Comparison of the macroinvertebrates communities in areas covered and devoid of the floating fern *Salvinia* spp. in Boquerón Wildlife Refuge (BWR) at Cabo Rojo, Puerto Rico

by

Nahíra Arocho Hernández

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Approved by:

Carlos J. Santos Flores, Ph. D. President, Graduate Committee	Date
Mónica Alfaro Lozano, Ph. D. Member, Graduate Committee	Date
Jaime Acosta Martínez, Ph.D. Member, Graduate Committee	Date
Lillian Ramírez Durand, M.S. Representative of Graduate Studies	Date
Nanette Diffoot-Carlo, Ph. D. Chairperson of the Department	Date

Abstract

Mangrove forests are recognized as highly productive ecosystems; nevertheless, their ecological values, biota, and food webs are complex and yet to be understood. Scientific publications and information on aquatic invertebrates in the mangroves of Puerto Rico are scarce and mostly outdated. The Boquerón Wildlife Refuge (BWR), located in Boquerón, Cabo Rojo, is the largest mangrove forest stand in Puerto Rico's west coast. Many of the mangrove channels in the BWR are invaded by thick mats of *Salvinia* spp. An assessment of the aquatic macroinvertebrate diversity in three mangrove areas in the BWR invaded by *Salvinia* spp. and three areas without these floating ferns was conducted once a month from June to November of 2013. Salinity and pH were measured for each sampling. Three sampling methods were used; aquatic light traps, Malaise traps, and D-net sweeps. Whole samples were screened and the organisms sorted and identified. A total of 21,305 invertebrates were collected and identified, at least to order-level. These organisms belong to 20 orders and 81 families. The most abundant order was Diptera. The groups with the greatest family richness were Diptera (21 families) and Coleoptera (12 families). The BWR showed a highly diverse ecosystem and the abundance of macroinvertebrates showed spatial and temporal variations. There was no clear relationship between the pH, salinity and precipitation and the abundance of organisms probably because the water level of the refuge is controlled manually through dikes. Mangrove areas with and without *Salvinia* behave as two distinctive habitats; similarity index values (Jaccard) were 0.5 which indicates that they share only around the half of their taxa (families). Each sampling method rendered a distinctive fauna. This study will serve as a baseline characterization for future studies, for the biomonitoring and specific management programs for the preservation of its biodiversity.

Resumen

Los manglares son considerados ecosistemas altamente productivos; sin embargo, su valor ecológico, biodiversidad, redes tróficas son complejas y poco entendidas. Las publicaciones científicas sobre los invertebrados acuáticos asociados a los manglares de Puerto Rico son escasas y no están actualizados. El Refugio de Vida Silvestre de Boquerón, ubicado en Boquerón, Cabo Rojo, es el bosque de manglar más grande del área oeste de Puerto Rico. Muchos de los canales del refugio han sido invadidos por el complejo de *Salvinia* spp. De junio a noviembre del 2013, se llevó a cabo una evaluación de la diversidad de macroinvertebrados acuáticos en tres zonas de manglares invadidas por *Salvinia* y tres áreas sin la presencia de la planta. La salinidad y el pH se midieron para cada muestreo. Se utilizaron tres métodos de muestreo: trampas acuáticas de luz, trampas de Malaise y redes de arrastre. Las muestras se examinaron completas y los organismos fueron separados e identificados por lo menos a nivel de orden. Se colectaron 21,305 invertebrados, estos se distribuyeron en 20 órdenes y 81 familias. El orden más abundantes fue Diptera. Los grupos con mayor riqueza a nivel de familia fueron Diptera (21 familias) y Coleoptera (12 familias). El bosque de manglar del refugio es bien diverso y se encontraron variaciones temporales y espaciales en la abundancia de los macroinvertebrados. No se encontró relación entre el pH, salinidad y precipitación con respecto a la abundancia de organismos posiblemente debido a que el nivel de agua del refugio se controla manualmente a través de los diques. Las zonas de manglar con y sin la presencia de *Salvinia* spp. se comportan como hábitats distintivos; esto se puede apreciar con los valores cercanos a 0.5 del Índice de Similaridad (Jaccard), lo que indica que las áreas comparten solo la mitad de sus taxones. Cada método de colecta representó una fauna distintiva. Este trabajo sienta las bases para estudios futuros y el desarrollo de programas de biomonitoreo y de manejo específicos para estos humedales.

Dedication

To God for loving me unconditionally, for being my strength and motivation in this whole process. For inspiring me through His Amazing Creation.

To Puerto Rico; I hope your beauty and yet to be discovered treasures continue to inspire the hearts and minds of future Puerto Rican scientists with a sincere compromise.

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Introduction

The term "mangrove" can be meant to apply to either a single tree or to a whole forest where one, several, or all of the species of mangrove trees known to exist within the given biogeographic area may occur (Cerame, 1973). Mangroves are among the most productive plants in the ocean (Duarte and Cebrian, 1996) and mangrove forests are recognized as highly productive ecosystems that provide large quantities of organic matter to adjacent coastal waters in the form of detritus and live animals (Holguin *et al.*, 2001). Recent advances in estimating photosynthetic production indicate that, on an area basis, mangroves are usually more productive than saltmarshes, sea grasses, macroalgae, coral reef algae, microphytobenthos, and phytoplankton (Alongi, 2002).

Mangrove forests are usually dominated by salt-tolerant trees and have complex food webs where detritus serves as base and nutrient source of the food web. They also provide shelter, feeding, nesting and breeding zones to amphibians, reptiles, crustaceans, mollusks, fish of commercial importance, and resident and migratory birds (Holguin *et al*., 2001). Many invertebrates, resident species and migrating bird species are totally dependent upon mangroves' productivity to survive and complete important life cycle functions. Even so, at least 35% of the worldwide area of mangrove forests has been lost in the past two decades, exceeding the loss of tropical rain forests and coral reefs (Valiela *et al*., 2001).

At present, there is a need of information to validate the conservation status of mangroves and understand in detail the aquatic ecology related to them. Knowledge on the inhabiting species and how the environmental variables influence them and the whole mangrove ecosystem is crucial to have a better sustainable use of these forests.

Ecologists recommend the use of resident organisms, such as invertebrates, as indicators of disturbances in aquatic environments. Understanding the relationship between habitat and aquatic biota is important for the development of new management techniques (Brasher, 2003). Scientific publications and information on aquatic invertebrates in the mangroves of Puerto Rico are scarce and outdated. These ecosystems in Puerto Rico are poorly understood and studied.

Lugo and Cintrón (1975) classified Puerto Rican mangroves into two general categories: south coast and north coast types. In general, south coast fringing mangroves grow by the edge of the sea; exhibit low structural complexity, low leaf fall, and low rate of tree growth; and are dominated by the red mangrove (*Rhizophora mangle*) (Cintrón *et al.*, 1978). The site of the study presented here is in the largest mangrove forest of the "south coast type of mangroves", the Boquerón Wildlife Refuge (BWR) at Cabo Rojo. There are four of the ten mangrove species that are distributed along the Atlantic coast in Puerto Rico and all of them are present in the reserve. These mangrove species are: red mangrove (*Rhizophora mangle*), black mangrove (*Avicennia germinans*), white mangrove *(Laguncularia racemosa*), and buttonwood (*Conocarpus erectus*).

In 1964, the BWR was declared a natural reserve and bird refuge for more than a 100 bird species, including the endemic and endangered bird species named "Mariquita" (*Agelaius xanthomus*). During 1965, 177 hectares of the reserve were heavily impacted by the construction of dikes to utilize this area as a bird refuge. The construction of dikes cut off circulation, causing long-term flooding, and thus mortality in all species of mangroves, and the replacement of the ecosystem (Breen & Hill, 1969; Lugo, 1981). Along with the existence of a nearby municipal dump and development of urban areas and tourism, this event led to a series of massive mangrove deaths and disturbances in the water quality. Stress has negative effects on biodiversity by reducing productivity, individual survival and colonization (Colinvaux, 1986). Mangrove coverage has decreased significantly throughout time. Altered wetlands often become dominated by non-native or invasive plant species, which can affect the biodiversity, causing accumulation of organic matter, eutrophication, and lower the amount of oxygen dissolved in the water.

Mangroves are poor competitors under non-saline areas where freshwater marsh plants easily outclass them (Kathiresan and Bingham, 2001). Several aquatic invasive plants have appeared in the reserve, like papyrus (*Cyperus odoratus*), cattail (*Typha domingensis*) and most recently the floating fern *Salvinia* spp. These floating ferns invade relatively calm and slow moving bodies of freshwater, multiplying rapidly and covering the water surface (USGS, 2005c), with floating mats sometimes as thick as 20-25 cm (Jacono *et al*., 2001).

Thick mats of *Salvinia* prevent sunlight from reaching submerged plants, changing the pH, lowering salinity and dissolved oxygen, thereby making it unsuitable for other life forms, and can provide safe haven to pest species such as mosquitoes (USGS, 2005c). Invasive plant species not only adversely impact the biodiversity and ecosystem of wetlands but also have a negative impact on their recreational use by humans (Zedler and Kercher, 2004). Therefore, there is a need to know and understand these changes, and know if the invasive aquatic plant *Salvinia* is affecting the aquatic biota of the mangrove forest. In the BWR many of the mangrove channels are invaded by thick mats made up by a mixture of at least two *Salvinia* species, but some mangrove areas lack these plants.

Lugo (2002) established that the biota of mangrove substrates is complex and largely unknown, and the complex food webs that converge on mangrove ecosystems are yet to be understood. Most of the literature is focused on the aboveground arboreal component of the ecosystem. Few attempts have been made to examine faunal abundance and species composition, but some studies have examined faunal changes in relation to development and age of forests (i.e. Suzuki et al., 1997, and Sasekumar and Chong, 1998, both cited in Alongi, 2002).

The present study focused on the assessment and comparison of the aquatic macroinvertebrate diversity in three mangrove areas in the BWR invaded by *Salvinia* spp. and three areas without these floating ferns. Aquatic macroinvertebrates were selected because these organisms have been greatly studied in other types of wetlands. Indeed, they are the most common, diverse and abundant animal group in freshwater to brackish wetlands. They are also sensitive to physiochemical changes and stressors in the water, therefore the presence, absence, diversity and abundance of certain groups give information of the water condition; and, besides, they are important in the food web of wetlands (Deliz, 2005).

Salinity plays a vital role in the distribution of species, their productivity and growth of the mangrove forest (Twilley & Chen, 1998). Thus, salinity (along with pH) were measured because it is known that macroinvertebrates respond at the community level to salinity impacts and, therefore, are an ideal taxonomic group to assess aquatic trends in salinity sensitivity.

In Puerto Rico there is little information on the macroinvertebrates associated to mangroves. The main goal of this study is to provide a basis of biodiversity of the macroinvertebrates associated to BWR mangroves and how the presence of *Salvinia* spp. correlates with this biodiversity. Additionally, it is intended to encourage: 1) the study of mangroves in Puerto Rico, especially their inland freshwater to brackish zones, 2) establish a baseline for future taxonomic work and ecological studies, and 3) promote biomonitoring and management of these unique wetlands. Mangroves deserve special protection because of their uniqueness and multiple ecological functions. Therefore, conservation and restoration strategies should not only focus on enlarging habitat areas and restoring a single habitat type, but also on conserving and strengthening landscape heterogeneity (Verberk *et al*., 2006).

Objectives

- Characterize the macroinvertebrate communities of the mangrove forest of the Boquerón Wildlife Refuge (BWR) in areas with and devoid of *Salvinia* spp.
- Determine a correlation between the composition of the taxonomic groups and the presence of the *Salvinia* spp.
- Determine the temporal variation in the taxonomic composition of the invertebrate communities.
- Determine the relation between the sampling gear and the achieved taxonomic composition in the samples.
- Present a pictorial catalogue of the macroinvertebrate diversity for the BWR's inland zones.

