UNIVERSIDAD SAN FRANCISCO DE QUITO USFQ

Colegio de Posgrados

Impactos de actividades antropogénicas discriminados por elementos mayores y traza en el camarón de río *Macrobrachium brasiliense* en la Amazonía ecuatoriana

Javier Patricio Mena Olmedo

Andrea C. Encalada, PhD.
Directora de Trabajo de Titulación

Laurence Maurice, PhD.
Valeria Ochoa-Herrera, PhD.
Co-directoras de Trabajo de Titulación

Trabajo de titulación de posgrado presentado como requisito
para la obtención del título de Master en Ecología, Estudios Amazónicos

Quito, 22 de diciembre de 2016

UNIVERSIDAD SAN FRANCISCO DE QUITO USFQ

COLEGIO DE POSGRADOS

HOJA DE APROBACIÓN DE TRABAJO DE TITULACIÓN

Impactos de actividades antropogénicas discriminados por elementos mayores y traza en el camarón de río *Macrobrachium brasiliense* en la Amazonía ecuatoriana

Javier Patricio Mena Olmedo

Firmas

Andrea Encalada, Ph.D.

Directora del Trabajo de Titulación

Laurence Maurice, Ph.D.

Co-directora del Trabajo de Titulación

Valeria Ochoa, Ph.D.

Co-directora del Trabajo de Titulación

Margarita Brandt, Ph.D.

Directora del Programa de Maestría en Ecología

Stella de la Torre, Ph.D.

Decana del Colegio de Ciencias Biológicas y Ambientales

Hugo Burgos, Ph.D.

Decano del Colegio de Posgrados

Quito, 22 de diciembre 2016

© Derechos de Autor

Firma del estudiante: ____

Nombre: Javier Patricio Mena Olmedo

Código de estudiante: 00127539

C. I.: 171904585-6

Lugar, Fecha: Quito, 22 de diciembre 2016

AGRADECIMIENTOS

Este trabajo fue desarrollado con el apoyo y bajo la dirección de Laurence Maurice, Andrea Encalada y Valeria Ochoa, profesoras de la USFQ. Agradezco a Jonathan Prunier, Christelle Lagane, Laure Laffont, Fausto Lopez y Phillipe Besson investigadores del Laboratorio Géosciences Environnement Toulouse (GET), Observatoire Midi Pyrénées, Université de Toulouse, por su valiosa ayuda con el trabajo de laboratorio en Francia. Además, agradezco al programa de investigación franco-ecuatoriano MONOIL "Monitoreo Ambiental, Salud, Sociedad y Petróleo en Ecuador" por permitirme ser parte de esta iniciativa. Así mismo, agradezco al Instituto Francés de Investigación para el Desarrollo Sostenible IRD, y a la Agencia Francesa de Investigación ANR (ANR-13 SOCENV-0003-01) por patrocinar el estudio. Emito un especial agradecimiento a Carlos Mena por el apoyo con parte del financiamiento por medio del Proyecto ABE, Biodiversidad Amazónica y Educación, coordinado por la USFQ y patrocinado por la Universidad de Carolina del Norte en Chapel Hill. Agradezco a Margarita Brandt, directora de la maestría, y al personal administrativo de la USFQ. Envío un agradecimiento especial al Ministerio del Ambiente del Ecuador por la autorizaciones y permisos concedidos al proyecto de manera cordial y oportuna. De la misma forma, agradezco a mis colegas Cristhian Moreno e Irina Cuesta por su apoyo físico y moral en el trabajo de campo; a Jonathan Valdivieso del Instituto Nacional de Biodiversidad-Museo Ecuatoriano de Ciencias Naturales por su apoyo en el campo; a Vladimir Carvajal del Museo de Historia Natural Gustavo Orcés-Escuela Politecnica Nacional, por su apoyo con información e identificación de los especímenes colectados;

a Jenny Ruales del Departamento de Ciencia de Alimentos y Biotecnología de la Escuela Politécnica Nacional por permitirme el acceso y uso de su equipo de laboratorio. Agradezco al Tncl. Valery Vásconez del Batallón de Selva 61 “Santiago” por su acogida en el destacamento militar durante nuestra campaña de muestreo en Morona Santiago. Envío un agradecimiento especial a todos los investigadores, asistentes, estudiantes y voluntarios que colaboraron en varias etapas de este trabajo, entre ellos a: Ana Dolenc y José Schrekinger del Laboratorio de Ecología Acuática-USFQ; Gisell Pazmiño, Esteban Tamayo y Verónica Yumiceba del Laboratorio de Ingeniería Ambiental-USFQ; a Mayra Molina, Norma Díaz y Magali Quimbita de la Facultad de Ingeniería Química y Agroindustria-Escuela Politécnica Nacional; Jonathan Martínez, Natalia Quezada, Diana Iza y Jorge Fuel, de la Facultad de Ciencias Químicas, Química Farmacéutica-Universidad Central del Ecuador; y para todos los asistentes de campo y dirigentes de las comunidades donde se realizó la campañas de muestreos.

Finalmente, agradezco a Ana María Olmedo y Mónica Arellano por su apoyo incondicional.

RESUMEN

Este estudio presenta, por primera vez, concentraciones de elementos mayores y metales traza en el camarón de agua dulce más abundante *Macrobrachium brasiliense* (Crustacea-Palaemonidae) en la Cuenca Hidrográfica Amazónica ecuatoriana. Nuestro trabajo se centra en la Amazonía ecuatoriana afectada por actividades antropogénicas. Seis ríos de la Amazonía norte ecuatoriana afectados por las actividades petroleras fueron muestreados y comparados con cuatro ríos geomorfológicamente similares en la Amazonía sur ecuatoriana, libres de actividades petroleras o mineras. Los elementos mayores y metales traza fueron cuantificados usando Espectrometría de Emisión Óptica de Plasma Acoplada Inductivamente (ICP-OES) y Espectrometría de Masa de Plasma Acoplada Inductivamente de Alta Resolución (HR-ICP-MS), respectivamente. Para el análisis de mercurio total en las muestras de tejido se utilizó Espectrometría de Absorción Atómica (AMA). Se registraron diferencias significativas entre lugares para varios elementos; sin embargo, las concentraciones realmente altas de Cr, Co y Ba en los camarones de los ríos Conde y Guashito sugieren que esos arroyos son los únicos que están fuertemente afectados directamente por la industria petrolera dentro de los sitios amazónicos del norte de Ecuador. De manera inesperada, *Macrobrachium brasiliense* de los ríos amazónicos del sur de Ecuador presentó las mayores concentraciones de Cs, Pb, As y Hg. Esta tendencia podría ser explicada por los procesos de liberación de metales originados por la erosión volcánica del suelo acelerada por los altos índices de deforestación medidos en la Provincia Morona-Santiago en el período 2008-2014. El mercurio no puede ser considerado como un proxy para el rastreo de las actividades petroleras; sin embargo, puede evaluar los impactos de la deforestación, especialmente en áreas tropicales donde los suelos andinos también pueden ser enriquecidos en mercurio por erosión de roca volcánica. Respecto a la evaluación del riesgo para la salud humana, las concentraciones de Pb y Cd en los camarones de los ríos Blanco, Yananas, Conde y Due excedieron los límites permitidos establecidos por la Organización Mundial de la Salud (OMS), la FAO, la Comisión Europea, los niveles máximos de contaminantes químicos en alimentos de Canadá , y el Ministerio de Salud Pública de Colombia (MSPS). Las concentraciones de mercurio no superaron los límites permitidos establecidos. Los camarones del río Basura presentaron altos contenidos de varios elementos tóxicos (Al, Co, V), probablemente debido a los efluentes de diversas actividades antropogénicas ubicadas aguas arriba de los puntos de muestreo, como compañías de extracción de aceite de palma o de fabricación de tuberías de petróleo. Para todos los metales pesados tóxicos evaluados, el riesgo para la salud humana por el consumo de camarón en los ríos muestreados es bajo. Sin embargo, de acuerdo con la ingesta diaria tolerable provisional de cromo (Cr PTDI) y los límites permisibles establecidos por el Instituto de Medicina - Academia Nacional de Ciencias de EE.UU., la población más expuesta se encuentra en el río Guashito para los tres grupos evaluados (hombres, mujeres y niños), siendo los niños los que merecen mayor atención.

Palabras clave: *Macrobrachium brasiliense*, metales traza, elementos mayores, mercurio, deforestación, Amazonía ecuatoriana, actividades antropogénicas, ingesta diaria.

ABSTRACT

This study presents, for the first time, concentrations of major elements and trace metals in the most abundant freshwater shrimp *Macrobrachium brasiliense* (Crustacea-Palaemonidae) in the Ecuadorian Amazon Basin. Our work focuses on the Ecuadorian Amazon affected by anthropogenic activities. Six rivers of the northern Ecuadorian Amazon affected by oil activities were sampled and compared with four geomorphological similar rivers in the southern Ecuadorian Amazon, free of oil or mining activities. The major elements and trace metals were quantified using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) and High Resolution Inductively Coupled Plasma Mass Spectrometry (HR-ICP-MS), respectively. For the analysis of total mercury in the tissue samples, Atomic Absorption Spectrometry (AMA) was used. There were significant differences between sites for several elements; however, the very high concentrations of Cr, Co, and Ba in shrimp from the Conde and Guashito rivers suggest that these streams are the only ones that are directly affected by the oil industry within the Amazonian sites of northern Ecuador. Unexpectedly, *Macrobrachium brasiliense* from the Amazon Rivers of southern Ecuador presented the highest concentrations of Cs, Pb, As and Hg. This trend could be explained by the release of metals caused by volcanic soil erosion accelerated by the high deforestation rates measured in the Morona-Santiago Province in the period 2008-2014. Mercury cannot be considered as a proxy for tracking oil activities. However, it can assess the impacts of deforestation, especially in tropical areas where Andean soils can also be enriched in mercury by volcanic rock erosion. Regarding the risk assessment for human health, the concentrations of Pb and Cd in shrimps of the Blanco, Yananas, Conde and Due rivers exceeded the limits allowed by the World Health Organization (WHO), FAO, European Commission, the maximum levels of chemical contaminants in food in Canada, and the Colombian Ministry of Public Health (MSPS). The mercury concentrations did not exceed the allowed limits established. Shrimp from the Basura River presented high contents of several toxic elements (Al, Co, V), probably due to effluents from various anthropogenic activities located upstream of the sampling points, such as palm oil extraction or oil pipelines. For all toxic heavy metals evaluated, the risk to human health from shrimp consumption in the sampled rivers is low. However, according to the provisional tolerable daily intake of chromium (Cr PTDI) and permissible limits set by the Institute of Medicine - National Academy of Sciences of the United States, the most exposed population is located on the Guashito River for the three groups (men, women and children), with children deserving more attention.

Key words: *Macrobrachium brasiliense*, trace metals, major elements, mercury, deforestation, Ecuadorian Amazon, anthropogenic activities, daily intake.

TABLA DE CONTENIDO

CHAPTER 1

Anthropogenic activities impacts on freshwater shrimp *Macrobrachium brasiliense* of the Ecuadorian Amazon discriminated by major and trace elemental signatures

Anthropogenic activities impacts on freshwater shrimp *Macrobrachium brasiliense* of the Ecuadorian Amazon discriminated by major and trace elemental signatures

[1,2]Javier P. Mena-Olmedo*, [3]Laurence Maurice, [3] Jonathan Prunier, [3]Christelle Lagane, [3] Laure Laffont,[1,4]Valeria Ochoa-Herrera, [1,5]Andrea C. Encalada

[1]Universidad San Francisco de Quito, Colegio de Ciencias Biológicas y Ambientales (USFQ-COCIBA)
[2]Maestría en Ecología - Estudios Amazónicos USFQ
[3]Géosciences Environnement Toulouse (GET) Laboratory, Observatoire Midi Pyrénées, IRD, CNRS, Université de Toulouse, Toulouse, France
[4]Colegio de Ciencias e Ingeniería, USFQ - Politécnico
[5]Laboratorio de Ecología Acuática, USFQ-COCIBA
*e-mail: pmenaolmedo@gmail.com

Abstract

This study presents, for the first time, concentrations of major and trace elements in the most abundant freshwater shrimp *Macrobrachium brasiliense* (Crustacean–Palaemonidae) in the Ecuadorian Amazon Basin. Our work focuses in the Ecuadorian Amazon basin affected by anthropogenic activities. Six rivers of the Northern Ecuadorian Amazon affected by oil activities were sampled and compared with four geomorphological similar rivers in the Southern Ecuadorian Amazon free from oil or mining activities. The major and trace metal elements were quantified using Inductively Coupled Plasma−Optical Emission Spectrometry (ICP-OES) and High Resolution Inductively Coupled Plasma−Mass Spectrometry (HR-ICP-MS), respectively. Significant differences were recorded among locations for several elements; however, really high concentrations of Cr, Co and Ba in the freshwater shrimps of Conde and Guashito Rivers, suggest that those streams are the only ones heavily affected directly by oil industry within the Northern Ecuadorian Amazon sites. Quite unexpectedly, the *Macrobrachium brasiliense* from the Southern Ecuadorian Amazon Rivers presented the highest Cs, Pb and As concentrations. This trend could potentially be explained by metal releasing processes originated from the volcanic soil erosion accelerated by the high deforestation rates measured in the Morona-Santiago Province within 2008-2014 period. Regarding the human health risk assessment, the Pb and Cd concentrations in the freshwater shrimps of the rivers Blanco, Yananas, Conde and Due exceeded the permissible limits established by the World Health Organization (WHO), FAO, European Commission (EC), the Health Canada's Maximum Levels for chemical contaminants in food and the Colombian Public Health Ministry (MSPS). The shrimps from the Basura River presented high contents of several toxic elements (Al, Co, V) probably due to the effluents from diverse anthropogenic activities located upstream the sampling point, such as a palm oil extraction or oil pipe fabrication companies. For all measured toxic heavy metals, the human health risk from shrimp intake in the sampled rivers is low. However, according to the chromium provisional tolerable daily intake (PTDI) and the established permissible limits from the Institute of Medicine – National Academics of Sciences US, the most exposed population are located in the Guashito River for the three evaluated groups of people (men, women, and children), being children who deserves more care.

Key Words: *Macrobrachium brasiliense*, metals, major elements, Ecuadorian Amazon, anthropogenic activities, daily intake.

Introduction

The bioaccumulation of heavy metals in the aquatic food chain of the Ecuadorian Amazon is not known despite of intense anthropogenic activities developed in this region; however, loss of quantity and quality of food resources in this area and consequently a decrease in productivity of these ecosystems (Lytle and Peckarsky 2001) have been attributed to human activities. In addition, a potential public human health risk directly linked to the consumption of the natural resources from these polluted environments has been recently identified in rural and urban areas (Wuana and Okieimen 2011).

There are many ways for metals to enter the aquatic ecosystems. Atmospheric deposition, rock weathering and soil erosion and anthropogenic inputs caused by industrial effluents, domestic sewage, nuclear testing or oil and mining wastes (Reddy et al. 2007) are the most common. Metals that are deposited in the aquatic environment may accumulate in the food chain and cause ecological damage also posing a threat to human health due to biomagnification over time and the trophic chain (Yilmaz and Yilmaz 2007). Aquatic organisms can accumulate heavy metals, such as Hg, As, Cd, Cr, Co, Pb, Sn, and Sb in their tissues several times above ambient levels (Canli and Atli 2003) increasing their concentration through time.

Since the 1960s, when the oil exploitation started in the Northern Ecuadorian Amazon (NEA), especially in Sucumbíos and Orellana provinces, oil industry has been considered as the main contaminating anthropogenic activity. In addition, it is also a driver for other polluting activities such as deforestation, urbanization, agriculture, cattle farming, etc. (Bass et al. 2010; Finer et al. 2008), which have brought significant negative environmental costs contaminating water resources, soils, and consequently the organisms that depend on them, including human beings (Finer et al. 2008; Bass et al. 2010; McCracken and Forstner 2014; Lytle and Peckarsky 2001; Dudgeon and Arthington 2006). Roads construction has opened new access for people to previous isolated areas where they have expanded their impact to pristine zones (Bilsborrow et al. 2004).

Being globally well known for having the greatest biodiversity in the world for the major groups of vertebrates and plants (Bass et al. 2010; Pitman et al. 2002; Finer et al. 2008), the Ecuadorian Northern Amazon is actually being threatened seriously by human activities. In addition, cultural goods and services by the presence of several indigenous communities with valuable ancestral knowledge, some even in voluntary isolation, have increased the importance of the region to be conserved not only nationally but also for the global community (Lu et al. 2011).

Most of the impacts of the oil industry and its indirect activities have not been descripted and accurately quantified regarding the multiple components of the ecosystem; however, it's generally known that this activity introduces in the environment toxic substances such as polycyclic aromatic hydrocarbons (PAHs) and heavy metals which are considered as a potential danger (Muniz et al. 2004) .

An efficient and practical way to evidence contamination in the environment is trough bioindicators (Hodkinson and Jackson 2005). Aquatic macroinvertebrates are considered as an appropriate bioindicator group par excellence. Due to the water dependency for their growth, water quality is also measured, as one of the main parameters to determine the habitat quality (Hodkinson and Jackson 2005). Macroinvertebrates constitute a group with high selectivity habitat and that can adapt to specific environment. Therefore, any change in the environmental conditions will be reflected in the structure and ecological composition of these communities (Terneus et al. 2012). Moreover, some organisms also called "sentinels" have certain characteristics such as representativeness, sedentariness, longevity, easy to be captured, big population size, widely study area distribution, and information availability which are preferably used for contamination studies (Hodkinson and Jackson 2005; Mcgeoch 1998; Reif 2011). Crustaceans and bivalves have been successfully studied as biological indicators for the determination and evaluation of effects of pollutants in the aquatic environment due to bioaccumulation processes. Some crustaceans such as shrimp and crab can be used for the application of biological and chemical tests that use an indicator organism to assess the environment (Darmono and Denton 1990; Kress et al 1998; Mantelatto et al. 1999).

In this study, we choose the freshwater shrimp *Macrobrachium brasiliense* for its widely distribution in South America (Anger 2013). It has been registered in inland rivers in Venezuela, Colombia, Guyana, French Guiana, Suriname, Ecuador, Peru, Bolivia and Brazil, in the Orinoco and Amazon basins and Guiana rivers that drain into the Atlantic Ocean (Pileggi et al. 2013; Rodriguez 1981; Holthuis 1959). This species is the most abundant freshwater shrimp in the Ecuadorian Amazon being registered from watersheds to sub-watersheds in Sucumbios, Orellana, Napo, Pastaza, Morona Santiago and Zamora Chinchipe provinces (MHNGO 2015). *M. brasiliense* is characterized by omnivorous feeding habits registering a huge diversity of organisms in their diet such as insects, diatoms, oligochaeta, plants, fungi, sand, from a content stomach analysis (Melo and Nakagaki 2013). This species occupies the median levels in the food chain, so it might probably be exposed to eventual contamination in river freshwater and sediments. Being one of the principal items found in the diet analysis of *M. brasiliense* (Melo and Nakagaki 2013), sand and sediments constitute 87.10 % and 90% respectively of their stomach content. Riverbed and bottom sediments are also where the contaminants are mostly stored in the rivers. This distribution, abundance and physiological characteristics make *M. brasiliense* a good study species to evaluate the environmental contamination risk in freshwater ecosystems.

Our main objective is to determine the concentrations of major and trace elements in the freshwater shrimp *Macrobrachium brasiliense* (Crustacea – Palaemonidae) that inhabits rivers of the Ecuadorian Amazon basin and identify the elements which are specifically linked to anthropogenic activities, such as oil industry, that can be classified as proxy for tracking these activities in natural ecosystems. Finally, we evaluated the human health risk based on shrimp regular consumption. We studied six rivers of the Northern Amazon affected by oil activities and compared them with four geomorphological similar rivers in the Southern Amazon area that are free from oil and mining activities.

Study Area

Two study areas in the Ecuadorian Amazon were selected considering the presence of the oil industry and its direct and indirect impacts. Northern Amazon (NA) was selected as the "affected area" due to its historic contamination issues while Southern Amazon (SA) was selected as the "control area" having no evidence of major anthropogenic activities related to extractive activities mainly oil industry (MAE-PRAS ZIL Maps 2015). The NA included Sucumbíos and Orellana provinces, while the SA area was located in the Morona Santiago province.

Ten sampling points (Table 1 and Figure 1, 2) were established for this study. Considering the areas with anthropogenic activities mainly originated from the oil industry and its indirect impacts, six sampling points were selected in the NA, each one of them located on different sub-watersheds of the Napo (M15-01 to M15-06 sampling points) and Coca rivers (M15-02 and M15-04). The NA sampling points were located in rivers where previous data (from the PRAS, and the MONOIL Program) evidenced contamination risks in the area. Rivers have also been selected for their geomorphological characteristics that allow to the development of the freshwater shrimp *Macrobrachium brasiliense*. On the other hand, four sampling points were established in sub-watersheds of the Santiago River in the SA (M15-08 to M15-11 sampling points), where there was no evidence of oil industry nor mining activities, upstream the Santiago River tributaries (ARCOM 2016). For this purpose, rivers with similar conditions to the NA Rivers as possible such as environmental, formation and structure were selected (Table 1, Figure 1).

The sampling campaign in the NA and in the SA was held during the dry season, from September 14th to 26th, 2015, and from November 10th to 15th, 2015, respectively. During these campaigns, fresh-water shrimp individuals of one single species *Macrobrachium brasiliense* were collected in all the sampling points along 150 m transect of each river. In addition, physico-chemical and microbiological parameters such as length, width and depth of the river, turbidity, fluvial habitat index (IHF), riparian habitat quality index (QBR), total suspended solids, nutrients, pH, conductivity, temperature, dissolved oxygen, alkalinity, ORP, COD and coliforms were measured.

Materials and methods

Physico-chemical and ecological parameters of the surface waters

The rivers depth and width and the number of meanders within 100 meters were measured at each sampling point. The Index of Fluvial Habitat Quality-IHF (Pardo et al. 2002) and the Index of Riparian Habitat Quality-QBR (Munné et al. 2003) were calculated using a variation (LEA-USFQ) of the protocols developed by the Freshwater Ecology and Management Research Group – Barcelona University.

The total suspended solids (TSS) were estimated using the Protocol for Seston Sampling in Streams and small rivers and the Standard Processing Protocols (Wallace 2013). Filters Whatmann (~0.7 µm porosity, 47 mm diameter, GF/F) were used for the water filtration process. The filtration procedure was performed in triplicate per each river.

An YSI–556 MPS multiparameters device was used to measure *in situ* pH, conductivity, temperature, dissolved oxygen, and ORP. In the USFQ Environmental Sciences Laboratory, the SM 5220 B ASTM 1995 method was performed to obtain chemical oxygen demand (COD). Nutrients analyses were achieved using a single surface water sample per river without filtration trough La Motte test kits Nitrate/Nitrite and Ammonium. Alkalinity measurements were realized in the field using the Gran method (Gran, et al. 1981).

Microbiological parameters

Bacteriological analysis of *Escherichia coli* and total coliforms was performed using the ®3M Petrifilm E. coli/Coliform count plates for triplicate per each river water sample (10 water samples total) in the field.

Shrimp samples were collected using small hand fishing net mainly during nighttime due to their nocturnal activity. Once captured, total body and chelas lengths, weight and also individual physiological characteristics such as sex, age, and reproductive state were measured. The individuals were labeled and stored in plastic coolers with dry ice and transported frozen to the laboratories where they were cleaned with deionized water and completely freeze-dried. The exoskeleton was not removed as individuals were quite small and generally local people consume the entire animals. The individuals were weighted again to know exactly the dry mass before their mineralization. The entire dried body was grinded and homogenized until obtaining a powder result. For individuals that didn't reach the minimum weight required (1.5 g dw), several freeze-dried individuals within the same range of size and wet weight were pooled until getting

1.5 g sample. Finally, nighty seven samples were obtained for the ten sampling points (with an average of 10 individuals per river).

For their mineralization, One gram of each freeze-dried sample was digested in hermetic Teflon vials using 5 ml nitric acid (14.6 N), 1 ml hydrogen peroxide (30%) and 2.5 ml hydrochloric acid (9.5 N) during 24 h at 120°C and 80 °C, respectively. Ultrasonic waves were applied to the samples to improve the process. Furthermore, blanks and certified reference material (TORT-3) samples were also treated in the same way in order to validate the method and evaluate the accuracy of the instruments. All this procedure was performed in ultra-clean room at the GET laboratory (Toulouse, France).

Concentrations of major elements were determined in the mineralized samples using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES, Horiba Hobin Yvon Ultima 2). Trace metals and metalloids (V, Cd, Mn and Ba) were quantified by Mass Spectrometry (ICP-MS, Thermo Scientific iCAP-Q), and High Resolution Mass Spectrometry (HR ICP-MS, Neptune Thermofinnigan) for Rare Earth Elements and all the other metallic elements. The accuracy per study element ranged from 1 to 5% while the average yield was 97 +/- 9%. When necessary, elemental concentrations were corrected from the CRM yields (Table 2).

Statistical Analysis

Data analysis was carried out using the SPSS statistical software. As variables presented normal distributions, one-way ANOVA and Pearson correlations were used between physico-chemical data of the rivers and chemical concentrations measured in shrimps.

Results and Discussion

The sampled rivers were selected according to their geomorphological and physico-chemical profiles. In order to facilitate the macro-invertebrates sampling, shallow water rivers draining forested area (with acidic pH and low conductivity) were preferentially chosen. The width of the study rivers varied between 6 m (for the Chichis and Due Rivers) and 12 meters being (for the Yananas R.). The deepest rivers were Río Blanco, Pacayaku and Kushapuku (1.5 m) whereas the shallowest one was Due (0.5 m). The river with less meanders within 100 meters was Due (1) and Basura (1) while with the highest were Río Conde and Chichis (3 each one) (Table 3).

The IHF and QBR indexes were calculated using the protocols developed by Pardo et al. (2002) and Munné et al. (2003), respectively. The IHF index is estimated using rivers physical characteristics such as stone embeddedness in riffles and runs, sedimentation in pools, riffle frequency, substrate composition, velocity/depth regime, shading of river bed, heterogeneity components, and aquatic vegetation cover while the QBR index uses characteristics of the vegetation cover and river structure such as total riparian cover, cover structure, cover quality, channel alteration, slope and form of the riparian zone, presence of one or several islands in the river, and presence of hard substrata. Both indexes score the status degree of each characteristic adding their values until getting the final score, which is 100 tops. The obtained index values were interpreted using their range quality level chart (Table 4).

All the NA Rivers appeared as disturbed, with IHF index ranging from 53 to 80. The river with the worst fluvial habitat quality “Strong alteration, bad quality” was the Blanco River (53) followed by the Conde River (55) (Figure 3). The rivers with the best fluvial habitat quality were Kusuimi (76) and Chichis (80), located in the SA.

The rivers with the best riparian habitat quality “Riparian habitat in natural condition” were Due (95) and Chichis (95) while the river with the worst fluvial habitat quality “Extreme degradation, very bad quality” was Basura (25) (Figure 4).

The average total suspended solids (TSS) concentrations in the river water samples was 9.8 $mg.l^{-1}$, and ranged from 4.0 to 24.4 $mg.l^{-1}$ corresponding to Yananas and Pacayaku rivers respectively.

