Regional Studies in Marine Science

Contaminants in Queen Conch (*Strombus gigas)* in Vieques, Puerto Rico

David Whitall[1*], Antares Ramos[2], Diane Wehner[3], Michael Fulton[4], Andrew Mason[1], Ed Wirth[4], Blaine West[4], Anthony Pait[1], Emily Pisarski[4], Brian Shaddrix[4], Lou Ann Reed[4]

*Corresponding author

[1]National Oceanic and Atmospheric Administration, National Centers for Coastal Ocean Science, Silver Spring, Maryland

[2] National Oceanic and Atmospheric Administration, Office of Coastal Management, Coral Reef Conservation Program, San Juan, Puerto Rico

[3]National Oceanic and Atmospheric Administration, Office of Response and Restoration, Assessment and Restoration Division, New York, NY

[4] National Oceanic and Atmospheric Administration, National Centers for Coastal Ocean Science, Charleston, SC

Abstract

Pollution has the potential to negatively alter coastal ecosystem health, including fisheries species, through direct impacts, food web effects and habitat degradation. Vieques is an island municipality of the Commonwealth of Puerto Rico, which lies off the east coast of the main island. In addition to normal pollution stressors associated with human activities, Vieques was also the site of a military bombing range from the 1940s until 2003. There is significant local concern about potential negative impacts of pollution from these and other activities on fisheries stocks, as well as seafood as a vector for toxic contaminants to enter the human food supply. In this study, queen conch (*Strombus gigas)* tissues were analyzed for a suite of contaminants: metals, the pesticide DDT (and its degradation products), and energetic compounds (associated with munitions) from three areas around the island. The magnitude of contamination found in queen conch was within the range of values reported in other studies in the

Caribbean, suggesting that the levels of these selected contaminants present in conch in Vieques are not unusual for the region.

Keywords: pollution, queen conch, munitions, metals, DDT

1.0 Introduction

Current and historical land use has the potential to impact the ecological health of marine ecosystems. Land based sources of pollution, such as nutrients, sedimentation and toxics, can affect critical habitats, alter food webs (e.g. by reducing reproductive success and therefore abundance), and directly affect the health of marine organisms. In addition to affecting ecological health, pollution can impact harvested species, both in terms of altering the stocks of the species, and also in terms of toxin body burdens which may affect the safety of the seafood for human consumption.

Vieques is an island municipality of the Commonwealth of Puerto Rico (United States) that lies 11 km off the east coast of the main island (Figure 1). It is 35 km long, is 7.2 km wide at its widest point, and is characterized by rolling hills with its highest point being Mount Pirata (301 m) on the western side of the island. There are numerous coastal lagoons and bays with varying degrees of connectivity to the ocean. Like mainland Puerto Rico, its climate is tropical (with temperatures ranging from 19^{o} to 32^{o} C) with a dry season (December to April) and a rainy season (May to November). There are two towns on the island, Esperanza on the south coast and Isabel Segunda on the north coast (Figure 1). The 2010 U.S. Census reported the population of Vieques to be 9,301 (U.S. Census, 2010).

Previous work (Bauer et al., 2010) showed that the benthic habitat in the Vieques marine environment was a mix of turf algae, macroalgae, gorgonians, crustose/calcareous algae, hard coral, and sponges. Turf algae made up the highest percentage of the bottom area. As is the case in much of the Caribbean, hard coral cover was generally low. The fish community observed in the study consisted of 34 taxonomic families and 110 species, with wrasses and damselfishes being the most abundant, and surgeonfishes and parrotfishes accounting for the highest proportion of biomass. One of the major findings of this previous study was that Vieques is similar in terms of benthic cover, total fish abundance and biomass to other nearby locations in Southwest Puerto Rico, St. Croix and St. John in the U.S. Virgin Islands (USVI). Longshore ocean currents generally move from east to west (Bauer et al., 2008).

Vieques has a varied and unusual land use history. Spanish colonists cleared much of the land for sugar cane production in the mid-1800s, and the sugar industry prospered into the early 1900s. Puerto Rico, including Vieques, became part of the United States in 1898 following the Spanish-American War. In the 1940s, the United States annexed approximately two-thirds of the land on Vieques for use by the Navy as a base and training facility. This included ammunition storage and disposal on the west end of the island and live fire training activities on the east end of the island, including air, sea, and maneuver warfare, air-to-ground bombing, amphibious landings, and artillery training operations (DON, 1979; DON, 1986; DON, 2001; GMI, 2003; CH2M HILL, 2004; GMI, 2005). Naval activities ceased in 2003 as controversy over the Navy's presence in Vieques escalated. In 2003, the Navy transferred most of this land to the Department of the Interior's Fish and Wildlife Service to create the Vieques National Wildlife Refuge. Public access to some of these lands is restricted due to safety concerns related to unexploded ordnance. In 2005, the former Navy areas of Vieques were designated as a "Superfund" site under the Comprehensive Environmental Response, Compensation, and Liability Act, requiring environmental investigation and cleanup by the Navy. Cleanup activities are expected to continue through at least 2025.

