

# Evaluating Wildlife Vulnerability to Mercury Pollution From Artisanal and Small-Scale Gold Mining in Madre de Dios, Peru

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## Abstract

Illegal, artisanal and small-scale gold mining (ASGM) often occurs in remote highly biodiverse areas, such as the Madre de Dios region of Peru. Mercury used in gold mining bioaccumulates in the environment and poses developmental, hormonal, and neurological threats to wildlife. The impact of ASGM on biodiversity remains largely unknown. We used geographic information science to create a spatial model of pollution risk from mining sites, in order to predict locations and species assemblages at risk. Multicriteria evaluation was used to determine how flow accumulation, distance from mining areas, total suspended sediment load, and soil porosity influenced the vulnerability of regions to mercury pollution. Results suggest that there is considerable opportunity for protection of areas with high biodiversity and vulnerability north of the Madre de Dios River where much of the land is not protected. Our study highlights the need for future ASGM research to consider more than deforestation risk alone while protecting the areas' unmatched biodiversity.

## Keywords

biodiversity, conservation planning, extinction risk, mining, protected areas

## Introduction

The Madre de Dios region, located in southern Peru (Figure 1), is one of the most biodiverse tropical regions of the world (Catenazzi et al., 2013; Ceballos & Ehrlich, 2006; Finer, Jenkins, Pimm, Keane, & Ross, 2008; Orme et al., 2005; ter Steege et al., 2003). Madre de Dios' biodiversity is threatened by resource extraction, particularly gold mining and the resulting pollution. Peru is the largest exporter of gold in South America and the fifth largest in the world ("Gold," 2017). While changes in land cover due to artisanal and small-scale gold mining (ASGM) are well studied (Asner, Lactayo, Tupayachi, & Luna, 2013; Elmes, Yarlequé Ipanaqué, Rogan, Cuba, & Bebbington, 2014), the impact mercury (Hg), used in the mining process, has on this region's biodiversity that remains unknown.

The Peruvian government defines artisanal and small-scale mining as concessions of up to 1,000 and 2,000 ha and production of up to 25 to 350 metric tons, respectively, but the impact of ASGM activity should not be underestimated, as it is the primary contributor to land change in the Southwest Amazon (Scullion, Vogt, Sienkiewicz, Gmur, & Trujillo, 2014). Liquid Hg is used

in the mining process to amalgamate and concentrate gold. This known pollutant is released into the atmosphere during burning and can also enter the environment through unmanaged tailings or mining by-products as is known to happen in ASGM in Madre de Dios (Fraser, 2009). Mixed with sand to form an amalgam, Hg is vaporized and whatever has not been separated from the gold is discarded, creating an opportunity for residual Hg to enter streams and rivers (Fraser, 2009). While formal, large-scale gold mining operations attempt to recover and reuse Hg, regulation of Hg used in artisanal mining is challenging in remote areas such as the Peruvian Amazon (Swenson, Carter, Domec, & Delgado, 2011; Veiga, Maxson, & Hylander, 2006). Enforcement of environmental regulations has largely been ineffective

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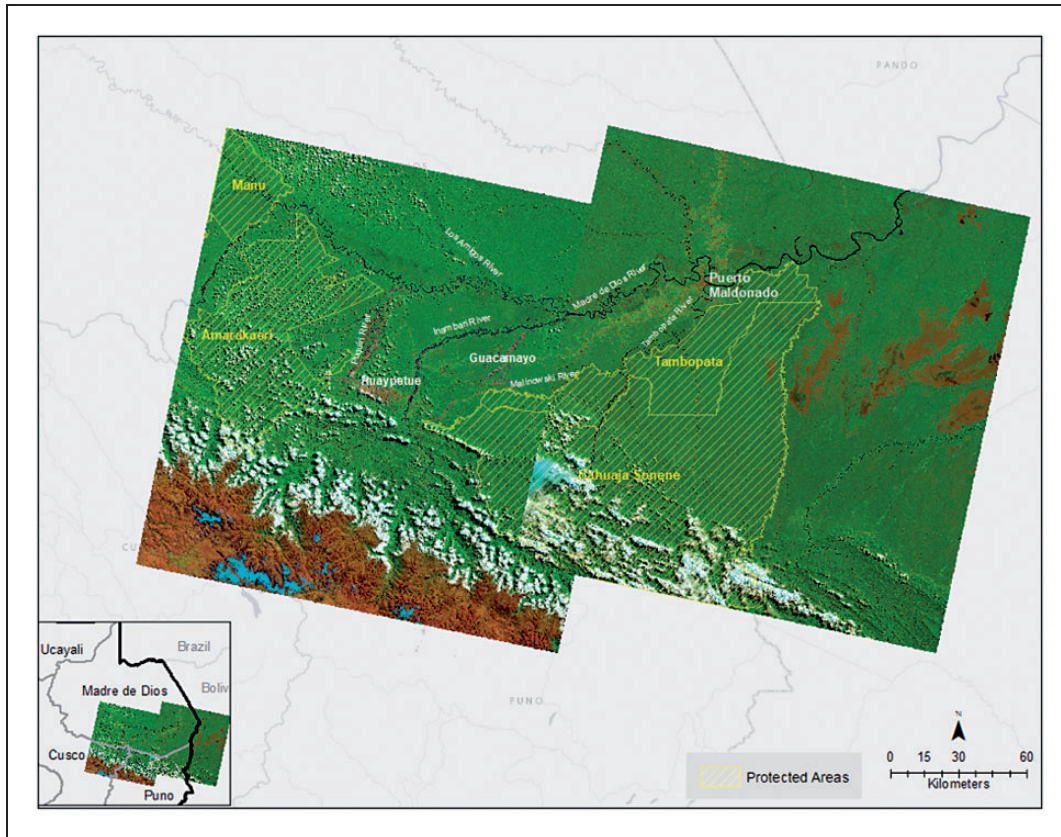
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**Figure 1.** False color composite of study region. Protected areas overlaid in yellow—provided by El Sistema Nacional de Áreas Naturales Protegidas por el Estado. The city of Puerto Maldonado is shown as well as the major mining areas of Huaypetue and Guacamayo.

