Impact of alluvial artisanal and small-scale gold mining in the Madre de Dios River Basin, Peru: total mercury levels in human and farmed fish populations

by

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Abstract

Alluvial gold mining in the region of Madre de Dios, Peru has caused extensive environmental degradation, human health risks, and social unrest. Poor legislation and strong political pressure has prevented the regional government from regulating artisanal small-scale gold mining. In March of 2012, local police clashed with 15,000 mining federation protesters in the capital city of Puerto Maldonado. Several smaller conflicts between military police and local populations have since ensued, yet little has been accomplished by the government in the way of legal reform. Meanwhile, artisanal small-scale gold mining in the region has continued. Large tracts of lowland rainforest have been transformed to desert-like landscapes, human populations have been exposed to mercury health risks, and the ecosystem has become increasingly impacted by the anthropogenic inputs of mercury into the environment. Humans, threatened species and fish in Madre de Dios have been found to have elevated mercury levels. In this study, samples of human hair showed that residency of participants was an important factor in predicting mercury levels. This is concluded to be at least partially a result of the proximity of a study site to mining activities. Samples of fish tissue from aquaculture ponds in Madre de Dios showed levels of mercury below the reference level. A linear correlation was found between length of time fish spent in pond and total mercury (reported in wet weight). There is a need for artisanal small-scale gold mining regulation. In addition, the underlying causes of continued unregulated alluvial mining in the area rests at least in part on the international community's failure to enact and enforce appropriate international environmental policies.

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Chapter 1: Literature Review

1.1 Introduction

The region of Madre de Dios in the Peruvian Amazon has seen a drastic increase in alluvial gold mining in the last three decades (Damonte et al., 2013). Development projects, the global increase in gold prices, and the lack of State enforcement and regulation have led to essentially uncontrolled gold mining in the region. Massive amounts of inorganic elemental mercury, used to amalgamate fine alluvial gold, have been released into the environment.

Accordingly, concerns about the impacts of mining activity have become an area of international attention and speculation. Unregulated use of mercury has led to speculation about the impacts on human health and environmental quality in the region. Mercury is a known causative agent of various types of disorders, including neurological, cardiac, motor, reproductive, genetic, renal, and immunological (Zahir, Rizwi, Haq and Khan, 2005). Additionally, mercury is a threat to the health of wildlife and bioaccumulates in the food chain.

In addition to environmental pollution from mercury used in the gold extraction process, social issues, deforestation, and legal conflicts have caused political unrest in recent years. Occurrences of violence and injustice surrounding alluvial gold mining in the region have gained attention from global media. Despite growing knowledge of and concern over the negative impacts of illegal and informal alluvial artisanal and small-scale gold mining in Madre de Dios, Peru, the mass exportation of illegal gold from Peru continues at an alarming rate.

This study examines the causes and impacts of artisanal small-scale gold mining (ASGM) in the Madre de Dios river basin of Peru. ASGM is the practice of extracting gold by individuals or small groups using mostly non-mechanical means. The terms and the distinction between small-scale and artisanal are discussed later. The purpose of Chapter 1 is to describe the political environment in which alluvial mining legal processes are being negotiated, the potentially devastating consequences of alluvial mining in Madre de Dios, and the historical context within which current conflicts are nested. The first chapter summarizes the recent trends in increased informal and illegal alluvial gold mining, environmental impacts, human health concerns, and various social and political aspects of ASGM.

Chapter 2 describes the author's research on human mercury levels in the region. Chapter 3 covers the author's research on mercury levels in fish in the region of Madre de Dios. The final Chapter of the report discusses the implications of the study findings and highlights areas in need of further research. Policy recommendations and instructions for the international gold consumer are briefly outlined.

1.2 Background Information

Alluvial artisanal small-scale gold mining overview

ASGM is a term broadly used to describe gold mining by individuals, families, or groups with minimal mechanization and often in the informal or illegal sector of the market (Hentschel, Hruschka, and Priester, 2002). ASGM is characterized by several conditions, including poor occupational safety and health care, inefficiency in gold recovery, exploitation of small deposits, lack of long-term mine planning, poor environmental considerations, and operational outputs that correspond to market price fluctuations.

Artisanal small-scale gold mining occurs in over 55 developing countries around the world (Spiegel and Veiga, 2010). It is estimated that 13 million people across the globe are employed in ASGM industries and a further 80-100 million people's livelihoods are directly reliant upon or impacted by ASGM activities (Hentschel, Hruschka, and Priester, 2002).

The method of gold extraction differs among ASGM groups around the world depending on characteristics of a deposit. In the Madre de Dios Region of Peru, the gold deposits are alluvial in nature. For millennia, gold from the eastern Cordillera in the central Andes of Peru has been transported downstream and deposited in the Amazon basin in the form of small flakes (Fornari and Hérail, 1991). Because gold is relatively denser than average river sediment, the deposits typically lay towards the bottom of these ancient alluvial deposits. Placer mining is the practice of recovering mineral alluvial deposits.

ASGM miners typically mine for gold using little equipment along exposed river banks or clear an area of forest to mine the sediment below. The top-layer of soil is removed and a large pit is dug to in the process of extracting the layer of earth containing the largest gold deposits. The excavated sand and gravel is transported to a type of sluice box where minerals (including gold) and sediment collect. The sluice box works using gravity to separate out less-dense materials from denser materials. A mixture of the excavated sediment and water is pumped to the top of the sluice box, usually several meters higher than the ground level.

Alternatively, some small-scale gold miners in Peru and other areas in the world use river dredgers to extract gold directly from the river bed (Mosquera César, et al. 2009). Other methods of mining exist in the practice of alluvial mining in the region and are used depending on season and deposit characteristics.

Both small-scale and artisanal alluvial gold mining methods in Peru use mercury to amalgamate smaller gold particles and increase recovery. It is estimated that in the years 2006 to 2009, 95% of Peru's mercury imports were used in ASGM (Superintendencia Nacional de Aduanas del Peru *in* USGS, 2007).

Alluvial artisanal small-scale gold mining in Madre de Dios, Peru

ASGM in the Peruvian Amazon has been a source of income for local populations for centuries (Damonte et al., 2013). In 2001, the department of Madre de Dios produced close to 70% of Peru's artisanal gold (United States Geological Survey, 2007). Due to the global economic crisis of the mid-2000s global gold prices began increasing sharply around 2003 (Swenson, Carter, Domec and Delgado, 2011). The average price of gold in (US dollars (USD) per ounce) increased by 50% between September 2007 and December 2008 (Shafiee and Topal, 2010). A 2011 study found that during the period of 2003-2009, deforestation from mining in Madre de Dios increased nonlinearly, while Peruvian national mercury imports increased exponentially during the same time (Swenson, Carter, Domec and Delgado, 2011).

The prospect of easy gold, along with the construction of the Interoceanic Highway, has contributed to a mass immigration to the region of Madre de Dios (see Table 1) (Instituto Nacional de Estadística e Informática, 2015). Completed in 2012, the Interoceanic Highway connects the Atlantic coast of Brazil to the Pacific coast of Peru, and travels through the heart of the Amazon rainforest, as well as through Madre de Dios. Prior to the construction of this highway, there was no paved road connecting the highland in the north to the interior Amazon region in Madre de Dios. Immigrants from areas such as Cusco have made their way by the thousands to Madre de Dios, lured by the prospect of gold earnings.

Table 1.1: Population trend in the District of Madre de Dios, Peru during the period of 1940-2015.

Year	**Population**	**Year**	**Population**
1940	4,950	2000	84,383
1961	14,890	2005	97,417
1972	21,304	2007	109,500
1981	33,007	2010	110,618
1993	67,008	2015	123,871

In the country of Peru, it has been estimated that there are more than 100,000 ASGM workers and an additional 400,000 workers employed in peripheral services (United States Department of Labor, 2012, United Nations Environment Programme, 2013b). The region of Madre de Dios continues to be heavily criticized on the local, national and international scale for failing to formalize and regulate the growing problem of illegal gold mining. The designation of ASGM workers as “informal” or “illegal” refers to the legal process of obtaining formal permitting from the government to mine alluvial gold. “Informal” miners are those ASGM workers who have formally registered for a permit to mine legally, but, due to several legislative complications, have not received their permit, but continue to mine. Illegal ASGM workers are those who never formally registered for a permit. Illegal miners do not pay taxes on their earnings from mining, and may operate in areas that are not specifically for, or even directly prohibit, mining activities (Veiga, 1997).

The Peruvian government has demonstrated a lack of political will and/or ability to develop effective ASGM legislation. Corruption, perceived threat to large-scale investment by multinational mining companies, unstable land tenure and difficulty of enforcement are all reasons why solid legislation has yet to be produced (UNEP, 2011) despite the intensifying levels of destruction from ASM. As Peru gains international attention on the issue of Amazon rainforest destruction, mercury contamination and social issues resulting from unregulated alluvial gold mining, tensions between government enforcement and illegal miners has increased. In March of 2012, local police clashed with 15,000 mining federation protesters in the capital city of Puerto Maldonado (Gardner, 2012). National police squads have attempted to gain control (Álvarez, Sotero, Egg and Peralta, 2011).

Before 2002, there was no legislation in Peru that recognized the existence of artisanal or small-scale mining. (United Nations Environment Programme, 2011). A piece of national legislation in 2002 (Law 27561, 2002) formally recognized the ASGM sector and defined the following two classifications:

1) Small-scale miners: Possesses any title for up to 2,000 hectares, and/or possess any title for capacity limitations of daily processing of materials.
2) Artisanal miners: Possesses any title for up to 1,000 hectares (or agreement with title holder), performs mining activities with manual methods and basic equipment, and is fully employed in mineral production.

Despite the legal framework set-forth by Peru in 2002, the government has yet to design and implement a successful process for formalizing and regulating the ASGM industry. In 2010, a 12-month Urgency Decree was issued that suspended new miner petitions, established mining exclusion zones, prohibited the operation of dredgers in rivers, prioritized the recovery and remediation of degraded areas by informal mining, and offered support to regional governments in complying with the Decree (United Nations Environment Programme, 2012). The decree was renewed for an additional 12 months in 2011, the same year in which Peru's Vice-Minister of Mines estimated that 97% of mining concessions in the Department of Madre de Dios were illegal (In Verité, 2013). That estimate is down only 2.9% from an estimate given by Peru's Environmental Minister two years earlier.

Currently, the Ministry of the Environment requires an environmental impact report to be undertaken in the approval process. Because there is little enforcement of mining laws, there is little incentive to perform the assessment or apply for permits. In 2012, President Ollanta Humala passed legislation that provided small-scale miners with a six-step formalization process. Many mine operators chose to submit formalization paperwork only to be met with a series of missed deadlines and empty direction from the government.

In 2014, the Government of Madre de Dios had yet to issue majority of the permits for which it received paperwork filing. When the regional government of Madre de Dios did not issue permits after the deadline set in April, angry miners clashed with militarized police in the mining town of Mazuko (one of the study sites visited in May 2014). Miner operators who filed for formalization before the April deadline were granted amnesty from raids carried out by the Peruvian army (Bloomberg News, 2014).

Environmental implications from alluvial artisanal small-scale gold mining in Peru

The use of mercury in alluvial gold mining poses a health risk to humans and ecosystems around the world. The environmental effects of ASGM and mercury use include deforestation, air and water pollution, habitat loss, and desertification (Veiga, Maxson, and Hylander, 2006). Since 2009, an estimated 1,500 liters of oil have been spilled in the region of Madre de Dios from motors in machines used in ASGM as well as increased river boat traffic (Álvarez, Sotero, Egg and Peralta, 2011). During the same period, 175,000 gallons of gasoline and diesel have been used in ASGM operations.