Total coliforms were presented in all the surface water samples of the ten rivers and ranged from 3 to 85 UFC/ml being Río Conde and Yananas the rivers with the lowest values (3 UFC/ml) while Guashito river reached the highest value (85 UFC/ml). Ranging from 1 to 82 UFC/ml, environmental coliforms were found in every single river, Yananas with the lowest value and Guashito with the highest one respectively. Most of the rivers presented *E. coli* except the Chichis River, ranging from 2 UFC/ml (Yananas) to 9 UFC/ml (Blanco) (Figure 4). The percentage of *E. coli* found of the total coliforms in the ten sampled rivers was widely variable from 70% (for the Yananas and Pakayaku rivers) to 0.0 % and 3.5 % for the Chichis and Guashito rivers respectively (Figure 5).

This method is a good approach for assessing the likelihood of risk for human health due to bacterial presence (Bruhm and Wolfson 2007) as *E. coli* may be a good sign of contamination by anthropogenic wastes. Being the river with the largest *E. coli* content, Río Blanco may be facing directly the effects of human settlings. The numerous villages along the river with an inadequate sanitary service may be the principal cause of these inputs. Pacayaku River is the second most contaminated by *E. coli*. However, this could be not only due to human direct effluents but also to aquatic fauna living here such as giant otters and caimans, which were registered species in this study. Not finding *E. coli* in the Chichis River appears to be due to the absence of contamination, not only anthropogenic but also produced by the natural fauna of the ecosystem. It seems to be that this river may be less affected by any harmful bacterial contamination. In addition, it also suggests that the aquatic fauna could be less abundant than the other rivers.

Chemical parameters

The pH values were similar among rivers ranging from 3.17 (Blanco) to 6.47 (Kushapuku) with a mean of 4.4 indicating a clear influence of the forest inputs in the drainage basins. The surface temperature was homogeneous among all rivers ranging from 23.4 ^{o}C (Yananas) to 27.5^{o}C (Guashito). The conductivity values among rivers were quite homogeneous ranging from 72 µS.cm^{-1} (Yananas) to 389 µS.cm^{-1} (Kusuimi). The dissolved oxygen values among rivers ranged from 3.42 mg.l^{-1} (40.5 % sat.) in Chichis River to 8.55 mg.l^{-1} (100% sat.) in Due River. The alkalinity was heterogeneous among rivers going from 8.13 mg.l^{-1} in Pacayaku River to 71.72 mg.l^{-1} in Río Blanco. Similarly, the ORP was quite different among rivers reaching 224.6 mV in Kusuimi River as maximum value and 30.6 mV in Pacayaku River as minimum. The COD among rivers ranged from 3.6 mg.l^{-1} (Yananas) to 37.6 mg.l^{-1} (Pacayaku). The nutrients ranged as following: ammonium from 0.27 mg.l^{-1} (Chichis) to 4.4 mg.l^{-1} (Pacayaku), nitrates from 0.00 mg.l^{-1} (Kusuimi & Chichis) to 11.9 mg.l^{-1} (Guashito), and nitrites from 0.16 mg.l^{-1} (Due) to 1.47 mg.l^{-1} (Guashito).

Pearson correlations showed that *E. coli* was highly and positively correlated with river depth, alkalinity with conductivity, nitrites with pH, total coliforms with nitrates and nitrites, and total suspended solids with COD and ammonium while number of meanders are negatively correlated with dissolved oxygen (Table 5).

Amazonian rivers are typically acids due to the presence of humic and fulvic acids leached from the forested soils erosion. However, the pH values of 3.17 and 3.28 in the Blanco and Conde rivers probably indicates some kind of affectation from discharges of chemicals from industrial processes nearby in the water turning it more acid. These two rivers are inside Indillana/15 oil field and Auca/61 oil field respectively. The high ammonium levels can be explained by farming practices and the use of fertilizers, which are common in the region. High conductivity values reflect the Andean origin of the rivers, due to volcanic rock weathering. The nitrification process can explain the positive correlation between nitrates and nitrites.

No significant differences between all physico-chemical parameters measured were found ($p>0.05$) indicating that the sampling points, both in the NA and in the SA, held similar characteristics.

Shrimp morphology

During this work, 443 individuals (mostly adults) were sampled and measured.
Total body lengths for adult shrimps varied from 16.8 mm (Basura and Pacayacu rivers) to 81.35 mm (Kushapuku), with an average of 52.34 mm (Figure 6). The average sizes coincide closely with the species description from Valencia and Campos (2007) where they measured 77.2 mm of maximum length, and are slightly larger than the individuals caught in the Ribeirão Claro stream, Sao Paulo- Brazil (Mantelatto and Barbosa 2005).

The shrimp weights vary from 0.73 g (Pacayaku) to 10.22 g (Kushapuku), with an average of 2.44 g. Individuals are slightly heavier comparing them with the individuals from the Ribeirão Claro stream, Sao Paulo, Brazil (Mantelatto and Barbosa 2005).

There was a strong correlation between body length and weight (Pearson: 0.931, N=443, p< 0.01) (Figure 7). Lengthis taken as a reference variable for discussion even if the weight could vary eventually with biological periods (reproduction, molt).

Significant difference in the average body length among the 10 sampling points were found (df=9, p<0.001), especially between Pacayaku and Kushapuku (df=72, *F*=45.27, *p*<0.001) (Figure 5). On the contrary, there is no significant difference in body length between NA and SA sampling points (df=442, f=6.3, p>0.01). The biggest and heaviest individuals were collected in the Kushapuku River (SA) while the shortest individuals were found in the Pacayacu R. (Fig. 7).

The significant difference in shrimp body length among individuals per rivers could be explained by several processes such as habitat characteristics, nutrition and food (resources availability), predation, social and sexual behavior and anthropogenic activities impacts.

Correlations among several bio-physico-chemical parameters, particularly pH, COD, river width and QBR index, with shrimp body lengths and weights were found (R=0.2-0.4, p<0.01) suggesting habitat characteristics are important factors for the species growth because being exposed to different environmental conditions may make the species to modify its physiological requirements expressing it in size (high genotypic and phenotypic plasticity).

Correlations among concentrations of Ammonium, Nitrates and Nitrites with shrimp body length and weight were found (Table 5) suggesting the availability of nutrients as a crucial factor for the species development. Similar findings were obtained comparing *M. amazonicum* populations of a Ibitinga Lake with downstream flowing waters suggesting its cause to ecological differences in the inhabited environments, specially in the availability of nutrients and the salinity were the first larval developed occurred (Taylor et al. 1862). Studies in a congener species *M. amazonicum* noted different biological characteristic regarding to size in different parts of a same river, for instance: a study comparing shrimp populations upstream, in a lake, and down stream along a Brazilian river attributing this to differences in the quantity and quality of food, due to flood cycle (Odinetz-Collart 1988). Since these parameters weren’t measured in this study, we could assume this could be one the main reasons to explain our findings. However, it is necessary for further researches to consider this hypothesis to be tested.

Big predators such as “black caiman” *Caiman niger* and “Giant Otter” *Pteronura brasiliensis* were registered in the Pacayaku River suggesting a possible negative effect in the development of the low-level organisms of the food chain, in this case shrimp. Unfortunately, there is a lack of strong predatory evidence that was not tested in this study. This could be an important topic for future research.

Studies in other Macrobrachium species suggest differences in size among individuals, especially in males, might be because of a social structure in the communities. Certain body and trait sizes could depend on the individual roles in the group (Pantaleão et al. 2014; Santos et al. 2006). Moreover, social activities such as stress competition for food, early sexual maturation, hyperactivity of subordinated individuals; ecdysis, aggressiveness and social hierarchy could be strongly influence morphologic growth and morphotypes of *M. amazonicum* (Karplus et al 1986, 2000).

Contaminating substances related with anthropogenic activities such as domestic wastes, fertilizers, polycyclic aromatic hydrocarbons (PAHs) and heavy metals drained into the aquatic ecosystems might interfere in the normal development and growth of the species. However, there are not studies to support this hypothesis in this species.

Chemical analysis

Forty-five shrimp samples (from 106 individuals total) were analyzed to determine major and trace metal concentrations using ICP-OES, ICP-MS and HR ICP-MS respectively, in six rivers, 4 in the NA and 2 in the SA.

The chemical elemental analysis results were grouped in 4 classes regarding their functionality : 1) major and trace elements necessary for the shrimp growth, 2) non toxic trace metals which are not necessary for the shrimp development, 3) toxic heavy metals and 4) rare earth elements.

1. Major and trace elements necessary for the shrimp growth: Ca, P, K, S, Na, Fe, Mn, Mg, Zn and Cu.

Because there is no information of the chemical composition of freshwater shrimps even in the Amazon Region, these results have been compared to several studies in marine and coastal shrimps.

The average concentrations of major elements in the freshwater shrimp *Macrobrachium brasiliense* are shown in the Table 6. The elemental concentrations exhibited the order Ca>P>K>S>Na>Fe>Mn>Mg>Zn>Cu. Concentrations of Ca, P, K, S, Na and Mg are also the highest compared to studies performed in marine shrimps such as *Penaeus notabilis* (Adeyeye et al. 2008), *P. indicus* (Ravichandran et al. 2009), two African species, and *Pandalus borealis* (Rødde et al. 2008), a Norwegian species.

Calcium represents the element with the highest content in *M. brasiliense* shrimp as observed in marine species (Adeyeye et al. 2008; Ameh and Isa 2013; Rødde et al. 2008). This element is present as calcium carbonate (30–55%), one of the main compounds of the exosqueleton of crustaceans in addition to chitin, both constituting the shrimp shell (Ladchumananandasivam 2012; Arbia and Arbia 2013). Concentrations of the other major elements are not significantly different between the six sampling points ($p>0.01$)

suggesting these elements are relatively stable in the organisms even though they had developed under different feeding pressures but comparable geochemical characteristics of the streams (Figures 15-22). Cooper and Zinc are elements known for being regulated by the organisms due to their physiological role. Both elements are involved in shrimp enzymatic and respiratory processes and gonads development (Kargın et al. 2001; Bay 1999). Cooper is related to the production of a respiratory protein called haemocyanin, the gills pigment (Bay 1999; Kargın et al. 2001). Cooper concentrations were significantly different among the six sampling points (f=4.767, df=44, p<0.01) but not significantly different between the NA and the SA (control) (f=2.332, df=44, p>0.01). Cu and Zn lowest concentrations were measured in shrimps of the Guashito River which coincidently is the river were collected the smallest individuals, inferring this could have happened due to Cu and Zn scarcity (environmental availability) or shrimp absorption mechanism failure. However, cooper concentrations didn't exceed 200 $\mu g.g^{-1}$ in the rivers which Bay considered lethal for the species (Bay 1999); the sampled population in the Ecuadorian Amazon is in the range 58.5-105.2 $\mu g.g^{-1}$ facing no critical panorama. The concentrations of the major elements are not significantly different between the NA and the SA suggesting similar amount of elements absorption and fixation by shrimp in both regions. Apparently, these elements are available in the aquatic ecosystems in both areas. In contrast, the S concentrations are significantly different between both areas (f=4.124, df=44, p<0.05) showing higher amounts in the Yananas River (SA). Sulfur is essential for protein synthesis and metabolism processes (RACI 2011) while Fe is related to biochemical and physiological functions in aquatic organisms such as enzymatic processes (Kargın et al. 2001). Iron concentrations are significantly different among the six sampled points (f=5.061, df=44, p<0.01) (Figure 24) suggesting differences in the environmental availability of this element or absorption by shrimps between sites. However, comparing to other studies in seawater shrimp species (Rødde et al. 2008; Kargın et al. 2001; Bat et al. 2013; Heidarieh 2013), the Iron concentrations in *M. brasiliense* shrimps were higher suggesting an inland factor, which can be explained by the exposure of natural higher concentrations in Amazon soils and surface water. Furthermore, iron concentrations in freshwater shrimps are not significantly different between the NA and the SA rivers (F=4.124, df=44, p>0.01) confirming this hypothesis. Highest concentrations of iron were measured in shrimps of the Basura River (800.91 $\mu g.g^{-1}$). That suggests an anthropogenic contamination source, like for example the presence of a small company producing metallic pipes close to the sampling point. In addition, iron concentrations were correlated with V (r=0.812, p<0.01) and Co (Pearson r=0.65, p<0.01), two toxic heavy metals related to anthropogenic inputs. Manganese concentrations are significantly different among the six sampled points (f=6.578, df=44, p<0.01) being Basura (1123.3 $\mu g.g^{-1}$) and Conde (406.7 $\mu g.g^{-1}$) the rivers with the highest concentrations score (Figure 25). Manganese is considered as a necessary element for producing haemocyanin as well as Cu, both involved in the respiration process, which can be regulated by the organisms (Kargın et al. 2001). Manganese concentrations are not significantly different between NA and SA streams (f=3.289, df=44, p>0.01).

2. No Toxic Trace Metals which are not necessary for the shrimp development

The concentrations of no toxic trace metals in the samples of the freshwater shrimp *Macrobrachium brasiliense* are shown in the Table 7.

The elements concentrations analyzed in the shrimp species *Macrobrachium brasiliense* collected from six rivers in the Ecuadorian Amazon exhibited the order Sr>Ba>Si>Al>Rb>Ti>B>Se>Mo>Zr>Cs>Ga>Nb>Tl>Hf>Th>U.

Strontium average concentrations in shrimps ranged from 777.8 $\mu g.g^{-1}$ in the Due River to 1586.1 $\mu g.g^{-1}$ in the Conde River. They are significantly different among the six sampling points (f=6.437, df=44, p<0.01) (Figure 18) evidencing the significant variability of this element in individuals; the highest contents have been measured in the Conde (1586.1 $\mu g.g^{-1}$) and Guashito (1533.4 $\mu g.g^{-1}$) rivers. Even though Sr concentrations are not significantly different between the NA and the SA rivers (f=3.835, df=44, p>0.05), it could be noticed that there may be a factor in the most NA rivers increasing Sr availability unlike Due (mean 777.8 $\mu g.g^{-1}$) and the SA River Yananas (mean 996.3 $\mu g.g^{-1}$), which are the rivers with the lowest Sr concentrations. Strontium is commonly found naturally abundant in the earth soil making it easy for mining exploitation. In biological organisms, strontium can substitute calcium in small extent and it's not considered a threatening element as it can increase bone density in humans (Cabrera et al. 1999).

Barium mean concentrations in *M. brasiliense* shrimps ranged from 124.0 $\mu g.g^{-1}$ (Blanco R.) to 755.2 $\mu g.g^{-1}$ (Conde R.). Ba concentrations are significantly different among the six sampled points (f=9.786, df=44, p<0.01) (Figure 23) suggesting differences in their bioavailability or in the absorption by shrimps, or differences in Ba sediment contents. Ba is naturally present in Amazonian soils; however, it's also found in formation waters and sludge oil drilling as it is commonly used to liquefy rocks during oil drilling operations. Consequently, high concentrations can be correlated with oil activities sources, which is the case of the Conde River where the Ba concentrations were much higher than the others (mean 755.2 $\mu g.g^{-1}$). Furthermore, considering all the study streams, Ba concentrations are not significantly different between the NA and the SA study rivers (f=4.124, df=44, p>0.01) (Figure 19).

Aluminum average concentrations in *Macrobrachium brasiliense* species ranged from 184 $\mu g.g^{-1}$ (Due R.) to 390 $\mu g.g^{-1}$ (Yananas R.). Aluminum is one of the most abundant elements in the earth's crust and can be released to the environment by both natural processes and anthropogenic sources, natural processes being the major contributor (FAO 2011). Aluminum anthropogenic mobilization occurs indirectly by the emission of acidifying substances to the atmosphere which can be accumulated in soil-derived dusts or by coal combustion, mining and agricultural activities (FAO 2011). Aluminum concentrations are not significantly different among the six sampled points (f=2.345, df=44, p>0.05) (Figure 20), which shows all rivers are probably facing similar effects.

Even though mean concentrations are the highest in the Yananas River, a control point, aluminum concentrations are not significantly different between the NA and the SA rivers (f=0.36, df=44, p>0.05) (Figure 20) suggesting that aluminum releases into the environment are not linked to the oil industry in the NA.

Titanium mean concentrations ranged from 3.6 $\mu g.g^{-1}$ (Due) to 17.0 $\mu g.g^{-1}$ (Guashito). Ti concentrations are significantly different among the six sampled points (f=8.218, df=44, p<0.01) (Figure 21). Titanium is widely used in several industries such as alloys for aerospatial parts, heat exchange and condenser, containers and apparatus manufacturing, extractive metallurgy, process and power generation, and petrochemical industry for corrosion resistance. Specifically, titanium is used today in refinery heat exchangers, vessels, scrubbers, column, piping systems, and other related equipment (Peters & Leyens 2003). Even though, Ti concentrations are not significantly different between the NA and the SA rivers (f=0.032, df=44, p>0.05). Guashito and Basura rivers may be the two most titanium concentrated rivers because the presence of many oil facilities in those areas (Block 7/Petroamazonas Coca-Payamino and PBHI oil camps). Titanium could be considered as proxy for oil industry occurrence. Furthermore, Ti has no biological role and is non-toxic (RSC 2016), so it may not represent a human health risk agent.

Selenium average concentrations in *Macrobrachium brasiliense* species ranged from 0.5 $\mu g.g^{-1}$ (Conde) to 1.3 $\mu g.g^{-1}$ (Guashito) and are significantly different among the six sampling points (f=9.147, df=44, p<0.01). Selenium is a natural occurring element in the earth's crust in inorganic forms such as selenide, selenite and senenite but these are quite rare minerals (Fordyce 2007). A way to incorporate Se in the aquatic environments is by rock and soil weathering which induces low Se concentrations in rivers and can be bio-accumulated by plants and aquatic organisms. Anthropogenic Se sources include coal and oil burning, mining and melting of sulfide ores. Selenium that may be present in fossil fuels combines with oxygen when burned, which may then react with water to form soluble selenium compounds (Agency for Toxic Substances and Disease Registry 2009). Selenium concentrations are not significantly different between the NA and the SA rivers (f=0.238, df=44, p>0.05) (Figure 22). In this case, the numerous lighters for oil burning in the Guashito River area may be an important source causing the highest Se concentrations in *M. brasiliense*. In contrast, concentrations in the other rivers from the impacted area may indicate the absence of this oil industry activity as it's evidenced in the sampling points oil industry impacts sources (Map 2).

Molybdenum mean concentrations ranged from 0.20 $\mu g.g^{-1}$ (Blanco) to 0.43 $\mu g.g^{-1}$ (Due). Molybdenum concentrations are significantly different among the six sampled points (f=7.287, df=44, p<0.01). Molybdenum is naturally found in earth's crust in ores as molybdenum disulfide (RSC 2016); however it's also used in several industrial processes such as making alloys for increasing resistance, anticorrosion, and hardness, electrical conductivity, heat resistant, lubricant additive and catalyst in the petroleum industry (RSC 2016). Consequently, high Mo concentrations in Due, Basura and Guashito may be due to industrial activities and oil industry. Molybdenum concentrations are not significantly different between the NA and the SA rivers (f=0.035, df=44, p>0.05) (Figure 23). However, Mo is proposed as proxy for anthropogenic activities including oil industry.

Cesium average concentrations in *Macrobrachium brasiliense* species ranged from 0.0243 $\mu g.g^{-1}$ (Guashito) to 0.6657 $\mu g.g^{-1}$ (Yananas). Their concentrations are significantly different among the six sampled points (f=130.624, df=44, p<0.01) but also significantly different between the NA and the SA rivers (f=165.539, df=44, p<0.01)(Figure 24), as its content is increased by 5 fold in the SA River. Cesium is a relatively rare element estimated to average 3 parts per million in the Earth's crust (Turekian et al. 1961). The largest present-day use of nonradioactive cesium is in cesium formate drilling fluids for the extractive oil industry (Butterman, W. 2004). However, oil industry is not actually occurring in the SA, where the highest Cs concentrations were found, discarding this possible affection. Nonradioactive cesium compounds are only mildly toxic and nonradioactive cesium is not a significant environmental hazard (Melnikov, P. 2010). Cesium is lately used as erosion tracker due to soil degradation. This element may be released into the aquatic environment by soils erosion, accumulating in the river sediments and consequently, available for aquatic organisms. Therefore, Cs in shrimp tissue may be evidence for recent soils erosion in the SA.

3. Toxic Trace Metals and Metalloids not necessary for the shrimp development

The average concentrations of toxic trace metals and metalloids in freshwater shrimp *Macrobrachium brasiliense* sampled in Ecuadorian Amazon Rivers are shown in the Table 8.

The elements concentrations in *Macrobrachium brasiliense* species exhibited the order V>Co>Cr>Pb>Cd>As>Sn. Since these elements cannot be excreted by these organisms and their concentrations mostly depend on their availability in the environment, they are commonly used as tracers of anthropogenic activities.

Vanadium mean concentrations in *Macrobrachium brasiliense* samples ranged from 0.56 $\mu g.g^{-1}$ in the Due River to 1.28 $\mu g.g^{-1}$ in the Basura River. They were much lower than the concentrations measured in soils and sediments of the Northern Ecuadorian Amazon (Lopez 2014) suggesting there was not a significant affection regarding this element. Vanadium concentrations are not significantly different among the six sampling points (f=1.524, df=44, p>0.01) neither between the NA and the SA (f=0.535, df=44, p>0.01) (Figure 25). It could be assumed that there is similar vanadium availability in both regions and this element is mainly coming from the soil erosion. Significant differences in V concentrations between rivers may suggest probable affections; however, there is no such evidence in this case.

Cobalt average concentrations in *Macrobrachium brasiliense* species ranged from 0.355 $\mu g.g^{-1}$ (Yananas) to 1.689 $\mu g.g^{-1}$ (Basura). This metal is commonly found in urban and industrial sewage as it is commonly used in alloys, batteries, pigments, fertilizers and animal feeding additives (INRS Toxicological Fiches 2012). The Co concentrations were within the previous ranges (Heidarieh 2013) published in marine shrimp species, and it's one of the trace metal measured in lowest concentrations as well. Cobalt concentrations are significantly different among the six sampling points (f=5.295, df=44, p<0.01) (Figure 26). In contrast, the Co concentrations are not significantly different between NA and SA (f=3.643, df=44, p>0.01); however, the lowest concentration (0,3 $\mu g.g^{-1}$) was found in the Yananas River suggesting there is not greatly affected by anthropogenic activities. High Co concentrations in the Conde River suggest a possible contaminating source as well and could be considered as proxy for industrial activities.

Chromium average concentrations in *Macrobrachium brasiliense* species ranged from 0.32 $\mu g.g^{-1}$ (Basura) to 1.98 $\mu g.g^{-1}$ (Guashito). The Guashito River, with the highest Cr values, is a sampling point characterized by intense oil activities nearby such as oil wells, lighters, stations and fields but also local contaminating sources such as oil pits and numerous oil spills (last one in 2007) (Block 7-Petroamazonas Coca-Payamino oil camp). Even though Cr concentrations were not significantly different among the six sampling points (f=4.830, df=44, p>0.01) neither between the NA and the SA (f=0.049, df=44, p>0.01) (Figure 27), the 'Guashito' River is probably facing some local contamination issues. In the same research Program MONOIL, very high concentrations of Cr were measured in sediments and surface waters of the Teaone River downstream the refinery (Lopez 2014), a highly contaminated river due to oil refining effluents. Therefore, Cr in shrimp tissue can be proposed as a proxy for tracking oil activities in rivers.
Lead mean concentrations in *Macrobrachium brasiliense* species ranged from 0.136 $\mu g.g^{-1}$ (Guashito R.) to 1.540 $\mu g.g^{-1}$ (Yananas R.). The highest concentrations were measured in the Yananas River of the control area. Lead is quite abundant in the earth's crust. It can be found in deposits such as hydrothermal vein, impregnation and replacement, and volcanogenic sedimentary deposits (U.S. Geological Survey, Mineral Commodity Summaries 2016). Widely used in a huge variety of industrial activities worldwide, lead exposure to people is relatively high, while nowadays, since a decade, in Northern countries, the exposure is decreasing. Lead concentrations are significantly different among the six sampling points (f=11.168, df=44, p<0.01) suggesting specific sources in the Blanco and Yananas rivers. In addition, Pb concentrations are significantly different between the NA and the SA rivers (f=7.727, df=44, p<0.01) (Figure 28). Being influenced by highland volcanic activity that could enrich Amazonian soils in heavy metals, the deforestation practices can increase the Pb inputs by accelerating the natural soil erosion process. High concentrations of Pb in the Yananas River freshwater shrimps allow us to consider Pb as a proxy for anthropogenic activities such as deforestation as well as mercury or cesium.

Cadmium average concentrations in *Macrobrachium brasiliense* species were quite heterogeneous between the 7 rivers and ranged from 0.24 $\mu g.g^{-1}$ (Blanco) to 1.27 $\mu g.g^{-1}$

(Due River). This element is widely used in various industrial processes such as coloring plastic and paints, stabilizing polymers and copolymers, anticorrosion coating of metals, constituting alloys, plastics stabilizing, and electric equipment fabrication (INRS Toxicological Fiches 2012), so the presence of this metal is definitely a sign of anthropogenic activities with contamination potential. There are not extensive oil activities in the Due River area. Even though some oil spills and derivative spills were registered from the crude pipeline crossing at 2 km from the sampling point, cadmium concentrations in this river cannot be only linked to these isolated events. The Due River may also be affected by industrial waste from the construction of a hydroelectric plant and a dam upstream. Being the river with the highest median Cd concentrations, Conde R. can be affected by industrial waste from the extensive oil activities in the area. The Cadmium concentrations are significantly different among the six sampling points ($f=17.780$, $df=44$, $p<0.01$) (Figure 29) probably because of the different affectation degree of anthropogenic activities in those environments. Furthermore, the Cd concentrations are also significantly different between NA and SA ($f=5.809$, $df=44$, $p>0.01$) showing clearly the difference between the affected and the control area, not contaminated by industrial activities. These results confirm that Cd can be used as a proxy for identifying industrial activities, not only oil exploitation but also by related oil industry activities.

Arsenic mean concentrations in *Macrobrachium brasiliense* species ranged from 0.13 $\mu g.g^{-1}$ (Guashito R.) to 0.58 $\mu g.g^{-1}$ (Yananas R.). These concentrations are significantly different among the six sampling points ($f=11.767$, $df=44$, $p<0.01$) suggesting different availability of this element among the rivers and they are also significantly different between the NA and the SA rivers ($f=35.497$, $df=44$, $p<0.01$). The highest concentrations of As in *Macrobrachium brasiliense* species were measured in the Yananas River, in the control area, which indicates clearly that this metalloid is not added in the environment by oil activities (Figure 30). It is commonly found in mineral bound form in the earth's crust, which is the main source originated in volcanic activity and the weathering of minerals, and it's also been released by several anthropogenic activities such as ore smelting, coal burning, pesticides and veterinarian food (FAO 2012). The As inputs to the watershed can also be influenced by highland volcanic activity, Arsenic may be mainly released by soil erosion events increased by specific agriculture practices. As mercury, cesium, and lead, arsenic can also be considered as a proxy for deforestation activities.

4. Rare Earth Elements (REEs)

The mean concentrations of rare earth elements in the samples of the freshwater shrimp *Macrobrachium brasiliense* are shown in the Table 9.

