University of Puerto Rico – Mayagüez Campus
College of Agricultural Sciences
Department of Agro-environmental Sciences

Final Project Report
Citizen Monitoring of Water Sanitation in a Rural Puerto Rico Watershed
QAPP title: Assessment of Water Quality and Efficacy of Water Treatment Infrastructure in Southwestern Puerto Rico

Cooperative Agreement no. 83553801 between US Environmental Protection Agency and the University of Puerto Rico Mayagüez Campus
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Submitted by:

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1. Executive Summary

This final report summarizes the activities carried out under Cooperative Agreement no. 83553801 between US Environmental Protection Agency (USEPA) and the University of Puerto Rico Mayagüez Campus (UPRM), College of Agricultural Sciences, encompassing the period from 1 January 2014 to 1 July 2016. The project had educational/outreach and research components. The educational/outreach aspect included the participation of UPRM Agricultural Extension Agents, UPRM Researchers, and UPRM Graduate Students as mentors, and citizen volunteers as mentees. The research component included the collaboration of citizen volunteers and project staff in carrying out a land-use/land-cover analysis and partial physical, chemical and biological characterization of stream waters. Stream-water quality was linked to land-use data in order to assess potential contributing sources.

Citizen Volunteers. The citizen volunteers were High School Students from UPRM-Agricultural Extension Service 4H youth program and University Students from UPR-Mayagüez. The volunteers were led through an initial formal training that for some culminated in their participation in equipment preparation, sample collection, and data gathering. Some of the citizen volunteers also participated in laboratory analysis and data management. The project had a high impact on citizen volunteers. All of the citizen volunteers that were part of the 4-H Youth Program have graduated from High School and most have continued studies leading to baccalaureate degrees. These students expressed that they were able to link information acquired in formal classroom setting to a real-life situations. Most communicated that after the experience they perceived environmental problems in a more rational and balanced perspective. The University students expressed deep satisfaction for their participation and their comments can be summarized as: (i) they became aware of the inter-disciplinary nature of the study; (ii) they learned the importance of receiving a broad science-based education; (iii) they valued working together with graduate students and professors; (iv) they realized the importance of team-work including scheduling, timeliness, organization, and communication; (v) the experience helped them focus on and identify future study or working areas.

Land-use/Land-cover analysis. The selected area was the eastern portion of the Lajas Valley watershed (ELVW), and four basins (Mondongo, Bárbara, Maginas and Cristales) within, were emphasized. Georeferenced land-use data from the Puerto Rico Governmental Portal for geographic data (gis.pr.gov) and from PR GAP project were used to establish the initial land-use/land-cover map. Ground truthing was used to validate the published classification and helped identify point sources of contamination such as over-flowing sewer conduits, illegal grey-water discharge pipes from homes, and other non-point sources. The resulting land use/land-cover map was substantially different from the original maps with four land use/land-cover classes namely urban land (UD), shrubland/forest, agriculture, and pond.

Overall, unmanaged shrubland/forest-land was the most dominant land use class within the ELVW, followed by agriculture and urban land use classes; with 61, 30 and 9% of total land area, respectively. The population was estimated at 33,936 covering an area of 14,668 ha (36,229 acres). The five basins ranged in area from 846 to 1,849 ha (2,090 to 4,568 acres) each, with a population range of 2,759 to 7,297 occupying an urban footprint from 131 to 1,581 ha

(324 to 640 acres), per basin. Mondongo basin clearly had the greatest land-area under agriculture, urban footprint and population, numerous passive and active discharge conduits, plus had a waste-water treatment plant (WWTP) that discharged 1.02 MGD of secondary/tertiary treated effluents for a total annual P load of 1,471 kg P, and estimated total N load of 6,034 kg total N/yr. In contrast, Cristales basins had the lowest land-area under agriculture, population, and urban footprint. In all basins (except Mondongo) unmanaged shrubland/forest was the dominant land use class with between 48 and 80% of the land area; followed by urban land with between 12 and 30%, and agriculture with between 7 to 28%. Within agricultural land-use important potential nutrient and fecal indicator contributing sources were grazed pastures and row crops encompassing a land area of 0.3 to 19% and from 2.5 to 20%, respectively.

Sampling and analysis. Twenty-three stations were established throughout the ELVW, Río Loco drainage outlet, and Guánica Bay and were sampled manually during low- to intermediate-hydrologic flows. The stations were sampled by both professionals and citizen volunteers. The stations were sampled up to five times from August 2014 to June 2015. Twelve stations that were established at the drainage outlet of five pre-defined basins (Mondongo, La Plata, Bárbara, Maginas, Cristales) were selected for further sampling during 2015 to 2016. Five of these stations were also used for storm-water sampling using passive-flow samplers.

In situ measurements of hydrologic stream-flow, pH, specific conductance, dissolved oxygen concentrations, and oxygen saturation were made. Grab samples were collected, transported to the laboratory and analyzed for total Kjeldahl nitrogen (TKN), dissolved NO_3-N, total nitrogen (N), total phosphorus (P), optical brighteners (OBs), total suspended solids (TSS), turbidity, enterococci, cattle and human bacteroides markers. Selected samples were analyzed for metals (Al, B, Ca, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Si, Zn). Storm event samples were analyzed for TKN, dissolved NO_3-N, total N, total P, enterococci, TSS, and turbidity.

Fecal indicators of contamination. The mean enterococci concentrations across sites and dates for all of the area was 338 (131)[1] MPN/100 mL. All stations had geometric means exceeding the suggested surface water EPA threshold of 35 MPN/100 mL. Only 4.4% of the samples had enterococci concentrations below the suggested EPA threshold. Overall, the % positive incidence for OBs, cattle bacteroides and human bacteroides was 13, 20, and 22%, respectively. The lowest enterococci concentrations were observed in Río Loco, Guánica Bay, and the four stations within the drainage channel, while the highest concentrations were observed in those stations draining high-density urban areas such as basins Mondongo (stations #13 and #4), Bárbara (station #10) and La Plata (station #8). Enterococci were not persistent at the outlet of the Lajas Valley watershed, Río Loco watershed and in Guánica Bay and the results were inconclusive to sustain that there is a consistent human and cattle contribution to fecal contamination and that fecal microorganisms are prevalent in coastal waters of Guánica Bay. Overall, enterococci concentrations were positively weakly correlated to nutrient data. Multiple

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[1] geometric mean ± 1 standard error

regression analysis relating enterococci concentrations to LU/LC data showed significant positive relationship with the proportion of urban land.

Nutrients, sediments and metals. The total N, dissolved NO_3-N and TSS concentrations in this study were similar to a companion study completed from 2011 to 2012 and total P concentrations were slightly higher, in waters draining the ELVW. The PR water quality standard and/or suggested levels of enrichment were exceeded in 30%, 43%, and 61% of the sampling stations, for total N, dissolved NO_3-N and total P concentrations, respectively. Within each basin, higher total N and dissolved NO_3-N concentrations were observed at upstream sampling stations, while higher total P concentrations were observed at downstream stations. In general, total N and dissolved NO_3-N were higher at urban-dominated basins, and were unaffected by stream-flow regime. Total P varied by basin and flow regime, while TSS was unaffected by flow regime, and both were higher at basins dominated by both urban and agricultural areas. The mechanisms driving inputs and losses of nutrients in stream-waters varied among basins and specific nutrients.

The waste water treatment plant (WWTP) in Mondongo basin is an important contributor of N and P to Mondongo stream as total N, dissolved NO_3-N and total P concentrations, immediately downstream of the WWTP discharge point, increased dramatically relative to an upstream station. Also, Mondongo Basin had significantly greater total P concentrations than other basins. Storm-event total N, total P and TSS (but not necessarily dissolved NO_3-N) concentrations were higher than overall means collected during base-flow and during low-intermediate flows from the ELVW.

Overall, stream total N was positively correlated to the proportion of basin area in urban land (UD) (r= 0.49), while negatively correlated to grazed pastures (GP) (r= -0.42), row crops (RC) (r= -0.29) and pond (P) (r= -0.31). The best fit geographically weighted regression (GWR) models showed positive relationships of total N with the proportion of UD and population. Stream total P was negatively correlated to unmanaged forest/shrubland (UF) (r= -0.40) while positively correlated to UD (r= 0.40), and hay and silage (HS) (r= 0.33). The overall GWR model showed that total P concentrations were negatively associated to all parameters because only one basin (Mondongo) had a positive association with UD and HS, while in the other basins these associations were negative. This suggests that there are spatially varying relationships influencing total P concentrations. Although dissolved NO_3-N was positively correlated to UD and negatively to all other LU/LCs, the GWR models included only negative relation with GP. Empirical scatterplots showed positive relationships between stream total N and UD for all basins and for total P relationships were positive with UD at Barbara and Mondongo, and negative at Cristales and Maginas.

It seems that the main sources contributing to stream N and P concentrations are the Lajas WWTP and northern urban areas of the ELVW, and to a lesser extent agricultural areas consisting primarily of row crops and grazed pastures. It seems that P concentrations from ELVW is potentially a more important contributor to Guánica Bay than N as a major portion of the latter was lost through the watershed drainage pathway. Also, the most important contributing basin to stream degradation within the ELVW was Mondongo by direct inputs from the WWTP, due to the high nutrient concentration and relative high contribution of discharge in Mondongo stream, and eventually Lajas Valley drainage channel. However, we recognize that in other basins point source inputs such as open conduits and possibly faulty septic systems, combined with agricultural areas such as runoff from grazed pastures and fertilized crops, may contribute to stream nutrient concentrations.

1.1. Project overview and objectives

The overall goal of the project was to: *Increase public awareness of sanitation issues in the Lajas Valley and Guánica/Río Loco watersheds by carrying out assessments of water quality and sewage infrastructure by professionals and citizen volunteers*. Specific objectives were:

1. Characterize the water quality for indicators of unsanitary conditions in streams and drainage channels draining selected watershed basins
2. Use GIS tools to identify point and non-point sources of contaminants
3. Use monitoring results in combination with GIS to link contaminants to specific sources
4. Improve public awareness of the threats of contamination and provide potential solutions to public health and environmental problems

1.2. Acknowledgements

The findings of this study are just another stone on the road towards exploring and understanding the dynamics of water quality in Puerto Rico. Our research group at UPRM continues to dig deeper beyond the surface as we continue to examine the role of agricultural activities on nutrients and fecal contaminants in water. This report summarizes work done my many individuals and organizations whom have contributed towards the success of this project. We acknowledge grant funding from USEPA and internal funds from the University of Puerto Rico, College of Agricultural Sciences Agricultural Experiment Station. We appreciate the interaction and support from USEPA project coordinator Bill Fisher, whom has guided us through the often intricate USEPA administrative network. We appreciate the participation and time of Agricultural Extension Service agronomists Isbeth Irizarry and Anibal Ruiz in mentoring a group of citizen volunteers. DSR especially recognizes project Co-PIs (Martínez and Pérez-Alegría) whom have provided enthusiasm and intellectual support since the preparation of the original proposal and throughout the project.

We appreciate the work done by three graduate students, Cristina López, Paloma Rodriguez, and Armando Román. All of the students collaborated in leading and organizing the volunteer groups, data analysis, data processing and laboratory and equipment maintenance. All three are currently completing their MSc thesis and throughout the duration of the project received complete or partial GRAs under the project auspices. Chapter 6 will be the subject of MSc thesis by C. López as she conducted much of the statistical analysis within and actively collaborated in its summary. A Mr. Hector Torres provided support in all field activities. We are especially grateful to the Soil and Water Chemistry Laboratory of UPR-Agricultural Experiment Station staff, Onilda Santana and Jose Luis Guzman whom analyzed all of the nutrient chemistry in water samples.

2. Professionals and citizen volunteers and outreach

2.1. Citizen volunteers and professionals

A group of *professionals* that included professors, researchers, graduate students, and technicians from UPR-Mayagüez was assembled. The selection of the graduate students was based on their personal interests, academic qualifications and academic experiences. The selection of Agricultural Extension Agents as *mentors* was based on their professional interests, geographic location, and professional expertise. We selected two groups (A and B) of *citizen volunteers*. Group-A consisted of 13 volunteers and were members of the Agricultural Extension Service (Cooperative Extension Service) 4-H Youth Program. Some of the students resided in the study area. The volunteers were in grades 10 to 12 (sophomores, juniors and seniors), ranged in age from 16 to 18 years, and were enrolled in two High Schools, *Escuela Superior Leonides Morales* in Lajas and *Áurea E. Quiles* in Guánica.

The Group-A volunteers were distributed among four sub-groups of between three and four students each, with the professionals and Agricultural Extension Agents serving as *mentors*. Agro. Isbeth Irizarry led three sub-groups and Agro. Anibal Ruiz led one group. Other collaborators that provided partial support were Agro. Jose A. Torre (Agronomist UPRM-AES), and Benerizael Anzueta (from the Puerto Rico Department of Education).

Group-B Citizen Volunteers was composed of twelve university students from UPR-Mayagüez. The students came from diverse academic backgrounds but most were from the Geology Department, which promotes among its undergraduates, internships and scientific collaborations with professors and researchers.

2.2. Training of professionals and citizen volunteers and follow-up meetings

All of the professional project participants were trained in field and laboratory procedures, equipment maintenance and laboratory analysis. Training was done by having the professionals read the project QAPP, followed by question-answer sessions and discussion prior to the two-day workshop held on 19 and 20 June 2014. The professionals also participated actively in the workshop, which reinforced the antecedent discussions. Further training was continued by formal and informal meetings throughout the project duration.

Group-A Citizen Volunteers were convened on two occasions. A two-day training was conducted at the UPR Agricultural Experiment Station in Lajas on 19 and 20 June 2014. There were 34 participants that included volunteers, mentors, professionals and graduate students. Presentations were given by project personnel and supporters on the following topics: (i) Watershed management; (ii) Water quality and its implications on health; (iii) Sources of water contamination; (iv) Lajas Valley and Río Loco watersheds; (v) Sampling techniques and monitoring stations. Diagnostic tests were given prior to workshop commencement and at the end to evaluate volunteer learning and training efficacy. The pre-workshop mean score was 5.8 and the post-workshop mean score was 11.6, out of 16 maximum points, which suggests that the students were able to improve their knowledge based of the selected topics. Each volunteer that completed the theoretical and experimental parts was certified with a diploma (Please see appendix for an example). All participants that completed the workshop received a Certificate of Participation. The project QAPP, including SOPs were made available to all participants at http://www.uprm.edu/waterquality.

The Group-A volunteers were convened for a second time at UPR Isla Magueyes Field Station on 21 November 2014. An oral and a scientific poster presentation was given. The presentations summarized results of the first two sampling rounds. The citizen volunteers were able to see how the data that they were helping to collect was being used. A discussion was carried out regarding the results obtained and their significance.

Group-B Citizen Volunteers were trained in field sampling procedures in a one-hour group orientation and a one-day laboratory and field workshop on 5 September 2015 in *Finca Alzamora* and *Quebrada de Oro* (stream) at UPR-Mayagüez. There were 19 participants in the workshop including graduate students and citizen volunteers. In all instances, we realized that in order for the participants to master the techniques, one training session was not going to be enough but rather an ongoing and continuous training was needed. To this end, during each activity (sampling and work session), the professionals took time to explain the background and details of each of the procedures and the corresponding rationale. A final presentation was offered to Group-B volunteers on 28 April 2016. In this session, the major highlights of the project results were communicated to the participants. Many of the participants expressed their opinions regarding their participation and how the experience influenced their outlook on environmental awareness of sources of contamination in the Lajas Valley.

2.3. Citizen volunteers activities

Sampling stations in stream-channels, corresponding to subbasin outlets throughout the Lajas Valley Watershed were established. For Group-A citizen volunteers, each sub-group was assigned two stations, which they sampled on pre-established dates. Mentors led citizen volunteers to the sampling stations where they met the professionals. Sampling was conducted with the supervision of their mentors and of UPRM professionals. Each station was sampled five times by the assigned group from August 2014 to July 2015. Participants were expected to fill out chain of custody sheets, data sheets, collaborate in data field physical and chemical water quality data collection. The landuse and landcover within each basin was discussed in the context of what they were visually observing and the water-quality results obtained.

For Group-B citizen volunteers, sampling was conducted on pre-established dates of Tuesday, Friday and Saturdays of selected weeks. Saturdays was selected because it was the day of the week in which there was less conflict among the participants. Participants did not visit the same stations repeatedly as in Group-A, but rather sampled a variety of stations based on the sampling schedule and their availability on particular dates. These volunteers also led a more active role in sampling, water quality data collection, data entry, pre-sampling instrument calibration and laboratory analysis.

2.4. Outreach

Five outreach activities, four training sessions and three professional presentations were carried out as part of the project activities. A web page was constructed for the project to provide educational material, project results and other information to citizen volunteers and the public (http://www.uprm.edu/waterquality). A *Twitter©* account was created to stimulate interaction and communication among citizen volunteers and project personnel @waterqualitypr. A *WhatsApp©* communicating group was created named "Citizen Monitoring" that served as communicating platform among citizen and professionals. Through this platform, participants could communicate sampling dates, certify attendance, and coordinate time and location of meeting, among other activities. Training videos and pictures that served to demonstrate sampling techniques and procedures were sent using the platform.

2.5. Sampling dates and participants

Five grab-sampling rounds were completed with Group-A citizen volunteers that were part of UPRM-AES 4-H Youth Program (Table 2.1).

Table 2.1. Group-A citizen volunteer participants during 2014-2015

Round #	Date	Participants
1	5 and 6 Aug 2014	12 citizen volunteers and 3 extension agents
2	29 September and 1 October 2014	8 citizen volunteers and 2 extension agents
3	12 and 19 January 2015	11 citizen volunteers and 3 extension agents
4	6, 16, and 20 April 2015	9 citizen volunteers and 2 extension agents
5	2 and 3 June 2015	6 citizen volunteers and 2 extension agents

Five grab-sampling rounds were completed with Group-B citizen volunteers that were undergraduate students at UPRM (Table 2.2).

Table 2.2. Group-B citizen volunteer participants during 2015-2016.

Round #	Date	Participants (citizen volunteers)
1	15, 18 and 19 September 2015	12
2	29 September, 2, 3 October 2015	14
3	13, 16 and 17 October 2015	11
4	27, 30 and 31 October 2015	9
5	2, 3 and 4 February 2016	3

2.6. Project impact on citizen volunteers and the public

The objectives of citizen volunteer participation was to: (i) promote environmental awareness of the threats of contamination and that they can eventually contribute towards potential solutions to public health and environmental problems of the Lajas Valley; (ii) improvement of participants' scientific literacy, increased critical thinking and confidence building skills; (iii) provide positive experiences that would guide them towards selection of future careers, study goals, community participation, and employment opportunities. This project provided a learning and research experience for the citizen volunteers where they learned technical field and laboratory skills, data entry and analysis techniques, QA/QC protocols, as well as communication and teamwork skills.

The Group-A volunteers had limited scientific literacy in the topics and little or no experience in handling water quality materials and equipment. Their knowledge-base of the role of nutrients in water quality maintenance and how fecal indicators of contamination can be used to assess the quality of a water body improved greatly. Based on our conversations, they were able to link the information that they had acquired in a formal classroom setting in their Biology and Earth Sciences courses, to what they were doing. This experience helped them to realize that water quality assessment can be done with the proper equipment and training and that important conclusions could be drawn from the data collected. This experience motivated them to continue their studies at the university level. Nine students graduated from High School in 2015, and four in 2016. Nine students are currently studying in various campuses of University of Puerto Rico, and one student is studying at the InterAmerican University.

The Group-B volunteers had an advanced scientific literacy level. Many of the students were sophomores and juniors in their respective science major (Geology, Agronomy, Horticulture) at UPRM. Yet, most of the participants had never had the opportunity to work with materials and equipment to assess water quality. At the end of the experience, most of the volunteers expressed deep satisfaction for their participation. A brief summary of their comments is: (i) they became aware of the inter-disciplinary nature of the study; (ii) importance of receiving a broad-based education; (iii) value of working with graduate students and professors; (iv) importance of team-work including scheduling, timeliness, organization, and communication; (v) how the experience had helped them focus on future study or working areas. Overall, the citizen volunteers were made aware of local environmental problems and were motivated towards improved environmental stewardship.

The three graduate students (C. López, P. Rodríguez and A. Román) that participated in the project are developing their MSc thesis within the overall project scope and were directly involved in training, educating and disseminating project results.

3. Project QC/QA summary

3.1. Selection of sampling sites and sampling completion

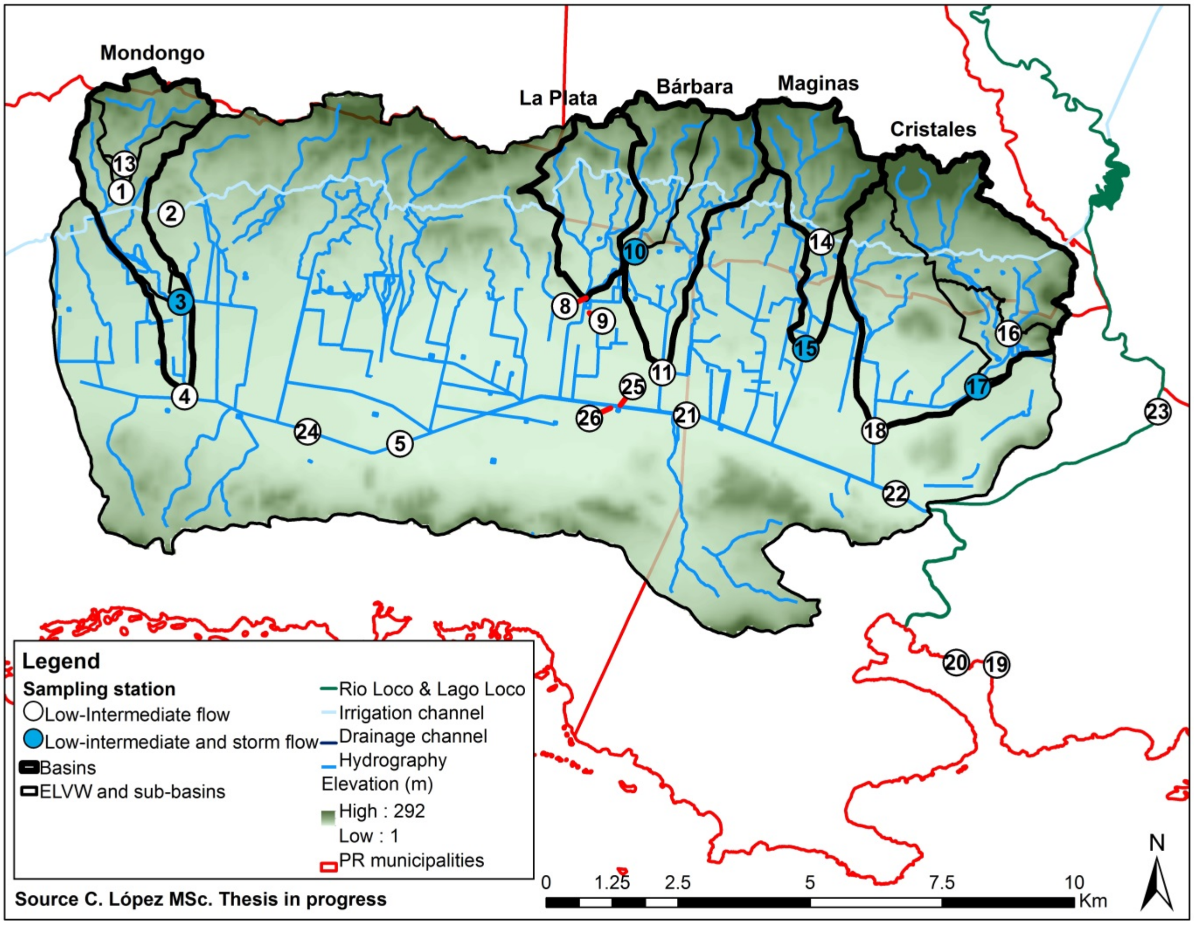
***Low-intermediate flow (grab) samplings*.** An attempt was made to sample all stations within a sample incursion encompassing a period of seven to ten days. Yet, this could not always be done because some stations could not be reached due to physical inaccessibility due to weather, we had to coordinate activities with the volunteers, or the stations did not meet the conditions for sampling.