ASGM is estimated to contribute 37% of total global mercury emissions (United Nations Environment Programme, 2012). While total global mercury emissions fell between 2005 and 2010, mercury emissions from ASGM doubled (United Nations Environment Programme, 2013a). Overall, approximately one-third of global mercury enters the environment as a result of ASGM activities (Telmer and Veiga, 2009).

The process of alluvial gold extraction involves extensive environmental degradation, as well as human and ecosystem health risks. The approximate ratio of mercury to gold amalgam is 2:1 (not a fixed ratio). Given that the region produces 16 tons of gold per year (Fraser, 2006) an estimated 32 tons of elemental mercury is released into the Madre de Dios fluvial system annually.

As observed during field visits in 2014, ASGM in Madre de Dios begins with the clearing of vegetation, often primary forest, through slash-and-burn methods. Studies using satellite mapping techniques as well as airborne field observations with CLASlite technology revealed that the level of deforestation to ASGM exceeds all other forms of deforestation in the region combined, including agricultural expansion, logging and ranching. (Asner, Llactayo, Tupayachi, and Ráez Luna, 2013). The same study reported 50,000 ha of primary rainforest destruction in the period of 1999 to 2012. Additionally, the authors reported an average deforestation rate of 6,145 ha per year from gold mining after 2008, triple the 1999 rate. These findings were more than double the predicted extent of forest destruction, as the extent of ASGM outside of formal mining zones was severely underestimated.

Personal field visits to active and abandoned mining illegal mining sites revealed the devastation that remains long after gold is mined from what once was forest (see Figure 1). One particular site, called Kilometro 108, after it’s location on the Interoceanic Highway, was particularly disturbing to witness. Much of the mining activity had been stopped, however temporarily, by the local government. This allowed the researcher to arrange for safe travel to view the area. As with much of ASGM activities in Madre de Dios, the presence of a massive illegal mining zone had been hidden from road and river view by preserving a thin barrier of trees on the perimeter.

The hour-long motorcycle ride followed what appeared to have previously served as a footpath for miners to move between the mining settlement near the highway and the illegal mining zone frontier. Only ten minutes into the motorcycle trip, the illusionary forest strip ended in what looked like a desert. After another 50 minutes or so of climbing sand dunes and passing the occasional dead tree still standing, the frontier of the mining area was in sight. Besides the mounds of sand left by miners, the available line of sight showed a flat, empty clearing surrounded on all sides by what appeared to be thick, undisturbed forest that expanded on all sides as far as the eye could see. Large pools of stagnant water lay scattered about, bordered by mounds of gravel-sized rocks and half-burned trees. The author noted the apparent lack of topsoil.

The trouble of deforestation due to ASGM activities is compounded by recent findings that miners have degraded areas designated as national reserves and buffer zones (DeRycke and Finer, 2015). The Tambopata National Reserve, Madre de Dios, is an area of unusually high biodiversity. The reserve contains the highest number, 210, of known species of herpetofauna, (Doan and Arriaga, 2002), more than 10,000 species of plants, 600 species of birds, 200 species of mammals, 1,000 species of butterfly, and countless insect species. Considered an ecological transition zone (subtropical to tropical) of great conservation importance, the Tambopata National Reserved Zone is a sanctuary for many threatened and endangered species, including giant otters (Pteronura brasiliensis), the lowland tapir (Tapirus terrestris), jaguar (Panthera onca), and other charismatic megafauna (SERNAP, 2015).

Figure 1.1: Photograph of ASGM environmental degradation at Km 108.

Socio-political implications of alluvial artisanal small-scale gold mining in Peru

The implications of environmental degradation from ASGM in Madre de Dios are rivaled only by the myriad social, economic, and political problems that have manifested in recent years. Land tenure insecurity is a fundamental cause of social injustice from ASGM. Miners have been reported to have encroached on un-titled, informally-recognized lands that are the historical home of different indigenous groups, including some of the last remaining uncontacted tribes in the world (Oliveira, et al., 2007). It is of note that the forest resources on the lands occupied by indigenous groups account for 55% of the carbon stored in the Amazon basin (Walker, et al. 2014).

The Peruvian Ministry of Labor has reported 48,000 forced laborers in Peru. Madre de Dios is one of the three areas with the highest incidences of forced labor. A report released by the international fair labor organization, Verité, reported serious instances of human rights abuse and breakdown of society, including exploitation of children, excessive alcoholism, lack of education and security, ungovernable areas, violent conflicts, loss of human life, corruption of government officials, and human sex trafficking rings (Verité, 2013).

Underlying most of the negative impacts of illegal and informal alluvial ASGM in Madre de Dios is the lack of enforcement capacity by the government. The lack of regulation has caused the regional and national Peruvian governments an estimated $305 million per year (Gardner, 2012). The number of ASGM prospectors immigrating to Madre de Dios, the rapid rate of population growth, and the enormity of the monetary incentives to mine are all factors that contribute to the current state of near-lawlessness in the region. The current gold rush has dwarfed more familiar problems of the area, such as illegal drug trafficking. Between June 2014 and May 2015, illegal mining accumulated $1.116 billion USD on the illegal market. During the same time period, illegal drug trafficking earned a mere $171 million USD in comparison (UIF, 2015).

Chapter 2: Mercury in human populations

2.1 Human mercury exposure introduction

Human health impacts of mercury vary depending on the chemical form of the mercury exposure, the dose and duration of exposure, the age and health of the exposed individual, as well as the route of exposure. Elemental mercury, such as that used in the amalgamation of gold in ASGM, primarily causes health problems when vapors are inhaled.

Informal and illegal miners are exposed to mercury vapors during the amalgamation process. Both ASGM workers and larger segments of the population are exposed to the most dangerous levels of mercury vapor in areas where gold is bought and sold. This is because the metallic mercury is burned off of amalgamated gold before sale to ensure that the weight of the product being purchased does not include mercury. Although some larger operations are properly out-fitted with fume hood condensers to capture and recycle mercury vapors, it is very common for ASGM workers to burn mercury in-doors, often in homes, with no ventilation protection. Symptoms of even low-level exposure to elemental mercury vapors include tremors, decrease in memory performance, and a decrease in autonomic nervous system functioning (Liang, et al., 1993).

After elemental mercury enters the atmosphere as vapor it may become oxidized and then deposit into nearby waterways. Elemental mercury also enters the fluvial system of Madre de Dios directly when miners spill or dump elemental mercury directly into the soil or water. An estimated 20% of mercury used by informal and illegal ASGM workers is directly lost to the aquatic ecosystem (Pfeiffer and Drude de Lacerda, 1988).

Once in an aquatic system, elemental mercury is biomethylated by bacteria into methylmercury, an organic compound that can enter the food chain and bioaccumulate. The concentration of methylmercury in a given ecosystem depends upon many factors, including the availability of sulfate-reducing bacteria in anoxic environmental conditions (Rudd, 1995) as well as the rates of methylmercury uptake and transfer between trophic levels (Chasar, et al. 2009). Fish and macroinvertebrates in aquatic systems accumulate mercury in their tissues over time, and levels increase further as one moves up the trophic levels.

Human populations in Madre de Dios depend heavily upon fish as a staple part of the diet. This poses regional health concerns as the concentration of mercury entering the environment increases each year due to both poor regulation from the government and accumulation of mercury in the ecosystem over time. Individuals are primarily exposed to methylmercury through the consumption of fish. Chronic exposure to methylmercury can cause damage to the central nervous system (CDC, 2009). Developing fetuses and young children are at a particularly high risk of adverse neurological effects from exposure to methylmercury (Gilbert and Grant-Webster, 1995). Because methylmercury can be transferred from a mother to a fetus in the womb or through breast milk, women of child-bearing age are also of greater exposure concern.

In addition to human health concerns, wildlife is also affected by increased levels of mercury in the environment. In the aquatic food web, apex predators and other carnivores are most vulnerable to the bioaccumulative effects of methylmercury. Giant river otters (*Pteronura brasiliensis*) are a major conservation concern and ecotourism attraction in Madre de Dios. The giant river otter is an endangered species that is found in the Madre de Dios River system and other parts of the Amazon basin (Carter and Rosas, 1997). The “lobo del rio”, or “river wolf”, has become a mascot of sorts for the booming ecotourism industry in Madre de Dios. The giant river otter’s diet consists almost exclusively of fish, including carnivorous species of fish (Gutleb, Schenck and Staib, 1997).

A research brief by the Carnegie Institution’s Department of Global Ecology (CAMEP, 2013b) reported that, in a sample of 226 adults from the capital city of Madre de Dios, Puerto Maldonado, the average hair mercury concentration was three times the hair USEPA reference dose of 1 ppm. The study also determined that women of child-bearing age and indigenous communities had the highest average mercury levels. At the time of authorship, the research from the CAMEP report had still not been published in a peer-reviewed journal.

A second 2012 study on mercury levels in human hair by a student at Stanford focused on comparing the levels of human hair total mercury in the town of Puerto Maldonado and a mining zone (Ashe, 2012). A sample size of 104 adults Puerto Maldonado showed that only 5% of the study population had a mercury level above the World Health Organization (WHO)'s minimum toxic level of 6 µg Hg/g hair (WHO, 2008). Furthermore, the mercury levels in samples taken from females within Puerto Maldonado (n=60) averaged 2.30 µg Hg/g hair, while the age of participants was found to have no significant relationship to mercury levels. The study found that residence location, frequency of fish consumption, and gender were correlated with higher mercury levels. Duration of residence and age of participant were not found to be significantly correlated with total mercury levels.

The studies outlined above serve as the main sources for information about human hair mercury levels in the region of Madre de Dios, Peru at the time of authorship. For unknown reasons, the CAMEP research brief is only available on the study's partially-funded NGO website, limiting the credibility of the information reported. Some findings, such as correlation between age and mercury level, are not in total agreement between the two studies outlined above (Ashe, 2012; CAMEP, 2013b). A third, more rigorous study (Yard et al., 2012) (outlined below), agrees with Ashe, 2012 in the occurrence of occupational mercury exposure.

A cross-sectional study that looked at mercury exposure among artisanal gold miners in Madre de Dios in 2010 measured mercury levels in human urine and blood (not hair). Mercury levels in urine are used to assess elemental mercury exposure and are useful in measuring mercury levels from exposure to burned mercury (usually in-doors before sale of gold amalgam). Blood mercury is useful for measuring methylmercury, which is accumulated through diet. The study concluded that all 103 participants from the mining town sampled had detectable levels of mercury in urine (≥7 ppm Hg), and 97% had detectable levels of blood methylmercury (≥6 ppm

MeHg) (Yard, et. al 2012). Increased fish consumption and exposure to heated gold-mercury amalgam were found to be correlated with higher mercury levels.

Empirical evidence of increased mercury levels, specifically due to ASGM activities, in human populations was lacking at the time of this study. Miners frequently state that they do not believe that mercury is toxic because they themselves tend to have no or minor health issues and they don't see evidence that their children are affected. Mistrust of the government reinforces the state of disbelief of mercury effects by miners. Policy experts agree that the state of low-governance in Madre de Dios makes some level of cooperation and compliance by miners a critical necessity for successfully implementing sustainable mining methods (United Nations Environment Programme, 2011). It is important to collect evidence using rigorous methods to assess the severity impacts of mercury contamination on human health so that the proper policy remedies and interventions are pursued.

2.2 Human data collection methods

Study scope, objectives and hypotheses

The purpose of this study was to scope for general trends of mercury levels in human hair in the Madre de Dios river basin. Specifically, the study aimed to broadly examine any obvious trends in the total mercury level of human populations along the length of the Madre de Dios River. By characterizing over-arching correlations between known influences of human mercury levels (i.e. diet, occupation, etc.) according to geographical distribution along a major river, the foundation will be laid for future studies to expound upon these correlations to begin to establish causation.