The REE concentrations ranges exhibited the order:
Ce>La>Nd>Eu>Gd>Pr>Sm>Dy>Er>Yb>Ho>Tm>Lu

The highest rare earth element mean concentration in all rivers is Cerium whereas the lowest is Lutetium. For all rare earth elements except one (Europium), in the Due River freshwater shrimps showed the lowest concentrations while they presented the highest concentrations in the Conde River. Concentrations of all rare earth elements are significantly different among the six sampling points (df=44, $p<0.01$). Moreover, concentrations of all rare earth elements are significantly different between the rivers Conde and Due (df=14, $p<0.05$), which indicates that a specific source in the Conde River is increasing REEs availability in the aquatic ecosystem. These concentrations are not significantly different between the NA and the SA rivers (df=44, $p>0.05$). (Figures 31-43).

REEs occur naturally in earth's crust and their concentrations are generally lower in soils compared to the other metals but this depends on the rock type and location (Hu et al. 2006). There are many causes for the REE to migrate and accumulate in soils such as lithologic discontinuities, the presence of aeolian or anthropogenic inputs (Aide and Aide 2012). REEs are widely used in several industrial, electrical and manufacture processes such as metallurgy, catalyst in chemical industry, coloring of glass and ceramics, productions of magnets and phosphorus, metal alloys for mechanical components, television and microwaves fabrication, batteries, lasers, glass, X-ray tubes, fiber optic technology, (McGill 2012), fertilizers and feed poultry food additive (Hu et al. 2006). Remarkably, REEs are used (as nitrates and chlorides) in zeolite cracker catalysts for the improvement of gasoline yields, reductions in the formation of coke and light hydrocarbons, and reforming of hydrocarbons (McGill 2012). These industrial sources linked to the oil industry can explain the highest concentrations measured in the freshwater shrimps of the Conde River.

They are generally low toxic due to their sparse distribution and low aqueous solubility (McGill 2012), so they are not considered as human health risk agents.

Human health risk assessment

Shrimps are considered as an important source of food consumed by humans and other organisms worldwide playing an important role in numerous animal diet because, besides from being a supply of high quality protein and vitamins, they also contain various minerals such as calcium, iron, iodine, etc. (Ravichandran et al. 2009; Baboli 2013) necessary for human nutrition. From a diet analysis of *M. brasiliense* (Melo and Nakagaki 2013) stomach content, sand and sediments, where the toxic elements are mostly stored, constituted 87.10 % and 90% of the items found representing an adequate species for bioaccumulation studies.

The Chromium concentrations in freshwater shrimps didn't exceed the maximum food levels for aquatic animals and their products (2.0 $\mu g.g^{-1}$ dw, GB 2762 2012). However, the chromium average concentrations were nearly close to the permissible limits in the Guashito River (1.98 ± 0.71 $\mu g.g^{-1}$ dw). In addition, according to the Provisional Tolerable Daily Intake (PTDI) for chromium established by the Institute of Medicine – National Academics of Sciences US (IOM 2001) (35 µg Cr/day for adult men, 25 µg Cr/day for adult women, and 15 µg Cr/day for children), the maximum daily and weekly intake shrimp (g) wet mass and the maximum daily estimated number of shrimps intake for adult and children per river were estimated. The river with the most elevated risk was the Guashito, with a maximum daily intake of shrimp in wet mass of 70.9 g for men, 50.6 g for women, and 30.4 g for children corresponding to a maximum daily intake of 27, 19 and 11 of fresh shrimps respectively.

Most of the shrimps in the sampled rivers didn't exceed the permissible limits for lead in crustaceans consumption (0.5 $mg.kg^{-1}$, European Commission 2010 & MSPS-Colombia 2016) (fish=0.3 $mg.kg^{-1}$, FAO 2011) except in the Blanco (NA) and Yananas (control river in SA) rivers with 0.959 and 1.54 $\mu g.g^{-1}$ dw, respectively. In addition, according to the Pb human health risk assessment (WHO/FAO in JECFA, 2011) (1.2 ug/kg/day for 1.0 mmHg increase in adult blood pressure and 0.6 ug/kg/day loss of 1 IQ point in children), maximum amounts of shrimp intake were calculated for all rivers. The most elevated risk were calculated for the Yananas and Blanco rivers where children are not recommended to eat daily more than 30 g and 50 g of fresh shrimp respectively, corresponding to a maximum daily intake of 18 and 21 of fresh shrimps respectively.

Cadmium concentrations in freshwater shrimps exceed the permissible limits established by the European Commission (2010), and the Colombian Public Health Ministry (2016) (0.5 $mg.kg^{-1}$) for crustaceans food intake in the Conde (1.2 $\mu g.g^{-1}$) and Due (1.3 $\mu g.g^{-1}$) rivers. In addition, according to the Provisional Tolerable Monthly Intake (PTMI) for cadmium (FAO 2011) (0.025 mg Cd/kg bw equivalent to 0.83 ug Cd/kg bw/day), maximum amounts of shrimp intake values were calculated for all rivers for men, women and children exposure. In the Due River, the maximum daily intake of shrimps is 65, 56 and 19 for men, women and children respectively.

Arsenic concentrations in all rivers exceeded the permissible limits established by the WHO (0.1 mg.kg-1, WHO-FAO 2011) for animal fat consumption. Moreover, according to the Provisional Tolerable Daily Intake (PTDI) for arsenic (0.003 mg As/kg bw/day) (FAO 2011), maximum amounts of shrimp intake values were calculated for all rivers for men, women and children. In the Yananas R. with the maximum As contents in shrimps, the maximum daily intake of fresh shrimps corresponds to 825, 707, and 236 for men, women and children, respectively.

Tin concentrations in *M. brasiliense* shrimps never exceed the permissible limits for luncheon meat and canned products (50 mg.kg-1, WHO-FAO 2011) nor the Provisional Tolerable Weekly Intake (PTWI) for tin (FAO 2011) (14 mg Sn/kg bw/week), for reasonable amounts of shrimp intake by local populations.

Regarding on the PTWI for Aluminum (2 mg Al/kg bw = PTDI 285.71 ug Al/kg bw, FAO 2011), human health risk caring especially in children is recommended in the Guashito River because the estimated amounts of maximum daily intake shrimp wet mass an the maximum daily number of shrimps for consumption are quite low (62.9 grams of shrimp

= 18 shrimps). However, due to the resident's low shrimp feeding habits, it may not be easy to exceed the recommended intake limits.

In general, based on the PTWI for each toxic element (Table 10), the fresh water shrimp consumption from the sampling rivers is relatively safe for adults. However, a special care is required for communities living close to the Guashito River, mainly for children. People should pay a special attention to the children diet; especially in Guashito, Yananas and Due rivers, where the daily safety values recommend for ingestion were the lowest (11, 18, and 19 freshwater shrimps respectively). In rivers where lead was taken as a reference element, total shrimp consumption has to be less than the suggested quantities because those calculated amounts are within the danger threshold for children (Daily Intake of 0.6 μg Cr/kg bw decreases 1 IQ in children).

According to the dietary habits of the people living in the communities of the sampling area, shrimp doesn't represent a major source of food in the daily life basis, unlike bush meat or fish. Moreover, informal conversations with habitants suggest that indigenous people from those areas can, for short periods of time monthly, only feed on freshwater shrimp because of other meat sources scarcity.

Conclusions

This is the first study on major and heavy metal bioaccumulation in the most abundant freshwater shrimp *Macrobrachium brasiliense* species of the Amazon basin (Ecuador). Major elements such as Ca, P, K, S, Na, Fe, Mn, Mg Zn and Cu were identified as essential for the shrimp growth due to their physiological importance in metabolism processes. Heavy metal concentrations in this species were very similar to concentrations measured in seawater shrimp species. One of the main anthropogenic activities responsible for releasing metals into the aquatic environment is soil erosion increased by deforestation practices and their proxy elements are Cs, Pb, and As. Therefore, the river categorized as mainly affected by deforestation is Yananas, in the Southern Ecuadorian Amazon basin not affected by extractive activities (oil industry and mining). Finally, our study evidenced several particular heavy metals found in the freshwater shrimps related to the oil industry: Ba, Ti, Se, V, Co, Cr, Cd and the 13 rare earth elements (Lanthanides). The most likely affected rivers by oil activities were the Conde, Basura, and Guashito rivers. According to the physico-chemical and microbiological (coliforms) parameters, Conde, Blanco, Basura and Guashito are the most altered conditions presumably from anthropogenic activities, which coincide with the heavy metal results.

The only two elements exceeding the permissible limits for crustacean intake are lead in the Blanco and Yananas rivers, and cadmium in the Conde and Due rivers. Based on the Provisional Tolerable Daily and Weekly Intake (PTDI – PTWI) established by FAO/WHO and IOM for toxic elements, the river with the lowest calculated amount for shrimp intake is the Guashito River being 71 g (27 shrimps), 50 g (19 shrimps), and 30 g (11 shrimps) the maximum daily number of shrimps intake for safety consumption for men, women and children, respectively. However, local people should pay a special attention to the children diet, especially from the Chichis and Kushapuku Rivers, where the safety values recommend for ingestion were the lowest.

Even though the human health risk assessment results from the fresh water shrimp *M. brasieliense* in this study are showing low risk for adults and special consideration for children, people living in the sampling areas who depend on the aquatic resources besides shrimp, may be facing much higher risk due to the exposure of more heavy metal sources such as water, plants and even more heavy metal concentrated animals such as piscivorous fish.

Similar studies not only in other organisms of the aquatic food chain but also in humans living in these environments are required in order to completely understand the heavy metals dynamic and fluxes. Considering all the variables involved in these complex processes will surely help us to know accurately the human health risk the people could be exposed in order to establish the best mitigating and preventing strategies against the potential contamination impacts from anthropogenic activities specially at big scale in the Amazon Region.

Acknowledgments

This work was developed in the frame of the French-Ecuadorian research program entitled MONOIL 'Environmental Monitoring, Health, Society and Oil in Ecuador'. This program was managed by the IRD, French Research Institute for the sustainable Development, and sponsored by the ANR, French Research Agency (ANR-13 SOCENV-0003-01). This specific study was also funded by the Project ABE, Amazonian Biodiversity and Education, coordinated by the USFQ and sponsored by the University of North Carolina at Chapel Hill. All biological samples received the authorization of the Ecuadorian Ministry of Environment for scientific purpose (N°15-IC-FAU/DPS/MA; No 014-15-EXP-IC-FAU-DNB/MA).

References

Adeyeye, E., H. O. Adubiaro, and O. J. Awodola. 2008. Comparability of Chemical Composition and Functional Properties of Shell and Flesh of Penaeus notabilis. Pakistan Journal of Nutrition 7.

Agency for Toxic Substances and Disease, R. 2009. 1 . PUBLIC HEALTH STATEMENT: Selenium:1–11.

Aide, M. T., and C. Aide. 2012. Rare Earth Elements: Their Importance in Understanding Soil Genesis. ISRN Soil Science 2012:1–11.

Ameh, A., and M. Isa. 2013. Kinetics of demineralization of shrimp exoskeleton in chitin and chitosan synthesis. Journal of Chemical … 4:32–37.

Anger, K. 2013. Neotropical Macrobrachium (Caridea: Palaemonidae): on the biology, origin, and radiation of freshwater-invading shrimp. Journal of Crustacean Biology 33:151–183.

Arbia, W., and L. Arbia. 2013. Chitin extraction from crustacean shells using biological methods-a review. Food Technology and … 9862:12–25.

Baboli, M. 2013. Determination of heavy metals and trace elements in the muscles of marine shrimp, Fenneropenaeus merguiensis from Persian Gulf, Iran. JAPS, Journal of Animal … 23:786–791.

Balfour, S., and N. Badrie. 2012. Seasonal influence and heavy metal analysis in marine shrimp (Penaeus spp.) sold in Trinidad, West Indies. Journal of Food … 1:193–199.

Bass, M. S., M. Finer, C. N. Jenkins, H. Kreft, D. F. Cisneros-Heredia, S. F. McCracken, N. C. a Pitman, P. H. English, K. Swing, G. Villa, A. Di Fiore, C. C. Voigt, and T. H. Kunz. 2010. Global conservation significance of Ecuador's Yasuní National Park. PloS one 5:e8767.

Bat, L., F. Şahin, and M. Sezgin. 2013. Heavy metals in edible tissues of the brown shrimp Crangon crangon (Linnaeus, 1758) from the Southern Black Sea (Turkey). Journal of Black … 19:70–81.

Bay, U. 1999. Heavy Metals in the Shrimp Xiphopenaeus kroyeri Sâo Paulo , Brazil 1995:152–159.

Betancourt, O., A. Narváez, and M. Roulet. 2005. Small-scale Gold Mining in the Puyango River Basin, Southern Ecuador: A Study of Environmental Impacts andHuman Exposures. EcoHealth 2:323–332.

Bilsborrow, R., A. Barbieri, and W. Pan. 2004. Changes in population and land use over time in the Ecuadorian Amazon. Acta Amazonica 34:635–647.

Bruhm, L., and L. Wolfson. 2007. Citizens Monitoring Bacteria : A training manual for monitoring E. coli.

Butterman, B. W. C., and R. G. Reese. 2003. Rubidium. Mineral Commodity Profiles:1–11.

Cabrera, W. E., I. Schrooten, M. E. D. E. Broe, and P. C. D. Haese. 1999. Strontium and Bone 14.

Canli, M., and G. Atli. 2003. The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. Environmental Pollution 121:129–136.

Dudgeon, D., and A. Arthington. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biological ….

European Commission. 2016. Food Safety Overview. In: http://ec.europa.eu/food/overview_en

FAO. 2011-2016. Codex Alimentarius. Secretariat of the Codex Alimentarius Commission Joint FAO/WHO Food Standards Programme Food and Agriculture Organization of the United Nations Viale delle Terme di Caracalla 00153 Rome, Italy.

Fieve, R., H. Meltzer, and R. Taylor. 1971. Rubidium Chloride Ingestion by Volunteer Subjects : Initial Experience. Psychopharmacologia 20.

Finer, M., C. N. Jenkins, S. L. Pimm, B. Keane, and C. Ross. 2008. Oil and gas projects in the Western Amazon: threats to wilderness, biodiversity, and indigenous peoples. PloS one 3:e2932.

Fordyce, F. 2007. Selenium Geochemistry and Health. AMBIO: A journal of the Human Environment 36:94–97.

Heidarieh, M. 2013. Evaluate of heavy metal concentration in shrimp (Penaeus semisulcatus) and crab (Portunus pelagicus) with INAA method. … 2:72.

Hodkinson, I., and J. Jackson. 2005. Terrestrial and aquatic invertebrates as bioindicators for environmental monitoring, with particular reference to mountain ecosystems. Environmental Management 35:649–66.

Holthuis, L. B. 1959. The Crustacea Decapoda of Suriname (Dutch Guiana) (Vol. 44). EJ Brill.

Hu, Z., S. Haneklaus, G. Sparovek, and E. Schnug. 2006. Rare Earth Elements in Soils. Pages 1381–1420 Communications in Soil Science and Plant Analysis.

INRS. 2012. Institute National de Recherche et e Sécurité. Fiches Toxicologiques. In: http://www.inrs.fr/publications/bdd/fichetox.html

IOM. 2001. Dietary Reference Intakes. Insitute of Medicine of the National Academics. Washington D.C.

JECFA. 2011, 2012, 2013. Evaluation of the Joint FAO-WHO Expert Commitee on Food Additives. In: http://apps.who.int/food-additives-contaminants-jecfa-database/search.aspx

Kargın, F., A. Dönmez, and H. Çoğun. 2001. Distribution of heavy metals in different tissues of the shrimp Penaeus semiculatus and Metapenaeus monocerus from the Iskenderun Gulf, Turkey: seasonal. Bulletin of Environmental Contamination … 66:102–9.

Karplus, I., Malecha, S., Sagi, A., 2000. The biology and management of size variation. In: New, M.B., Valenti, W.C. (Eds.), Freshwater Prawn Culture: the Farming of Macrobrachium rosenbergii. Blackwell, Oxford, pp. 254–289.

Ladchumananandasivam, R. 2012. The use of exoskeletons of shrimp (litopenaeus vanammei) and crab (ucides cordatus) for the extraction of chitosan and production of nanomembrane 2012:495–508.

Leyens, C. and M. Meyers. 2003. Titanium and Titanium Alloys: Fundamentals and Applications. DLR German Aerospae Center. Institute of Materials Research. Germany.

Lopez, Fausto. 2014. Impacts environnementaux des activité pétrolières actuelles et passées sur l’environnement en Équateur. Master 2 Thesis.

Lu, F., R. E. Bilaborrow, and A. I. Oña. 2011. Modos de vivir y sobrevivir. (Abya-Yala, Ed.). Quito-Ecuador.

Lytle, D., and B. Peckarsky. 2001. Spatial and temporal impacts of a diesel fuel spill on stream invertebrates. Freshwater Biology.

Ministerio del Ambiente del Ecuador. 2016. Proyecto de Remediación Ambiental y Social del Ecuador. Programa de Pasivos Ambientales. SUIA.

Ministerio del Ambiente del Ecuador. 2016. Direccion NAcional Forestal-SUIA. Tazas de deforestacion del Ecuador.

Mantelatto, F., and L. Barbosa. 2005. Population structure and relative growth of freshwater prawn *Macrobrachium brasiliense* (Decapoda, Palaemonidae) from São Paulo State, Brazil. Acta Limnologica Brasiliensia.

Martin, K. R. 2013. Interrelations between Essential Metal Ions and Human Diseases 13:451–473.

McCracken, S., and M. Forstner. 2014. Oil Road effects on the anuran community of a high canopy Tank Bromeliad (Aechmea zebrina) in the upper Amazon Basin, Ecuador. PloS one 9:e85470.

Mcgeoch, M. A. 1998. The selection, testing and application of terrestrial insects as bioindicators. Biological Reviews of the Cambridge ….

McGill, I. 2012. Rare Earth Elements. Ullmann's Encyclopedia of Industrial Chemistry.

Melnikov, P., & Zanoni, L. Z. 2010. Clinical effects of cesium intake. *Biological trace element research*, *135*(1-3), 1-9.

Melo, M. S. De, and J. M. Nakagaki. 2013. Evaluation of the feeding habits of *Macrobrachium brasiliense* (Heller, 1862) in the Curral de Arame stream (Dourados/Mato Grosso Do Sul, Brazil). Nauplius 21:25–33.

Moraes-Riodades, P. M. ., and W. C. Valenti. 2004. Morphotypes in male Amazon River Prawns, *Macrobrachium amazonicum*. Aquaculture 236:297–307.

Muniz, P., E. Danulat, B. Yannicelli, J. García-Alonso, G. Medina, and M. C. Bícego. 2004. Assessment of contamination by heavy metals and petroleum hydrocarbons in sediments of Montevideo Harbour (Uruguay). Environment international 29:1019–28.

Munné, a., N. Prat, C. Solà, N. Bonada, and M. Rieradevall. 2003. A simple field method for assessing the ecological quality of riparian habitat in rivers and streams: QBR index. Aquatic Conservation: Marine and Freshwater Ecosystems 13:147–163.

Nielsen, Forrest. 1997. Boron in Human and animal Nutrition. Plant and Soil. 193: 199.

Odinetz-Collart, O. 1988. Aspectos ecologicos do camarao *Macrobrachium amazonicum* (Heller, 1862) No baixo tocantins (Pa-Brasil).

Otten, J., J. Pitzi Hellwing, and L. Meyers. 2006. Dietary Reference Intakes DRI. Washington, D. C.

Pantaleão, J., G. Hirose, and R. Costa. 2014. Ocurrence of male morphotypes of *Macrobrachium amazonicum* (Caridea, Palaemonidae) in a population with an entirely freshwater life cycle. Brazilian Journal of Biology 74.

Pardo, I., M. Álvarez, J. Casas, and J. Moreno. 2002. El hábitat de los ríos mediterráneos. Diseño de un índice de diversidad de hábitat. Limnetica 21:115–133.

Leyens, C., & Peters, M. 2003. *Titanium and titanium alloys* (p. 8). Wiley-VCH, Weinheim.

Pileggi, L. G., C. Magalhaes, G. Bond-Buckup, and F. L. Mantelatto. 2013. New records and extension of the known distribution of some freshwater shrimps in Brazil. Revista Mexicana de Biodiversidad 84:563–574.

Pitman, N., J. Terborogh, M. Silman, P. Nunez, D. Neill, C. Ceron, W. Palacios, and M. Aulestia. 2002. A Comparison of tree species diversity in two upper amazonian forests. Ecology 83:3210–3224.

Ravichandran, S., G. Rameshkumar, and A. Prince. 2009. Biochemical composition of shell and flesh of the Indian white shrimp Penaeus indicus (H. milne Edwards 1837). … -Eurasian Journal of … 4:191–194.

Reif, J. 2011. Animal sentinels for environmental and public health. Public Health Reports 126:50–57.

Rødde, R. H., A. Einbu, and K. M. Va. 2008. A seasonal study of the chemical composition and chitin quality of shrimp shells obtained from northern shrimp (Pandalus borealis). Carbohydrate Polymers 71:388–393.

Rodriguez, G. 1981. Decapoda. Aquatic bioa of tropical South America, Part 1, Arthropoda.

Roulet, M., M. Lucotte, and R. Canuel. 2000. Increase in mercury contamination recorded in lacustrine sediments following deforestation in the central Amazon. Chemical ….

Royal Society of Chemestry. 2016. Periodic Table. in: http://www.rsc.org/periodic-table.

Santos, J., C. Sampaio, and A. S. Filho. 2006. Male population structure of the Amazon river prawn (*Macrobrachium amazonicum*) in a natural environment. Nauplius 14:55–63.

Taylor, P., J. A. F. Pantaleão, G. L. Hirose, and R. C. Costa. 1862. Invertebrate Reproduction & Development Relative growth , morphological sexual maturity , and size of *Macrobrachium amazonicum* (Heller 1862) (Crustacea , Decapoda , Palaemonidae) in a population with an entirely freshwater life cycle:37–41.

Terneus, E., K. Hernández, and M. J. Racines. 2012. Facultad de Ciencias Naturales y Exactas Universidad del Valle. Revista de Ciencias:31–45.

Turekian, K. K., & Wedepohl, K. H. 1961. Distribution of the elements in some major units of the earth's crust. *Geological Society of America Bulletin*, *72*(2), 175-192.

U.S. Geological Survey, 2016, Mineral commodity summaries 2016: U.S. Geological Survey, 202 p., http://dx.doi.org/10.3133/70140094.

Wallace, B., J. Hutchens, and J. Grubaugh. 2013. Transport and Storage of FPOM. Methods in Stream Ecology.

Wuana, R. a., and F. E. Okieimen. 2011. Heavy Metals in Contaminated Soils: A Review of Sources, Chemistry, Risks and Best Available Strategies for Remediation. ISRN Ecology 2011:1–20.

Yilmaz, a, and L. Yilmaz. 2007. Influences of sex and seasons on levels of heavy metals in tissues of green tiger shrimp (Penaeus semisulcatus de Hann, 1844). Food Chemistry 101:1664–1669.

Tables

Table 1. Geographical data of the ten sampling points in Northern (NA) and Southern Ecuadorian Amazon rivers (SA).

No.	Region	Province	River	Cod.	UTM/UPS			Altitude (masl)
1	North	Orellana	Conde	M15-01	18 S	291520	9923055	255
2	North	Sucumbios	Blanco	M15-02	18 S	318689	9961878	272
3	North	Sucumbios	Due	M15-03	18 S	233592	10003169	544
4	North	Sucumbios	Pacayaku	M15-04	18 S	325469	10000141	245
5	North	Orellana	Basura	M15-05	18 S	279648	9963505	284
6	North	Orellana	Guashito	M15-06	18 S	269232	9955379	269
7	South	Morona Santiago	Yananas	M15-08	18 S	168127	9664373	290
8	South	Morona Santiago	Kushapuku	M15-09	17 S	829986	9663818	332
9	South	Morona Santiago	Kusuimi	M15-10	18 S	177656	9668659	287
10	South	Morona Santiago	Chichis	M15-11	18 S	167464	9663947	289

Table 2. Analytical methods. Detection (DL) and quantification limits (QL), Accuracy, Reproducibility and Yield for the 47 analyzed elements.

Element:	B11	Rb85	Zr90	Nb93	Mo95	Cd111	Sn118	Cs133	Ba137	La139	Ce140	Pr141	Nd146	Sm147	Eu153	Gd157	Dy163	Ho165	Er166	Tm169	Yb172	Lu175	Hf178	Tl205
DL (µg.g-1)	0.1	0.003	0.001	0.005	0.007	0.06	0.07	0.0002	0.2	0.0002	0.0003	0.00001	0.0005	0.0003	0.0002	0.0004	0.0002	0.0001	0.0002	0.0001	0.0006	0.00002	0.002	0.00006
QL (µg.g-1)	0.4	0.010	0.004	0.017	0.024	0.19	0.22	0.0007	0.6	0.0007	0.0010	0.00002	0.0017	0.0010	0.0007	0.0012	0.0005	0.0005	0.0005	0.0002	0.0021	0.00008	0.008	0.00021
Accuracy (%)	29	2.1	42	43.02	3.04	12	-77.03	3	19	3	1	2	0.5	5	11	0.9	4.1	1	10	17	27	23	112	20
Reproducibility					-0.12	-0.1	-13.63		-0.2															
Yield (%)					88	90	-1263		83															

Element:	Pb208	Th232	U238	Na23	Mg24	Al27	Si28	P31	Ca44	Ti47	V51	Cr52	Mn55	Fe56	Co59	Cu63	Zn66	Ga69	Sr88	K39	As75	Se77	S32
DL (µg.g-1)	0.0008	0.0002	0.0001	51	0.5	0.3	5	32	430	0.06	0.06	0.004	0.14	1	0.0008	0.02	1	0.0004	0.05	140	0.007	0.07	60
QL (µg.g-1)	0.0027	0.0005	0.0002	170	1.56	1.0	18	108	1436	0.20	0.19	0.012	0.47	4.6	0.0027	0.08	4.24	0.0013	0.16	467	0.024	0.24	202
Accuracy (%)	2	12	4	0.9	3	60	-74	2	0.7	13	8	4	5	17	3	2	6	38	2	5	2	9	1
Reproducibility	-0.11			-0.03	-0.04			0.06	-0.1		-0.04	-0.14	0.1	-0.04	-0.06	-0.01	-0.01		-0.09	-0.1	-0.04	-0.20	0.02
Yield (%)	89			97	96			106	90		96	86	114	96	94	99	99		91	89	96	80	102

Table 3. Physico-chemical parameters (altitude, width, depth, temperature, pH, dissolved oxygen, trbidity, oxido-reduction potential, alkalinity, coliforms, total suspended solids, chemical oxygen demand, ammonium, nitrates, nitrites, meanders amount within 100m radius, IHF and QBR indexes) of the 10 sampling points (streams). Chemical analysis were measured in surface water samples.