Literature Review

Wetlands

Wetlands are found in every continent except Antarctica. Even though they have been poorly appreciated until recent times, an estimated 6% of the world's land surface is wetlands. Some of the major classes of wetlands: tidal salt and brackish marsh, freshwater marsh, prairie potholes, fens, bogs, swamp bottomland, and mangrove forest. These ecosystems are usually found at the interface between truly terrestrial ecosystems and truly aquatic systems (Mitsch and Gosselink, 1986). Since they are transition zones from uplands to deepwater aquatic systems, are considered as ecotones. They combine attributes, are highly dependent on both types of ecosystems. Among their many benefits to society and the environment, wetlands improve water quality, control erosion and flood, and provide unique habitats to support great diversity of plants and animals (Mitsch and Gosselink, 1986; Deliz, 2005).

In Puerto Rico there is a rich diversity of wetlands; nevertheless, they have been poorly studied. Little is known of their ecological value, biota, food webs, and complex water and land communities interactions. It is estimated that today there is only a fraction of the wetlands there used to be on the island, which have been constantly threatened by agricultural and urban development (Deliz, 2005). Most studies of Puerto Rican aquatic environments have focused their efforts on streams, rivers, lagoons, and other freshwater ecosystems; whereas there is a of lack ecological studies on important wetlands such as mangrove forests.

Mangroves

The term ‘mangrove’ has two general meanings in ecology. One refers to a group of halophytic species belonging to the tropical intertidal forest and the other refers to the forest itself, which is a complex of plant communities fringing tropical oceans (Cerame, 1973; Lugo and Snedaker, 1974; Cintrón *et al*., 1978; Tomlinson, 1986; Holguin *et al*., 2001; Martinuzzi *et al*., 2009). In 1968, term "mangal" was proposed as a term for the community that contains mangrove plants (Mitsch and Gosselink, 1986; Tomlinson, 1986).

Mangroves are the only forests situated at the interface between land and sea in the world's subtropics and tropics approximately 30° N and 30° S latitude (Alongi, 2002; Martinuzzi *et al*., 2009; Giri *et al*., 2011). As ecosystems, mangroves are considered estuarine habitats, transitional zones or ecotones between freshwater and the sea (Ward, 1992).

Four species of mangrove plants inhabit the Caribbean: *Rhizophora mangle* (Rhizophoraceae), *Avicennia germinans* (Avicenniaceae), *Laguncularia racemosa* and *Conocarpus erectus* (Combretaceae) (Tomlinson, 1986). These plants possess morphological and ecophysiological characteristics and adaptations that make them structurally and functionally unique (Alongi, 2002). They are considered pioneer species because of their ability to establish on otherwise non-vegetated substrates.

Mangroves are recognized as highly productive ecosystems (Holguin *et al.*, 2001; Alongi, op.cit.; Lugo, 2002; Giri *et al*., 2011), for they provide large quantities of organic matter to adjacent coastal waters in the form of detritus and live animals. They also help stabilize shorelines and reduce the impact of natural disasters such as tsunamis and hurricanes (Giri *et al*., 2011). Moreover, they improve water quality and clarity by filtering upland runoff.

In addition, mangrove ecosystems have an important role in nutrient recycling, and they function as a shelter, feeding, breeding and nursery zones for a wide variety of marine and estuarine vertebrates and invertebrates. In fact, most invertebrate and some resident vertebrate species are totally dependent upon mangroves to survive and complete important stages of the life cycle (Tomlinson, 1986). Many of the birds associated to the mangroves are migratory birds that depend on the high productivity of mangroves as an energy source.

Deforestation of mangrove communities is thought to be one of the major reasons for the decrease in coastal fisheries of many tropical and subtropical countries (Holguin *et al.*, 2001). Mangroves become more susceptible to diseases and pests when stressed by changes in salinity, tidal inundation, sedimentation and soil physicochemistry (Alongi, 2002). The area of the world's mangrove forests has decreased by 35% from 1980 to 2000 (Giri *et al*., 2011), at the rate of 2.1% per year, exceeding that of tropical rainforests and coral reefs (Valiela *et al*., 2001; Alongi, op. cit.; Duke *et al*., 2007).

Mangrove experts estimate that we will lose mangroves completely within the next 100 years. Agriculture, aquaculture, overexploitation, tourism and urban development have been the main cause of this forest decline. The ecological and economic values that justify mangrove conservation have been extensively highlighted and authors agree that comprehensive conservation strategies are vital to guarantee the future of mangroves (Valiela *et al*., 2001; Alongi, 2002; Lugo, 2002, Martinuzzi *et al*., 2009; Giri *et al*., 2011)). However, scientific understanding of mangroves is incomplete, in spite of the large bibliography on this ecosystem (Lugo, 1988; Lugo, 2002; Martinuzzi *et al*., op. cit.). Most of the literature has focused on floristic and structural topics (Mitsch and Gosselink, 1986) such as the description of species, their distribution and coverage. Lugo and Snedaker (1974) made a wide compilation of the historical perspective of previews works and classification mangroves in their work "Ecology of Mangroves". Fauna lists of mangroves have focused on crustaceans, mollusks, worms and birds, especially on marine biodiversity but little has been done on the biodiversity of the estuarine or freshwater-brackish portions of the mangroves. Faunistic studies have focused on changes in the composition of macroinvertebrates that colonize the roots of *Rhizophora mangle* (Reyes and Campos, 1992), and surveys on non-marine animal groups are meager. Very few attempts have been made to list insects in mangroves (Tomlinson, 1986).

Latin American and Caribbean mangroves have sustained human activity since pre-Columbian times; early use of mangroves was no threat to them. In recent years, though, the demands on mangrove forests have intensify. The mangroves of Latin America and the Caribbean, including the state of Florida, cover about 30 to 35 percent of the world's total mangrove area. On Caribbean islands, such as Puerto Rico, the area of mangroves has decreased to less than half of their original range (Lugo, 2002).

The Puerto Rican mangroves have been classified in two categories: south coast and north coast types. The determinant factors for the classification are: intensity of wave action, rainfall and freshwater runoff; factors considered in control of the development and structure of the mangrove (Pool *et al*., 1977; Cintrón *et al*., 1978; Martinuzzi *et al*., 2009). Basin and riverine mangroves predominate on the northern-coast type mangroves, while fringe mangroves predominate on the southern-coast type mangroves. Martinuzzi *et al*. (op. cit.) did a study combining historical information and long-term landscape analysis to relate land use change with the area of mangroves in Puerto Rico in the last 200 years. This study established that Puerto Rican mangroves have suffered and have been affected by the agricultural and economical changes the island went through; they were cut for the sugar cane fields, used for fuel wood, charcoal, and thus many coastal wetlands were drained. Protection of mangroves in Puerto Rico began in 1919, which marked a natural recovery of mangroves. Nevertheless, mangrove coverage rapidly declined between 1959 and 1971 due to rapid urbanization, urban drainages, channeling and garbage deposition. After that period, mangroves recovered thanks to protection in the rural sites.

Mangroves are highly productive ecosystems but have high mortality rates; as soon as the conditions change, their cost of survival is too high and massive mortality occurs. This high rate of mortality in mangroves takes place when salinity exceeds average levels, with the interruption of oxygen supply to roots, changes in water fluxes. Because they are highly productive and have pioneer species characteristics, they present resilience. They re-grow rapidly after floods but they are very vulnerable to changes in hydrologic fluxes, sedimentation, changes in temperature and modification of topography (Lugo, 2002).

The biota of mangrove substrates is complex and largely unknown, and the complex food webs that interact on mangrove ecosystems are yet to be understood. Mangroves are open systems with significant exchanges of materials and organisms with ecosystems in terrestrial, oceanic, estuarine and atmospheric environments; the study of such high levels of complexity is only beginning (Lugo, 2002). Most of the local studies of fauna, as elsewhere, have focused on the associations of organisms to the roots of the mangroves, especially in marine mangrove environments. In 1990, Rodríguez and Stoner published a study of the algal community associated with the roots of *Rhizopohora mangle* of the Laguna Joyuda estuary in Puerto Rico.

The Boquerón forest is located in the southeast of Puerto Rico. Included in the forest are the public beach of Boquerón and the Natural Reserve of Boquerón (Silander, 1986), as well as the Boquerón Wildlife Refuge (BWR). The refuge (BWR) is managed by the Department of

Natural and Environmental Resources of Puerto Rico and has approximately 182 hectares. Mangroves comprise around 58% of the lagoon; 11% is other types of wetlands, and 31% are open spaces, and it is surrounded by four kilometers of dikes. At the south of the reserve there are more wetlands, a small community, and an abandoned sanitary land field. The reserve receives fresh water from the Cartagena Lagoon through a creek belonging to the Lajas Valley Irrigation Project. During dry season, saltwater inflow is pumped into the reserve (Ruiz, 2012).

Macroinvertebrates

In contrast to temperate ecosystems, scientific literature on aquatic macroinvertebrates of the Caribbean islands' wetlands is scarce, especially concerning information of the macroinvertebrates of mangroves, which is almost non-existent. A review of the literature on aquatic invertebrates of temperate environments has demonstrated great differences between the kinds of macroinvertebrates present in the temperate zones and those in the Caribbean islands. On the other hand, Bass (2003) stated that most of the insect orders found in the temperate freshwater environments (mostly streams) are also present in the tropics; however, in different proportions.

Although less than 3% of all species of insects have aquatic stages, insects may comprise over 95% of total individuals or species of macroinvertebrates in inland aquatic systems (Ward, 1992). Therefore, in order to have a better understanding of the ecology of mangroves, and wetlands in general, it is imperative to produce taxonomic works of local aquatic insects in order to develop ecological and monitoring studies. In Puerto Rico, these types of extensive studies are lacking; most of the published works have been on specific taxonomic groups. One of the pioneers in entomological research in Puerto Rico was G. N. Wolcott. He made intensive collecting expeditions in Puerto Rico and the Caribbean and wrote a still-used famous guide, "Insects of Puerto Rico", in 1948 (Gutierrez *et al*., 2013). Some orders of insects have been, individually, very well studied, including Ephemeroptera (Traver, 1938) Odonata (Klots, 1932; García-Díaz, 1938; Paulson, 2011) and Trichoptera (Flint, 1964, 1992) (cited in Gutierrez *et al*., 2013). The orders Lepidoptera, Coleoptera and Diptera have been poorly studied aside from the general studies of Wolcott's in 1948.

A distinguished author who contributed to early aquatic entomology in Puerto Rico was Dr. Jenaro Maldonado Capriles. He published additions and corrections to Wolcott's "Insects of Puerto Rico" (Maldonado-Capriles and Navarro, 1967), and a summary of the works in insect taxonomy in Puerto Rico (Maldonado-Capriles, 1996). Additionally, he made most of the local studies on aquatic Hemiptera.

An important study on the aquatic insects in Puerto Rico, centered on the emergence patterns, composition, and temporal abundance of the aquatic insects of the stream Quebrada Prieta at El Verde (Masteller and Buzby, 1993). This was the first of a series of six publications of the same study area but focused on specific taxonomic groups in collaboration with other experts: Trichoptera (Flint and Masteller, 1993), Ephemeroptera (Pescador *et al*., 1993), Chironomidae (Ferrington *et al.*, 1993), Tipulidae (Gelhaus *et al*., 1993), Psychodidae (Wagner and Masteller, 1993) and Empididae, Ceratopogonidae and Simulidae (Masteller and Buzby, 1993). The most recent studies on aquatic insects in Puerto Rico are those by Deliz (2005), who studied the macroinvertebrates of the Cartagena Lagoon, and Gutiérrez *et al*. (2013), who published a list of aquatic insects of Puerto Rico, mostly insects of rivers and streams.

Invasive plant: *Salvinia* spp.