(Swenson et al., 2011), leading to haphazard handling of Hg and its release into the environment (Ashe, 2012). Elemental Hg is then methylated by aquatic organisms and bacteria, forming methylmercury (MeHg). MeHg is the most toxic organic form of Hg, as it bioaccumulates and biomagnifies through the aquatic food chain (Baeyens et al., 2003; Fitzgerald & Clarkson, 1991; Mason, Heyes, & Sveinsdottir, 2006).

Amphibians may be particularly at risk to Hg contamination given their current global decline and extinction rates (Stuart et al., 2004). Their permeable skin, exposure to aquatic environments during certain life stages, and unshelled eggs make amphibians sensitive to environmental contaminants including metals (Blaustein, Romansic, Kiesecker, & Hatch, 2003; Kerby, Richards-Hrdlicka, Storer, & Skelly, 2010). Hg pollution has been related to decreases in food consumption and body size and increases in mortality of amphibians (Bergeron, Hopkins, Todd, Hepner, & Unrine, 2011; Burke, Bergeron, Todd, & Hopkins, 2010; Todd, Willson, Bergeron, & Hopkins, 2012). Two-lined salamanders (*Eurycea bislineata*) collected at sites with high Hg levels consume half as much food as salamanders collected at uncontaminated sites and appear to have slower responses capturing prey and speed (Burke et al., 2010). American toads (*Bufo americanus*)

exposed to Hg through maternal transfer or through their diet as larvae are 7% smaller than control subjects, indicating Hg effects persist after metamorphosis (Todd et al., 2012). *Bufo americanus* larvae exposed to Hg through maternal transfer and diet experience 50% higher mortality than controls (Bergeron et al., 2011). Recent work examining Hg concentrations in an omnivorous and a herbivorous species of tadpoles found predictably higher total Hg levels in the omnivorous species, but suggested levels in both had the potential to be problematic (Boczulak, Vanderwel, & Hall, 2017). Higher total Hg concentrations were higher for the tadpoles than levels reported for invertebrates at comparable trophic levels, suggesting tadpoles may be important vectors of Hg (Boczulak et al., 2017).

Fish are also vulnerable to Hg contamination and experience negative effects on gonadal development, sex hormone production, reproduction, and behavior when exposed to MeHg in laboratory studies (Drevnick & Sandheinrich, 2003; Friedmann, Watzin, Brinck-Johnsen, & Leiter, 1996; Matta, Linse, Cairncross, Francendese, & Kocan, 2001; Webber & Haines, 2003). It has also been suggested that MeHg can affect reproduction in wild populations of fish (Scheuhammer, Meyer, Sandheinrich, & Murray, 2007).

Previous work in Madre de Dios has determined that Hg levels in water and soil are elevated in areas downstream of artisanal and small-scale mines (Diringer et al., 2015), that individuals living in mining areas have higher levels of Hg in blood and urine (Ashe, 2012), and that Hg levels in raptors are elevated (Shrum, 2009), so it is reasonable to expect that amphibians and aquatic organisms throughout the region are at some degree of risk. Remote sensing and geographic information system techniques have contributed significantly to models of Hg transport in aquatic environments for southwestern China (Lin, Larssen, Vogt, Feng, & Zhang, 2011) and South Carolina (Knights et al., 2014), and we will similarly take advantage of remote sensing data.

The objectives of this work are to identify locations where fresh water and amphibian species in Madre de Dios may be threatened by Hg pollution and to assess which protected areas are at risk. To achieve this objective, we asked (a) what areas are vulnerable to Hg pollution and (b) what areas are of highest conservation priority for freshwater and amphibian species? We answered these questions through the lens of remote sensing and spatial analysis using an advanced machine learning technique to quantify the extent of mining growth and multicriteria evaluation to model the spatial distribution of Hg vulnerability.

## Methods

### Study Area

The study region is a select portion of the Madre de Dios region of Peru (Figure 1). Madre de Dios is home to some of Peru's most well-known protected areas, such as Manú National Park, a United Nations Educational, Scientific, and Cultural Organization Natural World Heritage Site and Conservation International Biodiversity Hotspot. Manu National Park, the protected area with the highest species richness in the world for amphibians and reptiles (Catenazzi et al., 2013; Catenazzi & von May, 2014), is also in this region. Protected areas in the entire Madre de Dios region, as recognized by the National Service of Natural Protected Areas, total 37,716.8 kilometers and include Manu, Bahuaja Sonene, and Alto Purus National Parks, Tambopata National Reserve, and Amarakaeri and Purus Communal Reserves (Sistema de Areas Naturales Protegidas del Perú, 2018).