Twenty-six sampling stations were initially established throughout the Lajas Valley, lower portion of Río Loco watersheds, and Guánica Bay (Figure 3.1, Table 3.1). Stations #6 (Qbda. Jícara, El Salao) and #7 (Qbda. Jícara, Pagan Dairy, not shown in the map) were eliminated as these did not have a consistent stream-flow or the minimum to be measured, and stations #12 (Salinas Creek w. Bayer) was sampled only for exploratory purposes, thus a total of 23 stations were finally selected for grab sampling during low-intermediate hydrologic flows. From August 2014 to June 2015 five sampling incursions were done for a potential sampling total (sampling * station) of 115. This information was used to select twelve stations that were interesting from a water quality perspective, or were within a pre-defined basin or subbasin; these were sampled more intensively. The selected twelve stations were sampled from September 2015 to February 2016 in five sampling incursions for a potential sampling total of 60. Eight additional sampling events were made for exploratory purposes.

Storm-event (static bottles) samplings. Six stations were established for storm-event monitoring. We could not collect a storm-event water-sample from Station #6 Qbda. Jícara, El Salao, and this station was eliminated. Twenty-seven storm event samplings were completed and forty-two samples were collected.

Group-A volunteers sampled eight stations on five occasions each and Group-B volunteers participated in the sampling of the twelve stations selected for additional sampling (Table 3.2).

Figure 3.1. Sampling stations within the Lajas Valley Watershed, including stations within subbasins Mondongo, Bárbara, Magínas and Cristales. Additional stations in Guánica Bay (#19 and #20) and Río Loco watershed outlet are included. Sampling for station #7 (Qbda. Jícara, Pagán Dairy) was discontinued. A description of each station number is detailed in Table 3.1 (Source C. López MSc. Thesis in progress).



Source C. López MSc. Thesis in progress

Table 3.1. Description of sampling stations and associated subbasins.

Station ID	Basin	Station Name	Site description	Sampling Type	Geographic coordinates Latitude	Longitude
1	Mondongo	Lajas WWTP Outfall	Road PR-101, km 2.4 Lajas , east from the Lajas WWTP, upstream the bridge.	Professional (Grab)	18.0465	-67.0609
2		Irrigation Canal at Lajas	Road 116 Lajas, irrigation canal, left from the McDonalds.	Volunteer	18.0427	-67.052
3	Mondongo	Qbda. Mondongo I	Road 116, left from the gas station in Lajas.	Volunteer (Grab) +Professional (Storm)	18.0276	-67.0502
4	Mondongo	Qbda. Mondongo II	Road 116 Lajas, before the outlet into Caño de los Negros.	Professional (Grab)	18.0115	-67.05
5		Drainage Canal I	Road 116 Lajas, El Pulguero entry. At the end, downstream the drainage canal.	Professional (Grab)	18.0031	-67.0115
6		Qbda. Jicara (El Salao)	Intersection of Road PR-327 and Camino Minillas in Sabana Grande	Professional (Grab + Storm); eliminated	18.031	-66.9883
7		Qbda. Jicara (Pagan Dairy)	West end of Road PR-327 Lajas, to the south east of the Pagan Dairy	Pofessional (Grab); elminated	18.0175	-66.9895
8		Qbda. La Plata	Road 117 Lajas, enter Road PR-327 Second entry to the left. Upstream the bridge.	Volunteer (Grab)	18.0278	-66.9781
9		Caribbean Fisheries	Road 117 Lajas, enter Road PR-327 Second entry to the left. Stream that comes from the east.	Volunteer (Grab)	18.0274	-66.9779
10	Bárbara	Qbda. de El Rayo (El Tuque)	Road 117 Lajas, downstream the bridge in Road 2 (Road 2).	Professional (Grab + Storm)	18.0351	-66.9693
11	Bárbara	Qbda. Bárbara	Road 117, enter Road 2 (Road 2) Lajas. Third entry to the right in Francis Lopez Farm.	Professional (Grab + Storm)	18.0155	-66.9647

Station ID	Basin	Station Name	Site description	Sampling Type	Geographic coordinates Latitude	Longitude
12		Salinas Creek; West of Bayer, drainage from south	Road 116 Lajas, north west end of the Bayer Crop land, before the drainage canal	Pofessional (Grab); exploratory	18.0069	-66.9606
13	Mondongo	Upstream Lajas WWTP outfall	Road PR-1101 Lajas, downstream the bridge before intersection with Road Victoria.	Professional (Grab)	18.0511	-67.0601
14	Maginas	Qbda. Maginas I	Road 121 east Sabana Grande, entry though the sliding white gate after the bridge. Station located downstream abandoned bridge.	Professional (Grab)	18.0361	-66.9361
15	Maginas	Qbda. Maginas II	Road 121 east Sabana Grande, Station located in Guánica, inside Domenech private land, upstream the bridge.	Professional (Grab + Storm)	18.0185	-66.9392
16	Cristales	Qbda. Cristales I	Dowstream the bridge from the intersection between road 332 and road 3332, Guánica.	Volunteer (Grab)	18.0199	-66.9023
17	Cristales	Qbda. Cristales II	Road 332 Guánica, inside rice farm, northeast, to the left of the blue AAA water station, underneath the bridge.	Volunteer (Grab) + Professional (Storm)	18.0117	-66.9083
18	Cristales	Qbda. Cristalles III	Road 332 Guánica, southeast of the rice farm, where the drainage canal starts.	Volunteer (Grab)	18.005	-66.9269
19		Guánica town SW Runoff	End of road 332. East of Guánica bay, 100 feet from the shore.	Professional (Grab)	17.9642	-66.9075
20		Guánica WWTP Outfall	Guánica bay to the west, 100 feet from the WWTP discharge tube.	Professional (Grab)	17.9639	-66.9125
21		Drainage Canal II	Road 116 Lajas, northeast end of the Bayer Crop land, downstream the bridge in the drainage canal.	Professional (Grab)	18.0057	-66.9546

					Geographic coordinates	
Station ID	Basin	Station Name	Site description	Sampling Type	Latitude	Longitude
22		LV - Drainage Outlet	Road CII-1 Guánica, entering the fence, to the right from the floating boats.	Student (Grab)	17.9904	-66.9178
23		USGS station, Las Latas	Road 116-R Guánica , right into road 389 the left to Calle 1. South of the basketball courtyard.	Professional (Grab)	18.0069	-66.8761
24		Drainage Canal, downstream Artau	Road 116 to the east in Lajas, next entry to the left from road 305. At the end, upstream the bridge in the drainage canal	Professional (Grab)	18.0053	-67.0279
25		LV - Drainage Channel downstream of #26	Road 116, Cuesta Blanca Community, Lajas. At the end of Camino Juan Rivera, downstream the bridge in drainage canal	Professional (Grab)	18.0091	-66.9728
26		Outlet from Cuesta Blanca Community outlet to LV	Road 116, Cuesta Blanca Community, Lajas. At the end of Camino Juan Rivera, cement pipe southwest upstream the bridge in drainage canal	Professional (Grab)	18.0091	-66.9729

Table 3.2. Dates and time periods of sample incursions for water samples collected during low-intermediate flows (grab sampling).

Sampling incursion	Dates	Time period (days)
I	5 Aug to 3 Sep 2014	29
II	22 Sep to 22 Oct 2014	32
III	9 Jan to 24 Feb 2015	47
IV	6 to 24 Apr 2015	19
V	27 May to 5 Jun 2015	10
VI	15 to 19 Sep 2015	5
VII	29 Sep to 3 Oct 2015	5
VIII	13 to 17 Oct 2015	5
IX	27 to 31 Oct 2016	5
X	2 to 4 Feb 2016	3

3.2. Analysis completeness

A description of the number of grab samples collected during low-intermediate flows and analyzed during each sampling incursion is summarized in Table 3.3. Based on the 23 stations sampled during the synoptic survey and the 12 stations sampled more intensively thereafter, the goal was to have 175 data points for some of the parameters, excluding blanks and duplicates. The completeness rate was greater than 95% for water temperature, specific conductance, dissolved oxygen, nutrients (total N, nitrate-N, total P), suspended sediments, enterococci. The completeness rate was exceeded for optical brighteners (OBs), and bacteroidales. The completeness rate was below the target for elemental analysis of metals with a 63% completion rate.

The number of storm samples collected during storm-flow and analyzed for each parameter is summarized in (Table 3.4). The goal was to have 25 data points for nutrients, TSS and enterococci concentrations, as well as turbidity, from five passive rising-flow stream collecting stations (5 stations x 5 sampling events = 25 samples). A total of 42 stream storm-flow samples were collected, as in some sampling events more than one bottle may have been collected. The completeness rate was greater than 100% for all of the parameters.

Table 3.3. Percentage completion for each parameter for water samples collected during low-intermediate flows.

	Parameter [1]												
	Temp	SPC	DO	Flow	NO_3-N	TKN	Total P	OB	SS	Metals	Entero-cocci	Human bacter-oidales	Cattle bacter-oidales
Potential # samples	175	175	175	175	175	175	175	56	175	56	175	56	56
Completeness%	99.4	97.7	99.4	88.5	97.1	95.9	98.8	264.3	99.4	62.5	100.0	101.8	101.8

1 Temp is water temperature; SPC is specific conductance; DO is dissolved oxygen; TKN is total Kjeldhal nitrogen; total P is total phosphorus; SS is suspended solids; OB is optical brighteners.

Table 3.4. Percentage completion for each parameter for water samples collected during storm events.

	Parameter [1]						
Sampling Incursion	NO_3-N	TKN	Total P	OB	SS	Turbidity	Enterococci
# events	27	27	27	27	27	27	27
# samples	42	42	42	42	42	42	43
% completeness*	108	108	108	108	108	108	108

*completeness based on # of events sampled.

3.3. Sensitivity

Detection limits for all parameters were included in QAPP. The % of samples below method detection limits was 6.5, 10.4 and 3.9% for TKN, TN and total P. Enterococci concentration quantification using the Enterolert® system is based on the most probable number (MPN) method. The maximum MPN of a sample without dilution is 2,420 CFU/100 mL. The usual procedure was to run the sample based on a 1:2 dilution. But, when the results were expected to be higher than normal, samples were run based on 1:10 dilution. The % of samples that exceeded the method MPN maximum limit for each dilution was 17.0%. The % of samples that were below method detection limits was 2.7%. Trip blanks (distilled water) were 0 MPN/100 mL in all instances. All of the samples were analyzed within specified holding times.

3.4. Comparability

A comparability test for TKN and total P was run between UPRM-AES and USEPA Edison Laboratory. The inter-laboratory comparison for total P and nitrate- plus nitrite-N passed. The inter-laboratory comparison for TKN did not pass, possibly because of the small number of samples included in the analysis and many of the samples were near detection limits.

3.5. Precision

Triplicate analysis for TKN, total P, nitrate N, suspended sediments showed relative standard deviation (RSD) values of less than 20% (which is the limit specified in QAPP). Three triplicates were run for TKN and one triplicate was run for all other parameters. All three OB triplicates had a negative signal. Precision (RSD) for enterococci was calculated at 25% (29 April 2014) and 39% (6 May 2016).

4. Land-use/Land-cover analysis and sanitation survey

4.1. General description of the study area

The study area was the eastern Lajas Valley watershed, lower portion of Río Loco watershed, and Guánica Bay. The eastern Lajas Valley watershed (ELVW) (HUC-12) is a large plain with slopes ranging from 0 to 4% in the lower elevations that extends about 32 km from Río Loco in the east to Bahía de Boquerón in the west. It includes parts of the municipalities of Yauco, Guánica, Sábana Grande, Cabo Rojo, and Lajas. The width of the valley ranges from 1.6 to 4.8 km and is bounded to the north and south by a chain of hills of maximum altitude of 250 m. The land area of the ELVW, east of State Road 116 that drains towards Guánica Bay has a catchment area of 14,519 ha. The Río Loco watershed included three subbasins; the Río Loco at mouth, the Río Loco at Lajas Drainage channel outlet, and the coastal watershed east of Río Loco mouth for a total combined area of 5,608 ha. Further subdivisions of HUC-12 were done to segregate smaller subbasins and to select the sampling stations. Basins delineated five main streams of the ELVW: Mondongo, La Plata, Barbara, Maginas and Cristales.

The precipitation pattern in the study area has traditionally been a bi-modal distribution with rainfall predominating during the months of April to May and from late August to middle December. Annual precipitation ranges from 89 to 107 cm in the Lajas Valley to over 200 cm in the upper Río Loco watershed.

4.2. Land use / land cover assessment

Georeferenced land cover data by PR GAP project (2006) and Gould et. al. (2008) was obtained from the Puerto Rico Governmental Portal for geographic data (gis.pr.gov)[2] and used to establish the initial land cover map of the Lajas Valley (Figure 4.1) and of lower portion of the Río Loco watershed (Figure 4.2). The resolution of the PR GAP land cover data was 30mx30m. The PR GAP project has ten land-use/land-cover categories: (i) Artificial barren, (ii) Forest, (iii) Grasslands and pastures, (iv) Grazed pastures, (v) Hay, (vi) High-density urban, (vii) Low-density urban, (viii) Pond, (ix) Row crops, (x) Woods and shrubs Gould et al. (2004).

Ground-truthing excursions were made to validate the land-cover data of the Lajas Valley by PR GAP and translated into a land use category. Land use classification of validated areas was edited or confirmed in the attribute table of the PR GAP shapefile in ArcMap 10.0 (ESRI Inc, 2010). Land use classification corresponded to four main categories: (i) urban development, (ii) unmanaged shrubland/forest, (iii) agriculture, and (iv) pond (Figure 4.3 and Figure 4.4). These categories were determined based on potential contribution to stream water quality degradation. Urban development includes both low- and high-density urban classes described by PR GAP. Similarly, unmanaged shrubland/forest refers to all areas described as forest, woods and shrubs, pastures and grasslands by PR GAP. Within agriculture land use class three main agricultural subclasses were identified: (i) hay and silage, (ii) row crops, and (iii) grazed pastures, which differ substantially in terms of management and nutrient

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[2] Portal Datos Geográficos Gubernamentales. Available at: http://www2.pr.gov/agencias/gis/Pages/default.aspx.

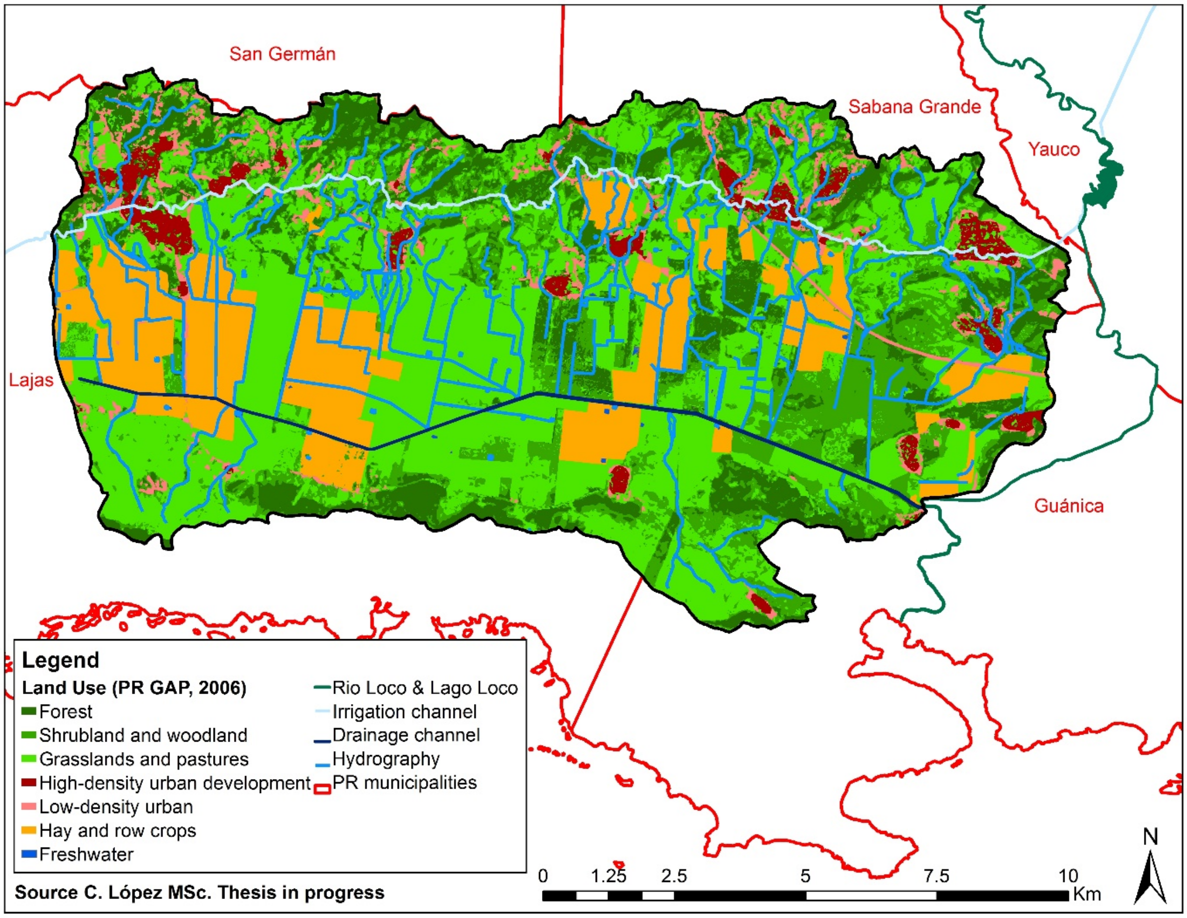
inputs and potential losses. Pond land-use class refers to clean water storage ponds in farms and water treatment plants, as well as aquaculture ponds and waste-water retention ponds in cattle farms.

Each of the three agricultural subclasses is hypothesized to contribute to stream quality degradation in different nature and magnitude. For example hay and silage (or haylage) production in the Lajas Valley is done using Pajón (*Dichancium annulatum)*, Huracán (*Bothriocloa pertusa*), Para *Panicum purpurascens*), and Carib (Malojillo, *Eriochloa polystachia*), and minor extents of Pangola (*Digitaria decumbens*), Pennisetum and Brachiarias. Based on previous research conducted (Sotomayor and Pérez-Alegría 2012) and informal follow-up visits we are aware of only one farmer in a 100 acre area that actively fertilizes with near 150 lb N/acre as urea. As a result, overall relative hay yields are near 60% of maximum with only two or three harvests per year. Other farmers may fertilize with 300 to 400 lb fertilizer/acre only on a sporadic basis (adding up to 60 lb N/acre and 20 lb P_2O_5/acre). The soils are generally deficient in P, thus based on this information, this hay and silage land-use subclass should contribute negligible amount of N and P in runoff.

Grazed pastures in the Lajas Valley are usually reserved for beef- or dairy-cattle. Grazed forage for beef-cattle is traditionally not fertilized, because the perception is that fertilizer cost to meat farm-gate price, makes it uneconomical. Thus, animals depend primarily on the consumption of forage-grass and the recycling of nutrients from animal-manure-forage to satisfy dietary nutrient requirements. As occurs in soils dedicated to haylage, the soils are generally deficient in P, thus this agricultural practice should contribute negligible amount of N and P in runoff. This area could contribute substantial levels of fecal indicators of contamination to runoff, in relation to the animal density in specific areas and if the areas are in proximity to drainage channels.

In contrast, grazed forage areas for dairy production, may contribute increasing amounts (relative to areas under beef-cattle grazing) of N and P to runoff. The areas are usually not fertilized, but animals are fed at least one-half of their daily caloric intake in the form of concentrate feed. Feeding occurs near milking parlors and thus manure is generally enriched in N and P, which is deposited to soils. Soil test P levels and soil N (in the form of nitrate and mineralizable-N) can be relatively high in specific areas where these animals predominate during a large part of the day. We identified four dairy production facilities in the study area of which one was inoperative during the study period (this facility did not have any animals in the milking parlor and grazing paddocks). Combined, these four dairy farms covered close to 1,165 acres of land, of which roughly 3 acres were occupied by active milking parlors, and hold close to 500 productive cows in various life stages each year. We estimate that near 98% of the land area classified as active dairy are grazed and of which about 20% of the area can have soil test P levels that could contribute significant amount of P to runoff (Sotomayor-Ramírez and Pérez-Alegría, 2011). The area under dairy-cattle grazing could contribute substantial levels of fecal indicators of contamination to runoff, in relation to the areas where dairy manure sludge is applied, animal density in specific areas and if the areas are in proximity to drainage channels.

Figure 4.1. Land use map of the Lajas Valley, based on PR GAP (2006).



Source C. López MSc. Thesis in progress

Figure 4.2. Land-use map of lower portion of the Río Loco watershed as described by PR GAP (2006).