Reported high cancer rates on the island (ATSDR, 2013) have led to widespread speculation that the marine environment is polluted from historical land uses and that seafood might be a vector for human exposure. More recently it has been hypothesized that contaminants have led to a decline in the Vieques fishery (Caribbean Business, 2013). A previous study (Pait et al., 2010) found relatively low levels of contaminants in marine surface sediments, with the exception of chromium (elevated at one site on the northeastern side of the island) and DDT (elevated at four sites on the southeastern side of the island).

In order to assess the hypothesis that contaminants are entering the marine food web at deleterious levels, this study reports contaminant concentrations in queen conch (*Strombus gigas)* collected from three areas around Vieques. Conch was suggested by local fishermen as an important species, and their habitat and feeding mechanisms are well suited to examining contamination. Queen conch are large marine soft bodied gastropod mollusks with a hard calcium carbonate shell. They are found throughout the tropical western Atlantic (Martin, 1995), generally in waters between 5 and 20 m, with their depth range is limited primarily by food sources (Sterrer, 1986). The habitat includes coral reefs, rocky shores, seagrass beds and patchy sand flats (Randall, 1964). Queen conch feed on algal plant material (e.g. Thalassia), epiphytes and benthic algae, may ingest sediment particles during this process (Randall, 1964). Conch can live up to 30 years (McCarthy, 2008) and reach up to 30 cm in length (Randall, 1964).

Individuals reach sexual maturity at 3 to 4 years of age (McCarthy, 2008) and are especially susceptible to predation as juveniles, i.e. prior to shell formation (Appeldoorn, 2013). Queen conch have relatively small home ranges (mean of 6 ha, Glazer et al., 2003). This small home range, in combination with its benthic habitat, make conch well suited for contaminant studies. Conch is an important food source across the Caribbean. Pollution has been shown to negatively impact queen conch populations in the Caribbean (Glazer et al., 2008; Spade et al., 2013).

Previous studies (Apeti et al., 2014, Rizo et al., 2010) have quantified contaminants in queen conch, and others (Glazer et al., 2008) have correlated the presence of contaminants (metals, PAHs, PCBs, DDT) with conch reproductive health. The conch tissues were analyzed for a suite of munitions-related (energetic) compounds, metals and DDT and its breakdown products.

2.0 Materials and Methods

2.1 Sampling Design

In April of 2014, scientists from NOAA and the Puerto Rico Department of Natural and Environmental Resources (DNER) consulted with the local fishing community to determine fishing areas of concern near the island. Federal resource managers and environmental regulators (US Fish and Wildlife Service and US Environmental Protection Agency) also were consulted. Two areas on the south shore of the island were identified as being of concern: Ensenada Honda and Bahia Salinas del Sur (Figure 1). Bahia Salinas del Sur is located adjacent to the former Live Impact Area (LIA), where most of the live bombing exercises occurred. Ensenada Honda, while not in the LIA, is adjacent to, and downstream from (based on the prevailing longshore current) the restricted area were military activities occurred. Additionally, the fishermen identified an area on the north shore, near Mosquito Pier (Figure 1), as an area relatively less impacted by pollution. While this “unimpacted” site is not a true control, it is useful for comparative purposes, as it is far less likely to have been affected by military activities. The benthic habitat of all three sampling areas contained a mix of coral reefs, seagrass beds and unconsolidated sediments. Within the two larger sampling areas (Ensenada Honda and Bahia Salinas del Sur), the sampling area was geographically divided into three sub-areas in order to ensure that fishing effort was spread evenly across the larger region. The sampling design called for three conch to be collected from each sampling area (21 conch total), with the results grouped within the three areas (Bahia Salinas del Sur, Ensenada Honda and Mosquito Pier).

2.2 Sample Collection Methods

Within each of the sampling strata, queen conch were collected by hand by SCUBA divers. Using search and rescue swimming patterns, divers searched the benthic habitat within each of the sampling sub-areas, looking for live conch of legal harvest size (>9 inches in total length). The total number of conch collected was limited to 15 because it was difficult to find conch large enough for legal extraction. Figure 1 shows the distribution of conch among the sub-areas. Nitrile gloves were worn during collection and handling to reduce the potential for contamination. Once aboard the boat, individual conch were placed in sealed plastic freezer bags and stored on ice. At the end of each field day, conch were frozen (-20^{o} C). After freezing for at least 24 hours, conch were partially thawed on ice which allowed the tissue to be extracted from the shell whole. The extracted tissue was then placed in pre-cleaned HDPE containers and re-frozen, prior to overnight shipment to the NOAA analytical lab (National Centers for Coastal Ocean Science) in Charleston, SC.

Conch tissue samples were analyzed for a suite of analytes (Table 1) including: energetic compounds (associated with munitions), metals and DDT (and its degradation products). DDT is reported as total DDT (sum of parent material and degradation products). Unless otherwise noted, metals are reported as total concentrations.

