LIMPIA GUERRERO 2013



13 December 2013	A Pilot Study of Environmental
Report No. 2013-38	Contaminants in México

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Front Photograph: The aftermath of Tropical Storm Manuel, September 2013; photograph $\mbox{$\mathbb{C}$}$ Santiago Lobeira.

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Table of Contents

1.	Abstract
2.	List of Figures4
3.	List of Tables5
4.	Introduction
5.	Marine Plastics Pollution
6.	Mercury and Petroleum Pollution15
7.	Summary
8.	Policy Recommendations
9.	Acknowledgements
10.	Literature Cited40
11.	Appendix 1: Aerial views of seven surveyed beaches for plastics pollution
12.	Appendix 2: "Evaluación de Mercurio" information card and questionnaire47
13.	Appendix 3: Mercury concentrations in 25 fish sampled in Guerrero
14.	Appendix 4: Numbers and species of birds sampled in Guerrero
15.	Appendix 5: Whole blood mercury concentrations in 45 birds sampled50
16.	Appendix 6: Hair mercury concentrations, age range, and frequency of fish consumption 52

ABSTRACT

In the fall of 2013, Biodiversity Research Institute (Gorham, ME, USA) partnered with Sustenta Soluciones S.A. de C.V. (México City, México) to conduct a three-week pilot environmental study along a 200-km stretch of coastline in the State of Guerrero. The focus was on three common pollutants often used as reliable predictors of ecological toxicity: plastics, mercury, and petroleum (PAHs). The pilot was a component of a much larger conservation campaign called Limpia Guerrero 2013 funded by the State of Guerrero.

Marine Plastics Pollution. Beach debris abundance was estimated from one-meter-wide belt surveys along 100-m stretches of beach on seven beaches between Acapulco to Zihuatenejo/Ixtapa, sorted and categorized by 10 types, and weighed. Nearly 9,700 pieces of litter were collected from a total sample area of 1.5 hectares. Litter density varied from 0.42 to 44.26 pieces per m². As a category of debris, plastics pollution varied from 0.05 to 4.44 pieces per m² of beach. The average proportion of plastics among total marine debris collected from the seven beaches of Guerrero was 24.81% (ranging from 1.10% to 69.72%), a low value when compared to the proportion globally among marine debris (60% to 80%) but high when compared to other recent surveys around the Pacific Ocean. Not surprisingly, beaches located in more public or lower income areas showed the highest density of marine debris and the highest density of plastics pollution. Tropical Storm Manuel in September likely confounded, even aggravated, the standing surface litter detected along the coastline of Guerrero.

Mercury (Hg) Pollution. Of 25 fish sampled during the study, four exceeded the U.S. EPA fish advisory concentration of 0.3 ppm. Based on the results of our pilot survey, we urge residents to abstain from eating Swordfish and sharks and limit their consumption of Horse-eye Jack (Caranx latus) and Manta Ray (Manta birostris). Some wading birds, songbirds, and other invertivorous birds sampled had blood Hg concentrations high enough to indicate concern about overall ecological health; however, the sample size was too small to make meaningful conclusions. From our limited number (13 individuals) of piscivorous birds sampled, two (Black Skimmer, Rynchops niger, and Royal Tern, Thalasseus maximus) exceeded the effect level established for fish-eating birds and wildlife. Over 70% of women of reproductive age sampled during our study exceeded the lowest observed adverse effect level of 0.3 ppm.

Petroleum (PAHs) Pollution. The use of FTA cards to detect PAHs in the blood of wildlife is a new approach for analyzing petroleum. We found detectable levels of PAHs ranging from 5.1 to 20.2 ng/mL in 11 out of 26 samples. Acenaphthene, one type of PAH cited as a hazardous substance by the U.S. EPA, was found in nine of those 11 samples. Preliminary results suggest invertivorous birds may be more susceptible to the biological intake of PAHs than fish-eating species. Further investigation is merited, especially given the potential of the FTA cards for long-term storage under field conditions for environmental emergencies.

Thus, based on the results of our three-week pilot study in Guerrero, we found unequivocal environmental signals for plastics, mercury, and petroleum pollution along the coastline and urge the state authorities to support long-term ecological research on these common contaminants to determine their sources and their potential solutions.



List of Figures

1.	Vegetation map of the State of Guerrero7
2.	Some of the dangers of marine debris to human health9
3.	The aftermath of Tropical Storm Manuel in Acapulco10
4.	Survey design for the one-meter-wide belt transects
5.	Locations of seven beaches analyzed for marine plastics pollution11
6.	An integrated statewide watershed-level approach to Guerrero's waste stream14
7.	Using a stainless steel biopsy punch to extract sample muscle plugs
8.	Whoosh-net, net-gun, and mist-nets17
9.	Sampler of aquatic and terrestrial habitats sampled for birds18
10.	Sampler of birds captured and diagnosed during the campaign
11.	A volunteer completes the questionnaire20
12.	Whatman FTA cards and capillary tubes for sampling bird blood22
13.	Angling for dorado in the waters off the coast of Ixtapa
14.	Samples sites for birds in Guerrero in 2013
15.	Mercury concentrations of Coryphaena hippurus (Dolphinfish)25
16.	Mercury concentrations of Lutjanus campechanus (Red Snapper)26
17.	Mercury concentrations of Xiphias gladius (Swordfish)27
18.	Mercury concentrations of Thunnus albacores (Yellowfin Tuna)
19.	Mercury concentrations of other fish species
20.	Mean blood mercury in fish-eating birds sampled in Guerrero29
21.	Mean hair mercury concentrations in women of reproductive age
22.	The Limpia Guerrero 2013 team of enthusiastic workers and researchers

List of Tables

1.	Comparison of the numerical importance of different litter categories	. 12
2.	Comparison of plastics density vs. overall debris density	.13
3.	Comparison between whole blood Hg and FTA card Hg	.24
4.	Detection of PAHs in birds using FTA cards	33

Limpia Guerrero 2013

INTRODUCTION

"Raising public awareness and persuading a change in attitude is the only guaranteed way of reducing the amount of waste reaching the sea and littering the shores."

- Gareth Rees and Kathy Pond, 1995, Marine Pollution Bulletin Located on the Pacific Ocean in southwestern México, Guerrero is one of 31 states which, with the Federal District, comprise the 32 federal entities of México. With a territory of 63,794 km² and a population of just over 3.44 million, the state has a density of 54 persons/km², ranking 16th for the country. Chilpancingo is the state capital and the second-largest city in the state. Guerrero also includes the high-density tourist destinations of Acapulco (the largest city), Zihuatenejo, and Ixtapa. Tourism is the principal economic factor of Guerrero though agricultural production, logging, fishing, and mining are locally important. Most of the state is mountainous with flat areas limited to small mesas and its 500 km long coastline. The climate is dominated by its rainy tropical areas along the coast and its rainy temperate areas in the sierras (Figure 1). Except for tropical storms and cyclones, most of the rainfall in Guerrero is produced by evaporation from the Pacific Ocean.

In cooperation with the State of Guerrero and in partnership with Sustenta Soluciones S.A. de C.V. (a green marketing and communications company headquartered in México City; www.sustenta.com), Biodiversity Research Institute (BRI; www.briloon.org) conducted a three-week pilot study in Acapulco, San Jerónimo de Juárez, and Zihuatenejo/Ixtapa from mid-October to early November 2013 to check for environmental signals in fish, birds, and humans for three common pollutants often used as reliable predictors of ecological toxicity in urban settings: plastics, mercury, and petroleum. The pilot was a component of a much larger and longer campaign called Limpia Guerrero 2013 (www.limpiaguerrero.com and www.facebook.com/limpiaguerrero), a Guerrerense initiative based on public education and communication to clean beaches, raise environmental awareness, and engage stakeholders from both the public and private sectors to find a sustainable solution to coastline pollutants in Guerrero.

The mission of BRI, now in its 15th year, is to assess emerging threats to wildlife and ecosystems around the world through collaborative research, and then to use its scientific findings to advance environmental awareness and to inform decision makers. Its research program encompasses a variety of ecological stressors: physical contaminants such as plastics pollution as well as chemical toxins such as mercury and petroleum pollution. As one of BRI's first projects in México, its small team of ecologists and wildlife biologists employed a unique combination of innovative wildlife science and contaminants research to assist the Limpia Guerrero 2013 campaign.¹



Figure 1: Vegetation Map of the State of Guerrero. Based on 2011 data, 31% of the state is covered by deciduous forest, 19% by a mix of coniferous and broadleaf forest, 16% by seasonal agriculture, 16% by cultivated grasslands, and 10% by broadleaf forest. The remainder – each equal to or less than 2.5% - are mesophyllic mountainous forest, coniferous forest, irrigated agriculture, water bodies, human settlement, hydrophilic vegetation, and other types of vegetation (Source: Instituto Nacional de Ecología y Cambio Climático, México City).

¹ Other BRI projects in México have included the February 2012 and 2013 Limpia Mahahual campaigns against marine plastics debris in the Yucatán Peninsula; for example, see <u>www.limpiamahahual.com</u> and <u>www.crowdrise.com/mahahual</u>.

"Guerrero needs our help. The situation there has been desperate. It's a beautiful region and a cultural hotspot in México. Our project will help Guerrero's citizens and its tourists live more sustainably with the environment in the future."

- Manolo Ruiz, Director General, Sustenta Soluciones S.A. de C.V., Limpiaguerrero.com

Pilot beach surveys were used to determine the amount and types of marine debris, including plastics, on a number of beaches some highly urbanized, some remote - in the State of Guerrero. The environmental impacts of marine debris are wide-ranging and can be both direct (e.g., ingestion or entanglement) and indirect (e.g., ecosystem alteration or introduction of invasive species). Marine debris can harm tourism, fishing, and navigation as well as endanger human health and safety (e.g., stepping on broken glass or discarded hypodermic needles; Figure 2). Plastics, human-made material that degrades but does not decompose, is considered a "toxic time bomb" by the United Nations Environment Programme (Kershaw et al 2011) because of its near-eternal lifetime, its ability to leach toxic additives (Cole et al 2011), and its tendency to adsorb waterborne environmental toxins such as pesticides, PCBs, and heavy metals (Cole et al 2011; Elliott and Elliott 2013). Thus, the beach surveys during this project yielded valuable, but preliminary, information about the standing stock of plastics and other surface litter along the coastline of Guerrero.

Two other beach contaminants were studied: mercury (Hg) and petroleum (PAHs). These are common toxic pollutants that often accumulate in aquatic organisms and can be used as reliable predictors of ecological toxicity in the environment, particularly in urbanized settings (e.g., see Van Metre et al 2000). Anthropogenic Hg in both aquatic and terrestrial systems is assumed generally to come from atmospheric fallout, sometimes traveling at great distances from its initial sources, primarily from coal-fired power plants and waste incineration. Mercury point sources such as artisanal gold mining and chlor-alkali plants can also have a detrimental effect locally. As a powerful neurotoxin, Hg has wide-ranging implications for the health and wellness of both humans and wildlife. The organic form of mercury that poses health risks is methylmercury (Evers et al 2011). Through an ecological process known as biomagnification, Hg accumulates in the tissues of high trophic-level organisms. Its impact has both economic and health effects. As a major component of petroleum, polycyclic aromatic hydrocarbons, or PAHs, are some of the most widespread pollutants in the environment often due to spills and the combustion of fossil fuels and organic waste. Studies show that certain metabolites of these hydrocarbons interact with DNA and are genotoxic, causing malignancies and heritable genetic damage in humans and other organisms. Heavy occupational exposure to PAHs entails a substantial risk of lung, skin, or bladder cancer. They can be absorbed by plants and can accumulate in soil and leach into water. Thus, Hg and PAHs are two common environmental contaminants in urbanized settings that require constant monitoring and evaluation.

