for several years after WWII, and parts of it were designated for the storage and disposal of bombs, shells and other munitions (AMPRO 2005). Primary contaminants of concern associated with such activities include heavy metals, particularly lead and mercury. Both elements are accumulative poisons and can occur in relatively high concentrations in aquatic species from contaminated waters (Denton et al. 2006). The impact of WWII activities on heavy metal profiles in sediments from the southern half of Saipan Lagoon has recently been demonstrated (Denton et al. 2014), and studies are currently underway to determine these effects on fisheries resources in this area. The study described herein proposes to determine the heavy metal concentration in biotic components living in shoreline sediments (i.e., infauna) traditionally harvested for food from AMME nearshore waters and evaluate any potential human health risks. The study is therefore seen as a useful extension of these earlier works and as providing information specifically about the AMME shoreline.

B. Objectives

Investigators from UOG and NPS staff will collaborate to accomplish the following specific objectives.

- Determine the spatial distribution of the bivalves and other macro-infauna along the shoreline of AMME.
- Design a contaminants sampling plan based on the distribution of macro-infauna, runoff areas and potential contaminant sources.
- Collect and process biota samples based on the sampling plan and determine heavy metal concentrations in their edible body tissue.
- Provide detailed and summarized information on heavy metal concentrations, distribution, and discernable trends to park staff that can be used determine if and what alternative shoreline management alternatives should be considered.

C. Public Purpose

The public will benefit from this project by learning if shoreline macro-infauna populations at AMME contain high enough concentrations and combinations of heavy metals to trigger consideration of alternative management strategies designed to protect human health. People who harvest animals at the park will benefit directly from learning about the concentrations of heavy metals in organisms and any associated health risks. Scientists, students, managers and interested people will benefit from learning how widely distributed, at what concentration, and in which species heavy metals occur. Students participating in the project will learn about heavy metal benefits, contamination and research methods. The public also will benefit by learning the amount of Selenium in body tissue of the park's shoreline infauna; small amounts of the heavy metal Selenium help protect humans against what has been referred to as mercury poisoning, and concentrations have not been examined in Saipan invertebrates. This project utilizes the UOG's expertise and experience to develop and conduct adequate and cooperative research and training about sampling the distribution of organisms harvested by park visitors and for testing the aquatic invertebrates for concentrations of heavy metals.

The research is likely to generate information about the nature and specific location of sensitive, rare and/or valuable park resources specified in, and information potentially withheld under, provisions of the National Parks Omnibus Management Act. Disclosure of information likely to be generated (concerning presence and distribution of some invertebrate species highly-prized as food) may create an unreasonable risk of harm, theft or destruction of park resources. Thus, the detailed and specific results of this research cannot be publicly released, published, or externally peer reviewed until they are reviewed by National Park Service authorities to assure the requirements of the National Parks Omnibus Management Act and other applicable laws are met. NPS employees will review the data and information generated through this CESU Task Agreement, determine which details and summaries are appropriate for public release based on applicable laws, and work to release the information to the public and scientific community independent and separate from this CESU Task Agreement. The benefits to park management will occur no matter when public release of information occurs.

ARTICLE II – STATEMENT OF WORK

A. The UOG will:
   1. Collaboratively undertake a project titled *American Memorial Park Shoreline Infauna and Heavy Metals Assessment* as described throughout this Task Agreement.
   2. Appoint Gary Denton, Ph.D., and Maria Celia Defrance Malay, Ph.D. as Principal Investigators (PIs).
   3. Hire or appoint other staff or students to the project as appropriate to achieve project objectives.
   4. Utilize the methods and processes as outlined in Attachment I: Methodology in conducting the project.
   5. Coordinate and oversee the following as responsibilities of Dr. Malay:
      a. Visual stratification of the American Memorial Park Shoreline based on sediment, habitat characteristics, and professional experience,
      b. Obtain permits and permissions to sample along the American Memorial Park shoreline to determine infaunal taxonomic composition, distribution and size,
      c. Random replicate-sample shoreline sediment/habitat strata for macro-invertebrates,
      d. Sort and classify the invertebrates into taxonomic groupings,
      e. Measure size in a representative subsample of each taxonomic group,
      f. Draft and submit a short report about the locations and success of field sampling,

   g. Draft and submit a short report including the abundance and size data of the taxa collected and including a compilation of invertebrate community characteristics by location and sediment/habitat strata.

6. Coordinate and oversee the following as responsibilities of Dr. Denton:
   a. Meetings, discussions and exchanges of a draft invertebrate-heavy metal sampling plan,
   b. Work with Mike Gawel to mutually identify and agree to the taxa to be analyzed and the specific heavy metals to be tested for concentration determination within the total minimum analysis numbers of this agreement (i.e., 54 infauna samples each tested for Lead, Mercury and Selenium or the equivalent e.g., 27 infauna samples tested for six metals costing equal amounts to Lead, Mercury and Selenium),
   c. Submit a written invertebrate-heavy metal sampling plan to NPS prior to field sampling,
   d. Obtain permits and permissions to sample and remove infauna along the American Memorial Park shoreline for determination of presence and amounts of selected heavy metals within representative infauna,
   e. Collect invertebrates from the shoreline of American Memorial Park in compliance with the sampling plan and permits,
   f. Process and analyze the selected invertebrate representatives for heavy metal analysis,
   g. Draft and submit a short report about the location and success of field sampling,
   h. Draft and submit a short report including the heavy metal concentrations found in invertebrate tissue from American Memorial Park and including a compilation of invertebrate heavy metal concentration characteristics by location.
7. Provide NPS copies of raw data, related meta-data, and summarized data as soon as possible after field sampling and at least twice monthly thereafter to provide isolated back-up NPS data copies.
8. Obtain written approval from the park Superintendent and NPS Public Information Officer(s) prior to releasing or publishing results from this project.
9. Cooperate with the NPS Agreement Technical Representative (ATR) to ensure that the conduct of the project complies with "Quality Control of Scientific and Other Scholarly Products in the Pacific West Region."
10. Ensure that reports and other formal materials (including publications and presentations) resulting from this collaborative project acknowledge the NPS and that the project was conducted through the Hawai'i-Pacific Islands Cooperative Ecosystem Studies Unit, and reference this Task Agreement number.
11. Upon request of the NPS, obtain digital photographs with captions of project activities and make these available to the NPS Hawai'i-Pacific Islands Cooperative Ecosystem Studies Unit Research Coordinator and others for use in presentations and reports.