2.3 Laboratory Methods

Conch were stored at -40°C prior to preparation for analysis. Briefly, tissues were prepared after thawing by homogenizing each conch in a Teflon container using a titanium handheld probe homogenizer (ProScientific, Inc.). Homogenized samples were sub-sampled for organic contaminant analysis (energetics, DDTs) and trace metals analysis (Balthis et al., 2012). Moisture content was determined by drying in an oven at 85°C (>24 hr) until constant mass was achieved. All data were validated by comparison with blanks, spikes (matrix and reagent spikes), and certified reference materials.

Trace metal determination used methods described in Reed et al. (2015). Briefly, homogenized tissue was microwave digested in nitric acid followed by peroxide addition. Analysis for 21 elements (Ag, Al, As, Ba, Be, Cd, Co, Cr, Cu, Fe, Li, Mn, Ni, Pb, Sb, Se, Sn, Tl, U, V, and Zn) was achieved using ICP-MS (Perkin Elmer Elan 6100), while Hg was determined by direct mercury analysis (DMA-80, Milestone Inc.).

DDTs were determined by GC/MS (Kimbrough et al., 2006) with slight modifications to the protocols. Briefly, samples were prepared for Accelerated Solvent Extraction (ASE) by grinding the conch homogenate with ~28 g anhydrous sodium sulfate in a mortar. Internal standards were added to the samples prior to extraction by ASE. Post extraction clean-up was achieved using gel permeation chromatography, and collected fractions were further processed using activated alumina. Final extracts were analyzed using an Agilent 6890 Gas chromatograph paired with an Agilent 5973 MS.

The determination of munition compounds in marine tissues was achieved by two separate extraction methods (Table 1), sonication (a modification of EPA 8330B, USEPA 2006)) and Accelerated Solvent Extraction (ASE, using a Dionex ASE 200). Samples were extracted by both methods and specifics of these protocols can be found in the supplemental information. Briefly, ~1.2 g lyophilized sample was placed into 50 mL solvent rinsed glass centrifuge tube and sonicated for 3hrs. Additionally, ~4.2g wet tissue was extracted using ASE. Final extracts were analyzed using LC/MS-MS (Agilent 1100 LC/AB Sciex API4000 MS) or Agilent 6890 GC/5973N MS). Data quality was determined by a series of blank, spike and standard reference material (SRM) analyses. Additional details on analytical methods are provided in the Supplemental Materials.

2.4 Statistical Methods

Data were analyzed using JMP statistical software. Because data were not normally distributed (Shapiro-Wilk test), non-parametric statistics (Dunn’s test) were used to evaluate differences between the sampling areas. Queen conch data from the St. Thomas East End Reserve (STEER) in the USVI were also collected by NOAA, allowing a statistical comparison to be done on the raw data between the data collected in Vieques In this study and the STEER data. Data were first made comparable by converting the STEER data to a wet weight basis, then compared using non-parametric tests (Wilcoxon, α=0.05).

3.0 Results

All data are presented on a wet weight basis and are available to the public via download through NOAA’s National Status and Trends Program data portal (NOAA, 2016). All munitions values were below the method detection limits. Only one sample had a detectable level of DDT; site BSS-7 in Bahia Salinas del Sur had a total DDT concentration of 0.194 ng/g. This total represents two breakdown products: 4,4'-DDD (.085 ng/g) and 4,4'-DDE (0.110 ng/g). Metal concentrations ranged from near the limits of detection (e.g. Ni, Pb) to over 100 μg/g for iron. Because metals occur naturally in the environment, values below the MDL are reported as the MDL (rather than as zero, because zero values are unlikely). Metals and DDT concentrations in conch are shown in Table 2.

For the majority of analytes, there were no statistically significant differences (Dunn's test, α=0.05) among the three sampling areas. However, Mosquito Pier showed higher concentrations than Bahia Salinas del Sur for Al (p=0.028) and Fe (p=0.031). Bahia Salinas del Sur was higher than Ensenada Honda for Zn (p=0.04), and Ensenada Honda was higher than Mosquito Pier for Cd (p=0.017). This collection of spatial results does not point to a consistent spatial pattern, i.e. no one sampling area is more polluted than another.

4.0 Discussion

Because of the low abundance of target organisms, the study could not be structure based on size class or age of the conch, both of which may have an effect on their potential to bioaccumulate contaminants. However, because the size of conch sample was not widely variable (9 to 12 inches), the impact of the size/age variable is hypothesized to be minimal compared to other explanatory variables (i.e. potential contaminant exposure).

