Metabolism and trophic status of streams draining basins with different land use in Southwest Puerto Rico

By

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Abstract

The eastern part of the Lajas Valley basin has an area of 14,519 ha, a population of about 33,000, and the predominant land use is 9.3% urban or sub-urban, 30% agricultural, and 61% forest / fallow. Quebrada Mondongo and Quebrada Bárbara are two sub-basins with predominant urban and agricultural land use, respectively. Quebrada Mondongo receives processed waters from a secondary sewage treatment plant (WWTP) and WTP and Quebrada Bárbara receives water from a WTP. Previous studies showed that the nutrient levels of streams in these sub-basins had concentrations of total nitrogen (TN) and total phosphorus (TP) in what is considered "enriched" and “impacted”. Assays of benthic metabolism were performed for net primary productivity (NPP) and respiration (R) using *in situ* recirculating metabolism chambers, and gross primary production (GPP) was computed as GPP = NPP- [-R]. Periphyton biomass, nutrient levels, physical and chemical parameter were also compared between sub-basins and among different points within the sub-basin. Differences between sub-basins were observed and the Quebrada Mondongo had significantly higher periphyton biomass, temperature, DO, specific conductance, flow, pH, TN, dissolved nitrate, TP, and dissolved phosphorus. NPP and R tended to be higher in Quebrada Mondongo, though not significantly. Quebrada Mondongo and the upstream of Quebrada Bárbara had GPP/R <1, suggesting that these benthic ecosystems are predominantly heterotrophic. The WWTP had no apparent effect in the stream ecology, as metabolism rates did not vary significantly from upstream to downstream stations. All sites were classified as either “enriched” or “impacted”, but the urban stream was greatly degraded.

Resumen

La parte este de la cuenca del Valle de Lajas tiene un área de 14,519 ha, una población de aproximadamente 33,000, y el uso predominante de la tierra es 9.3% urbano o suburbano, 30% agrícola y 61% bosque / barbecho. Quebrada Mondongo y Quebrada Bárbara son dos sub-cuencas con uso predominante de tierras urbanas y agrícolas, respectivamente. Quebrada Mondongo recibe aguas tratadas de una planta secundaria de tratamiento de aguas residuales (WWTP) y una planta de filtros y Quebrada Bárbara recibe agua de una planta de filtros. Estudios previos mostraron que los niveles de nutrientes de estas sub-cuencas tenían concentraciones de nitrógeno total (TN) y fósforo total (TP) en lo que se considera "enriquecido" e "impactado". Los ensayos de metabolismo béntico se realizaron para estimar la productividad primaria neta (NPP) y la respiración (R) utilizando cámaras de metabolismo de recirculación *in situ*, y la producción primaria bruta (GPP) se calculó como GPP= NPP- [-R]. La biomasa de perifitón, los niveles de nutrientes, los parámetros físicos y químicos también se compararon entre sub-cuencas y entre diferentes puntos dentro de la subcuenca. Se observaron diferencias entre sub-cuencas y la Quebrada Mondongo tuvo mayor biomasa perifitón, temperatura, oxígeno disuelto, conductancia específica, flujo, pH, TN, nitrato, TP y fósforo disuelto. NPP y R tienden a ser mayores en la Quebrada Mondogno, aunque no fue significativo. Quebrada Mondongo y aguas arriba de Quebrada Bárbara tenían GPP/R <1, lo que sugiere que estos ecosistemas bénticos son predominantemente heterótrofos. La WWTP no tuvo un efecto aparente en la ecología de la Quebrada Mondongo, ya que las tasas de metabolismo no variaron significativamente de las estaciones aguas arriba a las aguas abajo. Todas las estaciones se clasificaron como "enriquecidos" o "impactados", pero el flujo urbano se vio como el más degradado.

Dedication

To the Lajas Valley

Because you caught my attention from the first moment I saw you. You became my classroom without walls, where I learned to value your beauty, to be stronger, resistant, clever, patient and persevering. Because despite all the frustrations on the road, in you I got to see beautiful mornings and landscapes that I will never forget. Thank you for everything; this is little compared to how much you gave me in these last years.

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Introduction

Streams represent an important human water resource and an ecosystem for plants, fish and microbes. Regardless of their significance, streams are often threatened by contamination from anthropogenic activities like human water disposal, industrial activities, urban activities and agricultural activities. These activities could turn into sources of contamination if not well managed and could eventually affect the health of a stream. Stream health is defined by Meyer (1997) as "an ecosystem that is sustainable and resilient, maintaining its ecological structure and function over time while continuing to meet societal needs and expectations". Biological and chemical indicators, such as stream metabolism and trophic state have been used to monitor streams and their health.

Benthic stream metabolism is a process in which energy, in the form of carbon, is produced or consumed by biota and it can be assessed by quantifying rates of primary production and respiration. Benthic metabolism changes as a result of alterations in land uses, nutrient concentrations, light availability, and carbon input to a stream and serves as indicator of the magnitude and direction of energy flow (Bott and Newbold, 2013). For example, elevated nitrogen (N) and phosphorus (P) input from a wastewater treatment plant to a stream increased respiration rates downstream from the input source (Gücker et al., 2006). These increased nutrient levels have an impact in the trophic state of a stream.

The trophic state of a stream is greatly influenced by the nitrogen (N) and phosphorus (P) concentrations. Furthermore, the concentrations of N and P may limit or promote the heterotrophic and autotrophic activity of streams (Tank and Dodds, 2003). Benthic biomass has been related to trophic state of temperate and tropical streams (Lyon and Ziegler, 2009; Viggiano-Beltrocco, 2014). For example, an increase in nutrient concentrations will likely

increase primary productivity, resulting in higher algae production in the benthos and the water column (Bunn et al., 1999; Dodds et al., 2002). Heterotrophic activity is also related as nutrient enrichment with P can enhance microbial activity (Ramírez et al., 2003), which increases respiration rates.

Contamination into streams often comes from point sources such as wastewater treatment plants (WWTP) discharges and non-point sources like urban and agricultural runoffs. These can affect the health of a stream by changing nutrient concentrations, oxygen and light availability and metabolism rates. A point source of contamination, like a WWTP, has been found to increase the community respiration in downstream waters (Gücker et al., 2006; Ruggiero et al., 2006; Sánchez-Pérez et al., 2009) and also increase nutrient concentrations (Carey and Migliaccio, 2009; Sánchez-Pérez et al., 2009; Figueroa-Nieves et al., 2016; Rodríguez-Castillo et al., 2017). Agriculture related activities might also increase nutrient and sediment loads affecting stream nutrient concentrations and metabolism rates (Johnson et al., 2009). Agricultural streams seem to vary metabolism rates as some have found lower rates of respiration and higher rates of gross primary production (Gücker et al., 2009; Bernot et al., 2010), while others found high rates of respiration and lower rates of primary production (Frankforter et al. 2010; Hagen et al., 2010; Griffiths et al., 2013).

The Eastern Lajas Valley Watershed (ELVW) is an agricultural reserve that represents an extensive arable land in the southwestern part of Puerto Rico and contains important irrigation and drainage system built in the 1950's. This valley has varying land uses and potential point and non-point sources of contamination. Basins having agricultural and urban land uses have been identified in the ELVW (Sotomayor-Ramírez et al., 2015). The objective of this thesis was to assess the rates of primary production and respiration, benthic algae biomass (periphyton)

production and their relationship to the trophic state of streams draining different land uses. A secondary objective was to relate metabolism to sources of contamination in the ELVW. Knowing how point and non-point disturbances are affecting streams in the Lajas Valley is key to understanding the water quality in such an important agricultural reserve and the effects on the downstream waters.

Literature Review

Streams are an important resource for human enterprises such as recreation, drinking water, and agricultural and industrial activities; therefore streams need to be maintained healthy. A healthy stream has been defined by Mayer (1997) as "an ecosystem that is sustainable and resilient, maintaining its ecological structure and function over time while continuing to meet societal needs and expectations". Various parameters are used as indicators of the health of a stream to assess how it is responding to natural or anthropogenic disturbances. Some indicators of river health can be metabolism, benthic biomass, and nutrient concentrations. Furthermore, benthic metabolism and nutrient concentrations can be evaluated near point and non-point sources of contamination and they can be used to determine if those activities are affecting the stream ecosystem (Mulholland et al., 2005; Gücker et al., 2009; Sánchez-Pérez et al., 2009; Griffiths et al., 2013; Rodríguez-Castillo et al., 2017).

Stream metabolism

Stream metabolism has been used as a biological indicator of stream health by quantifying carbon concentrations or dissolved oxygen changes as indicators of energy fluxes. Primary production is the formation of organic matter from inorganic carbon by photosynthesizing organisms (Bott, 2006). The net energy that is stored as carbon is the net primary production (NPP) and can be quantified by the net changes of dissolved oxygen during the day. Energy in the form of carbon is also lost or consumed by heterotrophs and autotrophs in respiration (R). Respiration is quantified by changes in dissolved oxygen in the night or in the dark. The total energy that is produced and respired is the gross primary production (GPP) of an ecosystem (Odum, 1956). Also the GPP to R ratio (GPP/R) indicates the direction of energy movement or the relation between the production and consumption of energy in a system. If the

P/R ratio is more than 1 there is an addition of energy to the system, and if the ratio is less than 1, there is a loss of energy from the system (Bott, 2006). This ratio is indicative of the source of energy from the system; if autochthonous (GPP/R >1) or allochthonous organic matter (GPP/R <1). Autochthonous organic matter input is indicative of an autotrophic system while an allochthonous organic matter input is indicative of an heterotrophic system (Allan and Castillo, 2007).

Methods for determining stream metabolism vary depending on the equipment available and the stream characteristics. Metabolism can be measured using chambers or an open diel system. With an open system, a single or a dual (upstream-downstream) station can be installed in the stream to determine whole stream (benthic and column) metabolism with the diurnal dissolved oxygen change technique (Odum, 1956; Marzolf et al., 1994; Young and Huryn, 1998). This technique uses a dissolved oxygen meter to examine dissolve oxygen fluctuations over a period of 24h and takes into account the gas exchange between the water column and the atmosphere, called reaeration. A diel oxygen rate of change curve is created to determine the GPP and R for the 24h. McIntire et al. (1964) introduced the concept of measuring metabolism in respirometer chambers. Chambers have provided advantages in the metabolism study by: a) creating a way for laboratory controlled studies of metabolism, b) reducing the time of metabolism rates data collection *in situ* compared to the open diel method, and c) eliminating the need for calculating the reaeration constant of the stream.

The challenge of using metabolism chambers is creating a design that best simulates the natural conditions of a stream. Chambers could represent conditions occurring in the stream if light, flow and temperature are controlled. They have the advantage of screening out ultra violet (UV) light for assessing respiration in a shorter time span (Dodds and Brock, 1998). Although

some considerations for the use of chambers should be contemplated, such as nutrient limitation and internal temperature, this method eliminates the need for calculating reaeration fluxes of oxygen (Bott, 2006). Dodds and Brock (1998) introduced a portable chamber design that creates a laminar flow and minimizes several constraints; for example, UV light and solar irradiance attenuation were minimized using UV-transparent acrylic and the excessive increase in temperature and power consumption were reduced using an axial propeller instead of a centrifugal pump. This design was later modified to achieve a controlled flow, along with lower construction costs, lower internal temperature increase and increased portability for *in situ* measurements (Rüegg et al., 2015).

The metabolism of a stream responds to the trophic state of a river, especially carbon, nutrient concentrations, light and temperature. The trophic state can be classified as: 1) oligotrophic or nonproductive, 2) mesotrophic or medium productivity, or 3) eutrophic, which is highly productive (Dodds, 2007). A model of the response of benthic metabolism to drivers (Figure 1) can be created based on information from Young et al. (2008) and other studies such as Bernot et al. (2010). In an oligotrophic steam, nutrients and carbon are not in elevated concentrations; therefore GPP is higher than R and NPP. A system with high carbon will promote a higher respiration and a system with high nutrients will promote NPP, R and eventually GPP. There are some instances in which R production is much higher than GPP, and NPP becomes negative meaning a net heterotrophic metabolism. In an eutrophic system, where both nutrients and carbon are in high concentrations, R is expected to be higher than NPP and GPP is higher than both. Also, lower temperature can reduce metabolism rates and higher temperature is most likely to increase R and possibly GPP (Young et al., 2008). Light is especially important for autotrophs and an increase in light availability will most likely increase

NPP and GPP (Dodds, 2007; Young et al., 2008).

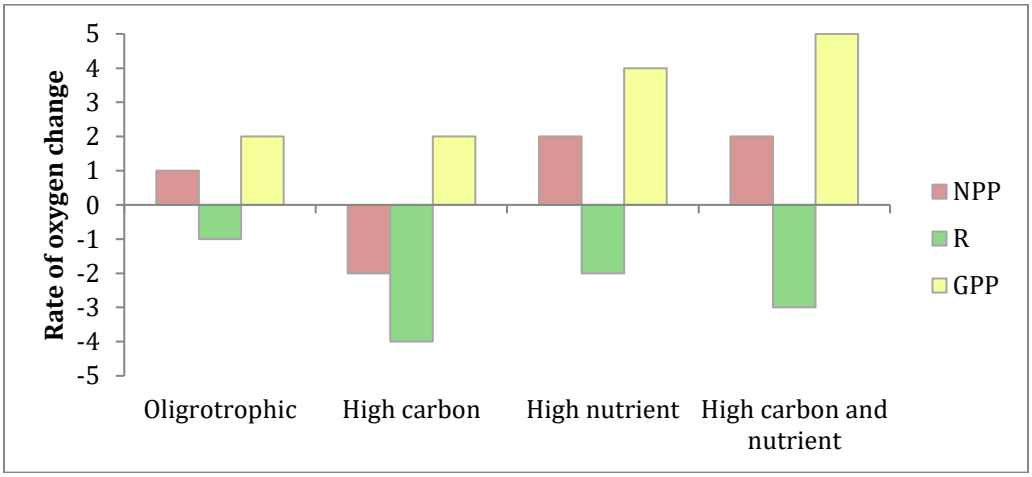


Figure 1. Conceptual diagram of respiration, net primary production and gross primary production in response to drivers.

Nutrients in streams

The trophic state of a stream is linked to the nutrient concentrations available. Nutrients, such as phosphorus (P) and nitrogen (N) in their different forms, can either limit or favor biological and chemical processes in a stream or lake (Stream Solute Workshop, 1990). A significant increase in their concentration can cause an environmental problem called eutrophication, in which the primary production is elevated leading to an excessive algae production that degrades the natural state of a stream (Dodds et al., 2002). With an excessive growth of algae, there is an increase in the biological demand of oxygen leading to a decrease in the oxygen concentration available for other organisms. This increase in nutrient concentration can come from point or non-point sources such as the WWTP, urban areas and agricultural activities, therefore judicious nutrient limits in permits are essential to maintain water quality.

Water quality standards in Puerto Rico are the chemical, physical or biological parameters and respective limits for the different types of water and classifications. For example, there are quantitative standards for dissolved oxygen, pH, turbidity, color, total dissolved solids,

nutrients and qualitative standards for Enterococci density. Due to the importance of nutrient concentration and the effect these have on streams, numeric criteria relevant to the tropical streams of Puerto Rico have been developed to establish background reference numeric concentrations (Sotomayor-Ramírez et al., 2011) (Table 1) and serve as the water quality standard for Class SD waters in Puerto Rico (PREQB, 2016). This allows for the assessment of stream water quality in Puerto Rico. It also allows classifying a stream by its trophic state in three categories: non-enriched, enriched or impaired. At the end, a better assessment serves to improve the recovery or maintenance of a stream.

Table 1. Numeric nutrient criteria suggested for the streams of Puerto Rico (Sotomayor et al., 2011)

Criteria	TN	NO_3-N	TP
	----------mg/L----------		
Non-enriched	<0.35	<0.25	<0.030
Enriched	0.35-1.70	0.25-0.97	0.030-0.160
Impaired	>1.70	>0.97	>0.160

TN is total nitrogen. TP is total phosphorus.

Benthic biomass

Benthic algae have also been used as water quality biological indicators. They are preferred since they have more time to adapt to the environment versus plankton, which flows rapidly through the stream (Bellinger and Sigee, 2010). The epilithic algal community biomass assemblage has been used to determine water quality due to its relationship with streams trophic state (Lyon and Ziegler, 2009; Gualtero-Leal et al., 2010; Viggiano-Beltrocco, 2014) and nutrient retention (Dodds, 2003). More specifically, periphyton biomass determination using chlorophyll-a has been related to P and N concentrations in temperate (Dodds et al., 2002) and tropical streams (Viggiano-Beltrocco, 2014). The quantification of the periphyton community biomass along with measuring benthic metabolism is crucial because periphyton influences the

water column interface, causing changes in dissolved oxygen (Dodds, 2003). Periphyton biomass can be estimated by scrapping a fixed area of rocks and cobbles from the stream or artificial substrates and biomass is quantified as the ash free dry mass (AFDM) and chlorophyll-a. Artificial substrates have an advantage over natural substratum due to the known accrual time of the community and increased reproducibility of an experiment (Viggiano-Beltrocco, 2014).

Sources of contamination

Contamination into streams comes from sources that are classified as point and non-point. Non-point sources are diffuse sources of contamination and the input may include nutrients that originate from fertilizers and organic residues from agricultural lands, oil and grease from urban runoff, sediments from erosion and construction sites, faulty septic tanks and acid from abandoned mines (USEPA, 2016). A point source of contamination is an identified source that can be credited as the producer of contaminants. Point sources can cause abrupt hydrological and chemical discontinuities along the stream in specific areas, while nonpoint sources are not as spatially discrete and cause more gradual changes (Merseburger et al., 2011).

Wastewater treatment plants (WWTP) are examples of point sources of contamination, but are essential to public health and communities. Water that comes into a WWTP is filtered from floating objects, pebbles and sand. The waters can pass through a Sequencing Batch Reactor (SBR) in which sewage is mixed with activated sludge, which are rich in specific bacteria, and is aerated and decanted. Water then passes through a disk filter, is disinfected using ultraviolet light and is returned into a stream. This complete process removes most organic and solid material that humans produce (Carey and Migliaccio, 2009).