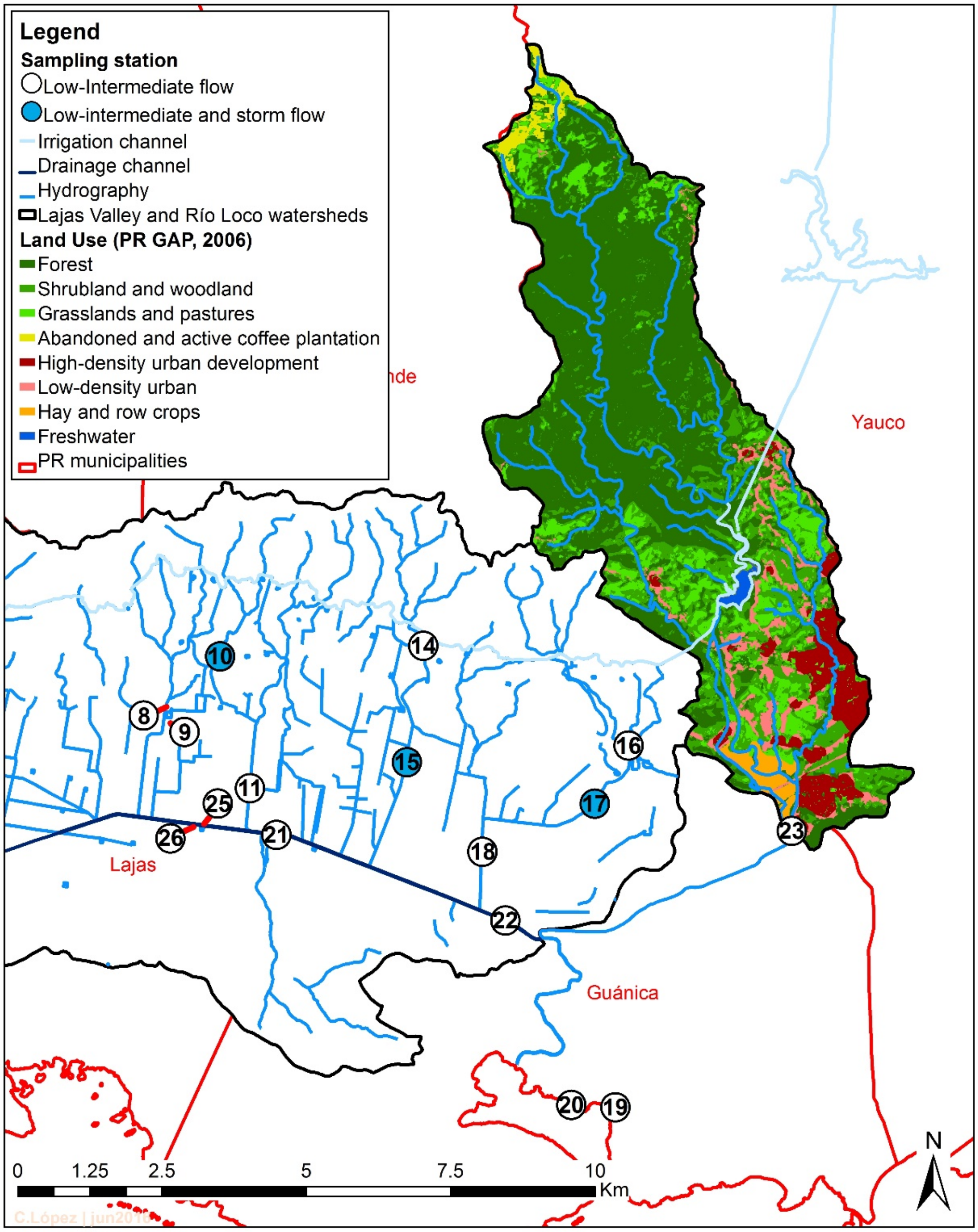
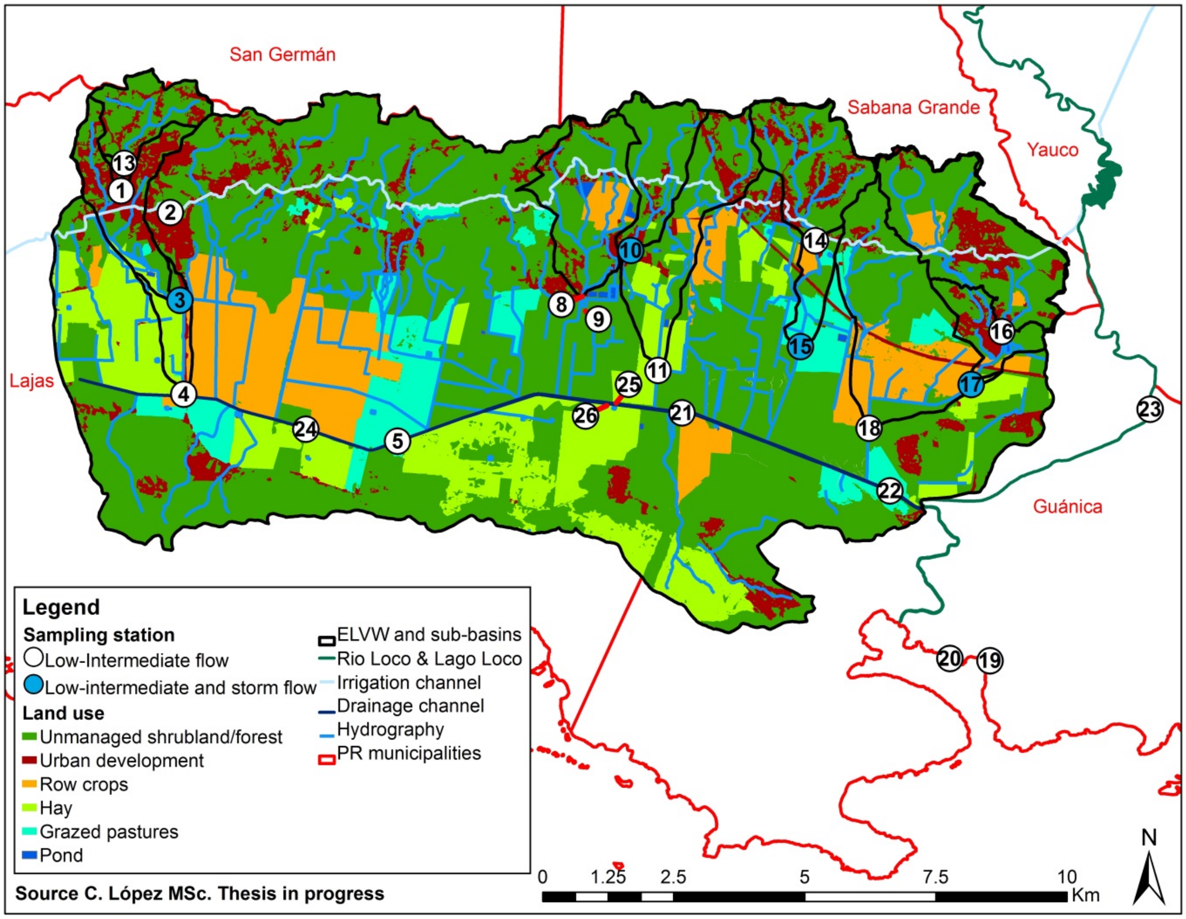
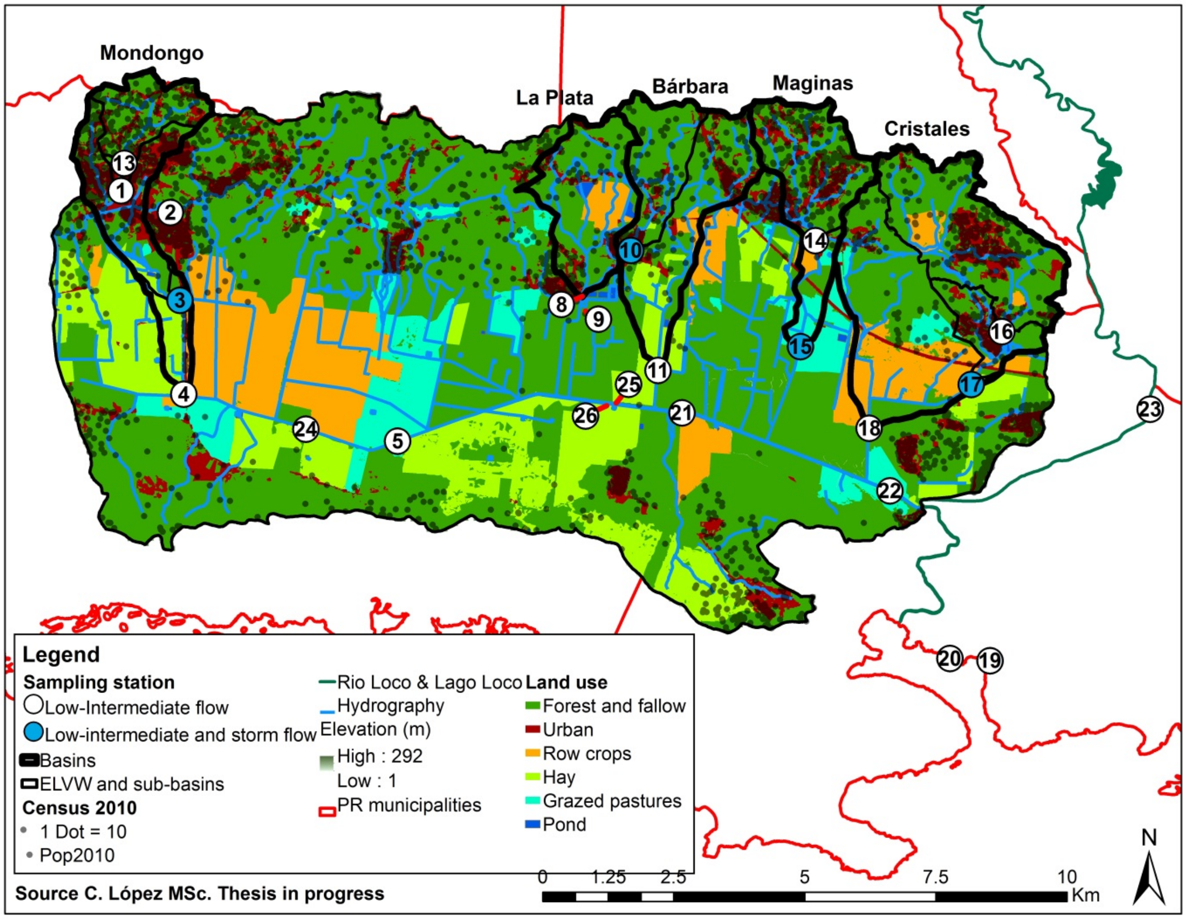


Figure 4.3. Revised land use map of the Lajas Valley, based on PR GAP (2006) and ground-truthing done in this project. The sampling stations are included for reference purposes.



Source C. López MSc. Thesis in progress

Figure 4.4. Land use map of the Lajas Valley including urban density, based on PR GAP (2006), and ground-truthing by the research group. There are an estimated 3,393 points, each of which represents 10 person/point.



Source C. López MSc. Thesis in progress

Row crop production in agronomic crops in the study area is limited primarily to rice, and minor extents of cotton and soybean for seed production. Usually only one cropping is done per year and the fields remain under bare-fallow in the crop production off-season. Although the initial intuition is to think that these areas can contribute high concentrations of N, P and sediment in runoff, this may not necessarily occur. Fertilizer of N, P and potassium to rice is done following recommendations by UPRM and are within nutrient rate recommendations published elsewhere. Further, the land area has minimum slopes so that excess precipitation is alleviated from fields as percolation, which may not contribute high levels of N and P due to the sorption, precipitation and/or microbial immobilization in the soil profile.

Row crop production in horticultural crops occurs for bananas, plantains, pineapple, citrus, tomato, pepper, and eggplant. These crops are intensively managed and fertilized. Fertilization levels are considered relatively high (as compared to other areas in the Lajas

Valley) but within ranges suggested by UPRM and as done in other similar areas. The soil-slope of these cropped areas is usually higher than other crop lands, the soils are shallower, thus these areas could contribute substantial amounts of N, P and sediment to runoff.

Within specific land uses we identified areas having sewage and non-sewage infrastructure (Areas) and potential agricultural and human sources of contamination. First, we interviewed PR Aqueduct and Sewer Authority Chief Engineer for Western Puerto Rico, Joel Lugo and Eng. Walmer Martínez (PRASA Compliance Officer). They described the sewer infrastructure in the area of interest which consisted of estimated population that were connected to the sewer system, geographic areas in which homesteads and buildings were connected, and geographic areas that had gravity and pump-based pipes. Further, they shared a GIS-based map which delineated gravity and pump-based pipes that conducted sewage to the Lajas Waste Water Treatment Plant (WWTP with NPDES PR0020575). The WWTP receives raw sewage from areas or *barrios* in Boquerón, Puerto Real, Lajas urban center (Mondongo basin) and La Parguera. The geographic areas that could potentially have sewer infrastructure were those within the barrios mentioned and which were in close proximity to the pipes within and between these areas.

In urban and suburban areas, potential point-source inputs were waste-water treatment plant discharge points, drinking water discharge points, and open pipe conduits. Potential non-point sources were urban and suburban animals (poultry, wildlife, dogs, cats), and homes/buildings with faulty septic tanks. In rural areas point sources were open pipe conduits, and non-point sources were faulty septic tanks, animal feeding operations, large animal production facilities, and grazing animal areas.

4.3. Climatic data

Historical precipitation (1981-2010) and daily precipitation data for 2014 and 2015 were gathered from NOAA-NCDC (http://www.ncdc.noaa.gov/cdo-web) for Lajas UPR-Agricultural Experiment Station (18.0328°N, 67.0725°W) and Santa Rita, Guánica 18.0097°N, 66.8847°W) (NOAA-NWS, 2016) (Figure 4.5). Daily precipitation data for Arenas, Guánica (18.0223°N, 66.9339°W) was collected from USGS (http://waterdata.usgs.gov/nwis/inventory/?site_no=180122066560300&agency_cd=USGS).

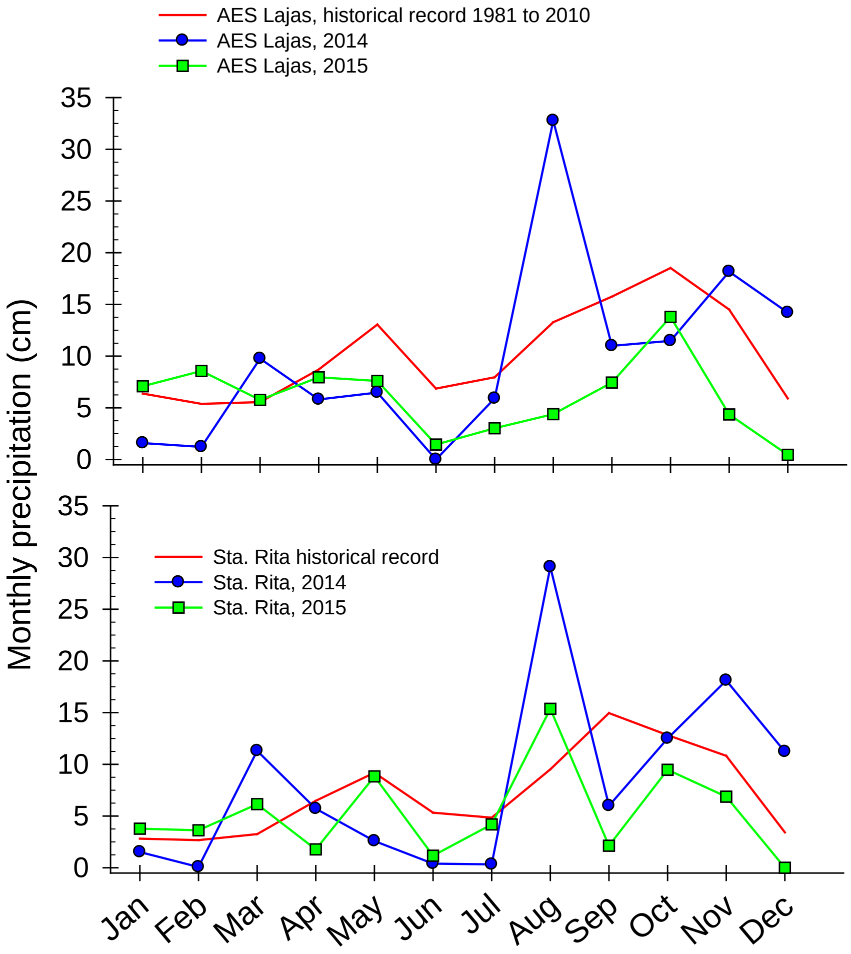
Historical mean annual precipitation for Lajas UPR-AES is 122 cm (48 inches) and for Santa Rita, Guánica is 86 cm (34 in) (Figure 4.5). Historical monthly precipitation peaks occur from September to November, where 40 to 44% of the total annual precipitation occurs in these three months. A second precipitation peak occurs in April to May where about 18% of the total annual precipitation occurs.

The mean annual precipitation difference relative to the historical average in Santa Rita for 2014 and 2015 was +12.7 cm and -22.8 cm, respectively; in Lajas AES for the same years it was –3.5 cm and -50 cm lower. Thus in the Lajas Valley, 2015 was a drier year than in 2014, and than the historical average. In Lajas AES and Santa Rita, the monthly patterns of 2015 were similar to the historical average albeit at lower magnitude in all months, except January and

February in Lajas AES and January to March in Santa Rita. Precipitation in Santa Rita was higher in August 2014 and 2015 than historical mean. In Santa Rita 2014, precipitation was greater than the historical average for the months of March, August, November and December.

During the stream sampling period of August 2014 to February 2016, greatest rainfall events occurred within the months of August and November 2014 and October 2015 at Lajas AES. Santa Rita followed this tendency with an additional precipitation event in early August 2015. August 2014 was exceptionally high precipitation at Lajas AES; precipitation was +19 and +11 cm greater than the historical average in Lajas AES and Santa Rita, respectively.

Figure 4.5. Historical monthly precipitation for Lajas Agricultural Experiment Station (AES) and Santa Rita Guánica, and monthly precipitation for Lajas AES during 2014 and 2015.



4.4. Land use

We re-classified the existing land-use/land-cover information (PR GAP 2006) of ELVW based on ground truthing excursions, but not from the Río Loco watershed (Figures 4.1 through 4.4). Unmanaged shrubland/forest-land was the most dominant land use class within ELVW, followed by agriculture and urban land use classes; with 61, 30 and 9% of total land area, respectively (Table 4.1). Within agriculture, hay and silage, row crops, and grazed pastures covered 13, 11 and 6% of total land area, respectively.

Contrasts among basins. Within Mondongo basin, the dominant land use class was agriculture, followed by unmanaged shrubland/forest and urban development covering 61, 30 and 9% of land area, respectively (Table 4.1). Within the agriculture class, hay and silage was the most dominant subclass, followed by row crops and grazed pastures, covering 31, 19 and 0.3% of total basin area, respectively. Row crops within this basin were mainly rice for seed production from a private seed-production company. Of the 727 acres under contract to the private seed company, approximately 225 acres are under rice production and the rest is fallow under a rice-fallow rotation of alternate years (J. Suárez, Personal Communication, 2014). Mondongo basin had the largest urban area and highest population and population density compared to the other basins with a carrying capacity of 1.9 persons/acre. Within Mondongo basin is the downtown Lajas area where most businesses are established, so that the area receives many daily transient visitors. Compared to other basins, Mondongo also had most land area and land proportion in hay and silage, while least land proportion in unmanaged shrubland/forest.

In Bárbara basin, unmanaged shrubland/forest was the dominant land use class followed by agriculture and urban, covering 65, 19 and 15% of total land area, respectively. Dominant agriculture subclasses were hay and silage followed by row crops, covering 13 and 6% of total basin land area, respectively. Grazing animals within Bárbara were scarce and limited to small amounts of free-ranging equine and cattle animals in woodlands and agricultural areas in fallow; these areas remained with an unmanaged shrubland/forest classification. Most agricultural activity was observed in the southern reaches of the Bárbara basin, with a few structures for animals rearing (i.e. fowl) observed among sub-urban households in the northern and mid-reaches of the basin and along Bárbara stream. An illicit animal feeding operation was identified within this basin, where pig rearing was occurring in which the structures were only 50 meters away from Bárbara stream.

Table 4.1. Land use and population within the eastern Lajas Valley watershed and basins (Source C. López MSc. Thesis in progress).

	Basins					
	ELVW [3]	Mondongo	Bárbara	Maginas	Cristales	La Plata
Area (acres)	36,229	3,812	3,786	2,090	4,568	2,822
Population [1]	33,936	7,297	3,403	3,818	5,190	2,759
Number of open conduits [2]	157	62	7	0	72	15
	-----------------------% of the total area within each basin----------------					
Urban development	9.34	21.08	15.65	30.17	12.85	11.48
Agriculture	29.90	50.24	19.34	21.81	27.75	7.16
• Row crops	10.93	19.08	6.08	2.49	19.92	5.92
• Hay	12.65	30.85	13.06	0.00	3.48	0.00
• Grazed pastures	6.33	0.30	0.20	19.32	4.35	1.24
Unmanaged shrubland/Forest	60.92	28.62	64.87	47.92	59.31	80.29
Pond	0.19	0.07	0.14	0.10	0.09	1.07

1 USDA-Census (2010)
2 Open conduits as documented in ground truthing excursions
3 ELVW is the eastern Lajas Valley watershed.

In Maginas, unmanaged shrubland/forest and urban development were the dominant land use classes, followed by agriculture, covering 48, 30 and 22% of total basin land area, respectively. Maginas ranked third in amount of population within the basin, yet had the largest proportion of land area in urban development with a carrying capacity of 1.8 persons/acre. The dominant agriculture subclass was grazed pastures followed by row crops. The area in grazed pastures was mostly due to a privately-owned cattle farm who followed best management practices (i.e. rotational grazing, decreased fertilization of pastures). This made Maginas the basin with greatest proportion of land area dedicated to animal grazing. Also, goat and horses were observed in the low-density urban areas of the upper stream reaches. Row crop agriculture in this area was primarily the horticultural crops of banana, plantains, pineapple and vegetables and these were intensively managed.

Within Cristales basin, unmanaged shrubland/forest and agriculture were the dominant land use classes, followed by urban development, covering 59, 28 and 13% of basin land area, respectively. Predominant agricultural subclass was row crops, due to a 600-acre state-owned rice-production farm. Grazing land areas were limited to small amounts of equine, cattle and fowl animals grazing freely in woodlands and agricultural land in fallow. Cristales basin was the largest of all basins yet had 29% less population than Mondongo and 26% more population than Maginas; the latter are the two basins with greatest proportion of land area in urban development (Source C. López MSc. Thesis in progress).

In La Plata basin, 80% of total land area was unmanaged shrubland/forest and 8% in agriculture, the latter mostly as row crops and grazed pastures. Hay and silage production was not observed in this basin. Row crops were predominantly *Musa sp.* (banana and plantain). Small scale breeding of goat, horses, and cows was observed throughout the basin. In fact, a group of goats would often be observed grazing in the vicinity of La Plata stream (near sampling station #8). Large ponds, covering close to 12 ha within La Plata basin and belonging to UPRM-AES, were dedicated to aquaculture in previous decades and now serve as storage ponds. This caused La Plata to be the basin with most land area in pond land-use class. La Plata basin also had the least population compared to all other basins within the ELVW, and had the least proportion of urban land (11%).

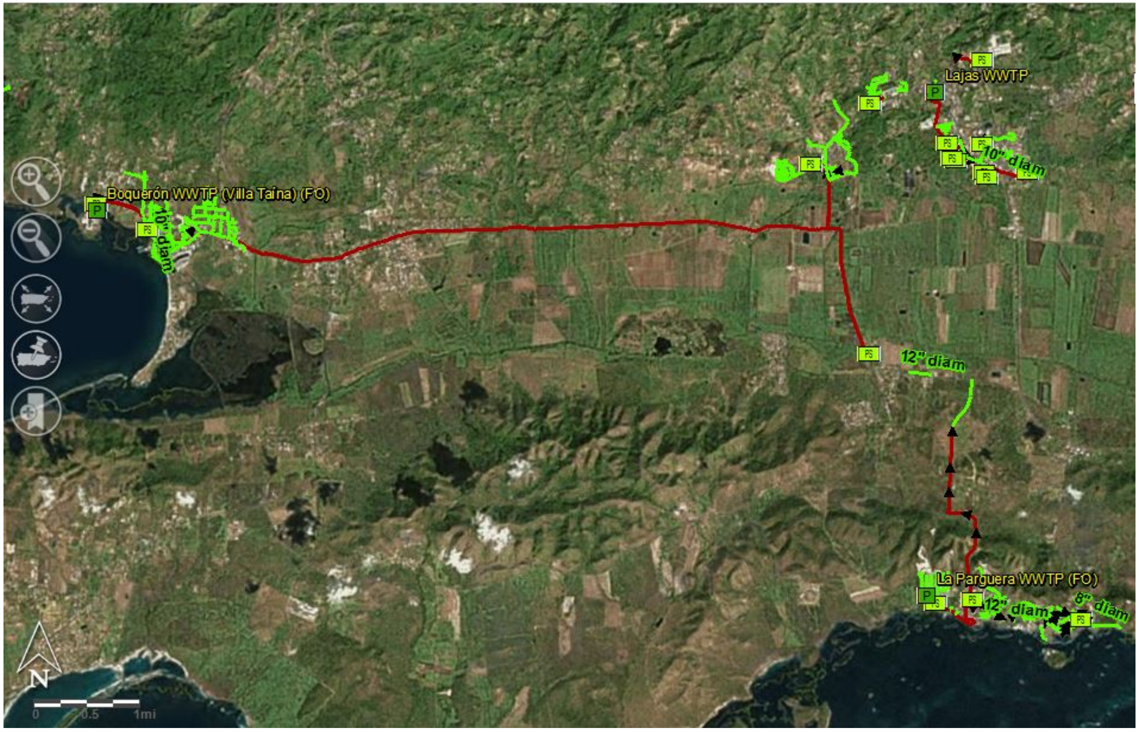
4.5. Sanitation survey

Point sources of contamination to streams in the eastern Lajas Valley watershed were identified and documented through ground truthing excursions in which pictures and GPS coordinates were taken. Most sources were open pipe outfalls with either passive or active discharges of domestic water directly into streams or drainage ditches. Passive discharges were pipes that did not have running water. In the case of active discharges, in most instances we could not distinguish raw sewage from bathrooms and septic tanks and grey water from laundry wash or kitchen sink wash. Small-scale artisanal rearing of goat, horses, cows, pigs, and fowl (i.e. chicken, turkey, guinea-hen) were observed as scattered small areas in low-density urban areas of the watershed. Goat, horses and fowl generally grazed freely in areas surrounding houses, shrublands and agricultural land in fallow; pigs were mostly confined in areas or sheds. Compared to other basins in the ELVW, Cristales and Mondongo had the highest number of domestic-water open-pipe outfalls, with a total of 72 and 62 documented domestic-water open-pipes, respectively (Table 4.1).