Even though 6% of the earth's land mass is wetland, 24% of the world's most invasive plants are wetland species. Many of these wetland invaders form monotypes, which alter available habitat, change biodiversity, and affect the nutrient cycling and productivity, thus altering diversity and modify food webs (Zedler and Kercher, 2004; Parys *et al*., 2013). Aquatic plants, particularly floating plants, can cause changes by reducing light availability and interfering with gas exchange at the water surface. Oxygen availability tends to be low in wetlands with dense floating vegetation (Batzer *et al*., 1999).

Salvinia, a free-floating fern, is a genus native to Southeastern Brazil (Pteridophyta: Salviniaceae). This genus reproduces both sexually and asexually. Its growth form consists of ramets connected by rhizomes forming matted colonies (Coelho *et al*., 2005). *Salvinia* grows best in still or slow moving water, has rapid growth rates (double its number and dry weight in less than three days) and forms dense mats that can be several layers of plants deep; thus, blanketing the surface of water (Julien *et al*., 2009).

Most studies examining insects associated with *Salvinia* species focused on identifying potential biological control agents (Bennett, 1966; Forno and Bourne, 1984; Parys *et al*., 2013). The majority of the studies deal with Giant Salvinia (*Salvinia molesta* Mitchell) as a potential serious aquatic fern, and center on the biological control agents (Forno, 1980; Room *et al*., 1984; Sands and Schotz, 1984; Thomas and Room, 1984; Doeleman, 1989; Miller and Wilson, 1989; Skeat, 1990; Oliver, 1993; Tipping and Center, 2003; Mcintosh *et al*., 2003; Flores and Carlson, 2006; Hennecke and French, 2008). Recent studies have examined macroinvertebrates associated to *Salvinia* in its native range (Herrera *et al*., 2000, Albertoni and Palma-Silva, 2006; Poi de Neiff and Neiff, 2006); while the work by Parys *et al*. (2013) is perhaps the first work that documents the diversity of invertebrates associated with *Salvinia* in the United States. They presented a list of adult insects (excluding Diptera and Lepidoptera) and Coleoptera was the most diverse order.

In Puerto Rico, Proctor (1989) reported *Salvinia minima* from Arecibo, Carolina and Mayagüez and *S. auriculata*, as a cultivated aquatic plant, in ponds near Fajardo. Axelrod (2011) reported *Salvinia auriculata* as an introduced and invasive plant in the Northern Coastal Lowlands (San Juan) and, also, the species *Salvinia minima* from Barceloneta, Arecibo and Mayagüez.

Materials and Methods

I. Study site

The mangrove forests of the Boquerón Wildlife Refuge (BWR) are located in Boquerón area at Cabo Rojo (18°00'55.2"N 67°09'34.4"W), in the southwest corner of Puerto Rico. The reserve has the largest mangrove stand in Puerto Rico's west coast (182 hectares). Dikes were constructed in this reserve in 1964 to control water flow and utilize the area as a bird refuge. It receives water from the Laguna Cartagena Wildlife Refuge which is located in the municipality of Lajas (18°01'N, 67°06'W).

Table 1: Precipitation values taken from the data base of the Natural Resources Conservation Service (NRCS) of Combate at Cabo Rojo, Puerto Rico

Sampling Months	Precipitation (cm)
June	50.5
July	56.2
August	58.5
September	75.4
October	5.1
November	14.7

II. Methodology

A. Sampling Locations

Samplings of aquatic macroinvertebrates were conducted once a month from June to November of 2013. Samples representing part of the dry season were taken from June to August, and those encompassing the rainy season were collected from September to November. There were six sampling areas: three with the a *priori* confirmed presence of *Salvinia* spp. (herein named stations 1, 3, and 6) and three others devoid of *Salvinia* spp. (named stations 2, 4 and 5) (Figure 4). The positions of the six sites were recorded using the global positioning system (GPS) unit; three sites where selected in each of the two trails of the reserve. Stations 1, 2, and 3 were on the left side trail (from the main entrance) and stations 2, 4 and 5 were on the right side trail (Figure 1).

Table 2: Sampling time table.

Traps placement	**Collection of samples**
June 7, 2013	June 9, 2013
July 3, 2013	July 5, 2013
August 16, 2013	August 18, 2013
September 24, 2013	September 26, 2013
October 24, 2013	October 26, 2013
November 18, 2013	November 20, 2013

Figure 1: Map showing the specific sampling sites in the Boquerón Wildlife Refuge (BWR). Red numbers indicate the sampling stations.

A

B

C

Figure 2: (A) *Salvinia* spp. mats in the mangrove channels; (B and C) colonies of the floating fern *Salvinia* spp.

Figure 3: Sampling stations. A. Station 1 (with *Salvinia*); B. Station 2 (devoid of *Salvinia*); C. Station 3 (with *Salvinia*); D. Station 4 (devoid of *Salvinia*); E. Station 5 (devoid of *Salvinia*); F. Station 6 (with *Salvinia*).

B. Sampling and identification of macroinvertebrates

In order to acquire extensive knowledge of the macroinvertebrate communities, the methodology was designed using three traps that are specific to certain groups of organisms or to their life stages. The sampling methods were the following:

(1) Aquatic light traps (Bioquip®) (volume of 4L) for organisms that live in the water column and close to the benthos. The aquatic light traps had a light stick inside lasting at least 48 hours to attract insects and other invertebrates.

(2) Two contiguous D-net (Nytex ® 500 µm netting; area of 454 cm^2) sweeps, made in a zigzag fashion along a 4.4 m stroke and, thus, filtering a volume 200L for each sweep; used to trap organisms living close to the surface, attached to the *Salvinia* mats or near the benthos.

(3) Malaise traps or Aquatic Insect Emergence Trap (Bioquip ®) (base area of $2m^2$) for the adult stages of mainly insects.

During each sampling date, the three sampling methods were employed in the six sampling sites. On each sampling date the Malaise and light traps were set ahead and two days later the trapped organisms were collected and the D-net sweeps were made. An average of the data of the two D-net sweeps was determined. A total of 144 samples were collected.

Figure 4: Trapping gears, A) Aquatic light trap, B) D-net, and C) Malaise trap

Samples from the aquatic light-traps and the dip-nets were washed to collect the organisms attached to the netting or surfaces. These samples were stored in plastic bags with 70% ethyl alcohol. The organisms in the Malaise trap were collected with a hand-held vacuum, stored in vials with ethyl-acetate-infused cotton, and taken to a refrigerator. In site salinity measures were taken using a refractometer and pH levels with a potentiometer.

In the laboratory, the whole sample was screened and the organisms were sorted from the detritus and undesired matter, were identified using a dissection microscope, and then stored into individual vials containing a mixture of 70% ethyl alcohol 95%, 27% water and 3% glycerol for preservation purposes. Taxonomic keys for aquatic invertebrates were used to identify the collected specimens to the family level, except for some taxonomic groups (Brown, 2009; Brown, 2010; Chu and Cutkomp, 1992; Epler, 2010; Fernández, F. and Sharkey, M. J., 2006;González & Carrejo, 1992; Heckman, 2011; Mari-Mutt, 1976; Marshall, 2006; Merrit, *et al*., 2008; Meurgey and Picard; 2011; Nieser and Alkins-Koo, 1991; Pointer, 2008; Thorp and Covich, 2010; Triplehorn and Johnson, 2005; Zumbado, 2006; Westfall and May, 1996). Photos were taken using a Focus Staking Digital Photography System to create a pictorial catalogue of the fauna.

C. Statistical analysis

Data was analyzed with the application of ecological indexes, Simpson Diversity Index [$D= \sum pi^2$] and Shannon-Wiener Index [$H'= - \sum pi \ln (pi)$], to compare diversity among stations with and without *Salvinia* over the six sampling months for each sampling method. The Jaccard Index of Similarity [$J= C / (A+ B + C)$] was applied to compare the areas with and devoid of *Salvinia* in terms of their invertebrates communities as obtained with each sampling method.

Nonparametric one way ANOVA and the Tukey tests were conducted using the program Infostat® in order to search for temporal and spatial differences in species richness, abundances of major taxonomic groups, and environmental parameters among the sampling sites and months; it was also used to determine the temporal and spatial interactions related to the total number of individuals collected for each taxon; most at family level and some at order. A log n+1 was used to attempt to normalize the data. Tukey tests were also applied to the mean pH and salinity values in order to search for temporal and spatial differences in the mean values among the sampling sites.

A multiple variable analysis was performed to the biodiversity data obtained with the three sampling methods. Principal component analysis (PCA) was conducted using the program Infostat®-version 2012 and applied in order to have a better two-dimensional perspective of the distribution and relationship among the most abundant taxonomic groups for the sampling months and for the sampling areas (with and without *Salvinia*). For the PCA, the data was standardized and represented in a biplot. This biplot demonstrated graphically the relationship of the samples (taxonomic groups), displayed as points, and the variables (the presence or absence of *Salvinia* spp. and the sampling months) displayed as vectors. Only the 15 most-abundant taxonomic groups for each sampling trap were used in the PCA.

RESULTS

I. Temporal variation of physicochemical parameters: pH, salinity and precipitation

Figures 5 and 6 show the values for the salinity and pH, respectively, in all six sampling sites. The areas devoid of *Salvinia* had the highest peaks of salinity, which is a major factor precluding in the distribution of the invasive freshwater plants into the mangrove channels. During the samplings of August and October 2013, the water levels changed dramatically with the opening of the dikes to flood the refuge which was a main factor determining these changes in salinity. The values of pH and salinity for all the samples are presented in the Appendix 1.

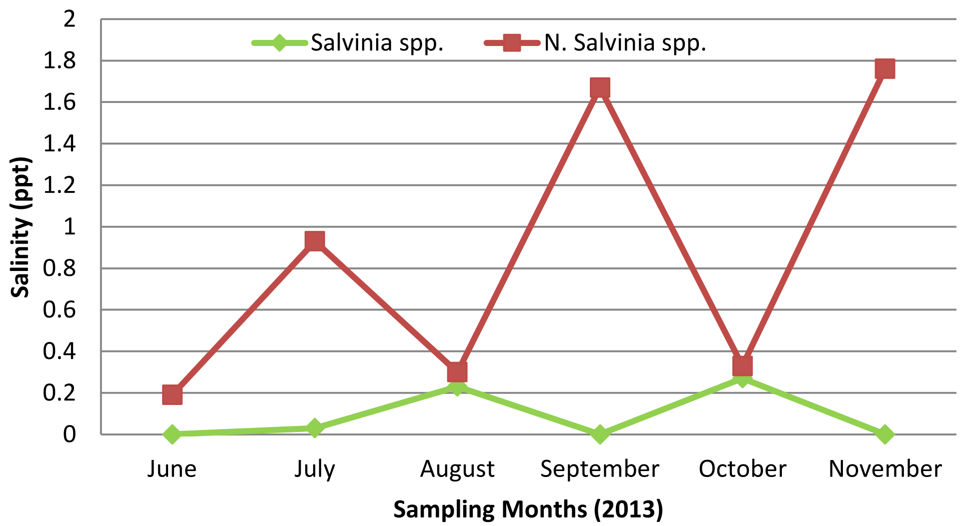

Figure 5: Mean salinity (0/00) for each sampling month in the areas with and devoid of *Salvinia* spp.

