

LET IT FLOW: DEFINING ENVIRONMENTAL STANDARDS FOR WATER
ABSTRACTION IN TROPICAL ANDEAN STREAMS

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ABSTRACT

The role that environmental flows (e-flows) have in aquatic ecosystems can be explained by the type and extent of processes that are promoted. The establishment of e-flow recommendations is necessary for the restoration and conservation of rivers and streams but requires interdisciplinary research with a strong ecohydrological perspective. At the currently rapid pace of water infrastructure development in Andean ecosystems, this goal will not be feasible without adaptive frameworks, cutting edge tools, and experiments to address ecological responses to flow alterations.

Environmental standards for water abstraction in most ecosystems require water managers involvement to ultimately implement guidelines and provide feedback for applied research. Thus, I initially assessed aquatic invertebrate and impacts associated with water abstraction controlled by a water supply company operating in tropical Andean streams of Ecuador. With their cooperation, I explored the opportunity to propose a framework for decision scaling based on research into “flow” thresholds for restoring and maintaining natural ecohydrological processes. Flow thresholds are critical breakpoints in flow-ecology relationships where fundamental ecosystem functions abruptly shift from natural conditions. Often biotic responses to flow changes incorporate large amounts of noise that make it difficult to discern thresholds or ecological limits of change.

I used an *in situ* experimental approach to identify e-flows thresholds for water abstractions. Thresholds for bioindicators of water quality are of utmost interest to water supply systems. So, in addition to using aquatic invertebrates, I evaluated the feasibility and reliability of an *in situ* fluorometer for assessing benthic algae and cyanobacteria. Results based on the fluorometer were comparable to more complicated and time-consuming standard laboratory-based methods. I designed, tested, and used a novel ecohydraulic stream-diversion structure to address the response of cyanobacteria to *in situ* flow alterations in an experimental stream reach. Through a series of systematic flow reductions and subsequent flow recoveries, I identified e-flow thresholds at a 60% reduction of natural flows at which point there were clear increases in cyanobacteria biomass. Interestingly, there was consummate reduction in cyanobacteria biomass when flow was restored to 60% of the natural flow. These results generally agree with a meta-analysis of global literature on flow-benthic biomass relationships. Thus, my research concludes that there are e-flow thresholds associated with benthic biomass and that these likely vary over a narrow range in alpine environments around the world. Such shifts in rivers are crucial for defining e-flows as standards for water abstractions, specifically in tropical Andean streams.

BIOGRAPHICAL SKETCH

Daniela “Dani” was born on a rainy night on November 24th, 1979 in Quito, Ecuador. Her parents, Rosy and Henry, decided to call her just Daniela without a middle name, unaware of the role Rosero would take as middle name in the future. After an exceptionally smooth birth, Rosy and Henry took Daniela to meet her three-year-old brother Henry Esteban. For her first four years, Daniela spent most of her time with her grandmother Laura, who taught Daniela how to take care of guinea pigs, rabbits, and chickens. Daniela always enjoyed taking care of them while trying to understand why they continually disappeared every other week right before the family enjoyed a great meal. At age three Daniela had another brother, Roberto Mauricio, and at age five a sister named Pamela Estefania. Daniela enjoyed playing with her siblings as real baby-dolls and, with them, she performed her very first engineering experiments with different designs for building slides and parachutes.

Before getting into pre-school, Daniela was committed to copying all the letters and numbers she could from old notebooks found around her grandmother’s shop. Daniela enjoyed school so much she never missed a day and, as a result of her stellar academic performance at the end of elementary school, she was appointed to carry the National Flag during the Annual Oath. For the next six years, she joined an International Baccalaureate program where she met her best friends, became captain of the basketball and soccer teams, and obtained a diploma in physics.

Daniela decided to study Environmental Engineering after convincing her parents that she would get a minor in economics. By the time she attended the Saint Estanislao de Kotska International University, USEK, her family and friends thought she was going to spend most of her time tying herself to trees as “environmentalists” did. Rather than tying herself to the university trees, she decided to study them. Daniela found the role of trees and the importance of ecosystem management to provide

services fascinating. Daniela increasingly leaned into ecology and soon she was given the opportunity to travel to the Galapagos Islands to volunteer at the Charles Darwin Research Station. For the next four months she learned to identify invertebrates by mounting ~35,000 specimens. This experience marked the beginning of Daniela's path towards a research career. After volunteering as field assistant on several projects, she had the opportunity to participate in a biological monitoring program using aquatic invertebrates for the Bavarian region and a marine mammal monitoring project on the North Sea with the University of Kiel in Germany.

Daniela built from her experiences to develop her bachelor's thesis on understanding the environmental fate of mercury in a lacustrine system of the Amazonian Rain Forest of Ecuador. After intense field work and completing her bachelor's degree, Daniela went backpacking for three months through the ruins of Peru, Bolivia, and Chile accompanying a group of graduate archaeologists.

Daniela funded, established, and created, with a group of friends/colleagues, a non-governmental organization (NGO) for the research of aquatic ecosystems in Ecuador. Daniela created a national water resources research and projects database. From her work with this NGO, Daniela saw the importance of combining engineering and aquatic ecology. Daniela found that combination in hydrology and enrolled herself in a master's degree program on Water Resources Management at the National Polytechnic School, EPN in Ecuador. During the program she was awarded a scholarship to finish her studies in operational hydrology in Sweden at the Swedish Institute for Hydrology and Meteorology, SIHM.

Daniela learned about the concept of environmental flows (e-flows) and realized that this was exactly what she was looking for, a perfect liaison between hydrology, engineering, and aquatic ecology. In 2004, Daniela presented the first environmental flows project proposal in Ecuador. The project was funded by the Water Fund for

Quito, FONAG, and explored methodologies for environmental flows determination in rivers altered by run-of-river hydropower.

Daniela married Rodrigo and had Barbara and Ezequiel, three years apart. Her children have joined Daniela on several fieldwork trips, sample collection campaigns, and even laboratory analyses. In 2008, Daniela started a second master's degree program on Tropical Ecology at the San Francisco University in Quito, USFQ, under the advisership of Dr. Andrea Encalada. Together, they assessed the impact of water abstractions in streams of the water supply system for Quito.

Before engaging with the Ph.D. program at Cornell University, Daniela worked as a professional engineer assessing local and national authorities regarding ecological responses to hydropower dams and water intake operations in streams and rivers. Early experiences ignited her interest to combine hydrology, hydraulics, and ecology to model ecosystems in Ecuador. In 2015 Daniela was awarded a national scholarship to pursue a doctoral program at Cornell University under the advisership of Dr. Todd Walter. Daniela's previous work on environmental flows with the French Institute for Research and Development (IRD) in Ecuador allowed her to pursue her research in her home-country. Under the guidance of Dr. Olivier Dangles in the CHALPI-FLOW Project, Daniela developed her research to provide management guidelines for Quito water resources. Daniela's research is applied in nature with the goal of improving water management to simultaneously achieve human and ecosystem needs. Daniela's work currently focuses on high-altitude tropical streams in the Andean region. Her research assesses abiotic and biotic ecosystem thresholds, water infrastructure impacts on aquatic ecology, new tools for aquatic biological monitoring, experimentally based improvements for e-flows guidelines, and flow-induced shifts in freshwater cyanobacteria. Ultimately, Daniela's main goal is to contribute with science – based criteria and evidence for sustainable management water resources in Ecuador.

To Rodrigo, Barbara, and Ezequiel, who made this Ph.D. possible.

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PREFACE

The steep increase in demand for water for human use and consumption represents a major threat to the integrity of aquatic ecosystems (Paerl *et al.*, 2018). Nowadays, managers face innumerable water conflicts, most of them related to overexploitation of water resources and commensurate degradation of aquatic ecosystems (Postel, 2000). Water shortages relate to the loss of aquatic biodiversity around the world (Jacobsen and Dangles, 2017). Many countries have encountered the premature need to manage water resources with a precautionary approach, in face of intense water shortages exacerbated by extreme climate events (Vörösmarty *et al.*, 2003). In contrast to overexploitation, some watersheds have promoted environmental flows assessments to return functions to rivers (Arthington, 2012). The environmental flows (e-flows) concept is intended to provide a framework to maintain ecosystem functions in space and time in order to protect ecosystem goods and services, which all living things depend on (Poff *et al.*, 2010; Arthington *et al.*, 2018). Although the systematic application of the e-flow concept is relatively new (Tennant, 1976; Arthington, 2012), most preliminary efforts are largely limited to hydropower systems, focusing on flow releases from hydropower dams (Mitrovic *et al.*, 2012; Olden *et al.*, 2014).

Environmental standards for water abstractions combines sensible areas of water resources management and ecosystems conservation. In face of climate change scenarios, aquatic ecosystems show increasing evidence of change driven by external conditions (i.e., temperature, water levels, nutrients, lights) (Vörösmarty *et al.*, 2003; Neif *et al.*, 2017; Huisman *et al.*, 2018; Ho *et al.*, 2019). In response to drivers change, cyanobacteria and benthic algae seem to proliferate threatening ecosystem services and functions, with potential consequences to other environmental compartments (e.g., increase on methane emissions) (Bižić *et al.*, 2020).

This dissertation research elucidated distinct e-flow “thresholds” for water infrastructure operational guidelines for tropical Andean streams. An adaptive framework, capable to incorporate results/experiences from flow – ecology relations using monitoring and experimental

data provide the opportunity to put in practice e-flows and assess the direction of responses. Using observations from monitoring and an experiment to manipulate flow covers a wide range of first-hand results to build the foundation to assess potential regime shifts in streams.

In **Chapter One**, I adapted the Eco-Engineering Decision Scaling framework (Poff *et al.*, 2016) conceived initially to analyse scenario/responses from multiple indicators previously defined from water manager consensus. To apply to part of the water supply system of Quito, Ecuador, I used feedback from engineers and water managers to develop a common set of metrics for defining flow-ecology relationships and assess managed-flow impacts on stream ecology. At 12 sites over three years, I collected flow and benthic invertebrate data (taxonomic richness, taxa important for fish, functional feeding groups, and water quality-sensitive taxa) during wet and dry seasons. I used these data to identify flow thresholds (relative to unmanaged flows) where flow withdrawal caused visible ecological impacts.

For this system, reduction of flow to 20% of the annual median was detrimental to benthic communities, while reductions to 40% of the annual median flow caused a variety of responses in the system (i.e., taxonomic richness decrease). A trade-off analysis of weighted metrics showed that a 50% benthic fauna richness could be sustained if dry season flows were maintained between 28% and 40% of the unmanaged median annual flow. This study provides a roadmap for bridging between eco-engineering theoretical frameworks and the adoption of the e-flows concept as actionable management thresholds.

Chapter Two assesses the feasibility of incorporating fluoroprobes, such as the BenthosTorch, to standard methods for monitoring benthic algal groups in order to develop early warnings for preventing algal proliferation. The current use of *in situ* methods like the BenthosTorch suffers from measurement heterogeneity and difficulties homogenizing samples. Here, we compared chlorophyll-a (Chl-a) readings from the BenthosTorch to standard laboratory methods to fully quantify and describe biomass and relative abundance of benthic algal groups on artificial and

natural substrates. We used the difference in Chl-a measurements between methods (n = 359) to evaluate the effects of environmental variables and we used artificial and natural substrates to determine if one reduced variation in BenthosTorch readings. Our findings demonstrate a general agreement between methods, but this relationship was stronger for artificial substrates. The interacting effects of light and benthic algal mat thickness indicated that artificial substrates are less susceptible to changes in these parameters than natural substrates. Artificial substrates provided significantly less variation among BenthosTorch readings for the same number of natural substrate samples. Overcoming differences on BenthosTorch readings seems plausible with the aid of artificial substrates in combination with proper calibration to standard laboratory methods. The ability of the BenthosTorch to produce immediate results facilitates replicate collections, which can reduce sample error and increase the area sampled on the same day. Understanding the presence of certain algal groups in a timely manner is a strategy worth incorporating into aquatic ecosystems monitoring.

In **Chapter Three**, I present an ecohydraulic structure designed for *in situ* flow manipulations. Controlled in-stream flow manipulations are challenging but necessary in order to assess the consequences of real-world flow alterations on aquatic ecosystems. We designed a double v-notch weir system, which was first prototype-tested in a laboratory flume and then field-tested. The device diverted instantaneous flows proportionally in a robust way (e.g., 10% of flow in the stream main channel and 90% of flow into the diversion channel). We tested diversions systematically and evaluated how well flows matched our target flow-percent. Although our field flow conditions did not vary a lot, the lab experiment showed similar performance for flow variations over about one order of magnitude. The experimental flow manipulation structure has the potential to divert streamflow while maintaining “natural” variations in flow conditions. The device is relatively easy to set up, it is long lasting, and an economical option for making e-flow

evaluations. This is one of the first ecohydraulic structures conceived and tested to experimentally manipulate e-flows in regulated upland streams.

Chapter Four presents the case of regime shift thresholds in streams using a whole ecosystem, flow manipulation experiment in conjunction with (1) data collected nearby from above and below water intakes and (2) data from a global literature survey. Identifying thresholds from flow–benthic fauna relationships constitutes an important advance in implementing sustainable principles for water infrastructure management, which has previously been difficult because there is a dearth of data associated with engineered hydrosystems. To quantify thresholds for water - withdrawals from Andean river networks, i.e., flow reductions at which streams' natural functioning, biodiversity and ecosystem services become degraded, we conducted a whole-ecosystem experimental flow manipulation. We reduced flow in a stream reach above a water intake from the supply system for the city of Quito, Ecuador.

During the low-flow season, we diverted water using a system of weirs to accommodate streamflow in complementary percentages (i.e., 90% flow deviation and 10% flow left in the stream – see chapter 3). We performed seven reductions and nine flow restitutions in systematic, seven-day intervals. During each flow-manipulation period, we measured flow, *in situ* physical and chemical parameters, and benthic algae biomass, with special interest on cyanobacteria.

Our experimental results show cyanobacteria biomass experience a sudden change with a 60% flow reduction. This threshold was correlated with a water temperature increase and an increasing trend on nitrate concentrations. We corroborated our experimental results with nearby streams undergoing flow reductions by water intakes (n = 1066), which showed shifts in cyanobacteria biomass similar to those generated in our experiment.

A global literature survey revealed results consistent with our local survey and our experiment, showing that cyanobacteria biomass increased significantly with flow reductions >72%. This study provides one of the first *in situ* demonstrations of regime shift thresholds in

natural running waters and unique experimental evidence of flow reductions through fixed percentages for e-flow management.

Related side projects

To propose a framework in partnership with the water supply company we analyzed information that was initially obtained in 2008 and that was intermittently used to provide guidelines for water operation. We consolidated a partnership between the water supply company EPMAPS, the Water Fund for Quito, FONAG, the French Institute for Research and Development IRD, and Cornell University to provide guidelines for water resources sustainable management in Quito: CHALPI-FLOW Project.

To understand the different effects of water abstraction on bioindicators of water quality from Chapter 1, co-authors, and I quantified the impact on aquatic invertebrates (Rosero-López *et al.*, 2019). Bioindicators of water quality (e.g., aquatic invertebrates) responded differently to the type of flow operation: water removal decreased taxa richness while regulation altered natural functions (e.g., phenology). We estimated water abstraction and regulation effects at the water supply system scale and related connectivity and aquatic invertebrate taxa diversity decrease to scenarios of intakes optimization to design a portfolio of eco-friendly water intake operation (González-Zeas *et al.*, 2019).

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CHAPTER 1

STREAMLINED ECO-ENGINEERING APPROACH HELPS DEFINE ENVIRONMENTAL FLOWS FOR TROPICAL ANDEAN HEADWATERS

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Abstract

1. Applying the environmental flows (e-flows) concept to human-altered lotic ecosystems continues to face many practical challenges and barriers. Here we modify a previously proposed framework, the Eco-Engineering Decision Scaling, for application to part of the water supply system of Quito, Ecuador.
2. Specifically, we used feedback from engineers and water managers to develop a common set of metrics for defining flow-ecology relationships and assess managed-flow impacts on stream ecology. We collected at 12 sites over three years, flow and benthic invertebrate data (taxonomic richness, taxa important for fish, functional feeding groups, and water quality-sensitive taxa) during wet and dry seasons. We then used these data to identify flow thresholds (relative to unmanaged flows) where flow withdrawal caused visible ecological impacts.
3. For this system, reduction of flow to 20% of the annual median was detrimental to benthic communities, while reductions to 40% of the annual median flow caused a variety of responses in the system. A trade-off analysis of weighted metrics showed that a 50% benthic fauna richness could be sustained if dry season flows were maintained between 28% and 40% of the unmanaged median annual flow. This study provides a roadmap for bridging between eco-engineering theoretical frameworks and the adoption of the e-flows concept as actionable management thresholds.

1. Introduction

Flow alterations are among the most pressing threats to the integrity of aquatic ecosystems. The ‘environmental flows’ (e-flows) concept has emerged as a management concept to establish the quantity and timing of water necessary to balance ecological and human needs (Arthington and Pusey, 2003; Tharme, 2003; Arthington, 2012; Poff, 2018). In the past 30 years, e-flows have become a key research area to develop sustainable water plans and have been recognized by many water resource managers worldwide (Poff *et al.*, 1997; Richter, 2010; Davies *et al.*, 2014). Importantly, the e-flow framework is an ongoing bridging between an engineering conceptualization of hydrological systems in water infrastructure projects (e.g., damming, withdrawal and diversions) and the ecological relevance of hydrosystems for biodiversity conservation and ecosystem functions (Bunn and Arthington, 2002; Poff *et al.*, 2010; Arthington *et al.*, 2018). E-flows provide a strong step in connecting these divergent perceptions; however, many goods and services provided by ecosystems remain underappreciated.

As a major conceptual and practical advance, Poff *et al.* (2016) proposed the Eco-Engineering Decision Scaling (EEDS) framework that allows for evaluation of trade-offs between engineering design and ecological performance for sustainable water infrastructure development. The EEDS is a stakeholder driven process for the analysis of water system development-sustainability trade-offs based on engineering and ecological thresholds defined at the initial stage of the project. The EEDS framework depends on climatic, hydrological and ecological data, which are used to parametrize hydrological models that simulate the functioning of the hydrosystems to be managed. In river basins with well-characterized hydroclimate and terrain data, an EEDS process can enable participants to identify and select feasible thresholds in ecological functions.

Building evidence-based decision tools for water management such as the EEDS is particularly challenging in regions with limited ecohydrological information and rapid infrastructure development (Auerbach *et al.*, 2016). Even in regions with copious environmental data, the paired flow-ecology data necessary for adopting an e-flows framework are generally

unavailable (e.g., Buchanan *et al.*, 2017). Data poor regions, such as the tropical mountains of Asia or South America, are, ironically, major areas of infrastructure development for water provision and hydropower generation (Jacobsen and Dangles, 2017; Anderson *et al.*, 2018). When the ecohydrological dynamics of a hydrosystem cannot be well-modelled, it is potentially advantageous to develop empirical datasets to identify thresholds that are acceptable in terms of both water needs and ecological health (Poff *et al.*, 2010), even if they span relatively short periods of time.

In this context, the main motivation of our study was to propose a general roadmap to adapt the EEDS framework to a data-scarce, hydro-development-prone region, where *a priori* thresholds cannot be well-defined. Our process began by developing key performance indicators for both ecological and engineering outcomes. Within the study area, incorporating ecological system indicators represented an advance toward more sustainable water management (Figure 1). We modified the EEDS process by coupling empirical data collection for use in threshold analyses, instead of building a system model and simulating water system vulnerabilities (see further details in the Material and Methods). The change in EEDS steps is rooted in objective-driven data collection of hydrosystems with limited *a priori* hydrological and ecological information, that, when available, have different time-frames.

Modeling hydrosystems under such circumstances is particularly difficult and provides coarse resolutions for Andean headwaters (Auerbach *et al.*, 2016; González-Zeas *et al.*, 2019). These highly diverse hydrosystems experience flow fluctuations that add complexity to already noisy ecological responses to flow (Jacobsen and Dangles, 2017). Also, Rosenfeld (2017) showed that benthic fauna metrics and other lotic indicators often show non-linear responses to flow alteration. For example, in tropical Andean streams, Cauvy-Fraunié *et al.* (2016) reported benthic algae and herbivores shifting from low to a high level of biomass with certain level of flow alteration. Changes in flow – benthic fauna relationships are known as thresholds and are of particular interest for managing water ecosystems (Andersen *et al.*, 2009; Dodds *et al.*, 2010; Baker and King, 2010). The analysis of thresholds in data-scarce hydrosystems offers the

possibility to understand potential shifts in ecosystem structure and functions resulting from human-induced changes on flow conditions (Cauvy-Fraunié *et al.*, 2016).

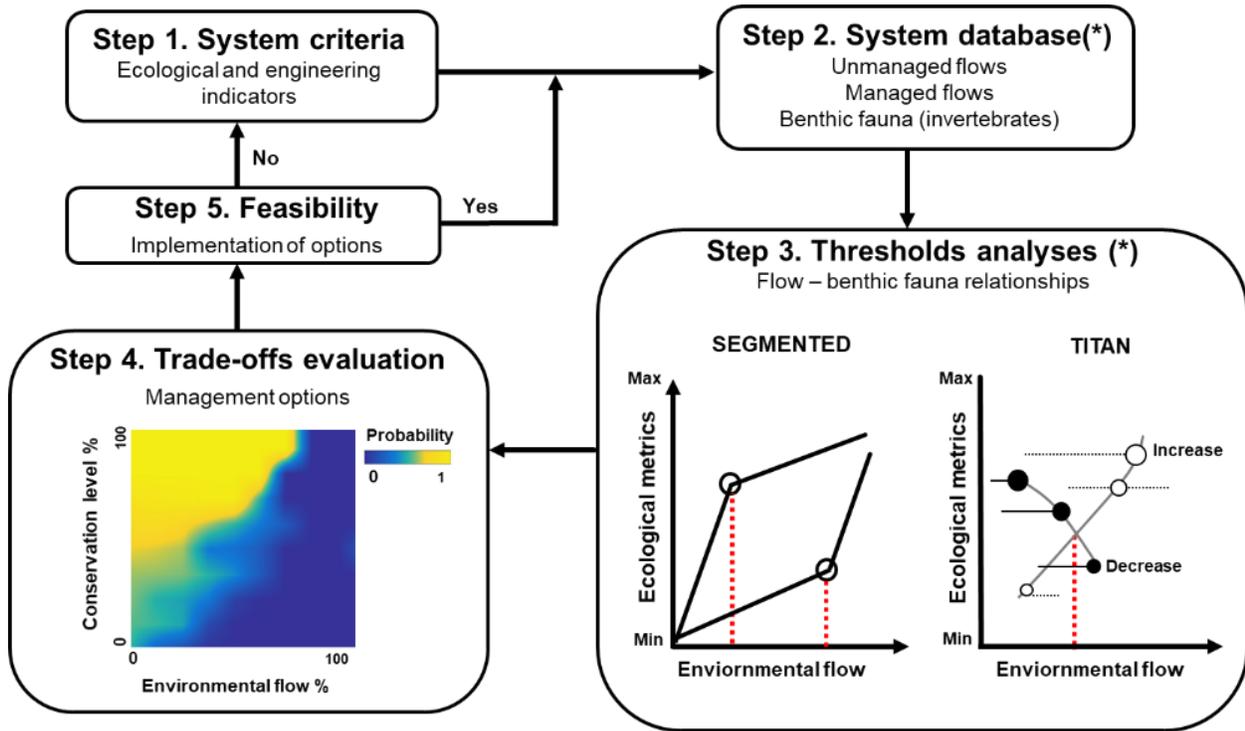


Figure 1. Methodological framework adapted from the Eco-engineering decision scaling (EDDS) for environmental flows definition on data-scarce hydrosystems.

We applied our roadmap to establish e-flows in several mountain streams providing potable water to Ecuador’s capital, Quito, with 2.7 million residents. As in the EEDS, we developed a collaborative initiative with the main stakeholders involved in water management: the metropolitan drinking water company of Quito (EPMAPS) that operates the system and the water fund for Quito (FONAG) in charge of catchment conservation. The water company and the water fund pioneered e-flows initiatives in Ecuador with activities targeted to promote research on flow-ecology relationships of which this is a principal study (pers. obs.). In collaboration with these organizations, we sampled over three years flow and benthic fauna in 12 headwater stream

sites of two of the main catchments providing water to Quito. Our preliminary hypothesis was that empirical data from managed and unmanaged streams offer the possibility to quantify thresholds for managing e-flows from water infrastructures. As an application of the EEDS, our objectives were threefold: 1) to evaluate benthic fauna response to flow in managed streams, 2) to identify thresholds in flow–benthic fauna relationships and 3) to identify trade-offs for acceptable eco-engineering management.

2. Methods and materials

2.1 Study area

The study system is located inside the Cayambe-Coca National Park and supplies about 16% of Quito's water demand. Managed streams are headwaters of the Chalpi and Papallacta watersheds, with drainage areas of 95 km² and 235 km², respectively. Both rivers are tributaries to the Amazon, but the supply system transports water across the Andean mountain range from an elevation of 3700 m above sea level to the city of Quito located in an inter-Andean valley at 2800 m. The water infrastructure (i.e., intakes) is mainly placed in first- and second-order headwater streams, comprising 12 intakes and two reservoirs. The land cover predominantly consists of high-altitude moorlands with herbaceous species (i.e., *Calamagrostis* sp.), and scattered patches of *Polylepis* forests with underlying thick and highly humic soils (Jacobsen and Dangles, 2017). The range of ambient temperature fluctuates from a minimum of 1.4°C to a maximum of 12.8 °C (EPMAPS). Two distinct climatic seasons occur: from March to August (high-flow period) with mean monthly precipitation of 176.8 mm, and from September to February (low-flow period) with mean monthly precipitation of 107.9 mm. The total annual precipitation in the catchment averages ~1700 mm. Between seasons, the length of the climatic transition varies from year to year (Jacobsen and Dangles, 2017), and within seasons the variability in precipitation and flow is higher in the high-flow period compared to the low-flow season. During low-flows, precipitation occurs almost exclusively as steady drizzle. Consequently, the in-channel response is damped by natural storage. Large storms accompany

steady drizzles in the high-flow season, generating rapid stream flow responses. In addition to supplying drinking water, the studied catchment provides highly valued ecosystem services, e.g., climate regulation, water purification, trout fishing for tourism, and aesthetics for recreation and conservation.

2.2. Eco-engineering decision scaling (EEDS)

2.2.1. Developing the System criteria (step 1)

To apply our modified EEDS framework, our water stakeholder group agreed to use the “annual median flow” measured in unmanaged streams as our flow metric and “benthic fauna” as the ecological indicator, based on the feasibility to conduct a flow – benthic fauna monitoring program. Capturing the critical magnitude and timing of flow requires the use of flow metrics to relate with observed flows during ecological surveys (Buchanan *et al.*, 2017). Here, the annual median flow is similar to the annual mean flow with the advantage that it is less responsive to extreme events. Consequently, the engineering/management indicator we used related observed flows with the annual median to obtain percent flows. We considered flows greater than the annual median to be "high-flows" and flows below the median to be "low-flows".