A wastewater treatment plant (NPDES PR0020575) and a public water supply plant (NPDES PR0022985) have been documented to discharge into Mondongo stream, while a public water supply plant (NPDES PR0026727) discharges effluents into Bárbara stream. Within Mondongo basin, the Lajas secondary wastewater treatment plant (WWTP) (NPDES Permit No. PR0020575) effluent contributes (three-year, 2013 to 2015, mean) 1.41 x 10^6 m^3/yr (or 0.7 million gallons per day, MGD) of secondary/tertiary wastewater contributing 1,500 kg of P, annually. Annual nitrate N effluent contribution was 1,135 kg NO_3-N. This plant serves between 1,736 to 2,000 households or between 6,944 to 8,000 people from downtown Lajas, Parguera and Boqueron sectors. Within Mondongo basin, only parts of downtown Lajas were connected to sewage systems; the rest of the basin did not have sewage connection (Figure 4.6). We estimate a total population of 4,000 in Mondongo basin is connected to the WWTP. Compared to other basins in the ELVW, Mondongo had the second highest amount of domestic-water open-pipe outfalls with a total of 62 documented domestic-water open-pipes. Many of these pipes were observed in the northern and southern reaches of Mondongo stream, along households in sub-urban communities in Barrios La Haya and El Tendal.

Within La Plata basin, none of the urban areas were connected to the WWTP, and 15 domestic water outfalls were documented in the upper reaches. Within Bárbara basin, none of the urban areas were connected to the WWTP, and 7 domestic water outfalls were documented in the upper reaches. The Maginas public water supply plant (NPDES 0026727) annually contributes close to 38,910 m^3/yr of discharge to Bárbara stream. Within Maginas, none of the urban areas were connected to a WWTP, and 17 domestic water outfalls were documented in the upper reaches. Within Cristales, the urban area of Barrio Susúa, Sabana Grande was connected to the sewage system but is pumped towards the WWTP in Yauco, out of the scope of this study. Cristales was the basin with most domestic outfalls documented through ground trothing excursions. A total of 72 open pipes of domestic water were documented throughout the upper reaches of Cristales basin, in Barrios Susúa, Sabana Grande and Susúa Baja, Guánica (Source C. López MSc. Thesis in progress).

Figure 4.6. Sewage connections to Lajas WWTP. Green lines indicate pipes fed by gravity while red lines indicate pumping pipes, and both transfer raw sewage to the Lajas waste-water treatment plant. Thus, only the geographical areas with tubes have the potential to be connected to the plant. PS refers to pumping stations (Source: *gis.pr.gov*).



5. Fecal indicators of contamination in the Lajas Valley Watershed

5.1. Materials and methods

A description of the study area, land-use/land-cover analysis and climatic information was done in Section 4.

Sampling. Twenty-three sampling stations were established within the Lajas Valley Watershed, lower portion of Río Loco Watershed, and Guánica Bay. The stations were selected based on a four-tiered targeted approach that included ease of access, land-use/land-cover information, sub-basin area, and potential sources of contamination. Some of the sites included areas up-and down-stream (representing a range of land use qualities) of potential problem areas such as waste-water treatment plants and animal feeding operations. Consideration was given to whether a particular land use activity or potential source of pollution was having an impact. An additional eight samples were collected from targeted areas, based on suspected sources of contamination.

Samples were collected from August 2014 to June 2015. Eleven stations that were established at the drainage outlet of pre-defined basins, and the reference station (#2) were selected for further sampling during 2015 to February 2016. Grab samples were collected during low- to medium-hydrologic flow regimes and storm events were collected during high-hydrologic flow regimes in selected stations. In some instances, there was a large time span during the sampling round. Some sites had to be re-visited because they did not have flowing water, or the sampling area could not be entered.

Each stream water grab sample was identified as corresponding to three flow regime classifications (base-flow, intermediate-flow, and high-flow) but were grouped into two classifications by combining intermediate- and high-flow data. Measured *in situ* stream discharge was compared with class discharge threshold values. Eleven years of stream discharge data was obtained for Río Guayanilla in Yauco, which was the closest gauged stream to the Lajas Valley. A c/h recession curve and an adjusted Riggs (1962) procedure were applied to Río Guayanilla stream discharge data to determine discharge threshold values at baseflow and for the 7-day, 2 year recurrence event (7Q2) which was classified as the maximum flow for intermediate-flow. Cross multiplication allowed estimation of threshold values for Lajas Valley stations.

Water sample collection. Surface water samples for microbial analysis were collected during low- to intermediate-hydrologic flows by a combination of volunteer monitors and professionals according to US EPA-approved procedures (USEPA, 1997; Sotomayor-Ramírez et al. 2011). Water samples were collected manually (grab) with previously sterilized 1-L polypropylene bottles. Bottles were submerged 10 cm beneath the water surface using aseptic techniques. Bottles were capped, sealed, placed on ice in a closed cooler, and transported to the laboratory for processing within 6 hours of collection. Additional water samples were collected for nutrient (total nitrogen, total phosphorus, and dissolved nitrate, metals, and other parameters and these were processed and analyzed as described in Section 6 of this report.

Water samples for storm events were collected using a passive rising-flow stream collectors (Gordon et al. 1992; Franklin et al. 2003). The sampler consists of a series of sterile 1-L Nalgene HDPE bottles (Nalgene Stormwater Samplers) placed at different stream-stage heights, and placed within a protective mounting kit. The bottles are filled during a storm event as the stream stage increases. After collecting a full liter of sample, the sampling mechanism closes to prevent cross-contamination with later water. Each bottle-kit combination at selected heights from the stream bottom corresponding to those resulting from storm events recurrence; based on relationships between stream height and stream-flow developed for each site as described by Sotomayor-Ramírez and Pérez-Alegría (2012). The collectors have been used successfully in the area by Perez-Alegría (unpublished data). Within 24 hours after a rainfall event, the collectors were inspected and samples collected. Samples were split into sub-samples for chemical and microbial analysis and transported to an analytical laboratory for analysis of chemical and microbial parameters as described previously.

Water sample analysis. Stream-water physico-chemical characteristics were measured *in situ* during manual sampling and included pH, temperature, specific conductance, and dissolved oxygen. Measurements were taken at mid-channel (at a depth of 15 cm from the water surface), with Multi-Probe System (YSI Inc., Ohio, USA). Prior to sampling, instrument settings were corroborated using buffers and standard solutions and calibrated as needed. Water velocity was measured with a Flow Probe Hand-held flowmeter (Global Water, College Station TX, USA) at each of the selected stream cross-sections and converted to hydrologic flow using depth x stream-width transects.

Enterococci as indicator of fecal indicators of contamination were enumerated using the Enterolert™ system (IDEXX Laboratories) (Kuntz et al. 2003; Sotomayor-Ramírez et al. 2006). When samples were suspected to have high enterococci concentrations, samples were diluted in sterile manufacturer-supplied 100-mL polystyrene bottles and mixed with manufacturer-supplied growth medium until dissolved. The contents of each bottle were poured into a sterile Quanti-Tray® panel containing 97 wells and heat-sealed. Quanti-Tray® panels for fecal enterococci enumeration were incubated at 41 ± 0.5^{o}C. The presence of fecal enterococci was determined by detection of UV fluorescence at 365 nm. The number of positive wells was converted to a most probable number (MPN) value based on the dilution factor and manufacturer supplied MPN tables.

The presence of *Bacteroidales* human specific marker HF183 was determined using quantitative polymerase chain reaction (PCR)-based analysis (Haugland et al. 2010), using modified methods (Rozier et al 2015). Duplicate water samples (100 mL) were passed through sterile, 0.22-µm-pore nitrocellulose membrane filters, the filters stored in sterile Whirl-Pak bags at -20^{o}C, and shipped on dry ice by overnight courier to Georgia College and State University (Milledgeville, GA). Filters were processed with a MoBio Ultraclean^{TM} Soil DNA Kit using a modification of the “Alternative Protocol” given by the manufacturer (Amador et al. 2008; Bachoon et al 2010). Extracted DNA was quantified using a Nanodrop ND-1000 spectrophotometer and visually inspected under UV light for integrity on a 2% agarose gel stained with ethidium bromide.

Microbial source tracking of fecal pollution, was performed by qPCR assays on a CFX 9600 (Bio RAD). The BacCow gene and HF183 gene qPCR assay used a modified protocol of Haugland *et al.* (2010). For HF183 gene the *Bacteroides dorei* DSM 17855 (DSMZ) was used as a positive control and *Escherichia coli* strain B from Sigma ® D48890-1UN as a negative control. The assay contained 1µM of each primer, 0.2 mg of cattle serum albumin (Sigma), 80 nM Fam^{TM} labeled Taqman^{R} probe, 9 µl of deionized water, and 1 µl of sample DNA. The samples were run at: 95°C for 15 min; 40 cycles at 95°C for 10s and 66.3°C for 40s (Haugland et al. 2010). The BacCow gene qPCR assay used DNA extracted from cow fecal samples as a positive control and DNA extracted from wild pig, fecal samples as a negative control. The assay contained 1µM of each primer, 0.2 mg of cattle serum albumin (Sigma), 80 nM Fam^{TM} labeled Taqman^{R} probe, 9 µl of deionized water, and 1 µl of sample DNA. The samples were run at: 95°C for 15 min; 40 cycles at 95°C for 10s and 58.0°C for 40s.

Water samples were analyzed for fluorescence from optical brighteners (OB) using a Turner Designs Model 10-AU-005 field fluorometer (Turner Designs, Sunnyvale, CA) fitted with filters for excitation (360 nm) and emission (436 nm) (Hartel et al. 2007a; 2007b). The fluorometer was calibrated prior to each analysis with a solution of known concentration (0.01 mg/L Ace Brisa Limpia Detergent for clothes). Three aliquots were taken from each water sample and allowed to reach room temperature (~75F). Each aliquot was irradiated under UV light for 0, 5 and 10 minutes, respectively. Fluorescence of each aliquot was measured with fluorometer and plotted. OB signal was determined positive when initial fluorescence was greater than 20 RFU and percent of reduction in RFU after 5 minutes under UV was greater than 30%. If the latter was between 6 and 30%, the ratio of percent reduction in fluorescence after 10 and 5 minutes of UV radiation was calculated and determined positive if ratio was less than 1.50.

Samples were analyzed for total Kjeldahl nitrogen (TKN) (EPA method 351.2), nitrate-N (EPA method 353.1), total phosphorous (EPA method 365.2). Samples for dissolved nitrate were passed through a 0.45-µm-pore size Gelman-Acrodisc filter before analysis. Selected samples were analyzed for metals following USEPA protocols by University of Georgia Soil and Waters Chemistry Laboratory (http://aesl.ces.uga.edu/). Samples for metals (Ca, Mg, K, Na, Fe, Mn, Zn, Cu, Al, Cd, Cr, As) were analyzed in selected samplings. Turbidity was measured using a model 2020 turbidity meter (LaMotte) in the laboratory.

Statistical analysis. Data was $\log_{10}$ transformed to achieve normality. Enterococci data was expressed as a geometric mean to assess spatial variation within the Lajas Valley. In some instances the Enterococci data exceeded the most probable number (MPN) and the data was expressed as the maximum value possible. Stations were grouped into basins (with some basins having two or more sampling stations) Maginas, Mondongo, Bárbara, and Cristales. Descriptive statistics such as mean, median, mode, standard deviation, interquartile ranges were computed as indicators of central tendency and variability.

Responses of fecal indicators of contamination to classes of qualitative variables such as land-use/landcover, subbasin, flow-regime was made using ANOVA. Pearson correlation analysis was made between Enterococci and other water quality parameters. Regression analysis that included simple linear, multiple and geographically weighted (See section 6) was made to examine relationships among quantitative variables.

5.2. Results and discussion

Enterococci, optical brighteners, cattle and human bacteroides markers

Water samples were collected from August 2014 to February 2016 from 23 water-quality stations distributed among the Lajas Valley watershed, Río Loco watershed drainage outlet, and Guánica Bay. A total of 175 water samples were collected during low- to intermediate flows of which 115 were collected from pre-defined basins of Mondongo, Bárbara, Maginas and Cristales. The remaining 60 samples were distributed among 25 samples from different points along the main drainage channel, 9 samples from the irrigation channel entrance to Lajas Valley (Reference Station #2), 8 samples from Guánica Bay, 3 samples from Río Loco, and 15 samples from La Plata Caribbean Fisheries and Cuesta Blanca stations. At least three samples were collected from all stations and the maximum number of samples collected was 13 for station #3 in Mondongo.

The Puerto Rico Environmental Quality Board (PREQB) does not have limits for class SD waters for fecal enterococci bacteria, but rather for fecal coliform bacteria. The PREQB (2014) has an enterococci limit of 35 CFU/100 mL for coastal waters and beaches. In terms of SD waters such as the ones we sampled, the PREQB regulation states that “these waters shall be free from other pathogenic organisms different to coliforms.” Our research (and supported by others) shows that fecal enterococci bacteria are fairly ubiquitous in streams throughout Puerto Rico, even in what would be considered reference stations. Figure 5.1 describes the spatial variation of enterococci concentrations as grouped into four categorical indices, to identify potential hot-spots. The lower limit of 35 MPN/100 mL was used because it is USEPA’s recommended §304(a) water quality criteria (US EPA, 2015).

The mean enterococci concentrations across sites and dates for all of the area was 338 ±131[3] MPN/100 mL. All stations had geometric means exceeding the suggested EPA threshold of 35 MPN/100 mL. Only 4.4% of the samples had enterococci concentrations below the suggested EPA threshold (Table 5.1). Overall, the % positive incidence for OBs, cattle bacteroides and human bacteroides was 13, 20, and 22%, respectively.

The lowest enterococci geometric means were in Río Loco, Guánica Bay, and the four stations within the drainage channel with concentrations of 63±316, 71±294, and 75±68 MPN/100 mL, respectively. (Figure 5.1). Mean Enterococci concentrations at the Lajas Valley drainage outlet (station #22) were 68 (223) MPN/100 mL. In Guánica Bay, excluding two samples collected in October, mean Enterococci concentrations were < 35 MPN/100 mL. Samples collected during October coincided with higher precipitation in the area.

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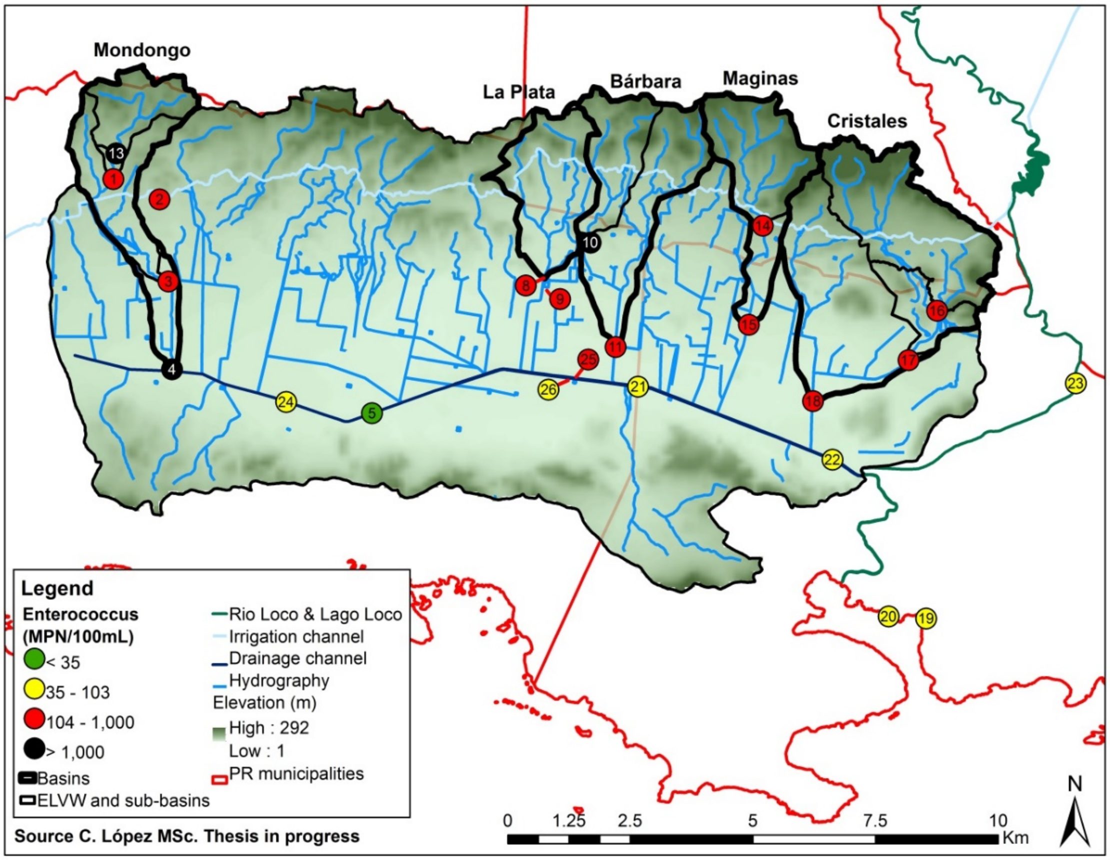
[3] geometric mean ± 1 standard error

Figure 5.1. Spatial variation of geometric means of fecal enterococci bacteria in selected basins and sampling stations of the Lajas Valley.



Source C. López MSc. Thesis in progress

Table 5.1. Percentage distribution of enterococci within our suggested class limits of stream waters in the Lajas Valley.

Enterococci class limit	Distribution
MPN/100 mL	%
<35	4.35
35 to100	30.43
100 to 1,000	52.17
>1,000	8.7

Enterococci concentrations decreased with increasing distance from the outlet of Mondongo (Station #4). Bacterial inactivation by solar radiation and adhesion to bottom sediments can reduce Enterococci counts (Marraccini et al. 2011). Based on the data collected we hypothesize that enterococci concentrations are reduced with increasing channel length by sorption to sediments and/or solar radiation (Figure 5.2).

The data suggests that Enterococci are not persistent at the outlet of the Lajas Valley watershed, Río Loco watershed and in Guánica Bay. The Guánica Bay had a positive OB signal near the outlet of the WWTP (Station #20) on 67% of the occasions sampled (Table 5.2; Figure 5.3). Both cattle and human markers were detected in only one of four occasions in which Guánica Bay was sampled (February 2015). Thus the results were inconclusive regarding the human and cattle contribution to fecal contamination or survival of fecal microorganisms in Guánica Bay.

Reference station #2, located within the irrigation channel that transports water into the Lajas Valley and into Lajas municipality, had mean enterococci concentrations of 241±119 MPN/100 mL. This station tested positive (22%, 2/9) for OBs but not for cattle or human bacteroides marker, which suggests that there may have been some occasional grey-water input at some point within the irrigation channel.

Overall, when the stations were analyzed as grouped by four basins, Bárbara and Maginas basins had concentrations that were significantly higher than Cristales and the latter were similar to those in Maginas (Table 5.3).

Table 5.3. Mean enterococci concentrations among basins.

Basin	Enterococci	
	MPN/100 mL	
Bárbara	1024	A [1]
Mondongo	736	A
Maginas	318	AB
Cristales	203	B

[1] Means with different letters are significantly different at $p<0.05$ as determined by Fisher's LSD test.

The highest enterococci concentrations were observed for those stations draining high-density urban areas, such as basins Mondongo (stations #13 and #4), Bárbara (station #10) and La Plata (station #8) with geometric mean concentrations of 1,601±438, 1,701±511, 1,271±492 and 956±693 MPN/100 mL. Caribbean Fisheries, which was the station that drained from the fish ponds (near La Plata), had mean concentrations of 566±731 MPN/100 mL. Except for station #4, these stations were all in the upper portion of the Lajas Valley watershed. Four of the top eight stations exhibiting highest enterococci concentrations were found in Mondongo basin.

Figure 5.2. Spatial variation of Enterococci upstream and downstream of the Lajas WWTP (upstream of station #1). From left to right, data points represent stations #13, 1, 3, 4, 24, 5, 25, 21, and #22.