The study area is home to several native communities including Infierno where members of the Ese Eja live, San Jacinto where the Shipibo-Conibo live, and multiple communities of Harakmbut, including Boca del Inambari, Puerto Luz, and Boca Isiriwe ("Comunidades Nativas," 2017). The city of Puerto Maldonado is included the study area and the Huepetuhe and Guacamayo mining areas. Madre de Dios generates the majority of Peru's artisanal gold with little enforcement of environmental

regulations and an unknown number of informal miners (Swenson et al., 2011). According to the Peruvian government's annual mining report, Madre de Dios legally employed 674 individuals across all sectors of mining in 2015 ("Empleo en Minería," 2016). Estimates in 2009 place 30,000 ASGM miners working in Madre de Dios (Fraser, 2009), and a raid of 200 ASGM camps in February 2016 in the Tambopata National Reserve was thought to be home to 5,000 illegal miners (Fernández Calvo, 2016). Since 2007, the primary contributor to land change in the Southwest Amazon region in Peru has been ASGM (Scullion et al., 2014). Madre de Dios is facing not only the ecological consequences of increased deforestation but also serious social conflicts, child labour, and sex trafficking due to ASGM activities (Verité, 2013; Society for Threatened People, 2014). Threats from Hg pollution are so serious that the Peruvian federal government declared a 60-day State of Emergency for the region in May 2016 (Taj, 2016). The Peruvian Ministry of the Environment reported in 2015 that the Madre de Dios rivers are contaminated with 40.5 tons of Hg annually (Madre de Dios, 2015).

### Identifying Mining Area

Remotely sensed imagery from Landsat 8 Operational Land Imager (OLI) was used to classify different land cover types in the region. Imagery with less than 20% cloud coverage and dating late August/early September 2015 were used. Dates coincide with the months in 2016 fieldwork was performed. Imagery and fieldwork occurred roughly in the middle of the dry season (April–November) for the region. Classification was performed on a combination of bands and indices selected for their capability to identify iron oxide, soil, vegetation, and moisture. Two iron oxide indices previously used to map gold mining areas (Gabr, Ghulam, & Kusky, 2010; Pour & Hashim, 2015) were included in order to differentiate sand and bare soil from mining (Table I in Supplementary Material II). Accuracy of land classifications was determined through visiting ground truth locations and using Google Earth imagery.

Random forest classification (Liaw & Wiener, 2002), a type of machine learning that uses ensembles of classification, and Mahalanobis Typicalities (Foody, Campbell, Trodd, & Wood, 1992), a distance-based classification approach, were used to discriminate mining sites. A classification threshold of 75% was used for random forest and a 40% threshold for Mahalanobis such that any pixel that did not meet the aforementioned thresholds remained unclassified. Areas identified as mining by both classifications were considered mining areas.

In using areas of agreement between random forest and Mahalanobis classifications, land stated here as mining represents what is likely a conservative estimate. In situ research and consultation with regional experts lead us

to state with reasonable certainty that such area does represent soils that are heavily disturbed and contaminated with Hg from burning the amalgam of gold and Hg.

### Vulnerability Modeling

For the purpose of this study, vulnerability is defined as risk of exposure to Hg. A multicriteria evaluation was developed to model the spatial distribution of Hg vulnerability by combining multiple environmental and chemical factors. Terrestrial and aquatic areas were modeled independently due to differences in the identification of factors related to Hg vulnerability within them. Vulnerability factors (Tables 1 and 2) were selected based on the availability of data and relevance (Lin et al., 2011; Ullrich, Tanton, & Abdrashitova, 2001). Factors were scaled into vulnerability scores from 0 to 100, with higher scores indicating higher vulnerability. For the purpose of this study, we considered vulnerability of 70 or greater as *high*. All models are for the dry season which in this region expands from April to November. Hg distribution and transport are affected by multiple variables, such as the dissolved oxygen content of water and prevailing wind patterns, and the hydrological system is very important (Moreno-Brush, Rydberg, Gamboa, Storch, & Biester, 2016).

Proximity to the identified mining areas was calculated through a cost distance analysis, with slope as the friction layer, as Hg pollution is assumed to be largely transported through runoff. Flow accumulation was generated through a runoff model in Terrset that generates a flow direction based on a surface image (in this case elevation;

Eastman, 2016). One drop of rainfall is assumed to fall on every pixel, and it continues to flow to a lower pixel until it reaches a pit or the boundary of the data. The algorithm is based on the study by Jenson and Dominigue (1988). Runoff processes are known to be an important input in related studies modeling pollutant transport (Bou Kheir, Shomar, Greve, & Greve, 2014; Lin et al., 2011).

As most Hg in water is bound to sediments (Andren & Harriss, 1975; Cossa, Sanjuan, & Noel, 1994; Mason et al., 1993), an average Normalized Difference Suspended Sediment Index (NDSSI) was created. Following the work of Azad Hossain, Chao, and Jia (2010), the near infrared (NIR) and red bands were used to generate the following equation,  $NDSSI = NIR - Red / NIR + Red$ . NDSSI indices were created for six Landsat scenes taken between the months of July and October (within the dry season) for the years of 2013 to 2016. These were then averaged and used as a factor in vulnerability modeling.

Sediment porosity was calculated from the World Soil Information's SoilGrids1km (Hengl et al., 2017). Soil characteristics such as soil porosity are used in related studies modeling pollutant transport or land suitability analysis (Bagdanavičiūtė & Valiunas, 2013; Bou Kheir et al., 2014).