B. The NPS will:
   1. Provide financial assistance to the UOG as provided in Article V. The Budget, included as Attachment II, is incorporated in this Task Agreement.
   2. Assign Sarah Allen, Pacific West Region Ocean and Coastal Coordinator, as the ATR. Mike Gawel will be the primary park contact, and Karl Brookins can assist with data and reports.
   3. Help UOG employees obtain permits and permissions for sampling along park shorelines.
   4. Provide overview and orientation associated with all park visits including: Briefings on safety procedures; introductions to park staff; and logistical support for field work operations, which include coordination with the park and making safety a priority.
   5. Assist with safe undertaking of fieldwork within the park and have appropriate NPS staff accompany recipient workers when needed in the field.
   6. Accept and store copies of project data and metadata.
   7. Assist with data analysis as requested and review and edit project report and sampling plan drafts in a timely manner.

8. Work with Dr. Denton to mutually identify and agree to the taxa to be analyzed and specific metals for heavy metal concentration determination within the total analysis limits of this agreement.
9. Consider and respond to requests to release data and results.
10. Cooperate with the UOG PI to ensure that the conduct of the project complies with "Quality Control of Scientific and Other Scholarly Products in the Pacific West Region." The ATR (or designee) is the peer review manager for this project.
11. Ensure that reports and other formal materials (including publications and presentations) resulting from this collaborative project acknowledge the UOG and that the project was conducted through the Hawai'i-Pacific Islands Cooperative Ecosystem Studies Unit, and reference this Task Agreement number.

ARTICLE III – TERM OF AGREEMENT

This Task Agreement is effective on August 18, 2014 and will extend through February 17, 2017.

ARTICLE IV – KEY OFFICIALS

A. For the NPS:
   1. ATR:
      Sarah Allen. Ph.D., Ocean and Coastal Resources Program Lead, Pacific West Region
      333 Bush Street Suite 500
      San Francisco CA 94104
      Phone: 415-623-2202 (o), 510-541-4241 (c)
      Email: sarah_allen@nps.gov

   2. Primary Park Contact:
      Mike Gawel Resource Chief, American Memorial Park
      War in the Pacific NHP
      135 Murray Boulevard, Suite 100
      Hagåtña, GU 96910
      Phone: 671-477-7278 x1010
      Fax: 671-477-7281
      Email: Mike_Gawel@nps.gov

   3. Other Project Participant:
      Karl Brookins Ph.D., Marine Fishery Scientist
      1201 Oakridge Dr. Suite 250
      Fort Collins CO 80525
      Phone: 970 267 7208
      Fax: 970 225 9965
      Email: karl_brookins@nps.gov

B. For the UOG:
   1. PI:
      Gary Denton, Ph.D., Professor of Environmental Toxicology
      Water and Environmental Research Institute, University of Guam
      UOG Stations
      Mangilao GU 96923

      Phone: 671-735-2690

Fax: 671-735-8890:
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2. PI:
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http://www.guammarinelab.com/machelmalay.html

C. Changes in Key Officials: Neither the NPS nor the UOG may make any permanent change in a key official without written notice to the other party reasonably in advance of the proposed change. The notice will include a justification with sufficient detail to permit evaluation of the impact of such a change on the scope of work specified within this Task Agreement. Any permanent change in key officials will be made only by modification to this Task Agreement.

ARTICLE V – AWARD AND PAYMENT

A. NPS will provide financial assistance to the UOG in an amount of $21,100 for the work provided herein. The chargeable appropriation and funding source for this Task Agreement is as follows:

2014	PPWONRADW0 PPMRSNR1Y.NM0000	$ 21,100

B. Payment Procedures: Advances/Reimbursements through the Automated Standard Application for Payments (ASAP) System

1. Method of Payment. Payment will be made to the recipient by advance and/or reimbursement through the Department of Treasury's ASAP system.

2. Requesting Advances. Requests for advances must be made through the ASAP system. The recipient may submit requests as frequently as required to meet its needs to disburse funds for the Federal share of project costs. If feasible, the recipient should time each request so that payment is received on the same day that funds are dispersed for direct project costs and the proportionate share of any allowable indirect costs. If same-day transfers are not feasible, advance payments must be as close to actual disbursements as administratively feasible.

3. Requesting Reimbursement. Requests for reimbursements must be made through the ASAP system. Requests for reimbursement should coincide with the recipient's normal billing pattern. Each request must be limited to the amount of disbursements made for the Federal share of direct project costs and the proportionate share of allowable indirect costs incurred during that billing period.

4. Adjusting payment requests for available cash. The recipient must disburse any funds that are available from repayments to and interest earned on a revolving fund, program income, rebates, refunds, contract settlements, audit recoveries, credits, discounts, and interest earned on any of those funds before requesting additional cash payments from National Park Service.

5. Payments. All payments are made by electronic funds transfer to the bank account identified on the ASAP Bank Information Form that the recipient filed with the U.S. Department of Treasury.

6. Supporting Documents for Agency Approval of Payments. When a recipient is determined "high risk" or has had performance issues. If the Agency approval requirement is in effect for this award, the ASAP system will indicate that Agency approval is required when the request for payment is submitted. The recipient must notify the Agreement Technical Representative (ATR) identified in this agreement that a payment request has been submitted. The payment authorizing official may request additional information from the recipient to support the payment requests prior to release of funds, as deemed necessary. The recipient is required to comply with these requests. Supporting documents include invoices, copies of contracts, vendor quotes, and other expenditure explanations that justify the reimbursement requests.

C. Financial Reports: The recipient shall submit a SF-425 Federal Financial Report on a quarterly basis.

1. The financial reports may be submitted using one of the following methods:
   a. One original mailed to: Contracting Division, National Park Service, Pacific West Regional Office, 333 Bush Street, Suite 500, San Francisco, CA 94104
   b. One scan emailed to PWR_Agreements@nps.gov
   c. Electronic submission through FedConnect

2. The recipient will report program outlays and program income on a cash or accrual basis.

3. Reports are due 30 calendar days after the end of each federal fiscal quarter which ends on December 31, March 31, June 30, and September 30. A Final report is required to be submitted 90 days after the end of the agreement period and will include all financial transactions for the life of the award.

4. In addition, a Final Financial Report will be submitted 90 calendar days after the end of the award period, at expiration, or upon termination. Transactions which occurred after the award expired will also be included in the final reports. These expenses shall include wrap-up activities incurred during the project period and where the transaction occurred after the award expired. Transactions for the entire award period will be included in this final report and will reflect the transactions for the entire award amount.