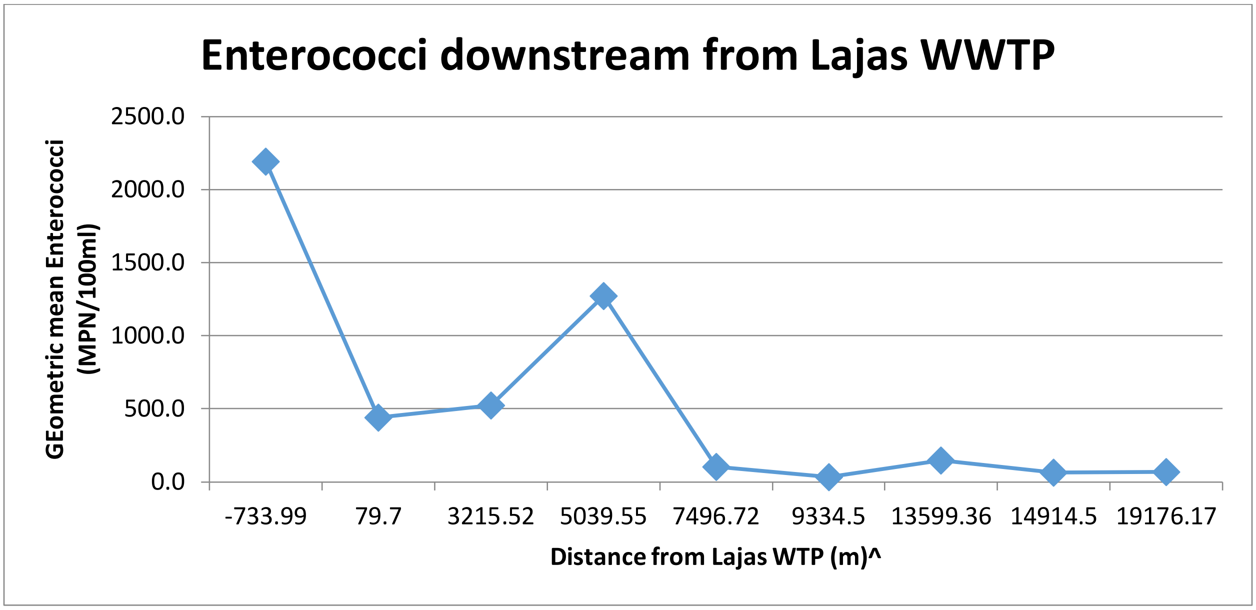
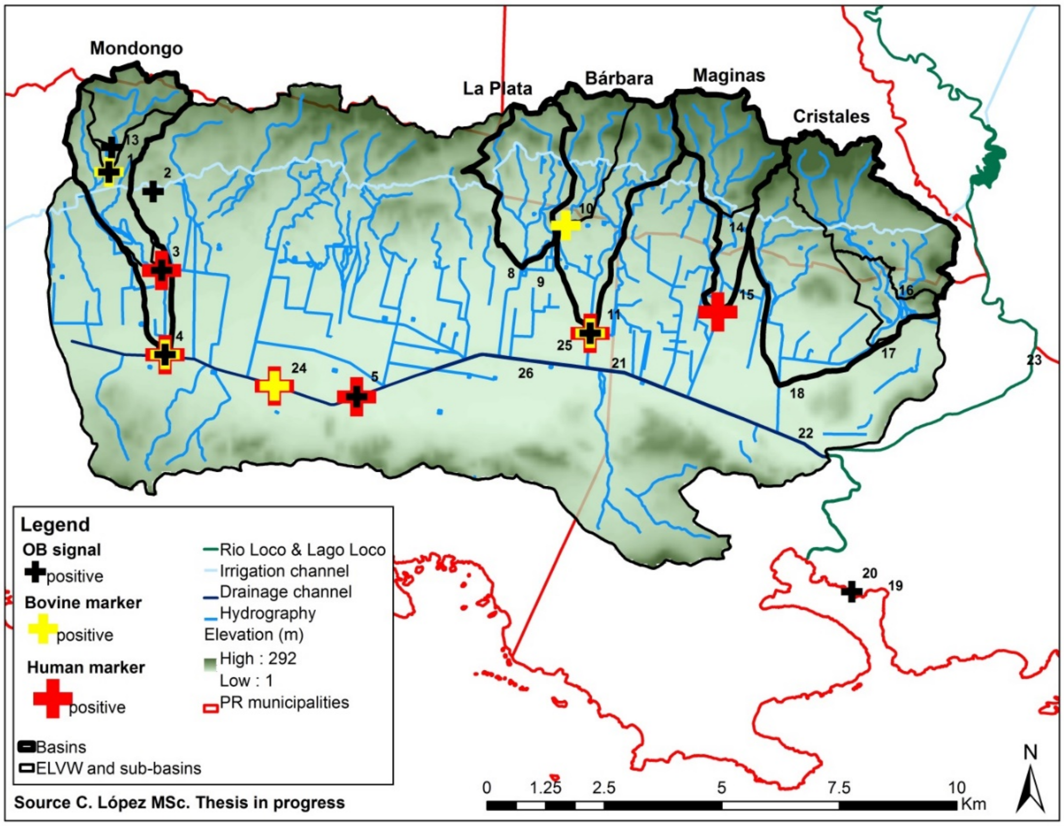


Table 5.2. Enterococci concentrations and % positive incidence for bacteroides and optical brighteners (OBs) in stations of the Lajas Valley watershed.

			Enterococcus		Positive incidence		
Basin or location [1]		Sub-basin	Geometric mean	stderr	Cattle bacteroides	Human Bacteroides	OBs
			-----------MPN/100 mL---		---------------------%-------------------		
ELVW		All sites	338	131			
Irrigation channel		2	241	119	0	0	22
Mondongo		1	442	491	40	20	20
Mondongo		3	522	445	0	40	27
Mondongo		4	1271	532	60	80	27
Mondongo		13	2190	611	50	0	29
Bárbara		11	362	482	40	40	18
Bárbara		10	1701	511	40	0	10
Maginas		15	329	836	20	40	0
Maginas		14	479	303	0	0	0
Cristales		16	134	212	0	0	0
Cristales		17	153	383	0	25	9.1
Cristales		18	163	624	0	0	0
	D.C.	5	32	29	0	75	40
	D.C.	21	64	136	0	0	0
	D.C.	22	68	223	25	0	0
	D.C.	24	102	86	75	50	0
	D.C.	25	147	192	0	0	0
	Bahía	20	65	52	0	0	67
	Bahía	19	77	595	50	50	0
	C.F.	9	563	731	0	0	20
	C.B.	26	76	44	50	0	0
	La Plata	8	955	693	0	0	0
	Río Loco	23	63	316	0	0	0

1 D.C. is the drainage channel; Bahía is Bahía de Guánica, C.F. is Caribe Fisheries, C.B is Cuesta Blanca.

Figure 5.3. Spatial variation of incidence of human bacteroides, cattle bacteroides and optical brighteners (OBs) in selected basins and sampling stations of the Lajas Valley.



Source C. López MSc. Thesis in progress

In Mondongo Basin, the station before the WWTP (#13) had highest enterococci concentrations, decreased downstream (stations #1 and #3), and increased again in #4. Among these stations, station #1 (Lajas WWTP outfall) did not have the expected “high” Enterococci concentrations, possibly due to an effective program for disinfection using UV light. Stations #1 and #3 had similar Enterococci concentrations during most sampling rounds.

Overall, all of the stations in Mondongo basin tested positive for OB on at least 20% of the occasions (Table 5.2). In one particular date (31 October 2015) all of the stations in Mondongo tested positive for OB. Station #13, which was upstream the WWTP or within the urban area, had the highest enterococci concentrations and tested positive on one of the sampling occasions for cattle but not for human bacteroides marker (this station was screened only twice for bacteroides markers). In station #1, which was immediately downstream of the WWTP, cattle and human bacteroides markers were observed 40 and 20% of the time, respectively. Station #4 had the highest incidence of cattle and human bacteroides with 60 and 80%, respectively.

Especially noteworthy was station #4 in Mondongo Basin, which 4 was downstream of an un-sewered community of 50 plus household structures, of which we observed at least 16 open pipes that presumably discharged into Mondongo drainage (C. López MSc Thesis in progress). Ruminants that were also observed near the households could also be contributing to the degradation of drainage waters via fecal contamination. This station had mean enterococci concentrations of 1,271 MPN/100 mL and 60, 80, and 27% incidence of cattle bacteroides, human bacteroides. As occurred in station #4 the presence of human and cattle bacteroides was also detected in station #24, albeit lower enterococci concentrations. Station #5 (which was downstream of a non-operational dairy facility) had enterococci concentrations < 100 MPN/100 mL, but had positive human bacteroides marker and OB signal. Enterococci concentrations increased further downstream in stations #25 and #21. Station #22 has a lower mean concentration of bacteria than those found in the preceding stations.

Figure 5.3 shows spatial variation in enterococci concentrations upstream and downstream of the Lajas WWTP. A contrast analysis among stations within Mondongo and reference station (#2) showed stations #13 and #4 were significantly greater enterococci concentrations than station #2. Station #13 was also significantly higher than station #3; there were no significant contrasts in enterococci concentrations among remaining stations.

In Bárbara basin, stations #10 and #11 had similar incidence of cattle marker and OB signal, but station #11 at the lower portion of the watershed also had a strong human bacteroides marker signal. This station is characterized as having potential waste-water discharges from illegally operated animal feeding operations.

In Maginas, the upper portion of the basin tested negative for cattle and human bacteroides and OB, but station #15 at the lower-portion of the basin had a weak bovine and strong human bacteroides signal.

In Cristales basin only station, #16, in the upper portion of the basin, had a weak human bacteroides marker and OB signal. When all of the subbasin stations were grouped, this basin had the lowest enterococci concentrations when compared with Maginas, Bárbara.

Storm events. A total of 44 stream samples (distributed among 23 events) were collected during storm flows with passive-rise stream flow collectors, and analyzed for enterococci concentrations (Table 5.4). Half of these samples exceeded maximum threshold values for Enterolert scale, even at 1 in 10 dilution; most of them occurred at station #10 in Bárbara basin and #3 in Mondongo. Excluding these samples, mean enterococci concentrations for storm samples was 1,121.0 MPN/100 mL. Note that mean values are underestimates of actual concentrations because half of these samples exceeded maximum threshold values for Enterolert scale. Storm event mean enterococci was greater (8.5x) than concentrations during low-intermediate flow (grab samples). Only 4.5% of the storm samples had enterococci concentrations below the suggested EPA threshold (35 MPN/ 100 mL).

Table 5.4. Summary of enterococci concentrations in streams and drainage network within the eastern Lajas Valley watershed, during storm flows.

		Enterococci	
Basin	Sub-basin	Mean	Stdev [1]
		--------mg/L---------	
ELVW	All storm sites	4,676	7
Mondongo	3	7,078	6
Barbara	10	7,762	4
Barbara	11	3,310	7
Maginas	15	2,238	1
Cristales	17	793	16

[1] Standard deviation

Station #15 at Maginas showed highest mean enterococci concentrations during storm flow, while station #17 at Cristales had lowest values. Station #15 was downstream a dense urban area, within a cattle farm. There was a tendency of greater enterococci concentrations with increase in stream stage. Bottles at highest stage had highest mean values across all sampling sites.

Associations among enterococci and water quality parameters

Overall, enterococci concentrations were positively weakly correlated ($p<0.05$) with dissolved nitrate-N (r=0.15), total N (r=0.19) and total P (0.17) and negatively associated with temperature (r=-0.19), specific conductance (r=-0.14) and the % area in grazed pasture (-0.21) (Table 5.5). The weak association among the parameters may be the result of an upper dilution factor limit, since the "true" MPN count could not be established in the most extreme cases.

Table 5.5. Pearson correlation coefficients and significance levels among enterococci concentrations and water quality parameters as grouped by subbasin or station. Stations having less than four data points are not presented.

Subbasin or station	n	Pearson r	p-value		Subbasin or station	n	Pearson r	p-value	
----------------------ELVW--------------------------									
Temperature	95	-0.23	0.0244	**					
Urban	91	0.22	<0.0001	**					
Grazed	91	-0.34	0.0009	**					
------------------BÁRBARA-----------------------					---------------------------------CRISTALES--------------------				
log10SPC	21	0.44	0.0468	**	Temp	32	-0.36	0.0455	**
log10TN	23	0.48	0.0215	**	log10TSS	32	0.33	0.0692	*
log10TP	23	0.59	0.0032	**	CANAL DRENAJE				
log10DP	19	0.56	0.0132	**	log10DP	18	-0.4	0.0984	*
Forest	23	0.48	0.022	**	log10TSS	24	0.47	0.0195	**
Urban	23	0.48	0.022	**	LA PLATA				NS
Pond	23	-0.48	0.022	**	MAGINAS				NS
Hay	23	-0.48	0.022	**	DO_ppm	16	-0.46	0.0737	*
grazed	23	-0.48	0.022	**	log10DP	11	0.55	0.0783	*
crops	23	-0.48	0.022	**	log10TSS	15	-0.49	0.061	*
pop2010	23	-0.48	0.022	**	----------------------------MONDONGO ----------------------				
areakm2	23	-0.48	0.022	**	pH	42	-0.26	0.0971	*
BAY					log10SPC	42	-0.34	0.0281	**
Temp	8	0.76	0.028	**	--------------------------CANAL RIEGO ---------------------				
log10SPC	8	-0.62	0.0993	*	log10SPC	10	-0.81	0.0049	**
--------------------CARIBE FISHERIES--------------------					log10turb	9	0.6	0.0881	*
log10TN	6	0.74	0.0922	*					

** is significant at p<0.05; and * is significant at p<0.1.

When the analysis was done separately by basins, weak correlations were found among enterococci and other water quality parameters for all basins except Caribe Fisheries, La Plata, and Maginas basins, in which there was no correlation. The exception was Bárbara, in which enterococci concentrations were positively correlated ($p<0.05$) to specific conductance, total N, total P, % forest, and % urban, and negatively correlated to other landuses such as % pond, % hay, % grazed, % crops, and population density.

Enterococci was significantly negatively correlated to specific conductance in Mondongo and *Canal de Riego* and to temperature in Cristales. Enterococci was significantly positively correlated to temperature and total suspended solids in Bahía and *Canal de Drenaje*, respectively.

Relationships among water quality and land use/land cover. Pearson correlation analysis was done to examine potential associations among stream enterococci concentrations with LU/LC characteristics for the 12 stations combined (labeled as ELVW in Table 5.6) and for individual basins. Overall, Enterococci was positively correlated to the proportion of basin area in urban development (UD) (r= 0.22), while negatively correlated to grazed pastures (GP) (r= -0.34). However, when analyzed by flow regime, these correlations were significant during intermediate but not low flow. Furthermore, population became significant ($p<0.05$) only during base flow, indicating urban areas are important contributors of enterococci during baseflow.

Table 5.6. Multiple linear regression (MLR) and geographically-weighted regression (GWR) models for stream enterococci concentrations among basins within the ELVW.

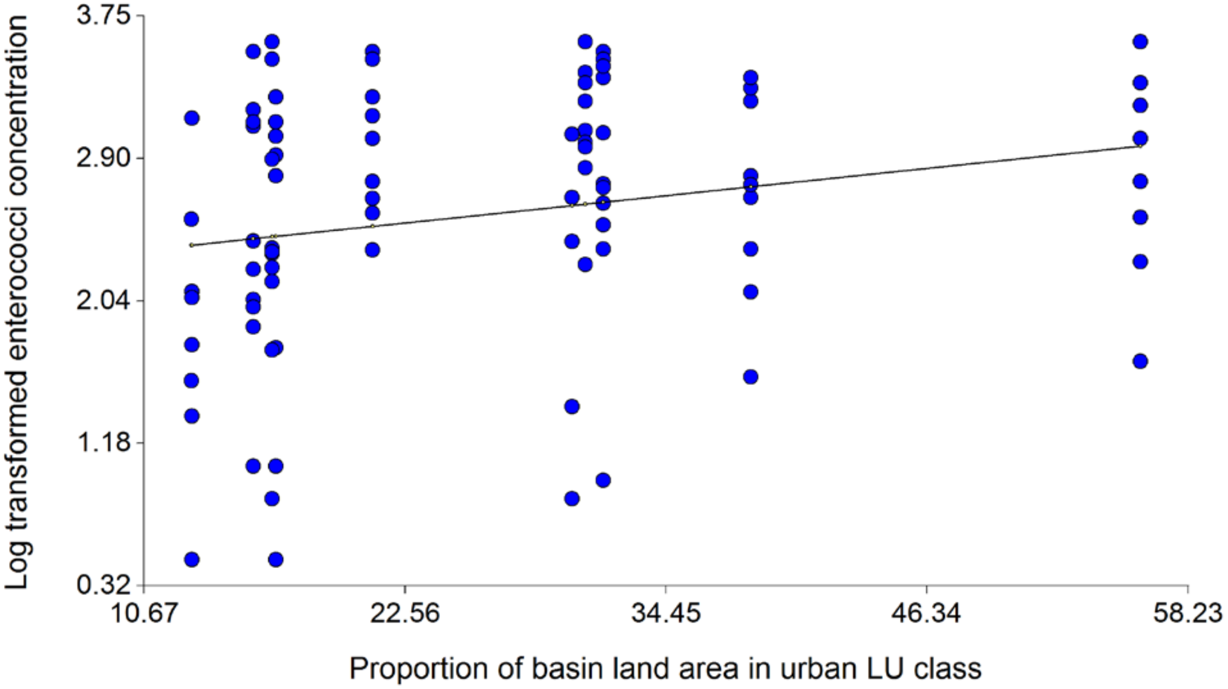
Parameter	Model type	Basin	Equation*	R^2 Adj
Enterococci	MLR	ELVW	2.197 + 0.014(UD)	0.05
	GWR	Barbara	1.994 + 0.022(UD)	0.08
	GWR	Maginas	1.739 + 0.020(UD)	0.07
	GWR	ELVW	2.193 + 0.012(UD)	0.03
	GWR	Cristales	1.972 + 0.011(UD)	0.02
	GWR	Mondongo	2.773 + 0.0005(UD)	0.00

UD – proportion of basin land area in urban development

Based on information generated from correlation analysis, simple and multiple linear regression models were constructed. GWR models for enterococci were considered a significant improvement over corresponding MLR models by showing higher R^2 and lower AICc values. Thus, MLR models are included in Table 5.6 for descriptive purposes only.

Stream enterococci concentrations showed significant linear relationships with proportion of basin area in UD and GP (data not shown). The best-fit MLR model included only positive relationship with UD (Table 5.6). This information is supported by previous quantitative observations in which enterococci concentrations were correlated with proportion of urban land and population. Figure 5.4 demonstrates that enterococci concentrations increase with the proportion of urban land. GWR model for enterococci had higher adjusted R^2 than corresponding MLR model (Table 5.6).

Figure 5.4. Scatterplot of significant linear relationship between stream enterococci concentrations within ELVW and proportion of basin land area in urban LU class.



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6. Nutrients, sediments, and metals in stream-waters of the Lajas Valley Watershed

6.1. Materials and methods

A description of the study area, land-use/land-cover analysis and climatic information is presented in Section 4.

Sampling. Twenty-three sampling stations were established within the Lajas Valley Watershed, lower portion of Río Loco Watershed, and Guánica Bay. The stations were selected based on a four-tiered targeted approach that included ease of access, land-use/land-cover information, sub-basin area, and potential sources of contamination. All of the sampling stations were linked to a defined sub-basin, which in turn is part of a basin within the ELVW (Figure 3.1). Some of the stations included areas up-and down-stream (representing a range of land use attributes) of potential problem areas such as point-sources, waste-water treatment plants and animal feeding operations; or upper and lower-portions of the basins. The geographic placement of the station included consideration of whether a particular land use activity or suspected source of pollution was having a potential impact on water quality. An additional eight samples were collected from targeted areas, based on suspected sources of contamination.

An initial round of five samples at each station were collected from August 2014 to June 2015. Eleven stations that were established at the drainage outlet of pre-defined basins and the reference station (#2) were selected for further sampling, from July 2015 to February 2016. Grab samples were collected during base flow, intermediate flow and high flows and these events were wadeable. Water samples were also collected during high-hydrologic flow storm events which occurred due to runoff and that caused an increase in stream stage from selected stations. During low-intermediate flow sampling, in some instances, there was a large time span between each sampling round. Some sites had to be revisited because they did not have flowing water or the sampling area could not be accessed.

Each stream water grab sample was identified as corresponding to three flow regime classifications (base-flow, intermediate-flow, and high-flow) but were grouped into two classifications by combining intermediate- and high-flow data. Measured *in situ* stream discharge was compared with class discharge threshold values. Eleven years of stream discharge data was obtained for Río Guayanilla in Yauco, which was the closest gauged stream to the Lajas Valley. A c/h recession curve and an adjusted Riggs (1962) procedure were applied to Río Guayanilla stream discharge data to determine discharge threshold values at baseflow and for the 7-day, 2 year recurrence event (7Q2) which was classified as the maximum flow for intermediate-flow. Cross multiplication allowed estimation of threshold values for Lajas Valley stations.

Water sample collection. Surface water samples for the analysis of total Kjeldahl nitrogen (TKN), dissolved nitrate, total phosphorus, suspended sediments and metals (Al, B, Ca, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Si, Zn) were collected during base-flow and low- to intermediate-hydrologic flows by a combination of volunteer monitors and professionals according to US EPA-approved procedures (USEPA, 1997). Water samples were collected manually (grab) with

1-L polypropylene bottles. Bottles were submerged 10 cm beneath the water surface using aseptic techniques. All samples were preserved in the field to pH<2 with H_2SO_4. Bottles were capped, sealed, placed on ice in a closed cooler, and transported to the laboratory for analysis within holding times specified in QAPP.

Water samples for storm events were collected using a passive rising-flow stream collectors (Gordon et al. 1992; Franklin et al. 2003). The sampler consists of a series of sterile 1-L HDPE bottles (Nalgene Storm water Samplers, Nalgene®, New York) placed at different stream-stage heights, and placed within a protective mounting kit. The bottles are filled during a storm event as the stream stage increases. After collecting a full liter of sample, the sampling mechanism closes to prevent cross-contamination with water. Each bottle-kit combination was placed at selected heights from the stream bottom corresponding to those resulting from storm events recurrence; based on relationships between stream height and stream-flow developed for each site as described by Sotomayor-Ramírez and Pérez-Alegría (2013). The collectors have been used successfully in the area by Perez-Alegría (unpublished data). Within 24 hours after a rainfall event, the collectors were inspected and samples collected. Samples were split into sub-samples for chemical or microbial analysis (See section 5.0); those for chemical analysis were acidified and managed as described previously.

Water sample analysis. Stream-water physico-chemical characteristics were measured *in situ* during manual sampling and included pH, temperature, specific conductance, and dissolved oxygen. Measurements were taken at mid-channel (at a depth of 15 cm from the water surface) with a YSI Professional Plus Multiparameter Water Quality Instrument (YSI Inc., Ohio, USA). Prior to sampling, instrument settings were corroborated using buffers and standard solutions and calibrated as needed. Water velocity was measured with a Flow Probe Hand-held flowmeter (Global Water, College Station TX, USA) at each of the selected stream cross-sections and converted to hydrologic flow using depth x stream-width transects.

Samples were analyzed for total Kjeldahl nitrogen (TKN) (EPA method 351.2), dissolved nitrate-N (EPA method 353.1), and total phosphorous (EPA method 365.2). Samples for dissolved nitrate were passed through a 0.45-µm-pore size Gelman-Acrodisc filter before analysis. Selected samples were analyzed for metals (Ca, B, Mg, K, Na, Fe, Mn, Zn, Cu, Al, Cr, Si, Mo, Ni) following USEPA protocols by University of Georgia Soil and Waters Chemistry Laboratory (http://aesl.ces.uga.edu/). Turbidity was measured using a model 2020 turbidity meter (LaMotte) in the laboratory. Total suspended sediments (TSS) were measured in the laboratory using SOP 015W (Based in EPA method No 160.2) and samples were filtered through a 0.7 µm pore size, 47 mm diameter Whatman glass fiber (GF/F) filter.

Statistical analysis. Nutrients, physico-chemical parameters, and flow data were tabulated and organized in MS Excel (Microsoft, Corp. Redmond Washington, USA). In instances where nutrient concentrations were below method detection limits (MDL), data was expressed as one-half of the MDL. Descriptive statistics such as mean, median, mode, standard deviation, interquartile ranges were computed as indicators of central tendency and variability. Histograms and boxplots indicated stream nutrient and sediment data was not normally distributed and this data was $\log_{10}$ transformed prior to subjecting to statistical analysis.

Nutrient and sediment data means in all tables were expressed as the inverse-log transformation of means.

Data from 10 stations were grouped into four basins (Mondongo, Bárbara, Maginas, Cristales), with some basins having two or more sampling stations. Statistical comparison of stream nutrient and sediment concentrations among basins were made with a two-way ANOVA in a repeated measures design. Two factors, basin and flow regime and their interaction acted as treatments and sampling dates as the repeated measures. It was hypothesized that the ANOVA would be able to separate out responses of stream nutrient and sediment concentrations to qualitative variables within each basin, such as relative proportions of land use/land cover (LU/LC).

Specific contrasts were made between: (i) urban (Mondongo and Maginas) and non-urban (Bárbara and Cristales) dominated basins; (ii) agriculture (Mondongo and Cristales) versus non-agriculture (Bárbara and Maginas) dominated basins; (iii) basins with greater rainfall and population (Mondongo versus others); (iv) grazed pastures dominated basins (Maginas versus others); (v) unmanaged shrubland (Bárbara versus others).