For a treatment plant to be operating within the United States it must have a National Pollutant Discharge Elimination System Permit (NPDES) emitted by the United States

Environmental Protection Agency (USEPA). This permit specifies the maximum physical, chemical and biological effluent concentrations and specifies how they are to be monitored. Although plants have these permits, they do tend to have an impact on the receiving stream. One of the major impacts is the significantly increased nutrient loading to that receiving stream (Marti et al., 2004; Haggard et al., 2005; Gücker et al., 2006; Rodríguez-Castillo et al., 2017). This was recently confirmed by a study in Puerto Rico in which the wastewater treatment plant increased the nutrient inputs to the stream and reduced the nutrient retention capacity of the benthos downstream of the effluent (Figueroa-Nieves et al., 2016). Nevertheless, little is known about how WWTP discharge affects the receiving streams' ecosystems (Gücker et al., 2006; Figueroa-Nieves, 2014; Rodríguez-Castillo et al., 2017).

Agricultural activities are an example of non-point sources of contamination. Bare agricultural soils can produce sediments that reach streams, which consequently reduces light in the water column (Gücker et al., 2009), and therefore reduces the primary productivity. Also, excess fertilizer and manure application to crops in agriculture can cause leaching and runoff of nutrients and alter water column background concentrations (Griffiths et al., 2013; Gücker et al., 2009; Jarvie et al., 2008). The input of nutrients and sediments from agricultural activities can affect streams by increasing primary production and algae biomass (Griffiths et al., 2013) or decreasing macroinvertebrates with increased sediment concentrations (Piggot et al., 2015).

The metabolism of a stream responds to both proximal and distant alterations within a watershed at a measuring point (Bott and Newbold, 2013), and can respond to point and non-point sources of contamination. For example, it was found that elevated total N and total P inputs from a WWTP to a stream increased respiration downstream from the plant (Gücker et al., 2006). WWTP have been found to increase dissolved organic carbon (DOC) that eventually increased

the heterotrophic microbial activity (higher respiration than primary production) downstream (Ruggiero et al., 2006). In agricultural land areas, there is a discrepancy, as some report higher primary production (Gücker et al., 2009; Bernot et al., 2010), while others report higher respiration rates (Frankforter et al., 2010; Hagen et al., 2010; Bott and Newbold, 2013; Griffiths et al., 2013). Metabolism rates might also differ within a stream as primary production and respiration rates increased from upper to lower reaches of a tropical stream as a result of labile organic carbon input and opening of the canopy (Ortiz-Zayas et al., 2005).

Eastern Lajas Valley Watershed

The Lajas Valley in the southwestern part of the main island is an important agricultural reserve for Puerto Rico. Different crops, including forage, are produced and livestock are bred. This watershed contains sub-basins that have a mix of urban, forest, rural and agricultural land uses that allows for the comparison between the different proportions of the urban, sub-urban and rural uses. The drainage waters from the Eastern Lajas Valley Watershed (ELVW) are directed to the Caño de los Negros drainage canal that flows into the Gúanica Bay, located in Guánica. A study suggested that the Guánica Bay received nutrients from the near watershed, rather than the near bay environment (Whitall et al., 2013), although this information has not yet been validated. The need for more information makes the study of the ELVW an important ecological assessment.

Mondongo and Bárbara are sub-basins with contrasting land use that are being studied within the ELVW (Table 2). Mondongo was found to have a higher urban land use and Bárbara is predominantly unmanaged shrubland and forest with agricultural areas (Sotomayor-Ramírez et al., 2016). These sub-basins contain point and non-point sources of contamination such as house drain pipes, cropped areas or WWTP's. Sotomayor-Ramírez et al. (2016) have shown that

Mondongo Sub-basin, which contains a WWTP, seemed to have higher total phosphorus (TP) and total nitrogen (TN) concentration than Bárbara Sub-basin, although these were not significantly different.

Table 2. Land use distribution of Mondongo and Bárbara sub-basins (Details on Table 3, p.15).

Sub-basin	**Area**	**Unmanaged shrubland/Forest**	**Urban**	**Agriculture**
	(km^2)	----------(%)----------		
Mondongo	7.086	51.97	31.43	16.45
Bárbara	4.89	60.69	13.32	25.56

Source: Sotomayor-Ramírez et al., 2016.

The main goal of this investigation was to assess of benthic metabolism rates, trophic state, benthic biomass and physical and chemical parameters in two sub-basins, Quebrada Bárbara and Quebrada Mondongo that have contrasting land uses. A secondary goal was to link these parameters to possible contamination sources in both sub-basins. Relationships among the parameters were examined in order to achieve a more comprehensive knowledge of the status of two streams that drain different land uses and flow into the Guánica Bay.

Objectives

1. Assess stream metabolism rates and benthic biomass in two sub-basins with contrasting land use and sources of contamination.
2. Evaluate how stream metabolism and benthic biomass change as a result of potential sources of contamination within a sub-basin.

Materials and Methods

Study sites

The study site is the eastern portion of the Lajas Valley (ELVW) that is part of the Lajas Valley watershed. This part of the watershed covers four municipalities including Lajas, Sabana Grande and Guánica. The Lajas Valley has a width that ranges from 1.6 to 4.8 km and is a uniform plain that extends east to west for more than 32 km and has slopes of 0 to 4% in the lower elevations (Sotomayor-Ramírez and Pérez-Alegría, 2011). The soils in the Lajas Valley are predominantly Mollisols and Vertisols (USDA-NRCS, 2015). The average annual precipitation ranges between 1016-1270 mm with a semiarid climate. The Lajas Valley became an important agricultural area for Puerto Rico since the 1950's construction of the irrigation and drainage infrastructure system called "Proyecto del Suroeste" and with legislation that created the first agricultural reserve in PR. This project consists of a main irrigation channel that moves water from east to west, with secondary irrigation channels that run from north to south. The drainage channel to the south, called Caño de los Negros, receives excess water draining east into the Guánica Bay. In 1999, the Lajas Valley was declared an Agricultural Reserve by Act 277.

Sampling sites

The first sub-basin is Quebrada Mondongo (Qbda. Mo) (Figure 2). This sub-basin is part of the urban areas of Lajas municipality and has an area of 7.086 km^2 [1] with a population of 7,297. The predominant soils in this sub-basin are Descalabrado series (Clayey, mixed, superactive, isohyperthermic, shallow Typic Haplustolls) and Jácana series (Fine, mixed,

[1] Al basins were delineated by Sotomayor-Ramírez and Pérez-Alegría (2011). Area calculated for Z-268 project (Sotomayor-Ramírez et al., 2016).

superactive, isohyperthermic Vertic Haplustolls) (USDA-NRCS, 2015). The land use is 52% forest, but has a 31.43% of urban cover (Table 3). The top part of the sub-basin is mainly unmanaged forests and fallows land, the middle part has the Lajas urban center and in the outlet there are some hay crop areas and houses.

Table 3. Detailed land use description of Quebrada Mondongo and Quebrada Bárbara sub-basins

	Sub-Basins		
	ELVW [2]	Qbda. Mondongo	Qbda. Bárbara
Area (km^2)	**146.34**	**7,086**	**4.89**
Population[1]	**33,936**	**7,297**	**3,818**
	----------% of the total area----------		
Urban development	**9.31**	**31.43**	**13.3**
Agriculture[3]	**29.63**	**16.45**	**25.56**
• **Row crops**	10.58	2.01	4.8
• **Hay**	12.70	13.78	20.19
• **Grazed pastures**	6.35	0.65	0.57
Unmanaged shrubland/forest	**60.82**	**51.97**	**60.68**
Pond	**0.24**	**0.15**	**0.43**

[1] USDA-Census (2010)
[2] ELVW is the eastern Lajas Valley watershed
[3] Agriculture is the sum of row crops, hay and grazed pastures

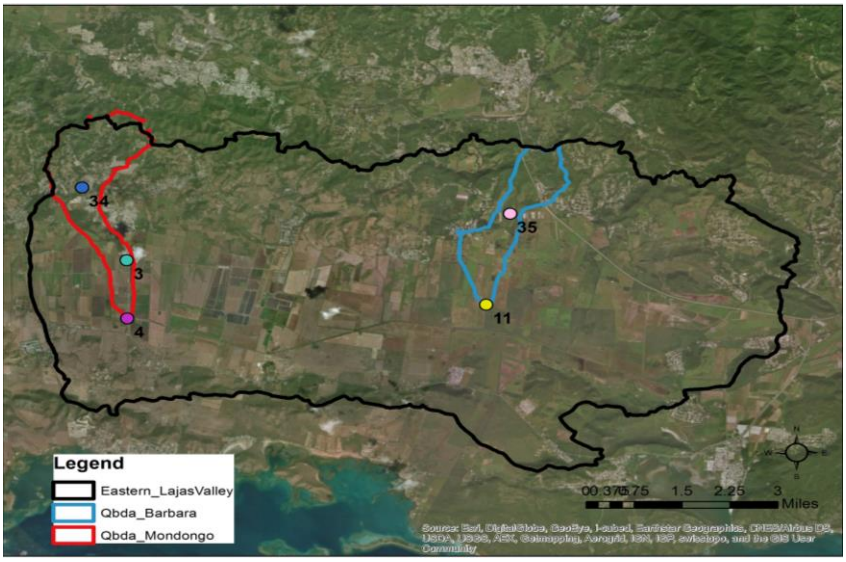


Figure 2. Map of the basins and stations in the Eastern Lajas Valley Watershed.

The Lajas municipality urban center is located near the middle of the sub-basin. In this area, the sub-basin receives point discharges of the Lajas WWTP at the outlet point 18.0503 N, -67.0617 W. This secondary treatment plant has a NPDES permit NO. PR0020575 to discharge a maximum of 1.2 million gallons a day (MGD) and has a monthly average of 0.733 MGD (Table 4) (USEPA, 2014). In the past years (2015-2017), this plant has been discharging concentrations close to their daily maximum effluent limits for most parameters. Not all of the parameters have had the same limit during these two years, such as TP, which increased from 1.0 mg/L to 8.26 mg/L and effluent flow which eliminated its limit of 1.2 MGD; both changes happened on May 2015 (ECHO, 2018). A filter plant known as the Lajas Water Treatment Plant (NPDES NO. PR002985) also discharges into the Quebrada Mondongo, downstream of the WWTP. The south part of the sub-basin consists of hay crops and isolated communities. This sub-basin discharges into the Caño de los Negros draining channel.

Table 4. Maximum daily concentrations permitted and reported in the outflow for the Lajas WWTP with NPDES Num. PR0020575.

Parameter	**Units**	**Daily maximum effluent[1]**	**Mean effluent discharge (Jan 2015 – June 2018)[3]**
Dissolved oxygen	mg/L	≥ 5.0	6.86
Effluent flow	MGD	1.2; no limit[2]	1.55; 1.06[4]
Temperature	˚C	32.2	29.72
NO_3-N + NO_2^--N	mg/L	10.0	0.93
NH_3 + NH_4^+	mg/L	6.0	2.27
Total phosphorus (P)	mg/L	1.00; 8.26[2]	2.28; 1.61[4]
Residual chlorine	mg/L	0.5	0.26

Source: [1]USEPA (2014). [2] New limits from May 2015. [3] Reports not available for Sept-Nov 2017, due to the impact of Hurricane María. ECHO (2018). [4] Mean effluent discharge from May 2015- June 2018.

There were three sampling sites in this stream (Figure 2). Station 34 (18.0477 N, -67.0609 W) (Appendix. 1 A) is located approximately 60 m upstream from the Lajas WWTP, but downstream from the urban center of Lajas Municipality. The sediments in this station were

sandy and abundant leaf litter was observed on most sampling dates. Riffles are observed upstream the station and a pond with fish was observed downstream of the station. The mean depth and width of the channel are 0.258 m and 1.0 m, respectively. Station 3 (18.0276 N, -67.0502 W) (Appendix. 1 B) is located 3,355 m downstream the Lajas WWTP and downstream of the Lajas WTP. This location had sandy sediments and pebbles. There is active erosion of the bank and the riparian vegetation and weeds are being controlled with herbicide by the Autoridad de Energía Eléctrica (AEE), which is the agency in charge of the maintenance of the drainage canal. The mean depth and width of the stream is 0.31 m and 1.47 m, respectively. Station 4 (18.0115 N, -67.05 W) (Appendix. 1 C) is located downstream of a community of approximately 54 houses where discharge pipes have been observed and are presumed to serve as conduits to Quebrada Mondongo. This station was also right before the outlet of the Quebrada Mondongo into the Caño de los Negros. Sediments in this station were sandy, it is completely unshaded and the riparian vegetation is also controlled with herbicides by the AEE. This station has a mean depth and width of 0.32 m and 1.59 m, respectively.

Quebrada Bárbara (Qbda. Ba) sub-basin is located between Lajas and Sabana Grande municipalities (Figure 2) and covers an area of 4.89 km^2, with a 60.69% of the area with unmanaged shrubland/ forest and a 25% of the area with agricultural land cover and a population of 3,403 (Table 3). The soils in this sub-basin are from Descalabrado series (Clayey, mixed, superactive, isohyperthermic, shallow Typic Haplustolls) and Fe series (Fine, smectitic, isohyperthermic Sodic Haplusterts) (USDA-NRCS, 2015). This stream also receives discharge from an upstream water treatment plant named Maginas WTP, NPDES permit No. PR0026727 (Table 5). The outlet of the effluent is located on 18.0438 N, -67.9500 W and USEPA has classified the discharge as minor with a limitation of 0.166 MGD.

Table 5. Maximum daily concentrations permitted and reported in the outflow for the Maginas WTP with NPDES Num. PR0026727.

Parameter	**Units**	**Daily maximum effluent[1]**	**Mean effluent discharge (Jan 2015 – June 2018)[2]**
Dissolved oxygen	mg/L	≥ 5.0	6.37
Effluent flow	MGD	0.166	0.36
Temperature	˚C	32.2	28.4
$NH_3 + NH_4^+$	mg/L	1.0	0.23
Total phosphorus (P)	mg/L	0.16	0.36[4]
Residual chlorine	µg/L	7.5[3]	17.78 mg/L[5]

Source: [1]NPDES, USEPA 2015. [3] Reports not available for Sept-Nov 2017, due to the impact of Hurricane María. ECHO (2018). [3]Limit reduced from 0.5mg/L to 7.5 µg/L in March 2016. [4] Outlier of 139 mg P/L on Feb 2016 removed from mean. [5]Reports until March 2016; reports after April 2016 were 0 µg/L.

There were two sampling sites in this sub-basin (Figure 2). The first is located before a banana orchard, Station 35 (18.0407 N, -66.9591 W) (Appendix. 1 D). This station had sandy sediments and rocks. This station has the PR-117 road upstream which has a culvert that allows water to pass from one end to the other of the road. The average depth and width of this station are 0.22 m and 0.79 m, respectively. The second site is located in a hay farm downstream from an observed hog production area, with no known operation permit, and downstream of the banana orchard (Station 11, 18.0155 N, -66.9647 W) (Appendix. 1 E). This station had sandy sediments and was observed to have algae mats floating on more than one occasion. Riparian vegetation is also controlled with herbicides by the AEE. The average depth and width of this station are 0.27 m and 0.86 m, respectively.

Fields measurements

Sampling was conducted from June 2016 until March 2017 (Appendix 2). On each sampling day, physical and chemical parameters were measured *in situ*. Dissolved oxygen, specific conductance, pH and temperature were measured using a YSI Pro-Plus Multiprobe

(Yellow Spring Instruments). Stream velocity was measured with a flow meter (Flowatch, JD Electronics SA) and stream discharge was determined as the product of stream velocity, depth and width.

Nutrient analysis

Water samples were sampled and tested for nutrient concentrations three times on each station. Stream surface water samples were collected using pre-washed 1-L Nalgene bottles. A 25ml subsample was filtered (Whatman GMF 0.45µm syringe), capped and stored. Samples were preserved to pH<2 with H_2SO_4 and transported to the laboratory in dark coolers at 4°C to be analyzed within the next 30 days. Total Kjeldahl nitrogen (TKN; EPA method 351.2) and total phosphorous (TP; EPA method 365.2) were measured from the unfiltered sample. NO_3-N (EPA method 353.1) and total dissolved phosphorus (TDP) were measured from the filtered sample. All nutrient analyses were done at the Soil and Water Chemistry Laboratory of the UPR-AES in Río Piedras, Puerto Rico, under the direction of Dr. Gustavo Martínez-Rodríguez.

Benthic stream metabolism

Benthic stream metabolism was measured at all stations in the two selected sub-basins of the ELVW using water-recirculating chambers. Based on the depth of the stream and water column, it was expected that the total stream metabolism (water column and benthos) was due to that occurring in the benthos, thus I used the term benthic stream metabolism. Recirculating metabolism chambers were designed according to the suggested dimensions of a 4:1, length and width ratio (Dodds and Brock, 1998). Chamber specifications were 0.152 m width, 0.686 m length and 0.161 m height, with an open bottom. They were constructed using 0.63 cm wide clear acrylic (92% transmittance of photosynthetically active radiation [PAR]) Plexiglas sheets for the exterior and assembled using nylon screws (Figure 3). A 12V DC motor (with the

capacity of 5152 rpm) was coupled to a 0.32 cm stainless still rod with a four blade plastic propeller to recirculate the water. Water recirculated from the bottom of the chamber to the top through a 10.16 cm PVC elbow. A YSI Pro-Plus Multiprobe (Yellow Spring Instruments) was installed into the chamber, across from the axial propeller. This design was based on the chamber design by Rüegg et al. (2015) and was modified and constructed with the help of Miguel Vázquez, laboratory technician at the UPR-AES in Río Piedras, Puerto Rico.

