BENTHIC MACROINVERTEBRATES AS BIOINDICATORS OF WATER QUALITY IN THE YAGUEZ RIVER, MAYAGUEZ, PUERTO RICO

by

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ABSTRACT

The objective of this study was to correlate the diversity of aquatic macroinvertebrates with water quality of the Yagüez River in Mayaguez, Puerto Rico. In the year 2005, for a period of eight months (January 27-December 12), three stations were randomly selected with three replicates establishing a longitudinal gradient along the river. Physicochemical variables such as pH, water temperature, stream flow, dissolved oxygen, conductivity, turbidity, and nutrients were measured at each site. Macroinvertebrates were collected with a D-net and preserved in 70% ethanol. The dominant orders found were Coleoptera (44.9%), Ephemeroptera (31.7%), and Prosobranchia (8.9%). The majority of the organisms found within these orders are indicators of intermediate and fair water quality. The Two-Way ANOVA indicated that seasonality was the variable that best explained the distribution of macroinvertebrates. According to the biological index "Biological Monitoring Working Party" (BMWP), stations A (downstream) and B (midstream) had acceptable water quality, and station C (upstream) had dubious water quality. The spatial distribution of the macroinvertebrates along the Yagüez River was determined by the seasonal rainfall pattern (dry and rainy season).

RESUMEN

El objetivo de este estudio fue relacionar la diversidad de los macroinvertebrados acuáticos con la calidad del agua del Río Yagüez en Mayagüez, Puerto Rico. En el año 2005, por un período de ocho meses (del 27 de enero al 12 de diciembre), tres estaciones en los rápidos fueron seleccionadas aleatoriamente con tres réplicas que establecían un gradiente longitudinal a lo largo del Río Yagüez. Las variables fisicoquímicas pH, temperatura del agua, corriente, oxígeno disuelto, conductividad, turbidez, y los nutrientes fueron medidos en cada sitio. Los macroinvertebrados fueron colectados con una red tipo D y llevados al Laboratorio de Entomología. Luego se clasificaron, identificaron y preservaron en etanol al 70%. Los órdenes dominantes encontrados fueron Coleóptero (44.9%), Efemeróptero (31.7%), y Prosobranchia (8.9%). La mayoría de los organismos encontrados dentro de estos órdenes son indicadores de una calidad del agua intermedia y pobre. La prueba de Análisis de varianza (ANOVA) a dos vías indicó que la estacionalidad es la variable que mejor explica la distribución de los macroinvertebrados. Según el índice de agua "Biological Monitoring Working Party" (BMWP), las estación A (río abajo) y B, (centro del río) ambas tenían condiciones aceptables en calidad agua y la estación C (río arriba) tenía condiciones dudosas de calidad del agua. La distribución espacial de los macroinvertebrados a lo largo del Río Yagüez fue determinada por patrones estacionales de lluvia (período seco y de lluvioso).

To my family and friends, for their love and support; without you this dream would not have been possible

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1 INTRODUCTION

During the past century water demand has increased, leading public agencies to develop larger supplies for domestic, irrigation, electricity, and industry usage (Áviles, 1969). The problem is that for a long time the management of our water resources has been dominated by obsolete legal policies, ineffective implementation of laws and focused in the chemistry of the waterbody (Karr and Chu, 1999). This has caused intensification of the levels of pollution and contaminants disposed into waterbodies. This impact has encouraged the government to create and implement new water resource management techniques.

Traditionally, the United States Environmental Protection Agency (USEPA) and states monitoring programs have relied on chemical analyses that measure the individual pollutant in the water column and physical variations (Ohio USEPA, 1990). Measuring chemical and physical perturbation have several advantages: 1) they are cost-effective; 2) rapid assessment; 3) in occasions the early detection of variations in physicochemical parameters and habitat prevents the alteration of the biological community; and 4) are also used as "diagnostic" indicators assisting in determining the causes and sources of problems affecting the biota (USEPA, 2007). Using only chemical and physical parameters it was found to be inefficient in detection of changes in the natural conditions of rivers because they only provide a fixed image of perturbation (Ohio USEPA, 1987; Alba-Tercedor, 1994; Segnini, 2003). A variety of methods are required to manage our water resources cost-effectively. This has led to the implementation of biological organisms (as bioindicators) to complement chemical and physical methodologies.

Biological monitoring measures and evaluates the condition of the biota and identifies ecological risks that are important for human health. This type of monitoring has been widely used in the evaluation of water quality (Hellawell, 1986; Cairns and Pratt, 1993; Alba-Tercedor, 1996; Figueroa et al., 1999; Guerrero-Bolaño et al., 2003) in Europe and the United States. This methodology detects the susceptibility of particular species, i.e., algae, benthic macroinvertebrates, and fish (Lenat and Barbour, 1994), to changes in their habitat (Segnini, 2003). The concept of bioindicator has been defined as a species, population, or community that has specific environmental requirements. An ideal indicator is one that has a low tolerance to environmental fluctuations (Zuñinga de Cardozo, 2000; Guerrero-Bolaño et al., 2003) that could affect the community structure of the river. Fluctuations in physicochemical parameters can cause a variation in the number, morphology, physiology or behavior of those organisms (Guerrero-Bolaño et al., 2003).

The most commonly used group of bioindicators are the benthic macroinvertebrates (Rosenberg & Resh, 1993; Resh & Jackson, 1993; Junqueira et al., 2000; USEPA, 2002; Kuhlmann et al., 2005; Silva et al., 2005). Among the macroinvertebrates several organisms have been suggested as indicators: mollusks (Mollusca), flat worms (Platyhelminthes), segmented worms (Annelida), and immature insects (Insecta) (Zuñiga, de Cardoso, 1994). Within the macroinvertebrates there is a group of species (e.g., Ephemeroptera, Plecoptera and Trichoptera (EPT taxa)) or individual orders (e.g., Mayflies larvae - Order Ephemeroptera) that are used in an effort to assess water quality conditions (USEPA, 2007). Their usage includes several advantages: facilitating comparisons among several bodies of water, limited mobility, wide distribution in a variety of local habitats, and simple equipment can be used to sample macroinvertebrates (Hawkes, 1979; Penny, 1985; Hellawell, 1986; Abel, 1989; Figueroa et al., 1999; Reece and Richardson, 1999). Their life cycles are relatively shorter when compared to fish and they have a wide sensitivity to different levels of pollution (Hellawell, 1986; Rosenberg & Resh, 1993; Reece and Richardson, 1999; Silva et al., 2005). Benthic macroinvertebrates live and feed in sediments where toxins are likely to accumulate. They also serve as a primary food source for many fish (Barbour et al., 1999), thereby providing sources for bioaccumulation of toxins in fish. These organisms have a historical record in field studies which analyze community structure, including being used in many biotic and diversity indices (Hilsenhoff, 1988; Alba-Tercedor, 1996).

Benthic macroinvertebrates have been used since the early 1900's as a biomonitoring tool in temperate zones. Early studies took place in the Illinois River analyzing the presence of organisms that had various tolerance levels to waste decomposition (Forbes and Richardson, 1913; Richardson, 1928). In Europe, Kolkwitz and Marsson (1908, 1909) developed the *Saprobiensystem* for the assessment of organic pollution. This system recognizes three zones that describe the severity of organic pollution and characteristics of fauna and flora (Goodnight, 1973). During the 1980's, in the United States government implemented the Rapid Bioassessment Protocols (RBPs) nationwide. The RBP's were created as cost-effective techniques to assess and monitor its streams. Benthic macroinvertebrates are the most popular bioindicators implemented by the states (Barbour et al., 1999).

Europe and the United States have developed protocols where they implemented biological tools like macroinvertebrates. The U.S. Environmental Protection Agency (USEPA, 2002) did a study using artificial substrates to sample benthic macroinvertebrates of the Ohio River from 1965-1971 and compared it with a repetition of the study in 2002. In Great Britain; Armitage et al. (1983) ordered the macroinvertebrates in tolerance groups generating a gradient of the less to the greatest in tolerance contamination. With this system it was easier to compare the sampling sites. Then Spain adapted this system of tolerance ranges to develop their own family index where new organisms are added to the original table and the tolerance values for some changed (Alba-Tercedor and Jimenez-Millan, 1987). However, in Neotropical zones fewer studies have been done using macroinvertebrates as water quality indicators when compared to temperate zones.

In the Neotropical zones the implementation of biological monitoring using macroinvertebrates has been limited due to the lack of specialists in this area and the few studies that are available that describe the taxonomic composition of the species (Figueroa et al., 1999). In Mexico, Hurtado et al. (2005) analyzed the structure and ecological changes the in San Juan river basin using the macroinvertebrate bioindicators to develop conservation plans. Also in Colombia several studies have macroinvertebrates to determine water quality of rivers. Zuñiga de Cardoso et al. (1994) conducted a study in the basins of the Cali and Meléndez rivers in the department of Valle, which included not only physicochemical and bacteriological parameters but biological variables (benthic macroinvertebrates) that identified factors that affected the water quality. However, more research regarding macroinvertebrates as bioindicators is necessary.

There are few studies of benthic macroinvertebrate communities in streams of Puerto Rico. The majority of these studies was conducted in the Caribbean National Forest and was focused on the impact of gastropods in lotic environments (Covich and McDowell, 1996). Additional information regarding benthic macroinvertebrate communities of the streams was gathered in a study by Covich (1994). Although his observations were based on qualitative sampling (i.e.made by picking specimens from rocks) he noted that there was considerable variation in taxa composition of the invertebrate communities in different streams. Understanding the relationship between habitat and aquatic biota is important for the development of new management techniques (Brasher, 2003). This can be accomplished by studying additional stream ecosystems.

Mayagüez is located on the western coast of Puerto Rico and has a land area of 78 mi^2 with a population of about 114,000 residents (USGS, 2004). It has three main rivers running through it, including the Yagüez River. The Yagüez River is being considered as a water supply because of increased demands by the city's population. The aquatic organisms of these rivers have not been systematically surveyed or recorded.

This information is crucial since there is a growing concern regarding the deterioration of the public water supply system affecting the number of housing and industries that can be constructed (USGS, 2004). The principal source of potable water from runoff surface water for this city is the use of filtration plants (USDC, 1998). To satisfy the water demand, the municipal government requested the U.S. Geological Survey (USGS) to conduct an assessment of the surface, ground water resources, and the sanitary quality of the streams within the city limits (USGS, 2004). An effective management plan for this river will require information about its aquatic invertebrate communities.

The goal of this study was to validate established methodologies that could be used for long-term ecological monitoring. We sampled the Yagüez River during eight months to: (1) examine the spatial and temporal variations of the benthic macroinvertebrate communities, (2) determine whether there are differences in taxonomic composition of invertebrates communities along the longitudinal gradient of the river, (3) assess channel characteristics, and (4) analyze relationships between water quality and the tax composition and abundance of macroinvertebrates as bioindicators.

2 LITERATURE REVIEW

2.1 Biotic and abiotic factors that influence the spatial and temporal distribution of aquatic macroinvertebrates

In ecological research, a major challenge is to understand the spatial and temporal patterns that affect diversity in nature (Fenoglio et al., 2004). In lotic waters, the physical environment imposes certain challenges to the organisms that live there (Allen, 2001), thus studying the environmental variability in lotic systems will provide us with an idea of how the organisms are distributed.

It is well known that severe physical disturbances (i.e. increments in discharge) reduce the density and diversity of benthic macroinvertebrates, but that they recover rapidly from such disturbances (Death et al., 1995). Many tropical streams are subject to increments in discharge (Power et al., 1988); this physical disturbance has been postulated as having a strong influence in changing the community structure or nutrient availability in rivers and streams (Lake and Barmuta, 1986, Resh et al., 1988).

The impact of lesser disturbances, such as periodic increases in flow following showers or changes in season, on macroinvertebrate community structure is not clear. In freshwater ecosystem from the temperate region, New Zeland, Death et al. (1995) found a strong relationship between environmental stability and macroinvertebrate diversity. They suggested community structure was kept by the combination of low levels of disturbance and habitat patchiness.

2.1.1 Factors that affect species zonation in Neotropical region

Aquatic macroinvertebrate distributions in lotic systems are generally considered to be affected by environmental factors at different spatial scales due to environmental heterogeneity (Heino et al., 2003, 2004). Studies demonstrated that across-scale variation takes place at a small scale when analyzing diversity within the same stream reach (Downes et al., 1993; Boyero et al., 2001; Li et al., 2001). The macroinvertebrate spatial variation in small scale studies is affected by hydraulic fluctuations in microhabitat, within a single riffle; by current velocity, composition of the substratum, and water depth; and by biotic factors like competition and predation (Minshall, 1984; Barmuta, 1990; Malmqvist and Mäki, 1994; Downes et al., 1998; Heino et al., 2004; Brooks et al., 2005).

In contrast to temperate ecosystems, the study of tropical macroinvertebrates is rather scarce. In terms of insects, there is seen to be a pattern of patchy distribution (Pringle et al., 1988). Habitat plays an important role in the composition of the macroinvertebrate community by providing food and shelter from predators. For instance, in some tropical streams, leaf accumulation on the stream bottom sustains a high density and diversity of insects (Ramírez et al., 1998). Riffle and pool areas also provide different habitat structure.

Another important factor determining the composition and density of insect assemblages in the tropics is the occurrence of stream macrobiota (fishes and decapods) (Ramírez and Hernández-Cruz, 2004). In tropical streams, predation from fish and omnivorous shrimps is important in structuring the macroinvertebrate community (Covich, 1988; Pringle and Hamazaki, 1998; Pringle et al., 1999; Crowl et al. 2001). Ramirez and Hernández-Cruz (2004) examined the effects of shrimp on insect assemblages in the Luquillo Experimental Forest in northeastern Puerto Rico in two second-order streams (Prieta and Bisley-3). They concluded that local geomorphology played a major role in determining the structure and composition of the aquatic insect assemblages. Either by predation or competition, shrimps could also be considered important regulators of insect community structure and composition.

In temperate regions the River Continuum Concept (RCC) was proposed by Vannote et al. (1980), this concept proposes that the structure and function of communities along a river is influenced by the gradient of physical factors formed by fluvial geomorphic processes creating a continuous gradient from headwaters to mouth. Few tropical systems have been studied to fit this model. Within the RCC, the longitudinal pattern is influenced by the availability of coarse organic matter in lotic food webs this could affect the composition of macroinvertebrates in Neotropical regions (Vannote et al., 1980). Greathouse and Pringle (2006) concluded from their studies of the Río Mameyes within the Luquillo Experimental Forest (LEF), that the RCC applies to lotic systems of tropical islands. However, an exception was found in this model that it does not apply for the filter-feeding groups of macroinvertebrates due to the fish or shrimp predation. Thus in the tropics, the RCC model seems to be potentially influenced by the top-down (i.e. predation) trophic control (Posey et al., 2002).

Fenoglio et al. (2004) studied the micro-distribution of stream invertebrates in a riffle of a Neotropical rainforest stream. They considered small-scale environmental characteristics such as current velocity, water depth, position of the riverbed, and characterization of the substratum. They found that faster current velocity increases the invertebrate density and richness because is related with water oxygenation and nutrient availability (Fenoglio et al., 2004. The position in the streambed was an important factor to those aquatic communities with lateral shallow parts having the lowest density and richness.

However, the habitat substrate composition also influences the type of dominant functional group. Greathouse and Pringle (2006) found that in boulder-bedrock pools, the diversity of functional group was affected since shredders decreased and scrapers increased. Also, Fenoglio et al. (2004) found that the substratum composition influenced both abundance and richness of the aquatic invertebrates. Boulders and cobbles showed more richness than sandy microhabitats.