Pearson correlation analysis was made between nutrient and sediment concentrations with other water quality parameters, as well as LU/LC characteristics within basins. Linear and multiple regression models (MLR) were evaluated with SAS (SAS Inc., 2013), using stream nutrient (total N, dissolved NO_3-N, total P) and TSS concentrations as dependent variables, and land use/land cover (LU/LC) characteristics within basins as independent variables. Stream data from the upper basins (Mondongo, Barbara, Maginas, Cristales) and station #22 was used in models. LU/LC classes included grazed pastures (GP), hay and silage (HS), row crops (RC), urban development (UD), unmanaged pastures/shrubland (UP) and pond (P). Conclusions from these global MLR models were used to develop geographically-weighted regression (GWR) models in ArcMap 10.4 (ESRI Inc., 2015). An adaptive kernel bandwidth was used because sample density varied across the study area, and optimal bandwidth was determined by minimizing the corrected Akaike Information Criterion (AICc) (Fotheringham et. al., 2002).

6.2. Results and discussion

***Physical and general stream characteristics*.**

Mean streamflow at the time of water sampling at each station, during the study period across all stations and dates including Río Loco was 0.029 m^3/s; for Lajas Valley it was 0.022 m^3/s and for Río Loco it was 0.198 m^3/s. The ranking of the magnitude of streamflow for the various sites and basins was in the following order: Irrigation channel > Río Loco> Drainage channel> Mondongo> Cristales> La Plata> Bárbara> Maginas (Table 6.1).

Table 6.1. Summary of physico-chemical parameters in streams and drainage network within the Lajas Valley, during low-intermediate flows during 2014 to 2016 (Source C. López MSc. Thesis in progress).

			Temperature		pH		Dissolved oxygen		Specific conductance		Discharge	
			------°C---------				-------mg/L------		-------dS/m---------		-----m3/s-------	
Basin or location [1]		Sub-basin	mean	stdev [2]	mean	stdev	mean	stdev	mean	stdev	mean	stdev
ELVW	All sites[2]		26.7	2.0	7.9	0.2	6.2	1.7	1,023.3	1.9	0.022	5.888
Irrigation channel		2	25.7	1.4	7.8	0.1	7.0	0.5	257.0	1.2	0.650	3.214
Mondongo		13	25.4	1.7	7.8	0.1	5.9	0.9	724.4	1.2	0.009	3.296
Mondongo		3	26.9	1.7	8.0	0.2	7.6	0.9	1,819.7	2.0	0.106	1.726
Mondongo		4	27.0	2.1	8.0	0.2	7.4	0.7	2,187.8	1.8	0.110	1.905
Mondongo		1	27.8	0.1	7.8	0.1	5.4	0.9	2,570.4	1.8	0.019	3.214
Bárbara		11	26.9	2.2	8.0	0.2	8.1	0.9	812.8	1.4	0.021	3.214
Bárbara		10	27.1	2.5	7.8	0.2	6.8	1.7	933.3	1.4	0.007	3.006
Maginas		14	25.3	1.4	7.8	0.2	6.1	1.0	660.7	1.2	0.004	5.702
Maginas		15	27.3	1.5	8.0	0.3	6.0	2.9	741.3	1.3	0.006	2.249
Cristales		16	25.6	1.4	7.9	0.2	5.3	0.9	891.3	1.2	0.008	2.061
Cristales		18	26.6	2.0	7.7	0.2	4.6	1.5	708.0	1.4	0.041	1.854
Cristales		17	26.7	2.0	8.0	0.2	6.0	2.1	676.1	1.3	0.023	6.531
	D.C.	5	26.6	2.4	7.9	0.2	7.4	1.8	1,621.8	1.4	0.072	3.112
	D.C.	25	27.1	1.3	7.7	0.2	5.4	2.2	1,023.3	1.3	0.071	1.531
	D.C.	21	27.1	1.1	7.8	0.2	3.8	1.6	1,148.2	1.5	0.261	1.762
	D.C.	22	27.6	2.2	7.8	0.1	5.0	1.7	794.3	1.8	0.557	2.128
	D.C.	24	28.3	3.1	8.0	0.1	7.4	0.6	1584.9	1.4	0.113	1.343
	Bahía	19	29.4	1.6	8.1	0.1	6.0	0.4	51,286	1.1	N/A	N/A
	Bahía	20	29.5	1.2	8.0	0.1	5.1	1.0	51,286	1.1	N/A	N/A
	C.B.	26	24.2	1.9	7.7	0.1	6.1	0.1	1,380.4	1.7	0.000	2.512
	La Plata	8	25.2	1.5	7.8	0.2	6.3	0.8	524.8	1.5	0.015	5.023
	Río Loco	23	25.8	2.1	7.5	0.2	6.5	1.6	575.4	2.5	0.198	19.634
	C.F.	9	27.8	1.1	7.7	0.1	5.0	0.8	380.2	1.2	0.008	3.373

1 D.C. is drainage channel, Bahía is Bahía de Guánica, C.F. is Caribe Fisheries, C.B is Cuesta Blanca. ; 2 Standard deviation

Flow analysis showed that 30% of the grab samples were collected during baseflow while 70% were collected during intermediate-high flows (Table 6.2). Of the samples classified as intermediate-high flows, 36% were above the intermediate-flow threshold value Discharge data at the irrigation channel near the Lajas water filter plant (WFP) (Station #2) was obtained from USGS (2016) and annual discharge was estimated at 7.9 X 10^6 m^3/yr (or 5.72 MGD). Discharge data from the WWTP and the WFP were obtained from ECHO (2016), with mean daily discharge of 1.02 MGD (3.86 x 10^6 L/d) and 0.35 MGD (1.32 x 10^6 L/d), respectively.

Table 6.2. Percentage distribution of flow regimes during grab sampling in stream waters of the Lajas Valley, as grouped within class limits established by this study.

Flow regime [1]	Mean discharge	Distribution
	m^3/s	%
Base-flow	0.005	30.1
Intermediate-high flow	0.072	69.9

1 The intermediate-flow class corresponds to discharge data above base flow and up to the maximum value of the 7-day, 2 year recurrence event (7Q2); high-flow class corresponds to higher discharge than the intermediate-flow limit.

The mean instantaneous discharge measured in stations #3 and #4 was 0.106 and 0.11 m^3/s. This is in accord with what was expected as the stations are separated by 1.82 km, and have similar contributing basin characteristics. Only where runoff occurs (or during low-intermediate flows) is station #4 expected to have higher flow (Table 6.3). There are two contributing point-sources upstream of station #3, which are the WWTP and the WFP. The WWTP has a continuous discharge but the WFP contribution is intermittent, subject to when the filters are cleaned (J. Lugo, Personal Communication, 2016). Based on published daily discharge data (ECHO, 2016) we calculated that the mean WWTP contribution to Mondongo stream discharge at station #3 is 42% and the combination of the WWTP and WTP is 57%. This same calculation at station #1 gave grossly erroneous data, thus the discharge data in station #1 needs to be revised. All of the pre-defined basins (Mondongo, Bárbara, Maginas and Cristales) had higher streamflow at stations closest to basin outlets (stations #4, #11, #15 and #18, respectively).

The stream-water mean pH across all stations and dates and in all stations of the Lajas Valley was 7.85. Mean stream-water temperature across all stations and dates was 26.75°C. Mean stream-water dissolved oxygen (DO) across all stations and dates was 6.23 mg/L, corresponding to a dissolved oxygen percent saturation of 78.4%. Mean DO ranked streams and drainage network in the following order (mean DO oxygen percent saturation value in parenthesis): Bárbara (93.2%)> Irrigation channel (87.1%)> Mondongo (83.9%)> Río Loco (80.1)> La Plata (76.9%)> Maginas (75.2%)> Guánica Bay (87.4%)> Drainage channel (73.4%)> Cristales (66.5%). Mean stream-water specific conductance (SPC) across all stations and dates was 1,122 dS/m. Mean SPC in wadeable streams of the Lajas Valley and irrigation channel (#2) were 1,023.3 and 257.0 dS/m, respectively. Mean SPC at Río Loco and Guánica Bay were 575.4 and 51,286.1 dS/m, respectively.

Table 6.3. Mean hydrologic discharge data collected in stations # 13, 1, 3 and 4 of Mondongo streams, the waste water treatment plant (WWTP) and the water filter plant (WTP), and the relative stream discharge contribution of the WWTP and WTP to Mondongo stream.

Station	Mean	Base flow	High
	-------------------x 10^6 L/d------------		
4	9.50	4.97	14.67
3	9.16	5.09	9.69
13	0.78	0.46	3.44
1	1.64	1.04	5.45
WWTP	3.86	3.86	3.86
WTP	1.33		
Relative contribution (%)			
WWTP	42.1	75.9	39.8
WWTP plus WTP	56.7	102.0	53.5

Nutrients. A total of 169, 170 and 173 samples from throughout the ELVW, Río Loco and Guánica Bay were analyzed for total N, dissolved nitrate, and total P concentrations, respectively, in low-intermediate flows (grab) (Table 6.4). The mean total N, dissolved nitrate-N and total P concentrations across all stations and dates during low-intermediate flow were 1.35, 0.692, 0.186 mg/L, respectively. The mean total N, dissolved nitrate-N and total P concentrations across all stations and dates of the Lajas Valley were 1.48, 0.776 and 0.219 mg/L, respectively. The PR water quality standards for class SD waters for total P have recently been revised from 1 mg P/L to 0.160 mg P/L (PREQB, 2014). For the same waters, the PREQB does not have standards for total N, rather a limit of 10 mg NO_3-N is established. Sotomayor-Ramírez and Martínez (2011) have suggested numeric nutrient criteria and provided limits for enriched and degraded waters (Table 6.5), and the measured concentrations were used to categorize the data into three classes of “Reference”, “Enriched” or “Impaired”.

Total N. Seven of the 23 sampling stations (30%) exceeded PREQB water quality standards for class SD waters and were impaired in terms of total N concentrations (Table 6.4; Figure 6.1). The specific areas that were impaired were all of the stations within Mondongo basin, two stations within the drainage channel (#24 and #25), and the station in the upper portion of Maginas basin (#14). The highest total N concentrations were observed for stations draining high-density urban areas within Mondongo (station #1) and Maginas (station #14) basins, with concentrations of 3.19±2.38[4] and 2.75±1.84, respectively (Table 6.4). Guánica Bay had lowest total N concentrations, followed by reference station #2 located within the irrigation channel, with means of 0.417±2.24 and 0.646±1.32 mg/L, respectively. The ranking of stream-water total N concentrations for the various sites and basins was in the following order: Mondongo> Maginas> Drainage channel> Bárbara> Caribe Fisheries> Cristales> Cuesta Blanca> La Plata> Irrigation channel> Guánica Bay.

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[4] mean ± 1 standard deviation

Table 6.4. Summary of nutrient and sediment concentrations in streams and drainage network within the eastern Lajas Valley watershed, during low-intermediate flows during 2014 to 2016 (Source C. López MSc. Thesis in progress).

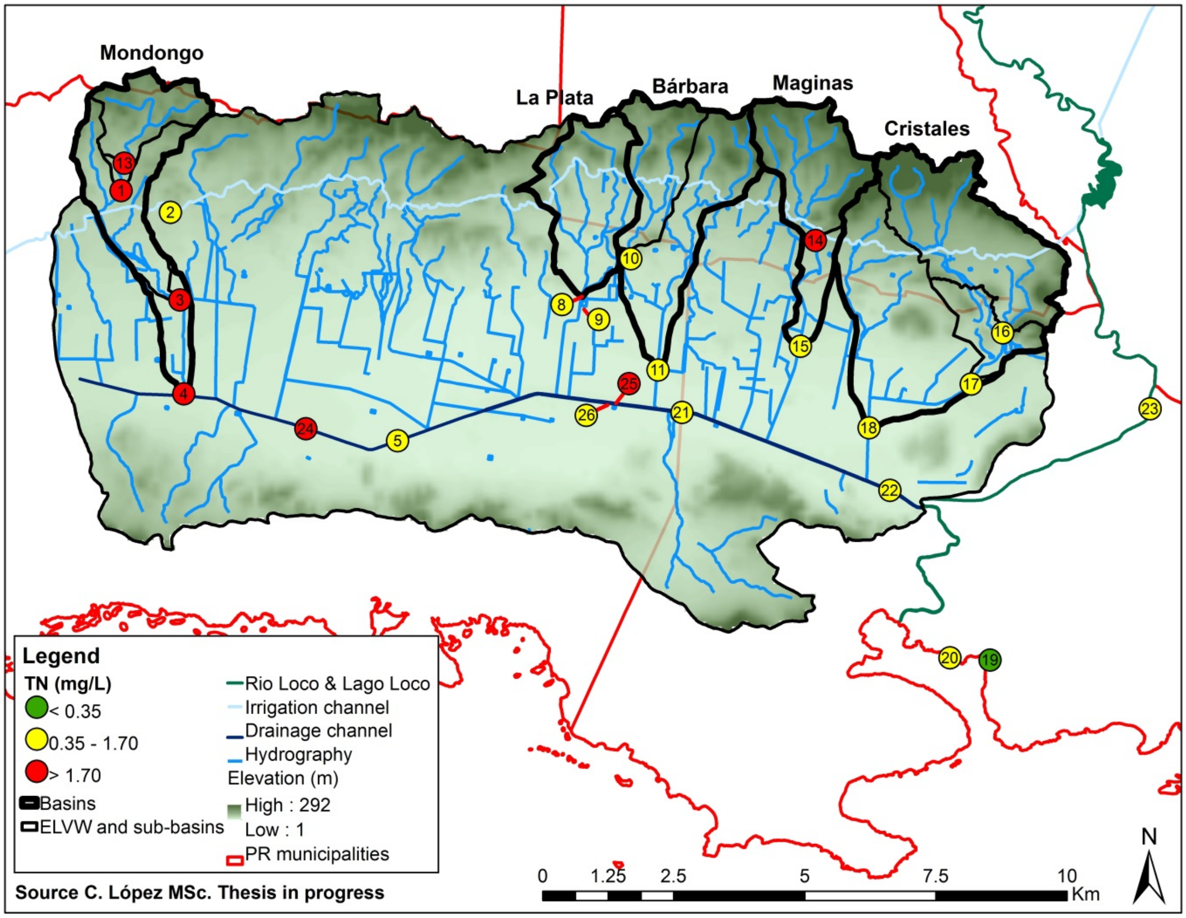
			Total N		Dissolved NO_3-N		Total P		Suspended sediments	
Basin		Sub-basin	Mean	Stdev [2]	Mean	stdev	Mean	stdev	Mean	stdev
			----------------	----------------	----------------	----mg/L----	----------------	----------------	----------------	----------------
ELVW		All sites	1.48	2.04	0.776	2.95	0.219	2.29	18.2	5.13
Irr. channel		2	0.646	1.32	0.309	1.66	0.062	1.41	20.89	2.4
Mondongo		4	2.02	1.76	1.27	1.95	0.490	1.46	34.36	3.42
Mondongo		3	2.16	1.85	1.39	1.82	0.526	1.85	38.9	2.96
Mondongo		13	2.20	1.37	1.97	1.39	0.112	1.23	2.29	3.67
Mondongo		1	3.19	2.38	1.30	1.82	0.632	1.63	5.01	4.09
Bárbara		11	1.20	1.78	0.804	1.45	0.068	3.30	18.16	5.13
Bárbara		10	1.39	1.79	0.776	4.73	0.235	1.44	11.94	8.81
Maginas		15	0.946	2.15	0.543	3.04	0.180	1.70	4.01	5.53
Maginas		14	2.75	1.84	2.55	1.88	0.087	1.38	1.59	3.64
Cristales		18	0.679	1.79	0.182	2.33	0.209	1.40	73.62	3.43
Cristales		17	0.798	2.06	0.535	3.05	0.140	1.57	19.28	3.49
Cristales		16	1.69	1.37	1.16	1.46	0.191	1.34	28.51	1.8
	D.C.	22	1.20	1.46	0.522	1.60	0.174	1.95	52.36	2.19
	D.C.	21	1.37	1.59	0.476	1.96	0.215	1.45	23.93	2.3
	D.C.	5	1.66	1.81	1.05	1.68	0.299	1.48	28.84	1.89
	D.C.	25	1.81	1.38	0.995	1.38	0.245	1.22	36.14	1.29
	D.C.	24	1.85	1.68	1.05	1.68	0.363	1.40	89.54	1.77
	Bahía	19	0.269	2.08	0.032	5.09	0.019	2.07	20.04	2.08
	Bahía	20	0.652	2.18	0.2	8.00	0.079	3.08	18.79	1.62
	La Plata	8	0.661	1.74	0.275	2.88	0.120	1.35	21.88	1.58
	C.B.	26	0.708	1.18	0.05	1.26	0.457	1.12	16.98	1.91
	C.F.	9	1.29	1.41	0.389	1.29	0.263	2.57	85.11	2.45
	Río Loco	23	1.62	2.40	1.32	2.88	0.078	2.14	15.85	8.91

1 D.C. is drainage channel, Bahía is Bahía de Guánica, C.F. is Caribe Fisheries, C.B is Cuesta Blanca; 2- Standard deviation

Table 6.5. Percentage distribution of total N and total P concentrations of stream waters collected during low-intermediate flows in stream waters in the Lajas Valley, as grouped within class limits of the PREQB water quality standard for Class SD waters or nutrient criteria according to Sotomayor-Ramírez et al. (2011).

	Total N	Distribution	Total P	Distribution
	mg/L	%	mg/L	%
Reference	<0.35	4.4	<0.030	4.4
Enriched	0.35 to 1.7	65.2	0.030 to 0.16	34.8
Impaired	>1.7	30.4	>0.16	60.9

Figure 6.1. Spatial variation of total N concentrations in streamwaters of the Lajas Valley and Río Loco watersheds and Guánica Bay during low-intermediate flows. Concentrations were categorized according to Table 6.5.



Source C. López MSc. Thesis in progress

Total N was significantly affected by basin ($p<0.05$) but not by flow regime and flow-regime*basin interaction ($p>0.05$). Total N concentrations were greater in Mondongo than in Cristales, the latter which was similar to Maginas and Bárbara (Table 6.6). Urban-dominated basins (Maginas and Mondongo) contrasted significantly with non-urban dominated basins with mean values of 2.10 versus 1.10 mg N/L, respectively ($p<0.05$). All other total N contrasts were non-significant ($p>0.05$). Numerous open conduits (not necessarily flowing) were observed throughout Cristales, Mondongo and Maginas basins. Spatial data that describes the sewage infrastructure and connections to the sewage connections in the Lajas Valley demonstrates that most of the urban areas are not connected to the Lajas WWTP (NPDES PR0020575) and households and buildings depend on septic tank systems.

Table 6.6. Comparisons of main effects of basin and season for nutrient and sediment concentrations during low-intermediate flows of the Lajas Valley during 2014-2016. The numerical values are the inverse-log transformation of means.

	Total N[1]		NO_3-N[2]		Total P[1]		TSS[2]	
	mg/L							
	Basin							
Mondongo	2.49	A	1.97	A	0.41	ns	12.88	AB
Maginas	1.68	AB	1.24	AB	0.12		2.92	B
Bárbara	1.29	AB	0.79	AB	0.13		19.63	AB
Cristales	0.97	B	0.48	B	0.18		34.84	A
Mean	1.51		0.93		0.21		13.80	
	Flow regime[3]							
Base flow	1.52	ns	1.01	ns	0.17	ns	16.89	ns
Intermediate to high flow	1.50		0.96		0.20		9.35	

1 Means within a column with different letters are significantly different (p<0.05) as determined with Fisher's LSD test.

2 Means within a column with different letters are significantly different (p<0.10) as determined with Fisher's LSD test.

3 The intermediate-flow class corresponds to discharge data above base flow and up to the maximum value of the 7-day, 2 year recurrence event (7Q2); high-flow class corresponds to higher discharge than the intermediate-flow limit.

Dissolved nitrate-N. Ten of the 23 sampling stations (43%) were considered impaired, with dissolved NO_3-N concentration above 0.97 mg/L. This included all stations within Mondongo, the three stations within the drainage channel closest to Mondongo (#24, #5 and #25) and the upper station in Maginas basin (#14) (Table 6.4; Figure 6.2). Four stations (17%) were classified as either non-enriched or reference and had mean dissolved NO_3-N concentrations below 0.25 mg/L.

Dissolved nitrate-N was significantly influenced by basin ($p<0.10$) but not by flow regime and flow-regime*basin interaction ($p>0.10$). As occurred for total N, dissolved NO_3-N concentrations were greater in Mondongo than in Cristales, the latter which was similar to Maginas and Bárbara (Table 6.5). Lowest dissolved NO_3-N concentrations were observed in Guánica Bay, Cuesta Blanca (#26) and lower Cristales basin (#18), with concentrations of 0.095±7.71, 0.050±1.26, and 0.182±2.33 mg/L, respectively. Urban-dominated basins (Maginas and Mondongo) contrasted significantly with non-urban dominated basins with mean values of 1.38 versus 0.60 mg NO3-N/L, respectively ($p<0.05$).Total N and dissolved nitrate-N inputs were similar as a result of flow regime suggesting that there are continuous N inputs into drainage areas by open conduits or septic tank seepage.

Total P. Fourteen stations (61%) were classified as impaired and the remainder were enriched in terms of total P concentrations. Highest total P concentrations were observed for stations draining Mondongo basin, Cuesta Blanca (#26), and Caribe Fisheries (#9), with mean concentrations of 0.407±2.19, 0.457±1.12 and 0.263±2.57, respectively (Figure 6.3; Table 6.3). Lowest total P concentrations occurred at Irrigation channel (#2), Guánica Bay and Río Loco, with concentrations of 0.062±1.41, 0.035±3.09 and 0.078±2.13 mg/L, respectively.

Total P concentrations were significantly affected by the basin*hydrologic flow ($p<0.05$) interaction but not by the main effects ($p>0.05$) (Table 6.7). Total P concentrations were higher during intermediate flow at Bárbara ($p>0.05$). There were trends for higher total P concentrations during low flow at Maginas and Mondongo and higher total P during intermediate flow at Cristales and Bárbara. The potentially higher concentration during low flow in the urban dominated basins suggests that there are point source inputs and when there is increased discharge due to runoff or subsurface flow contribution to streams.

Contrast analysis revealed that Mondongo had greater total P concentrations than other basins ($p<0.05$). Further contrast revealed that Mondongo and Cristales had higher total P concentrations as compared to the other basins with mean values of 0.28 versus 0.13 mg P/L, respectively ($P<0.10$). The higher concentration in Mondongo can be attributed to the point source input of the WWTP and the observed conduits from households. In Cristales, increased P concentration can be attributed to enhanced P mobilization due to land preparation during tillage and land-leveling and to a lesser extent fertilizer-P application. Cristales basin possibly has point source contribution from household open conduits (See Table 4.1).