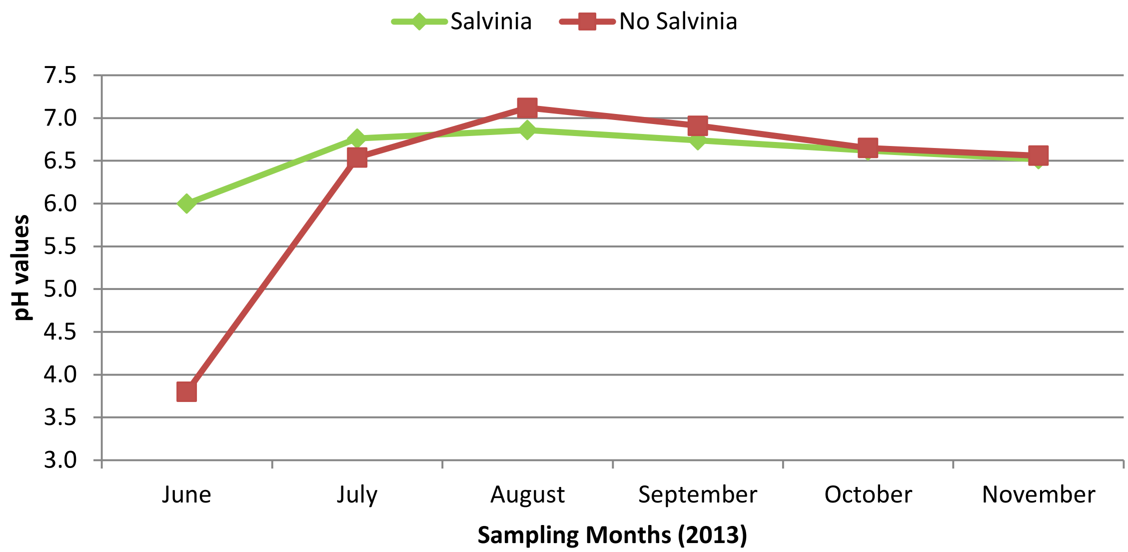

Figure 6: Mean pH measurements for each sampling month in the areas with and devoid of *Salvinia* spp.

II. Biodiversity

Taxonomic composition: general abundance and biodiversity

A total of 21,305 organisms were collected between the months of June and November of 2013 at Boquerón Wildlife Refuge the invertebrates were distributed into 20 orders and 81 families. The macroinvertebrate communities were composed mostly by aquatic insects, crustaceans and gastropods. The insect orders Diptera was the most representative group (with 21 families), followed by Hemiptera, Odonata, Coleoptera, Collembola, Hymenoptera and Lepidoptera. These groups represented about 41% of all individuals collected. Of the non-insect fauna, the gastropods represented 5%, the Decapoda 19% (mostly due to blooms of larval stages; i.e. zoea), Acari 15%, and the rest were comprised by a variety of microcrustaceans also comprised

19% [ostracods (4%), cladocerans (12%) and copepods (3%)]. Amphipods, isopods and other rare orders represented only 1% of the collected fauna.

The groups with the greatest family richness were Diptera (with 21 families) and Coleoptera (with 12 families) (Table 4, Fig. 23). The sampling months that rendered more collected individuals were September and October. Overall, sampling sites with *Salvinia* were represented by 55 families, while the sampling sites without *Salvinia* presented 57 families.

Table 4: General diversity and abundance of the principal taxonomic groups. Except the subphylum Crustacea.

Taxonomic Group	Families represented	Total number of individuals
Diptera	21	6,900
Coleoptera	12	464
Hemiptera	11	724
Odonata	4	389
Collembola	5	141
Hymenoptera	4	4
Lepidoptera	1	95
Gastropoda	5	1,151
Acari	Undetermined	3,249

III. Sampling Traps

Aquatic Light traps

There were higher abundances of invertebrates in the aquatic light traps in the areas without *Salvinia* spp. The groups with the greatest abundance were from the Subphylum Crustacea, and out of the five groups with the highest accumulative total number of individuals, four were crustaceans (Zoeae, Cladocera, Copepoda and Ostracoda). Acari were also very abundant. The orders with greatest family representation were Coleoptera and Diptera; being Chironomidae the group with highest abundance of larvae.

Light traps are targeted towards the organisms that live in the water column. There was a difference of the abundance of total number of individuals in the areas without *Salvinia* spp. specifically in the rainy months (September and October). These months had isolated blooms of small-bodied animals such as Cladocera (September and October), copepods (September) and zoeae (August, September, and October).

The total numbers of individuals in areas with *Salvinia* were mostly similar. Areas with this invasive fern have very dark water columns and are full of dense litter. Figure 9 presents the distribution of the different taxonomic groups over time in areas with and devoid of *Salvinia* spp. Mites dominated the areas with *Salvinia*, while in areas devoid *Salvinia* have great abundance of Cladocera, Copepoda, Diptera and Gastropoda. The most abundant type of microcrustaceans in areas with *Salvinia* were the ostracods.

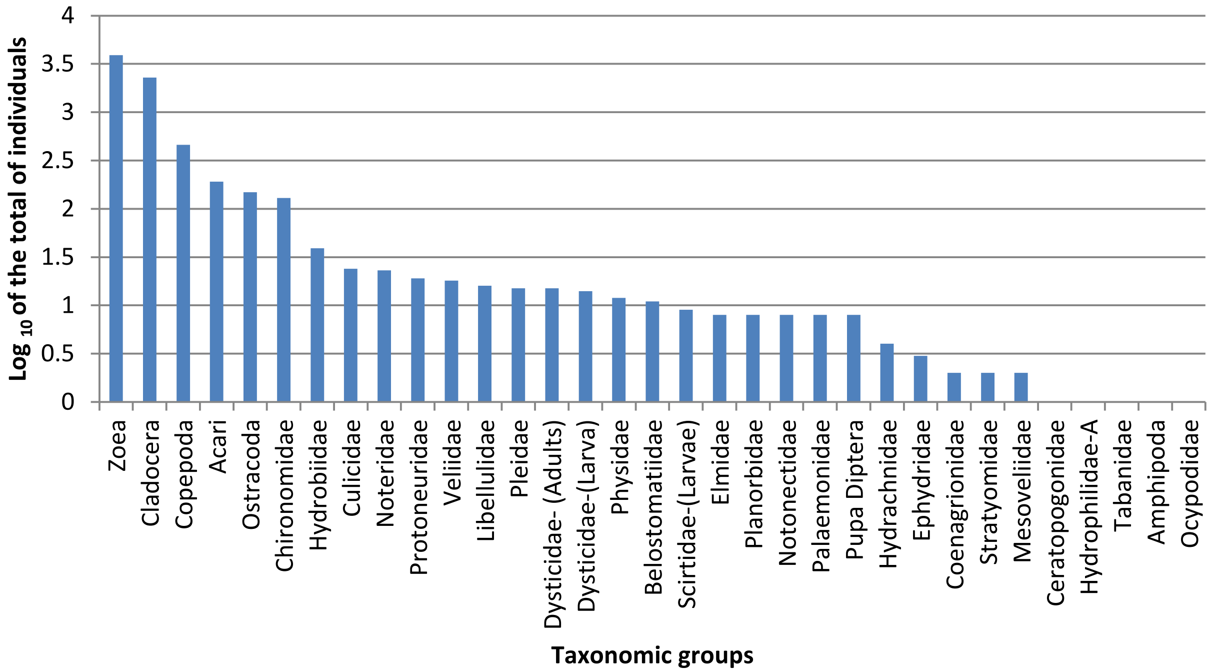

Figure 7: Log $_{10}$ of the accumulative total number of individuals of the taxonomic groups represented in the aquatic light traps (per 4L; n=36). Taxonomic groups were arranged in order of abundance, groups to the right are represented by one individual because Log $_{10}$ of one equals zero. Diptera and Coleoptera individuals are larval stages unless stated (except for noterids and elmids).

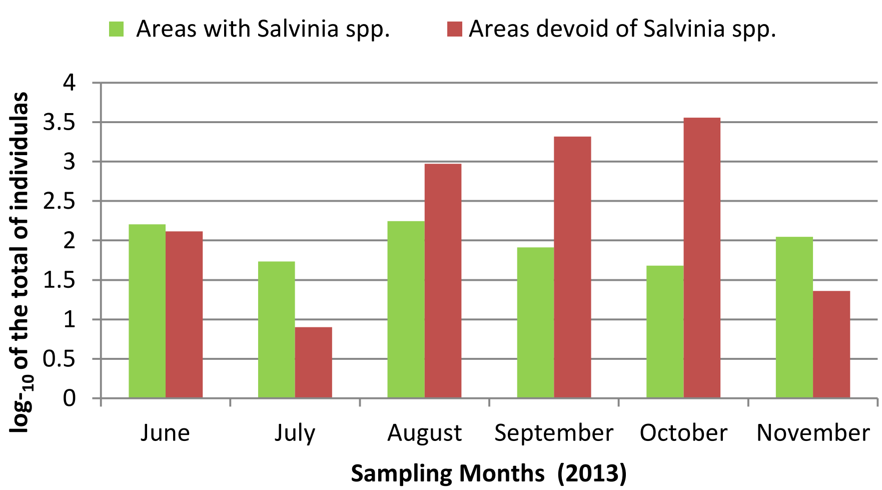

Figure 8: Log $_{10}$ of the accumulative number of individuals collected in the aquatic light traps (per 4L; n=36) for areas with and devoid *Salvinia* spp.

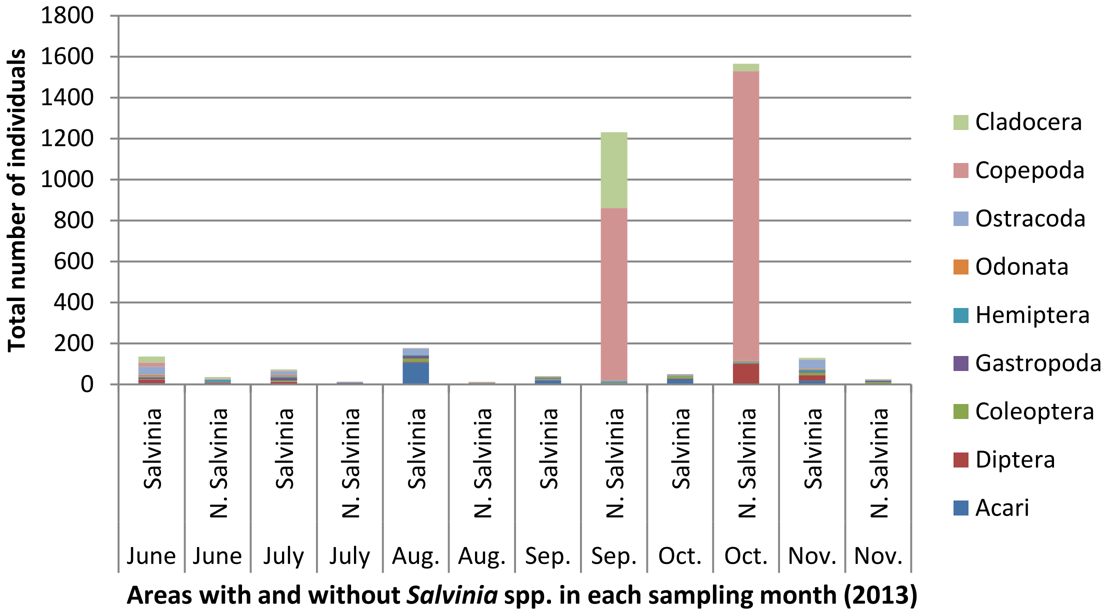

Figure 9a: Comparison of the total number of individuals within the dominant groups of invertebrates in the sampling sites with the presence and the devoid of *Salvinia* spp. collected with aquatic light traps (per 4L; n=36).