2.1.2. Anthropogenic factors influencing the aquatic community structur**e**

The rapidly growing human population is causing stream communities to become at risk (Covich et al., 2003). Anthropogenic impacts like alteration in the land usage by domestic and agricultural usage change the biotic communities (Brasher, 2003). These alterations modify the aquatic habitats, changes the community structure, and affect the migratory patterns of organisms (McDowell, 1995; Smith et al., 2003), A major anthropogenic factor that is impacting stream ecosystems is worldwide urbanization. It creates changes in land coverage (Allan and Johnson, 1997; McCully and Pringle, 2000; Paul and Meyer, 2001) that increase the accumulation of toxic substances and water demand (Roy et al., 2003; Oscoz et al., 2006). Roy et al. (2003) found that catchments areas associated to urbanization developments showed a decrease in diversity and an increase in the abundance of more tolerant organisms.

Climate variability has caused prolonged droughts or floods in different parts of the world, affecting the habitats and ecosystems. This extended drought, in addition to increased population growth, has generated a water demand which affects the biota of aquatic ecosystems. The decrease in the water volume lowers the levels of dissolved oxygen and increases the accumulation of nutrients and the amount of toxins (Covich et al., 1999, 2003). Rapid human growth limits the available space in island ecosystems affecting their characteristics and increasing the water demand (Smith et al., 2003). Governments are under pressure to optimally use freshwater sources and are exploiting water resources with minimal planning.

Increase of human activities like recreation and consumption of stream waters can intensify the effects of natural low water periods. These periods are becoming more persistent and thus promote a decrease in migratory amphidromous populations such as (Benstead et al., 1999, 2000; Pringle et al., 2000; Covich et al., 2003). Covich et al. (2003) studied the 1994 prolonged drought in locality, where some headwater riffles dried out, thereby decreasing the densities of *Xiphocaris*. This specie study suggested that current climate change could cause alterations in aquatic populations and communities.

March et al. (2003) discussed the ecological problems caused by building dams on stream habitats. Tropical island streams are dominated by amphidromous fauna that migrate up and down the streams; thus dams represent obstacles that fragment aquatic habitats. In Puerto Rico there are large dams without spillways, becoming impenetrable barriers for native fish and shrimp (Holmquist et al., 1998). However, dams with spillways provide the means for certain native fish and shrimp to overcome the barrier. Small dams have the same effect as large dams by interfering with the migration patterns of certain organisms (Benstead et al., 1998; Fievet et al., 2001). In a study done by Benstead et al. (1999) in Puerto Rico, a low-head dam acted as a partial barrier to upstream migration, but it created a bottleneck effect during the low river flow. The bottleneck effect results in a concentration of predators and prey just below the obstacle (dam) that translates into higher mortality rates for prey (Benstead et al., 1999). During low river flow, shrimp larvae were entrained into the water intake of the dam, having mortality rates of 34% and 62% (Benstead et al., 1999).

Another important factor that influences benthic community structure and ecosystem processes in freshwater and marine ecosystems is the fluctuation in interhabitat coupling (Palmer et al., 2000; Levin et al., 2001; Austen et al., 2002; Covich et al., 2004). According to Covich et al. (2004), interhabitat coupling is the transport of nutrients, organic matter, and sediment among aquatic and terrestrial habitats and within the aquatic ecosystem. Nutrients influence aquatic community structure because they are the resources used by primary producers and their fluctuations affect the food web. Although, nutrient enrichment could enhance the growth of primary producers its major effect was on primary consumers (Ramírez and Pringle, 2006). Primary consumers have been found to respond positively to nutrient-enriched detritus-based ecosystems by increasing insect density and physiology (Pearson and Connolly, 2000; Robinson and Gessner, 2000; Rosemond et al., 2002; Cross, 2004; Ramírez and Pringle, 2006). Ramírez and Pringle (2006) found that larval Chironomidae (Diptera) under high phosphorous conditions matured more rapidly; this fast biomass turnover assures a constant food supply to predators. They came to the conclusion that primary consumers could move significant amounts of energy to upper trophic levels and found similarity in larval biomass among streams as phosphorus increases.

In Puerto Rico there has been an increase in the levels of eutrophication in the surface waters. Phosphate input to rivers in Puerto Rico could originate from different sources, but the main ones are from organic wastewater discharge and from inorganic crop fertilizers. This induces the growth of undesirable algae and aquatic weeds and affects the concentration of oxygen when they decompose (Sharpley et al., 2000). Phosphorus is the limiting nutrient in freshwater (Correl, 1998) and thus an excess accelerates eutrophication. Sotomayor et al. (2001) studied eleven streams in Puerto Rico and found that four had median phosphorus levels (0.04-029 mg) ; six had at least 25% of the sampling episodes exceeding 0.1 mg P/L, with only the Guajataca River not indicating this trend. The Yagüez River was among the six rivers with at least 25% of their sampling above 0.1 mg P/L. However, the Loíza, Coamo, and Guanajibo Rivers had the highest concentration of total phosphate (TP), at least 75% of their samples were above 0.1 mg P/L.

2.2 Bioassessment of Water Quality

2.2.1 Benthic macroinvertebrates in streams employed as biomonitoring tools

An alternative method to determine water quality is studying organisms like macroinvertebrates. Using macroinvertebrates as biomonitoring tools provides a general view of what has occurred in the waterbody (Alba-Tercedor, 1994; Ohio USEPA, 1987). Because they are affected by various environmental stressors at different life stages (Barbour et al., 1999).

Moreover, benthic macroinvertebrates serve as primary food source for many fish that are key components of a healthy food web.

Benthic macroinvertebrates have been used since the early 1900's as a biomonitoring tool. Early studies took place in the Illinois River analyzing the presence of organisms that had various tolerance levels to waste decomposition (Forbes and Richardson, 1913; Richardson, 1928). In Europe, Kolkwitz and Marsson (1908, 1909) developed the *Saprobiensystem* for the assessment of organic pollution. This system recognizes three zones that describe the severity of organic pollution and characteristics of fauna and flora (Goodnight, 1973). In the United States a similar methodology was created by Wilber (1969), but with less structure.

In the mid-1980's, the Rapid Bioassessment Protocols (RBPs) were created as a cost-effective technique to assess and monitor the extensive kilometers of streams in the United States (Barbour et al., 1999). The RBPs were made for three aquatic assemblages (i.e., periphyton, benthic macroinvertebrates, and fish) and are a combination of previous methods used by several state water resource agencies. To assist in managing our water resources the government recognized that it was necessary to find a rapid way to analyze data.

Currently, benthic macroinvertebrates are preferred and widely used as a biomonitoring tool throughout the world. There are several advantages when using these organisms: they are easy to collect, sampling has been standardized, equipment is cost-effective, their life cycles are sufficiently long to detect changes in its environment, they are widely distributed, comparisons can be made between aquatic systems, and they have different ranges of tolerance to contaminants (Hellawell, 1986; Alba-Tercedor, 1996). Although the practice of biomonitoring has been around for more than 100 years in temperate zones of Europe and the United States (Ghetti, 1986; Hellawell, 1986; De Pauw and Vanhoren, 1983; Rosenberg and Resh, 1993; Roy et al., 2003), this approach is relatively novel in Neotropical regions.

In the Neotropics undergoing studies are being conducted to determine the structure and ecological importance of macroinvertebrates as indicators of water quality in Mexico (Figueroa and Pinzón, 2000; Hurtado et al., 2005), Chile (Figueroa et al., 2003, 2005), Panama (Medianero and Samaniego, 2004), Puerto Rico (Collazo, 2001), Costa Rica (Ramírez and Pringle, 2006), Brazil (Cleto-Filho and Walter, 2001; Marques and Barbosa, 2001), Peru (Lloyd et al., 1992), Argentina (Paggi, 2003), Colombia (Asprilla et al., 1998; Roldán 1999; Posada et al., 2000; Guerrero-Bolaño et al., 2003; Rincón 2003; Gutiérrez et al., 2004), and Venezuela (Correa, 2000; Segnini, 2003).

2.2.2 Biotic Index

Biotic index are based on the presence of certain organisms and are related to their life cycles. Biotic indices are independent of seasonality and provide information regarding the macroinvertebrate assemblage. The biological indices take into consideration not only the concepts of the "Saprobiensystem" and the diversity, but integrate the adaptability of the different taxonomic groups providing information about aquatic community structure (Segnini, 2003).

Their early development was associated with the first studies done in Europe by Kolwitz and Marson (1908, 1909) cited by Goodnight (1973). Different biotic indexes have been developed in Europe (Table 2.1) and in the United States (Table 2.2). However, in Neotropical regions both the BMWP' (Figueroa et al., 1999; Guerrero-Bolaño et al., 2003; Pino-Chala et al., 2003) and the FBI (Vergara-Ruiz et al., 1994; Figueroa et al., 2003) are being used because these indices enable rapid identification (family level) and assessment.

Table 2.1. Biotic indexes used in Europe.

Index	Authors	Usage	Methodology
Saprobien System	Kolwitz and Marsson (1908, 1909)	This system was widely use for the assessment of organic pollution.	It recognizes three zones decreasing in severe conditions having in each containing characteristics of animals and plants (Goodnight, 1973).
Beck's Biotic Index	Beck (1955)	It was the first simple method for illustrating biological data.	It divides macroinvertebrates in two categories: Class I: those that can tolerate no significant amounts of pollution. Class II: those that can tolerate moderate organic pollution but disappear in conditions that are anaerobic or nearly so (Goodnight, 1973).
Trent Biological Index (BI)	Woodiwiss (1964)	It was developed for the freshwater invertebrates found in the River Trent.	It determines the pollution level by the presence/absence of certain invertebrates (Woodiwiss, 1964).
Extended Biotic Index (EBI)	Guetti (1986)	Used for the evaluation of water quality. It's a modification of the Trent Biological Index.	It is based on the study macrozoobenthonic community (Guetti, 1986).
Biological Monitoring Working Party (BMWP)	Armitage (1983)	It was published in 1980 for a national survey of water by the National Water Council (NWC). To determine how polluted the water body is by organic pollution.	It assigns scores to certain types of invertebrates depending on the tolerance/intolerance to organic pollution (Armitage, 1983).
Biological Monitoring Working Party (BMWP')	Alba-Tercedor and Sánchez-Ortega (1988)	To assess organic pollution using macroinvertebrates.	The biotic index scale oscillates from 1 to 10 (sensitive to tolerant) and correlates the index with five different degrees of contamination (Alba-Tercedor and Sánchez-Ortega, 1988).

Table 2.2. Biotic indexes used in the United States.

Index	Authors	Usage	Methodology
Family Biotic Index (FBI)	Hilsenhoff (1977, 1987, 1988)	Based on family-level identification of stream arthropods and is a quantitative method.	High values indicate high pollution tolerance and low values indicate pollution intolerance (Hilsenhoff, 1988)
Invertebrate Community Index (ICI)	Ohio EPA (1987)	This methodology compares sampling sites to relatively undisturbed sites that have similar geographical features.	Depending of the undisturbed site comparison, ICI can receive a score of 6,4,2, or 0 (Ohio EPA, 1987)
National Sanitation Foundation Water Quality Index (NSF WQI)	Brown et al. (1970)	Systemize a series of tests in which it can monitor for patterns and changes over time in the water quality.	Nine water quality parameters were selected to be included in the index: dissolved oxygen, fecal coliforms, pH, biochemical oxygen demand, temperature change, total phosphate, nitrate, turbidity and total solids. The water quality has a range from 0 (worst) to 100 (best) (Brown et al., 1970).

2.3 Previous studies made on macroinvertebrates in streams of Puerto Rico

Few data exist concerning the benthic macroinvertebrate communities in streams of Puerto Rico. García-Díaz (1938), as a result of an ecological survey, reported a list of the genera of aquatic insects found in Puerto Rico. Denning (1947, 1948) described six new species and recorded two additional continental species of Trichoptera. Most of the local studies on freshwater invertebrates have mainly focused in the Luquillo Experimental Forest (LEF) and are mostly related to functional assemblages of invertebrates in lotic environments (Covich and McDowell, 1996). Other records of the benthic macroinvertebrate communities of the forest were included in a study by Covich et al. (1994). These observations were made by picking specimens from rocks rather than by systematic sampling. Covich et al. (1994) noted, however, that there was considerable variation in the diversity of the invertebrate communities in different streams of the LEF.

Based on the Theory of Island Biogeography (MacArthur and Wilson, 1967), the total numbers of macroinvertebrates species in Puerto Rico would be expected to be comparatively small. This theory applies to the caddisfly (Trichoptera) in Puerto Rico. Caddisfly have been used a bioindicators of water quality in temperate zone. There have been reported 42 caddisfly species from Puerto Rico compared with about 90 in Cuba, 39 in Jamaica, and 30 in Hispaniola (Flint, 1992; Flint, 1993).

Flint and Masteller (1993) did extensive work on the composition and phenology of Trichoptera in El Verde, Puerto Rico, which is located within the Caribbean National Rainforest. They came to the conclusion that the factors controlling emergence patterns of aquatic insects in tropical environments are still poorly known (Flint, 1993). They stated that the Trichoptera of Puerto Rico and the other Antillean islands are still scarcely studied.

Ferrington et al. (1993) studied the on the composition of Chironomidae (Diptera) at El Verde. Revealing that species richness, taxonomic composition, and relative abundances of Chironomidae emerging from Quebrada Prieta were similar to a study made by Lehmann (1979, 1981) in two low latitude streams in Zaire. The importance of this study was that it represented the first study of Chironomidae for a low-order neotropical rainforest stream. In Lehmann's (1979, 1981) studies, the abundant species were considered to be multivoltine throughout the year as is the case of the stream Quebrada Prieta (Ferrington et al., 1993). Suggesting that Chironomidae reproduces several time throughout the year.

Pescador et al. (1993) did a study on the composition and phenology of mayflies in Quebrada Prieta in the LEF in Puerto Rico. This stream has a low diversity of mayflies in comparison to higher latitude streams with two dominant families, Baetidae and Leptophlebiidae (Pescador et al., 1993). The family Baetidae had one genus *Cloeodes* and Leptophlebiidae had two genera, *Borinquena* and *Neohagenulus*.

2.3.1 Previous studies of the Yagüez River

The city of Mayagüez has an increasing population size. Large extensions of land have been paved for the development of urbanizations. This leads to a reduction in the permeability of the soil increasing runoff toward the river. Erosion near the river bed has increased the amount of sediments that get into it. The river has also been canalized, changing the river's natural basin causing floods and finally the river has been used as a clandestine sewer (Santiago-Vázquez et al., 1965).

The Engineering School sponsored by the Water Resources Research Institute-University of Puerto Rico at Mayagüez did a preliminary study of the Yagüez River and provided recommendations to prevent future floods. The recommendations made by this study were: regulating tanks, a pipe to run the river water through the city, defensive dikes in the margins, canalization and cleaning of the river bed (Santiago-Vázquez et al., 1965). This report also pointed out that floods were not the only problem the river faced, since during the dry seasons organic contamination is also a problem.

Another research on residential water usage pattern in Mayagüez was conducted by the Water Resources Research Institute (WRRI)-University of Puerto Rico. The objective of this research was to develop a model that relates residential water usage and property value, enabling developers to estimate water requirements. It was found that the family income status was not an adequate variable to estimate water usage. Instead, the number of bedrooms in a household has a relationship to water usage (Guilbe, 1969). Another research completed in the Yagüez River watershed was a hydrologic study to obtain sufficient data to propose water management plans (Nuñez, 1971). However, there have not been studies done in the Yagüez River that describe the benthic macroinvertebrates.

3 MATERIALS AND METHODS

3.1 Study Area

The Yagüez River passes across the city of Mayagüez located on the west side of Puerto Rico. The headwater originates in the mountains of Uroyoán, located to the southwest of the town of Las Marías and to the northwest of the town of Maricao at 365.76 m (1200 ft) above sea level. The Yagüez River has a total length of approximately 13 miles or 20.8 kilometers (García, 1938). It runs from east to west and its final part was canalized and located in the urban zone. According to the National Oceanic and Atmospheric Administration (NOAA) the mean annual precipitation is 1,766 mm (69 in) for the Mayagüez city during the period between 1948 and 2005.