These three pollutants – plastics, mercury, and petroleum – are just several toxins in a panoply of human waste to contaminate

coastal waters globally. After studying marine litter monitoring programs around the world, some researchers have concluded that "Raising public awareness and persuading a change in attitude is the only guaranteed way of reducing the amount of waste reaching the sea and littering the shores" (Rees and Pond 1995). In other words, scientific studies are only part of the solution for our environmental problems; we must seek long-term behavioral changes in our interactions with the natural world around us. Ultimately, it is for our own health and well-being. We hope that the scientific analysis from this pilot study in Guerrero will help México and other nations in the Western Hemisphere deal responsibly and sustainably with their waste streams.



Figure 2: Some of the dangers of marine debris to human health – a soda bottle filled with more than 100 discarded hypodermic needles and syringes discovered during the 2013 campaign on a remote beach south of Acapulco. Photograph © H. Bruce Rinker.

MARINE PLASTICS POLLUTION

Plastics are among the primary pollutants in coastal sediments, surface and pelagic waters, and the benthos (Graham and Thompson 2009) and, hence, are the dominant type of anthropogenic debris found throughout the marine environment (Eriksen et al 2013). Further, the accumulation of marine litter in the oceans, particularly plastics, is a growing problem worldwide (Topçu et al 2013). As noted by many observers (e.g., Cole et al 2011; Ryan et al 2009), coastlines receive plastic litter from both terrestrial and marine sources. Terrestrial sources of litter typically dominate close to urban areas, sites of tourism, and near river outflows whereas marine debris is often deposited along shorelines when caught in near-shore currents. Society's reliance upon and failure to manage plastics materials has resulted in a proliferation of persistent synthetic trash in the environment (Cole et al 2011; Gassel 2013; Goldstein et al 2012) to the point where the novel ecological interactions caused by the introduction of plastic particles in oceanic ecosystems are now termed the "plastisphere" (Goldstein et al 2012).

For the coastline of Guerrero, confounding and even aggravating the background deposit of marine debris was the aftermath of Tropical Storm Manuel in September 2013. The storm killed dozens, evacuated thousands, and destroyed infrastructure and the livelihoods of countless residents throughout the region (e.g., see <u>www.accuweather.com/en/weather-news/deadly-manuel-mexico-acapulco/17897158</u>). Mountains of debris – from plastics and metals to medical waste and wood – descended from the sierras into coastal waterways and then washed ashore into a spectrum of urbanized and remote settings. For weeks afterward, municipalities throughout the coastal region hastily removed the debris with heavy equipment without regard to category (Figure 3), hauling the rubbish to temporary

dump sites and even burning it. Thus, BRI's surveys of beach debris were conducted on shorelines from Acapulco to Zihuatenejo/Ixtapa with varying degrees of cleanup in progress: from high-density tourist locations with incumbent intense cleaning to remote and uninhabited locations with no cleaning whatsoever prior to the Limpia Guerrero campaign. However, all shorelines showed substantial effects, including beach erosion and litter accumulation, from the recent tropical storm.



Figure 3: The aftermath of Tropical Storm Manuel in Acapulco – (Left to Right) (a) A fallen bridge over the Papagayo River in Acapulco; (b) Heavy equipment removing beach debris; (c) Plastic debris as a primary pollutant on Guerrero beaches. Photographs a and b © H. Bruce Rinker, Photograph c © Alfredo Blasquez.

Methods

Using a "one-meter-wide belt transect" (Figure 4; Velander and Mocogni 1999), trained temporary workers characterized standing surface litter by marking off 10 one-meter-wide transects perpendicular to the shoreline carried out at 10 m intervals from the vegetation to the bottom strand line on the beach (including both wet and dry areas). The two end points of the 100-m line adjacent to the vegetation were archived via a GPS unit for future reference, and total area of the study site was determined. Project workers collected all surface litter in each transect and then sorted into 10 categories: plastics, Styrofoam, fabrics, glass/ceramics, metals, paper, rubber, wood, medical waste, and other/unknown. Litter was counted, weighed on-site, and then disposed of properly. All data were recorded and filed.

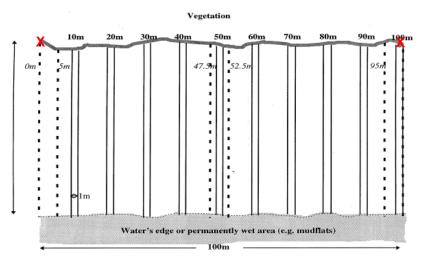


Figure 4: Survey design for the one-meter-wide belt transects; "X" denotes archived end points (Source: Velander and Mocogni 1999).

Results

A location map of the seven different beaches surveyed for marine plastics pollution is provided as Figure 5. The distance between the most southerly survey location (Acapulco) and the most northerly site (Zihuatenejo/Ixtapa) was approximately 200 km of coastline.

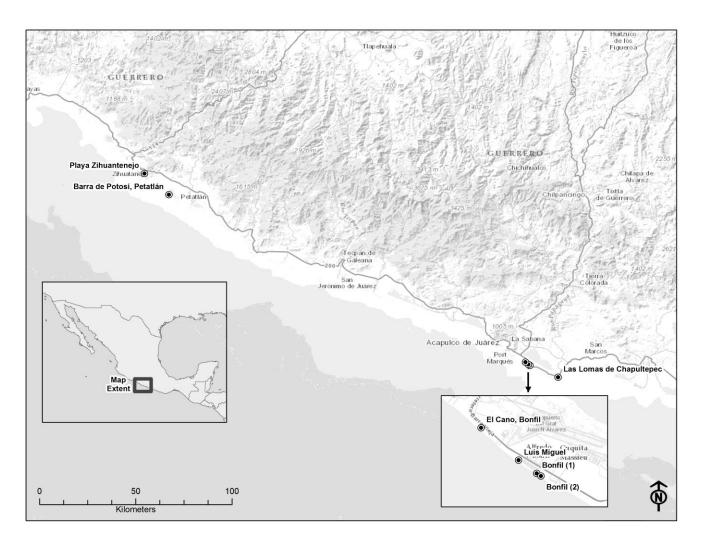


Figure 5: Locations of seven beaches analyzed for marine plastics pollution during the Limpia Guerrero 2013 campaign. These were a subset of the 25 beaches cleaned from October to December (nine in the Acapulco area, six in San Jerónimo de Juárez, and 10 in Zihuatenejo/Ixtapa).

The litter levels of each location, as measured by one-meter-wide belt transects, represented a total sample size of just over 1.5 hectares. Only macro-litter was included (defined as that which had at least one side more than 5 cm in length). For this relatively small collection area, nearly 9,700 items of litter belonging to 10 categories were counted: plastics, Styrofoam, fabric, glass/ceramics, metal, paper, rubber, wood, medical waste, and other/unknown (Table 1). Initial surveys did not include wood and also

combined recyclable PET litter with plastics. The wood included both natural (e.g., tree limbs and tree trunks) and human constructs (e.g., crates and household debris), some of which was too large or unwieldy for removal by hand. Aside from the wood collected and counted, representing over 70% of the total, the next highest category was plastics debris (including notable numbers of 1-L plastic bottles and bottle caps). The least common categories were fabric and paper. Though medical waste represented a comparatively small percentage of the total items collected throughout the campaign, each item was viewed as a serious potential health hazard for wildlife and humans (e.g., hypodermic needles and syringes, some of which contained unknown liquids, and partly opened pharmaceutical bottles). Campaign workers ignored litter items beyond the transect lines until the completion of the survey at which time they collected and removed all manageable remaining debris from the beach location.

Category #	Category Description (English/Español)	# Counted	% of Total	
1	Plastics/Plásticos	1434	14.81	
2	Styrofoam/Espuma Plástica	397	4.10	
3	Fabric/Tela	33	0.34	
4	Glass and Ceramics/Vidrio y Cerámica	453	4.68	
5	Metal/Metal	113	1.17	
6	Paper/Papel y Cartón	47	0.49	
7	Rubber/Hule	136	1.40	
8	Wood/Madera	6911	71.39	
9	Medical Waste/Basura Médica	66	0.68	
10	Other/Otro	90	0.93	
	ΤΟΤΑΙ	9680	100	

Table 1: Comparison of the numerical importance of different litter categories for all seven beaches analyzed for the Limpia Guerrero

 2013 campaign; category number simply refers to the order of listing on the data sheets.

Table 2 shows a comparison of the density of plastics debris (number of items per m²) to overall debris (number of items per m² for all categories combined) for each of the seven beaches in the Limpia Guerrero study. In order of highest to lowest density of plastics, the beaches were Bonfil(2), Playa Zihuatenejo, Bonfil(1), Luis Miguel, Las Lomas de Chapultepec, El Cano, and Barra de Potosí. In order of highest to lowest density of overall debris, the order changed slightly: Bonfil(2), Playa Zihuatenejo, Bonfil(1), Barra de Potosí, Luis Miguel, Las Lomas de Chapultepec, and El Cano. However, in terms of percentage of overall debris collected and analyzed, plastics pollution was highest for Luis Miguel (70%). The other beaches ranked in order of highest to lowest percentage were Las Lomas de Chapultepec (45%), Bonfil(1)(22%), Playa Zihuatenejo (13%), El Cano (13%), Bonfil(2)(10%), and Barra de Potosí (1%). Not surprisingly, beaches located in more public or lower-income areas (e.g., Playa Zihuatenejo and Bonfil, respectively) tended to have the highest density of marine debris and the highest

density of plastics pollution. This was likely due, at least in part, to local wind and water currents as well as to local attitudes toward beach cleanups and other investments of time and resources for conservation.

The density of debris for Guerrero beaches averaged 11.42 pieces per m² (ranging from 0.42 to 44.26 pieces per m²), a relatively high value when compared to other locations around the world (e.g., 0.085 to 5.058 per m² in Topçu 2013). As a category of debris, plastics averaged 1.55 pieces per m² (ranging from 0.05 to 4.44 pieces per m²). When the proportion of plastics among total marine debris for Guerrero was compared to the results of other recent surveys in the Pacific Ocean (e.g., Barnes 2009; Derraik 2002), the state showed a high value more like those reported from the Caribbean, North Atlantic, or Mediterranean, probably due in large part to the deposit of rubbish from the September storm. However, the average for Guerrero (24.81% with a range of 1.10% to 69.72%) was far below the proportion globally among marine debris (60% to 80%; Moore 2008).