Name	COD.	Date	Region	Altitude (masl)	Width (m)	Depth (m)	T (°C)	Conductivity (µS/cm)	pH	Dissolved O2 (mg/l)	Dissolved O2 (%sat)	Turbidity (ntu)	ORP (mV)	Alkalinity (mg/l)	Coliforms (UFC/ml)	E. coli (UFC/ ml)	Total coliforms (UFC/ ml)	TSS (mg/L)	COD (mg/L)	Ammonium (mg/L)*	Nitrates (mg/L)*	Nitrites (mg/L)*	# Meanders within 100m	IHF	QBR
Conde	M15-01	9/14/15	Norte	255	9	0.75	26.28	77	3.28	-	-	8.83	90	10.91	2	0.3	3	7.9	12.6137	0.3250	1.4960	0.1837	3	55	45
Blanco	M15-02	9/24/15	Norte	272	10	1.50	25.94	273	3.17	5.88	75.00	9.4	165.6	71.72	30	9.3	39	12.5	10.1542	0.3380	4.0187	0.2750	2	53	70
Due	M15-03	9/17/15	Norte	544	6	0.50	25.33	112	4.6	8.55	100.00	1.3	85	34.99	9	1.7	10	5.6	16.8301	0.2730	4.0187	0.1606	1	67	95
Pacayaku	M15-04	9/19/15	Norte	245	10	1.50	25.27	77	4.54	5.94	85.60	21.3	30.6	8.13	12	6.7	19	24.4	37.5605	4.3810	0.4987	0.3883	2	65	35
Basura	M15-05	9/22/15	Norte	284	8	1.00	26.06	73	3.69	7.02	86.50	10.6	173.7	12.72	12	3.3	15	15.9	17.1814	0.5850	1.5547	0.2112	1	61	25
Guashito	M15-06	9/26/15	Norte	269	7	1.20	27.51	253	6.07	5.40	67.90	24.18	42	22.78	82	3.0	85	7.6	11.5596	0.4030	11.9973	1.4740	2	66	90
Yananas	M15-08	11/12/15	Sur	290	12	0.70	23.36	72	4.29	7.14	83.80	4.82	168.6	21.14	1	2.3	3	4.0	3.6246	0.4030	5.2507	0.4169	2	69	75
Kushapuku	M15-09	11/11/15	Sur	332	7	1.50	26.55	209	6.47	6.50	79.00	2.35	80.7	21.53	30	4.0	34	7.3	0.0000	0.3510	4.9427	0.6941	2	66	75
Kusuimi	M15-10	11/10/15	Sur	287	8	0.60	24.25	389	3.68	6.80	80.40	6.33	224.6	57.03	15	3.0	18	5.90	11.4441	0.3380	0.0000	0.4752	2	76	60
Chichis	M15-11	11/14/15	Sur	289	6	0.60	23.86	115	4.25	3.42	40.50	0.73	156.9	36.48	30	0.0	30	6.4	5.0463	0.2730	0.0000	0.2816	3	80	95

Table 4. Fluvial and riparian habitat quality level categories (Pardo et al. 2002).

FLUVIAL AND RIPARIAN HABITAT QUALITY LEVEL		
CATEGORIES	*Value*	*Color*
Riparian habitat in natural condition	≥ 95	Blue
Some disturbance, good quality	75-90	Green
Disturbance important, fair quality	55-70	Yellow
Strong alteration, bad quality	30-50	Orange
Extreme degradation, very bad quality	≤ 25	Red

Table 5. Pearson correlations between microbiological and physico-chemical parameters of the study Rivers.

Parameters	Depth	Conductivity	pH	Dissolved oxigen	Coliforms	Total coliforms	TSS	COD	Nitrates
Alkalinity		.727*							
E. coli	.797**								
COD							.832**		
Ammonium							.849**	.854**	
Nitrates					.734*	.727*			
Nitrites			.711*		.878**	.864**			.814**
# Meanders within 100m				-.862**					

** Significant correlation at the level 0.01 (2 tails)

* Significant correlation at the level 0.05 (2 tails)

Table 6. Means and standard errors (SE) of the concentrations of major elements found in the shrimp species *Macrobrachium brasiliense* collected in six rivers in the Ecuadorian Amazon. Unit (μg.g^{-1} dw). * SA River as a control.

River	Ca	P	K	S	Na	Fe	Mn	Mg	Zn	Cu
Conde	137126 ± 8332	10928 ± 634	8833 ± 610	4589 ± 437	4782 ± 347	327 ± 52	406.70 ± 86.76	2565 ± 228	113 ± 9	64.81 ± 5.52
Blanco	158543 ± 9139	8930 ± 667	8551 ± 487	4548 ± 371	5327 ± 249	328 ± 30	148.89 ± 32.42	2534 ± 144	114 ± 7	98.53 ± 5.47
Due	103849 ± 23372	11167 ± 793	9426 ± 1434	6311 ± 796	5368 ± 584	320 ± 87	123.10 ± 52.34	1978 ± 364	139 ± 8	113.17 ± 21.96
Basura	115467 ± 17954	10964 ± 851	9464 ± 551	5435 ± 570	4684 ± 303	801 ± 166	1123.26 ± 333.55	2123 ± 128	135 ± 7	72.54 ± 4.42
Guashito	132467 ± 12832	10223 ± 573	7995 ± 819	5140 ± 525	5153 ± 289	438 ± 45	294.83 ± 236.73	2118 ± 133	96 ± 8	64.42 ± 5.02
Yananas*	118734 ± 7595	10828 ± 728	10566 ± 489	6152 ± 400	5509 ± 294	309 ± 83	56.03 ± 6.07	2363 ± 112	131 ± 5	97.37 ± 5.79

Table7. Means and standard errors (SE) of the concentrations of No Toxic Trace Metals found in the shrimp species *Macrobrachium brasiliense* collected from six rivers in the Ecuadorian Amazon. Units = $\mu g.g^{-1}$. * SA River as a control.

River	Sr	Ba	Si	Al	Rb	Ti	B	Se	Mo	Zr	Cs	Ga	Nb	Tl	Hf	Th	U
Conde	1586.1 ± 95.8	755.2 ± 90.9	260 ± 36	276 ± 35	20.327 ± 0.860	5.4 ± 0.8	3.4 ± 0.2	0.5 ± 0.03	0.21 ± 0.01	0.141 ± 0.012	0.0402 ± 0.0014	0.060 ± 0.008	0.04 ± 0.01	0.0609 ± 0.0042	0.026 ± 0.002	0.0233 ± 0.0034	0.0106 ± 0.0020
Blanco	1312.9 ± 71.9	124.0 ± 12.2	416 ± 91	308 ± 21	14.381 ± 0.633	12.4 ± 1.2	3.7 ± 0.6	1.1 ± 0.07	0.20 ± 0.00	0.297 ± 0.040	0.0267 ± 0.0025	0.061 ± 0.005	0.07 ± 0.01	0.0248 ± 0.0009	0.034 ± 0.004	0.0096 ± 0.0017	0.0126 ± 0.0015
Due	777.8 ± 156.7	398.6 ± 107.7	157 ± 43	184 ± 40	29.048 ± 3.799	3.6 ± 0.5	4.0 ± 0.5	1.2 ± 0.08	0.43 ± 0.05	0.071 ± 0.024	0.0712 ± 0.0094	0.039 ± 0.008	0.03 ± 0.01	0.0681 ± 0.0092	0.011 ± 0.003	0.0113 ± 0.0055	0.0123 ± 0.0053
Basura	1036.3 ± 84.2	374.4 ± 29.6	471 ± 87	349 ± 50	27.146 ± 0.740	16.5 ± 3.6	2.7 ± 0.3	0.8 ± 0.10	0.33 ± 0.05	0.202 ± 0.131	0.0834 ± 0.0020	0.077 ± 0.011	0.07 ± 0.02	0.0451 ± 0.0069	0.027 ± 0.017	0.0146 ± 0.0065	0.0071 ± 0.0009
Guashito	1533.4 ± 164.0	247.7 ± 89.8	491 ± 51	363 ± 29	12.216 ± 1.609	17.0 ± 2.0	4.0 ± 0.8	1.3 ± 0.19	0.27 ± 0.02	0.313 ± 0.066	0.0243 ± 0.0059	0.079 ± 0.006	0.09 ± 0.01	0.0191 ± 0.0044	0.035 ± 0.006	0.0156 ± 0.0010	0.0116 ± 0.0017
Yananas*	996.3 ± 214.7	482.2 ± 60.8	397 ± 132	390 ± 125	31.861 ± 8.039	14.3 ± 6.5	4.6 ± 0.7	0.9 ± 0.19	0.28 ± 0.07	0.252 ± 0.080	0.6657 ± 0.1549	0.081 ± 0.030	0.08 ± 0.03	0.0602 ± 0.0151	0.038 ± 0.012	0.0233 ± 0.0085	0.0179 ± 0.0080

Table 8. Means and standard errors (SE) of the concentrations of Toxic Trace Metals found in the shrimp species *Macrobrachium brasiliense* collected from six rivers in the Ecuadorian Amazon. Unit ($\mu g.g^{-1}$ dw). * SA River as a control.

River	V	Co	Cr	Pb	Cd	As	Sn
Conde	0.65 ± 0.11	1.280 ± 0.247	0.52 ± 0.04	0.282 ± 0.038	1.20 ± 0.10	0.24 ± 0.02	0.22 ± 0.01
Blanco	0.92 ± 0.11	0.662 ± 0.109	0.61 ± 0.03	0.959 ± 0.122	0.24 ± 0.03	0.16 ± 0.02	0.62 ± 0.28
Due	0.56 ± 0.16	0.365 ± 0.120	0.72 ± 0.32	0.202 ± 0.034	1.27 ± 0.31	0.23 ± 0.05	0.17 ± 0.04
Basura	1.28 ± 0.32	1.689 ± 0.409	0.32 ± 0.07	0.276 ± 0.075	0.45 ± 0.08	0.17 ± 0.02	0.12 ± 0.004
Guashito	1.04 ± 0.13	0.503 ± 0.162	1.98 ± 0.71	0.136 ± 0.006	0.33 ± 0.03	0.13 ± 0.05	0.11 ± 0.01
Yananas*	1.05 ± 0.31	0.355 ± 0.033	0.93 ± 0.28	1.540 ± 0.629	0.25 ± 0.02	0.58 ± 0.15	0.15 ± 0.05

Table 9. Means and standard errors (SE) of the concentrations of Rare Earth Elements found in the shrimp species *Macrobrachium brasiliense* collected from six rivers in the Ecuadorian Amazon. Unit ($\mu g.g^{-1}$). * SA River as a control.

River	Ce	La	Nd	Eu	Gd	Pr	Sm	Dy	Er	Yb	Ho	Tm	Lu
Conde	0.8783 ± 0.1764	0.4258 ± 0.0533	0.331 ± 0.053	0.3492 ± 0.0417	0.082 ± 0.012	0.08222 ± 0.01402	0.068 ± 0.011	0.0437 ± 0.0076	0.0222 ± 0.0043	0.016 ± 0.003	0.0081 ± 0.0015	0.0031 ± 0.0006	0.00252 ± 0.00049
Blanco	0.2288 ± 0.0338	0.1314 ± 0.0174	0.120 ± 0.017	0.0483 ± 0.0033	0.030 ± 0.003	0.03001 ± 0.00396	0.023 ± 0.003	0.0143 ± 0.0019	0.0073 ± 0.0009	0.005 ± 0.001	0.0025 ± 0.0004	0.0010 ± 0.0001	0.00089 ± 0.00013
Due	0.1516 ± 0.0791	0.0880 ± 0.0367	0.083 ± 0.046	0.1030 ± 0.0184	0.027 ± 0.013	0.01988 ± 0.01038	0.023 ± 0.012	0.0130 ± 0.0077	0.0060 ± 0.0034	0.003 ± 0.002	0.0024 ± 0.0014	0.0007 ± 0.0003	0.00053 ± 0.00032
Basura	0.3975 ± 0.0693	0.2065 ± 0.0337	0.176 ± 0.030	0.1162 ± 0.0058	0.044 ± 0.006	0.04534 ± 0.00756	0.036 ± 0.006	0.0192 ± 0.0035	0.0101 ± 0.0019	0.008 ± 0.002	0.0033 ± 0.0007	0.0013 ± 0.0002	0.00121 ± 0.00019
Guashito	0.3319 ± 0.0549	0.2180 ± 0.0328	0.178 ± 0.029	0.0634 ± 0.0138	0.042 ± 0.006	0.04577 ± 0.00734	0.033 ± 0.005	0.0191 ± 0.0038	0.0093 ± 0.0016	0.007 ± 0.001	0.0032 ± 0.0005	0.0012 ± 0.0002	0.00129 ± 0.00022
Yananas*	0.3487 ± 0.1204	0.2568 ± 0.0856	0.204 ± 0.068	0.2174 ± 0.0522	0.053 ± 0.016	0.05178 ± 0.01777	0.044 ± 0.015	0.0287 ± 0.0102	0.0144 ± 0.0048	0.010 ± 0.004	0.0052 ± 0.0019	0.0019 ± 0.0006	0.00162 ± 0.00061

Table 10. Maximum daily estimated number of shrimps suggested for consumption per river based on the tolerable weekly or daily intake per reference element (Cr PTDI = 0.5 μg/kg bw, IOM 2001) (Pb PTDI= 0.6 μg/kg bw decrease 1 IQ in children, 1.6 μg/kg bw increase 1 mmHg of blood pressure in adults, JECFA 2011) (Cd PTMI = 25 μg/kg bw, FAO 2011) for average adults men (70 kg), women (60 kg) and children (20 kg) and the concentrations (μg.g-1 ± SE) of the elements found in the freshwater shrimp species *Macrobrachium brasiliense* collected from six rivers in the Ecuadorian Amazon (* SA River as control).

River	reference element	ug.g-1		SE	average shrimp weight (g)	maximum daily estimated number of shrimps
			MEN			
Conde	Cd	1.20	±	0.10	2.0	96
Blanco	Cr	0.61	±	0.03	2.4	96
Due	Cd	1.27	±	0.31	2.8	65
Basura	Cr	0.32	±	0.07	1.7	261
Guashito	Cr	1.98	±	0.71	2.7	27
Yananas*	Cr	0.93	±	0.28	1.8	85
			WOMEN			
Conde	Cd	1.20	±	0.10	2.0	82
Blanco	Cr	0.61	±	0.03	2.4	69
Due	Cr	0.72	±	0.32	2.8	50
Basura	Cr	0.32	±	0.07	1.7	187
Guashito	Cr	1.98	±	0.71	2.7	19
Yananas*	Cr	0.93	±	0.28	1.8	61
			CHILDREN			
Conde	Cd	1.20	±	0.10	2.0	27
Blanco	Pb	0.96	±	0.12	2.4	21
Due	Cd	1.27	±	0.31	2.8	19
Basura	Cd	0.45	±	0.08	1.7	90
Guashito	Cr	1.98	±	0.71	2.7	11
Yananas*	Pb	1.54	±	0.63	1.8	18

Figures

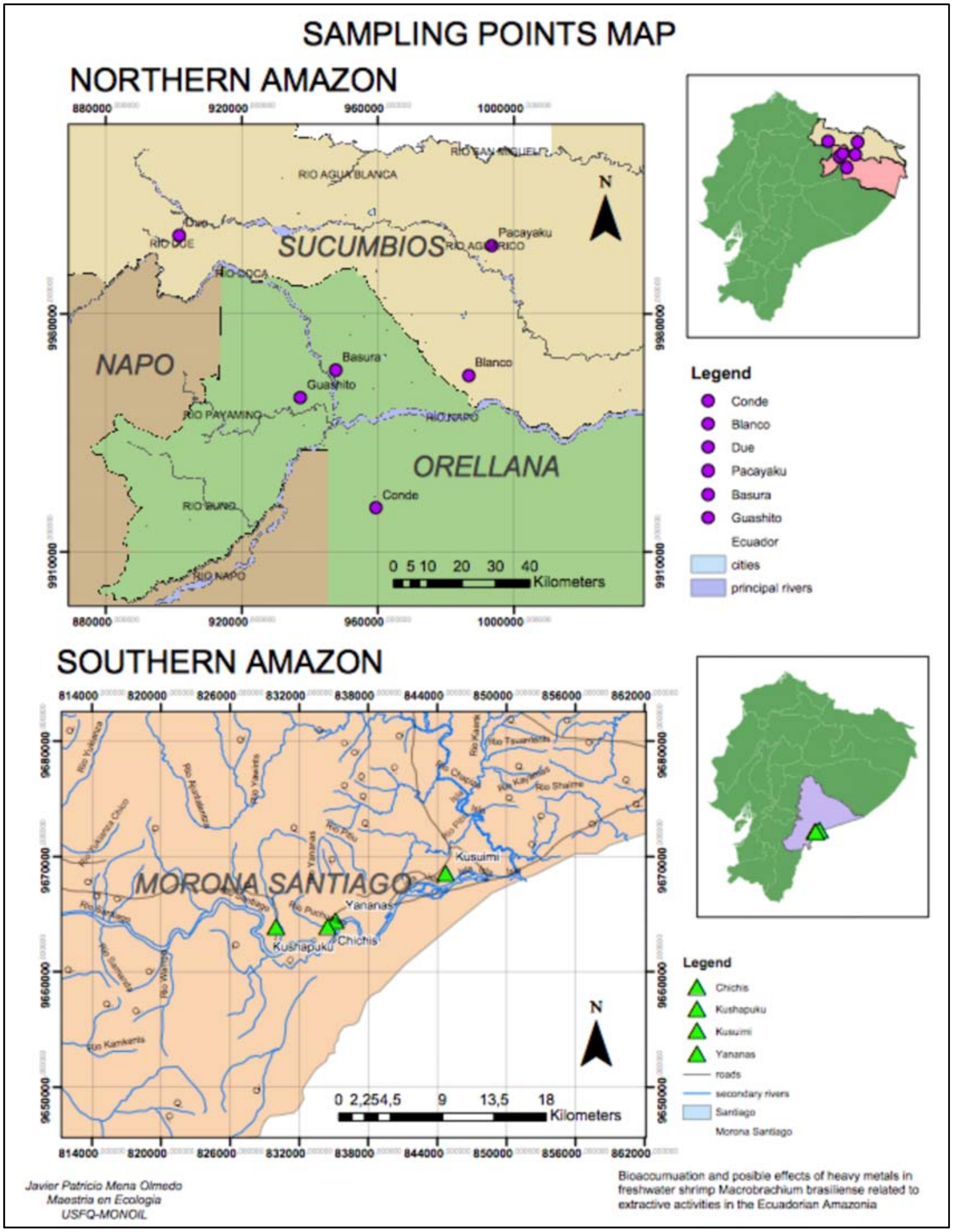


Figure 1. Sampling Points Maps. A). Northern Ecuadorian Amazon sampling points (NA). B) Southern Ecuadorian Amazon sampling points (SA).

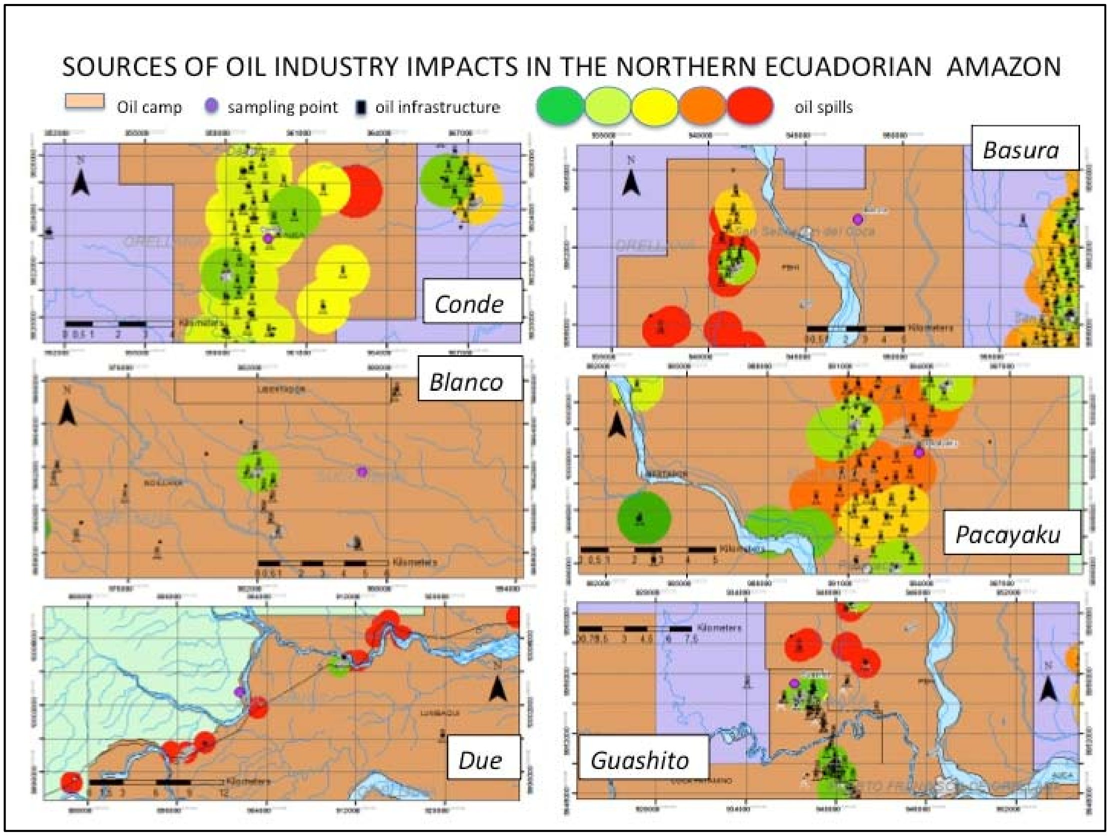


Figure 2. Northern Ecuadorian Amazon Sampling Points Map. Oil camps, oil infrastructure (oil wells, lighters, stations, oil pits and spills).

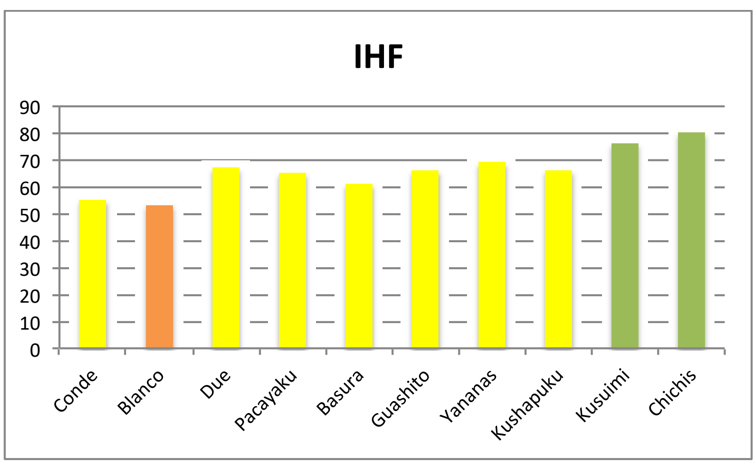


Figure 3. Fluvial habitat index values per evaluated river. The colors indicate the quality of the habitat based on the categories table.

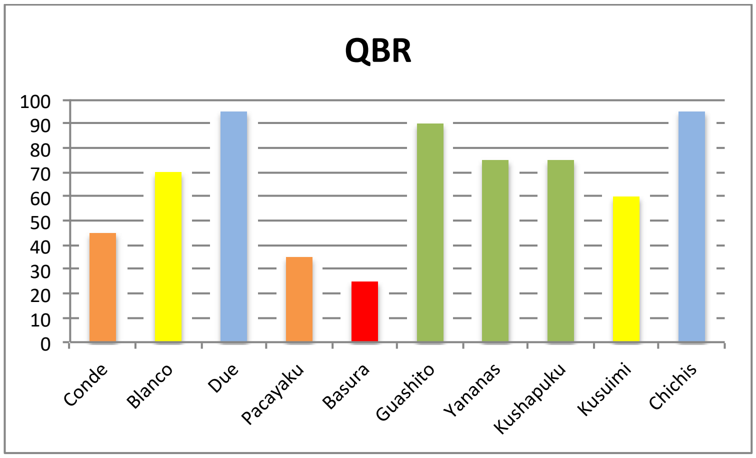


Figure 4. Riparian habitat quality index values per evaluated river. The colors indicate the quality of the riparian habitat indicated in the categories table.

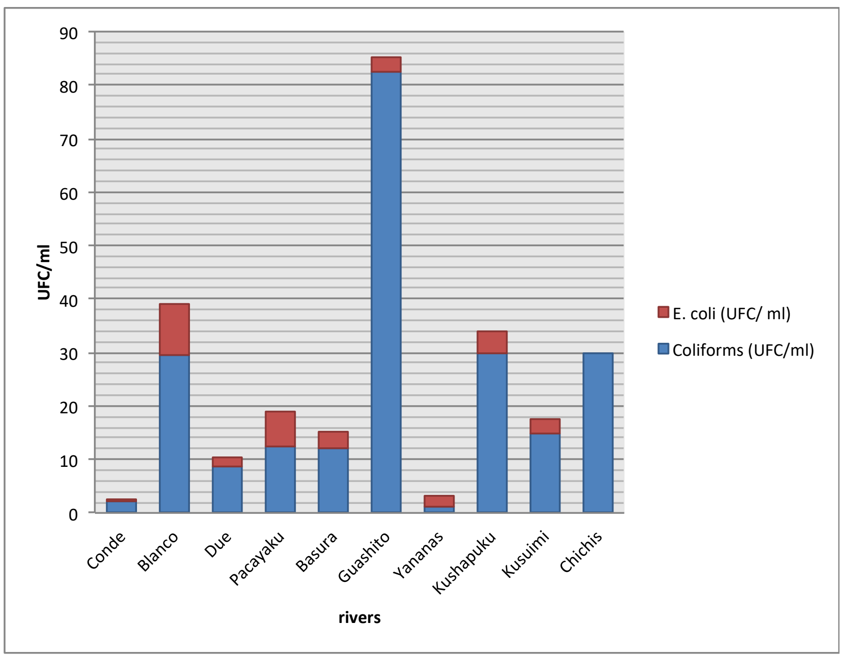


Figure 5. Total coliforms (environmental coliforms + *E. coli*) measured in surface water samples of the ten sampling streams in the Ecuadorian Amazon. UFC/ml= unities of forming colonies per milliliter.

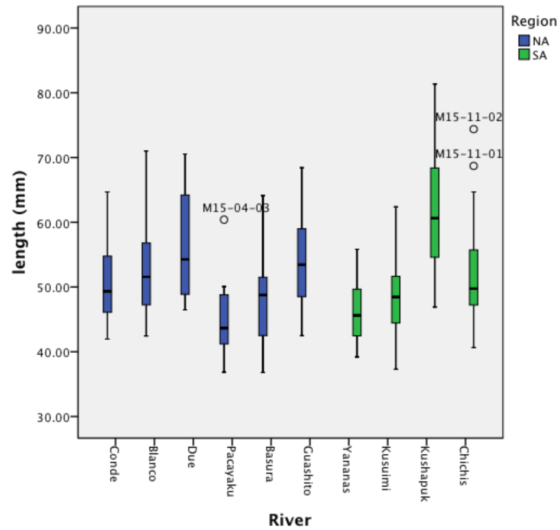


Figure 6. Minimum, maximum and median of the shrimp body lengths per sampled river. In blue the Northern Amazon Rivers (NA) and in green the Southern Amazon Rivers (SA).