In order to run a regression and test for correlations among specific mercury risk variables among the communities, the researcher operated on a null hypothesis that for each variable, there would be no significant difference between the mercury levels of each community and the variable. Alternatively, it was expected that communities that consume more fish, that are farther downstream, and where known mining activities occur would show higher mercury levels.

Study Sites

The region of Madre de Dios in the Peruvian Amazon is touted as one of the world's greatest biodiversity hotspots (Olsen and Dinerstein, 1998). The Madre de Dios River is 1,060 km in length and has its source in the Cordillera de Vilcanota, a southern branch of the Eastern Cordillera of the Andes (71°12'W; 13°25'S) (Ziesler and Ardizzone, 1979). Major tributaries include Manú, Tambopata, Piedras, Inambari and Heath. In Bolivia, the Madre de Dios River empties into the Beni, and eventually the Amazon River. A map of the study area is included in Appendix 1.

Field data was collected during the months of May, June and July, 2014. The Principle Investigator, Aubrey Langeland, met with the Peruvian Ministry of Health for the District of Madre de Dios, Peru, as well as other appropriate governmental agencies, to gain proper documentation and permission for the collection and exportation of samples. Field sites were chosen based on distance from the headwaters of Madre de Dios River, safety risks to researcher, and remoteness of location (Table 2.1). The four field sites visited for collection of human hair samples and survey administration include Bajo Madre de Dios (BMDD), Boca Amigo (BA), Mazuko and Pilcopata.

The study sites lie at varying distances from the headwaters of the Madre de Dios River. Pilcopata is chosen as a type of control site. It is located just off of the Marcachea River approximately 2.4 km before it empties into the Madre de Dios. At the time of field research, there was no or little reported mining behavior in Pilcopata according to the residents and adjacent communities (Personal correspondences, June 2014). As a rough verification of this fact, satellite images for the region for the years 2005, 2010, and May of the study year revealed no obvious, major forest disturbance from ASGM (NASA Landsat Program, 2005; NASA Landsat Program, 2010; NASA Landsat Program, 2014).

Table 2.1: Overview of study sites selected for human hair sample collection and administration of semi-structured survey.

Study Site	Bajo Madre de Dios	Boca Amigo	Mazuko	Pilcopata
GPS	12º 35’ 639” S 69º 09’ 197” W	12º 36’ 161” S 70º 05’ 288” W	13º 06’ 558” S 70º 22’ 770” W	12º 54’ 367” S 71º 24’ 178” W
Distance[1] to headwaters	228.1 Km	142.8 Km	69.39 Km	53.44 Km
District	Tambopata	Madre de Dios	Inambari	Kosñipata
Province	Tambopata	Manu	Tambopata	Puacartambo
Region	Madre de Dios	Madre de Dios	Madre de Dios	Cusco
Illegal mining	Yes	Yes	Yes	No
Remoteness[2]	Moderate	High	Low	Moderate

Semi-structured survey

In each of the four locations presented in Table 2.1, participants were randomly selected to participate in an informal survey to assess dietary habits and livelihood characteristics. In order to participate, subjects needed to be at least 18 years of age and must have lived in the research site for at least six months (see Appendix 2). In the communities of Pilcopata, BMDD, and BA, every household easily accessed (i.e. located in the main town center) was visited, and an adult from each household was asked to participate. The illegal nature of mining and political circumstances prevented many households from participating; therefore each willing household was used in the sample. The potential impact of this reality is noted in the discussion section below.

[1] Calculated in ArcGIS10 software using a Haversine formula to calculate the great-circle distance between the study site coordinates and those of the Madre de Dios river origin (ignores topography and river bends; “As the crow flies”).

[2] For the purposes of this study, remoteness is defined by ease of access, such that a high level of remoteness indicates no road access, a moderate level indicates road access but rural or difficult to reach, and low levels of remoteness indicate an easily accessible urban center.

In the much larger town of Mazuko, the town was mapped and divided using a grid. Households were selected from 2 random grid spaces and an adult from each household was asked to participate. Each survey took approximately 20 minutes and was conducted by the P. I. Research protocol involving human subjects was approved by the University of Michigan IRB (HUM00086592).

Human hair sample collection

Total mercury in hair samples is a good indicator of long-term mercury exposure, particularly through ingestion of methylmercury from contaminated fish (Veiga and Baker, 2004). It can be assumed that 95% of the total mercury present in fish tissue is methylmercury (Bloom, 1992). The World Health Organization has set a minimum toxic concentration (the concentration at which negative health effects begin to manifest) of mercury in human hair at 6µg/g (World Health Organization, 1976).

Human hair was collected from each survey participant. Approximately 100 strands of hair were collected from the occipital region of the skull. Samples were taken with stainless steel scissors as close to the scalp as was safely possible. The scissors were sterilized with rubbing alcohol between participant sample collections. The hair was stored between sheets of adhesive paper, and the sample end closest to the scalp was marked for later identification and analysis purposes. The samples were then labeled with sample ID number corresponding to survey ID number, and stored individually in double plastic Ziploc bags.

Upon arrival to the United States in August, all samples were placed in a freezer until analysis was possible in January 2015. Prior to analysis, samples were trimmed so that the 4 cm length of hair closest to the scalp would be analyzed. Hair grows at an average rate of approximately 4 cm per month (WHO, 2008), and therefore the mercury analysis would evaluate total mercury exposure for the 4 months preceding analysis. The hair was analyzed using a Milestone Direct Mercury Analyzer (DMA-80). The procedure for sample analysis followed standard protocol according to EPA method 7473 (EPA, 1997).

Chemical Analysis

A sample weight of 50 to 55 milligrams was measured out for analysis. Three different readings were taken for each sample, and the average of the three runs was used as the reported total mercury concentration for each sample. Prior to analysis, the DMA-80 equipment was primed using a custom calibration curve and tested using three separate standards (see Appendix 3). Quality control measures included random testing of reference materials, blank measurements every 10th reading, and random checks of previously measured samples. Recovery rates of 95-100% were accepted for reference material tests.

Statistical Analysis

Statistical analysis for Chapter 2 of this thesis was performed using RStudio (RStudio, 2015). All reported values from the sample populations are represented by the box plots. A histogram of the data points for hair total mercury shows that the distribution is skewed right. To increase normalcy, the data underwent a logarithmic transformation before further statistical analysis. In addition, one outlier (value larger than three standard deviations from the mean) was dropped, giving a total of 80 observations for analysis.

The data was displayed in a box-and-whiskers plot. The data was grouped by study site location. The values represented in the box plots are the first quartile, median, and third quartile, with the upper and lower adjacent values shown as whiskers (McGill, Turkey, and Larson, 1978).

A generalized linear regression model was fit to the logarithmic-transformation of mercury hair levels for the 80 included observations. The model was used to determine if the fresh fish consumption, farmed fish consumption, or canned/frozen fish consumption impacted hair mercury levels. The multivariate ANOVA revealed that none of the above mentioned variables was significant in predicting mercury levels of participants (Multiple R-squared: 0.04361, Adjusted R-squared:-0.007399).

The model was then modified and a second multivariate ANOVA was run on the logarithmic-transformed dataset including the previous four categorical variables along with a fifth categorical variable: location. Four regressions were performed so that each of the four study locations served as the reference group, to which the other three locations were compared. The regression used the model: log(Hg) ~ GROUP + EAT + Farm + CSM + SEX. A summary of results is given in Table 2.6. Variable coding and explanation is summarized in Table 2.2.

Table 2.2: Summary of variable coding for regression analysis.

Variable Description	**Variable Name**	**Type**	**Code**
Consumption of any fresh fish	FISH	Binomial	0 = Consumes fresh fish ≤ 1x per month. 1 = Consumes fresh fish > 1x per month.
Consumption of farmed fish	FARM	Binomial	0 = Consumes farmed fish ≤ 1x / month 1 = Consume farmed fish > 1x / month
Consumption of canned, smoked or frozen fish	CSF	Binomial	0 = Consumes CSF fish less than or equal to one time per month. 1 = Consumes CSF fish more than one time per month.
Gender of participant	GENDER	Binomial	0 = Male. 1 = Female.
Study site of participant	GROUP	Ordinal	1 = Bajo Madre de Dios 2 = Boca Amigo 3 = Pilcopata 4 = Mazuko

One last multivariate ANOVA regression was designed and run using the following equation: log(Hg) ~ GROUP + EAT2 + Farm + CSM + SEX + TIME. The variables are explained in Table 2.2 above and Table 2.3 below. The final regression differs from the previous two in its breakdown of the variable EAT, as well as the addition of the variable TIME, which is a proxy for residence time in the study site. Statistical analysis and tests were performed using R Studio software.

Table 2.3: Description of ordinal variables used in third round of ANOVA tests.

Variable Description	**Variable Name**	**Type**	**Code**
Consumption of fresh fish	EAT2	Ordinal	0 = Never consumes fresh fish 1 = Consumes fresh fish 1-2 time/month 2 = Consumes fresh fish one time/week 3 = Consumes fresh fish 2-3 times/week 4 = Consumes fresh fish 4+ times/week
Residence time (uninterrupted) at study site	TIME	Ordinal	1 = 6-12 months 2 = 13-23 months 3 = 2-5 years 4 = 6-10 years 5 = 11-20 years 6 = 21+ years

2.3 Results of hair analysis

Descriptive statistics results

Table 2.4 shows the descriptive statistics output for human hair mercury levels in the study populations. The average mean between the four study sites show that the selected control site, Pilcopata, had the lowest mean (0.93 µg Hg/g hair). This is roughly half of the next lowest mean of Mazuko (1.67 µg Hg/g hair). The highest mean total mercury level was found in the study site BA (5.04 µg Hg/g hair), followed by BMDD(4.273 µg Hg/g hair).

Notably, the control town of Pilcopata also had the lowest standard deviation (0.50 µg Hg/g hair), roughly a fourth that of BA (2.29 µg Hg/g hair), and Mazuko (2.32 µg Hg/g hair), and one fifth the standard deviation of BMDD (2.76 µg Hg/g hair). There were no samples from the study population in Pilcopata that had mercury readings above the WHO minimum toxic level of 6 µg Hg/g hair. Two, seven and seven participants had levels higher than the minimum toxic level in Mazuko, Bajo Madre de Dios, and Boca Amigo, respectively.

Table 2.4: Descriptive statistic summary for mercury concentration (µg Hg/g hair) of human hair samples from the four study sites. CI = 95%.

Site	n	Mean	Median	Range	SD	No. above WHO min. toxic level (6 µg Hg/g)
BMDD	21	4.27±0.41	3.97	0.59-10.97	2.76	7 (35%)
BA[3]	17	5.04±0.39	5.20	2.13-10.13	2.29	7 (41%)
Pilcopata	23	0.93±0.13	0.85	0.29-2.33	0.50	0 (0%)
Mazuko	20	2.55±0.35	1.67	0.59-9.21	2.32	2 (10%)

Descriptive statistics were also produced to examine the differences between men and women at each study site (table 2.5). Means were similar for both males and females in Pilcopata (0.91 and 0.95, respectively). In Bajo Madre de Dios, 2 of the 7 individuals affected with mercury levels above the minimum toxic level were female, while in Boca Amigo 5 of the 7 affected were female. Therefore, in Boca Amigo, 5 of the 10 women sampled, or 1 in 2, have mercury levels above the WHO minimum toxicity level of 6 µg Hg/g hair. In Bajo Madre de Dios, 2 of the 8 women sampled were affected. In Mazuko, 1 in 14 women sampled were affected.

Table 2.5: Comparison of mean mercury concentration (µg Hg/g hair) of human hair samples from the four study sites displayed by male (m) and female (f) participants.

