NATURE BASED LIVING LAB:

AN UNDERGRADUATE RESEARCH EXPERIENCE AT THE UPPER AMAZONIA

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NATURE-BASED LIVING LAB (NB LAB):

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Editors: Luis-Miguel Quishpe, Pilar Aramburuzabala, H. Mauricio Ortega-Andrade, Carlos Ávila, Rodrigo Espinosa, Miguel Herrera-Robledo.

Technical Review: Editorial CEDIA
Style Correction: Laura Malache Silva
Translation: Editorial CEDIA
Design and layout: Paz Cordero González

A publication by the Editorial CEDIA, arbitrated by double-blind academic pairs.

cedia

CEDIA
Gonzalo Cordero 2-111 y J. Fajardo
Cuenca – Ecuador
info@cedia.org.ec

FIRST EDITION
ISBN: 978-9942-7178-3-2
DOI: https://doi.org/10.48661/r1kv-fn29

Tena, Ecuador
February, 2024.

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UCTION

Nature-based living lab (NB LAB): an undergraduate research experience at the Upper Amazonia

NB-LAB as undergraduate research experience at the Upper Amazonia

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Rainforests play a practical role in keeping our planet healthy by absorbing carbon dioxide and releasing the oxygen that humans depend on for survival. Absorption of carbon dioxide also helps to stabilize the climate of Earth. In addition, rainforests help to maintain the world water cycle by adding water to the atmosphere through the process of transpiration, which creates clouds.

Get wild about saving the Amazonian Rainforest! Our initiative creates space for young researchers from all over the world who want to contribute to its protection. NB-LAB = Nature-based living lab for interdisciplinary practical and research semester on sustainable development and environmental protection in the Amazonas Rainforest.

The main aim of NB–LAB is "Setting up capacities for user-driven applied research and innovation through an interdisciplinary research group of Latin American and European universities that will contribute to sustainable rural community development under preservation and responsible use of the natural resources in the Amazonian Region".

The Sub aims are: [1] Establish two nature-based living labs (NB-Labs) in Tena and Iquitos with modern lab facilities for experimental learning and research activities situated in the natural habitat of the Amazonian Rainforest directly connected to the rural communities and indigenous people from the Kokama Kukamiria, Iquito and Matsès nationalities in Peru and Kichwa or Shuar nationalities in Ecuador. [2] Establish international interdisciplinary research and development teams in the scope of mutually recognized student internships.

A summer school (SS) was designed and conducted at Ikiam University as a crucial step to complete the aims. Summer school included six weeks of immersion in real–life research environments, although the involvement of exchange students started two months before they traveled to Ecuador (Table 1).

Table 1. Summary of the pre-travel study program for NB lab prospects.

First contact *[2nd week of June, 2022]*	Focus – Explanation of the technical aspects of the research problem to solve Ikiam advisors chat with the prospects and explain the problem to unveil the hypothesis they have designed to address the challenges.
Second contact *[10 days after the first contact]*	Focus – The materials and the methodology to follow Ikiam advisors chat with the prospects and explain the materials and methodological insights to use in the research that they will perform.
Third contact *[10 days after the second contact]*	Focus – The serving–learning component Ikiam advisors describe the challenges the local communities face and discuss with the prospects several strategies to help solve them.
Fourth contact *[10 days after the third contact]*	Focus – Integration of a research plan Ikiam advisors chat with the prospects to explain how to merge previous analyses into a research plan. The final homework will be to integrate a professional plan to perform during the in–situ exchange program at Ikiam.

This academic period spanned from June 13th to July 29th, 2022, and was conducted online using teleconferences.

During the pre-travel stage, faculty and staff from Ikiam co-designed with the exchange students a research plan that the latter will perform when visiting Ikiam. Preparation activities were standardized by using guidelines and templates to develop a research plan. The tailor-made plans were reviewed by a committee of tutors to: (a) Enhance the scope and strategy; (b) Identify risks and milestones of the program, and (c) Organize the subjects, courses, equipment, and reactants required for the research.

So, the ultimate goal of the pre-travel stage was to develop a specific research plan for each exchange student. *Before arriving at Ikiam they knew what they must perform to complete the research goals.*

The second stage started once the students arrived in Ecuador. The core of the second stage was research platforms (See below), in which Ikiam staff and students trained the exchange students to use cutting-edge, real-time monitoring tools and experimental design. In this stage, the students also were assisted in processing discovery and data analysis. Table 2 describes the main features of each of the six weeks of the 2022 NB-LAB summer school. Summer school also was designed to test a service-learning approach, which means offering students a valuable form of experiential learning while also helping to meet the needs of local communities. The in-situ research experience during the deployment and utilization of cutting-edge techniques over biodiversity spots at participatory science-oriented research stations surely will be inspirational for the students to pursue STEM-oriented graduate programs in the short-term, that was one of the aims of the program.

Table 2. Summary of the research stage of the 2022 NB–LAB Summer School.

Week 1 *[01 – 07 August, 2022]* **Site – Central campus UTE, Quito.** The intercultural week. Was held at Equinoctial Technological University [UTE].It included visits to museums, historical sites, and symposia.	**Week 2** *[08 – 14 August, 2022]* **Site – Ikiam University and Colonso Chalupas Biological Reserve, Upper Amazonia** Scientific training and capacity building;Masterclasses;Workshops; Preparation of field trips [Bootcamp 1]; celebration and reflection activities.	**Week 3** *[15 – 21 August, 2022]* **Sites – Ikiam University and fieldwork in "Minga lodge and reserve", located in Chontapunta, Napo.** Scientific fieldwork in lower zones of Napo river basin. Master classes and lectures.
Week 4 *[22 – 28 August, 2022]* **Sites – Ikiam University and Colonso Chalupas Biological Reserve, Upper Amazonia** Scientific training and capacity building; Masterclasses; Workshops; Preparation of field trips [Bootcamp 2]; celebration and reflection activities.	**Week 5** *[29 August – 04 September, 2022]* **Sites – Ikiam University and fieldwork in "Sumak Kawsay in situ", located in the surroundings of Mera Town, Pastaza.** Fieldwork in the Andean-Amazon transition zone. Masterclasses and lectures.	**Week 6** *[05 – 10 September, 2022]* **Sites – Ikiam University and Colonso Chalupas Biological Reserve, Upper Amazonia** Data analysis and curation. External evaluation. Master classes and lectures; Evaluation Seminar. Celebration and demonstration activities.

The research platforms

The background of the undergraduate research platforms conducted during the 2022 NB LAB summer school is described below.

Research platform 1:
Exploring the biodiversity of the Amazon landscape.

The Ecuadorian region of the Amazon Basin (EAR) is a biodiversity hotspot explored just partially. Led by Prof. Mauricio Ortega-Andrade, several Ikiam University faculty, staff, and students have contributed to elucidate some of this diversity by describing new metabolites and toxins associated with fungi, plants, reptiles, and birds as well as performing the first campaign of DNA barcoding in the region during the 2022 NB-LAB summer school (Quilumbaquin et al., 2023). Based on the concept called next generation of natural history (Tosa et al., 2021), the overall goal of this research platform currently is to explore representative environments in real-time, from trophic networks in aquatic systems using terrariums and mesocosms to automatizing ecological monitoring. The tools used by Ikiam specialists to perform their sampling campaigns include (but not are limited to): genetics, genomics, and bioinformatic methods; aircraft- and satellite-based remote sensing; camera-traps and acoustic recorders; mesocosms and the very last generation of real time DNA sequencing tools.

Research platform 2:
Effects of global warming at different altitude gradients.

Ikiam University scientists led by Prof. Pablo Meneses have access to perform research in the Colonso-Chalupas biological reserve (CCBR), a national park that comprises more than 94 000 ha and has an extraordinary altitude gradient of 477 to 4480 masl. This research platform aimed to generate basic ecological information placed on different altitudinal gradients to support policy decision-making linked to global warming mitigation and adaptive processes (Moulatlet et al., 2021). In this context, Pablo Meneses and his team analyzed the fragmentation dynamics of the ecosystems over the last decade as well as the environmental temperature and amphibians variation in function of different land uses in the buffer zone of the CCBR (Santos et al., 2019). In addition, they use ecological niche modeling to predict the potential geographic distribution of four invasive species under different climate change scenarios. The use of (I) ecological niche modeling focused on conservation and (II) remote sensing by drones to predict optimal regional planning in different global warming scenarios (i.e., flooding by glacier melting) is also in focus for the staff and students involved in this platform.

Research platform 3:
Geo-physical, chemical, and morphological insights on the northern Andean Volcanic Belt.

Much of the richness found in the Ecuadorian sections of the Amazon Basin originates from the Andean mountains. Hundreds of tons of sediment and millions of cubic meters of water are transported from the Andes to the Amazon Basin every week. To elucidate the connections and effects of geological issues (such as seismic and volcanic activity, sediments and nutrient transport, geochemical cycles, and the evolution of soil) over the biodiversity, hydrology, and urban planning of the EAR has been the major goal of this research platform.

The primary goal of this research was to develop scattered research stations (seismographs), and another goal pursued by this platform was to deploy them within Colonso-Chalupas National Park and its surroundings. The mobile seismic tomography equipment acquired through NB-LAB is helping in the quantification of several properties of the subduction of the Nazca plate and volcanic activity in the zone (Araujo et al., 2023; Espin and Araujo, 2022; Araujo et al,. 2021). The expertise in the use of cutting-edge geomorphology, geochemical, and sedimentology techniques is also a competitive advantage of the personnel that works in this research platform, which is coordinated by Professors Sebastián Araujo, Oswaldo Guzmán, Santiago Bálcazar, and Corina Campos.

Research platform 4:
Microbial ecology and ecotoxicology of fluvial systems.

A considerable strain is being exerted on the fluvial systems in the EAR. Unplanned urban growth, illegal mining, inadequate design of landfill assets, and de facto reuse of wastewater are the main factors that are exerting unstoppable pressure on the water bodies. Led by Professors Marcela Cabrera and Rodrigo Espinosa, a set of Ikiam faculty and students are researching on the following issues: Evaluation of the ecological risk and ecotoxicology on aquatic environments (Changay et al., 2021: Capparelli et al., 2021; Rosero et al., 2021); identification of organic compounds and microplastics in surface water and wastewater (Villegas et al., 2021; Villegas et al., 2022; Cabrera et al., 2023; Galarza et al., 2023). During the 2022 NB–LAB summer school exchange students were involved in monitoring the fate of emerging pollutants in aquatic trophic networks.

Research platform 5:
Removal and inactivation of compounds of emerging concern by photocatalytic membrane reactors.

The overall goal of this research platform, led by Prof. Miguel Herrera-Robledo, is to develop photocatalytic membrane reactors (PMR), small disinfection devices based on membrane separations coupled to advanced oxidation processes (AOP) that are capable of removing and inactivating viruses and oxidizing most of the compounds of emerging concern (CEC). The strategy to develop these membrane photocatalytic reactors includes the following stages: (a) the synthesis and instrumental characterization of novel visible light active photocatalysts (such as bismuth oxyhalides, BiOX; in which X = Iodine, Chlorine, Bromine) (Zuarez et al., 2023); (b) its deposition on porous (ceramic membranes) and non-porous substrates (walls, floors) (Zuarez et al., 2022b); (c) the testing of its efficiency against viruses and CEC (Tuba–Guamán et al., 2022); and (d) analysis on BiOX cytotoxicity versus different cell lineages (Zuarez et al., 2022a).

Research platform 6:
Sustainable architecture design.

Led by Prof. Irene Acosta, this platform aims to: (I) innovate in the design of structures and buildings used by Amazonian communities through the use of active and passive bioclimatic design techniques; (II) insert the utilization of ecological construction materials with low environmental impact, recycled or of local origin; and (III) study the life cycle of building materials and the potential effect of their degradation on the environment and biodiversity. During the 2022 NB–LAB summer school, this platform was composed only of Ikiam faculty and students and was in charge of the reengineering of the Ikiam research station (IRS).

This book describes the paths and outcomes of the research efforts conducted during the first exchange program in Tena, Ecuador. Read and enjoy the starting paths of this nature-based living laboratory. If you are interested in the next stages in the quest to consolidate the NB LAB program, save time to analyze the epilogue.

↘ *Link to "Student Guide of 2022 NB–LAB Summer School"*

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CHAP

OTER 1

Nature-based living lab (NB LAB): an undergraduate research experience at the Upper Amazonia

Exploring the biodiversity of the amazon landscape from a genetic ecology approach

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WQ (Walter Quilumbaquin) and MO (Mauricio Ortega) contributed to the concept and ideas on the manuscript design and analysis. WQ, MO, AC (Andrea Carrera), KA (Katherine Apunte), MG (Moisés Gualapuro), MS (Mónica Sánchez) and AA (Alex Arias) collaborated in the logistics and organization of the workshops. WQ, MO, JC (Jhoanna Cortegano), PG (Paula García) and GS (Goomaral Sukhbold) analyzed data, designed figures and tables, and wrote the draft version of the manuscript. All authors reviewed, commented, and approved the final version of the manuscript; all authors declared not conflict of interest.

Abstract

The Tena River basin is a partially explored biodiversity hotspot; however, it has been affected by anthropogenic activities and global warming. Observing the declines and extinctions in the wide range of Amazonian biodiversity before they went extinct is the primary purpose of research work in Ecuador. For this, complete information on the diversity of species coexisting in these areas is relevant because it enables the generation of strategies for conserving the environment and the species they harbor. Thus, the objective of the NB Lab 1 platform, Exploring the biodiversity of the Amazon landscape, was to contribute to the knowledge of the batrachofauna (amphibian assemblage) of the Tena River basin using DNA barcoding, environmental-DNA (eDNA) metabarcoding-based techniques, Nanopore sequencing, and automation of bioinformatics processes. This was carried out through three processes: 1) fieldwork: observation of specimens in their habitat and collection of environmental samples; 2) laboratory work: molecular assays for processing amphibian tissues and eDNA samples, library preparation, and ONT sequencing; 3) data analysis: use of bioinformatics tools such as NGSpeciesID for DNA barcoding data, amplicon_sorter for eDNA metabarcoding reads and bash for computational process automation. Forty amphibian 16S rRNA gene sequences were generated. The eDNA approach was used for biomonitoring amphibian populations, and four species were detected: *Scinax ruber, Pristimantis malkini, Rhinella marina, and Pristimantis* sp. Furthermore, a bash script based on the Linux platform was designed to reduce the data processing time of DNA barcoding and eDNA metabarcoding. Each biodiversity assessment project is a step towards a better knowledge of local biodiversity, which is fundamental at the governmental level to implement better management plans that further benefit local communities regarding environmental conservation.

Keywords

Diversity, Metabarcoding, Amphibian, Oxford Nanopore Technology, Biomonitoring

Resumen

La cuenca del río Tena es un lugar importante de biodiversidad parcialmente explorado, que se ha visto afectado por las actividades antropogénicas y el calentamiento global. Disponer de información completa sobre la diversidad de especies que coexisten en estas zonas es relevante porque permite generar estrategias de conservación del medio y de las especies que albergan. Así, el objetivo de la plataforma 1 del NB Lab: Explorando la biodiversidad del paisaje amazónico fue contribuir al conocimiento de la batracofauna (conjunto de anfibios) de la cuenca del río Tena usando ADN barcoding, técnicas basadas en eDNA metabarcoding, secuenciación Nanopore y automatización de procesos bioinformáticos. Esto se llevó a cabo mediante tres procesos: 1) trabajo de campo: observación de ejemplares en su propio hábitat y colecta de muestras ambientales; 2) trabajo de laboratorio: ensayos moleculares para el procesado de tejidos de anfibios y muestras de eDNA, preparación de librerías y secuenciación ONT; 3) análisis de datos: uso de herramientas bioinformáticas como *NGSpeciesID* para datos de ADN barcoding, amplicon sortes para lecturas de metabarcoding de eDNA y bash para la automatización de procesos computacionales. Se generaron 40 secuencias del gen 16S rRNA de anfibios. Se utilizó el enfoque de eDNA para el biomonitoreo de poblaciones de anfibios y se detectaron cuatro especies: *Scinax ruber, Pristimantis malkini, Rhinella marina* y *Pristimantis* sp. Además, se diseñó un script bash basado en la plataforma Linux para reducir el tiempo de procesamiento de datos de ADN Barcoding y eDNA metabarcoding. Cada proyecto de evaluación de la biodiversidad es un paso hacia un mejor conocimiento de la biodiversidad local, lo cual es fundamental a nivel gubernamental para aplicar mejores planes de gestión que beneficien aún más a las comunidades locales en términos de conservación del medio ambiente.

Palabras clave

Diversidad, Metabarcoding, anfibios, Tecnología Oxford Nanoporos, Biomonitoreo

1.Introduction

Studies focused on species diversity are especially relevant for unexplored areas such as the Tena River basin (TRB), which harbors populations of diverse taxonomic groups (Celi & Villamarín, 2020; Ordónez et al., 2011). Among them are amphibians, which have aroused great interest due to their alarming population decline: about 40.7% of all species are threatened (Luedtke et al., 2023). They have also aroused interest in the environmental field due to their contribution to the functioning of ecosystems and medicine due to bioactive molecules in their skin (Ortega-Andrade et al., 2021; Pereira, 2014; Proaño-Bolaños et al., 2016).

Ecuador has the third-highest amphibian diversity, with 676 formally described species (Ron et al., 2019). Traditional metrics of diversity and composition of amphibian communities have been generated using classical techniques such as observations, specimen collections, and taxonomic identification through morphological characteristics; their application requires expertise and many hours of work (Aguirre León, 2009; Heyer et al., 2001). These factors are challenging in the documentation and detection of amphibians and the development of conservation strategies (Brozio et al., 2017; Gascon et al., 2007). DNA barcoding and environmental DNA (eDNA) can be implemented to address these problems.

DNA barcoding is a tool that employs short DNA sequences from a specific genetic region and functions as a unique identifier for each species (Sigwart & Garbett, 2018). This approach has contributed to the knowledge of amphibian diversity since it provides preliminary information on populations that present cryptic species, immatures, invaders, etc. (Vences et al., 2012; Vences et al., 2005). This information is used to create genetic databases, which are necessary for developing conservation strategies and essential for applying biomonitoring techniques such as eDNA (Lopes et al., 2017; Zangl et al., 2020).

eDNA is an indirect, rapid, and non-invasive sampling technique used to assess and monitor biodiversity by capturing and processing cells and tracing DNA present in the environment (Taberlet et al., 2018). This

approach has been applied to monitoring invasive species, endangered species, amphibian diversity, and species with low population density (Brozio et al., 2017; Lopes et al., 2017). Environmental samples (soil, water, or air) may contain the genetic information of many organisms living in an ecosystem, as they release DNA molecules in the organic waste (skin, gametes, and feces) that we discard into the environment (Ficetola et al., 2008; Taberlet et al., 2018). This information can now be retrieved and analyzed more quickly due to advances in sample preparation and the development of cheaper and more affordable sequencing technologies, such as MinION from Oxford Nanopore Technologies (ONT) (Maestri et al., 2019).

Nanopore technology sequencing has made it possible to digitize the information contained in biological samples in a faster and more complete way, as it generates raw data ranging from long reads (genomes) to short reads (amplicons) (De Coster et al., 2018). For ONT data analysis, algorithms capable of correcting sequencing errors and generating robust consensus sequences have been designed. For example, NGSpeciesID is an algorithm designed to process ONT-generated data, as it has bioinformatics packages for read clustering, consensus formation, and consensus polishing of sequences (Sahlin et al., 2021). On the other hand, Amplicon_sorter is an algorithm used for metabarcoding data analysis, as it separates a mixture of amplicons into groups based on similarity and length to generate a robust consensus sequence (Vierstraete & Braeckman, 2022).

The generation of information on the biodiversity of the Amazon landscape is essential. Therefore, the objective of this chapter was to contribute to the knowledge of the batrachofauna of the Tena River basin using DNA barcoding, eDNA-based techniques, Nanopore sequencing, and improving the automation of bioinformatics processes.

2.Methods

2.1. Sampling site

The study site is within the TRB (Napo Province, Ecuador), in the Amazon Piedmont. It has an altitudinal range of 600 to 710 masl and an annual precipitation of 3 500 mm. eDNA samples were collected at six points all along the Tena River in August 2022: low, median, and high basin (Fig. 1). The Tena River flows through diverse ecosystems, from primary and secondary forests to urban areas, which makes it a body of water that harbors a wide biodiversity of plants and animals, among others.

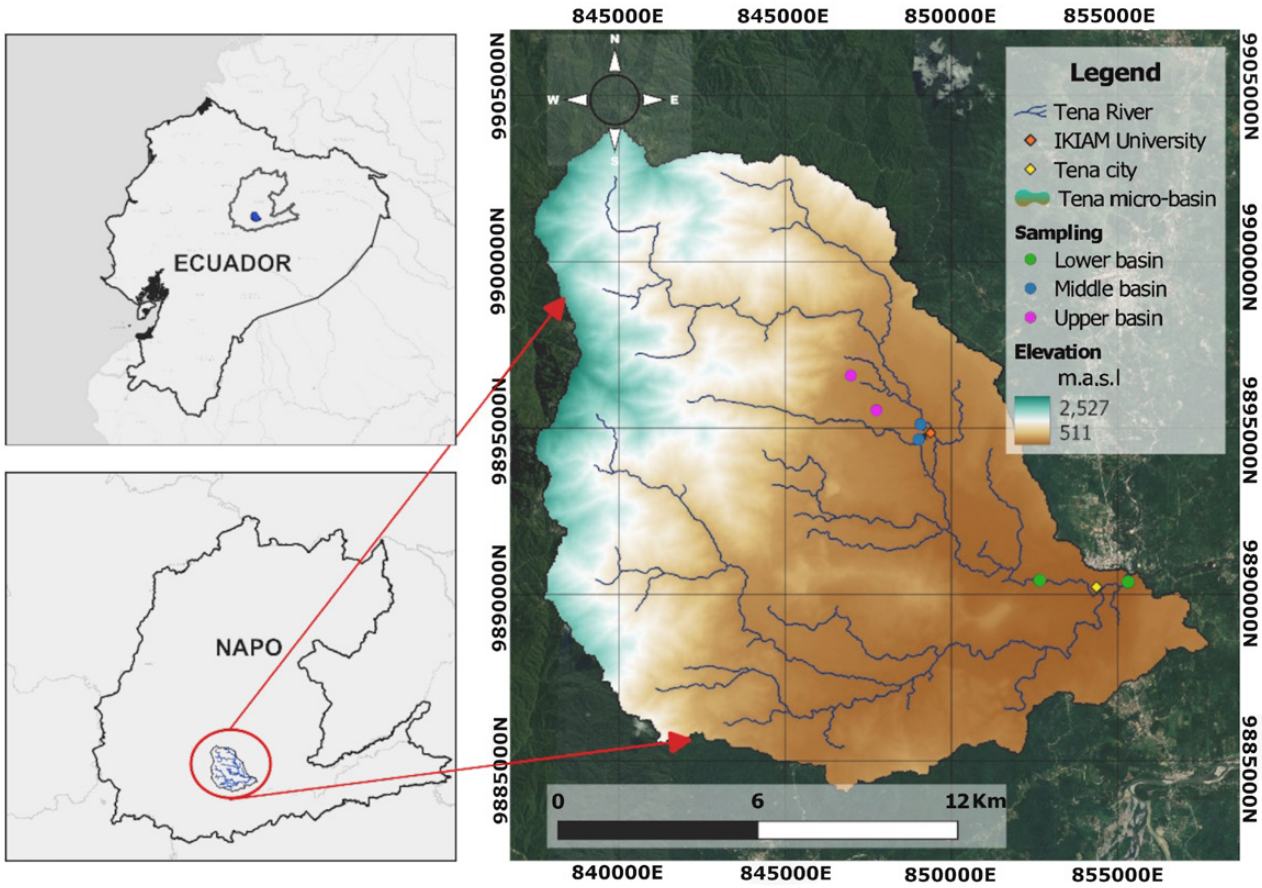


Figure 1. Sampling site in the Tena River Basin, Napo, Ecuador.

It consists of six points along the Tena River: lower (green), middle (blue), and upper basin (purple). Two reference points were considered: the Amazon Regional University Ikiam (Orange) and the city of Tena (Yellow).

2.2. Molecular assays for DNA Barcoding

This process began with compiling all unprocessed tissues from the Ikiam amphibian gene bank. A total of 40 ethanol-preserved specimens were obtained from the Ikiam collection. Subsequently, DNA extraction was performed using the Wizard® Genomic DNA Purification Kit (Promega, USA) with 5-10 mg of muscle tissue, following the manufacturer's protocol. The quantification was performed with the NanoDrop™ One/One Microvolume UV-Vis (Thermo Fisher Scientific, USA) to determine the purity and quantity of genomic DNA. The 16S ribosomal RNA gene (16S rRNA, approx. 650bp) was amplified with Veriti 96 well Thermal Cycler (Thermo Fisher Scientific, USA). The reaction volume was 25µL and consisted of 1X GoTaq Green Master Mix (Promega, USA), nuclease-free water, 0.2 µM of the markers 16sSar-L (5'-TTT CTG TTG GTG CTG ATA TTGC CGC CTG TTT ATC AAA AAC AT-3'), 0.2 µM of 16sSbr-H (5'-ACT TGC CTG TCG CTC TAT CTTC CCG GTC TGA ACT CAG ATC ACGT-3') (Vences et al., 2005), and 50 ng/uL of genomic DNA. PCR reactions were run using the following thermal profile: 5 min for 95 °C, 35 cycles of 95 °C for 30s, 59 °C for 30s, 72 °C for 45s, a final extension of 5 min at 72 °C and cooling to 4 °C. PCR products were visualized on 1.5% agarose gels with the ENDURO™ GDS Touch Gel Documentation System (Thermo Fisher Scientific, USA).

2.3. Molecular assays for Environmental DNA

At each sampling point, three liters of water were collected downstream (1L), upstream (1L), and in the middle river (1L). Water samples were filtered at the Ikiam laboratory. This process was carried out using a sterilized glass filtration system (DURAN, Germany) and nitrocellulose membranes (PORAFIL NC) with a pore size of 0.45 µm and a 47 mm diameter. The eDNA samples (filters) were carefully cut into small pieces with sterile scalpels (No. 11), placed in sterile 2 mL microtubes, and stored at -20 °C. The eDNA extraction was performed using the method described in Baetens (2019) with modifications. The eDNA samples were quantified using the Qubit 4 Fluorometer (Invitrogen, USA). The 16S rRNA gene (approx. 300bp) was amplified with a Veriti 96-well Thermal Cycler (Thermo Fisher Scientific, USA). The reaction volume was 25µL and contained 10-25 ng/µL extracted DNA, 1X KAPA2G Robust HotStart Ready Mix (Merck, Darmstadt, Germany), nuclease-free water, 0. 2 µM of Vert 16S eDNA-F1 (5'-TTT CTG TTG GTG CTG ATA TTGC AGA CGA GAA GAC CCY DTG GAG CTT-3'), 0.2 µM of Vert 16S eDNA-R1 (5'-ACT TGC CTG TCG CTC TAT CTTC GAT CCA ACA TCG AGG TCG TAA-3') (Vences et al., 2016) and 50 ng/µL bovine serum albumin (BSA, Invitrogen, USA). The thermal profile used was as follows: 3 min for 94 °C, 30 cycles of 94 °C for 30s, 58 °C for 60s, 72 °C for 90s, a final extension of 10 min at 72 °C and a cooling to 4°C. PCR products were visualized on 2% agarose gels in an ENDURO™ GDS Touch Gel Documentation System (Thermo Fisher Scientific, USA).

2.4. Nanopore DNA Sequencing

The first step in this process was purifying all PCR products from DNA and eDNA samples using Exonuclease I and Shrimp Alkaline Phosphatase (New England BioLabs, USA). Subsequently, library preparation (Barcode adapters attachment, DNA repair and end-prep, and sequencing adapters attachment) was performed using the Barcoding Expansion Pack 1-96 PCR kit (EXP-PBC096, ONT, UK), NEBNext FFPE DNA repair mix (M6630), NEBNext Ultra II End repair/dA-tailing module (E7546), and ligation sequencing kit (SQK-LSK109, ONT, UK). Sequencing was performed on a MinION MK1B (portable sequencer, ONT, UK) and the flow cell R9.4.1 (FLO MIN106, ONT, UK) for 15 hours.

2.5. Data analysis

Raw sequence data from DNA barcoding was processed with the bioinformatic tool NGSpeciesID. This tool filters reads based on quality scores (Q > 12), finds and removes adapters and primers, polishes the data with Medaka to correct sequencing errors, and generates a robust consensus sequence for each sample. Sequences obtained were compared with the National Center for Biotechnology Information (NCBI) database through the BLAST+ v2.13.0 algorithm to assign the genus (threshold 90-96%) and species (threshold >97%).

eDNA Fastq files were processed with Python v3.10.1. and a docker image in Docker Desktop 4.1. Visualization, cleaning, polishing, and taxonomic assignment of eDNA data were carried out with the following algorithms: NanoPlot v1.20.0, NanoFilt v2.5.0, Cutadapt v4.1, Amplicon_sorter, and BLAST+ v2.13.0. The analysis time of the Nanopore sequencing data was optimized by creating a bash script. This script allowed anchoring the command lines used to manipulate and analyze the eDNA data (decompress, concatenate, filter, polish, generate a robust consensus sequence based on similarity and length, and taxonomic assignment) in a single script.

3.Results and Discussion

3.1. Barcoding

The genetic information of the batrachofauna from the Ikiam scientific collection was completed with the sequencing of 40 specimens. These corresponded to 33 species of amphibians and belonged to nine families (**Fig. 2**), Craugastoridae being the most numerous, with 19 species, followed by Hylidae with eight species.

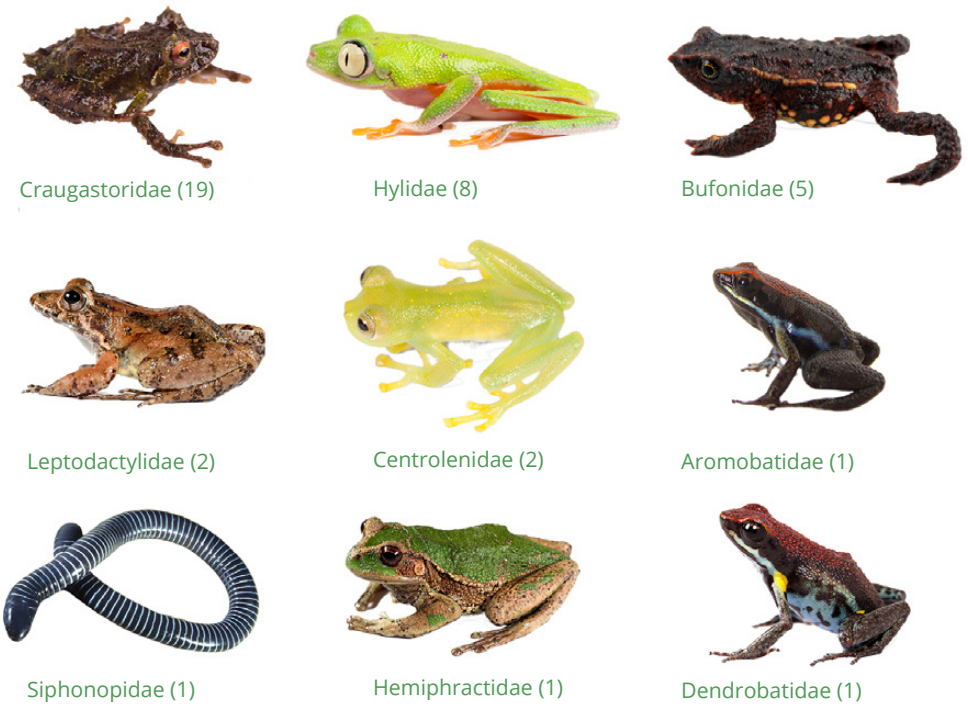


Figure 2. Amphibian families recorded by genetic analysis.

Photographs: https://bioweb.bio/.

These amphibian specimens were identified genetically by DNA barcoding to generate more sources of evidence and corroborate their morphological identification, resulting in 19 matches (both methods identified the same species) (Table 1). As mentioned by Vences et al. (2005), DNA barcoding is an effective tool for species identification; however, due to the genetic structure of batrachofauna, more sources of information are necessary for reliable identifications. The identification of amphibian diversity in areas such as TRB, which has few studies on the subject, is essential to fill in the gaps in information on their composition and distribution, as well as to detect the presence of possible new species such as those of the genus Pristimantis (Table 1) (Angulo et al., 2006; Quilumbaquin et al., 2023).

Table 1. List of amphibians identified by morphological characteristics and DNA barcoding.

Barcode Sequencing	Family	Genus	Species Identified Morphologically	Specimen Code	Species Identified Genetically (NCBI)	Accession (NCBI)	Identify Percentage (%)
B02	Craugastoridae	Pristimantis	*Pristimantis conspicillatus*	HMOA 1898	*P. conspicillatus*	MW567377	97.18
B03	Craugastoridae	Pristimantis	*Pristimantis altamnis*	HMOA 2179	*P. mallii*	MZ429999	98.67
B04	Hylidae	Boana	*Boana calcarata*	HMOA 2443	*Hypsiboas sp.*	JN970538	99.82
B05	Craugastoridae	Pristimantis	*Pristimantis brevicrus*	HMOA 2163	*P. brevicrus*	MF118690	100
B06	Craugastoridae	Pristimantis	*Pristimantis eriphus*	HMOA 2098	*P. eriphus*	EU186671	98.3
B07	Craugastoridae	Pristimantis	*Pristimantis gladiator*	APA 003	*P. gladiator*	ON468348	99.65
B08	Bufonidae	Osornophryne	*Osornophryne cf simpsoni*	EC-250	*O. simpsoni*	JX411995	99.315
B09	Hylidae	Scinax	*Scinax garbei*	HMOA 2499	*Scinax garbei*	ON907637	99.82
B10	Centrolenidae	Teratohyla	*Teratohyla midas*	HMOA 2390	*C. durrellorum*	KF534356	99.64
B11	Hylidae	Agalychnis	*Agalychnis hulli*	HMOA 1974	*Agalychnis hulli*	GQ366226	99.83

Matches between the two methods are underlined (green).