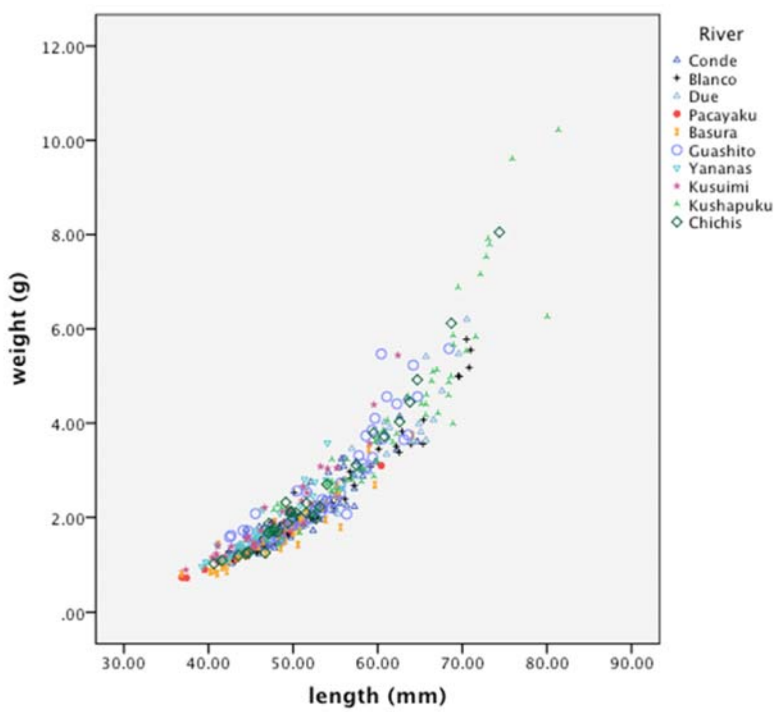
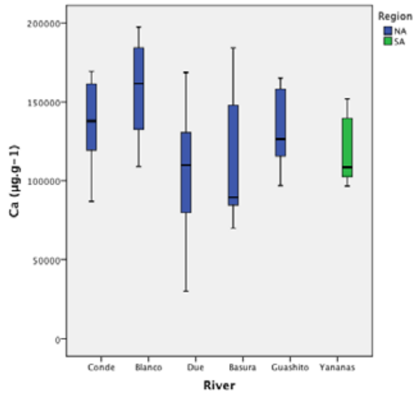
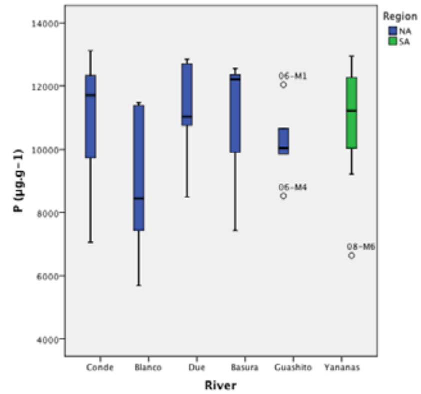


Figure 7. Individuals shrimp body length vs weight collected in the ten sampling points in the Ecuadorian Amazon. Each color and symbol represents individuals in a single sampled river. The difference in sizes among them is remarkable especially between the individuals in the Pacayaku River and the Kushapuku River.

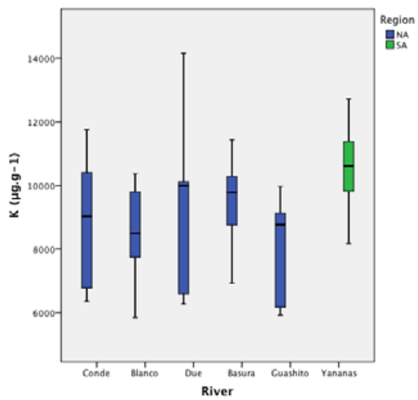
8.


Region
NA
SA
Ca (µg.g-1)
River
Conde
Blanco
Due
Basura
Guashito
Yananas

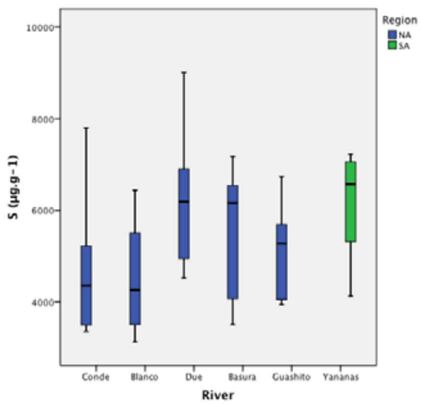
9.


Region
NA
SA
P (µg.g-1)
06-M1
06-M4
08-M6
River
Conde
Blanco
Due
Basura
Guashito
Yananas

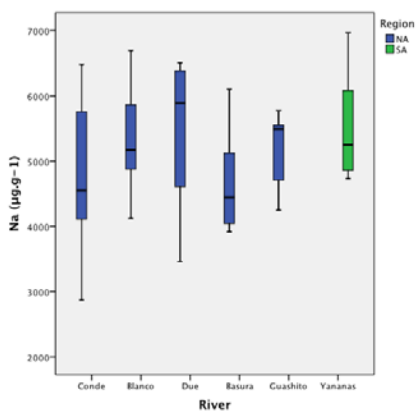
10.


Region
NA
SA
K (µg.g-1)
River
Conde
Blanco
Due
Basura
Guashito
Yananas

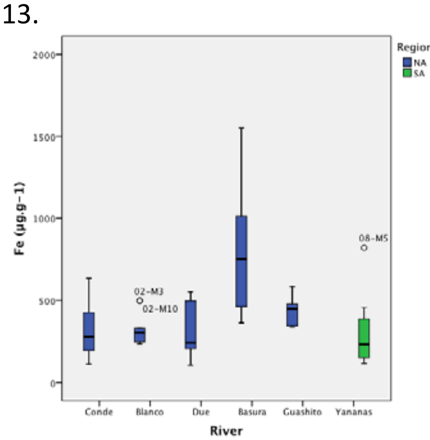
11.


Region
NA
SA
S (µg.g-1)
River
Conde
Blanco
Due
Basura
Guashito
Yananas

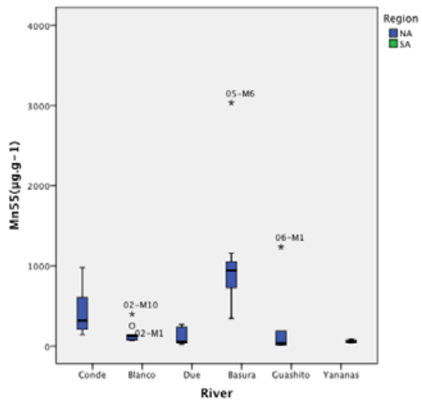
12.


Region
NA
SA
Na (µg.g-1)
River
Conde
Blanco
Due
Basura
Guashito
Yananas

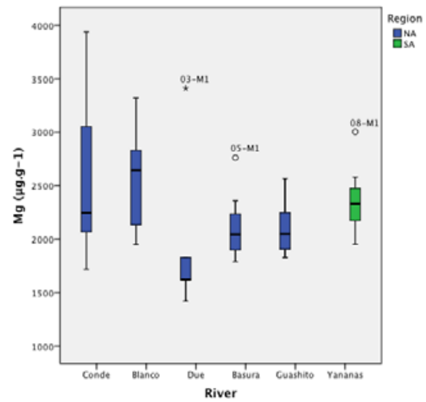
13.


Region
NA
SA
Fe (µg.g-1)
02-M3
02-M10
08-M5
River
Conde
Blanco
Due
Basura
Guashito
Yananas

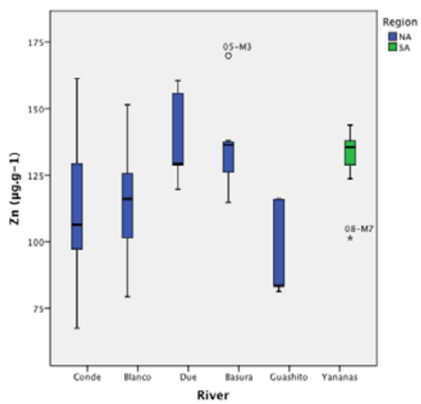
14.



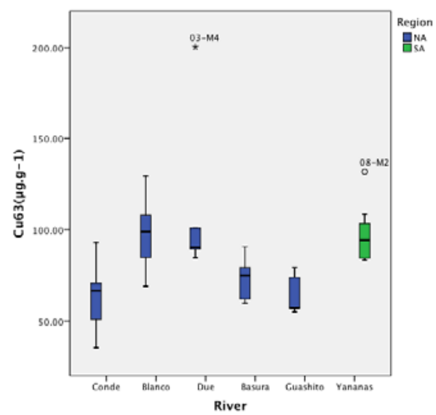
15.



16.



17.



Figures 8-17. Box plots of the major elements concentrations analyzed in the shrimp species *Macrobrachium brasiliense* collected in six rivers in the Ecuadorian Amazon. Unit (µg.g^{-1} dw). In blue the Northern Amazon Rivers (NA) and in green the Southern Amazon River (SA)(control).

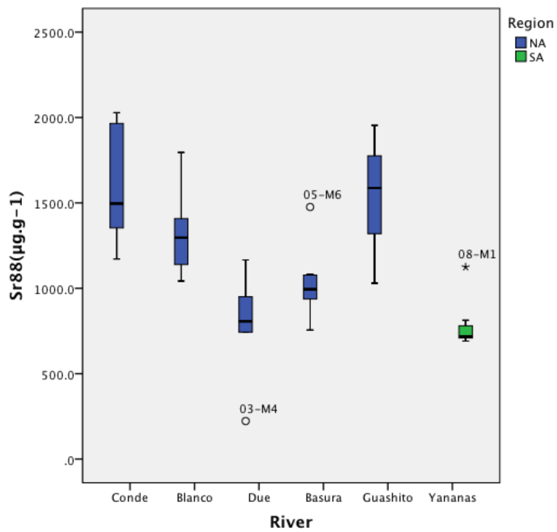


Figure 18. Minimum, maximum and median of Sr concentrations found in the shrimp species *Macrobrachium brasiliense* collected from six rivers in the Ecuadorian Amazon. Unit (µg.g^{-1} dw). In blue the Northern Amazon Rivers (NA) and in green the Southern Amazon River (SA) (control).

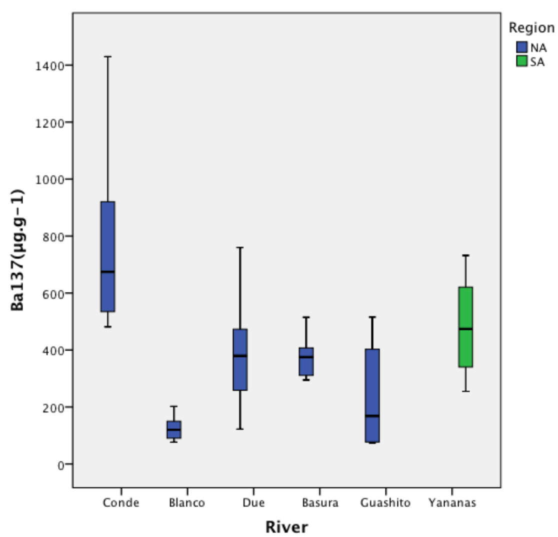


Figure 19. Minimum, maximum and median of Ba concentrations found in the shrimp species *Macrobrachium brasiliense* collected from six rivers in the Ecuadorian Amazon. Unit (ug.g^{-1}). In blue the Northern Amazon River s (NA)and in green the Southern Amazon River (SA)(control).

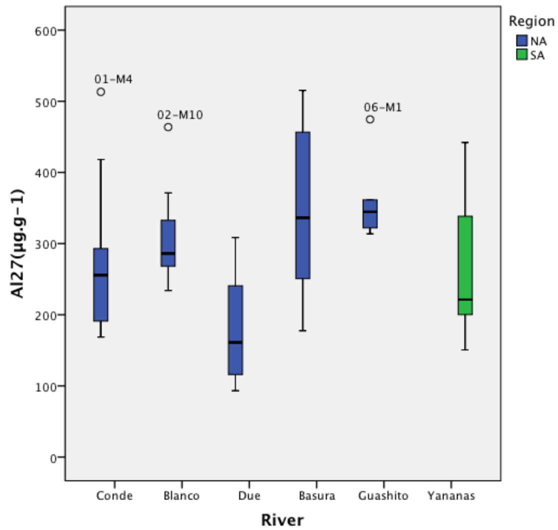


Figure 20. Minimum, maximum and median of Al concentrations found in the shrimp species *Macrobrachium brasiliense* collected from six rivers in the Ecuadorian Amazon. Unit (ug.g^{-1} dw). In blue the Northern Amazon Rivers (NA) and in green the Southern Amazon River (SA) (control).

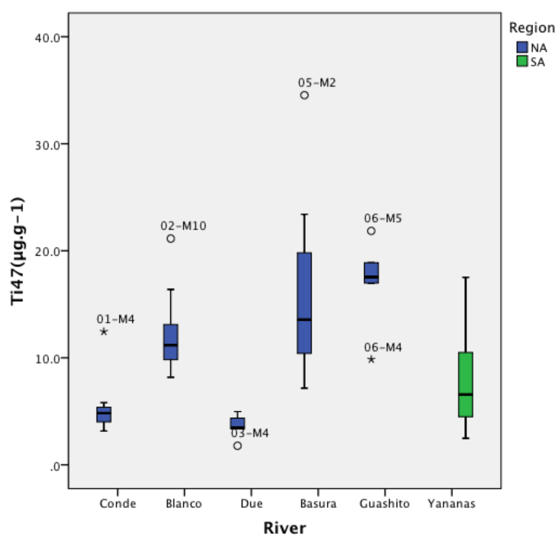


Figure 21. Minimum, maximum and median of Ti concentrations found in the shrimp species *Macrobrachium brasiliense* collected from six rivers in the Ecuadorian Amazon. Unit (ug.g^{-1}). In blue the Northern Amazon Rivers (NA) and in green the Southern Amazon River (SA)(control).

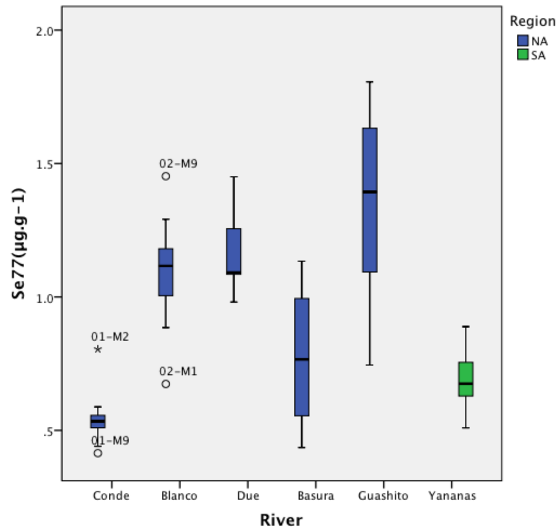


Figure 22. Minimum, maximum and median of Se concentrations found in the shrimp species *Macrobrachium brasiliense* collected from six rivers in the Ecuadorian Amazon. Unit ($ug.g^{-1}$). In blue the Northern Amazon Rivers (NA) and in green the Southern Amazon River (SA)(control).

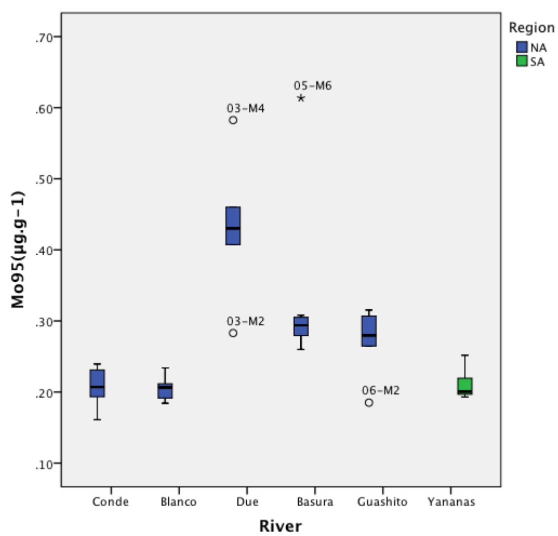


Figure 23. Minimum, maximum and median of Mo concentrations found in the shrimp species *Macrobrachium brasiliense* collected from six rivers in the Ecuadorian Amazon. Unit ($ug.g^{-1}$). In blue the Northern Amazon Rivers (NA) and in green the Southern Amazon River (SA) (control).

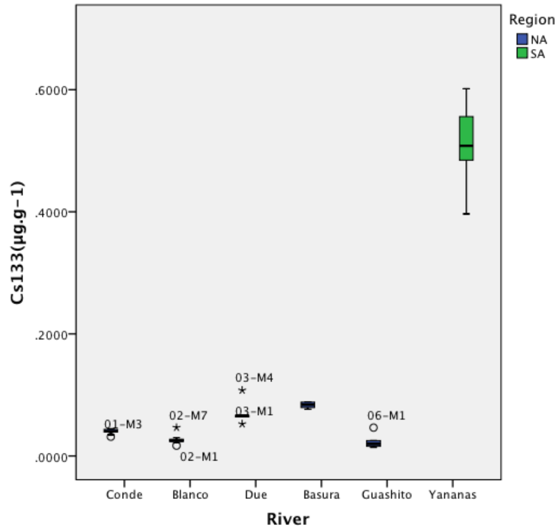


Figure 24. Minimum, maximum and median of Cs concentrations found in the shrimp species *Macrobrachium brasiliense* collected from six rivers in the Ecuadorian Amazon. Unit (ug.g^{-1}). In blue the Northern Amazon Rivers (NA) and in green the Southern Amazon River (SA) (control).

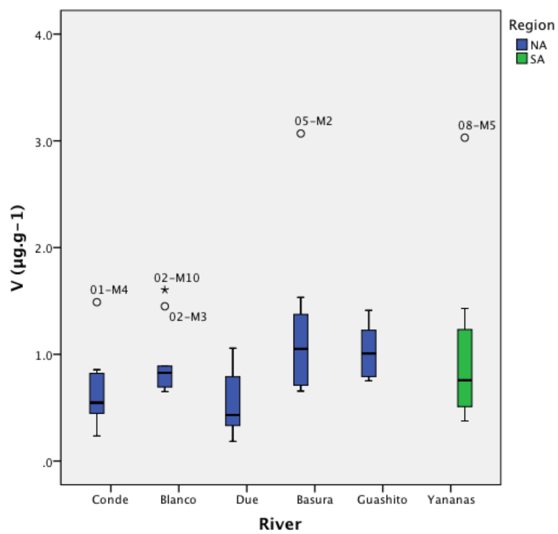


Figure 25. Minimum, maximum and median of V concentrations found in the shrimp species *Macrobrachium brasiliense* collected from six rivers in the Ecuadorian Amazon. Unit (ug.g^{-1} dw). In blue the Northern Amazon Rivers (NA) and in green the Southern Amazon River (SA)(control).

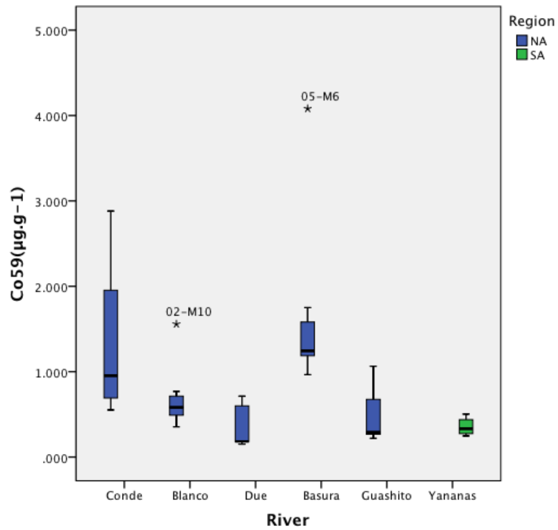


Figure 26. Minimum, maximum and median of Co concentrations found in the shrimp species *Macrobrachium brasiliense* collected from six rivers in the Ecuadorian Amazon. Unit (µg.g^{-1} dw). In blue the Northern Amazon Rivers (NA) and in green the Southern Amazon River (SA) (control).

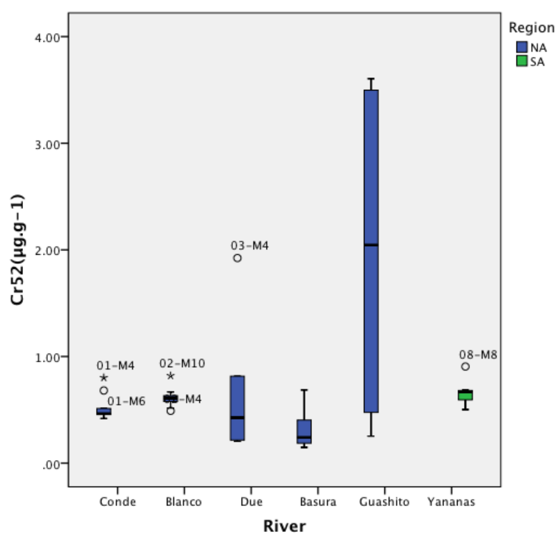


Figure 27. Minimum, maximum and median of Cr concentrations found in the shrimp species *Macrobrachium brasiliense* collected from six rivers in the Ecuadorian Amazon. Unit (ug.g^{-1}). In blue the Northern Amazon Rivers (NA) and in green the Southern Amazon River (SA)(control).

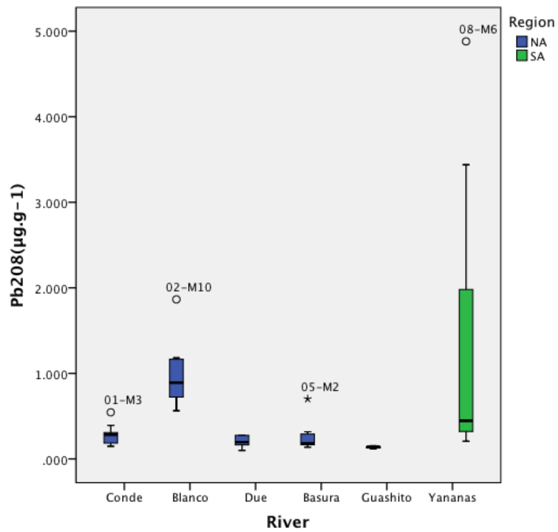


Figure 28. Minimum, maximum and median of Pb concentrations found in the shrimp species *Macrobrachium brasiliense* collected from six rivers in the Ecuadorian Amazon. Unit (ug.g^{-1} dw). In blue the Northern Amazon Rivers (NA) and in green the Southern Amazon River (SA)(control).

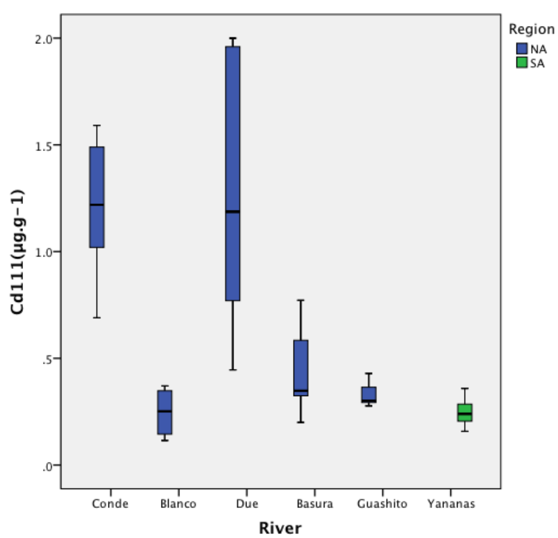


Figure 29. Minimum, maximum and median of Cd concentrations found in the shrimp species *Macrobrachium brasiliense* collected from six rivers in the Ecuadorian Amazon. Unit (ug.g^{-1} dw). In blue the Northern Amazon Rivers (NA) and in green the Southern Amazon River (SA)(control).

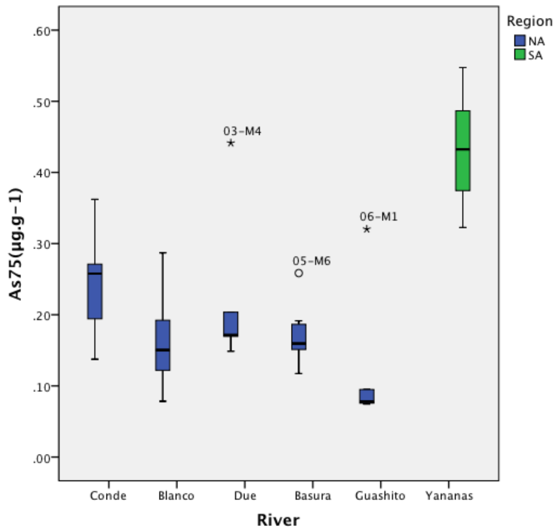
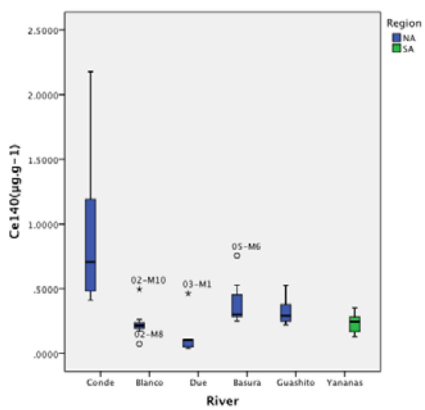
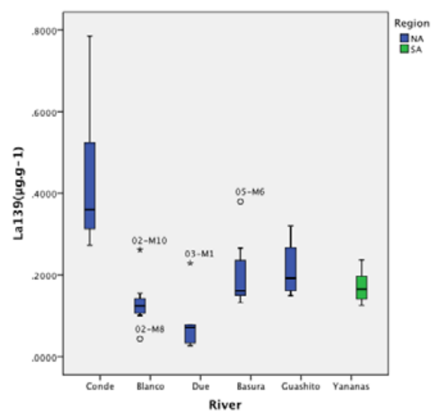


Figure 30. Minimum, maximum and median of As concentrations found in the shrimp species *Macrobrachium brasiliense* collected from six rivers in the Ecuadorian Amazon. Unit (ug.g^{-1} dw). In blue the Northern Amazon Rivers (NA) and in green the Southern Amazon River (SA)(control).

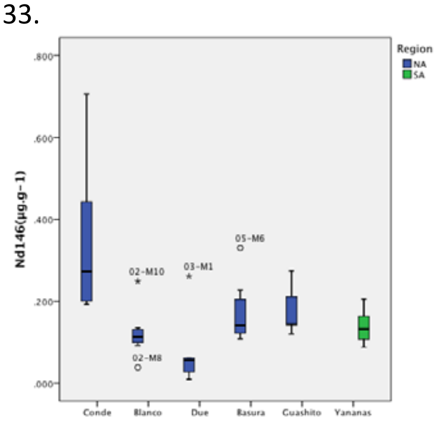
31.



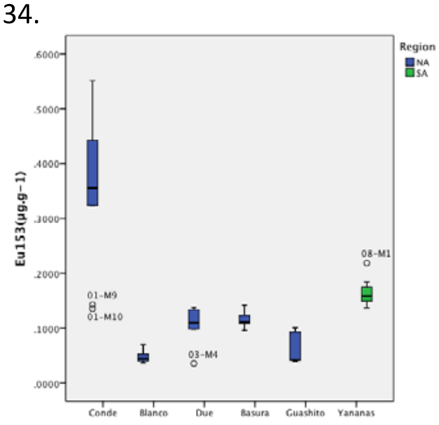
32.



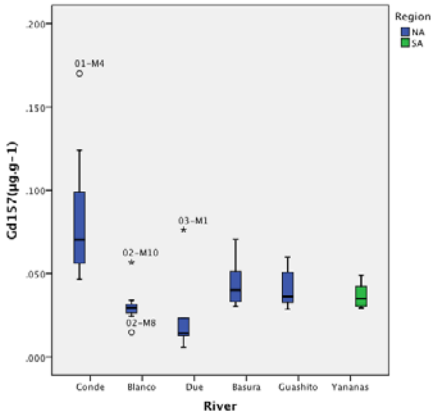
33.