After freshwater ecologists presented an array of potential metrics related to benthic fauna, water managers in our stakeholder group were particularly interested in four: 1) the diversity of benthic invertebrates (as expressed by the total number of taxa or taxonomic richness); 2) benthic invertebrates considered important food for fish (expressed as the number of taxa e.g. *Chironomidae* and *Andesiops*, found in the gut of exotic trout, the only fish reported for high-altitude tropical streams in the northern Andes, see Vimos-Lojano *et al.*, 2015); 3) the functional diversity of organisms, which is crucial to maintain key ecological functions in the stream (as measured by the number of functional groups, Tomanova *et al.*, 2006; Príncipe *et al.*, 2010), and 4) the benthic organisms that can be considered as bio-indicators of the water quality (measured as the number of families from EPT orders, which are three orders known to be more sensitive to pollution: Ephemeroptera, Plecoptera, and Trichoptera, Lenat, 1998; Vimos-Lojano *et al.*, 2017).

2.2.2. Developing the database system (step 2)

To compensate for the lack of hydrological and ecological understanding of the water system, both managers and scientists agreed to establish a hydrological-ecological monitoring program in 12 sites located in the Chalpi and Papallacta watersheds. Six sites corresponded to unmanaged, free-flowing streams, with characteristically narrow and incised channels, located approximately 200 m above water intakes. The other six sites corresponded to managed streams subjected to water withdrawals, with prominent modified-open channels located approximately 200 m below intakes. In these sites, streamflow at downstream sites was the result of water releases from intakes. The 12 sites were equipped with stream discharge gauging stations installed for continuous readings using pressure transducers informed by a manually-derived rating curve. To build our database, first, we measured streamflow during the sampling of benthic invertebrates. To measure streamflow, we applied the integrated velocities method with the aid of a micro-propeller current meter (Agrisearch Equipment Eijkelkamp, Netherlands). We measured velocity and depth at several verticals-increments over the stream depth separated by a large range of distances across the stream width.

Second, we sampled benthic fauna every three months, on 11 occasions for three years from June 2006 to December 2008. Samples were taken at upstream and downstream sites of intakes at the same day in months that included both high- and low-flows seasons. Benthic macroinvertebrates were sampled using a Surber sampler (area = 0.0625 m², mesh size = 500 μm) placed on three habitats in two different reaches (six samples in total) and disturbing the substrate for 60 s. Reaches in sites were separated by either a wetland area or small tributaries that contributed a variety of flow conditions. After preservation in 4% formaldehyde, specimens were identified to the lowest practical taxonomic level (usually genus/family except for Oligochaetes and Chironomidae) using a 10X-magnification stereoscope and regional taxonomic keys (Fernández and Domínguez, 2001).

Finally, flows measured during the benthic survey (instantaneous discharge) were

normalized by the median of the period of record (2006-2008) and expressed as percent of flow. “Flow” from here on refers to the percent of observed flow normalized by the annual median, e.g., 20% flow = 20% of the median annual flow. A total of 264 pairs of flow–benthic data comprised the database for the threshold analysis.

2.2.3. *Threshold analysis (step 3)*

In most hydrological systems, deleterious ecological impacts of human activities are concentrated during low-flow periods (Vörösmarty *et al.*, 2010). In our analysis, we used the paired flow-ecological data from the entire dataset (264 observations) and also from the low-flows season only (146 observations). We calculated thresholds in flow-benthic fauna relationships within the range of flows using two methods. First, we used linear regression models to estimate breakpoints in flow-benthic fauna relationships using ecological metrics in separated datasets of managed and unmanaged streams. For this analysis we used data only from the low-flow season. We ran the Segmented package version 5.5, aimed to estimate one or more segmented relationships in the linear predictor using bootstrapped starting values to calculate breakpoints with a 95% confidence interval (Muggeo, 2003; R Core Team, 2017). Second, we used the entire dataset to apply an analytical approach to find community thresholds in response to the flow gradient using the magnitude and direction in the responses of individual members from the benthic community (Baker and King, 2010). We used the Thresholds Indicators of Taxonomic Analysis (TITAN) results as evidence of the potential community threshold response to flow. The analysis identifies, through bootstrapping, flow at which large, disproportionate increases or decreases of abundance and frequency of tolerant and sensitive taxa occur across the flow gradient (0 to 100%). The outcome is based on z scores for sensitive taxa (z-) and tolerant taxa (z+), where the maximum z score for each taxon is the change point, and the flow maximizing taxon scores is the community threshold (King and Baker, 2010). We built matrices of taxa with frequencies > 3 occurrences and abundances > 5 individuals (see Baker and King, 2010). After eliminating taxa and observations not meeting the criteria, we retained 63 taxa for

managed streams and 68 taxa for unmanaged streams (Table 1). We ran the TITAN package version 2.0, in the R statistical environment (Baker and King, 2010; R Core Team, 2017).

Table 1. Taxa used for the TITAN analysis after selection of frequencies > 3 occurrences and abundances > 5 individuals. Taxa present in managed and only in unmanaged streams (*).

Abb.	Taxa	Abb.	Taxa	Abb.	Taxa	Abb.	Taxa
ALL	<i>Alluaudomyia</i>	HEH	<i>Hexanchorus</i>	TRI	<i>Tricorythodes</i>	TIP	<i>Tipula</i>
ANA	<i>Anacroneuria</i>	MET	<i>Metrichia</i>	ORT	<i>Orthoclaadiinae_03</i>	ZUM	<i>Zumatrighia</i>
ANC	<i>Anchyrtarsus</i>	NEC	<i>Nectopsyche</i>	CHE	<i>Chelifera</i>	OCH	<i>Ochrotrichia</i>
AND	<i>Andesiops</i>	HEM	<i>Hemerodromia</i>	LIS	<i>Listronotus</i>	OEC	<i>Oecetis</i>
ANO	<i>Anomalocosmoecus</i>	NEO	<i>Neoelmis</i>	LYM	<i>Lymnaea</i>	AUB	<i>Aubertoperla</i>
ATO	<i>Atopsyche</i>	HET	<i>Heterelmis</i>	MAC	<i>Macrelmis</i>	GYR	<i>Gyrulus</i>
AUS	<i>Austrelmis</i>	HEX	<i>Hexacylloepus</i>	MAY	<i>Mayobaetis</i>	TAB	<i>Tabanus</i>
CHO	<i>Chironomidae</i>	CLA	<i>Claudioperla</i>	GRU	<i>Grumichella</i>	CLA	<i>Orthoclaadiinae_02</i>
CON	<i>Contulma</i>	LET	<i>Leptophlebiidae</i>	PIS	<i>Pisidium</i>	ATA	<i>Atanatolica</i>
CYP	<i>Cyphon</i>	NEY	<i>Neocylloepus</i>	PSH	<i>Psephenidae</i>	AUT	<i>Austrotinoides</i>
ELO	<i>Elodes</i>	PSE	<i>Pseudodisersus</i>	SIM	<i>Simulium</i>	ATR	<i>Atrichopogon</i>
FAR	<i>Farrodes</i>	POL	<i>Polycentropus</i>	CHR	<i>Chrysops</i>	AED	<i>Aedes</i>
GIG	<i>Gigantodax</i>	RHD	<i>Rhaphilum</i>	HYA	<i>Hyaella</i>	LPT	<i>Leptonema*</i>
GLO	<i>Glossiphoniidae</i>	DIS	<i>Disersus</i>	PLA	<i>Planariidae</i>	SMC	<i>Smicridea*</i>
HYD	<i>Hydroptilidae</i>	HEL	<i>Helicopsychidae</i>	BAE	<i>Baetodes</i>	MOR	<i>Mortoniella*</i>
LEP	<i>Leptohyphes</i>	ALI	<i>Alisotrichia</i>	CAI	<i>Cailloma</i>	OLI	<i>Oligoneuridae*</i>
LIM	<i>Limonia</i>	TAN	<i>Tanypodinae</i>	HAP	<i>Haplotaxida</i>	LIC	<i>Limonicola*</i>

2.2.4. Evaluation of trade-offs and feasibility (steps 4 and 5)

To evaluate trade-offs between water withdrawn for human supply and needed flow to conserve benthic fauna, we calculated the frequency with which ecological metrics in low-flows from managed streams equaled conditions in unmanaged streams. We used 264 pairs of flow and benthic data to run probit models for a binary response, classifying ecological metrics that meet each category or not. The probit models were then applied to all levels of e-flow percent to estimate ecological metrics at all sites. Data were then standardized relative to their maximum value (e.g., 50% of total taxa richness) thereby defining conservation levels. The trade-off analysis offers three types of inputs: the potential flow left in the stream (as a percent of median annual flow), the targeted conservation level, and the probability of reaching the target. The efficiency frontier corresponds to the point/region where both entries reach the higher probability. We evaluated trade-offs using individual metrics and weighted the contribution to

overall ecosystem integrity metrics. We graphed trade-offs using Plotly (Plotly Technologies Inc. Montreal, QC 2018).

3. Results

3.1. System criteria and database

The eco-engineering indicators selected in the system criteria effectively described the range of conditions in unmanaged streams (low-flows and high-flows) and in managed streams (artificial low-flows). Gauged flows and flows from the benthic survey in managed streams were always lower than the annual median of natural flows, while in unmanaged streams, both flows (i.e., gauged and surveyed) were predominantly above the median value during the high-flow season (e.g., June, July, August) (Figure 2). In managed streams all metrics from the benthic fauna indicators were lower than unmanaged streams, despite the flow seasonality.

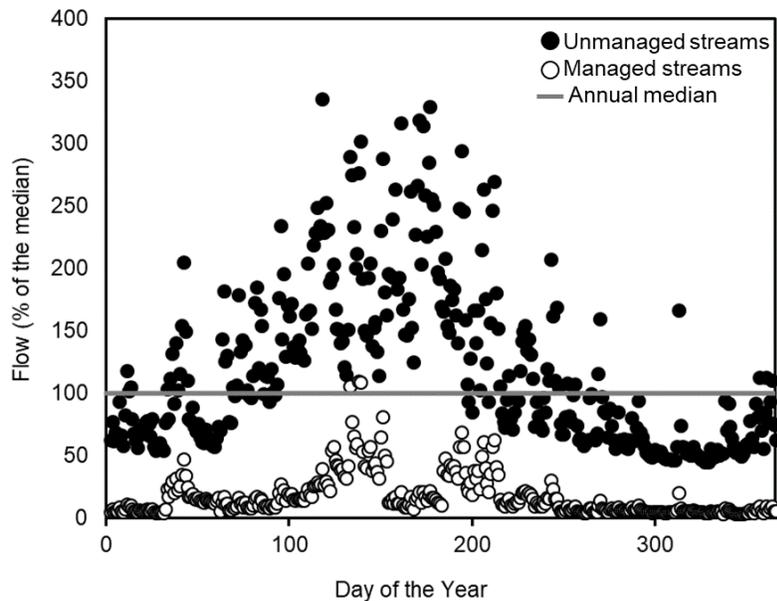


Figure 2. Characteristic hydrograph of streams in the headwaters of Chalpi and Papallacta watersheds in Ecuador, showing flow as the percent of the median for unmanaged and managed streams; points include 264 discharge measurements made in conjunction with benthic samples.

3.2. Thresholds analyses

Ecological metrics in low-flows of managed streams had highly variable responses in contrast to

unmanaged streams (Figure 3). In managed streams, flow – benthic fauna relationships showed groups of data following systematic trends, i.e., as flows increased so did most ecological metrics in a consistent manner. Unmanaged low-flows generated no significant responses for the four benthic fauna metrics (Table 2).

Table 2. Results of flow - benthic fauna relationships in low-flow conditions for managed and unmanaged streams of tropical Andes.

Ecological metric	<i>Managed streams</i>			<i>Unmanaged streams</i>		
	<i>df</i>	R²	p	<i>df</i>	R²	p
Taxonomic richness	88	0.28	0.21	41	0.27	0.217
Food for fish	88	0.64	2.76e-9	41	0.32	0.141
Functional feeding groups	88	0.46	3.4e-12	41	0.11	0.611
EPT water quality	88	0.40	0.006	41	0.12	0.492

In linear relationships, thresholds appeared as breakpoints where relationships changed according to the percent of annual median flow in the stream (Table 3). In managed streams the diversity of benthic taxa experienced an abrupt change when flows reached 58% of the annual median (Figure 3a). Flow thresholds were 50% for taxa considered important for food for fish and for functional feeding groups, and 20% for EPT taxa (Figure 3c, 3e, 3g). In unmanaged streams, naturally occurring extreme low-flow events were scarce and contributed few data for those conditions (Figure 3b, f). Interestingly, most ecological metrics had much higher variability below thresholds than above, suggesting that a certain level of low-flow avoids drops in the benthic community integrity of streams.

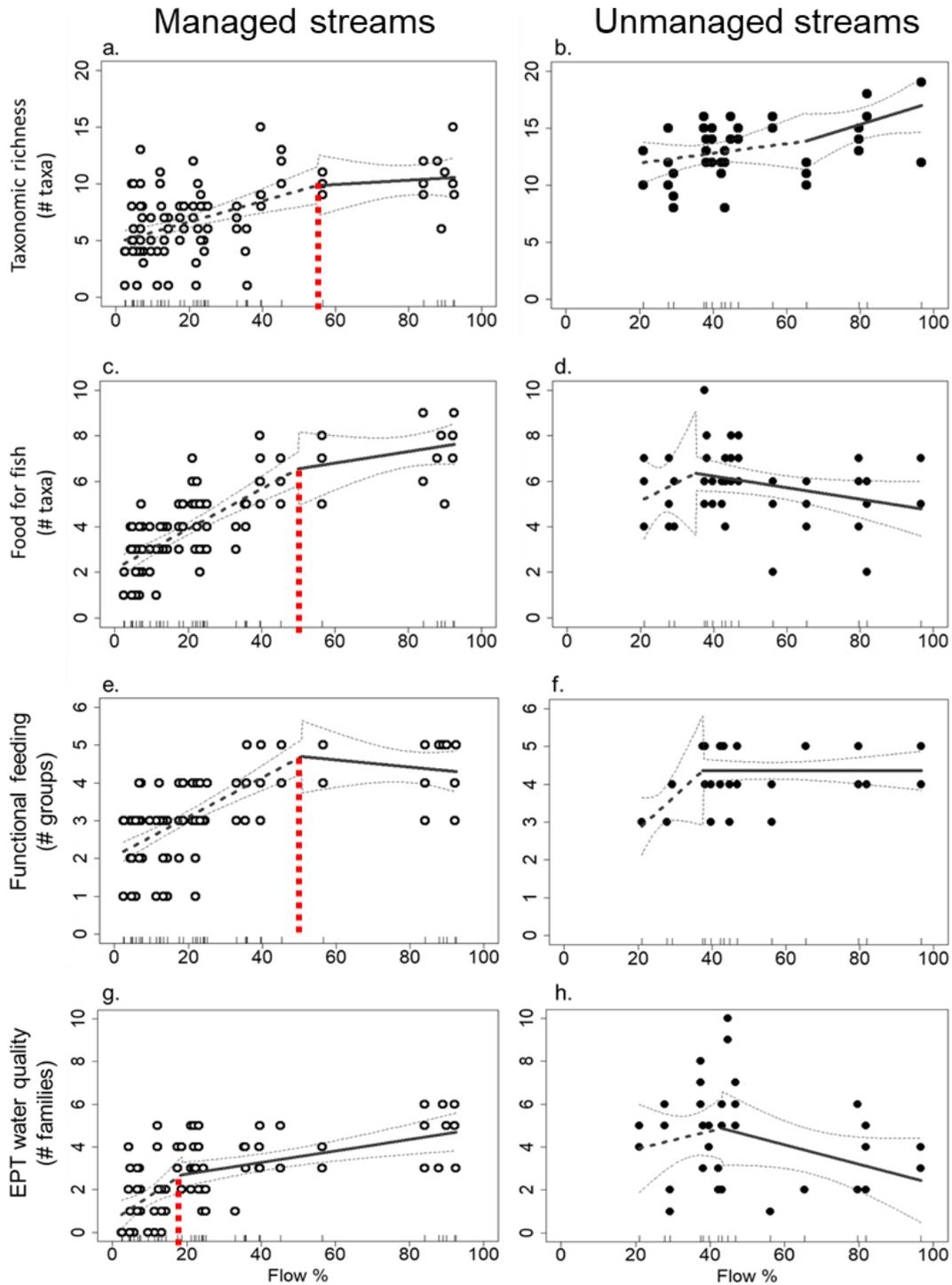


Figure 3. Scatter plots where red lines indicate thresholds in flow – benthic fauna relationships during low-flow conditions in managed (left panels) and unmanaged streams (right panel), using the percent of median annual flow (Flow %) and ecological metrics of taxonomic richness (a, b), taxa considered food for fish (c, d), functional feeding groups (e, f), and families from the Ephemeroptera, Plecoptera and Trichoptera orders (g, h).

Table 3. Results of thresholds as breakpoints in flow where ecological metrics change in pattern.

Ecological metric	Managed streams		Unmanaged streams	
	Breakpoint	Std. Error	Breakpoint	Std. Error
Taxonomic richness	54.23	12.53	65.56	41.28
Food for fish	50.41	14.19	37.47	8.18
Functional feeding groups	50.72	10.44	36.66	16.61
EPT water quality	18.70	5.94	43.30	12.75

Our TITAN analysis revealed several change points for tolerant and sensitive taxa with percent of the median annual flow (Figure 4). In managed streams the distributions of change points for tolerant and sensitive taxa intersected in a threshold of 22% of the annual median flow, while in unmanaged streams the distribution of change points for tolerant and sensitive taxa intersected in a threshold of 48% (Figure 4).

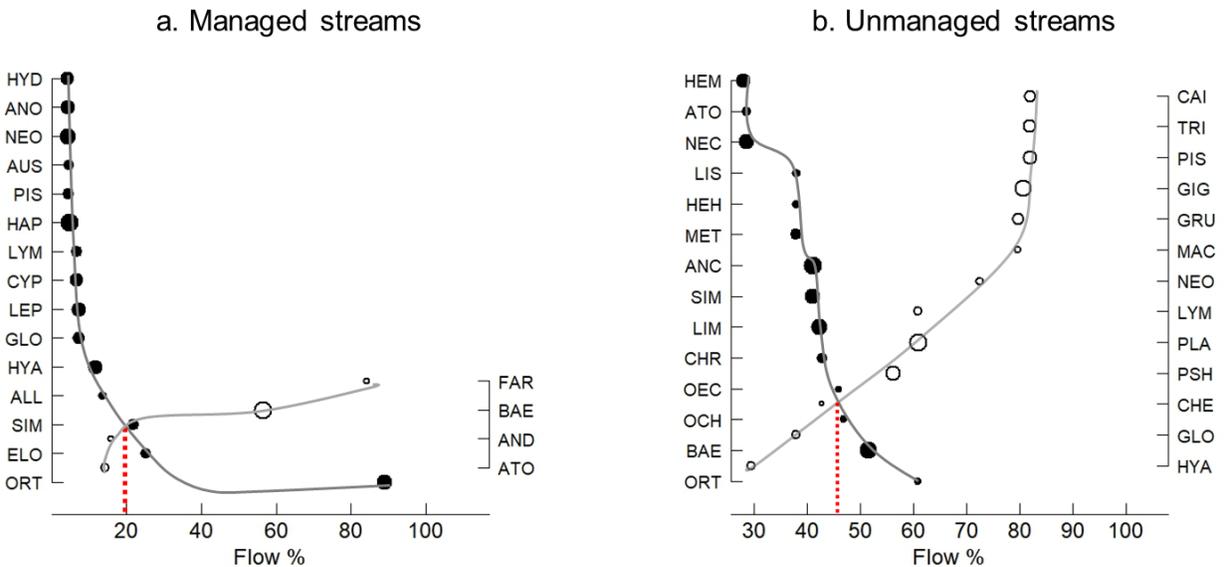


Figure 4. Results of change points from the thresholds analysis with TITAN for managed (a) and unmanaged streams (b), where filled circles are decreasing taxa (left), open circles are increasing taxa (right), circle size indicates taxa abundance, and thresholds corresponds to the intersection of distributions with the percent of median annual flow (Flow %) (red dotted line).

3.3. Evaluation of trade-offs and feasibility

Our thresholds analyses revealed that, overall, observed benthic invertebrate integrity was maintained when managed flows equaled or exceeded 40% of median annual flows. Releasing this percent of water volume from infrastructure constrains the capability of water managers to cope with Quito's water demand. To evaluate trade-offs, stakeholders need a tool to visualize plausible flows and the probability to achieve targeted conservation levels (Figure 5).

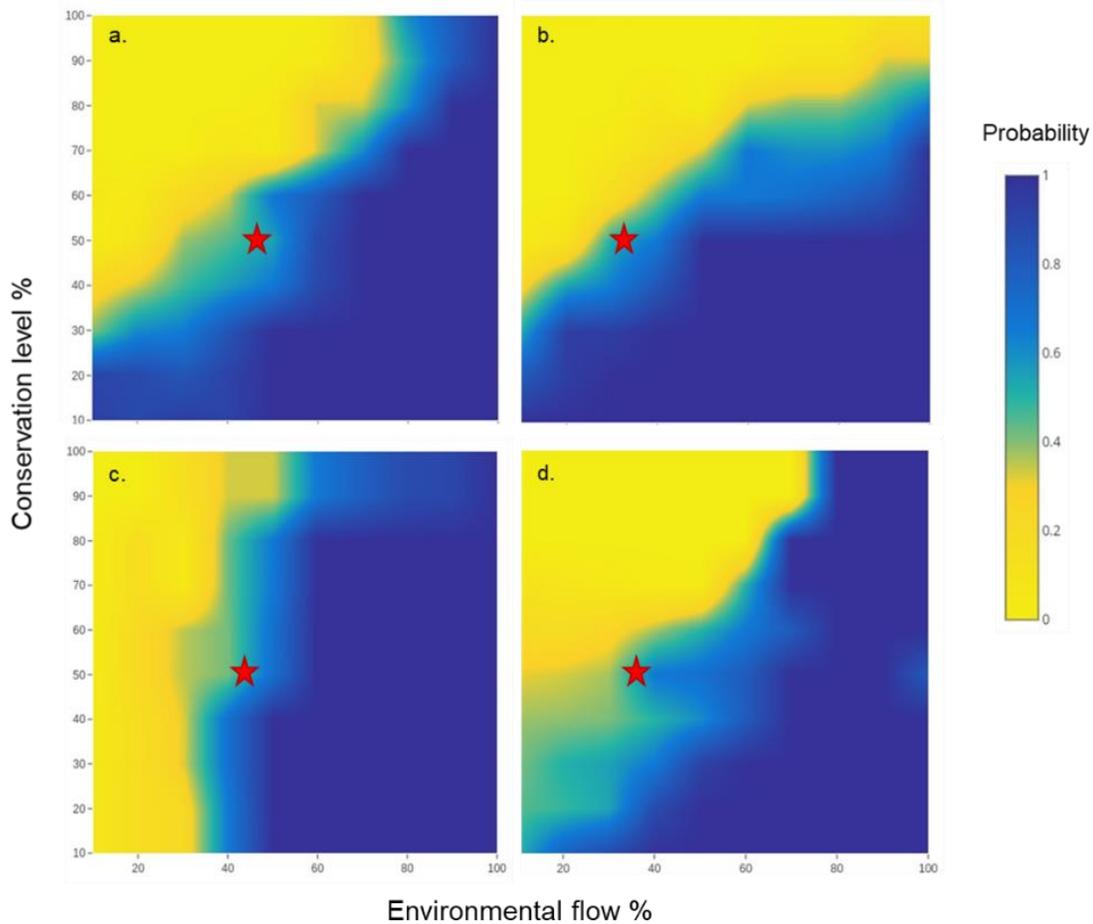


Figure 5. Trade-offs evaluation between flow left in streams and conservation levels of benthic fauna. Plots indicate for the four studied ecological metrics, the probability of a given conservation level as a function of the percent of flow left in streams: taxonomic richness (a), taxa considered food for fish (b), functional feeding groups (c), and invertebrates from the Ephemeroptera, Plecoptera and Trichoptera orders (d). The red star represents a hypothetical conservation level of 50% (5 out of 10 invertebrate taxa), that would have a 0.5 probability or occur in half the sites in the watershed, when flow left in the streams is 35%.

This tool works by showing how a conservation level, such as 50% (i.e. preserving 50% of a benthic invertebrate metric), would have a 0.5 probability (i.e. occurring in half of the sites in the watersheds) with 47% of the annual median flow for taxa richness, 38% for taxa considered important food for fish and EPT taxa, and 42% for functional feeding groups (Table 4).

Table 4. Percent of flow for ecological metrics calculated with benthic fauna on the threshold analyses and the trade-off analysis for a 0.5 probability of occurrence (i.e., half of streams).

Ecological metric	Thresholds analyses		Trade-off analysis
	<i>Segmented</i>	<i>TITAN</i>	<i>Conservation level 50%</i>
Taxonomic richness	58%	22%	47%
Food for fish	50%	n/a	38%
Functional feeding groups	50%	n/a	42%
EPT water quality	20%	n/a	38%

For a combined analysis of all metrics we weighted metrics equally and calculated the probabilities to achieve a 50%-level of benthic fauna conservation as a minimum in managed streams. A 50% conservation level considering weighted metrics would have a 0.5 probability of occurrence with 35% of the annual median flow left in streams (Figure 6). A range of flows between 28% and 40% of the annual median flow provides a wide spectrum of probabilities 0.3 and 0.7, respectively, for the evaluation of trade-offs (Figure 6).

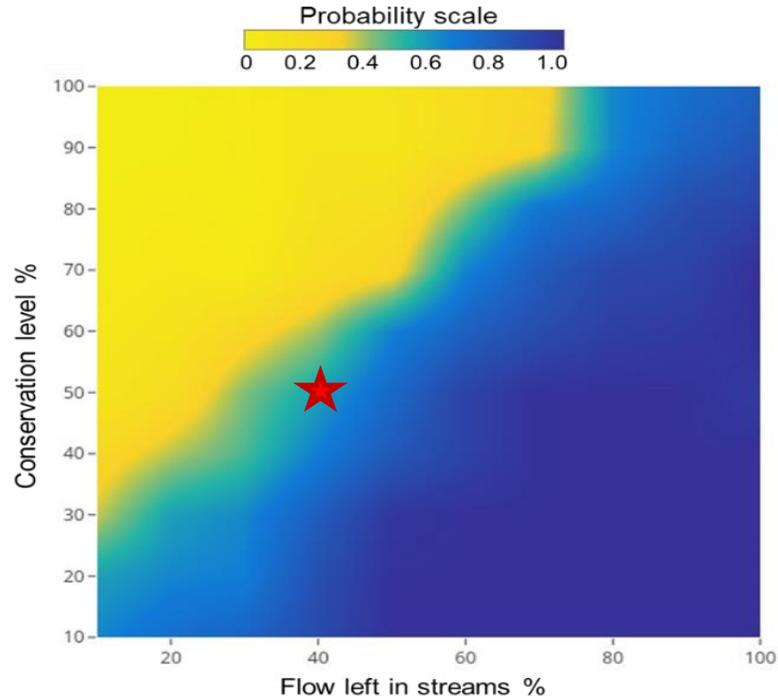


Figure 6. Trade-off evaluation for weighted ecological metrics in managed tropical Andean streams, where the probability of a given conservation level varies with the percent of flow left in streams by water managers.