Barcode Sequencing	Family	Genus	Species Identified Morphologically	Specimen Code	Species Identified Genetically (NCBI)	Accession (NCBI)	Identify Percentage (%)
B12	Hylidae	Osteocephalus	*Osteocephalus verruciger*	HMOA 2376	*O. verruciger*	KF002167	98.98
B13	Bufonidae	Osornophryne	*Osornophryne cf simpsoni*	EC-249	*O. simpsoni*	JX411995	99.31
B14	Bufonidae	Osornophryne	*Osornophryne cf simpsoni*	EC-251	*O. simpsoni*	JX411995	99.31
B15	Hylidae	Phyllomedusa	*Phyllomedusa vaillantii*	HMOA 1968	*P. vaillantii*	AY549363	96.28
B16	Craugastoridae	Pristimantis	*Pristimantis carvalhoi*	HMOA 2060	*P. carvalhoi*	KY652651	97.9
B17	Hylidae	Hyloscirtus	*Hyloscirtus albopunctulatus*	HMOA 2227	*H. alytolylax*	KT279541	91.11
B18	Leptodactylidae	Leptodactylus	*Leptodactylus discodactylus*	HMOA 2366	*L. wagneri*	MW291404	99.68
B19	Craugastoridae	Pristimantis	*Pristimantis delius*	HMOA 2375	*P. jimenezi*	MW291404	92.33
B20	Aromobatidae	Allobates	*Allobates zaparo*	TWQ_045	*Allobates zaparo*	AY364579	99.67
B21	Craugastoridae	Pristimantis	*Pristimantis omeviridis*	KDLC 005	*P. enigmaticus*	MT636520	99.174

Barcode Sequencing	Family	Genus	Species Identified Morphologically	Specimen Code	Species Identified Genetically (NCBI)	Accession (NCBI)	Identify Percentage (%)
B23	Craugastoridae	Pristimantis	*Pristimantis thymelensis*	APA 001	*P. thymelensis*	EF493516	99.01
B24	Craugastoridae	Pristimantis	*Pristimantis sp.*	HMOA 2239	*Pristimantis sp.*	MZ430052	97.2
B25	Craugastoridae	Pristimantis	*Pristimantis llanganati*	HMOA 2112	*P. bromeliaceus*	EF493351	88.68
B26	Craugastoridae	Pristimantis	*Pristimantis sp.*	HMOA 2232	*Pristimantis sp.*	MZ430052	97.2
B27	Craugastoridae	Pristimantis	*P. croceoinguinis*	HMOA 2095	*Pristimantis mallii*	MZ429999	99.22
B28	Siphonopidae	Siphonops	*Siphonops annulatus*	HMOA 2130	*S. annulatus*	EU753986	99.61
B29	Bufonidae	Osornophryne	*Osornophryne cf simpsoni*	EC-248	*O. simpsoni*	JX411995	99.31
B30	Craugastoridae	Pristimantis	*Pristimantis llanganati*	HMOA 2185	*P. eriphus*	EU186671	98.41
B31	Craugastoridae	Pristimantis	*Pristimantis variabilis*	HMOA 2160	*P. malkini*	ON907632	100
B32	Hylidae	Phyllomedusa	*Phyllomedusa tomopterna*	HMOA 2495	*P. tarsius*	KY576698	98.6

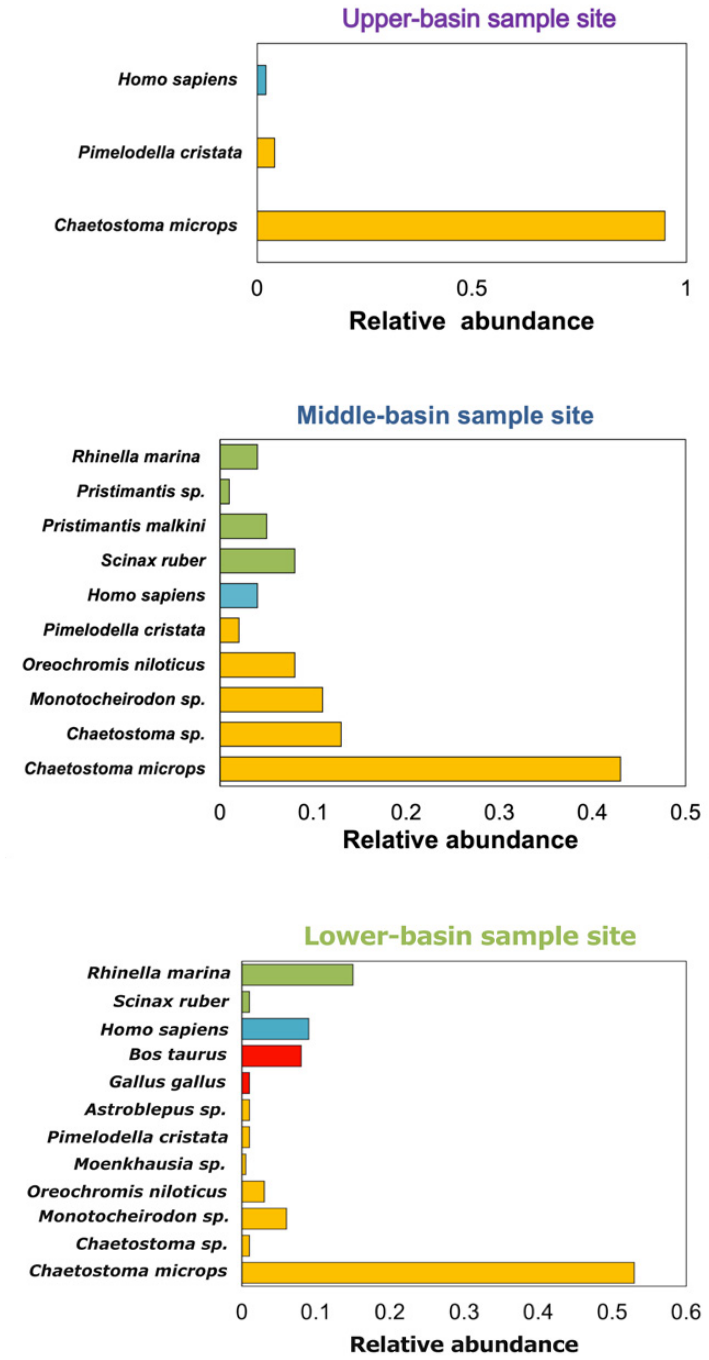
Barcode Sequencing	Family	Genus	Species Identified Morphologically	Specimen Code	Species Identified Genetically (NCBI)	Accession (NCBI)	Identify Percentage (%)
B33	Craugastoridae	Pristimantis	*Pristimantis martiae*	HMOA 2506	*P. unistrigatus*	EF493387	92.16
B34	Leptodactylidae	Adenomera	*Adenomera hylaedactylus*	HMOA 2442	*A. andreae*	ON907619	100
B35	Hylidae	Hyloscirtus	*Hyloscirtus phyllognathus*	HMOA 1902	*H. phyllognathus*	KT279548	97.89
B36	Craugastoridae	Pristimantis	*Pristimantis sp.*	TWQ_046	*P. unistrigatus*	EF493387	90.27
B37	Hemiphractidae	Gastrotheca	*Gastrotheca pseustes*	APA 002	*G. pseustes*	KJ489491	99.82
B38	Craugastoridae	Pristimantis	*Pristimantis malli*	HMOA 2379	*P. jimenezi*	MK881466	92.33
B39	Bufonidae	Rhaebo	*Rhaebo ecuadorensis*	HMOA 2220	*R. ecuadorensis*	ON907634	100
B40	Centrolenidae	Nymphargus	*Nymphargus siren*	HMOA 2081	*N. cochranae*	EU663061	99.65
B41	Craugastoridae	Pristimantis	*Pristimantis thymelensis*	HMOA 2246	*P. aff thymelensis*	EF493516	95.57
B49	Dendrobatidae	Ameerega	*Ameerega biliguis*	TWQ_044	*Ameerega biliguis*	HQ290996	99.34

Pristimantis frogs exhibited the highest richness, with 19 recorded species, but we only identified six by both methods (Table 1). Likewise, we observed more significant difficulties during morphological identification. It agrees with several studies that indicate that this high richness (or species-rich) group is a real challenge at the time of traditional identification (morphological visualization) because several species may present similar morphological characteristics, which generates a high proportion of cryptic species (Arteaga-Navarro & Guayasamin, 2011; Grosjean et al., 2015; Reyes-Puig et al., 2019; Mebert et al., 2022). The remaining specimens of this genus that were genetically and morphologically analyzed and not assigned to a specific species were identified only at the genus level (Table 1). This problem is evident not only in this study but also in many research and scientific collections, which has led to a greater interest in the classification of species of this group, generating a high percentage of new species descriptions in recent years (Ron et al., 2020; Ortega, Brito, & Ron, 2022).

In this study, 40 amphibian 16S rRNA gene sequences were generated by barcoding and included in the local reference databases of Ikiam and NCBI. Completing the genetic information of all organisms in the databases is essential for more accurate identifications and use in eDNA biomonitoring.

3.2. eDNA Metabarcoding

The after-analysis results expressed as relative abundance (RA), i.e., the number of specific copies over the total, are summarized in Figure 3. We did not detect amphibian species using metabarcoding in the upper basin. At the same time, the lower basin, just in and after the city, shows two amphibian species: *Rhinella marina* (15% RA) and *Scinax ruber*, which is less represented (1%). The middle basin shows a higher diversity of amphibians, where *R. marina* and *S. ruber*'s DNA are also represented in water samples. However, this time *S. ruber* was detected in a more considerable amount (less than 4% RA respect 8%) and *Pristimantis malkini* (5%). Other species, like the direct-developing frog *Pristimantis* (1%), were detected with low frequency in the middle-basin samples.



Green bars represent amphibian species, while yellow bars represent fish species; the blue one corresponds to human DNA, and red bars correspond to feedstock. Note the higher abundance of C. microps in every sample site.

Figure 3. A bar chart summarizing the relative abundance of eDNA detected through metabarcoding according to each sample site.

Biomonitoring using eDNA has proven to be an effective alternative for detecting amphibians in areas with high biodiversity (Ficetola et al., 2008; Brozio et al., 2017; Lopes et al., 2017). In this study, we recorded four species of amphibians using the eDNA method. It coincides with those detected by Quilumbaquin et al. (2023) in the areas surrounding our sampling site, except O. Simpson (Table 2). These amphibian species were detected from free DNA molecules in water samples from rivers and ponds. This demonstrates that the eDNA approach is a non-invasive tool and does not require direct interaction with the organism of interest (Taberlet et al., 2018).

Table 2. Detection of eDNA through metabarcoding of each amphibian species according to the sampling site.

Amphibian species	Upper basin	Middle basin	Lower basin	Negative control
Scinax ruber	No	Yes	Yes	No
Pristimantis malkini	No	Yes	No	No
Pristimantis sp.	No	Yes	No	No
Rhinella marina	No	Yes	Yes	No
*Osornophryne simpsoni**	Yes	Yes	Yes	Yes

**Suspected cross-contamination among the samples.*

The species *S. ruber* and *R. marina* were detected in the middle and lower parts of the TRB, evidencing a high probability of detection compared to other species. The study by Quilumbaquin et al. (2023) mentions that these two species have a probability of detection of around 100%; i.e., it is possible to record these species from a water sample (from rivers or ponds) with the eDNA technique. This detection may also be possible because they have a wide distribution and are closely related to bodies of water, as they spend part of their life cycle in them (Ron et al., 2019). On the other hand, the amphibian species only detected in the middle part of the basin were of the genus Pristimantis. The detection of these amphibian species with the eDNA approach may be affected by specific characteristics of the group, such as their reproductive biology, as they have direct development (absence of tadpoles) and are more related to terrestrial environments (Ron

et al., 2020; Ortega, Brito & Ron, 2022; Mebert et al., 2022). However, species such as *P. malkini* have been frequently recorded near streams or rivers, allowing their monitorization using water samples (Ron et al., 2019; Quilumbaquin et al., 2023).

To evaluate the process, during research with the eDNA method (field and laboratory), it is necessary to have several controls to generate robust and reliable information. The presence of the amphibian *O. simpsoni* at all sites and even in the negative control sample (i.e., deionized water only) is alarming since the detection of this species demonstrates that cross-contamination with DNA from this organism occurred at any step of the assays. The presence of cross-contamination during eDNA biomonitoring programs can generate biased information with erroneous records (Taberlet et al., 2018). Some studies recommend proper sterilization of all materials used in environmental sampling and the rest of the process (Baetens, 2009; Riascos et al., 2018). Likewise, for effective detection using the eDNA approach, it is advisable to repeat the collection of environmental samples several times, as eDNA is not homogeneous in the environment, so with single monitoring, it is likely that species with higher population density or none will be recorded (Brozio et al., 2017; Taberlet et al., 2018; Quilumbaquin et al., 2023).

Regarding other vertebrate species detected, the relative abundance of human DNA increases from 0.1% in the upper basin to 10% in the lower basin along the Tena River. Likewise, the number of vertebrates increased, with three records in the upper basin, 10 in the middle basin, and 12 in the lower basin, where feedstock DNA (chicken and bovine DNA) was detected (Fig. 3). The eDNA method can detect most organisms in a water sample (depending on the primers). The increase of organisms in the lower basin may be due to several factors, one of them being the contamination of the river water by sewage (Wingfield et al., 2021) and the other being the characteristics of lotic systems; the DNA molecules of organisms can be carried downstream by the river flow (Wacker et al., 2019; Lozano-Mojica & Caballero, 2021).

This study used universal genetic markers for vertebrates to detect several species of amphibians associated with aquatic environments and other groups such as fish, mammals, and birds (Piñol et al., 2019). The groups with the highest prevalence during eDNA biomonitoring were fish and amphibians, which coincides with the results obtained in other eDNA studies (Evans et al., 2016; Vences et al., 2016). The correct selection of markers is fundamental in eDNA metabarcoding since using a single marker can detect several taxonomic groups or interfere in populations with low population density.

The information generated by the eDNA technique is an essential contribution to understanding the biodiversity harbored by aquatic ecosystems. These results could complete the diversity inventories of the Tena River basin since there are areas with information gaps (Quilumbaquin et al., 2023). Likewise, knowing the diversity of species associated with bodies of water can be a critical factor in estimating the health of ecosystems (amphibians are sensitive to pollutants in their environment) and identifying the presence of invasive and domestic species (Saber et al., 2017; Taberlet et al., 2018).

3.3. Pipeline design for bioinformatics

Automation of biological data analysis is essential to reduce data processing time and generate diverse information more quickly. We designed a bash script from a series of individual command lines to analyze ONT sequencing data for DNA barcoding and eDNA metabarcoding (Fig. 4).

The bash script was tested with two data sets of DNA barcoding and eDNA metabarcoding. The script was first tested with raw data from four previously analyzed species, and similar results were obtained. It was then evaluated with data from 40 specimens processed in this study, and 33 species were identified. Similarly, this algorithm was run for the raw data of the environmental samples generated in the study by Quilumbaquin et al. (2023), detecting only the species with the highest population density. This result is mainly due to the lack of processing power, as the analysis was performed on an HP laptop with 4GB of RAM and two cores. When working with environmental sample data, millions of reads are generated for all organisms present in the sample, which requires more processing power (64 GB of RAM and 16 or more cores), especially to identify reads for species with low population density (Vierstraete & Braeckman, 2022).

```
#! /bin/bash

Mkdir merging cutting binning annotating consensus

# Concatenate fastq format files

Cat  filename/*.fastq > merging/merged.fastq

#Search and cutting of adapters and primers; [Adapters.fa] list of primers

Cutadapt -g file: Adapters.fa merging/merged.fastq > cutting/
filename_cut.fastq

# Quality control 12

NanoFilt –q 12 cutting/filename_cut.fastq > binning/filename_bin.fastq

Cut merging/merged.fastq > cutting/filename_cut.fastq

bin merging/merged.fastq > binning/filename_bin.fasta

annotate binning/filename_cut.fastq > binning/filename_bin.fasta

#Script used to generate consensus sequences based on size and similarity

Python3 amplicon_sorter.py -I binning/filename_bin.fastq -o binning/
filename_binR -np 8 -min 400 -max 700 -ra -maxr 1000

mv binning/filename_binR/filename_bin_consensussequences.fasta ~/home
directory

#Taxonomy assignment with NCBI

apt-get install ncbi-blast+

makeblastdb -in DatabaseReference.fasta -dbtype nucl -out Data

Blastn -db Data -query filename_bin_consensussequences.fasta -
num_alignments 1 -outfmt "qseqid stitle pident length" – out Bin.out
```

Figure 4. A bash script was used for the analysis of the eDNA metabarcoding data obtained from the ONT sequencing platform.

4.Conclusions

Information about each country's biodiversity is essential for designing strategies to conserve and recover the ecosystems and the organisms they harbor. The Tena River Basin is an area rich in diversity and little explored, where tools such as DNA barcoding and eDNA metabarcoding can help continue to fill in the information gaps in this region. The advantage of these two techniques lies in the simplicity of their implementation, which generates robust and valid results. Likewise, these approaches complement classic sampling techniques, which have generated much of our information.

The genetic information generated by the DNA barcoding technique is essential for identifying species (especially groups with cryptic species) and generating reference databases, which is critical for the taxonomic assignment of eDNA metabarcoding data. The biodiversity sampling of the TRB with eDNA metabarcoding developed in this work showed that there are still aspects to work on to improve the application of this technique, but the amount of information recovered per sampling site evidenced the great potential of this technique, mainly for sites that are difficult to access.

Developing a project to discover Ecuador's biodiversity through the eDNA metabarcoding approach can be a real challenge, depending on the type of ecosystems to be studied, the organisms, and the complexity of the bioinformatics processes. However, each biodiversity assessment project is a step towards a better understanding of local biodiversity, which is essential at the government level to implement better management plans that will further benefit local communities in environmental conservation.

5.Acknowledgment

We would like to express our sincere gratitude for the generous support provided by the Erasmus+ grant: 619346-EPP-1-2020-1-DE-EPPKA2-CBHE-JP. This funding has been instrumental in the successful development of the research project, enabling us to explore and present our findings in this chapter.

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CHAP

OTER 2

Nature-based living lab (NB LAB): an undergraduate research experience at the Upper Amazonia

Effects of global warming on landscape and biodiversity:

Study cases of forest fragmentation dynamics and the potential geographic distribution of invasive species in global warming scenarios in Ecuador

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Abstract

Climate change, land use changes, pollution, and invasive species are some of the main threats to ecosystems and human sustainability worldwide. In this chapter, we present two study cases: one related to forest fragmentation dynamics in the Tena River basin located in western Amazonia and another about the potential geographic distribution of four key invasive exotic species under different climate change scenarios in Ecuador. Forest fragmentation dynamics were analyzed by combining remote sensing and geographic information systems; ecological niche models were used to assess the spatial distribution of the ecological niche of four invasive species under different climate change scenarios. The results expose decreases in the forest area and increases in the levels of forest fragmentation over the period 2017-2021 in the Tena River basin. We also found significant changes in the ecological niches of invasive species under future climate change scenarios in mainland Ecuador. Several protected natural areas were to be affected by changes in the environmental suitability of invasive species. These results underscore the importance of considering potential changes in land use, fragmentation, and potential distribution of invasive species when developing conservation and management strategies for natural areas. Our findings provide valuable insights to formulate effective policies and actions to mitigate the negative impacts of landscape fragmentation and invasive species on biodiversity in mainland Ecuador.

Keywords

Tena River basin, Habitat Fragmentation, Niche Modeling, Western Amazonia

Resumen

El cambio climático, los cambios en el uso del suelo, la contaminación y las especies invasoras son algunas de las principales amenazas para los ecosistemas y la sostenibilidad humana a nivel mundial. En este capítulo presentamos dos casos de estudio: uno relacionado con la dinámica de fragmentación del bosque en la cuenca del Tena ubicada en la Amazonía occidental y otro sobre la distribución geográfica potencial de cuatro especies exóticas invasoras clave bajo diferentes escenarios de cambio climático en Ecuador. La dinámica de fragmentación de los bosques se analizó mediante la combinación de sensores remotos y sistemas de información geográfica; y se utilizaron modelos de nicho ecológico para evaluar la distribución espacial del nicho ecológico de cuatro especies invasoras bajo diferentes escenarios de cambio climático. Los resultados exponen disminuciones en el área forestal y aumentos en los niveles de fragmentación forestal durante el período 2017-2021 en la cuenca del Tena, Amazonía de Ecuador. También encontramos cambios significativos en los nichos ecológicos de las especies invasoras bajo futuros escenarios de cambio climático en el Ecuador continental. Se descubrió que varias áreas naturales protegidas podrían verse afectadas por cambios en la idoneidad ambiental de las especies invasoras en el futuro. Estos resultados resaltan la importancia de considerar los cambios potenciales en el uso del suelo, la fragmentación y la distribución potencial de especies invasoras al desarrollar estrategias de conservación y manejo para áreas naturales protegidas y sus alrededores. Los hallazgos de este trabajo brindan información valiosa para la formulación de políticas y acciones efectivas destinadas a mitigar los impactos negativos de la fragmentación del paisaje y las especies invasoras sobre la biodiversidad en el Ecuador continental.

Palabras clave

Cuenca del Río Tena, Fragmentación del Hábitat, Modelado de nicho ecológico, Amazonía occidental.

1.Introduction

Climate change could exacerbate the effects of land use change over local temperatures, producing adverse effects on human health, agriculture, water resources, and biodiversity worldwide (Prevedello et al., 2019). Climate change will particularly affect species-rich biomes such as the Amazon. Indeed, Amazonia is one of the most important and threatened ecosystems on the planet (Ferraz et al., 2003). It hosts the greatest biodiversity in the world and plays essential roles in climate regulation and biogeochemical cycles at different scales (Albert et al., 2023; Hoorn et al., 2010). However, human pressures on Amazon ecosystems in the last century have been increasing, giving rise to threats related to land use changes, alterations in water use, and climate change. Additionally, pollution and the introduction of invasive exotic species pose further threats (Albert et al., 2023). About 20% of the original forest has been transformed into other land uses, especially for agriculture (Lapola et al., 2023, Ferraz et al., 2003). Furthermore, the rapid human pressures that sprawl over Amazon ecosystems cause habitat losses and degradation, affecting their resilience capacity against climate change (Malhi et al., 2008).

The Global Climate Change model scenarios for the Amazon region forecast an increasing occurrence of droughts, especially in the dry season (Cochrane and Barber, 2009). Also, temperatures in the Amazon basin have increased at 0.25°C per decade, expected to reach 3.3°C by the end of this century (Malhi et al., 2008). In this context, species responses to climate change could include modifications in morphology, phenology, and geographic distribution range (Weiskopf et al., 2020). Deforestation is one of the most important human pressures in Amazonia, whose effects include habitat reduction and forest fragmentation with increasing edge formations (Haddad et al., 2015; Fahrig, 2017), exposing vegetation to higher temperatures, lower humidity, and consequently a greater risk of desiccation (Laurance et al., 2002). Therefore, habitat fragmentation has significant negative effects on biodiversity and ecosystem functions. In addition, forest fragmentation reduces the capacity of ecosystems, such as carbon and nitrogen retention, productivity, and pollination, reducing species richness as well as impacting nutrient retention and trophic dynamics (Haddad et al. 2015).

One of the main objectives of conservation biology is to assess, understand, and mitigate threats to biodiversity (Robinson, 2006). Climate change can alter the distribution patterns of invasive species and expand their range of dispersal; therefore, understanding their biogeography is essential to conserving biodiversity and maintaining the integrity of ecosystems (Gurevitch and Padilla, 2004; Cordier et al., 2020).

Climate change also opens new pathways for the introduction and expansion of the distribution area of already introduced species because it creates new habitat conditions where there were none for invasive species (Tylianakis et al., 2008; Bradley et al., 2010).

Invasive species are known for causing significant negative impacts on ecosystems and people (Hanley and Roberts 2019), mainly on provisioning, regulatory, and cultural ecosystem services (Pejchar and Mooney 2009). Recognizing the economic benefits of controlling invasive species provides vital information for policymakers and professionals to take control actions (Hanley and Roberts 2019). As the number of invasive species increases, efforts to develop appropriate policies and management responses to control them have also risen (Roberts et al., 2018). In this context, several species have been registered as invasive in Ecuador. Some of them, such as *Lithobates catesbeianus* (bullfrog), *Procambarus clarkii* (river crayfish), *Oncorhynchus mykiss* (rainbow trout), and *Oreochromis niloticus* (Nile tilapia), are widely distributed. Mapping the expansion of the area occupied by invasive species is crucial for biodiversity conservation. Depending on the rate of invasion, invasive species may displace native species, potentially leading to local extinctions (Luque et al., 2014).

Several methods have been developed to estimate species distribution areas using Ecological Niche Modelling (ENM), based on the correlation of known occurrences with environmental variables (Sillero et al., 2021). The use of these methodologies has improved our knowledge of the biogeography of species and responses to their current and future threats, especially for endangered species (Elith et al., 2006; Lobo et al., 2010; Romero et al., 2014). The application of technology for the modeling of ecological niches and the prediction of geographic distributions have been a useful tool to define core areas of species diversity, study the effect of climate change on species distributions, and develop conservation strategies for the protection of species (Pearson, 2006; Hannah et al., 2007).

The research platform "Effects of global warming at different altitude gradients" aimed to explore the (a) forest fragmentation dynamics in the Tena River Basin and (b) model suitability of environmental ecological niches and their relationship with protected areas of four key invasive exotic species under different climate change scenarios in Ecuador.

2. Methods

2.1. Forest fragmentation dynamics in the Tena River Basin

2.1.1. Land cover classification

Supervised classification techniques on the Google Earth Engine platform were used to identify forest land cover in three years: 2017, 2019, and 2021 (Figure 1). 2A processing level Sentinel2 composite and mosaicking images were generated for each year (Medina-Lopez, 2020). We opted to use multitemporal composite images to avoid gaps and inconsistencies due to the high concentration of clouds throughout the year in the study area (Carrasco et al., 2019). As a consequence of the high cloud cover in the study area, the years of analysis were selected according to the three years with the highest cloud-free area coverage. A random Forest algorithm was applied to classify land cover in binary maps of Forest and Non-Forest. This algorithm has proven to be an efficient classifier for land cover classification using Sentinel2 images (Thanh Noi and Kappas, 2017). The Forest/Non-Forest map accuracy assessment used a confusion matrix and evaluated the Kappa index.

2.1.2. Fragmentation Assessment

The fragmentation index (FRAGINDEX) is a metric obtained by combining three landscape metrics: the percentage of intervened areas, the percentage of edges, and interspersion. This metric estimates and analyzes fragmentation phenomena at a regional scale (Butler et al., 2004). In this case, we used QGIS software to calculate a fragmentation index using a regular grid of 1 km x 1 km, a grid size deemed adequate for the landscape interpretation of fragmentation (Long et al., 2010; Moulatlet et al., 2021). The Fragmentation Index metric allows the spatial distribution identification of forest fragmentation at the class level. We applied this metric to three land cover maps obtained for 2017, 2019, and 2021. Changes in forest fragmentation over the period 2017-2021 were identified using geoprocessing tools in QGIS. Furthermore, to identify global changes in fragmentation parameters in our study area, six class-level metrics were calculated using the Landscape Ecology Statistics Phyton plugin for QGIS software (QGIS, 2023): land cover, landscape proportion, number of patches, mean patch area, edge length, and overall core area (Figure 1).

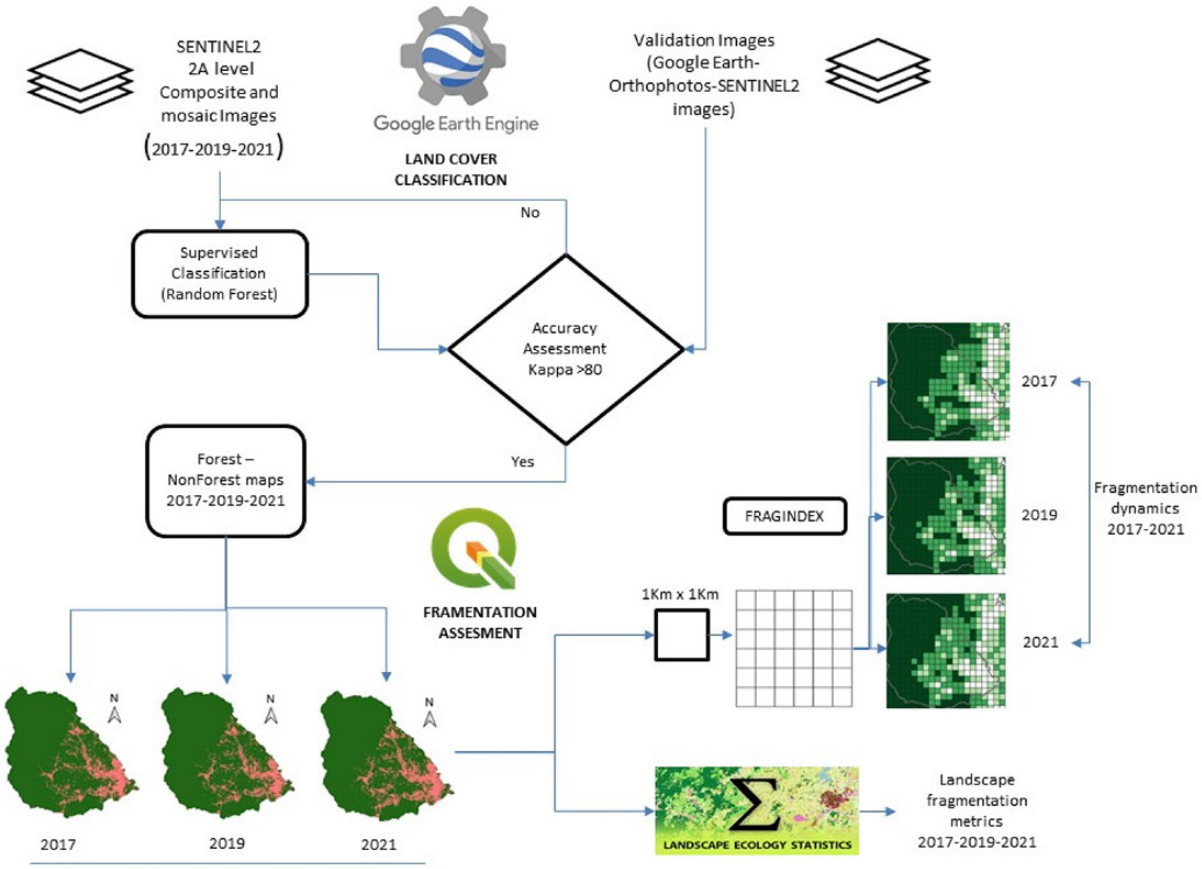

Figure 1. Methodological workflow to analyze the forest fragmentation issue in the Tena Basin.

The integration of supervised classification techniques on the Google Earth Engine platform has proven instrumental in delineating forest land cover using remote sensing. In our study, the generated binary maps of forest and non-forest have served as a foundation for calculating class-level metrics, enabling a detailed analysis of forest fragmentation processes within the Tena River Basin over of five years.

2.2. Environmental suitability modeling for invasive species

2.2.1 Occurrence data

The study conducted in mainland Ecuador assessed the environmental suitability for four invasive species: *Lithobates catesbeianus* (amphibian, American Bullfrog), *Procambarus clarkii* (crustaceous, Red Swamp Crayfish), *Oncorhynchus mykiss* (fish, Rainbow Trout), and (fish, Nile Tilapia) (Figure 2). To gather distribution data for those species, we compiled occurrence records along the complete distributional range of each species from the Global Biodiversity Information Facility (GBIF; https://www.gbif.org), iNaturalist (https://www.iNaturalist.org), VertNet (https://www.vetnet.org), Batrachia (https://www.batrachia.com), Anfibios del Ecuador (Ron et al. 2020), and Museo de Zoología de la Pontificia Universidad Católica del Ecuador (QCAZ; https://bioweb.bio). The final dataset included data compiled up to August 20, 2022.

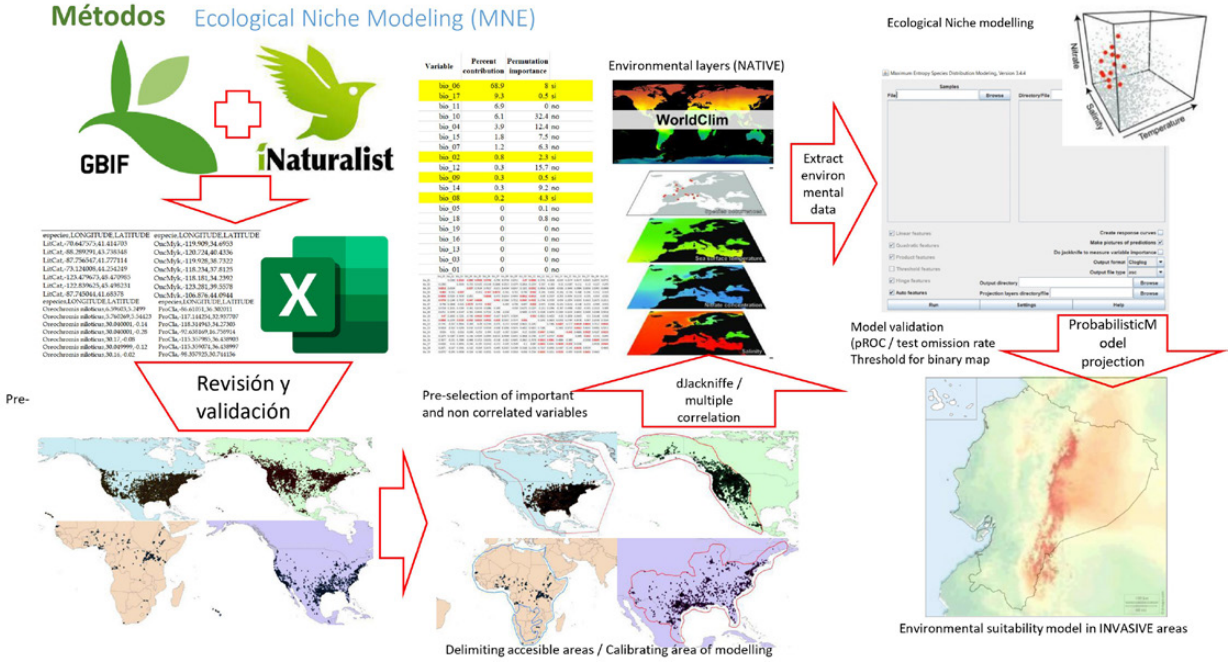

Figure 2. Methodological workflow for modeling the potential geographic distribution in Ecuador of four key invasive exotic species under different climate change scenarios.