On Vieques, the use of energetic compounds was explicitly associated with military activities (i.e. the use of munitions). The absence of munitions constituents in conch tissues is consistent with previous studies of marine sediments (Pait et al., 2010), which also did not detect these compounds in the marine environment of Vieques. While there is no debate that munitions were used in large quantities on Vieques, these compounds do not seem to be prevalent in the coastal ecosystems. This is consistent with what has been reported at other former military sites (ATSDR, 2007, Cox et al., 2007). Some data suggests that energetic compounds in marine environments are rapidly degraded by bacteria (Montgomery et al., 2008). Lotufo et al. (2013) concluded that ecological risks from munitions constituents in marine systems are negligible. These conclusions were based on the fact that energetic compounds are not considered to be bioaccumulative, that aqueous exposure rather than dietary uptake is the dominant route of exposure, and that high elimination rates for these compounds resulted in an essentially complete loss of body residue within hours to days of when the source of contamination was removed (Lotufo et al., 2013).

DDT was widely used as a pesticide, including for mosquito control, until it was banned in 1972. Because of its environmental persistence, it is not unusual to find it in the marine environment (Pait et al., 2010, Pait et al., 2012, Whitall et al., 2014). In this study, DDT was only detected in one (of fifteen) samples, so it may not be widespread in the conch population. This detection was of breakdown products of DDT, not the parent material, suggesting that this is not "new" DDT (i.e. illegally applied or leaking from a barrel). Given that previous studies have found high DDT in the marine environment of Vieques (Pait et al., 2010), additional sampling of marine biota for DDT may be warranted. Concentrations of DDT reported here are below what has been determined to have effects on queen conch reproduction (Glazer et al., 2008).

Unlike energetic compounds or DDT, metals have both natural (crustal erosion) and anthropogenic sources. The mere presence of metals in biological tissues does not indicate that the biota are contaminated. In fact, many metals (e.g. Fe, Mn, Ni, Se, and Zn) are essential micronutrients for animal health. Additionally, since metals are elemental, they do not break down in the environment the way many organic contaminants do. The presence of some major crustal elements (e.g. Al and Fe) may be due to residual sediment particles in the guts of the conch, as a result of their feeding method. A robust discussion of the effects of these metals on tropical marine ecosystems is available elsewhere (e.g. Pait et al., 2010, Whitall and Holst 2015). Previous research has shown that, based on the munitions used in Vieques, the metals most likely to be elevated are: iron, aluminum, copper, manganese, zinc, and lead (ATSDR, 2013). Evaluating metals data requires looking for outliers, both within this study and when comparing these data to other published datasets.

As is with any environmental study with limited available study organisms (i.e. relatively small n value), Type II error cannot be ruled out when considering the comparisons between sampling areas. However, the lack of a consistent spatial pattern for metals within the study area suggests there is no "hotspot" among the three sampling areas. This is not what might be expected if the bombing activities on the east end of the island resulted in contamination of the marine food web, for example with metals and energetics residues. This study only reports data for queen conch; other species may bioaccumulate contaminants at different rates, so these data should not be extrapolated to the Vieques fishery as a whole.

The concentrations reported for conch in this study are compared with other values from the literature in Table 3 (Glazer et al., 2008, Rizo et al., 2010, Apeti et al., 2014). In general, concentrations of metals and DDT measured in Vieques were within the range of values reported elsewhere in the Caribbean where munitions are not present (Florida, US Virgin Islands, Cuba, Table 3). This suggests that metals and DDT concentrations in conch in Vieques are not unusually high for the Caribbean region. This is consistent with previously reported sediment contaminant data (Pait et al., 2010) for Vieques.

Statistically (Wilcoxon test, α=0.05), Cu, Pb, Sn and Zn concentrations in the STEER (USVI) exceeded those in Vieques. Conversely, concentrations of Cr, Ni and Se were higher in Vieques than in the STEER. This does not suggest that Vieques has unusually high pollution for the region.

Although this study was not designed to evaluate conch from a seafood safety perspective, these data can be compared with previously published seafood consumption guidelines. In the interest of being as conservative as possible, the most stringent available guidelines were selected. These guidelines for sport and subsistence fishing (USEPA 2000) assume frequent consumption of seafood (sixteen 8 ounce meals per month), meaning that the suggested acceptable tissue concentrations are very low. Guidelines do not exist for all measured analytes. In order to compare arsenic data from this study (total arsenic) to the guidelines (only inorganic arsenic is toxic), inorganic arsenic was assumed to make up 2% of the arsenic in the conch tissue (USEPA, 2012). Comparing the conch concentration data from this study with these USEPA guidelines (Table 4) demonstrates that based on the concentrations of arsenic and cadmium, sixteen servings of conch per month would be unsafe. Based on maximum values of these metals measured in Vieques, four meals per month of conch would be considered acceptable levels of metal intake. Additionally, it should be noted that the levels reported here to exceed USEPA guidelines are not unique to Vieques. Using these conservative guidelines, conch from Cuba, Florida and St Thomas all have concentrations that suggest a risk from conch consumption (see Tables 4 and 5).