4. Discussion

4.1. Eco-engineering decision scaling in scarce data hydrosystems

In this study, we propose an application of the EEDS framework (Poff *et al.*, 2016) to define e-flows using empirical data from managed streams for water supply in data-poor catchments. Because the empirical data gathering was rooted in a stakeholder driven process (Acreman *et al.*, 2014), it evolved from a monitoring program into a collaborative initiative between the water supply company (EPMAPS), as the primary user, and the water fund for Quito (FONAG), as the conservation counterpart. In cases like the studied system, where water managers are aware of the ecological role of biotic components of streams, it was still challenging to jointly define suitable indicators to relate with some characteristics of the flow regime. This is a common obstacle reported in the e-flows management literature (e.g., Overton *et al.*, 2014). The point of view of the collaboration in the studied system targeted the potential restoration in streams of

currently operational structures (Arthington, 2012). Such application of the EEDS approach could potentially incorporate abiotic indicators of water quality or be used to evaluate land use change and other anthropogenic alterations.

4.2. System criteria database

In our study, managed streams had flows that were always below the annual natural median. These flows, as minimum releases from intakes, proceeded from a stationary management perspective in contrast to an adaptive or continuous management approach. It allowed us to understand, to some extent, the mechanisms behind flow–benthic fauna relationships under flow regulation (Davies *et al.*, 2014; Rosenfeld, 2017). The percent of annual median flow from unmanaged streams that is released into managed streams offered a certain degree of stability for some taxa, i.e., a condition highly beneficial for these organisms in naturally harsh environments (Jowett and Biggs, 2009). The flow-benthic fauna relationships assessed in this research, although providing a snapshot of flow values, depicted complex patterns that incorporate some of the intrinsic noise and variability that characterize tropical hydrosystems (Jacobsen and Dangles, 2017).

We were able to generate relationships covering extreme low-flows found in managed and unmanaged streams. This indicates that a stationary metric such as a percent of some flow condition (e.g., annual median, seasonal median, etc.) could provide a range of variation depending on streams hydrological regimes (Buchanan *et al.*, 2017). In managed streams, low-flows showed slight variability reflecting, among others, the climatic conditions in the drainage area and the seepage from impoundments. The empirical data obtained in flow releases were possible thanks to the water company interest to identify thresholds for managing e-flows in streams. Such flow releases did not respond to any ecological condition previously defined, instead were intended to provide some “minimum-flow” condition for monitoring benthic fauna and to comply with Ecuadorian water law. E-flow thresholds obtained in this research are three times higher than those promoted by the law in Ecuador.

4.3. Thresholds analyses

Several ecological responses to flow reduction in temperate and tropical streams have elucidated the magnitude of change to a percent reduction of the mean annual flow (Pringle *et al.*, 2000; Poff and Zimmerman, 2010). Obtaining thresholds may not always be possible, therefore, using the right analyses beyond the effects of flow reduction is imperative for Andean tropical streams (Andersen *et al.*, 2009). The community taxa analysis (TITAN) provided one flow threshold while the ecological metrics analysis (Segmented) provided several. With both approaches it was possible to capture the high variability of the data that did not only show the effect of flow decrease but, also the variety of benthic fauna responses to changing conditions.

Overall benthic fauna metrics related negatively to flow decreases as reported extensively for other locations (Dewson *et al.*, 2007; James *et al.*, 2008; Poff and Zimmerman, 2010). The number of Ephemeroptera, Plecoptera and Trichoptera families in the studied system showed a similar response to reduced flows from mountain streams managed for water withdrawals in New Zealand (Dewson *et al.*, 2007). The number of taxa considered food for fish in this study was dominated by the Chironomidae family, a potential result of drift from flashy floods as reported in low land managed streams (Tonkin *et al.*, 2009). Death *et al.* (2009) reported that flow reductions up to 80% affected mainly filter feeders in managed streams, while we found a decrease on the number of functional feeding groups when flows reached 20% of the annual median.

Extreme low-flow conditions (< 20% of median annual flow) in managed streams simulated patterns extensively reported for intermittent streams (Stubbington *et al.*, 2017; Datry *et al.*, 2018), where habitats such as pools provide, to some extent, favourable conditions between flow releases. Andean streams with unmanaged conditions are known for their high levels of dissolved oxygen and low nutrient concentrations, providing suitable conditions for benthic fauna colonization (Ríos-Touma *et al.*, 2011). Changing from perennial to intermittent conditions would require Andean streams surpassing thresholds of hydrosystem function and

structure (Cauvy-Fraunié *et al.*, 2016), a potential scenario if managed streams undergo sustained flow reductions beyond the observed minimum flow condition. On the one hand, an e-flow threshold of 40% in managed streams coincided with flows where we found the bulk of benthic fauna in unmanaged streams. On the other hand, an e-flow threshold of 20%, a predominant condition in managed streams, coincided with minimum flows registered in unmanaged streams where we found low values of benthic fauna metrics. These findings suggest that e-flows thresholds of 40% of the annual median is necessary for the recovery of the benthic community in managed streams (Table 3), and an e-flow of 20% might be the lowest condition these hydrosystems can withstand prior to collapse.

4.4. Evaluation of trade-offs

Our framework analysed the performance of eco-engineering indicators in trade-offs of what will be achieved with different flow releases from water intakes regarding conservation levels of ecological metrics, either individually or combined. The proposed framework identifies a set of conditions to define minimum flow requirements and maximize conservation levels. As we were aware that our proposed 40% of annual median flow as an e-flow threshold for these streams would compromise the water company's capability to cope with the urban population demand, a trade-off analysis was developed to identify effects of further flow reductions. Our aim was to present a guideline to water managers that would allow them to evaluate probabilities of reaching certain levels of benthic fauna conservation as a function of flows left in streams. For instance, the weighted metrics trade-off analysis (Figure 6) corroborates that higher percent of flow (25 – 30% of annual median) left in streams will have a higher probability to achieve at least a 50% conservation level of benthic fauna in managed Andean tropical streams. These percentages are two and threefold higher than minimum percentages formerly proposed by Tennant (1976) and widely used in e-flows assessments for water infrastructures (drinking supply and hydropower generation) (Tharme, 2003). Subjecting streams to a stationary proportion of a flow component jeopardizes the intrinsic variability of streams, therefore managing e-flow thresholds above a

minimum condition provides at least the confidence that a certain level of conservation can be achieved. The efficiency frontier composed of trade-offs with flow thresholds is translated into allowable percentages of flow deviation from the unmanaged/natural condition, to maintain in streams as part of the water management for human supply.

4.5. Feasibility

Visualizing trade-offs as efficiency frontiers of several management options in the studied system still requires the feasibility analysis proposed in the last step of the framework (step 5, Figure 1). After a feasibility assessment, water managers, as stakeholders, still have the opportunity to create a new system database to refine thresholds through monitoring or to restate the system criteria to evaluate different eco-engineering indicators.

5. Conclusions

The consequences of reduced stream flow on benthic fauna are consistent with other findings in different latitudes and documented for high-altitude tropical streams (see Jacobsen and Dangles, 2017). Alterations of flow regimes in the studied system show that below the minimum e-flow threshold (<20% of median annual flow) conditions are distinctly unnatural, therefore surpassing this threshold will likely lead to a basal change in the hydro-ecosystem characteristics (Acreman *et al.*, 2014; Cauvy-Fraunié *et al.*, 2016).

We argue that under the e-flows concept, a threshold of flow for ecological indicators (e.g., benthic fauna) represents the potential restoration for hydrosystems. Applying e-flows from thresholds and trade-offs bridge the gap between the engineering and ecological perspectives for balancing ecological and human needs on integrated water resources management (Arthington, 2012; Overton *et al.*, 2014; Acreman *et al.*, 2014; Poff, 2018). Although our findings are constrained to the empirical data generated from monitored Andean tropical streams, they provide one of the first attempts to implement the eco-engineering decision scaling framework to

define e-flows. Thresholds from flow–benthic fauna relationships in the light of data-scarce hydrosystems constitute an advance in implementing sustainable principles for water infrastructure management.

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CHAPTER 2

STANDARDIZING INSTANTANEOUS MEASUREMENTS OF BENTHIC ALGAL BIOMASS AND COMPOSITION IN STREAMS

Abstract

Incorporating fluoroprobes, such as the BenthosTorch, to standard methods for monitoring benthic algal groups amplifies opportunities to respond promptly and to develop early warnings for preventing algal proliferation. Yet, the current use of *in situ* methods like the BenthosTorch suffers from measurement heterogeneity and difficulties homogenizing samples. Here, we compared Chl-a readings from the BenthosTorch to standard laboratory methods to fully quantify and describe biomass and relative abundance of benthic algal groups on artificial and natural substrates. We used the difference in Chl-a measurements between methods (n = 359) to evaluate the effects of environmental variables and we used artificial and natural substrates to determine if one reduced variation in BenthosTorch readings. Our findings demonstrate a general agreement between methods, but this relationship was stronger for artificial substrates. The interacting effects of light and benthic algal mat thickness indicated that artificial substrates are less susceptible to changes in these parameters than natural substrates. Artificial substrates provided significantly less variation among BenthosTorch readings for the same number of natural substrate samples. Overcoming differences on BenthosTorch readings seems plausible with the aid of artificial substrates in combination with proper calibration using standard laboratory methods. The ability of the BenthosTorch to produce immediate results facilitates replicate collections, which can reduce sample error and increase the area sampled on the same day. Understanding the presence of certain algal groups in a timely manner is a strategy worth incorporating into aquatic ecosystems monitoring.

1. Introduction

The monitoring of benthic algae can provide key insights into the structure and function of stream ecosystems. Under specific conditions (e.g., stable-flowing water, high temperatures, and labile nutrients), benthic algae can proliferate, potentially reducing water quality and altering ecosystems functions (Scanlan *et al.*, 2010; Ceola *et al.*, 2013). Quantifying benthic algal groups on different time and spatial scales is economically and logistically challenging but necessary for monitoring aquatic ecosystems under changing conditions, both natural (e.g., climate) and human induced (e.g., nutrient loading) (Wu *et al.*, 2001; Echenique-Subiabre *et al.*, 2016).

Until recently, the quantification of algal biomass, pigment concentrations, and algal taxonomic groups, has relied on standard laboratory methods. In addition to laborious sample preparation and the high level of taxonomic expertise required, standard methods pose logistical constraints for replication while maintaining accuracy (Steinman *et al.*, 2017). As an alternative to time-consuming laboratory methods, *in situ* techniques (e.g., fluoroprobes: BenthosTorch) emerged in the last decade to rapidly estimate Chl-a as a surrogate of algal accrual, phytoplankton and benthic algal characteristics (Catherine *et al.*, 2012), which can increase temporal and spatial measurements.

Comparative studies have employed different methods to assess the reliability of *in situ* versus standard laboratory methods and have shown better agreement between measurements when algal concentrations are relatively low (Table 5). (Steinmann *et al.*, 2017). All of these studies used natural substrates and generally found it challenging to standardize Chl-a readings for benthic algal biomass and relative abundance from mature mat formations. In terms of Chl-a, BenthosTorch and standard methods were r correlated for benthic algal found in oligotrophic streams (Kahlert and McKie, 2014). For an urban Kansas stream (USA), Harris and Graham (2015) found strong correlations between the BenthosTorch and standard methods when Chl-a concentrations were lower than 4 $\mu\text{g}/\text{cm}^2$. Echenique-Subiabre *et al.* (2016) found low correlations in eutrophic streams when benthic algal mats were relatively thick. In terms of relative abundance of benthic algal and primary producers, Echenique-Subiabre *et al.*, (2016),

Kahlert and McKie (2014), and Harris and Graham (2015) presented contradictory results for diatoms and cyanobacteria (Table 5). Kaylor *et al.* (2018) recently addressed the sensitivity of BenthosTorch readings to light exposure, suggesting that assessing the reliability of Chl-a readings from the BenthosTorch should consider physical factors that influence benthic algal biomass proliferation (i.e., light, temperature, water velocity, nutrients).

Table 5. Previous comparisons of *in situ* (BenthosTorch) and standard methods (laboratory) for chlorophyll-a concentrations and relative abundance of benthic algae and primary producers (i.e., diatoms, green algae, cyanobacteria).

Reference	<i>n</i>	Chl-a concentration	Laboratory method	<i>n</i>	Relative abundance
Kaylor et al. 2018	50	BenthosTorch underestimated Chl-a when laboratory measures where $> 4\mu\text{g}/\text{cm}^2$	95% acetone, Fluorometer	-	-
Echenique-Subiabre et al. 2016	288	High correlation of methods for thin biofilms $< 4\mu\text{g}/\text{cm}^2$, ($R^2 = 0.60$, $p < 0.001$) Low correlation of methods for biofilms $> 4\mu\text{g}/\text{cm}^2$, ($R^2 = 0.23$, $p = 0.008$)	90% absolute methanol, Spectrophotometer	120	High correlation for cyanobacteria when proportion $< 50\%$ ($R^2 = 0.53$, $p < 0.001$)
				64	High correlation for diatoms when proportion $> 40\%$ ($R^2 = 0.54$, $p < 0.001$)
				117	Low correlation for green-algae when proportion $< 20\%$ ($R^2 = 0.10$, $p < 0.001$)
Harris and Graham 2015	30	High correlation for concentrations $< 4\mu\text{g}/\text{cm}^2$ ($R^2 = 0.47$, $p < 0.01$)	96% heated ethanol, Fluorometer	6	BenthosTorch underestimated diatoms by 5.4X and green algae by 1.3X
				6	BenthosTorch overestimated cyanobacteria
Kahlert and McKie 2014	24	BenthosTorch not significantly different from laboratory method	90% acetone, Spectrophotometer	24	BenthosTorch estimation of diatoms ~85% and laboratory ~35%
				24	BenthosTorch estimation of green algae ~11% and laboratory ~27%
				24	BenthosTorch estimation of cyanobacteria ~4% and laboratory 32%

Despite general agreement among previous studies about the relationships between the BenthosTorch readings and standard methods, there is a need to reduce the variability in Chl-a readings and relative abundance analysis of major algal groups. We hypothesized that substrate variability may substantially contribute to differences in measurements and we hypothesize that standardized substrates will decrease variability. Artificial substrates of known size and texture have been demonstrated to provide similar responses to natural substrates (Cattaneo and Amireault, 1992; Tuchman and Stevenson, 1980). Artificial, standardized substrates facilitate

monitoring different stages of benthic algal mat formation and, together with uniformed texture, may offer a more robust comparison of Chl-a readings between the BenthoTorch and standard methods.

Our study assessed the performance of the BenthoTorch compared to standard laboratory methods using artificial and natural substrates to understand conditions under which the BenthoTorch is least sensitive. Specifically, we aimed to: 1) compare total Chl-a concentration and relative abundances of algal groups between the BenthoTorch and standard methods on artificial and natural substrates, 2) evaluate environmental effects of light, temperature, algal mat thickness, depth, velocity, and discharge on Chl-a readings from the BenthoTorch and laboratory methods, and 3) assess BenthoTorch readings with standardized, artificial substrate to evaluate variability of readings compared to natural substrates. The use of standardized, artificial substrates to compare BenthoTorch and standard methods has not been previously recorded in the literature. Thus, the results of this research may have implications for improving the reliable use of the BenthoTorch via the use of artificial substrates for monitoring biomass of benthic algal and primary producers on aquatic ecosystems.

2. Materials and methods

2.1. Study area

The study area is the headwaters of the Chalpi Grande River watershed, basin area = 95 km², located inside the Cayambe-Coca National Park in the northern Andes of Ecuador at an elevation of 3835 m (S 0°16' 45", W 78° 4'49") (Figure 7). Streams are predominantly fed by rainfall draining volcanic geology with substrates varying from boulders to silt and tussock grasslands covering stream banks. Located in the highest part of the Chalpi Grande watershed is Quito's water supply system. Low head dams are placed throughout the stream network to facilitate water withdrawals to the system of water supply pipes (water intakes). The Chalpi Norte stream is a main headwater tributary and the presence of small dams provide an ideal setting to study benthic algae in natural (free flow) and altered (regulated flow) stream reaches because

hydrological conditions (i.e., streamflow) vary over short spatial and temporal scales (Jacobsen and Dangles, 2017; Rosero-López *et al.*, 2019a). Intricate mats of benthic algae are readily found in free-flowing conditions while in regulated flow conditions iron-oxidizing bacteria accumulate beneath benthic algae, which might influence the performance of the BenthoTorch method.

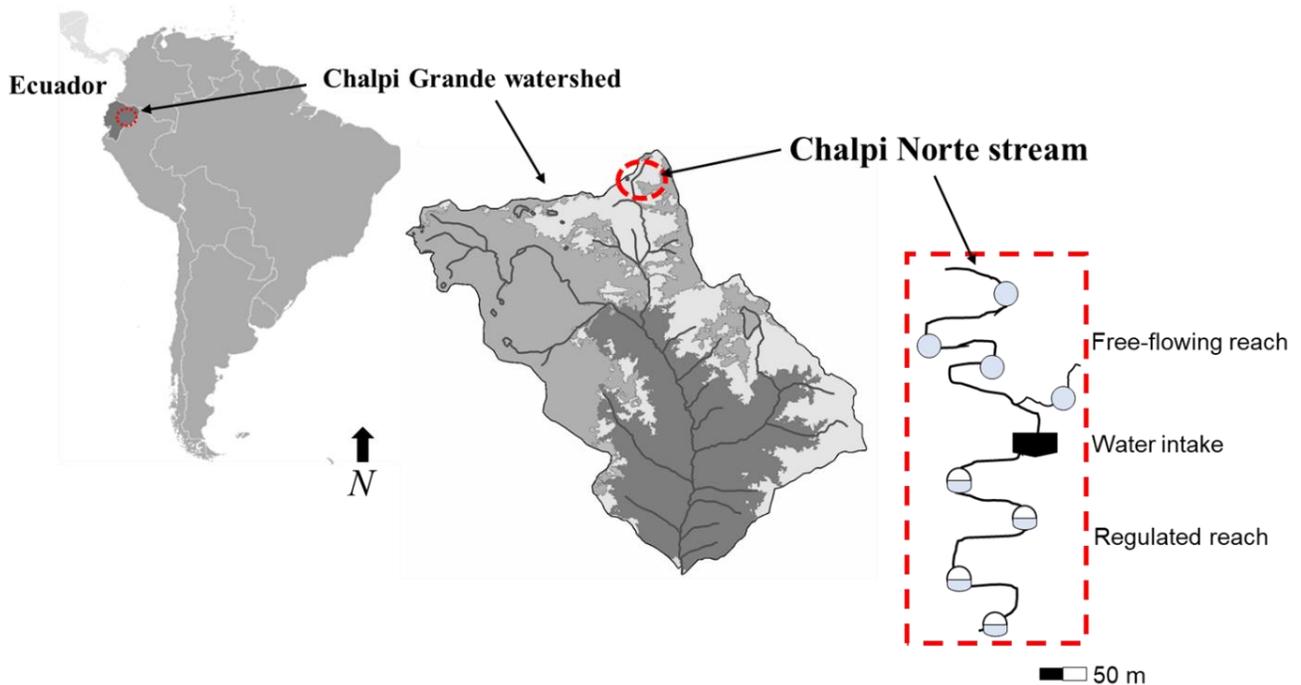


Figure 7. Chalpi Norte stream study sites above (free-flowing reach) and below the water intake (regulated reach) in the Chalpi Grande watershed, north -east of Ecuador.

2.2. Sampling design

We conducted our sampling during the 2017 high-flow season, July – September (Supplementary Material FS1). To characterize the spatial and temporal dynamics of benthic algae, we measured Chl-a as a surrogate of algal accrual on artificial substrates and biomass on natural substrates (Figure 8). We selected four sites in free-flowing conditions (upstream of water intakes) and four sites with regulated flow conditions (downstream of water intake).

Except for one site in an unaltered tributary (control site), all sites were in the Chalpi Norte stream (Figure 8).

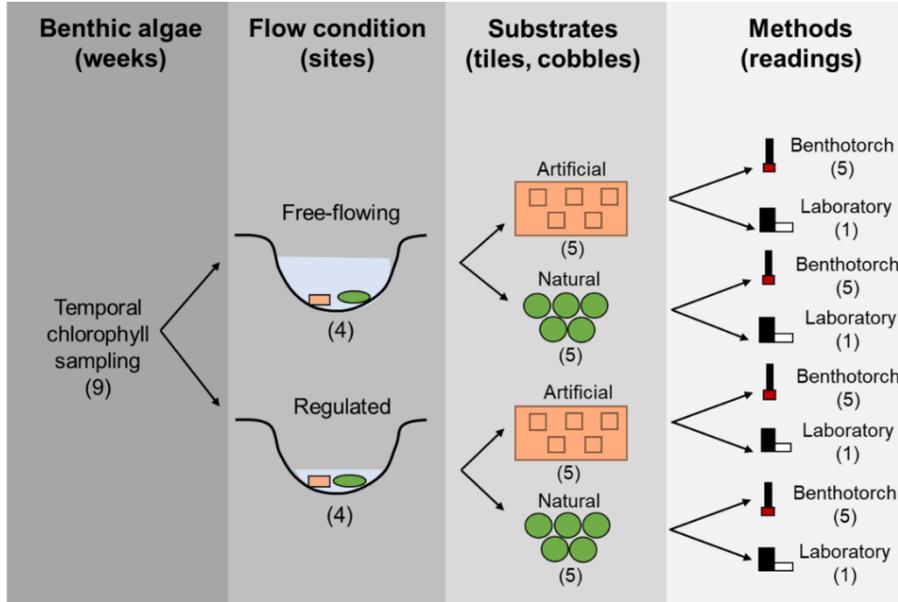


Figure 8. Survey design for methods comparisons of chlorophyll-a concentration and benthic algal relative abundance on artificial and natural substrates under different flow conditions: free-flowing and regulated flow, number in parenthesis indicate replicates.

Artificial substrates were created by cementing unglazed ceramic tiles (4 x 4 x 0.5 cm) to clay bricks (15 x 25 cm). In the field, we placed 10 bricks with five tiles at every site arranged in two parallel rows perpendicular to the current. We collected samples on days 0, 7, 14, 21, 28, 35, 42, 49, 56 and 63 with no replacement: five tiles per site (artificial substrates, total n = 359) and five cobbles per site (natural substrates, total n = 355, diameter = 5 – 9 cm). For each set of samples, we applied two methods for benthic algal Chl-a determination and for relative abundance of algal taxonomic groups (see Section 2.3). We measured physical variables on artificial and natural substrates (see Section 2.4). Based on previous findings (see Rosero-López *et al.*, 2019b) these headwater streams show low aquatic invertebrates' density in high-flow season; therefore, we assumed the grazing effect was negligible.

2.3. Measuring benthic algal on artificial and natural substrates

We used a fluoroprobe (BenthoTorch) to make *in situ* measurements of Chl-a concentrations of each algal group. We applied standard methods for laboratory Chl-a extraction and analysis, and for taxonomic identification.

2.3.1. In situ method: BenthoTorch

The BenthoTorch fluorometer (BG36700-V, bbe Moldaenke GmbH Schwentental, Germany), is a deployable pulse-amplitude-modulated (PAM) spectrofluorimetric tool, that uses predefined algorithms to instantaneously identify the Chl-a fluorescence signal of benthic algae and primary producers such as diatoms, cyanobacteria, and green algae (Carpentier *et al.*, 2013). This instrument emits light pulses at 470, 525, 610, and 700 nm and records Chl-a response at 690 nm (Kahlert and McKie 2014). We placed the BenthoTorch on artificial and natural substrates avoiding light entering the algae's surface area of excitation (Kaylor *et al.*, 2018). The sampled area where the beam of light excited the Chl-a was 1 cm². Ten seconds of exposure provides Chl-a concentrations for diatoms, cyanobacteria, and green algae. To reduce variation, we performed three readings for each measurement, these were averaged, as suggested by the manufacturer (bbe Moldaenke, 2013).

2.3.2. Standard methods: Laboratory readings and taxonomic identification

We measured Chl-a concentrations following the DIN 38 412-L16 laboratory method (Deutsches Institut für Normung, 2015). We specifically followed the manufacturer's laboratory method for Chl-a extraction with ethanol and quantification using a spectrophotometer to avoid variances caused by different solvents and equipment (i.e. acetone and methanol, see Steinman *et al.*, 2017). Following the bbe Moldaenke (2013) protocol, samples (algae and substrate) were stored in foil bags with a moist sponge and placed in isolated boxes with ice packs (T = ~7 °C) for transportation to the laboratory. Under minimal light exposure, benthic algae were removed from

substrates using a nylon brush and collected in a tray using filtered water. The sample slurry of each substrate was homogenized and placed in a graduated cylinder to measure total volume (V_P). We calculated the scraped area (A_{SCR}) on natural substrates using ImageJ2 software (Rueden *et al.*, 2017). We delineated the contour of the substrate surface colonized by benthic algal using a known reference area for scale. We filtered 2.5 ml of the sampled slurry (V_E) using glass microfiber filters in a funnel (Whatman GF/F 0.7 μm nominal pore size). Filters were introduced into dark film canisters filled with 10 ml of 90% buffered ethanol, ensuring complete submersion. Canisters were closed and incubated for 12 hours at room temperature. After incubation, we poured 3 ml of the extract into a cuvette and used a spectrophotometer (Agilent 8453, Thermo Scientific) equipped with UV-visible spectroscopy software running on a PC (bbe Moldaenke, 2013). We read samples at a 665 nm wavelength followed by a reading at 750 nm, both against the offset of reference 90% ethanol solution (DIN 38 412-L16). Then we used the 750 nm measurement for the compensation of the sample turbidity and, lastly, added 3M HCl (10 μl for 10 ml of extraction volume) to the filtered extract. After a 10 min incubation in darkness, we measured Chl-a converted to phaeophytin under the 665 and 750 nm wavelengths. We calculated Chl-a concentrations using:

$$[\text{Chl} - \text{a}] = 29.6 \cdot [(A_{v665} - A_{v750}) - (A_{n665} - A_{n750})] \cdot \frac{V_E}{A_{SCR} \cdot d} \quad (1)$$

Where, Chl-a is the chlorophyll a concentration ($\mu\text{g}/\text{cm}^2$), A_v and A_n are the absorptions at 665 and 750 nm before (v) and after acidification (n), V_E is the extracted volume, A_{SCR} is the scraped area, and d is the cuvette width (cm).