5. All financial and programmatic records submitted by recipients, supporting documents, statistical records, and other grants-related records shall be maintained in accordance with 43 CFR §12.82 or §12.953, as applicable.

D. The result of work under each phase of this Task Agreement is considered to be independently useful. The data obtained from one phase, however, may be utilized for future phases, subject to satisfaction with the data, desirability for additional data, and available funding. Any future phase would be added through the issuance of a written modification to this agreement.

ARTICLE VI – PROJECT PRODUCTS

A. Schedule/Milestones/Dates
   1. April 30, 2015 - Field Sampling of invertebrate distribution and abundance completed; draft Field Sampling Report 1 due to Mike Gawel and Sarah Allen within 72 hours of returning from the sampling trip.

2. July 15, 2015 - The draft Invertebrate Abundance, Size and Distribution Report due to Mike Gawel and Sarah Allen from Dr. Malay. NPS will review and return to Dr. Malay within 5 working days.
3. August 3, 2015 - Final Invertebrate Abundance, Size and Distribution Report submitted to Mike Gawel and Sarah Allen.
4. October 14, 2015 - The draft *Invertebrate-Heavy Metal Sampling Plan* is due to Mike Gawel. Final sampling plan due to Mike Gawel and Sarah Allen prior to conducting field sampling.
5. February 15, 2016 - Field Sampling of invertebrates for heavy metal analysis completed. Field Sampling Report 2 due to Mike Gawel and Sarah Allen within 72 hours of returning from the sampling trip.
6. January 30, 2017 - The draft Heavy Metals Concentration and Distribution Report due to NPS from Dr. Denton to Mike Gawel and Sarah Allen. NPS will review and return within 5 working days.
7. February 17, 2017 - The final Heavy Metals Concentration and Distribution Report submitted to Mike Gawel and Sarah Allen.

B. Description of Project Products/Reports
1. Field Sampling Report 1: Approximately 1-3 pages: backup copies of all field data should be included as appendix (and not be included in the page count) if not submitted earlier. Details to include the locations sampled and the relative abundance of invertebrates (e.g., low, moderate, high abundance). Any significant findings, changes to methods, or problems encountered during the field work should also be summarized.
2. Invertebrate Abundance, Size and Distribution Report: 2 or more pages. The report should include a list of taxa of macro-invertebrates identified and their relative abundance and size at each location. Mean and a measure of variance in abundance and size should be included for each sediment/habitat strata sampled. Methods used would be included by reference to other publications and Field Sampling Report 1, unless unpublished methods are used.
3. Invertebrate-Heavy Metal Sampling Plan: Written plan listing the locations and amounts of each invertebrate taxon to be collected. This plan should also specify how many, and which, invertebrates are mutually agreed to and will be used in determining heavy metal concentration (e.g. pooled samples of three individuals), the number of invertebrate samples to be analyzed, and the identity of heavy metals to be analyzed from the invertebrate samples.
4. Field Sampling Report 2: Approximately1-3 pages; backup copies of all field data should be included as appendix (and not be included in the page count) if not submitted earlier. Details to include the locations sampled and the number and types of invertebrates collected. Any significant findings, changes to methods, or problems encountered during the field work should also be summarized.
5. Heavy Metals Concentration and Distribution Report: A written report listing the concentrations of heavy metals found in each invertebrate sample tested from each location, as well as the mean and a measure of variance from replicates and habitats sampled. Any significant documented trends, such as decreasing concentrations moving away from a specific location, will also be included in the report. Methods used will be included by reference to other publications and Field Sampling Report 2, unless unpublished methods are used.

C. Delivery of Project Products
1. One electronic (pdf) of all products five drafts and five final documents will be submitted to the ATR Sarah Allen and Mike Gawel at the addresses shown in Article IV, Key Officials; hard copies will be delivered if requested in writing.
2. One electronic (pdf) copy of the final *Invertebrate Abundance, Size and Distribution Report* and the *Heavy Metals Concentration and Distribution Report* (if available as a version determined by the park as acceptable for public release) will be submitted to the NPS Hawai'i-Pacific Islands

CESU Research Coordinator Darcy Hu, PO Box 52, Hawai'i National Park, HI 96718; darcy_hu@nps.gov.

3. One electronic (pdf) copy of final *Invertebrate Abundance, Size and Distribution Report* and the *Heavy Metals Concentration and Distribution Report* (if available as a version determined by the park as acceptable for public release) will be submitted to the HPI CESU University Lead Sharon Ziegler-Chong, Office of Research – HPI CESU, 200 West Kawili Street, Hilo, HI 96720; ziegler@hawaii.edu.
4. One electronic (pdf) copy of the final *Invertebrate Abundance, Size and Distribution Report* and the *Heavy Metals Concentration and Distribution Report* (if available as a version determined by the park as acceptable for public release) will be submitted to the following address: Carol Simpson, Technical Information Center, Denver Service Center, National Park Service, PO Box 25287, Denver, CO, 80225, carol_simpson@nps.gov.

ARTICLE VII – PRIOR APPROVAL

The recipient shall obtain prior approval for budget and program revisions, in accordance with 2 CFR § 215.25 or 43 CFR § 12.70, as applicable.

ARTICLE VIII – CLOSEOUT PROCEDURES

A. This Task Agreement shall be closed out in accordance with the procedures stated in 2 CFR §§ 215.70-73 or 43 CFR §§ 12.90-92, as applicable.

B. The recipient shall submit, within 90 calendar days after the end date of the award, all financial, performance, property, and other reports as required by the terms and conditions of the award. NPS may approve extensions when requested by the recipient.

C. Unless NPS authorizes an extension, the recipient shall liquidate all obligations incurred under the award not later than 90 calendar days after the end date of the agreement.

D. The recipient shall promptly refund any balances of unobligated cash that NPS has advanced or paid and that are not authorized to be retained by the recipient for use in other projects.

E. The recipient shall account for any real and personal property acquired with Federal funds or received from NPS in accordance with 2 CFR §§ 215.31-37 or 43 CFR §§ 12.71-74, as applicable.

ARTICLE IX – SPECIAL PROVISIONS

A. Central Contractor Registration and Universal Identifier Requirements (2 CFR § 25)

1. Requirement for Central Contractor Registration (CCR). Unless you are exempted from this requirement under 2 CFR 25.110, you as the recipient must maintain the currency of your information in the CCR until you submit the final financial report required under this award or receive the final payment, whichever is later. This requires that you review and update the information at least annually after the initial registration, and more frequently if required by changes in your information or another award term.