Site	n(m)	Mean (m)	No. (m) > min toxic level	n(f)	Mean (f)	No. (f) > min toxic level
BMDD	13 (62%)	4.42±0.60	5 of 7 (71%)	8 (38%)	4.03±0.49	2 of 7 (28%)
BA[4]	7 (42%)	4.89±0.49	2 of 7 (28%)	10 (58%)	5.05±0.60	5 (71%)
Pilcopata	13 (57%)	0.91±0.11	0 (0%)	10 (43%)	0.95±0.79	0 (0%)
Mazuko	6 (30%)	3.08±0.86	1 of 2 (50%)	14 (70%)	2.32±0.36	1 of 2 (50%)

[3] Boca Amigo had one observation with an average total mercury content of 30.12 µg Hg/g hair. This outlier was not included in any of the above statistical calculations except for the toxic level count.
[4] Boca Amigo had one observation with an average total mercury content of 30.12 µg Hg/g hair. This outlier was not included in any of the above statistical calculations except for the toxic level count.

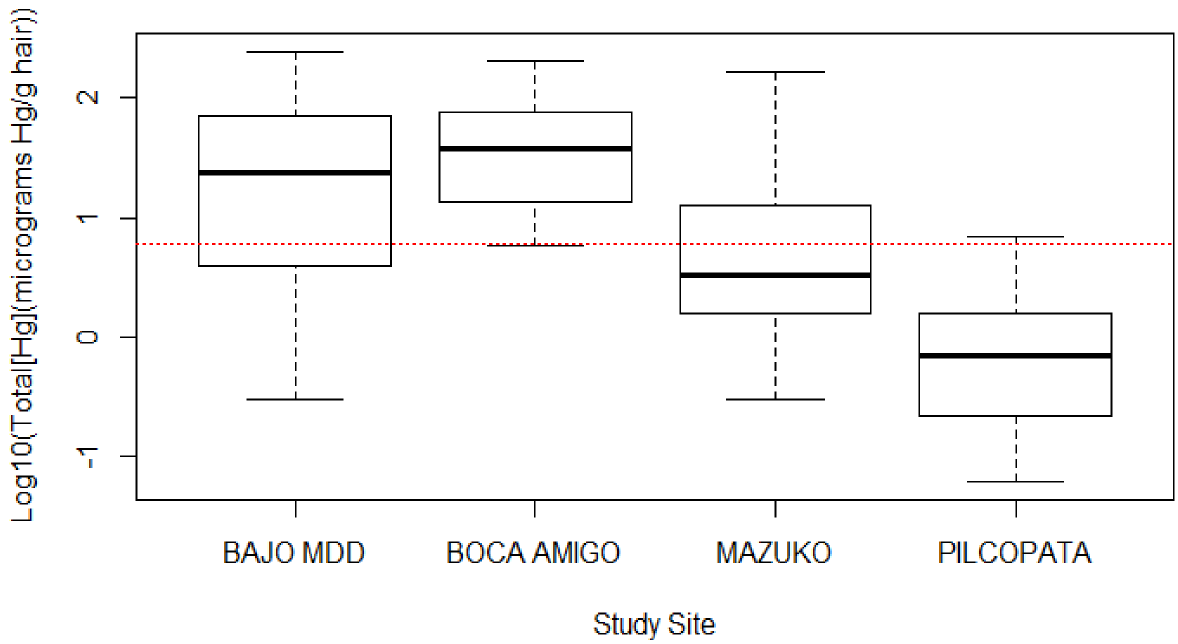

Figure 2.1: Box-and-whisker plot showing the logarithmically-transformed total Hg levels in subject populations by study location. The horizontal line represents the logarithmically-transformed WHO minimum toxicity level (in log form, equals 0.78).

As shown in the box and whisker plot above, the logarithmic-transformation places the mean mercury concentration of Bajo Madre de Dios and Boca Amigo well above the minimum toxic level. Mazuko's logarithmically-transformed data has a mean value just below the minimum toxicity level with some Log10(Total[Hg]) values far above the toxicity level. Pilcopata is almost entirely below the minimum toxicity level.

Regression Results

Table 2.6: Output of multivariate ANOVA showing the results according to reference group. Note: The results for variables FISH, FARM, CSF and GENDER does not change when reference group is changed.

Reference Group	**Coefficients**	t value	Pr(>|t|)
Bajo Madre de Dios	Intercept	4.062	0.0001 ***
	Boca Amigo	1.204	0.2325
	Pilcopata	-6.943	1.41e-09 ***
	Mazuko	-3.106	0.0027 **
Boca Amigo	Intercept	4.302	5.24e-05 ***
	Bajo Madre de Dios	-1.204	0.2325
	Pilcopata	-7.885	2.5e-11 ***
	Mazuko	-4.045	0.0001 ***
Mazuko	Intercept	0.852	0.3970
	Bajo Madre de Dios	3.106	0.0027 **
	Boca Amigo	4.045	0.0001 ***
	Pilcopata	-3.659	0.0005 ***
Pilcopata	Intercept	-2.390	0.0194 *
	Bajo Madre de Dios	6.943	1.4e-09 ***
	Boca Amigo	7.885	0.0001 ***
	Mazuko	3.659	0.0005 ***
All study sites	FISH	0.613	0.5421
	FARM	1.677	0.0979
	CSF	1.272	0.2073
	GENDER	-0.014	0.9891

*** Significant at the 0.01 level; ** Significant at the 0.05 level; * Significant at the 0.10 level.
Residual standard error: 0.654 on 72 degrees of freedom
Multiple R-squared: 0.549, Adjusted R-squared: 0.505
F-statistic: 12.51 on 7 and 72 DF, p-value: 2.26e-10

The residence location of the participant was a significant predictor of mercury levels found in hair samples (see table 2.5).Using the general linear framework described above, the model demonstrated that fish consumption levels (using categorical assignment from either table 3 or table 4), gender, consumption of farmed fish, consumption of canned/smoked/frozen fish, and residency time were not statistically significant in predicting total mercury levels in the study population (α =

0.05). The t statistic for the variable “EAT2” (ordinal variable representing frequency of fresh fish consumption) did not show improvement from the initial model that used the variable FISH (binary variable representing frequency of fresh fish consumption).

Comparison of the different residence locations was found to be a significant predictor of mercury levels (table 2.5). The model used for the data output in table 2.6 had a F-value of 12.51 indicating that the null hypothesis (there is no difference between mercury levels among the four groups) is false. The ANOVA has an adjusted R-squared value of 0.505. Together with significance values from the series of linear regression tests in table 2.6, the R-squared indicates that location is the primary explanatory variable in predictability of participant mercury level.

The study site Bajo Madre de Dios was significantly different from Pilcopata ($p<0.01$). Accounting for the logarithmic transformation, the total mercury level in Pilcopata was predicted to be roughly a quarter that of the predicted level in Bajo Madre de Dios. Besides Pilcopata, the population of Bajo Madre de Dios also had a mercury level that is significantly different from that of Mazuko ($p<0.05$). Residents of Mazuko had a predicted mercury levels of approximately half that of Bajo Madre de Dios.

Boca Amigo was shown to have a significantly different total mercury level than both Pilcopata ($p<0.01$) and Mazuko ($p<0.01$). The total mercury level for a resident of Pilcopata was predicted to be 0.18 times that of a resident of Boca Amigo. The level for Mazuko was predicted to be 0.385 times that of Boca Amigo.

The ANOVA results for the regression run with Mazuko designated as the reference site showed that all three sites were significantly different from the predicted total mercury level for Mazuko. The results for Mazuko compared with Bajo Madre de Dios and Boca Amigo are reported above. Compared to Pilcopata, Mazuko had a total mercury level that was significantly different ($p<0.01$), and predicted to be 0.46 times higher than the predicted level for a resident of Pilcopata. As demonstrated above, Pilcopata was significantly different ($p<0.01$) from the other three study sites, and was predicted to have a mercury level that is lower than each.

The categorical variable “FARM”, which reported the consumption of fish sourced from agricultural farms, had a p-value of 0.09. This correlation is not statistically significant, but is notably more significant than other variables measured in the regressions.

2.4 Discussion – Sample site health

The multiple regression shows that the study’s control site, Pilcopata, had a predicted human hair total mercury value that was significantly different than the other three sites ($p<0.01$). A resident of Pilcopata was predicted to have a hair total mercury level that was 0.75, 0.82 and 0.54 times lower than Bajo Madre de Dios, Boca Amigo, and Mazuko, respectively. Because Pilcopata is at the headwaters of the Madre de Dios River, it can be inferred that there is a possible connection between the location of a community along a river where major mining activities occur.

There are several confounding factors, however, that would need further analysis before a conclusive correlation can be drawn between the location of Pilcopata and the significantly lower mercury levels found in the study population. As stated above, the illegal nature of mining and political circumstances prevented many households from participating; therefore each willing household was used in the sample. It should be noted that this may therefore confound results as households that engaged in mining behavior may have been less likely to participate. This would likely have been especially true for the current study, as the Peruvian military had actively raided mining operations shortly before the research period. Therefore, some hostility towards researchers was observed in mining communities and in conversation with several miners, themselves.

Field research corresponded to the end of the rainy season in the region. This usually results in flooding of local rivers, and creates suboptimal fishing conditions. Therefore, the sample populations may not have consumed as much fish during the study period (or the four months prior, see below) as might be consumed during other times of the year. This would be particularly true for more remote study sites where river fish is depended upon more heavily as a basic diet staple. There are more species of river fish consumed than the typical availability of farmed fish species (only 2-3 farmed fish species common). Different species have different levels of mercury (see Chapter 3), and so populations consuming river fish during the dryer months of the year may experience a temporal increase in total mercury levels in the hair.

River chemistry is an important factor in the consideration of patterns of mercury contamination in river basins. There is some debate about the natural, pre-anthropogenic levels of mercury in the Madre de Dios region, as well as the Amazon River basin in general. Hair mercury levels have been shown to be positively correlated with river pH and dissolved organic carbon in the Amazon basin (Silva-Forsberg, Forsberg and Zeidemann, 1999). A study on the Madeira River in Brazil, to which the Madre de Dios River is a major tributary, found that elevated mercury levels in the region are largely due to natural sources and biogeochemical processes (Lechler, et. al, 2000). The impacts of anthropogenically-sourced mercury from mining was shown to be localized. While the environment in direct proximity to mining sites was shown to be elevated, the authors concluded that there was no evidence of down-stream elevation from fluvial transport as a result of mining activities.

The results of this study seem to support the notion of a localized effect as residents of the mining town Boca Amigo, upstream from both Bajo Madre de Dios and Mazuko, had the highest mean concentration of hair mercury. Pilcopata had a mean level of 0.93 µg Hg/g dry hair. This level is well below the WHO minimum toxic dose of 6 µg Hg/g dry hair. Larger studies that measure sediment mercury levels with a core plug analysis would be needed to make a more definitive conclusion about the natural level of mercury in the Madre de Dios River basin. If, however, one were to assume that the relatively lower level of total mercury in human hair represented in Pilcopata were indicative of base-levels of mercury concentration in the fluvial system, then the elevated levels of mercury concentrations of human populations elsewhere along the Madre de Dios River would be more likely to be a direct result of anthropogenic mercury inputs into the system. Yet, there are other factors to consider.