We subsampled 2 ml of scrubbed benthic algal slurry (see below) and placed it into a labeled round bottom cryogenic vial. Samples were preserved with Lugol's iodine solution and kept at room temperature. We left samples to settle for at least 8 hours and started identification

no later than 24 hours after collection. We identified and enumerated benthic algal groups on random fields using an Utermöhl sedimentation chamber on an inverted microscope (40-45x objective, 400 – 450 total system magnification, Zeiss Axio Vert. 40, Germany). We enumerated 300 natural units in a minimum of 10 and a maximum of 100 fields on a 12 x 12 mm grid. All analyses were carried-out at the laboratory of the Quito water supply company, *Empresa Pública Metropolitana de Agua Potable y Saneamiento* (EPMAPS). We grouped taxa to calculate the relative abundance of benthic algal groups (i.e., diatoms, cyanobacteria, green algae).

2.4. Physical variables (light, temperature, algal mat thickness, depth, velocity, and flow)

We performed our study during high-flow conditions in July and August of 2017 (Table 6). We registered environmental light intensity five times, every 10 s for 60 s each time, at a standard height of 1.6 m from the ground (LI-200R, Pyranometer sensor LI-COR®, USA). For the instantaneous water temperature, we used a thermo-probe at five points around artificial and natural substrates (VWR Thermometer probe, VWR Scientific™, USA). On substrates, we measured the benthic algal mat thickness (Digital caliper, Mitutoyo 500-196-30 CAL, USA). We measured water velocity twice (Acoustic digital current meter OTT Hydromet, Germany) at a 2 cm depth and water column depth with the current meter wading rod. We also measured integrated velocities at 20, 60 and 80% of the total depth, at a minimum of 6 locations across the stream, using the current meter to calculate discharge at the reach scale (i.e., using the area-velocity method).

Table 6. Mean and ranges of physical variables registered on sites from the Chalpi Norte stream with different flow conditions during July and August 2017.

Site identification	Flow condition	Dominant substrate	Benthic algal mat thickness (mm)		Light (Watt/dm ²)	Water temperature (°C)	Water velocity at 2 cm from bottom (m/s)	Water depth (m)	Stream flow (m ³ /s)
			Artificial subs.	Natural subs.					
Chalpi 01	Free-flowing	Gravel (Pebble - Cobble)	1.5 (0 - 3)	5.9 (1.67 - 9.5)	1.91 (0.55 - 4.02)	8.41 (7.02 - 8.99)	0.71 (0.31 - 1.08)	0.15 (0.14 - 0.19)	0.34 (0.17 - 0.54)
Chalpi 02	Free-flowing	Gravel (Pebble - Cobble)	1.4 (0 - 5)	6.5 (0.5 - 13.1)	2.39 (0.4 - 3.99)	8.86 (8.21 - 9.25)	0.68 (0.34 - 0.97)	0.15 (0.13 - 0.21)	0.35 (0.17 - 0.55)
Chalpi 03	Free-flowing	Gravel (Pebble - Cobble)	1.2 (0 - 4)	8.1 (1.4 - 14.3)	2.65 (1.55 - 4.22)	8.43 (8.01 - 8.99)	0.86 (0.21 - 1.42)	0.21 (0.18 - 0.27)	0.38 (0.18 - 0.56)
Chalpi 04*	Free-flowing	Gravel (Pebble - Cobble)	0.9 (0 - 2)	7.1 (1.8 - 12.7)	1.73 (0.55 - 3.72)	8.14 (7.44 - 9.93)	0.67 (0.33 - 0.99)	0.14 (0.11 - 0.21)	0.04 (0.03 - 0.21)
Chalpi 05	Regulated	Gravel (Cobble)	1.2 (0 - 4)	5.8 (0.4 - 13.9)	2.38 (0.33 - 4.55)	9.61 (8.54 - 10.09)	0.32 (0.21 - 0.59)	0.10 (0.09 - 0.13)	0.016 (0.012 - 0.034)
Chalpi 06	Regulated	Gravel (Cobble)	0.9 (0 - 2.5)	6.5 (1.3 - 13.1)	1.91 (0.56 - 3.99)	9.22 (9.05 - 9.84)	0.57 (0.32 - 0.88)	0.12 (0.07 - 0.15)	0.025 (0.015 - 0.038)
Chalpi 07	Regulated	Gravel (Cobble)	1.4 (0 - 3.5)	5.8 (0.4 - 11.4)	1.63 (0.45 - 4.81)	9.35 (8.09 - 9.93)	0.72 (0.29 - 0.99)	0.14 (0.07 - 0.16)	0.092 (0.024 - 0.101)
Chalpi 08	Regulated	Gravel (Cobble)	1.1 (0 - 3.5)	7.3 (2.2 - 14.0)	1.89 (0.45 - 4.21)	9.16 (8.64 - 9.77)	0.61 (0.25 - 0.99)	0.13 (0.07 - 0.16)	0.191 (0.088 - 0.391)

* this site corresponds to a tributary of the Chalpi Norte stream

2.5 Statistical analysis

We applied the Bland-Altman test, also known as the Tukey, mean-difference plot to evaluate the interval of agreement between two methods (Giavarina, 2015; Datta, 2017). It is important to note that high correlations between two datasets is an insufficient criterion to assess method comparability (Giavarina 2015). While correlation analyses are often used to assess the relationship between methods, this approach does not provide the degree of replicability between methods; thus, our choice to use the Bland-Altman test. According to the test, normally distributed data from a method comparison are in acceptable agreement if 95% of the data falls within ± 1.96 standard deviations from the line of equality (Bland and Altman 1999). To identify the distribution of data within confidence intervals and along the line of equality, Bland and Altman proposed a graphical analysis based on the plot of the average of paired data (on the x-axis) and the difference of paired data (i.e., difference = laboratory measurement – field measurement) (y-axis). We assigned the laboratory method as the reference from which we compared the BenthosTorch. The distance of the mean of differences to the line of equality corresponds to the potential bias between methods and, in our case, indicates if the BenthosTorch underestimates (+ bias) or overestimates (- bias) measurements from the laboratory method. The mean of differences could be systematic and statistically significant when the line of equality is not within the limits of the interval of agreement. To test the significance of differences, a two-tailed test of means was necessary. We ran a Pearson correlation to complement our methods agreement analysis, which we performed using the “blandr” package (Datta, 2017; R Core Team,

2017), with un-transformed data of Chl-a concentrations ($\mu\text{g}/\text{cm}^2$) and relative abundance (%) of benthic algal groups (i.e., diatoms, cyanobacteria, green algae) for both substrates.

To determine the effect that physical variables have on the accuracy of Chl-a readings we modeled variables known to be related to benthic algal accrual and biomass in streams (Grubicic *et al.*, 2010). We evaluated benthic algal mat thickness, water temperature, light, water velocity, depth, and streamflow registered at sites with different flow conditions (free-flowing and regulated) (Table 6).

We assessed the difference between methods on each substrate with a linear mixed-effects model that allows correlation patterns to be explicitly modeled (Bates *et al.*, 2015). We used the difference of paired readings as the response variable, physical variables as fixed effects and flow condition (free-flowing vs. regulated), sites, and sampling dates as random effects as follows:

$$\Delta_{(laboratory-Benthotorch)} = \beta_0(\text{flow}) + \beta_1(\text{velocity}) + \beta_2(\text{temperature}) + \beta_3(\text{depth}) + \beta_4(\text{benthic algal mat thickness}) + \beta_5(\text{light}) + (1|\text{flow condition}) + (1|\text{site}) + (1|\text{date}) \quad (2)$$

We assumed a Gaussian distribution of differences and visually checked residuals goodness-of-fit as well as homoscedasticity. After testing for colinearity, we compared models with all terms and a model with the interaction of significant variables. We selected models for each substrate using p-values obtained by the likelihood test and Akaike information criteria (AIC); a lower AIC value indicates a better model fit. We built models with the “lmer” function in the lme4 package v.3.2.3 (Bates *et al.*, 2015; R Core Team, 2017). To visualize the distribution of differences, we plotted a heat map with physical variables of significant effects (Plotly Technologies Inc. Montreal, QC 2018).

We assessed BenthoTorch readings with substrate standardization first by evaluating biomass accrual through time for diatoms, cyanobacteria and green algae found on artificial substrates from sites with different flow conditions (free flowing vs. regulated). Second, we used

a separate data set collected in the Chalpi Norte stream in 2018 to apply a rarefaction to BenthosTorch readings from artificial and natural substrates. We calculated the coefficient of variation from 5 BenthosTorch readings taken on 5 artificial and 5 natural substrates from 5 different sites of an experimental stream reach ($n = 25$). To calculate the coefficient of variation according to the number of samples, we used a randomization of data ($n = 25$) then averaged the obtained values and calculated standard error. We plotted the coefficient of variation against the number of samples for artificial and natural substrates (R Core Team, 2017).

3. Results

3.1. Agreement of methods

3.1.1 Chlorophyll a (Chl-a) concentrations

On artificial substrates, concentrations of Chl-a ranged from 0 to 6 $\mu\text{g}/\text{cm}^2$ ($n = 359$) (Figure 9a) and concentrations on natural substrates ranged between 0 and 15 $\mu\text{g}/\text{cm}^2$ ($n = 355$) (Figure 9b). Among all sampling events and both free-flowing and regulated condition, the correlation between the BenthosTorch and the standard method was significant for both substrates (artificial: $R^2 = 0.84$, $p = 0.0074$; natural: $R^2 = 0.85$, $p = 0.0032$). On artificial substrates the systematic difference analysis showed that $> 3 \mu\text{g}/\text{cm}^2$ the BenthosTorch underestimated laboratory Chl-a concentrations by 11.4% (Figure 9c). The systematic difference analysis on natural substrates revealed the BenthosTorch underestimated algal biomass by 31% when compared to standard readings, with higher bias obtained for Chl-a values $> 5 \mu\text{g}/\text{cm}^2$ (Figure 9d).

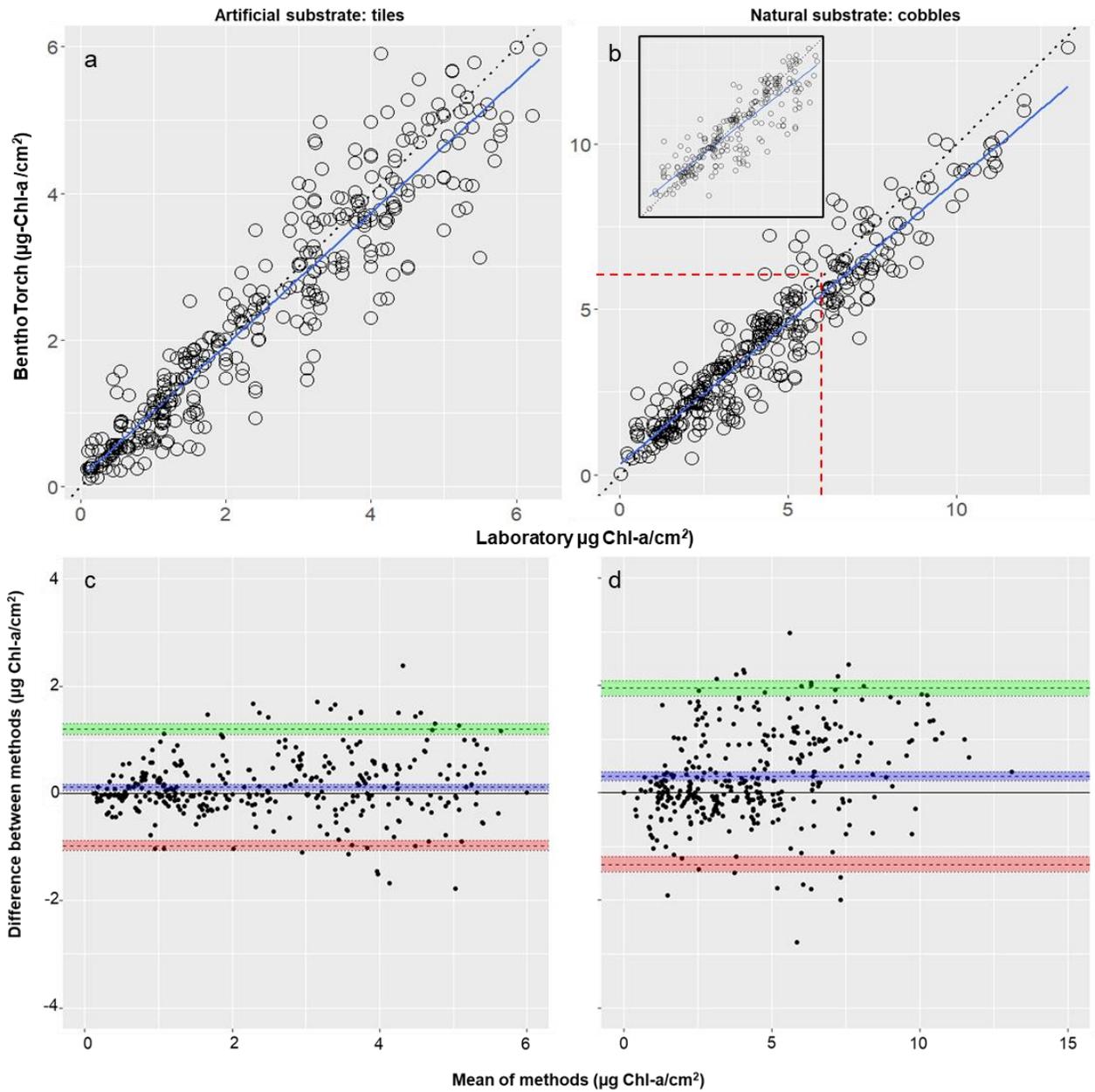


Figure 9. Results of chlorophyll-a (Chl-a) concentrations on artificial and natural substrates measured with the BenthosTorch (in situ) and the laboratory methods (standard). Pearson correlations between paired measurements, the blue line indicates the deviance to the line of equality (dotted line), small plot on natural substrates indicates the regression relationship for the range found on artificial substrates (0 – 6 $\mu\text{g/cm}^2$) (red dotted lines) (a, b). Bland and Altman intervals of agreement and systematic differences (c, d).

3.1.2 Relative abundance of benthic algal groups

On artificial substrates we found significant positive correlations between BenthoTorch and standard laboratory readings ($n = 359$) for diatoms ($R^2 = 0.76$; $p = 0.018$), cyanobacteria ($R^2 = 0.65$, $p = 0.004$), and green algae ($R^2 = 0.89$, $p = 0.007$, Figure 10a, b, c). When the relative abundance of diatoms exceeded 40%, the BenthoTorch underestimated diatoms concentration by $\sim 2\%$. Similarly, when the relative abundance of cyanobacteria exceeded 40%, the BenthoTorch underestimated concentrations by $\sim 2.8\%$ (Figure 10d, e). The BenthoTorch underestimated green algae by 1.5% when the relative abundance surpassed 50% (Figure 10f).

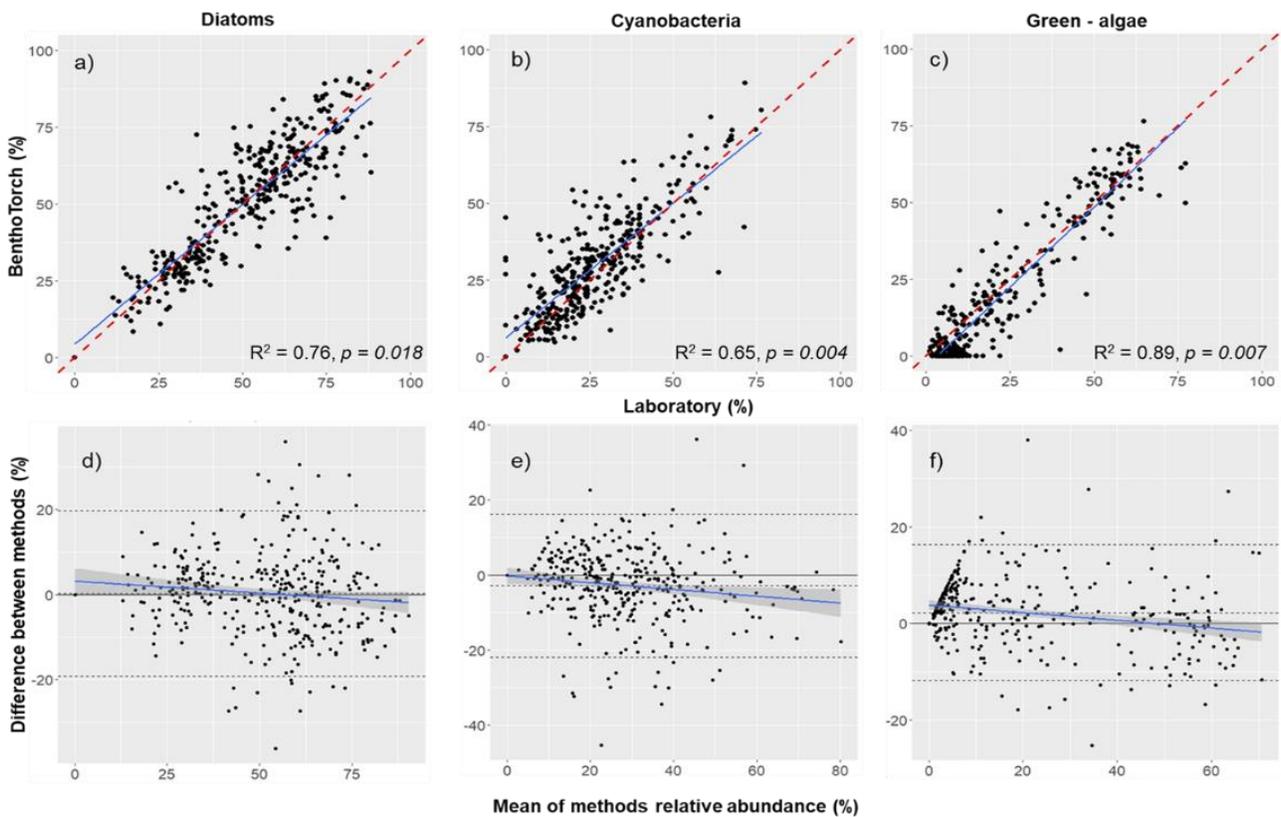


Figure 10. Results for the relative abundance (%) of diatoms (a, d), cyanobacteria (b, e), and green algae (c, f) measured with the BenthoTorch (in situ) and laboratory (standard) methods on artificial substrates $n = 359$. Top panels show regressions lines (blue) concerning the 1:1 relation line (red). Bottom panels show bias between methods agreement (black) with the 95% confidence intervals (dotted lines) and regression lines (blue).

We found that 95.4% of the diatoms data, 95.3% of cyanobacteria, and 95.1% of green algae fell within the 95% confidence limits (Figure 10). These results indicated that the BenthosTorch performed congruently with measurements obtained from the standard identification analysis on artificial substrates. Contrary to artificial substrates, on natural substrates the correlation between the BenthosTorch and the standard method was not significant for diatoms ($n = 144$) ($R^2 = 0.49$, $p = 0.132$), and marginally significant for cyanobacteria ($R^2 = 0.39$, $p = 0.07$) and green algae ($R^2 = 0.65$, $p = 0.05$) (Figure 11a, b, c). We found that above a threshold of 25% on natural substrates, the BenthosTorch underestimated the relative abundance of cyanobacteria and green algae relative to standard laboratory identifications (Figure 11e, f).

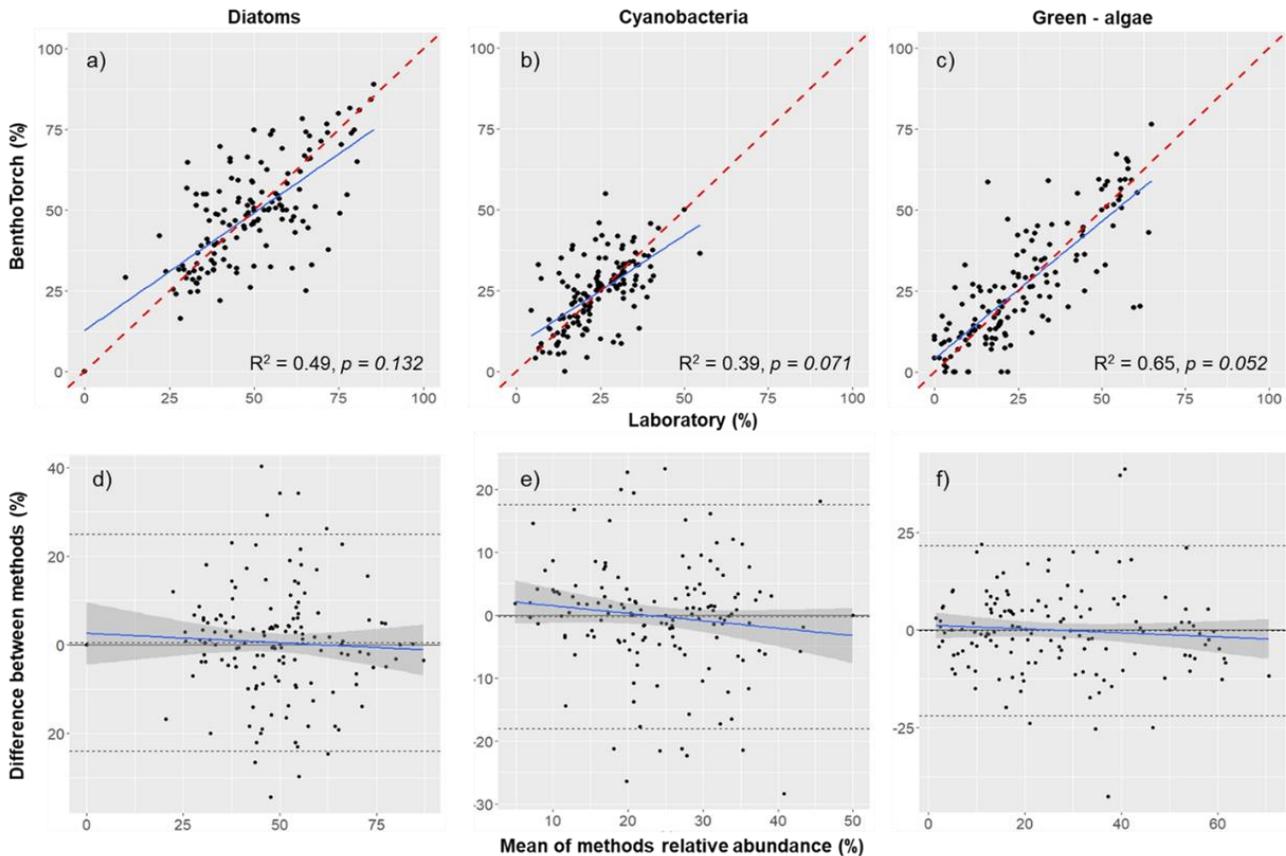


Figure 11. Results for the relative abundance (%) of diatoms (a, d), cyanobacteria (b, e), and green algae (c, f) measured with the BenthosTorch (in situ) and laboratory (standard) methods on natural substrates $n = 144$. Top panels show regressions lines (blue) concerning the 1:1 relation line (red). Bottom panels show the bias between methods agreement (black) with the 95% confidence intervals (dotted lines) and regression lines (blue).

From the systematic difference analysis, we found that 95.8%, 93.7%, and 91.6% of green algae, diatoms, and cyanobacteria data, respectively, fell within the 95% confidence intervals. These results indicated that, overall, the BenthosTorch performed accordingly to the standard method for taxonomic identification and performance was better on artificial substrates.

3.2 Physical variables and the differences in methods

We found that mat thickness and light quantity were the only significant ($p < 0.05$) predictors of the bias in Chl-a concentrations on artificial and natural substrates (Table 7). The general linear model comparison between substrates was significant ($\chi^2(1) = 61.25$, $p < 0.0001$), which indicated that on artificial substrates only mat thickness exerted an effect on the bias while mat thickness and light explained the bias on natural substrates. We found no consistent bias between measurements methods on artificial substrates for mat thicknesses between 1 to 5 mm and a range of light from 500 to 1500 $\mu\text{mol}/\text{m}^2 \text{ s}$.

Table 7. Results of the linear mixed model for the difference between chlorophyll-a readings (difference = laboratory - BenthosTorch) and the effects of light and benthic algal mat thickness measured on artificial substrates and natural substrates.

	Artificial substrates $n = 359$				Natural substrates $n = 355$			
<i>Fixed effects</i>	Estimate	Std. Error	t value	<i>p</i>	Estimate	Std. Error	t value	<i>p</i>
(Intercept)	-0.07	0.13	-0.55		0.92	0.20	4.65	
Light	0.04	0.05	0.83		-0.25	0.08	-2.99	0.001
Thickness	-0.09	0.04	-2.06	0.05	-0.31	0.03	-11.18	0.0001
Light*Thickness	0.01	0.02	0.62		0.09	0.01	8.18	0.0001
<i>Random effects</i>	Variance	Std. Dev.			Variance	Std. Dev.		
Date	0.000	0.000			0.000	0.000		
Site	0.054	0.233			0.000	0.000		
Flow condition	0.000	0.000			0.000	0.000		
Residual	0.312	0.559			0.619	0.780		

On the contrary, on natural substrates within this range of light, the bias decreased when mat thickness increased from 2 to 5 mm (Figure 12). The BenthosTorch underestimated (+ bias) Chl-a concentrations on artificial substrates when light decreased below 460 $\mu\text{mol}/\text{m}^2 \text{ s}$. The

BenthosTorch overestimated Chl-a concentrations on natural substrates when light increased above 1400 $\mu\text{mol}/\text{m}^2\text{s}$, for benthic algal mat thicknesses of 3 to 5 mm (Figure 12).