2. Requirement for Data Universal Numbering System (DUNS) Numbers. If you are authorized to make subawards under this award, you:

a. Must notify potential subrecipients that no entity (see definition in paragraph 3 of this award term) may receive a subaward from you unless the entity has provided its DUNS number to you.
b. May not make a subaward to an entity unless the entity has provided its DUNS number to you.

3. Definitions. For purposes of this award term:
   a. *Central Contractor Registration (CCR)* means the Federal repository into which an entity must provide information required for the conduct of business as a recipient. Additional information about registration procedures may be found at the CCR Internet site (currently at http://www.sam.gov).
   b. *Data Universal Numbering System (DUNS)* number means the nine-digit number established and assigned by Dun and Bradstreet, Inc. (D&B) to uniquely identify business entities. A DUNS number may be obtained from D&B by telephone (currently 866–705–5711) or the Internet (currently at http://fedgov.dnb.com/webform).
   c. *Entity*, as it is used in this award term, means all of the following, as defined at 2 CFR part 25, subpart C:
      i. A Governmental organization, which is a State, local government, or Indian Tribe;
      ii. A foreign public entity;
      iii. A domestic or foreign nonprofit organization;
      iv. A domestic or foreign for-profit organization; and
      v. A Federal agency, but only as a subrecipient under an award or subaward to a non-Federal entity.
   d. *Subaward*:
      i. This term means a legal instrument to provide support for the performance of any portion of the substantive project or program for which you received this award and that you as the recipient award to an eligible subrecipient.
      ii. The term does not include your procurement of property and services needed to carry out the project or program (for further explanation, see Sec. II.210 of the attachment to OMB Circular A–133, "Audits of States, Local Governments, and Non-Profit Organizations").
      iii. A subaward may be provided through any legal agreement, including an agreement that you consider a contract.
   e. *Subrecipient* means an entity that:
      i. Receives a subaward from you under this award; and
      ii. Is accountable to you for the use of the Federal funds provided by the subaward.

ARTICLE X – ATTACHMENTS

The following attachments are hereby incorporated into this Task Agreement. In the event of any apparent conflict between the terms of the Task Agreement and the attachments, the terms of the Task Agreement, including its designations and modifications, will prevail.

I. Project Overview
II. Budget
III. Standard Form 424, Application for Federal Assistance (incorporated by reference)
IV. Standard Form 424A, Budget Information (incorporated by reference)
V. Standard Form 424B, Assurances – Non-Construction Programs (incorporated by reference)
VI. Standard Form 425, Federal Financial Report (incorporated by reference)

Attachment I –Project Overview

UOG researchers, assisted by park staff, will stratify American Memorial Park shoreline based on sediment/habitat characteristics. UOG research staff will randomly replicate-sample each shoreline sediment/habitat strata for macro-invertebrates, sort and classify the invertebrates into taxonomic groupings, measure the size of a representative subsample of each taxonomic group, and draft and submit to NPS two short reports: one short report about the locations and success of field sampling, and a second report on the abundance and size data of the taxa collected including a compilation of invertebrate community characteristics by location and sediment/habitat strata. Copies of raw data and related meta-data, as well as summarized data, will be provided to the park as soon as possible and frequently to provide an isolated back-up NPS data copy. NPS employees will assist with obtaining permits and permissions, data analysis and presentations as requested by UOG, and provide written editorial comments of draft project reports. UOG staff will obtain written approval of the Park Superintendent and NPS Public Information Officers prior to releasing or publishing results from this project.

UOG researchers and park staff will meet and discuss, and/or exchange and discuss written draft(s) of invertebrate-heavy metal sampling plans based on the distribution of macro-infauna, runoff areas and potential contaminant sources. The sampling plans will include 54 samples of biota (individuals or groups of individuals) tested for three heavy metals each, unless Gary Denton and Mike Gawel mutually agree to a different combination of 162 or more heavy metal determinations. A written sampling plan, including specific locations, species and numbers of each species, will be provided to NPS for review and mutual concurrence before conducting field collections.

University of Guam Water and Environmental Research Institute staff will collect invertebrate samples from the shoreline of American Memorial Park, process and analyze the biota for the heavy metals as described and mutually agreed to in the invertebrate-heavy metal sampling plan listed above. University of Guam Water and Environmental Research Institute staff will draft and submit to NPS two short reports; one short report about the locations and success of field sampling, and a second report including heavy metal concentrations in the invertebrate taxa tissue collected and a compilation of invertebrate community heavy metal characteristics by location. Copies of raw data and related meta-data as well as summarized data will be provided to the park as soon as possible and frequently to provide an isolated back-up NPS data copy. NPS employees will assist with obtaining permits and permissions, data analysis and presentation as requested by UOG and provide written editorial comments of draft project reports. UOG staff will obtain written approval of the Park Superintendent and NPS Public Information Officers prior to releasing or publishing results from this project.

Project methodology for heavy metal analysis is given in Denton et. al., 2008 Water and Environmental Research Institute of the Western Pacific, University of Guam Technical Report No.123. http://www.weriguam.org/docs/reports/123.pdf

Project methodology for documentation of mangrove in-fauna is given in:
Sasekumar A. 1984. Methods for the study of mangrove fauna. In: S & J Snedaker, eds. The Mangrove Ecosystem: Research Methods.

Sasekumar A and V. Chong. 1998. Faunal diversity in Malasian Mangroves. Global Ecology and Biogergraphy Letters 7:57-60.

Alfaro, A. 2006. Benthic macro-invertebrate community composition within a mangrove/seagrass estuary in northern New Zealand. Estuarine Coastal and Shelf Science 66:97-110.

References

AMPRO (2005). Unexploded Ordnance, Historical Research and UXO Assessment. Saipan Commonwealth of the Northern Mariana Islands. *Report prepared by AMPRO Ordnance and Explosives Consultants for the CNMI Division of Environmental Quality,* December 10, 2005, 58 pp.

Denton, G.R.W., L.P. Concepcion, H.R. Wood and R.J. Morrison (2006). Trace Metals in Marine Organisms from Four Harbours in Guam, *Marine Pollution Bulletin* 52:1784–1832.

Denton G, Brian Bearden, P. Houk, J. Starmer, and H. Wood. 2008. Heavy metals in biotic representative from the intertidal zone and nearshore waters of Tanapag Lagoon, Saipan, Commonwealth of the Northern Mariana Islands (CNMI). Water and Environmental Research Institute of the Western Pacific, University of Guam Technical Report No.123. http://www.weriguam.org/docs/reports/123.pdf

Denton, G.R.W., Emborski, C.A., Habana, N., and Starmer, J.A. (2014). Influence of Urban Runoff, Inappropriate Waste Disposal Practices and World War II on the Heavy Metal Status of Sediments in the Southern Half of Saipan Lagoon, Saipan, CNMI. *Marine Pollution Bulletin,* 81:276-281.