During 2005, for a period of eight months (January 27-December 12), three stations were randomly selected along the riffles establishing a longitudinal pattern in the Yagüez River. At each station, three replicate plots that had similar physical characteristics like: depth, velocity and surface flow were sampled with an area of 1 m^2 (Brooks et al., 2005). The position of the three sites was taken using the global positioning system (GPS) unit. Station A was located in the Balboa sector at (18°12’37.3” N and 067°07’19.8” W), downstream of the Yagüez River with an elevation of 19 m. The station B was in the Batelles sector, midstream of the Yagüez River (18°12’37.6” N and 067°07’19.6) with an elevation of 72 m. Station C was located at Franklin Ortiz sector, upstream of the Yagüez River, (18°12’03.7” N and 067°03’00.9” W) with an elevation of 310 m (Fig.3.1 and Fig. 3.2).

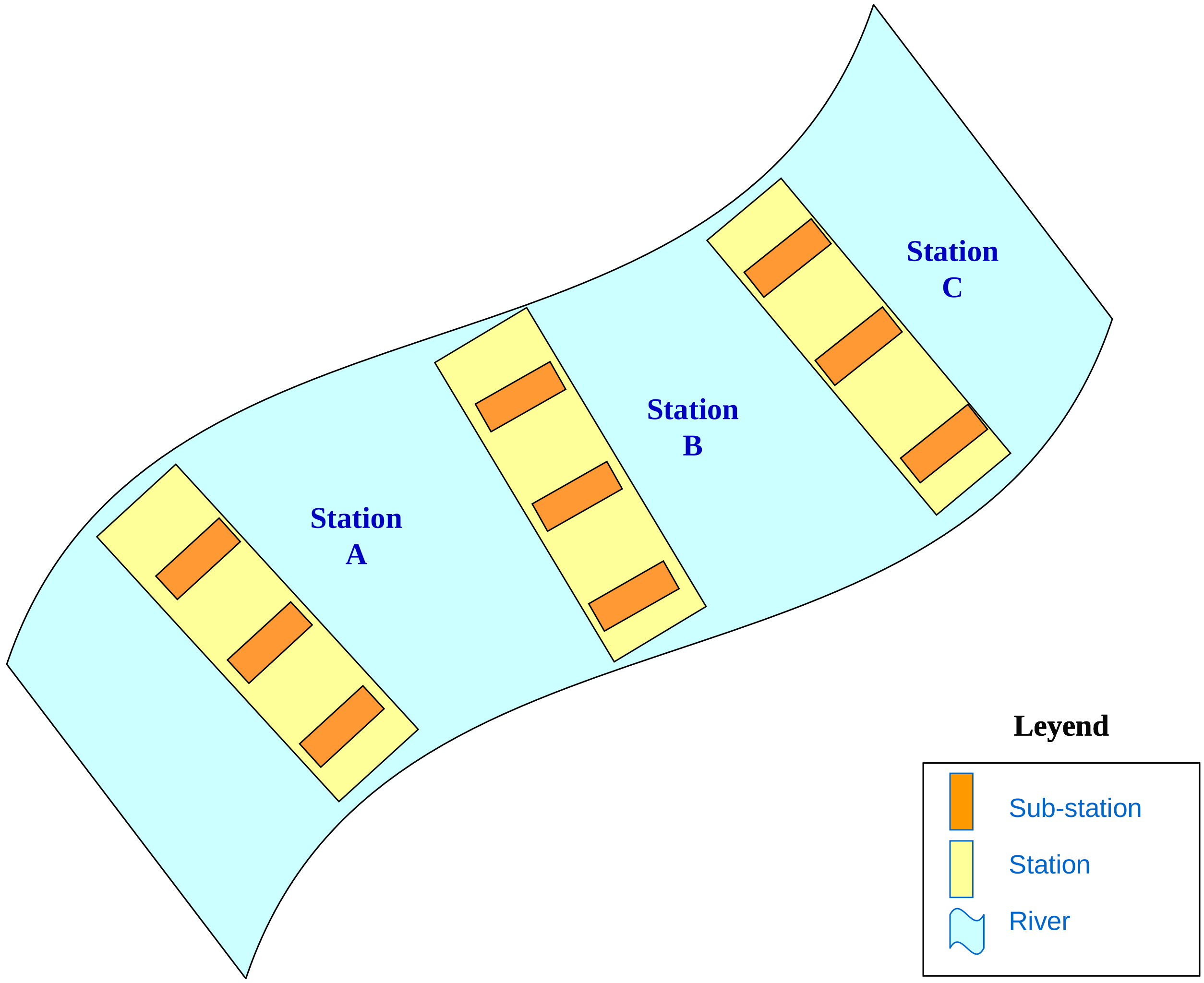


Fig. 3.1. The sampling arrangement for the three stations along the Yagüez River. The transect areas varied according to stations (A:32.40m^2, B:55.88m^2 and C:29.73m^2). The mean distance from shore to shore in each station was A=9.80 m, B=8.27 m, and C=4.80 m. In each station three substations were selected with a 1 m^2 area. The distance between stations A-B: 3,500 m and B-C: 4,500 m.

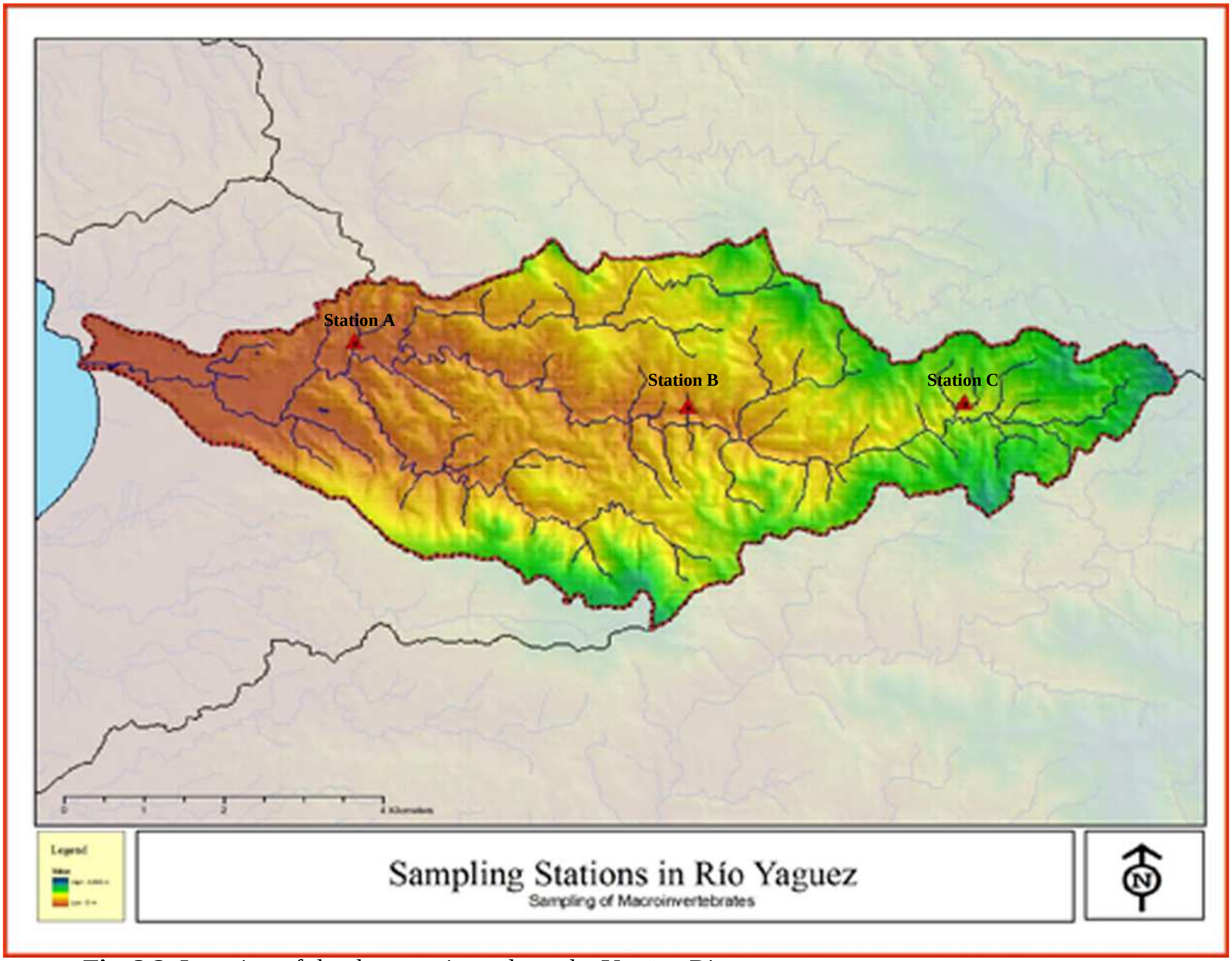


Fig. 3.2. Location of the three stations along the Yagüez River.

3.2 Physicochemical & nutrient sampling

At each sampling site, the physicochemical parameters were sampled once a month for a period eight months. The physicochemical parameters of the water were measured *in situ* using a *Hanna® pHep Waterproof pH Meter* for pH and temperature (°C); an *Oaklon® Waterproof Data*

Meter DO 300 Series for dissolved oxygen (mg/L); a *Hanna® DiST 1 Conductivity Meter* for conductivity (ppm) and *Pro Tape® Industrial Grade Model* was used to estimate depth (cm). The sensors used to measure the parameters of turbidity (NTU) [*Vernier® Turbidity Sensor*] and streamflows (m/s) [*Vernier® Flow Rate Sensor*] were connected to a *Texas Instruments® CBL2* and a graphic calculator *Texas Instruments® TI-83 Plus*. A *Garmin® GPS 12 Channel* was used to determine the position of the sampling sites. The mean precipitation was calculated three days before the sampling date.

Within the three stations, the nutrients were sampled by taking water from each plot using a sterile and Environmental Protection Agency (USEPA) pre-cleaned, wide-mouth, borosillicated 500 mL jar (Ben Meadows Company). These samples were stored in a cooler at 4^{o}C and taken to the Entomology Laboratory (UPR-M) for further analysis. The nutrients were analyzed in the laboratory using the LaMotte Smart 2 Colorimeter in order to determine the concentrations of phosphate, nitrate, and nitrite (it is subtracted from the nitrate values in order to eliminate the reduced nitrate to nitrite).

3.2.1 Cross sectional profile

Two random sites in pools within stations were selected as a representative of the reach for the month of June 2005. Vertical measurements were taken systematically at different points along the water surface making a horizontal line. At each point within the horizontal line we measured the vertical distance from the streambed and the water depth (Gordon et al., 1992).

3.3 Invertebrate Sampling

Invertebrate samples were collected from the fast-flowing riffle zone which is associated with high diversity (Plafkin et al., 1989). All the sites had a riparian covering consisting of bamboo but mainly arboreal. These samples provided estimates of the organisms' relative abundances in order (Fig.1.1) to compare the three sites. The kick sampling method was used in this study because is the most common way to sample benthic macroinvertebrates. A *kick* is a stationary sampling where the net is positioned and a disturbance (i.e., with the toe or heel of the boot the upper layer of the cobble is dislodged and the underlying bed is scraped) is generated in an area upstream of the net (Barbour et al., 1999).

The samples were collected using a D-frame kick net with a 210-mm mesh size (Brasher, 2003). Each station had three plots with an area of 1.5 m^2 each. Within each station sampling began at the downstream end of the reach and proceeded upstream of the riffle zone. Using a series of kicks of the substrate we sampled at various velocities three times, for one minute, in the riffle. This was called a composite sample.

The samples were removed from the D-frame kick net, placed on plastic containers, and stored in a cooler at 4^oC. Samples were taken to the Entomology Laboratory (UPR-Mayagüez), and were sorted and preserved in 70% ethyl alcohol with glycerin. The invertebrates in the samples were identified under a stereomicroscope with a 40x maximum magnification. The organisms were photographed and identified to family, using specialized taxonomic keys (Flint, 1964; Pennak, 1978; Vélez, 1985; Meritt and Cummins, 1996; Thorp and Covich, 2001). The macroinvertebrates were also classified into trophic groups (filterers, shredders, scrapers, collectors and predators) according to the criteria established by Barbour et al. (1999).

3.4 Measurement of the macroinvertebrates diversity

Many indices have been created for measuring species diversity; however there is still a polemic in determining which one is the best (Death and Winterbourn, 1995). In this study we used several indices to determine different aspects of the macroinvertebrate diversity.

1) Species number (S)

2) Total abundance

3) Dominance (d)

4) Species richness (D) by means of the Margalef's index (Clifford and Stephenson, 1975):

$$D = (s-1)/\ln N$$

D = diversity
s = number of individual per species
N = total number of individuals

Margalef's index has values that vary from 0 to 5. Values that are lower than 1 suggest contaminated water, values between 1 and 3 moderately contaminated habitats, and values over 3 correspond to clear waters (Merritt and Cummins, 1978 cited from Vergara et al., 1994).

5) The alpha by Fisher is another diversity index (Medianero and Samaniego, 2004) calculated by the statistical program PAST (Hammer et al., 2001), unlike the Shannon-Weaver and Simpson's, this index is independent of the sample size and does not give too much weight to the most common species in the sample (Wolda, 1983; Flowers, 1991).

$$\alpha = N(1-x)/x$$

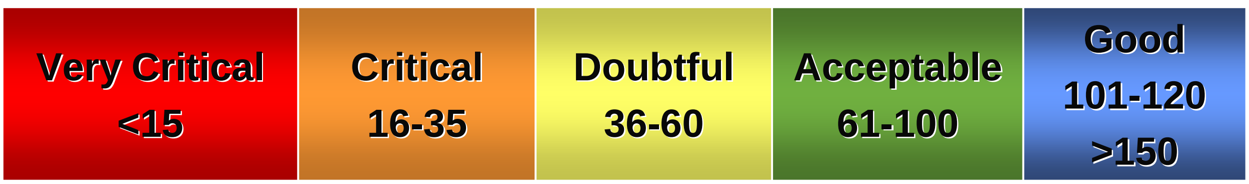
α = diversity index
N = total number of individuals
x = number of species

3.5 Biological Indices

I used the Biological Monitoring Working Party (BMWP') (Alba-Tercedor and Sánchez-Ortega, 1988). This index is based in the identification of families as biological indicators of water quality assigning tolerance values to pollution (Table 4.3) (Segnini, 2003). This index gives a value of 1 to 10 according to the tolerance of the taxa to organic pollution. The sum of all the tolerance values of each taxa provides the value for the BMWP. This value is then compared to five categories of water quality for a waterbody (Table 3.1).

Table 3.1. Water quality classifications and color for the Biological Monitoring Working Party (BMWP') index (Alba-Tercedor, 1996).

Class	Quality	Value	Explanation	Color
I	Good	>150	Very clean waters	Blue
		101-120	No contaminated or non altered waters	
II	Acceptable	61-100	There evidence of some contamination effect	Green
III	Doubtful	36-60	Contaminated waters	Yellow
IV	Critical	16-35	Very contaminated waters	Orange
V	Very Critical	<15	Heavily contaminated waters	Red



3.6 Data Analyses:

We analyzed the data to determine if it followed a normal distribution using the Shapiro-Wilks test (InfoStat, 2004). The Non-metric Multidimensional Scaling test (NMS) and the Principal Component Analysis (PCA), both multivariate analyses, were used to analyze habitat (physicochemical) and macroinvertebrate data (PC-ORD for Windows, Version 4.41, McCune and Mefford, 1999). The difference between these two multivariate tests is that the NMS integrates the biological data and how it responds to fluctuations in the physicochemical parameters. Instead, the PCA provides information of which physicochemical parameter has the greatest effect in the variance and provides a distribution pattern of the sites and sampling dates.