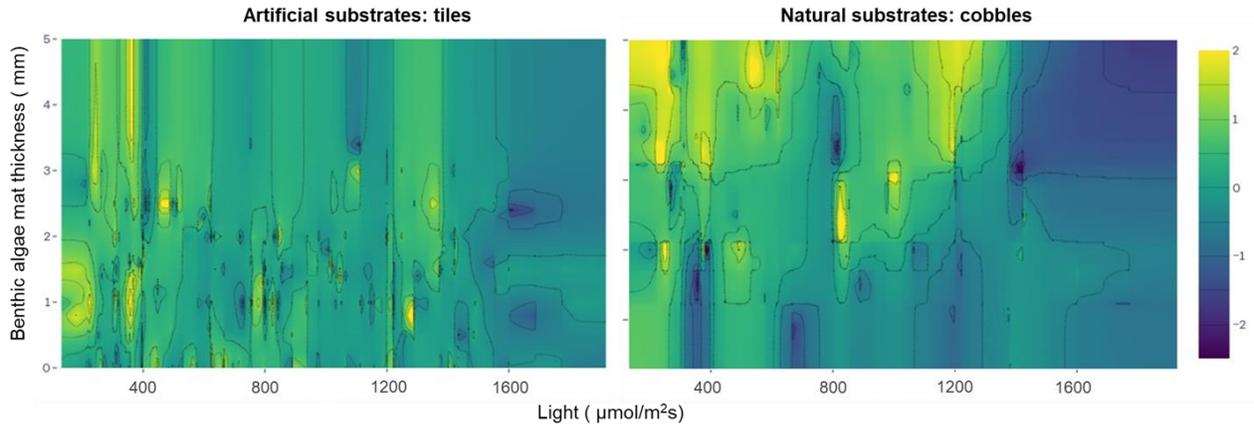


Figure 12. Difference of chlorophyll-a readings (Laboratory - BenthosTorch) from benthic algae found on artificial and natural substrates according to mat thickness and light measured under different flow conditions (free-flowing and regulated) in the Chalpi Norte stream during July and August 2017.

3.3 Standardization of substrates and BenthosTorch readings

BenthosTorch readings on artificial substrates provided information on biomass accrual for diatoms, cyanobacteria and green algae in both free-flowing and regulated conditions (Figure 13).

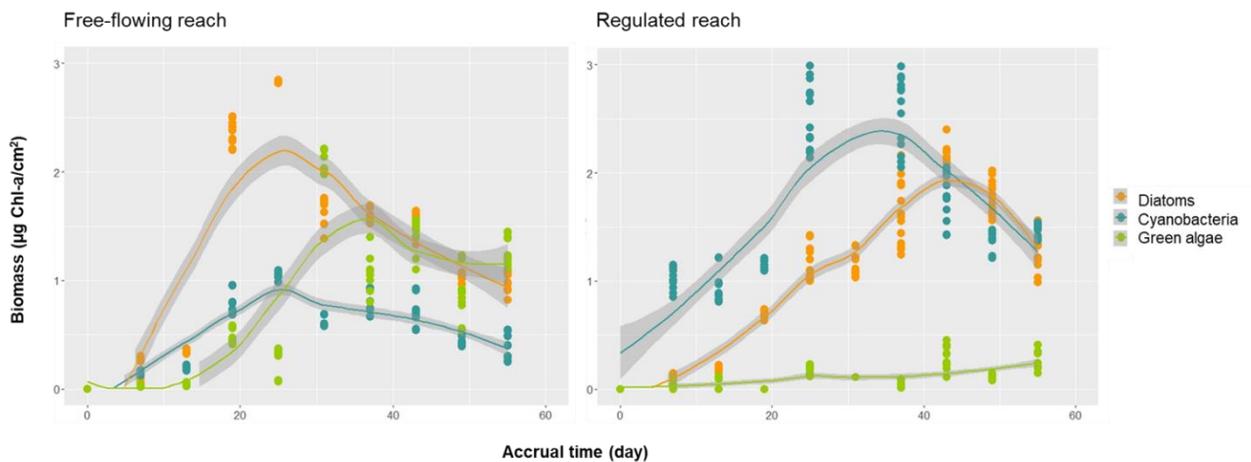


Figure 13. Biomass of benthic algal and primary producers (i.e., diatoms, cyanobacteria, green algae) registered weekly during 55 days of accrual on artificial substrates ($n = 20$) from free flowing (a) and regulated (b) reaches in the Chalpi Norte stream, Ecuador.

In free-flowing conditions, the biomasses of diatoms and cyanobacteria peaked simultaneously but with different values on day 24. Green algae peaked on day 36 with lower biomass than diatoms but greater than cyanobacteria. Cyanobacteria presented the lowest biomass from of three groups (Figure 13a). Under regulated flow conditions cyanobacteria peaked on day 36 with higher biomass than diatoms, which peaked on day 42 (Figure 13b). Green algae biomass was lower and presented no obvious peak of biomass accrual (Figure 13b).

The rarefaction analysis showed that the coefficient of variation for BenthosTorch readings from artificial and natural substrates decreased with the number of samples (Figure 8). The range of variation of BenthosTorch readings on artificial substrates was lower than natural substrates. With the same number of samples, we obtained different coefficient of variations for artificial substrate (5%) and natural substrate (21.3%). To obtain a 5% coefficient of variation with natural substrates we need to sample 90 cobbles (Figure 14).

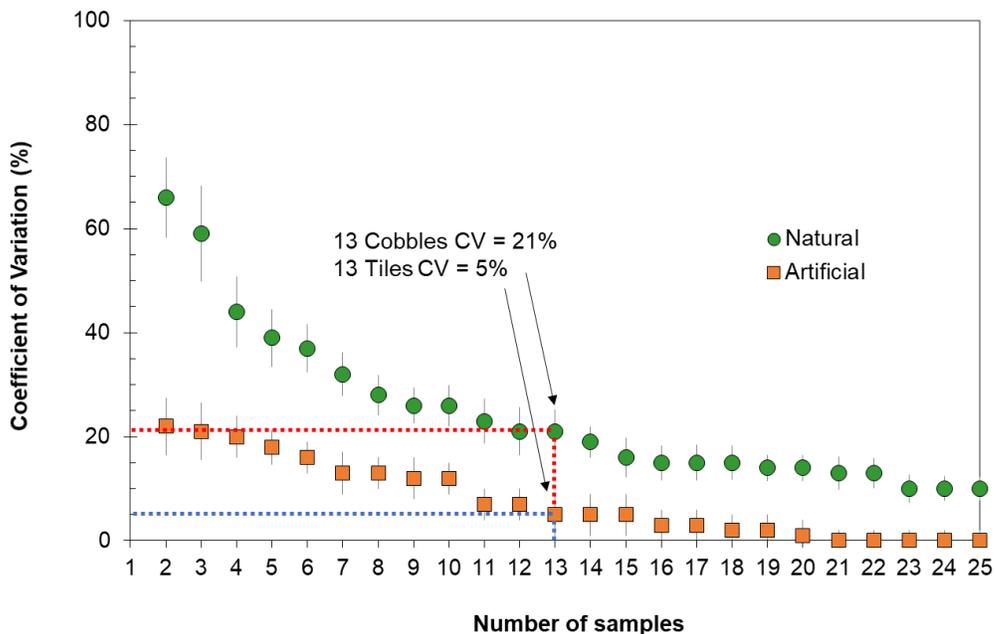


Figure 14. Rarefaction analysis of the coefficient of variation of BenthosTorch readings for an equal number of samples (n = 13) for artificial (blue dotted line) and natural (red dotted line) substrates.

4. Discussion

The main goal of this study was to assess the performance of the BenthosTorch compared to the standard laboratory method using artificial and natural substrates. We assessed: (1) Chl-a and relative abundance of algal groups, (2) the effect of physical variables on BenthosTorch and laboratory readings, and (3) the potential use of artificial substrates to standardize BenthosTorch readings.

Previous comparisons between the BenthosTorch and laboratory methods have been performed exclusively on natural substrates with confounding results for Chl-a and relative abundance of algal groups (Table 1). In our study, BenthosTorch readings obtained on artificial substrates under different flow conditions demonstrated the BenthosTorch provides comparable results to standard laboratory Chl-a concentrations. Overall, there was higher agreement between methods for Chl-a on artificial substrates than on natural substrates.

On both substrates the BenthosTorch underestimated Chl-a concentrations compared to standard measurements. Our findings are consistent with Harris and Graham (2015) and Kaylor *et al.*, (2018) who found the BenthosTorch underestimated Chl-a readings for concentrations $> 4 \mu\text{g}/\text{cm}^2$, although our threshold was $3 \mu\text{g}/\text{cm}^2$. The high correlation between methods that we found for Chl-a concentrations on artificial substrates is consistent with previous studies (Kaylor *et al.*, 2018, Echenique-Subiabre *et al.*, 2016, Harris and Graham, 2015), which found that the BenthosTorch worked best for thin films and low concentrations. This pattern has been extensively attributed as the cause of discrepancies between readings on natural substrates (Harris and Graham, 2015; Echenique-Subiabre *et al.*, 2016).

The relative abundance of algal groups between the two methods was more comparable on artificial substrates than on natural substrates. This could be explained by the physiological state of photopigments of diatoms in the upper layers of biofilms that form on artificial substrates as opposed to the distinct layers that form of natural substrates (Escoffier *et al.*, 2014). Our results for artificial substrates are consistent with findings from Harris and Graham (2015) and Echenique-Subiabre *et al.*, (2016) for natural substrates with early-stage algal mat formations,

which likely have a similar morphology to the algal mats on our artificial substrates. The relative abundance of cyanobacteria was overestimated by the BenthosTorch (~ 5%) when the proportion was more than 40% of the community on artificial substrates, which agrees with findings on natural substrates by Harris and Graham (2015). However, this result contrasts with findings from Echenique-Subiabre *et al.*, (2016) and by Kahlert and McKie (2014) who found the BenthosTorch underestimated the abundance of cyanobacteria in natural substrates. Echenique-Subiabre *et al.*, (2016) attributed an underestimation of cyanobacteria to higher abundance of phycoerythrin-containing cyanobacteria that fluoresce at a different wavelength than cyanobacteria without this pigment. A further assessment of phycoerythrin-containing cyanobacteria on artificial substrates could elucidate the confounding results with previous research. However, despite the systematic overestimation (~2.8%) of cyanobacteria on artificial substrates, results showed strong agreement between methods. We found similar results on artificial substrates for green algae as reported for eutrophic and oligotrophic streams where the BenthosTorch systematically underestimated green algae (Echenique-Subiabre *et al.*, 2016; Harris and Graham, 2015; Kahlert and McKie, 2014).

The modeled differences between methods revealed a weak association with mat thickness on artificial substrates, while on natural substrates the association was strong. Kaylor *et al.*, (2018) and Echenique-Subiabre *et al.*, (2016) have described the importance of light and benthic algal mat thickness, respectively, in influencing the BenthosTorch performance. Kaylor *et al.*, (2018) found that the light environment prior to BenthosTorch measurements can impact Chl-a estimates and, to reduce this error, they moved rocks into a shaded environment. However, this increases sampling time and logistical complexity. Evaluating the relationship between the light environment and Chl-a readings relative to laboratory methods may be a way to reduce logistical constraints of using the BenthosTorch for Chl-a readings.

We demonstrated that artificial substrates are a resourceful complement for the standardizing BenthosTorch readings, allowing the study of the benthic algal temporal dynamics and providing less variation on Chl-a readings with a lower number of samples. Any inefficiency

for registering incoming, reflecting signals from irregular substrates explains part of the bias in observed measurements, although this has been addressed in recent BenthosTorch versions (Escoffier *et al.*, 2014). Artificial substrates provided data on benthic algal colonization despite potential detachment caused during high-flows, which might have limited Chl-a yield on artificial substrates compared to natural substrates (Graba *et al.*, 2014). Indeed, the uniformity of surface on artificial substrates has been shown to reduce benthic algal self-shadow, favoring pigment fluorescence (Carpentier *et al.*, 2013). In contrast, irregularities on natural substrates increase biomass accumulation in crevasses that could be responsible for affecting the fluorescence of algae in the underlying layers (Hauer and Lamberti, 2011). Overcoming differences in BenthosTorch readings seems plausible with the aid of artificial substrates and facilitate calibration with standard laboratory methods. Substrate standardization enhanced BenthosTorch readings, showing low variation between measurements and providing the opportunity to sample extensively with in-field replication.

5. Conclusions

Benthic algal and microbial communities are key basal resources in aquatic food webs as they are a main source of energy for higher trophic levels (Battin *et al.*, 2016; Besemer *et al.*, 2018). Understanding the presence and abundance and the temporal dynamics of certain algal groups in a timely manner is a strategy worth incorporating into aquatic and marine ecosystems monitoring. Factors triggering aquatic algal groups have challenged the current body of standard methods available to provide succinct responses to changing environmental conditions (Watson *et al.*, 2008; Vorösmaty *et al.*, 2010; Franks, 2018). Here, we highlight the advantage of using the BenthosTorch and artificial substrates compared to standard methods and natural substrates for assessing chlorophyll a concentrations and relative abundance of benthic algal groups. The ability of the BenthosTorch to produce immediate results facilitates replicate collections, which can reduce sample error and increase the area sampled on the same day. BenthosTorch Chl-a readings allowed to capture the dynamic of benthic algal accrual on artificial substrates. This

monitoring tool, like most methods, should always be accompanied by a routine comparison with standard methods. When the BenthosTorch was compared to different standard laboratory methods reported in the literature, neither provided an unequivocally correct measurement. For methods comparison, an analysis of agreement could provide a better understanding of methods performance than correlation analysis, especially in the interest of deciphering conditions responsible for differences between methods. Incorporating fluoroprobes, such as the BenthosTorch, to standard methods for monitoring benthic algal groups amplifies the opportunities to respond promptly and develop early warning systems for algal proliferation prevention.

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CHAPTER 3

DESIGN OF A PAIRED WEIR SYSTEM FOR EXPERIMENTAL MANIPULATION OF ENVIRONMENTAL FLOWS IN STREAMS

Abstract

Controlled in-stream flow manipulations are challenging but necessary to implement in order to assess the consequences of real-world flow alterations on aquatic ecosystems. We designed a double v-notch weir system, which was first prototype-tested in a laboratory flume and then in the field. The device diverted instantaneous flows proportionally in a robust way (e.g., 10% of flow in the stream main channel and 90% of flow into the diversion channel). We tested diversions systematically and evaluated how well flows matched our target flow-percent. This is one of the first ecohydraulic structures conceived and tested to experimentally manipulate environmental flows (e-flows) in regulated streams at upland stream scales.

1. Introduction

Environmental flows (e-flows) is a stream management concept with the goal to restore and maintain freshwater ecosystems for society and ecological processes (2007 Brisbane Declaration: Arthington, 2012). E-flows assessment requires new experimental advances to complement current frameworks and methods in order to provide scientifically defensible tools that inform environmentally sustainable water management (Arthington et al., 2018; Rosero-López *et al.*, 2019a). E-flows recommendations rely on understanding how stream ecology, hydrology, and hydraulics respond to anthropogenic flow alteration (Poff and Zimmerman, 2010; Jacobsen and Dangles, 2017). However, it is not trivial to infer how physical alterations (e.g., flow rate, sediment transport, nutrient cycling, dissolved oxygen, conductivity), which are relatively easy to monitor, influence biological indicators (e.g., fish, macroinvertebrates, plants, benthic algae) of ecosystem health. Flow-ecology relationships are often cited as a cornerstone of e-flows development, although such relationships are difficult to establish (e.g., Buchanan *et al.*, 2017).

Research into e-flows, and the relatively new field of ecohydraulics, continues evolving to evaluate the impacts on downstream ecosystems due to human water resources utilization (Nestler *et al.*, 2016; Katopodis, 2005), including designing specialized flow manipulation structures. Most operational hydraulic structures (e.g., dams, water intakes) are designed without considering e-flows, especially structures designed for water diversion, i.e., as opposed to those used for flow regulation (Rosero-López *et al.*, 2019b). Thus, e-flows recommendations utilize managing hydraulic structures currently built with non-ecosystem-based purposes (e.g., spillways, side channels). Research conducted downstream of hydraulic structures has taken advantage of settings where streams have already been altered to understand ecosystem response to flow manipulations (e.g., Konrad *et al.*, 2011, Buchanan *et al.*, 2017).

Flow manipulations at mesoscales have been assessed in laboratory flumes, artificial channels, and stream reaches providing valuable insights about biological responses to specific

flow alterations (Poff *et al.*, 1990; Biggs, 1995; Uehlinger *et al.*, 2003; Dewson *et al.*, 2007a; James *et al.*, 2008). Experimental flow manipulations in natural environments are difficult to perform. Cauvy-Fraunié *et al.*, (2016) used rock dams to simulate a decrease in glacier melt water input and assess stream-level consequences on biota, but this approach can only be used in relatively small streams. Experimental approaches to address flow-ecology relationships remain understudied principally because of the challenges to the control of *in situ* flows at realistic temporally-, spatially-, and ecologically-relevant scales (Lancaster, 2018).

We propose an experimental flow manipulation structure designed to maintain fixed diversion percentages of upstream flow (natural conditions) at a whole ecosystem scale. Our objectives were to: 1) design and test a paired-weir system to systematically alter streamflow, and 2) assess manipulated flows and associated changes in stream channel morphology. The structure design and field test presented in this research are part of an ecosystem-scale experimental flow manipulation study performed in our experimental reach. This study presents first-hand guidelines for developing field experiments to assess e-flows releases for current and future water resources infrastructure.

2. Methods and materials

2.1 Experimental stream reach

The experimental reach is in the Chalpi Norte stream located in the Cayambe-Coca National Park in the northern Andes of Ecuador at an elevation of 3835 m (S 0°16' 45", W 78° 4'49"). The Chalpi Norte is a second-order stream in the headwaters of the 96 km² Chalpi Grande watershed. The experimental reach's drainage area is 5.4 km² with a predominantly volcanic geology. The stream runs through tussock grasslands that cover the stream banks and bottom substrates range from boulders to silt. We selected a stream meander as our experimental reach that allowed us to set up a relatively short flow diversion structure (Figure 15). The experimental

reach was 97 m long with an average width of 1.5 m (min= 0.7 m, max= 2.3 m) and ~1% slope. We monitored discharge in the upstream reach for 12 months to assess streambank stability during low and high flows (Figure 15). Streamflow monitoring provided valuable information of the suitable period for e-flows manipulations (Supplementary Material, FS1).

2.2 V-notch paired-weir design and laboratory testing

We designed a system of two triangular weirs (a.k.a., v-notch weirs) with different angle openings to work simultaneously. We built a lab-scale plywood prototype with two opening angles to divert 30% and 70% of the flow, respectively, through each weir (Figure 16). We used the sharp-crested, v-notch weir equation:

$$Q = C_d \frac{8}{15} \sqrt{2g} \tan\left(\frac{\theta}{2}\right) h^{5/2} \quad (1)$$

where: Q is the discharge, C_d (0.62, Henderson, 1966) is the non-dimensional discharge coefficient, θ is the angle of the v-notch (see Figure 17b), h is the water head above the weir vertex, and g is the acceleration of gravity (Shen, 1981; Martinez 2005). For our prototype, weir angles were determined via a set of two equations, i.e., equation (1) for 30% of total flow and equation (1) for 70% of total flow. We tested the prototype in a small hydraulic flume in the DeFrees Hydraulics Laboratory, Cornell University. We measured the height of water in the flume before diverting the water and the height of water over the v-notch with a point-gauge device. Flow in the laboratory flume varied between 1.0 and 9.6 l/s as measured by a modified venturi flow meter (Lo-Loss PMT-IP series, Badger Meter Inc.) where the differential pressure is measured with a pressure transmitter (Rosemount 3051S series with HART protocol Emerson Process Management) sampled at 1/3 Hz and averaged over three measurements for each case.



Figure 15. *Top:* aerial image of Chalpi Norte stream in the Cayambe-Coca National Park, Ecuador, dotted yellow line indicates the place where the pipe for diversion was buried; *center:* experimental reach during low flows (photo taken on January 15, 2017), *bottom:* experimental reach during high flows (photo: taken on July 24, 2017).

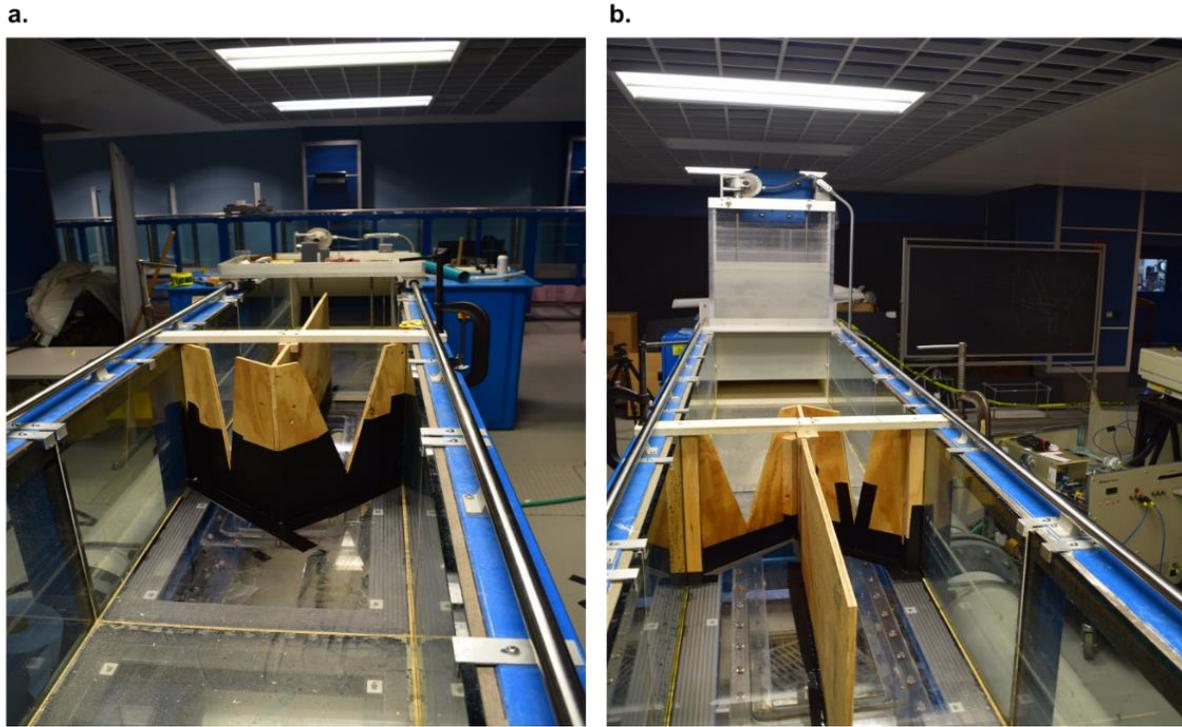


Figure 16. Upstream (a) and downstream (b) views of the two v-notch paired-weir prototype system tested in a flume, De Fries Hydraulic Laboratory, Cornell University.

We conducted 11 experiments, each at a different total flow rate, and assessed the paired-weir system performance by measuring cross-sectional area and velocity to calculate flows on each channel downstream of the diversion. Flows were measured with an acoustic digital current meter (Acoustic Digital Velocity meter ADC OTT Hydromet, Germany), sampled at 6 Hz and averaged over three measurements.

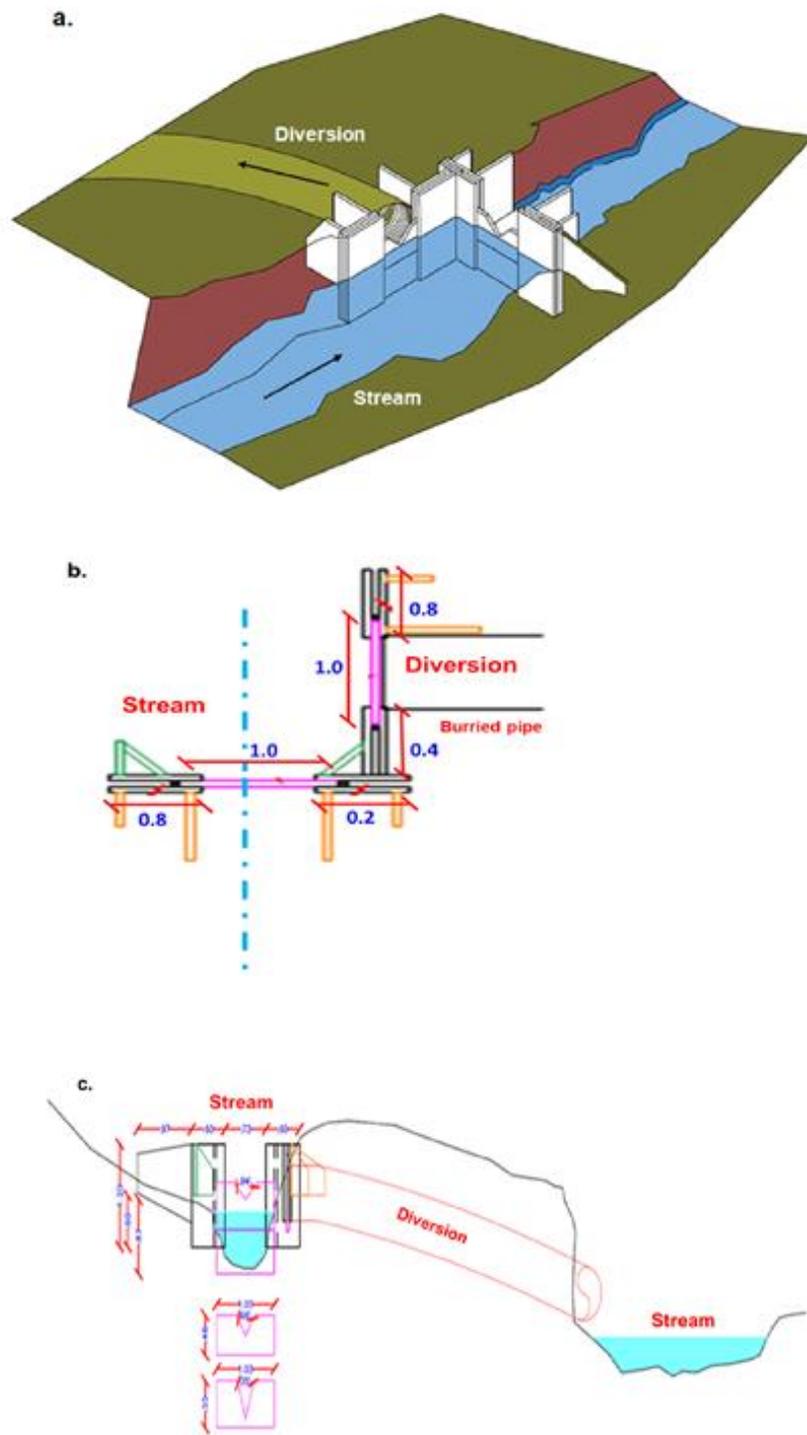


Figure 17. Experimental flow manipulations structure designed and installed in the Chalpi Norte stream (a), paired-weir system top view of stream and diversion channel (b), transverse view of stream weir and buried pipe that ends in the stream (c).