Appendix 1 provides aerial views of the seven beaches surveyed for plastics pollution during the Limpia Guerrero 2013 campaign. The associated graphs show the quantity of beach debris collected per category from the one-meter-wide belt transects. In all cases except for Barra de Potosí, Petatlán, plastics dominated the beachscape as an anthropogenic class of debris along the coastline of Guerrero.

Site	Name	Area (ha)	Debris Density (#/m ²)	Plastics Density (#/m ²)	% Plastics	
1	Bonfil (1)	0.09	10.79	10.79 2.34		
2	Bonfil (2)	0.12	44.26	44.26 4.44		
3	Luis Miguel	0.17	1.66	1.16	69.72	
4	El Cano, Bonfil	0.48	0.42	0.05	12.75	
5	Las Lomas de Chapultepec	0.43	0.78	0.35	45.27	
6	Playa Zihuatenejo	0.13	18.82	2.48	13.19	
7	Barra de Potosí, Petatlán	sí, Petatlán 0.08 3.2		0.04	1.10	
		TOTAL = 1.5	\overline{X} = 11.42	\overline{X} = 1.55	\overline{X} = 24.81	

Table 2: Comparison of plastics density vs. overall debris density for seven beaches analyzed during the Limpia Guerrero 2013 campaign.

Discussion

The standing abundance of marine debris, especially plastics pollution, along the coast of Guerrero reflects a worldwide tendency of communities toward contaminants: lack of an integrated approach on the scale of an entire drainage basin to control and reverse diffuse sources of pollution (Boesch 2001). The consequences of such habits are often aggravated by major storm events (such as Tropical Storm Manuel in September 2013 in Guerrero) that gather up debris from various watersheds

and then deposit that same debris along shorelines. An integrated statewide watershed-level approach to Guerrero's waste stream could effectively reduce or even eliminate the 10 categories of debris used in this study (Figure 6). Given the value of tourism, both domestic and international, for Guerrero, the merits of such an approach for long-term investments should be obvious.² Yet the state's extraordinary environment has an intrinsic value that far outweighs its value as a tourism asset. From rare species recognized internationally for conservation (e.g, sea turtles and shorebirds) to stunning landscapes (from its sierras to its seashores), Guerrero has an striking inventory of natural resources diminished daily by overexploitation and neglect.



Figure 6: An integrated statewide watershed-level approach to Guerrero's waste stream could effectively reduce or even eliminate the 10 categories of debris noted in this study. Photograph © H. Bruce Rinker.

This three-week pilot study looked briefly at garbage along 200 km of seashore between Acapulco and Zihuatenejo/Ixtapa. Beaches to the north and south of this line were not examined, thereby neglecting nearly 300 km of coastline for the State of Guerrero. Future analyses of marine debris in the northern and southern parts of the state will address current gaps in our knowledge about its coastline contaminants.

Finally, the lack of an integrated drainage-basin approach for Guerrero (again a typical problem for communities around the world) contributes to a "tragedy of the commons" for its shorelines and waterways. Unregulated development, poor enforcement of standing environmental laws and regulations, and unsustainable pressures from society at-large all contribute directly to this decrease in both the quantity and quality of its coastal biodiversity. As Hardin (1968) noted, "Freedom in a commons brings ruin to all." Until this lack of an integrated approach is addressed, Guerrero will continue to lose its resiliency in dealing effectively with natural disasters.

² In 2008, Guerrero attracted USA\$278.8 million of private investments into the tourism sector of the economy with most of it invested in Acapulco and Zihuatenejo/Ixtapa (see press release, "Guerrero, Estado atractivo para la inversión turística," Secretaria de Turismo Estado de Guerrero, 9 September 2008).

"Limpia Guerrero has the potential to transform the people and the landscape to promote healthy ecosystems that will attract tourists from all over the world."

- Santiago Lobeira, Socio Fundador y Consejero, Sustenta Soluciones S.A. de C.V.

MERCURY AND PETROLEUM POLLUTION

Mercury is a well-recognized environmental contaminant that adversely affects the reproduction, behavior, and physiology of birds and other organisms. In marine systems, Hg contamination occurs through atmospheric deposition, input from rivers, and point sources. Fish-eating birds living in marine environments are exposed to Hg primarily through diet where the degree of exposure generally increases with trophic position. Bioaccumulation of methylmercury, the most toxic form of the pollutant, is of particular concern for piscivorous species of birds and certain mammal species, given their position as top-level consumers and the high proportion of methylmercury in their diet of fish. Dietary exposure and sensitivity to Hg, however, can vary within foraging guilds and species due to differences in diet composition, foraging habitat selection, and environmental conditions (Eagles-Smith et al 2009). Understanding variation in Hg exposure due to wildlife foraging ecology and location will help us to identify which species are at greatest risk of negative effects from Hg exposure and where to focus our conservation efforts.

Methods

The field methodology employed for Hg contamination in fish and birds was identical to that used to detect petroleum (PAHs) pollution and, thus, is detailed below as a combined description. The laboratory analysis of samples, however, was conducted by two separate facilities: BRI's Wildlife Mercury Research Lab (Gorham, ME) and the Center for Environmental Sciences and Engineering at the University of Connecticut (Storrs, CT). BRI analyzed the fish muscle, bird blood, and human hair for Hg contamination. The University of Connecticut examined the bird blood for petroleum signals (PAHs).

Fish

Selecting a sample site. The sampling site was an area where fishing and fish consumption were routinely conducted (e.g., a coastal/marine area), thereby exposing individuals engaged in these activities to potentially adverse health effects associated with Hg in the diet.

Selecting target fish species. When selecting target fish species, it was important to match the consumption patterns of the community with the fish species sampled. Target fish species were

mostly predatory fish. This type of fish is considered the best indicator of Hg biomagnifications in aquatic ecosystems. Such fish are often important food source for human populations. The predominant route for Hg exposure in humans is through fish consumption so establishing the links between Hg contamination in aquatic ecosystems and the potential risks of exposure in humans was an important consideration for selecting target species. Thus, selected fish species were top-level predators that are also economically important and locally available (not imported).

Sampling target fish species. During the sample collection, it was important to maintain a clean working environment to prevent any potential contamination of the sample from external sources. Each fish sampled was photographed, its length measured to the nearest 0.1 cm, weighed to the nearest 1.0 g, and all data recorded. A few scales were removed from the dorsal side of the fish behind the gills. The skin was punctured with a sterile disposable 5- or 8-mm stainless steel biopsy punch to secure a single muscle plug (Figure 7). The plug was transferred with clean forceps to a labeled sterile cryovial. All samples were placed in a small Ziploc bag and put into a cooler with ice while in the field. They were transferred later to a freezer until shipment and laboratory analysis.



Figure 7: Using a stainless steel biopsy punch to extract sample muscle plugs from a small shark donated by a local fisherman. Photograph © H. Bruce Rinker.

Birds

Bird sampling occurred opportunistically in October and November 2013 near beach clean-up sites. Non-lethal capture methods were used for the sampling of all birds, depending on local conditions such as available species and habitat type (Figure 8):

• Mist-nets (four-paneled nets of fine thread and trammel lines six, nine, or 12 m in length with 36 mm mesh; deployed in multiple sites including along forest edges and in forest corridors)

• Whoosh-nets (bungee-powered rectangular nets 6 m x 4.25 m and 8 m x 4.25 m each with 55 mm mesh; deployed with decoys on flat open beaches)

• Net-gun (bolt-action .308 rifle modified with four barrels and a center basket for holding a net and weighted foam floats with a range of eight to 16 m; deployed in open spaces such as beaches and open water)

Each technique required a unique skill (e.g., the safe removal of birds captured and tangled in mist-nets or firearms safety for the net-gun) along with all the proper paperwork, including collection permits, and sampling equipment and materials such as bird field-guides and data sheets.



Figure 8: (Clockwise from top) Operating a whoosh-net, deploying a whoosh-net, waiting with net-gun, setting mist-nets. Photographs © H. Bruce Rinker.

A key starting point for all three techniques was reconnaissance to note the abundance and diversity of birds present in the area (Figures 9 and 10). Once a likely hotspot was identified, then a convenient location safe for processing animals was established nearby and the appropriate sampling technique(s) deployed at the location.



Figure 9: Sampler of aquatic (freshwater, brackish, and saltwater) and terrestrial habitats sampled for birds along 200 km of coastline in the State of Guerrero. Photographs © H. Bruce Rinker.

Feather samples. Two outer tail feathers (for shorebirds and songbirds) or second secondary feathers (for waterbirds and seabirds) were plucked or clipped symmetrically and placed in a labeled paper envelope for storage and later analysis for heavy metals.

Blood samples. Small amounts of blood were extracted from birds using a 23-, 25-, or 27-gauge needle in order to identify the recent dietary uptake of mercury. If the bird was small (e.g., Spotted Sandpiper), then one to three capillary tubes of blood were collected from the pierced cutaneous ulnar vein. Blood was then transferred to FTA cards (Fast Technology for Analysis of Nucleic Acids; GE Healthcare Life Sciences, Piscataway, NJ; Shlosberg et al 2011) and/or sealed with Critocaps® for archival purposes. The blood spots on the cards were allowed to air-dry, and then the cards were wrapped in aluminum foil and placed in individual Ziploc bags. The tubes were placed inside a labeled vacutainer. All samples were put on ice after collection. If the bird was large (e.g., Brown Pelican), then blood was drawn directly from the tibial vein of the right leg with a manual syringe and transferred to FTA cards and/or stored in heparinized or no-additive microtainers. No more than 1% of the bird's body weight in blood was collected. After proper labeling, samples were placed in a small Ziploc bag in a cooler with ice and then frozen within six hours of collection. All birds were checked to ensure that bleeding had stopped prior to their release.

FTA cards were employed experimentally to measure possible Hg and petroleum (PAHs) contamination – a fairly new approach for Hg analysis that also destroys the viral pathogen for Newcastle's Disease and an as-yet untested approach for PAH analysis. Since the latter approach provided discernible signals during our pilot study, the outcome will be a notable contribution to field biology, especially with worries about the transference of wildlife pathogens across international borders.

Bird measurements. Using a portable metric balance and technical calipers, the following standard morphometrics were recorded: date, sample number, location, species name, weight (g), wing chord (mm), tail length (mm), bill length (mm), bill culmen (mm), bill width (mm), bill depth (mm), tarsus length (mm), and head-to-tip-of-bill length (mm). Such measurements are often used to determine sex, age, and overall health of individual birds. Once all measurements were logged, birds were released unharmed and away from any possible ground predators, usually within 10 to 15 minutes of capture.