5.0 Conclusions

Although the concentrations of some metals (Cr, Ni, and Se) were statistically higher in conch in Vieques than in the nearby St. Thomas East End Reserves marine protected area, the conch tissue data presented in this study fall within the range of reported values for the Caribbean. This, combined with the observation that there was no clear "hot spot" of contamination between the three sample areas, suggests that former land uses on Vieques, including military activities, have not resulted in unusually high contamination in conch. In addition, no energetics were found above the level of detection in any of the samples analyzed. Using very conservative seafood advisory guidelines, more than four servings of conch per month would exceed safe levels of metal intake; however, using these same guidelines, other published concentration data suggest that conch from Cuba, Florida and St. Thomas also present consumption risks. Site specific information about the amount of seafood consumed by the residents of Vieques would allow for a more accurate assessment of seafood safety.

This type of study could be applied to additional species, including higher tropic levels which might be susceptible to bioaccumulation, that are important both from an ecological and a fisheries perspective. Understanding the magnitude and extent of contaminants in seafood allows for more holistic fisheries management and allows consumers to make informed dietary decisions.

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List of Tables and Figures

Figure 1: Site map with labels. MP=Mosquito Pier, EH=Ensenada Honda, BSS=Bahia Salinas del Sur. Locations of former military activities are also shown, including live fire range on the eastern end of the island and waste disposal (including munitions) on the western end.

Table 1: Calculated MDL and data assurance parameters for DDTs, munitions and inorganic elements.

Table 2: Summary statistics for total metals and total DDT in queen conch in Vieques. No energetic compounds were detected. Values are wet weight as ng/g for DDT and μg/g for metals. For all analytes, n=15.

Table 3: Comparison of queen conch contaminant values from Vieques, Puerto Rico (this study) with queen conch data from Cuba (Rizo et al., 2010), St. Thomas, USVI (Apeti et al., 2014) and Florida (Glazer et al., 2008) Values are wet weight as ng/g for DDT and μg/g for metals.

Table 4: Comparison of data from queen conch in Vieques, Puerto Rico (this study) with US Environmental Protection Agency seafood consumption guidelines (USEPA 2000). Values are wet weight.

Table 1. Calculated MDL and data assurance parameters for DDTs, munitions and inorganic elements

Analyte	**Average MDL Dry**	**Average MDL Wet**	**Average Spike Recovery**	**Average Matrix Spike Recovery**	**SRM Recovery**	**NIST SRM**
Units	*ng/g*	*ng/g*	*%*	*%*	*%*	
2,4'-DDD	0.031	0.005	92.8	101	101	1974c
2,4'-DDE	1.17	0.218	90.4	89.6	83.6	1974c
2,4'-DDT	0.163	0.029	99.8	141	18.0	1974c
4,4'-DDD	0.011	0.002	92.8	114	99.4	1974c
4,4'-DDE	0.064	0.011	93.9	100	107	1974c
4,4'-DDT	0.011	0.002	95.9	114		1974c
1,3,5,7-tetranitro-1,3,5,7-tetrazocane	14.0	2.42	99.4	97.5		
1,3,5-trinitroperhydro-1,3,5-triazine	14.0	2.42	101.0	107		
2,2’,6,6’-tetranitro-4,4’-azoxytoluene	14.0	2.42	106.9	36.2		
2,4-dinitrotoluene	32.2	12.8	78.0	70.0		
2,6-dinitrotoluene	32.2	1.79	76.7	62.0		
2-amino-4,6-dinitrotoluene	11.1	1.79	106.0	108		
2-nitrotoluene	12.9	1.79	93.2	104		
3-nitrotoluene	12.9	2.42	84.7	79.5		
4-amino-2,6-dinitrotoluene	11.1	2.42	87.9	69.9		
4-nitrotoluene	12.9	2.42	87.4	104.3		
nitrobenzene	64.5	2.42	99.5	35.8		
pentaerythritoltetranitrate	14.0	6.06	99.9	86.9		
tetryl	14.0	6.06	64.1			
trinitrotoluene	11.1	2.42	106.5	101		
Units	*ug/g*	*ug/g*	*%*	*%*	*%*	
Silver (Ag)	0.145	0.039	97.4	102.6	81.4	1566b
Aluminum (Al)	0.844	0.786	123	81.6	101	1566b
Arsenic (As)	0.178	0.328	108	87.9	87.5	1566b
Barium (Ba)	0.042	0.007	102	99.9	79.6	1566b
Beryllium (Be)	0.050	0.009	111	101.6		1566b
Cadmium (Cd)	0.128	0.069	99.9	102.3	84.5	1566b
Cobalt (Co)	0.172	0.032	96.0	96.4		1566b
Chromium (Cr)	0.039	0.072	101	97.8		1566b
Copper (Cu)	0.467	0.929	102	94.3	88.3	1566b
Iron (Fe)	0.807	0.752	99.3	43.2	96.1	1566b
Mercury (Hg)	0.109	0.001			111	1566b
Lithium (Li)	0.302	0.056	106	97.5		1566b
Manganese (Mn)	0.171	0.095	90.0	107	96.9	1566b
Nickle (Ni)	0.112	0.062	97.7	95.2	87.4	1566b
Lead (Pb)	0.026	0.015	103	97.4	85.2	1566b
Antimony (Sb)	0.828	0.154	107	106	4019	1566b
Selenium (Se)	0.206	0.115	96.6	97.9	96.9	1566b
Tin (Sn)	0.132	0.024	104	105.1	151	1566b
Thallium (Tl)	0.074	0.013	104	98.2		1566b
Uranium, (U)	0.187	0.110	104	98.1	87.2	1566b
Vanadium (V)	0.055	0.093	103	97.9	93.4	1566b