Factor weights (Table 1) were calculated based on an Analytical Hierarchy Process that compared the relative importance of all pairwise combination of variables (Saaty, 1977). The consistency ratio for both set of factors (land and rivers) was acceptable (0.06 in both cases). Previous work in this region has shown that Hg concentrations are higher downstream of mining activities compared with upstream (Diringer et al., 2015), and Hg from ASGM

**Table 1.** Factors Used in Modeling Land Vulnerability With Weights and Explanation of Effect on Vulnerability.

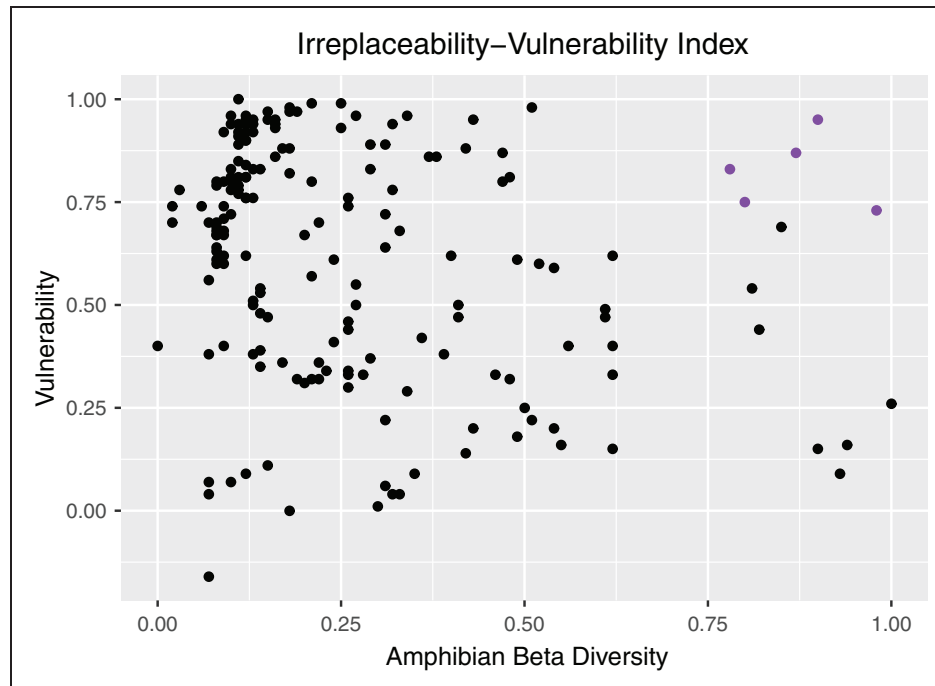
Factor	Explanation	Weight
Distance from mining area	Cost distance from area classified as mining. ↓ distance = ↑ vulnerability	.6491
Digital elevation model	Elevation inverted. ↓ elevation = ↑ vulnerability	.2790
Soil porosity	↑ soil permeability = ↑ vulnerability	.0719

**Table 2.** Factors Used in Modeling River Vulnerability With Weights and Explanation of Effect on Vulnerability.

Factor	Explanation	Weight
Distance from mining area	Cost distance from area classified as mining. ↓ distance = ↑ vulnerability	.5660
Runoff	Calculated surface runoff. ↑ runoff = ↑ vulnerability	.2674
Mean suspended sediment (NDSSI)	Average suspended sediment index. ↑ suspended sediment = ↑ vulnerability	.1267
Soil porosity	↑ soil permeability = ↑ vulnerability	.0399

Note. NDSSI = Normalized Difference Suspended Sediment Index.





**Figure 2.** Example of an Irreplaceability–Vulnerability Index using beta diversity of amphibians and vulnerability of land area. Per watershed, the vulnerability score is shown with beta diversity for amphibian species. Both beta diversity and vulnerability have been standardized. Purple dots represent the five watersheds with the greatest combined beta diversity and land vulnerability scores, thus identifying high-priority watersheds.

activities is thought to remain close to emission source or is transported downstream adsorbed to suspended matter (Moreno-Brush et al., 2016). Accordingly, distance from mining area and runoff/elevation were highly weighted.