Spatialized data per species was revised in QGIS 3.4.14, along with geospatial data such as watersheds, digital elevation models, and base maps, to assess data consistency (Chapman 2005). Problematic occurrence data, either at the georeferenced or taxonomic level, were removed from the dataset. This process aimed to obtain a clean and debugged database that met appropriate standards for ecological niche modeling (Barve et al., 2011; Peterson and Soberón, 2012; Bedia et al., 2013) and biogeographic analyses (Pearman et al., 2008; Broennimann et al., 2012).

2.2.2. Environmental variables

Climate variables for current and future scenarios were downloaded from the WorldClim2 database (Fick y Hijmans 2017) (http://www.worldclim.org). We obtained 15 climatic variables at a 30-second (~1 km^2) spatial resolution. To characterize future climate conditions, we used data for two IPCC representative concentration pathway emissions scenarios from the Hadley Center General Circulation Model (GCM, HadCM2, and HadCM3), which are based on emissions scenarios that approximate the IPCC Business-as-Usual (BAU) scenarios. Therefore, the corresponding projected bioclimatic variables were obtained under the SSP4.5 and SSP8.5 scenarios with a horizon of 2040-2060 from the HadGEM3-GC31-LL model.

2.2.3 Ecological Niche Modeling

Using ecological niche modeling (ENM) and the Maximum Entropy Algorithm (Maxent) (Elith et al., 2011), environmental suitability models were created based on species occurrences, geographic data, and current environmental variables. This approach has been widely applied to study the potential impacts of climate change on biodiversity, conservation prioritization, niche evolution, factors governing species distributions, and the geographic ecology of invasive species, etc. (Pearson & Dawson, 2003; Pearson et al., 2006; Peterson et al., 2011).

The Maximum Entropy Algorithm (MAXENT) was used based on its highest AUC values: maximum number of background points = 1000, number of iterations = 500, convergence threshold = 0.00001, regularization value = 1, but with five replicates using subsample as the run type and without artificial extrapolations, for both conservative and non-conservative data. Each species was allowed to occupy a suitable climate at each time step within a dispersal radius defined by literature or knowledge values. The Jackknife procedure and the correlation statistics (-0.8 to 0.8 in *Pearson r* values) were used to assess the importance of the variables in a first run with all values by default. Regarding the data to be used, 75% were used for model calibration, 25% for model evaluation to generate the receiver operating characteristic curve (ROC) and evaluate the area under it (AUC), and the partial ROC (pROC) to provide a more robust assessment of the predictions of the resulting ENMs. Then, the maps were reclassified using a threshold considering an omission error of 10% permissible.

2.2.4. Environmental expansion and contractions of the niche and the relationship with protected areas.

Current (Presence = 1) and future (Presence = 10) ecological models were reclassified to define suitable and unsuitable (absence = 0) environmental areas and to delimit and quantify the increase (expansion) or reduction (contraction) from one scenario to another in Ecuador. After obtaining the reclassified map of the potential distribution of invasive species under current and future scenarios of climate change, we proceeded to intercept them with the maps of the natural protected areas (MAATE 2023).

3. Results and Discussion

3.1. Case study 1. Forest fragmentation dynamics in the Tena River Basin

Land cover and land use transformations are drivers of habitat loss and forest fragmentation. In this study, landscape fragmentation metrics at the class level show an important reduction in the forest area in the Tena River Basin. Over 2017-2021, 6.7 square kilometers of forests have been transformed into other land uses. Furthermore, an approximate rate of gross forest loss of 134 hectares per year was recorded over five years (Table 1.). The overall forest land cover proportion in 2021 decreased by about 2% compared to 2017. The findings indicated a consistent forest area during the 2017-2019 period and alterations in the forest fraction in the subsequent 2019-2021 period.

Table 1. Landscape fragmentation metrics for the study area in the Tena River basin, Napo, Amazonia of Ecuador.

Class Metric	2017	2019	2021
Land cover	215.6 km^2	215.9 km^2	208.9 km^2
Landscape proportion	53.32 %	53.40 %	51.65 %
Edge length	1 333.22 km	1 368.36 km	1 501.58 km
Number of patches	663	702	843
Mean patch area	32.5 ha	30.7ha	24.8 ha
Overall core area	202.5 km^2	202.6 km^2	194.4 km^2

Several studies have reported that habitat loss and forest fragmentation result in a reduction of forest area, an expansion of the forest edge, and the isolation of fragments (Fischer and Lindenmayer 2007, Haddad et al. 2015, Hadley and Betts 2016, Fahrig 2019). In the Tena River basin, the decrease in forest land cover has led to an increase in the number of forest patches, resulting in an important change in the mean patch area metric, which was reduced from 32.5 hectares in 2017 to 24.8 hectares in 2021. Consequently, there has been an increase in edge lengths and a reduction in the overall core area. In tropical ecosystems, small

fragments and long edge lengths have impacts on the physical environment, entailing adverse effects on the composition of certain species (Haddad et al., 2015). On a landscape scale, the diminishing forest area and disrupted connectivity among fragments heighten population isolation and impede natural movement and gene flow, ultimately resulting in localized extinctions and a diminished likelihood of successful recolonization (Fischer and Lindenmayer 2007). In this context, formulating land-use planning policies that aim to enhance the structural connectivity between non-protected habitat patches and the forested areas within the RBCC could facilitate linkages among habitat patches, reducing habitat isolation (Taylor et al., 2006; Ford et al., 2020). This approach promotes favorable population dynamics and mitigates the risk of species extinction (Beier and Noss, 1998).

The FRAGINDEX metric for 2017, 2019, and 2021 exhibited high values of forest fragmentation in places near the principal human settlements. In addition, the results evidenced active forest fragmentation processes located in the middle basin and the buffer zone of the Chalupas Colonso Biological Reserve, where about 22 indigenous communities live and where the main water sources for the city of Tena are located. In contrast, the Colonso Chalupas Biological Reserve and certain areas characterized by low spatial accessibility exhibited an absence of forest fragmentation (Figure 3). This is an expected result for Ecuador. Recent studies demonstrated the highest rates of deforestation along the first five kilometers, starting from the external border of protected areas (Kleemann et al., 2022). In this study, these areas correspond to the buffer zone of the Colonso Chalupas Biological Reserve.

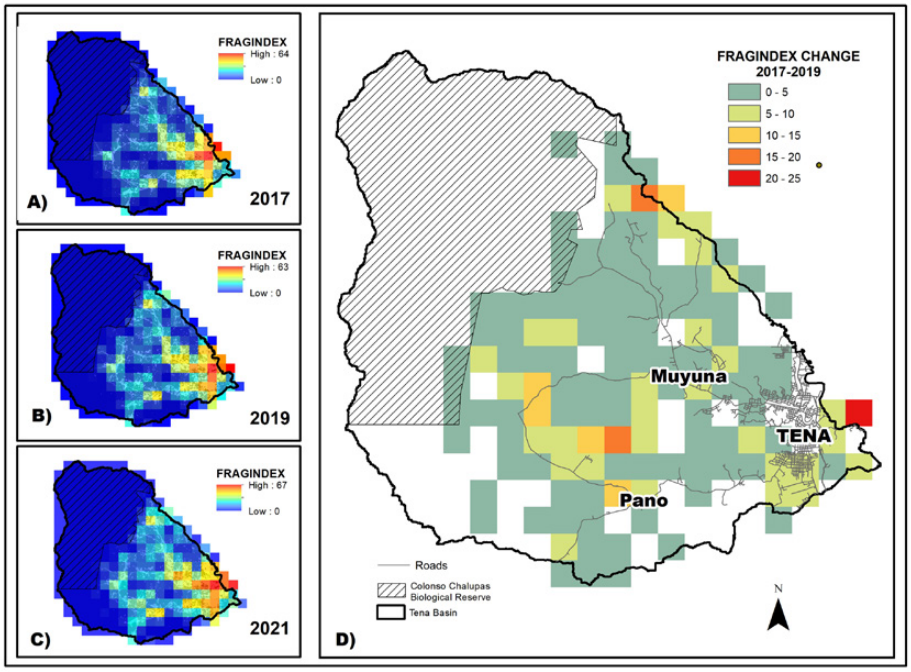

Figure 3. FRAGINDEX spatial distribution and change 2017-2021 in the Tena River basin, Napo, Ecuador.

Previous studies about deforestation in the Ecuadorian Amazon suggest that the causes of deforestation are related to demographic, socioeconomic, and geographic processes (Mena, 2010; Sellers, 2017; Hänggli et al., 2023; Pan W K Y, and Bilsborrow R E, 2005). Population density, geographic accessibility, and topographic conditions are the main factors in land use change in Ecuador (Marquette, 1998; Mena et al., 2006; Sierra, 2013; Hänggli et al., 2023). The study found evidence of notable increases in the FRAGINDEX metric in rural areas where local governments have been promoting road network expansion processes. The results are consistent with other studies that have reported that the opening of roads increases the probability of land use changes related to forest loss (Mena, 2010; Sellers, 2017; Hänggli et al., 2023; Pan W K Y, and Bilsborrow R E, 2005) and the significant role of protected areas in preventing deforestation-active processes (Sierra 2013, Kleemann et al., 2022; Van der Hoek, 2017). Roads sprawling into undisturbed landscapes in the middle Tena River Basin not only have the potential to initiate substantial land use changes, but they can also serve as access corridors for poachers, invasive species, and squatters, thereby posing threats to the pristine nature of these areas (Sage, 2020), increasing human pressures on the Colonso Chalupas Biological Reserve, and jeopardizing the maintenance of the multiple ecosystem services provided by the forest land cover.

Natural ecosystems in the RBCC and its buffer zone provide multiple ecosystem services to rural communities and urban settlements within the Tena River basin (Cuenca et al., 2019). The forest offers a range of essential ecosystem services, including the provision of food, water, medicines, and raw materials for construction. These services play a crucial role in sustaining the livelihoods of rural indigenous families. In addition, the forest located in these areas provides services for the regulation of floods, soil erosion, and the local climate (Cuenca et al., 2019). A recent study highlighting the significance of the extent and spatial distribution of forest fragmentation in regulating floods within the Tena River basin has revealed that the most severe flood scenarios, considering return periods of 1, 10, and 100 years, occur when forest fragmentation concentrates in the upper river basin (Hurtado-Pidal, J. et al., 2022). This underscores the critical importance of prioritizing reforestation and conservation efforts within the upper and middle basins as a key strategy for sustaining the livelihoods of rural indigenous families and mitigating flood risks.

3.2. Case Study 2. Environmental suitability models and the relationship with protected areas for four invasive species in scenarios of climate change:

The results obtained indicate that 11% of Ecuador has the environmental conditions to hold *Lithobates catesbeianus* (American Bullfrog). Under the current scenario, suitable areas for the Bullfrog are spatially concentrated in the Andean zone and within 30 Protected Natural Areas (NPA), including 37% of the Colonso Chalupas Biological Reserve. The potential spatial distribution using RCP 4.5 and RCP 8.5 scenarios shows an important reduction in the ecological niche of *Lithobates catesbeianus* in the future, limiting their geographic distribution to elevations above 4000 meters above sea level. Also, the climate change scenarios for 2050 show a migration of suitable environmental conditions to another eleven protected areas located in the Andes (Figure 4 A-B).

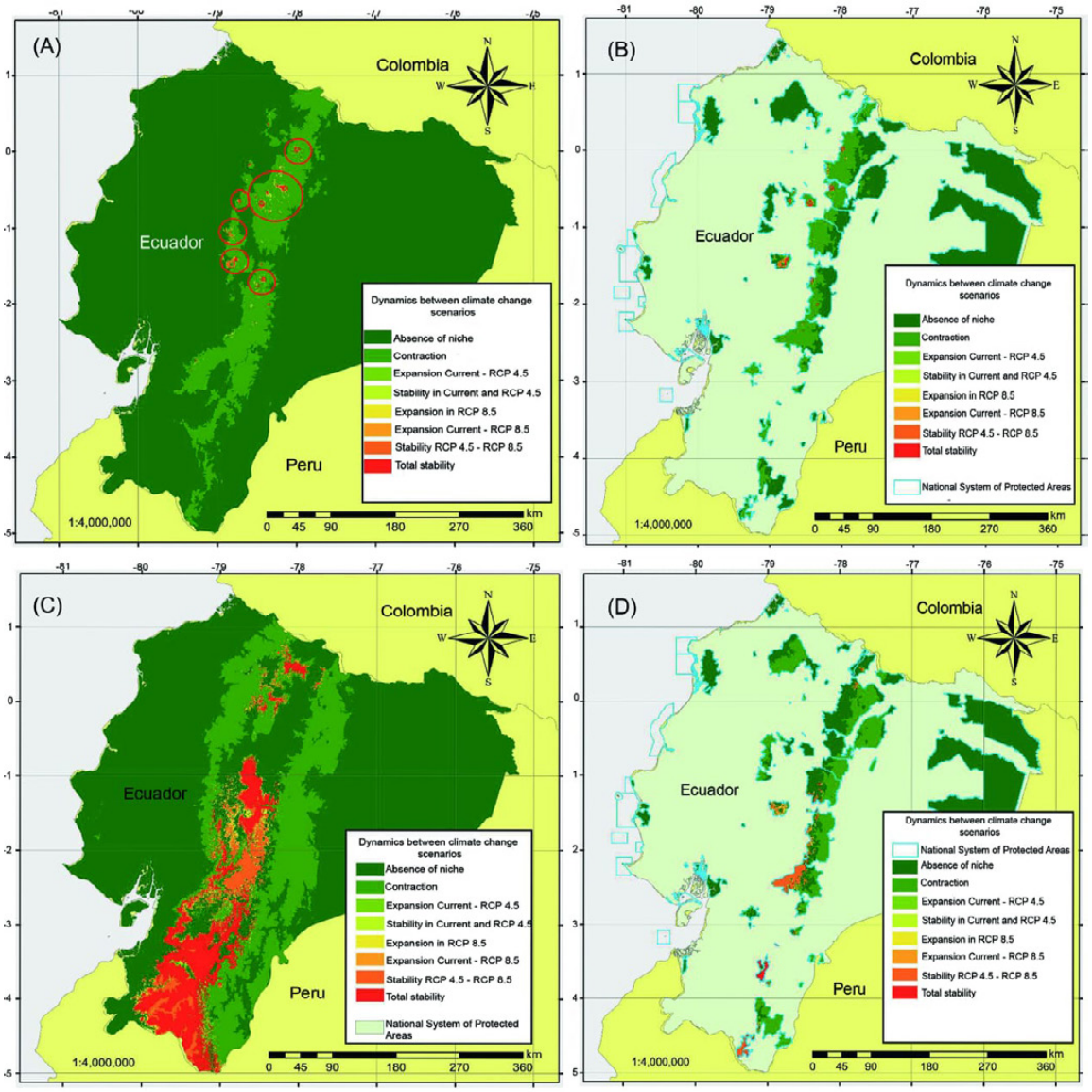

Figure 4. Suitability areas of *Lithobates catesbeianus* (A-B) and *Oncorhynchus mykiss* (C-D), under (A, C) the current scenarios, RCP4.5 and RCP8.5 in continental Ecuador, and (B, D) their relation with the Protected Natural Areas.

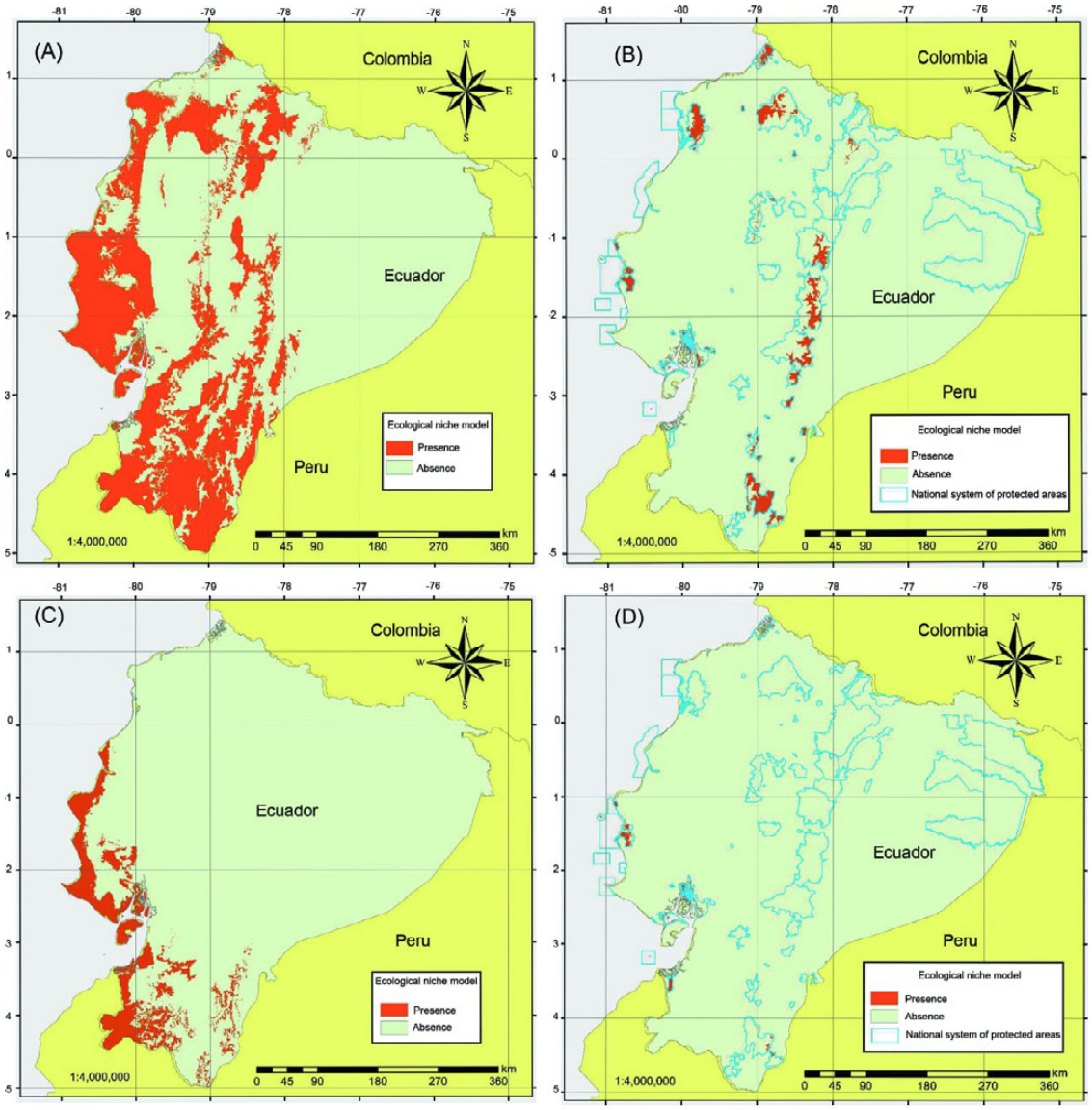

Figure 5. Suitability areas of *Oreochromis niloticus* (A-B) and *Procambarus clarkii* (C-D), under (A, C) the current scenarios, RCP4.5 and RCP8.5 in continental Ecuador, and (B, D) their relation with the Protected Natural Areas.

The niche model form results for *Oncorhynchus mykiss* (Rainbow Trout) exhibited suitable environmental conditions for the species in 30% of Ecuador.}, with these areas mainly concentrated in the southern and central Andes lowlands. Also, 32 Natural Protected Areas currently have suitable conditions for the potential distribution of the Rainbow Trout, including around 74% of the Colonso Chalupas Biological Reserve area. Future scenarios suggest a potential reduction in the species' distribution to areas around 500 meters above sea level. Under future scenarios, RCP4.5, and RCP8.5, the ecological niche model for O. mykiss shows less occurrence in natural protected areas. The models from 2050 show suitable environmental conditions for these species inside 21 NPA and 22 NPA, respectively (Figure 4 C-D).

About *Oreochromis niloticus* (Nile Tilapia), this invasive species has the potential for a geographic distribution in approximately 29% of Ecuador. The current ecological niche of this fish is along the Andes and coastal areas, around an altitudinal range ranging from 0 to 3134 meters above sea level. Forty-seven Natural Protected Areas have suitable conditions for the species, with El Pambilar, El Zarza, El Quimi, Parque Lago, and Pululahua being the protected areas with the largest surface area suitable for the Nile Tilapia. Under future scenarios, RCP4.5 and RCP8.5 potential areas of distribution were not identified, which suggests there could not be adequate environmental conditions in the future for the presence of the species (Figure 5 A-B).

The current potential geographic distribution of *Procambarus clarkii* (American Prawn) covers 6% of the Ecuadorian area. This species is found mainly in the southern coastal areas, in an altitudinal range from 0 to 2623 meters above sea level. Under future scenarios RCP4.5 and RCP8.5, the results identified the absence of environmental conditions in the presence of the American Prawn. Regarding the spatial distribution of the ecological niche in protected areas, we found suitable environmental conditions in 15 Natural Protected Areas, mainly located in coastal areas (Figure 5 C-D).

4. Conclusions

In the last five years, the tropical forest fragmentation in the Tena River Basin has increased. Although forest fragmentation is relatively low compared to other river basins in Ecuador, it could increase in the coming years due to the expansion of human borders towards the eastern edges of the Colonso Chalupas reserve. Our results exhibit the potential of remote sensing techniques and geographic information systems to understand the spatial dynamics of fragmentation as a substantive element to delineate public policies to mitigate the effects of climate change. However, more studies are needed to understand the principal forest fragmentation drivers and land use change effects on local climate.

We found significant changes in the ecological niches of invasive species under future climate change scenarios in mainland Ecuador. The study also examined the overlap between the distribution areas of invasive species and existing Protected Natural Areas on mainland Ecuador. We found that several Natural Protected Areas could be affected by changes in the environmental suitability of invasive species. Consequently, specific areas requiring focused attention for invasive species management and control were identified to safeguard ecosystem integrity and biodiversity conservation. These results underscore the importance of considering potential changes in the distribution of invasive species when developing conservation and management strategies for protected natural areas. The findings provide valuable insights for the formulation of effective policies and actions aimed at mitigating the negative impacts of invasive species and preserving biodiversity on mainland Ecuador.

5. Acknowledgments

We express our sincere gratitude for the generous support provided by the Erasmus+ grant: 619346-EPP-1-2020-1-DE-EPPKA2-CBHE-JP. This funding has been instrumental in the successful development of the research project, enabling us to explore and present our findings in this chapter.

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CHAP

TER 3

Nature-based living lab (NB LAB): an undergraduate research experience at the Upper Amazonia

Seismic surveys and inverse problems regularization in the Ecuadorian Amazon

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SA (Sebastián Araujo) wrote the manuscript. Oswaldo Guzmán (OG) worked on the geomorphological interpretation of the tomography images. Roy Rubio (RR) drew the seismic line maps. Pedro Guerrero (PG), Erika Rojas (ER), Walter Moposita (WM), Isabel García (IG), María Fernanda Puetate (MFP), SA, and RR did the seismic survey, and they processed the signal to obtain the tomographic results. Anderson Guamán (AG) coordinated the equipment used. All authors reviewed, commented on, and approved the final version of the manuscript; all authors declare no conflict of interest.

Abstract

We used the two-dimensional travel-time seismic tomography method to investigate three different geological settings in the Northern Amazon of Ecuador. This region includes the transition between the Eastern Andes Cordillera and the Oriente Basin. This transition is not continuous and is interrupted by the Eastern Sub-Andean Belt. We carried out seismic studies in the foothills of the Eastern Cordillera, in a fault zone in the Colonso-Chalupas Reserve, and on the banks of the Tena River. In the Oriente Basin, we made the third line on the banks of the Napo River. The inverse tomography problem was regularized using the L-curve by three types of norms: the Euclidean norm, the taxi norm, and the infinite norm. The use of these three different norms ensures better error control and, therefore, a more precise determination of the L-curve corner, which is the point that gives the optimal result. The L-curve regularization is also a method to ensure the correct resolution of the inverse problem. We interpret the regularized model in each case to define the different structural units and the geomorphological variations. In the case of the fault in the Colonso-Chalupas Reserve, we can infer the splay of the Talag fault at a depth of 10 meters. On the banks of the Tena River, there are no appreciable changes in the tabular stratification up to 10 meters deep. On the banks of the Napo River, we can see traces of river erosion in the old channel at a depth of 10 meters.

Keywords

seismic tomography, inverse problem regularization, Colonso-Chalupas Reserve, Tena River, Napo River, Talag Fault.

Resumen

Hemos usado la tomografía por tiempos de viaje en dos dimensiones para investigar tres sitios geológicos diferentes en la Amazonía Norte de Ecuador. Esta región incluye la transición entre la Cordillera Oriental hacia la Cuenca Oriente. Esta transición no es continua y está interrumpida por el Cinturón Subandino Oriental. Hemos realizado estudios sísmicos en el piedemonte de la Cordillera Oriental en una zona de fallas de la Reserva Colonso-Chalupas y en la orilla del río Tena. En la Cuenca Oriente, hicimos una tercera línea sísmica en la orilla del río Napo. El problema inverso de tomografía fue regularizado usando la curva-L con tres tipos de normas: euclídea, taxi e infinita. El uso de estos tres tipos de diferentes normas asegura un mejor control del error; por lo tanto, una determinación más precisa del vértice de la curva-L que es el punto que da el resultado óptimo. La regularización mediante la curva-L es también un método que asegura la resolución correcta del problema inverso. Hemos interpretado el modelo regularizado en cada caso para definir las unidades estructurales y las variaciones geomorfológicas. En el caso de la falla en la Reserva Colonso-Chalupas, podemos inferir las fallas subsidiarias de la falla Tálag a una profundidad de 10 metros. En la orilla del río Tena, no hay cambios apreciables en la estratificación tabular hasta los 10 metros de profundidad. En la orilla del río Napo podemos observar las huellas de la erosión del río en el antiguo canal a una profundidad de 10 metros.

Palabras clave

tomografía sísmica, regularización del problema inverso, reserva Colonso-Chalupas, río Tena, río Napo, falla Tálag.

Introduction

Geoscience research in the Ecuadorian Amazon region has been guided by the search for natural resources, particularly oil. Much of the knowledge we currently possess about the geology of the Ecuadorian Amazon provinces comes from that initial exploration (Tschopp, 1953). Numerous additional campaigns throughout oil exploration have completed our regional picture of the Oriente Basin in the disciplines of tectonics, petrography, sedimentology, and geophysics (Baby et al., 2004).

Within this context of exploration and exploitation of hydrocarbons, there have been uninvestigated areas within the geodiversity of the Amazonian landscape. One of these zones is the foothills of the Colonso-Chalupas Reserve, whose territory extends between the highlands of the Western Cordillera and the intrusion of the Abitagua granite. This reserve area has been preserved without traces of human occupation and with almost non-existent research due to the isolation generated by the granite wall between the Amazonian plains and the inter-Andean provinces.

The Colonso-Chalupas Reserve was one of the reasons that prompted the creation of the Ikiam University, which is located precisely on the edge of the Colonso-Chalupas Reserve in the transition zone between the granite and alluvial valleys near the city of Tena in the province of Napo (Wise & Carrazco Montalvo, 2018).

The Geosciences career at Ikiam University raises several priority research topics that must be addressed in addition to engineering teaching activities. These issues begin with the study of geodiversity due to the granite intrusion mentioned above and the Napo Uplift generated by the compression of the Nazca plate with the South American Plate. This uplift is part of the Eastern Sub-Andean Belt that runs through Ecuador from north to south and forms a third mountain range with the sections of Napo-Galeras in the north and Cutucú in the south (Alvarado et al., 2016). In the northern zone, active volcanism also develops in the Eastern Subandean Belt, with active points in the Reventador and Sumaco volcanoes. The Napo uplift also, combined with the tropical conditions in the Amazonian rainforest, causes a karst landscape that, together with the Sumaco volcano, gives rise to the Napo Sumaco Geopark initiative (Simbaña et al., 2018; Vera et al., 2023).

Another research topic for Geosciences is natural hazards due mainly to earthquakes, mass movements, and flooding. The Colonso Chalupas Reserve is crossed by a segment of the Puná-Pallatanga-Cosanga-Chingual mega fault (Alvarado et al., 2016). The activity of the Cosanga segment in the reserve area has not presented significant historical earthquakes, so there remains the possibility that small earthquakes dissipate the seismic energy and do not accumulate stresses as if they occurred in the northern segment of Cosanga, where they originated two earthquakes of magnitude 6.1 and 6.9 on March 6, 1987 (Kawatsu & Cadena, 1991). On the other hand, the characteristics of the relief combined with the intense tropical weathering and the rainfall regimes cause frequent mass movements that block first-order communication and trade routes, as well as flooding of populated sectors located in the active flood plains of the fluvial systems of the piedmont.

The third topic on which Ikiam's academic activity is developed is non-renewable natural resources. In this area, mainly metallic deposits are presumed to exist due to the intrusion of the Abitagua granite (Chiaradia et al., 2004). The current exploitation in

Figure 1. The NBLAb Geosciences team of the four universities developed the seismic surveys.

our study area is alluvial gold in auriferous places that have already been accurately identified in the Napo, Anzu, and Jatunyacu rivers (Barragán et al., 1991). Since the origin of the university, work on issues related to mining has been highly critical from a simplistic perspective where geosciences are seen as a tool in the exploitation of finite resources (Wilson & Bayon, 2017). The exploitation of natural resources must instead be based on the complexity of the transformations of nature and the raw materials that come from them (Linera, 2013). Thus, extractivism is taught in Ikiam as a technical system rather than a production mode (Linera, 2013).

To incorporate most of these research and teaching topics within the NBLab program, a transversal discipline such as seismic exploration was chosen, and in this discipline, a particular technique is seismic tomography. The Geosciences career at Ikiam has already developed several investigations in the Amazon region using seismic tomography to obtain images of the Amazonian subsoil (Araujo et al., 2021; Paredes & Araujo, 2021; Espín & Araujo, 2022; Araujo et al., 2023). The perspective of seismic survey integration within the study of the Colonso Chalupas Reserve and the Amazon was based on the original idea of monitoring the reserve through sensors and electronic devices for automated data collection.

The objective of this research is to use seismic tomography to describe the morphological and structural features of three different sites within the geodiversity of the Northern Amazon in Ecuador: a fault zone within the Colonso-Chalupas Reserve, the bank of the Tena River on the Ikiam University campus, and the bank of the Napo River in the Oriente Basin.

There are no previous studies that allow seismic images to compare the structures of these three areas. Therefore, the relevance of our research is based on describing geodiversity to later relate it to the biological diversity and the diversity of the native peoples who have populated the region.

The members of the Geosciences team consist of four students from Ikiam University. We also have the participation of two professors and a laboratory technician. External members come from Ernst-Abbe-Hochschule Jena University, Universidad de la Amazonía Peruana, and Universidad Tecnológica Equinoccial (Figure 1).

Site description

From a geodynamic context, the region where we conduct the seismic survey is on the edge between the South American Plate and the Norandean Sliver (Alvarado et al., 2016). The Norandean Sliver is an accretion zone with a northwestward displacement of 9 mm per year concerning the South American Plate (Nocquet et al., 2014). This displacement is caused by the oblique subduction of the Nazca Plate under the South American Plate.

The escape to the northeast of the Norandean Sliver causes a series of faults grouped into four main systems: Puná, Pallatanga, Cosanga, and Chingual. Our study site is precisely in the Cosanga segment, expressed by a series of transpressive faults running through the granitic mountains in the Colonso Chalupas Reserve (Fig. 2).

The convergence of the South American and Nazca Plates also makes a third mountain range appear parallel to the Western and Eastern branches of the Andes: The Eastern Subandean Belt (Fig. 2). This creates a Subandean Zone before the relief completely disappears in the Oriente Sedimentary Basin.

The main hydrographic basins of the study site are those of the Tena River, which rises in the Colonso mountains, and the Napo River, which collects tributaries of the Llanganates Reserve and rivers of the Eastern Andes (Fig. 2).

Amid of this geodynamic diversity, we chose three sites to carry out the seismic lines of our study. The first is on the access road to the Colonso Chalupas Reserve, where we are interested in observing the Cosanga fault zone. The second point is the bank of the Tena River, where we investigated the strata's geometry below the Ikiam infrastructure. A third point is on the banks of the Napo River, where we seek to understand fluvial dynamics and potential flood zones in the Oriente Basin.