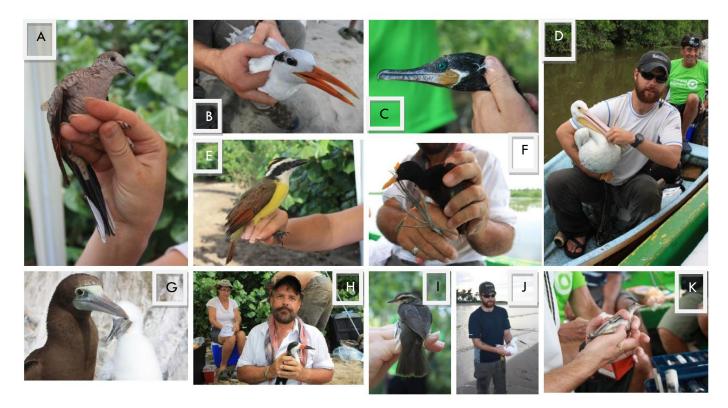


Figure 10: Sampler of birds captured and diagnosed during the Limpia Guerrero 2013 campaign (a total of 45 individual birds representing 19 species). A = Inca Dove (Scardafella inca); B = Royal Tern (Thalasseus maximus); C = Neotropic Cormorant (Phalacrocorax brasilianus); D = American White Pelican (Pelecanus erythrorhynchos); E = Great Kiskadee (Pitangus sulphuratus); F = Northern Jacana (Jacana spinosa); G = Brown Booby (Sula leucogaster); H = Black Skimmer (Rynchops niger); I = Northern Waterthrush (Parkesia noveboracensis); J = Snowy Egret (Egretta thula); K = Spotted Sandpiper (Actitis macularius). Photographs © Alfred Blasquez (F, H); H. Bruce Rinker (A-E, G, I-K).

Humans

Selecting human subjects for sampling. The community to be sampled lived near an active fishing area. Individuals participating in the study were all females of reproductive age (i.e., between 18 and 45 years old) from communities where fish were also sampled.

Completing the questionnaire. Individuals who donated hair filled out a short questionnaire (Appendix 2), writing legibly and completing all questions including details on how to contact them for their sample results (Figure 11).



Figure 11: A volunteer completes the questionnaire before donating her hair sample for mercury analysis. Photograph © Alfredo Blasquez.

Collecting hair samples. All samples were collected in a clean and safe manner. During the sample collection, a sanitary working environment was maintained to prevent any potential contamination of samples from external sources. An alcohol wipe was used to clean the surfaces of the stainless steel scissors. A bundle of hair (approximately 30 strands) was grasped in the occipital region of the head (i.e., near the nape of the neck) and cut as close as possible to the scalp. The hair sample was then secured with a small self-adhesive tape to the collection card indicating the direction of the scalp. The collection card and hair sample were sealed inside a small Ziploc bag until sample analysis in BRI's Wildlife Mercury Research Lab.

Sample Analysis

Fish muscle, bird blood, and human hair. Fish muscle, bird blood, and human hair samples were analyzed for total Hg concentrations at BRI's Wildlife Mercury Research Lab. Samples included whole bird blood, FTA bird blood cards (Figure 12), fish tissue plugs, and human hair. Samples were placed into nickel sample boats, weighed, and analyzed for total Hg concentration using a thermal decomposition

"Pollution on beaches and in bays is an unfortunate reality that afflicts us, no matter our geographical boundaries and social levels, and requires complex multidisciplinary solutions that include technical and scientific diagnosis and socioenvironmental models of education, accompanied by effective communication We are all responsible."

- Tulio Estrada Apátiga, Secretario de Medio Ambiente y Recursos Naturales del Estado de Guerrero technique and atomic absorption spectroscopy with an automated direct Hg analyzer (DMA 80: Milestone Incorporated, USA) and the U.S. EPA Method 7473.³ At the start of analysis, we included two samples each of two standard reference materials (Dorm-3 and Dolt-4), five method blanks, and two sample blanks. After every 20 samples, a duplicate was analyzed along with two samples each of two standard reference materials (Dorm-3 and Dolt-4), four method blanks, and one sample blank. Mercury results were reported in parts per million (mg/kg) wet weight (ww) for blood and fish, mg/kg dry weight (dw) for FTA blood cards, and mg/kg fresh weight for hair samples.

To convert the Hg results of these experimental FTA cards for dry blood into ww concentrations for comparison with our database (blood Hg concentrations traditionally reported as ww), we found an average multiplication factor from paired samples and then applied that factor to our FTA card dw Hg concentrations to obtain a ww result. We believe that the results calculated in this manner were accurate to +/- 0.1 to 0.4 ppm.⁴ Using FTA cards for contaminants research is a novel approach undergoing evaluation at this time. Thus, so far as we know, this is the first use of FTA cards for both Hg and PAH contamination in wildlife under field conditions.

FTA cards for PAHs. Samples were analyzed in the University of Connecticut's Center for Environmental Sciences and Engineering (Storrs, CT). A 1-cm diameter biopsy punch (GE Healthcare Life Sciences, Piscataway, NJ) was utilized to remove a standardized spot area from the Whatman FTA card. One or two spots were excised per card, depending on availability, and placed in an extraction vial, and then 200 μ l of formic acid was added. The samples were sonicated for 2 minutes, and 5 ml of acetonitrile was added, followed by an additional sonication of 2 minutes. The samples were then cleaned up prior to extraction to remove phospholipids, concentrated, and analyzed using a gas chromatograph/tandem mass spectrometer (GC/MS/MS). Results were reported in ng/ml (or ppb) since 50 μ l of blood was contained in each completely filled punch circle.

³ <u>www.epa.gov/osw/hazard/testmethods/sw846/pdfs/7473.pdf</u>.

 $^{^4}$ Throughout this report, we use ppm, mg/kg, and $\mu g/g$ interchangeably.



Figure 12: Whatman FTA cards and capillary tubes for sampling bird blood during the Limpia Guerrero 2013 pilot study.

MERCURY POLLUTION

Results for Mercury Pollution

Mercury in fish samples. During this study, we sampled 25 fish representing 15 species from angling and fish markets in Acapulco, San Jerónimo de Juárez, and Zihuatenejo/Ixtapa to correspond approximately to the campaign's beach clean-up efforts in those locations (Figure 13). The Hg concentrations in these fish, ranging from 0.024 ppm in Red Snapper to 1.642 in Swordfish, are given in Appendix 3.



Figure 13: Angling for dorado in the waters off the coast of Ixtapa. Photograph © H. Bruce Rinker.

Mercury in bird samples. During this study, we sampled 45 birds representing 19 species from three different coastal locations in the State of Guerrero: Acapulco, San Jerónimo de Juárez, and Zihuatenejo/Ixtapa (Figure 14). Appendix 4 provides the number of birds per species, and Appendix 5 shows the whole blood Hg concentrations of the birds ranging from 0.007 ppm in an Inca Dove to 2.185 ppm in a Royal Tern. One goal of our study was to measure total Hg concentrations in piscivorous bird species such as the Brown Pelican (*Pelecanus occidentalis*) and Black Skimmer (*Rynchops niger*) and then compare these data to those collected in other regions in order to understand the risk level for Hg contamination in waterbirds in Guerrero (Figure 20). However, our sample set of birds included individuals from a variety of coastal lowlands (such as lagoons and mangrove forests) as well as the marine environment.



Figure 14: Sample sites for birds in Guerrero in 2013.

Whole blood Hg vs. FTA card Hg for birds. During the pilot study, we collected paired samples (i.e., both whole wet blood in capillary tubes and blood spots on FTA cards) from 12 birds representing seven species. To convert the Hg results of the FTA cards for dry blood, we multiplied the FTA concentration by the average ratio of wet-to-dry blood Hg concentration obtained from the analysis of

paired samples. When comparing the whole blood Hg to the FTA card Hg, we found differences ranging from 3.7% for a Black Skimmer to 41.9% for a Great Kiskadee with a mean for all 12 birds of 12.7% (Table 3). Though our results appear ambiguous at first glance, they do seem to validate our assertion that FTA cards can be used as a substitute for traditional field sampling techniques (i.e., the collection and treatment of whole wet blood samples) to determine concentrations of Hg in blood from recently sampled birds during environmental emergencies. However, further research on this application is strongly recommended.

Table 3: Comparison between whole blood Hg (wet weight) and FTA card Hg (dry weight converted to wet weight) for 12 birds from

 Guerrero.

Scientific Name	Common Name	Whole Blood Hg (ppm, wet weight)	FTA Card Hg (ppm, calc. wet weight)	Percent Difference
Ardea alba	Great Egret	0.567	0.543	4.3
Himantopus mexicanus	Black-necked Stilt	0.133	0.098	30.3
Jacana spinosa	Northern Jacana	0.343	0.306	11.4
Pelecanus erythrorhynchos	Am. White Pelican	0.132	0.116	12.9
Pitagnus sulphuratus	Great Kiskadee	0.049	0.075	41.9
Pitagnus sulphuratus	Great Kiskadee	0.050	0.047	6.2
Pitagnus sulphuratus	Great Kiskadee	0.071	0.075	5.5
Rynchops niger	Black Skimmer	0.305	0.293	4.0
Rynchops niger	Black Skimmer	0.816	0.908	10.7
Rynchops niger	Black Skimmer	2.241	2.326	3.7
Rynchops niger	Black Skimmer	0.698	0.796	13.1
Sula leucogaster	Brown Booby	0.162	0.177	8.8
				Mean = 12.7%

Mercury in human hair samples. During this study, we collected hair samples from 38 women of reproductive age: 15 women from Acapulco, 15 from Llano Real near San Jerónimo de Juárez, and 8 from Petatlán outside Zihuatenejo (Appendix 6). Mercury levels ranged from 0.06 to 49.3 ppm. Seventy-one percent (27 of 38) of these individuals had Hg concentrations that exceeded the known lowest observed adverse effect level (LOAEL) of 0.3 ppm (Schoeman et al 2010). Concentrations of Hg exceeding this level have been shown to affect fetal development and alter neurological development (Schoeman et al 2010). One individual even showed a level of Hg in her hair over 164 times the LOAEL.

Discussion for Mercury Pollution

Mercury in fish samples. Four commonly marketed fish sampled during our study are highlighted below, followed by an analysis of other species sampled from angling or markets in Guerrero. The Hg concentration of each species is provided for comparison to the U.S. EPA recommended fish Hg criterion of 0.3 μ g/g (U.S. Environmental Protection Agency 2001).

Dolphinfish (Mahi-mahi; Dorado; Coryphaena hippurus)

Dolphinfish adults are typically found in open waters and occasionally near the coast. They feed on most types of fish and zooplankton, but they will also consume crustaceans and squid (Eschmeyer et al 1983). Adults reach sexual maturity at approximately 4-5 months (Randall 1995). A heavily commercialized fishery of very high value exists with fish marketed fresh or frozen. Most contaminant studies have found consistently low levels of Hg in Dolphinfish muscle tissue. Cai et al (2007) reported a mean muscle Hg value of 0.07 μ g/g in the Gulf of México. Kaneko et al (2007) analyzed Hg in muscle tissue of 30 individuals and reported a mean of 0.13 μ g/g (ww) off the coastal waters of Hawaii in 2006. Another study from the southeastern coast of the United States and the Gulf of México found Hg concentrations of 0.10 and 0.13 μ g/g, respectively (Adams 2009). García-Hernández et al (2007) reported a mean Hg concentration of 0.05 μ g/g in 14 individuals collected in the Gulf of California. Five individual Dolphinfish were sampled and analyzed in our study (Figure 15). The mean Hg concentration for these fish was 0.131 μ g/g with a range of 0.075 to 0.179. All of these individuals were below the U.S. EPA recommended fish Hg criterion of 0.3 μ g/g.