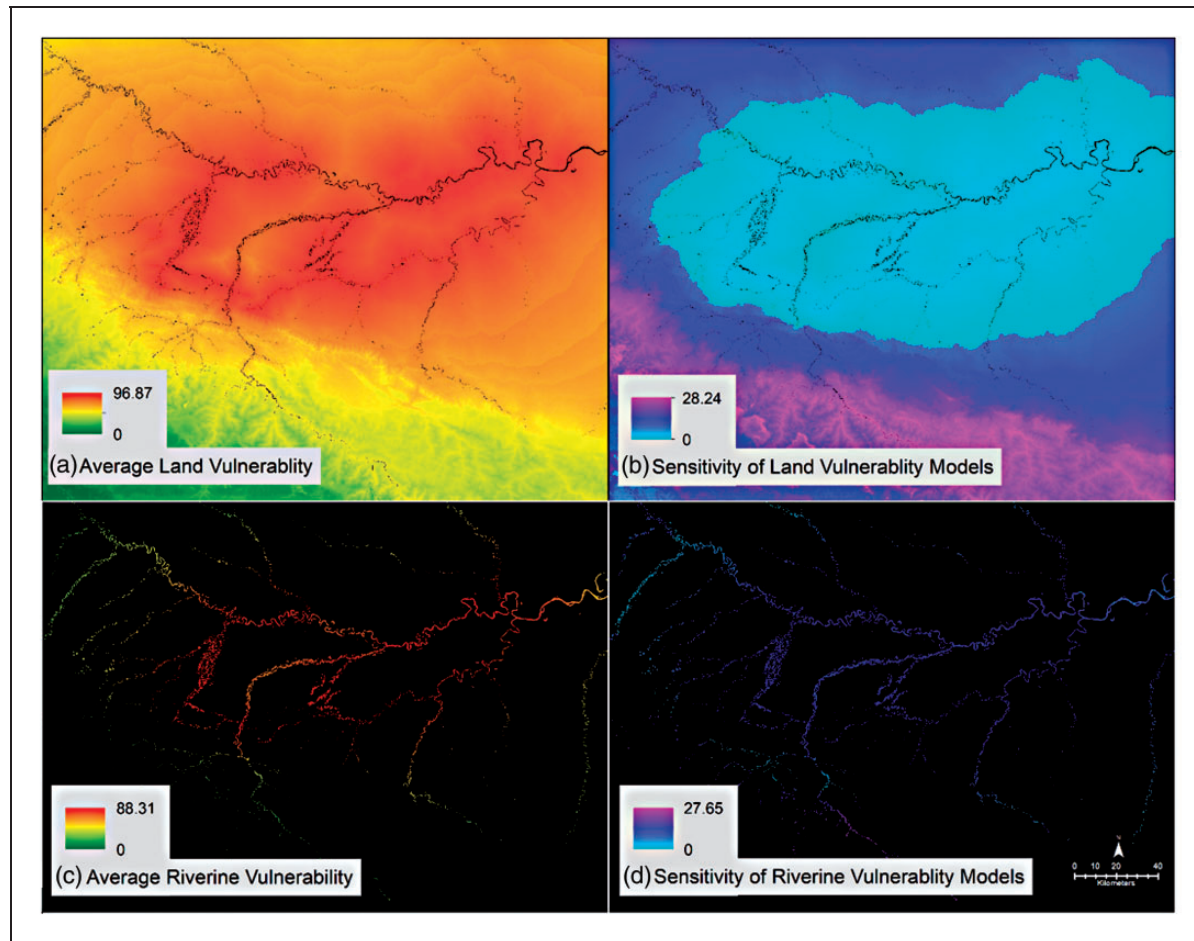
To account for different levels of uncertainty in the aggregation of factors, an ordered weighted average approach was used, which varies the level of ORness (or risk) in the aggregation process. An ORness of 0 corresponds to a risk-averse aggregation, while an ORness of 0.5 corresponds with intermediate risk and an ORness of 1 correspond to a risk-taking aggregation. Models were run with ORness levels 0, 0.25, 0.5 (a weighted linear combination), 0.75, and 1.0 for both land and rivers in order to evaluate the effect of factor aggregation in the identification of vulnerability to Hg pollution. A nonlinear model was used to determine weights that maximize entropy while observing the predetermined ORness (Malczewski et al., 2003). The sensitivity of the vulnerability model to the level of risk included in the aggregation was determined by calculating the standard deviation for all models.

### Biodiversity Risk Assessment

To determine which areas are most at risk, watersheds were assessed using irreplaceability–vulnerability plots (Margules & Pressey, 2000). Irreplaceability–vulnerability analysis has been used previously to inform and prioritize protection (Linke, Pressey, Bailey, & Norris, 2007; Noss,

1999; Sangermano, Toledano, & Eastman, 2012). Beta diversity calculated as gamma diversity divided by the average alpha diversity (species richness) within a region (x-axis) was plotted against vulnerability (y-axis) at the watershed level (Figure 2). The analysis was also done using gamma diversity, or regional diversity, as irreplaceability and using the Range Restriction Index (RRI) which compares the area over which the species is found to the full study area, with higher RRI values indicating the majority of species have restricted ranges (Whittaker, 1972). Prioritization using gamma diversity identifies areas where the largest number of species will be affected. Prioritization using beta diversity identifies areas with high-species turnover. Identifying watersheds based on high RRI values prioritizes watersheds in which species have relatively small ranges when compared with the study area. The five watersheds with the highest vulnerability and beta (Figure 5(a) and (c)) and gamma (Figure 5(b) and (d)) diversity were identified and mapped.

Regions for the calculation of regional diversity (gamma), turnover (beta), and RRI were defined as watersheds derived from a digital elevation model. Irreplaceability scores and vulnerability scores were standardized by area. All measures of diversity were based on the distribution of amphibian and freshwater (crab, crayfish, fish, insect, and mollusk) and the International Union for Conservation of Nature species ranges and calculated using TerrSet's Habitat and Biodiversity Modeler (Eastman, 2016).



**Figure 3.** Average vulnerability of land area (a) and riverine area (c) and model sensitivity of land (b) and riverine area (d).