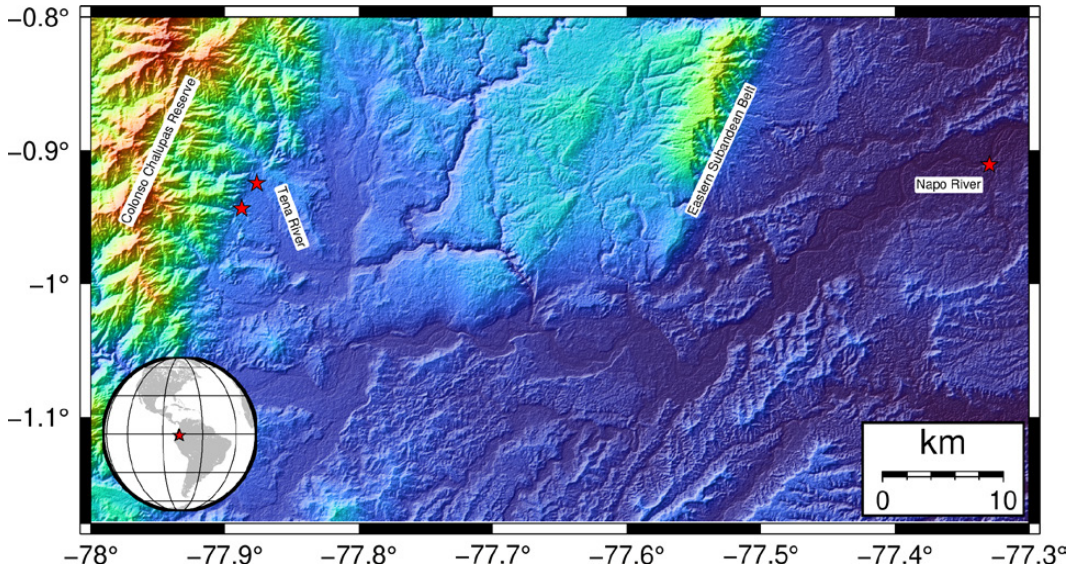

Figure 2. The map shows the locations of the three seismic lines (red stars).

The westernmost line is on the road to the Colonso-Chalupas Reserve. The line to the banks of the Tena River is at the Ikiam campus. The survey on the Napo River is in the plain to the east of the Eastern Subandean Belt.

Surveys methods

We used a Geometrics ES-3000 seismometer with vertical component geophones and a natural frequency of 4.5 Hz to collect the survey data. The elastic waves in the ground were generated by the percussion of a 6 kg hammer on an aluminum plate. 2 cm thick. These waves travel through the medium, and their times are recorded on geophones installed on the ground surface, following a straight line. Depending on the terrain, this line can be flat or have a slope that follows the topography of the study area.

Seismic waves are refracted in the various geological formations of the subsoil since each formation has a different propagation velocity.

Seismic tomography tries to find the seismic wave velocity and the arrival times of the waves. Tomography is an inverse problem whose solution begins by proposing an a priori model. This model can be obtained from previous seismic or geological studies or, as in our case, through a heuristic that tests various values of speed and depth. Seismic rays propagate on the a priori model, and then the theoretical propagation time of these rays is calculated. This process consists of solving the direct problem:

$$d = G \cdot m \quad (1)$$

Where **d** is the vector that contains the data obtained by applying the linear relationship **G** to the parameters **m** of the initial model, in the case of seismic tomography, the matrix **G** is obtained by measuring the length of the seismic ray in each of the cells into which the workspace has been subdivided.

It is now a question of minimizing the residual between the theoretical and experimental times, done using an adjustment by least squares. From this minimization, a posteriori velocity model is obtained by solving the inverse problem:

$$m = (G^t \cdot G)^{-1} \cdot G^t \cdot d \quad (2)$$

This new model is used as a priori for iterations that increasingly minimize the residue. This minimization is the fit of the data to the theoretical rays and is calculated with the **RMS** (root mean square), which is the Euclidean distance between the fit of the model **G*m** and the data **d**:

$$RMS = |G * m - d|_2 \quad (3)$$

Equation (2) allows solving the inverse problem of tomography, and equation (3) measures the fit quality obtained. However, the work still needs to be finished since inverse problems in physics are ill-posed problems where their solution is unstable and highly dependent on the starting data (Tikhonov et Arsenin, 1976).

To obtain a physical interpretation of the solutions to an inverse problem, these unstable solutions must be regularized using the Tikhonov method (Zhdanov, 2015). We must find the smoothing parameter alpha that minimizes the functional:

$$P^{\alpha}(m,d) = |G \cdot m - d|_2^2 + \alpha \left|m - m_{apri}\right|^2 \quad (4)$$

In equation (4), the first parametersummand is the RMS setting (3). The second summand is the norm between the resulting model **m** and the *a priori* model.

To find alpha, we use the L-curve graphic method (Hansen, 1992; Zhdanov, 2015). This method has been successfully applied to two-dimensional seismic tomography cases (Araujo et al., 2023) and involves using three types of standards for the distance between the models. We compute the Euclidean norm, the taxi-cab norm, and the infinity norm to account for the various types of errors that may be implicit in the data (Tarantola, 2005). These three types of norms have been initially used in analyzing L-curves in regional tomography (Potin, 2016) and successfully applied in near-surface seismic tomography cases (Araujo et al., 2023).

The Euclidean norm is calculated as the average of the squared distances between the resulting model and the prior model:

$$l_2 = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(m_i - m_i^{apri}\right)^2} \qquad (5)$$

The taxicab norm is the average of the distances computed using the absolute value between the resulting model and the a priori model:

$$l_1 = \frac{1}{n}\sum_{i=1}^{n}\left|m_i - m_i^{apri}\right| \qquad (6)$$

Finally, the infinity norm is the maximum value of all the absolute distances between the resulting model and the a priori model:

$$l_\infty = \left\{\left|m_i - m_i^{apri}\right|\right\} \qquad (7)$$

The Seisimager software has two regularization parameters for the tomography: vertical smoothing and horizontal smoothing. As we show in Araujo et al., (2023), the best strategy is to keep the vertical smoothing fixed and vary the horizontal one to have better detail in the lateral variations of the velocity model. We then plot the RMS versus the three norms to obtain the L-curve. The corner of the L-curve gives the value of the regularization parameters, thus obtaining the resulting tomographic images.

A qualitative interpretation of the L-curve graph is to see that the vertical axis gives the RMS values to fit the data to the theoretical model. The more we descend vertically on the graph, the lower the RMS and, therefore, the better the data fit. At one point, however, we did not descend further, and the graph began to go to the right of the horizontal axis, where we have the norm values. The zero value of the norms would be produced if the model was identical to the a priori model of flat layers. However, this is not the case, and the posterior model has a suitable distance from the prior model with a non-zero norm value. There is no point in increasing the values of the norm since we are making a model with more significant irregularities, many of which are artifacts and do not exist in the natural geological environment. The L-curve corner, therefore, expresses the optimal compromise between the smallest error of data fit and the model that contains the irregularities well resolved from the initial model of flat layers.

The L-curve also becomes a method to test the quality of our tomographies because if there are too many errors in the experiments and data processing, we will not obtain the L-curve graph.

Results and Discussion

The seismic line in the Colonso-Chalupas Reserve was carried out on the access road to the reserve at -0.945498 S and -77.890955 W (Fig. 3). At this point, there is a drinking water distribution station, and the line is placed on a slope where the existence of the Tálag fault is presumed (Costa et al., 2020). We installed 12 geophones 5 m apart for an entire seismic line of 55 m. We defined the survey length to cover the road slope around the presumed fault. We made shots with the hammer every 5 m, beginning 2.5 m before the first geophone and continuing between each pair of geophones until ending 2.5 m after the last geophone.

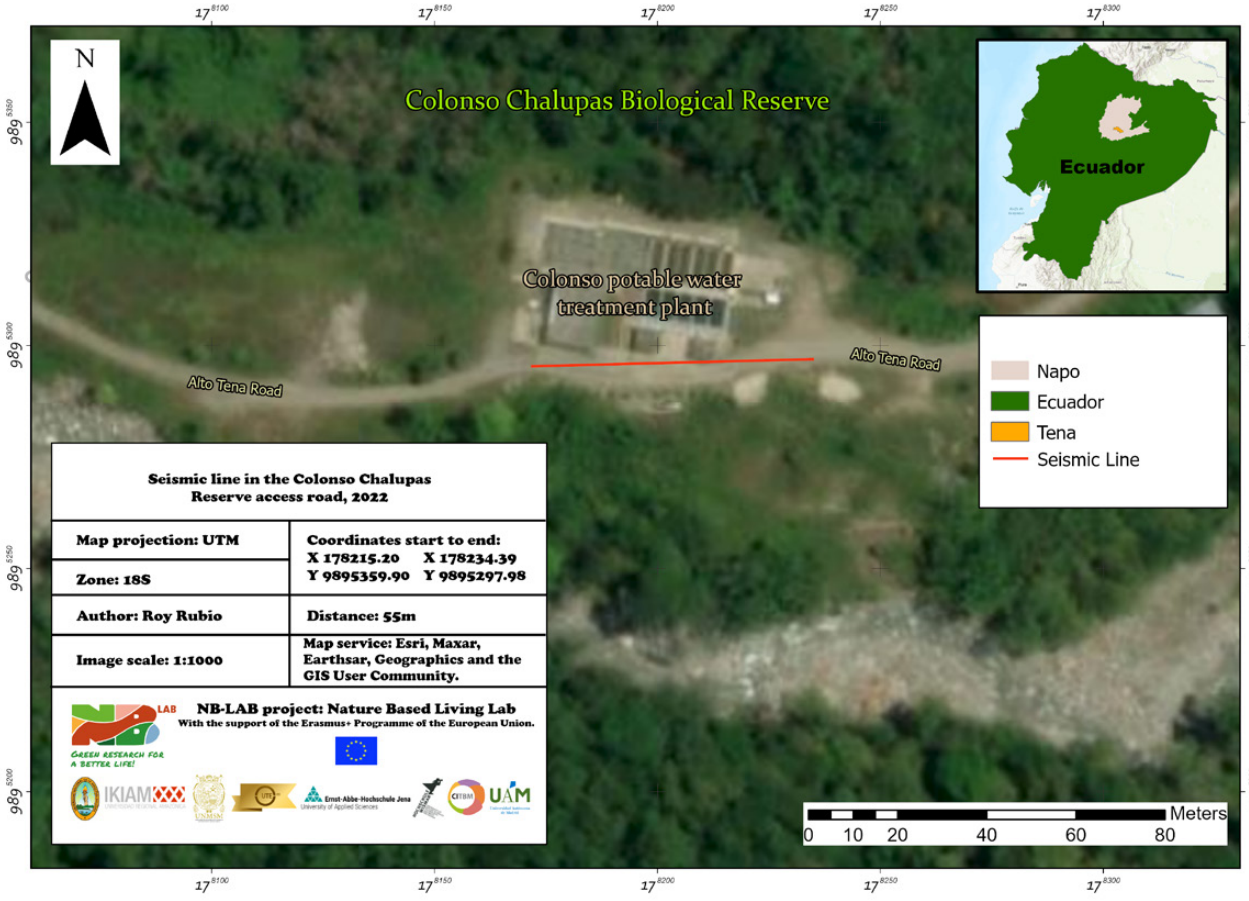

Figure 3. The seismic survey (red line) in the Colonso-Chalupas Reserve.

The line length is 55 m on an 18 % slope.

For the seismic line in the alluvial plain of the Tena River, the right bank of the river was chosen, where the Ikiam University campus is located (Fig. 4). At this point, -0.947732 S and -77.862999 W, there is an area of land that has been cleared of vegetation for existing buildings. Here, we installed 8 geophones 5 m apart on a line of 35 m total length. The length of the line was limited to the area without vegetation. We made the hammer shots in a similar way to the previous line.

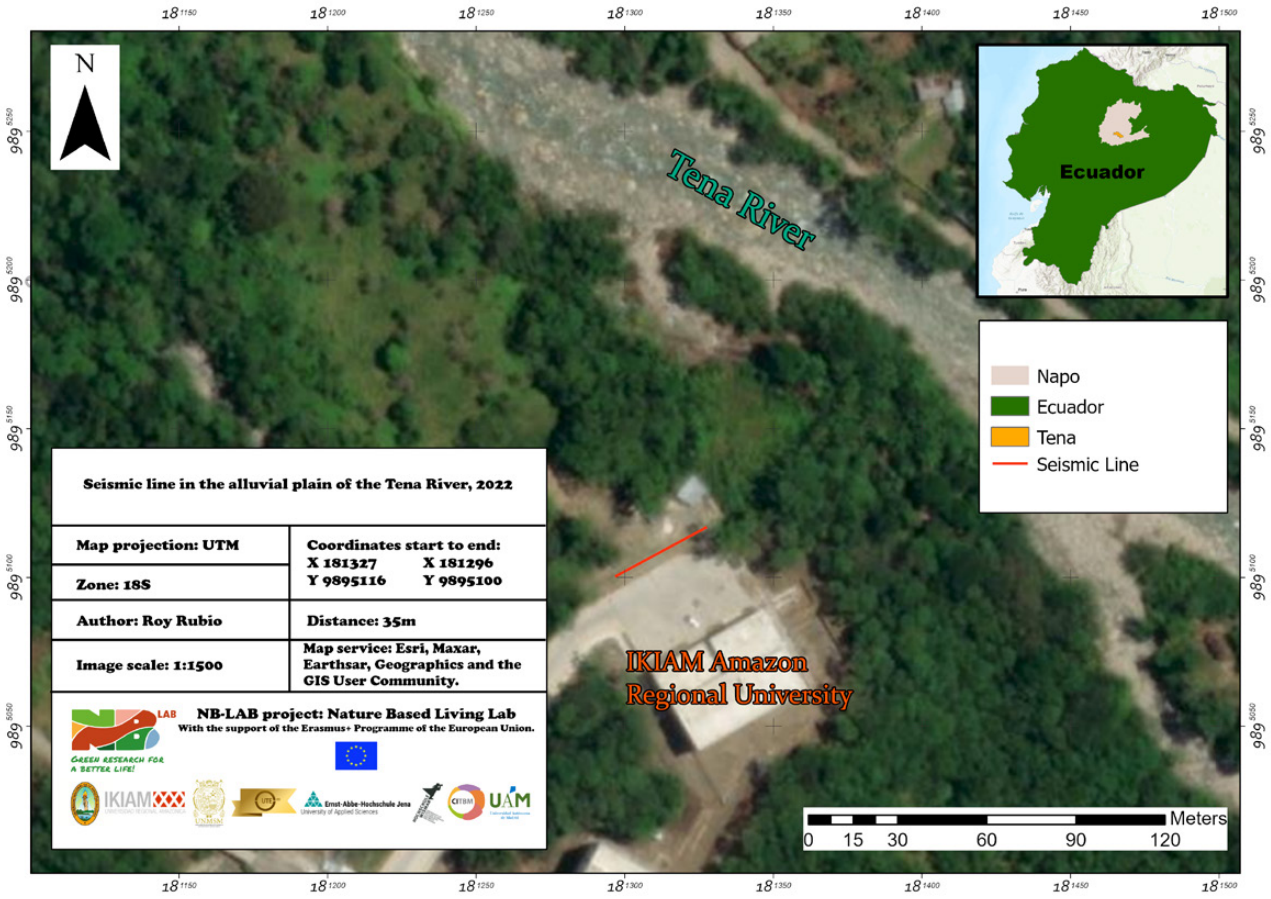

Figure 4. The seismic survey in the Tena River bank (red line).

The line is 35 meters long on the Ikiam University campus.

The seismic line in the Napo River alluvial plain was installed on the right bank at -0.908188 S and -77.333646 W (Fig. 5). At this point, we take advantage of an area cleared of vegetation for agricultural work. In the same way as the line in the Tena River, the surface free of vegetation defined the maximum length of the experiment. In this case, we installed 12 geophones with a separation of 5 m for a total length of 60 m. We proceeded with the shots in the same way as the two previous lines.

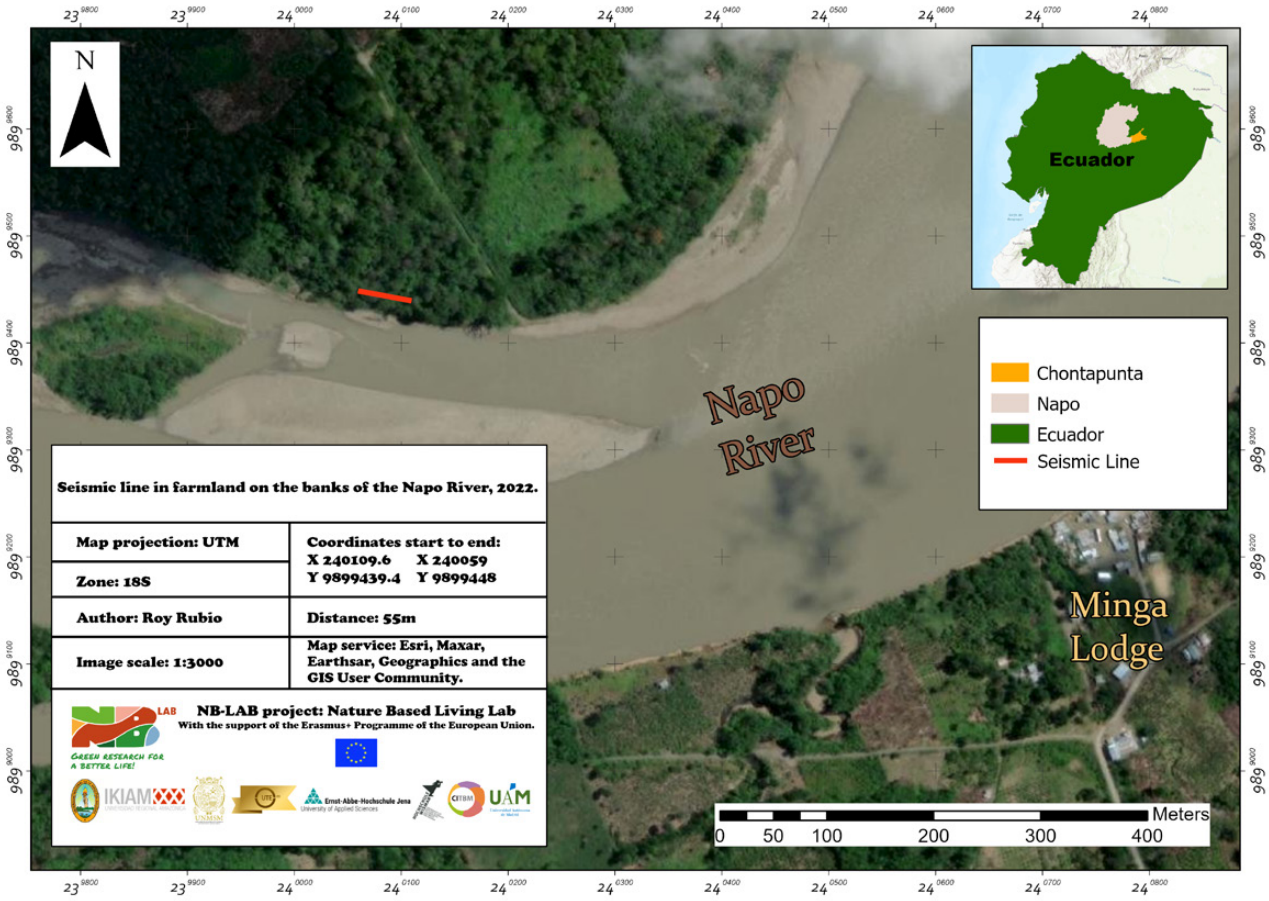

Figure 5. The seismic survey in the Napo River (red line).

The line is 55 meters long on the left bank of the river.

The seismic signal processing consists, first of all, of correctly placing the position of the various shots made in the survey. Then, we proceeded to delete the geophones that were not used in the survey. We can then start picking the first arrivals using the PickWin software. For some signals, it is necessary to use a low-pass filter to eliminate the influence of the sound wave that can mask the P-wave.

With the first arrivals picked, we obtained the travel-time curves, which we can correct, erasing the erroneous data that may come from the impossibility of accurately picking the arrival times.

Travel-time curves allow building the a priori model by testing various values of maximum speed, minimum speed, and depth and then tracing the seismic rays to achieve their best coverage in depth.

The regularization process starts with the a priori model and tests the different vertical smoothing values, keeping the horizontal smoothing constant. In each test, the correct convergence of the PlotRefra algorithm must be monitored by plotting the RMS versus the number of iterations (Fig. 6). There must be a correct convergence of the algorithm before proceeding to draw the L-curves.

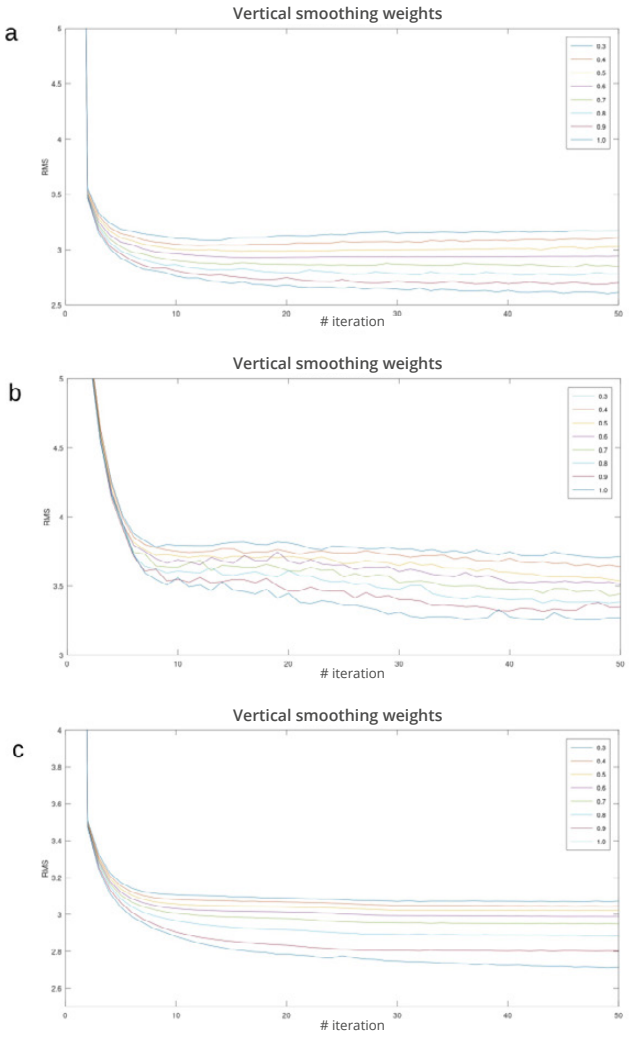

Figure 6. We show the tomography algorithm convergence for the three seismic lines: a) Colonso-Chalupas road, b) Tena River bank, and c) Napo River bank. In the three cases, we use 50 iterations and plot the RMS on the vertical axis.

The colors give the values of the vertical smoothing parameter setting constant for the horizontal parameter: a) 1.0, b) 0.8, and c) 1.0.

As was pointed out in the previous section, to better define the lateral discontinuities of the geological medium, it is necessary to set the vertical smoothing and vary the horizontal one to obtain the L-curves. In the case of our seismic survey, the main objective is to observe the vertical differentiation of the strata rather than detailing the diversity of faults or folding. For this reason, we use the opposite strategy: fix the horizontal smoothing and vary the vertical to draw the L-curves.

Figure 7 shows the L-curves for the optimal horizontal parameter in each case: 1.0 for the Colonso-Chalupas Road, 0.8 for the Tena River Bank, and 1.0 for the Napo River Bank. To plot the L-curves, we use the RMS value on the vertical axis, and we calculate the three norms defined in equations 5, 6, and 7 to obtain the values of the horizontal axes. We show the different values of the vertical smoothing parameter with a color scale.

The analysis of the L-curves allows obtaining the value of the vertical parameter by observing the L-curve corner that is indicated by a red arrow (Fig. 7). For the line on Colonso-Chalupas Road, the vertical parameter is 0.9 in the three norms of Figure 7a. The L-curves for the line in the Tena River bank have values of vertical smoothing (1.0 and 0.9) that are left out of the analysis. However, we can observe in the three norms an L-curve corner at 0.7 (Fig. 7b). For the line on the Napo River bank in two of the norms (the Euclidean and the taxicab), the L-curve corner is at 0.9 vertical (Fig. 7c). In contrast, for the infinity norm, the result is not conclusive.

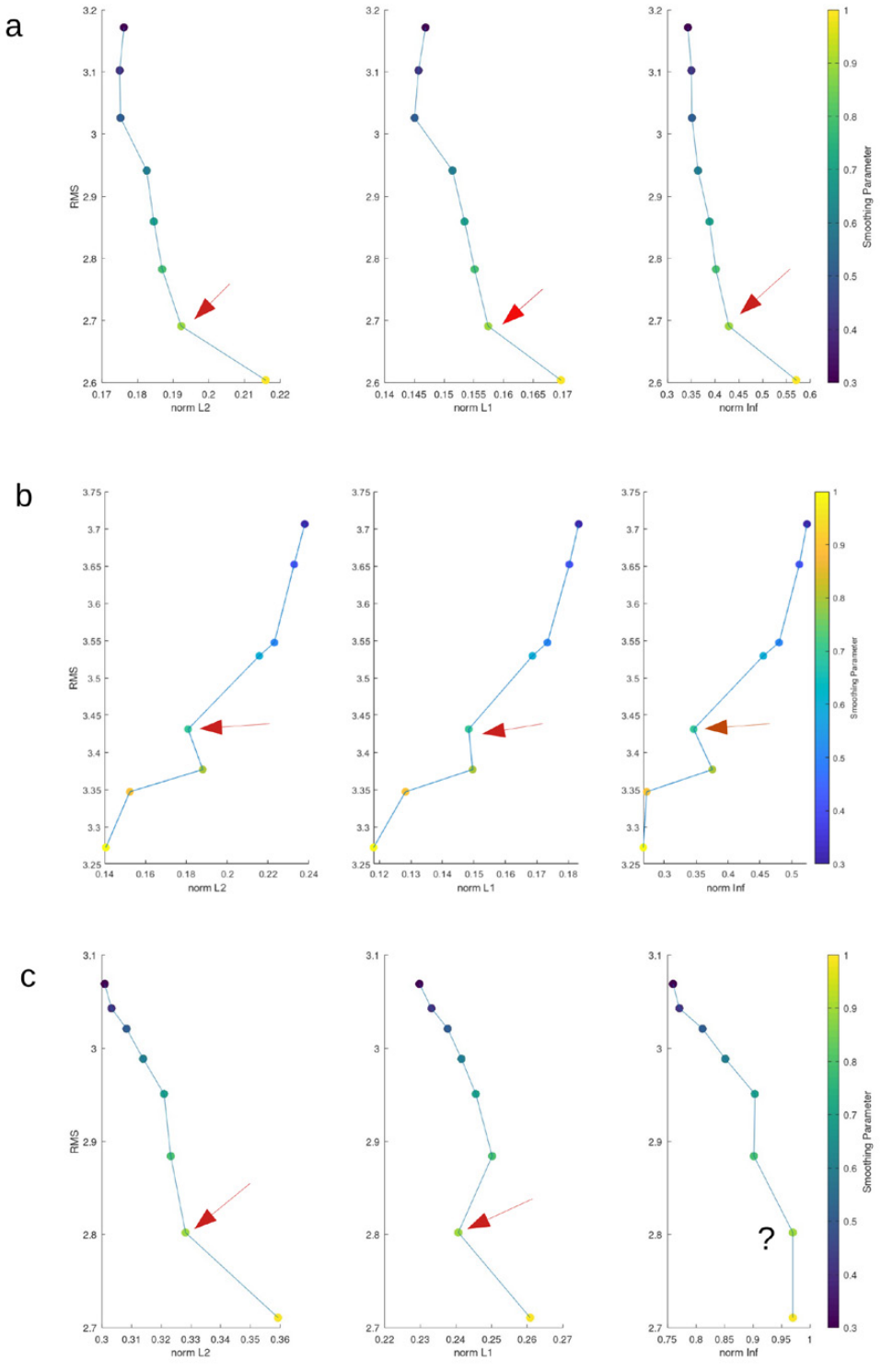

Figure 7. The L-curves for the three seismic lines: a) the Colonso-Chalupas Road, b) the Tena River bank, and c) the Napo River Bank.

We plot on the vertical axis the RMS and, on the horizontal axis, the values of the Euclidean norm, the taxicab norm, and the infinity norm. The red arrow signals the L-curve corner, and gives the values for the regularization parameters.

We obtain the optimal solutions in the three seismic lines with correctly defined regularization parameters (Figures 8, 9, and 10). The regularization parameters for the Colonso-Chalupas and Napo River seismic lines are 1.0 horizontal and 0.9 vertical for both cases. For the line in the Tena River, the regularization is 0.8 horizontal and 0.7 vertical.

In the line on the Colonso-Chalupas road (Fig. 8), three seismic stratigraphic units can be identified. The first superficial up to 2 m depth with speeds of the P wave between 0.3 km/s and 0.5 km/s (Unit 3), the second between 2 and 8 m depth with speeds from 0.5 km/s to 0.9 km/s (Unit 2), and finally a unit with velocities around 1 km/s from 8 m depth to 15 m (Unit 1) exceeded the resolution limit of the experiment. Units 2 and 3 are wedge-shaped, being thicker to the west than the eastern zone, corresponding to the proximity to the sediment source of the Colonso-Chalupas Reserve. Both units are interpreted as wedges of alluvial fans (Fig. 8b).

All units are affected by structural deformations, and this is evidenced in the discontinuity that occurs at 10 m on the horizontal scale and propagates to the upper one, affecting at least the base of Unit 3. This deformation can be caused by a high-angle reverse fault at the base and a low-angle towards the top. Moreover, it could be interpreted as a frontal splay of the Talag fault (Fig. 8b). This possible sign of failure cannot be an artifact caused by the methodology since the a priori model is of uniform flat layers and the entire area had regular coverage from the geophones.

The Talag fault has been described as a reverse Quaternary fault that runs at the foot of the Abitagua granite in the area of the Colonso-Chalupas reserve adjacent to the Ikiam University (Costa et al., 2020). It is approximately 15 km long, but its fault trace is blind (Costa et al., 2020). Therefore, our images constitute the first evidence of their activity in the area.

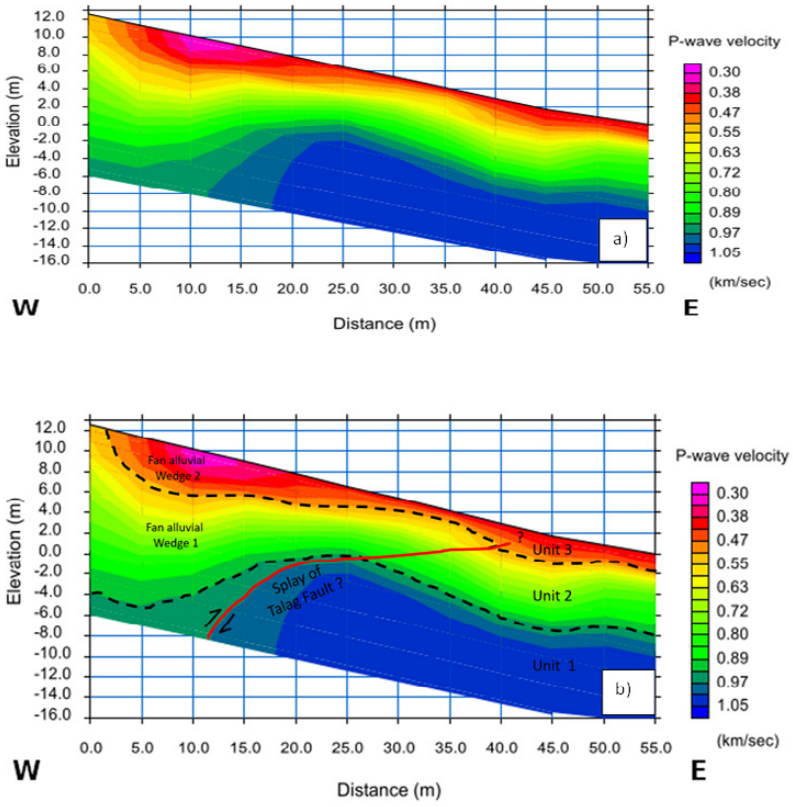

Figure 8. The tomography results for the seismic line on the Colonso-Chalupas road: a) Without interpretation. b) With seismic stratigraphic and structural interpretation.

The color scale gives the value of the P-wave velocity. The line goes from west to east

For the seismic survey on the banks of the Tena River (Fig. 9), two seismic stratigraphic units can be identified (Units 2 and 3). The deep velocity anomaly with a velocity greater than 1 km/s in the southwest cannot be considered for the analysis because it is in a low seismic ray coverage zone. Units 2 and 3 tend to be parallel, denoting that the contacts between the units are tabular and have not suffered any structural affectation up to 10 m deep. The boundary between these units occurs at a depth of 4 m, where speeds of 0.9 km/s begin. Conversely, the speed values on the surface, 0.1 km/s, are lower than those observed on the Colonso-Chalupas road. These low speeds correspond to the area of soil that has not been removed, as in the road case.

On this site, we have as a reference the technical report carried out for building the Ikiam University campus. For this report, five seismic lines were made. The velocity structure of the area is described in six lithological units up to 50 m deep. In this technical report, there are two units up to 10 m deep with speeds of 0.9 m/s and 1.3 m/s, respectively. Our study obtains values of 0.9 m/2 and 1.1 m/s in these same lithological units. However, our results are more detailed in the shallower unit up to 3 m deep, which the technical report does not describe.