The behavior of mercury in a stream environment is not as well understood as mercury in a lake or wetland ecosystem. As of 2011, the Madre de Dios River contains an estimated 294 km^2 of peatland, mostly in the meandering belt (Householder, Janovee, Tobler, Page and Lähteenoja, 2012). However, the area of peatland may be significantly lower at the present time due to destruction from ASGM activities. Presence of wetlands, including peatland, has been shown to have a positive correlation with methylmercury levels (Bringham, Wentz, Aiken and Krabbenhoft, 2009). There are many more examples of both biotic and abiotic characteristics of fluvial systems that impact mercury behavior in the ecosystem, such as pH, geomorphology, sediment type, and rate of atmospheric deposition of mercury (Tsui, Finlay and Nater, 2009). Total mercury levels in human populations may also vary along the Madre de Dios River through its many major tributaries where mining is known to take place, sometimes extensively. Further investigation on the biogeochemical processes of the Madre de Dios River and tributaries would be helpful in determining site-specific causation of elevated mercury.

The results of this study do not match similar studies in concluding that the amount of fish consumed correlates with increased mercury levels. It is possible that due to the timing of this study, fish was not currently in season and had not been for several months. Therefore, the level of total mercury may be higher in the study populations during the dry season when fishing is more lucrative and diets depend more heavily upon river fish as a staple.

Other studies have shown a correlation between gender and total mercury levels (Ashe, 2012; CAMEP, 2013b). The current study found no such correlation in the regression tests. It is possible that the results may have been different had more miners and family members of miners been willing to participate. Additionally, the levels measured in this study were of total mercury. Methylmercury, which measures the amount of mercury from diet, may yield different results. Exposure to atmospheric mercury is not reflected in hair sample analysis. This type of exposure needs to be analyzed through blood and/or urine samples.

2.5 Conclusion – Human health implications

Mercury contamination is a real and growing threat to human populations in the region of Madre de Dios, Peru. The impacts of mercury can be expected to continue to affect local populations as the harmful effects of mercury in the ecosystem biomagnify over time and with ever-increasing anthropogenic inputs (Telmer and Veiga, 2009; Swenson, Carter, Domec, and Delgado, 2011). Human health of current and future generations face a growing exposure risk unless the proper policy interventions are implemented in an expedient manner. Populations of particular locations, especially known illegal and legal ASGM settlements, are predicted to have higher levels of total hair mercury concentrations. This indicates a higher risk for mercury exposure overall, and therefore higher risks of negative health effects and environmental contamination of resources.

This study has shown evidence that there is an increased risk of mercury exposure in the region of Madre de Dios dependent upon location. Further research on historical mercury levels in sediments throughout the river basin, localized biotic and abiotic factors affecting environmental chemical processes, and analysis of similar populations along the main channel of the Madre de Dios River as well as its tributaries would help to definitively quantify the impact that anthropogenic releases of mercury from ASGM has on the human population and ecosystem.

It is important to distinguish that while this study has identified elevated levels of total mercury concentration in human hair, this study was not designed to definitively identify the cause of said mercury level elevation. Low levels of total mercury in the study population near the headwaters suggests that there may be a correlation between mercury levels and down-river accumulation. However, the data presented in this study is limited in scope and no causation can be drawn from the findings.

Chapter 3: Total mercury in aquaculture-sourced fish

3.1 Introduction to mercury in fluvial systems

Elemental mercury enters aquatic ecosystems through atmospherically deposited sources as well as through direct anthropogenic activities (Tsui, Finlay and Nater, 2009). In the river basin of the Madre de Dios River in Peru, human populations are engaged in alluvial ASGM operations. The process of recovering alluvial gold involves the use of mercury to amalgamate the gold flecks. Mercury is burned off of the gold amalgam before sale using a small retort to ensure a pure gold product. Because mercury has a lower latent heat of vaporization, heating the gold amalgam will cause the mercury to distill off as a vapor (Drake, et al., 2000). Mercury enters the environment in Madre de Dios directly through spills and waste poured out by miners, and through atmospheric deposition during the heating of the gold amalgam.

Elemental mercury undergoes methylation by bacteria in aquatic ecosystems to become the bioavailable form of mercury, methylmercury (Winfrey and Rudd, 1990). The bioaccumulation of mercury in an aquatic system varies considerably depending on characteristics of the particular ecosystem. The central determinants of the process include the food web structure, especially rates of methylmercury uptake and trophic transfer (Chasar, et al., 2009), the rate of transformation of elemental mercury to methyl-mercury by bacteria in anoxic conditions (King, Harmon, Fu, and Gladden, 2002), and elemental mercury deposition (Harris, et al., 2007). Other important factors of mercury concentrations in aquatic ecosystems include total dissolved organic carbon and water pH (Driscoll et al., 1995).

The Madre de Dios River carries a high load of suspended sediments (Puhakka et al., 1992), most of which enters the system through tributaries, which bring sediment loads from the Andes that settle on the flood plains. Soils textures vary from clay to sand and show abrupt changes that correspond with remnant fluvial features of the floodplain (Hamilton, Kellndorfer, Lehner and Tobler, 2007). The pH of the Madre de Dios River is acidic (Osher and Buol, 1998). The river varies by several meters in depth depending on the time of year as the rainy season causes intense flooding of plains.

Information on mercury levels in the Madre de Dios River is limited. A 1997 study inspired by concern over the increasing threat towards giant river otters from ASGM activities, the study measured the level of mercury and methylmercury in samples from seven fish families, including *Serasalmus*, in the nearby Manu River. The study reported that total mercury levels in samples of *Piaractus brachypomus* were found to be 0.053 and 0.067 µg Hg/gram wet weight (Gutleb, Schenck, and Staib, 1997) for average-sized fish that otters may prey on. River otters are considered a useful sentinel species to proximate mercury and human health effects from fish species (Basu et al., 2005).

Communities along the Madre de Dios River are heavily dependent upon river fish as a dietary staple and therefore are at an increased risk of exposure to easily absorbed methylmercury from contaminated fish. The United States Environmental Protection Agency (EPA) set a methylmercury reference level for fish tissue intended for human consumption at 0.3 µg MeHg/g fish tissue (wet weight) (EPA, 2001). The EPA estimates that between 10 and 25% of total mercury present in fish tissue is methylmercury. Therefore, the reference dose for total mercury in fish tissue is between 0.333 µg Hg/g fish tissue and 0.4 µg Hg/g fish tissue (wet weight). The *Piaractus brachypomus* samples from Gutleb, Schenk and Staib, 1997 fell below the safe levels set forth by the EPA.

A more recent study of one of the main tributaries to the Madre de Dios River, the Tambopata River, measured methylmercury concentrations in fish tissue from members of the family *Serraselmus* in an oxbow lake (Roach, et. al., 2013). An oxbow lake is formed when the main river channel changes course at a river bend and leaves behind a curved lake. The oxbow lake is often connected to the main channel, but the river no longer flows through that area. These lake formations are common in the Madre de Dios River basin and are favorite fishing spots of local populations. The average total mercury concentration of the two species belonging to the *Serraselmus* family was found to be 0.320 ± 0.142 µg Hg/g fish tissue (wet weight).

Methylmercury levels were reported as 0.304$\pm$0.138 μg MeHg/g fish tissue (wet weight) (Roach et al., 2013). The concentrations reported are above the EPA mercury criteria for freshwater fish. The authors found that overall, mercury levels in fish of the oxbow lake were higher than mercury levels of fish from the main channel. One explanation for this finding is that lakes are more likely to promote anoxic conditions necessary for the conversion of elemental mercury to methylmercury due to stagnation.

A research project was undertaken by the Carnegie Amazon Mercury Environment Program (CAMEP) measured total mercury concentrations of 15 commonly consumed species of fish. The samples were purchased from markets in the capital of Madre de Dios, Puerto Maldonado. Of the 15 species, 9 were found to have total mercury levels at or above the EPA reference level for methylmercury (CAMEP, 2013a). Interestingly, three of the five species with the lowest reported total mercury levels are commonly used in agricultural fish farms in the region. The CAMEP brief, however, did not clarify if the samples purchased from the market for the study were wild-caught or raised in agriculture.

In recent decades, conservation efforts have implemented pond aquaculture practices in the Peruvian Amazon (USAID, 1997). The species most commonly farmed in Madre de Dios is known locally as “paco”. Aquaculture has several benefits that have made the establishment of fish farms in Madre de Dios a successful intervention and development project. By reducing pressure on wild stocks through provision of farmed fish, aquaculture improves natural fluvial stocks and has a positive impact on biodiversity conservation (Diana, 2009). Conservation organizations such as the Amazon Conservation Association (ACA), and its Peruvian sister organization, La Asociación para la Conservación de la Cuenca Amazónica (ACCA), have helped communities throughout Madre de Dios establish aquaculture ponds. Some of the benefits in the Madre de Dios region include providing income alternatives to poor immigrant families who might otherwise turn to mining, as well as providing educational opportunities to local communities and school about the importance of conservation (ACA, 2015; ACA 2012). A report co-sponsored by

ACCA and CAMEP in 2012 reported elevated mercury levels in fish from the Madre de Dios River (Fernandez, 2012). Local people and the ACCA used results from the report to promote aquaculture as a “mercury-free” alternative to river fish. Unfortunately, it is unclear if the fish purchased from the market in the 2012 study was sourced from rivers or fish farms, nor is it possible to discern how far away the market fish was sourced.

Interviews[5] with owners of three of the larger fish vendors in Puerto Maldonado revealed that paco was a commonly purchased fish at their booth. When asked where the paco sold at their counter came from, each vendor had different sources from different areas, suggesting that fish purchased from the central market in Puerto Maldonado cannot be assumed to be from the Madre de Dios River, nor from near Puerto Maldonado. The locations and sources given by the vendors were: Iberia (Madre de Dios region) aquaculture, Iñapari (Madre de Dios region) aquaculture, and Brazil aquaculture. The vendors have been in business for 15-30 years each.

As mentioned earlier, one of the most commonly consumed fish in the region is paco (*Piaractus brachypomus*, aka *Colossoma bidens*). Known in English as the “red-bellied pacu”, *P. brachypomus* is from the same family as the piranha, *Serrasalmidae* (Goulding, 1980). The paco is a mainly herbivorous, largely opportunistic feeder that grows rapidly. A typical aquiculture pond receives fingerling paco from a breeder or NGO and can produce fish large enough for harvest in one year (IIAP, 2002). To date, little information has been produced on the health comparison between fish farmed in aquaculture versus wild fish captured from local rivers. In order to assess the best possible strategy to promote and institute fish ponds in Madre de Dios, answers to critically important questions about human health risks and mercury are needed. Aquaculture projects in the region continue to be supported and implemented by limited resources of NGOs like ACCA. To maximize the benefit and sustainability of these programs, empirical research is needed.

[5] Anonymous fish vendors in Puerto Maldonado in discussion with the author, June 2014.

3.2 Fish tissue collection and analysis methods

Study scope, objectives and hypotheses

The purpose of this research is to measure the mercury levels of paco raised in fish ponds in Madre de Dios to determine if there is evidence that aquaculture-sourced fish contain mercury levels different from river-sourced fish. One possibility is that paco sourced from aquaculture contain lower levels of total mercury than wild paco. Paco sourced from aquaculture are typically significantly smaller than paco fished from rivers. Larger fish are known to have higher levels of mercury due to the biomagnification of mercury in the ecosystem (Balshaw, Edwards, Daughtry, and Ross, 2007). Additionally, whereas paco in rivers are opportunistic feeders and will therefore feed on whatever is available, paco grown in fish ponds are fed a mostly vegetarian diet. Wild paco may opportunistically consume other organisms. The consumption of other organisms by an individual of a higher trophic level is known to correlate with higher mercury levels. Therefore, the pond-dwelling fish that are fed mostly vegetarian diets are more likely to have less mercury exposure and correspondingly lower levels of mercury in their tissues.

A second possible outcome from this research may show that aquaculture fish have mercury levels that are higher than those of river fish. Fish ponds are stagnant, a condition more conducive to the production of methylmercury than a river with a strong current. Also of consideration is the location of the aquaculture ponds. For maximum impact, pond projects are often implemented in sites that are near or in mining areas. The presence of mercury in the proximal environment may have an impact on fish mercury levels in aquaculture-sourced paco.