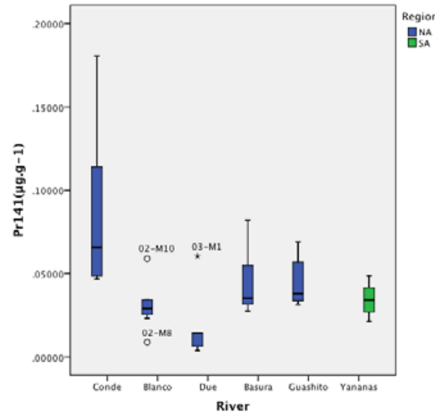
34.



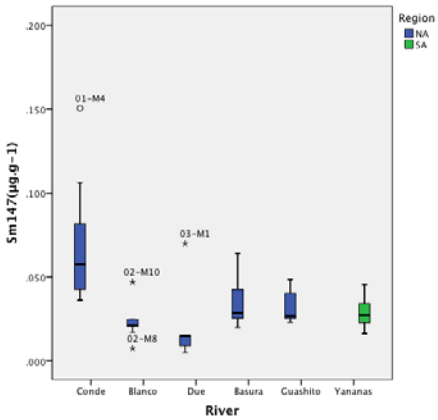
35.



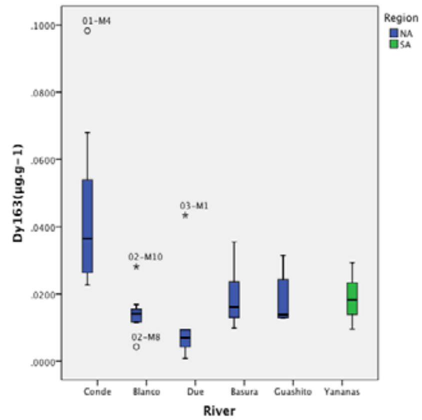
36.



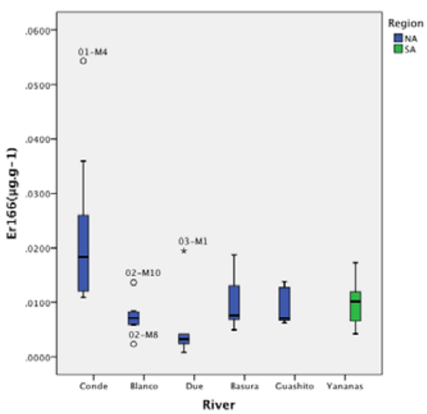
37.



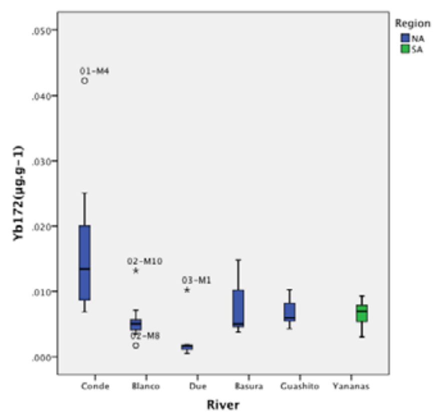
38.



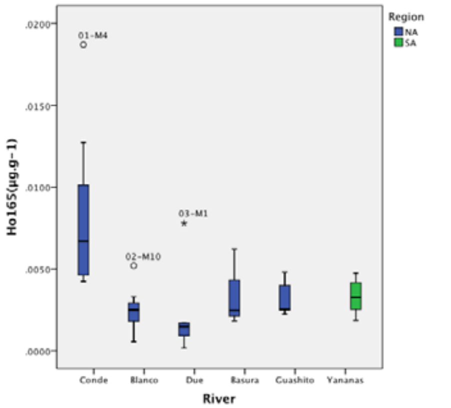
39.



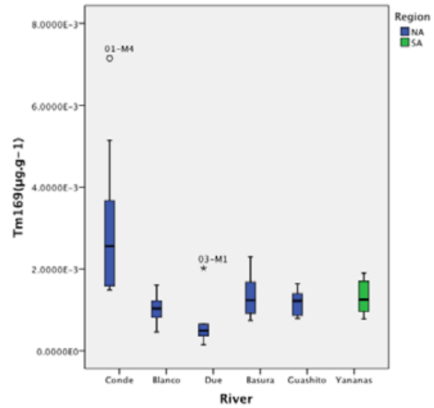
40.



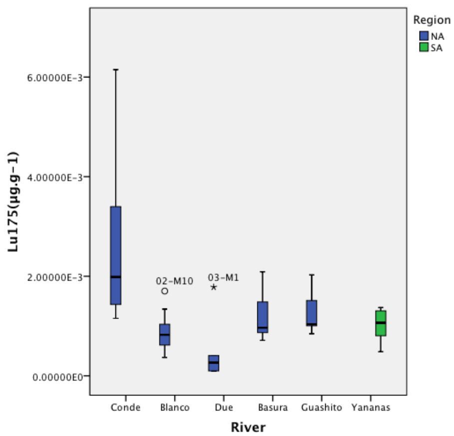
41.



42.



43.



Figures 31-43. Minimum, maximum and median of REE concentrations found in the shrimp species *Macrobrachium brasiliense* collected from six rivers in the Ecuadorian Amazon. Unit (ug.g^{-1} dw). In blue the Northern Amazon Rivers (NA) and in green the Southern Amazon River (SA) (control).

CHAPTER 2

Deforestation impacts in the Ecuadorian Amazon discriminated by total mercury concentrations in freshwater shrimp *Macrobrachium brasiliense*

Deforestation impacts in the Ecuadorian Amazon discriminated by total mercury concentrations in freshwater shrimp *Macrobrachium brasiliense*

[1,2]Javier P. Mena-Olmedo*, [3,4]Laurence Maurice, [3] Laure Laffont,[1,4]Valeria Ochoa-Herrera, [1,5]Andrea C. Encalada

[1]Universidad San Francisco de Quito, Colegio de Ciencias Biológicas y Ambientales (USFQ-COCIBA)
[2]Maestría en Ecología - Estudios Amazónicos USFQ
[3]Géosciences Environnement Toulouse (GET), Observatoire Midi Pyrénées, IRD, CNRS, Université de Toulouse, Toulouse, France
[4]Colegio de Ciencias e Ingeniería, USFQ-Politécnico
[5]Laboratorio de Ecología Acuática USFQ-COCIBA
*e-mail: pmenaolmedo@gmail.com

Abstract

This study presents, for the first time, total mercury concentrations in the most abundant freshwater shrimp *Macrobrachium brasiliense* (Crustacean– Palaemonidae) in the Ecuadorian Amazon basin. In this study, six rivers of the Northern Ecuadorian Amazon affected by oil activities were sampled and compared with four geomorphological similar rivers in the Southern Ecuadorian Amazon free from oil or mining activities. Total mercury concentrations in entire body ranged from 0.012 to 0.142 $\mu g.g^{-1}$ (dry weight). Quite unexpectedly, the *Macrobrachium brasiliense* sampled in the Southern Ecuadorian Amazon Rivers presented the highest Hg concentrations. This trend could be explained by the high deforestation rates measured in the Morona-Santiago province within the 2008-2014 period (7925 $ha.y^{-1}$ against 4251 $ha.y^{-1}$ for the Northern provinces) (MAE-PRAS 2014). Mercury cannot be considered as a proxy for oil activities tracing; however, it can evaluate deforestation impacts, especially in tropical areas where Andean soils can also be enriched in mercury by volcanic rock weathering. Regarding the human health risk assessment, shrimp consumption proposed based on international standards (WHO/FAO JECFA 2003; PTWI = 1.6 $\mu g.kg^{-1}$ body weight); it appears that in all the sampled rivers, the regular freshwater shrimp consumption is safe. In addition, the mercury concentrations didn't exceed the permissible limits established by the European Commission (EC 2010), the Health Canada's Maximum Levels for chemical contaminants in food (2016) and the Colombian Public Health Ministry (MSPS-Colombia 2016); however, especial awareness in the consumption by children is recommended especially in the rivers Chichis and Kushapuku, in the Southern Ecuadorian Amazon, where the safety amounts recommend for ingestion were the lowest from all rivers.

Key Words: *Macrobrachium brasiliense*, total mercury, deforestation, Ecuadorian Amazon, anthropogenic activities.

Introduction

The Hg dynamic in the Ecuadorian Amazon is greatly influenced by the Andean cordillera (Maurice-B. et al. 2003). Andes are considered as one of the most active belt of mercury in the world due to the influence of tectonic and volcanic activity, which could release significant concentrations of inorganic mercury to the aquatic ecosystems from soil erosion (Mainville et al. 2006; Fostier et al. 2000; Roulet 2001; Roulet et al. 2000). In addition, Napo headwaters could contain high concentrations of elemental mercury coming from highlands (Mainville et al. 2003).

Land use and land cover change in tropical forest has been attributed to complex dynamics and synergic factors driven mainly by deforestation (Lambin et al. 2003). Since the beginning of the oil exploration and the land reform in the mid 60's, many people have migrated to the Ecuadorian Amazon in the aim of colonizing new lands, changing drastically the original land use and natural cover. Moreover, indigenous peoples living in those areas have changed not only their practices but also the surface of the land use due to new technologies availability and roads construction for oil exploitation (Walsh et al. 2008). Complex systems such as socio-economical status, communities location, demographic explosion, house holding issues, agricultural colonization, etc. has been identified as drivers for deforestation among indigenous and colonists in Northern Ecuadorian Amazon (Bilsborrow et al. 2004; Walsh et al. 2008; Bilsborrow et al. 2004). Several satellite images based studies showed critical implications from deforestation and forest degradation in the Northern Ecuadorian Amazon (Sierra 2000; Walsh et al. 2008; Mena 2008). Deforestation data from 1986 to 2014 in the Ecuadorian Amazon showed different patterns in the North compared to the South (Sierra 2000; MAE-SUIA 2014). The average deforestation rate peak in Orellana and Sucumbios, provinces of the Northern Ecuadorian Amazon, was 9496 and 12552 ha/year respectively from the 1990 to 2000, basically driven by the establishment of the oil industry activities and decreased afterwards in 2000 to 2014 period (3087 and 5416 ha/year) due to environmental awareness (MAE-SUIA 2014). In Morona Santiago, a province located in the Southern Ecuadorian Amazon, the average deforestation rate peak was 9378 – 7925 ha/year from 2000 to 2014 unlike 6225 ha/year during 1990-2000 period (MAE-SUIA 2014). There is no data available from the last two years (2015-6); however, increasing rates until nowadays are expected because of a continuous trend.

Several studies on mercury in the Amazon Region have been developed lately to pointing out awareness of exposure due to various toxic consequences especially for the human health (Maurice-Bourgoin and Quiroga 2002; Mainville et al. 2006; Webb et al. 2004; Webb 2004). Some authors have shown the release of mercury into the aquatic ecosystem due to soil erosion from deforestation in the Amazon as a big issue to be considered as priority (Fostier et al. 2000). Some have focused not only in abiotic components such as soil, water and sediments but also in organisms such fish and focused in human health risk due to fish consumption being a principal food resource (Maurice-Bourgoin and Quiroga 2002; Mergler et al.,). Recent studies showed higher mercury concentrations in human hair and urine of most exposure groups living near oil camps (Webb et al. 2016). Others authors have shown irreversible neurobehavioral effects on adults and children with high mercury concentrations in hair (Webb et al. 2004; Webb 2004) .

Once sorbed in sediment particles or biofilms, inorganic mercury can be transformed into methylmercury (MeHg) which bioavailability allows this species to be absorbed by primary consumers organisms entering in the aquatic food chain (Roulet et al. 2000; Pfeiffer and Lacerda 1989). Moreover, methyl mercury is biomagnified from the secondary consumers to the top predators, especially the piscivorous organisms such as predatory fish, birds, big mammals and humans (Webb et al. 2004; Maurice-Bourgoin and Quiroga 2002; Maurice-bourgoin et al. 2000).

Aquatic invertebrates, particularly Crustaceans, are considered one of the best bio-indicator potentially used for environmental characterization (Hodkinson and Jackson 2005). The freshwater shrimp *Macrobrachium brasiliense* is widely distributed in South America (Anger 2013) being registered in inland rivers in Venezuela, Colombia, Guyana, French Guiana, Suriname, Ecuador, Peru, Bolivia and Brazil, in the Orinoco and Amazon basins and Guiana rivers that drain into the Atlantic Ocean (Pileggi et al. 2013; Rodriguez 1981; Holthuis 1959). This species is the most abundant freshwater shrimp in the Ecuadorian Amazon distributed along watersheds in Sucumbios, Orellana, Napo, Pastaza, Morona Santiago and Zamora Chinchipe provinces (MHNGO 2015). *M. brasiliense* is characterized by omnivorous feeding habits registering a huge diversity of organisms in its diet such as insects, diatoms, oligochaeta, plants, fungi, sand, from a content stomach analysis (Melo and Nakagaki 2013) and many decomposed vertebrates corpses occupying the medium levels in the aquatic food chain. It might be exposed to an eventual contamination of the rivers by feeding on river sediments. Being one of the principal items found in the diet analysis of *M. brasiliense* (Melo and Nakagaki 2013), sand and sediments constitute 87.10 % and 90% respectively of their stomach content. Species feeding habitats, riverbeds and bottoms, coincides to where the contaminated sediments are mostly stored. Shrimp as human food resource is very important for people in the Amazon region when other sources such as bush meat and fish decrease. It may not be the principal protein source by people but it is always included as food complement regularly.

This study presents the total mercury concentrations in the most abundant freshwater shrimp *Macrobrachium brasiliense* (Crustacea – Palaemonidae) of the Ecuadorian Amazon basin and aims to evaluate the impact of the deforestation rates on the Hg enriched soil erosion to hydrosystems and the bioaccumulation risks in macroinvertebrates. The second aim of this study is to identify whether or not mercury can also be considered as a proxy for tracing oil industry activities impacts on the aquatic food chain. Finally, the human health risk based on the regular consumption of freshwater shrimp was assessed. Six rivers of the Northern Amazon affected by oil activities were studied and compared with four geomorphological similar rivers in the Southern Amazon, free from oil or mining exploitation.

Study Area

Amazonian soils (oxysols) are considered extremely poor in nutrients, acid, with short horizon A with little space for the vegetation to growth. In addition, this region is highly influenced by volcanic activity from the highlands adding particular elements in the food chain. The main rivers originate in the “sierra” region which enriches with minerals the lowlands. There is not a big distinction between wet and dry seasons in the region.

Two study areas in the Ecuadorian Amazon were selected considering the presence of the oil industry and its direct and indirect impacts. Northern Amazon (NA) was selected as the “affected area” due to its historic contamination issues while Southern Amazon (SA) was selected as the “control area” having no evidence of major anthropogenic activities related to extractive activities mainly oil industry (MAE-PRAS ZIL Maps 2015). The NA included Sucumbíos and Orellana provinces, while the SA the Morona Santiago province only.

Ten sampling points were established for this study, six in the NA and 4 in the SA (Table 1). Considering the areas with anthropogenic activities mainly originated from the oil industry, each of the 6 sampling points were located in sub-watersheds of the Napo R. (Conde R./M15-01, Due R./M15-03, Basura R./M15-05 and Guashito R./M15-06) and Coca river (Blanco R./M15-02 and Pacayaku R./M15-04). The 4 control sampling points were located in sub-watersheds on the left bank of the Santiago River (Yananas R./M15-08, Kushapuku R./M15-09, Kusuimi R./M15-10, and Chichis R./M15-11), where neither oil industry nor legal mining activities upstream were identified (ARCOM 2016). The 10 surveyed rivers presented similar geomorphological and physico-chemical characteristics (Figure 1, Table 1).

The sampling campaigns were held from September 14th to 26th, 2015, in the NA and from November 10th to 15th, 2015, in the SA. Fresh-water shrimp individuals of one single species *Macrobrachium brasiliense* were collected in each surveyed river along 150 m transect, mainly during the night. In surface waters, physico-chemical and microbiological parameters such as total coliforms, turbidity, fluvial habitat index (IHF), riparian habitat quality index (QBR), total suspended solids, nutrients, pH, conductivity, temperature, dissolved oxygen, alkalinity, ORP, and COD were measured.

Materials and methods

The rivers depths and widths were measured in each sampling location as well as the number of meanders within 100 meters. The Index of Fluvial Habitat Quality-IHF (Pardo et al. 2002)and the Index of Riparian Habitat Quality-QBR (Munné et al. 2003) were calculated using the protocols developed by the Freshwater Ecology and Management Research Group – Univeristy of Barcelone (2012-3).

The total suspended solids (TSS) concentrations in surface waters were measured in triplicate by filtration on GF/F (©Whatmann) membranes (Wallace, B. 2013). An YSI – 556 MPS multiparameters device was utilized to measure *in situ* pH, conductivity, temperature, dissolved oxygen, and the oxidation-reduction potential (ORP). A single measurement was done per each river. In the USFQ Environmental Sciences Laboratory, the SM 5220 B ASTM 1995 method was performed to analyze chemical oxygen demand (COD). Nutrients analyses were achieved using a single surface water sample per river without filtration trough La Motte test kits Nitrate/Nitrite and Ammonium. Alkalinity analysis was achieved using the Gran method (Gran, et al. 1981). A bacteriological counting of *Escherichia coli* and total coliforms was performed using the ®3M Petrifilm in triplicate for each surface water sampling point.

Shrimp samples were collected using small hand fishing net during the night because of their nocturnal activity peaks. Once captured, total body length, chelas length and weight were measured in the field as well as several standard morphological parameters such as sex, age (juvenile, adult), and reproductive state. The measured individuals were labeled and stored in plastic coolers with dry ice till the laboratory where they were frozen. Before chemical analysis, individuals were cleaned up with deionized water and freeze-dried. The exoskeleton was not removed as individuals were quite small and generally local people use to consume the entire animals. One point five grams powder sample per individual was weighted for total mercury analysis in triplicate. For individuals that didn't reach the minimum weight required, several freeze-dried individuals within the same range of size and wet weight were pooled until getting 1.5 g sample. Finally, nighty seven samples were obtained for the ten sampling points. Total Hg analyses were realized by Atomic Absorption Spectrometry with an Advanced Mercury Analyzer (AMA) in the Geosciences Environnement Toulouse (GET) Laboratory in Toulouse, France. Only adult individuals were analyzed. Fifty-eight blanks and 30 certified reference material samples (NIST ®SRM 2976, Mussel Tissue) were analyzed to validate the method and evaluate its accuracy (3%). The long-term external reproducibility of the measurement was 0.6% (2SD standard deviation, n=96) and the mean yield 101% (Table 2).

Data analysis was carried out using the SPSS statistical software. One-Way ANOVA and Pearson Correlations were used to determine significant differences between data sets in the rivers and correlations between parameters and chemical concentrations.

Results and Discussion

Environmental Parameters

The sampled rivers were selected according to their geomorphological and physico-chemical profiles. In order to facilitate the macro-invertebrates sampling, shallow water rivers draining forested area (with acidic pH and low conductivity) were chosen. Conductivity ranged from 72 to 389 $\mu S.cm^{-1}$, and pH between 3.17 and 6.47.

The study rivers varied between 6 (Chichis and Due R.) and 12 meters width (Yananas R.). The deepest rivers were Blanco, Pacayaku and Kushapuku (1.5 m) whereas the shallowest one was Due (0.5 m). The river with less meanders within 100 meters was Due (1) and Basura (1) while with the highest were Río Conde and Chichis (3 each one) (Table 3).

The IHF index is estimated using rivers physical characteristics such as stone embeddedness in riffles and runs, sedimentation in pools, riffle frequency, substrate composition, velocity/depth regime, shading of river bed, heterogeneity components, and aquatic vegetation cover while the QBR index uses characteristics of the vegetation cover and river structure such as total riparian cover, cover structure, cover quality, channel alteration, slope and form of the riparian zone, presence of one or several islands in the river, and presence of hard substrata. Both indexes score the conservation degree of each characteristic adding their values until getting the final score, which can be 100 tops. The obtained index values were interpreted using their range quality level color chart (Table 4).

All the NA Rivers were disturbed, with IHF index ranging between 53 and 80. The river with the worst fluvial habitat quality "Strong alteration, bad quality" was the Blanco River (53) followed by the Conde River (55) (Figure 1). The rivers with the best fluvial habitat quality were Kusuimi (76) and Chichis (80), both located in the SA.
The rivers with the best riparian habitat quality (QBR)"Riparian habitat in natural condition" were Due (95) and Chichis (95) while the rivers with the worst fluvial habitat quality "Extreme degradation, very bad quality" were Basura (25), Pacayacu and Conde (Figure 2).

The total suspended solids (TSS) were quite low and ranged from 4.0 to 24.4 mg.l^{-1} corresponding to Yananas and Pacayaku rivers respectively (Figure 3).

The pH values were similar among rivers ranging from 3.17 (Blanco) to 6.47 (Kushapuku) with a mean of 4.4 indicating a clear influence of the forest inputs in these small drainage basins. The surface temperature was homogeneous among all rivers ranging from 23.36ºC (Yananas) to 27.51ºC (Guashito). The conductivity values among rivers were quite homogeneous ranging from 72 µS.cm^{-1} (Yananas) to 389 µS.cm^{-1} (Kusuimi). The dissolved oxygen values among rivers ranged from 3.42 mg.l^{-1} (40.5 % sat.) in Chichis River to 8.55 mg.l^{-1} (100% sat.) in Due River. The alkalinity was heterogeneous among rivers going from 8.13 mg.l^{-1} in Pacayaku River to 71.72 mg.l^{-1} in Río Blanco. Similarly, the ORP was quite different among rivers reaching 224.6 mV in Kusuimi River as maximum value and 30.6 mV in Pacayaku River as minimum. The COD among rivers ranged from 3.6 mg.l^{-1} (Yananas) to 37.6 mg.l^{-1} (Pacayaku). The nutrients ranges were: ammonium from 0.27 mg.l^{-1} (Chichis) to 4.4 mg.l^{-1} (Pacayaku), nitrates from 0.00 mg.l^{-1} (Kusuimi & Chichis) to 11.9 mg.l^{-1} (Guashito), and nitrites from 0.16 mg.l^{-1} (Due) to 1.47 mg.l^{-1} (Guashito).

Small Amazonian rivers are typically acidic due to the presence of humic and fulvic acids leached from the forested soils erosion. However, the pH values of 3.17 and 3.28 in the Blanco and Conde rivers probably indicate possible chemical discharges from industrial processes nearby. These two rivers are inside Indillana/15 oil field and Auca/61 oil field respectively. The high ammonium levels found could possibly be due to livestock farming practices and fertilizers use, which are common from subsistence economic practices in the region. Unlikely to the lowlands black water rivers of the Amazon basin, conductivity values were quite high because of the Andean origin of the rivers, and more specifically the volcanic rocks weathering process which enrich the surface waters in major ions. Nitrates and nitrites high correlation is because of the nitrification process. Dissolved oxygen in some sampled rivers could have depended by the amount of water entering in a certain river section by both flow rate and amount of tributaries. In this case, meanders within 100 meters were contributing to the oxygenation of the rivers.

Total coliforms were evidenced in all the surface water samples and ranged from 3 to 85 UFC/ml being Río Conde and Yananas the rivers with the lowest values while Guashito river reached the highest content. Ranging from 1 to 82 UFC/ml, environmental coliforms colonies (no threatening bacteria) were present in all rivers. *E. coli* colonies were found in every single river except in the Chichis. Conde and Due rivers presented the lowest *E. coli* counts being 0.3 and 1.7 UFC/ml respectively while Blanco and Pacayaku were the rivers with the highest *E. coli* values 9.3 and 6.7 UFC/ml. respectively (Table 2, Figure 4). The percentage of *E. coli* of the total coliforms was widely variable. Yananas and Pakayaku showed the highest percentages (70% and 35% respectively), while Chichis and Guashito had the lowest (0.0 % and 3.5 % respectively) (Figure 4).

Even tough the presence of environmental coliforms in the streams doesn't necessarily mean that they are contaminated by human effluents, this method is a good approach for assessing the likelihood of risk for human health due to bacterial presence (Bruhm and Wolfson 2007). On the other hand, *E. coli* may be a good sign of contamination by anthropogenic wastes. Being the rivers with the largest *E. coli* count, Río Blanco may be facing directly the effects of human settlings. The numerous villages settled along the river with an inadequate sanitary service may be the principal cause of these inputs. Pacayaku River is the second most contaminated river by *E. coli*. However, this could be not only due to direct human effluents but also to the presence of aquatic predators such as giant otters and caimans, observed in the field. Not finding *E. coli* in Chichis River appears to be due to the absence of biological contamination, not only anthropogenic but also produced by the natural fauna of the ecosystem. It seems to be that this river might be less affected by any harmful bacterial contamination. In addition, it also suggests that the aquatic fauna could be less abundant than in the other rivers.

E. coli was highly correlated with river depth, alkalinity with conductivity, nitrites with pH, total coliforms and nitrates, number of meanders with dissolved oxygen, and total suspended solids with COD and ammonium (Table 5).

No significant differences between all physico-chemical parameters measured were found (One-way ANOVA, $p>0.05$) confirming that the sampling rivers, both in the NA and in the SA, held similar characteristics.

During this work, 443 individuals (mostly adults) were collected and measured in the field. Total body lengths for adult shrimps varied from 16.8 mm (in the Basura R.) to 81.35 mm (in the Kushapuku R.), averaging 52.34 mm, while the smallest median length was found in the Pacayaku River (average=44.67 mm) (Figure 5). The average measured sizes coincide closely with the species description from several localities in Colombia, 77.2 mm maximum (Valencia & Campos 2007), and are slightly larger than the same fresh water shrimp species individuals from Sao Paulo, Brazil (Mantelatto and Barbosa 2005). However, there's no much morphological information to compare with.

The shrimp wet weights varied from 0.73 g (Pacayaku) to 10.22 g (Kushapuku), with an average of 2.44 g. Individuals were slightly heavier comparing them with the individuals from Sao Paulo, Brazil (Mantelatto and Barbosa 2005). The heaviest individuals were found in the Kushapuku River (average = 4.05 g) while the lightest individuals were found in Pacayaku River (average=1.37 g) (Figure 6).

There is a strong correlation between body length and wet weight of the freshwater shrimps *M. brasiliense* sampled in Ecuadorian Amazon rivers (Pearson: 0.931, N=443, p< 0.01) (Figure 7). Length will be taken as a reference variable for discussion as body weight could be suitable to vary during specific biological periods of the individuals (reproduction, molting, eggs incubation or laying, etc.).

The difference in total length among all samples is remarkable. Significant difference in the average body length among the 10 sampling points were found (df=9, p<0.001), especially between the Pacayaku and the Kushapuku rivers (df=72, f=45.27, p<0.001) (Figure 5). However, there is no significant difference in body length between NA and SA sampling points (df=442, f=6.3, p>0.01) (Figure 5). The biggest and heaviest individuals were collected in the Kushapuku River (SA) while the shortest individuals were found in the Pacayacu R. (Fig. 7).

Total mercury results

Nighty-six shrimp composed samples (25 mg weight) were analyzed in triplicate for total mercury concentrations which belonged to 211 individuals of ten rivers (Table 6).