Sasekumar A. 1984. Methods for the study of mangrove fauna. In: S & J Snedaker, eds. The Mangrove Ecosystem: Research Methods --> but I currently don't have a copy of this reference on me.

Sasekumar A and V. Chong. 1998. Faunal diversity in Malasian Mangroves. Global Ecology and Biogergraphy Letters 7:57-60.

Attachment II. Budget

Budget **American Memorial Park Shoreline Infauna and Heavy Metals Assessment** **Principal Investigators: Drs. Gary Denton and Maria Celia Defrance Malay** **8/18/2014 – 2/17/2017**	
Budget Item	**Requested Funds**
A. DIRECT COSTS	
SALARIES:	
PI Dr. Gary Denton @ $2,393/wk for 3.5 wk	8,376
PI Dr. Malay @ $338.4/day for 7 days	2,369
SUBTOTAL SALARIES	10,745
BENEFITS	
PI Dr. Denton (36.48%)	3,055
PI Dr. Malay (36.48%)	864
TRAVEL	
1 RT airfare Guam to Saipan ($300) for each PI Denton	300
Lodging and per diem for Saipan for Dr. Denton: ($236/day for 7 days)	1,652
Car rental for Dr. Denton, 7 days on Saipan ($31/day)	217
1 RT airfare Guam to Saipan ($300) for each PI Malay	300
Car rental for Dr. Malay, 3 days on Saipan ($31/day)	93
Lodging and per diem for Saipan for Dr. Malay ($236/day for 3 days)	708
SUPPLIES	
Field and laboratory supplies for Dr. Denton (sampling and preservation material, etc.)	1,025
Field supplies (notebooks, measuring tapes, etc.)	261
B. SUBTOTAL DIRECT COST	19,220
C. SUBTOTAL MODIFIED TOTAL DIRECT COST (Salaries only)	10,744
C. INDIRECT COST (17.5% on MTDC)	1,880
D. TOTAL COST OF PROJECT	**$21,100**

APPENDIX B.

(Task Agreement P14ACO1579, Cooperative Agreement P14AC00637, Modification 01)

HAWAI'I-PACIFIC ISLANDS COOPERATIVE ECOSYSTEMS STUDIES UNIT
TASK AGREEMENT P14AC01579
COOPERATIVE AGREEMENT P14AC00637
MODIFICATION 01

BETWEEN THE

UNITED STATES DEPARTMENT OF THE INTERIOR
NATIONAL PARK SERVICE

AND

UNIVERSITY OF GUAM
DUNS NO.: 779908151
UNIVERSITY STATION
MANGILAO, GU 96923

CFDA: 15.945, Cooperative Research and Training Programs – Resources of the National Park System (CESU)
PROJECT TITLE:American Memorial Park Shoreline Infauna and Heavy Metals Assessment
PREVIOUS FEDERAL FUNDING:$21,100.00
FEDERAL FUNDS OBLIGATED BY THIS ACTION:$0.00
TOTAL AMOUNT OF FEDERAL FUNDS OBLIGATED: $21,100.00
TOTAL AMOUNT OF AWARD: $21,100.00
PERIOD OF PERFORMANCE:08/18/2014 – 06/30/2017

GENERAL: The purpose of this modification is to update the project schedule, project products and original budget. Extreme impacts of Typhoon Soudelor on the shoreline resources of American Memorial Park, the loss of one of the PIs on this project (Dr. Malay has left UoG), and allowance for peer review necessitates changes to permit the remaining PI (Dr. Denton) to expand his role to complete the overall objective of determining best organisms to sample and testing them for heavy metals concentrations. Dr. Denton's expanded role allows the project to use his expertise to conduct additional analyses and provide more detailed conclusions, as stated in revised specific project objectives and reflected in revised schedule, products and budget. This modification also replaces the original ATR with a newly-certified individual.Finally, this modification changes the chargeable appropriation used to fund this agreement, in order to correct an error made at the time of initial award.

MODIFICATION:

1. **ARTICLE I.B. – BACKGROUND AND OBJECTIVES**, is modified to read as:

   B. Objectives

   Investigators from UOG and NPS staff will collaborate to accomplish the following specific objectives.

   - Determine the spatial distribution of selected heavy metals in surface sediments and potentially useful bio-indicators (e.g., algae, seagrass and bivalve mollusks) along the shoreline of AMME.
   - Design a contaminant sampling plan based on access and availability of all selected biotic and abiotic components of interest, runoff areas and potential sources of heavy metal pollution.
   - Differentiate between total and biologically available heavy metal loads from a comparative assessment of the biotic and abiotic data and highlight any abnormalities in edible species analyzed that could be important from a public health standpoint.
   - Provide detailed and summarized information on heavy metal concentrations, distribution, and discernible trends in all matrices examined to park staff that can be used determine if and what alternative shoreline management strategies should be considered.

2. **ARTICLE II – STATEMENT OF WORK**, is modified to read as:

   A. The UOG will:

      1. Collaboratively undertake a project titled *American Memorial Park Shoreline Infauna and Heavy Metals Assessment* as described throughout this Task Agreement.
      2. Appoint Gary Denton, Ph.D. as Principal Investigator (PI).
      3. Hire or appoint other staff or students to the project as appropriate to achieve project objectives.