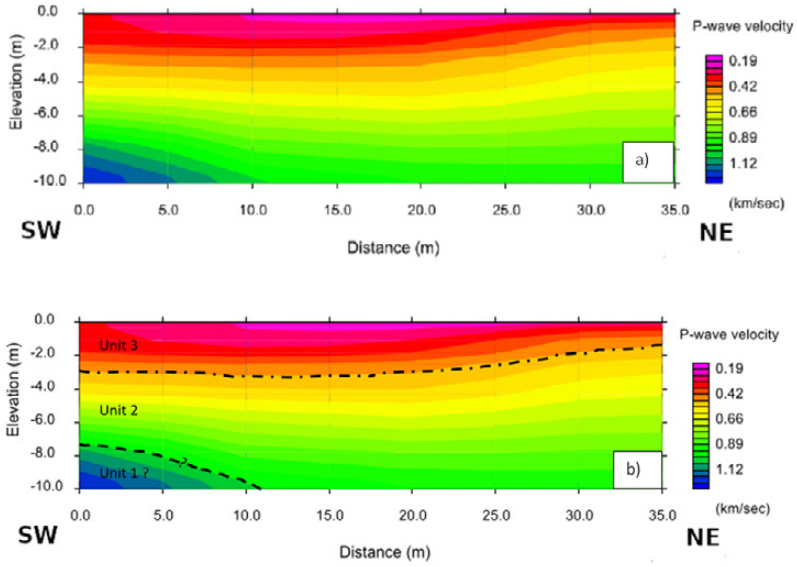

Figure 9. The tomography results for the seismic line in the Tena River bank: a) Without interpretation. b) With seismic stratigraphic and structural interpretation.

The color scale gives the value of the P-wave velocity. The line goes from the Ikiam University campus in the southwest toward the river in the northeast.

In the seismic line in the Napo River (Fig. 10), three seismic stratigraphic units can be identified. The first superficial up to 4 m depth with speeds of the P wave between 0.1 km/s and 0.8 km/s (Unit 3), the second between 4 and 12 m depth with speeds from 0.8 km/s to 1.9 km/s (Unit 2), and finally the basal one from 12 m to the resolution limit of 15 m with velocities higher than 2 km/s for the P wave (Fig. 10b). As in the line of the Tena River bank, the seismic line in the Napo River shows a layer of velocity 0.1 km/s at 1 m depth.

This layer is caused by the area of agricultural exploitation where the line was made. Units 1 and 2 show lateral discontinuities that reach their maximum ex-

pression at 15 and 30 m on the horizontal scale. The surface that constitutes the discontinuity is continuous laterally and is interpreted as a basal surface of fluvial erosion through which the main channel of the Napo River circulated (Fig. 10b). By contrast, unit 3 shows a parallel stratification, which can be interpreted as the product of the filling of the fluvial channel of the river in periods of flooding once it displaces its active channel.

Therefore, this line denotes the migration of the Napo fluvial system in a northwest-southeast direction and the identification of an active flood zone in the line's acquisition area.

There are no references to seismic studies on the banks of the Napo River, at least in its upper channel. However, our results allow us to correlate P wave velocities with the erodability of the river banks since precise knowledge of the composition of the river banks in their percentages of bedrock, cobble boulder, gravel, sand, and silt is essential to determining the stability of the river banks against future floods (Celi, 2002; Celi, 2014).

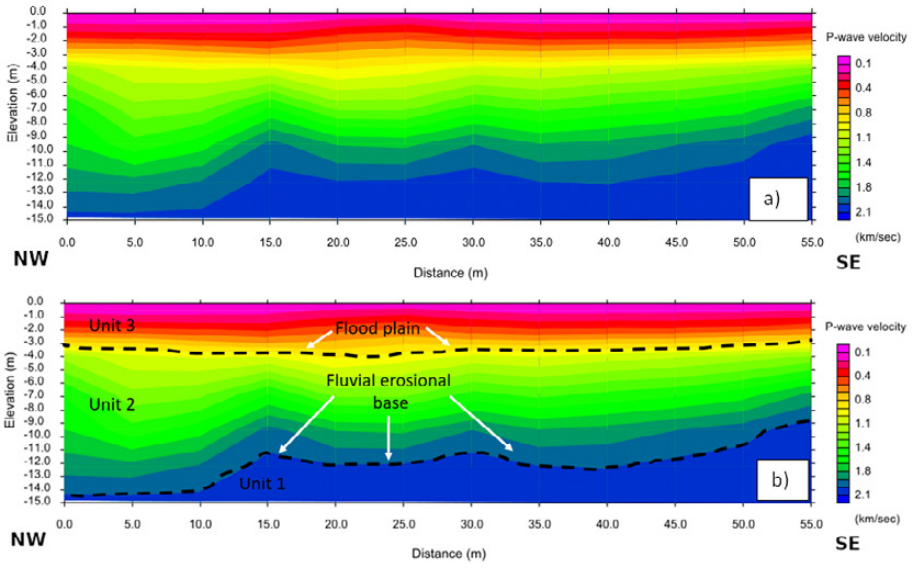

Figure 10. The tomography results for the seismic line in the Napo River bank. a) Without interpretation. With seismic stratigraphic and structural interpretation.

The color scale gives the value of the P-wave velocity. The line goes from northwest to southeast in the downstream direction.

Conclusions

The regularization methodology of the inverse problems could be correctly explained and applied in the case of near-surface seismic tomography. We have successfully used this methodology to carry out three seismic lines in different areas of the Ecuadorian Amazon within the region of influence of Ikiam University.

After the resolution and regularization of the tomography problem, we obtained the three images representing the value of the velocity of the P wave. These images have allowed us: i) to identify the possible presence of a frontal splay of the Talag fault and its probable affectation of the Quaternary alluvial fan; ii) to understand that the layers in a sector adjacent to the Ikiam infrastructure are pseudo-horizontal and without tectonic affectation; and iii) to identify the migration direction and active flood zones of the Napo fluvial system.

In the future, we will complete these seismic wave velocity models with other seismic images and geological data to complete and validate the proposed interpretation.

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CHAP

PTER 4

Nature-based living lab (NB LAB): an undergraduate research experience at the Upper Amazonia

Microplastics and emerging pollutants:

A study of contamination in aquatic ecosystems of the Ecuadorian Amazon

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Author contribution

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Abstract

The rapid growth of the population, the development of the industry, and changing needs over time have led to an increase in the use of polymer-based products, as well as others such as cleaning products, antibiotics, hormones, pesticides, etc. However, poor handling and disposal of their residues have led to a problem of environmental contamination. Due to the risk, they represent for ecosystems and human health, an evaluation of these emerging pollutants (EPs) is necessary, especially in vulnerable areas such as the Ecuadorian Amazon, which is one of the most biodiverse regions of the planet and in which different anthropogenic activities are developed. Therefore, the present study evaluated the contamination caused by emerging contaminants such as diclofenac sodium, nicotine, sulfamethoxazole, acetaminophen, trimethoprim, caffeine, and microplastics (MPs) in streams of the Napo-Ecuador province with different degrees of anthropogenic pressure. The study sites for the analysis of microplastics in water and fish samples were the Napo and Huachiyacu rivers, while for the analysis of diclofenac sodium, nicotine, sulfamethoxazole, acetaminophen, trimethoprim, and caffeine, only water samples were taken from the Mamallacta stream and the Ikiam University water treatment plant. The extraction of MPs was carried out by chemical digestion techniques of fish tissues (gills, muscle, and gastrointestinal tissue) and filtered water samples, which were subsequently quantified and classified. The extraction of EPs was performed by solid-phase extraction (SPE) and identification and quantification by UPLC-QTOF-MS. It was determined that 59.65% of fish (n = 57) presented MPs. In the Napo River, the highest presence of MPs was an abundance of 4,686 items/fish (occurrence factor of 80%) and 609 MPs/L of water. The MPs found were fibers, fragments, sheets, and microspheres, with a greater proportion of <500 µm sized particles. Likewise, acetaminophen and caffeine had the highest concentrations of contaminants found in the samples from the Mamallacta stream (0.9470 and 0.8078 µg/L) and the Ikiam treatment plant (0.6854 and 0.7602 µg/L). According to these results, the study showed that the greater the anthropogenic pressure, the greater the contamination.

Keywords

Anthropic pressure, streams, fish, Napo.

Resumen

El rápido crecimiento de la población, el desarrollo de la industria y las necesidades a lo largo del tiempo han conllevado a un aumento en el uso de los productos a base de polímeros, así como otros, tales como productos de limpieza, antibióticos, hormonas, pesticidas, etc. Sin embargo, el mal manejo y la mala disposición de sus residuos han conllevado a un problema de contaminación ambiental. Debido al riesgo que representan para los ecosistemas y para la salud del ser humano, es necesario una evaluación de estos contaminantes emergentes (EPs), especialmente de zonas vulnerables, tal es el caso de la Amazonía ecuatoriana, que es una de las regiones más biodiversas del planeta, y en donde se desarrollan diferentes actividades antropogénicas. Debido a ello, el presente estudio evaluó la contaminación causada por contaminantes emergentes como diclofenaco sódico, nicotina, sulfametoxazol, acetaminofén, trimetoprima, cafeína, y microplásticos (MPs), en riachuelos de la provincia de Napo-Ecuador con diferente grado de presión antrópica. Los sitios de estudio para el análisis de microplásticos en muestras de agua y peces fueron el río Napo y el río Huachiyacu, mientras que, para el análisis de diclofenaco sódico, nicotina, sulfametoxazol, acetaminofén, trimetoprima, cafeína se tomaron sólo muestras de agua en el riachuelo Mamallacta y la planta de tratamiento de agua de la Universidad Ikiam. La extracción de MPs se realizó mediante técnicas de digestión química de los tejidos de peces (branquias, músculo, tejido gastrointestinal) y muestras de agua filtradas, que posteriormente fueron cuantificados y clasificados. La extracción de EPs se realizó mediante extracción en fase sólida (SPE) y la identificación y cuantificación mediante UPLC-QTOF-MS. Se determinó que el 59.65% de peces (n=57) presentaron MPs. En el río Napo se determinó mayor presencia de MPs con una abundancia de 4.686 ítems/pez (factor de ocurrencia de 80%), y 609 MPs/L de agua. Los MPs encontrados fueron fibras, fragmentos, láminas y microesferas, en mayor proporción partículas de tamaño <500 µm. Asimismo, acetaminofén y cafeína fueron los contaminantes de mayor concentración encontrados en las muestras del riachuelo Mamallacta (0.9470 y 0.8078 µg/L) y la planta de tratamiento de Ikiam (0.6854 y 0.7602 µg/L). De acuerdo con estos resultados, el estudio demostró que, a mayor presión antrópica, mayor será la cantidad de contaminación encontrada.

Palabras clave

Presión antrópica, riachuelos, peces, Napo.

1.Introduction

From a global perspective, rapid urbanization and population growth have led to an increase in the production and consumption of plastics around the world (Jambeck et al., 2015). The need to use resistant, lightweight, and non-corrosive materials has led to this increase (Jain et al., 2021). However, excessive consumption and poor waste management constitute a potential pollution problem. Studies estimate that by 2030 there will be a twofold increase in the amount of plastic waste (Patrício Silva et al., 2021). These products end up decomposing into millions of microplastics (Cózar et al., 2014) due to their non-biodegradability, insolubility, small particle sizes, and bioavailability to life forms. They generate negative effects on biological ecosystems (Wright et al., 2013) and constitute a potential risk to human health (Li et al., 2015).

Microplastics (MPs) are synthetic solid particles or polymeric matrices (Frias & Nash, 2019) whose size is < 5 mm (Alprol et al., 2021); they present different shapes, sizes, colors, or types of polymers (Jain et al., 2021). They can enter water bodies through different pathways due to anthropogenic activities (Alprol et al., 2021). In freshwater ecosystems such as surface waters (rivers and streams), MPs act as natural conduits linking terrestrial, freshwater, transitional, and marine systems (Windsor et al., 2019); therefore, they represent the main long- and short-range transport pathways, contributing to the storage of MPs in some benthic habitats, floodplains, or riparian habitats (Horton & Dixon, 2018). In this sense, fish may come to ingest MPs accidentally or intentionally while feeding in the water column or benthic habitats (Browne et al., 2011). As for ichthyofauna, evidence shows that due to the consumption of MPs by aquatic species, these particles can be retained in the gills and gastrointestinal tract (GIT), causing physical and chemical effects on the organisms (Liedermann et al., 2018).

It is estimated that 92% of the 5.25 million particles in the marine realm correspond to microplastics (Eriksen et al., 2014), making the aquaculture sector one of those affected by MP contamination because these particles can accumulate in the products generated by this sector, thus affecting aquatic ecosystems. They have been detected in bivalves (Li et al., 2015) and other species such as mussels, shrimp, crabs, and fish (Rezania et al., 2018). There are two sources of microplastics: an external one through rivers, seas, land, or atmosphere (industrial effluents, sewage treatment plants, agricultural activities, textile waters, etc.) and an internal one introduced during the aquaculture process due to the aging or wear of plastic fishing gear, feeding, and packaging of aquaculture products (R. Kumar et al., 2021; H. Wu et al., 2023).

A particularity of MPs is that because of their particle size (large contact surfaces) and due to their hydrophobic surface, they can be loaded with hydrophobic organic compounds (HOCs), organochlorine pesticides, polychlorinated biphenyls (PCBs) (Cai et al., 2017), heavy metals, antibiotics, etc. (Alprol et al., 2021), constituting an even greater risk to the environment. For example, in aquaculture products they can cause oxidative stress, affect their behavior, growth, reproduction, and even death of these

species (H. Wu et al., 2023). Likewise, microplastics also constitute a potential health risk to humans as they accumulate and amplify in the food chain (Tang et al., 2021). Therefore, the consumption of aquaculture products constitutes a source of microplastics in human intake (Walkinshaw et al., 2020).

Similarly, anthropogenic activities such as wastewater discharge, agricultural runoff, use of agrochemicals, etc., have influenced the increase in the concentration of emerging contaminants in water, such as personal care products, pharmaceuticals, hormones, flame retardants, pesticides, and detergents, generating risks to ecosystems and human health (M. Kumar et al., 2020; Saquib et al., 2021). They have been detected in significant quantities in water bodies such as surface water, groundwater, domestic and municipal wastewater, and industrial effluents (Chander et al., 2016), causing adverse effects on aquatic ecosystems (J. le Wu et al., 2023).

The Ecuadorian Amazon has been characterized as one of the most biodiverse areas on the planet (Myers et al., 2000). Napo is among the Amazonian provinces with a population of around 131,675 inhabitants (Quillupangui, 2023) and is made up of watersheds such as the Misahualli River, Napo River, Quijos River, etc. In these areas, anthropogenic activities have also affected freshwater ecosystems. Studies have reported the presence of emerging contaminants in this province. For example, on the beach of Puerto de Misahuallí, MPs were found in sediments whose average concentration was 987 itemskg-1 (particle size between 0.5-2 mm) and 761 itemskg-1 (particle size > 2 and < 5 mm) (Lucas-Solis et al., 2021). According to the authors, this contamination may be influenced by hydrological dynamics, such as proximity to populated areas, industrial activity, or the location of treatment plants (Klein et al., 2015; Lucas-Solis et al., 2021; Yang et al., 2021). In other regions of the South American Amazon, the presence of MPs has also been reported, such as in Brazil, where the Negro River, near Manaus, was the area where the highest concentration of MPs in sediments was reported (Gerolin et al., 2020).

Other emerging contaminants have also been studied in water bodies in the Ecuadorian Amazon, such as caffeine, triclosan, estradiol, acetaminophen, nicotine, and ibuprofen (Cipriani-Avila et al., 2023). This study was carried out in the city of Tena, Napo Province, where caffeine was the main compound found, with concentrations ranging from 19 to 31.5 ugL-1, acetaminophen (50.5 ugL-1) and trimethoprim (2 ugL-1) were also found (Capparelli et al., 2021).

The present study focuses on evaluating the presence of emerging contaminants (microplastics, diclofenac sodium, nicotine, sulfamethoxazole, acetaminophen, trimethoprim, and caffeine) in aquatic ecosystems of the north-central zone of the Ecuadorian Amazon, specifically in the city of Tena, Napo province, and determining whether anthropogenic activities influence the presence of these contaminants in water bodies and if there is a presence of MPs in fish tissues (gills, muscle, gastrointestinal tissue (GIT)).

2.Materials and Methods

2.1 Study area

The study area is in the province of Napo, canton Tena. This region, like others in the Amazon, is subjected to intense anthropogenic pressures, such as large-scale deforestation, mining, overfishing, invasive species, and pollution on several fronts (Aguirre et al., 2021). The Napo River is one of the main tributaries of the Amazon River that originates in the eastern slopes of the Andes of Ecuador and Colombia and flows along 1130 km to its mouth in the Amazon in Loreto (Peru) (Nugra et al., 2016). The Napo is the largest river in Ecuador, where the waters of the thawing Antisana, Sincholagua, Cotopaxi, and Llaganates converge (Pomboso et al., 2006) and travels 450 km through Ecuadorian territory (Cevallos & Ruales, 2005).

Fish samples for analysis of MPs were taken at two points in the Napo River and three points in the Huachiyacu River, considering different types of anthropogenic intervention. Water samples for the analysis of diclofenac sodium, nicotine, sulfamethoxazole, acetaminophen, trimethoprim, and caffeine were taken at two points in the city of Tena: at the treatment plant of the Universidad Regional Amazónica Ikiam and in the Mamallacta stream (Figure 1).

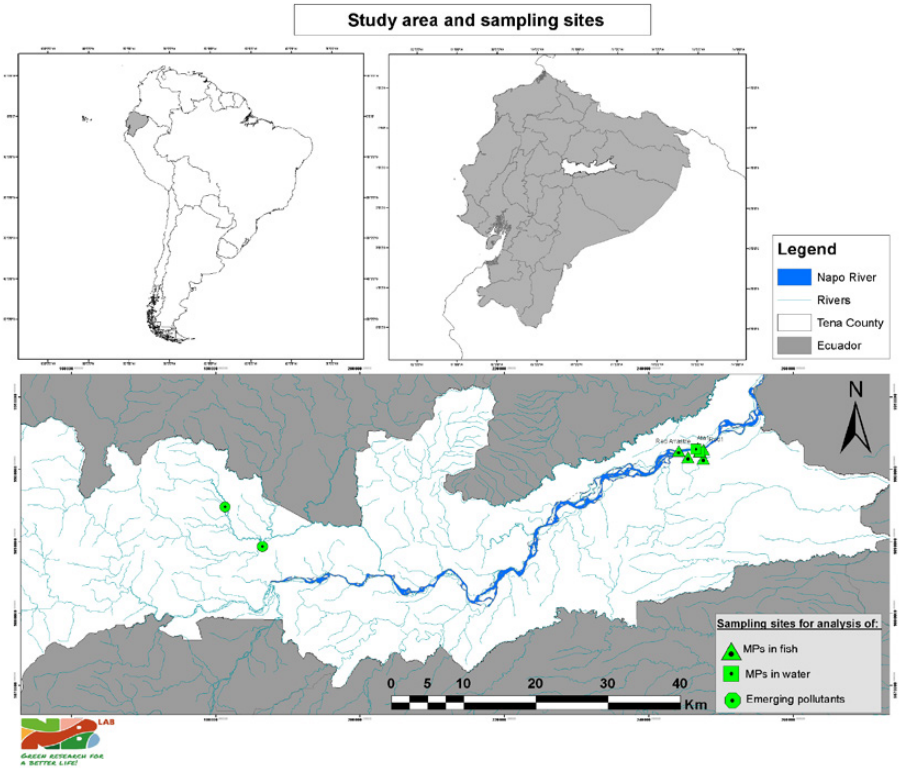

Figure 1. Study area and sampling points of microplastic and emerging pollutants experiments.

2.2 Materials and reagents

Hydrogen peroxide (30 %, USP) was used for sample digestion, sodium chloride (≥99.0%) was used to allow microplastics to float. All reagents were analytical grade, obtained from Sigma-Aldrich.

For the analysis of emerging contaminants, high purity standards of diclofenac, sodium, nicotine, sulfamethoxazole, acetaminophen, trimethoprim, and caffeine, obtained from Sigma-Aldrich and Supelco, were used.

2.3 Sampling

a) Natural Water

Two water samples were taken in the tributaries of the upper Napo River basin during August 2022 for the analysis of MPs, diclofenac sodium, nicotine, sulfamethoxazole, acetaminophen, trimethoprim, and caffeine. A 500 mL sample was collected in amber glass bottles previously washed with deionized water and ethanol. They were transported to the National Water Reference Laboratory under refrigerated conditions and stored at 4°C until analysis.

b) Fish

A total of 57 fish specimens were obtained by direct capture (20 in the Napo River and 37 in the Huachiyacu River). Instruments used were electrofishing backpacks, hand nets, trawl nets, waiting nets with different mesh sizes, *atarrayas*, and bait traps, considering the size of the water body and the capacity of the instrument. Samples were obtained in August 2022; all specimens were preserved in 96% ethanol and subsequently stored at -4 °C until morphological analysis. Table 1 compiles information on the study fish, their diet, and their trophic position.

Table 1. Description of fish sampled.

Local name	Scientific name	Fish number	Feeding habit	Trophic position
Bagre Bico de pato	*Sorubim lima*	1	Piscivorous, insectivorous	4.1 ±0.71
Bocachico	*Prochilodus nigricans*	2	Detritivorous	2.4 ±0.18
Carachama	*Chaetostoma sp*	14	Detritivorous	no data
Raspabalsa	*Pseudohemiodon lamina*	3	Insectivorous, detritivorous	2.5 ±0.2
Sábalo	*Salminus hilarii*	1	Piscivorous	2.7 ±0.3
Tararira	*Hoplias malabaricus*	1	Piscivorous, Insectivorous	4.5 ±0.0
Chuti	*Crenicichla proteus*	1	Piscivorous	3.2 ±0.5
Bío del río	*Sternopygus macrurus*	1	Insectivorous	3.2 ±0.40
Sardina de río	*knodus gamma*	10	Omnivorous with a preference for invertebrates	3.0 ±0.4
Sardina de río	*Creagrutus kunturus*	11	Insectivore and detritivore	2.2 ±0.1
Sardina de río	*Astyanax bimaculatus*	10	Omnivorous (zooplankton, detritus, higher plants and sometimes on the scales of fish)	2.4 ±0.1
Vieja	*Cichlasoma bimaculatum*	1	Insectivorous	3.6 ±0.53
S/N	unknown	1	no data	no data

Information taken from www.fishbase.org.

2.4 MPs extraction

a) Specimen samples

Fish were identified by species level following taxonomic keys (Bogotá et al., 2006; Barriga, 2012) and the species list (Anaguano, 2014). Each specimen was measured for total length (TL) and standard length (SL) (0.1 cm accuracy) and weighed with a spring balance (0.3 g accuracy). The gastrointestinal tract (esophagus, stomach, and intestine), gills, and a portion of muscle tissue were removed from each specimen. All samples were transferred to Erlenmeyer flasks and subjected to heating for 48 hours at 50°C. Subsequently, the dried tissues were weighed on a digital balance (precision 0.01 g) and crushed with glass rods and mortars.

Selected organs and tissues from each specimen were chemically digested using 5 to 25 mL of 30% hydrogen peroxide (previously filtered) and kept at a temperature of 65°C for 48 hours.

The separation of the MPs from the liquid was performed by density difference, for which 15 mL of a saturated solution of sodium chloride (density 1.2 g/mL) was added, twice the amount of hydrogen peroxide used was added to each sample, and it was left to stand for 48 h. The settleable solids were separated from the remaining liquid with the microparticles, and the supernatant was vacuum filtered through a 0.45 µm cellulose nitrate filter and placed in a Petri dish for subsequent quantification and identification.

b) Natural water samples

To extract MPs from natural water samples, we followed the procedure indicated by Capparelli et al. (2021). The samples were filtered through a sieve with a pore size of 63 µm, and a series of washes were performed with deionized water (ultrapure Milli-Q), with which the particles retained in the sieve were transferred to an Erlenmeyer flask. The samples were brought to dryness by heating at 60°C in an oven. Subsequently, the sample was chemically digested using 15 mL of 30% hydrogen peroxide, heated to a temperature of 60°C, and left to stand for 72 hours.

We added 15 mL of a saturated sodium chloride solution (density 1.2 g/mL) to separate the MPs from the liquid by density differences, transferred to a separatory funnel, and left to stand for 24 h. The settleable solids were separated. Finally, the supernatant with MPs was vacuum filtered through a 0.45 µm membrane filter and placed in a Petri dish for subsequent quantification and identification.

For control of MPs analysis in water and tissues, blanks were used by placing filters in Petri dishes in areas close to the sample treatment site throughout the assay until microscopic observation. In addition, a sample blank consisting of 1 L of deionized water was used, for which the entire extraction process was carried out to rule out any interference.

2.5 Identification and validation of MPs

The visual identification and measurement of MPs was performed according to the guidelines of the *"Microplastics sampling and processing guidebook: sampling and processing guidebook"* of Mississippi State University, USA (Sartain et al., 2018). MPs were quantified and identified with the help of an electronic stereoscope with an Olympus SZX7 camera (Olympus Corp., Tokyo, Japan) that allows particles to be seen within the necessary range (≤ 5 mm). The entire filter area was analyzed, and each microplastic was quantified. Data on color and size were taken. A classification was made according to shape into fragments, fibers, films, and spheres.

To rule out the overestimation of MPs due to possible contamination of the work area, a blank sample consisting of a membrane filter placed in a Petri dish, exposed to laboratory conditions during stereoscopic observation, was used as a control.

2.6 Occurrence and abundance analysis

Microplastic occurrence was determined for each stream sampled by the percentage frequency of occurrence, which was calculated by the following formula: FO% = (Ni/N) ×100, where FO% = frequency of occurrence of microplastic particles; Ni = number of fish with microplastics; and N = total number of fish examined. The average abundance (MPs/fish) was obtained by dividing the total number of MPs by the weight of both the individual and the tissue analyzed. Additionally, a Welch's t-test was performed with Rstudio software (Core R Team, 2019) and GraphPad Prism version 8.02 to compare the means of MPs in fish from the two water bodies, since the sample sizes and variances are unequal due to the differences between the rivers.

2.7 Quantification of emerging compounds

Water samples were treated by solid-phase extraction (SPE) according to the methodology outlined by Glassmeyer et al. (2017) and Cipriani-Avila et al. (2023). Before extraction, 500 mL of sample was filtered using 0.45 µm pore glass fiber filters and passed through Waters OASIS HLB cartridges (200 mg, 6 mL), which were previously conditioned with 4 mL of methanol at a flow rate of 10 mL/min and 6 mL of deionized water (ultrapure Milli-Q) in the washing step. After loading the 500 mL of sample, the cartridges were allowed to dry under vacuum for 10 minutes. Subsequently, the analytes were eluted using 6 mL of methanol, and the samples were concentrated to solvent dryness under a stream of nitrogen gas (5.0) and reconstituted with 0.5 mL of methanol. Finally, it was analyzed using a Waters Model I-Class ultra-high-efficiency liquid chromatograph coupled to a Waters Model Xevo G2 QTOF mass spectrometer (UHPLC-QTOF-MS). Equipment conditions were column C18 (1.7 µm, 100 mm × 2.1 mm i.d.), mobile phases A (Water) and B (Acetonitrile) with 0.1% formic acid, and an elution gradient (B = 5% 1 min, 5-100% 9 min, 100-5% 2 min, column re-equilibration 5% 3 min). Mass spectrometer conditions were determined according to the study of Cipriani-Avila et al. (2023).

3.Results and discussion

3.1 Abundance of MPs

Figure 2 shows that there is a difference between the abundance of MPs in water samples and fish samples, with a higher abundance in the water samples from the Napo River Basin because pollution with MPs is frequent in the food webs of urban rivers (McNeish et al., 2018). Fish inhabiting freshwater near urbanized regions and their surroundings are mainly exposed to MPs contamination (Hossain et al., 2019; Parvin et al., 2021).

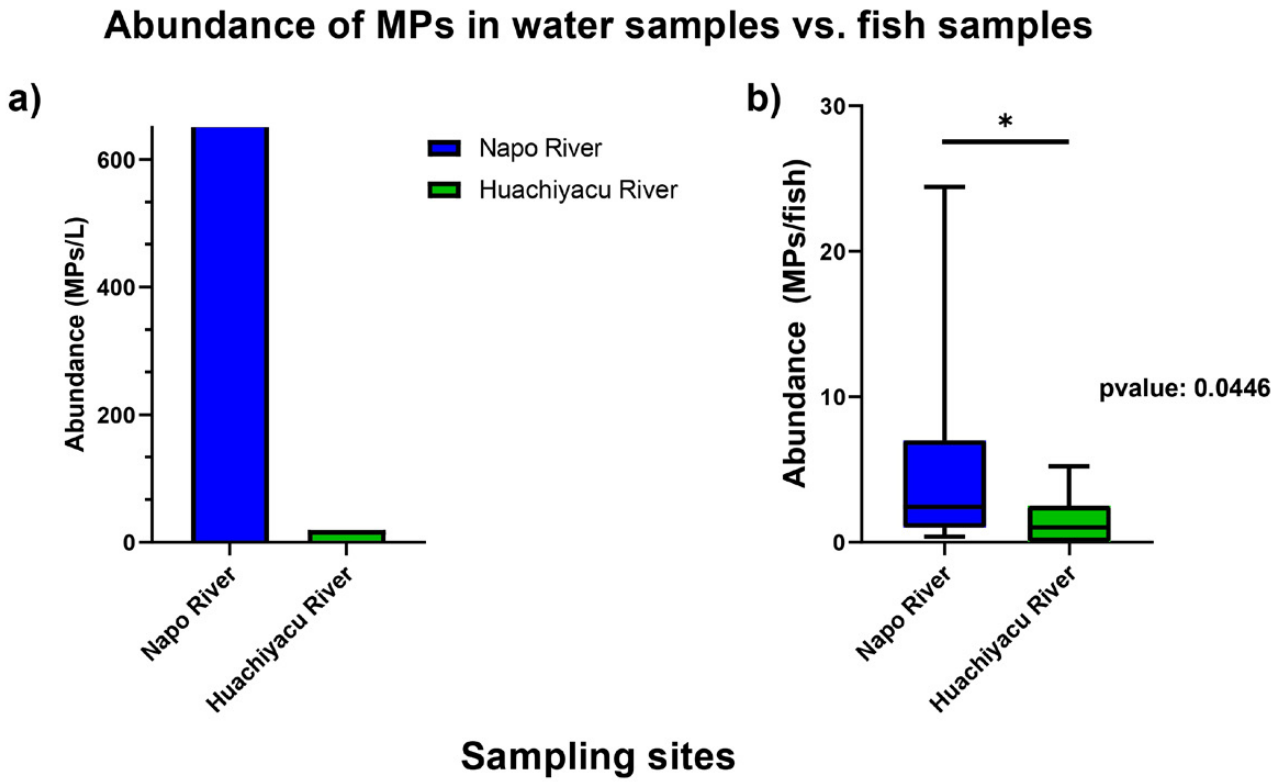

Figure 2. Abundance of MPs in the sampling sites: a) water samples; b) fish samples, mean number ± standard deviation of MPs per individual.

** Indicates significant differences ($p < 0.05$).*

The abundance of MPs in the water samples is related to the abundance of MPs in fish, where the Napo River shows greater contamination by this type of emerging pollutant.

It was determined that 59.65 % (n = 57) of the fish under study were contaminated with MPs. The highest abundance of MPs per individual was observed in the Napo River (4.686 items/fish). In the undeveloped streams (Huachiyacu), the mean abundance was 1.355 items per fish.

Concerning mean abundance per gram of tissue, a value of 1.936 items/g was determined in the Napo River and 0.631 items/g in the Huachiyacu stream. The difference in the abundance of MPs in the two water bodies is statistically significant (p-value:0.0446) as shown by the unpaired t-test with Welch's correction, since the difference between the means is marked. The same happened with the comparison of variances (p-value < 0.0001), so we can ensure that there is a significant difference between the two variances of MPs in fish (Table 2). A higher MPs occurrence factor was observed in the Napo River, with percentages higher than 80% (Table 3).

Table 2. Welch's t-test of the abundance of MPs in the sampled sites.

Unpaired t test with Welch's correction	
P-value	0.0446
P-value summary	*
Significantly different (P < 0.05)?	Yes
One or two-tailed P-value?	Two-tailed
Welch-corrected t, df	t=2.183, df=15.70
How big is the difference?	
Mean of column A	4.687
Mean of column B	1.355
Difference between means (B - A) ± SEM	-3.779 ± 1.731
95% confidence interval	-7.454 to -0.1034
R-squared (eta squared)	0.2329
F-test to compare variances	
F, DFn, Dfd	18.69, 15, 36
P-value	<0.0001
P-value summary	****
Significantly different (P < 0.05)?	Yes

Table 3. Abundance and occurrence of MPs in the sampled sites.

River	Abundance (MPs/L)	Abundance (items/fish)	Abundance per gram (items/g)	N (# Total fish)	Ni (# Fish with MPs)	FO (MPs Occurrence) (%)
Napo	609	4.686	1.936	20	16	80.00
Huachiyacu	8	1.355	0.631	37	18	48.65

3.2 Characterization of MPs

In the water samples from the Napo River, a greater presence of plastic films was found, while in the Hauchiyacu River, a greater quantity of fragments was found (Figure 3). The MPs found in the fish tissues of the Napo River were mostly fibers, followed by fragments, films, and microspheres (Figures 3 and 4). Several studies have reported that fibers are the most commonly found microplastic morphotype (Saemi-Komsari et al., 2023), and it has also been recognized that this type of MPs has a high potential for uptake by marine organisms (Browne et al., 2011; Pappoe et al., 2022). The fibers can enter aquatic ecosystems from sources such as household sewing waste, laundry, fishing gear, ropes, etc. (Pradit et al., 2023). Therefore, clothing and household discharges are responsible for a considerable fraction of fibers in aquatic biota. In this sense, MPs such as fibers could represent a problem for the health of marine species, water quality, and human health (eye, skin, and tract irritation).