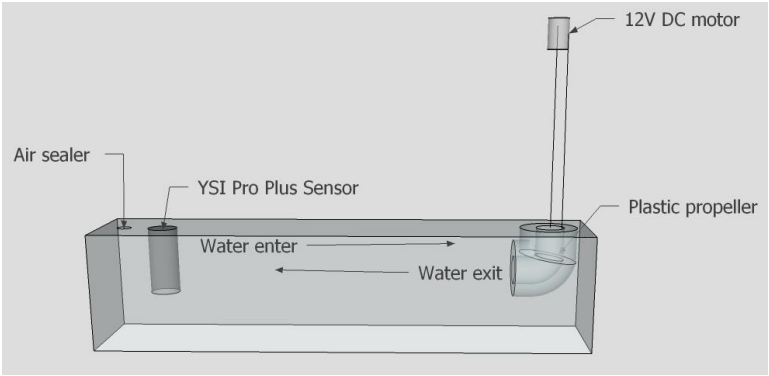


Figure 3. Metabolism chamber construction design.

Two chambers were used simultaneously to assess stream metabolism. Primary production rates were measured using an uncovered chamber and respiration rates were measured using the same chamber, covered with impermeable gray plastic fabric to block light. Both chambers were pushed 2.54 cm into the stream sediment. Temperature and DO concentrations were measured inside of the chamber using a YSI Pro-Plus Multiprobe at 1min intervals for a period of 1 hour, as suggested by Ramírez et al. (2003). Measurements were made at least 4 times at each station (n=4) in the hours from 10:00 am to 2:00pm when the light intensity was expected to be at its maximum. Light intensity (Lux) was measured inside of the uncovered chamber using a light sensor (HOBO Pendant Temperature/Light Data Logger 8K).

Measurements of the net change of dissolved oxygen in light and darkness were used to

estimate the NPP and R, respectively. Rates of NPP and R were achieved by using Equation [1], from Bott (2006):

$$\text{NPP or R } (g\ O_2/\ m^2 * h) = \frac{\text{slope } O_2\left(\frac{mg}{L}\right) * \text{Volumen in chamber (L)} * 60\ \text{min/h}}{\text{substratum area } (m^2) * 1000 mg/g} \qquad [1]$$

were slope was the rate of change of the dissolved oxygen concentration in 1 hour, chamber volume was 11.1 L and 11.15L for uncovered chamber and covered chamber, respectively, and surface area was 0.089 m^2. GPP was calculated as the sum of the NPP and R [2].

$$GPP = NPP - [- R] \qquad [2]^2$$

A production versus consumption ratio, known as P/R was calculated as GPP/R in order to classify the station as heterotrophic or autotrophic. Rates were also converted into carbon units for GPP [3], where PQ is the photosynthetic quotient which is the moles of O_2 released during photosynthesis or moles of CO_2 incorporated. A value of 1.2 is used as the PQ because it is the molar ratio between O_2 released and the CO_2 assimilated during photosynthesis (Wetzel, 2001). Carbon units of R were calculated using equation [4], were RQ is the respiratory quotient which is the moles of CO_2 released or moles of O_2 consumed. A value of 0.85 is used as RQ because it is the molar ratio between CO_2 released and the O_2 consumed during respiration (Wetzel, 2001; Pennington et al., 2018). The ratio 12/32 is the atomic weight of C to that of O_2 (Bott, 2006).

$$g\ C\ m^{-2}\ h^{-1} = g\ O_2 \times 1/PQ \times 12/32 \qquad [3]$$

$$g\ C\ m^{-2}\ h^{-1} = g\ O_2 \times RQ \times 12/32 \qquad [4]$$

The temperature inside of the chambers, especially the uncovered chamber, increased during the

[2] The known metabolism equation is GPP=NPP + R, but because R is a rate of consumption it is a negative value, which means that the equation is actually GPP = NPP – [-R].

1-hour assays (Appendix 6). A temperature correction was applied to both NPP and R measurements using van't Hoff-Arrhenius coefficient to account for this increase in temperature during incubation. Parkhill and Gulliver (1999) equation used by Riley and Dodds (2013) was modified to normalize essays for the initial temperature on each chamber [5]:

$$\text{Rate corrected} = \text{Rate}/\ (1.045^{[T_{avg} - T_i]}) \qquad [5]$$

where Rate is the rate of metabolism calculated (NPP or R) , T_{avg} is the average internal chamber temperature in ˚C measured during each 1-hour assay and T_i is the initial chamber temperature during the assay.

Benthic biomass determination

Benthic biomass was quantified from the periphyton biomass that occurred in a 7-day assay using artificial substrates (Viggiano-Beltrocco, 2014). The blocks used were the same used by Viggiano-Beltroco (2014). Twelve concrete blocks with dimensions of 10 cm long, 8 cm wide and 8 cm height (640cm^3) were placed in the streams in groups of 4, for 7 seven days. After the 7 days, the blocks were randomly taken from the stream into 4 groups and each group was a 3-block composite sample. Increased flow was experienced due to heavy rain events during some incubation periods; therefore some blocks were lost in the current or covered by sediment, thus some stations had 3 and others had 4 replicates. Three sampling events were performed in the Quebrada Mondongo stations. Quebrada Bárbara Station 35 had 4 events and Station 11 had 3 events. Periphyton samples were scrubbed off the blocks from a 5.75 cm diameter ring area into a 500mL amber bottle using a soft toothbrush and stream water. Samples were transported in coolers to the laboratory to be analyzed within the next 21 days.

These samples were analyzed for chlorophyll-a with a Turner 10-AU fluorometer (Welschmeyer, 1994). The periphyton biomass was calculated using equation [6]:

$$\mathrm{Chl-a_{final}}\left(\frac{\mathrm{mg}}{\mathrm{m^2}}\right) = \frac{\mathrm{Chl-a_{raw}\ x\ V_{sample}\ x\ V_{extracted}\ x\ F.D.}}{\mathrm{V_{filtered}\ x\ n\ x\ Area}} \qquad [6]$$

where Chl-a_{final} is the final value in mg/m^2; Chl-a_{raw} is the fluoresce reading in the fluorometer in µg/L; V_{sample} is the sample collected (500mL); $V_{extracted}$ was the acetone extracted (0.015 L); F.D. is the dilution factor; $V_{filtered}$ is the total sample filtered (20mL); n is the number of blocks used; Area is the scrapped area of the blocks (0.002597 m^2).

Ash free dry mass (AFDM) procedure was performed by filtering 250 mL of the sample thru a 47 mm glass microfiber filter and measuring the weight loss after incineration at 500°C for 4 hours. AFDM was calculated as [7]:

$$\mathrm{AFDM}\left(\frac{\mathrm{g}}{\mathrm{m^2}}\right) = \frac{\mathrm{(W_1\text{-}W_2)\ x\ V_{sample}}}{\mathrm{V_{filtered}\ x\ n\ x\ Area}} \qquad [7]$$

where W_1 is the weight of the crucible + filter + filtered sample after dried in oven for 24 h; W_2 is the weight of crucible + filter + sample after incineration; V_{sample} is the total sample volume of 500 mL; $V_{filtered}$ is the volume of sample filtered (250 mL); n is the number of blocks used; Area is the area scrapped from the blocks (0.002597 m^2).

Both analyses were performed in the Soils and Water Laboratory in University of Puerto Rico at Mayagüez. An autotrophic index (AI) was calculated as the ratio between AFDM/Chl-a (USEPA, 2000). Increased ratio means that the larger percent of AFDM are heterotrophs, rather than autotrophs (Weber, 1973).

Data analysis

An ANOVA Fisher LSD ($p<0.05$) was used to compare physical and chemical analysis, benthic biomass and benthic metabolism means between sub-basins and between stations within the sub-basins. Data for stream flow, light intensity, temperature, specific conductance, pH and dissolved oxygen were log-transformed when needed to homogenize the variance. The data were corrected for normality using the Shapiro-Wilks test. T-student test were also performed among

indicators between basins and between stations within the basins. Only significant difference for this test are shown. Contrast analyses were performed to compare physical and chemical analysis, benthic biomass and benthic metabolism means between sub-basins including Stations 3 and 4 from Quebrada Mondongo versus Stations 35 and 11 from Quebrada Bárbara. Station 34 of Quebrada Mondongo was left out of this analysis, as it was considered to be an upstream station. Pearson correlation analysis was generated to establish relations among variables in both sub-basins. Data were processed with Infostat Software (National University of Córdoba, Argentina).

Results

Physical and chemical parameters

Physical and chemical parameters such as stream flow, temperature, specific conductance, pH and DO varied significantly ($p<0.05$) between Quebrada Mondongo and Quebrada Bárbara (Table 6). Quebrada Mondongo had higher temperature, specific conductance and stream flow, while Quebrada Bárbara had higher DO and pH. Light intensity (Lux) did not vary significantly between sub-basins.

Table 6: Comparison of physical and chemical parameters between Quebrada Mondongo and Quebrada Bárbara sub-basins.

Sub-basin	Flow	Intensity	Temperature	SEC	pH	DO
	(m^3/min)	Lux	(ºC)	(µS/cm)		mg/L
Qbda. Mo	3.99*[1]	159,861 ns[2]	29.01*	1733.17*	7.90*	5.46*
Qbda. Ba	1.61	157,738	24.98	587.88	8.55	7.25
Mean	3.02	158993	27.48	1,300.0	8.14	6.13

[1]Values with * are significantly different ($p<0.05$) between sub-basins. [2]ns denotes that values are not significantly different. Qbda. Mo = Quebrada Mondongo; Qbda. Ba = Quebrada Bárbara.

Quebrada Mondongo had three stations along the stream. Station 34 was upstream of the WWTP and Station 3 and 4 where downstream of the WWTP. Mean stream flow varied among stations, but was lower upstream of the WWTP (1.33 m^3/min) than downstream, where it was almost four times higher (4.74 m^3/min) ($p<0.05$) (Table 7). Light intensity, temperature, pH, SEC and DO were significantly different in Station 34 than Station 3 and Station 4. Mean temperature increased by 2 ºC, SEC was 5 times higher and DO increased almost twice downstream from the WWTP. Stream flow, temperature, SEC, and DO were similar between Station 3 and 4 and were higher downstream of the Lajas WWTP. Water pH was significantly different among stations, with the highest mean value found on Station 4 (8.07) and the lowest in Station 34 (7.63).

Table 7. Means of chemical and physical parameters measured in stations within Quebrada Mondongo.

Sub-basin	Station		Flow		Intensity		Temp[1]		SEC[2]		pH		DO[3]
		n=	(m^3/min)	n=	Lux	n=	(ºC)	n=	(µS/cm)	n=		n=	(mg/L)
Qbda. Mo	**34**	7	1.33 a[4]	4	126,930.21 a	7	27.03 a	7	417.97 a	7	7.63 a	7	3.48 a
	3	9	4.74 b	4	185,378.89 b	9	29.34 b	9	2264.56 b	9	7.88 b	9	6.29 b
	4	12	5.25 b	5	165,792.62 b	12	29.91 b	12	2101.83 b	12	8.07 c	12	5.99 b
Mean			3.99		159,861.5		29.01		1733.17		7.90		5.46

[1]Temperature, [2] Specific conductance, [3] Dissolved oxygen. [4]Values with different letters or * are significantly different ($p<0.05$). Qbda. Mo = Quebrada Mondongo.

Table 8. Means of chemical and physical parameters measured in stations within Quebrada Bárbara.

Sub-basin	Station		Flow		Intensity		Temp[1]		SEC[2]		pH		DO[3]
		n=	(m^3/min)	n=	Lux	n=	(ºC)	n=	(µS/cm)	n=		n=	(mg/L)
Qbda. Ba	**35**	10	1.89 ns[4]	4	167,963.70 ns	7	25.83 ns	7	419.27*[5]	7	8.30*	7	6.42*
	11	10	1.27	5	149,558.76	10	24.38	10	705.90	10	8.73	10	7.83
Mean			1.61		157,738.7		25.00		587.88		8.55		7.25

[1]Temperature, [2] Specific conductance, [3] Dissolved oxygen. [4] ns denotes that values are not significantly different. [5]Values with * are significantly different ($p<0.05$). Qbda. Ba = Quebrada Bárbara.

Quebrada Bárbara drains water from a predominantly rural sub-basin and had two stations along the stream. Station 35 is upstream of a banana orchard and Station 11 is downstream, but inside a hay farm. Stream flow, temperature and light intensity were not significantly different between Station 35 and Station 11 ($p < 0.05$) (Table 8). Station 11 had significantly higher water pH, SEC and DO than Station 35.

Nutrient concentrations

Total N, NO_3-N, TP and DP were significantly different ($p<0.05$) between Quebrada Mondongo and Quebrada Bárbara. Means were found to be higher in Quebrada Mondongo and most were more than twice higher than the mean of Quebrada Bárbara (Table 9).

Table 9. Mean nutrient comparison among Quebrada Mondongo and Quebrada Bárbara sub-basins.

Sub-Basin	**TN**	**NO_3-N**	**TP**	**DP**
	----------mg/L----------			
Qbda. Mo	4.19*[1]	2.01*	0.96*	0.81*
Qbda. Ba	1.50	0.65	0.31	0.05

[1]Values with * are significantly different ($p<0.05$) between sub-basins. Qbda. Mo = Quebrada Mondongo; Qbda. Ba = Quebrada Bárbara.

Nutrient concentrations did not differ significantly between stations within each sub-basin, with the exception of NO_3-N. NO_3-N was significantly higher downstream from the WWTP ($t(4)=2.95$, $p<0.05$) (Figure 4). Although other nutrient means were not found significantly different upstream vs. downstream from the WWTP in Quebrada Mondongo, we observe a numerical increase in the mean concentrations of TN, TP and DP in the downstream waters. The mean concentrations of TN and TP were three times higher, NO_3-N was twice higher and DP was five times higher downstream from the WWTP. In Quebrada Bárbara, NO_3-N was higher in Station 11 (1.04 mg/L) than Station 35 (0.26 mg/L) (Figure 5). The mean TP and DP

concentrations substantially decreased from Station 35 to Station 11, and a mean NO_3-N increased from Station 35 to Station 11, but these means were not significantly different.

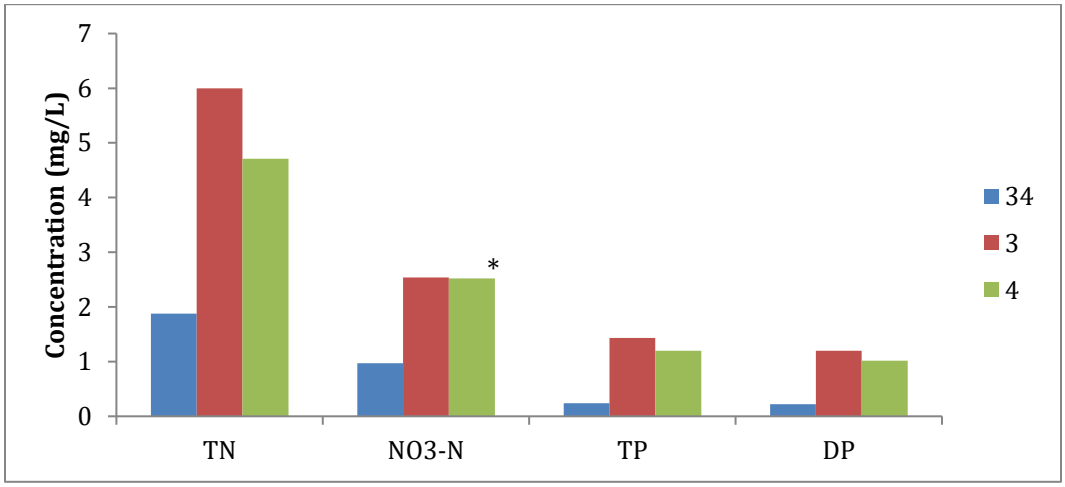


Figure 4. Mean nutrient concentrations in stations within Quebrada Mondongo. Each value is the mean of three replicates. Values with * are significantly different ($p<0.05$).

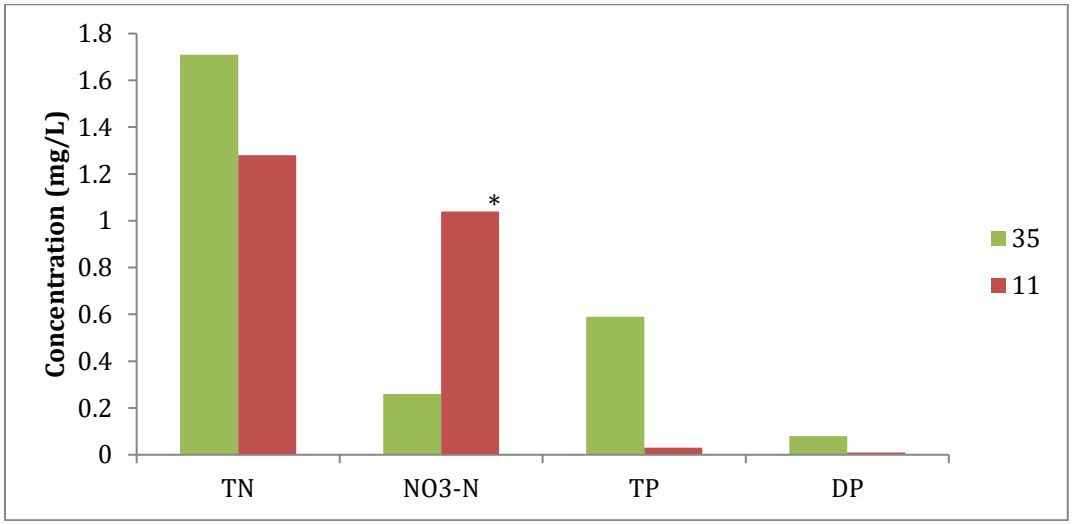


Figure 5. Mean nutrient concentrations in stations within Quebrada Bárbara. Each value is the mean of three replicates.Values with * are significantly different ($p<0.05$).

Comparing the mean values with the numeric nutrient criteria suggested by Sotomayor-Ramírez et al. (2011) (Table 1), 40% of the water samples in both streams were classified as "enriched" and 60% of the samples were classified as Impaired for TN (Table 10). Of the waters sampled for NO_3-N and TP, 73% were classified as "impaired". The only samples classified as

"non-enriched" were found for TP in Station 11 and for NO_3-N in Station 35. As for Quebrada Bárbara, the waters in Station 35 were classified as "impaired" for TN and TP and "enriched" for NO_3-N. In Station 11, the waters were classified as "impaired" for NO_3-N, "enriched" for TN and "non-enriched" for TP.