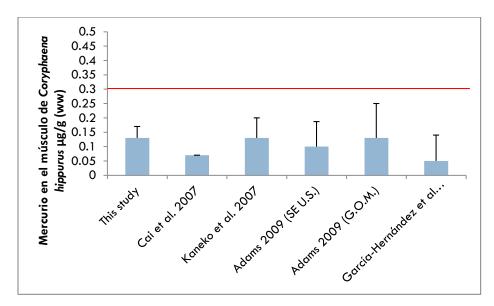


Figure 15: Mercury concentrations of Coryphaena hippurus (Dolphinfish; Mahi-mahi; Dorado) sampled in this study compared with background concentrations from other literature. Red line indicates the U.S. EPA recommended fish mercury criterion of 0.3 ppm.

Red Snapper (Huachinango; Lutjanus campechanus)

The Red Snapper is a heavily-commercialized fish found in the Western Atlantic and Gulf of México. Individuals sold and consumed on the Pacific coast of México are most likely transported from the Gulf Coast. Adults are found over rocky bottoms and feed mostly on fish, shrimp, crabs, cephalopods, worms, and plankton (Frimodt 1995). Studies typically show relatively low levels of Hg in muscle tissue from this species. A study of fish caught off the coast of New Jersey in 2003 reported a mean Hg level of 0.20 μ g/g (Burger and Gochfeld 2005). Bank et al (2007) reported a mean concentration of 0.06 μ g/g in Red Snapper caught in the Gulf of México off the coast of Louisiana. Liang et al (2011) sampled Red Snapper from five mariculture sites around Hong Kong and found muscle Hg concentrations ranging from 0.0759 to 0.105 μ g/g. In this study, we sampled three Red Snapper individuals with a mean Hg concentration of 0.088 μ g/g with a range of 0.024 to 0.157 (Figure 16). Each of these individuals was below the U.S. EPA recommended fish Hg criterion of 0.3 μ g/g.

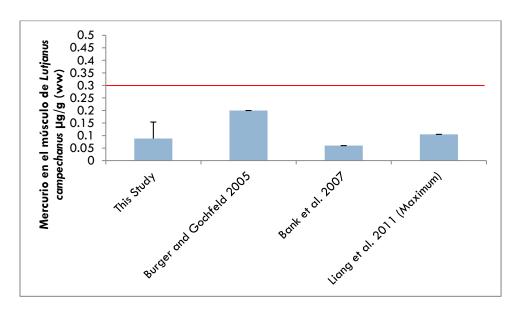


Figure 16: Mercury concentrations of *Lutjanus campechanus* (Red Snapper; Huachinango) sampled in this study compared with background concentrations from other literature. Red line indicates the U.S. EPA recommended fish mercury criterion of 0.3 ppm.

Swordfish (Xiphias gladius)

The Swordfish is a high trophic-level, oceanic fish. Adults are opportunistic feeders that consume mainly fish such as Atlantic Mackerel, Barracuda, Hake, Redfish, Herring, and Lanternfish (Scott and Scott 1988). This species has a widely-developed fishery and is often marketed for sashimi, teriyaki, or fillets (Collette 1995). Contaminant studies on this species frequently report muscle Hg levels well above the U.S. EPA limit for safe consumption. Kaneko et al (2007) reported a mean Hg concentration of 1.07 μ g/g from 50 individuals sampled off the coast of Hawaii in 2006. A study by Chen et al (2007) compared Hg concentrations in Swordfish from both the Indian and Atlantic oceans. Mean values were 1.47 and 1.20 μ g/g, respectively. However, maximum Hg concentrations in that study were as high as 3.97 μ g/g. Cortes and Fortt (2007) sampled six swordfish from fish markets in Chile and reported Hg values ranging

from 1.25 to 1.7 μ g/g. Another study in the southwest Atlantic Ocean sampled 192 Swordfish and reported Hg concentrations ranging from 0.04 to 2.21 μ g/g with a mean of 0.62 (Mendez et al 2001). Only one Swordfish was sampled during our study (Figure 17). This individual, however, had a muscle Hg concentration of 1.642 μ g/g, a level well above the U.S. EPA recommended fish Hg criterion of 0.3 μ g/g.

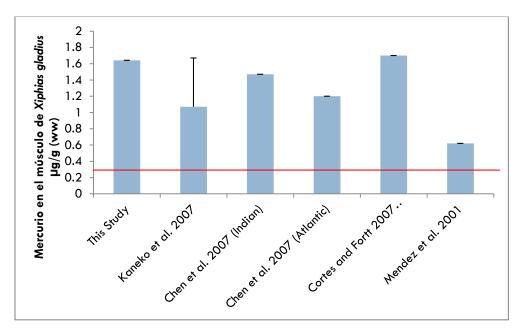


Figure 17: Mercury concentrations of Xiphias gladius (Swordfish) sampled in this study compared with background concentrations from other literature. Red line indicates the U.S. EPA recommended fish mercury criterion of 0.3 ppm.

Yellowfin Tuna (Thunnus albacares)

The Yellowfin Tuna is another high trophic-level fish found in ocean waters around the globe. Adults feed mostly on fish, crustaceans, and squid. Commercial fishing vessels typically employ large encircling nets to catch near-surface schools. Yellowfin Tuna meat is highly marketed frozen and canned, but is also highly valued for sashimi (Smith 1997). Several studies have reported muscle Hg values in tuna at or above recommended thresholds. Kaneko et al (2007) sampled 50 individuals in Hawaii and reported a mean value of 0.30 μ g/g. A study of market fish in New Jersey in 2003 sampled 50 individuals and reported a mean of 0.65 μ g/g (Burger and Gochfeld 2005). Another study sampled tuna from fish markets in the central and southern regions of Japan, reporting a mean Hg value of 0.33 μ g/g (Hisamichi et al 2010). In this study, one Yellowfin Tuna was sampled and analyzed (Figure 18). This individual had a muscle mercury concentration of 0.135 μ g/g that is below the U.S. EPA recommended fish Hg criterion of 0.3 μ g/g.

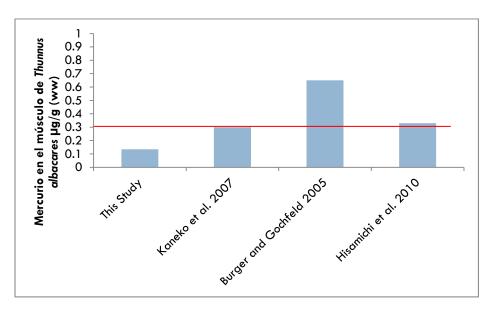


Figure 18: Mercury concentrations of *Thunnus albacares* (Yellowfin Tuna) sampled in this study compared with background concentrations from other literature. Red line indicates the U.S. EPA recommended fish mercury criterion of 0.3 ppm.

Other Species

Several other species were sampled and analyzed in this study (Figure 19). These species included Sea Bass spp., Dogfish (Squalus acanthias), Manta Ray (Manta birostris), Common Snook (Robalo; Centropomus undecimalis), Porgo Blanco (spp. Unknown), Agujon Needlefish (Tylosurus pacificusi), Tilapia spp., Pacific Sierra (Sierra; Scomberomorus sierra), Roosterfish (Gallo; Nematistius pectoralis), Horse-eye Jack (Ojon; Caranx latus), Shark spp., and Star-studded Grouper (Bofa; Epinephelus niphobles). Little to no background Hg data exist for these species. Both the Manta Ray and a Horse-eye Jack exceeded the U.S. EPA recommended fish Hg criterion of $0.3 \,\mu g/g$ with concentrations of 0.372 and $0.432 \,\mu g/g$, respectively. In future studies, higher sample sizes within species will help provide a more complete picture of mean Hg concentrations and the frequency at which these exceed safe consumption levels.

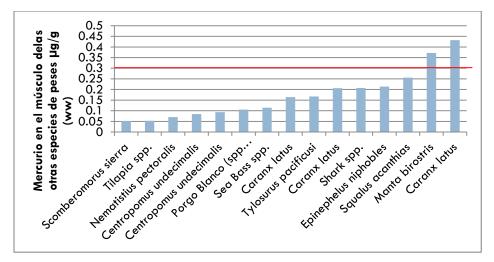


Figure 19: Mercury concentrations of other fish species sampled throughout this study. Red line indicates the U.S. EPA recommended fish mercury criterion of 0.3 ppm.

Mercury in bird samples. We compared blood Hg results in fish-eating birds sampled in Guerrero with the same species sampled for other BRI studies in Nicaragua and the United States. Most fish-eating birds tested in Guerrero had higher concentrations of mercury in their blood than birds sampled in the United States (Figure 20). In general, mercury concentrations for most marine piscivores were relatively low. However, using a blood Hg concentration of $1.0 \,\mu\text{g/g}$ (Evers et al 2008; Heinz et al 2009) as an adverse effect level threshold for reproductive success in skimmers, we found that Black Skimmers and Royal Terns (assuming that terns have similar Hg threshold levels as skimmers) may be at risk from elevated Hg exposure. One Black Skimmer (out of four) sampled from Hacienda de Cabañas had a blood Hg concentration of 2.24 ppm. Similarly, a Royal Tern from Barra Vieja in Acapulco had a concentration of 2.19 ppm. Further study is urgently needed to increase our sample size to evaluate Hg risks for waterbirds in Guerrero and to explore potential environmental factors for such elevated values observed in our study.

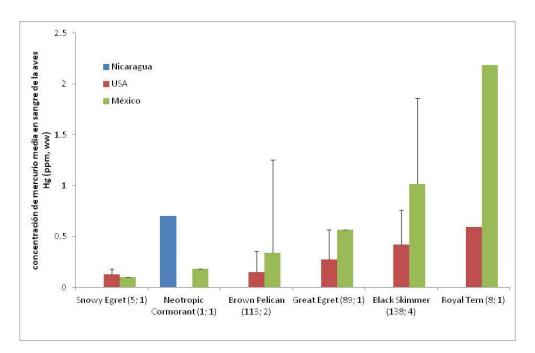


Figure 20: Mean blood mercury (ppm, wet weight) in fish-eating birds sampled in Guerrero, México in 2013 as compared to the same species sampled in the United States in 2010 (Eggert 2012) and Nicaragua in 2012 (Lane et al 2013).

Brown and White Pelicans, cormorants, egrets, terns, and Black Skimmers are fish-eating species, a predisposition that places them at risk for methylmercury bioaccumulation. Differences in diet composition (e.g., prey species and size) may influence the degree of exposure. For example, Brown Pelicans feed primarily on Menhaden (*Brevoortia* spp.), but also consume Mullet (*Mugil* spp.) and Anchovy (*Anchoa* spp.). The diet of skimmers is generally more varied and may include Killifish (*Fundulus* spp.), Silverside (*Menidia* spp.), Sheepshead Minnow (*Cyprinodon* variegates), Mullet, and Menhaden. Thus, the predominance of Menhaden, a filter feeder on mostly phytoplankton, in the diet of pelicans may lower their risk to Hg exposure. On the other hand, the risk of Hg exposure increases for terns and skimmers because they utilize near-shore and estuarine habitats in which the methylation of inorganic Hg by anaerobic bacteria is favored. Within coastal areas, however, Hg is likely found in higher concentrations in tidal creeks, shallow estuarine waters, and water-land margins than in deep waters and open bays. Because of this, and their overlapping foraging preferences, species such as Black Skimmers and Royal Terns may show a greater uptake of Hg in their diets than Brown and White Pelicans.