A Non-metric Multidimensional Scaling (NMS) test was used to observe how the biological data was distributed in a "physicochemical space" (pers. com. Ramirez, 2007). This test allowed us to detect changes in the biota relative to variations in the physicochemical environment.

Spearman rank correlations were used to identify relationships between physicochemical parameters and the diversity of the organisms with the water quality index (BMWP'). Using a confidence margin of $\alpha=0.05$. Tests were run using the statistical program InfoStat, version 2004 (InfoStat, 2004). The Spearman rank correlation was also used to examine potential relationships between the diversity indexes (Margalef and alpha Fisher) and the water quality index (BMWP'). This test provides information regarding the strength and direction of the relationship between two variables. The Spearman correlation has a rho (r_s) values between 1 and -1.

To determine the similarity that could exist in macroinvertebrate species composition among stations, a cluster analysis using the Bray-Curtis distance was employed. Kruskal-Wallis was used to determine if there were significant differences in the species abundances and the physicochemical parameters between the three sampling stations.

4 RESULTS

4.1 Physicochemical parameters

Among the chemical parameters measured the pH values were within the limits established by the EQB which are between 6 and 9 (Fig. 4.1a) for a class SD. A class SD indicates surface water intended for use as a raw source of public water supply, recreation and the preservation of species (EQB, 2003). The lowest pH for the three stations was measured in March (A=6.71 ± 0.01, B=6.71 ± 0.00, and C=6.66 ± 0.04). The highest pH for the three stations was reported in November (Fig. 4.1a). There were significant differences found for pH between stations and sampling dates (Kruskal-Wallis, $H = 62.87$ and $p < 0.0001$).

The dissolved oxygen measured from all stations was within the standard established by the EQB, which is 5.0 mg/L (Fig. 4.1b) for a class SD. A lower borderline value was observed during the month of June. According to a Kruskal-Wallis test, the dissolved oxygen presented significant differences between stations and sampling dates ($H = 68.63$ and $p < 0.0001$).

Turbidity (NTU) was significantly higher at all the stations in May (Fig. 4.1c). Differences of turbidity between samplings and stations were statistically significant (Kruskal-Wallis, $H = 49.16$ and $p = 0.0012$) within the three stations.

Conductivity was lowest at the three stations during (Fig. 4.1d) the month of June. There were significant differences between stations and samplings found along the Yagüez River with $H = 70.62$ and $p < 0.0001$.

Water temperature did not surpass the maximum standard temperature of 32.2 ^{0}C established by EQB. The temperature was highest during June (station A=25.33 ± 0.1^{0}C, B=25.13 ± 0.1^{0}C, and C=24.27 ± 0.1^{0}C) (Fig. 4.2a). There were statistically significant differences found between the three stations during all monthly sampling with H = 69.61 and $p < 0.0001$.

Stream flow was highest during the month of May (Fig. 4.2b). There was also an increased during October and November. The stream flow presented significant differences within the stations sampled H = 42.03 and p = 0.0090.

Depth was similar among the stations during the month of April with stations A=139.70 ± 49.5 mm, B=127.00 ± 34.1 mm, and C=127.00 ± 45.4 mm (Fig.4.2c). There were no significant differences in depths between stations and sampling dates (Kruskal-Wallis, H = 34.57 and p = 0.0562).

Rainfall (mm) had a maximum 14.65 ± 1.11, minimum 0.00 ± 1.11 and a mean 2.97 ± 1.11 for all the stations along the stream. There were two significant rainfall events, during the months of April and May (Fig.4.2d). There were no significant differences found for precipitation among the Yagüez River H = 40.78 and $p < 0.0001$.

Table 4.1. Physicochemical parameters measured in the three stations of the Yagüez River.

Parameter	**Stations**		
	A **Mean ± SD** **(high/low)**	**B** **Mean ± SD** **(high/low)**	**C** **Mean ± SD** **(high/low)**
Conductivity	335.57 ± 19.95 (500.00, 170.37)	332.84 ± 24.14 (540.00, 170.15)	307.15 ± 14.64 (387.40, 171.48)
pH	7.56 ± 0.10 (8.52, 6.70)	7.46 ± 0.14 (8.62, 6.46)	7.63 ± 0.10 (8.66, 6.64)
Turbidity (NTU)	53.38 ± 7.70 (147.00, 15.00)	61.92 ± 7.95 (162.00, 26.00)	57.50 ± 7.99 (147.00, 21.00)
D.O. (mg/L)	6.99 ± 0.22 (8.58, 5.03)	7.23 ± 0.25 (9.32, 5.13)	7.37 ± 0.24 (8.97, 5.06)
Streamflow (m/s)	0.40 ± 0.06 (1.28, 0.14)	0.38 ± 0.06 (1.08, 0.05)	0.34 ± 0.04 (0.94, 0.02)
Depth (mm)	154.28 ± 12.89 (285.75, 38.10)	195.66 ± 16.21 (342.90, 85.73)	147.90 ± 12.09 (304.80, 63.50)
Temperature (0C)	22.82 ± 0.27 (25.40, 20.90)	22.45 ± 0.30 (25.20, 20.00)	22.13 ± 0.26 (24.30, 20.20)
Rainfall (mm)	2.97 ± 1.11 (14.65, 0.00)	2.97 ± 1.11 (14.65, 0.00)	2.97 ± 1.11 (14.65, 0.00)
Phosphate (mg/L)	0.58 ± 0.08 (2.31, 0.11)	0.77 ± 0.13 (2.89, 0.00)	0.54 ± 0.08 (2.08, 0.00)
Nitrate (mg/L)	1.55 ± 0.20 (5.81, 0.00)	1.23 ± 0.14 (2.78, 0.00)	1.45 ± 0.21 (4.26, 0.00)

4.2 Nutrients

During the months of February (A=0.84 ± 0.25, B=2.13 ± 0.29 and C=1.17 ± 0.32) and October (A=1.09 ± 0.27, B=1.11 ± 0.22 and C=0.65 ± 0.14) the concentrations of phosphate were higher than during other sampling dates (Fig. 4.3a). There were significant differences between stations and sampling dates found along the Yagüez River ($H = 63.04$ and $p < 0.0001$). The total phosphate concentration standard established by the EQB is 1 mg/L (EQB, 2003).

The mean nitrate (mg/L) concentrations measured at the stations are found in Table 4.1. Statistically significant differences in nitrate concentration were found among sampling stations during every sampling event (Kruskal-Wallis, $p=0.0001$). Nitrate concentrations were below the USEPA standard for drinking water (10 mg/L; Fig. 4.3b) (USEPA, 2003). The highest concentrations of nitrate were measured during January at stations C (upstream) and B (midstream). Meanwhile, the highest concentration for station A (downstream) was measured in October.

4.3 River Channel Characterization

A morphometric cross-section of the Yagüez River was constructed during June 2005. From Fig. 4.4 it is evident that stream flow created different passages within the river, widening downstream. Arrows represents stream flow as it goes through each station. This pattern varied throughout the year due to the annual rainfall that occurs in the river.

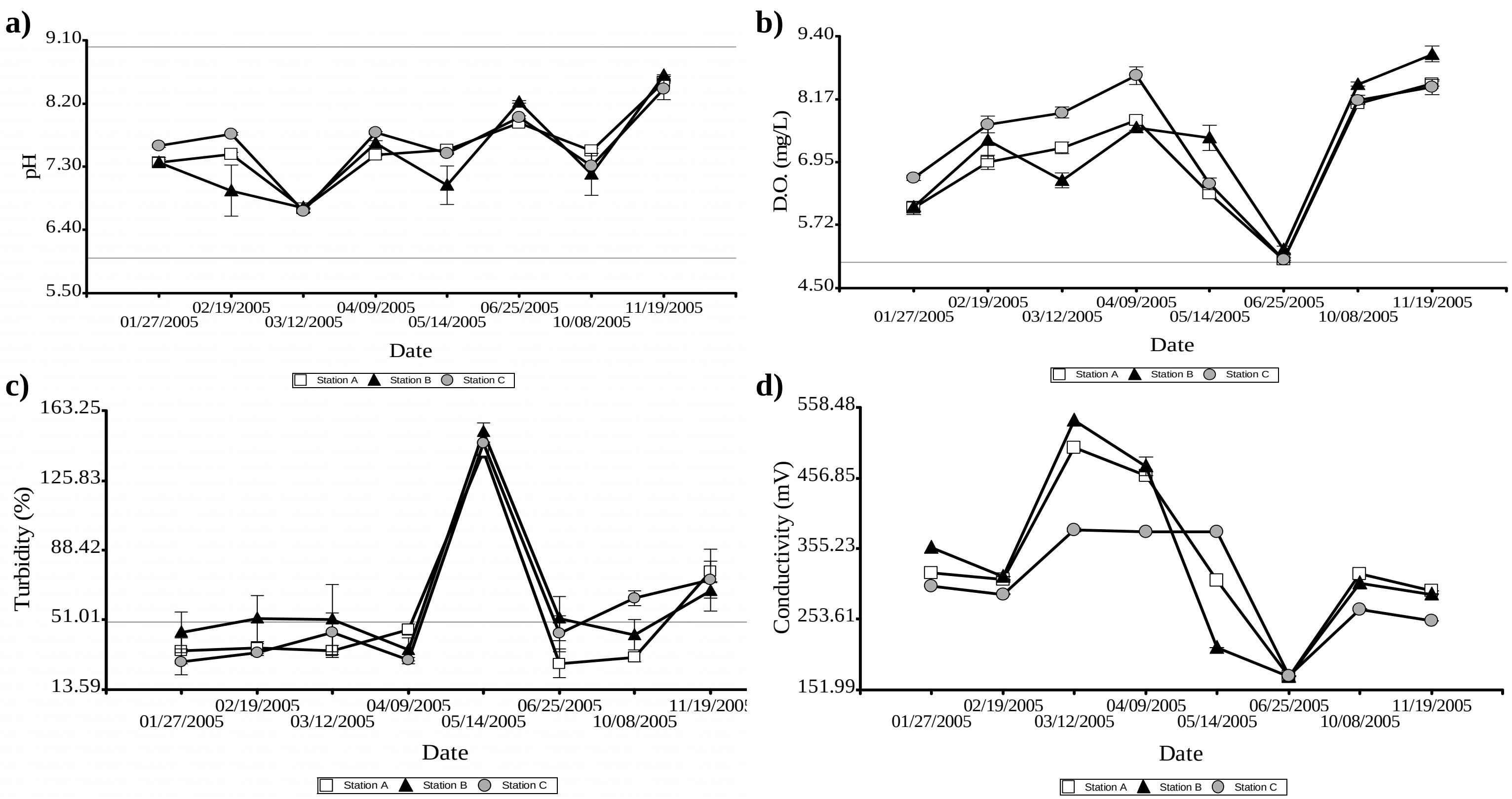


Fig. 4.1. Temporal variations of chemical parameters: (a) pH, (b) dissolved oxygen, (c) turbidity, and (d) from the conductivity in the Yagüez River, Puerto Rico (January-November, 2005). Bars represent standard errors from the mean (SEM) and horizontal lines indicate standard parameter.

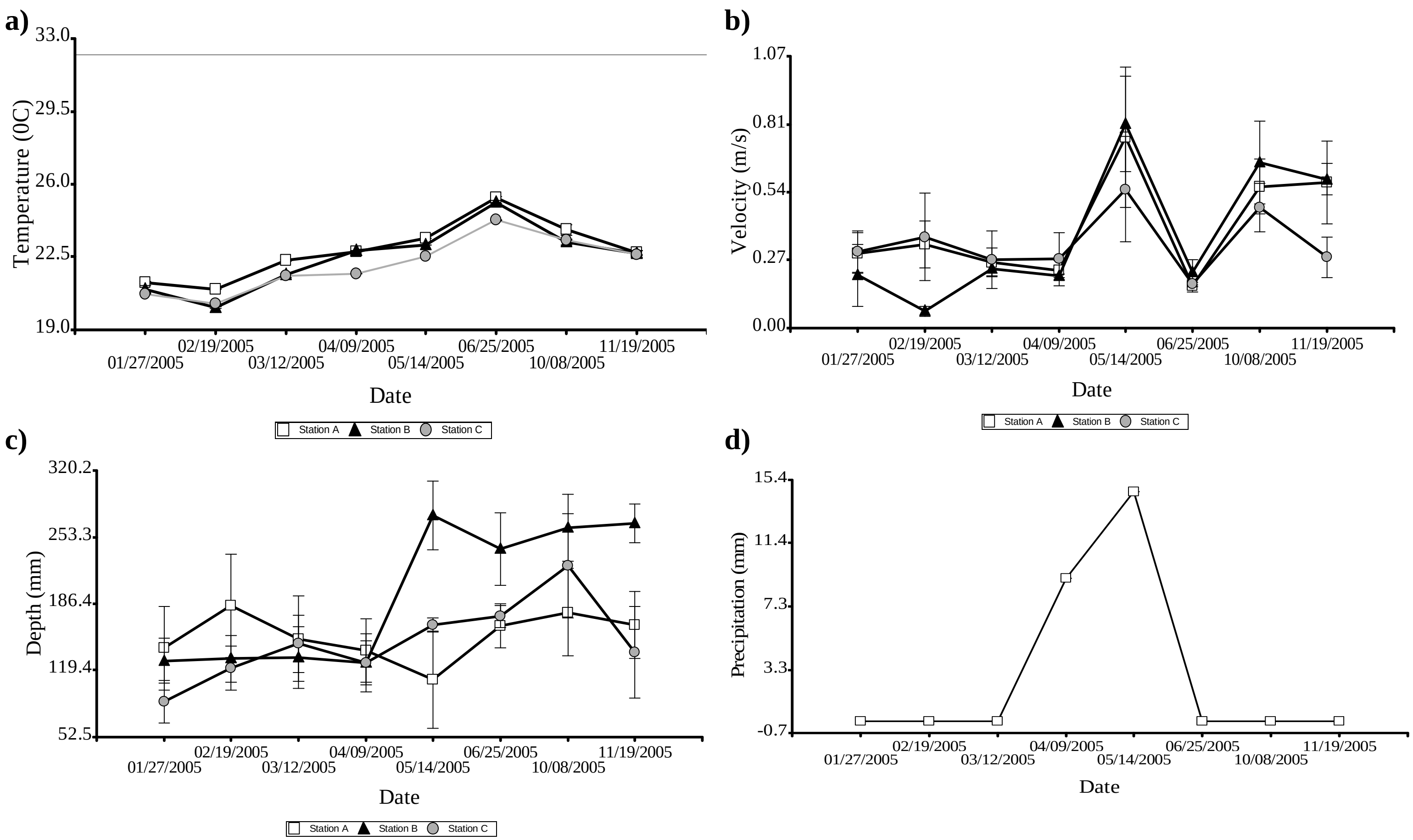
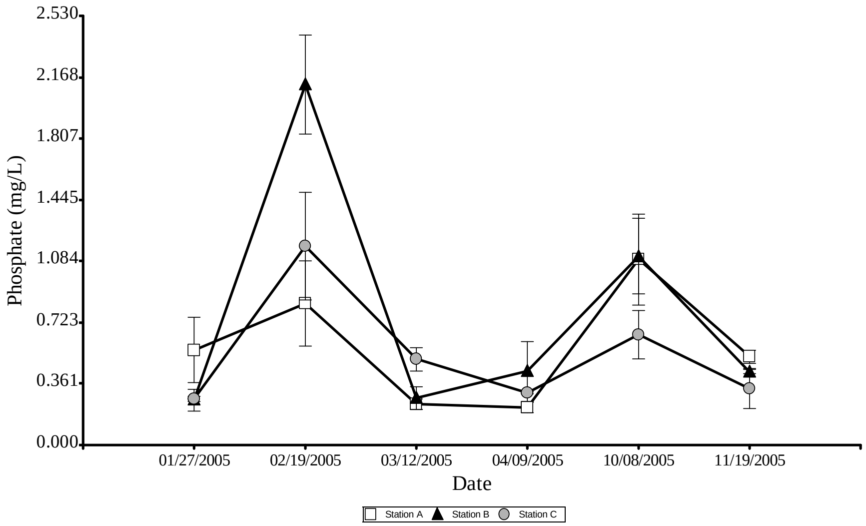


Fig. 4.2. Temporal variations of physical parameters: (a) temperature, (b) velocity, (c) depth, and (d) rainfall in the Yagüez River, Puerto Rico (January-November of 2005. Bars represent standard errors of the mean (SEM) and horizontal lines indicate standard parameter.

a)



b)

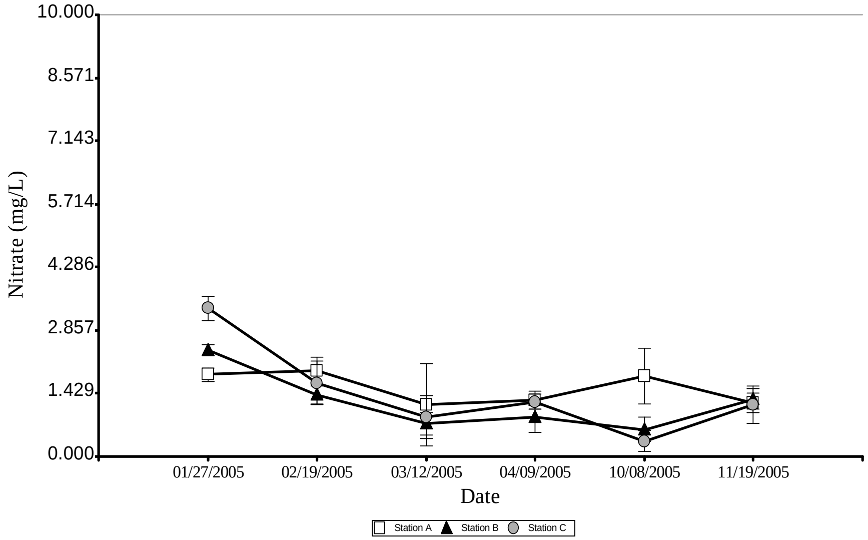


Fig. 4.3. Temporal variation of nutrient concentrations (mg/L): (a) phosphate and (b) nitrate measured at three stations in the Yagüez River (January-November of 2005). Bars represent standard errors of the mean (SEM) and horizontal lines indicate standard parameter.