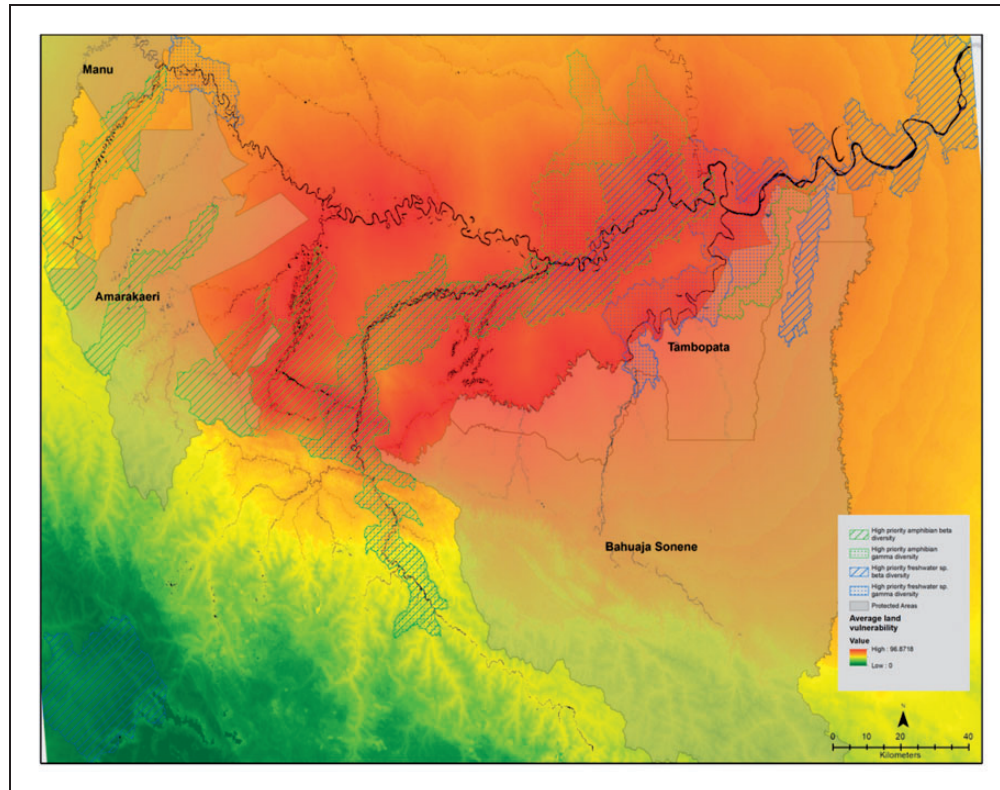
## Results

Much of the region is potentially vulnerable to Hg pollution. We identified 121.65 km<sup>2</sup> of land as active mining area and we assume this area to contribute to Hg pollution (Figure I in Supplementary Material II). While this represents a small portion of Madre de Dios' 85,301 km<sup>2</sup> of land, Hg from these sites may have a large spatial impact. On average, 23,250 km<sup>2</sup> of land (27% of the study region) presented a high vulnerability, identified as 70 or higher using a scale of 0 to 100 with 100 as maximum vulnerability (Figure 3). Vulnerable area totaling 10,795 km<sup>2</sup> extends into Tambopata National Reserve, Bahuaja-Sonene National Park, and Amarakaeri Communal Reserve. Land within Manu National Park was considered low risk for amphibians. There were scattered areas of high risk found along the Madre de Dios River in the northern portion of the study area. Some areas of high vulnerability were over 25 km from identified mining area. High land vulnerability was largely concentrated around rivers, particularly the Inambari, the Puquiri, and selected portions of the Madre de Dios and Tambopata Rivers.

Vulnerability for riverine area reached as high as 89 for locations adjacent to identified active mining areas. Of the 70,565 km<sup>2</sup> of freshwater areas considered in calculations, 66,263 km<sup>2</sup> vulnerability scores an average of 70 or higher. Almost the entire portion of the Madre de Dios River within the study area was highly vulnerable with scores declining west of where the river intersects with the Los Amigos River. Much of the Inambari River was also highly vulnerable. Active mining was identified along the majority of the Puquiri River, and thus vulnerability was found to be high for this river also. A few scattered mines were identified along the Malinowski River adjacent and within Tamboata National Reserve. All along the eastern border of Tambopata the river presented high vulnerability.

Models were sensitive to the ORness level selected, but sensitivity increased outward from riverine/mining areas (Figure 3), meaning that for areas near riverine and mining locations model results were consistent across aggregation methods.

Identifying high-priority watersheds using amphibian beta diversity or species turnover as irreplaceability prioritized watersheds primarily in the eastern portion of the



**Figure 4.** High-priority watersheds. Watersheds identified as having high land vulnerability and high amphibian irreplaceability are in green. Watersheds identified as having high riverine vulnerability and high freshwater species irreplaceability are in blue. High-priority watersheds in which irreplaceability was measured as beta distribution are lined and those using gamma distribution are dotted. Protected areas are outlined in grey.

study region (Figure 5(a)). The highest, second highest, and fourth highest priority watersheds were adjacent to one another between Amarakaeri Communal Reserve and Bahuaja Sonene National Park. Active mining was not identified in the third and fifth highest priority watersheds. Prioritizing gamma diversity and land vulnerability, high-priority watersheds were found in west of Puerto Maldonado along and just north of the Madre de Dios River (Figure 5(b)). Four of these watersheds were adjacent to one another and outside of nationally protected areas with the fifth highest priority watershed overlapping with Tambopata National Reserve. No active mining areas were identified in the fourth watershed. None of the high-priority watersheds identified using either beta or gamma diversity contained many species with highly restricted ranges, as indicated by the low RRI values.