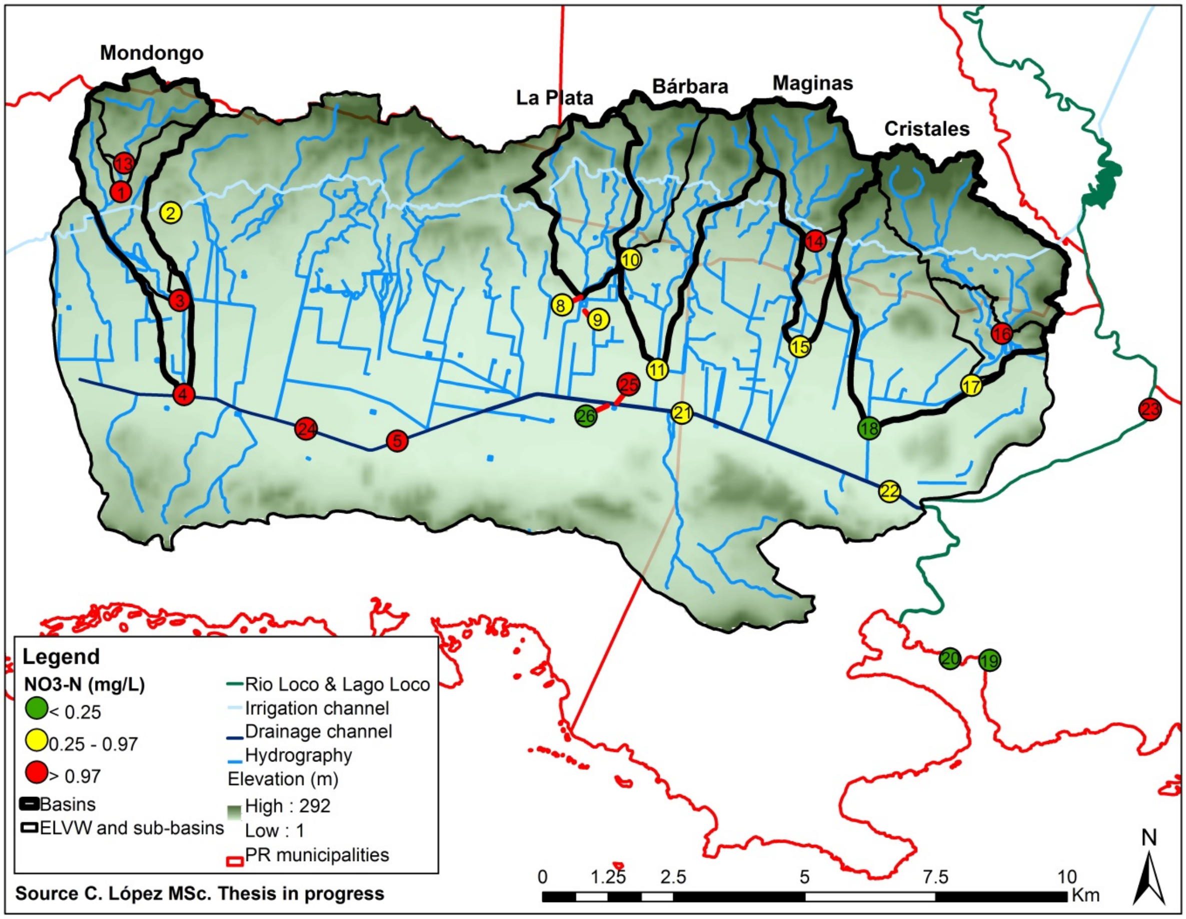
Table 6.7. Comparisons of significant interactions (basin*flow regime) for nutrient and sediment concentrations during low-intermediate flows of the Lajas Valley during 2014-2016.

		TP	
		----- mg/L ------	
Mondongo	Low	0.47	A [1]
Mondongo	Intermediate	0.35	AB
Cristales	Intermediate	0.19	ABC
Bárbara	Intermediate	0.18	ABC [2]
Cristales	Low	0.16	ABC
Maginas	Low	0.13	ABC
Maginas	Intermediate	0.12	BC
Bárbara	Low	0.09	C [2]

[1] Means within a column with different letters are significantly different ($p<0.05$) as determined with Fisher's LSD test.

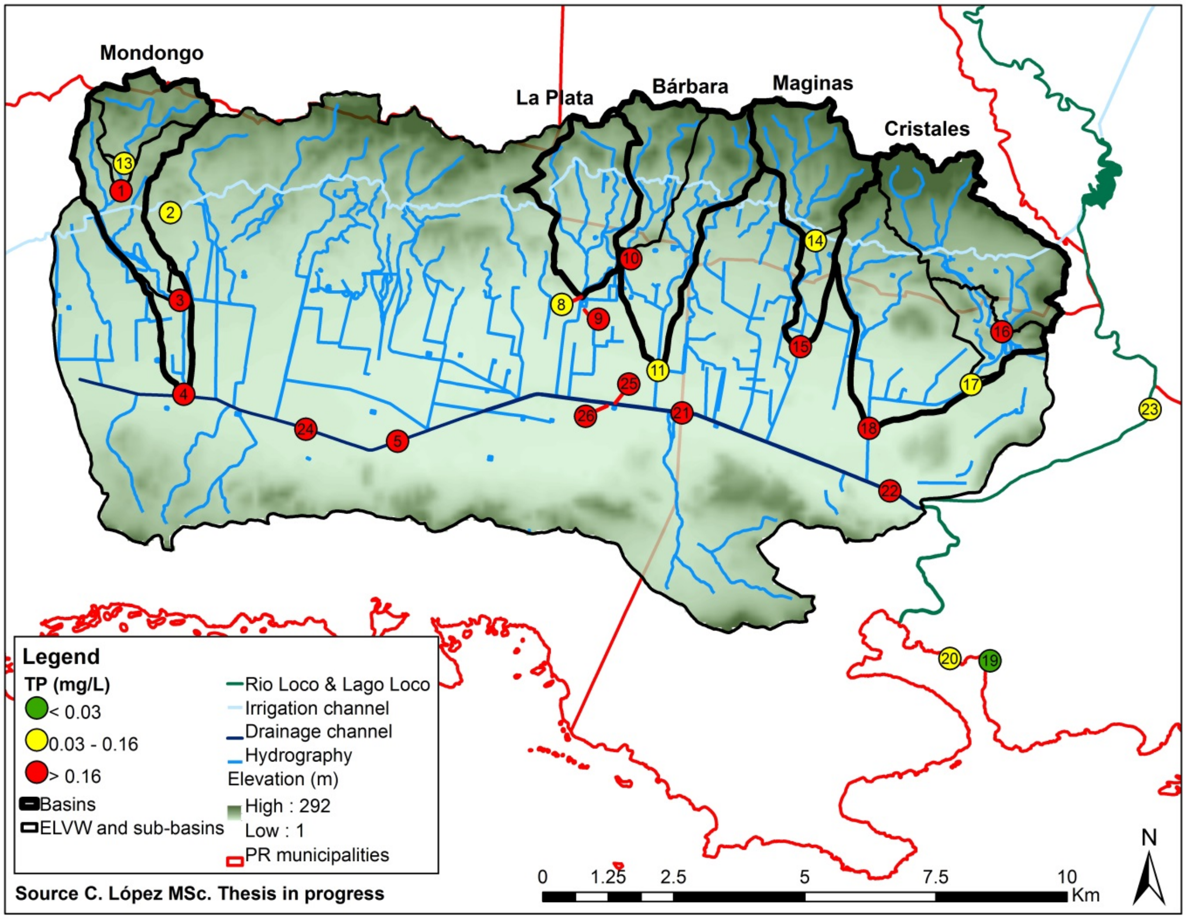
[2] Even though letters are not different, the comparison between Bárbara intermediate flow and Bárbara Low-flow are significantly different.

Figure 6.2. Spatial variation of dissolved NO_3-N concentrations in the Lajas Valley and Río Loco watersheds and Guánica Bay during low-intermediate flows. Concentrations were categorized according to Table 6.5.



Source C. López MSc. Thesis in progress

Figure 6.3. Spatial variation of total P concentrations in the Lajas Valley and Río Loco watersheds and Guánica Bay during low-intermediate flows. Concentrations were categorized according to Table 6.5.



Source C. López MSc. Thesis in progress

Mondongo and Cristales basins had higher proportion of land area dedicated to agriculture, compared to other basins. Mondongo had the greatest land proportion under urban LU class and had higher population, and Cristales had the largest proportion under row crops and a greater drainage area. Land area under urban and row crops may be contributing to increased P concentrations and would be expected to predominate during the wet period or that occurring during intermediate-high flow. Row crops in Cristales and other basins are primarily rice production which, to the best of our knowledge, apply near recommended fertilizer-P rates to crops (Sotomayor-Ramírez and Pérez-Alegría, 2013), but as explained previously land leveling, tillage and land clearing operations may mobilize soil P.

We noticed that station #13 in Mondongo had notably lower concentrations than the rest of the stations in this basin. In fact contrast analysis showed significantly higher concentrations ($p<0.05$) than other stations in Mondongo. Thus the ANOVA was redone excluding station #13. In this analysis, stream total P concentrations were significantly affected by basin ($p<0.05$) but not by flow-regime and by the interaction ($p<0.05$). Mondongo Basin had significantly greater total P concentrations than other basins.

***Total suspended sediments*.** A total 174 stream samples were analyzed for total suspended sediments (TSS). Mean TSS concentration across all sites and dates for all of the study area was 18.20 mg/L. Mean TSS concentration in wadeable streams of the Lajas Valley for all dates was 18.20 mg/L. Mean TSS concentration for Río Loco, Guánica Bay and irrigation channel were 15.85 ± 8.91[5], 19.50 ± 1.78 and 20.89 ± 2.40 mg/L. Highest TSS concentrations occurred at stations in the drainage channel and lower reaches of basins (#18, #3 and #4). Lowest TSS concentrations occurred at stations in the upper reaches of basins (#13, #14, #10). As expected TSS concentrations were relatively low as most grab samples were collected during low- or low-intermediate flows when there was low probability of runoff occurring.

TSS concentrations were relatively low compared to storm events. TSS were significantly affected by basin but not by flow regime or basin*hydrologic flow interaction. TSS concentrations were significantly higher (12x) in Cristales than in Maginas, the latter was not different to Barbara or Mondongo ($p<0.10$) (Table 6.6). Contrast analysis indicated significantly lower TSS concentrations occurred in urban-dominated basins (Mondongo and Maginas). TSS concentrations were hypothesized to be higher during runoff events due to erosive forces in bare soil or with lower ground cover and to stream-bed scouring. Urban areas have greater impervious surface areas that will contribute greater precipitation as runoff potentially contributing to erosive forces in stream-beds in anthropogenically modified stream channels such as the ones in Lajas Valley streams beds. Our sampling strategy during grab-sampling events were designed so that we could be characterizing the water quality during low flows (base flow) or during intermediate to high flows (between base flow and storm flows). Intermediate-high flows could be the result of sampling the descendent limb of the hydrograph from antecedent storm events, which tends to dilute sediment concentrations. The rising limb of the hydrograph tends to have greater suspended sediment concentrations.

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[5] Mean ± 1 standard deviation

Our data supports observations by Sotomayor-Ramírez and Perez-Alegria (2013) whom reported higher nutrient concentrations in drainage waters from the Lajas Valley relative to Río Loco. They found that total N and total P concentrations in the Lajas Valley drainage channel were 1.25 and 0.24 mg/L, respectively; in Rio Loco, mean concentrations were 0.85 and 0.05 mg/L, respectively. Our total N (1.20 mg N/L) and total P (0.174 mg P/L) concentrations in the Lajas drainage channel at station #22 were very similar to those reported by Sotomayor-Ramírez and Perez-Alegria (2013), yet total N (1.62 mg N/L) and total P (0.078 mg P/L) concentrations in Rio Loco were higher in this study than reported by Sotomayor-Ramírez and Perez-Alegria.

Water quality variation within specific basins

Mondongo. The sampling stations corresponding to Mondongo Basin were #13, #1, #3, and #4. Station #2 is outside the Mondongo basin but within the main-stem of the Lajas Valley Irrigation channel and thus serves as a reference station for comparison. We wanted to explore (i) the relative role of the Lajas Wastewater treatment plant (WWTP, NPDES PR0020575) to the quality of the water of Mondongo Stream. The sampling stations were established prior to and after the WWTP. Station #13 is located 734 m upstream the WWTP. Stations #1, #3 and #4 are located 80 m, 3.2 km, and 5.0 km downstream, respectively of the WWTP.

Water pH was not affected by the WWTP with pH in the range of 7.6 to 8.2 (Figure 6.4). Water specific conductance increased immediately downstream of the WWTP with values 4x and 11x higher than the upstream station (mean of 737 µS/cm) and the reference station (mean of 258 µS/cm), respectively. Dissolved oxygen concentration and DO saturation followed the same trend, so that DO saturation will be presented. DO saturation was similar in stations #1 and #2, with a mean of 71% saturation, possibly due to the fact that the stations were located near shaded areas with less direct incident solar radiation as stations #3 and #4. As the WWTP contributes near 30% of the flow to Mondongo stream the quality of this water will have a large influence in the overall Mondongo water quality. The water within Mondongo stream, up-stream of the WWTP may have greater carbonaceous compounds that increase oxygen demand and reduce oxygen concentrations or that have lower DO concentrations. Yet, at this moment the relative role of the WWTP on Mondongo stream DO concentrations cannot be ascertained, but will be the focus of MSc thesis by P. Rodriguez[6]. Water turbidity and total suspended solid (TSS) concentrations followed the same trend. The mean turbidity in reference station #2 was 21.7 NTU as compared to that in station #1 (mean of 3.7 NTU), which demonstrates that the WWTP does not contribute TSS to Mondongo stream. Turbidity was higher in stations downstream of the WWTP possibly as a result of stream-bed scouring and possible hydrologic flow-input from the wash-off and excess water of PRASA Water Filter Plant (NPDES PR0022985).

As has been shown previously, the WWTP is an important contributor of N and P to Mondongo stream as total N concentrations immediately downstream of the WWTP discharge

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[6] P. Rodríguez. 2016. Unpublished MSc thesis draft. University of Puerto Rico, Department of Agro-environmental Sciences; whom is quantifying GPP, NPP and respiration rate in the stream-channel benthos.

point, increased 6x and 11x with respect to stations #13 and #2 (reference). Total P concentrations, increased 2x and 6.6x with respect to stations #13 and #2 (reference) (Figure 6.5). Dissolved nitrate-N concentrations in all stations within Mondongo basin were higher than the reference station #2. About one-half of the total N concentrations in the Lajas Valley irrigation channel is in nitrate form, yet about 90% of the total N concentration in station #13 is in nitrate form (very little organic-N or ammonium-N). Approximately 34 and 62% of the total N was in dissolved nitrate form for stations # 1 and the combination of stations #3 and #4, respectively. Prior to station #13, Mondongo stream passes through a major part of the Lajas urban center, where we observed direct inputs from households. It appears that sub-urban areas upstream of station #13 are contributing to decreased stream water quality.

The waters in reference station #2 are enriched (see Table 6.5 for class limits) in terms of total N, dissolved nitrate-N, and total P. But, the waters upstream and downstream of the WWTP are all impaired in terms of total N and dissolved nitrate-N concentrations. The waters upstream of the WWTP are enriched in terms of total P, but are impaired downstream of the WWTP. Contrast analysis showed all stations in Mondongo had significantly higher total N and dissolved nitrate-N concentrations than station #2. Contrasts also showed stations #1, 3 and 4 had significantly higher TP concentrations than stations #13 and #2. The relative role of nutrients on the metabolism of the stream benthic ecology will be explored by P. Rodriguez in MSc thesis[2].

The Lajas WWTP is designed to treat up to 1.5 MGD with peak flow of 3.5 MGD. It is currently operating at a mean 1.02 MGD capacity (ECHO, 2016), serving a total population of 4,042 or roughly 1,010 household units (J. Lugo, 2016 personal communication). Most of these households are located outside of the eastern portion of the Lajas Valley, and these are primarily from Boqueron, Cabo Rojo and La Parguera, Lajas. Households within the ELVW connected the Lajas WWTP are limited to the Lajas urban center within Mondongo basin. Further, they have a current interim total P discharge limit of 8.26 mg P/L. According to ECHO (2016)[7], the mean monthly total P concentrations (mg/L) for 2015 was 1.05 mg P/L. Based on reported monthly discharge and total P concentrations for the years 2013 to 2015 we calculated a total P discharge mean of 123 kg P/month or 1,471 kg P/year to Mondongo stream. Said amounts are equivalent to 7,323 kg of fertilizer triple superphosphate and if applied to cropland could potentially provide phosphorus to crops in about 84 ha of cropland annually.

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[7] USEPA NPDES Enforcement and Compliance reporting data (https://echo.epa.gov)

Figure 6.4. Frequency distribution of general water-quality parameters of stations within Mondongo basin and a reference station.

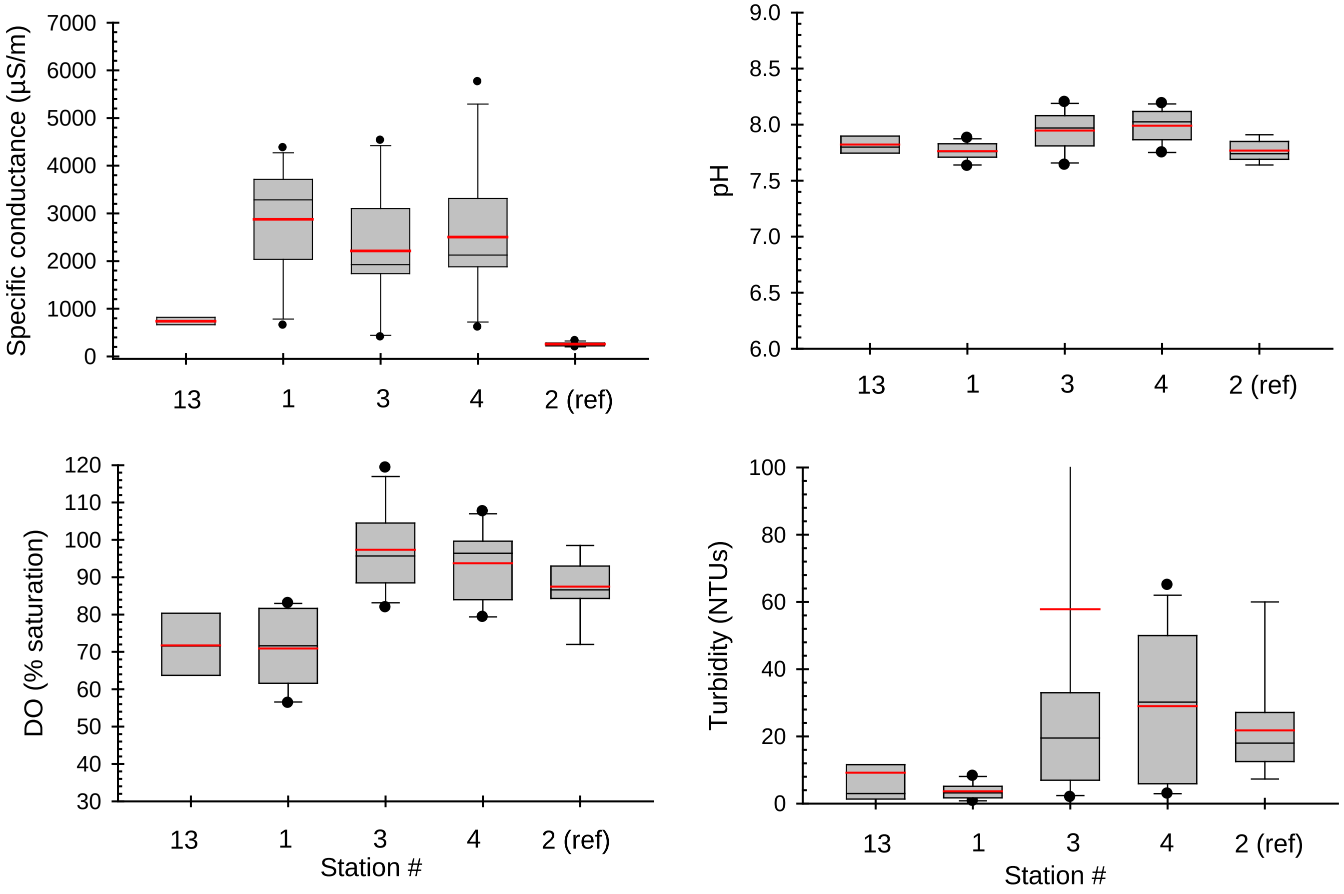
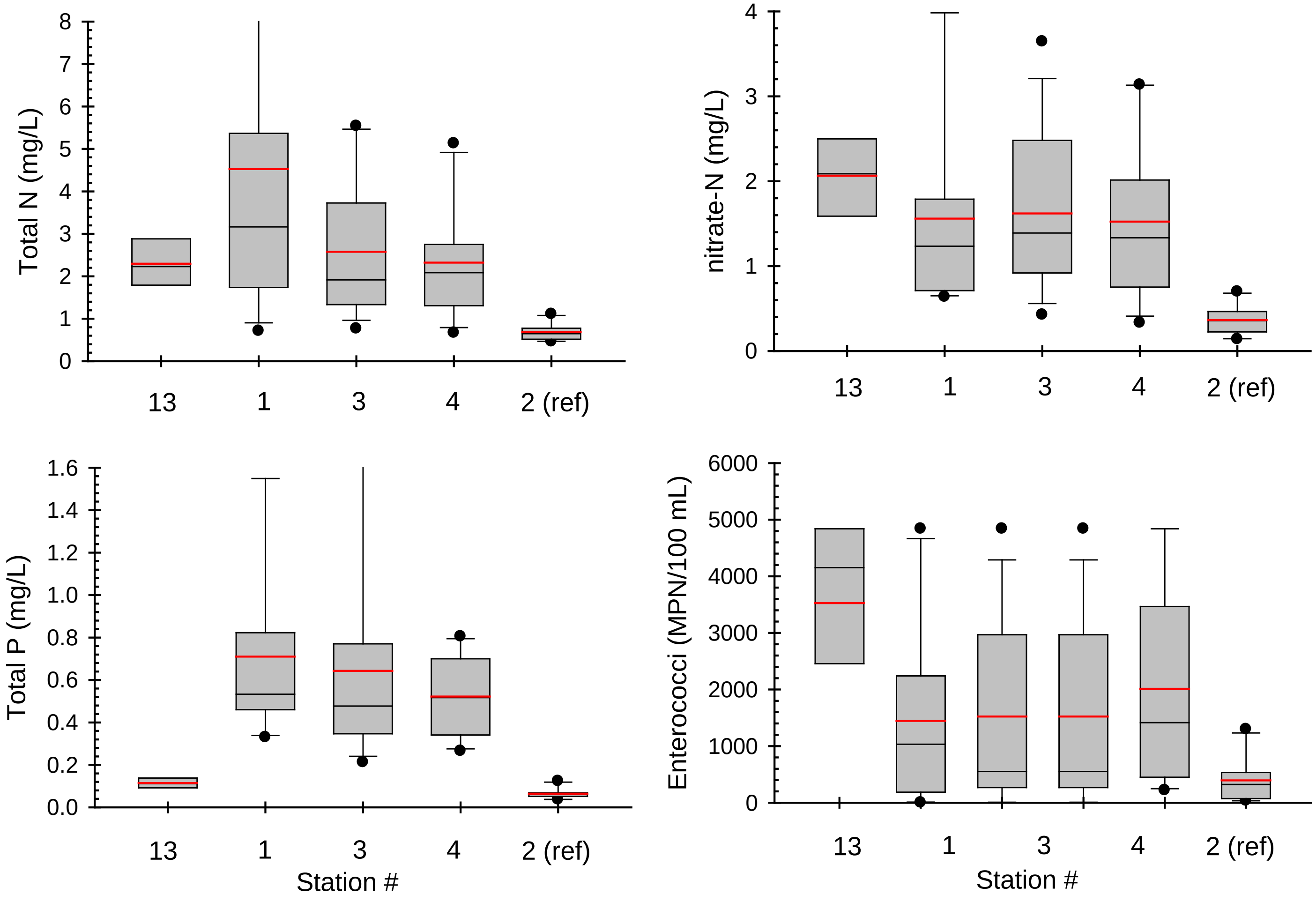


Figure 6.5. Frequency distribution of water-quality parameters associated with nutrients and fecal indicator bacteria of stations within Mondongo basin and a reference station.