Table 10. Relative distribution of the mean nutrient concentration values as classified using suggested nutrient numeric criteria for Puerto Rico (Sotomayor-Ramírez et al., 2011).

Criteria	TN	Distr.[1]	NO_3-N	Distr.	TP	Distr.
	mg/L	%	mg/L	%	mg/L	%
Reference	<0.35	0	<0.25	13.3	<0.030	13.3
Enriched	>0.35-1.70	40	>0.25-0.97	13.3	0.030-0.160	13.3
Impaired	>1.70	60	>0.97	73.3	>0.160	73.3

[1]Frecuency Distribution

Benthic biomass

Periphyton biomass varied significantly between Quebrada Mondongo and Quebrada Bárbara. Quebrada Mondongo had significantly higher periphyton biomass ($p<0.05$), but Quebrada Bárbara had higher Ash Free Dry Mass (AFDM) ($p<0.05$) (Table 11). The Autotrophic Index was also significantly different between Quebrada Mondongo and Quebrada Bárbara ($p<0.05$).

Table 11. Mean concentrations of periphyton biomass between Quebrada Mondongo and Quebrada Bárbara.

Sub-Basin	Chl-a	AFDM	AI
	----------mg/m^2----------		
Qbda. Mo	57.14*[1]	7,157.48*	383.74*
Qbda. Ba	8.81	17,982.82	2,973.75

[1]Values with * are significantly different ($p<0.05$) between sub-basins. Qbda. Mo = Quebrada Mondongo; Qbda. Ba = Quebrada Bárbara.

The highest periphyton biomass in Quebrada Mondongo was found in Station 4 (78.94 mg/m^2) (Table 12) and was about two times higher than upstream the WWTP, but it was not different from the other stations. The highest (AFDM) mean value was found on Station 4, but

was not significantly different from Station 3. Also, the Autotrophic Index was not significantly different among stations in Quebrada Mondongo, although the mean of Station 34 was 5 times higher than the mean of Station 4. In Quebrada Bárbara, the lowest mean value of periphyton biomass was found in Station 11 (4.27 mg/m^2) and was significantly lower that Station 35 (13.34 mg/m^2) (Table 13). Station 11 had higher AFDM, but was not significantly different from Station 35. The Autotrophic Index was significantly higher in Station 11, and was about 4 times higher than Station 35.

Table 12. Mean concentration of periphyton biomass in stations within Quebrada Mondongo.

Sub-basin	Station	Chl-a	AFDM	Autotrophic Index
		----------mg/m^2 ----------		
	34	33.80[1] ns[2]	5,873 ns	767.28 ns
Qbda. Mo[1]	3	58.68	6,863	232.95
	4	78.94	9,038	151.00
Mean		57.14	7157.48	383.74

[1] Values are means of three replicates. [2]ns denotes that values are not significantly different. Qbda. Mo = Quebrada Mondongo.

Table 13. Mean concentration of periphyton biomass in stations within Quebrada Bárbara.

Sub-basin	Station	Chl-a	AFDM	Autotrophic Index
		----------mg/m^2 ----------		
Qbda. Ba[1]	35	13.34*[2]	15,963 ns[3]	1,208.14*
	11	4.27	20,001	4,739.36
Mean		8.81	17,982.82	2,973.75

[1] Values are means of three replicates. [2]Values with * are significantly different ($p<0.05$). [3]ns denotes that values are not significantly different. Qbda. Ba = Quebrada Bárbara.

For benthic biomass samples it is usual to see periphyton biomass increase as AFDM increases. This was observed in Quebrada Mondongo numerically and visually (Appendix 3: A and B), but was not the case for Quebrada Bárbara, as periphyton biomass decreased, but AFDM increased numerically and visually (Appendix 3: C). I observed that the periphyton community that grew on the substrates of Station 11 were darker, did not show high chlorophyll biomass and were dense. This allowed for a higher AFDM, but low chlorophyll-a numbers.

Benthic metabolism of the creeks

Benthic metabolism rates were compared between sub-basins when normalized to their initial chamber temperature during each assay. NPP values ranged from -0.23 to 0.25 g O_2/ m^2*h, were higher in Quebrada Bárbara, but were not significantly different between both sub-basins ($p<0.05$) (Table 13). R rates ranged from 0.07 to 0.40 g O_2/ m^2*h. Higher numerical R rates were found in Quebrada Mondongo, but were not significantly different from the rates found in Quebrada Bárbara ($p<0.05$). GPP rates ranged from -0.09 to 0.38 g O_2/ m^2*h and were not significantly different between sub-basins ($p<0.05$). During the measurements, temperature inside the chambers increased approximately 1.77˚C and 0.51˚C in the light and dark chambers, respectively (Appendix 6, Table 2).

Table 14. Comparison of mean net primary production, respiration and gross primary production between Quebrada Mondongo and Quebrada Bárbara.

Sub-Basin	NPP	R	GPP	GPP/R
	---------g O_2/ m^2*day-------			
Qbda. Mo	-2.95 ns[1]	4.28 ns	1.34 ns	0.24 ns
Qbda. Ba	-0.95	3.17	2.22	0.65
Mean	-2.13	3.83	1.70	0.41

[1]ns denotes that values are not significantly different. Qbda. Mo = Quebrada Mondongo; Qbda. Ba = Quebrada Bárbara.

When comparing among stations within sub-basins, none of the parameters were significantly different ($p<0.05$) within Quebrada Mondongo sub-basin (Figure 6). Nevertheless, some trends could be identified on both sub-basins. In Quebrada Mondongo, rates of NPP, R and GPP reflected a numerical increase from upstream of the WWTP to downstream of the WWTP. Downstream in Station 4, rates stopped increasing as R and NPP became smaller, therefore reducing GPP rates as well (Table 14). Station 3 exhibited higher carbon units for both R and GPP than the other sub-basin stations. The ecosystem reflected an heterotrophic state throughout

the whole stream (GPP/R <1). On the other hand, all rates numerically increase in Quebrada Bárbara (Figure 7). Furthermore, the system changed from GPP/R of <1 to GPP/R >1, indicating a change from a heterotrophic to an autotrophic environment. GPP/R ratio was found to be <1 in Stations 34, 3, 4 and 35 indicating that these ecosystems are heterotrophic on those stations and Station 11 was the only one with a GPP/R relationship of >1, suggesting that it is an autotrophic ecosystem.

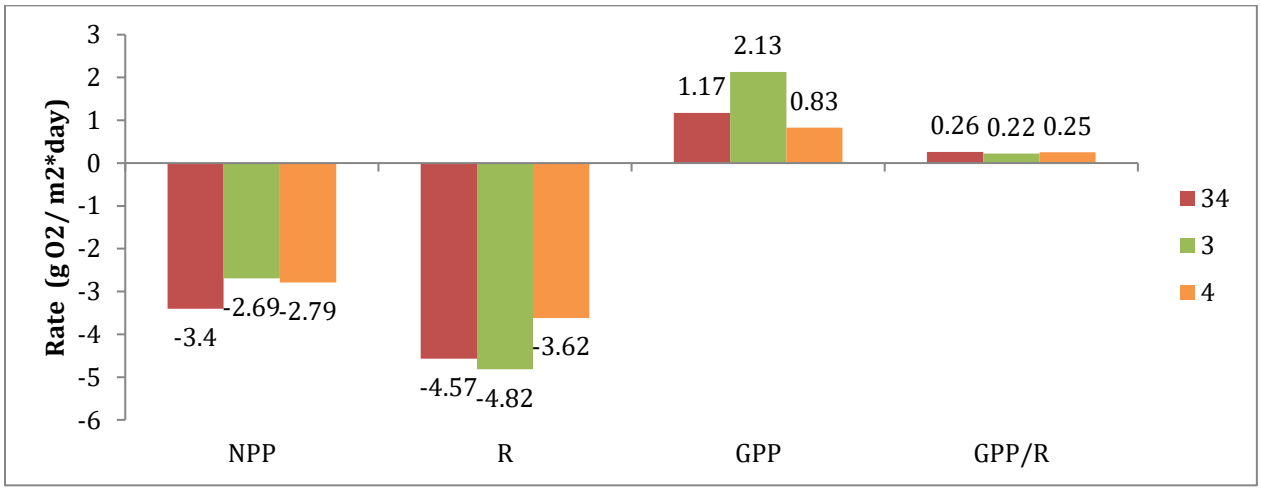


Figure 6. Metabolism rates measured on each station of Quebrada Mondongo.

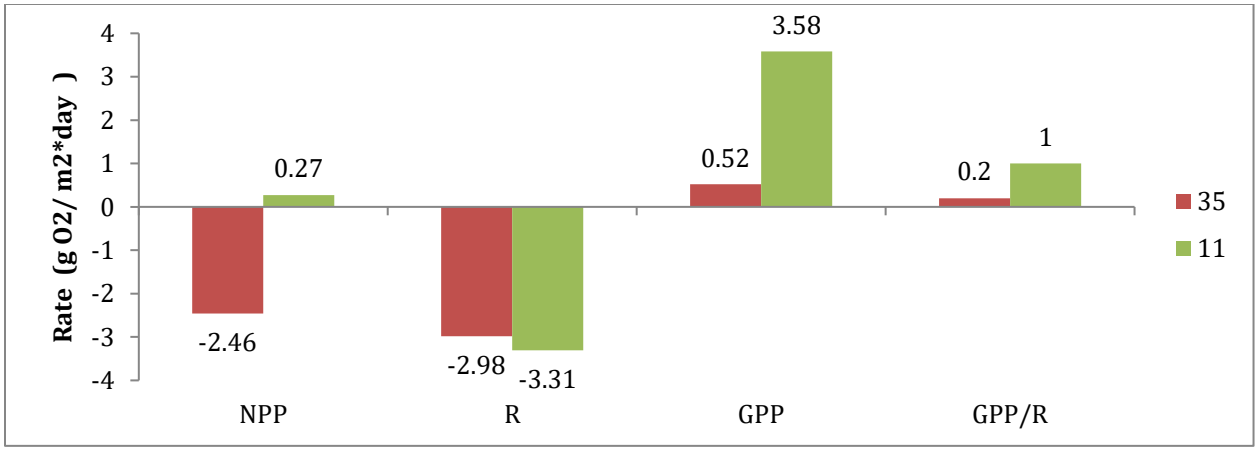


Figure 7. Metabolism rates measured on each station of Quebrada Bárbara.

Contrast analysis

A contrast analysis was done with downstream stations of both sub-basins, meaning the exclusion of Station 34 in Quebrada Mondongo. This analysis revealed that downstream stations at Quebrada Mondongo had higher temperature, specific conductance, stream flow, TN, NO_3-N, TP, DP and periphyton biomass (Chl-a) ($p<0.05$). Quebrada Bárbara had higher DO, pH, AFDM and AI ($p<0.05$). Light intensity, NPP, R and GPP were not different between both sub-basins ($p<0.05$).

Correlation analysis

A Pearson's Correlation analysis was generated for all values in both sub-basins. Stream flow was correlated to SEC ($r=0.91$, $p<0.05$) NO_3-N ($r=0.81$, $p<0.05$), DP ($r=0.60$, $p<0.05$), TN ($r=0.63$, $p<0.05$) and temperature ($r=0.68$, $p<0.05$). SEC was correlated to TN ($r=0.69$, $p<0.05$) and NO_3-N ($r=0.84$, $p<0.05$). Periphyton biomass was positively correlated with TN ($r=0.71$, $p<0.05$), NO_3-N ($r=0.61$, $p<0.05$), TP ($r=0.64$, $p<0.05$), DP ($r=0.61$, $p<0.05$), temperature ($r=0.65$, $p<0.05$) and SEC ($r=0.55$, $p<0.05$). GPP was positively correlated to both NPP ($r=0.79$, $p<0.05$) and R ($r=0.45$, $p<0.05$). NPP ($r=0.73$, $p<0.05$) and GPP ($r=0.47$, $p<0.05$) rates were both correlated to pH. NPP and GPP were positively correlated to AI ($r=0.60$, $p<0.05$) and ($r=0.53$, $p<0.05$).

Discussion

Quebrada Mondongo and Quebrada Bárbara were studied for a period of 9 months. Both streams are geographically close, but stream water drains from sub-basins with different land uses and with potential point and non-point sources of contamination. Quebrada Mondongo was identified as the sub-basin with the larger urban area and population density and Quebrada Bárbara was identified as a forest and agricultural activity sub-basin. Both sub-basins were compared with each other and compared among established sampling stations.

As Quebrada Mondongo and Quebrada Bárbara differ in land use, they also differed in most of the chemical and physical parameters. Quebrada Modongo had higher streamflow, and was probably due to the input of the WWTP, which had been calculated to be approximately 42% of the total stream discharge (Sotomayor-Ramírez et al., 2016) and was measured to constitute approximately 72% of the total stream discharge in this study. The Lajas WWTP plus the WTP discharges a higher water volume than does the Maginas WTP and other sources discharging into Quebrada Bárbara. Additionally, Quebrada Mondongo had a higher urban area, which tends to increase runoff into the stream. Quebrada Mondongo actually fits characteristics expected for an urban stream, as urban streams tend be have higher water temperature, specific conductance and nutrient concentrations as urbanization increases (Ramírez et al., 2009). Light intensity conditions were considered previously to establishing sampling stations; light conditions did not vary between sub-basins.

Urban streams, especially those receiving WWTP inputs tend to have high nutrient concentrations. Quebrada Mondongo receives high concentrations of nutrients from the WWTP (Table 4), in addition to the potential discharge of the surrounding communities with documented discharging household pipes. Quebrada Bárbara has a WTP, but it has been

recorded to discharge fewer amounts of nutrients into the stream (Table 4). It also has an area that may contribute relatively low nutrient concentrations, other than NO_3-N. All of this resulted in Quebrada Mondongo having significantly higher TN, NO_3-N, TP and DP concentrations than Quebrada Bárbara. Higher nutrient concentrations, especially N and P, have been related to higher algae in streams (Dodds, 2003; Lyon and Ziegler, 2009; Gualtero-Leal et al., 2010; Viggiano-Beltrocco, 2014); this was observed in the results as Quebrada Mondongo had higher nutrient concentrations, which led to higher periphyton biomass (Chl-a). Also, urbanization of basins tends to increase benthic algae biomass due to higher light availability and increase nutrient levels (Alberts et al., 2018) and can increase nutrient concentrations measured in the biofilm (Qu et al., 2017). This was confirmed as periphyton biomass was positively correlated with total P, NO_3-N, TP and DP. However, Quebrada Bárbara experimented higher AFDM concentration, probably due material being deposited in the cement block over the 7-day incubation period (observe picture in Appendix 3:C). This resulted in differences in the Autotrophic Index between both streams, suggesting that Quebrada Bárbara was more heterotrophic that Quebrada Mondongo.

Benthic stream metabolism rates were numerically different for Quebrada Mondongo and Quebrada Bárbara, but did not show significant differences when normalized to initial chamber temperature. Even so, high rates of R were observed in Quebrada Mondongo which are expected in urban streams, especially those receiving treated waters, due to the inputs of carbon and nutrients (Paul and Meyer, 2001; Izagirre et al., 2008; Rodríguez-Castillo et al., 2017). On the other hand, higher rates of primary production are usually expected in stream draining agricultural basins due to the input of nutrients and reduced riparian shading. Quebrada Bárbara did not have the highest nutrient concentrations, but did have relatively high NPP rates, which

have been observed in other studies of agricultural basins (Bernot et al. 2010; Griffiths et al., 2013). GPP is a calculated value that depends on the measured R and NPP rates; therefore, the lack of significant difference resulted in a lack of difference in GPP rates between both sub-basins, even though Quebrada Bárbara seemed to have higher rates. Both sub-basins had mean GPP/R ratios of <1, suggesting heterotrophic states. This implies that both sub-basins are receiving high amounts of allochthonous energy, probably coming from the WWTPs in the case of Quebrada Mondongo, as it was expected. No significant correlations were found between NPP, R and GPP with other chemical or physical parameters, as reported by Hagen et al. (2010), except for NPP and GPP with AI and pH.

A limited amount of studies have examined on metabolism rates in the tropics and in Puerto Rico, but rates found on this study were within the range in other studies in tropical and subtropical region, especially Puerto Rico (Figure 6 and 7). For Puerto Rico, rates of stream metabolism have been studied, although must studies have been performed in El Yunque National Rainforest, which is a natural reserve located in the east part and receives high amounts of rainfall. Rates of R and GPP varied from 2.44 to 4.96 and 0.19 to 1.77 g O_2/ m^2*d, respectively (Ortiz-Zayas et al., 2005). Later, rates of R and GPP were documented to vary from 0.2 to 21.2 and 0.1 to 5.8 g O_2/ m^2*d, respectively (Koenig et al., 2017). In another eastern stream in Puerto Rico, R rates were reported at near 7 g O_2/ m^2*d, NPP of -2.5 g O_2/ m^2*d and GPP of 3.5 g O_2/ m^2*d (Bernot et al., 2010). Higher rates of R have been recorded in other tropical and subtropical streams, as R varied from 1.9 to 67.9 g O_2/ m^2*d in Costa Rica (Ramírez et al., 2003). In Peru, R varied from 1.88 to 15.02 g O_2/ m^2*d, NPP from -14.8 to -1.8 g O_2/ m^2*d and GPP from 0.07 to 0.189 g O_2/ m^2*d (Bott and Newbold, 2013). All of these studies usually report higher rates of R than GPP, and negative rates of NPP, as occures this study.