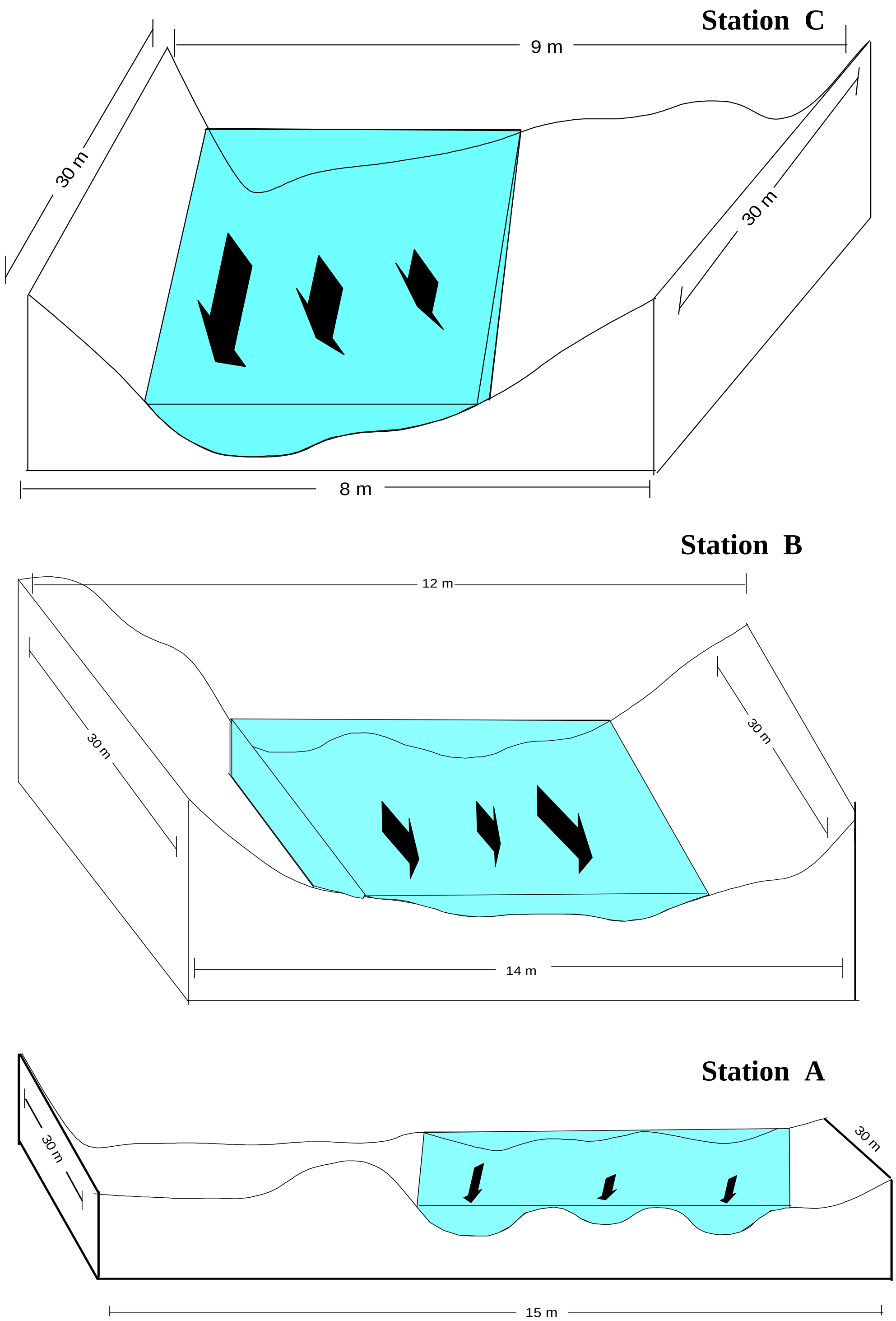


Fig. 4.4. Cross-sections of the river channel at three stations of the Yagüez River. This is a representation of the river channel geomorphology during the month of March 2005 (dry season). The arrows represent water flow across each channel providing an overview of the erosion and deposition process that occurs in lotic systems.

4.4 Biodiversity

4.4.1 Taxonomic composition

A total of 1,395 macroinvertebrates were collected. These specimens were identified and distributed in 12 orders and 23 families (Table 4.2). The macroinvertebrate communities of the sampling sites at the Yagüez River were composed by three major groups: aquatic insects, decapods and gastropods. Aquatic insects, with 18 families (89% of total organisms), were the dominant component of the stream benthos. Coleopterans are the most prominent order of macroinvertebrates collected, with 44% of the total. Within this order, the most abundant family was Elmidae. Ephemeroptera were the second most abundant order in the riffle river habitat with a relative abundance of 32%. The numerically dominant family in the order Ephemeroptera was Leptophlebiidae. The Dipterans represented a relative abundance of 7.6%. Within this order the dominant family was the Chironomidae.

Table 4.2. Abundance of macroinvertebrates collected at the Yagüez River sampling stations during January-November, 2005.

Classification		**Site**		
Order	Family	A Abundance per family ± S.D.	B Abundance per family ± S.D.	C Abundance per family ± S.D.
Class Hexapoda (Insecta)				
Coleoptera	Gyrinidae	---	---	12 ± 1.41
	Limnichidae	---	2 ± 0.00	---
	Staphylinidae	10 ± 2.52	1 ± 0.00	---
	Elmidae	206 ± 17.11	248 ± 8.91	148 ± 7.32
Diptera	Empididae	---	1 ± 0.00	---
	Psychodidae	---	1 ± 0.00	---
	Simuliidae	1 ± 0.00	1 ± 0.00	---
	Tipulidae	2 ± 0.00	1 ± 0.00	---
	Chironomidae	40 ± 5.65	50 ± 3.66	9 ± 0.84
Ephemeroptera	Caenidae	2 ± 0.00	23 ± 6.95	1 ± 0.00
	Baetidae	34 ± 4.49	14 ± 1.16	1 ± 0.00
	Leptophlebiidae	125 ± 13.75	166 ± 8.78	76 ± 6.53
Hemiptera	Veliidae	2 ± 0.00	7 ± 0.96	2 ± 0.00
Lepidoptera	Pyralidae	20 ± 12.73	---	---
Odonata/Anisoptera	Libellulidae	---	2 ± 0.00	1 ± 0.00
Odonata/Zygoptera	Coenagrionidae	---	1 ± 0.00	---
Trichoptera	Philopotamidae	19 ± 5.17	5 ± 0.50	1 ± 0.00
Class Ostracoda				
Podocopida	Cypridae	2 ± 0.00	---	---
Class Malacostraca				
Decapoda	Pseudothelphusidae	---	1 ± 0.00	1 ± 0.00
	Atyidae	1 ± 0.35	---	3 ± 0.00
	Palaemonidae	3 ± 0.71	8 ± 0.00	---
Class Gastropoda				
Neritopsina	Neritidae	13 ± 1.07	4 ± 1.41	---
Prosobranchia	Thiaridae	83 ± 10.41	40 ± 4.72	1 ± 0.00
Total		563 ± 11.14	576 ± 7.06	256 ± 5.99

4.4.2 Macroinvertebrates Abundance and Richness

The abundance and richness for stations A and B were similar. Instead for station C (upstream) both abundance and richness were low (Table 4.3).

Table 4.3. Macroinvertebrates richness and abundance measured in the three stations at the Yagüez River.

Macroinvertebrates	Stations		
	A **Mean ± SD** **(high/low)**	**B** **Mean ± SD** **(high/low)**	**C** **Mean ± SD** **(high/low)**
Richness	6.00 ± 3.74 (12.00, 1.00)	6.00 ± 2.60 (10.00, 3.00)	3.00 ± 1.75 (7.00, 1.00)
Abundance	70.00 ± 26.18 (229.00, 8.00)	68.00 ± 14.51 (147.00, 19.00)	32.00 ± 6.95 (60.00, 2.00)

When comparing the relative abundance of organisms between stations, the Kruskal-Wallis test revealed that there were no significant difference between the macroinvertebrate among stations (H= 3.16, P= 0.2058) (Table 4.4).

Table 4.4. The Kruskal-Wallis for the relative abundance of macroinvertebrates in the three stations at the Yagüez River.

Variable	Station	N	Mean	S.D.	H	p
Abundance	A	8	70.38	74.05	3.16	0.2058
Abundance	B	8	67.63	41.04		
Abundance	C	8	32.00	19.66		

4.4.3 Diversity indices

Station B located in the mid section of the river presented the highest diversity (e.g. Margalef, 2.83 and Fisher, 3.77) (Table 4.5). There was no correlation found between the diversity indices (Margalef and alpha Fisher) and the water quality index (BMWP') (Spearman rank correlation, $p>0.05$).

Table 4.5. Biodiversity indices of macroinvertebrates sampling from three stations along the Yagüez River, Puerto Rico during January-November 2005.

Diversity Index	**Stations**		
	A	**B**	**C**
Taxa S	16	19	12
Total Abundance	563	576	256
Margalef	2.37	2.83	1.98
alpha Fisher	3.07	3.77	2.61

4.4.4 Functional feeding groups

The numerically dominant functional feeding group in the three stations was the collectors-gatherers which represented 74%, 89%, and 94% of the total individuals collected at the stations (Fig 4.5). Scrappers were the second dominant functional feeding group in stations A (downstream) with 96 individuals, representing 17% of the total and B (midstream) with 44 individuals, representing 8% of the total. Predators had the highest abundance in station C with 15 individuals (Fig. 4.5), representing 6% of the total. Shredders (A = 20, B = 2 and C = 1 individuals) and filterers (A = 1, B = 0 and C = 0 individuals) were nearly absent along the Yagüez River with very few individuals by stations, representing less than 7% of the total individuals collected (Table 4.6).

Table 4.6. Functional groups found in the three stations in the Yagüez River.

Functional Group	Order	Family
Filterer	Trichoptera	Philopotamidae
Shredders	Decapoda	Atyidae
	Decapoda	Pseudothelphusidae
	Diptera	Tipulidae
	Lepidoptera	Pyralidae
Scrappers	Coleoptera	Elmidae
	Neritopsina	Neritidae
	Prosobranchia	Thiaridae
Collectors-gatherers	Coleoptera	Limnichidae
	Diptera	Chironomidae
	Diptera	Psychodidae
	Ephemeroptera	Baetidae
	Ephemeroptera	Caenidae
	Ephemeroptera	Leptophlebiidae
Collectors-filterers	Diptera	Simuliidae
Predators	Coleoptera	Gyrinidae
	Coleoptera	Staphylinidae
	Hemiptera	Veliidae
	Odonata	Libellulidae
	Odonata	Coenagrionidae

Information taken from Merritt and Cummins, 1996.

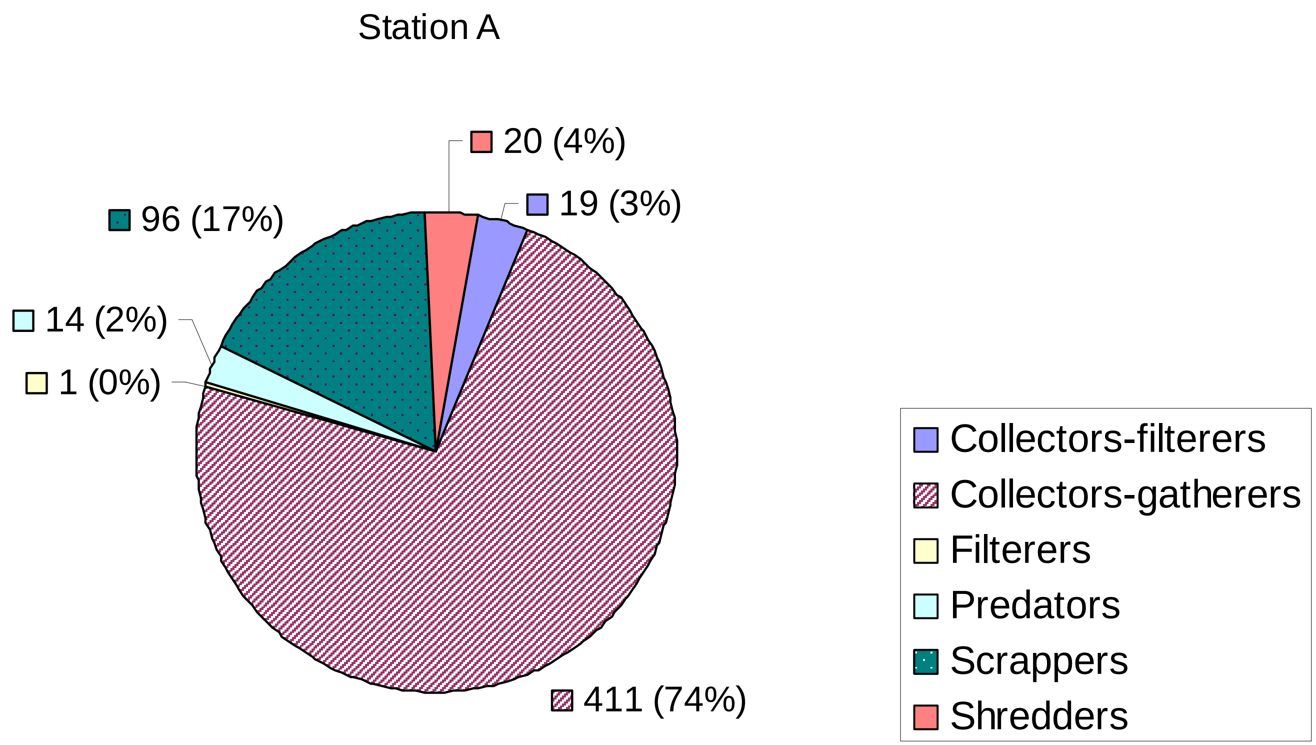


Fig.4.5. Relative abundance of macroinvertebrate trophic-groups from collections at station A in the Yagüez River during January-November, 2005.