4. Utilize the methods and processes as outlined in the revised project overview, included as Attachment VII, in conducting the project.
5. Coordinate and oversee the following as responsibilities of Dr. Denton:
   a. Meetings, discussions and exchanges of a draft invertebrate-heavy metal sampling plan,
   b. Work with Mike Gawel to mutually identify and agree to the taxa to be analyzed and the specific heavy metals to be tested for concentration determination within the total minimum analysis numbers of this agreement (i.e.,30 sediment and 54 infauna samples each tested for Lead, Mercury and Selenium or the equivalent; e.g., 15 sediment and 27 infauna/flora samples tested for six metals costing equal amounts to Lead, Mercury and Selenium),
   c. Submit a written heavy metal sampling plan to NPS prior to field sampling,
   d. Obtain permits and permissions to sample and remove infauna along the American Memorial Park shoreline for determination of presence and amounts of selected heavy metals within representative infauna,
   e. Collectall samples from the shoreline of American Memorial Park in compliance with the sampling plan and permits,
   f. Process and analyze the selected samples for heavy metal analysis,
   g. Draft and submit a short report about the location and success of field sampling,
   h. Draft and submit a short report including the heavy metal concentrations found in sediments and biota analyzed from American Memorial Park and including a compilation of heavy metal data for similar matrices from elsewhere for comparative purposes.
6. Provide NPS copies of raw data, related meta-data, and summarized data as soon as possible after field sampling and at least twice monthly thereafter to provide isolated back-up NPS data copies.
7. Obtain written approval from the park Superintendent and NPS Public Information Officer(s) prior to releasing or publishing results from this project.
8. Cooperate with the NPS Agreement Technical Representative (ATR) to ensure that the conduct of the project complies with "Quality Control of Scientific and Other Scholarly Products in the Pacific West Region."
9. Ensure that reports and other formal materials (including publications and presentations) resulting from this collaborative project acknowledge the NPS and that the project was conducted through the Hawai'i-Pacific Islands Cooperative Ecosystem Studies Unit, and reference this Task Agreement number.
10. Provide digital photographs with captions of project activities and permission to use all photos to the NPS Hawai'i-Pacific Islands Cooperative Ecosystem Studies Unit Senior Science Advisor and others for use in presentations and reports.

B. The NPS will:

1. Collaboratively undertake a project titled *American Memorial Park Shoreline Infauna and Heavy Metals Assessment* as described throughout this Task Agreement.
2. Provide financial assistance to the UOG as provided in Article V. The revised budget, included as Attachment VIII, is incorporated in this Task Agreement.
3. Assign Justin S. Mills,Assistant Data Manager for the Northern Great Plains Inventory and Monitoring Network, as the ATR. Mike Gawel will be the primary park contact, and Karl Brookins can assist with data and reports.
4. Help UOG employees obtain permits and permissions for sampling along park shorelines.
5. Provide overview and orientation associated with all park visits including: Briefings on safety procedures; introductions to park staff; and logistical support for field work operations, which include coordination with the park and making safety a priority.
6. Assist with safe undertaking of fieldwork within the park and have appropriate NPS staff accompany recipient workers when needed in the field.
7. Accept and store copies of project data and metadata.
8. Assist with data analysis as requested and review and edit project report and sampling plan drafts in a timely manner.
9. Work with Dr. Denton to mutually identify and agree to the taxa to be analyzed and specific metals for heavy metal concentration determination within the total analysis limits of this agreement.
10. Consider and respond to requests to release data and results.
11. Cooperate with the UOG PI to ensure that the conduct of the project complies with "Quality Control of Scientific and Other Scholarly Products in the Pacific West Region." The ATR will designate a peer review manager for this project.
12. Ensure that reports and other formal materials (including publications and presentations) resulting from this collaborative project acknowledge the UOG and that the project was conducted through the Hawai'i-Pacific Islands Cooperative Ecosystem Studies Unit, and reference this Task Agreement number.

ARTICLE III – TERM OF AGREEMENT, is modified to read as:

This Task Agreement is effective on August18, 2014 and will extendthroughJune 30, 2017.

4. **ARTICLE IV.A.1. – KEY OFFICIALS**,ismodified to read as:

   1. ATR:
      Justin S. Mills,Assistant Data Manager
      Northern Great Plains Inventory and Monitoring Network (NGPN)
      231 East Saint Joseph St.
      Rapid City, SD 55701
      Phone:605-341-2804
      Email: Justin_S_Mills@nps.gov

5. **ARTICLE IV.B. – KEY OFFICIALS**,is modified to read as:

   B. For the UOG:

      PI:
      Gary Denton, Ph.D., Professor of Environmental Toxicology
      Water and Environmental Research Institute, University of Guam
      UOG Stations
      Mangilao GU 96923
      Phone: 671-735-2690
      Fax: 671-735-8890:
      Email: gdenton@triton.uog.edu or gdenton@uguam.uog.edu
      http://www.weriguam.org/faculty/gary-denton/page/research-interests.html

6. **ARTICLE V.A. – AWARD AND PAYMENT**, is modified to read as:

   A. NPS will provide financial assistance to the UoG in the amount of $21,100 for the work provided herein, and in accordance with the approved budget (as revised, Attachment VIII). The chargeable appropriations and funding sources for this Task Agreement are as follows:

Fiscal Year	Cost Structure	Fund Source	Amount
2014	PPWONRADW0 / PPMRSNR1Y.NM0000	ONPS	$21,000
2017	PPPWWAPAN0 /PPMPSAS1Z.Y00000	ONPS	$100

7. **ARTICLE VI – PROJECT PRODUCTS**, is modified to read as:

   A. Schedule/Milestones/Dates

      1. October 14, 2015 - The draft *Invertebrate-Heavy Metal Sampling Plan* is due to Mike Gawel.Final sampling plan due to Mike Gawel and Justin Mills prior to conducting field sampling.
      2. August 15, 2016 - Field Sampling for heavy metal analysis completed. Field Sampling Report due to Mike Gawel and Justin S. Mills within 72 hours of returning from the sampling trip.
      3. February 27, 2017 - The draft Heavy Metals Concentration and Distribution Reportdue to NPS from Dr. Denton to Mike Gawel andJustin S. Mills. NPS will review and return within 5 working days.
      4. March10,2017 - Submit draft report to NPS for peer review, which will be completed by May 31, 2017.
      5. June 30, 2017 - The final Heavy Metals Concentration and Distribution Reportsubmitted to Mike Gawel andJustin S. Mills.

   B. Description of Project Products/Reports

      1. Heavy Metal Sampling Plan: Written plan listing the sampling locations and amounts of representative taxon to be collected. This plan should also specify how many, and which, biotic components are mutually agreed to and will be used in determining heavy metal concentration (e.g. pooled samples of three individuals), the number of samples and the identity of heavy metals to be analyzed from all samples.
      2. Field Sampling Report: Approximately1-3 pages; backup copies of all field data should be included as appendix (and not be included in the page count) if not submitted earlier. Details to include the locations sampled and the number and types of biotic representatives collected. Any significant findings, changes to methods, or problems encountered during the field work should also be summarized.
      3. Heavy Metals Concentration and Distribution Report: A written report listing the concentrations of heavy metals found in sediments and each biotic component tested from each location, as well as the mean and a measure of variance from replicates and habitats sampled. Any significant documented trends, such as decreasing

concentrations moving away from a specific location, will also be included in the report. Methods used will be included by reference to other publications unless unpublished methods are used.