Quebrada Mondongo

Quebrada Mondongo was expected to behave as an urban stream, as it drains through the Lajas Municipality urban center, receives water from a WWTP and runoff of all the urban areas. Streamflow was expected to increase due to the WWTP, which discharges a maximum of 1.2 million gallons a day and has a mean maximum discharge of 710,000gal/day (ECHO, 2018). The WWTP has been calculated to contribute 42% of the total discharge in Station 3 (Sotomayor-Ramírez et al., 2016), and was calculated to be 72% of the stream discharge during the time period of this study, which means that the streamflow to this point is greatly impacted by the effluent emission. As flow significantly increased downstream from the WWTP, so did temperature and specific conductance. Temperature increased by 2˚C and SEC increased by ~1,800 µS/cm as expected and observed for other streams receiving WWTP discharges in Puerto Rico (Figueroa-Nieves et al. 2014). Moreover, this WWTP has been discharging water with temperatures of over 2˚C higher than upstream waters and suspended and dissolved sediments, into the Quebrada Mondongo, which could explains these upstream downstream differences. Dissolved oxygen concentrations also increased and it might be due to the WWTP effluents. The effluent water from the WWTP passes through a series of “stair like” aeration steps before entering the Quebrada Mondongo, which creates turbulence and increases the DO concentration. This great volume of water, that has been reported to have a mean DO concentration of 6.86 mg/L from 2015-2018 (Table 3), is then mixed with the stream water probably increasing its overall downstream DO concentrations, as observed upstream waters have really low DO concentrations.

Lower total N, total P and dissolved P concentrations were found upstream of the WWTP (Station 34), although they were not statistically different due to variation in concentrations

between sampling events. The WWTP did seem to increase the NO_3-N concentration downstream of the outlet. The mean nutrient concentrations found in this study in all stations from Quebrada Mondongo sub-basin were higher than other mesotrophic and eutrophic stream streams in Puerto Rico, such as Río Guanajibo or Río Piedras (Viggiano-Beltrocco, 2014), and from those reported by Sotomayor-Ramírez et al. (2016) on this same stream. Samples in this study were only gathered during low base flow, unlike the previous study, which also included storm event samples. Mean nutrient concentrations are probably higher during base flow as a result of point sources inputs being diluted with runoff from storm events (McCarty and Haggard, 2016). Nutrient concentrations were also correlated to base flow, suggesting than an increment in stream flow led to an increase in nutrient concentrations. Lajas WWTP effluent had a higher mean nutrient concentration than the upstream waters; therefore an increase nutrient concentration in the downstream stations was expected (Carey and Migliaccio, 2009; Sánchez-Pérez et al., 2009; Figueroa-Nieves et al., 2016; Rodríguez-Castillo et al., 2017). After the WWTP discharge, downstream of Station 3, the TN and TP concentrations decreased. Because NO_3-N is not changing from Station 3 to Station 4, it might suggest that organic nitrogen or NH_4^+ are being buried in the sediments or NH_4^+ is being utilized by microbes or algae (Bernot and Dodds, 2005). Phosphorus is either retained in the substrates and sediments or is being removed via uptake by periphyton communities (Dodd, 2003).

Beyond the records and observations in this investigation, the Lajas WWTP has been contributing a mass amount of nutrients into Quebrada Mondongo for several years (ECHO, 2018). The WWTP was estimated to contribute 1,645 kg P/ year on 2013-2015 (Sotomayor-Ramírez et al., 2016). Updated data indicates that the WWTP contributed an annual average of 1,794 kg P/ year on 2013 until June 2018. There were two years in which the plant contributed

excessive amounts of P: from January to December 2016 was 2,874 kg P/ year and from January until June 2018, there were 2,540 kg P. Nitrate-N annual contribution from the plant was calculated to be 819 kg/year from 2013-2015 (Sotomayor-Ramírez et al., 2016) and calculated to be 511 kg/year on 2013 until February 2018 (data was only reported once every 3 months since January 2015). Also, effluent concentrations are so high that, even with the dilution in the stream water, the nutrient concentrations still surpass the limits of the Water Quality Standards for SD water classification according to the Environmental Quality Board of Puerto Rico (JCA, 2016). For example, the WWTP is permitted to discharge up to 6 mg/L of NH_3-N + NH_4^+-N and 10 mg/L of NO_3-N + NO_2-N, and after mixing, it still surpassing the 1.70 mg/L TN limit on SD waters. As for P, on May 2015 the TP limit discharge concentration was changed from 1.00 mg P/L to 8.26 mg P/L; after mixing, the stream water has a higher concentration than the 0.16 mg/L TP limit for SD waters. This effluent discharge, plus the already high concentrations in the upstream, has resulted in an impaired classification for TN, NO_3-N and TP of most Quebrada Mondongo stations according to the suggested numeric criteria (Table 10) (Sotomayor-Ramírez et al., 2011).

The increase in numerical nutrient concentrations and light availability downstream from the WWTP occurred as AFDM, benthic biomass, and NPP rates increase were also observed (Table 11), suggesting that this higher nutrient concentration might be causing an increase in periphyton biomass (Dodds et al., 2002; Alberts et al., 2018) and resulting in higher net primary production. The 7-day benthic biomass and AFDM in this stream are much higher than other studies in streams in the east and center of Puerto Rico (Tank and Dodds, 2003; Gualtero-Leal et al., 2010; Viggiano-Beltrocco, 2014), reflecting the also higher nutrient concentrations.

Stations in Quebrada Mondongo behaved similar to the urban and agricultural streams studied by Bernot et al. (2010) across the United States and Puerto Rico and like other tropical streams (Bott and Newbold, 2013; Koenig et al., 2017; Saltarelli et al., 2018,) with negative NPP, and higher rates of R than GPP. In fact, they behaved just as "high carbon" response of the stream metabolism model (Figure 1). The addition of nutrients by the WWTP, higher temperatures and an increment in light availability, appeared to increase the numerical GPP, R and NPP rates in the Quebrada Mondongo from upstream to downstream (Table 14). This has also been observed for nutrient enrichment in streams (Young et al., 2008), increased urbanization (Bernot et al., 2010; Alberts et al., 2017) and for streams receiving water from WWTP (Gücker et al., 2006). This contrasts with a study in Spain in which GPP tended to decrease downstream a WWTP (Rodríguez-Castillo et al., 2017). Respiration rates were similar in Station 34 and 3, but decreased in Station 4 as both rates of NPP and GPP declined. Even with the increments in NPP, the stream remained consistent in negative NPP, and higher R than GPP, meaning GPP/R ratio of <1, suggesting that the systems are heterotrophic and are mostly receiving energy rather than producing it (Bott, 2006) (Table 11). Nevertheless, the difference between stations was not significant and no clear correlation was observed between metabolism rates and nutrient levels, suggesting that the ecology of the stream is not necessarily affected by the WWTP inputs and that the behaved really different between sampling dates.

Quebrada Bárbara

Quebrada Bárbara was a stream with a high forest area and higher agricultural activity. This stream received water from a small urban community and agricultural lands that included grazing areas, a banana orchard, and hay crop area. It also receives water from the Maginas Water Treatment Plant located northeast of the sub-basin. Stations within the stream showed no

difference in flow and temperature, as it apparently did not receive significant external inputs of water from the upstream station to the downstream station. Specific conductance, pH and dissolved oxygen increased downstream from Station 35 possibly as result of runoffs from the orchard and hay crop area.

Nutrient concentrations in Quebrada Bárbara were not as high as the urban stream, but were high as expected for an agricultural stream (Griffiths et al., 2013; Gücker et al., 2009; Jarvie et al., 2008; Qu et al., 2017). Also, nutrient contribution from the Maginas WTP was calculated as 881 kg P/ year from September 2013 to June 2018 and N in the form of ammonia contribution was 64 kg N/ year (NH_3 + NH_4^+) from August 2014 to June 2018. Total nitrogen, TP and DP concentration tended to decrease downstream, except for NO_3-N. Station 11 was recently studied for TN and TP and its mean concentrations were found to be similar, although NO_3-N was found to be half of the reported mean concentration (Sotomayor-Ramírez et al., 2016). A numerical decrease in TN was observed while NO_3-N significantly increased, indicating that organic-N or ammonium are being consumed or are being nitrified and converted into nitrate, which has been documented in other agricultural land streams (Kemp and Dodds, 2002). Also, TP drastically decreased, suggesting that it might be absorbed by the periphyton community or is sorbed in the sediments (Dodds, 2003). This concurs with the increase in periphyton biomass that we see from Station 35 to Station 11 (Table 10) and has been previously correlated with agricultural lands (Bechtold et al., 2012). This might indicate that the nitrogen and phosphorus are being consumed by the system to produce more biomass.

It was interesting to observe that the chlorophyll-a concentrations were actually decreasing from Station 35 to Station 11, while AFDM was increasing. This is not common, as Chl-a and AFDM are expected to respond simultaneously. This increase and the high difference

between Chl-a and AFDM values led to a high Autotrophic Index, suggesting that it was a very heterotrophic system. AFDM considers all of the periphyton community, not only the chlorophyll producing algae. The incineration also included all of the material and detritus that were deposited during the 7-day incubation period. As noted in Appendix 3:C, blocks in Station 11 were covered by what it looks like non-green algae and heavy amount of sediments on more than one occasion. We even had to remove some blocks from incubation and eliminate assays due to sediments being deposited as stream flow receded on Station 35 (Appendix 3. D). Sadly, the Maginas WTP only kept record of suspended and dissolved sediments for July 2015-January 2016 and turbidity was reported low, as they only report once a month; therefore, no analysis could be achieved between discharged sediment concentrations and AFDM concentrations. The accumulation in the block may have shifted the AI to an heterotrophic value as it may have been artificially inflated by this non-living organic detrital material (USEPA, 2000). This contrasted with the results of the stream benthic metabolism.

Benthic metabolism rates were similar to those found on other studies for agricultural areas. Station 35 was heterotrophic, with negative NPP and higher R than GPP. Upstream from this station, the stream is almost completely shaded, and flows into an agricultural area, which has an open canopy. This could explain the heterotrophic behavior. After this, all rates increase on Station 11. In fact, Station 11 was the only station that reflected an autotrophic behavior (GPP/R >1) and this coincides with the positive mean NPP, and lower R that GPP. Respiration and NPP rates in this station were higher than upstream, therefore increasing its GPP mean rates, behaving like the “high carbon and nutrients” model of benthic metabolism (Figure 1). This resulted in a shift from heterotrophy to autotrophy in the stream, which has been observed before in agricultural intensive areas (Hagen et al., 2010). This increase in GPP has also been found in

other agricultural areas in temperate streams due to decrease canopy cover and increase in nutrient availability (McTammany et al., 2007; Gücker et al., 2009; Bernot et al., 2010; Griffiths et al., 2013).

Ecosystem metabolism drivers

As seen before, ecosystem metabolism has been related to many stressor and drivers such as light availability (Dodds, 2007; Young et al., 2008; Bott and Newbold, 2013), carbon fluxes (Ortiz-Zayas et al., 2005), nutrient additions (Gücker et al., 2006; Johnson et al., 2009; Bott and Newbold, 2013; Griffiths et al., 2013) and temperature change (Young et al., 2008; Demars et al., 2015; Song et al., 2018). In this study, ecosystem metabolism rates were not correlated to any parameter, other than NPP and GPP with pH and AI. However, we observed that rates usually increased when light availability increased and when nutrients concentrations were increasing. We also observed that rates increased at the same time as temperature increased, at least on Quebrada Mondongo, as happened from Station 34 to Station 3 and 4, downstream fro the WWTP. This was not observed in Quebrada Bárbara as rates increased numerically, but there was no difference in temperature between Stations 35 and 11. Yet, no overall conclusion can be made from these inferences.

Conclusions

For this study, two streams with contrasting land use sub-basins were selected within the Eastern Lajas Valley Watershed. The Quebrada Mondongo was the predominant urban sub-basin with and important point sources (WWTP and WTP) and the Quebrada Bárbara was the predominant agricultural sub-basin. Each sub-basin also had different potential point and non-point sources of contamination that were assessed as upstream and downstream stations.

Nutrient concentrations were higher in Quebrada Mondongo and mean values of both sub-basins were either enriched or in violation with the Water Quality Standard of Puerto Rico. Benthic biomass was also higher in Quebrada Mondongo, but AFDM was higher in Quebrada Bárbara. Benthic stream metabolism essays were not significantly different among basins, because even with slight differences, both basins were primarily heterotrophic, indicating that land use did not affect stream metabolism.

Quebrada Mondongo experimented an increase in NO_3-N downstream from the WWTP and WTP, as also happened in Quebrada Bárbara downstream from the agricultural area. All nutrient concentration values measured on Quebrada Mondongo and most of Quebrada Bárbara were in violation of the Water Quality Standard of Puerto Rico. Benthic biomass did not change in Quebrada Mondongo, but did decrease in Quebrada Bárbara. No differences were observed in benthic metabolism downstream from the WWTP and TWP in Quebrada Mondongo. Benthic metabolism did shift from heterotrophy to autotrophy in Quebrada Bárbara, but no significant differences were observed. I suggest that the benthic metabolism, benthic biomass and nutrient concentrations are to be monitored for continuous periods of more than a year and compared among seasons for a better differentiation among sub-basins and among stations within sub-basin.

References

Alberts, J.M., J.J. Beaulieu and I. Buffam. 2017. Watershed land use and seasonal variation constrain the influence of riparian canopy cover on stream ecosystem metabolism. Ecosystems. 20(3):553-567.

Alberts, J.M., K.M. Fritz and I. Buffam. 2018. Response to basal resources by stream macroinvertebrates is shaped by watershed urbanization, riparian canopy cover, and season. Freshwater Science. 37(3):640-652.

Allan, J.D. and M. Castillo. 2007. Stream Ecosystem Metabolism. *In* Allan, J.D. and Castillo, M. (ed.). Stream Ecology: Structure and function of running waters. 2nd ed. Springer, Netherlands.

Bechtold, H.A., A.M Marcarelli, C.V. Baxter and R.S. Inouye. 2012. Effects of N, P and organic carbon on stream biofilm nutrient limitation and uptake in semi-arid watershed. Limnology and Oceanography. 57(5):1544-1554.

Bellinger, E. and D. Sigee. 2010. Algae as bioindicators. p. 121. *In* Bellinger, E. and Sigee, D. (ed.). Freshwater algae: Identification and use of Bioindicators. Wiley-Blackwell, UK.

Bernot, M.J., D.J. Sobota, R.O. Hall, P.J. Mulholland, W.K. Dodds, J.R. Webster, J.L. Tank, L.R. Ashkenas, L.W. Cooper, C.N. Dahm, S.V. Gregory, N.B. Grimm, S.K. Hamilton, S.L. Johnson, W.H. McDowell, J.L. Meyer, D. Peterson, G.C. Poole, H.M. Valett, C. Arango, J.J. Beaulieu, A.J. Burgin, C. Crenshaw, A.M. Helton, L. Johnson, J. Merriam, B.R. Niederlehner, J.M. O'Brien, J.D. Potter, R.W. Sheibley, S.M. Thomas and K. Wilson. 2010. Inter-regional comparison of land-use effect on stream metabolism. Freshwater Biology. 55:1874-1890.

Bernot, M.J. and W.K. Dodds. 2005. Nitrogen retention, removal, and saturation in lotic ecosystems. Ecosystems. 8(4):442-453.

Bott, T. 2006. Primary productivity and community respiration. p. 663-690. *In* Hauer, F. and G. Lamberti. (ed.). Methods in stream Ecology. Academic Press, New York.

Bott, T. and J.D. Newbold. 2013. Ecosystem metabolism and nutrient uptake in Peruvian headwater streams. International Review of Hydrobiology. 98:117-131.

Bunn, S., P. Davies and T. Mosisch. 1999. Ecosystem measures of river health and their response to riparian and catchment degradation. Freshwater Biology. 41:333-345.

Carey, R.O. and K.W. Migliaccio. 2009. Contribution of Wastewater treatment Plant Effluents to Nutrient Dynamics in Aquatic Systems: A Review. Environmental Management. 44:205-217.

Demars, B.O.L, J. Thompson and J.R. Manson. 2015. Stream metabolism and the open diel oxygen method: Principles, practice, and perspectives. Limnology and Oceanography: Methods. 13(7):356-374.

Dodds, W. 2003. The role of periphyton in phosphorus retention in shallow freshwater aquatic systems. Journal of Phycology. 39:849-849.

Dodds, W. 2007. Trophic state, eutrophication and nutrient criteria in streams. Trends in Ecology and Evolution. 22(12):669-676.

Dodds, W. and J. Brock. 1998. A portable flow chamber for *in situ* determination of benthic metabolism. Freshwater Biology. 39:49-59.

Dodds, W., V. Smith and K. Lohman. 2002. Nitrogen and phosphorus relationships to benthic algal biomass in temperate streams. Canadian Journal of Fisheries and Aquatic Sciences. 59:865-874.

ECHO (Enforcement and Compliance History Online). 2018. Effluent charts: PRASA LAJAS WWTP [Online]. Available at **www.echo.epa.gov/effluent-charts#PR0020575** (verified 20 Jan. 2018)

Figueroa-Nieves, D., W. McDowell, J. Potter and G. Martínez. 2016. Limited uptake of nutrient input from sewage effluent in a tropical landscape. Freshwater Science. 35(1):12-24.

Frankforter, J.D., H.S. Weyers, J.D. Bales, P.W. Moran and D.L. Calhoun. 2010. The relative influence of nutrients and habitat on stream metabolism in agricultural streams. Environmental Monitoring Assessment. 168:461-479.

Griffiths, N., J. Tank, T. Royer, S. Roley, E. Rosi-Marshall, M. Whiles, J. Beaulie, and L. Johnson. 2013. Agricultural land use alters the seasonality and magnitude of stream metabolism. Limnology and Oceanography. 58(4):1513-1529.

Gualtero-Leal, D., G.A. Martínez, C. Santos-Flores and D. Sotomayor-Ramírez. 2010. Benthic algal diversity and biomass as indicators to establish reference streams in Puerto Rico. Verhandlungen des Internationalen Verein Limnologie. 30:1567-1572.

Gücker, B., I. Boëchat and A. Giani. 2009. Impacts of agricultural land use on ecosystem structure and whole-stream metabolism of tropical Cerrado streams. Freshwater Biology. 54:2069-2085.