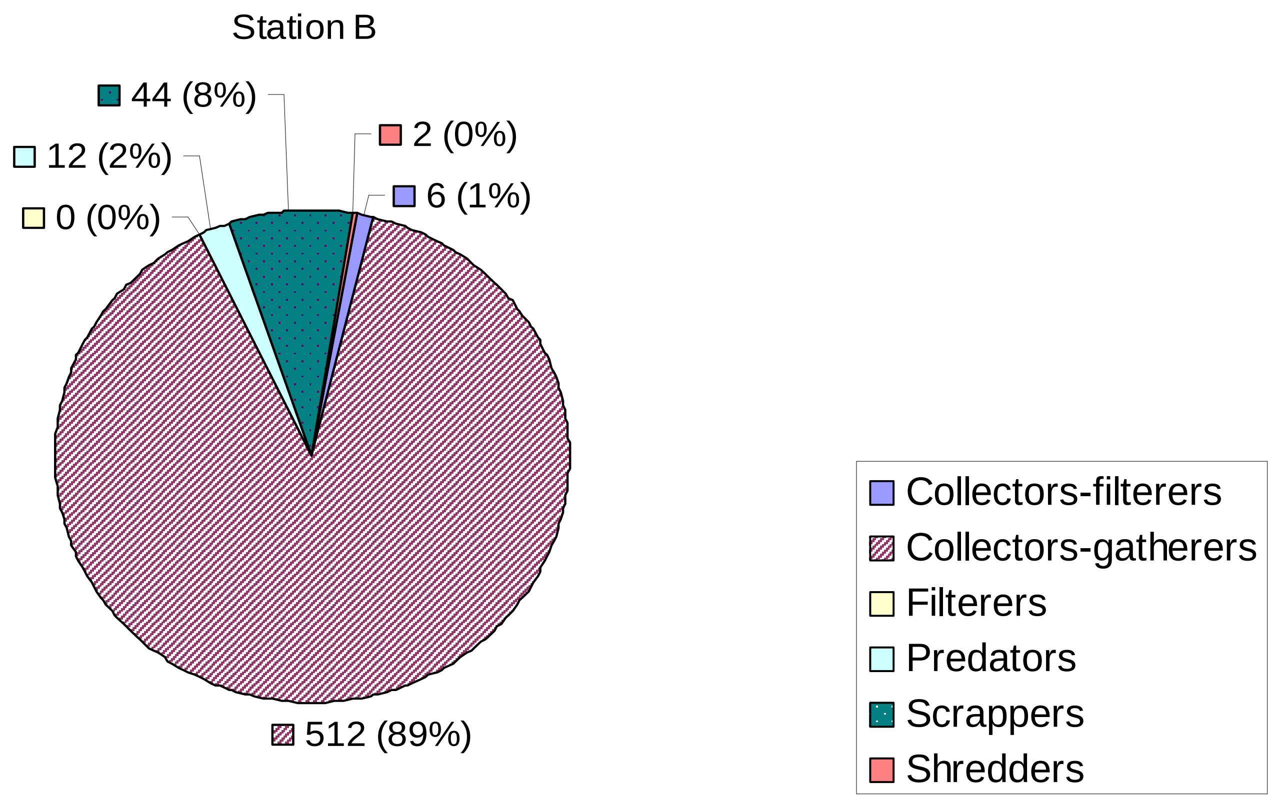


Fig.4.6. Relative abundance of macroinvertebrate trophic-groups from collections at station B in the Yagüez River during January-November, 2005.

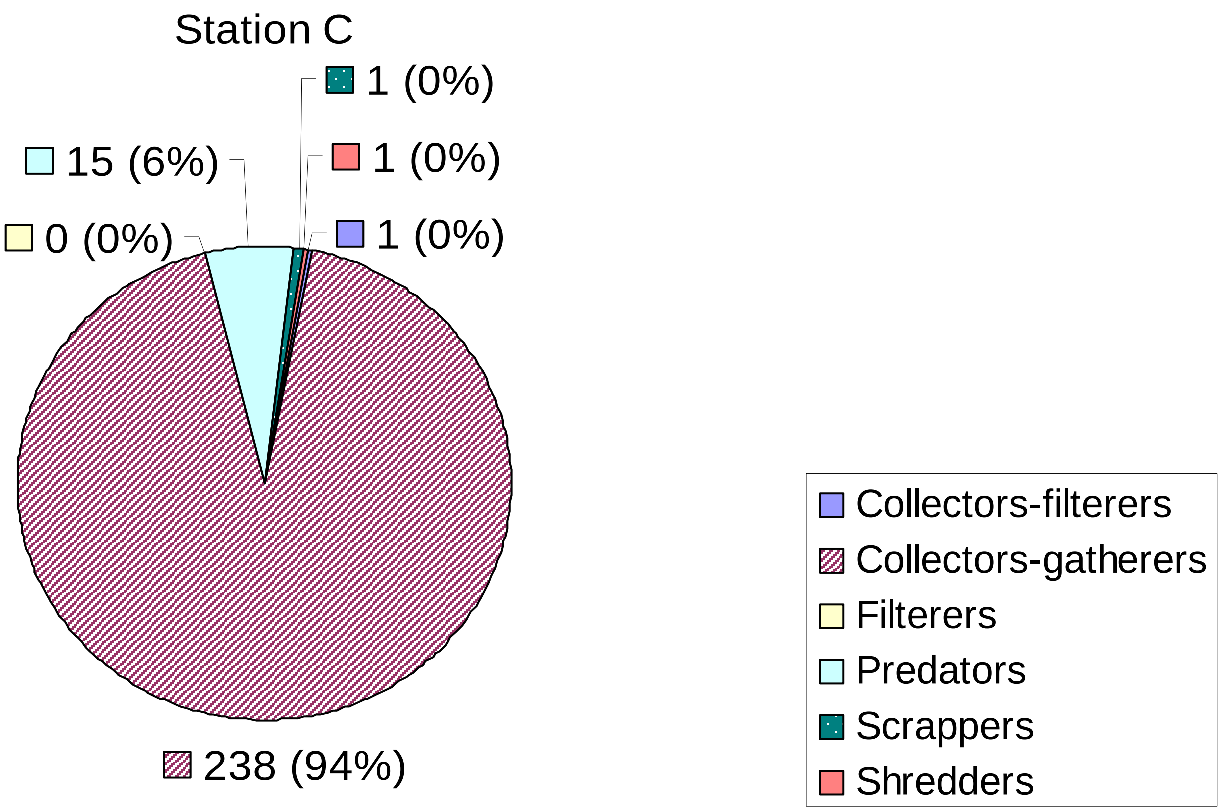


Fig. 4.7. Relative abundance of macroinvertebrate trophic-groups from collections at station C in the Yagüez River during January-November, 2005.

4.5 Biological Indices

The water quality analysis of the Yagüez River samples was done using the Biological Monitoring Working Party (BMWP') that provides tolerance/intolerance values for certain families of macroinvertebrates (Table 4.7). The BMWP' suggests organic pollution along the Yagüez River. Station A (downstream), with a value of 61, and B (midstream), with a value of 76, presented "acceptable" (class II) water quality (Table 4.8). At station C the BMWP' ranged within the "doubtful" (class III) water quality.

Table 4.7. Water quality ratings for macroinvertebrates collected based on the BMWP' index by Alba-Tercedor (1988) in the Yagüez River.

Order	Family	Tolerance/Intolerance Measures BMWP[1]
Class Insecta		
Coleoptera	Elmidae	5
	Gyrinidae	3
	Limnichidae	---
	Staphylinidae	---
Diptera	Chironomidae	2
	Empididae	4
	Psychodidae	4
	Simuliidae	5
	Tipulidae	5
Ephemeroptera	Baetidae	4
	Caenidae	4
	Leptophlebiidae	10
Hemiptera	Veliidae	3
Lepidoptera	Pyralidae	---
Odonata/Anisoptera	Libellulidae	8
Odonata/Zygoptera	Coenagrionidae	6
Trichoptera	Philopotamidae	8
Class Ostracoda		
Podocopida	Cypridae	3
Class Malacostraca		
Decapoda	Atyidae	6
	Palaemonidae	---
	Pseudothelphusidae	---
Class Gastropoda		
Prosobranchia	Thiaridae	6
Neritopsina	Neritidae	6

Table 4.8. Water quality using the BMWP' index by Alba-Tercedor (1988) in the three stations in the Yagüez River

Station	BMWP' value	Water Quality	Class
A	61	Acceptable	II
B	76	Acceptable	II
C	53	Doubtful	III

4.6 Data Analyses

The classification of similarities among macroinvertebrates groups collected from the three stations in the Yagüez River was based on the cluster analysis of Bray-Curtis (single linkage) (Fig. 4.6). The three stations were clustered in two groups (with an arbitrary line in the 60% of similarity). The first group was composed of only station C (doubtful) and the second group is composed of station A (acceptable) and B (acceptable) having 78% of similarity.

A two-way ANOVA was done to compare conductivity (F=13.23, p=0.0018), pH (F=5.11, p=0.0357), and total abundance (F=14.22, p=0.0013) between the dry and rainy seasons (appendix III). These physicochemical parameters and the abundance were log (x+1) transformed and they had a normal distribution.

4.6.1. Multivariate techniques used in determining the relationship between the physicochemical parameters and the biota

Based on the Non-metric Multidimensional Scaling (NMS), presented that seasonality was the variable that best explained the distribution of macroinvertebrates (4137 = Seed for random number generator). In November, stations A and C, the values went into the extreme of the graph (Fig. 4.7). The Principal Component Analysis (PCA) grouped the sites based on environmental characteristics. The first PCA axis accounted for 75% of the variance and was largely influenced by conductivity (Fig. 4.8).

Correlations between physicochemical factors and the water quality (BMWP') index in the Yagüez River were not statistically significant. At station B, depth (r_s=-0.89, p=0.02) and streamflow (r_s=-0.90, p=0.02) presented a strong negative correlation with the water quality index. There was no correlation found between the abundance and the water quality index (BMWP') throughout the stations (Table 4.6).

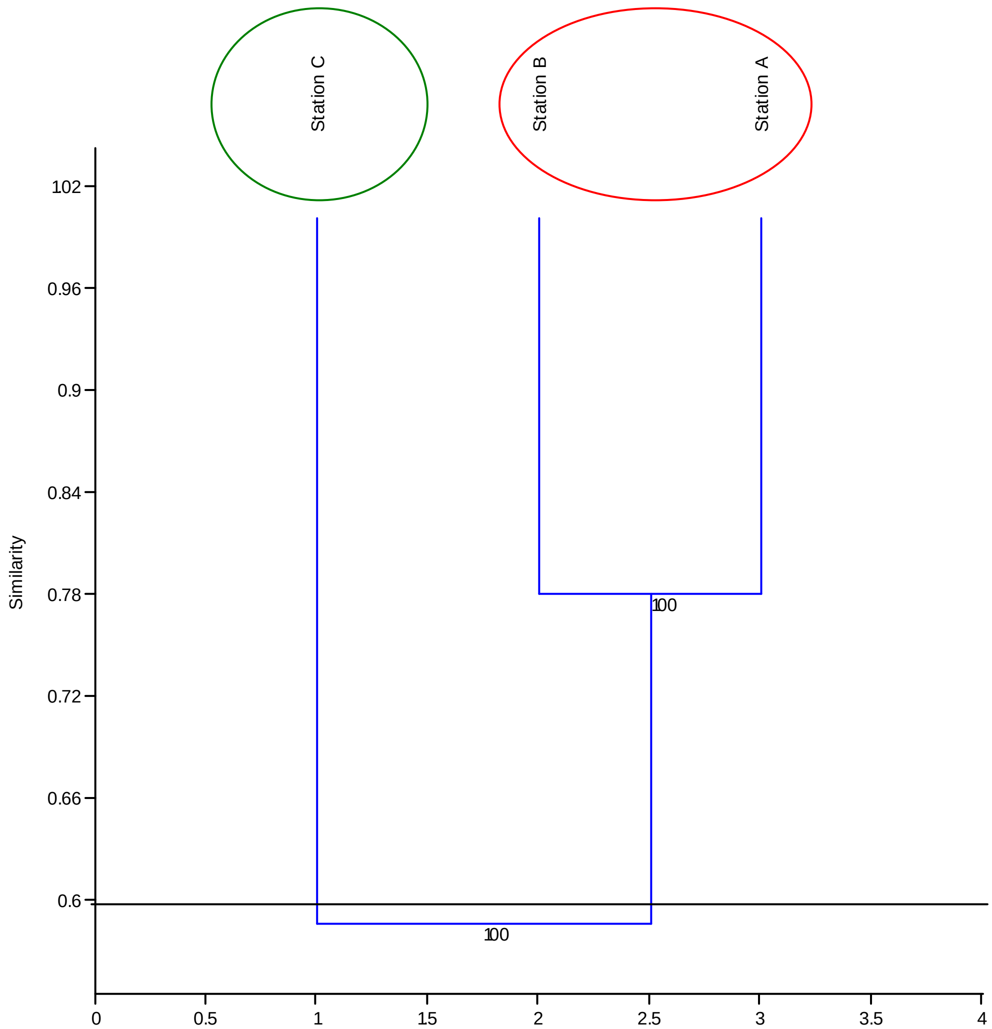


Fig. 4.8. Cluster analysis based on the Bray-Curtis similarity test of the three stations along the Yagüez River.

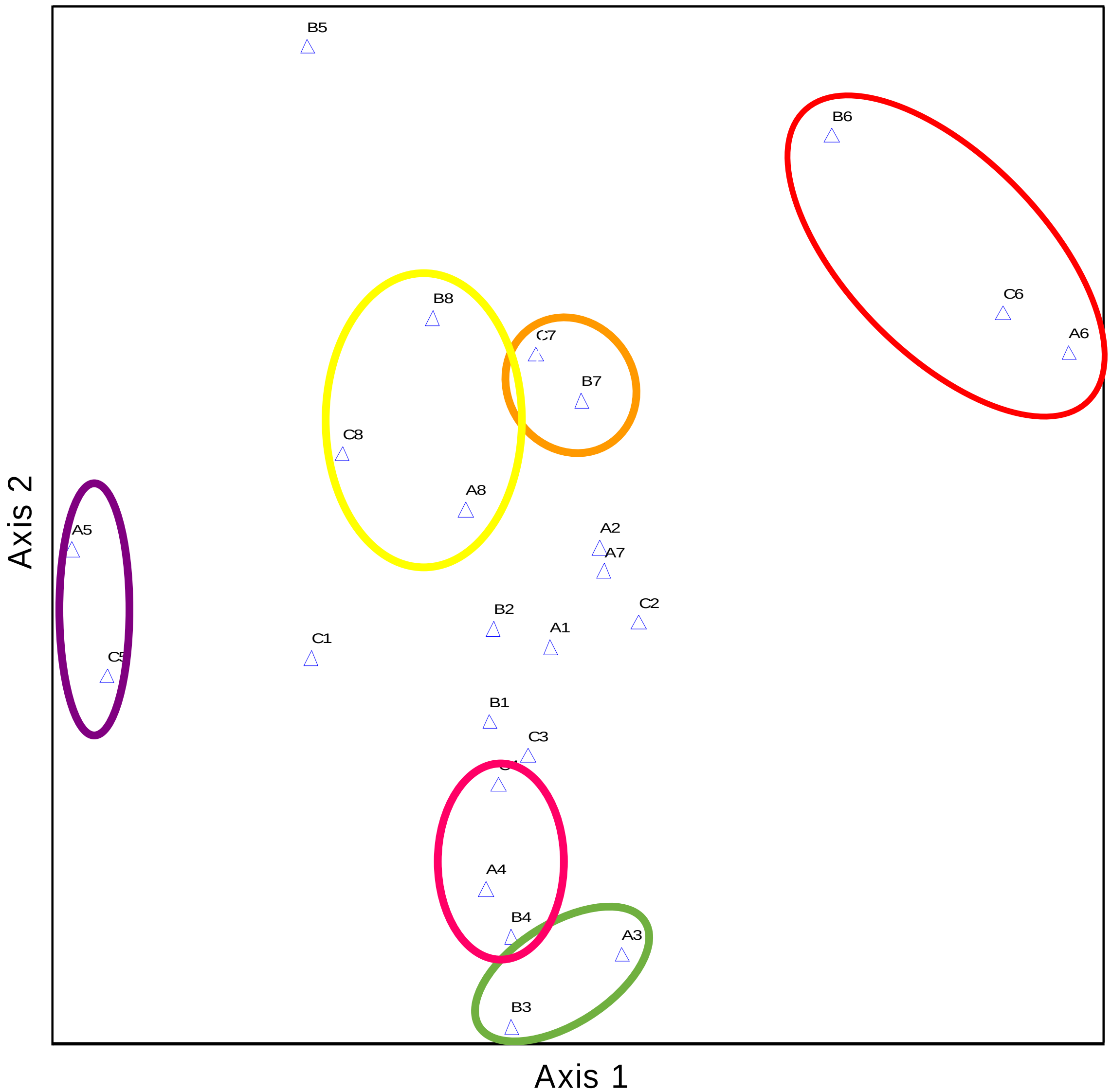


Fig. 4.9. A non-metric multidimensional scaling test showing the relationship of the families with the physicochemical parameters.