2.3 Field installation and testing

We buried a diversion pipe (Figure 15 top, dashed line), designed with a capacity approximately equal to the estimated bankfull stream discharge and installed perpendicular to stream to avoid potential backwater effects and clogging. The diversion pipe was constructed of corrugated plastic pipe with a slope of 3% and interior diameter of 0.50 m. We conducted a topographical survey of the experimental stream reach ($L = 97$ m) to assess stream morphology and stream cross-sections between the diversion pipe inlet and outlet. Every 1.5 m we measured channel width, thalweg elevation, location within the reach, longitudinal slope, and lateral slopes (Total Station, Northwest Instrument 10836 NTS03). We installed pressure transducers 8 m upstream and 12 m downstream of the paired v-notch structure, and 3 m downstream from the diversion outlet (HOBO U20L-04, Onset® Computer Corporation, USA). We scaled-up our laboratory-tested structure such that a stream weir allowed some Chalpi Norte stream flow to enter the experimental stream reach and a diversion weir diverted some flow to a diversion pipe (Figure 17). The stream weir was approximately perpendicular to the diversion weir ($\beta_{\text{field}} = 87^\circ$) (Figure 17b), which was similar to our lab test ($\beta_{\text{lab}} = 90^\circ$) (Figure 16). The pipe entrance was secured in a concrete box with a fitting that allowed the weirs to be inserted. The outflow-end of the pipe was armored with cobbles to dissipate energy in order to reduce downstream erosion (Figure 17c). The experimental device setup needed 75 person-hours, including the excavation for the diversion pipe and the structure stabilization.

We selected materials resistant to water pressures, sediment abrasion, slightly acidic water conditions, and the presence of iron reducing bacteria. Specifically, we selected ASTM galvanized steel sheets (thickness = 10 mm) to build 10 v-notch weir plates with different angles. We designed a wooden structure to hold weir plates and allow for manual weir interchange; rails sealed with rubber were used to avoid leakage (Figure 18). We designed the system of weirs to accommodate flow diversions through combinations of paired weirs (e.g., 90% flow going into the experimental reach and 10% flow into the diversion pipe). We used 5 sets of plates: 10-90%,

20-80%, 30-70%, 40-60%, and 50-50%, and *vice versa*. We used the 50% probability of exceedance flow from the Chalpi Norte stream discharge record between 2011 and 2017 (Supplementary Material, FS2) to calculate angles based on equation 1.

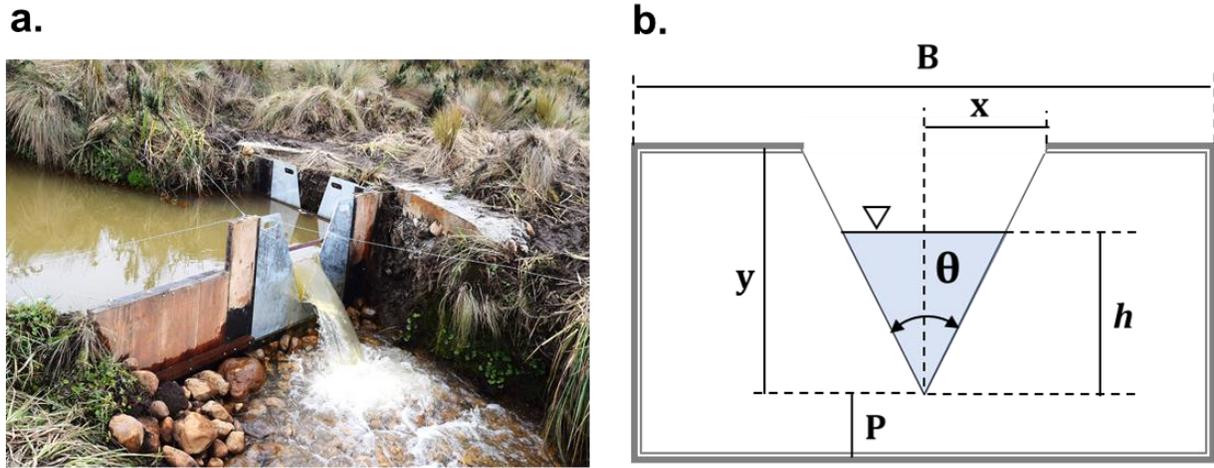


Figure 18. Experimental flow manipulation structure with combined v-notch operating simultaneously (a), schematic design of sharp crested plates with different openings angles (θ) to measure water height on stream and diversion weirs (b).

In the field-test we measured water height over the weirs in the experimental reach and in the diversion pipe. We performed hourly measurements for eight hours over five days, with two sets of plates each day. We left each set of complementary plates in place for four hours and we started manipulations by gradually reducing flow in the experimental stream reach and then systematically restored flows in the same way. We conducted streamflow measurements 8 m upstream and 12 m downstream from the paired-weir structure. The field testing was performed between November 15-19, 2018, prior to the ecosystem-scale experimental flow manipulation that took place afterwards and lasted until April 2019 (unpublished data). In the experimental reach, we made measurements every 1.5 m to record depth profiles and vertical velocities over the cross-sections (e.g., width = 1 m ~ 10 velocity profiles); we used a wading rod and an ADC velocimeter (Acoustic Digital Current meter OTT Hydromet, Germany).

We used the HEC-RAS 5.0 model (US Army Corps of Engineers) to simulate two-dimensional flows. We developed a 2D hydraulic routing model using a digital elevation model (DEM, in ArcGIS), georeferencing an aerial image (DJI Phantom 4 Pro V2.0, USA), and using the stream reach topography for establishing the stream geometry in the RAS Mapper tool in HEC-RAS 5.0. We created 0.3 m x 0.3 m cells by running the geometric pre-processor in RAS Mapper to test hydraulic conditions (i.e., velocity, depth, wetted perimeter) during controlled flow alterations.

3. Results

3.1 Laboratory and field tests

Laboratory testing allowed us a higher degree of flow and pressure to control than field conditions. We tested only one set of weir plates in the laboratory to assess the reliability of diverting flow based on the weir-opening angles determined from equation 1. Our design was for a 30-70% partitioning of flow through the two weirs. Laboratory tests indicated that for all flows the paired-weir device diverted flow close to 30:70 but the majority of ratios were under the 30:70 line (Figure 19).

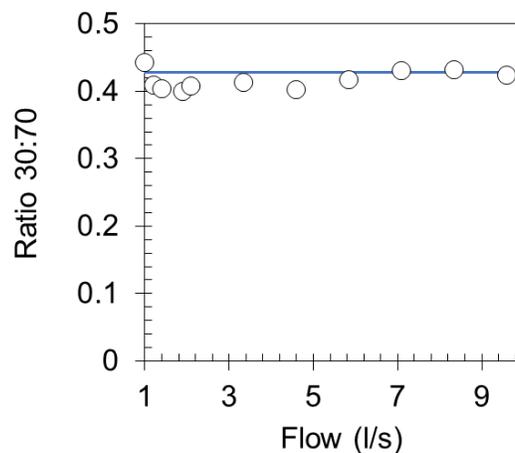


Figure 19. Flow ratios with respect to the 30:70 ratio line (blue) for different flows diverted by a two-weir system tested at a laboratory flume.

In the field we tested the five sets of weir plates with different dimensions to divert natural flow at fixed percentages (Table 8). Over five days of field tests, the average flow in the Chalpi Norte stream was 56.1 l/s (min = 53.8 l/s, max = 58.4 l/s), which corresponded to a 99% probability of exceedance calculated from the flow series for the Chalpi Norte stream between 2011 and 2018 (Supplementary Material, FS2). Tests started with a targeted reduction of 10%, allowing 90% of flow to the experimental stream reach (Figure 20). Under this flow reduction, the experimental reach flows varied between 89 and 94% from the upstream (natural) flow (Figure 20). We continued to consecutively reduce flow by 10% increments from 20 to 90%. Then we gradually re-diverted flows in the same 10% increments back into the experimental stream reach (recovery). Targeted partitioning between the experimental reach and the diversion were in close agreement with field measurements (Figure 20).

Table 8. Triangle weirs plate dimensions for the experimental reach (1) and diversion channel (2), flow manipulation (%), opening angles (θ), width from the notch to the edge of the opening (x), width of the plate (B), for a dimensionless discharge coefficient $C_d = 0.62$, a height from the notch to the edge of the plate $y = 0.8$ m, and a height from the bottom edge of the plate to the notch $P = 0.2$ m, (see Figure 18).

Flow (%)		θ (°)		x (m)		B (m)	
(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
10	90	20	100	0.1	1.0	0.3	1.9
20	80	30	95	0.2	0.9	0.4	1.7
30	70	45	90	0.3	0.8	0.7	1.5
40	60	62	88	0.5	0.8	1.0	1.6
50	50	85	85	0.7	0.7	1.5	1.5

3.2 Experimental Stream Reach Response

Field tests showed the paired-weir system diverted flows as expected with deviations of 2 to 12% with respect to targeted (predicted or designed) flows (Figure 20). In general, the observed flows were less sensitive to changes in upstream stream height than predicted. Observed flows during reductions of 10 and 20% were most similar to predicted flows and the largest differences were

for reduction of 40 and 70% (Figure 20). In general, higher variations between predicted and observed flows occurred on weirs with wide angles (low degree of reduction) compared to narrow angles.

While performing experimental flow manipulations we observed eddy formation around v-notches of wider-notch weirs, which may account for some flow deviations relative to calculated flows. Wider weirs also generated shorter down-stream supercritical flow sections compared to narrow angle weirs, so some downstream armoring may be suggested for the future. Experimental flow reductions influenced channel depth, width and velocity distributions along the manipulated reach (Table 9).

Table 9. Reductions in flow and morphological variables in the experimental stream reach.

<i>Flow</i>	Reduction (%)			
	<i>Depth</i>	\pm	<i>Width</i>	\pm
10	1	2	3	1
20	3	3	11	4
30	9	3	22	8
40	13	4	29	10
50	19	5	35	11
60	25	7	43	15
70	37	11	51	19
80	45	9	59	18
90	56	8	66	22

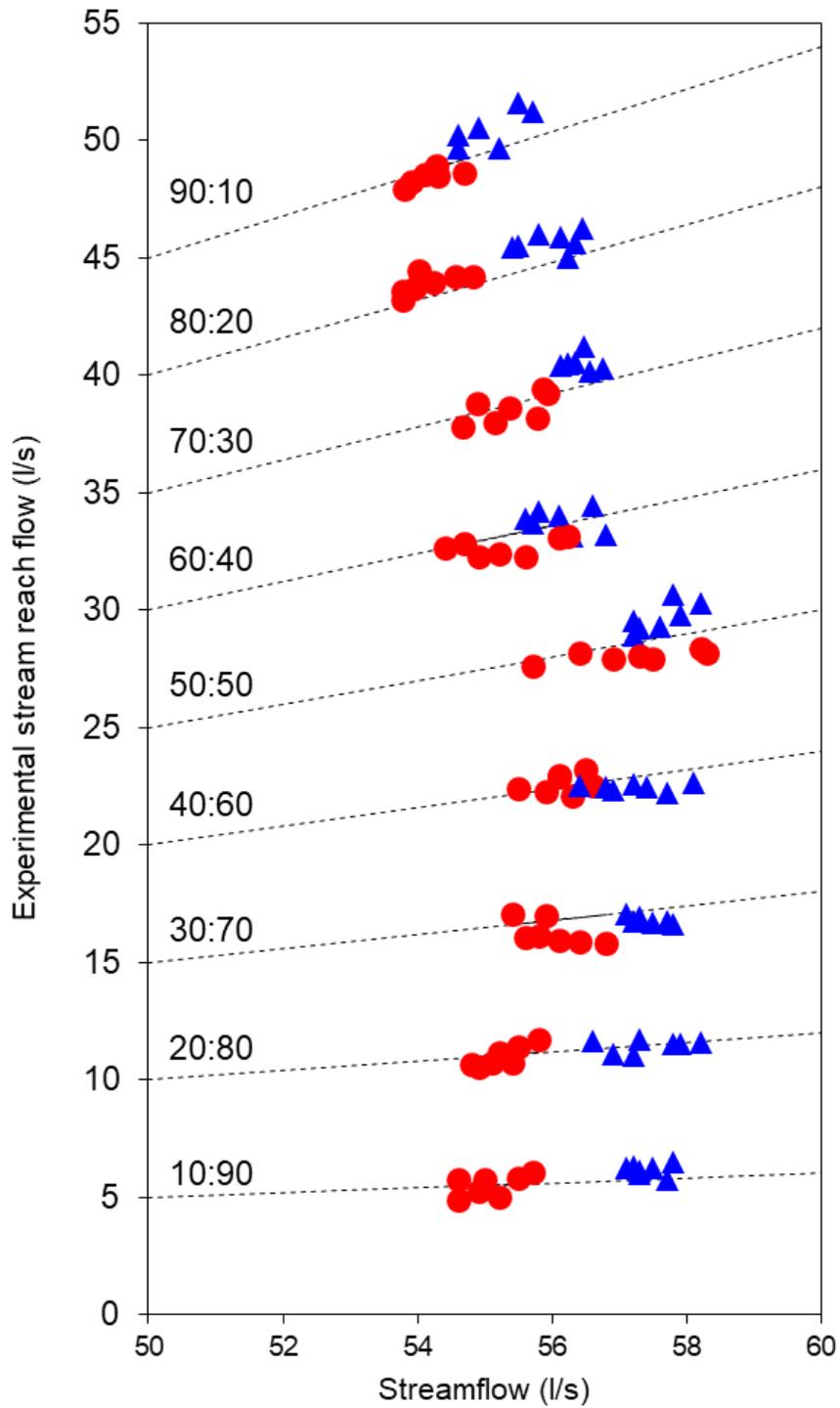


Figure 20. Flow reductions (red circles) and recovery (blue triangles) on the experimental stream reach according to target manipulations of flow (dotted lines).

We observed a decrease of 19% in depth and 35% in channel width with a flow reduction of 50%. A flow reduction of 90% reduced depth by 56% and width by 66%. The 2D flow simulations showed that a 50% flow reduction maintained relatively high velocities >0.75 m/s in the stream channel and medium velocities close to the stream banks >0.25 m/s (Figure 21a). For a 90% flow reduction, stream channel width decreased and velocities were dramatically reduced to <0.25 m/s (Figure 21b).

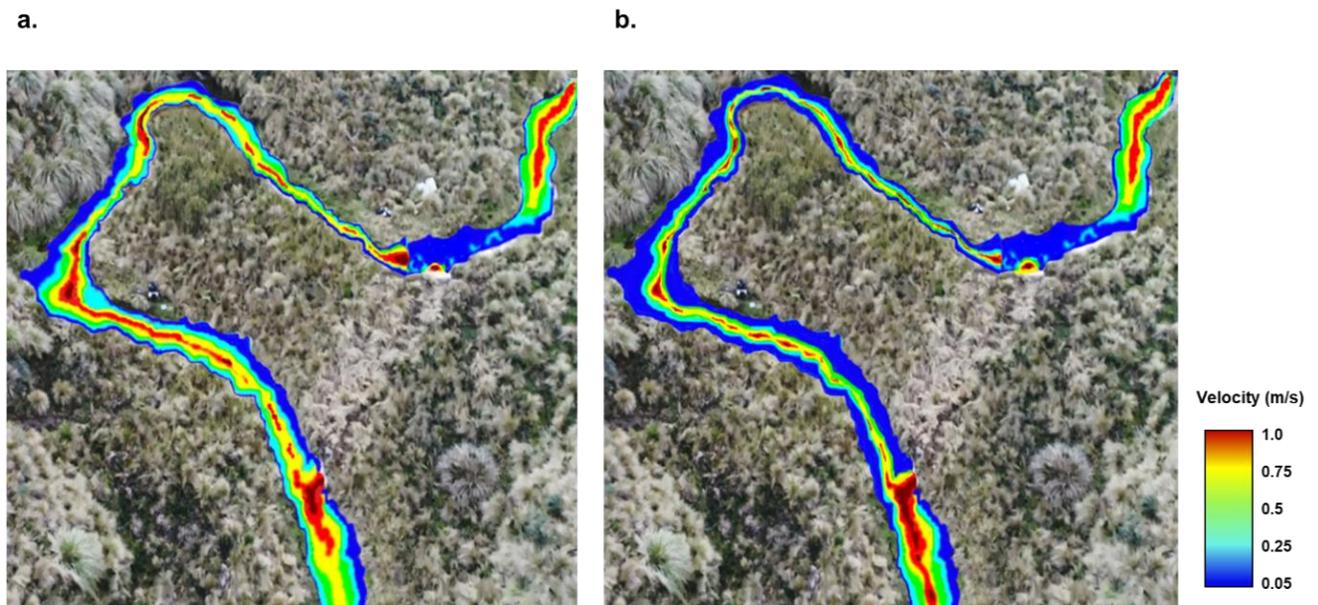


Figure 21. HEC-RAS 2D velocity simulations in the experimental stream reach of the Chalpi Norte stream undergoing reductions of 50% (a) and 90% (b) of the flow through the operation of a two v-notch weir system for e-flows research at ecosystem scale.

4. Discussion

The purpose of this study was to demonstrate the efficacy of an experimental flow manipulation structure designed specifically to divert streamflows while maintaining variations in flow conditions. We conceived of a v-notch weir system to reduce and restore flow to an experimental reach as part of an ecosystem-scale project to investigate experimental e-flows releases. With

this structure we sought to create flow manipulations that would allow researchers to evaluate the impacts of flow reductions on downstream stream ecosystems (Konrad *et al.*, 2013; Arthington, 2012; Poff and Zimmerman, 2010). Laboratory testing demonstrated the efficacy of a paired-weir system to accommodate flow diversions over an order-of-magnitude-range of flow rates. Laboratory flume tests demonstrated the potential accuracy of diversions over a range of controlled flows (Martinez, 2005). We designed our structure to perform flow manipulations to provide valuable results for the period of critical supply (González-Zeas *et al.*, 2019). During high flows, severe rainstorms overflowed water supply infrastructure in the area (observation-unpublished data).

In the field, we found the paired v-notch weir system generally performed according to hydraulic designs and generated the expected effects on downstream physical characteristics (Francoeur and Biggs, 2006). Although one would have expected lateral inflows and outflows along a natural stream reach, we found little evidence of substantial influence of such flows along the experimental reach. This is probably because stream flow during the five days of field testing was extremely low (i.e., 99% probability of exceedance); however, this facilitated relatively rapid installation. After our field test, flow increased as response to precipitation as we started the ecosystem-scale flow manipulation that lasted for 175 days.

Current ecohydraulic infrastructure faces the challenge of estimating the proportion of flows passing through fish ladders, passages, and screens while streams are being diverted (Katopodis, 2005). This creates a cumbersome suite of responses that translates into inoperable guidelines for water managers (Kaur *et al.*, 2017; Kaur *et al.*, 2019). A common finding from previous research has highlighted the confusing effects of flow alterations and other human activities occurring at the same time (e.g., land-use change, climate change) (Dewson *et al.*, 2007b; Sabaton *et al.*, 2008; James *et al.*, 2008; Konrad *et al.*, 2011; Maazouzi *et al.*, 2013; Elbrecht *et al.* 2016;). Based on the location of our experimental stream reach, we can attribute observed changes in the stream meander exclusively to flow manipulations (see Figure 1).

Contrary to hydraulic infrastructure for flow release and biota transport (Katopodis, 2005), the experimental flow manipulation structure presented here allowed us to control for instantaneous flows in percentages that can be translated into operational guidelines. This structure can be used to relate gradual flow reductions and increases to longstanding e-flows criteria proposed by Tennant (1976) and implemented through legislation in several countries (Gore, 1977; Tharme, 2003; Arthington, 2012). This device can also be used to improve thresholds on flow-ecology relationships and hydraulics of habitats at different scales (Buchanan *et al.*, 2017; Lamouroux *et al.*, 2010; Lancaster and Downes, 2010; Lancaster, 2018). We are currently using this device and field site to experimentally inform e-flows criteria for upland high-altitude tropical streams. However, the utility of flow manipulations includes a wide range of applications from e-flow management to research on ecological perspectives of flow disturbance and resilience of ecosystems to flow alterations under climate change.

5. Conclusions

The experimental flow manipulations presented here demonstrated the capability of our paired-weir system to provide first-hand quantitative criteria for operating water infrastructure in an e-flows context. Our device allows for controlled ecosystem-scale flow alterations to assess percent of instantaneous flow rather than the typical, static hydrological metrics used for environmental flows assessment (i.e., mean annual flow, minimum flow). The paired v-notch weir system diverted flow from the stream to the experimental reach and to a diversion proportionally. The structure allowed us to calculate flow using hydraulic principles that were corroborated with downstream flow measurements. For experimental executions and ecosystem scale response analysis, we expected proportional flow variations within 10% and our tests showed variations generally within this limit. Although our field flow conditions did not vary a lot, the lab experiment showed similar performance for flow variations of about one order of magnitude. The experimental flow manipulation structure has the potential to divert streamflow

while maintaining “natural” variations in flow conditions. The device is relatively easy to set up, it is a long lasting, and an economical option for making e-flow evaluations.

6. Supplementary Material – Chapter 3

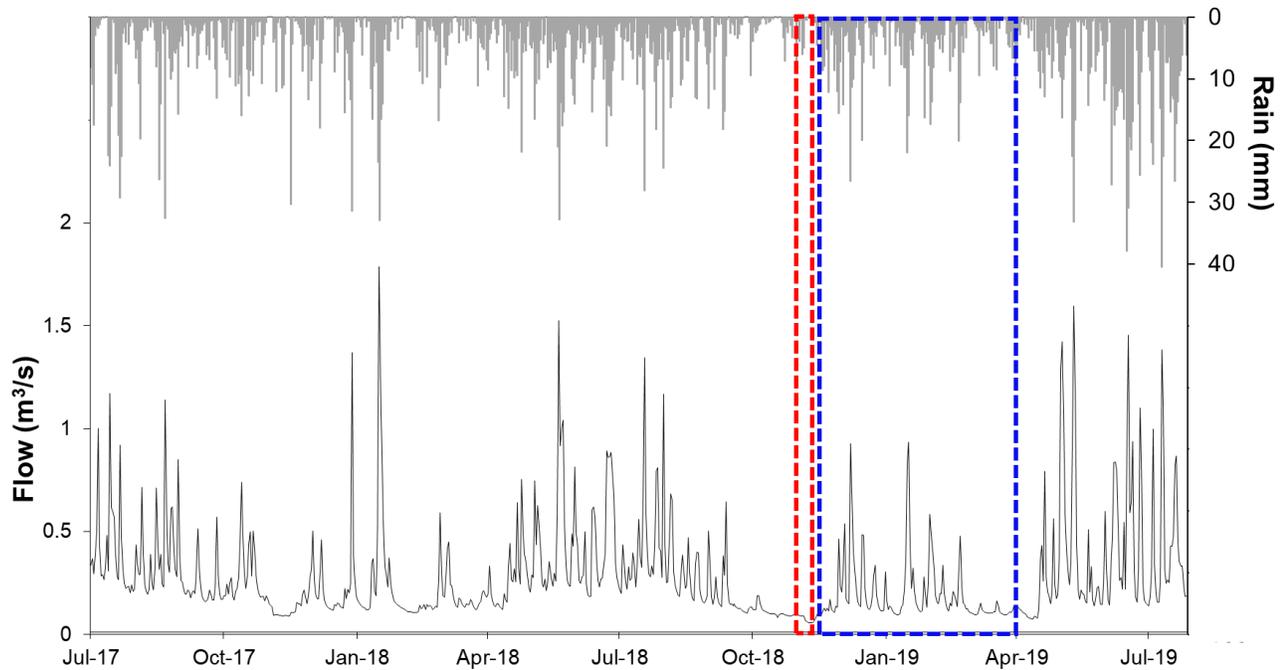


Figure 22. Averaged daily discharge (flow) and precipitation (rain) recorded for the Chalpi Norte stream from July 2017 to July 2019 where the dotted red rectangle indicates the period of field test of the structure for experimental manipulations of environmental flows and the dotted blue rectangle indicates the ecosystem-scale experiment.

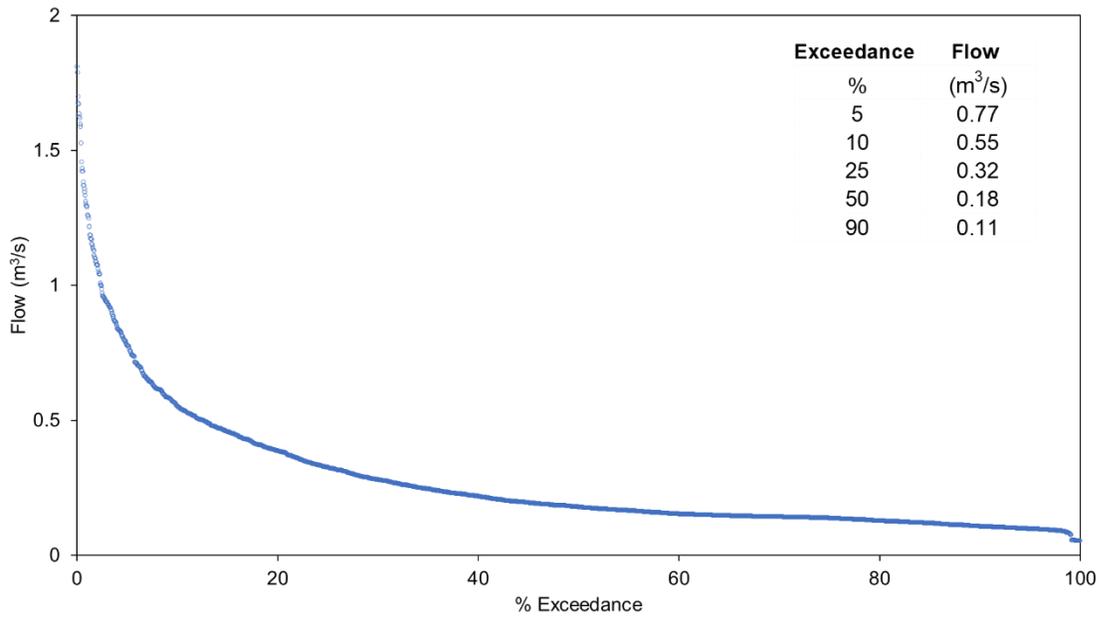


Figure 23. Chalpi Norte stream Flow-Duration Curve calculated from streamflow records obtained between 2011 and 2017 at the nearest hydrological station, 120 m upstream the experimental reach; upper right values calculated from maximum exceedance series with Log Pearson III.

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CHAPTER 4

FLOW-INDUCED SHIFTS IN RIVER CYANOBACTERIA BIOMASS: A WHOLE ECOSYSTEM EXPERIMENT

Abstract

Our results present the case of regime shift thresholds in streams using a whole ecosystem experimental flow manipulation with comparisons to data based on nearby samples above and below water intakes and data from a global literature survey. We induced a cyanobacteria biomass shift through flow manipulations showing that flow exerted a significant change in water temperature primarily and potential combinations of environmental variables: nitrate concentration, dissolved oxygen, light that seemingly relate to cyanobacteria biomass. We observed, during a 35-day period of maximum flow reduction (i.e. 90%), that cyanobacteria (CyanoHABs) proliferation was arrested, probably due to limitations associated with low water flows. Although our experiment idealized flow reductions in fixed percentages, our intention was to show flow as the driver of change and associate an ecological meaning to a flow threshold. The effect of flow and environmental variables over cyanobacteria biomass showed two main thresholds: first, cyanobacteria increase with a 50-60% flow reduction and, second, cyanobacteria biomass was near natural conditions with ~60% flow recovery. These findings have important implications for water management as cyanobacteria proliferation in rivers can threaten downstream lakes and reservoirs, consequently affecting more than one type of water supply source usually interconnected in provision systems. Importantly, our results from mountain streams agree with observations and experiments realized in freshwater systems around the world, supporting a global shift in cyanobacteria biomass with reduced flows. With respect to flow, our study provides one of the first *in situ* demonstrations of regime shift thresholds in natural running waters.