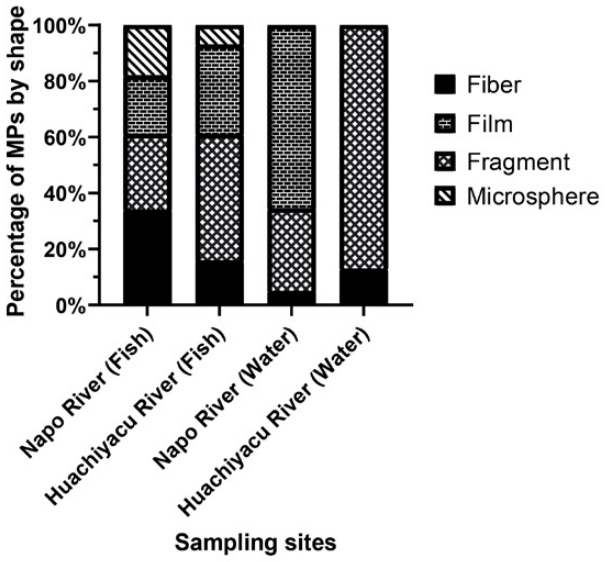

Figure 3. Percentage of MPs found in fish tissues and water samples according to their morphotype: fibers, fragments, films, and microspheres.

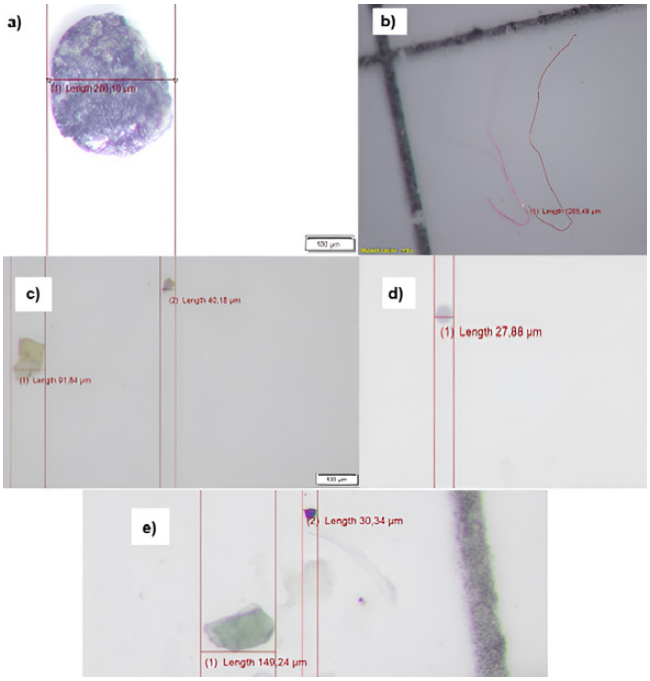

Figure 4. Photographs showing different morphotypes and colors of microplastics found in fish: a), c), and e) fragments; b) fiber; and d) sphere, detected with stereomicroscopy.

In terms of size, particles smaller than 500 µm (56%), between 500 µm and 1 mm (32%), and only 12% with a size between 1 mm and 5 mm were found (Figure 5).

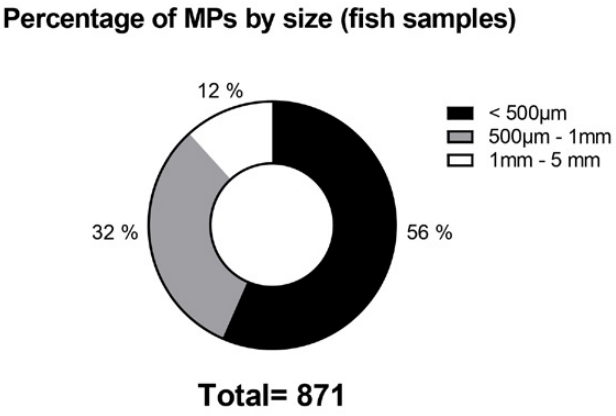

Figure 5. Percentage of MPs by size for each sampling site.

In general, the color of MPs does not show any connection to their origin; however, it allows for easy visual inspection (Jain et al., 2021). In this study, the most frequent colors were blue, transparent, white, purple, and black (Figure 6).

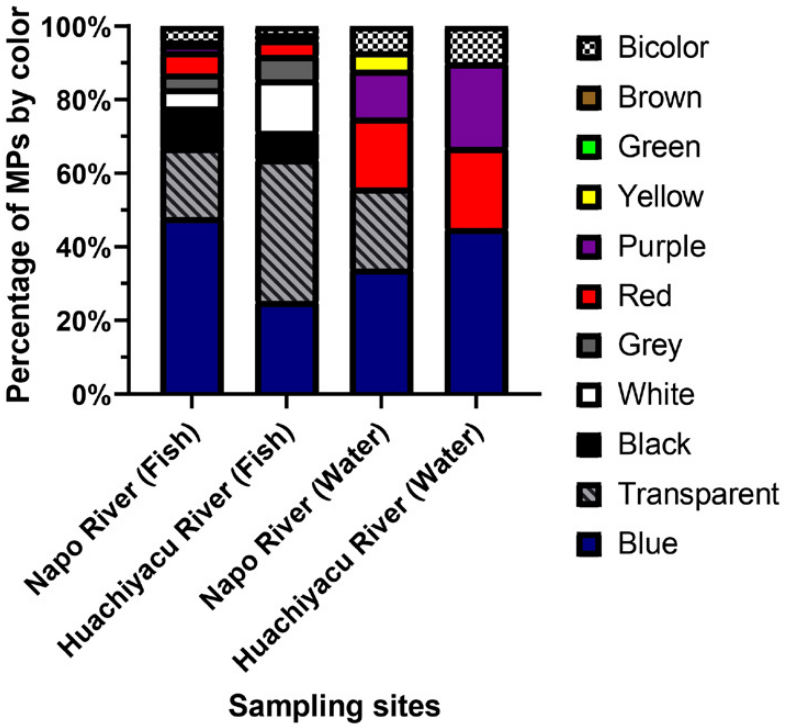

Figure 6. Percentage of MPs in fish tissues and water samples according to their color for each sampling site.

Figure 7 shows the number of MPs particles in each organ analyzed: the GIT (gastrointestinal tract), gills, and muscle tissues of the fish. A higher number of particles were found in the GIT and gills.

Other studies also report a significant presence of MPs in the digestive tract of fish (R. Kumar et al., 2021). However, they can also adhere to the skin and gills due to their movement and respiration (Sembiring et al., 2020).

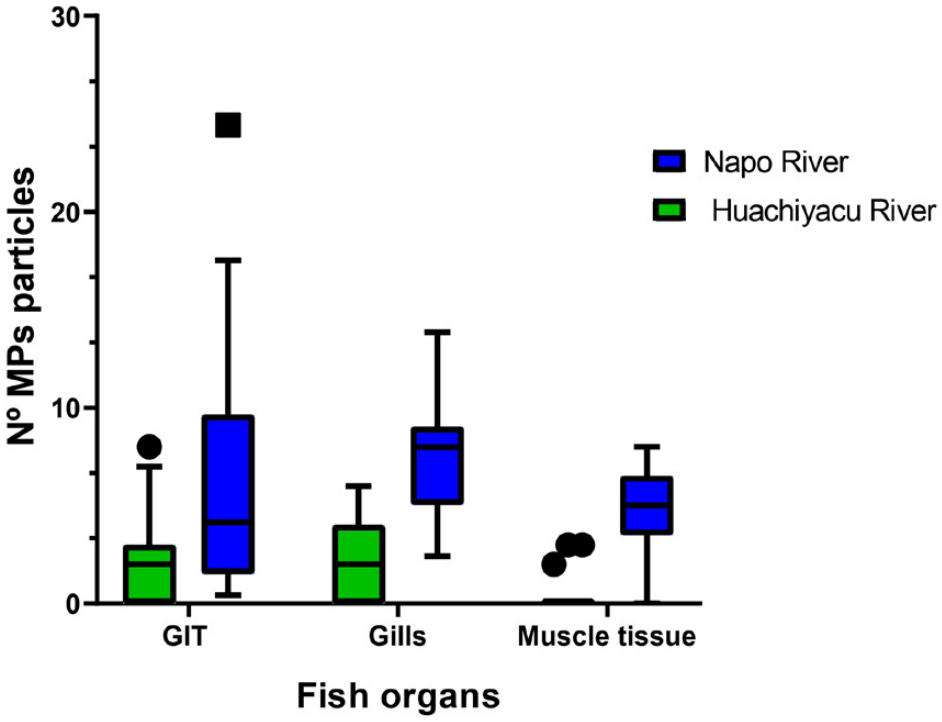

Figure 7. Quantity of MPs in the different organs of the fish analyzed in each stream sampled.

3.3 Analysis of emerging compounds in the water samples

The analyses indicated that caffeine and three of the five drugs studied were detected in water samples taken from the Mamallacta stream and the Ikiam treatment plant. However, in the latter area, a higher concentration of diclofenac sodium, sulfamethoxazole, acetaminophen, and caffeine was evidenced (Table 3). It is known that these types of emerging contaminants can enter water bodies because of urine or feces excreted by humans (Wu et al., 2023).

Table 3. The concentration of emerging pollutants in water samples.

Sampling sites	Concentration (µg/L)					
	Diclofenac Sodium	Nicotine	Sulfamethoxazole	Acetaminophen	Trimethoprim	Caffeine
Ikiam Treatment Plant	0.0680	ND	0.0259	0.9470	ND	0.8078
Mamallacta stream	0.0261	ND	0.0006	0.6854	ND	0.7602

In this study, acetaminophen was found in higher concentrations in both the Ikiam treatment plant and the Mamallacta stream, with values between 0.9470 µg/L and 0.6854 µg/L, respectively. This drug, also called paracetamol, is registered in Ecuador's National Table of Basic Medicines (Consejo Nacional de Salud, 2022) and is commonly used as an antipyretic and analgesic for humans. Research indicates that high concentrations of acetaminophen and other drugs in bodies of water can cause negative effects in some aquatic organisms (Wu et al., 2023); for example, in the freshwater fish *Oreochromis mossambicus,* liver damage at the tissue level has been observed (Kavitha et al., 2011). Likewise, both paracetamol and diclofenac have been shown to interfere with neurotransmission in *Daphnia magna* by inhibiting cholinesterases (ChEs) (Oliveira et al., 2015).

Another of the emerging contaminants found in higher concentrations was caffeine (0.7602-8078 µg/L), which is also included in the National Table of Basic Medicines of Ecuador (2022). This substance is also present as an adjuvant in some drugs (Cipriani-Avila et al., 2023). However, it is also an active ingredient of guayusa, which is one of the plants cultivated in the Amazon region, such as in the canton of Tena, and its infusion is generally consumed by the population as an energizing drink (*Wayusa Upina: Una Tradición Ancestral Que Se Mantiene Hasta Nuestros Días*, 2020).

4.Conclusions

The results obtained in the present work are an indication that inadequate final disposal of commonly used products such as plastics and pharmaceuticals constitutes a contamination pathway for aquatic ecosystems in the study areas in the north-central Ecuadorian Amazon. The extraction methodologies and analytical techniques used, such as UHPLC-QTOF-MS, allowed the identification and quantification of emerging contaminants (diclofenac, sulfamethoxazole, acetaminophen, and caffeine) due to their high sensitivity. Regarding the analysis of microplastics, it would be advisable to carry out a characterization by infrared spectroscopy to identify the functional groups associated with the different polymers present in the samples and thus know the sources of origin of these MPs.

According to the results, there is a direct correlation between increased anthropogenic pressure and higher levels of contamination, i.e., anthropogenic activities around the streams have contributed to the generation and accumulation of microplastics in the study areas (Napo and Huachiyacu rivers), thus affecting the water bodies and consequently the aquatic biota, such as fish. In addition, the presence of commonly used drugs such as diclofenac, sulfamethoxazole, acetaminophen, and caffeine were detected in the Mamallacta stream and the Ikiam treatment plant, with the last two having the highest concentrations. Because these emerging contaminants tend to bioaccumulate, they are a potential problem for the environment and human health.

This study seeks to encourage the scientific community to develop more research on the contamination of water bodies caused by different emerging pollutants in various areas of the Ecuadorian Amazon since it is one of the most biodiverse regions of the planet and it is necessary to care for and protect it. With the results obtained and future research in the environmental area, it will be possible to establish strategies for better management of plastic waste and water treatment plants, as well as raise public awareness of the negative consequences of poor waste management.

5. Acknowledgments

We would like to express our sincere gratitude to Mariana Capparelli for her invaluable expertise and meticulous review of this book chapter. Her insightful feedback and constructive comments have greatly enriched the content and quality of this work. We would also like to acknowledge the generous support provided by the Erasmus+ Programme of the European Union grant: 619346-EPP-1-2020-1-DE-EPPKA2-CBHE-JP. This funding has been instrumental in the successful development of the research project, enabling us to explore and present our findings in this chapter.

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CHAP

OTER 5

Nature-based living lab (NB LAB): an undergraduate research experience at the Upper Amazonia

Towards the application of photocatalytic materials in environmental remediation

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Abstract

Water pollution is a major environmental concern in contemporary society. As the global population grows, new water contaminants arise, which represents additional threats to the protection and conservation of this vital resource. Thus, platform 5 of NB-LAB, the removal and inactivation of compounds of emerging concern and waterborne pathogens through photocatalytic membranes, has taken up the challenge of providing clean water using BiOI micro/nano photocatalysts as a tool to degrade and inactivate the water pollutants. In this sense, BiOI flower-like microspheres were synthesized and characterized using a set of instrumental techniques, such as x-ray diffraction, scanning electron microscopy, energy-dispersive x-ray spectroscopy, and transmission electron microscopy, for the degradation of chemical pollutants and waterborne microbial pathogens. The results reveal that the preparation of BiOI with a flower-like morphology was carried out satisfactorily. The photocatalytic tests showed that BiOI microspheres could degrade up to 90% of Bisphenol A, reduce the bacterial concentration from 107 to 106 CFU/mL and inactivated almost 70% of the cyanobacterium *Microcystis sp.* The results of the toxicity tests indicate that the examined nanostructures do not exhibit damaging effects on blood cells; however, the potential impact on various cell lines remains uncertain since they might provide positive or negative outcomes. Finally, based on the above, it is concluded that BiOI photocatalysts could be excellent candidates for the degradation and inactivation of contaminants of emerging concern present in aquatic effluents.

Keywords

Photocatalysis, BiOI, Bisphenol A, *Escherichia coli, Microcystis sp*., Toxicity

Resumen

La contaminación del agua es una preocupación ambiental importante que crece a nivel mundial. Al crecer la población surgen nuevos contaminantes del agua, lo que representa amenazas adicionales a la protección y conservación de este recurso vital. Esto ha motivado la investigación en la plataforma 5 de NB-LAB para la eliminación e inactivación de compuestos de preocupación emergente y patógenos transmitidos por el agua a través de membranas fotocatalíticas, asumiendo el desafío de proporcionar agua limpia utilizando micro/nano fotocatalizadores BiOI como herramienta para degradar e inactivar los contaminantes del agua. En este sentido, se sintetizaron y caracterizaron microesferas BiOI de tipo flor utilizando un conjunto de técnicas instrumentales como: difracción de rayos X, microscopía electrónica de barrido, espectroscopia de rayos X de energía dispersiva y microscopía electrónica de transmisión, para la degradación de contaminantes químicos y patógenos microbianos transmitidos por el agua. Los resultados revelan que la preparación de BiOI con morfología similar a una flor se realizó satisfactoriamente. Las pruebas fotocatalíticas demostraron que las microesferas de BiOI podían degradar hasta el 90% del Bisfenol A, reducir la concentración bacteriana de 107 a 106 UFC/mL y el 70% de la cianobacteria *Microcystis sp.* Los resultados de las pruebas de toxicidad indican que las nanoestructuras examinadas no presentan efectos dañinos en las células sanguíneas; sin embargo, el impacto potencial en varias líneas celulares sigue siendo incierto. Finalmente, con base en lo anterior, se concluye que los fotocatalizadores BiOI podrían ser excelentes candidatos para la degradación e inactivación de contaminantes de preocupación emergente presentes en efluentes acuáticos.

Palabras clave

Fotocatálisis, BiOI, Bisfenol A, *Escherichia coli, Microcystis sp*., Toxicidad

1. Introduction

The world faces the degradation of aquatic ecosystems with the consequent effects on human health due to emerging contaminants. This group of contaminants includes antibiotics, pharmaceuticals, personal care products, hormones and artificial sweeteners (Tran et al., 2017). These pollutants are not eliminated in conventional treatment processes and are also present in water bodies; they can bioaccumulate in macroinvertebrates, aquatic organisms of the food chain and humans (Rodríguez-Narváez et al., 2017). Wastewater treatment in the Ecuadorian Amazon region is deficient; up to 56% of wastewater is discharged into rivers without any treatment and barely 25% is treated (Capparelli et al., 2021). The northern Amazon of Ecuador has been widely recognized for its distinguished biodiversity at the global level (De la Torre et al., 2012; Viteri-Salazar and Toledo, 2020). However, in recent years, this invaluable biological wealth has faced a specific threat: the need to ensure the purity of drinking water in the region. (M. Pelaez et al., 2012). Therefore, the presence of ECs in the tributaries of Amazonian rivers represents a serious threat to biodiversity and human beings; therefore, it is essential to seek innovative and effective solutions for the conservation of this delicate ecosystem.

Nanotechnology has arisen as an excellent opportunity to face environmental pollution, with the development of some nanomaterials such as nanoadsorbents, nanocomposites, nanomembranes, and nano photocatalysts (Mbarek et al., 2022). Among them, micro- and nano-photocatalysts have exhibited outstanding properties to be used for environmental remediation. They are semiconductors that can absorb either UV, visible, or near-infrared light (NIR) to carry out chemical reactions (Simonsen, 2014). It means that sunlight can be used as a renewable energy source to help mitigate environmental pollution.

The photocatalytic reactions begin with the absorption of light by the semiconductor material; this chemical process results in the formation of a photogenerated electron/hole pair that migrates from the bulk to the photocatalyst surface to react with chemical species (Hernández-Ramírez & Medina-Ramírez, 2015). These reactions can lead to the formation of reactive oxygen species (ROS), which can transform toxic compounds into non-hazardous reaction by-products for the environment and living beings. Therefore, during the last few years, several visible-light-driven (VLD) semiconductor structures have been developed for photocatalytic applications.

Bismuth oxyiodide (BiOI) is a VLD semiconductor that has attracted great interest for photocatalytic applications. This semiconductor has a narrow band gap of 1.7-2.0 eV (Hu et al., 2014; H. Li et al., 2013; Liu et al., 2013; Zhang et al., 2016), which makes it suitable for conducting photocatalytic reactions with visible-light wavelengths smaller than 620 nm. Moreover, this semiconductor can efficiently promote the separation of charge carriers, thanks to its layered structure (Li et al., 2018), which is fundamental to achieving a high degradation rate of pollutants. Due to these facts, several nanostructured BiOI materials (e.g., flower-like structures, nanoplates, nanosheets, and nanofilms) have been synthesized and used for organic pollutant degradation (Bárdos et al., 2019; Jia et al., 2015; Mahmoodi et al., 2018; Wang et al., 2017), bacteria inactivation (Jamil et al., 2015; Jiang et al., 2017; Long et al., 2016; Sun et al., 2020), air purification (Huang et al., 2018), and energy production (Zhao & Dai, 2015).

On the other hand, assessing the potential toxicity of nanostructured microspheres is crucial to avoid adverse effects on aquatic and human life (Ganguly et al., 2018). When bulk materials are reduced from micro to nanoscale, their toxicity levels may vary depending on their chemical composition, size, structure and morphology type (van der Merwe & Pickrell, 2018). Bi-based materials, for instance, have been considered toxic due to the potential for acute and chronic effects with long-term exposure (Yan and Sun, 2011). However, it is essential to note that the toxicity of Bi alone cannot be extrapolated to Bi-based nanostructured photocatalysts, as their behavior and effects can undergo significant changes when integrated into specific nanomaterials (Ganguly et al., 2018). Therefore, the systematic evaluation of these materials is necessary to understand their impact on the environment. Thus, to qualify a photocatalyst for practical applications, several stages must be followed, including (a) the synthesis of nanostructures, (b) efficiency testing against representative pollutants, and (c) an assessment of toxicity.

To date, there has not been a complete evaluation of BiOI microspheres in the degradation of chemical pollutants, bacteria and cyanobacteria inactivation. Moreover, there is a lack of information about their toxicity to organisms and ecosystems to demonstrate their practical application. So, this work aims to evaluate the efficiency and toxicity of BiOI microspheres to ensure their practical application in water remediation.

2. Materials and Methods

2.1 Synthesis of bismuth oxyiodide (BiOI) flower-like microspheres.

BiOI flower-like microspheres were synthesized by a modified solvothermal method (Zuarez-Chamba et al., 2022a). The procedure for the preparation of BiOI powders was as follows: A solution A was prepared by dissolving 3 mmol (1.455 g) of Bi(NO3)3·5H20 in 30 mL of ethylene glycol with sonication for 30 minutes and then under constant stirring (500 rpm) for 30 min. Simultaneously, solution B was prepared by dissolving 3 mmol (0.498 g) of KI in 30 mL of ethylene glycol under continuous stirring (500 rpm). Afterward, solution B was added drop by drop (1 mL/min) to solution A under constant stirring (500 rpm). The mixed solution was let sit for 30 minutes at constant stirring (500 rpm) and then transferred into a 100 mL Teflon-lined autoclave. The autoclave was placed in a reactor and then in an oven and heated at 126 °C for 18 hours. After being cooled to room temperature, the resulting precipitate was collected by vacuum filtration and washed three times with DI water, three times with ethanol, and again two times with DI water. Finally, the product was dried at 60 °C for 24 hours.

2.2 Instrumental characterization of BiOI microspheres

BiOI structures were characterized by X-ray diffraction (XRD) to evaluate the crystalline structure using a Malvern Panalytical Empyrean X-ray diffractometer equipped with a copper X-ray tube (Kα radiation, λ = 1.54056 Å). The XRD data was collected in the 2Θ range from 5° to 90° with a scan rate of 0.01° at 45 kV, and 40 mA. The morphology of the powder was observed by scanning electron microscopy (SEM) in a Tescan Mira 3 scanning electron microscope. The microstructure of the powders was observed by transmission electron microscopy (TEM) in an FEI-Tecnai G20 Spirit Twin transmission electron microscope equipped with an Eagle 4k HR camera. The elemental composition of the powders and films was analyzed by energy-dispersive X-ray spectroscopy (EDS) using a Bruker X-Flash 6-30 detector with a resolution of 123 eV at Mn Kα.

2.3 Efficiency testing against representative water pollutants

2.3.1 Bisphenol A degradation

A photocatalysis test was performed in a photoreactor equipped with two fans, a 500 mL beaker (6 cm high), a stirring plate, and one white-light LED lamp (LEDEX B4665, 50W, 5000 Lm, 6000 K daylight, 100-200 V) placed at 26 cm from the beaker. 100 mg of powdery BiOI structures were dispersed by sonication into 350 mL of Bisphenol A (BPA) solution (10 mg/L) for 10 min. Subsequently, this solution was stirred in the dark at 300 rpm for 1 h to achieve the adsorption/desorption equilibrium between the pollutant and the photocatalyst surface. Then, it was irradiated with visible light for 3 hours under constant stirring. During this time, 26 mL of the solution was taken every 20 minutes and the BPA concentration was measured using a UV-vis spectrophotometer at 276 nm.

2.3.2 Microbial inactivation

2.3.2.1 Bacteria culture and preparation

Escherichia coli ATCC 25922™ was cultured in Mueller Hinton Broth (MHB) for 18 to 24 h at 37 °C with shaking at 150 rpm. Then, 0.5 mL of the bacterial culture was taken into sterile MHB. It was incubated at 37°C with shaking for 90 min to reach bacterial cells in exponential phase bacterial growth (OD 0.4). *E. coli* cells were collected by centrifugation at 4000 rpm for 15 min. After centrifugation, the MHB was discarded, and the pellet was washed two times with a NaCl 0.9 % (m/v) solution. In the last wash, the saline solution was discarded and the pellet was resuspended with a 0.5 mL NaCl 0.9% solution.

2.3.2.2 Photocatalytic inactivation performance

The inactivation performance of BiOI microspheres was performed using a photoreactor, as seen in Figure 1, that consisted of a white LED lamp (400W, 6500 K) as the visible light source and a cooling system composed of two fans to remove the heat produced by the lamp. Thus, the inner temperature, in the photoreactor was maintained at 25 °C.

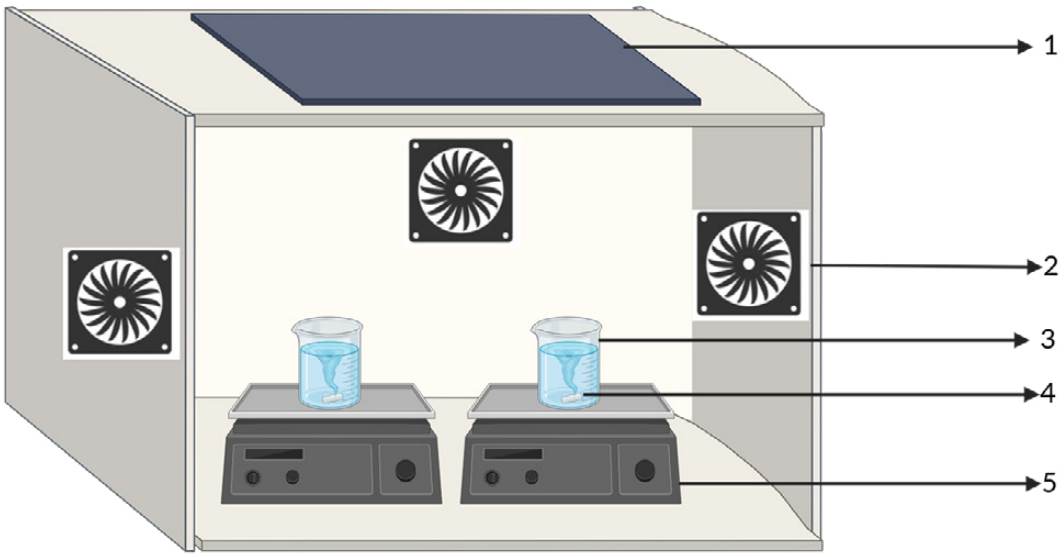


Figure 1. Schematic diagram of the experimental photoreactor for photocatalytic inactivation, which is composed of a 400W white light LED lamp (1), three fans (2), two 100 mL beakers (3), two magnetic stirring bars (4), and two magnetic stirrers (5).

All glassware, magnetic stir bar and micropipette tip rack were sterilized in an autoclave at 121 °C for 15 min before each photocatalytic inactivation experiment. A 100 mL beaker was used to carry out the photocatalytic reactions. For this, 15 mg of BiOI microspheres were added to 49.5 mL of NaCl 0.9% solution and 0.1 M NaOH was used to adjust the pH to 7.0 to ensure the survival of the bacterial strain. Then, the solution was sonicated for 10 minutes to disperse the photocatalyst. After that, the entire pellet of bacterial cells previously washed was added to the solution to complete a volume of 50 mL. The density of viable cells in the solution was approximately 1x107 CFU/mL. Subsequently, the solution was stirred at 300 rpm throughout the experiment to ensure contact between the bacterial cells and the photocatalyst surface. In a typical procedure, the experiment solution was stirred in dark conditions for 30 min to ensure the adsorption-desorption equilibrium between the photocatalyst and bacteria cells. After that, the suspension was stirred under visible light irradiation for 90 min. Aliquots of 100 µL were taken every 30 minutes, and serial dilutions were subsequently prepared. The density of viable cells was determined by the plate count method. For this, the cell suspensions from each serial dilution were spread on Mueller Hinton Agar (MHA) using a Digralsky handle and then incubated at 37 °C for 24 h. Finally, the number of colonies formed was counted to obtain the bacterial density (CFU/mL). The negative control consisted of a bacterial suspension (1x107 CFU/mL) in NaCl 0.9% solution. Two replicates were made for each serial dilution and the assay was performed in triplicate.

2.3.3 Cyanobacteria inactivation

The experiments were performed in a photoreactor composed of a 250 ml Erlenmeyer flask and a 400W lamp (Figure 1). The cyanobacterium *Microcystis sp.* was grown in Blue Green 11 (BG-11) medium for two weeks. The BG-11 medium was changed to a 0.9% saline solution by centrifugation, and the residual medium was discarded. 50 mg of BiOI powder was added and dispersed in 150 mL of cyanobacteria biomass (85 µg/L of chlorophyll-a). After, it was stirred for 30 minutes in dark conditions to allow a complete interaction of the photocatalyst surface with the cyanobacteria biomass. Subsequently, the lamp was turned on and the solution was irradiated for 390 min. A negative control test was performed without microspheres under the aforementioned conditions.

The amount of chlorophyll-a was quantified as an indicator of cell inactivation (Song et al., 2018). Aliquots of 10 mL of the sample were taken each hour and then filtered by vacuum filtration using a glass fiber membrane (0.47 µm in diameter). After, the membrane was placed in a 15 mL falcon tube containing 6 mL of acetone, and it was refrigerated overnight at 4°C (5-18 h) for chlorophyll-a extraction. The chlorophyll-a absorbance was measured using a UV-VIS spectrophotometer, and the concentration was calculated using the following equation (Fan et al., 2020):

$$chlorophylll_a \left(\frac{mg}{L}\right) = \frac{[11.85(OD_{664} - OD_{750}) - 1.54(OD_{647} - OD_{750}) - 0.08(OD_{630} - OD_{750})]V1}{2V_2L}$$

Where OD is the optical density, V1 represents the volume of the extract (mL), V2 is the volume of the water sample (mL), and L is the optical path of the cuvette (cm).

Finally, to determine the inactivation percentage the following equation was used (Fan et al., 2022):

$$Cyanobacteria\ inactivation\ percentage\ (\%) = \frac{C_i - C_f}{C_i} * 100$$

Where Ci represents the initial chlorophyll-a concentration (µg/L), and Cf represents the final chlorophyll-a concentration (µg/L).

2.3.4 Toxicity assays

2.3.4.1 Hemolytic assay

In this experimental phase, 2 mL of horse blood was placed into a 50 mL conical tube, followed by centrifugation at 1000 rpm for 5 minutes. Upon completion of centrifugation, the supernatant was carefully discarded, and the red blood cells (RBCs) were meticulously washed using 30 mL of sterile PBS solution. This washing process was repeated until the supernatant became clear. Subsequently, the RBCs were combined with PBS to achieve a final volume of 50 mL, with a RBC concentration of 4% (Neun et al., 2018). For the preparation of serial dilutions of the BiOI nanostructure, sterile 2X PBS was employed. Various concentrations (2000, 1000, 500, 250, 125, 62.5, 31.25, 15.62, 7.81, and 3.90 µg/mL) were established and distributed into microtubes, following the methodology proposed by Zgoda et al., in 2001. Each serial dilution was performed in quintuplicate. Specifically, 200 µL of the corresponding nanostructure dilution was combined with 200 µL of the 4% RBC solution. As part of the experiment, a positive control was implemented, involving 200 µL of the 4% RBC solution mixed with 195 µL of PBS and 5 µL of Triton X-100. Conversely, a negative control was established, comprising 200 µL of the 4% RBC solution and 200 µL of PBS alone. Subsequently, all test tubes were incubated at 37°C for 120 minutes in a dark environment, with the microplate shielded by aluminum. Following incubation, they were subjected to centrifugation at 1000 rpm for 5 minutes, after which 200 µL of each supernatant was transferred into a 96-well microplate (Neun et al., 2018).

Plates were finally read at OD 570 nm in a microplate reader. It was calculated using the following equation:

$$hemolysis\ (\%) = \frac{(A - AO)}{(AX - AO)} * 100$$

Where A is the nanostructure solution reading, AO is the negative control reading, and AX is the positive control reading (Lippi et al., 2014).

2.3.4.2 Micronucleus assays

The *Oreochromis niloticus* fry was divided into two experimental groups and a control group, each consisting of 5 fry. The specimens were kept in an aquarium with five liters of water and an internal temperature of 27°C ± 3°C. After a 2-week acclimation period, the fish were fed three times per day with an artificial diet. The two experimental groups started receiving

doses of BiOI (0.176 g/portion) mixed with the food (0.5 g) for 5 days. As in Figure 2, blood samples were randomly collected after 24, 48, and 72 h of exposure to BiOI microspheres by cardiac puncture using a heparinized 2 ml needle flushed with EDTA.

The dermis, intestine and gills were analyzed to verify the bioaccumulation of BiOI microspheres using an optical microscope. For each treatment sample, a thin blood smear was placed on a pre-cleaned microscope slide, which was then immersed in 96% ethanol for 15 minutes. The slides were air-dried, stained with 6% Giemsa stain for 15 min, rinsed with tap water, allowed to dry, and examined under a microscope at 40X magnification. Circular or oval bodies, smaller than the main nucleus, non-refractive, with the same staining pattern and focus as the main nucleus, were scored as micronuclei.