The concentrations of total mercury in the freshwater shrimp *Macrobrachium brasiliense* samples are shown in the Table 14. Mercury concentrations ranged from 0.025 $\mu g.g^{-1}$ (dry weight, dw) in the Guashito River to 0.094 $\mu g.g^{-1}$ (dw) in the Chichis River. Since there is no data of total mercury concentrations in freshwater shrimp species in the Amazon Region, it's not possible to compare our findings; however, two marine shrimp analysis show similar concentrations values as following: 0.032 $\pm$ 0.002 $\mu g.g^{-1}$ in *Fenneropenaeus merguiensis* from the south Persian Gulf (Baboli 2013), and 0.08 $\mu g.g^{-1}$ in *Panaeus spp.* from the Caribbean Sea, Trinidad and Tobago islands (Balfour and Badrie 2012). All the highest average Hg concentrations in shrimp tissue among the ten sampling points were found in the SA Rivers, Yananas, Kushapuku, Kusuimi and Chichis (Figure 8).

Impacts of human activities in mercury concentrations in freshwater shrimps

The total mercury concentrations in freshwater shrimps *M. brasiliense* among all the sampled rivers are significantly different (f=12.759, df=96, p<0.001). Similarly, the mercury concentrations between the sampled rivers in the NA and the SA are significantly different (f=44.082, df=96, p<0.01) with mean concentrations of 0.043 $\mu g.g^{-1}$ and 0.074 $\mu g.g^{-1}$ in the

NA and SA rivers, respectively, suggesting an enrichment in Hg of the southern hydro-ecosystems and/or environmental or human factors that increase the bioavailability of Hg in the SA comparatively with the NA.

Even though Hg is used in a huge variety of industrial processes such as electrical, metallic, or chemical, etc. (INRS Toxicological Fiches 2012), none of them could actually explain such concentrations in shrimp sampled in the SA Rivers. There haven't been reported these kind of activities in the region and there is no big cities that could contaminate the aquatic ecosystem. Mercury is a metal highly concentrated in Andean tropical soils (Mainville et al. 2003; Mainville et al. 2006; Fostier et al. 2000) which is probably the main natural source in this region. Even in non-impacted areas by oil industry in the Southern Ecuadorian Amazon, Hg concentrations in sediments ranged between 6 $ng.g^{-1}$ (Due and Basura R.) and 79 $ng.g^{-1}$ (Pacayacu R.), 50 $ng.g^{-1}$ being the average of the natural geochemical background in the Ecuadorian Amazon. Another possible source of mercury is gold and rock mining activities as reported in Western Ecuador, in the Puyango River Basin (Betancourt et al. 2005); however, there is no evidence of such activities upstream nor surrounding the sampled points in the Santiago River tributaries (MAE-PRAS 2015; ARCOM 2015). However, itinerant artisanal gold mining in the sampled area but there was none evidence during the sampling period as confirmed by the total Hg concentrations measured in sediments of the sampled rivers. On the other hand, it has been demonstrated that soil erosion increased by deforestation is the main process responsible for Hg surface sediment enrichment in Amazonian Rivers, such as the Tapajos, Arapiuns and Amazon rivers (Roulet et al. 2000) suggesting that similar events could have happened in the Ecuadorian SA. Morona Santiago province presents the highest annual deforestation rate being 7925 ha/year from 2008 to 2014 (-0.44% annual change rate) (MAE-DNF 2016) (Table 7, Figure 9, 10). In contrast, the NA has reduced the Average Change Rate ACR (-0.16%, -0.39%) in the same period comparing to previous ones, which is clearly noted in the difference total Hg concentrations between both study regions. Anthropogenic activities in the NA could have also released mercury concentrations to the environment during the deforestation peak driven by oil exploitation (1990-2000); however, this impact could not be measured nowadays because the contaminated sediments were probably already drained downstream trough rivers. Consequently, mercury is not considered as a proxy for oil industry itself but it is for deforestation in areas with similar soil geochemical.

Environmental and biological factors in the Hg bioaccumulation in freshwater shrimps

Comparing the total Hg concentrations in the freshwater shrimp *Macrobrachium brasiliense* with the concentrations found in the sediments, in a parallel study from the same sampling points, there was no correlation in order to explain dependency (r=-0.047) (Figure 11). This may be due to the feeding habitats of the shrimp, which despite the literature says this species one of the principal item found in stomach content studies are sand and sediments constituting 87.10 % and 90% respectively (Melo and Nakagaki 2013), might be and exception in the shrimp communities from this environments. *M. brasiliense* may develop mainly in the coarse substrate made of gravels that don't accumulate inorganic mercury while THg analysis were performed in fine sediments. To clarify this hypothesis, stomach content studies in this species should be realized. Consequently, this species Hg concentrations may not depend directly from sediments as thought but it surely bioaccumulate Hg from other sources principally predatory fish corpses in decomposition and algae, which are not certainty know yet.

Hg concentrations in organisms truly depends on its bioavailability in the aquatic ecosystem produced by bacterial methylation, so this process may be different in all rivers. Because of the high genotypic and phenotypic plasticity attributed to *Macrobrachium species*, they are probably modifying their metabolisms and physiology in order to be less affected by toxic heavy metals. This could be noticed in the speeding of the excretion rate when exoskeleton molting, a process to eliminate threatening elements. Finally, sediment sampling methods and design has to be reviewed in order to make sure the samples representativeness of the rivers.

Human health risk assessment from freshwater shrimp consumption

The total mercury concentrations found in *freshwater shrimps from* all the rivers (Figure 12) don't exceed the permissible limits for crustaceans intake (0.5 $mg.kg^{-1}$) established by the European Commission (EC 2010), the Health Canada's Maximum Levels for chemical contaminants in food (2016) and the Colombian Public Health Ministry (MSPS-Colombia 2016), (fish intake = 0.5 $mg.kg^{-1}$ WHO-FAO 2011). In addition, according to the Provisional Tolerable Weekly Intake (PTWI) of mercury established by the WHO/FAO JECFA (2003) (1.6 $\mu g.kg^{-1}$), the maximum amounts of shrimp intake were calculated for men, women and children living close to the study rivers. The river with the lowest shrimp intake values was the Chichis R. in the SA, with 683 g for men (70 kg), 585 g for women (60 kg), and 195 g for children (20 kg) as the maximum daily intake shrimp wet mass, corresponding to 266, 228, 76 the maximum daily number of shrimps intake respectively for each group.

For all the study rivers, the shrimp consumption is safe when referred to the mercury PTWI of the FAO-WHO (JECFA 2003) (Table 8).

According to the dietary habits of the people living in the sampling areas, shrimp doesn't represent a main source of food, unlike bush meat or fish. Moreover, from informal conversations with habitants in the sampling points area, it was suggested that indigenous people can only sustain themselves fishing freshwater shrimps when other protein sources are missing, which can happen during short periods of time along the year.

Conclusions

This first study on total mercury concentrations in the most abundant freshwater shrimp species of the Amazon basin, in Ecuador, showed that Hg concentrations were very similar to those previously measured in seawater shrimp species (Baboli 2013; Balfour and Badrie 2012). Average total mercury concentrations in *Macrobrachium brasiliense* adults ranged from 0.025 $ng.g^{-1}$ in the Guashito River, in the Napo R. basin, to 0.094 $ng.g^{-1}$ in the Chichis River, in the Santiago R. basin, at the South. A significant difference in Hg accumulation in shrimps has been observed between the Northern Ecuadorian Amazon, in oil exploited areas, and the Southern Ecuadorian Amazon, with a higher enrichment in Hg of the southern hydro-ecosystems. Our results confirm that mercury can not considered as a proxy for oil exploitation activities while a recent study in the Peruvian and Ecuadorian Amazon (Webb et al., 2016) detected increased levels of mercury in urine for men involved in oil spill remediation while indigenous people living near oil production sites generally had urine mercury levels within the global background standard suggested by the World Health Organization. Our results confirm that soil erosion is the main process that release mercury into the aquatic environment, process which can be increased by agricultural practices, such as deforestation. Mercury in the Amazon region can be considered as a proxy for tracing deforestation.

For all the study rivers, the shrimp consumption by local people is safe when referred to the mercury PTWI of the FAO-WHO (JECFA 2003). In addition, the mercury concentrations in *M. brasiliense* didn't exceed the permissible limits established by the European Commission (EC 2010), the Health Canada's Maximum Levels for chemical contaminants in food (2016) and the Colombian Public Health Ministry (MSPS-Colombia 2016)(0.5 $mg.kg^{-1}$ WHO-FAO 2011). However, the most exposed group to Hg is children and young women living in remote areas of the Amazon region, who highly depend on natural resources for their diet. Anthropogenic activities based on land use change such as monoculture farming or oil and mining industry and their indirect activities, involve deforestation that accelerate the soil erosion process and the enrichment of the aquatic food chain in mercury. These results can help to promote more sustainable agriculture practices preserving part of the forest cover and the health of riparian communities depending on aquatic resources to live.

Acknowledgements

This study is developed under the Franco-Ecuadorian research program entitled MONOIL 'Environmental Monitoring, Health, Society and Petroleum in Ecuador'. This program is managed by the IRD, French Research Institute for the Development, and sponsored by the ANR, French Research Agency (ANR-13 SOCENV-0003-01). This specific study has also be funded by the Project ABE, Amazonian Biodiversity and Education, coordinated by the USFQ and sponsored by the University of North Carolina at Chapel Hill.

References

Agencia de Regulación y Control Minero. 2015. ARCOM. Catastro Minero de Ecuador.

Anger, K. 2013. Neotropical Macrobrachium (Caridea: Palaemonidae): on the biology, origin, and radiation of freshwater-invading shrimp. Journal of Crustacean Biology 33:151–183.

Baboli, M. 2013. Determination of heavy metals and trace elements in the muscles of marine shrimp, Fenneropenaeus merguiensis from Persian Gulf, Iran. JAPS, Journal of Animal … 23:786–791.

Balfour, S., and N. Badrie. 2012. Seasonal influence and heavy metal analysis in marine shrimp (Penaeus spp.) sold in Trinidad, West Indies. Journal of Food … 1:193–199.

Betancourt, O., A. Narváez, and M. Roulet. 2005. Small-scale Gold Mining in the Puyango River Basin, Southern Ecuador: A Study of Environmental Impacts andHuman Exposures. EcoHealth 2:323–332.

Bilsborrow, R., A. Barbieri, and W. Pan. 2004. Changes in population and land use over time in the Ecuadorian Amazon. Acta Amazonica 34:635–647.

Bruhm, L., and L. Wolfson. 2007. Citizens Monitoring Bacteria : A training manual for monitoring E. coli.

European Commission. 2016. Food Safety Overview. In: http://ec.europa.eu/food/overview_en

FAO. 2011-2016. Codex Alimentarius. Secretariat of the Codex Alimentarius Commission Joint FAO/WHO Food Standards Programme Food and Agriculture Organization of the United Nations Viale delle Terme di Caracalla 00153 Rome, Italy.

Fostier, A., M. Forti, and J. Guimaraes. 2000. Mercury fluxes in a natural forested Amazonian catchment (Serra do Navio, Amapa State, Brazil). Science of the Total ….

Hodkinson, I., and J. Jackson. 2005. Terrestrial and aquatic invertebrates as bioindicators for environmental monitoring, with particular reference to mountain ecosystems. Environmental Management 35:649–66.

Holthuis, L. B. 1959. The Crustacea Decapoda of Suriname (Dutch Guiana) (Vol. 44). EJ Brill.

INRS. 2012. Institute National de Recherche et e Sécurité. Fiches Toxicologiques. In: http://www.inrs.fr/publications/bdd/fichetox.html

JECFA. 2011, 2012, 2013. Evaluation of the Joint FAO-WHO Expert Commitee on Food Additives. In: http://apps.who.int/food-additives-contaminants-jecfa-database/search.aspx

Lambin, E. F., H. J. Geist, and E. Lepers. 2003. D Ynamics of L and -U Se and L and -C Over C Hange in T Ropical R Egions. Annual Review of Environment and Resources 28:205–241.

Ministerio del Ambiente del Ecuador. 2016. Proyecto de Remediación Ambiental y Social del Ecuador. Programa de Pasivos Ambientales. SUIA.

Ministerio del Ambiente del Ecuador. 2016. Direccion Nacional Forestal-SUIA. Tazas de deforestacion del Ecuador.

Mainville, N., J. Webb, M. Lucotte, R. Davidson, O. Betancourt, E. Cueva, and D. Mergler. 2006. Decrease of soil fertility and release of mercury following deforestation in the Andean Amazon, Napo River Valley, Ecuador. The Science of the total environment 368:88–98.

Mainville N, Webb J, Lucotte M, Mergler D, Davidson R, Bet- ancourt O, et al. 2003. Evaluation of the impacts of defores- tation on mercury levels in soils, fish and humans in the Ecuadorian Amazon. IDRC International Forum on Ecosystem Approaches to Human Health, Montre ́al, Que ́bec, Canada, May 18–23, Parallel Sessions Abstracts:12 Malm.

Maurice-Bourgoin L. and Quiroga I., 2002. Total mercury distribution and importance of the biomagnification process in rivers of the Bolivian Amazon. In: *The Ecohydrology of South American Rivers and Wetlands* (McClain M., Ed.), IAHS Special Publication nº. 6, pp. 49-66.

Maurice-Bourgoin L., Quiroga I., Chincherros J. and Courau P., 2000. Mercury distribution in waters and fishes of the Upper Madeira rivers and mercury exposure in riparian Amazonian populations. *Science of the Total Environment,* 260: 73-86.

Maurice-Bourgoin L, Alanoca L, Fraizy P, Vauchel P. 2003. Sources of mercury in surface waters of the upper Madeira erosive basins. J Phys IV France 2003; 107:855–8.

Melo, M. S. De, and J. M. Nakagaki. 2013. Evaluation of the feeding habits of *Macrobrachium brasiliense* (Heller, 1862) in the Curral de Arame stream (Dourados/Mato Grosso Do Sul, Brazil). Nauplius 21:25–33.

Mena, C. F. 2008. Trajectories of Land-use and Land-cover in the Northern Ecuadorian Amazon : Temporal Composition , Spatial Configuration , and Probability of Change 74:737–751.

Munné, a., N. Prat, C. Solà, N. Bonada, and M. Rieradevall. 2003. A simple field method for assessing the ecological quality of riparian habitat in rivers and streams: QBR index. Aquatic Conservation: Marine and Freshwater Ecosystems 13:147–163.

Pardo, I., M. Álvarez, J. Casas, and J. Moreno. 2002. El hábitat de los ríos mediterráneos. Diseño de un índice de diversidad de hábitat. Limnetica 21:115–133.

Pfeiffer, W., and L. de Lacerda. 1989. Mercury concentrations in inland waters of gold-mining areas in Rondonia, Brazil. Science of the Total … 88:233–240.

Pileggi, L. G., C. Magalhaes, G. Bond-Buckup, and F. L. Mantelatto. 2013. New records and extension of the known distribution of some freshwater shrimps in Brazil. Revista Mexicana de Biodiversidad 84:563–574.

Rodriguez, G. 1981. Decapoda. Aquatic bioa of tropical South America, Part 1, Arthropoda.

Roulet, M., M. Lucotte, and R. Canuel. 2000. Increase in mercury contamination recorded in lacustrine sediments following deforestation in the central Amazon. Chemical ….

Roulet, M. 2001. ‘‘Le mercure: son cycle bioge´ochimique et sa re´partition aux e´chelles plane´taire et amazonienne’’. In: Le Mercure en Amazonie: Ro ˆle de I'Homme et de l'Environnement, Risques Sanitaires, Carmouze J-P, Lucotte M, Boudou A (edi- tors), Paris: IRD Editions, pp 81–120.

Sierra, R. 2000. Dynamics and patterns of deforestation in the western Amazon: the Napo deforestation front, 1986–1996. Applied geography 20:1–16.

Wallace, B., J. Hutchens, and J. Grubaugh. 2013. Transport and Storage of FPOM. Methods in Stream Ecology.

Walsh, S., J. Messina, and C. Mena. 2008. Complexity theory, spatial simulation models, and land use dynamics in the Northern Ecuadorian Amazon. Geoforum 39:867–878.

Webb, J. 2004. Use of the Ecosystem Approach to Population Health: The case of Mercury Contamination in Aquatic Environments adn Riparian Populations, Andean Amazon, Napo RIver Valle, Ecuador. Canadian Journal of Public Health 96:44–47.

Webb, J., O. T. Coomes, N. Ross, and D. Mergler. 2016. Mercury concentrations in urine of amerindian populations near oil fields in the peruvian and ecuadorian amazon. *Revue*, 151:344–350.

Webb, J., N. Mainville, D. Mergler, M. Lucotte, O. Betancourt, R. Davidson, E. Cueva, and E. Quizhpe. 2004. Mercury in Fish-eating Communities of the Andean Amazon, Napo River Valley, Ecuador. EcoHealth 1:SU59–SU71.

Tables

Table 1. Geographical data of the ten sampling points in Northern (NA) and Southern Ecuadorian Amazon rivers (SA) and oil spills, infrastructure and environmental liabilities in 5 km radius distance from the each sampling point. Average change rate (ACR %) and Average deforestation rate (ADR ha/year) for each province within 2008-2014 (PRAS-MAE 2016).

No.	Region	Province	River	Cod.	UTM/UPS			Altitude (masl)	Oil spills	oil wells	lighters	stations	pools	pits	towers	ACR (%)	ADR (ha/year)
1	North	Orellana	Conde	M15-01	18 S	291520	9923055	255	87	53	5	1	54	12	45	-0.16	3087
2	North	Sucumbios	Blanco	M15-02	18 S	318689	9961878	272	2	13	0	3	0	0	11	-0.39	5416
3	North	Sucumbios	Due	M15-03	18 S	233592	10003169	544	1	0	0	0	0	0	0	-0.39	5416
4	North	Sucumbios	Pacayaku	M15-04	18 S	325469	10000141	245	42	73	6	3	76	171	66	-0.39	5416
5	North	Orellana	Basura	M15-05	18 S	279648	9963505	284	0	0	0	1	0	0	0	-0.16	3087
6	North	Orellana	Guashito	M15-06	18 S	269232	9955379	269	12	25	19	1	13	0	19	-0.16	3087
7	South	Morona Santiago	Yananas	M15-08	18 S	168127	9664373	290	0	0	0	0	0	0	0	-0.44	7925
8	South	Morona Santiago	Kushapuku	M15-09	17 S	829986	9663818	332	0	0	0	0	0	0	0	-0.44	7925
9	South	Morona Santiago	Kusuimi	M15-10	18 S	177656	9668659	287	0	0	0	0	0	0	0	-0.44	7925
10	South	Morona Santiago	Chichis	M15-11	18 S	167464	9663947	289	0	0	0	0	0	0	0	-0.44	7925

Table2. Analytical methods (Detection (DL) and quantification limits (QL), Accuracy, Reproducibility and Yield) for total mercury measurement with Atomic Absorption in the Advanced Mercury Analyzer (AMA).

Element:	**Hg**
DL (µg.g-1)	0.02
QL (µg.g-1)	0.08
Accuracy (%)	3
Reproducibility	0.006
Yield (%)	101

Table 3. Physico-chemical parameters (altitude, width, depth, temperature, pH, dissolved oxygen, trbidity, oxido-reduction potential, alkalinity, coliforms, total suspended solids, chemical oxygen demand, ammonium, nitrates, nitrites, meanders amount within 100m radius, IHF and QBR indexes) of the 10 sampling points (streams). Chemical analysis were measured in surface water samples.

Name	COD.	Date	Region	Altitude (masl)	Width (m)	Depth (m)	T (°C)	Conductivity (µS/cm)	pH	Dissolved O2 (mg/l)	Dissolved O2 (%sat)	Turbidity (ntu)	ORP (mV)	Alkalinity (mg/l)	Coliforms (UFC/ml)	E. coli (UFC/ ml)	Total coliforms (UFC/ ml)	TSS (mg/L)	COD (mg/L)	Ammonium (mg/L)*	Nitrates (mg/L)*	Nitrites (mg/L)*	# Meanders within 100m	IHF	QBR
Conde	M15-01	9/14/15	Norte	255	9	0.75	26.28	77	3.28	-	-	8.83	90	10.91	2	0.3	3	7.9	12.6137	0.3250	1.4960	0.1837	3	55	45
Blanco	M15-02	9/24/15	Norte	272	10	1.50	25.94	273	3.17	5.88	75.00	9.4	165.6	71.72	30	9.3	39	12.5	10.1542	0.3380	4.0187	0.2750	2	53	70
Due	M15-03	9/17/15	Norte	544	6	0.50	25.33	112	4.6	8.55	100.00	1.3	85	34.99	9	1.7	10	5.6	16.8301	0.2730	4.0187	0.1606	1	67	95
Pacayaku	M15-04	9/19/15	Norte	245	10	1.50	25.27	77	4.54	5.94	85.60	21.3	30.6	8.13	12	6.7	19	24.4	37.5605	4.3810	0.4987	0.3883	2	65	35
Basura	M15-05	9/22/15	Norte	284	8	1.00	26.06	73	3.69	7.02	86.50	10.6	173.7	12.72	12	3.3	15	15.9	17.1814	0.5850	1.5547	0.2112	1	61	25
Guashito	M15-06	9/26/15	Norte	269	7	1.20	27.51	253	6.07	5.40	67.90	24.18	42	22.78	82	3.0	85	7.6	11.5596	0.4030	11.9973	1.4740	2	66	90
Yananas	M15-08	11/12/15	Sur	290	12	0.70	23.36	72	4.29	7.14	83.80	4.82	168.6	21.14	1	2.3	3	4.0	3.6246	0.4030	5.2507	0.4169	2	69	75
Kushapuku	M15-09	11/11/15	Sur	332	7	1.50	26.55	209	6.47	6.50	79.00	2.35	80.7	21.53	30	4.0	34	7.3	0.0000	0.3510	4.9427	0.6941	2	66	75
Kusuimi	M15-10	11/10/15	Sur	287	8	0.60	24.25	389	3.68	6.80	80.40	6.33	224.6	57.03	15	3.0	18	5.90	11.4441	0.3380	0.0000	0.4752	2	76	60
Chichis	M15-11	11/14/15	Sur	289	6	0.60	23.86	115	4.25	3.42	40.50	0.73	156.9	36.48	30	0.0	30	6.4	5.0463	0.2730	0.0000	0.2816	3	80	95

Table 4. Fluvial and riparian habitat quality level categories (Pardo et al. 2002).

FLUVIAL AND RIPARIAN HABITAT QUALITY LEVEL		
CATEGORIES	*Value*	*Color*
Riparian habitat in natural condition	≥ 95	Blue
Some disturbance, good quality	75-90	Green
Disturbance important, fair quality	55-70	Yellow
Strong alteration, bad quality	30-50	Orange
Extreme degradation, very bad quality	≤ 25	Red

Table 5. Pearson correlations (*r* value) between microbiological and physico-chemical parameters of the study Rivers.

Parameters	Depth	Conductivity	pH	Dissolved oxigen	Coliforms	Total coliforms	TSS	COD	Nitrates
Alkalinity		.727*							
E. coli	.797**								
COD							.832**		
Ammonium							.849**	.854**	
Nitrates					.734*	.727*			
Nitrites			.711*		.878**	.864**			.814**
# Meanders within 100m				-.862**					

** Significant correlation at the level 0.01 (2 tails)

* Significant correlation at the level 0.05 (2 tails)

Table 6. Total mercury concentrations averages and standard errors (SE) measured in the freshwater shrimp species *Macrobrachium brasiliense* collected in ten rivers of the Ecuadorian Amazon. Unit = $\mu g.g^{-1}$ dw. * SA Rivers as control. N= Number of collected individuals and samples per river.

River	Hg (ug.g-1±SE)	N	samples (25 mg)
Conde	0.042 ± 0.005	23	10
Blanco	0.036 ± 0.002	19	10
Due	0.063 ± 0.006	19	10
Pacayaku	0.059 ± 0.008	12	8
Basura	0.038 ± 0.003	28	10
Guashito	0.025 ± 0.002	16	10
Yananas*	0.071 ± 0.008	32	8
Kushapuku*	0.057 ± 0.006	10	10
Kusuimi*	0.074 ± 0.008	30	10
Chichis*	0.094 ± 0.007	22	10
	Total	**211**	**96**

Table 7. Average change rate (ACR) & Average deforestation rate (ADR) ha/year in Morona Santiago, Orellana and Sucumbios provinces during 2000-2014 (MAE-SUIA 2016).

Province	2008-2014		2000-2008		1990-2000	
	ACR	ADR ha/year	ACR	ADR ha/year	ACR	ADR ha/year
Morona Santiago	-0.44%	7925	-0.51%	9378	-0.32%	6225
Orellana	-0.16%	3087	-0.16%	3154	-0.48%	9496
Sucumbíos	-0.39%	5416	-0.23%	3429	-0.81%	12552

Table 8. Means and standard errors (SE) of the total Hg concentrations in the freshwater shrimp species *Macrobrachium brasiliense* collected in ten rivers of the Ecuadorian Amazon (* SA Rivers as control). Maximum weekly and daily intake shrimp dry and wet mass (g) and maximum daily estimated number of shrimps suggested for consumption per river based on the tolerable weekly intake (Hg PTWI = 1.6 μg/kg, JECFA 2003) for average adults men (70 kg), women (60 kg) and children (20 kg).