Realistically, this study cannot expect to accurately compare the average mercury levels of paco from fish farms in the region of Madre de Dios to wild paco caught from local rivers. There is not enough evidence of mercury levels in wild fish available, and age, diet and growth potential would make comparisons futile. It is therefore the objective of this study to compare mercury levels in paco from regional fish farms to the EPA criterion of 0.3 μg Hg/g fish (wet weight).

Study sites

The author worked with various governmental and research agencies to collect fish samples from aquaculture farms in Madre de Dios. These groups and areas include:

1) Researchers and staff at ACCA in Puerto Maldonado, Peru. Samples of *Piaractus brachypomus*, were collected from 17 fish farms located in seven communities in Madre de Dios near the Interoceanic Highway.
2) The Instituto de Investigaciones de la Amazonía Peruana (IIAP). Samples of *Piaractus brachypomus*, were collected from three fish ponds in Iberia and three fish ponds in Puerto Maldonado.
3) The Ministry of Health. One fish pond was visited to collect samples of *Piaractus brachypomus.*

All sites were located within the region of Madre de Dios, Peru. Iberia served as a control site given the location far from the Madre de Dios River, near the Brazilian border. At the time of the study, no ASGM activities were believed to occur on any large scale in Iberia.

Information on the location and samples taken from each of the aquiculture facilities is reported in table 3.1 below. No approval was needed from the University of Michigan's Animal Use and Care Compliance Program.

Table 3.1: Overview of the different study sites for fish sample collection.

Site	Code	No. of farms	No. of sample	GPS
Iberia	Iberia	3	15	11º 24” 629” S, 69 º 23” 026’ W
Puerto Maldonado[6]	PM	3	10	29 º 39” 033’ S, 69 º 19” 290’ W
Maz	Maz	1	5	13 º 03” 968’ S, 70 º 21” 145’ W
Nueva Generación	NG	1	5	12 º 50” 225’ S, 70 º 11” 766’ W
Primavera Alta	PA	1	5	12 º 54” 765’ S, 70 º 09” 002’ W
Primavera Bajo	PB	4	17	12 º 54” 805’ S, 70 º 10” 285’ W
Santa Rosa	SaRo	2	10	12 º 53” 227’ S, 70 º 18” 669’ W
Santa Rita Alta	SRA	3	14	12 º 54” 556’ S, 70 º 14” 529’ W
Santa Rita Bajo	SRB	2	4	12 º 54” 814’ S, 70 º 12” 360’ W
Virgen de la Candalaria	VC	2	8	12 º 52” 735’ S, 70 º 01” 987’ W
Villa Santiago	VS	2	10	13 º 01” 621’ S, 70 º 20” 954’ W
Total	**11**	**24**	**149**	

Sample collection

Sample collection took place during July 2014. ACCA arranged with fish farm owners to allow visits from the researcher and a ACCA organization representative to collect five *Piaractus brachypomus* specimen. The owners were asked to randomly obtain the samples (ie the first 5 they could catch). Given that fish are added to ponds as fingerlings and harvested together one-year later, all fish were considered to be of the same age and roughly the same size.

The fish were deceased upon arrival, having been collected the morning of the visit by the owner using the methods used before sale at local markets. Data was collected on the fish including number of months in the pond, length (cm),

[6] One outlier was removed from the samples collected from this study site.

approximate weight (kg) and GPS coordinates of the individual pond. A tissue sample weighing approximately 50 grams was taken from the side of the fish, over the lateral line just anterior to the tail. The fish sample was placed in a Ziploc bag and labeled. The samples were frozen in a storage freezer in Puerto Maldonado before being transported to Lima, Peru at the end of July. The samples were then stored in an industrial freezer at a local university before arrangements could be made to have the samples shipped to the University of Michigan, Ann Arbor in the United States on dry ice.

The remainder of the fish after samples were removed was given back to the pond owners for consumption. All fish were donated for analysis except for the specimen collected in Mazuko, which were purchased by the researcher. The results of individual aquaculture ponds were reported back to the appropriate organizations for dissemination to pond owners.

Figure 3.1: An aquaculture pond visited during this study. The pond is typical of the area. Sample shown is a pond funded by ACCA.

Chemical analysis

Upon arrival to the U.S., samples were removed from shipping box and dry ice, still in double Ziploc bags. The samples were placed in an insulated box and placed in a freezer to ahead of analysis. Each sample was measured on a balance while still frozen to 0.0001g. Care was taken to ensure no sample was removed from the freezer for more than a few minutes while measurements were taken. Samples were kept in an insulated box with ice packs while outside of the freezer for added protection. After the initial weight was recorded, samples were placed into individual Whirl Pak bags and labeled. Samples were dehydrated using a vacuum freeze dryer dehydrator. The samples were dehydrated in two batches, with each batch remaining in the dehydrator for at least 48 hours.

Final weights were recorded for each sample using a balance after dehydration to 0.0001g. The dry weight was, on average, approximately 25-22% of the wet weight. The fish samples were then pulverized to a fine powder using a disposable spatula. Samples were analyzed using a Milestone Direct Mercury Analyzer-80 at the University of Michigan Biostation in Pellston, MI using EPA method 7473 (United States Environmental Protection Agency, 2007). The same calibration curve created to analyze total mercury in human hair in Chapter 2 of this study was used for fish analysis. Several reference materials were tested before, during and after analysis of the fish tissue samples (See Appendix 3). Recoveries between 90 and 110% were accepted to validate the calibration. All samples were run in batches that contained blanks, duplicates and triplicates as well as random standard reference material checks for accuracy. Blank readings were consistently below 0.0005 µg Hg/g. All samples were evaluated in March and April of 2015. All samples had two or three measurements taken. The reported values are an average of these values.

Statistical analysis

All conversions and statistical analysis for Chapter 3 of this thesis was performed using RStudio statistical software (RStudio, 2015) and Microsoft Excel (Microsoft, 2010). The standard format for reporting of mercury concentration in fish tissue is to convert from the dry weight concentration, equivalent to the DMA output, to the wet weight concentration. All dry weight concentrations were converted to wet weight concentrations. This is accomplished using equation [1] below.

Equation [1]: Conversion of dry weight [Hg] (Dw) to wet weight [Hg] (Ww)

$$Ww[Hg] = (Dw\ [Hg] \times (1-\Delta w))/100$$

The distribution was checked using a histogram, and one outlier was removed from the dataset. The wet weight mercury concentration was plotted against the dry weight concentration to check conversion accuracy. The conversion has a correlation of close to 1:1 (See figure 3.2 below).

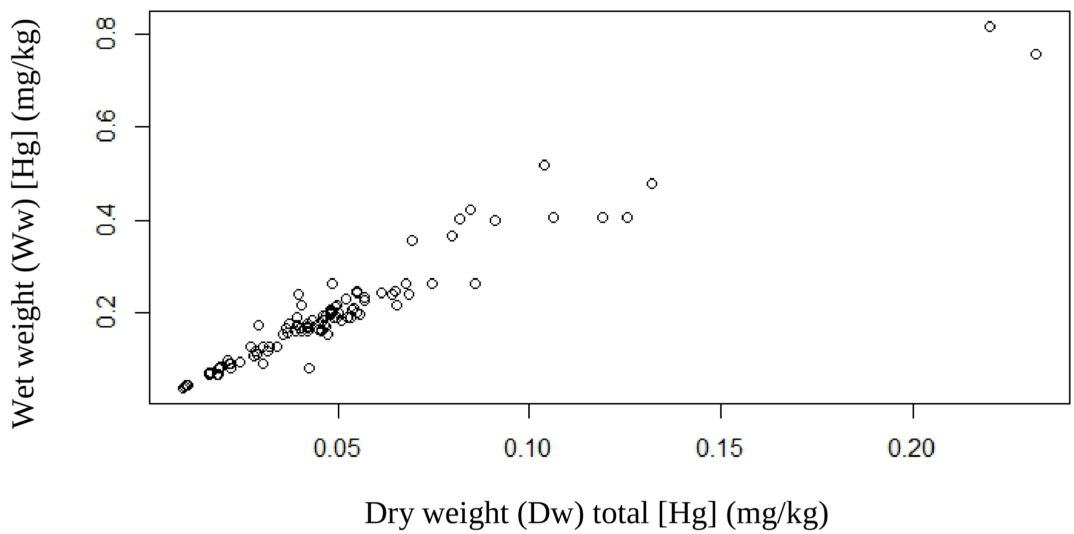

Figure 3.2: Scatterplot of dry weight mercury concentration converted to wet weight mercury concentration.

Total mercury ([Hg] mg/kg dry weight) was plotted against fish specimen weight mercury (figure 3.3). The R-squared value and slope were determined using statistical software. Simple descriptive statistics were running using Microsoft Excel (Microsoft, 2010) functions. The statistic of interests was the mean wet weight mercury concentration (mg/kg) by study site. The range, standard deviation, 95% C.I. and mean were all calculated and reported in table 3.2 and figure 3.4 in the results.

3.3 Results – Mercury in farmed fish

The weight of fish specimen was positively correlated with mercury (mg Hg/kg dry fish tissue) (See figure 3.3 below). The R^2 value of the relationship between fish specimen weight in kg and total mercury concentration in dry fish tissue was 0.932.

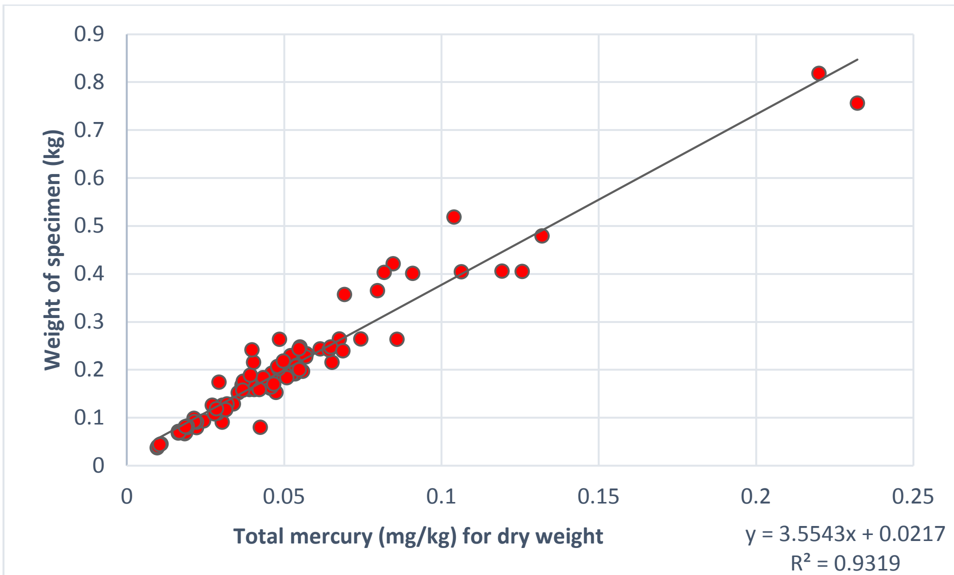

Figure 3.3: Plot of total mercury concentration (dry weight) against fish weight for all samples.

The mean total mercury concentration for all study sites was below the EPA reference dose of 0.3 mg/kg. Of all the samples, only two fish from Virgen de la Candalaria had mercury concentrations near this value (0.232 and 0.220 mg Hg/kg wet weight fish tissue).

As shown in Table 3.2 and Figure 3.4 below, Mazuko had the lowest mean mercury concentration for both wet weight and dry weight (0.028 mg/kg and 0.106 mg/kg, respectively). The study site with the highest mercury concentration was Virgen de la Candelarai (Ww = 0.116 mg/kg Hg and Dw = 0.400 mg/kg Hg). The control site of Ibería had a mean wet weight of 0.035, making it the third lowest concentration.