As previously mentioned in section 4, annual nitrate N effluent contribution was 1,135 kg NO_3-N. Total N concentrations and loads were not measured, but the N:P ratio of station #13 upstream of the WWTP was 19.6 and in station #1, 5 and in station #3, and 4.1 in Station #4. This fact demonstrates how streamwater is being enriched more by P than by N. Further, using the N:P ratio of station #3, the total N contribution is estimated at 6,034 kg total N/yr. Thus we estimate that the nutrient contribution of the WWTP to Mondongo stream is 1,471 kg P/yr and 6,034 kg N/yr of which 19% is in nitrate form.

The temporal variation in total N and total P concentrations in Mondongo basin are shown in Figure 6.6 and serve to describe particular events when there are note-worthy changes in the nutrient concentrations. Of particular importance is total N concentrations of 16.9 mg N/L on 14 April 2015.

Other basins (Bárbara, Maginas and Cristales). Within Bárbara, Maginas and Cristales basins, there was a decrease in total N concentrations of 14, 66, and 60% in stations that are in the lower portion of the watershed as compared to those in the upper portion of the basins. The stations were the highest total N concentrations were observed were those immediately downstream unsewered urban communities and where greatest population is observed. In Maginas and Cristales basins, dissolved NO_3-N concentrations decreased by 79, and 85% downstream. In contrast, within Bárbara basin dissolved NO_3-N concentrations were similar between upstream and downstream stations. Within Bárbara and Cristales basins, total P concentrations were similar between upstream and downstream stations (differing by only up to 9%). Within Maginas, mean total P concentrations increased by 52% downstream. We believe that a cattle farm located between upstream and downstream stations in Maginas may be partly responsible for the increase in total P concentrations to Maginas stream.

Storm events. A total of 43 stream samples (distributed among 23 events) were collected during storm flows with passive-rise stream flow collectors and analyzed for nutrient and sediment concentrations. Mean values for total N, dissolved NO_3-N, total P and TSS across all storm stations and dates were 3.72, 0.347, 1.20 and 693 mg/L, respectively (Table 6.8). Storm-event total N, total P and TSS concentrations were higher than overall means collected during low-intermediate flows from the eastern Lajas Valley (see Table 6.2).

Station #15 in Maginas stream showed highest mean total N and dissolved NO_3-N during, while station #3 in Mondongo had highest total P and TSS concentrations. Overall, nutrient and sediment concentrations mean values were higher at each station during storm flow, compared to their corresponding grab sample mean values. The exception was dissolved NO_3-N which consistently showed lower values during stormflow, except at stations #15 and #17 who showed higher mean NO_3-N during stormflow. Total N, total P and TSS were 60, 82 and 97% higher during stormflow, respectively. NO_3-N was 55% higher during low-intermediate flow.

Figure 6.6. Temporal variation of total N and total P concentrations in Mondongo Basin and reference station #2. Total N concentration in station #1 of 14 Apr 2015 is 17.0 mg N/L.

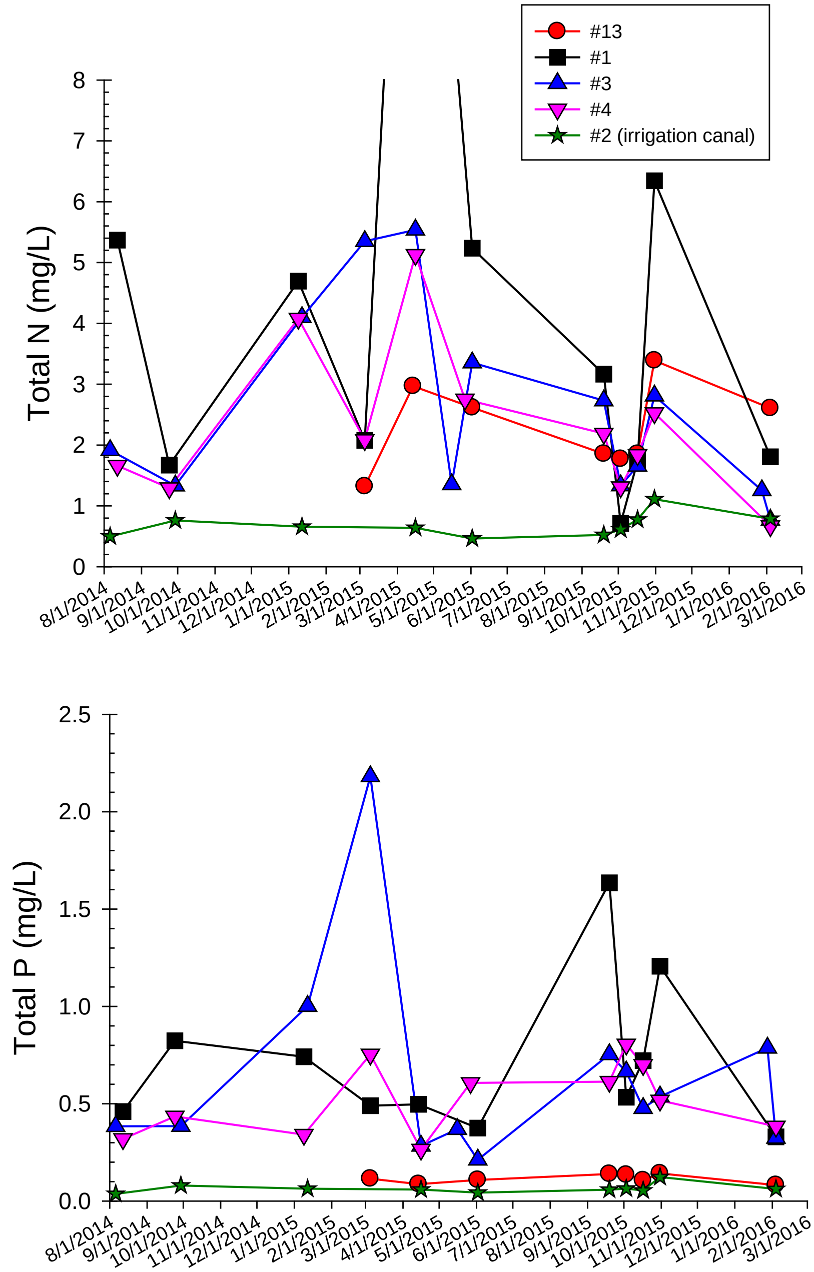


Table 6.8. Summary of nutrient and sediment concentrations in streams and drainage network within the eastern Lajas Valley watershed, during storm flows (Source C. López MSc. Thesis in progress).

		Total N		Dissolved NO_3-N		Total P		Suspended sediments	
Basin	Sub-basin	Mean	Stdev[1]	Mean	stdev	Mean	stdev	Mean	stdev
		-----------------------------------mg/L---							
ELVW	All storm sites	3.72	1.95	0.35	6.03	1.20	2.63	691.8	4.57
Mondongo	3	5.13	1.74	0.54	6.76	2.45	2.46	1819.7	3.89
Barbara	10	3.02	1.51	0.14	8.32	0.78	1.74	288.4	3.47
Barbara	11	4.90	3.02	0.58	1.95	1.51	3.55	1071.5	5.01
Maginas	15	5.62	2.09	1.02	1.58	1.74	2.75	1174.9	4.90
Cristales	17	2.14	1.58	0.68	1.82	0.59	2.09	512.9	3.98

[1] Standard deviation

Sotomayor-Ramírez and Pérez-Alegria (2013) reported nutrient and sediment concentrations during storm flow at Lajas Valley drainage channel and Río Loco. Mean values at Lajas were 3.55, 0.33, 0.75 and 459.7 mg/L for total N, NO3-N and total P, respectively. Mean values at Río Loco were 1.19, 0.50, 0.13 and 59 mg/L, respectively. The total N, dissolved NO3-N and TSS concentrations in this study were similar, and total P concentrations were slightly higher than those reported by Sotomayor-Ramírez and Pérez-Alegria (2013)

The data collected during base-flows, low-intermediate flows, and storm events can be used in future modeling studies to provide new estimates of total N and total P exports from watersheds as done by Sotomayor-Ramírez and Pérez-Alegría (2013).

***Relationships among water quality parameters*.** Pearson correlation analysis was done to evaluate quantitative relationships among stream nutrient and sediment concentrations with other water quality (WQ) parameters (Table 6.9). Total N was positively correlated ($p<0.05$) with SPC, dissolved NO_3-N, total P and negatively weakly correlated ($p<0.1$) with SS. Dissolved NO_3-N was positively correlated with pH, DO, and SPC, while negatively correlated to temperature and TSS. Total P was correlated positively correlated with discharge, temperature, and SPC. Suspended sediments was correlated positively with stream discharge and total P, while showing negative correlation with dissolved NO_3-N.

***Relationships among water quality and land use/land cover*.** Pearson correlation analysis was done to examine potential associations among stream nutrient and sediment concentrations with LU/LC characteristics for the 12 stations combined (Table 6.9). Overall, stream total N was positively correlated to the proportion of basin area in urban development (UD) (r= 0.49), while negatively correlated to grazed pastures (GP) (r= -0.42), row crops (RC) (r= -0.29) and pond (P) (r= -0.31). As for total N, dissolved NO_3-N was also positively correlated to UD (r= 0.41) and negatively correlated with GP (r= -0.45), RC (r= -0.32) and Pond (r= -0.30).

Stream total P was negatively correlated to unmanaged forest/shrubland (UF) (r= -0.40) while positively correlated to UD (r= 0.40), and hay and silage (r= 0.33). TSS was positively correlated to RC (r= 0.39), HS (r= 0.31), and GP (r= 0.24) while negatively correlated with urban development (r= -0.43).

Based on information generated from correlation analysis, simple and multiple linear regression models were constructed. For all nutrient and sediment data, global GWR models were considered a significant improvement over corresponding MLR models by showing higher R^2 and lower AICc values. Yet, basin-specific GWR models sometimes did not agree with the overall global GWR models. We recognize that the overall significance of the basin-specific GWR models with lowest R^2 values still needs to be explored further (C. López, MSc Thesis).

Stream total N concentrations showed significant linear relationships with proportion of basin area in UD, GP, RC, and P (data not shown). But the best-fit global MLR model included only positive relationship with UD and negative with GP (Table 6.10). This information is supported by previous quantitative observations in which higher total N concentrations were associated with basins having greater proportion of urban land and population. Figure 6.7 demonstrates that total N concentrations increase with the proportion of urban land. This is especially dramatic for Cristales and Maginas, as both basins showed greater total N concentrations at upstream sites, while having similar proportion of urban area with downstream sites. Cristales has the lowest proportion of urban land while Maginas has the largest proportion of urban land. Mondongo and Barbara also had high and low urban land area, respectively; yet both streams were influenced by water treatment plants. The global GWR model had an improved fit over global MLR model (with higher R^2), yet the UD coefficient in the GWR model approximated zero. All basin specific GWR models showed positive relationship of total N concentrations with UD and negative with GP, except Cristales which had positive relationship with GP. This may be the result of one station (i.e. Cristales stations #16 and #17) influencing the direction of the overall basin model. As mentioned previously, the overall significance of the basin-specific GWR models with lowest R^2 values still needs to be explored further. But, a possible interpretation is that holding the proportion of UD constant, greater proportion of GP will result in reduced total N concentrations. This may be expected as grazed pastures with at least two animals per hectare should receive at least 200 kg N/ha to achieve maximum productivity. As these grazed pastures are N limited any N input will improve plant N uptake and growth.

For dissolved NO_3-N, simple regression models showed significant linear relationships with proportion of basin area in UD, GP, RC, and P. But, best fit MLR model included only negative relationship with GP. The global GWR model showed a negative relationship with GP, yet in basin-specific models the GP was both positive and negative depending on the basin. The best fit among the basin specific models corresponded to Maginas in which GP was negative. A possible interpretation is that greater proportion of GP will result in reduced dissolved NO_3-N concentrations.

Table 6.9. Pearson correlation coefficients and significance levels among nutrient and SS concentrations with land use classes and other water quality parameters, for all streams within the eastern Lajas Valley watershed during low-intermediate flow. (Source C. López MSc. Thesis in progress).

Nutrient or SS concentration	Correlated WQ or LU Parameter*	N**	Pearson r	p-value
Total N	SPC	144	0.42	<0.05
	NO_3-N	145	0.84	<0.05
	TP	145	0.28	<0.05
	SS	144	-0.16	0.06
	UD	117	0.49	<0.05
	GP	117	-0.42	<0.05
	P	117	-0.31	<0.05
	RC	117	-0.29	<0.05
NO_3-N	temperature	145	-0.18	<0.05
	pH	145	0.18	<0.05
	DO	145	0.24	<0.05
	SPC	144	0.33	<0.05
	TSS	144	-0.29	<0.05
	GP	117	-0.45	<0.05
	UD	117	0.38	<0.05
	RC	117	-0.32	<0.05
	Pond	117	-0.30	<0.05
TP	Discharge	139	0.32	<0.05
	Temp	145	0.26	<0.05
	SPC	144	0.58	<0.05
	TN	145	0.28	<0.05
	UF	117	-0.40	<0.05
	UD	117	0.40	<0.05
	HS	117	0.33	<0.05
	Pond	117	-0.20	<0.05
TSS	Discharge	139	0.37	<0.05
	NO3-N	144	-0.29	<0.05
	TP	144	0.26	<0.05
	HS	117	0.31	<0.05
	UD	117	-0.43	<0.05
	RC	117	0.39	<0.05
	GP	117	0.24	<0.05

* UF is unmanaged forest/shrubland, UD is urban development, GP is grazed pastures, HS is hay and silage, RC is row crops, P is Pond. **Correlations with WQ parameters was done with all stream stations while correlation with LU/LC classes was done only for 11 stations within upper basins and ELVW outlet (#22).

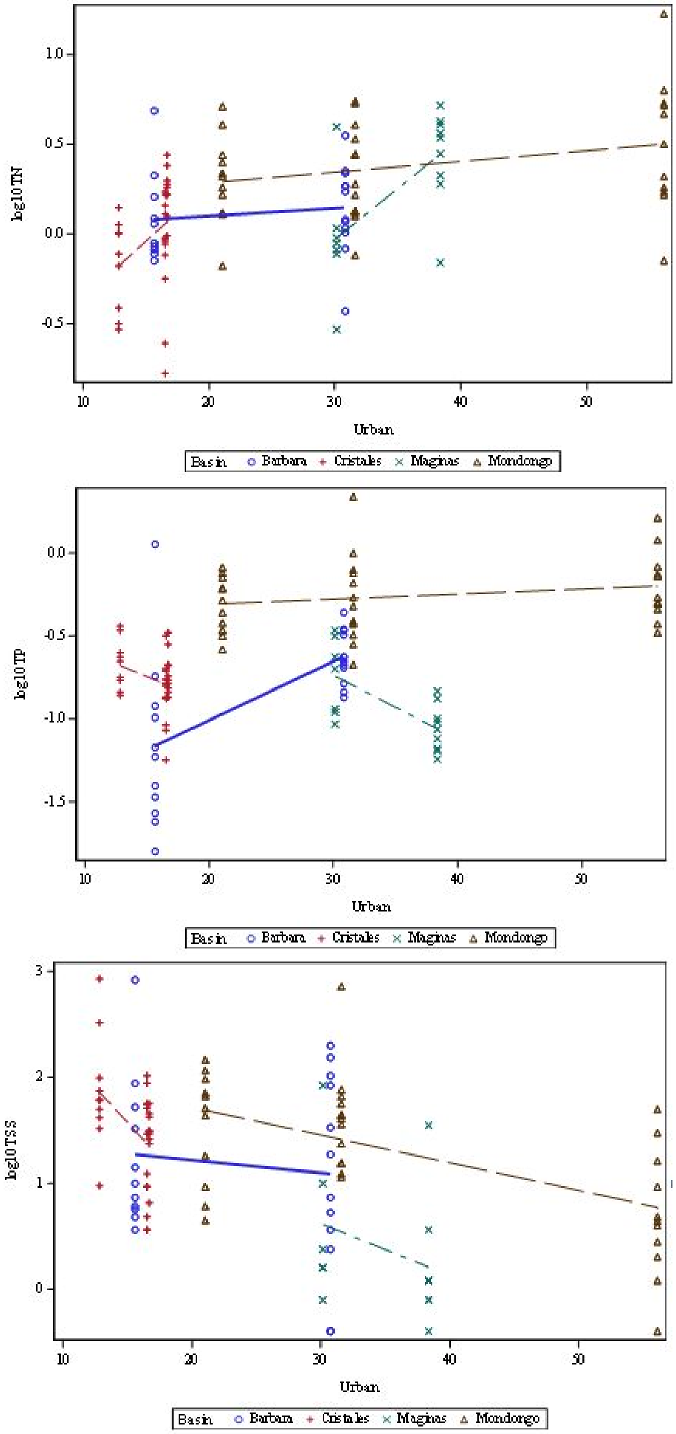
Table 6.10. Multiple linear regression (MLR) and geographically-weighted regression (GWR) models for basins within the ELVW.

Parameter	Model type	Basin	Equation [1]	R^2 Adj
Total N	MLR	ELVW	0.047 + 0.008(UD) - 0.082(GP)	0.33
	GWR	ELVW	0.0192 - 0.002(UD) - 0.081(GP)	0.34
	GWR	Barbara	-0.058 + 0.008(UD) - 0.056(GP)	0.18
	GWR	Maginas	-0.058 + 0.009(UD) - 0.056(GP)	0.18
	GWR	Mondongo	0.298 + 0.004(UD) - 0.2358(GP)	0.07
	GWR	Cristales	-0.247 + 0.004(UD) + 0.024(GP)	0.03
Disolved NO_3-N	MLR	ELVW	0.11 - 0.151(GP)	0.27
	GWR	ELVW	-0.006 - 0.041 (GP)	0.29
	GWR	Maginas	0.322 - 0.196(GP)	0.42
	GWR	Barbara	0.86 - 0.050(GP)	0.02
	GWR	Cristales	-0.573 + 0.017(GP)	0.01
	GWR	Mondongo	0.11 + 0.041(GP)	0.00
Total P	MLR	ELVW	-0.99 + 0.011(UD) – 1.608(P) + 0.017(HS)	0.32
	GWR	ELVW	-0.056 – 0.007(UD) – 0.957(P) – 0.024(HS)	0.59
	GWR	Mondongo	-1.40 + 0.022(UD) – 2.741(P) + 0.027(HS)	0.48
	GWR	Barbara	-0.140 – 0.019(UD) – 0.021(P) – 0.056(HS)	0.30
	GWR	Maginas	-0.207 – 0.020(UD) – 0.019(P) – 0.051 (HS)	0.30
	GWR	Cristales	-0.369 – 0.016(UD) – 0.010(P) – 0.041(HS)	0.24
TSS	MLR	ELVW	1.637 – 0.021(UD) + 0.016(HS)	0.21
	GWR	ELVW	1.946 - 0.036(UD) – 0.009(HS)	0.34
	GWR	Cristales	2.233 – 0.048(UD) – 0.020(HS)	0.35
	GWR	Maginas	2.264 – 0.048(UD) – 0.021 (HS)	0.34
	GWR	Barbara	2.347 – 0.050(UD) – 0.024(HS)	0.29
	GWR	Mondongo	1.110 – 0.006(UD) + 0.022(HS)	0.27

[1] UF is unmanaged forest/shrubland, UD is urban development, GP is grazed pastures, HS is hay and silage, RC is row crops, P is Pond.

Stream total P showed significant linear relationships with proportion of basin in UD, UP, HS, and pond (data not shown), thus the global MLR model for TP included these LU/LCs (Table 6.10). Scatterplots showed positive relationships between stream total P and UD at Barbara and Mondongo, and negative relationship at Cristales and Maginas (Figure 6.7). The basin-specific GWR models confirmed the spatial variation among these relationships, with positive Bi values for UD and HS at Mondongo basin, yet negative Bi values at all other basins (Table 6.8). Mondongo also showed best fit to this model (highest R^2), thus confirming the strong relationship and contribution this basin has to stream TP.

Figure 6.7. Scatterplots comparing relationships between stream water parameters and land use classes, among basins, using simple linear regressions.



Stream TSS correlated significantly with proportion of basin area in UD, RC, HS, and GP (Table 6.9). Significant simple linear relationships were also observed between TSS with these LU classes (data not shown). Global MLR model determined negative relationships between stream TSS and UD, while positive with HS (R^2_{adj}=0.21). The global GWR model showed negative relationships between TSS with UD and HS (Table 6.10). Basin-specific GWR models were similar to the global GWR model, except Mondongo where relationships with HS was positive.

Metals. A total of 23 samples were analyzed for elemental analysuis of metal concentration, corresponding to one sampling round at each station, and three rounds at the Guanica Bay. Mean concentrations of metal concentrations are summarized in Table 6.11.

Table 6.11. Summary of some metal concentrations in streams and drainage network within the Lajas Valley, during low-intermediate flows.

		Al		B		Ca		Fe		K		Na		Si	
		--mg/L--													
Basin or location [1]	Sub-basin	mean	stdev	mean	stdev	mean	stdev	mean	stdev	mean	stdev	mean	stdev	mean	stdev
ELVW	All sites	0.38	0.70	0.69	1.05	1203.4	5291.8	0.18	0.16	1164.5	5003.1	23002.7	145713.1	11.94	6.81

6.3. Conclusions

It was observed that upstream stations within Mondongo and Maginas in which the proportion of urban land was greatest were impaired by stream N concentrations (total N and dissolved NO_3-N). Cristales was also impaired by dissolved NO_3-N at the upstream station (station #16). These stations were all located in northern portion of the eastern Lajas Valley watershed, downstream of or within predominantly urban areas.

Statistical analysis demonstrated that basin characteristics significantly affected stream nutrient and sediment concentrations. Mondongo, the basin with most land area in urban development and with most population and population density, proved to have significantly higher total N concentrations than Cristales, which had least population and land area in urban development. Correlation and regression analysis demonstrated that nutrient and sediment concentration in streams of the ELVW are primarily positively related to anthropogenic activities, such as urban development and to a lesser extent agricultural activities.

Thus, it seems that the main sources of stream contamination by N and P are urban areas. The analysis suggests that agricultural activities could be a contributing source to stream degradation, yet of lower magnitude in comparison to urban development (UD). Furthermore, relationships between agricultural activities and stream degradation were positive for some basins and negative for others, indicating relationships are not consistent spatially and thus GWR models were appropriate. In fact, all GWR models showed higher R^2 values than corresponding MLR models. However, the overall significance of the basin-specific GWR models still needs to be explored further (C. López, MSc Thesis).

A decrease in stream N concentrations, particularly NO_3-N was observed along streams and drainage network of the ELVW. Denitrification may be responsible for decrease in total N concentrations and dissolved NO_3-N concentrations along streams and drainage channels of the eastern Lajas Valley watershed. Decreases in phosphorus concentrations along streams may be due to sediment adsorption in channel beds, which may later be re-suspended into the water column during events of high flow.

It appears that urban centers and the Lajas WWTP are important nutrient sources to the Lajas Valley stream and drainage network, and ultimately to the Guánica Bay. In terms of N, coastal waters will be more affected by drainage waters from Mondongo and Maginas basins because a greater proportion of total N was in dissolved form compared to other basins. In term of P, coastal waters may be more affected by drainage waters from Mondongo and Cristales basins.

6.4. Selected references

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