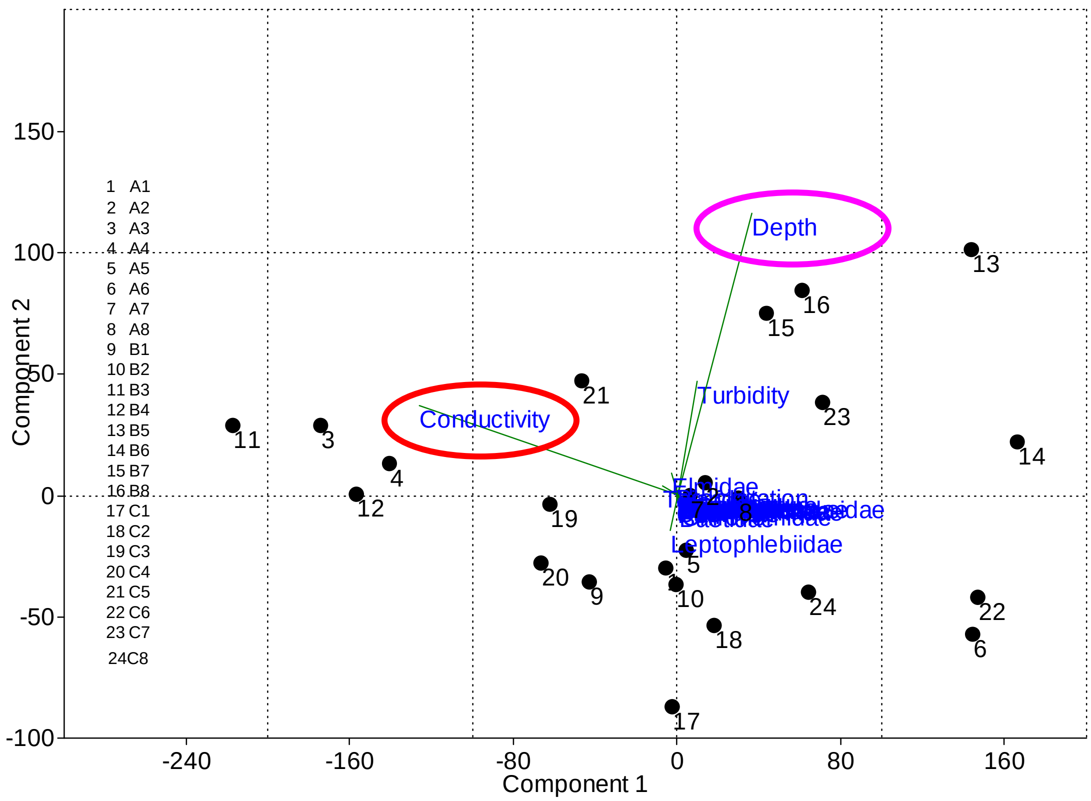


Fig. 4.10. Principal Component Analysis (PCA) of the physicochemical parameters and the relative abundance of the macroinvertebrates. The numbers correspond to the stations and months of sampling in the Yagüez River.
* A, B, C= correspond to the stations along the river; 1-8=the sampling months

Table 4.9. Spearman rank correlations of the physicochemical parameters and macroinvertebrates vs. the water quality (BMWP') index for each station along the Yagüez River.

Stations	Physicochemical & BMWP'	Spearman's (rho)	p-values
A	pH	-0.63	0.10
downstream	Dissolved Oxygen	-0.39	0.31
	Turbidity	-0.48	0.21
	Temperature	-0.55	0.14
	Velocity	-0.23	0.54
	Depth	0.38	0.32
	Conductivity	0.39	0.31
	Precipitation	-0.17	0.66
	Phosphate	0.54	0.15
	Nitrate	0.61	0.10
	Organisms	0.74	0.05*
B	pH	0.11	0.76
midstream	Dissolved Oxygen	-0.40	0.29
	Turbidity	-0.38	0.32
	Temperature	-0.46	0.23
	Velocity	-0.90	0.02*
	Depth	-0.89	0.02*
	Conductivity	0.47	0.21
	Precipitation	0.24	0.53
	Phosphate	0.10	0.79
	Nitrate	0.56	0.14
	Organisms	0.63	0.10
C	pH	-0.41	0.28
upstream	Dissolved Oxygen	-0.65	0.09
	Turbidity	-0.26	0.50
	Temperature	-0.24	0.52
	Velocity	-0.35	0.36
	Depth	0.04	0.91
	Conductivity	0.21	0.57
	Precipitation	-0.05	0.90
	Phosphate	-0.06	0.87
	Nitrate	-0.11	0.78
	Organisms	0.47	0.21

* Statistically significant with $\alpha \leq 0.05$

5 DISCUSSION

5. 1. Physicochemical parameters and water quality

The physicochemical characteristics of waterbodies have been found to influence the community structure of macroinvertebrates. Fluctuations of these parameters could provide information of the water quality of the place. The Environmental Quality Board (EQB) of Puerto Rico has established standards for some of these parameters to ensure the water quality for human consumption and for the conservation of our water resources.

pH

The pH values reported for the Yagüez River ranged between 7.56-7.63. The pH values found in the Yagüez River was within the limits that macroinvertebrate organisms tolerate (Guerrero-Bolaño, 2003).

Dissolved oxygen

During June DO measurements in the three stations were almost reached the border line of the acceptable value (5 mg/L) established by the EQB. According to Machado and Roldán (1981) the amount of oxygen could be affected by several factors like: the characteristics of the channel, fluctuations in water turbulence, variations in temperature and the biochemical processes such as respiration and photosynthesis. A reduction of the dissolved oxygen during that period could had been caused by different factors: there was a decrease in the stream flow and depth of the river, a short dry season period had begun and also the highest temperature (25.3^{0}C) for the river was recorded in that day for the three stations. In the summer, as temperature increases the availability of oxygen decreases due to the higher rates of decomposition. The decomposition processes results in intense oxygen consumption.

Significant differences were found for the dissolved oxygen within stations and sampling dates. Station C showed a higher OD concentration during the dry season this could be explained because at higher elevation the diffusion of oxygen is higher. The opposite occur for the rainy season were the OD concentrations decreases, this could be due to the input of organic material from the runoffs.

The dissolved oxygen concentrations in a study done in Chile in 14 stations located throughout the Damas River basin varied greatly between sampling stations having a range between 3-8.8 mg/L. The stations that had low levels of dissolved oxygen, below 5 mg/L, the dominant species of macroinvertebrates demonstrated a great tolerance to pollution. The organism presented include: Oligochaeta (*Tubifex*), Hyrudinea, Diptera (Chironomidae spp.) and Gastropoda (*Physa*), all of which are capable of living in very low oxygen concentrations (Figueroa et al. 2003). This suggests that the prevalence of low oxygen concentration have an effect in the community structure of macroinvertebrates. In the Yagüez River, the macroinvertebrate orders present during the month that had lowest dissolved oxygen values were: Chironomidae and Gastropoda. These organisms are able to endure low oxygen concentrations.

Turbidity

The higher record for this parameter was during May. This occurred after a short rainy season period with a maximum precipitation recorded for all the stations of 14.65±0.0 mm. This rainy season caused an increase in stream flow. Rainfall, runoff, and high stream flow could have caused erosion that led to higher concentrations of suspended particles, resulting in increased in turbidity.

An increase in turbidity can affect the aquatic organisms. Fine particulate sediment reduces the amount of light that can penetrate and this will lead to a reduction in the photosynthesis rate and in dissolved oxygen available.

Streamflow

Current velocity is an essential property of streams because it influences the water temperature, concentration of substances, distribution of habitats and organisms throughout the stream. During May, the Yagüez River had the highest stream flow. A similar trend is observed in the LEF stream where the highest stream flow is during the months of May and September (Garcia-Martino et al., 1996). This could be explained because it was the beginning of the rainy season. The rainfall causes stream bank and channel erosion that could have an effect in the habitat and invertebrate distribution. In a study done by Fenoglio et al. (2004), it was reported that small-scale variations caused by: current velocity, position of the streambed and the substrate composition will influence the density and richness of invertebrates. They found that collectors were the most represented functional feeding group and shredders were almost absent. Along the Yagüez River we also found a major representation of collectors and very few shredders.

The only two parameters that presented a correlation with the BMWP' was depth and streamflow in station B. The other parameters did not present a significant difference with the BMWP' in our study. These can be due to different characteristics found in

Caribbean rivers like: water temperature, stream flow rates, distinctive benthic macroinvertebrate, and geomorphology (USEPA, 1994).

Temperature

It is known that water temperature has a major influence on organism activity and growth. High water temperature increases the biological activity but if it exceeds the organism's range of tolerance it could be lethal. Several factors could influence water temperature: tropical rivers are dependant in the elevation above sea level (Machado and Roldán, 1981 cited by Guerrero-Bolaño et al., 2003), variations throughout the day, and extend of the riparian ecosystem and thus shading of the stream. The temperature for this waterbody exhibits a seasonal slight thermal pattern, where there are small fluctuations along the sampling dates, with the highest temperature registered in June.

An increase in temperature during the month of June may possibly be enhanced by runoff from roads and parking lots nearby. The temperatures found in the headwater stream of Rio Mameyes at the LEF is relatively consistent throughout the year and ranges between 18^{o} and 24^{o} C. When compared with the Yagüez River the range slightly varies 20.4^{o} and 24.9^{o} C. These temperature differences could be caused by elevation variation that exists between the Yagüez River and the LEF streams. According to Ortiz-Zayas et al. (2005) the Rio Mameyes is strongly influenced by elevation.

5.2 Nutrients

Phosphate is a key nutrient for the growth of plants, phytoplankton and bacteria. If the phosphate concentration exceed the level that streams can hold it could cause a bloom of organisms that affect the water quality. For our study we measure orthophosphate. The month of February had the highest levels of phosphate for station B. In the month of February there were no major events of rainfall and the water velocity was relatively low, this could have an effect in the concentration of phosphate making it higher. Also it could come from the decomposition of organic material, because patches of algae were observed during the month of February. In the Rio Mameyes was measured the total dissolved phosphate and the concentration was above detection levels. The lowest concentrations for the total dissolved phosphate occurred during the months of November and March when the water flow was lowest (Ortiz-Zayas, 2005). The measurements of two different types of phosphate (total phosphate and ortho-phosphates) can lead to the differences found between the two rivers.

Nitrate

The nitrate levels along the three stations were below the standards for primary drinking water (10 mg/L) (USEPA, 2003). We observed the nitrate levels for January were the highest for the three stations, the oxygen was low and the pH was relatively more alkaline being in the range of 7.36 and 7.60. In a study done by Oscoz et al. (2006), they found that the degradation of organic matter causes a decrease in the pH (a range of 7.15-7.63); a decrease in the dissolved oxygen; and increase in the nitrogen levels (Winkler, 1999; Timm et al., 2001; Rueda et al., 2002). This type of pattern was observed during the month of January for the Yagüez River. These variations could be caused by organic input that comes from the accumulation of vegetation mainly composed of bamboo leaves.

These same factors affected the Río Rosario at a site denominated “pasture” that was studied by Collazo-Vázquez (2001). This type of organic enrichment has been associated with the increment of collectors. The dominant group was the collectors along our three stations, suggesting that there is a great availability of organic material (Resh & Jackson, 1993; Metcalfe-Smith, 1994; Del Moral et al., 1997; Bonada et al., 2000). It is important to consider that throughout the month of January the precipitation was low and also the water velocity and this might have led to an increase in the availability of organic matter.

5.3 River Channel Characterization

The Yagüez River has a geomorphology in which the water velocity has created different passages within the river. These passages in the river provide different habitats that create small-scale spatial variation within the stations. This plays a major factor in determining the macroinvertebrate aquatic structure. The physicochemical parameters presented a significant difference between stations and sampling dates

5.4 Biodiversity

5.4.1 Species composition and abundance

When compared to Ramírez and Pringle (1998) the insect assemblage of Yagüez River was characteristic of Neotropical streams. The majorities of the insect families were found in all the three stations for the exception of several bedrock specialists like: Pyralidae (Lepidoptera) and Empididae (Diptera). The insect orders Megaloptera and Plecoptera were not found in the Yagüez River. Ramírez and Hernández-Cruz (2004), indicated that in streams of Puerto Rico these two orders have not been reported. The insect composition was similar to those previously reported for nearby rivers like: Río Culebrinas and Río Grande de Añasco (USEPA, 1994) (see Appendix V) and the Río Rosario (Collazo-Vázquez, 2001).

In previous studies it has been found that fluctuations in abundance of some mayflies (Ephemeroptera) in the tropics have been attributed to rainfall (Bishop, 1973; Bright, 1982). Also Bright (1982) found that in the Palau stream apparently there is a correlation of current velocity and discharge of the river. In Río Quebrada Prieta in the Luquillo Mountains, the emergence of mayflies does not correlate with rainfall (Pescador et al., 1993). In this river ecosystem, Baetidae and Leptophlebiidae were the only two families found in Quebrada Prieta. This low diversity contradicts the assumption that the diversity of aquatic insects in the tropics is greater than in temperate regions (Stout and Vandermeer, 1975). Certain studies have suggested that the variation in diversity between tropical streams within a region is similar to the effect observed in latitudinal gradient between temperate and tropical streams (Flowers, 1991). According to Pescador et al.

(1993), the low diversity could be in relation to the relatively small size and insular nature in Quebrada Prieta.

In our study, we found both families Leptophlebiidae and Baetidae, and a third family Caenidae. In the western area it has been reported by USEPA (1994) that these three families appeared in both, the Rio Grande de Añasco and Rio Culebrinas, Mayagüez. Also it has been found for macroinvertebrates that the substrate type is essential for oviposition (Ward, 1992). Substrate type could provide means of protection from predation and water flow. Most of mayflies' nymphs show a preference for two types of substrate detritus, e.g. deposits and stones that support algae and trap of organic particles (Goulart et al., 2005). There could also be an evolutionary factor that could play a role of faunal composition in site establishment. For example, the body of certain mayflies is adapted to colonize certain types of substrate. The Leptophlebiidae have a flattened body that provides the adaptations necessary to colonize gaps between rock fragments and smaller particles like gravel and sand (Goulart et al., 2005).