Gücker, B., M. Brauns and M. Pusch. 2006. Effects of wastewater treatment plant discharge on ecosystem structure and function of lowland streams. Journal of the North American Benthological Society 25(2): 313–329.

Hagen, E.M., M.E. MacTammany, J.R. Webster and E.F. Benfield. 2010. Shifts in allochthonous input and autochthonous production in streams along an agricultural land-use gradient. Hydrobiologia. 655:61-77.

Haggard, B., E. Stanley and D. Storm. 2005. Nutrient retention in a point-source-enriched stream. Journal of the North American Benthological Society 24(1):29-47.

Hall, R.O. and E.R. Hotchkiss. 2017. Stream Metabolism. p. 219-233. *In* Hauer, F. and G. Lamberti. (3rd ed.). Methods in stream Ecology Vol 2: Ecosystem Function. Academic Press, New York.

Izagirre, O., U. Agirre, M. Bermejo, J. Pozo and A. Elosegi. 2008. Environmental controls of whole-stream metabolism identified from continuous monitoring of Basque streams. Journal of the North American Benthological Society 27(2):252–268.

Jarvie. H.P., P.J.A. Withers, R. Hodgkinson, A. Bates, M. Neal, H.D. Wickham, S.A. Harman and L. Armstrong. 2008. Influence of rural land use on streamwater nutrients and their ecological significance. Journal of Hydrology. 350:166-186.

JCA (Junta de Calidad Ambiental de Puerto Rico). 2016. Puerto Rico Water Quality Standards Regulations. Available at: https://www.epa.gov/sites/production/files/2014-12/documents/prwqs.pdf (Verified 5 May 2018)

Johnson, L., J. Tank and C. Arango. 2009. The effect of land use on dissolved organic carbon and nutrient uptake in streams. Freshwater Biology. 54:2335-2350.

Kemp, M.J. and W.K. Dodds. 2002. Comparison of nitrification and denitrification in prairie and agriculturally influenced streams. Ecological Applications. 12(4):988-1009.

Koenig, L.E., C. Song, W.M. Wollheim, J. Rüegg and W.H. McDowell. 2017. Nitrification increases nitrogen export from a tropical river network. Freshwater Science. 36(4):698-712.

Lyon, D. and S. Ziegler. 2009. Carbon cycling within epilithic biofilm communities across a nutrient gradient of headwater streams. American Society of Limnology and Oceanography. 54(2):439-449.

Marti, E., J. Aumatell, L. Gode, M. Poch and F. Sabater. 2004. Nutrient retention efficiency in streams receiving inputs from wastewater treatment plants. Journal of Environmental Quality 33:285–293.

Marzolf, E.R., P.J. Mulholland and A.D. Steinman. 1994. Improvements to the diurnal upstream-downstream dissolved oxygen change technique for determining whole-stream metabolism in small streams. Canadian Journal of Fisheries and Aquatic Sciences. 51:1591-1599.

McCarty, J.A. and B.E. Haggard. 2016. Can we manage nonpoint-source pollution using nutrient concentrations during seasonal baseflow? Agricultural & Environamental Letters. 1:160015.

McIntire, C.D., R.L. Garrison, H.K. Phinney, and C.E. Warren. 1964. Primary production in laboratory streams. Limnology and Oceanography 9:92-102.

McTammany, M.E., E.F. Benfield and J.R. Webster. 2007. Recovery of stream ecosystem metabolism from historical agriculture. Journal of the North American Benthological Society. 26(3):532-545.

Merseburger, G., E. Martí, F. Sabater and J. Ortiz. 2011. Point-source effects on N and P uptake in a forested and an agricultural Mediterranean streams. Science of the Total Environment. 409:957-967.

Meyer, J. 1997. Stream health: Incorporating the human dimension to advance stream ecology. Journal of the North American Benthological Society. 16(2):439-447.

Mulholland, P.J. J.N. Houser and K.O. Maloney. 2005. Stream diurnal dissolved oxygen profiles as indicators of in-stream metabolism and disturbance effects: Fort Benning as a case study. Ecological Indicators. 5:243-252.

Odum, H.T. 1956. Primary production in flowing waters. Limnology and Oceanography 1(2):102–117.

Ortíz-Zayas, J.R., W. M. Lewis, J.F. Sounders, J.H. McCutchan and F.N. Scatena. 2005. Metabolism of a tropical rainforest stream. Journal of the North American Benthological Society. 24(4):769-783.

Parkhill, K. L., and J. S. Gulliver. 1999. Modeling the effect of light on whole-stream respiration. Ecological Modeling. 117:333-342.

Paul, M. J., and J. L. Meyer. 2001. Streams in the urban landscape. Annual Reviews in Ecology and Systematics 32:333–365.

Pennington, R., A. Argerich and R. Haggerty. 2018. Measurement of gas-exchange rate in streams by the oxygen-carbon method. Freshwater Science. 37(2):222-237

Piggott, J., C. Townsend and C. Mattahaei. 2015. Climate warning and agricultural stressors interact to determine stream macroinvertebrate community dynamics. Global Change Biology. 21:1887-1906.

PREQB. 2016. Puerto Rico Water Quality Standards Regulations. 40-43. Available at: **https://www.epa.gov/sites/production/files/2014-12/documents/prwqs.pdf** (verified 24 Feb. 2018)

Qu, X., Z. Ren, H. Zhang, M. Zhang, Y. Zhang, X. Liu and W. Peng. 2017. Influences of anthropogenic land use on microbial community structure and functional potentials of stream benthic biofilms. Nature: Scientific reports. 7(1).

Ramírez, A., C. Pringle and L. Molina. 2003. Effects of stream phosphorus levels on microbial respiration. Freshwater Biology. 48:88-97.

Ramírez, A., R. De Jesús-Crespo, D.M. Martinó-Cardona, N. Martínez-Rivera and S. Burgos-Caraballo. 2009. Urban streams in Puerto Rico: what can we learn from the tropics? Journal of the North American Benthological Society. 28(4):1070-1079.

Rodríguez-Castillo, T., J. Barquín, M. Álvarez-Cabria, F.J. Peñas and C. Álvarez. 2017. Effects of sewage effluents and seasonal changes on the metabolism of three Atlantic rivers. Science of the Total Environment. 599-600:1108-1118

Riley, A.J. and W.K. Dodds. 2013. Whole-stream metabolism: strategies for measuring and modeling diel trends of dissolved oxygen. Freshwater Science. 32(1):56-69.

Rüegg, J. J.D. Brant, D.M. Larson, M.T. Trentman and W.K. Dodds. 2015. A portable, modular, self-contained recirculating chamber to measure benthic processes under controlled water velocity. Freshwater Science. 34(3):831-844.

Ruggiero, A., A.G. Slimini and G. Carchini. 2006. Effect of a wastewater treatment plant on organic matter dynamics and ecosystem functioning in a Mediterranean stream. Ann Limnol. –Int. J. Lim. 42(2):97-107.

Saltarelli, W. A., W.K. Dodds, F. Tromboni, M.C. Calijuri, V. Neres-Lima, C.E. Jordão, J.C.P. Palhares and D.G.F. Cunha. 2018. Variation of stream metabolism along a tropical environmental gradient. Journal of Limnology. (in press). https://doi.org10.4081/jlimnol.2018.1717

Sánchez-Pérez, J., M. Gerino, S. Sauvage, P. Dumas, É. Maneux, F. Julien, P. Winterton and P. Vervier. 2009. Effects of wastewater treatment plant pollution on in-stream ecosystems functions in an agricultural watershed. Annuales de Limnologie - International Journal of Limnology. 45:79-92.

Song, C. , W.K. Dodds, J. Rüegg, A. Argerich, C.L. Baker, W.B. Bowden, M.M. Douglas, K.J. Farrell , M.B. Flinn, E.A. Garcia, A.M. Helton, T.K. Harms, S. Jia, J.B. Jones, L.E. Koenig, J.S. Kominoski, W.H. McDowell, D. McMaster, S.P. Parker, A.D. Rosemond, C.M. Ruffing, K.R. Sheehan, M.T. Trentman, M.R. Whiles, W.M. Wollheim and F. Ballantyne IV. 2018. Continental-scale decrease in net primary productivity in streams due to climate warming. Nature Geoscience. 11:415-420.

Sotomayor-Ramírez, D. 2015. Summary report for project: Citizen Monitoring of Water Sanitation in a Rural Puerto Rico Watershed.

Sotomayor-Ramírez, D. G. Martínez and L. Pérez-Alegría. 2011. Determination of numeric nutrient criteria in rivers and streams of Puerto Rico. Final Report submitted to PREQB as per Agreement 000080395 between UT Batelle, LLC Oak Ridge National Laboratory and University of Puerto Rico, Agricultural Experiment Station. 58 pp. with appendices.

Sotomayor-Ramírez, D. and L. Pérez-Alegría. 2011. An assessment of agricultural activities in the eastern portion of the Lajas Valley Agricultural Reserve, within Lajas and Guánica

municipalities. University of Puerto Rico, Mayagüez, Agricultural Experiment Station, Unpublished Manuscript. 16pp.

Sotomayor-Ramírez, D., G. Martínez and L.R. Pérez-Alegría. 2016. Citizen Monitoring of Water Sanitation in a Rural Puerto Rico Watershed. Final Project Report submitted to USEPA as per Agreement 83553801 between US Environmental Protection Agency and the University of Puerto Rico Mayagüez Campus.

Stream Solute Workshop. 1990. Concepts and Methods for Assessing Solute Dynamics in Stream Ecosystems. Journal of the North American Benthological Society. 9(2):95-119.

Tank, J. and W. Dodds. 2003. Nutrient limitation of epilithic and epixylic biofilms in 10 North American streams. Freshwater Biology. 48:1031-1049.

USEPA. 2000. Nutrient Criteria Technical Guidance Manual: Rivers and Streams. Chapter 3: Select Variables. p. 38. Available: **https://nepis.epa.gov** (verified 5 May 2018)

USDA-NRCS. 2015. Web Soil Survey. Available at **http://websoilsurvey.nrcs.usda.gov/app**. (verified 24 Jan. 2018)

USEPA. 2014. Lajas Wastewater Treatment Plant NPDES Fact Sheet [Online]. Available at **www3.epa.gov/region02/water/water_permits/lajas_wwtp_fact_sheet.pdf** (verified 7 Feb. 2016).

USEPA. 2015. Maginas WTP NPDES [Online] Available at **https://www3.epa.gov/region02/water/water_permits/maginas_wtp_npdes_draft_permit_08_2015.pdf** (verified 5 Jan. 2018)

USEPA. 2016. Polluted Runoff: nonpoint source pollution [Online]. Available at **www.epa.gov/polluted-runoff-nonpoint-source-pollution/what-nonpoint-source** (verified 7 Feb. 2016).

Viggiano-Beltrocco, M. 2014. Benthic Algae as biological indicators of trophic status in tropical streams. MS Thesis. University of Puerto Rico, Mayagüez.

Weber, C.I. 1973. Biological field and laboratory methods for measuring the quality of surface waters and effluents. U.S. Environmental Protection Agency Report 670 / 4 / 73 / 001.

Welschmeyer, N. 1994. Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and pheopigments. Limnol. Oceanogr. 39(8): 1985-1192.

Wetzel, R.G. 2011. Limnology: Lake and River Ecosystem. Elsevier, San Diego, California, USA. pp. 148, 758.

Whitall, D., L.J. Bauer, C. Sherman, K. Edwards, A. Mason, T. Pait and C. Caldow. 2013. Baseline Assessment of Guánica Bay, Puerto Rico in Support of Watershed Restoration.

NOAA Technical Memorandum NOS NCCOS 176. Prepared by the NCCOS Center for Coastal Monitoring and Assessment Biogeography Branch. Silver Spring, MD. 169 pp.

Young, R.G. and A.D. Huryn. 1998. Comments: Improvements to the diurnal upstream-downstream dissolved oxygen change technique for determining whole-stream metabolism in small streams. Canadian Journal of Fisheries and Aquatic Sciences. 55:1784-1785.

Young, R.G., C. Matthaei and C. Townsend. 2008. Organic matter breakdown and ecosystem metabolism: functional indicators for assessing river ecosystem health. Journal of the North American Benthological Society. 27(3):605-625.

Appendices

Appendix 1. Pictures of study sites:

A) Station 34 Quebrada Mondongo, **B)** Station 3 Quebrada Mondongo, **C)** Station 4 Quebrada Mondongo, **D)** Station 35 Quebrada Bárbara, **E)** Station 11 Quebrada Bárbara

A)

B)

C)

D)

E)

Appendix 2. Summary of sampling dates in all stations

Table 1: Sampling dates according to each station

	Quebrada Mondongo			Quebrada Bárbara	
Sampling dates	34	3	4	35	11
6/27/2016	x				
6/28/2016		x	x		
6/30/2016			x		
7/1/2016	x		x		
7/4/2016	x				
7/5/2016		x	x		
7/14/2016			x		
7/18/2016	x	x	x		
7/19/2016		x			
7/20/2016	x	x			
7/25/2016	x				
7/26/2016		x	x		
7/27/2016			x		
7/29/2016	x				
7/30/2016		x			
8/17/2016	x	x	x		
8/24/2016	x	x	x		
9/15/2016			x		
9/28/2016			x		
10/20/2016				x	
10/26/2016				x	
10/31/2016				x	
11/10/2016				x	
11/16/2016				x	
12/15/2016				x	x
1/18/2017					x
1/23/2017					x
1/25/2017				x	
1/30/2017					x
2/1/2017				x	x
2/13/2017				x	x
3/8/2017					x
3/15/2017					x
3/20/2017				x	x
3/29/2017					x

Appendix 3.Examples of periphyton biomass blocks from different stations

A)

B)

C)

D)

A) Station 34 Quebrada Mondongo (6/27/16); **B)** Station 4 Quebrada Mondongo (6/28/16); **C)** Station 11 Quebrada Bárbara (2/13/17); **D)** Station 35 Quebrada Bárbara (10/21/16) Blocks had to be removed due to temporary flow decrease in the stream and sediment deposit from Maginas WTP in the benthos and over blocks

Appendix 4. Field recommendations and possible pitfalls of this metabolism chamber technique

Many considerations were taken when using this stream metabolism chamber technique. Possible pitfalls and implementation indication are listed here and are discussed more clearly here and in the next appendices:

1. Chamber placement into stream and sediments
2. Limitation on material and replicates
3. Water infiltration (Appendix 4)
4. Chamber internal temperature (Appendix 6)
5. Slope analysis (Appendix 7)

The chambers used in this project were modified from their original form to an open bottom chamber due to the stream and benthos characteristics. Since these are not completely sealed chambers, some precautions are to be taken into account when implementing the method. First, the chambers need to be placed in the sediment down to 2.54 cm in the most subtle way possible to avoid large benthos disturbances and sediment dispersion. Once placed, one must make sure that there are not water bubbles, therefore, it is recommended to turn the motor and axial propeller on to assure none were caught in the system. After this, let the system run for at least 3 minutes, program the YSI to read in 1-minute intervals, seal the chamber and start counting time.

Another important factor with this method is that the chamber is directly placed into the sediment; consequently, mayor precautions after placing and before finishing the reading time are to be taken into account. It is important to always enter the stream from downstream before placing the chamber, during the 1-hour period and after to avoid sediment dispersion. Also, it is extremely important not to walk around the chambers once they are placed, to avoid any oxygen or water intrusion into the chamber from underneath it. This can also be detected in the data,

once graphed. Any oxygen intrusion can imply mayor pitfalls in the system and overestimating the oxygen content, and reading/assay must be discarded.

One of our limitations during this study was that of materials and replicates. Due to the amount of chambers constructed, DO loggers, day light period in which we wanted to perform the assay, expected rain in the afternoons and physical labor required, it was impossible for us to measure stream metabolism on all stations at the same time or at the same day, not even on the same sub-basin. For this reason, replicates are from different days and vary within months in basins. Also, we used a dark chamber and a clear chamber, and decided to use both at the same time to measure R and NPP in the same time span. Therefore, we had to assume that the respiration in the dark chamber was that of the respiration in the light chamber. Anyhow, even in whole stream metabolism analysis, R during the day is impossible to separate from the measurements in production (Hall and Hotchkiss, 2017), and the respiration during the nighttime, or in this case the dark chamber, is assumed to be the respiration during the 24h.

Appendix 5. Water filtration in metabolism chamber

One of the challenges using an open bottom metabolism chamber was to assure that no external water was entering the chamber during the metabolism assessment. To verify this, two tests were performed on June 3, 2016 using a solution of 100mL of water with 10 drops of red vegetable gel dye. Fist, 8mL of vegetable red dye solution was injected into the sediment on 6 locations around the chamber that was installed 2.54 cm into the stream. The chamber was observed for 10 minutes and no color was observed inside the chamber. Secondly, 50 mL of the red vegetable dye solution were added inside the chamber. The motor was turned on for water recirculation and the chamber was observed for 20 minutes with all of the systems working as an actual reading. No dye was observed around the box or in the sediment during the test time, even with the motor and the plastic propeller on. Pictures demonstrate chamber at 0 min (A), 10 min (B) and 20 min (C).

A)

B)

C)

Appendix 6. Water temperature inside metabolism chamber

One of the primary concerns regarding metabolism chambers is the water temperature change in a controlled system. This was one of the main criteria in the design and construction of the recirculating metabolism chamber that Rüegg et al. (2015) developed and they adapted a system that used axial propellers, instead of the common recirculating pump. During the measurements, we observed that temperature inside the chambers increased approximately 1.77˚C and 0.51˚C in the light and dark chambers, respectively. We normalized each measurement by using van't Hoff-Arrhenius equation [5] on page 22.