1. Introduction

Understanding the variation in ecosystem responses to environmental disturbance is crucial for making robust ecological predictions in an increasingly stressed world (Scheffer *et al.*, 2001). While some ecosystems respond gradually, others undergo sudden shifts in structure and function after disturbance exceeds a critical threshold (Ho *et al.*, 2019). Such shifts can result in ecosystems transitioning to states where recovery is generally slow (e.g., the system displays hysteresis) or even impossible (the system moves to an alternative stable state) (Pace *et al.*, 2019). Lake eutrophication is one of the best-studied examples of ecological transitions, where clear waters can suddenly shift to algal-dominated systems (Scheffer *et al.*, 2001; Ibanez *et al.*, 2010; Pace *et al.*, 2019). Such system state shifts may have dramatic consequences for biodiversity and the provisioning of vital ecosystem services for humans (i.e., potable water supply, fisheries, aesthetics, and conservation); therefore, the study of state changes is crucial to developing sustainable natural resources management plans (Paerl *et al.*, 2018; Wurtsbaugh *et al.*, 2019).

Whole-ecosystem experimental manipulations have been fundamental to our comprehension of trophic-state shifts in lakes (Robinson and Uehlinger, 2008; Pace *et al.*, 2019). In comparison to lakes, there is much less research on regime shifts in running waters because ecosystem-scale flow manipulations of rivers have proven logistically challenging (James *et al.*, 2008; Cauvy-Fraunié *et al.*, 2016; Halliday *et al.*, 2016; Neif *et al.*, 2017). Instead, our understanding of flow-ecological state relationships in lotic systems has mostly relied on correlations using field data from largely uncontrolled stream reaches or data from experiments performed in artificial channels and flumes (Rosenfeld, 2017; Rosero-López *et al.*, 2019). As a consequence, the management framework used to establish the quantity and timing of water necessary to balance ecological and human needs in river systems, i.e., environmental flows (e-flows), is currently based on poorly integrated system shift predictions (Arthington *et al.*, 2018). Predicting ecology-flow thresholds in running waters is important to maintain flows for

livelihoods and ecosystems (Gain *et al.*, 2013). Moreover, for a variety of reasons, lotic systems could provide warnings for managing water resources at watershed scales because the relatively smaller water volume in rivers may be sensitive indicators of larger system shifts than lakes.

Defining flow-induced shifts in rivers appears particularly crucial in the case of harmful algal blooms of cyanobacteria (CyanoHABs), where several taxa (e.g. *Mycrocystis*) produce toxins with potentially severe effects on human and wildlife health (Mitrovic *et al.*, 2011; Paerl *et al.*, 2011; Huisman *et al.*, 2018). CyanoHABs are a global phenomenon, whose emergence in freshwaters is often triggered by decreases in water levels (Paerl *et al.*, 2009) (Figure 24a). CyanoHABs are expected to become more frequent and more impactful due to climate change, global increases in water use, and decreased water quality, which promotes eutrophication (Paerl *et al.*, 2018; Wurtsbaugh *et al.*, 2019). While eutrophication has been well-addressed in lakes, our understanding is relatively poor regarding how flow changes may result in favorable CyanoHABs conditions in rivers (i.e., high temperature, low pH, low dissolved oxygen) (Pace *et al.*, 2019; Guillet *et al.*, 2016; Halladay *et al.*, 2016) and whether changes are gradual or more sudden (Güven and Howard, 2006; Bowling *et al.*, 2013).

Here, we address regime shifts using a whole-ecosystem experiment in a mountain river system of the tropical Andes (Figure 24b-d). This is a highly biodiverse region that is particularly impacted by the rapid proliferation of water infrastructure (Jacobsen and Dangles, 2017; González-Zeas *et al.*, 2019). Using a Before-After-Control-Impacted (BACI) design (Fisher *et al.* 2019) and a series of weirs (Chapter 3), we gradually diverted 20 to 90% of a river into a downstream experimental stream reach (manipulated) while a similar adjacent reference river remained undisturbed.

The river manipulation consisted of three stages: (1) establishment of baseline conditions under unaltered stream flow (12 weeks); (2) experimental diversion of water flow, inducing systematic flow reductions in the downstream reach (14 weeks; the upper reach was kept

undisturbed); and (3) gradual reset to initial flow conditions in the experimental reach (12 weeks).

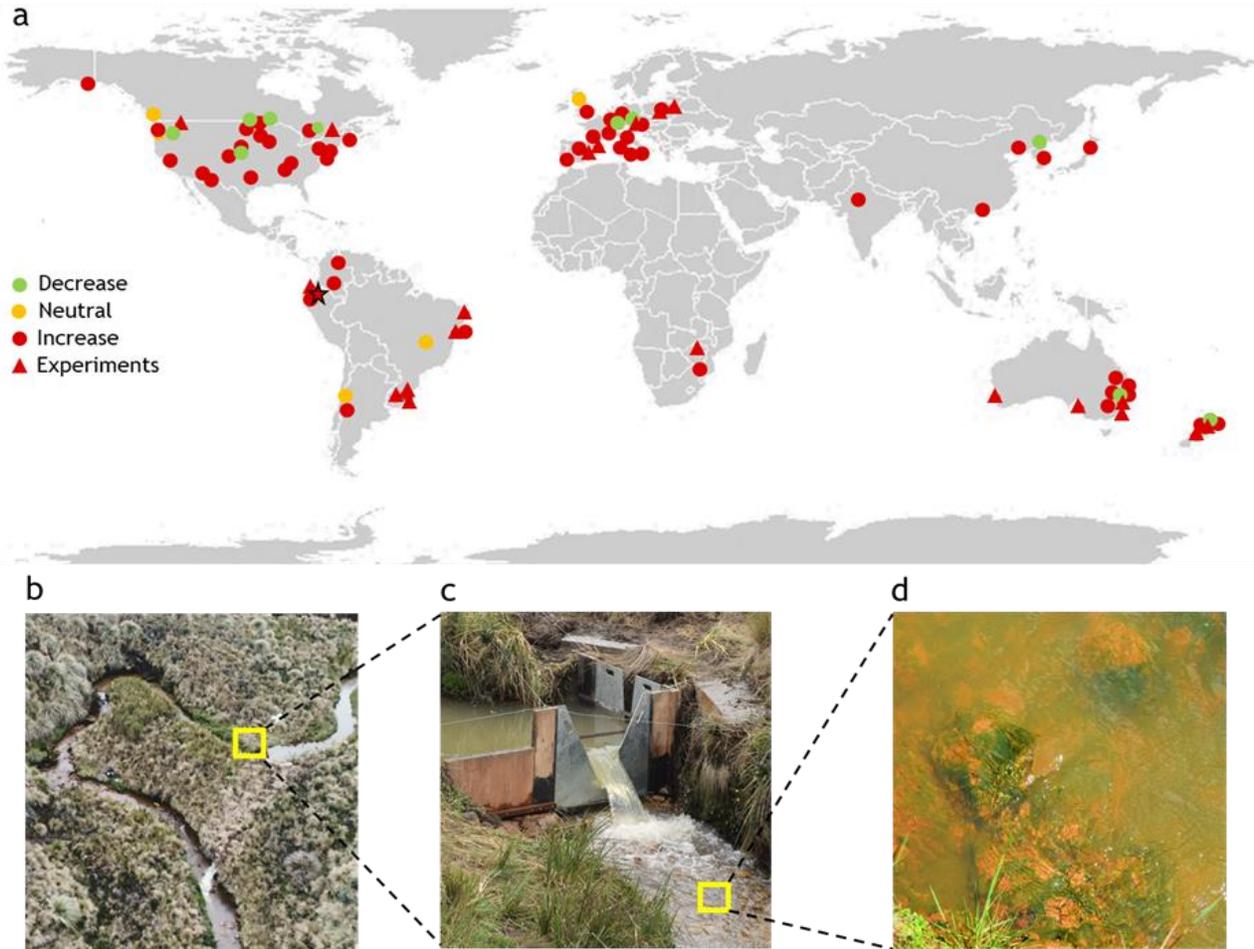


Figure 24. Cyanobacteria relations to flow (rivers) and water level (lakes). a) Worldwide qualitative (circles, $n = 79$) and quantitative (triangles, $n = 21$) studies reporting cyanobacteria biomass decrease (green), neutral response (yellow) or increase (red) to flow/water level reduction, current research (red star), b) experimental flow manipulations in a high-altitude stream reach, c) using a series of v-notch weirs to reduce natural flow in fixed percentages, d) showed an increase in cyanobacteria levels.

This experimental design allowed us to: 1) assess the response of benthic communities to flow reduction, with a special focus on cyanobacteria biomass, and quantify potential flow thresholds that trigger system shifts, and 2) evaluate the ecosystem resilience, defined as the

capacity of the benthic communities to return to their initial configuration after flow disturbance ceases. To determine whether our experimental results would be corroborated across a spatial gradient in water flow reduction, we further analyzed the spatial variability in cyanobacteria biomass across 26 stream sites for 41 weeks (1066 sampling points in total), in the same general study area. Also, we performed a global survey of quantified cyanobacteria biomass response to river flow. Comparison among these studies could help predict future temporal ecological variability under reduction in river flow.

2. Methods

2.1 Ecuadorian Study area

The study area is the headwaters of the Chalpi Grande River watershed, 95 km², located inside the Cayambe-Coca National Park in the northern Andes of Ecuador at an elevation range of 37890 to 3835 m (S 0°16' 45", W 78° 4'49"). Within this watershed is situated the primary water supply system for Quito. The system is comprised of two reservoirs and ten water intakes placed on first and second order streams that altogether provide 39% of Quito's water supply (González- Zeas *et al.*, 2019). For 12 weeks we monitored the baseline Chalpi Norte stream conditions where we conducted our 25-week experiment. For 41 weeks, we monitored the natural flow and flow recovery at different locations in the Chalpi Norte stream and six streams undergoing water abstractions (Figure 25).

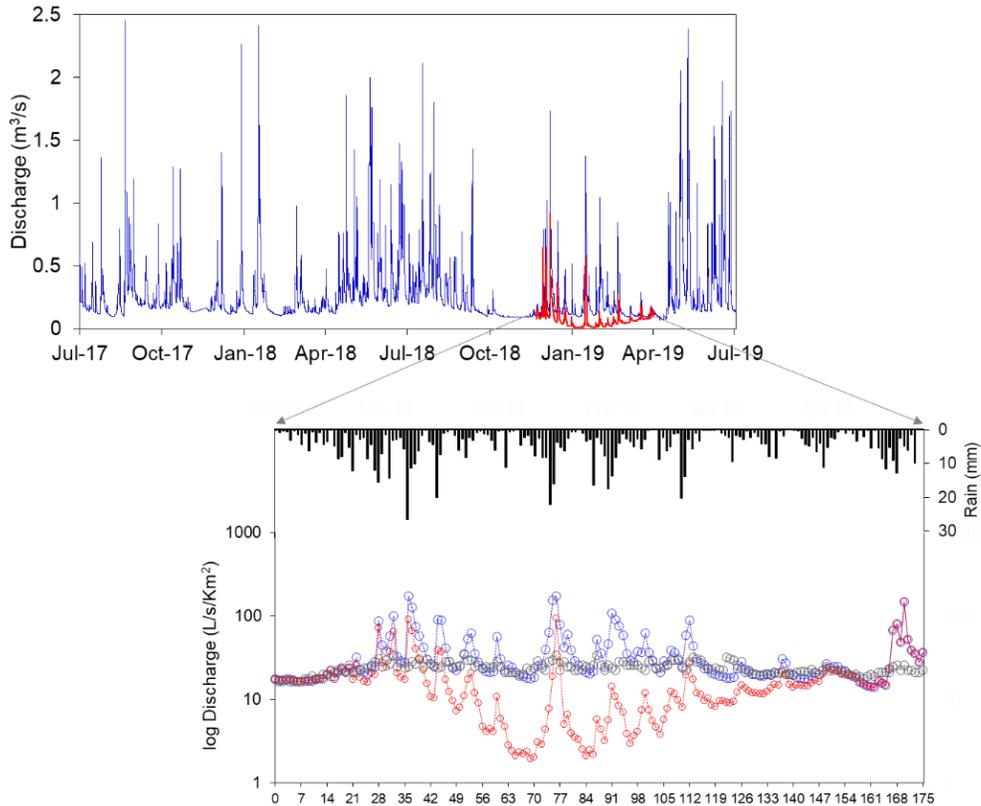


Figure 25. Chalpi Norte stream hydrograph from 10-min water levels converted to discharge for the baseline (blue line), manipulation (red line), and recovery (blue line) periods. Close-up to flow manipulation experiment show daily averaged precipitation in the top (bars) and drainage-area normalized discharge for the upstream (blue open circles) and downstream site (red open circles) from the diversion in the Chalpi Norte stream, and the reference stream (gray open circles).

2.2 Experiment for flow manipulation and monitoring flow reduction and recovery

We set up a full Before-After/Control- Impact (BACI) design (Fisher *et al.*, 2019) to measure ecosystem variables under natural and manipulated flow conditions. We conducted the experiment between November 2018 and April 2019. We identified a stream reach on the Chalpi Norte that was above any water intakes that allowed us to divert flow from a meander, and return it to the stream below the meander (Figure 26). We monitored an upstream site, diversion 12 m above the ecohydraulic structure (Chapter 3). The experimental reach (downstream) corresponded to a meander portion of the Chalpi Norte stream ($L = 97$ m). In addition to the stream reach above our experimental reach, we monitored a reference stream that was a tributary to the Chalpi Norte, with upstream and downstream sites separated by a distance of 16 m. We

manipulated instantaneous flow in the Chalpi Norte stream through a series of fixed percent using different v-notch weir pairs (Chapter 3). We started diversions to maintain in the meander 80, 60, 50, 40, 30 and 20% of the incoming flow for 7-day periods, then we maintained 10% of the flow for 36 days. We started to return flow incrementally to recover 20, 30, 40, 50, 60, 70, 80, 90 and 100% of the flow. In response to a natural peak in flow during the 90% reduction, the manipulated flow briefly increased above the targeted reduction (i.e., 46 % of flow in the experimental stream reach or a 54% flow reduction) (Figure 25). We registered the same peak of natural flow on the upstream site of the experiment and the control stream.

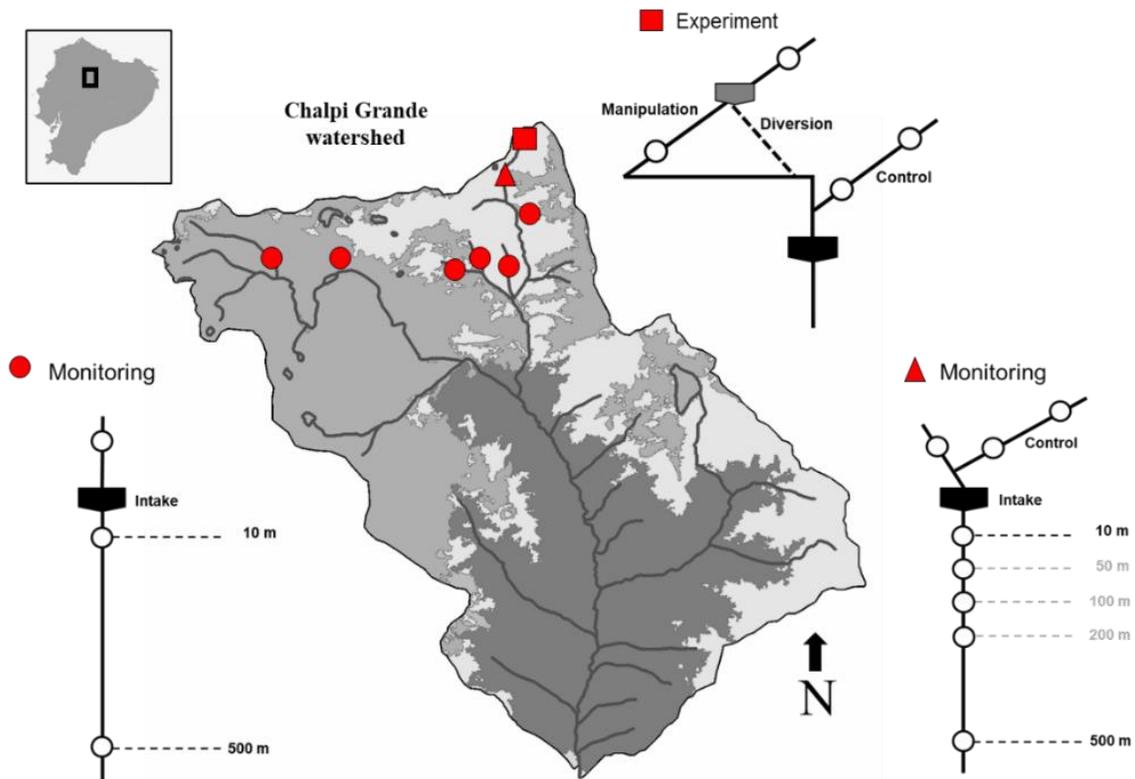


Figure 26. Ecuadorian study sites located in the headwaters of the Chalpi Grande watershed. The experiment for flow manipulation (red square) in the Chalpi Norte stream, with an upstream site (open circle) above the artificial diversion (dotted line), and a downstream site (open circle) in the manipulated reach below the diversion; the Control stream with upstream and downstream sites used for reference conditions, the experiment was located before the water intake for the supply system (black connector). Spatial monitoring of flow recovery in the Chalpi Norte stream (red triangle) at different distances (10, 50, 100, 200, 500 m) from the water intake, and in headwater streams from the supply systems (red circles) at two distances (10 and 500 m).

2.3 Local stream monitoring below water intakes

Several stream sites were monitored weekly between July 2018 and July 2019. We included the upstream site of the water intake on the Chalpi Norte stream and five sites downstream the water intake at 10 m, 50 m, 100 m, 200 m, and 500 m (Figure 26). We selected four streams (Gonzalito, Quillugsha 3, Venado, Guaytaloma) with water intakes placed on the main channel. Two streams (Quillugsha 1 and 2) had a water intake placed in the junction of streams where we sampled one site upstream and two sites downstream at 10 m and 500 m. See section 2.5 for measurement method details.

2.4 Global literature survey

We performed a literature search to explore benthic algae responses to flow alterations (increase or decrease), focusing on cyanobacteria and streams, although, we also used reports from lakes (n = 100). We used ISI Web of Science, Google Scholar and Google Search for the words: “cyanobacteria” + “stream”, “algal bloom” + “flow”, “cyanobacteria” + “lakes” (Supplementary Material). We used published and/or publicly available data to calculate the percent of flow remaining in streams and a factor of cyanobacteria increase or decrease according to reported base or previous conditions. Four studies reported no change of cyanobacteria biomass and 10% of the studies reported a decrease in cyanobacteria biomass attributable to flow reduction (Figure 24a). Most research (65%) reported biomass increases with flow reductions or, in the case of lakes, water level decreases (n = 59); all described the water budget as one of the environmental drivers responsible for cyanobacteria blooms (Figure 24a). We found experiments conducted in streams (21%) that related cyanobacteria biomass with flow reductions due to dam releases and/or water intakes.

2.5 Abiotic and biotic variables sampling and analyses at our Ecuadorian sites

We had two classes of study sites: an “experimental” site where we conducted controlled flow diversions and several “monitoring” sites that were located near dams or water intakes. For

abiotic parameters we measured precipitation from a rain gage (HOBO Onset USA) installed in the Chalpi Norte stream. On all sites we installed water level sensors (HOBO U40L, Onset USA) where we conducted multiple wading-rod flow measurements to convert water level into discharge via stage-discharge relationships. Beside vertically distributed velocities for discharge calculation, we also measured bottom velocity at 50 mm from the substrate (ADC current meter, OTT Hydromet, Germany). Streamwater's physical and chemical *in situ* parameters (i.e., pH, temperature, conductivity, dissolved oxygen) were measured using a portable sonde (YSI, Xylem, USA). We collected 500 ml samples to analyze nutrients (i.e., nitrate and phosphate) at the water supply company's (EPMAPS) laboratory.

Our biotic variables included cyanobacteria, benthic algae (diatoms and green algae), and aquatic invertebrates biomass. For cyanobacteria and benthic algae we used a BenthosTorch (bbe Moldaenke GmbH, Germany) to measure chlorophyll-a on artificial substrates (Chapter 2). We installed unglazed ceramic plates (200 mm x 400 mm) with a grid of squares of 2500 mm². We allowed 21 days for colonization and then performed five readings on five squares of each plate/substrate. To consider the effect of grazers, we sampled aquatic invertebrates using a Surber net (mesh size = 250 µm, area = 0.0625 m²). For the experiment we performed all measurements on five sites distributed on the stream reach (n = 25), and we measured cyanobacteria, benthic algae, physical and chemical *in situ* parameters every two days, and nutrients and invertebrates every seven days. For monitoring sites we measured cyanobacteria, benthic algae, discharge, and physical and chemical *in situ* parameters every seven days and nutrients and invertebrates every 30 days. To evaluate differences we calculated mean abiotic and biotic variables during the experimental phases (BL: baseline, FR: flow reduction, FI: flow re-instate as flow recovery) in four experimental reaches (upstream and downstream site) in two streams (manipulated and reference) and we applied a paired one-tail t-test at $\alpha = 0.05$ (Table 10).

To quantify the relationships between environmental variables and cyanobacteria biomass under manipulated and natural flow conditions we used multivariate autoregressive state-space modelling (MARSS) (Holmes *et al.* 2014; Cauvy-Fraunié *et al.*, 2016). We fitted

models to time-series data using the MARSS R-package (Holmes *et al.*, 2012), which provides support for fitting autoregressive state-space models via maximum likelihood. We fitted univariate autoregressive state – space models with Gaussian errors for flow, conductivity, pH, water temperature, nitrate, phosphate, cyanobacteria biomass, and benthic algae biomass time series. The observed time series Y_t was described by an underlying process, the true state process X_t , evolving through time, as follows.

$$X_t = BX_{t-1} + U + W_t \quad W_t \sim (0, Q) \quad (1)$$

$$Y_t = X_t + V_t \quad V_t \sim (0, R) \quad (2)$$

with X_t as a vector of states at time t , Y_t a vector of observations at time t , W_t a vector of process errors (normally distributed with mean 0 and variance Q), V_t a vector of observation errors (normally distributed with mean 0 and variance R). B is the coefficient of autoregression in the state vectors through time. U describes the mean trend. The best fitting model was identified as having the lowest Akaike Information Criterion adjusted for small sample sizes (AICc) (Holmes *et al.*, 2012; 2014). To detect structural breaks in cyanobacteria biomass time series we calculated the differences between the smoothed state estimates at time t and $t-1$, sudden changes in level were detected when the standardized smoothed state residuals exceed the 95% confidence interval for a t -distribution. We tested the strength of environmental variables on cyanobacteria biomass and identified the best model with the lowest AICc and no convergence issue, models were fitted independently for each stream site.

To analyze cyanobacteria across a gradient of flow alterations we compared weekly data pairs ($n = 637$) from upstream and downstream monitoring sites. For cyanobacteria we scaled the biomass measured at the downstream site(s) to the biomass measured at the upstream site and calculated an increase/decrease factor. We determined the fraction of the instantaneous flow in the downstream sites relative to the instantaneous flow in the upstream sites. We applied the same analysis to data from experiments obtained on the web search. We applied the Ramer-

Douglas-Peucker (RDP) algorithm to find a breakpoint and the best line of fit for the local and global survey data distribution, we used the *kmlShape-R* package (Visvalingam and Whyatt, 1990; Genolini *et al.*, 2016).

3. Results and Discussion

3.1 Whole-ecosystem experiment

We conducted our experiment during the dry-season months of October through April characterized by stable low flows (Jacobsen and Dangles, 2017; González-Zeas *et al.*, 2019). The analysis of environmental variables showed that flow, temperature, and nitrate concentration were significantly different in the experimental reach for the flow reduction and recovery compared to base flow conditions (Table 10).

Table 10. Mean abiotic and biotic variables during the experimental phases (BL: baseline, FR: flow reduction, FI: flow re-instate as flow recovery) in four experimental reaches in two streams (manipulated and reference). * indicates significant differences in parameter value when compared to values from the baseline prior alteration (significant P values at 0.05 alpha level from a paired one-tail t-test).

	Manipulated stream						Reference stream					
	Upstream site			Downstream site			Upstream site			Downstream site		
	BL	FR	FI	BL	FR	FI	BL	FR	FI	BL	FR	FI
Discharge ($\text{m}^3 \cdot \text{s}^{-1}$)	0.188	0.211	0.159	0.188	0.087*	0.103*	0.019	0.021	0.023	0.019	0.022	0.024
(% CV)	57	82	69	57	99	91	19	30	24	18	29	22
Temperature ($^{\circ}\text{C}$)	7.4	7.7	8.1	7.4	10.1*	9.5*	6.9	6.9	7.1	7.1	6.9	7.1
(min - max)	4.1 - 10.5	4.7 - 10.6	5.1 - 11.1	4.3 - 10.5	5.6 - 11.7	5.1 - 11.1	4.1 - 9.4	4.1 - 9.3	4.2 - 9.6	4.2 - 9.5	4.1 - 9.5	4.1 - 9.3
pH	6.9	6.8	6.8	6.9	7.2	6.8	6.8	6.8	7	6.8	6.9	6.9
(\pm SD)	0.3	0.3	0.4	0.5	0.6	0.4	0.3	0.4	0.3	0.4	0.3	0.2
Conductivity ($\mu\text{S} \cdot \text{cm}^{-2}$)	40.03	44.05	42.01	44.77	48.23	43.07	38.55	38.39	41.01	39.74	37.88	40.71
(\pm SD)	2.33	3.21	2.81	2.88	4.91	3.71	1.55	2.11	1.65	1.56	2.11	1.65
Dissolved Oxygen (mg . L)	7.9	7.7	8.2	7.9	7.6	7.8	8.1	7.8	7.7	7.7	7.9	7.5
(min - max)	6.1 - 9.9	6.9 - 9.7	6.2 - 9.9	6.6 - 10.3	6.2 - 9.2	6.1 - 9.4	6.3 - 9.4	6.5 - 9.9	6.4 - 10.1	6.3 - 9.8	6.5 - 9.9	6.5 - 10.1
Nitrate ($\text{mg} \cdot \text{L}^{-1}$)	0.34	0.24	0.34	0.26	0.36*	0.48*	0.16	0.17	0.18	0.16	0.16	0.18
(\pm SD)	0.04	0.07	0.02	0.06	0.13	0.05	0.05	0.03	0.09	0.06	0.03	0.09
Phosphate ($\mu\text{g} \cdot \text{L}^{-1}$)	0.07	0.07	0.09	0.09	0.08	0.08	0.05	0.06	0.05	0.07	0.07	0.08
(\pm SD)	0.004	0.005	0.003	0.005	0.005	0.006	0.002	0.002	0.004	0.005	0.003	0.006
Cyanobacteria ($\mu\text{g Chl-a} \cdot \text{cm}^{-2}$)	1.86	1.89	1.63	1.22	3.95*	3.23*	0.99	1.04	1.08	0.95	0.97	1.05
(\pm SD)	0.39	0.39	0.32	0.45	2.12	2.25	0.07	0.21	0.22	0.09	0.19	0.17
Invertebrates ($\text{g DM} \cdot \text{m}^{-2}$)	3.85	4.01	4.61	3.94	3.63	4.11	2.99	2.34	2.94	2.83	3.02	2.54
(\pm SD)	0.51	0.69	0.97	0.79	1.02	0.89	0.54	0.43	0.78	0.48	0.64	0.73

During flow manipulations there were some unusually large rain events in the second half of our experiment (Figure 27a). Our experimental flow reduction raised water temperature

markedly at the 50% flow reduction point (Figure 27c). After the sudden temperature jump, gradual flow reductions sustained elevated temperatures for 100-days. We observed a converse temperature drop with a 50% flow recovery (Figure 27d) similar to rivers experiencing flow releases from dams (Halladay *et al.*, 2016, Neif *et al.*, 2017; Ho *et al.*, 2019).