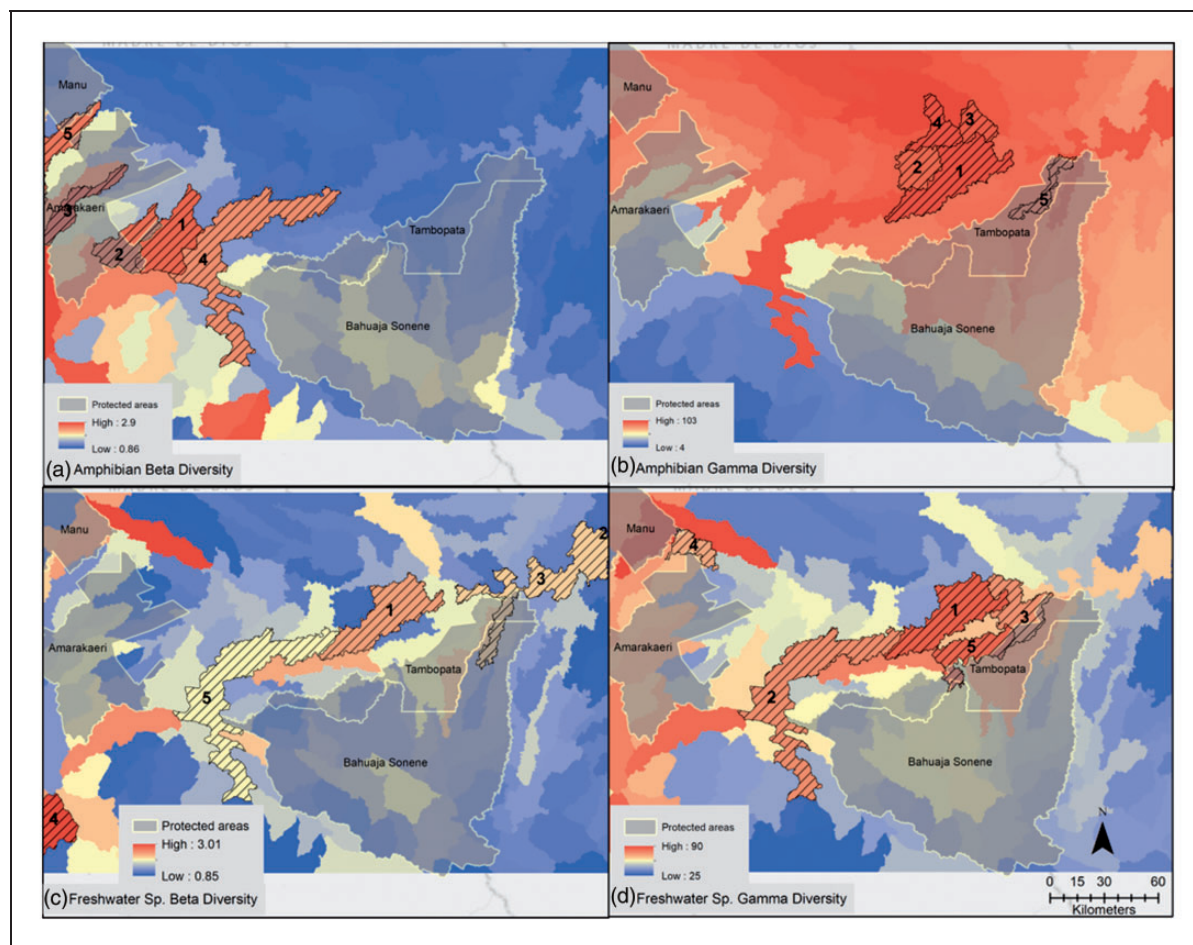
When considering riverine vulnerability and beta diversity of freshwater species, high-priority watersheds were spread out across the study area (Figure 5(c)). The highest priority watershed contains a portion of the Madre de Dios River and it is outside nationally protected areas. No active mining was identified in the fourth watershed, located in the southwestern portion

of the study region. Watersheds that were of high priority when considering gamma diversity were more clustered than when considering beta diversity (Figure 5(d)). No active mining was identified in the fourth-ranking watershed, adjacent to Manu National Park and removed from the other high-ranking watersheds. Freshwater species found within the fifth highest priority watershed considering gamma diversity had high restricted ranges, as indicated by RRI values of 0.61. The same watershed along the Madre de Dios ranked highest in priority when either beta or gamma diversity were used as the measure of irreplaceability.

## Discussion

Considerable attention has been paid to mining-related deforestation in Madre de Dios, but the extent which Hg pollution is affecting wildlife remains largely unknown. At an ORness level of 0.75, an intermediate-high level of risk allowing a significant amount of trade-off in factors contributing to vulnerability, we found that 23,250 km<sup>2</sup> of land are vulnerable to Hg pollution from ASGM activity. Because this high ORness level allows for high values in one factor to balance low values in a separate factor





**Figure 5.** Beta diversity, also known as species turnover, for amphibians (a) and freshwater species (c) for the study area are shown. Also shown is gamma diversity, or regional diversity, for amphibian (b) and freshwater (d) species. High-priority watersheds are striped and numbered according to their relative irreplaceability–vulnerability score. Note in panel (c) that the watershed with the second highest priority has a very small area and is barely visible at this scale in the upper right-hand corner of panel (c).

contributing to vulnerability, our estimates may be quite conservative if this flexibility is not present in the natural system.

Reported estimates represent the potential vulnerability for the dry season only. Vulnerability likely differs during the rainy season due to hydrological factors, such as the increase in flooded area as well as potential variance in suspended sediment loads.

Our results are particularly worth noting in regard to amphibian conservation, for which this region is renowned. Waste from mining activities is one of the most important sources of water contamination for Peruvian amphibians (Catenazzi & von May, 2014). A recent reassessment of Peruvian amphibians identified habitat loss and pollution as the main threats (Jarvis et al., 2015). Of the 163 amphibian species present within the study area, 119 or 73% are at least partially aquatic. Amphibian species within the area identified as high risk include *Dendropsophus allenorum* and *Ameerega simulans*, endemic to Peru, as well as

species labeled as data deficient from the International Union for Conservation of Nature such as *Hamptophryne alios*. Watersheds identified as high priority and that were identified as having active mining include Watersheds 1, 2, and 4 in Figure 5(a) and Watersheds 1, 2, 3, and 5 in Figure 5(b) with no overlap.