Table 2: Temperature comparison and analysis from each metabolism chamber

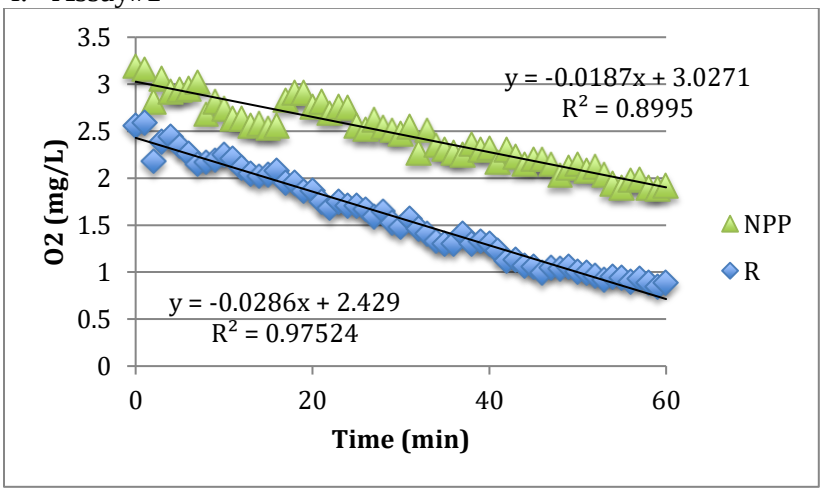
Station	Assay	Light chamber initial (˚C)	Light chamber final (˚C)	Light chamber change (˚C)	Dark chamber initial (˚C)	Dark chamber final (˚C)	Dark chamber change (˚C)
34	1	28.4	29.8	1.4	27.9	27.8	-0.1
34	2	26.7	26.9	0.2	26.5	26.5	0
34	3	28	28.7	0.7	27.7	27.7	0
34	4	26.7	27	0.3	26.5	26.7	0.2
3	1	30.5	31.6	1.1	29.6	29.7	0.1
3	2	30	32.3	2.3	29.5	30.2	0.7
3	3	28.4	30.5	2.1	27.7	28	0.3
3	4	31.1	33.9	2.8	30.6	30.9	0.3
4	1	34.2	36.8	2.6	34	35.5	1.5
4	2	28.7	30.7	2	28.3	29.1	0.8
4	3	32.3	34.6	2.3	31.6	32.5	0.9
4	4	30	32.9	2.9	29.6	30.6	1
4	5	33.3	36.5	3.2	32.7	34.6	1.9
35	1	26.9	28.1	1.2	26.4	26.7	0.3
35	2	27	27	0	26.6	26.6	0
35	3	26.3	27.3	1	25.9	26.2	0.3
35	4	27	28.1	1.1	26.6	26.7	0.1
11	1	23.1	25.3	2.2	22.6	23.3	0.7
11	2	23.6	25.2	1.6	22.5	22.9	0.4
11	3	23.5	26.2	2.7	23.1	23.9	0.8
11	4	24.7	27.5	2.8	24.3	25	0.7
11	5	27.8	30.2	2.4	27.3	27.6	0.3
Mean (˚C):				1.768			0.509

Appendix 7. Stream metabolism assay slope comparison

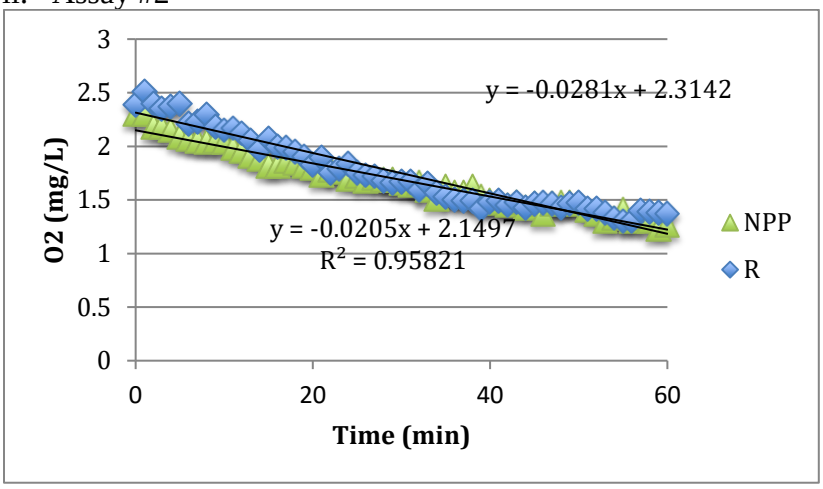
Stream metabolism assays were performed during a period of 1-hour and a regression line was generated to obtain the slope, using only assays that reflected an R^2 grater than 0.50 (Appendix 6). Comparisons of the slopes, between assays and within each assay through time, were planned, but after conversations we understood that it could not be achieved for several reasons. First, due to the fact that all of the data from a single assay were values that depended on each other, a comparison between the slopes of different times was not possible. Also, the 1-hour time range resulted in a higher R^2 than the R^2 from the first 15 minutes or first 30 minutes (Table 3, appendix). Furthermore, this behavior could possibly imply that the 1-hour assay softens the possible disturbance effect that was created by installing the metabolism chamber into the stream. Lastly, we wanted to approach every single assay with the same standards, meaning the 1-hour reading, therefor regulating the analysis of all assays.

Here is included all of the essay readings from which the rates were calculated and Table 3 which compares all R^2 and their slopes.

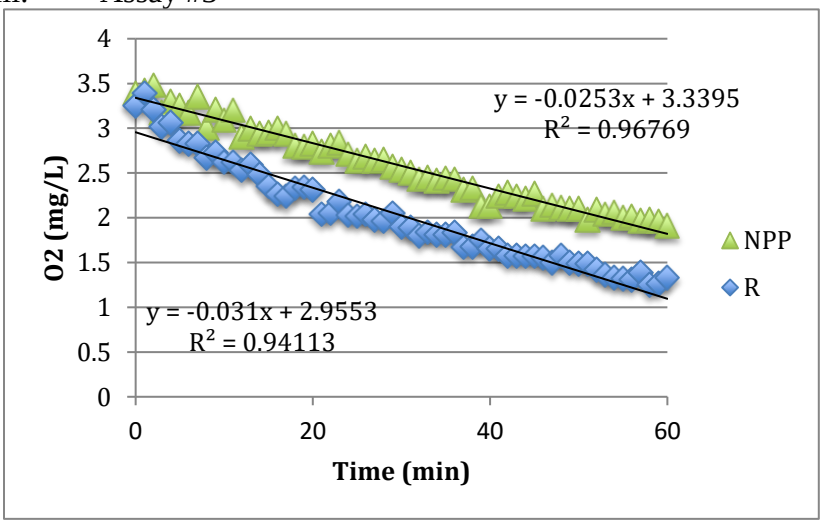
a. NPP and R measurements per visit on Station 34 of Quebrada Mondongo
   i. Assay#1