C. Delivery of Project Products

1. One electronic copy of all draft (Word format) and final (pdf format) documents will be submitted to the ATR Justin S. Mills and Mike Gawel at the addresses shown in Article IV, Key Officials; hard copies will be delivered if requested in writing.
2. One electronic (pdf) copy of the final *Heavy Metals Concentration and Distribution Report* (if available as a version determined by the park as acceptable for public release) will be submitted to the NPS Hawai'i-Pacific Islands CESU Senior Science Advisor. Contact information is available from the ATR.
3. One electronic (pdf) copy of final *Heavy Metals Concentration and Distribution Report* (if available as a version determined by the park as acceptable for public release) will be submitted to the HPI CESU University Lead Sharon Ziegler-Chong, Office of Research – HPI CESU, 200 West Kawili Street, Hilo, HI 96720; ziegler@hawaii.edu.
4. One electronic (pdf) copy of the final *Heavy Metals Concentration and Distribution Report* (if available as a version determined by the park as acceptable for public release) will be submitted to the following address: Carol Simpson, Technical Information Center, Denver Service Center, National Park Service, PO Box 25287, Denver, CO, 80225, carol_simpson@nps.gov.

8. **ARTICLE X – ATTACHMENTS**, is modified to add at the end the following:

VII. Revised Project Overview (Modification 01)
VIII. Modification 01 Rebudget of FY2014 Funds

9. **All other terms and conditions remain unchanged.**

IN WITNESS WHEREOF, the parties hereto have executed this modification on the date(s) set forth below.

UNIVERSITY OF GUAM

Robert A Underwood

Dr. Robert Underwood
President, University of Guam

MAR 2 0 2017
Date

Victorina M.Y. Renacia

Victorina M.Y. Renacia
Legal Counsel, University of Guam

3/17/17
Date

NATIONAL PARK SERVICE
PACIFIC WEST REGIONAL OFFICE

William W. Nash

William W. Nash
Financial Assistance Officer

03/21/2017
Date

VII. Revised Project Overview (Modification 01)

UOG researchers, assisted by park staff, will meet and discuss, and/or exchange and discuss written draft(s) of the heavy metalsampling plans based on the distribution of macro-infauna/flora, runoff areas and potential contaminant sources. The sampling plans will include 30 sediment and 54 samples of biota (individuals or groups of individuals) tested for three heavy metals each, unless Gary Denton and Mike Gawel mutually agree to a different combination of 162 or more heavy metal determinations. A written sampling plan, including specific locations, species and numbers of each species, will be provided to NPS for review and mutual concurrence before conducting field collections.

University of Guam Water and Environmental Research Institute staff will collect sediment and biotic samples from the shoreline of American Memorial Park, process and analyze them for the heavy metals as described and mutually agreed to in the heavy metal sampling plan listed above. University of Guam Water and Environmental Research Institute staff will draft and submit to NPS two short reports: one short report about the locations and success of field sampling (Field Sampling Report), and a second report including heavy metal concentrations in the sediments and biotic representatives collected and an assessment of any spatial differences and trends that emerge from the data (Heavy Metals Concentration and Distribution Report). Copies of raw data and related meta-data as well as summarized data will be provided to the park as soon as possible and frequently to provide an isolated back-up NPS data copy. NPS employees will assist with obtaining permits and permissions, data analysis and presentation as requested by UOG and provide written editorial comments of draft project reports. UOG staff will obtain written approval of the Park Superintendent and NPS Public Information Officers prior to releasing or publishing results from this project.

Project methodology for heavy metal analysis is given in Denton et. al., 2008. Water and Environmental Research Institute of the Western Pacific, University of Guam Technical Report No.123. http://www.weriguam.org/docs/reports/123.pdf

References

AMPRO (2005). Unexploded Ordnance, Historical Research and UXO Assessment. Saipan Commonwealth of the Northern Mariana Islands. *Report prepared by AMPRO Ordnance and Explosives Consultants for the CNMI Division of Environmental Quality,* December 10, 2005, 58 pp.

Denton, G.R.W., L.P. Concepcion, H.R. Wood and R.J. Morrison (2006). Trace Metals in Marine Organisms from Four Harbours in Guam, *Marine Pollution Bulletin* 52:1784–1832.

Denton G, Brian Bearden, P. Houk, J. Starmer, and H. Wood. 2008. Heavy metals in biotic representative from the intertidal zone and nearshore waters of Tanapag Lagoon, Saipan, Commonwealth of the Northern Mariana Islands (CNMI). Water and Environmental Research Institute of the Western Pacific, University of Guam Technical Report No.123. http://www.weriguam.org/docs/reports/123.pdf

Denton, G.R.W., Emborski, C.A., Habana, N., and Starmer, J.A. (2014). Influence of Urban Runoff, Inappropriate Waste Disposal Practices and World War II on the Heavy Metal Status of Sediments in the Southern Half of Saipan Lagoon, Saipan, CNMI. *Marine Pollution Bulletin,* 81:276-281.

VIII. Modification 01 Rebudget of FY2014 Funds

Modification01Rebudget of FY2014 Funds
"American Memorial Park Shoreline Infauna and Heavy Metals Assessment"
Principal Investigator: Dr. Gary Denton
08/18/2014 – 06/30/2017

Budget Item	Requested Funds
A. DIRECT COSTS	
SALARIES:	
PI Dr. Gary Denton @ $2,393/wk for 5 wk	11,965
BENEFITS	
PI Dr. Denton (36.48%)	4,365
TRAVEL	
1 RT airfare Guam to Saipan ($300) for each PI Denton	300
Lodging and per diem for Saipan for Dr. Denton: ($236/day for 7 days)	1,652
Car rental for Dr. Denton, 7 days on Saipan ($31/day)	217
SUPPLIES	
Field and laboratory supplies (analytical standards and reference materials, etc.)	507
B. SUBTOTAL DIRECT COST	19,006
C. SUBTOTAL MODIFIED TOTAL DIRECT COST (Salaries only)	11,965
D. INDIRECT COST (17.5% on MTDC)	2,094
E. TOTAL COST OF PROJECT	**$21,100**

APPENDIX C

(Meta Data: Lat-long Coordinates for Biota Sampling Sites 1-11)

Shoreline Coordinates for AMME Biotic Sampling Sites

Site	Map Coordinates[a]	
	Longitude	Latitude
1	145.7162150	15.21786831
2	145.7171400	15.21952000
3	145.7186151	15.21962365
4	145.7202667	15.21965601
5	145.7213370	15.21954778
6	145.7221260	15.21870326
7	145.7213138	15.21777680
8	145.7249506	15.21758650
9	145.7264535	15.21776610
10	145.7293845	15.21863126
11	145.7313104	15.21999882

[a]Courtesy Justin Mills: U.S. NPS

APPENDIX D

(Supplementary Information: Biomonitors and Biomonitoring Considerations)

BIOMONITOR PREREQUISITES AND PROGRAM DESIGN IMPERATIVES

The idea of using aquatic biota to monitor pollutant levels in aquatic systems came about in the 1960s and was primarily conceived to monitor radionuclide abundance in the environment (e.g., Seymour 1966). Since then, the technique has been adapted for monitoring stable heavy elements, and other persistent pollutants known to impact biological systems. Classic papers dealing with essential design imperatives for aquatic monitoring programs include the excellent reviews of Phillips (1977, 1978, 1980, 1986, 1990), Philips and Rainbow (1993), and Phillips and Segar (1986). Key points drawn from these works are briefly discussed below.