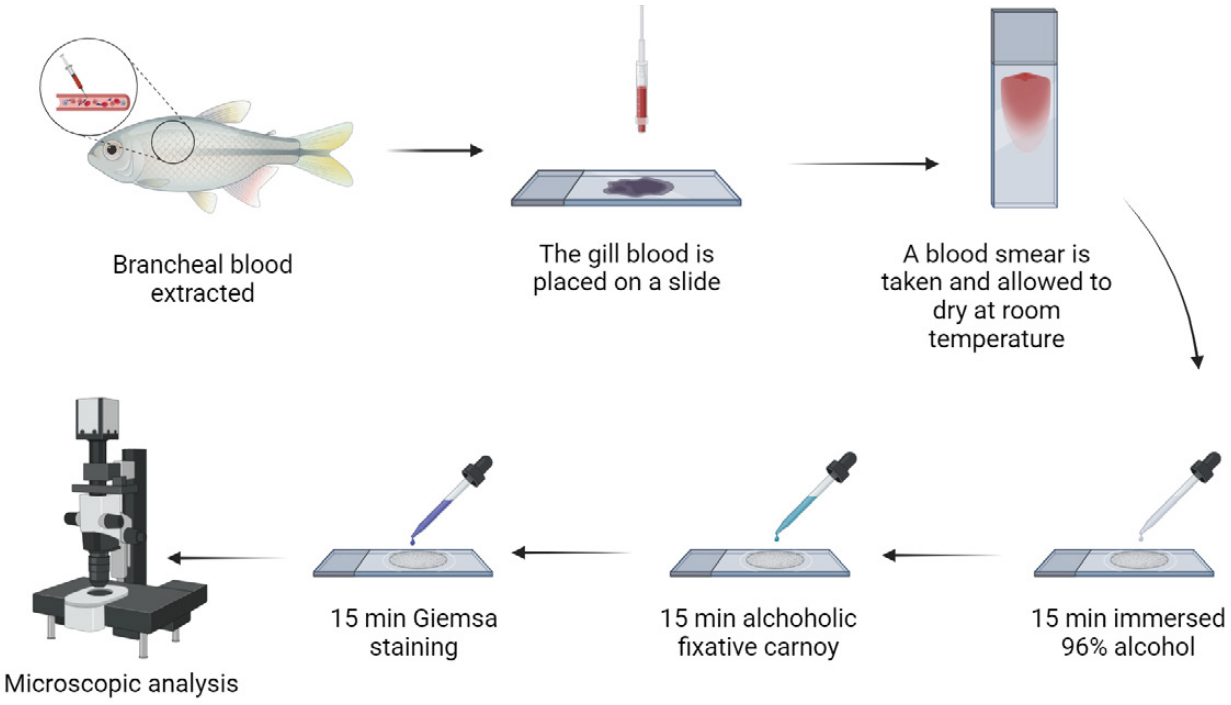


Figure 2. Giemsa staining process for the micronucleus assay.

3. Results and Discussion

3.1 Structural, morphological, and chemical characterization

The crystal structure of BiOI-flower-like microspheres was investigated by XRD. As can be seen in Figure 3a, the diffraction peaks of the as-prepared sample are identical to the XRD patterns of the tetragonal structure of BiOI reported in the ICSD card #073-2062. The main diffraction peaks are at 2Θ: 8.85°, 29.03°, 31.72°, 45.52°, 54.95°, and 66.52°, which are according to the results reported in a previous study (Zuarez-Chamba et al., 2022a). The high intensity of the peak located at 31.72° indicates that there was preferential growth in the direction of the crystalline plane (110). Also, the high intensity of the diffraction peaks reveals that the sample is highly crystalline. Moreover, no additional diffraction peaks corresponding to impurities or another crystalline phase were observed, which means a pure sample.

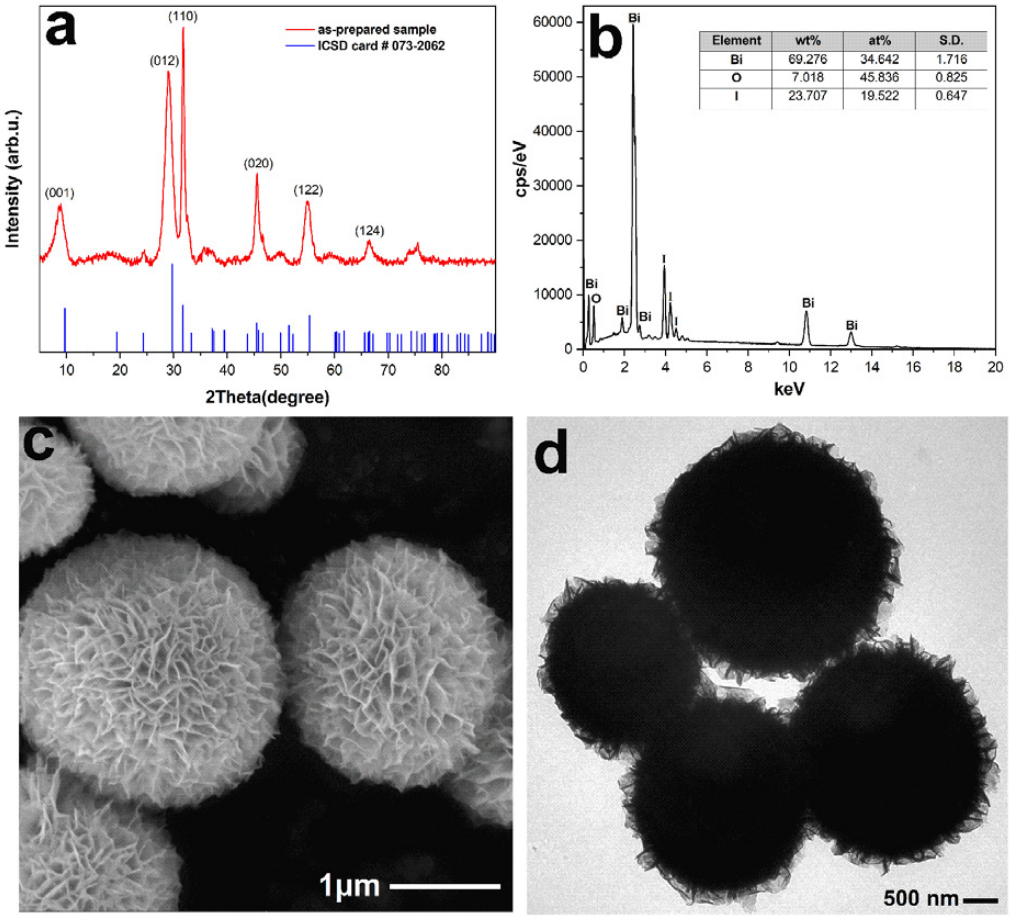


Figure 3. XRD patterns (a), EDS analysis (b), SEM (c) and TEM (d) images of BiOI flower-like microspheres.

The elemental chemical composition was analyzed by EDS. As is shown in Figure 3b, the prepared sample is only composed of Bi, O, and I elements, which confirms that the sample is pure. On the other hand, the SEM image depicted in Figure 3c reveals that the sample is composed of microspheres with an average diameter of 1.534 ± 0.524 μm, and its surface is composed of hundreds of nanosheets with a thickness of a few nanometers. The TEM image in Figure 3d confirms that the microspheres are surrounded by nanosheets.

3.2 Photocatalytic activity

Raw water can contain organic matter, ions, organic and inorganic chemicals, and microbial pollutants (e.g., bacteria, cyanobacteria). For this reason, micro- and nano-photocatalysts must be able to degrade several types of pollutants. In this sense, the photocatalytic efficiency of BiOI flower-like microspheres was evaluated using an endocrine disruptor compound (BPA) as a chemical pollutant, and *E. coli* and cyanobacteria as microbial pollutants.

As is shown in Figure 4a, BiOI flower microspheres could degrade up to 90% of BPA after 3 hours of visible light irradiation. The reduction in bacterial cell viability was also evident under visible light irradiation conditions, in which 0.3 mg/mL of BiOI microspheres decreased the bacterial density from 1 x 107 to 1.19 x 106 CFU/mL in 120 min (Figure 4b). This result was consistent with a previous study in which 0.5 mg/mL of BiOI microspheres presented photocatalytic activity against E. coli, achieving the inactivation of 20% of bacterial cells in 120 min (Wang et al., 2015). On the other hand, chlorophyll-a was used as an indicator of the photocatalytic inactivation of cyanobacteria (Fan et al., 2020). As seen in Figure 4c, the initial concentration of chlorophyll-a was 85 μg/L and it was diminished to 25.32 μg/L in the presence of BiOI microspheres. This indicated that 70.21% of cyanobacteria were inactivated after 390 min of visible light irradiation. This inactivation percentage is higher compared to previous studies of MOF-235 photocatalyst, in which 26.4% of *M. aeruginosa* was inactivated at the same time (He et al., 2021).

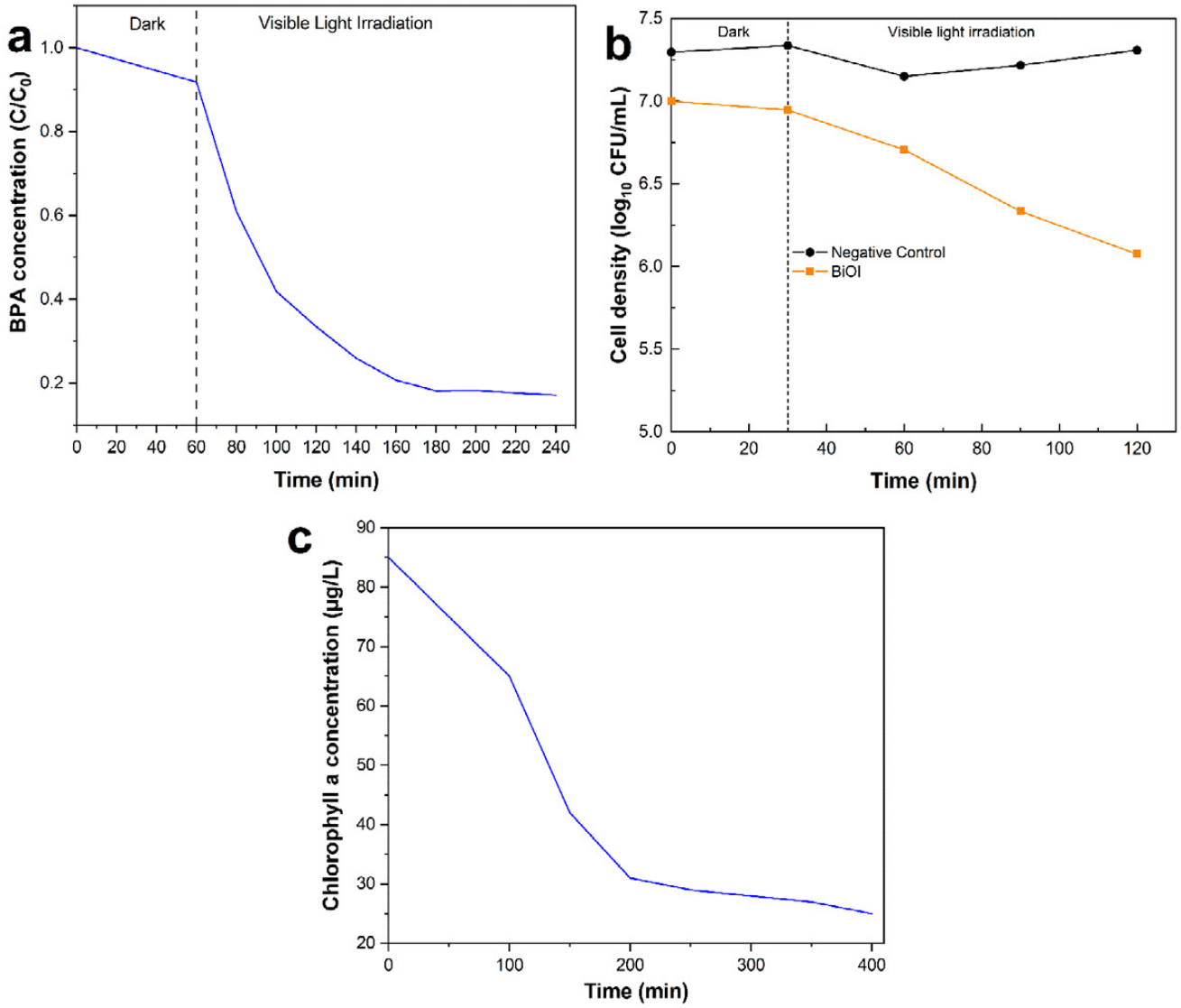


Figure 4. BPA degradation (a), bacteria (b) and cyanobacteria inactivation (c) by BiOI flower-like microspheres.

The inactivation process of bacteria and cyanobacteria begins with the activation of the semiconductor material. When the photocatalyst is exposed to visible light irradiation, an electron in the valence band (VB) is excited with a photon from the light (Simonsen, 2014). The photoexcited electron jumps to the conduction band (CB), leaving a hole in the VB. After that, the photoexcited electron and hole can react with acceptor and donor electron species, respectively, to form ROS (Hernández-Ramírez & Medina-Ramírez, 2015). In solution media, the photoexcited electrons can react with dissolved oxygen, resulting in the formation of a superoxide radical($\cdot O_2^-$) Meanwhile, holes can react with water molecules or hydroxyl ions to form hydroxyl radicals ($\cdot OH$) (Zuarez-Chamba et al., 2022b).

Organic compounds and biomolecules (i.e. lipids, proteins, and DNA) are highly susceptible to being broken down by the attack of ROS, holes, or photoexcited electrons (Zuarez-Chamba et al., 2022b). In this sense, bacteria and cyanobacteria can be inactivated through two routes: a direct route and an indirect route. Through the direct route, the damage to the outer and inner cell components occurs mainly by the oxidation or reduction conducted by ($\cdot OH$) and ($\cdot O_2^-$) radicals, respectively. Through the second one, the inactivation occurs via direct oxidation with holes and reduction with photoexcited electrons. However, for inactivation to occur via this route, the bacteria and cyanobacteria must be in close contact with the photocatalyst surface (Zuarez-Chamba et al., 2022b).

3.3 Toxicity tests

Most photocatalysts used in different research groups are powders dispersed in solution. Owing to this fact, the complete recovery of the powder from the treated water body is difficult in some cases, and consequently, a certain amount is discharged to water effluents (Zuarez-Chamba et al., 2022b). Therefore, to mitigate negative effects on ecosystems and human health after their use, it is important to first evaluate their cytotoxicity and ecotoxicity.

When assessing the cytotoxic impact of BiOI microspheres on erythrocytes in light and dark conditions, it becomes evident that the extent of hemolysis remains below 5% even at the highest concentration, as depicted in Figure 5a. Conducting these analyses in both light and dark environments is crucial due to the photosensitive nature of microparticles, which may yield varying effects on different components of their surroundings (Romano et al., 2021; Zuarez-Chamba et al., 2022b). In this instance, the microparticles may exert distinct impacts on erythrocytes when exposed to light as opposed to darkness. According to the results shown in Figure 5a, no negative effects of BiOI microspheres on erythrocytes were observed in dark conditions. It is important because blood cells cannot be damaged by interactions with the microsphere surface. Moreover, by increasing the concentration of BiOI microspheres, the hemolysis percentage was close

to zero both in dark (Figure 5a) and light conditions (Figure 5b). This suggests that even at a concentration of 2000 ug/ml BiOI microspheres are not harmful to blood cells. However, the hemolysis test plays a pivotal role in cytotoxicity assessments, serving as the initial indicator of how these microspheres might interact in subsequent cell line assays or with tissues and organs. To obtain a deeper understanding on nanoparticle cytotoxicity, it is imperative to conduct further tests on cell lines and model organisms (Ribeiro et al., 2020).

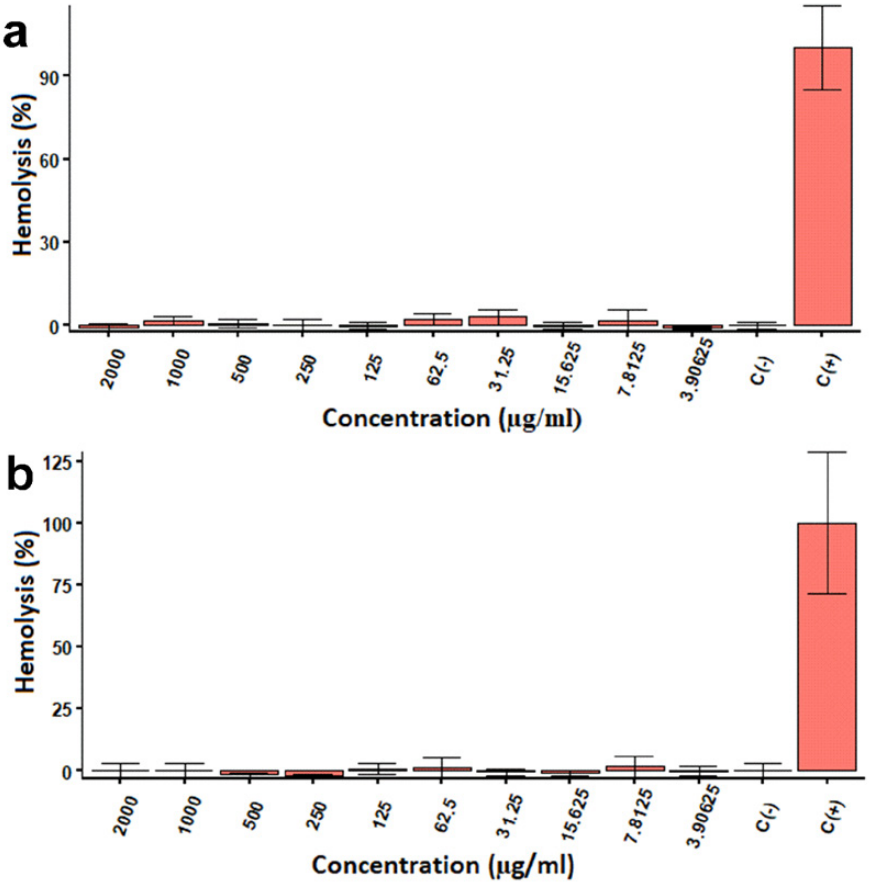


Figure 5. Hemolysis assays using different concentrations of BiOI microspheres at dark conditions (a) and under visible light irradiation (b). C(-): negative control; C(+): positive control.

On the other hand, genotoxic damage in fish occurs because of DNA structure modification due to xenobiotic agents (chemical, physical or biological). At the nuclear level, the formation of micronuclei, nucleoplasmic bridges (NP) and nuclear buds (NB) during cell division is considered a biomarker of genotoxic damage (Roldan, 2023). In this sense, as is shown in Figure 6A, fingerlings exposed to 0.176 g of BiOI died progressively during the period of the experiment.

Even though, at 48 hours, a fingerling was found with their organs outside of the body, analysis of peripheral blood smears from the studied fish showed that the erythrocytes formed nuclear abnormalities: small circular or ovoid particles that resemble a nucleus but vary in size and position in the cytoplasm (Figure 6B). The characteristic orange color of the BiOI microspheres allowed the bioaccumulation to be distinguished in the different tissues extracted from the fingerling. It was observed that BiOI microspheres were able to bioaccumulate in the gut, gills and dermis (Figure 6C-E).

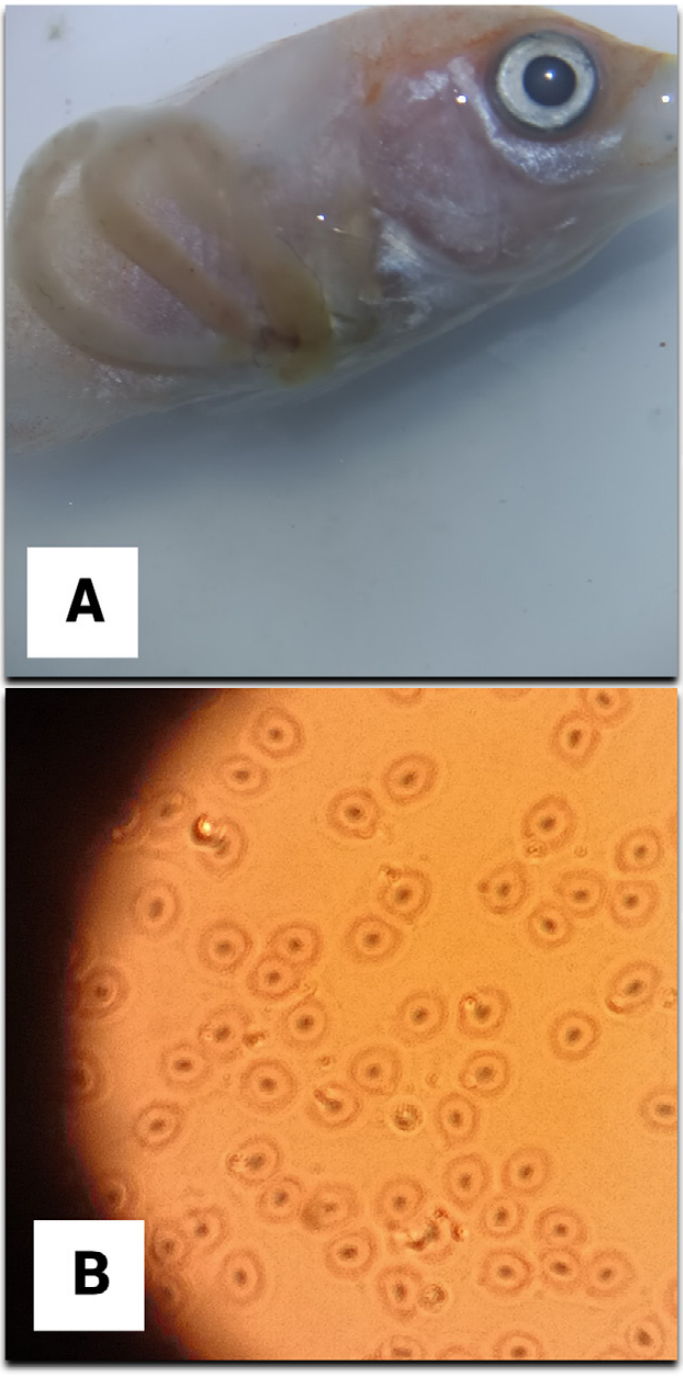
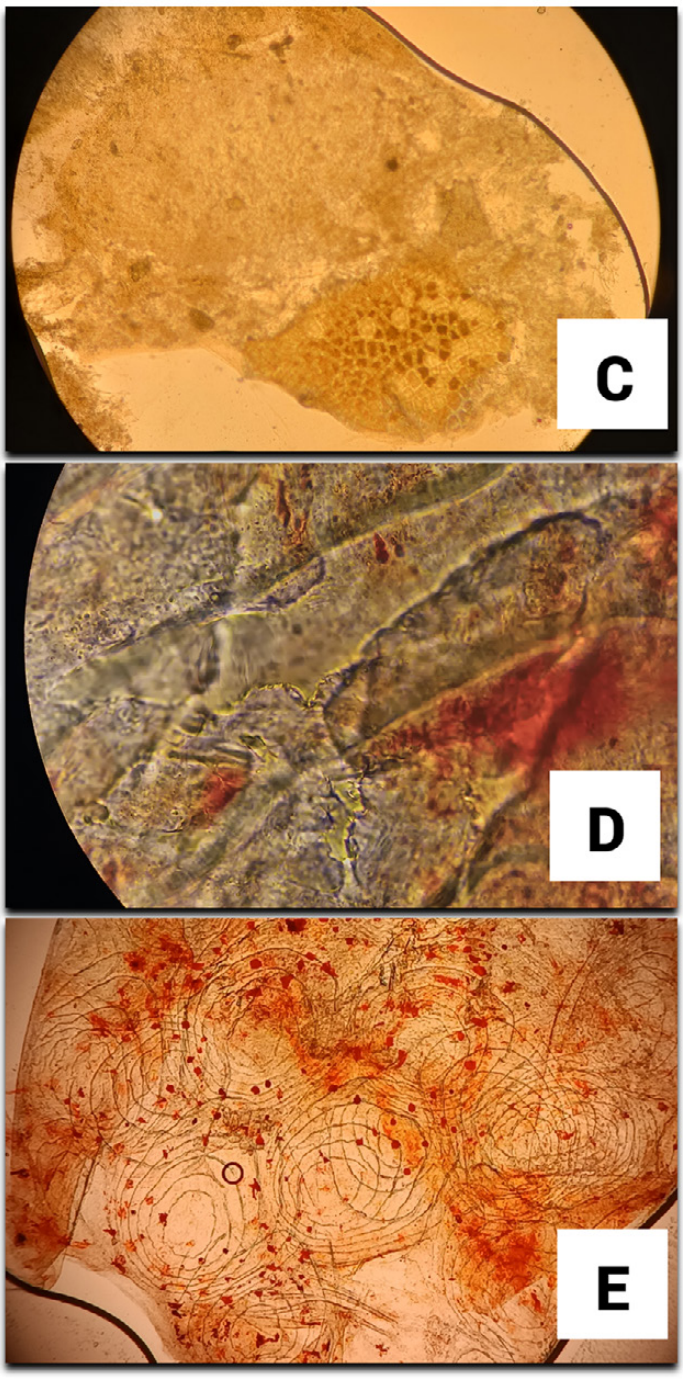


Figure 6. Ecotoxicity test. (A) Fingerling *O. niloticus* exposed to BiOI flower-like microspheres during 48 h. (B) Blood cell micronuclei. (C) Accumulation in the intestine. (D) Accumulation on gills. (E) Accumulation in the dermis.



The toxicity effect of BiOI flower-like microspheres observed on horse blood cells and tilapia fingerlings may be attributed to several factors. Firstly, there may be significant differences in cellular composition and response mechanisms (Roldan, 2023). Additionally, red blood cells are highly specialized for oxygen transport and do not have a nucleus, whereas fish cells are nucleated. This difference in cellular structure and function can influence susceptibility and response to toxic micro- and nano-structures (Roldan, 2023).

On the other hand, the exposure and absorption of the BiOI microspheres by horse blood cells and fish cells may follow different pathways. In the hemolysis assay, blood cells were directly exposed to BiOI microspheres. Meanwhile, in the micronucleus assay, the BiOI microspheres must first be absorbed by the fish through the water and the food. After the absorption, BiOI microspheres accumulate and interact with cells from different tissues.

Moreover, the detoxification and elimination mechanisms for toxic substances differ between horses and fish. It is possible that fish, such as tilapia fingerlings, have less efficient defense and excretion mechanisms to eliminate BiOI microspheres when they live in contaminated water (DaSilva Pierri et al., 2021). Thus, there is an accumulation in different tissues such as the gills, intestines, and dermis. Therefore, this could explain the presence of micronuclei in tilapia fingerlings and the finding of a dead fingerling with its organs protruding.

4. Conclusions

The solvothermal method has been demonstrated to be an efficient method to obtain BiOI flower-like microspheres. These micro- and nano-structures exhibited outstanding properties for water sanitation. These structures could successfully degrade BPA and waterborne microorganisms like *E. coli* and toxic cyanobacteria. However, it is still necessary to improve the efficiency of BiOI flower-like microspheres to achieve complete inactivation of the pollutants in a shorter length of time and to reduce their toxicity in some types of cells from fish. In this sense, we consider that more research is needed to understand the effects of BiOI flower-like microsphere accumulation in fish tissues. On the other hand, the low toxicity that BIOI showed on blood cells, even at 2000 ug/ml, is a great first step to continuing cytotoxicity research in cell lines. Nonetheless, BiOI flower-like microspheres could be a good option for water treatment.

5. Recommendations

Finally, this chapter establishes a roadmap for continuing research focused on the development of BiOI and other photocatalytic materials, as well as assessing their feasibility in environmental applications. Future studies are recommended to optimize current synthesis protocols that enhance the engineering of crystal facets and morphology to produce materials with improved properties.

6. Acknowledgments

We would like to express our sincere gratitude for the generous support provided by the Erasmus + grant: 619346-EPP-1-2020-1-DE-EPPKA2-CBHE-JP. This funding has been instrumental in the successful development of the research project, enabling us to explore and present our findings in this chapter.

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CHAP

PTER 6

Nature-based living lab (NB LAB): an undergraduate research experience at the Upper Amazonia

Architecture for research: Reengineering of a scientific station on Amazonian biodiversity

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All authors reviewed, commented, and approved the final version of the manuscript; all authors declare no conflict of interest.

Abstract

There is a need to redesign the research station of the Universidad Regional Amazónica Ikiam located in the Colonso Chalupas National Park (PNC) to convert the current deteriorated and poorly managed station into a habitable, functional, efficient, and sustainable place for visitors and researchers. The first step to achieving this objective was to conduct a bibliographic analysis of similar environments, identifying architectural solutions applicable to the case study. After analyzing of existing resources, the next step was to apply surveys and interviews to determine the needs and experiences that would ensure a design that would meet the appropriate requirements for users. Finally, reengineering of the station was proposed: the design promotes an environment conducive to scientific research as well as conservation of the natural environment through minimization of environmental impact and responsible resource use practices. Most of the fieldwork was carried out by undergraduate university students under the supervision of Ikiam faculty.

Keywords:

Architecture; research; reengineering; scientific station; undergraduate research experience

Resumen

Existe la necesidad de rediseñar la estación de investigación de la Universidad Regional Amazónica Ikiam ubicada en el Parque Nacional Colonso Chalupas (PNC) para convertir la actual estación deteriorada y mal administrada en un lugar habitable, funcional, eficiente y sostenible para visitantes e investigadores. El primer paso para lograr este objetivo fue realizar un análisis bibliográfico de entornos similares, identificando soluciones arquitectónicas aplicables al caso de estudio. Tras realizar un análisis de los recursos existentes, el siguiente paso fue aplicar encuestas y entrevistas para determinar las necesidades y experiencias que garanticen un diseño que cumpla los requisitos adecuados para los usuarios. Por último, se propuso una reingeniería de la estación: el diseño promueve un entorno propicio para la investigación científica, así como la conservación del medio natural mediante la minimización del impacto ambiental y prácticas responsables de uso de los recursos. La mayor parte del trabajo de campo fue realizado por estudiantes universitarios bajo la supervisión del profesorado de Ikiam.

Palabras clave:

Arquitectura; investigación; reingeniería; estación científica; experiencia investigadora universitaria

Introduction

The Colonso Chalupas Scientific Station has an area of 75.93m² (Cuenca, 2019). Currently, the activities and visits of researchers are restricted due to the deterioration of its infrastructure. The reactivation of this space requires a reengineering through a flexible structural system that optimizes resources through modularity, an innovative construction system that uses materials efficiently, an active bioclimatic system that takes advantage of renewable energy sources, and a passive bioclimatic system that takes advantage of the environment for the natural balance of comfort. Figure 1 shows the location of the intervention site at the coordinates latitude: 0°56'7.20 "S, longitude: 77°55'36.39".

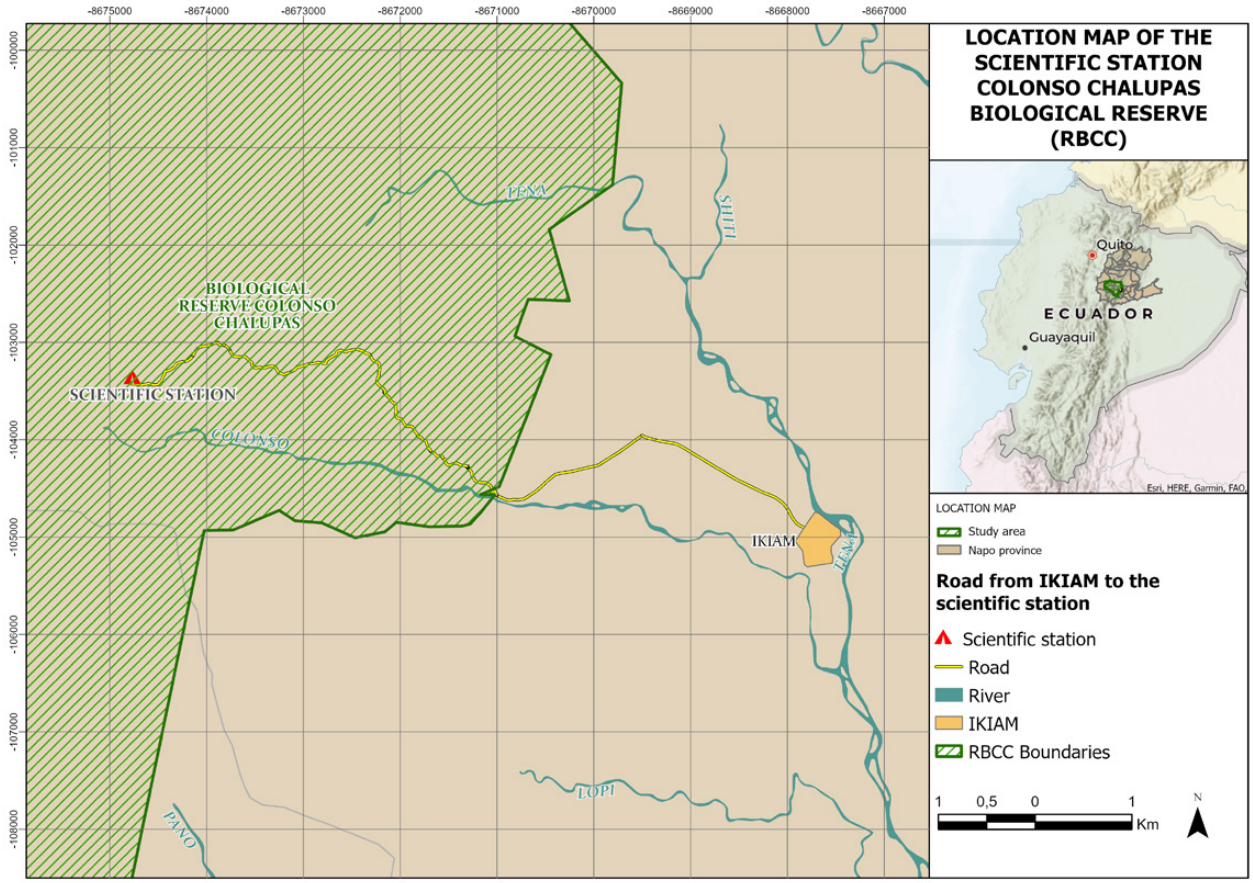

Figure 1. Shows the location of the current scientific station.

In this context, the research described the following objectives:

1. Develop a literature review that includes a comparative analysis of facilities in similar contexts to determine active and passive bioclimatic design strategies.
2. Conduct a complete diagnosis of existing facilities to identify areas for conservation, improvement, or restoration.
3. Apply surveys and interviews to collect qualitative and quantitative data to gain a deep and diverse understanding of user perceptions and experiences.
4. Define a comprehensive architectural program for the redesign of the science station, ensuring adequate functionality and spatial hierarchy to facilitate research and habitability.
5. Design the architectural re-engineering of the science station, innovating in the design of structures through the use of active and passive bioclimatic design techniques, and incorporating the use of low-impact green building materials to create spaces that foster research and provide sustainable, state-of-the-art solutions.