Table 3.2: Descriptive statistics reported for each study site

Site	n	Median Ww[Hg] (mg/kg)	Range Ww[Hg] (mgkg)	StDev Ww[Hg] (mg/kg)	95% C.I.
Iberia	10	0.035	(0.010, 0.091)	0.030	0.035±0.007
Mazu	5	0.028	(0.019, 0.032)	0.005	0.028±0.002
PM	9	0.192	(0.016, 0.104)	0.036	0.048±0.008
NG	5	0.065	(0.051, 0.065)	0.021	0.071±0.007
PA	5	0.048	(0.039, 0.050)	0.004	0.046±0.001
PB	17	0.0431	(0.027, 0.074)	0.113	0.043±0.019
SaRo	10	0.03	(0.030, 0.046)	0.011	0.030±0.002
SRA	14	0.045	(0.019, 0.068)	0.013	0.042±0.002
SRB	4	0.054	(0.040, 0.086)	0.022	0.058±0.008
VC	7	0.119	(0.047, 0.232)	0.072	0.116±0.017
VS	10	0.052	(0.047, 0.061)	0.005	0.052±0.001

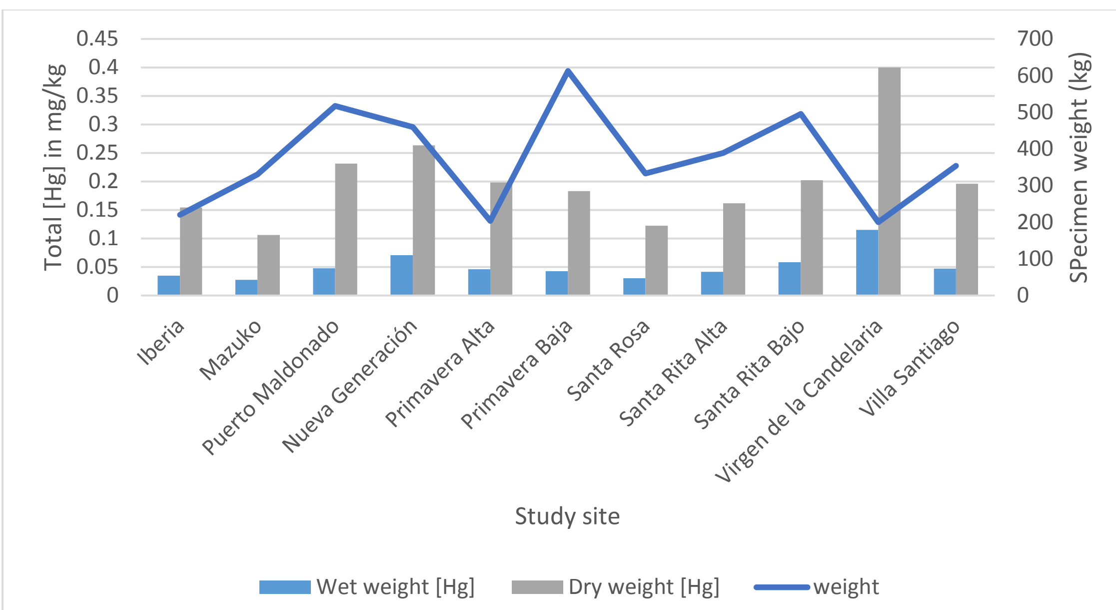

Figure 3.4: Comparison by study site: mean wet weight mercury concentration (mg/kg), mean dry weight mercury concentration (mg/kg), and mean specimen weight (kg).

It is notable that Primavera Baja, despite having the largest number of samples, had a standard deviation 178% larger than the next largest value (Virgen de la Candalaria, n=7), and more than 200% larger than the other nine sites. Table 3.3 below looks at the statistics for the four aquaculture farms visited within Primavera Baja.

Table 3.3: Individual statistics for study site Primavera Baja.

Primavera Baja Site Number	**Number of Samples**	**Mean [Hg] Ww (mg/kg)**	**Range [Hg] Ww mg/kg**	**Variance**
1	2	0.050	(0.046, 0.050)	4.0e-5
2	5	0.041	(0.027, 0.074)	3.9e-4
3	5	0.041	(0.037, 0.049)	2.5e-5
4	5	0.045	(0.040, 0.055)	4.0e-5
ALL (1-4)	**17**	**0.0431**	**(0.027, 0.074)**	**1.2e-4**

The results for Primavera Baja show considerable variation within a small area, such as a community. Primavera had more samples and more study sites than any other study site, as well as a larger standard deviation. The largest variance is seen in site 2 (3.9e-4), which is larger than the variance between all 17 samples. This may be a result of one outlier (the maximum value) in site 2. If this observation is removed, then the variance between samples is reduced to 5.0e-5. However, the range of samples in other sites, as well as the small sample sizes, does not warrant throwing this observation out.

3.4 Discussion – Farmed fish

In all 149 observations of farmed fish in the region of Madre de Dios, there were no unsafe levels of mercury using the EPA reference limit. This finding, however, should be corroborated with more research. There are a few confounding factors.

The most important consideration is the age of the fish in the sample groups. Due to the time of the field study, the aquaculture fish were only 4-8 months old, depending on location. Within a community, the age of fish may vary by each pond as a strategy to stagger harvests. Table 3.4 below shows the different mean mercury concentrations calculated by age group. The relationship in table 3.4 shows that, on average, an individual fish increased mercury content by approximately 0.01 mg/kg. If the trend continued, then by the time a paco fish reaches one year of age, it would likely have a total mercury content of 0.123 mg/kg. This value would be well below the EPA reference level of 0.3 mg/kg, though more actual data would be needed for corroboration, as it is not known if a paco fish would continue to increase mercury content at a linear rate. Atmospheric and mining conditions alike would influence rates of mercury methylation and uptake, as well as possible metabolic processes.

Table 3.4: Mean mercury concentration by age of fish specimen at time of sampling.

Age (months)	4	6	8
Mean Ww [Hg] (mg/kg)	0.036	0.053	0.079

A second important consideration to contemplate when drawing conclusions is that the fish ponds sampled were relatively new (a few years). It is likely that, as the ponds age, methyl-mercury will continue to accumulate through transformation after atmospheric deposition in the stagnated pond water. A follow-up longitudinal study, including aquaculture pond sediment samples, would help clarify whether the concentration of bioavailable mercury in the ponds would change over time.

As shown in table 3.4, the youngest fish in the study were a little over four months old (fingerling transplant + four months in pond). The mercury concentration was already at 0.036. A thorough analysis of the source of fingerlings in the region to see what type of mercury is in the sediment, stock fish and fingerlings at time of transport would provide a base-level upon which to analyze the level of mercury fish accumulate in the aquaculture pond.

Lastly, a study that involved the comparison of mercury concentration in naturally-caught river paco to the levels in market-ready farmed paco would allow a comparison between what levels of mercury may be attributed to regional environmental conditions, and what levels are caused by other factors, such as species or age. This would require detailed analysis as river paco were much larger than farmed paco.

3.5 Conclusion – Fish consumption

The level of mercury in fish raised in aquaculture in the region of Madre de Dios, Peru appear to be below the EPA reference level of 0.3 mg/kg mercury (wet weight). Assumptions made in this study should be more carefully studied in the future, particularly with regard to the linear accumulation of mercury over time by the species paco. This study was to serve as a scoping study for general mercury levels in farm-raised fish at approximately half-stage of production.

Introduced as a conservation strategy, the importance of fish farms in the region of Madre de Dios continues to grow. By providing livelihood options, sustainable projects like fish farms help to reduce the pressure on natural fish populations, thus improving biodiversity (Diana, 2009). Based on results from the survey discussed in Chapter 2 of this thesis (See Appendix 2), the provision of a sustainable meat source can also be expected to reduce pressure on hunting for bush meat by local populations. The income generated by these activities also encourage sustainable development projects and livelihood improvement within the local community (ACCA, 2012). Perhaps most importantly, successful sustainability projects that improve local livelihoods and provide options help to discourage poor immigrants from ASGM and the environmental degradation that ensues.

Chapter 4: The "curse" of Madre de Dios

Weak governance and environmental (in)justice

The threat of deforestation and land degradation is far from the only factor threatening the sustainability and future of the Madre de Dios region in Peru. Decades of failed timber extraction and forestry laws in the region have contributed to a downward spiral of new logging legislation and failed enforcement (Smith et al., 2006). The government is not able to enforce the laws it creates and has lost credibility.

The history of distrust between authority and the governed runs deep in Peru. Corrupt leaders and perverse incentives can be found in many sectors involving the extraction of natural resources. For example, the Yanacocha Gold Mine in Peru is a large-scale multinational operation that contributed more than $1.150 billion USD in tax revenues to the Peruvian government since it was established in 1996 (International Finance Corporation, 2008). As in many democratic societies, the corporations that are profitable for the government tend to have a large influence on policy and government officials. Local residents, who have seen a decrease to natural and social resources since mining operation began, have protested the American-owned Yanacocha Gold Mine to no avail (Bury, 2005). The profits and power are too great, and ultimately the government has protected the interests of the international corporation over the interests of local people.

Despite the mineral boom increasing resource extraction in Peru in recent decades, the local governments have not been able to capture control and profit from the lucrative industries (Arelllano-Yangua, 2008). Furthermore, ASGM and other extractive industries have done little to reduce poverty. Throughout Peru's highlands, violations against the land and water rights of local communities have gained attention as mining undergoes a revival in Peru. Some communities have been able to fight back, and sometimes win, against large mining companies extracting resources from vein deposits, despite the Peruvian government ignoring the law of prior consent.

The law of free and prior consent states that any decisions made about indigenous lands must have community consent first. In addition to the highlands, this law applies to indigenous groups all over Peru including Madre de Dios. Unlike the highlands, however, the nature of valuable natural resources and the vast, seemingly endless Amazon frontier have rendered enforcement difficult at best. Similarly, many indigenous communities within Madre de Dios have not been able to fend off trespassers searching for gold, who degrade their environment and settle their lands. A 239% increase in artisanal and small-scale gold mining was found to be the main factor driving land-use and land-cover change in the period 2006-2011 (Scullion et al., 2014). Besides threatening tenure security, preliminary results have shown that indigenous groups are more vulnerable to mercury contamination as their diet relies heavily upon fish (CAMEP, 2012b).

There are many threats to the indigenous communities of Madre de Dios. From miners, to loggers to an expanding agricultural frontier and growing population, the need to secure land titles, and have this right enforced, is dire. The United Nations' Declaration on the Rights of Indigenous Peoples, which Peru voted in favor of upon adoption in 2007, states that the rights of indigenous peoples, including the right to the natural resources on traditional indigenous lands, should be left to the control and discretion of those groups (United Nations Human Rights Council, 2007). There is a clear and devastating violation of the nationally and internationally recognized rights of the indigenous groups of Madre de Dios.

Over the last century, anthropogenic emissions of elemental mercury have tripled in concentration in the atmosphere and ocean (Mason, Fitzgerald, and Morel, 1993). After fossil-fuel fired power plants, the next largest contributor to global mercury emissions is small-scale gold mining activities across the globe (Pirrone, et al., 2009). In October 2013, the Peruvian government signed the United Nations' Minamata Convention, an international treaty negotiated to reduce anthropogenic mercury emissions and general mercury release into the environment in order to protect human health (United Nations Environment Programme, 2013c). Yet, Peru's mercury imports have *increased* in recent years, largely for the purposes of small-scale gold mining (Swenson, et al., 2011).