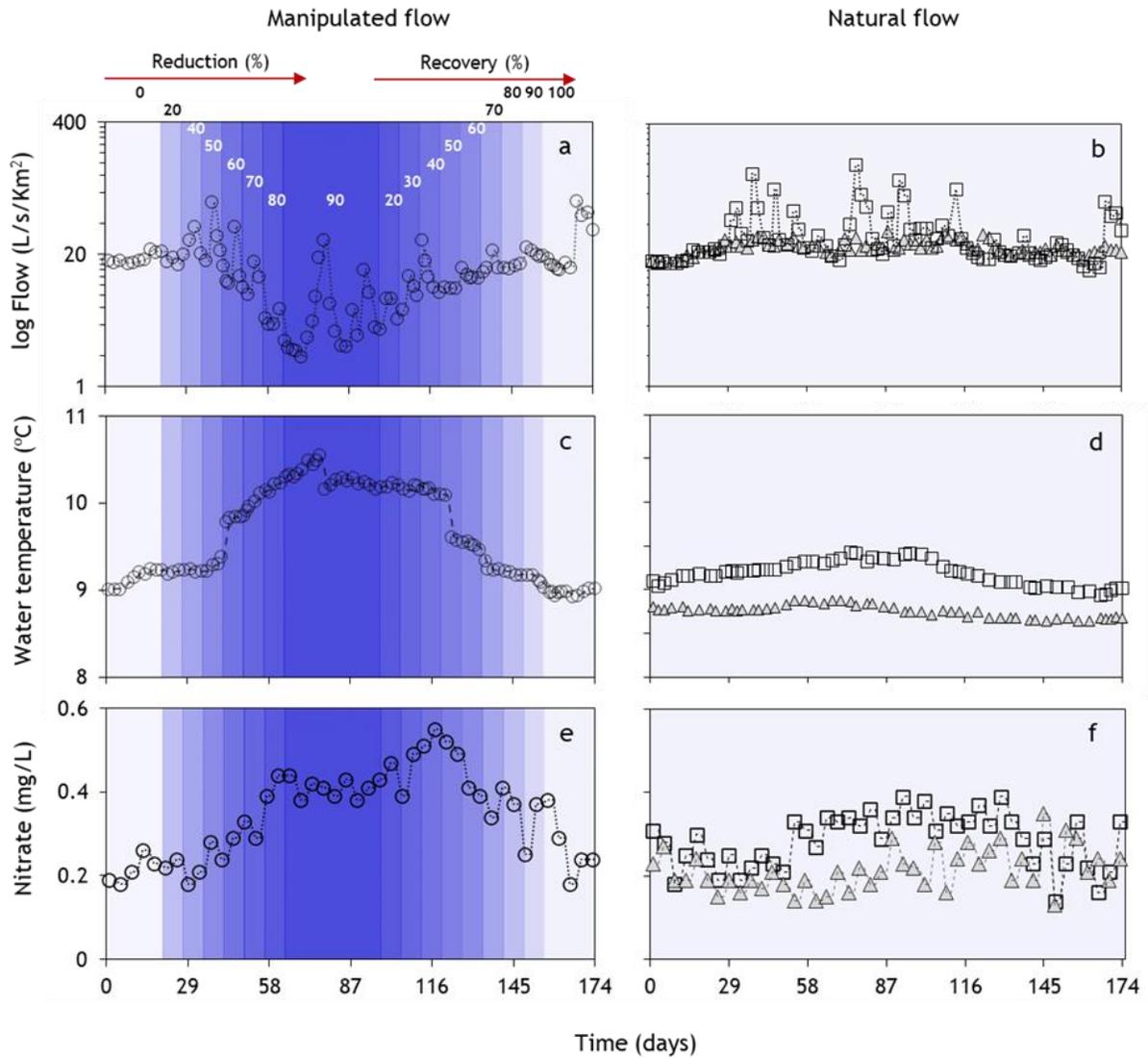


Figure 27. Environmental variables during experimental flow manipulations and natural flow. Time - series (open symbols) and smoothed-state estimates (dotted lines) for the experimental stream reach (circles), the manipulations of flow targeted reductions of 20, 40, 50, 60, 70, 80 and 90% and recoveries of 20, 30, 40, 50, 60, 70, 80, 90 and 100% (blue shades), while maintaining daily fluctuation (a, c, e), natural flow conditions for the upstream site of the experiment (squares) and the control stream (triangles (b, d, f).

An increase in nitrate (NO_3^-) concentrations occurred over the flow reduction period and continued to rise during the first part of flow recovery (Figure 27e). The rapid NO_3^- increases and decreases around days 58 and 120, respectively, occurred in both the experimental and natural upstream reaches (Figure 27 e,f, squares). The NO_3^- concentration during flow manipulations generally followed an inverse pattern to flow although there was a notable concentration jump ($\text{NO}_3^- = 0.46 \text{ mg} \cdot \text{L}^{-1}$) at 80% flow reduction and a peak ($\text{NO}_3^- = 0.58 \text{ mg} \cdot \text{L}^{-1}$) at 40% flow recovery. A two-fold increase in NO_3^- concentration may indicate a shift in N-limited headwater streams due to non-fixer cyanobacteria proliferation (Neif *et al.*, 2017; Paerl *et al.*, 2018; Wurtsbaugh *et al.*, 2019).

The 60% flow reduction was concurrent with a two-fold increase in cyanobacteria biomass and a 90% flow reduction corresponded to a four-fold biomass increase (Figure 28a). Flow reductions up to 50% did not correspond to notable changes in cyanobacteria biomass relative to control conditions (upstream and reference stream sites) (Figure 28b) (Guillet *et al.*, 2016). Multivariate autoregressive state-space models (MARSS) (Holmes *et al.*, 2012; Holmes *et al.*, 2014; Cauvy-Fraunié *et al.*, 2016; Dong *et al.*, 2017) analysis of cyanobacteria biomass with flow revealed two significant shifts during the experiment: cyanobacteria biomass increased with a 60% flow reduction from natural flows (after ~35 days/5 weeks of gradual manipulations) and biomass decreased with 40% flow recovery (after ~42 days/6 weeks of restoring natural flows) (Figure 28a, red curve). During the recovery period strong precipitation led to flow increases, which facilitated cyanobacteria to return to natural levels (Scheffer *et al.*, 2001; Mitrovic *et al.*, 2011).

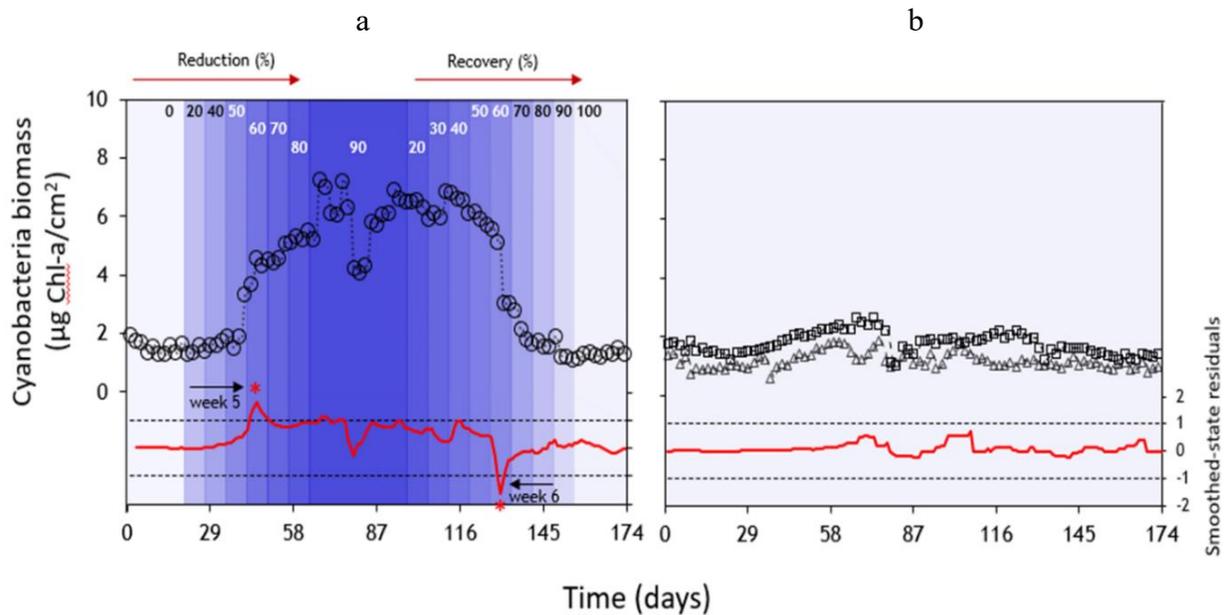


Figure 28. Cyanobacteria biomass response to manipulated and natural flow. Time-series and smoothed-state estimates (dotted lines) for the experimental reach (open circles) undergoing manipulated flow (blue shades) **(a)** and for the upstream site of the experimental reach (squares) and the control stream (triangles) under natural flow **(b)**. Red curves correspond to standardized smoothed-state residuals from state-space models, dashed black lines are the 95% confidence intervals, and stars indicate when standardized smoothed-state residuals are beyond the dashed – line confidence interval levels.

The cyanobacteria biomass also responded to the distinct temperature increase around 50-60% flow reduction, which was expected (Figure 28a). During the recovery period, the jump in NO_3^- concentration at 40% flow recovery correlated with cyanobacteria biomass decrease, the strength of this interaction was also expected (Maier *et al.*, 2001; Guven and Howard, 2006; Paerl *et al.*, 2009; Guillet *et al.*, 2016). Our experiment provided thresholds for cyanobacteria biomass at ~50-60% flow reduction, which represented a shift on biomass that was sustained in high levels (i.e., four-fold compared to reference conditions), through the period of maximum flow reduction. In the reference stream, cyanobacteria biomass fluctuated in low proportions within benthic algae community (Figure 29) (Paerl *et al.*, 2018).

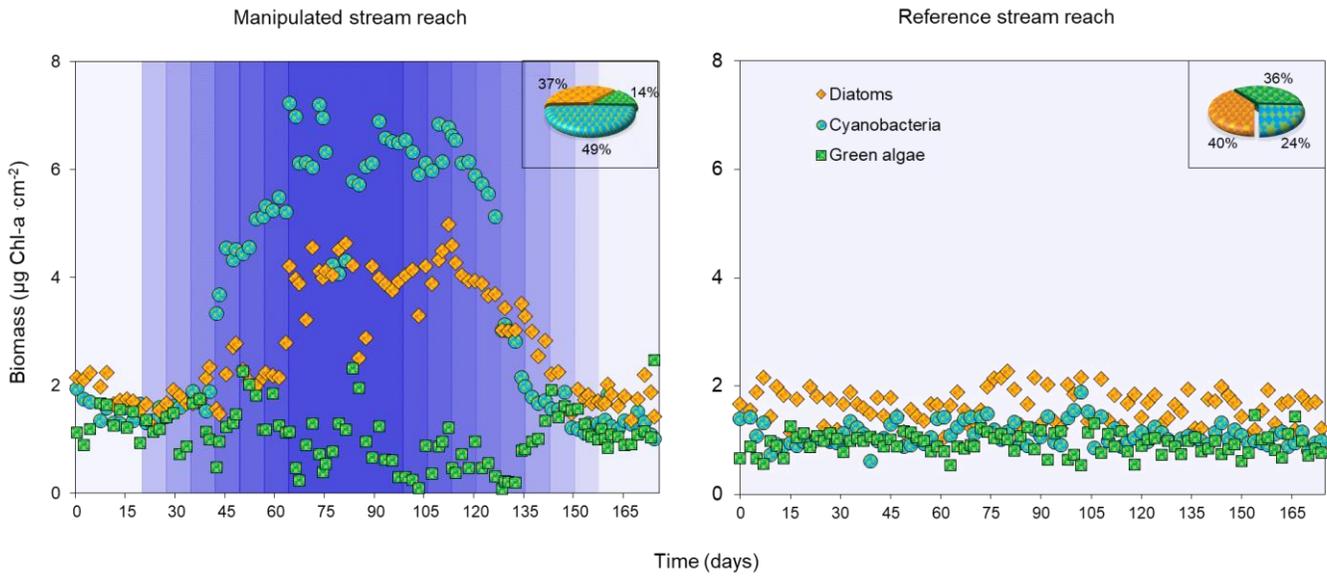


Figure 29. Cyanobacteria (turquoise circles), diatoms (orange rhomboids), and green algae (green squares) biomass measured in time (days) in the manipulated stream reach (flow reductions and recovery -blue shades, see Fig 27a) and the reference stream reach, right frames show relative abundance (%) of color-coded groups.

Studies based on experimental flow releases from dams corroborate our flow recovery findings, where flow increases and associated water velocities limit cyanobacteria biomass settlement and proliferation (Guillet *et al.*, 2016). Additionally, previous findings from dams' releases showed cyanobacteria biomass decrease after flow pulses followed by biomass proliferation during flow recession (Bushaw-Newton *et al.*, 2002; Olden *et al.*, 2014).

3.2 Local and global scale surveys

Nearby streams undergoing flow reductions by water intakes (n = 637) showed shifts in cyanobacteria that were similar to those generated in our experiment (Figure 30a, gray colored circles). Impacted sites close to water intakes (10 m) with flow reductions of >90% showed cyanobacteria biomass increases of up to four times the reference conditions (i.e., upstream) (Figure 30a). Cyanobacteria biomass just below water intakes experienced stable-low flow conditions until an operational release flushed benthic biofilms and sediments at the site

(personal observation). Overflow from water intakes occurred sporadically in response to extreme precipitation events, allowing sites within 10 m of water intakes to maintain dominant-stable conditions. Impacted sites further away from water intakes (500 m) with small flow reductions, 10-25%, showed small cyanobacteria biomass increases relative to reference conditions. Between 30 and 50% flow reductions, cyanobacteria biomass also resembled reference conditions. Flow reductions from 50% to 90% generally showed cyanobacteria biomass increases between one and four-fold, relative to reference conditions (Figure 30a). Evidence of cyanobacteria biomass increasing with flow reduction caused by dams in low land rivers show a trend that was similar to our findings (Mitrovic *et al.*, 2006; 2011). Comparing our experimental flow reduction results to the local survey, we can see that a 60% flow reduction corresponded to a two-fold cyanobacteria biomass increase relative to the upstream site for our high-altitude tropical stream experiment (Figure 30a, blue colored circles). The local survey plus our experimental results show that cyanobacteria biomass fluctuates near natural/reference conditions and flow reductions between 5 – 50%. Cyanobacteria shifted with a two and three-fold increase in biomass compared to reference conditions when a flow reduction changed from 40 to 50%.

Our global literature survey revealed results consistent with our local survey and our experiment, showing low cyanobacteria biomass increases, less than a factor of two, with flow reductions between 5 and 50% (Figure 29b) (Paerl *et al.*, 2018; Ho *et al.*, 2019). Cyanobacteria biomass had the highest increase factor (i.e., four-fold) with flow reductions >58% (Figure 30b). Few global data <25% showed no increase on cyanobacteria biomass with higher relative percentage flow reduction. Experimental dam releases have reported no increase in cyanobacteria biomass during flow recession (Olden *et al.*, 2014). Our paired cyanobacteria biomass and flow reduction data provided us with a range of responses to flow changes. The distribution of data from further downstream of water intakes show that recharge of flow is driven by space and that, consequently, cyanobacteria biomass decreases as response to flow increase.

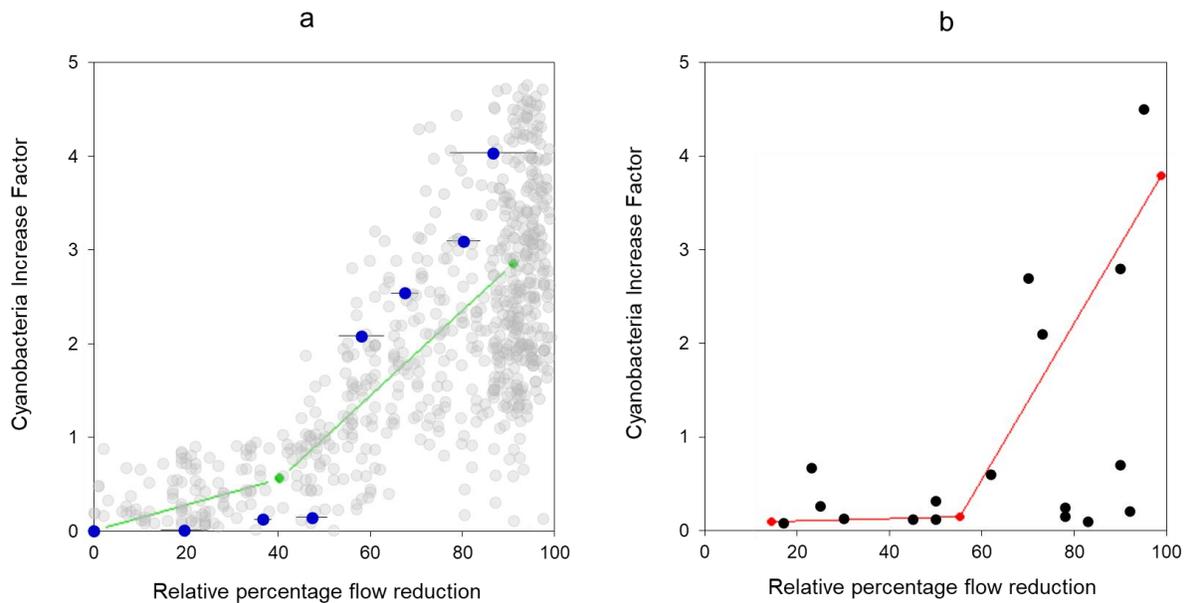


Figure 30. Cyanobacteria increase factor according to the relative percentage of flow reduction. **a)** Measurements (gray circles) of paired cyanobacteria-flow monitoring data ($n = 697$) at the time of sample in one location upstream the water intake and five locations downstream the water intake in the Chalpi Norte stream (10, 50, 100, 200 and 500 m) and at two locations (0 and 500 m) in six streams from the water supply system; experimental results (blue circles) of cyanobacteria increase with targeted flow reductions including variations \pm SE (black arrows). Douglas-Peucker model (RDP) (green line) fitted to monitoring data showing a breakpoint for cyanobacteria increase with a 40% flow reduction. **b)** Global survey of cyanobacteria-flow data ($n = 21$) showing two distributions fitted with RDP models: cyanobacteria increase after a breakpoint of 50% flow reduction (red line), and cyanobacteria show no change with flow reduction (blue line).

4. Conclusions

Our results present the case of regime shift thresholds in streams using a whole ecosystem experimental flow manipulation with comparisons to data based on nearby samples above and below water intakes and data from a global literature survey. Although our experiment idealized flow reductions in fixed percentages, our intention was to show flow as the driver of change and associate an ecological meaning to a flow threshold. We induced a cyanobacteria biomass shift through flow manipulations showing that flow exerted a significant change in water temperature primarily and potential combinations of environmental variables: nitrate concentration, dissolved oxygen, light that seemingly relate to cyanobacteria biomass (Guyen and Howard, 2006; Ibanez

et al., 2012; Bowling *et al.*, 2013; Cauvy-Fraunié *et al.*, 2016). The effect of flow and environmental variables over cyanobacteria biomass showed two main thresholds: first, cyanobacteria increase with a 50-60% flow reduction and, second, cyanobacteria biomass was near natural conditions with ~60% flow recovery. We observed, during a 35-day period of maximum flow reduction (i.e. 90%), that cyanobacteria (CyanoHABs) proliferation was arrested, probably due to limitations associated with low water flows (Pace *et al.*, 2019; Paerl *et al.*, 2018). These findings have important implications for water management as cyanobacteria proliferation in rivers can threaten downstream lakes and reservoirs, consequently affecting more than one type of water supply source usually interconnected in provision systems (Jacobsen and Dangles, 2017; González- Zeas *et al.*, 2019). Research on lakes has demonstrated a disproportionate increase of cyanobacteria compared to other phytoplankton in North America and Europe (Paerl *et al.*, 2018). A similar trend might be occurring in rivers when flow reductions pass natural thresholds of relative abundance within the benthic community (Bowling *et al.*, 2013; Ho *et al.*, 2019; Wurtsbaugh *et al.*, 2019). Importantly, our results from mountain streams agree with observations and experiments realized in freshwater systems around the world, supporting a global shift in cyanobacteria biomass with reduced flows. With respect to flow, our study provides one of the first *in situ* demonstrations of regime shift thresholds in natural running waters (Scheffer *et al.*, 2001; Robinson and Uehlinger, 2008; Ibanez *et al.*, 2012; Neif *et al.*, 2019), and unique experimental evidence of flow reductions through fixed percentages for e-flow management.

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CHAPTER 5

CONCLUDING THOUGHTS AND NEXT STEPS

The use of experiments to manipulate flow in streams allows us to evaluate ecosystem response under controlled conditions where flow is the main driver of change. Defining environmental standards for water abstraction is in much need of new approaches to identify shifts on flow – ecology relations. Over many years, several researchers have analyzed observations from monitored streams providing important insights of flow alteration impacts. However, the temporal effect of water operation and climate conditions associated with ecosystems can obscure the nature of flow – ecology responses and limit our understanding of thresholds of change. Researchers working on defining environmental standards and proposing e-flows guidelines have struggled with methods that generate results that can be extrapolated other situations. Although this has challenged water resources management, it has also created momentum to adapt to changing conditions. The research presented in this dissertation outlines approaches, or perhaps combines several approaches, to define environmental standards that can further help water managers closely assess ecosystem changes associated to water abstraction to maintain environmental flows (e-flows).

With this dissertation I described a path for environmental flows assessment (EFA) considering temporal and spatial datasets from both, 1) observations on the local water system and 2) experimentation at a whole ecosystem scale. The involvement of water managers in defining environmental standards is cornerstone for implementation in any system. Particularly in the water system for the city of Quito, e-flows management is up to the water supply company. Finding a science-based approach to work under the premise of securing water demand challenged some aspects of environmental flow assessment (e.g., impacts *versus* optimization) and made me aware of the compromises that water managers have to consider. However, including an *in situ* experiment in our assessment gave to water managers the opportunity to obtain first-hand results for the systems they operate and a front-row seat to watch

the experiment in action. This resulted in a trade-off analysis and ultimately in a science-based decision made by water managers. The intention to adapt a framework to a context that fits most water systems in the tropics where increasing water demand jeopardizes science-based decisions. The success of e-flows implementation will ultimately rely on the feasibility of the water system to incorporate environmental standards that let rivers flow.

Evidence of decreasing water quality in different aquatic ecosystems around the world has triggered an alarm alerting us to the importance of assessing abiotic and biotic water characteristics. Aquatic ecosystems in the Andes of Ecuador already show evidence of harmful algal blooms in water supply reservoirs. The emergence of this situation just as I was beginning my dissertation research provided a timely opportunity to search for tools and strategies to assess water quality focusing on benthic algal and cyanobacteria in streams, lakes, and reservoirs. As I intend to demonstrate in Chapter 2, extending the area and frequency of sampling with the aid of a fluorometer, in this case, the BenthoTorch, could be beneficial for any monitoring program that needs to consider *chlorophyll a* as surrogate of algal and cyanobacteria biomass, i.e., as indicators of potential toxic bacteria. I showed the advantage of covering a major sampling area on a water system using artificial substrates to overcome the problem of highly variable readings from mature algal mats on natural substrates. I intend to extend my research to understand the temporal dynamics of benthic algal and cyanobacteria using artificial substrates and the BenthoTorch in tributaries of reservoirs that have shown evidence of cyanobacteria blooms. Since water managers are aware of the susceptibility of their systems, they have considered water quality-early warnings a path worth to strengthening. Assessment of the monitoring program will result in science-based decisions that ultimately will contribute the knowledge framework for continuing to develop environmental standards for water abstraction.

To complement regular monitoring, this dissertation presented the importance of experimental approaches to manipulate flow with the objective to assess first-hand ecosystem responses to flow alteration. Including experimental components in future projects will provide

clearer insights towards assessing a wide range of external factors (or stressors) including those associated with climate change, e.g., increased weather variability. Collaborative initiatives, like the Chalpi Flow Project, are probably the best ways to involve water managers in making research improvements that will lead to reasonably rapid implementation of a dynamically evolving e-flows framework.

I am interested in future research that employs experimentation at a water intake scale. Difficulties of this type of project will be the ensuring a secure water supply while implementing environmental standards. Regardless, such a project would provide valuable insights into the operational challenges for implementing e-flows standards. I believe some areas of e-flows still need to address external factors that have not been previously considered. This will require researchers and managers to dynamically adapt to emerging conditions. For example, I think there are shifts in the natural flow's regime other than changes in flow magnitude that requires a better understanding of the interactions between hydrological processes and ecological responses in time. I intend to pursue a repeatable experiment that considers the transition periods, where peak flows combine with prolonged dry days, amplify the range of potential ecosystem responses. The ultimate result will provide a time series of environmental standards to follow according to seasonality and water demand. Finally, I would like to continue collaborating for future implementation of environmental standards and to science-based feedback to water managers to develop strategies that fit different scenarios.