			Men (70 kg)				***Women (60 kg)***				***Children (20 kg)***			
River	**Hg (ng.g-1±SE)**	average shrimp weight (g)	maximum daily intake shrimp (g) dry mass	maximum weekly intake shrimp (g) dry mass	maximum daily intake shrimp (g) wet mass	maximum daily estimated number of shrimps	maximum daily intake shrimp (g) dry mass	maximum weekly intake shrimp (g) dry mass	maximum daily intake shrimp (g) wet mass	maximum daily estimated number of shrimps	maximum daily intake shrimp (g) dry mass	maximum weekly intake shrimp (g) dry mass	maximum daily intake shrimp (g) wet mass	maximum daily estimated number of shrimps
Conde	0.042 ± 0.005	2.03	384	2688	1536	756	329	2304	1317	648	110	768	439	216
Blanco	0.036 ± 0.002	2.37	446	3122	1784	753	382	2676	1529	645	127	892	510	215
Due	0.063 ± 0.006	2.81	256	1790	1023	364	219	1534	877	312	73	511	292	104
Pacayaku	0.059 ± 0.008	1.37	271	1896	1083	790	232	1625	929	678	77	542	310	226
Basura	0.038 ± 0.003	1.66	422	2956	1689	1015	362	2533	1448	870	121	844	483	290
Guashito	0.025 ± 0.002	2.67	644	4507	2575	964	552	3863	2208	826	184	1288	736	275
Yananas*	0.071 ± 0.008	1.76	225	1574	900	511	193	1349	771	438	64	450	257	146
Kushapuku*	0.057 ± 0.006	4.05	282	1977	1130	279	242	1695	969	239	81	565	323	80
Kusuimi*	0.074 ± 0.008	2.14	217	1516	867	404	186	1300	743	347	62	433	248	116
Chichis*	0.094 ± 0.007	2.56	171	1195	683	266	146	1024	585	228	49	341	195	76
	Mean	*2.3*	***332***	***2322***	***1327***	***610***	***284***	***1990***	***1137***	***523***	***95***	***663***	***379***	***174***

Figures

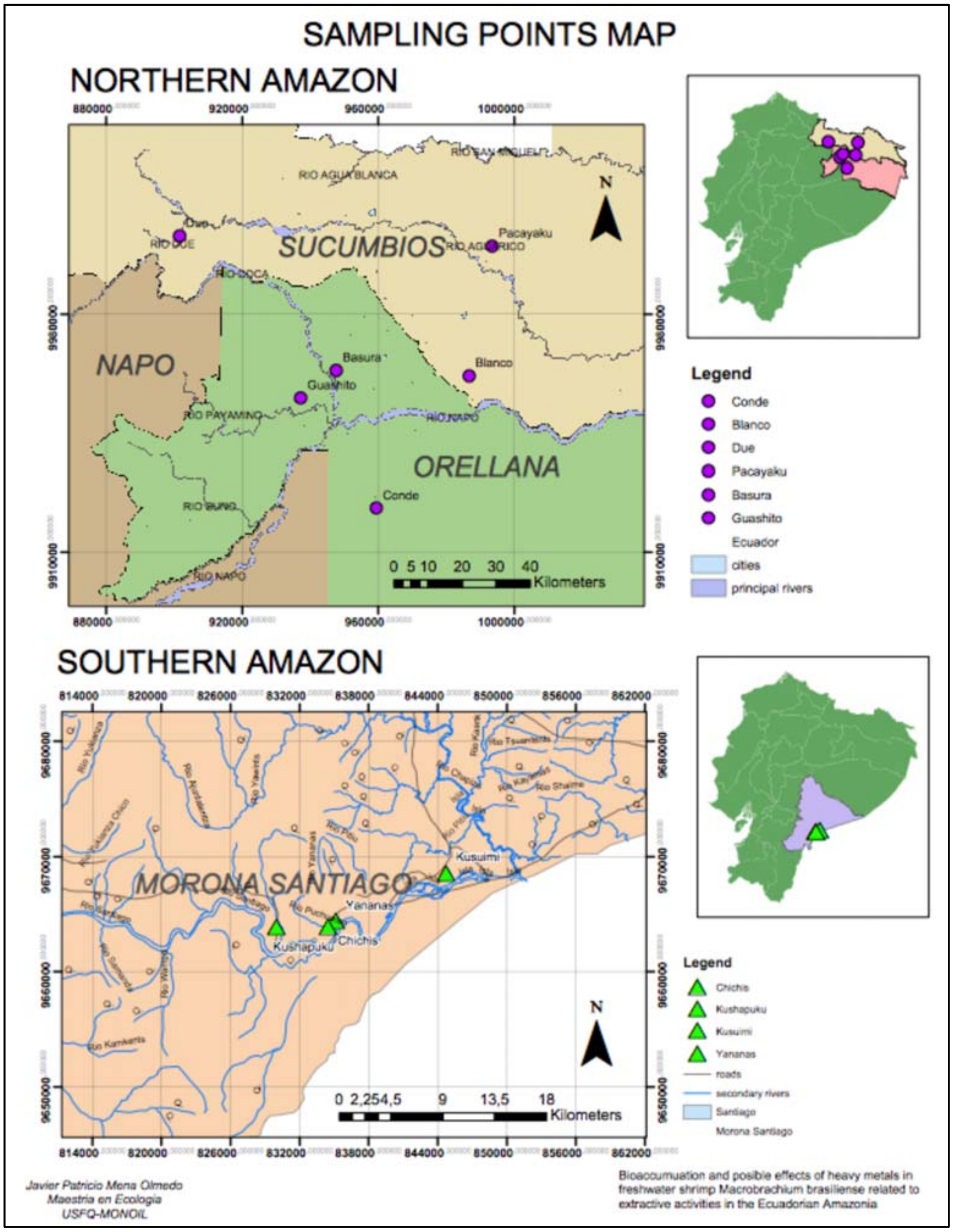


Figure 1. Sampling Points Maps. A). Northern Ecuadorian Amazon sampling points (NA). B) Southern Ecuadorian Amazon sampling points (SA).

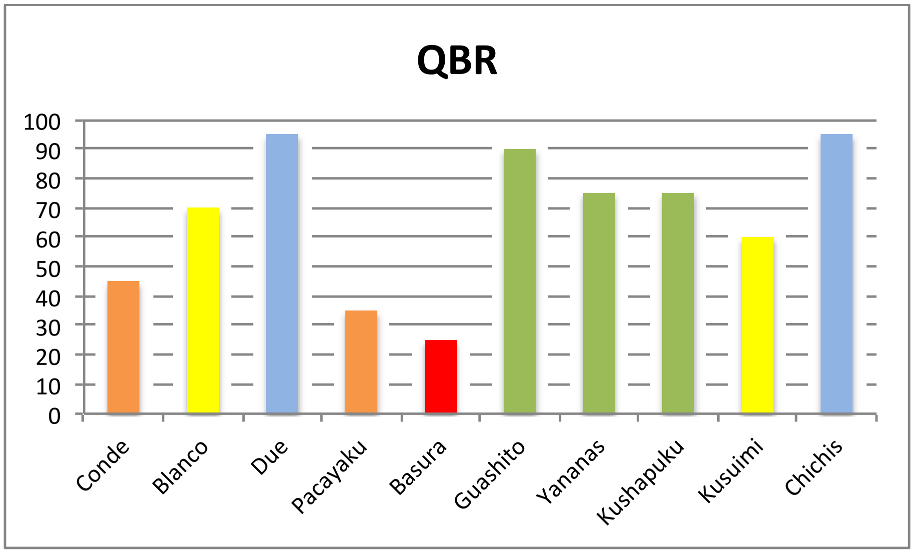


Figure 2. Riparian habitat quality index values per evaluated river. The colors indicate the quality of the riparian habitat indicated in the categories table.

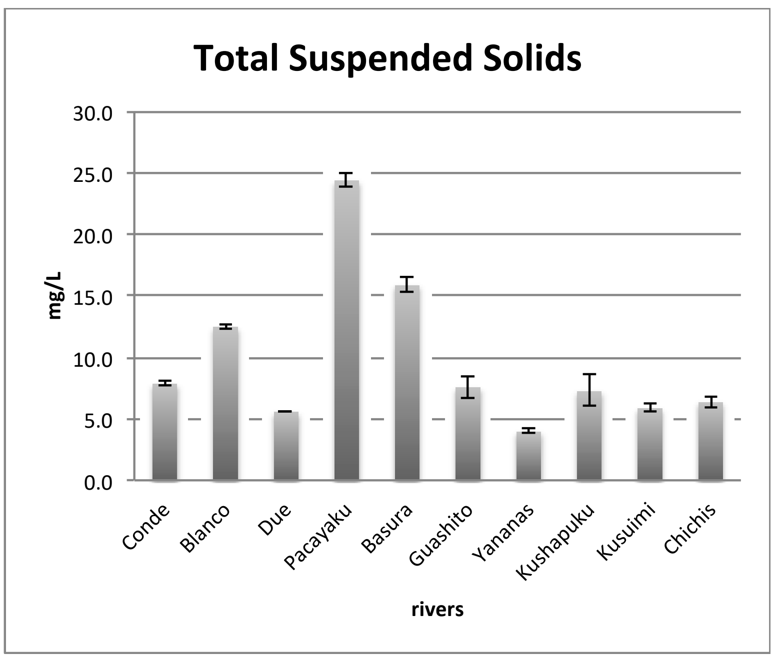


Figure 3. Total suspended solids (mg/L) measured in surface water samples from the ten sampling points (streams) (n=3 filters).

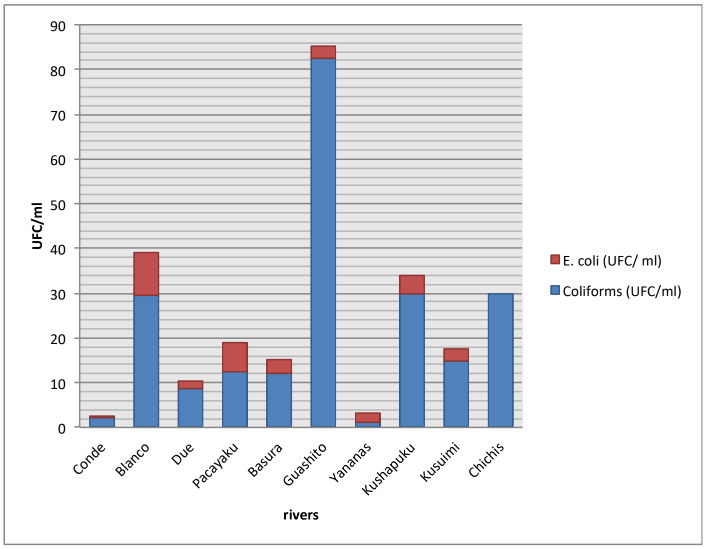


Figure 4. Total coliforms (environmental coliforms + *E. coli*) measured in surface water samples of the ten sampling streams in the Ecuadorian Amazon. UFC/ml= unities of forming colonies per milliliter.

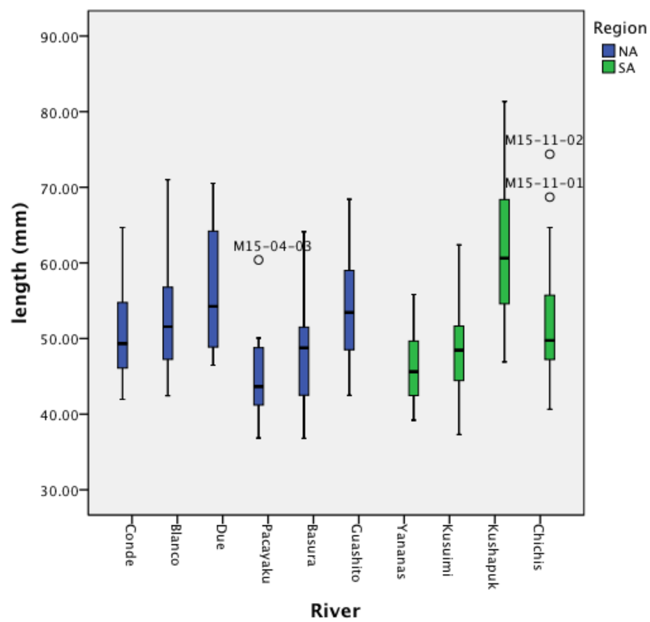


Figure 5. Minimum, maximum and median of the shrimp body lengths per sampled river. In blue the Northern Amazon Rivers (NA) and in green the Southern Amazon Rivers (SA).

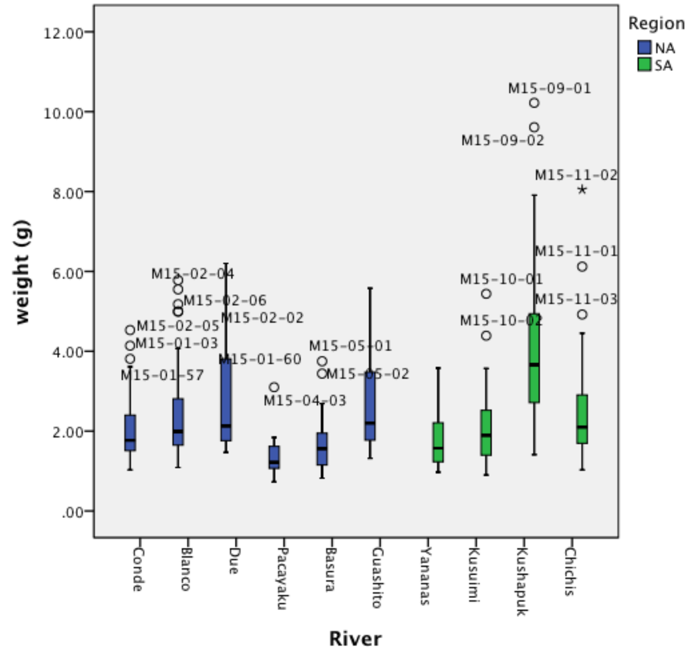


Figure 6. Minimum, maximum and median of the shrimp weights per sampled river. In blue the Northern Amazon Rivers (NA) and in green the Southern Amazon Rivers (SA).

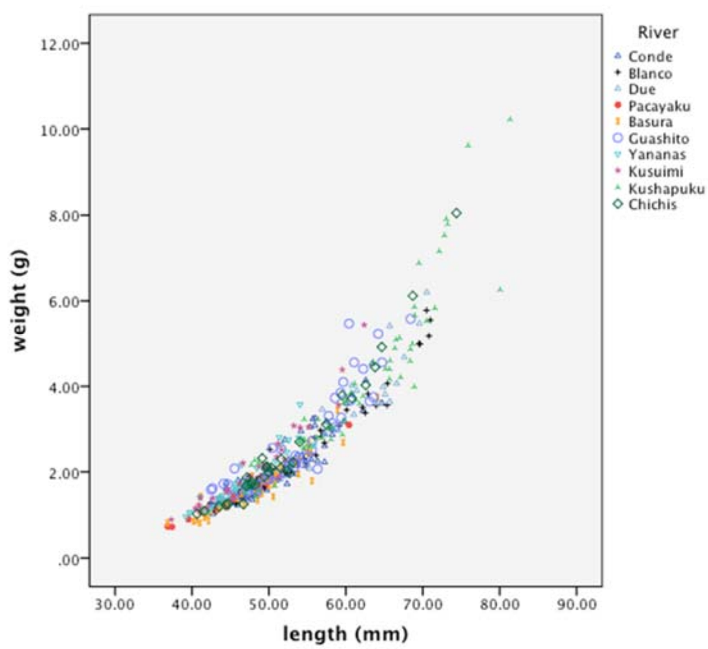


Figure 7. Individuals shrimp body length vs weight collected in the ten sampling points in the Ecuadorian Amazon. Each color and symbol represents individuals in a single sampled river. The difference in sizes among them is remarkable especially between the individuals in the Pacayaku River and the Kushapuku River.

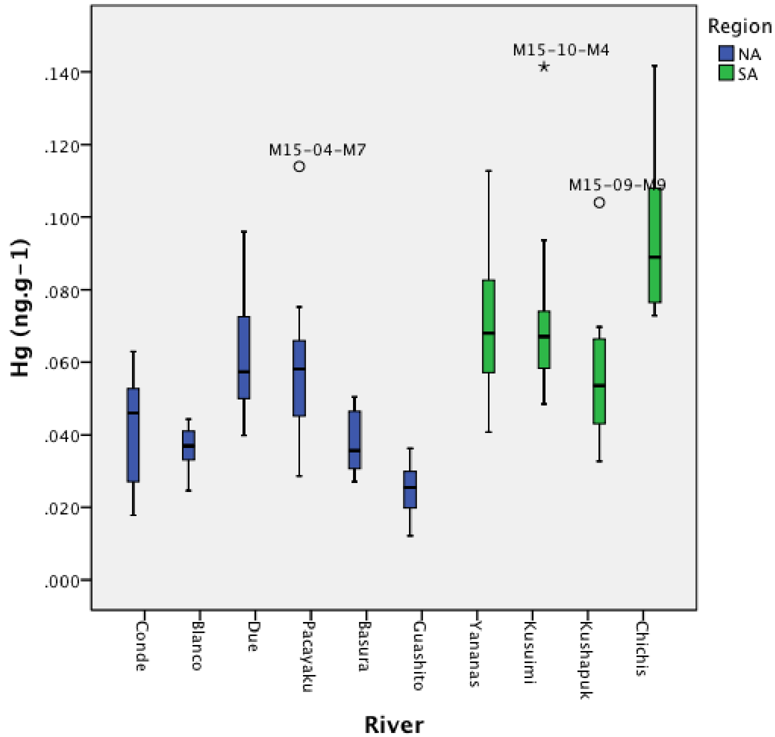


Figure 8. Minimum, maximum and median of total Hg concentrations in the freshwater shrimp species *Macrobrachium brasiliense* collected from ten rivers in the Ecuadorian Amazon. Unit (µg.g^{-1}). In blue the Northern Amazon Rivers (NA) and in green the Southern Amazon River (SA)(control area).

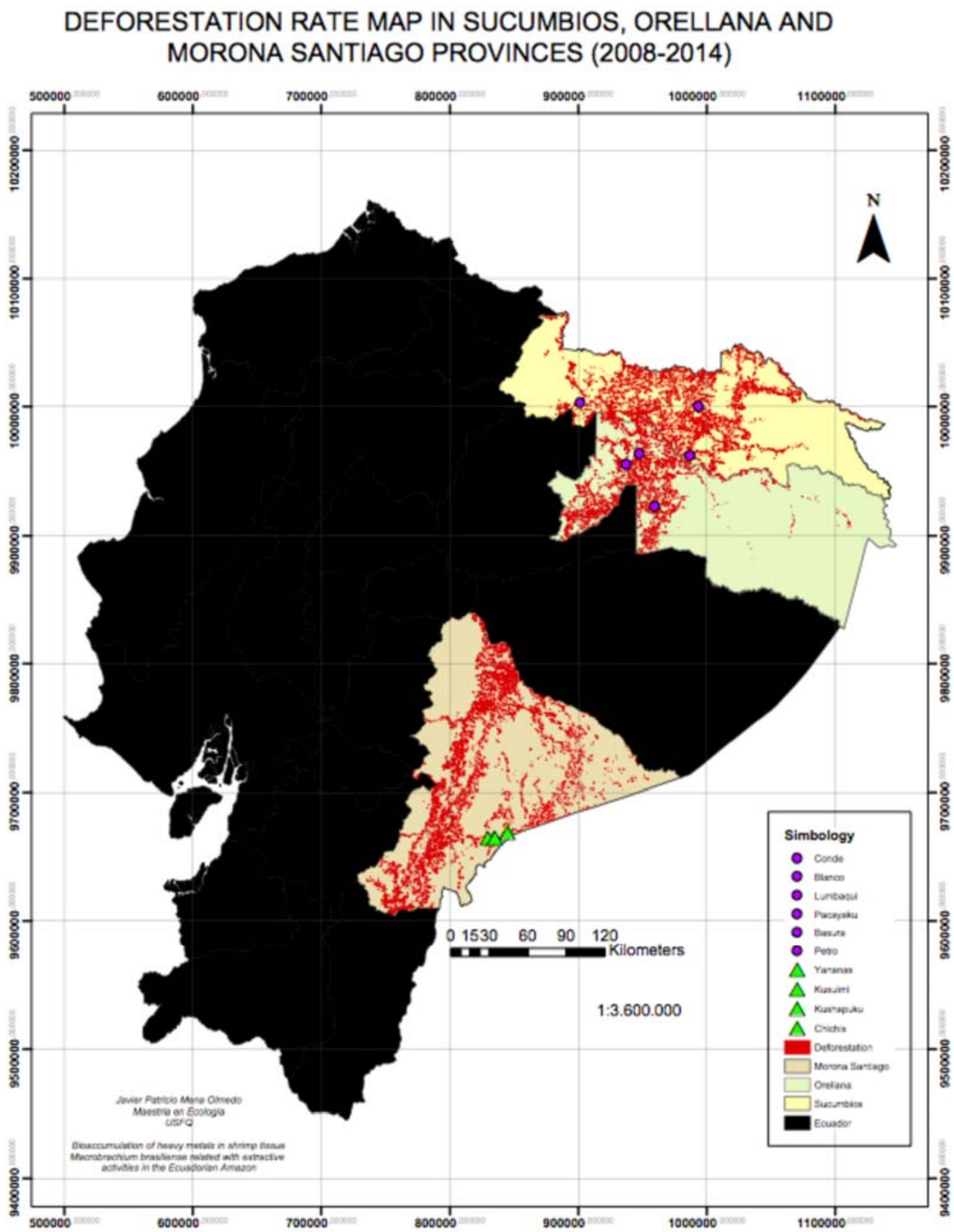


Figure 9. Deforestation rate map in Sucumbío, Orellana and Morona Santiago Provinces in the Ecuadorian Amazon in small scale. In purple the NA sampled points, in green the SA sampled points. Source: SUIA-MAE 2016.
deforestation in areas with similar soil geochemical.

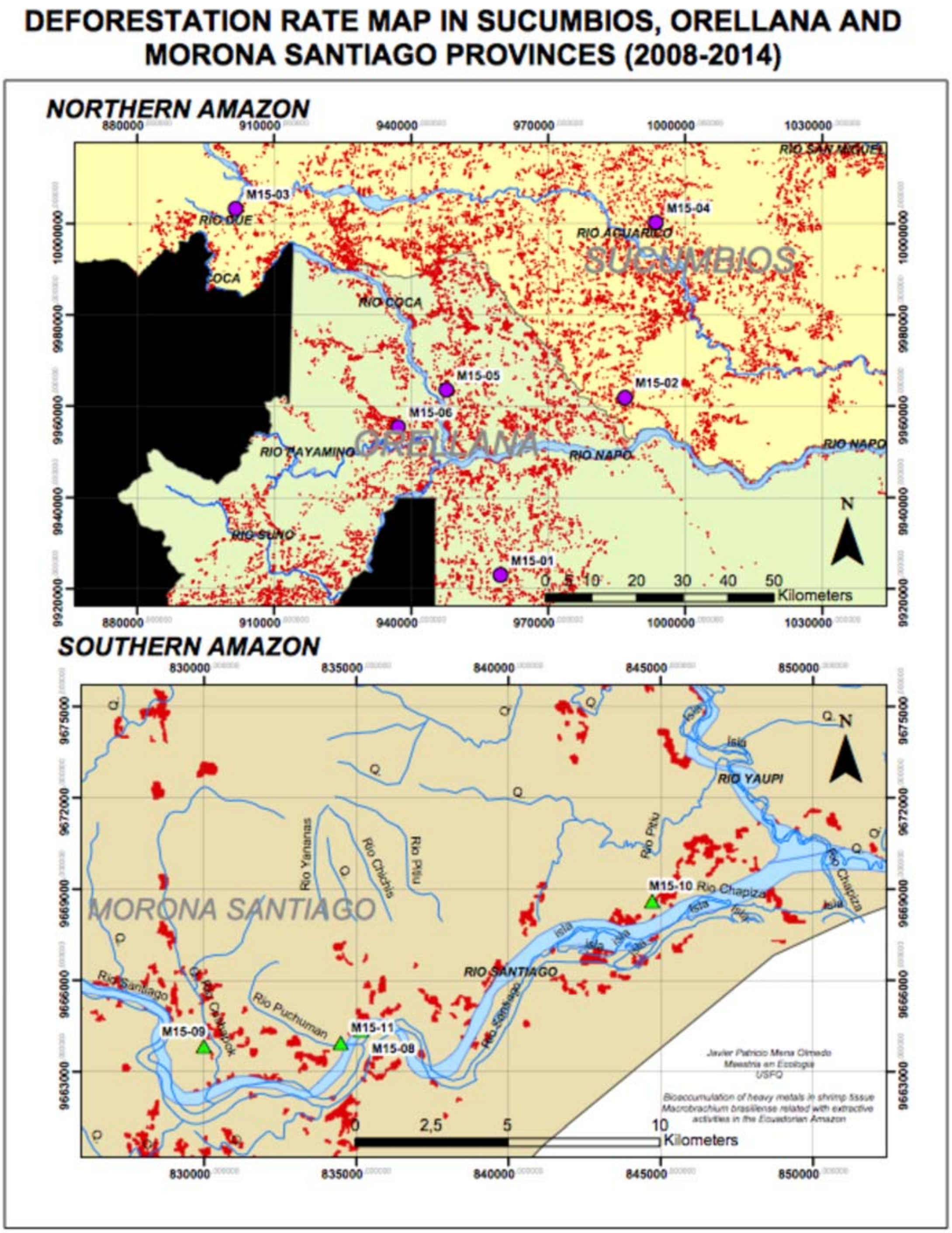


Figure 10. Deforestation rate map in Sucumbío, Orellana and Morona Santiago Provinces in the Ecuadorian Amazon. In purple the NA sampled points, in green the SA sampled points. Source: SUIA-MAE 2016.

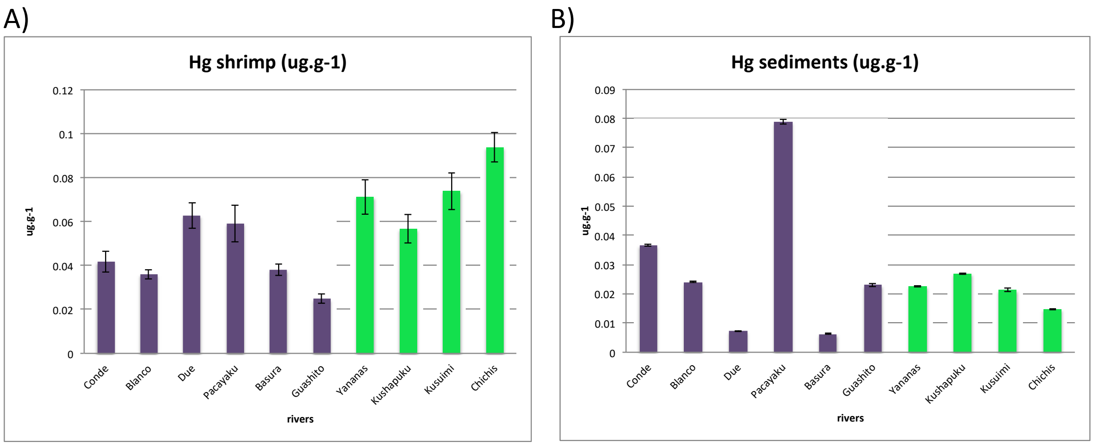


Figure 11. A) Total Hg concentrations (ug.g-1) in 10 Ecuadorian Amazon Rivers in freshwater shrimp *Macrobrachium brasiliense* and B) sediments (MONOIL 2015-6). In purple the Northern Amazon Rivers while in green the Southern Amazon Rivers.

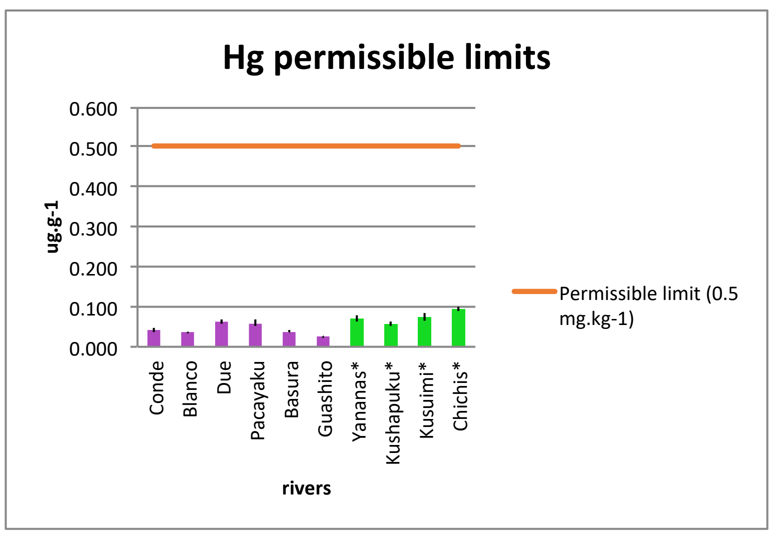


Figure 12. Total mercury concnetrations in *Macrobrachium brasiliense* freshwater shrimp from 10 sampling points in the Ecuadorian Amazon and the Hg permisible limit 0.5 mg Hg $.kg^{-1}$dw established by the European Commission (2010), Health Canada's Maximum Levels for chemical contaminants in food (2016) and Colombian Public Health Ministry (MSPS-Colombia 2016).