Attempts by the regional and federal Peruvian governments to halt illegal gold mining in Madre de Dios, have failed. Attempts to cut off supply lines and to regulate resources needed for mining, such as gasoline and mercury, have failed. Legislation banning the purchase, sale or export of illegal gold, has failed. Even military operations – Peruvian militants bombing and burning Peruvian communities – have failed. If effective policy is to be drafted and enforced to regulate the destructive pattern of illegal gold mining in Peru, then these failures must be understood.

Local inhabitants of Madre de Dios have historically been cut off from the larger world economy. It wasn't until an airport was constructed in the region in recent decades that tourism became popular in the area. In fact, before the construction of the airport in the capital of Puerto Maldonado, the only way to reach this town would have been to traverse upstream starting at the mouth of the Amazon River near the Atlantic Ocean or to descend from the Andes. Now, a person can feasibly take a bus, relatively inexpensively, from Lima to Puerto Maldonado in a matter of days.

Immigrants who travel to Madre de Dios in search of their gold fortunes can make \$150 to \$200 in a day. In most areas of the Peruvian highlands, jobs that pay \$10-20 per day can be hard to come by[7]. Meanwhile, the price of gold remains high, and buyers on the global market happily buy up whatever gold they can. As such, it comes as no surprise that the Peruvian government, corruption aside, has been unable to control the growing trend of illegal mining. Indeed, there are few governments who can defeat the global, capitalistic market.

The best hope for effective policies start with the education of consumers and enforcement of corporate social responsibility. Large firms in Europe and North America end up purchasing the illegal gold produced in Peru. Although some companies claim to enforce standards on sustainably-sourced gold, the mass export of gold from Peru every year to the global economy proves that there are some gaps in

[7] Personal communication with miner encountered in Boca Amigo (July, 2014).

the supply chain. Policies on the international level enforcing the production of sustainably-sourced gold in ASGM would likely be of the greatest impact possible short of a crash in gold prices.

Locally, NGOs, government agencies and mining collaborations should seek alternative, sustainable methods for recovering gold and remediating the environment. It is important that technical solutions take into account the views of miners, as a large decrease in production or high overhead costs would likely fail to be implemented (Hentschel et al., 2002).

On the part of the Peruvian government, one of the most important policy actions is to legally recognize and enforce indigenous land rights and tenure. Additionally, a system that effectively issues, monitor, and enforces permits would allow for better regulation on the currently un-regulated industry.

A common narrative in literature that examines Peru's resources is that of the "resource curse". Some compare the existence of valuable minerals and natural resources in Peru to a curse because of the historical turmoil and human loss it has caused. The Spanish Conquistadores defeated the well-developed and highly technologically innovative civilization of the Incans. For gold. Canadian and U.S. mining conglomerates have displaced indigenous groups from their ancestral homes in the Andes. For gold. And now, the forces of capitalism and globalization are encouraging the deforestation of one of the most important biodiversity hotspots on our planet. For gold.

The resource curse is a scape-goat. The true curse is that of greed, which has driven the actions of the global north for centuries, and which has promoted the persecution of small-scale miners, who seek stable livelihoods but struggle in poverty. Assigning sole blame for the destruction of the Peruvian Amazon to these miners ignores a simple fact: illegal gold is exported. Someone is buying it. Someone is encouraging the trend.

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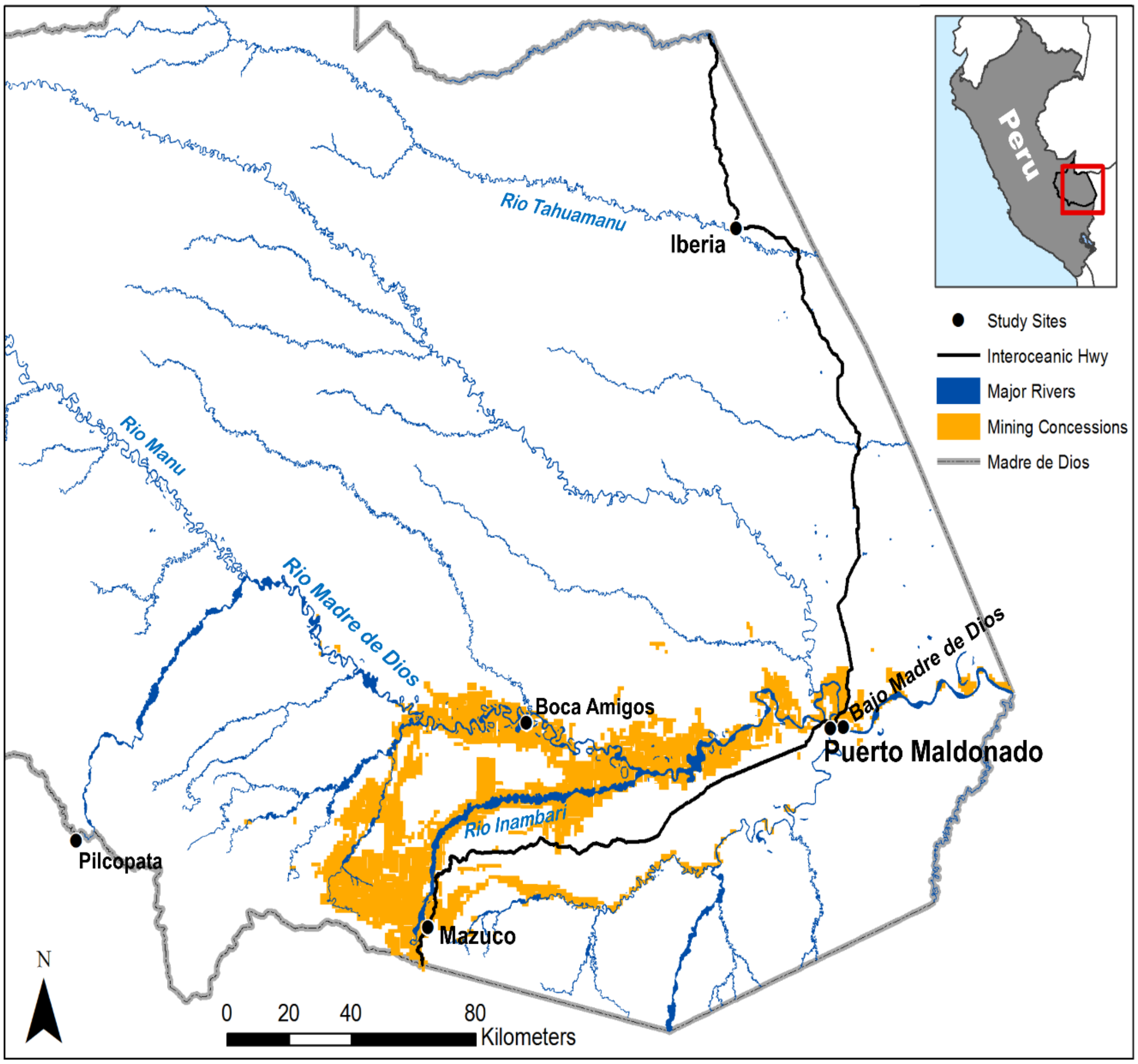
Appendix 1: Map of study site locations.



Map created with ESRI ARCGIS software.

By: David X. J. Gonzalez, M.S.

Appendix 2: Study questionnaire on diet.

Note: Survey edited for space.

Mercury Study: Survey Questions Site:________________

ID Number: __________________ Time of Interview: _____________

Date of Interview:______________

1) Date of birth ______
2) How often do you usually eat fish?

   ≤ 1x per month ≤ 1x per week ≤ 3x per week ≤1x per day

   >1x per day
3) A serving size of fish is equal to the size of a deck of cards. How many servings of fish have you consumed in the last three days?
4) What other types of animal protein do you consume?
5) How many days have you eaten those proteins during the last week?

Hunting/trapping on your land	List protein type
Hunting/trapping on nearby land	
Hunting/trapping on land away from home	
Purchase at market	
Purchase from neighbor who hunted	
Purchase from neighbor who raises meat/farms	
Home-raised/farmed	
Trading for goods/services	
Other:	

6) How do you procure these additional protein types?

7) Which species of fish do you prefer to eat? (List below, or write "no preference" if you have no preference)
8) What species of fish do you normally eat? (If you don't know, write "I do not know")

9) Where do you obtain the fish you eat? (Fished by yourself or family, purchased from another fisherman, purchased from market…)

10) Do you know where the fish was caught?

If so, where?

11) Do you eat fish that is dry, smoked or canned?

If yes, how often?

Any particular types of fish that you prefer to eat these ways?

12) Do you consider fish an important food source for your family?

If Yes, please circle the all reasons that apply why and briefly explain:

Easy to access   Inexpensive   Best source of protein available Tradition/Beliefs

Taste preference

13) How many people are in your household?

14) How many children are in your household?

15) Are you currently pregnant or breastfeeding?

16) Have you ever given birth?

If yes, how old is your youngest child? ______________________

17) Do/Did you eat the same amount of fish when pregnant?

If no, did you eat   More   Less   fish than you do now?

18) Do/Did you eat the same amount of fish when breastfeeding?

If no, did you eat   More   Less   fish than you do now?

19) If you have had more than one pregnancy, is this pattern the same for all previous pregnancies?

If no, how have your eating habits changed?

20) How long have you continuously lived in this community?________________

21) Where did you live before? ______________________________________

22) Have you ever attended school? If yes, what is the highest level of education you completed?

No   Yes, Level completed:___________________

Appendix 3: DMA-80 calibration and standard testing.

Calibration curve set using a liquid standard of 1 mg/mL Hg in 10% HNO_3.

Cell 1

Hg [ng]	**Height**	**ΔE (A)**
1.0e-5	4.0e-4	0.03
1.00	8.0e-4	-0.01
3.83	0.11	-0.01
5.05	0.18	0.02
9.84	0.36	0.01
19.92	0.68	-2.9e-3

6-point linear curve
$R^2 = 0.995$
A = -0.01 Hg
0.04 Hg

Cell 2

Hg [ng]	Height	ΔE (A)
39.50	0.03	-2.0e-4
78.30	0.07	1.0e-4
98.30	0.08	-1.0e-4
148.60	0.13	2.0e-4
197.50	0.16	1.0e-5

5-point linear curve
$R^2 = 0.998$
A = 5.72e-3 Hg
7.92e-4 Hg

Weight (g)	**Con [ng]**	**[ ] St (ppm)**	**Cell Number**
0.0385	4.0	0.1	1
0.0453	5.0	0.1	1
0.0966	10.0	0.1	1
01958	20.0	0.1	1
0.0384	40.0	1.0	1,2
0.0778	80.0	1.0	2
0.0968	100.0	1.0	2
0.1461	150.0	1.0	2
0.1972	200.0	1.0	2

Reference material IAEA-407, Fish homogenate, Hg value: 0.222 µg/g.
95% C.I. (0.216-0.228) µg/g.

Test No.	Expected [Hg] µg/g	DMA-80 read [Hg] µg/g	% recovery
1	0.222	0.229	103%
2	0.222	0.232	104%
3	0.222	0.227	102%

Reference material IAEA-436, Tuna fish flesh homogenate, Hg value: 4.19 µg/g
95% C.I. (4.04-4.34) µg/g.

Test No.	Expected [Hg] µg/g	DMA-80 read [Hg] µg/g	% Recovery
1	4.19	4.316	103%
2	4.19	4.000	95%
3	4.19	4.100	97%

Reference material IAEA-086, Human hair, Hg value: 0.573 µg/g.
95% C.I. (0.543-0.612) µg/g.
Calibration range:1-100 ng.

Test No.	Expected [Hg] µg/g	DMA-80 read [Hg] µg/g	% recovery
1	0.573	0.632	110%
2	0.573	0.600	105%
3	0.573	0.595	103%