Zinc (Zn)	0.217	0.449	101	108	82.8	1566b

Table 2: Summary statistics for total metals and total DDT in queen conch in Vieques. No energetic compounds were detected. Values are wet weight as ng/g for DDT and μg/g for metals.

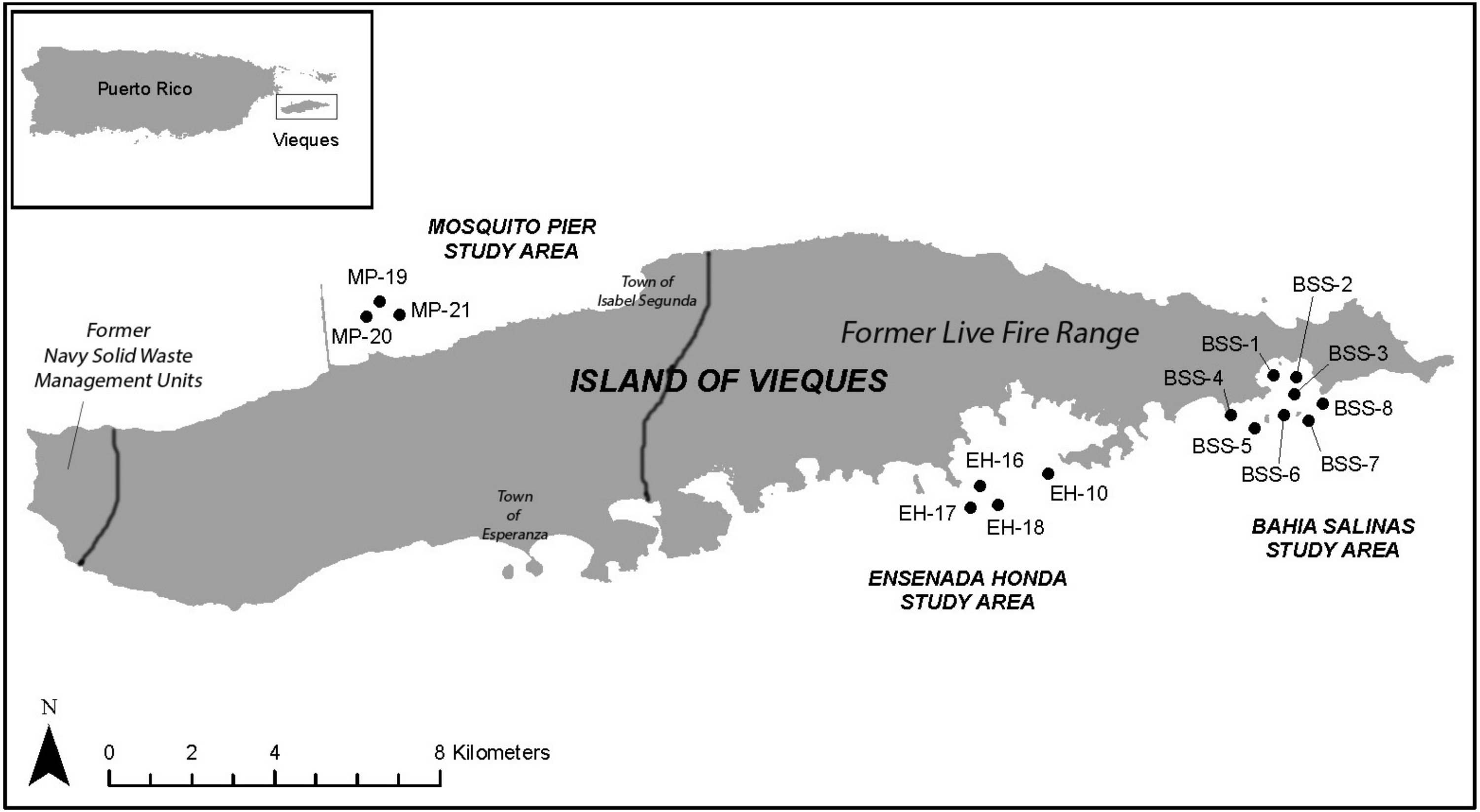
Analyte	min	max	mean	median
Ag	0.043	0.206	0.109	0.095
Al	13.1	61.2	33.725	28.35
As	4.18	20.2	9.504	8.655
Ba	0.036	0.285	0.180	0.178
Be	0.008	0.009	0.009	0.009
Cd	0.097	0.688	0.350	0.338
Co	0.03	0.045	0.033	0.032
Cr	0.948	2.08	1.449	1.365
Cu	4.77	26.3	11.25	9.435
Fe	59.5	197	128.031	116.5
Hg	0.017	0.045	0.028	0.023
Li	0.067	0.118	0.090	0.087
Mn	9.3	63.9	33.893	31.15
Ni	1.13	3.81	2.108	2.015
Pb	0.024	0.072	0.053	0.046
Sb	0.144	0.166	0.153	0.152
Se	0.492	0.967	0.652	0.604
Sn	0.02	0.042	0.034	0.035
Ti	0.012	0.014	0.013	0.013
U	0.136	0.827	0.492	0.470
V	0.896	5.53	2.877	2.45
Zn	7.51	46.3	25.632	20.3
DDT	0	0.195	0.013	0