In regard to aquatic species, several endemic or threatened freshwater fish may be at risk including *Apistogramma urteagai* (Kullander, 1986), *Ancistrus heterorhynchus* (Fisch-Muller, 2003), *Hyphessobrycon nigricinctus* (Eschmeyer, 2005). With almost 2,200 recognized species of fish, the Amazon Basin is the most diverse in terms of fish fauna (Albert & Reis, 2011). Amazonian freshwater ecosystems have received little attention, and data needed to manage these ecosystems are largely unavailable (Junk & Piedade, 2004), making vulnerability assessments and identification of management priorities difficult (Castello et al., 2013). To conserve freshwater species within the region, Watershed 3 in Figure 5(c) should be prioritized for its high gamma diversity and



high RRI. The same watershed ranks highest for both gamma and beta diversity and thus should be prioritized (Figure 5(c) and (d)). It currently falls outside protected areas.

Along the Madre de Dios River, between the city of Puerto Maldonado and where the Inambari River joins the Madre de Dios river, vulnerability to Hg pollution is high. Hg is likely transported along this portion of the Madre de Dios river (MdD) from the Guacamayo mining area (Figure 1), where a substantial amount of ASGM activity occurs, as well as from pockets of ASGM activity along the MdD itself. This area is largely unprotected except for a few ecotourism concessions.

Our method for identifying areas of active mining and Hg pollution was conservative; the intersection of two classification methods was ultimately used to identify mining area. We assume all 21.65 km<sup>2</sup> of land identified as mining activity (Figure I in Supplementary Material II) equally contributes to Hg pollution, but this may not be the case. While, future work ideally should further calibrate and validate the spectral signature of mining where Hg is being released, this may not be possible in Madre de Dios until conditions are safe. Area adjacent to the Puquiri River provides potential opportunities for model calibration, if safe and reliable access can be obtained along the river. The Guacamayo mining area may provide more logical calibration/validation sites given road access in this region.

Results are limited by our ability to verify sites where Hg was released into the environment, as visiting sites with active mining proved unsafe. This represents a limitation of our validation, but one we do not believe greatly affected our results, as the final classification map used to determine inputs to the vulnerability model was verified by consulting local experts. We did not model atmospheric transport of Hg, which may also affect accuracy. However, analysis of Hg released from 3,000 sites associated with Hg production and use suggests that the majority of Hg emissions is not from atmospheric emissions but is transported from sites through hydrological processes (Kocman, Pirrone, & Cinnirella, 2013). Estimates of Hg release specifically from ASGM also show that most Hg is released into the hydrosphere (Telmer & Veiga, 2009). Seasonal migrations of species were not considered in this study. While a species home range may cover areas of high and low or no risk, potential exposure is only expected to occur when the individual occupies areas of high risk. Trophic level effects were beyond the scope of this research, and it is logical to expect species preying upon Hg-exposed species could have elevated Hg levels. This study does not capture such effects. It is also important to note that only conservation areas formally protected under the federal government were included in this study.

In summary, our work suggests Hg pollution from ASGM is at such a spatial extent that it could threaten biodiversity in Madre de Dios. Vulnerability extends into protected areas. Amphibian gamma diversity is vulnerable in unprotected areas north of the Madre de Dios River, and aquatic gamma diversity is threatened within watersheds along the Inambari and Madre de Dios rivers (Figure 5).

## Implications for Conservation

In addition to deforestation from ASGM, high levels of Hg found in raptors and fish are thought to be linked to ASGM in Madre de Dios (Diringer et al., 2015; Shrum, 2009). A 2012 study found that fish consumption and location of residence are significant risk factors for elevated Hg levels, with households in mining zones at increased risk (Ashe, 2012). Knowledge of the spatial extent of Hg vulnerability could aid in our decision-making process when considering how to balance the economic needs of humans with biodiversity. Many threatened amphibians occur outside of protected areas, and there is a recognized need to improve protection and further research population status and threats (Catenazzi & von May, 2014; Jarvis et al., 2015).

The vulnerability models presented in this article suggest that the largest mining operations on the landscape may not always be the worthiest of attention in regard to amphibian alpha and gamma diversity. Some of the watersheds identified as high priority are those where ASGM activity is less obvious in comparison with the Huepetuhe and Guacamayo mining areas. In general, many of the watersheds considered do not overlap with protected areas (Figures 4 and 5).

For freshwater species, Watershed 3 in Figure 5(c) should be prioritized for its high gamma diversity and high RRI. The same watershed ranks highest for both gamma and beta diversity (Figure 5(c) and (d)). Amphibian gamma diversity high-priority Watershed 3 overlaps with area previously identified as a critical management zone (Thieme et al., 2007). The highest priority watershed for amphibian gamma diversity, which is also the highest priority watershed for freshwater beta diversity, also overlaps with area classified as a potential threat mitigation zone previously (Thieme et al., 2007). In general, the mainstream Madre de Dios and its floodplains have been recognized as a habitat class lacking protection in this region (Thieme et al., 2007).

Managing of mining concessions should consider potential vulnerability and areas of high biodiversity to safeguard the region's unmatched biodiversity. We suggest watersheds identified in this study be protected using an adaptive comanagement approach in which participants share knowledge, and training is provided for regional and local stakeholders (Armitage et al.,

2009). Areas outside the high priority watersheds indicated should not be entirely disregarded, as our methods did not consider species' population size, rates of decline, and existing threats.

ASGM in Madre de Dios is largely unregulated and illegal, thus legalizing and formalizing existing mining efforts should be considered. This could ensure that workers have the proper tools and training to protect themselves and the surrounding ecosystems from the harmful effects of Hg and could have a real impact for both the industry and the biodiversity currently threatened.

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### Supplemental Material

Supplemental material for this article is available online.

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