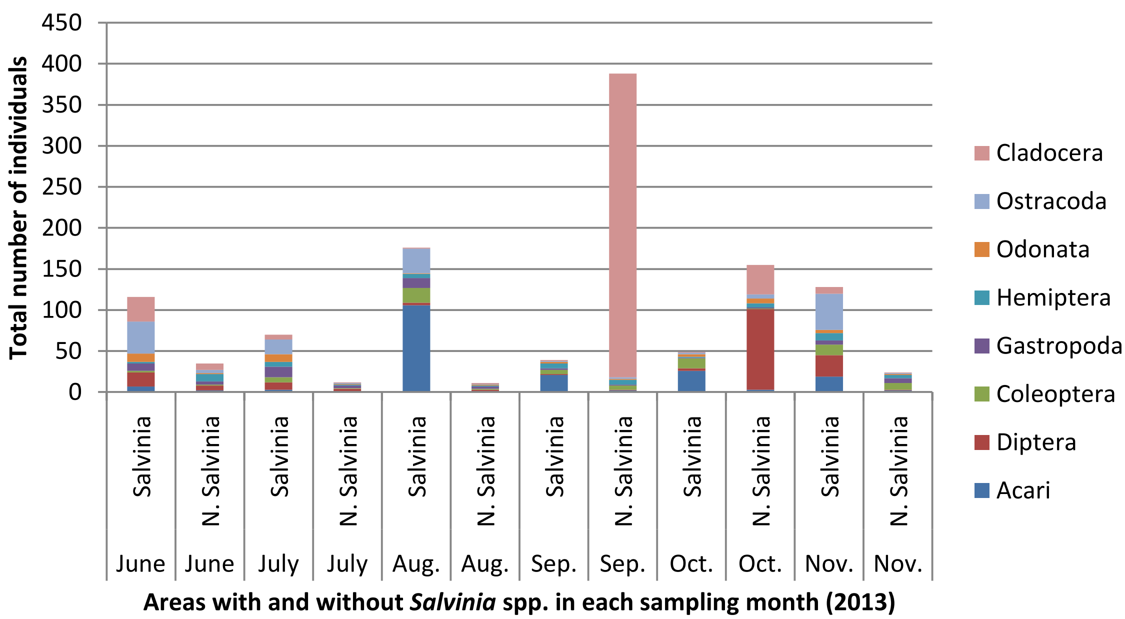

Figure 9b: Comparison of the total number of individuals within the dominant groups of invertebrates (excluding copepods) in the sampling sites with the presence and the devoid of *Salvinia* spp. collected with aquatic light traps (per 4L; n=36).

A clear pattern of spatial distribution was observed with the aquatic light traps, in which areas covered by *Salvinia* were dominated by Acari and Ostracoda, while those devoid of *Salvinia* spp. were dominated by Cladocera, Gastropoda (Hydrobiidae), and Diptera (especially members of Chironomidae and Culicidae), suggesting that many invertebrates in these habitats have their own niche as seen in all aquatic ecosystems Regardless of the presence of *Salvinia*, microcrustaceans were the most abundant group in the aquatic light traps, followed by Acari and the Chironomidae larva.

The PCA for the taxonomic groups collected with aquatic light traps in areas with the presence and absence of *Salvinia* spp. the 55 % of the variance (PC1) presented a separation of the areas with and devoid *Salvinia* spp. The group that determined the greatest variability of the data for the areas with *Salvinia* was Acari, and for the areas without *Salvinia* were the Chironomidae. The PC2 explained 45% of the variability compounded with rest of the taxonomic groups (Figure 10). In figure 11, the PC1 of the sampling months for the aquatic light traps presented all the sampling months for 49%. The months of June and July demonstrated similarity, as also did the months of August and September, in terms of their variances.

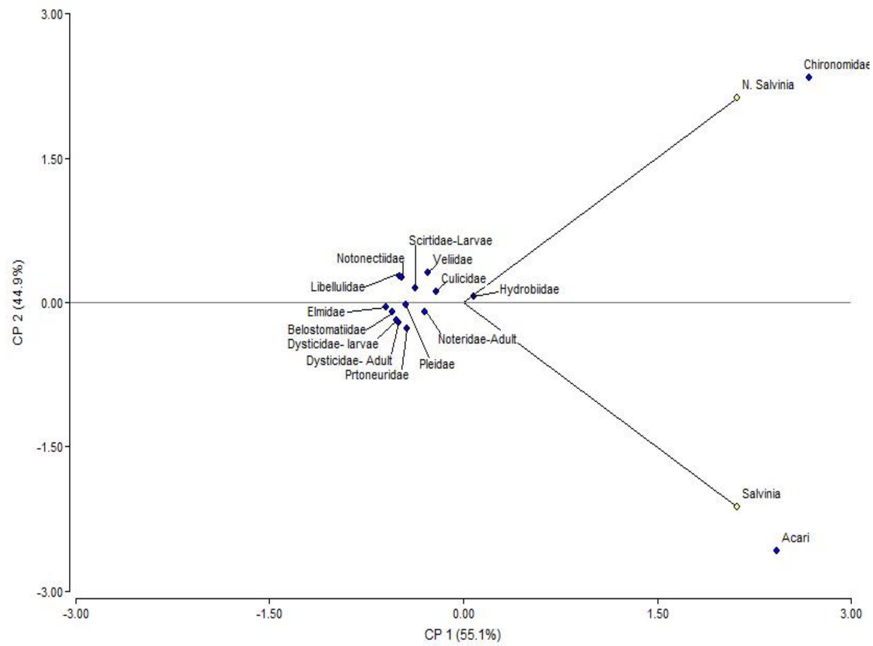

Figure 10: PCA of the most abundant taxa collected with the aquatic light traps in the areas with and devoid of *Salvinia* spp. (CP= Principal component or PC)

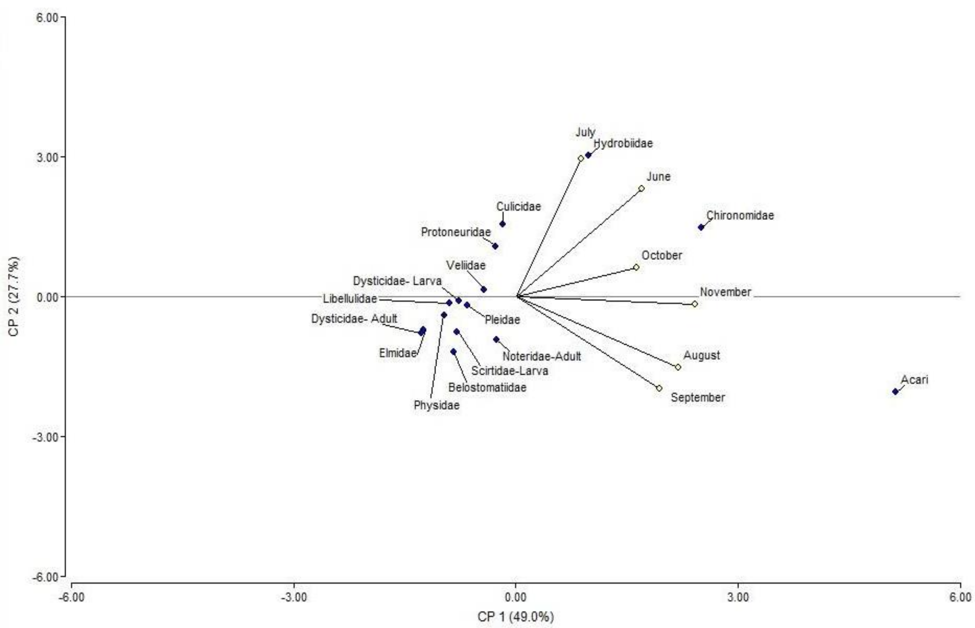

Figure 11: PCA of the most abundant taxa collected with the aquatic light traps in the six sampling months. (CP= Principal component or PC)

Malaise Traps

For the emergence trap the overall dominant groups collected were Diptera represented by 21 families (Fig. 13). The areas without *Salvinia* spp. overall had the highest abundance of individuals mostly Diptera. In the month of July there was high abundance of Ephydridae and Culicidae in September, both families were present in all the samples (Fig. 14). A particular phenomenon observed in the Malaise traps was that the adult odonates collected that belong to the family Coenagrionidae (*Ischnura capreolus, Telebasis coralina* and *Enallagma civile*) (Appendix V, Figs.A85-A87), while the nymphs in the D-net sweeps aquatic light traps were mostly from the family Protoneuridae. Another particular group were the larvae of Scirtidae (Coleoptera) that were abundant in areas without *Salvinia* in the month of June.

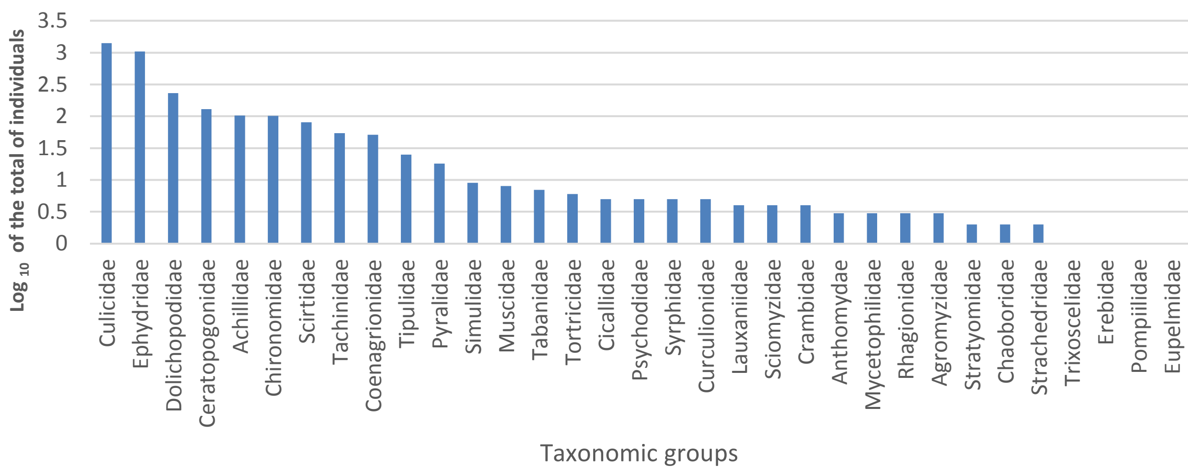

Figure 12: Log_{10} of the total number of individuals of the taxonomic groups represented in the Malaise traps. Taxonomic groups were arranged in order of abundance, groups to the right-most half are represented by one individual because Log_{10} of one equals zero (n=36).

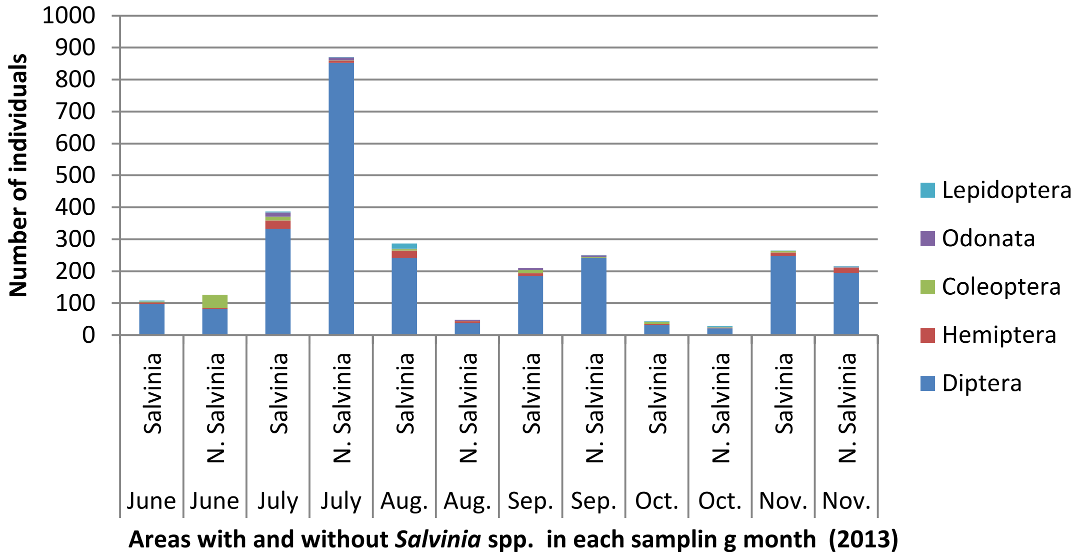

Figure 13: Comparison of the total number of individuals within the five most dominant groups of macroinvertebrates collected with Malaise traps in the sampling sites with and devoid *Salvinia* spp. (n=36).