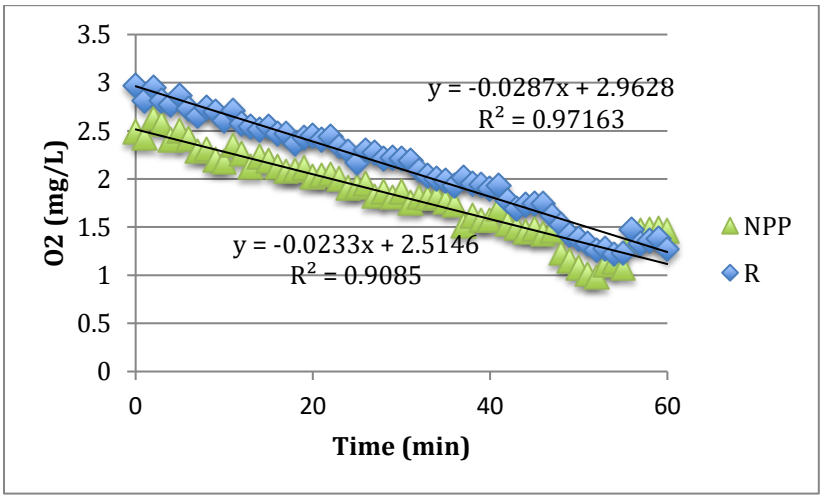
ii. Assay #2



iii. Assay #3

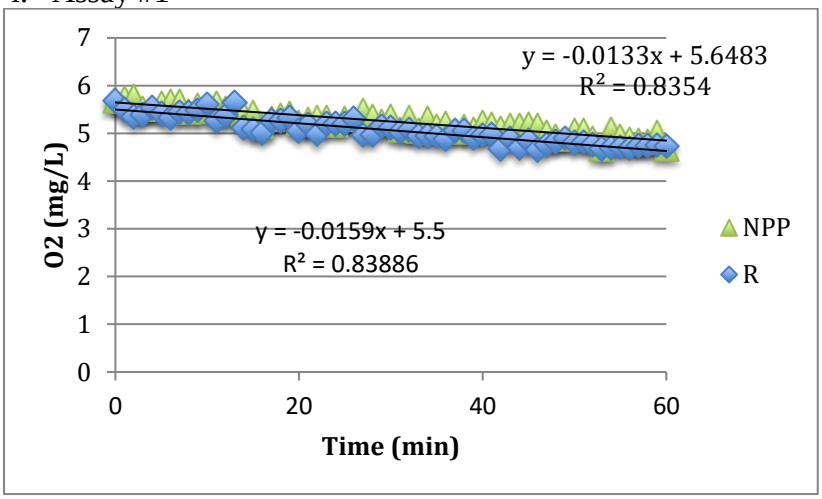


iv. Assay #4

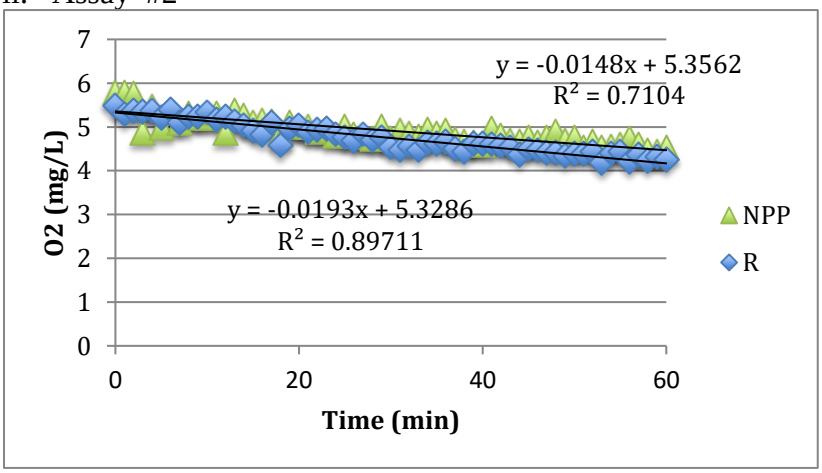


b. NPP and R measurements per visit on Station 3 of Quebrada Mondongo

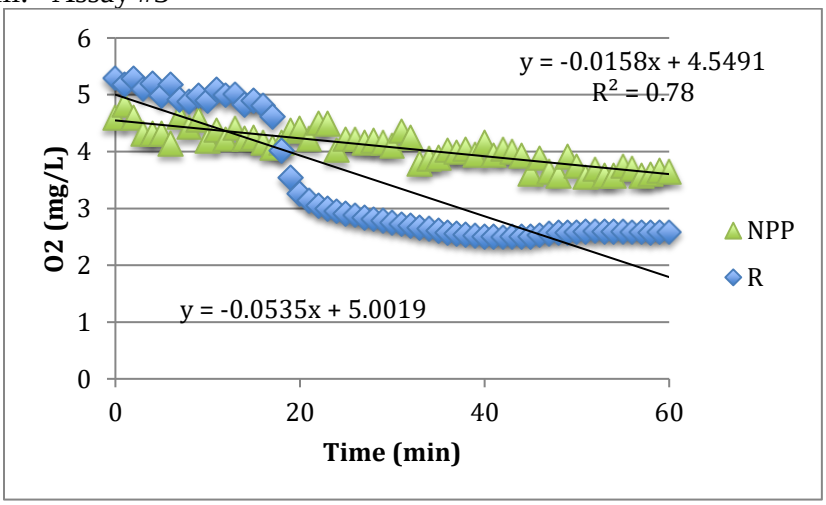
i. Assay #1



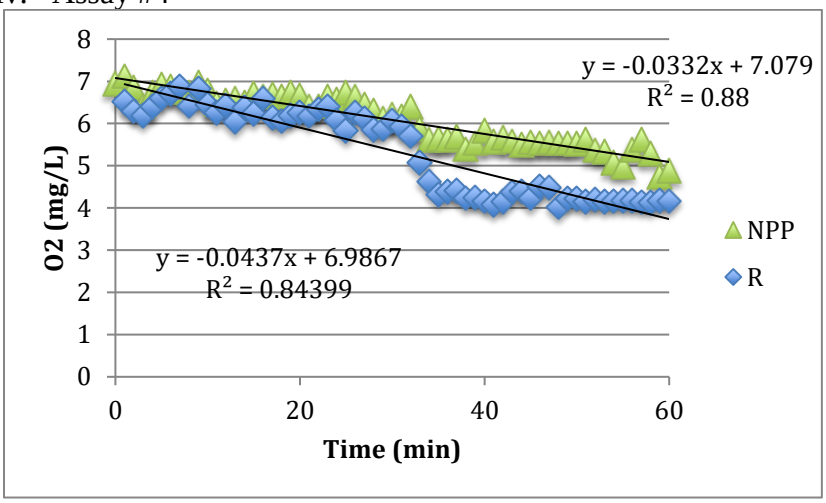
ii. Assay #2



iii. Assay #3

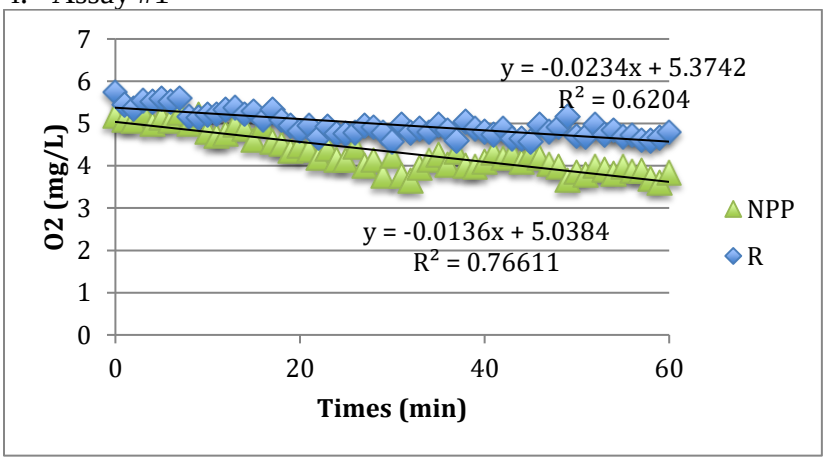


iv. Assay #4

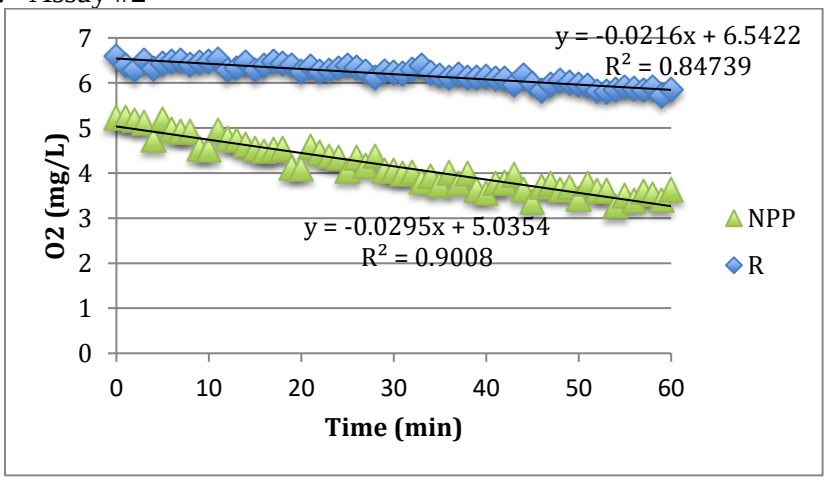


c. NPP and R measurements per visit on Station 4 of Quebrada Mondongo

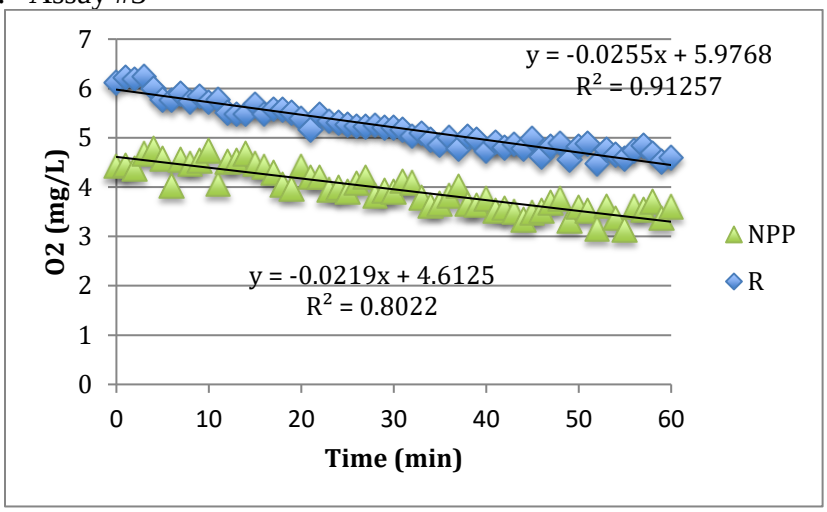
i. Assay #1



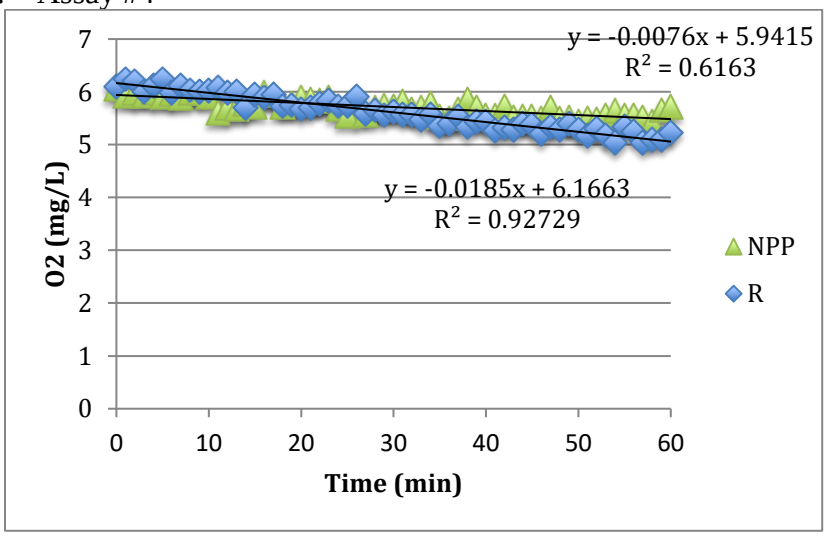
ii. Assay #2



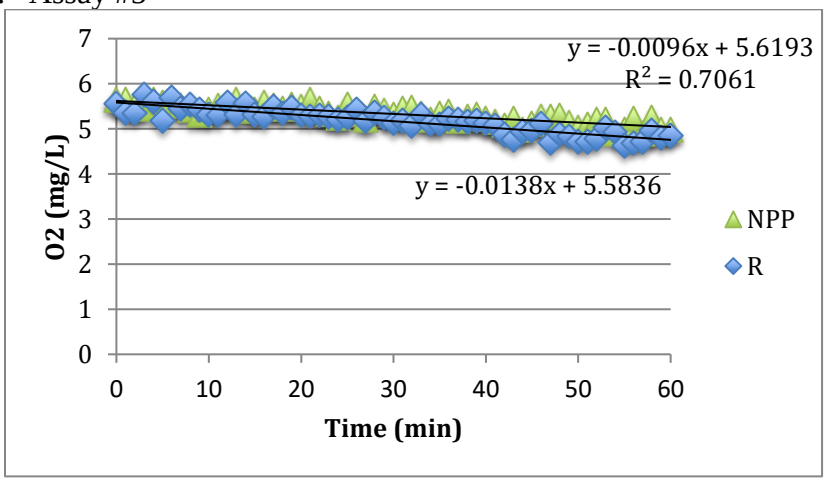
iii. Assay #3



iv. Assay #4

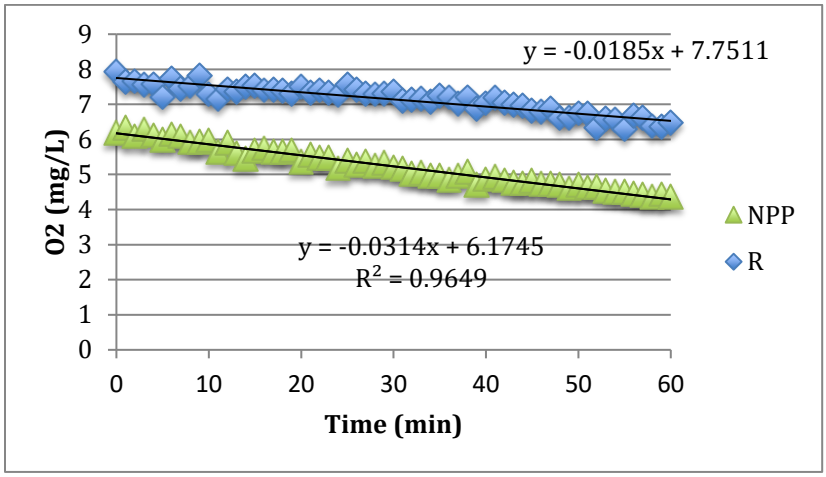


v. Assay #5

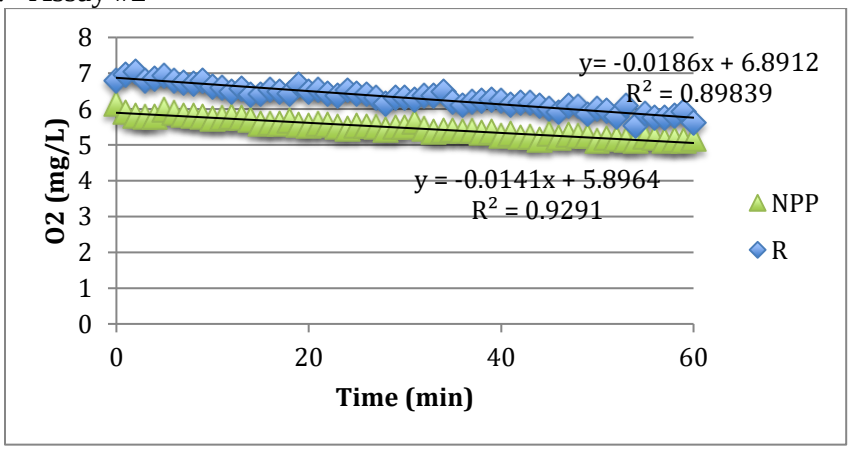


d. NPP and R measurements per visit on Station 35 of Quebrada Bárbara

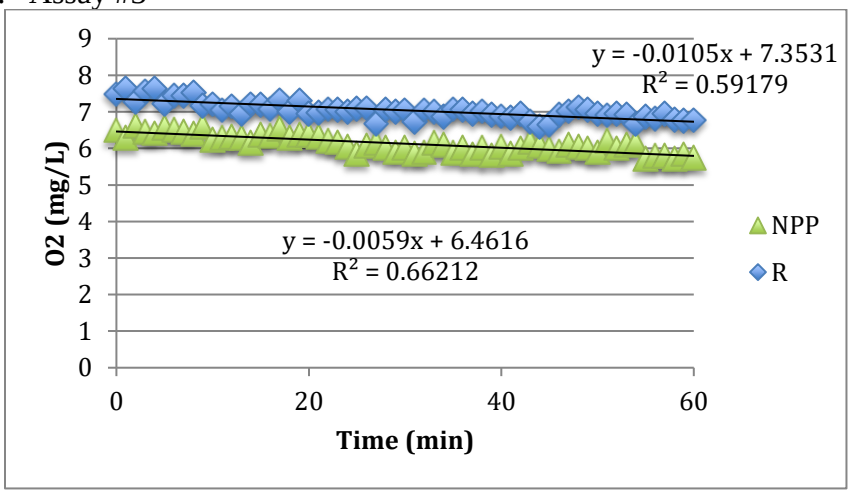
i. Assay #1



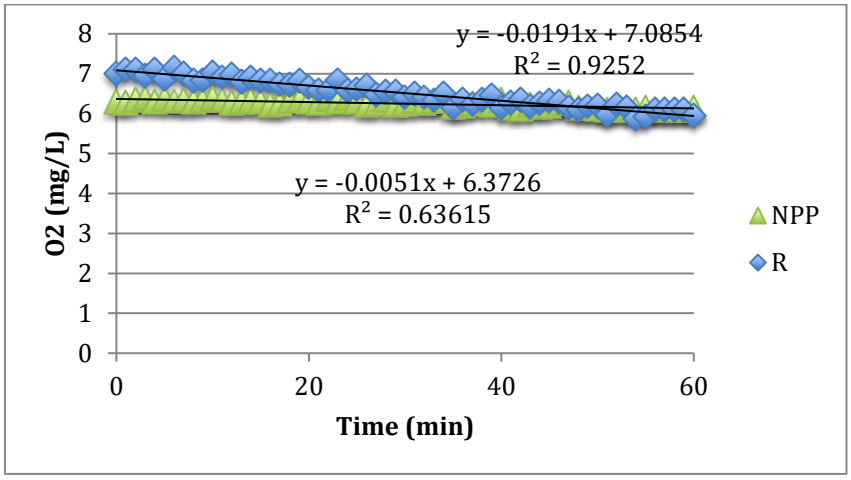
ii. Assay #2



iii. Assay #3

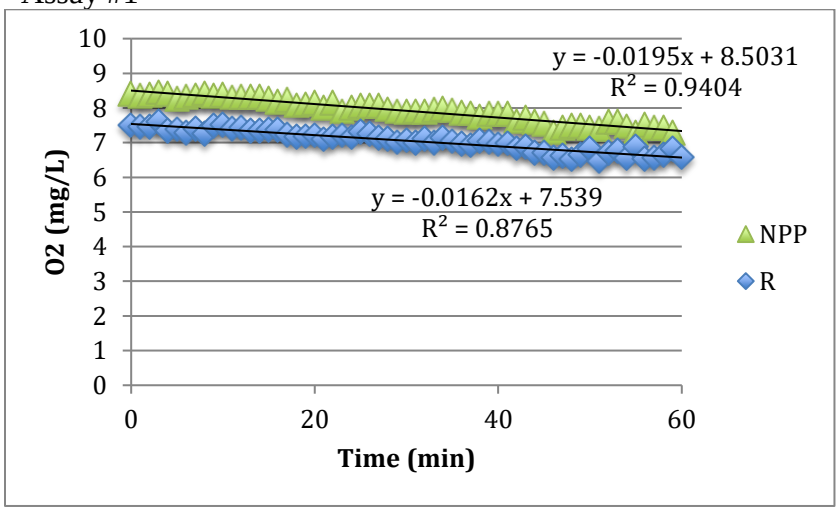


iv. Assay #4

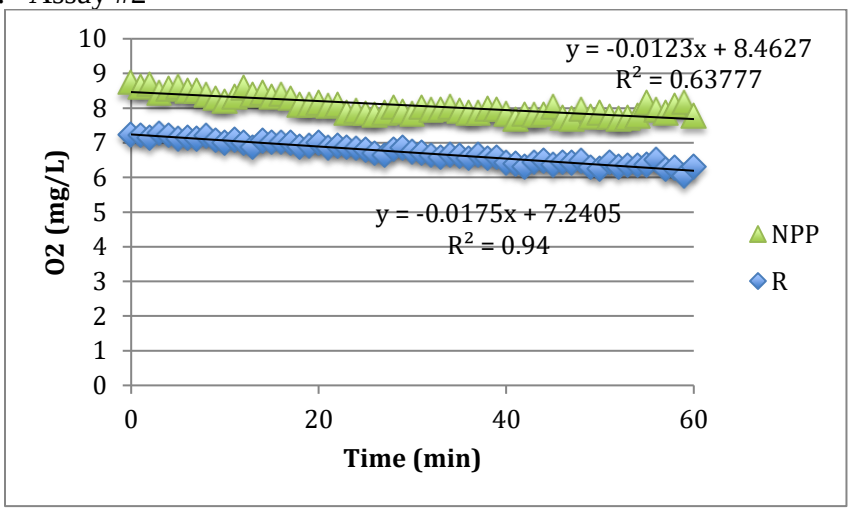


e. NPP and R measurements per visit on Station 11 of Quebrada Bárbara

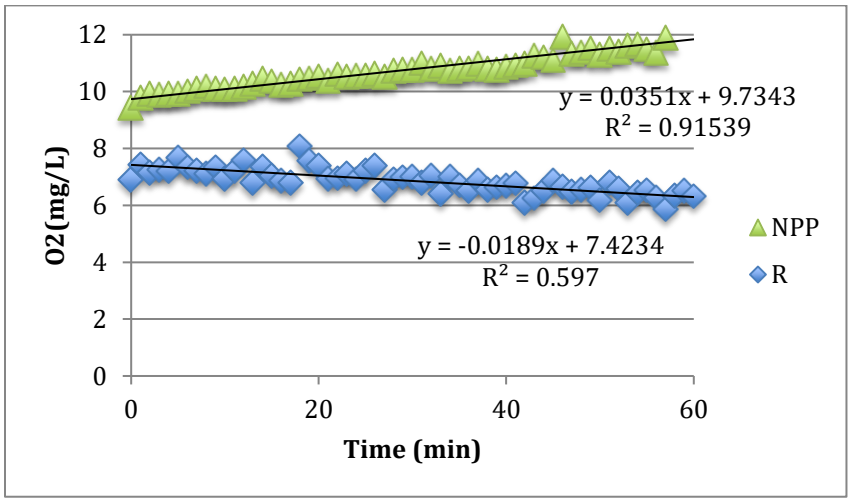
i. Assay #1



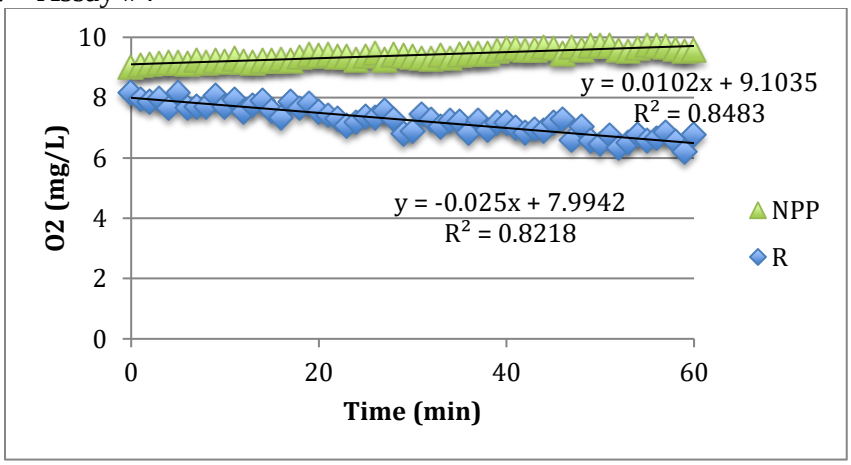
ii. Assay #2



iii. Assay #3



iv. Assay #4



v. Assay #5

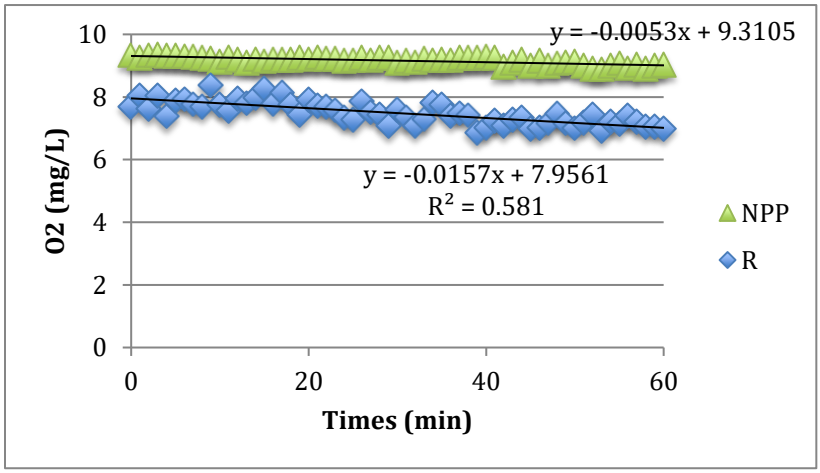


Table 3: Comparison of the R^2 and slopes from each metabolism assay

		NPP Assays (light chamber)						Respiration Assays (dark chamber)					
Station	Assay	1 hour	Slope	30 min	Slope	15min	Slope	1 hour	Slope	30min	Slope	15min	Slope
34	1	0.90	-0.019	0.50	-0.015	0.82	-0.041	0.98	-0.029	0.94	-0.032	0.74	-0.032
34	2	0.96	-0.021	0.95	-0.015	0.95	-0.028	0.92	-0.028	0.97	-0.028	0.88	-0.030
34	3	0.97	-0.025	0.92	-0.028	0.73	-0.033	0.94	-0.031	0.94	-0.045	0.93	-0.061
34	4	0.91	-0.023	0.92	-0.024	0.71	-0.026	0.97	-0.029	0.94	-0.025	0.85	-0.028
3	1	0.84	-0.013	0.63	-0.015	0.52	-0.018	0.84	-0.016	0.60	-0.015	0.58	-0.023
3	2	0.71	-0.015	0.57	-0.025	0.27	-0.032	0.90	-0.019	0.81	-0.026	0.63	-0.025
3	3	0.78	-0.016	0.32	-0.012	0.29	-0.022	0.77	-0.054	0.86	-0.107	0.59	-0.024
3	4	0.88	-0.032	0.49	-0.017	0.30	-0.023	0.84	-0.044	0.43	-0.020	0.07	-0.013
4	1	0.77	-0.014	0.85	-0.043	0.52	-0.030	0.62	-0.023	0.81	-0.031	0.42	-0.025
4	2	0.90	-0.030	0.81	-0.037	0.64	-0.043	0.85	-0.022	0.40	-0.008	0.07	-0.005
4	3	0.80	-0.022	0.51	-0.022	0.00	-0.002	0.91	-0.026	0.88	-0.033	0.80	-0.047
4	4	0.62	-0.008	0.39	-0.010	0.61	-0.020	0.93	-0.019	0.79	-0.019	0.48	-0.019
4	5	0.71	-0.010	0.26	-0.007	0.20	-0.010	0.78	-0.014	0.34	-0.010	0.05	-0.007
35	1	0.97	-0.031	0.89	-0.035	0.79	-0.047	0.84	-0.019	0.30	-0.011	0.24	-0.022
35	2	0.93	-0.014	0.85	-0.017	0.50	-0.014	0.89	-0.019	0.79	-0.021	0.81	-0.036
35	3	0.66	-0.006	0.62	-0.017	0.31	-0.016	0.58	-0.011	0.59	-0.019	0.53	-0.033
35	4	0.64	-0.005	0.12	-0.002	0.02	-0.001	0.93	-0.019	0.82	-0.020	0.40	-0.015
11	1	0.94	-0.019	0.85	-0.018	0.34	-0.007	0.88	-0.016	0.69	-0.014	0.14	-0.007
11	2	0.64	-0.012	0.86	-0.029	0.47	-0.022	0.94	-0.018	0.85	-0.017	0.66	-0.018
11	3	0.92	0.035	0.91	0.034	0.82	0.046	0.60	-0.019	0.07	-0.009	0.01	-0.005
11	4	0.84	0.010	0.73	0.001	0.58	0.012	0.82	-0.025	0.65	-0.031	0.24	-0.021
11	5	0.59	-0.005	0.32	-0.004	0.68	-0.014	0.58	-0.016	0.20	-0.014	0.08	-0.016
Average:		0.81	-	0.65	-	0.50	-	0.83	-	0.67	-	0.46	-