The order Coleoptera at the Yagüez River showed a greater diversity when compared to two rivers closed by Río Grande de Añasco and Rio Culebrinas. The only family found at the three rivers was Elmidae it also was the only coleopteran family reported by USEPA (1994).

The order Diptera had the Chironomidae family found at Río Espiritu Santo, Río Grande de Loiza, Río Grande de Añasco, Río Cibuco, Río Culebrinas, Río de Bayamón, Río Guajataca, Río Grande de Manatí, Río de la Plata, Río Mameyes and Río Grande de Arecibo. This wide distribution is due to the ability of these organisms to resist any type of conditions; this is the reason for being considered tolerant to contaminants. Simuliidae has a wide distribution but is not reported for the Río Cibuco and Río Grande de Manatí. The Tipulidae family has a limited distribution and been observed it was found at the Yagüez River, Río Grande de Loíza, Río Grande de Añasco, Río Culebrinas, and Río de Bayamón (USEPA, 1996).

Based on Ramírez and Hernández-Cruz (2004) geomorphology is the most critical parameter to explain aquatic insect assemblage structure and composition in the Yagüez River as it was in the case of two second-order streams Prieta and Bisley-3 in LEF. According with USEPA (1994), in Puerto Rico, the elevation does not play an important role determining aquatic structure composition.

5.4.2 Species richness and composition

Total insect species richness was similar for station A and C except for bedrock habitat at station B (acceptable) which supported higher abundance, maximum species richness and diversity (α-Fisher) (Table 4.3). Station B presented several characteristics that encourage the development of aquatic insects: 1) bedrock habitat and 2) fast flowing water current. The bedrock habitat did not have shrimp providing, a relatively predator free and stable environment for insects. Also, it had fast flowing water current that supply particles for food.

In general, the richness and composition of the orders traditionally used in determining water quality (Ephemeroptera, Plecoptera and Trichoptera) is low in the Yagüez River when compared with temperate regions (Covich, 1988 cited by USEPA, 1994). However, there are many endemic species found in neotropical streams from the order Diptera and Odonata (USEPA, 1994). Another component particular to neotropical streams, including Yagüez River, are the freshwater crab *Epilobocera sinuatifrons*, the atyid shrimp, palaemonid shrimp and the potamonid crabs (USEPA, 1994).

5.4.3 Functional feeding groups

It has been found that in tropical lotic systems the decomposition of plant material to fine particulate organic matter is operated by macroconsumers (crustaceans and fish) or by microbial activity (Pringle et al., 1993; Graca et al., 2001; Dobson et al., 2002; Fenoglio and Cucco, 2004). Therefore, our results support this hypothesis about the processing in the tropics of allochtonous coarse particulate organic matter. Macroinvertebrate shredders were nearly absent, only represented by 1.6% of the total of organisms. Meanwhile, collectors-gatherers and collectors-filterers (both feed on fine particle organic matter) were dominant.

This tendency was observed in a study done in the central highland region of Kenya by Dobson et al. (2002) were he found that shredders does not operate in East African streams. Instead in North Temperate Zone this functional feeding group is a key component of the streams. This difference between tropical and temperate zones could be enhanced by microbial activity in higher temperatures. Another factor that could affect the shredders abundance in tropical streams is the result of low palatability of detrital input that comes from the dominant riparian trees of the region.

5.5 Water Quality

In general, water quality in the Yagüez River is acceptable based on the biological index BMWP'. The BMWP' presented a similar water quality condition for station A and B. These had acceptable water quality with a class II where there is evidence of some contamination effect. The upstream area station C presented "doubtful" water quality this could be explained by the land usage because the Yagüez River surroundings were used for agriculture (personal observation). It has been reported that agricultural runoffs is one of the major sources of pollutants of aquatic habitats; because they are responsible for 46% of the sediment, 47% of total phosphorus, and 52% of the total nitrogen (Gianessi et al., 1986; Allan, 2001).

However, this was a surprising finding because usually upstream of a river is expected to have a better water quality. For example in the basins of the Cali and Melendez rivers in the department of Valle, Colombia the upstream stations had the highest water quality index with values ranging (75.7-77.2) (Zuñiga et al. 1994). The downstream part of the Cali River had the lowest water quality index (32.5) because this area is the reservoir of domestic and industrial discharges. The Melendez River instead presented acceptable water conditions similar to our findings in the Yagüez River.

With the biological indices, the families were clustered in different levels of tolerance (Table 4.5). In the BMWP' the families that were dominant for both stations A and B were bioindicators of "less sensitive to pollution" and "pollution tolerant". Six families were identified by being the "less sensitive to pollution" (i.e. Elmidae, Simuliidae, Tipulidae, Neritidae, Thiaridae found in both stations) and "tolerant to pollution" (i.e. Chironomidae, Baetidae, Caenidae, Veliidae found in both stations) (Appendix V). Only two families that were "sensitive to pollution" were found in station A (i.e. Leptophlebiidae and Philopotamidae), and three of these were found in station B (i.e. Leptophlebiidae, Libellulidae and Philopotamidae).

5.6 What Could Cause Spatial and Temporal Variation in Community Assemblage throughout the River?

Our results demonstrated that the factors that affect the annual distribution of the macroinvertebrates in the river were the seasonality, conductivity and the precipitation. The results were obtained by using different multimetric variable approaches. The NMS presented the seasonality was the variable that explained the distribution of macroinvertebrates. The first PCA axis was largely influenced by the conductivity. However, the two-way ANOVA established that the total abundance and the conductivity presented significant differences between the dry and rainy seasons.

Other factors that could influence the macroinvertebrate assemblage and that could explain such a low abundance in the upstream part of the Yagüez River where we would expect the greatest diversity and richness because there is less accessibility. Throughout the past years the land surrounding this area was used for agricultural purposes like coffee and plantains growth. The addition of fertilizers to this area and the runoffs produced by the precipitation causes that it discharges on the river. The natural disturbances caused by rainfall and the anthropogenic impact for land development affect the fauna in that place lowering the richness and diversity of the place.

The macroinvertebrate structure and abundance in the Yagüez River was influenced by several factors such as: hydrological regime, substrate, the type and abundance of the trophic resources or by the land usage near the streambed. However, the water quality of the Yagüez River in overall has acceptable water conditions that could harbor a diversity of organisms. Before using it as a water supply an exhaustive water assessment should be done.

6 CONCLUSIONS

- Dominant families of the macroinvertebrates along the Yagüez River were: Elmidae (Coleoptera), Leptophlebiidae (Ephemeroptera), and Chironomidae (Diptera).
- The Coleoptera order demonstrated the higher amount of individuals represented by four families (Elmidae, Gyrinidae, Limnichidae, and Staphylinidae).
- The order Diptera was represented by 5 families (Chironomidae, Empididae, Psychodidae, Simuliidae, and Tipulidae). The chironomid were the most tolerant to organic material according to the BMWP‘.
- The families found are similar to the ones reported previously in other rivers in the western region, like Río Culebrinas, Río Grande de Añasco, and Río Rosario.
- The water quality of the Yagüez River based on the biological index BMWP‘ is acceptable for the midstream and downstream section of the river. The upstream section of the river contained more organic material according to the biological index.
- The spatial distribution of the macroinvertebrates along the Yagüez River is determined by the seasonality which is influenced by the rainfall (dry and rainy season) in tropical streams.
- The macroinvertebrate species of the Yagüez River richness and abundance was low for the orders Ephemeroptera, and Trichoptera.

- There were no correlations found between the physicochemical parameters and the water quality index (BMWP') among station with the exception of stream flow and depth for station B.
- The elevation among the three stations did not affect the distribution of benthic macroinvertebrates.

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Appendix A: Statistical Analysis and Macroinvertebrate Tolerance Levels

Appendix I: Descriptive Statistic of the Yagüez River for each station.

Station	Family	Variable	n	Average	S.D.	Sum
a	Atyidae	Abundance	1	1.00	0.00	1.00
a	Baetidae	Abundance	7	4.86	4.49	34.00
a	Caenidae	Abundance	2	1.00	0.00	2.00
a	Chironomidae	Abundance	12	3.33	5.65	40.00
a	Cyclophoridae	Abundance	6	8.50	15.55	51.00
a	Elmidae	Abundance	24	8.58	17.11	206.00
a	Leptophlebiidae	Abundance	16	7.81	13.75	125.00
a	Neritidae	Abundance	7	1.86	1.07	13.00
a	Palaemonidae	Abundance	2	1.50	0.71	3.00
a	Philopotamidae	Abundance	4	4.50	5.69	18.00
a	Pyralidae	Abundance	2	10.00	12.73	20.00
a	Simulidae	Abundance	1	1.00	0.00	1.00
a	Staphylinidae	Abundance	3	3.33	2.52	10.00
a	Thiardae	Abundance	7	4.57	2.64	32.00
a	Tipulidae	Abundance	2	1.00	0.00	2.00
a	Veliidae	Abundance	2	1.00	0.00	2.00
b	Baetidae	Abundance	8	1.75	1.16	14.00
b	Caenidae	Abundance	5	4.60	6.95	23.00
b	Chironomidae	Abundance	14	3.50	3.74	49.00
b	Coenagrionidae	Abundance	1	1.00	0.00	1.00
b	Cyclophoridae	Abundance	2	9.50	12.02	19.00
b	Elmidae	Abundance	31	6.90	8.83	214.00
b	Empididae	Abundance	1	1.00	0.00	1.00
b	Leptophlebiidae	Abundance	16	10.38	8.78	166.00
b	Libellulidae	Abundance	1	1.00	0.00	1.00
b	Limchinidae	Abundance	1	2.00	0.00	2.00
b	Neritidae	Abundance	2	2.00	1.41	4.00
b	Palaemonidae	Abundance	1	8.00	0.00	8.00
b	Philopotamidae	Abundance	1	2.00	0.00	2.00
b	Pseudothelphusidae	Abundance	1	1.00	0.00	1.00
b	Psychodidae	Abundance	1	1.00	0.00	1.00
b	Simulidae	Abundance	1	1.00	0.00	1.00
b	Staphylinidae	Abundance	1	1.00	0.00	1.00
b	Stenelmis	Abundance	2	17.00	2.83	34.00
b	Thiardae	Abundance	11	1.91	1.58	21.00
b	Tipulidae	Abundance	1	1.00	0.00	1.00
b	Veliidae	Abundance	4	1.75	0.96	7.00
c	Atyidae	Abundance	3	1.00	0.00	3.00
c	Baetidae	Abundance	1	1.00	0.00	1.00
c	Caenidae	Abundance	1	1.00	0.00	1.00
c	Chironomidae	Abundance	6	1.50	0.84	9.00
c	Cyclophoridae	Abundance	1	1.00	0.00	1.00
c	Elmidae	Abundance	22	6.73	7.32	148.00
c	Gyrinidae	Abundance	4	3.00	1.41	12.00
c	Leptophlebiidae	Abundance	13	5.85	6.53	76.00
c	Pseudothelphusidae	Abundance	1	1.00	0.00	1.00
c	Veliidae	Abundance	2	1.00	0.00	2.00

Appendix II: Shapiro-Wilks for the abundance for three stations along the Yagüez River

Station	Variable	n	Mean	S.D.	W	p-value* (one tail)
a	Abundance	100	5.62	11.19	0.46	<0.0001
b	Abundance	111	5.19	7.06	0.65	<0.0001
c	Abundance	56	4.57	5.99	0.66	<0.0001

*If the p-value ≤ 0.05 then the sample could not have been selected from a normal distribution.

Appendix III: Two Way ANOVA: Variance Analysis for the physical-chemical parameters for the Yagüez River.

Conductivity:

Variable	N	R^2	R^2 Aj	CV
Conductivity	24	0.43	0.31	25.30

F.V.	SC	gl	CM	F	p-value
Model	97086.69	4	24271.67	3.61	0.0236
Site	8269.64	3	2756.55	0.41	0.7473
Season	88817.05	1	88817.05	13.23	0.0018
Error	127570.51	19	6714.24		
Total	224657.20	23			

pH:

Variable	N	R^2	R^2 Aj	CV
pH	24	0.31	0.16	6.57

F.V.	SC	gl	CM	F	p-value
Model	2.07	4	0.52	2.10	0.1211
Site	0.81	3	0.27	1.09	0.3763
Season	1.26	1	1.26	5.11	0.0357
Error	4.68	19	0.25		
Total	6.75	23			

Temperature:

Variable	N	R^2	R^2 Aj	CV
Temperature	24	0.63	0.55	4.16

F.V.	SC	gl	CM	F	p-value
Model	28.38	4	7.09	8.12	0.0005
Site	2.24	3	0.75	0.86	0.4812
Season	26.14	1	26.14	29.91	<0.0001
Error	16.60	19	0.87		
Total	44.98	23			

Nitrate:

Variable	N	R^2	R^2 Aj	CV
Nitrate	24	0.13	0.00	22.85

F.V.	SC	gl	CM	F	p-value
Model	0.02	4	0.01	0.73	0.5804
Site	0.02	3	0.01	0.92	0.4482
Season	1.1E-03	1	1.1E-03	0.16	0.6919
Error	0.13	19	0.01		
Total	0.15	23			

Total Abundance:

Variable	N	R^2	R^2 Aj	CV
total abundance	24	0.65	0.58	16.94

F.V.	SC	gl	CM	F	p-value
Model	2.58	4	0.65	8.79	0.0003
Site	1.54	3	0.51	6.97	0.0024
Season	1.05	1	1.05	14.22	0.0013
Error	1.40	19	0.07		
Total	3.98	23			

Appendix IV: A comparison of the aquatic insects found in the Yagüez River (RY) with other rivers in the western region (Río Grande de Añasco [RGDA], Río Culebrinas [RC] and Río Rosario [RR]) of Puerto Rico.

Classification		Site			
Order	Family	RGDA[1]	RC[1]	RR[2]	RY[3]
Class Insecta					
Coleoptera	Elmidae	X	X	X	X
	Gyrinidae	---	---	---	X
	Limnichidae	---	---	---	X
	Staphylinidae	---	---	---	X
Diptera	Chironomidae	X	X	X	X
	Empididae	X	---	---	X
	Psychodidae	X	---	X	X
	Simuliidae	X	X	X	X
	Stratiomyidae	X	---	---	X
	Tipulidae	X	X	X	X
Ephemeroptera	Baetidae	X	X	X	X
	Caenidae	X	X	X	X
	Leptophlebiidae	X	X	X	X
Hemiptera	Veliidae	---	---	X	X
Lepidoptera	Pyralidae	X	X	X	X
Odonata/Anisoptera	Libellulidae	X	X	---	X
Odonata/Zygoptera	Coenagrionidae	---	---	X	X
Trichoptera	Hydroptilidae	X	X	X	---
	Philopotamidae	X	X	X	X
	Hydropsychidae	X	X	X	---
	Glossomatidae	X	X	X	---
	Helicopsychidae	X	---	X	---
	Hydrobiosidae	X	---	X	---
	Xiphocentronidae	X	---	---	---

1. Families of aquatic insects reported by USEPA, 1994
2. Families of aquatic insects reported by Collazo, 2001
3. Families of aquatic insects reported by Ruperto, 2008

Appendix V: Families classified by tolerance levels to determine water quality along the Yagüez River.

Station	Good	Intermediate	Fair
A	Leptophlebiidae	Elmidae	Chironomidae
	Philopotamidae	Simuliidae	Baetidae
		Tipulidae	Caenidae
		Atyidae	Veliidae
		Neritidae	Pyralidae
		Thiaridae	Ostracoda
B	Leptophlebiidae	Elmidae	Empididae
	Libellulidae	Simuliidae	Chironomidae
	Philopotamidae	Tipulidae	Psychodidae
		Coenagrionidae	Baetidae
		Neritidae	Caenidae
		Thiaridae	Veliidae
C	Leptophlebiidae	Elmidae	Chironomidae
	Libellulidae	Atyidae	Baetidae
	Philopotamidae	Thiaridae	Veliidae
			Gyrinidae