Species Selection:

The basic premise underlying the biomonitor concept is that contaminants accumulate in the tissues of the biomonitor at rates that are proportional to concentrations in the surrounding water. Tissue residue levels are, therefore, a time-averaged indication of each contaminant's biological availability at that particular location and point in time. According to Butler *et al.* (1971), Haug *et al* (1974), and Phillips (1977), an ideal indicator has the following attributes and should:

- accumulate the pollutant without being killed by the levels encountered in the environment,
- be sedentary in order to be representative of the area in which it is collected,
- be abundant throughout the study area, easily recognized, and readily sampled,
- be of sufficient size to provide adequate tissue for analysis,
- be relatively long-lived to permit sampling over several months or years,
- be amenable to translocation,
- demonstrate a simple correlation between pollutant levels accumulated in its tissues and the average pollutant concentration in the surrounding water.

The latter prerequisite is of overriding importance because it requires that the biomonitor of choice possesses little or no ability to metabolically regulate pollutant levels in its tissues. Another highly desirable characteristic is that the biomonitor should exhibit a high affinity (concentration capacity) for the contaminant in question. Some of the early studies with heavy metals were compromised by insufficient attention to metabolic control and the flawed assumption that high tissue concentrations of a particular element were a sign of biomonitoring potential. Crustaceans for example are naturally high in copper and zinc and many representative species of this group regulate tissue levels of both metals within relatively narrow limits (Bryan 1964). Hence, they are of no practical use as indicators for these elements. Zinc regulation has also been observed in a number of other invertebrate groups that accumulate this metal to relatively high levels (Bryan and Hummerstone 1973), including several species of bivalves (Phillips and Yim 1981, Klumpp and Burdon-Jones 1982, current study).

A number of other important considerations present themselves when selecting candidate species for heavy metal monitoring purposes. One obvious consideration that is often overlooked is that heavy metals occur naturally in the environment (albeit at low levels) and different species have evolved widely differing capacities to accumulate them. Consequently, even closely related species sometimes have metal profiles that are very different from one another. Moreover, some metals are biologically essential and are regulated in certain species but not in others. Again, such differences can occur within as well as between biotic groups. The simple fact of the matter is that no single organism will satisfy the monitoring needs for all heavy metals of environmental concern.

Crucial factors that affect heavy metal levels within and between species are largely related to age, growth, microhabitat, and the interactive effects of season and sexual development. Choosing the correct biomonitor or suite of biomonitors, and refining sampling parameters and protocols to accommodate these variables is, therefore, of paramount importance, if spatial and temporal differences in metal abundance are to be accurately assessed and interpreted correctly.

In temperate regions, a considerable amount of research has focused on the biomonitoring ability of a select group of organisms (mostly brown algae, bivalve mollusk especially mussels and oysters, and various fish). In contrast, relatively little attention has been directed towards the utility of tropical species for monitoring purposes. As a consequence, preliminary monitoring programs, like the one undertaken here, may be forced to include hitherto 'untested' species that are only distantly related to well-established monitoring organisms from other regions of the world. This particular problem is compounded somewhat by the fact that, while species diversity is characteristically high in tropical waters, species abundance is often not very great.

Sample Variability:
How well a biomonitor reflects changes in the ambient availability of a contaminant is determined largely by the degree of variability encountered in the population sampled. The more variable the tissue levels, the less reliable the organism becomes, and the greater the number of individuals required to detect a given level of change. Such variability can essentially be divided into two broad categories, namely that which can be reduced or eliminated by the investigator, as opposed to that which cannot. Controllable variations include parameters such as the age/size, growth, fitness, sex and reproductive condition of the individuals sampled, in addition to differences related to their position on the shore and/or in the water column. Uncontrollable variations may be ascribed to regional and seasonal differences in temperature and salinity, and includes the inherent, natural variability normally encountered between individuals of the same species as a result of subtle variations in genetic make-up, metabolic efficiencies, health and well-being. Failure to address these variables during the initial design phase of a monitoring program can produce data that are extremely noisy and often highly misleading.

Program Design:
Pollution monitoring programs involving the use of bioindicators generally have one or both of the following objectives:

- To identify spatial difference in contaminant abundance within an area or region, including the delineation of 'hot-spots'
- To evaluate short- and long-term temporal changes in contaminant abundance within any particular site or area

Both objectives are separate from one another and have specific requirements (Phillips and Segar 1986). For example, if the primary goal is to delineate spatial difference in contaminant bioavailability, it is important to adopt a synchronous sampling regime to ensure that temporal fluctuations in pollutant availability at each of the sites studied do not interfere with the data. On the other hand, monitoring temporal trends in pollutant abundance within any particular site requires a sampling frequency that is determined by the biological half-life of the contaminant of interest if an uninterrupted record of its biological availability is to be obtained. In addition, the influence of seasonal changes in temperature, salinity and reproductive status on pollutant levels within the bioindicator needs to be addressed in order to identify ‘real’ changes in a contaminant’s ambient availability.

Both objectives also have a number of common requirements that must be met in order to optimize the survey design. For example, it is customary to standardize on a specific size or size range of individuals in order to eliminate any possible age-dependant variability in contaminant levels (e.g., mercury in fish). This can be done in one of two ways, either by selecting a specific size range, or by taking what is available and normalizing the data to a specific size by regression techniques. Another requirement common to both monitoring objectives calls for the standardization of collection sites on the shore or in the water column. This is particularly important in areas receiving freshwater inflow or in waters that are highly stratified. Finally, it is necessary to identify the inherent variability of pollutant levels in the biomonitor of choice in order to optimize sample sizes for the desired degree of statistical resolution over space and time.