Some bird species may be viewed as indicator species of environmental contaminants such as mercury. Based on research by Heinz et al (2009), the Brown Pelican (Pelecanus occidentalis), Great Egret (Ardea alba), and Royal Tern (Thalasseus maximus) are species categorized as having medium sensitivity to Hg exposure and the Snowy Egret (Egretta thula) as having high sensitivity to mercury. Further study in Guerrero on these species, other piscivores, and invertivores will address gaps in our knowledge about the movement of Hg throughout aquatic and terrestrial ecosystems in the state. We know that certain environmental conditions may influence Hg exposure in organisms, but the processes related to the production and biomagnification of methylmercury under such conditions are not as well understood in estuarine and marine waters as they are in freshwater systems (Fitzgerald 2007). Based on the results of our pilot study in Guerrero, long-term ecological research is called for to understand Hg cycling in the region and the interchange of contaminants between marine and terrestrial environments.

Mercury in human hair samples. Most human exposure to Hg comes from the consumption of contaminated fish that are high on the trophic pyramid such as Tuna, Shark, and Swordfish. Methylmercury, the predominant form of mercury in fish and the most toxic to wildlife and humans, is known to impact neurological development in infants and is also linked to cardiovascular disease in adults (Clarkson et al 2003; Valera et al 2011). High Hg exposure to offspring during pregnancy can be especially problematic as Hg concentrations in cord blood average twice that of maternal blood concentrations at the time of birth (Hightower and Moore 2003).

Studies have long focused on the effects of maternal fish consumption and related Hg intake during pregnancy. While it is generally agreed that moderate fish consumption is healthy for pregnant women, and even beneficial to embryonic development, the risks posed by certain types of fish often outweigh these benefits. Oken et al (2005) demonstrated that higher maternal

"Our waste streams are diminishing biodiversity around the world. Ultimately, it's all about a change in attitude."

- H. Bruce Rinker, Ph.D., Director of Scientific Advancement, Biodiversity Research Institute fish consumption correlated with higher Hg levels and was thus associated with lower offspring cognitive scores. At the same time, however, higher maternal fish consumption was associated with increased infant cognition, predominantly in infants whose mothers had consumed fish with lower mercury levels. Similarly, Oken et al (2008) found that pregnant women who consumed more fish had higher Hg levels. Among the children of these women, higher Hg exposure was positively correlated with lower developmental test scores at 3 years of age. In the same study, however, maternal fish intake of more than twice per week was associated with improved performance on language tests and visual motor skills. While the benefits of moderate fish consumption in daily diets and during pregnancy are obvious, these results highlight the importance of avoiding or limiting consumption of high trophic-level fish such as Swordfish, tuna, and sharks, species of fish known to contain high concentrations of Hg via biomagnification.

All 38 women sampled during the BRI study also filled out a survey documenting the frequency at which they consumed fish and whether or not they had children in their home, were nursing, or were considering getting pregnant. Seven out of the 31 women who addressed the question about getting pregnant affirmed that they were considering the possibility. All but one of these women had a hair Hg concentration that exceeded the LOAEL of 0.3 ppm (Schoeman et al 2010; Figure 21). The one individual considering having children whose Hg level was below 0.3 ppm was also the only person who declared that she "rarely" consumed fish.

These results highlight the urgent need for improved education and community awareness regarding the safe consumption levels and acceptable frequencies of eating fish, especially those species known to carry high levels of mercury.

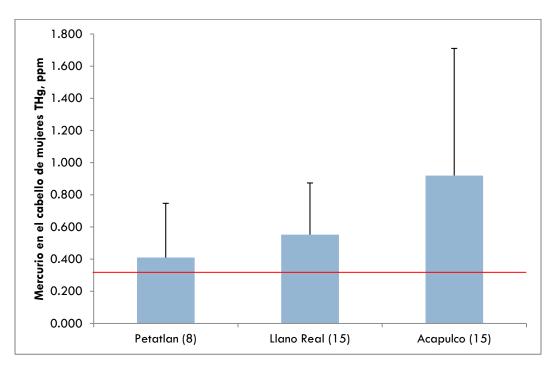


Figure 21: Mean hair mercury concentrations in women of reproductive age sampled in Guerrero in 2013. Red line represents LOAEL of 0.3 ppm. The outlier value of 49.3 ppm from Petatlán was excluded from the mean.

"The Limpia Guerrero initiative helps continue BRI's interest to understand and minimize the effects of pollutants like plastics, mercury, and petroleum to both wildlife and humans in the Western Hemisphere."

- Dr. David C. Evers, Executive Director, Biodiversity Research Institute

PETROLEUM POLLUTION

Results for Petroleum Pollution

The results of the analysis show that employing blood spot/FTA cards can be used to determine concentrations of PAH compounds in blood collected from birds. We were able to detect PAHs in blood in 11 out of a possible 26 individuals (42%)(Table 4). Concentrations were low and, in many cases, were slightly above the reporting limit of 3 ng/ml (ppb) with acenaphthene being detected in nine of the 11 positive samples. Based on data from this pilot study, we did not detect any trends for petroleumrelated pollutants. In other words, no specific bird taxa showed (or, conversely, did not show) detectable levels of PAHs in blood in particular sampled environments.

The low sample concentrations could be partially related to the small sample mass/volume used for analysis. Each blood spot contained 50 μ l of blood so, in the best case scenario of being able to use 2 spots, a total of 100 μ l of blood was used for analysis. This is significantly less volume than typically used by the University of Connecticut for blood analysis (500 to 1000 μ l), which can impact the sensitivity of the analysis. The limitation of sample mass is greatly outweighed; however, by the ability to collect samples from areas endemic for the Newcastle's Disease and not have to use treatment methods that will render any analysis useless. In this study, the Connecticut lab was able to mitigate slightly the low sample mass by using the highly sensitive and selective GC/MS/MS for analysis that can provide a 10 to 100x increase in sensitivity compared to traditional analytical techniques.

Discussion for Petroleum Pollution

Though blood from birds has been used previously as a monitoring tool for oil exposure (Pérez et al 2008), the use of FTA cards to detect PAHs in the blood of wildlife is a new approach for analyzing petroleum. Since we validated that these cards can be used to determine concentrations of Hg and PAHs in blood from recently sampled birds, it would be advantageous to determine the long-term life of these cards for contaminant determination. If it is demonstrated that the concentration of contaminants that are in blood bound within the card matrix are stable, then these cards can be used to archive samples and create a reference collection. This will provide tremendous flexibility to create a historical collection with minimal sample storage requirements, which otherwise may not be available, to determine impact related to an environmental emergency. Further, we recommend that future sampling and analysis using the FTA cards include other heavy metals (e.g., cadmium, lead, and arsenic) and pesticides used along the coast and elsewhere in the State of Guerrero. Pesticides and PCBs will likely lend themselves to this type of assessment technique since they are much more stable than PAHs and will be less likely to be lost due to handing and sublimation.

Scientific Name	Common English Name – Sample Number	Total PAH (ng/mL)	Napthalene (ng/mL)	Acenaphthene (ng/mL)	Benzo(b)fluoranthene (ng/mL)	Indeno(1,2,3-cd)pyrene (ng/mL)	Dibenzo(a,h)anthracene (ng/mL)	Benzo(g,h,j)perylene (ng/mL)
Actitus macularius	Spotted Sandpiper – 1	5.3	ND*	5.3	ND	ND	ND	ND
Actitus macularius	Spotted Sandpiper – 1	12.6	12.6	ND	ND	ND	ND	ND
Ardea alba	Great Egret – 1	5.8	ND	5.8	ND	ND	ND	ND
Jacana spinosa	Northern Jacana – 3	20.2	ND	ND	3.7	5.5	5.5	5.5
Pitagnus sulphuratus	Great Kiskadee – 2	15.5	ND	5.2	3.4	3.9	ND	3.0
Rynchops niger	Black Skimmer – 2	5.1	ND	5.1	ND	ND	ND	ND
Rynchops niger	Black Skimmer – 3	8.4	ND	8.4	ND	ND	ND	ND
Rynchops niger	Black Skimmer – 4	6.4	ND	6.4	ND	ND	ND	ND
Scardafella inca	Inca Dove – 1	5.7	ND	5.7	ND	ND	ND	ND
Scardafella inca	Inca Dove – 2	9.4	ND	9.4	ND	ND	ND	ND
Scardafella inca	Inca Dove – 3	6.9	ND	6.9	ND	ND	ND	ND

Table 4: Detection of PAHs in birds from Guerrero by using FTA cards: 11 out of 26 samples tested for PAHs.

* ND = Not Detected.

Most previous studies that analyzed PAHs in bird tissues focused on egg and embryo tissues as these are more likely to contain detectable levels of PAHs. Other research has demonstrated, however, that analyzing PAHs in bird blood will effectively reveal recent uptake of these compounds (Pérez et al 2008). Studies on young birds have shown that exposure to PAHs reduces growth and immune system function and increases metabolic and endocrine system activity. Experiments with adult birds have revealed that certain PAH compounds can reduce egg production, hatching success, and immune system function (Albers 2006).

For our Guerrero study, 11 samples out of a possible 26 showed detectable levels ranging from 5.1 to 20.2 ng/mL for total PAH. One PAH used to make dyes, plastics, and pesticides, acenaphthene was detected in nine of the 10 samples positive for PAH signals. Most of these individual birds were not piscivorous, but instead had diets consisting mainly of insects and other invertebrates. These preliminary results suggest that invertivores may be more susceptible to biological intake of PAHs than fish-eating species. Further investigation of these compounds, along with sampling more appropriate tissues such as eggs and muscle, will give us a more complete understanding of the dynamics of PAH pollution in the study area. Comparisons among previous studies are difficult, given the variability of PAHs analyzed and tissues sampled. Thus, continued sampling of similar species across different capture sites and sampling periods will likely give us the best mode of comparison and potential for detecting temporal and spatial differences in PAH contamination throughout the study area.

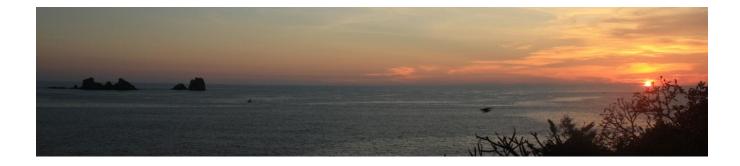
SUMMARY

With its widely recognized expertise in innovative wildlife science and contaminants research, BRI conducted a three-week pilot study in Guerrero in the fall of 2013 to check for environmental signals in fish, birds, and humans for three common pollutants often used as reliable predictors of ecological toxicity: plastics, mercury, and petroleum. The signals were unequivocal and strongly indicate the necessity of (1) long-term ecological research in Guerrero and (2) an integrated follow-up response by the state to address their causes and solutions. Such pollutants are widespread contaminants of the coastal and marine environments that often produce discernible adverse effects on ecosystems only in limited areas around population centers and ports (Boesch et al 2001). That is, until they are measured and observed over a period of time, they sometimes remain "hidden risks" in wildlife and humans (Osborne et al 2011). However, reversing and controlling diffuse sources of pollution, such as those measured during this study, requires an integrated approach on the scale of an entire drainage basin (Boesch et al 2001).