Methodology

The methodology is divided into five fundamental stages: (i) benchmark analysis; (ii) analysis of the existing; (iii) surveys and interviews; (iv) definition of the architectural program, functionality, and spatial hierarchy; and (v) development of the reengineering proposal.

This orderly process allows us to have a solid proposal that meets proven design standards, is consistent with the particularities of the area, and is based on an approach to users and their experiences.

For the development of the project, there is a population of 50 users, including research professors and national and foreign students.

Benchmark analysis

Following the approach proposed by Gastón and Rovira (2007), the analysis was carried out in two stages. The first stage involved collecting data from similar buildings to observe the overall characteristics of the study object,

along with critiquing the information to filter substantial data about the building. The second stage focused on the analysis of architectural projects to define indicators and quantitative evaluation criteria. These indicators have been adapted from LEED certification (Morales, 2017) for the application of sustainable practices.

Analysis of existing

An architectural survey, photographic record, digitalization of the current state of the scientific station, and development of pathology files were carried out (Sánchez, 2013). With this, a morphological and constructive baseline of the building was established (Almagro, 2004) to determine the state of conservation of each element, the causes, and the intervention to be implemented. Complementarily, the analysis of location, environment, and ethnography was incorporated (Gallardo, 2015).

Surveys and Interviews

The surveys and interviews focused on essential variables to capture the user experience. These include length of stay and specific use of spaces, identifying key areas for improvement; size of the visitor group, relevant for spatial planning; type of activities performed, crucial for adapting the functionality of the station; perceived quality of spaces and adequacy of furniture and lighting, providing insights on comfort and efficiency. In addition, we inquired about the difficulties experienced, to identify and address specific problems, and the type of clothing used, reflecting the adaptability of users to the station environment. These variables, when analyzed, offer a detailed guide to optimizing the station, aligning it with the real needs of its most frequent users.

To calculate the optimal sample size in a context where the population is relatively small (50 users), the formula for simple random sampling with correction for finite populations was applied; this formula adjusts the sample size to take into account that the population is finite, which is especially relevant in small populations:

$$n = \frac{N \times \frac{Z^2 \times p \times (1-p)}{e^2}}{N - 1 + \frac{Z^2 \times p \times (1-p)}{e^2}}$$

Where:

n is the sample size.
N is the size of the population (50 users).
Z is the value corresponding to the desired confidence level (1.96 for 95% confidence).
p is the estimated proportion of the attribute present in the population (0.5).
e is the tolerable margin of error (0.05 for 5%).

The sample size calculated for a population of 50 users, with a confidence level of 95% and a margin of error of 5%, is 40; therefore, 40 users were surveyed. Of this 100%, 82% were students, and 18% were faculty researchers.

As part of the redesign project of the scientific station, a series of interviews were conducted to gather detailed information and concrete perspectives on the use and needs of the intervened space. We interviewed 100%

of the researchers, that is, the 7 professors, who are characterized by frequent use of the facilities. This selection is justified for the following reasons:

1. They possess significant knowledge and practical experience, which provides a perspective on the current strengths and weaknesses of the station.

2. As regular users, they can identify a wide range of needs and expectations that may not be evident to less frequent users.

3. By understanding the needs of more frequent users, we can ensure that the redesign not only solves current problems but also fosters long-term sustainability and effective use of the station.

In addition, a semi-structured survey was implemented to combine multiple-choice, open-ended, opinion scale-based questions to obtain quantitative data and capture qualitative perspectives on user experiences, needs, and perceptions. The multiple-choice questions facilitated the collection of specific responses on functional and operational aspects of the station, while the open-ended and opinion-scale questions provided a comprehensive understanding of the redesign project.

Finally, in the case of interviews, considering that the objective is to obtain detailed information from frequent users, semi-structured interviews were applied, which are ideal for exploring participants' thoughts and experiences but have a guide for key issues and questions.

Definition of the architectural program, functionality, and spatial hierarchy.

Quantitative and qualitative data were collected through surveys and interviews (Gallardo, 2015). The interviews highlight three aspects: characterization of the age group and gender; use of space, routines, and schedules; and opinion on improvements. The surveys include questions related to functional perception, spatial comfort, the improvement of living space, and time spent at the scientific station.

Development of the reengineering proposal

In the book "Architecture as Science", Araujo (2019) refers to the criteria governing architectural projects consisting of six plans: spatial, constructive, structural, energetic, geometric, and plastic, to develop the graphic solution of the architectural project using Autodesk Revit software, a 2D and 3D modeling tool that generates floor plan drawings, architectural sections, volumetry to obtain graphic representations, generation of architectural documentation, and quantification of materials (Sanchez et al., 2019). Once the 3D model is obtained, it is imported into the Twinmotion software, where visualizations are generated with real-time rendering for the creation of interactive graphics and complete virtual tours of the architectural project to improve the communication of ideas (Iglesias, 2021).

Results and Discussion

Benchmark analysis

The comparative analysis identified seven top-rated projects (Figure 2) that present essential characteristics for the reengineering proposal. The seven selected projects present essential features of bioclimatic architecture adapted from LEED certification. They take advantage of the local climate to achieve optimal thermal and visual comfort, using solar energy and other environmental sources for air conditioning and lighting. In addition, non-invasive modular formal elements are incorporated that integrate and blend in with the surroundings, providing landscape value through their facades. These projects also have functional elements that guarantee dynamism, flexibility, and optimal use of the architectural space. As for the construction elements, dry construction strategies are applied to reduce costs and promote sustainability. Priority is given to the use of active and passive bioclimatic systems. Figure 2 shows the benchmark analysis before the proposal approach.

Figure 2. Benchmarking of the seven analyzed projects.

The Forestry Foundation Administrative Headquarters Juréia-Itatins project (Figure 2A), by 23SUL architects, incorporates as its main strategy an interior corridor for air exchange, cantilevers for climate protection, and to protect the facade from rain and generate shade (23 SUL Arquitectos, 2021).

The Chocolab Integration Center Project, by MEC Arquitectura (Figure 2B) uses a low-tech permeable facade system that allows natural ventilation and light to enter the interior of the building. It uses a dry construction technique where materials are assembled on-site (MEC Arquitectura, 2019).

Jones Beach Energy and Nature Center, by nARQCHITECTS architects (Figure 2C), incorporates reused material from the existing building and incorporates active and passive bioclimatic systems for energy efficiency (nArchitects, 2020).

Coastal Marine Research Station, by Martín Hurtado Arquitectos (Figure 2D). It uses a load-bearing construction system, which allows for the optimization of materials through its modular system, thus allowing the integration of the volumetry with the landscape (Martin, 2010). The visitor center, Panama Jungle, by Brenda Gotti and colleagues (Figure 2E) incorporates a steel structure and wood panels. The structure adapts to the topography using piles and ramps to connect the spaces (ENSITU, 2005).

Macquarie University Incubator, by Architectus (Figure 2F), implements galleries to connect spaces. The facades are permeable to take advantage of natural lighting and ventilation; they use prefabricated modular elements (Architectus, 2017).

Cabin-tower Karadya BIO-RESERVA 2012, by Borrachia Architects Studio (Figure 2G), incorporates rainwater harvesting, geothermal heating, solar panels, and a roof that has an air chamber to ensure ventilation, and the construction does not exceed the height of the trees (Estudio Borrachía Arquitectos, 2012).

Analysis of existing

At the same time, an in situ diagnosis of the scientific station was carried out, prioritizing the evaluation of both structural and non-structural elements of the building. This process made it possible to determine that the reinforced concrete foundation (Figure 3A) maintained as it did and did not present pathologies that would indicate a structural risk. However, the bamboo columns and beams show considerable damage as a result of their constant exposure to external factors such as rain and weather (Figure 3B). Therefore, they can be recycled, to manufacture furniture, and replaced with new structural elements.

As for the non-structural elements, such as interior and exterior partitions, floors, and bamboo roofs (Figure 3E), significant deterioration was observed due to humidity and leaks in the roof. The current conditions indicate a critical situation that requires replacement. The plumbing system is obsolete since the water collection channels (Figure 3F) are broken and the water storage tank and toilet are out of service (Figure 3C). Meanwhile, the electrical system, such as the solar panels, is in disuse due to inadequate orientation for proper solar collection and power supply. To address these issues, the project proposed an action plan that includes structural repairs, improvement of the rainwater harvesting systems, and replacement of the partitions (Figure 3D) to ensure the long-term integrity and functionality of the scientific station.

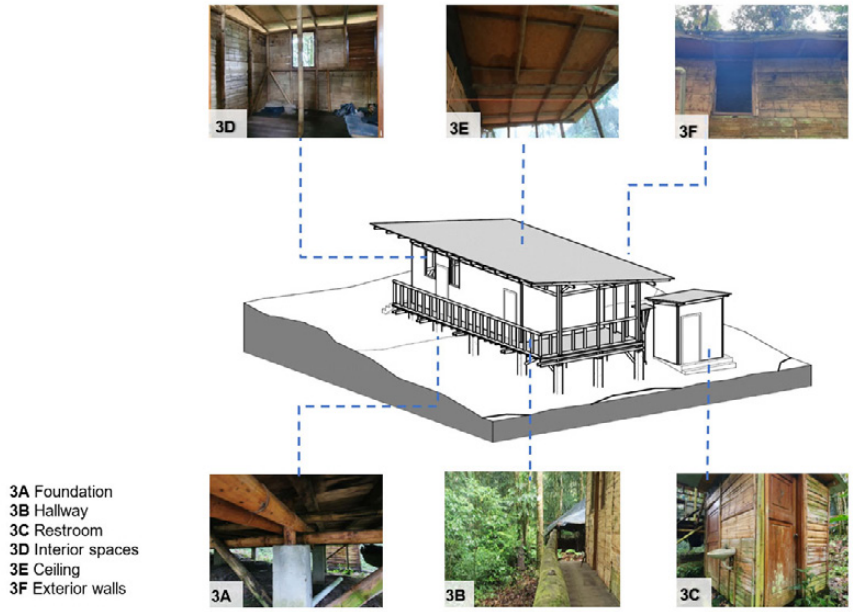

Figure 3. Current status of the Scientific Station (PNC).

Surveys and Interviews

Surveys and interviews show that 90% of visits to the scientific station usually last from 1 to 5 days, while the remaining 10% correspond to more than 5 days. As for visitor groups, the majority consist of between 10 and 20 people, although smaller groups of up to 4 people are also recorded. In addition, it was detected that the station does not have an area for unloading equipment upon arrival. It lacks a workspace with adequate lighting and storage furniture. Lacks a designated area for unloading equipment upon arrival. The meeting and breakout area is makeshift and has limited capacity. Additionally, sanitary facilities are separate from the station.

This information was incorporated into the architectural program of the new building: an arrival area at the entrance, the work area was expanded, the kitchen area was concentrated in a single space, and a meeting area was conceived with the creation of a rest area.

Based on the information gathered in the literature review, surveys, and interviews, we proceed to the following approach:

First of all, the principles that will govern the project, which are mainly based on the analysis of references, are made evident. The following strategies are com-

piled and incorporated from the projects studied: dry and modular construction systems, incorporation of prefabricated elements, recycling of existing construction elements, use of piles to adapt to the topography of the site, creation of corridors to ensure ventilation, extensive cantilevers to protect the natural elements, integration of the volumetry with the landscape, use of natural resources, water collection, solar panels, ventilation through the roof.

The second part of the architectural program lists the zones of the new scientific station: collaborative work areas, rest areas, and flexible interaction areas that adapt to different needs and activities. A central core is planned that separates the work area from the service area and serves to store laboratory equipment. In addition, the central core houses the stairs that connect to the rest area on the upper floor. In short, a hierarchical reorganization is proposed for the arrival of users, allowing adequate space for unloading luggage. In addition, the design allows access to the main floor, where there are flexible work areas for research activities. Finally, on the top floor is the rest area, conceived as a free space.

Figure 4 contains a QR that stores information on the re-engineering of the science station, plans and video of the existing one, plans, and video of the proposed one.

Development of the reengineering proposal

The architectural plan of the reengineering proposal shows three main zones: collaborative work areas, rest areas, and flexible interaction zones that can adapt to different needs and activities. A central core is incorporated to separate the work and service areas, functioning as a storage area for laboratory equipment. The central core houses the stairs connecting to the upper-floor rest area.

Figure 4.QR Existing and Proposal
Graphic Documentation.

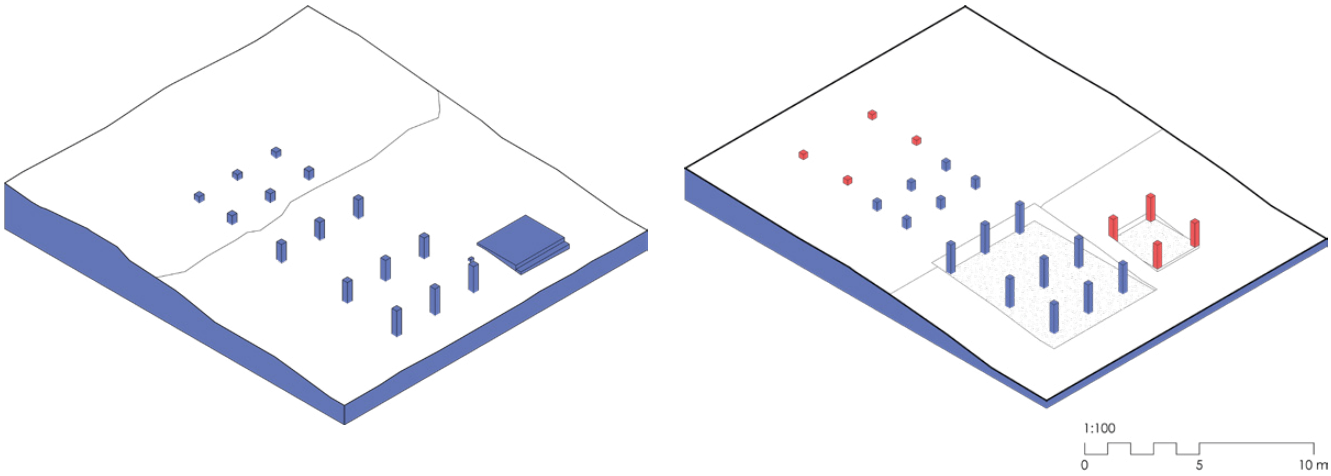

Figure 5A. Intervention made on the structural plan between the existing and the proposal.

Structural plan

Foundation reuse: After assessing the current condition of the site, the reuse of the reinforced concrete foundation provides a base for the scientific station's structural system. This approach saves costs and reduces the environmental impact associated with a new foundation. Sustainable and modular materials are used for the scientific station expansion area, making it easy to transport and erect.

Regarding the existing bamboo columns, the current assessment highlights moisture stains and cracks that put the structural integrity at risk. The proposal involves the columns complete replacement with a steel frame system.

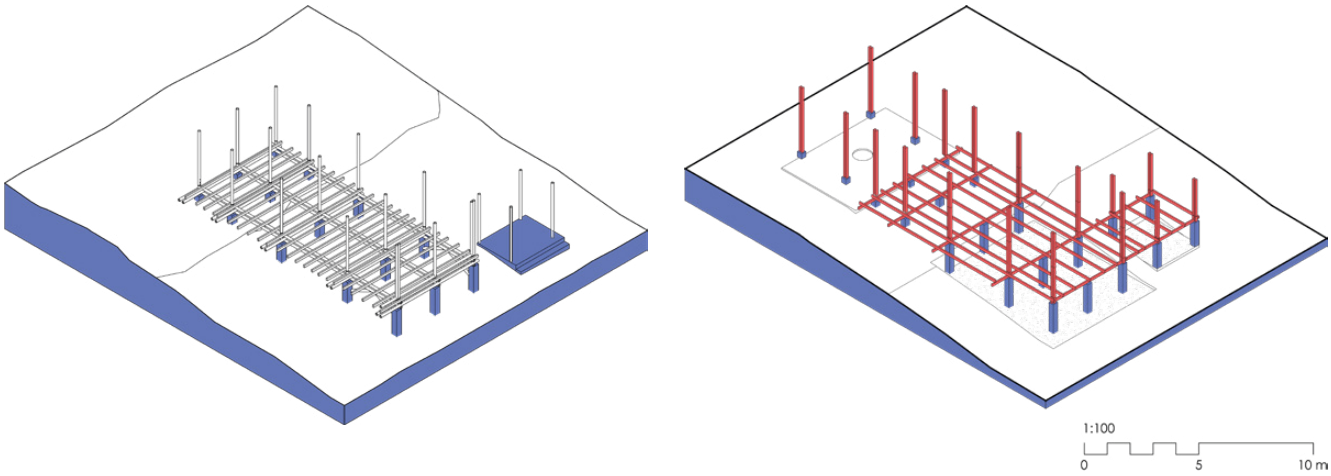

Figure 5B. Intervention made on the construction plan between the existing and the proposal.

Construction plan

The current condition of the floor decking shows wear and damage in several areas due to constant exposure to adverse weather conditions. The integral proposal involves the complete replacement of the floor with a reinforced, rain-resistant PVC wood material, thus offering a solution that ensures greater durability and safety.

It is crucial to highlight that the environmental conditions require the choice of low-maintenance materials, as users visit the station sporadically and for short periods, without carrying out any maintenance. In this context, a steel frame was chosen for the interior partitions, providing durability and resistance. Likewise, the roof structure is made of steel frames, thus protecting the entire building, especially its envelope of exterior panels made of bamboo slats.

This strategic choice of materials not only meets the need for resistance to adverse weather conditions but also ensures minimum maintenance, meeting the specific requirements of an environment where user interaction is sporadic.

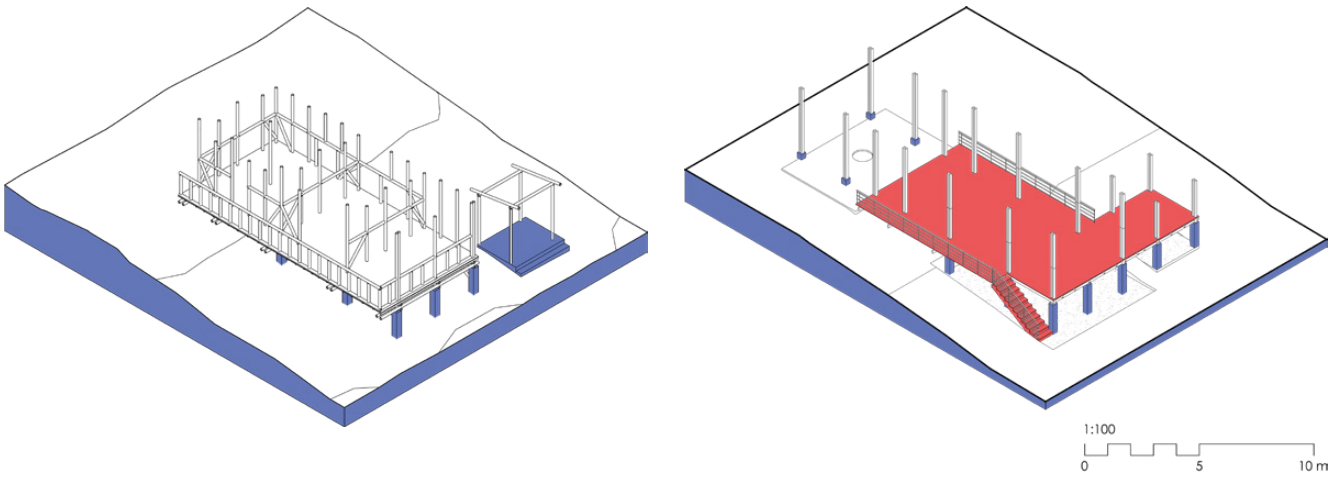

Figure 5C. Intervention made on the geometric plan between the existing and the proposal

Geometric plan

The form is defined through the measures and lines that configure the spaces of the project, giving it shape, unity, modularity, proportion, and geometric measure. This is considering the pre-existing conditions of the foundations, materials, and site.

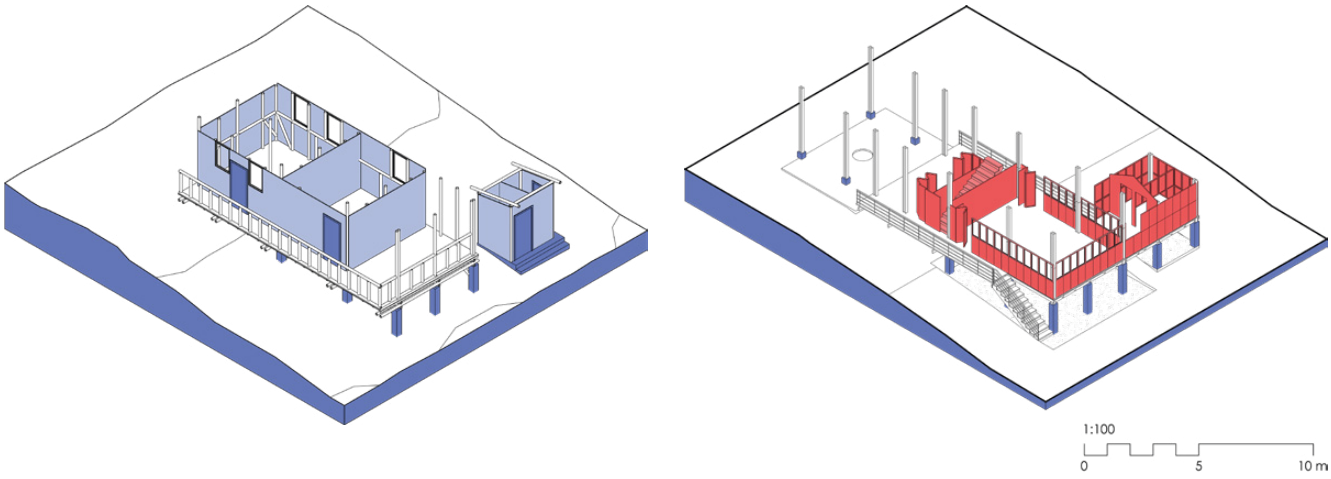

Figure 5D. Intervention made on the space plan between the existing and the proposal.

Space Plan:

Functional redistribution: the distribution of three areas is optimized: work, rest, and service. This reorganization improves the flow of activities and habitability in this environment.

Open and flexible spaces: a strategy designed to adapt the work to the different needs and research activities, considering variable periods of stay. The project has been configured in such a way that the spaces relate to the immediate surroundings and the users.

Relationship with the natural context: integrates the exterior landscape with the scientific station and dynamizes the living spaces.

Recycling of the building envelope: this is a strategy to make the best use of existing material. Bamboo will be used in interior structures, partitions, and furniture design instead of being discarded. A structure based on the steel frame system is proposed because of its efficiency in transporting and assembling elements onsite, its maintenance-free nature, and its durability in adverse climates.

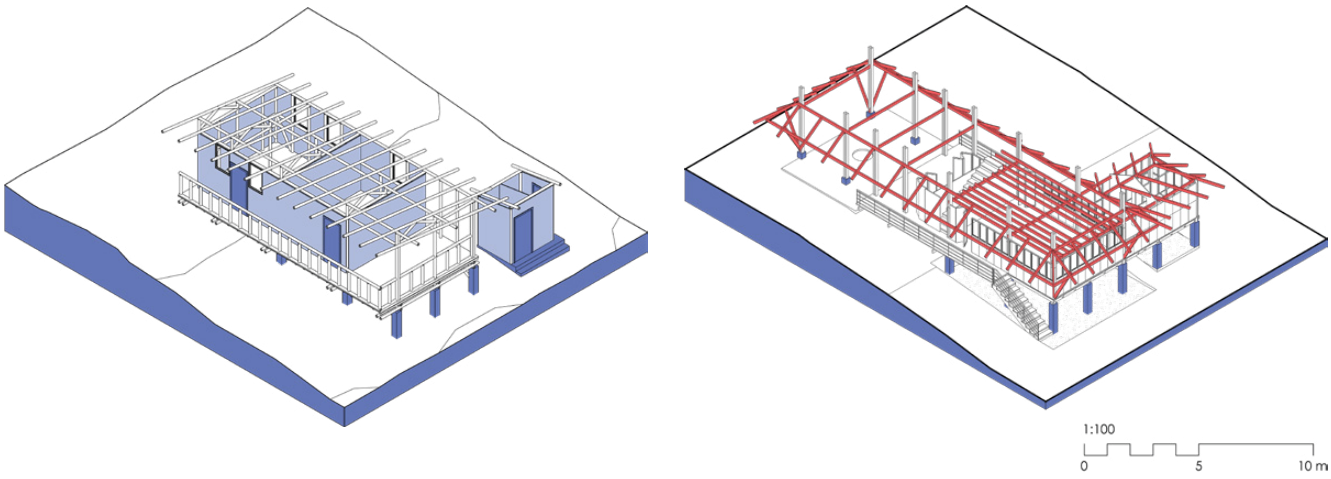

Figure 5E. Intervention made on the energy plan between the existing and the proposal.

Energy plan

Implementation of active and passive systems: Strategies are being developed to minimize the use of mechanical ventilation systems and artificial lighting, taking into account the climatic conditions. The scientific station does not have potable water, so a rainwater collection and storage system is planned for non-potable use.

Renovation of the roof: it is completely replaced by two hipped roofs with wider eaves to protect the station from constant rainfall, constant cloud cover, and falling leaves or tree branches. It also facilitates the ventilation of the interior spaces and integrates harmoniously into the landscape and the existing structure.

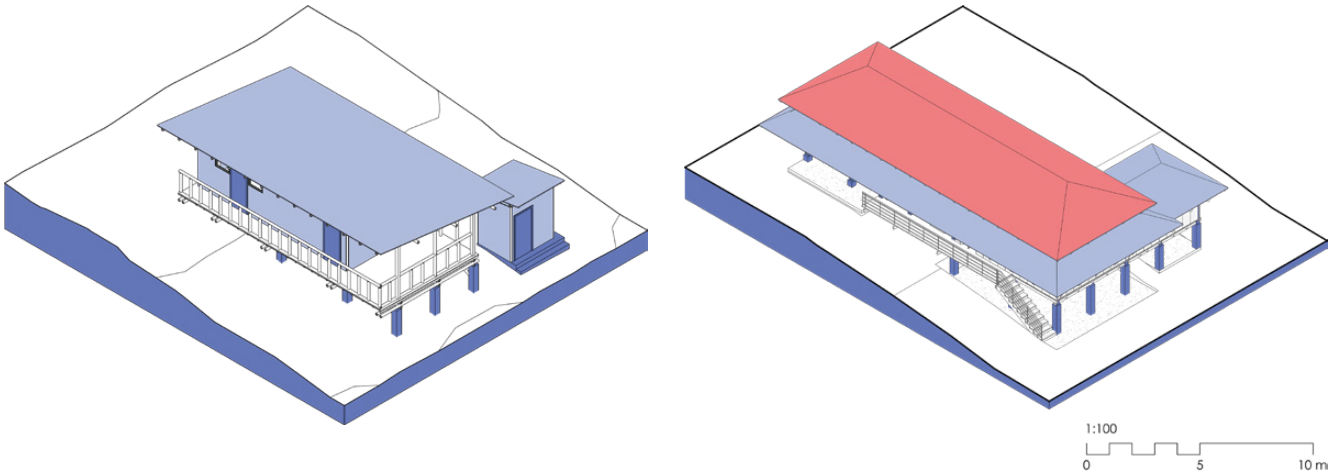

Figure 5F. Intervention made on the plastic plan between the existing and the proposal.

Plastic plan

Shape as a result of construction: visually integrates all previously analyzed successful decisions from an environmental, architectural, constructive, and structural point of view. The QR code stores the digital result of the re-engineering of the scientific station.

The final volumetry preserves the foundations while introducing technical, functional, and aesthetic advances. The objectives are to improve spatial efficiency and integrate the station into the natural environment. Sustainable design elements, such as solar panels and rainwater harvesting systems, will be included to make the station environmentally friendly and energy efficient. In short, the redesign of the scientific station not only improves functionality and safety but also aligns with its surroundings for a more harmonious integration and emphasizes sustainability.

Conclusions

A reengineering design was carried out for the research station of the Universidad Regional Amazónica Ikiam located in the Colonso Chalupas National Park (PNC) in four phases: bibliographic analysis of references, diagnosis of pre-existing resources, surveys, and interviews to determine the architectural program, and development of plans: structural, energetic, geometric, plastic, constructive, and spatial. The main differences between the current design and the proposal are based on bioclimatic strategies to increase thermal comfort, the change of the roof morphology to improve the protection of the architectural object, and a hierarchical reorganization of the spaces, separating the users' activities into three levels. The first level is intended for the arrival of users, allowing adequate space for unloading luggage. The second level consists of versatile work areas for research and various activities. Finally, the third level houses the rest area, conceived as an open space suitable for camping. The materialization of the design promotes the minimization of environmental impact.

Reengineering in architectural projects highlights its value by incorporating bioclimatic strategies, evidencing a strong commitment to environmental sustainability, and reflecting a contemporary perspective in architecture. The close relationship between design and a thorough understanding of user needs culminates in a functional and versatile environment. The proposed methodology demonstrates an innovative way to approach reengineering projects. The combination of bibliographic analysis and resource diagnosis constitutes a comprehensive and balanced approach, ensuring a holistic understanding of the context and needs involved.

However, limitations are identified, mainly the limited availability of information on similar interventions under specific conditions, such as tropical climates, and limited accessibility. In addition, there is a lack of current responses in terms of materiality in such contexts.

These limitations point to future research with a specific focus on minimizing environmental impact through sustainable architectural interventions. It is also suggested to explore the long-term effectiveness of bioclimatic strategies and materiality and assess user acceptance, thus providing valuable direction for future research.

Acknowledgments

We would like to express our sincere gratitude for the generous support provided by the Erasmus+ grant: 619346-EPP-1-2020-1-DE-EPPKA2-CBHE-JP. This funding has been instrumental in the successful development of the research project, enabling us to explore and present our findings in this chapter.

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EPILO


OGUE

Nature-based living lab (NB LAB): an undergraduate research experience at the Upper Amazonia

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Board of directors of the Nature-Based Living Lab for an interdisciplinary practical and research semester on sustainable development and environmental protection in the Amazon Rainforest.

The NB-Camps project is a continuation and extension of the NB-LAB project, funded by the Erasmus+ Capacity Building for Higher Education program between 2020 and 2023 (http://www.nb-lab.info/). While NB-LAB established the international cooperation and mobility agreements, the physical research facilities and methodologies for user-driven applied research at the partner universities, NB-Camps aims at their validation, curricular and institutional integration, and transfer to further higher education and research institutions in the partner country, Ecuador. The NB-Camps project targets the Amazon and Andean regions in Ecuador that have been involved in the NB-LAB project and extends the outreach to the Galapagos, where two new partners now joined the consortium. In doing so, the NB-Camps project will intensify the existing international cooperation between the partner universities in Ecuador and Germany. This will contribute to the structural strengthening of the established research and teaching capacities at the partner universities, promoting a sustainable and reliable partnership with a common pool of expertise collaborating on solutions for global societal and environmental challenges.

In the decade of climate change, there is a need for more applied research and challenge-based learning provided by universities. The role of higher education institutions (HEI) is shifting towards empowering students to create tools that deal with pressing societal challenges. This requires the availability of more research and development facilities that will act as innovation ecosystems for students, researchers, businesses, and society working together. Being faced with real-life problems, students should get away from the role of education recipients and become active co-creators of knowledge. Since an individual or a single professional domain will not solve complex challenges such as climate change, food security, or social inequity, there is a need for mainstreaming sustainability in interdisciplinary education through cross-professional cooperation team projects. To address these needs, suitable research and education structures and mechanisms must be put into place to respond to the growing demand for sustainability-driven and impact-oriented higher education. A coherent and direct integration of both educational and research processes in the partner HEI will be pursued through the application of two interrelated approaches:

- Participatory research in a nature-based living lab camp setting from the scientific perspective
- Community-service learning from an educational perspective.

Both approaches will be combined and applied by mixed teams of students and researchers working together on a specific problem during a four-week research camp in different biological reserves, such as the Galapagos Islands and the Cuyabeno biological

reserve placed within the Yasuní National Park. The evaluation of the four planned nature-based camps will provide evidence for the benefits of these approaches for students, academia, and society, paving the way to mainstream them in existing teaching and research structures and initiate transfer to further higher education institutions. The camps will also act as a meeting point for researchers from various professional domains, revealing the opportunity for 4-week research endeavors and expeditions with international peers. This is an opportunity to generate research-informed content for the subjects they teach and establish long-lasting cooperation links organized around the research topics of sustainable living, biodiversity conservation, and environmental protection.

NB-Camps ties up the preliminary results from the nature-based living lab pilot and the lessons learned from the initial community service-learning projects to explore options to embed these novel participatory research and experimental learning approaches into the existing teaching and research structures of the partner universities. The intended nature-based camps within the NB-Camps project will accommodate students and researchers applying combined approaches in mixed interdisciplinary teams working on practicable solutions for the local communities living in the unique ecosystems in the Amazon Rainforest, Andes Mountains, and Galapagos Islands.

Due to budgetary restrictions of the funding agency call (DAAD), the civil conflict situation in Peru and the specific cooperation opportunities with the partners from Galapagos, resulted in the end of the NB-LAB project, and the current NB-Camps project shall be implemented in Ecuador only. Where possible, the relevant expertise from NB-LAB members will be involved in the NB-Camps project (e.g., training on community service learning for scientific staff, a living lab participatory research approach, or guest lectures on biodiversity topics). As some of the trainers come from partner universities in Peru, the south-south cooperation link can be retained despite the budgetary limitations of the project.

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ISBN: 978-9942-7178-3-2