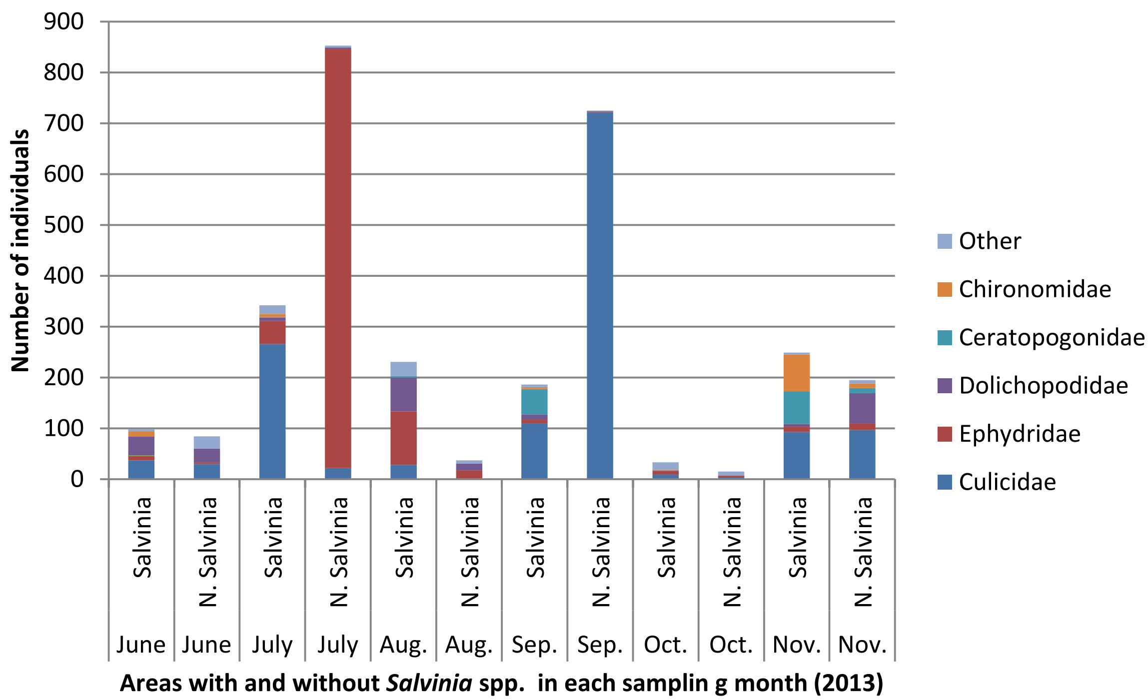

Figure 14: Comparison of the total number of individuals in the dominant families of Diptera collected with the Malaise traps in the sampling sites with and devoid of *Salvinia* spp. (n=36).

The first component of the figure 15 explains 92% of the variance of the taxonomic groups for the areas with and devoid *Salvinia* spp. For this PC1 presents a clear separation of the vectors of areas with and devoid *Salvinia*. For the areas without *Salvinia* spp. the presence of ephydrids is the factor that most influences the variability of the data for this areas. The PCA for the sampling months, the PC1 (57%) presents all the sampling months. A group was formed by the months of July and August (similar in their variance) influenced by the presence of Ephydridae, and another group of September and November influenced by the presence of Culicidae; meanwhile, the months of October and June behaved as an intermediate of the two groups (Fig. 16).

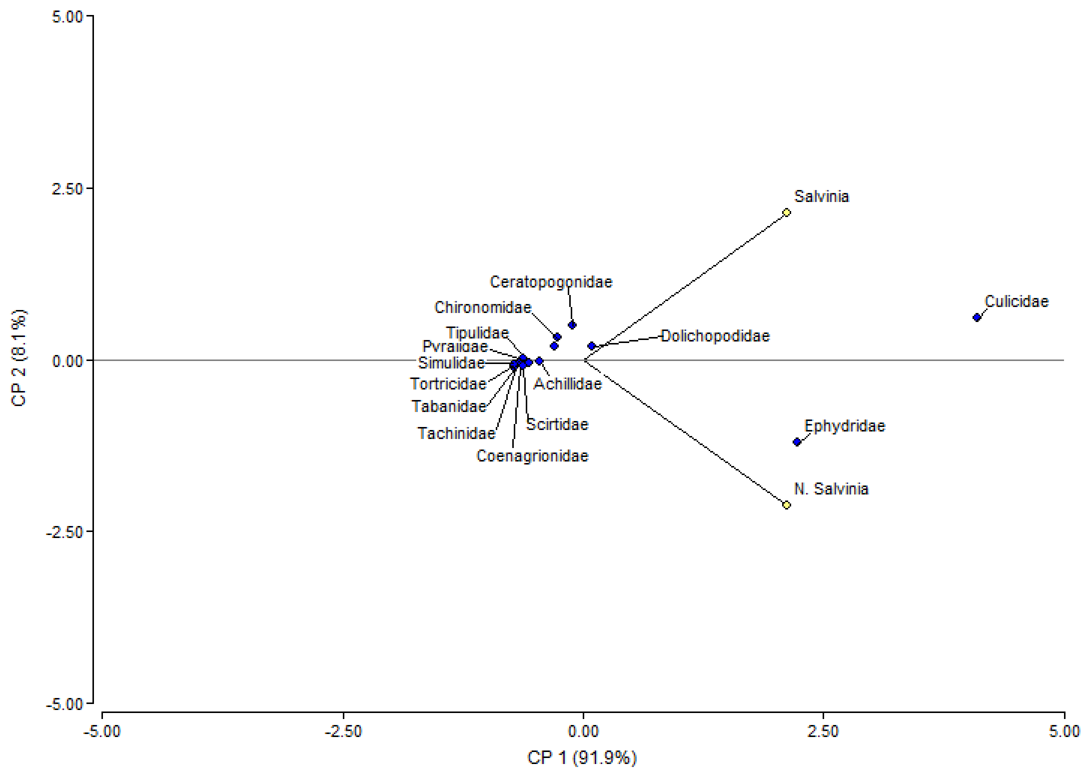

Figure 15: PCA of the most abundant taxa collected with the Malaise traps in the areas with and devoid of *Salvinia* spp. (CP= Principal component or PC).

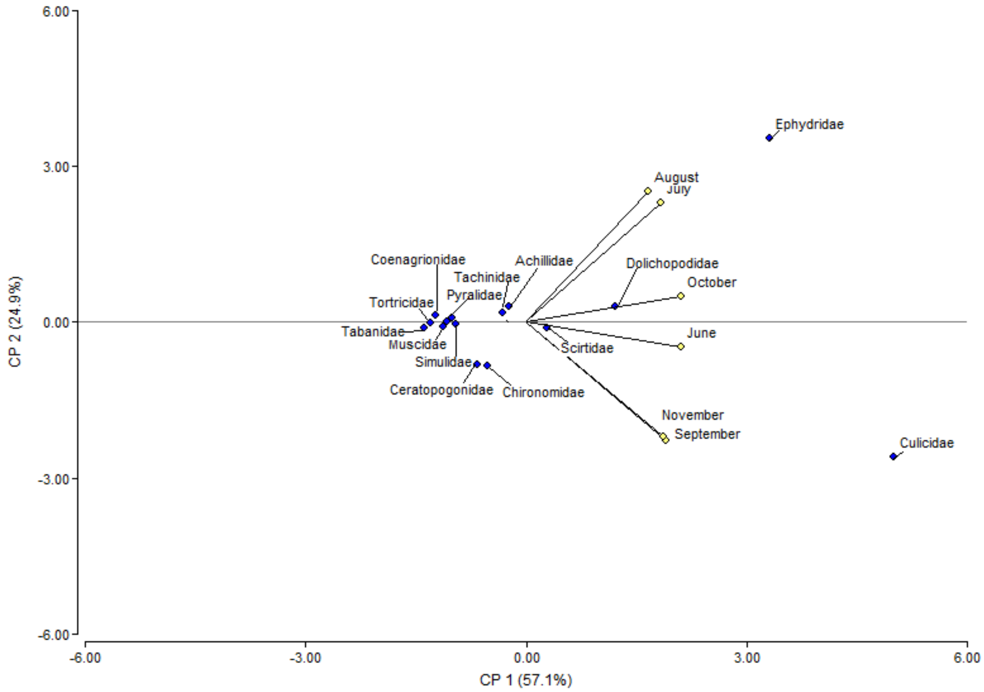

Figure 16: PCA of the most abundant taxa collected with the Malaise traps in the six sampling months. (CP= Principal component or PC).

D-net sweeps

The taxonomic group with the highest total abundance in the D-net sweeps from areas with *Salvinia* spp. was Acari, followed by Diptera (Chironomidae). Taxonomic groups that were well represented in the D-net sweeps but rarely captured in the other sampling methods, were the Odonata nymphs (Protoneuridae), Hemiptera, Collembola and freshwater mollusks (Physidae) (Fig. 17). Hemiptera and Collembola find refuge in the leaves of *Salvinia*. The month of July presented peaks in both areas with and without *Salvinia*. The lowest abundances were obtained in August because for that sampling date the refuge was mostly dry, and just two days after the day of sampling day the dikes were opened and the refuge was flooded. Another high abundance peak was obtained in November, specifically due to Chironomidae.

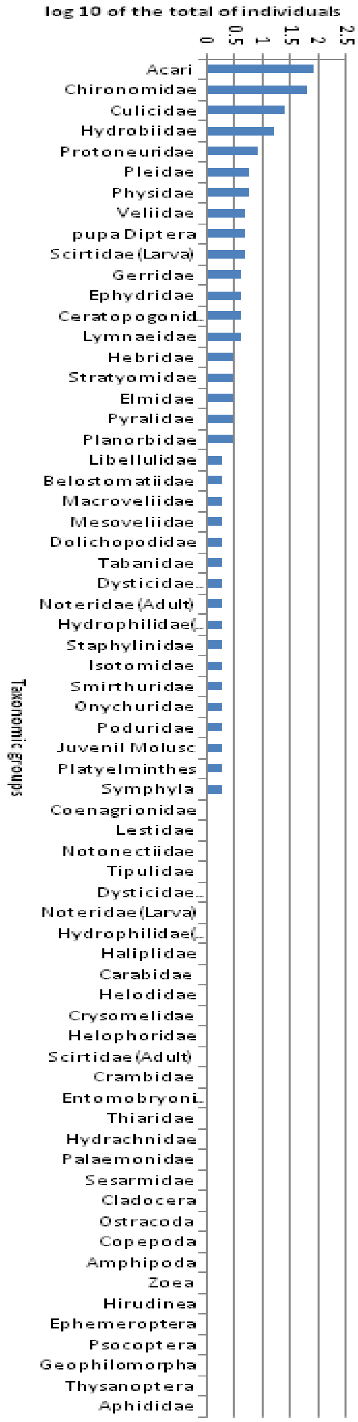

Figure 17: Log_{10} of the accumulative total number of individuals of the taxonomic groups represented in the D-net sweeps (per 200L; n=36). Taxonomic groups were arranged in order of abundance, groups to the right-most half are represented by one individual because Log_{10} of one equals zero. Diptera and Coleoptera individuals are larval stages unless stated (except Carabidae, Chrysomelidae).