For this study, we focused on fish, birds, and people in the coastal lowlands and the marine environment of Guerrero. Yet we observed plastics and other beach debris washed into this region from the state's uplands, much of which was likely deposited from the sierras during the September 2013 tropical storm. To the best of our knowledge, no one had measured standing surface litter for the state until this study so the density of plastics and other marine debris prior to Tropical Storm Manuel remains unknown. Thus, our observations beg the question: Could some or all of this debris have been avoided without what appears to be an increasing rate of deforestation and erosion in the highlands over the past decade?⁵ The sources of the mercury and petroleum contamination that we detected are also unknowns at this time. How might these pollutants have been influenced by the recent storm or have changed over time? These and many other questions have emerged as a consequence of our pilot study,

⁵ See an interactive tool for global forest change, including the Mexican State of Guerrero, during the past decade published by the University of Maryland: <u>http://earthenginepartners.appspot.com/science-2013-global-forest</u>.

but our research denotes an important starting point to address these concerns and their solutions – a starting point that also represents a model for other coastal states in México. Future studies may include a much broader timeframe and longer coastline, other taxonomic groups (e.g., plants, invertebrates, and bats), upland environments, and a more comprehensive array of contaminants (e.g., other heavy metals and pesticides).



POLICY RECOMMENDATIONS

As a consequence of BRI's three-week pilot study, and general observations by its researchers in the field, we offer the following policy recommendations to the State of Guerrero for immediate consideration (not given in order of priority but should be considered *in toto*):

Pollutants

- 1. Improve all infrastructures throughout the coastal area for gathering and recycling debris (including well-labeled and easily accessible trash cans and PET depositories).
- 2. Work with hoteliers, restaurateurs, and other stakeholders in the regional tourism industry to establish policies against single-use plastic products such as plastic drinking straws and plastic shopping bags.
- 3. Promote and enhance proper land-based waste management including landfills and sewage treatment facilities; coastal and riverside communities should make sure that open landfills for household waste and/or industrial waste are eliminated as part of their overall waste management strategies.
- 4. Work to reduce the generation of marine litter from merchant ships, offshore platforms, fishing vessels, and pleasure crafts; waste should be stored aboard and discharged onshore in proper reception facilities.
- 5. Issue public alerts to avoid the consumption of Swordfish and limit the consumption of Manta Ray and Horse-eye Jack; distribute educational pamphlets about sustainable seafood choices (see <u>www.seafoodwatch.org</u>) and include information about plastics, mercury, and petroleum pollution in the science curriculum for local schools and universities.

Fish and Wildlife

- 1. Train Mexican scientists and graduate students in the art and craft of wildlife capture and sampling in order to remain vigilant about the protection of regional fish and wildlife; this can be achieved, e.g., via a memorandum of understanding between BRI and the Universidad Autónoma de Guerrero to help establish undergraduate and graduate studies focused on regular monitoring and sampling of the biodiversity in Guerrero.
- 2. Enforce the anti-motorized-vehicle (including anti-ATVs) regulations for the beaches of Guerrero, especially El Revolcadero and Playa Bonfil, to protect nesting sea turtles and foraging shorebirds; consideration should also be given to the negative effects of night lighting and poaching on sea turtles attempting to access local beaches for nesting.
- 3. Monitor and strictly manage the degree of gill-netting in the Tres Palos Lagoon outside Acapulco to maximize the local fisheries for both humans and wildlife.
- 4. Expand contaminant analyses of fish and wildlife to include stable isotope analysis; mercury stable isotope analysis in particular is an increasingly important tool (though costly) in identifying sources of mercury contamination in the environment.
- 5. Conduct further scientific investigations focused on fish-eating wildlife and other taxa to assess the risks from exposure to mercury and other heavy metals in the regional environment.

Conservation

- Conduct a biological inventory and an economic assessment on tourism of the Tres Palos Lagoon outside Acapulco in the spring or summer 2014 with possible extensions that focus on other nearby site-specific resources such as the Santuario Murciélago near Copalillo.
- 2. Enforce existing legislation to protect the lagoons, mangroves, and coastal waterways of Acapulco and throughout the State of Guerrero from all illegal encroachments (including, but not limited to, burning marshes, cutting mangroves, and overfishing).
- 3. Improve the conservation/education message in the sierras to stress the links between upland and lowland areas of Guerrero; reforest denuded highlands as a first step toward protecting the upper reaches of watersheds vulnerable to landslides and flooding during major storm events.
- 4. Invest in the long-term conservation education of resident youth and adults to protect the natural resources of Guerrero; create an incentives program for youth volunteers that will provide deferred credits toward higher education focused on conservation, a kind of civilian youth conservation corps or "cuerpo de conservación civil juventud."
- 5. Establish efficient and sustainable ecotourism packages in partnership with local and regional governments, businesses, NGOs, and educational institutions for domestic and international visitors to capitalize on the extraordinary and irreplaceable value of Guerrero's natural resources.
- 6. Promote regular beach cleanup operations and campaigns throughout the region via local authorities, volunteers, and/or NGOs.

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BRI also appreciates the abiding support, counsel, and friendship of Tulio Ismael Estrada Apátiga (Minister of the Environment, State of Guerrero, Chilpancingo, México) and Manolo Ruiz Ingelmo (Director General, Sustenta Soluciones S.A. de C.V. and Limpiaguerrero.com, México City) throughout the Limpia Guerrero 2013 campaign. From the project's inception in March 2013 to its completion in mid-December 2013, BRI's work in Guerrero would not have been possible without the unfailing commitment of these two dedicated individuals to conservation in México and beyond.

BRI wishes to express its thanks to Santiago Lobeira, Sergio San Miguel, and Angel Trejo for their outstanding assistance, much of it "behind the scenes," throughout the project: from the acquisition of scientific permits to the logistics of transportation, housing, and finances. The fact that the project moved along flawlessly is due in large part to their thoughtfulness and stewardship.

The authors are deeply grateful for the kindnesses of Olivia Espinosa and Santiago Lobeira, and Samar Ingelmo and Manolo Ruiz, for the "loan" of their beautiful apartments in Acapulco and Ixtapa, respectively, throughout our stay in Guerrero. Accommodations for fieldwork cannot get any better than these superb homes for renewal and relaxation!

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The authors were moved profoundly by the generosity, hospitality, and kindness of the nearly 600 temporary workers from the municipalities of Acapulco, San Jerónimo de Juárez, and Zihuatenejo/Ixtapa for the Limpia Guerrero 2013 campaign (Figure 22). The charm and loveliness of these coastline cities were exceeded only by the charm and loveliness of its residents. We extend our heartfelt thanks to each and every dedicated worker for the success of the campaign.

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It should be noted that BRI's general scientific work in México falls in part under the authority of its official international agreements with two federal agencies of the Mexican Government: a carte de *intención* with CONANP (Comisión de Áreas Naturales Protegidas) and a convenio de concertación, or memorandum of understanding, with INECC (Instituto Nacional de Ecología y Cambia Climático).



Figure 22: The Limpia Guerrero 2013 team of enthusiastic workers and researchers for Zihuatenejo/Ixtapa, part of a small army of over 600 people to study and clean the beaches of Guerrero. Photograph © Alfredo Blasquez.

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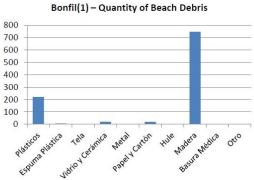
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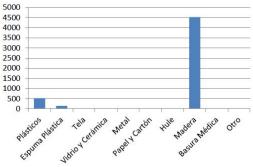
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Appendix 1: Aerial views of seven beaches surveyed for plastics pollution during the Limpia Guerrero 2013 campaign; the associated graphs show the quantity of beach debris collected per category from the one-meter-wide belt transects.



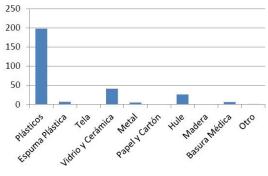


Bonfil(2) – Quantity of Beach Debris





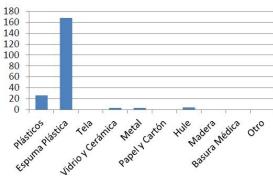
Luis Miguel – Quantity of Beach Debris



Appendix 1, continued.

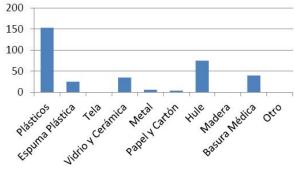


El Cano, Bonfil – Quantity of Beach Debris





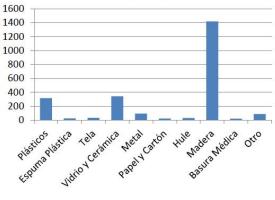
Las Lomas de Chapultepec – Quantity of Beach Debris



Appendix 1, continued.

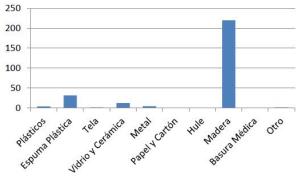


Playa Zihuatenejo – Quantity of Beach Debris

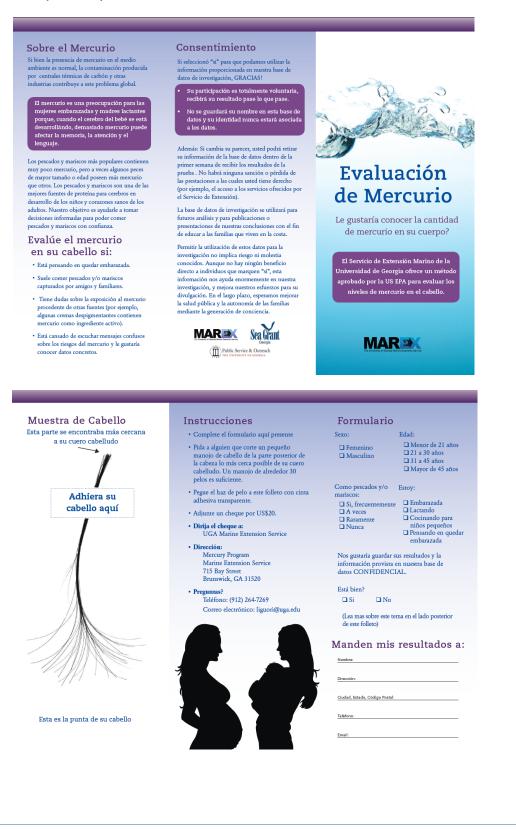




Barra de Potosí, Petatlán — Quantity of Beach Debris



Appendix 2: "Evaluación de Mercurio" information card and questionnaire provided to each volunteer for hair sampling during the Limpia Guerrero 2013 campaign; as a part of funding for the study, all fees for volunteers for sample analysis were waived.