Table 3: Comparison of queen conch contaminant values from Vieques, Puerto Rico (this study) with queen conch data from Cuba (Rizo et al., 2010), St. Thomas, USVI (Apeti et al., 2014) and Florida (Glazer et al., 2008) Values are wet weight as ng/g for DDT and μg/g for metals.

	Vieques	Vieques	Cuba	Cuba	St Thomas	St Thomas	Florida	Florida
Analyte	min	max	min	max	min	max	min	max
Ag	0.043	0.206	--	--	0.039	0.9	1.03	2.54
Al	13.1	61.2	--	--	10.164	191.268	--	--
As	4.18	20.2	--	--	4.319	13.085	--	--
Ba	0.036	0.285	--	--	--	--	--	--
Be	0.008	0.009	--	--	--	--	--	--
Cd	0.097	0.688	--	--	0.209	1.046	2.62	24.14
Co	0.03	0.045	--	--	--	--	--	--
Cr	0.948	2.08	--	--	0.345	1.295	--	--
Cu	4.77	26.3	6.4	32.6	10.044	28.798	14.06	84.34
Fe	59.5	197	--	--	74.976	353.43	--	--
Hg	0.017	0.045	--	--	0.0139	0.212	0.01	0
Li	0.067	0.118	--	--	--	--	--	--
Mn	9.3	63.9	--	--	10.622	84.49		
Ni	1.13	3.81	--	--	0.813	1.961	16.28	9.59
Pb	0.024	0.072	0.2	2.3	0.056	0.314	--	--
Sb	0.144	0.166	--	--	--	--	--	--
Se	0.492	0.967	--	--	0.117	0.571	--	--
Sn	0.02	0.042	--	--	0.010	3.353	--	--
Ti	0.012	0.014	--	--	--	--	--	--
U	0.136	0.827	--	--	--	--	--	--
V	0.896	5.53	--	--	--	--	--	--
Zn	7.51	46.3	20.4	31.1	27.918	326.43	30.53	660.32
DDT	0	0.195	--	--	0	0	2.02	7.57

Table 4: Comparison of data from queen conch in Vieques, Puerto Rico (this study) with US Environmental Protection Agency seafood consumption guidelines (USEPA 2000). Values are wet weight.

Analyte	VQS max	VQS mean	VQS median	EPA Guideline
Inorganic Arsenic (ug/g)	0.404	0.190	0.173	0.18
Cd (ug/g)	0.688	0.350	0.338	0.18
Hg (ug/g)	0.045	0.028	0.023	0.059
Se (ug/g)	0.967	0.652	0.604	2.9
DDT (ng/g)	0.195	0.013	0	29



Puerto Rico
Vieques
MOSQUITO PIER
STUDY AREA
MP-19
MP-21
MP-20
Former
Navy Solid Waste
Management Units
Town of
Isabel Segunda
Former Live Fire Range
ISLAND OF VIEQUES
BSS-2
BSS-1
BSS-3
BSS-4
BSS-8
BSS-5
BSS-7
BSS-6
EH-16
EH-10
EH-17
EH-18
Town
of
Esperanza
BAHIA SALINAS
STUDY AREA
ENSENADA HONDA
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Supplemental Information

Methods

Methods were developed for the analysis for energetic/munition associated compounds (Table S1). Munitions compounds were evaluated using two extractions (a modified US EPA 8330 sonication method and ASE) and two analytical protocols (GC/MS and LC/MSMS) (Table S1). For sonication, homogenized sample aliquots (~10 g wet) were lyophilized in amber vials for two days prior for water removal. After lyophilization, samples were transferred to hexane rinsed mortar bowls and ground into a fine powder. Samples were returned to their respective vials and stored in a foil covered desiccator until extraction. Tissues samples of ~1.2 g dry (corresponding to ~4.5 g wet) were placed into 50 mL glass, solvent rinsed centrifuge tubes. The internal standards $^{13}C_7$, $^{15}N_3$-TNT; $^{13}C_4$, $^{15}N_4$-HMX; $^{13}C_3$-RDX; d_5-Nitrobenzene and 3,4-Dinitrotoluene were added followed by 15 mL of 50:50 dichloromethane/acetone. Centrifuge tubes were capped and vortexed for 1 minute then placed into a chilled sonicator bath for 3 hours with temperature controlled not to exceed 30°C.