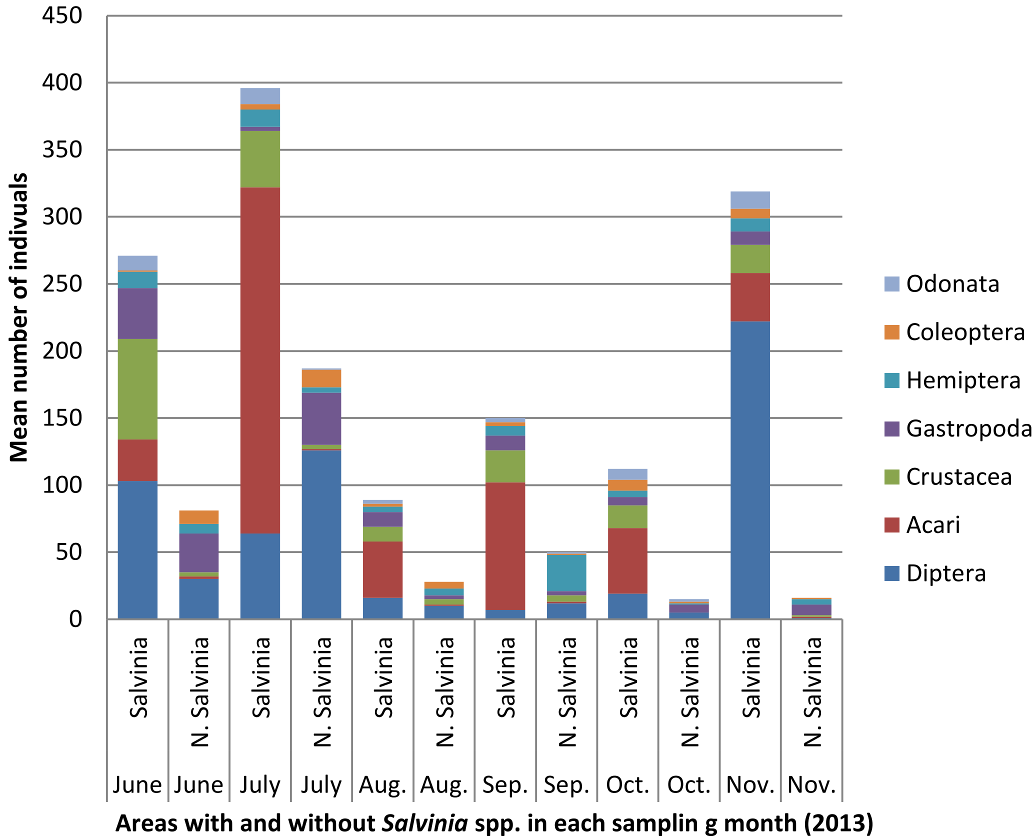

Figure 18: Comparison of the mean number of individuals of macroinvertebrates (dominant groups only) collected with D-net sweeps in the sampling sites with the presence and without *Salvinia* spp. (200L; n=36).

The PCA for the variable "presence of the plant *Salvinia*" for the D-net sweeps demonstrated that the first component explained 56% of the distribution of the taxonomic groups of the areas devoid *Salvinia* spp. were dominated Culicidae and Hydrobiidae. The PC2 (44%) explained the variance of the areas with the presence of the plant that was influenced by the presence of Acari and Chironomidae (Fig. 19). For the sampling months (Fig. 20), the PC1 explained 70% of the temporal distribution of the taxonomic groups, grouping the months of July, August, September and October, and another group by June and November. This phenomenon was mainly due to the presence of high numbers of water mites in July and of chironomids in November.

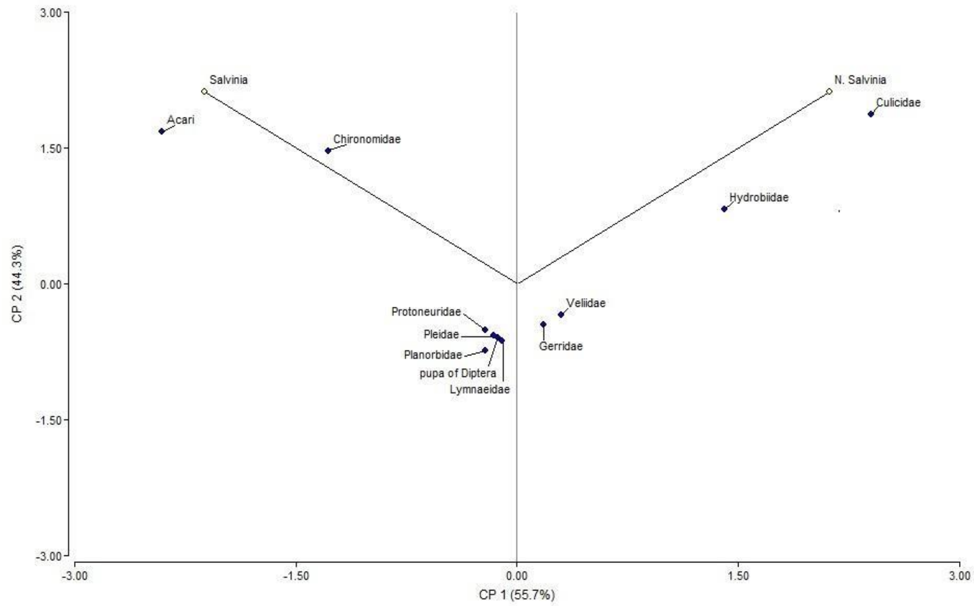

Figure 19: PCA of the most abundant taxa collected with the D-net sweeps in the areas with and devoid of *Salvinia* spp. (CP= Principal component or PC).

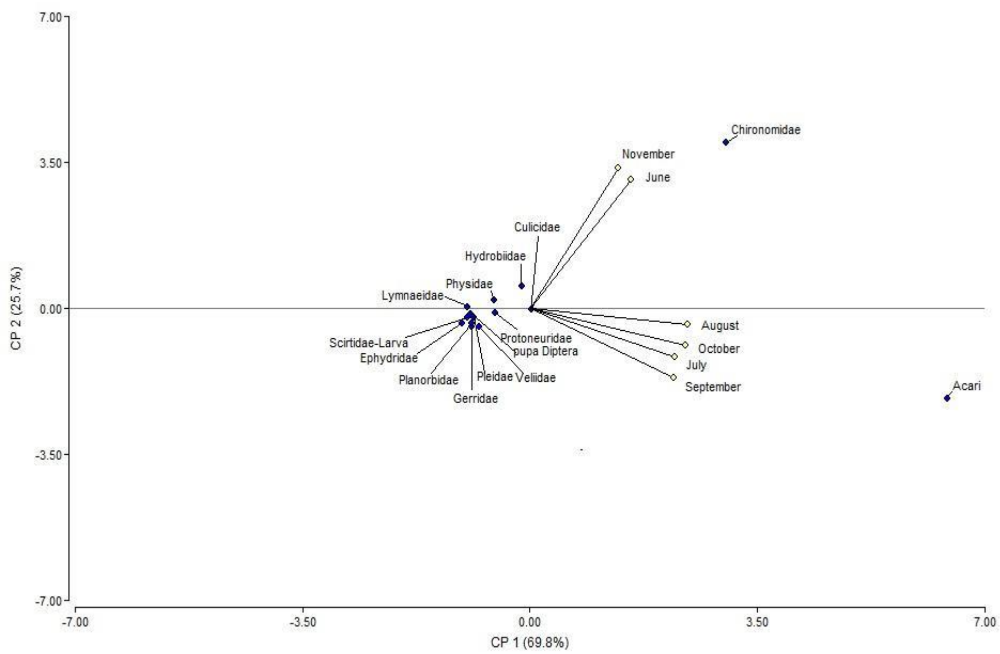

Figure 20: PCA of the most abundant taxa represented in the D-net sweeps during the six sampling months. (CP= Principal component or PC).

Statistical Analyses

For the pH values, no significant differences between areas with *Salvinia* spp. and devoid of *Salvinia* spp., neither between months. Additionally, there were no correlations among pH, salinity and precipitation values.

In a paired T-test of the salinity values for the areas with and devoid of *Salvinia*, there were no significant differences; however, when the unpaired T-test was applied there were significant differences ($p=0.03$).

ANOVA detected significant differences in the total number of macroinvertebrates per taxonomic group among sampling months ($p=0.0111$) and between sampling sites with and without *Salvinia* spp. ($p= 0.0071$). However, there were no significant difference for the interaction of the factors “months” and “sampling sites”.

According to the Tukey test, July was significantly different from the rest of the sampling months based on total number of macroinvertebrates per taxonomic group ($p>0.05$). These significant differences in the month of July are due to a high abundance of Diptera (Ephydridae) in the Malaise traps in areas devoid *Salvinia* spp., and also due to the great abundances of Acari in the D-net sweeps in areas with *Salvinia* spp. Regarding the mean number of macroinvertebrates per sampling site, station 3 (with *Salvinia* spp.) was significantly different ($p>0.05$) from station 2 (without *Salvinia* spp.).

Diversity Indexes

Jaccard's index was used to compare the macroinvertebrate faunas of the areas with and devoid of *Salvinia* spp. Among the three traps, the Jaccard's indexes were quite similar (Table 3). Regardless of the type of trap used, the Jaccard index values were around 0.5, meaning that only half of the species were shared between the two types of habitat (*Salvinia* vs. no *Salvinia*).

The Shannon-Wiener Index (= Diversity Index) calculated for the aquatic light traps had the highest values in the areas with *Salvinia* spp., with the highest peak in diversity in November (H'= 2.20). That represented a marked increase after the low values obtained for the other two "rainy season" months of September (H'= 0) and October (H'= 0.3). In the areas devoid of *Salvinia* the highest peaks in Diversity Index-values were also for the "dry" months, specifically in July (H'= 2.16), and lowest in the rainy months; the sampling area with the lowest values was station 4.

For the Malaise traps, the areas with *Salvinia* exhibited Diversity Index-values usually greater than 1 (one), except for the "rainy" months of October (station 1) and November (station 3). In areas without *Salvinia* spp., diversity values were greater than 1 (as in station 2), or lower or higher than 1 (stations 4 and 5). Dipteran families had high abundance peaks, with greatest abundance for Ephydridae in July (station 4; H'= 0.05; D= 0.98) and for Culicidae in September, (also in the station 4; H'= 0.02; D= 0.99). Diversity indexes calculated with data from the D-net sweeps showed values above 1, regardless of the presence of *Salvinia*, except for the station 4 in November (H'= 0) (Fig.21).

Simpson's Dominance Indexes showed that the aquatic light traps and the Malaise traps were higher in the areas without *Salvinia* spp., especially for the stations 4 and 5. For these two types of traps the highest values of dominance for the areas with *Salvinia* spp. were observed in the rainy months. The highest dominance value determined with the data from the D-net sweeps was obtained in July (station 1; D= 0.68) in the areas with *Salvinia*. Areas without *Salvinia* spp. had similar Dominance-values for the all sampling stations and months, except for station 4 in November (D=1) (Fig.22). The highest diversity values for the D-net sweeps were determined in June in areas with *Salvinia* spp. (station 1, H'= 2.1; station 6, H'= 2.5). For the areas without *Salvinia* spp., the highest diversity values were obtained in July (station 2, H'= 2.5) and August (station 5, H'= 2.6). A similar behavior was seen also in the PCA of the temporal distribution of the taxonomic groups, in which the high number of Acari in the areas with *Salvinia* spp. in the months of July-October was the main explanatory factor (Fig. 20).

Table 3. Jaccard's similarity index calculated comparing the taxa of all the stations with and devoid of *Salvinia* spp. for each sampling method.