Appendix 3: Mercury concentrations in 25 fish sampled in Guerrero in 2013, representing 15 species. Note the value of swordfish compared to all other samples.

Location	Spanish Name	English Name	Latin Name	Hg (ww ppm)
Acapulco	Dorado	Mahi-mahi	Coryphaena hippurus	0.075
Acapulco		Red Snapper	Lutjanus campechanus	0.085
Acapulco		Sea Bass		0.114
Acapulco		Dogfish	Squalus acanthias	0.256
Acapulco		Manta Ray	Manta birostris	0.372
Acapulco		Swordfish	Xiphias gladius	1.642
Llano Real	Robalo	Common Snook	Centropomus undecimalis	0.084
Llano Real	Robalo	Common Snook	Centropomus undecimalis	0.093
Llano Real	Porgo blanco			0.105
Llano Real	Agujon	Agujon Needlefish	Tylosurus pacificus	0.167
Llano Real (Laguna de Mitla)	Tilapia	Tilapia		0.053
Ixtapa	Dorado	Mahi-mahi	Coryphaena hippurus	0.145
Ixtapa	Dorado	Mahi-mahi	Coryphaena hippurus	0.149
Isla de Barra de Potosí, Petatlán	Sierra	Pacific Sierra	Scomberomorus sierra	0.052
Isla de Barra de Potosí, Petatlán	Gallo	Roosterfish	Nematistius pectoralis	0.070
Zihuatenejo	Huachinango	Red Snapper	Lutjanus campechanus	0.024
Zihuatenejo	Dorado	Mahi-mahi	Coryphaena hippurus	0.106
Zihuatenejo		Yellow-fin Tuna	Thunnus albacares	0.135
Zihuatenejo	Huachinango	Red Snapper	Lutjanus campechanus	0.157
Zihuatenejo	Ojon	Horse-eye Jack	Caranx latus	0.164
Zihuatenejo	Dorado	Mahi-mahi	Coryphaena hippurus	0.179
Zihuatenejo	Ojon	Horse-eye Jack	Caranx latus	0.206
Zihuatenejo		Shark spp.	Unk.	0.207
Zihuanenejo	Bofa	Star-studded Grouper?	Epinephelus niphobles?	0.214
Zihuatenejo	Ojon	Horse-eye Jack	Caranx latus	0.432

Latin name	Common Name (Español)	Common Name (English)	Number
Actitis macularius	Playero Alzacolita	Spotted Sandpiper	6
Ardea alba	Garza Grande	Great Egret	1
Egretta thula	Garza Nivea	Snowy Egret	1
Geothlypis trichas	Mascarita Común	Common Yellowthroat	1
Himantopus mexicanus	Candelero Americano	Black-necked Stilt	1
Icteria virens	Gritón Pechiamarillo	Yellow-breasted Chat	1
Jacana spinosa	Jacana Mesoamericana	Northern Jacana	5
Melanerpes chrysogenys	Carpintero Cachetidorado	Golden-cheeked Woodpecker	1
Mniotilta varia	Reinita Trepadora	Black-and-white Warbler	1
Parkesia noveboracensis	Chipe-suelero Charquero	Northern Waterthrush	1
Pelecanus erythrorhynchos	Pelícano Blanco Americano	American White Pelican	1
Pelecanus occidentalis	Pelícano Café	Brown Pelican	2
Phalacrocorax brasilianns	Cormorán Neotropical	Neotropic Cormorant	1
Pitangus sulphuratus	Bienteveo Grande	Great Kiskadee	3
Quiscalus mexicanus	Zanate Mayor	Great-tailed Grackle	7
Rynchops niger	Rayador Americano	Black Skimmer	4
Scardafella inca	Tórtola Colilarga	Inca Dove	5
Sula leucogaster	Bobo Vientre-blanco	Brown Booby	2
Thalasseus maximus	Golondrina-marina/Pagaza Real	Royal Tern	1

Appendix 4: Numbers and species of birds sampled in Guerrero in 2013.

Appendix5: Whole blood mercury concentrations in 45 birds sampled in Guerrero in 2013, representing 19 species.

Location	Site	Blood Hg (ppm ww)	Species
Acapulco	Laguna de Tres Palos	0.132	American White Pelican
Acapulco	Barra Vieja	0.018	Great-tailed Grackle
Acapulco	Barra Vieja	0.027	Great-tailed Grackle
Acapulco	Barra Vieja	0.030	Great-tailed Grackle
Acapulco	Barra Vieja	0.032	Great-tailed Grackle
Acapulco	Barra Vieja	0.035	Great-tailed Grackle
Acapulco	Barra Vieja	0.036	Great-tailed Grackle
Acapulco	Barra Vieja	0.047	Great-tailed Grackle
Acapulco	Laguna de Tres Palos	0.005	Inca Dove
Acapulco	Laguna de Tres Palos	0.005	Inca Dove
Acapulco	Laguna de Tres Palos	0.016	Inca Dove
Acapulco	Laguna de Tres Palos	0.081	Northern Jacana
Acapulco	Laguna de Tres Palos	0.135	Northern Jacana
Acapulco	Laguna de Tres Palos	0.249	Northern Jacana
Acapulco	Laguna de Tres Palos	0.343	Northern Jacana
Acapulco	Laguna de Tres Palos	0.560	Northern Jacana
Acapulco	Barra Vieja	2.185	Royal Tern
Acapulco	Barra Vieja	0.102	Snowy Egret
Acapulco	Laguna de Tres Palos	0.180	Spotted Sandpiper
Acapulco	Laguna de Tres Palos	0.195	Spotted Sandpiper
Acapulco	Laguna de Tres Palos	0.315	Spotted Sandpiper
Acapulco	Laguna de Tres Palos	0.511	Spotted Sandpiper
Acapulco	Laguna de Tres Palos	0.797	Spotted Sandpiper
Acapulco	Laguna de Tres Palos	lost	Spotted Sandpiper
Hacienda de Cabañas	Playa Paraiso	0.305	Black Skimmer
Hacienda de Cabañas	Playa Paraiso	0.698	Black Skimmer
Hacienda de Cabañas	Playa Paraiso	0.816	Black Skimmer
Hacienda de Cabañas	Playa Paraiso	2.241	Black Skimmer
Hacienda de Cabañas	Playa Paraiso	0.257	Black-and-white Warbler

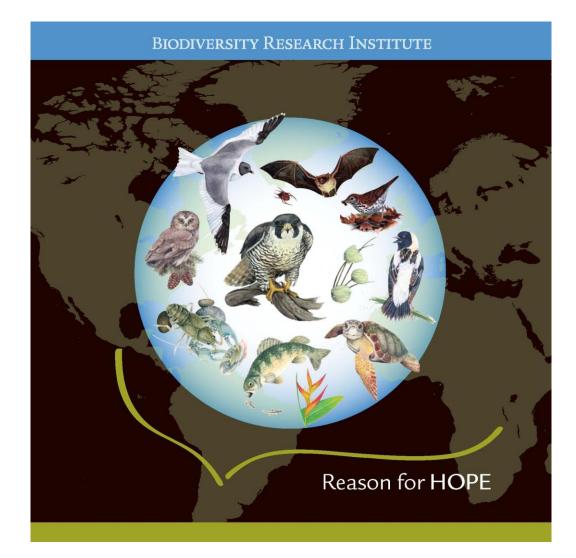
Appendix 5 continued.			
Location	Site	Blood Hg (ppm ww)	Species
Hacienda de Cabañas	Playa Paraiso	0.133	Black-necked Stilt
Hacienda de Cabañas	Playa Paraiso	0.109	Common Yellowthroat
Hacienda de Cabañas	Playa Paraiso	0.026	Golden-cheeked Woodpecker
Hacienda de Cabañas	Playa Paraiso	0.567	Great Egret
Hacienda de Cabañas	Playa Paraiso	0.049	Great Kiskadee
Hacienda de Cabañas	Playa Paraiso	0.050	Great Kiskadee
Hacienda de Cabañas	Playa Paraiso	0.071	Great Kiskadee
Hacienda de Cabañas	Playa Paraiso	0.007	Inca Dove
Hacienda de Cabañas	Playa Paraiso	0.008	Inca Dove
Hacienda de Cabañas	Playa Paraiso	0.115	Yellow-breasted Chat
Ixtapa	Isla de Morro Sacatoso	0.162	Brown Booby
Ixtapa	Isla de Morro Sacatoso	0.177	Brown Booby
Petatlán	Isla de Barra de Potosí	0.180	Neotropical Cormorant
Petatlán	Isla de Barra de Potosí	0.152	Northern Waterthrush
Zihuatanejo	Playa Principal	0.202	Brown Pelican
Zihuatanejo	Playa Principal	0.479	Brown Pelican

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Appendix 6: Hair mercury concentrations, age range, and frequency of fish consumption of all women sampled in Guerrero in 2013.

Location	Cabello Hg [mg/kg]	Edad	La Frecuencia
Acapulco	0.060	31-45	Frecuentemente
Acapulco	0.182	21-30	A veces
Acapulco	0.260	21-30	A veces
Acapulco	0.396	31-45	Frecuentemente
Acapulco	0.426	21-30	A veces
Acapulco	0.439	no info	no info
Acapulco	0.564	no info	no info
Acapulco	0.598	21-30	Frecuentemente
Acapulco	0.625	21-30	Frecuentemente
Acapulco	0.632	no info	no info
Acapulco	1.334	21-30	Frecuentemente
Acapulco	1.624	31-45	A veces
Acapulco	2.042	<21	Frecuentemente
Acapulco	2.061	21-30	Frecuentemente
Acapulco	2.549	>45	Frecuentemente
Llano Real	0.226	21 - 30	A veces
Llano Real	0.252	31-45	Frecuentemente
Llano Real	0.265	< 21	Frecuentemente
Llano Real	0.274	21-30	A veces
Llano Real	0.301	21- 30	Raramente
Llano Real	0.389	21 - 30	Frecuentemente
Llano Real	0.401	21 - 30	A veces
Llano Real	0.449	31 -45	Frecuentemente
Llano Real	0.458	31- 45	A veces
Llano Real	0.495	21 - 30	Raramente
Llano Real	0.658	21-30	A veces
Llano Real	0.957	21-30	Frecuentemente

Appendix 6 continued.			
Location	Cabello Hg [mg/kg]	Edad	La Frecuencia
Llano Real	0.984	< 21	Frecuentemente
Llano Real	1.013	< 21	Frecuentemente
Llano Real	1.165	21 - 30	Frecuentemente
Petatlán	0.163	21-30	Raramente
Petatlán	0.186	>45	Raramente
Petatlán	0.246	31-45	A veces
Petatlán	0.297	<21	Frecuentemente
Petatlán	0.306	21-30	Frecuentemente
Petatlán	0.337	no info	A veces
Petatlán	0.556	<21	A veces
Petatlán	1.116	31-45	Frecuentemente
Petatlán	49.312	21-30	A veces



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