Roughly 4.2 g of wet tissue sample was placed into a solvent rinsed mortar bowl containing ~27 g of anhydrous sodium sulfate. Samples were ground thoroughly and transferred into 33 mL ASE cells. Samples were spiked with the internal standards and extracted using an ASE 200 using a 50:50 dichloromethane/acetone mixture at 1000 psi. Calibration standards, reagent spikes, and matrix spikes were extracted by ASE in addition to the samples (range: 10-250 ng).

After extraction (sonication or ASE) samples were filtered through sodium sulfate into 200 mL TurboVap tubes and concentrated under a stream of nitrogen (pressure did not exceed 1.1 bar, water bath temperature = 25°C). Samples were concentrated to 0.5 mL and solvent exchanged with dichloromethane once. Samples were again concentrated to 0.5 mL and were transferred to glass culture tubes. TurboVap tubes were rinsed three times with dichloromethane and added to the sample extracts (F_v=2 mL). Samples were then cleaned up using a J2 Scientific Gel Permeation Chromatography (GPC) system (J2 Scientific Biobead column with 100% dichloromethane as the mobile phase). After GPC cleanup samples were concentrated to 0.5 mL and solvent exchanged to methanol twice. Extracts were concentrated to 0.5-1 ml and filtered through 0.45µm PTFE filters into amber sample vials. Recovery standards were added prior to instrumental analysis (1,2-dinitrobenzene and d_4-17β estradiol) .

Sample extracts were analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS) and gas chromatography mass spectrometry (GC/MS since several analytes would not ionize on the LC-MS/MS platform). While EPA methods used HPLC-DAD (diode array detector), initial work showed multiple interferences with conch tissue. Table 2 details the analytes determined on each instrument. For LC-MS/MS, an Agilent 1100 Series HPLC/AB Sciex API 4000 tandem mass spectrometer, operated in negative electrospray ionization with scheduled multiple reaction monitoring, was used. Separation was performed by a Phenomenex Synergi 4µ Hydro-RP 80A column using a methanol/water gradient. For GC/MS analysis, an Agilent GC/MS (6890/5973N) operated in selected ion monitoring was used. Samples were injected onto a Restek DB-225ms column (30m x 0.25µm x 0.25mm) through a split-splitless injector. Calibration curves (10-250 ng) were prepared for each batch of samples (from 8 to 13 samples). For ASE samples, the extracted curve was used, while sonication extracted samples used a calibration curve prepared directly from a stock solutions.

All spiking/calibration and internal standard stocks were stored refrigerated in glass amber bottles. Working stocks for spiking and calibration were volumetrically prepared weekly from concentrated stocks in methanol to minimize degradation. Standards for the native compounds were purchased from AccuStandard, while stable isotope-labeled standards were purchased from AccuStandard and Cambridge Isotope Laboratories.

Table S1: Extraction, instrumentation, standards and method detection limits for energetic compounds analyzed.

Analyte	ASE	Sonication	LC-MS/MS	GC/MS	Internal Standard Used	Calibration Range (ng)
1,3,5,7-tetranitro-1,3,5,7-tetraazacyclooctane (HMX)	X		X		$^{13}C_4$, $^{15}N_4$-HMX	10-250
1,3,5-trinitroperhydro-1,3,5-triazine (RDX)	X		X		$^{13}C_3$-RDX	10-250
Tetryl	X		X		$^{13}C_7$, $^{15}N_3$-TNT	10-250
Nitrobenzene	X			X	d_5-Nitrobenzene	50-250
2,4,6-trinitrotoluene		X	X		$^{13}C_7$, $^{15}N_3$-TNT	10-250
4-Amino-2,6-dinitrotoluene		X	X		$^{13}C_7$, $^{15}N_3$-TNT	10-250
2-Amino-2,4-dinitrotoluene		X	X		$^{13}C_7$, $^{15}N_3$-TNT	10-250
2-Nitrotoluene	X			X	d_5-Nitrobenzene	10-250
3-Nitrotoluene	X			X	d_5-Nitrobenzene	10-250
4-Nitrotoluene	X			X	d_5-Nitrobenzene	10-250
Pentaerythritol tetranitrate (PETN)	X		X		$^{13}C_4$, $^{15}N_4$-HMX	10-250
2,4-dinitrotoluene	X			X	3,4-Dinitrotoluene	25-250
2,6-dinitrotoluene	X			X	3,4-Dinitrotoluene	25-250
2,2’6,6’-tetranitro-4,4’-azoxytoluene	X		X		$^{13}C_4$, $^{15}N_4$-HMX	10-250