Trap	**Jaccard's Index**
Light traps	J= 0.52
Malaise Traps	J= 0.62
D-nets sweeps	J= 0.52

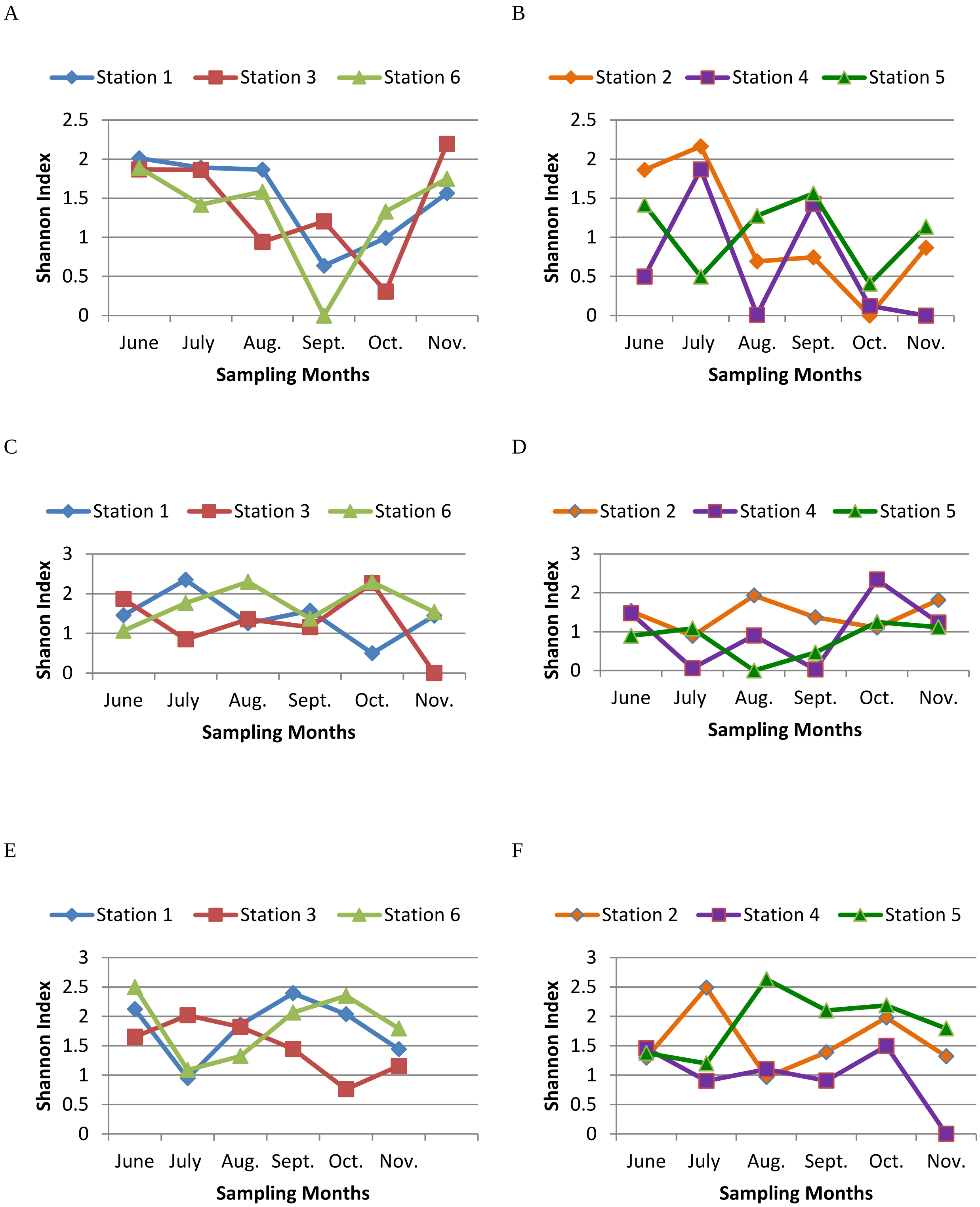

Figure 21: Temporal variations of the Shannon Diversity Index for the stations with and without *Salvinia* spp. for each sampling method in 2013. Stations with *Salvinia* spp.: A. Aquatic light traps; C. Malaise traps; E. D-net sweeps. Stations without *Salvinia* spp.: B. Aquatic light traps; D. Malaise traps; F. D-net sweeps.

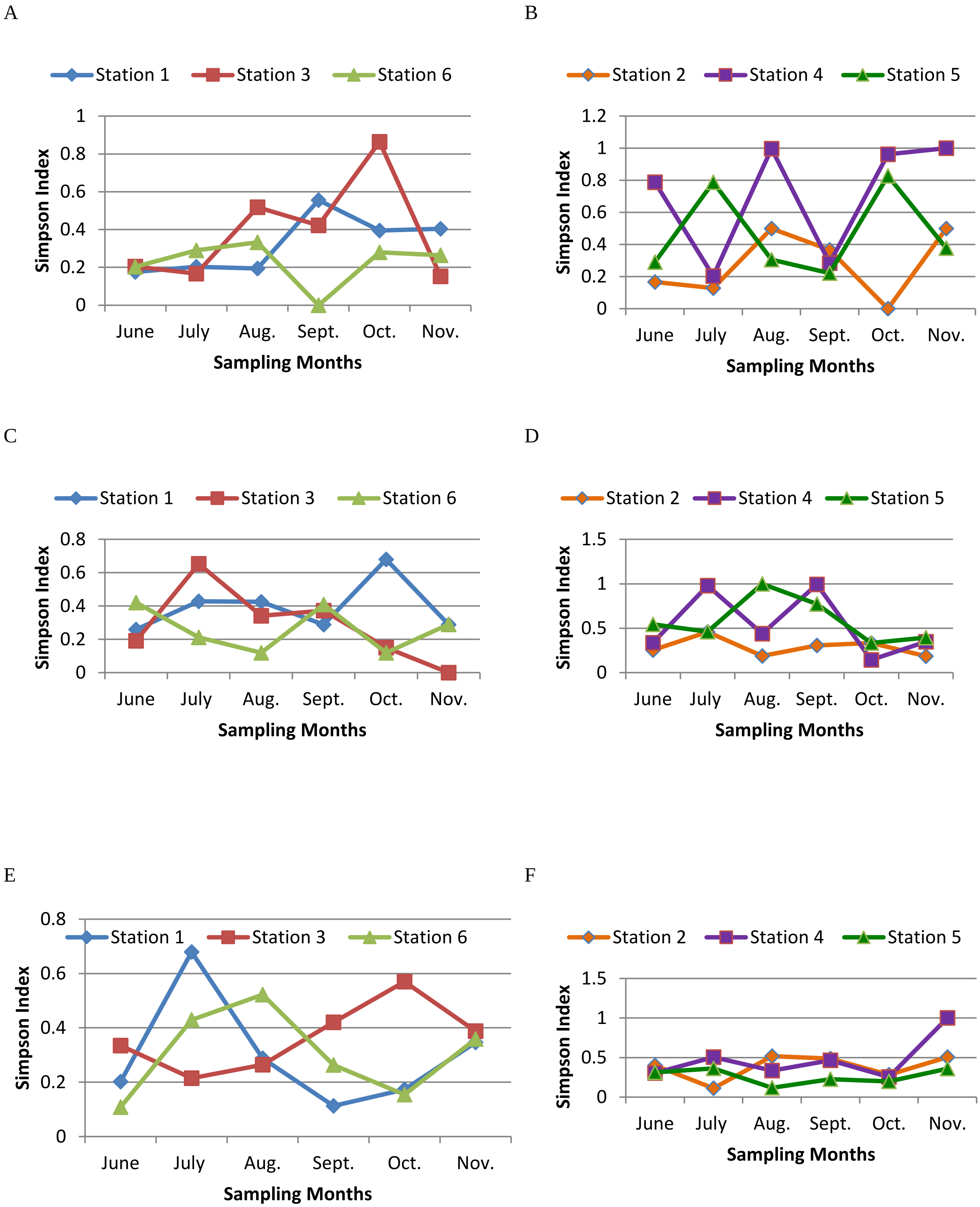

Figure 22: Temporal variations for the Simpson Dominance Index in the stations with and without *Salvinia* spp. for each sampling method in 2013. Stations with *Salvinia* spp.: A. Aquatic light traps; C. Malaise traps; E. D-net sweeps. Stations without *Salvinia* spp.: B. Aquatic light traps; D. Malaise traps; F. D-net sweeps.

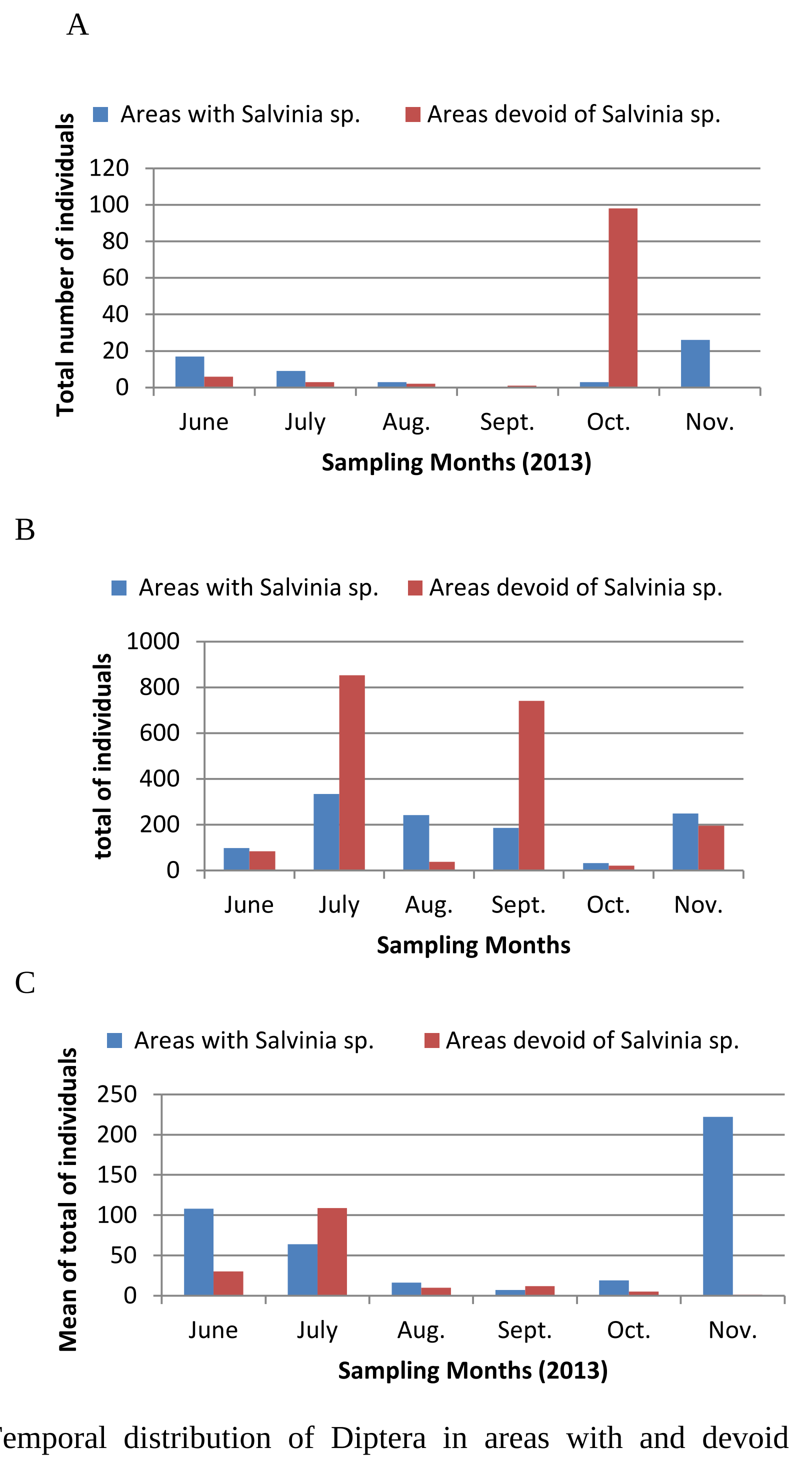

Figure 23: Temporal distribution of Diptera in areas with and devoid of *Salvinia* spp., as determined with the three sampling methods (n=36). A. Total number of Diptera collected per month in the light traps, B. Total number of Diptera per month collected in the Malaise traps, C. Mean of the total number of Diptera collected per month in the two D-net sweeps.

Discussion

The lack of any relationship between the pH, salinity and precipitation in a temporal and spatial scale regarding the abundance of organisms is probably due to the manually controlled water level at the refuge, which is done by the opening of the dikes and permitting the inflow of water from the Cartagena Lagoon or from the refuge's lagoon (Laguna Rincón; salt water). This event was drastically noticed in the months of August and October. Changes in water level directly affect entomofauna by shaping the littoral zone and altering chemical parameters (Ward, 1992).

It is known, that organisms that live in the water column are preferentially attracted to the light and greater numbers of organisms were found with the aquatic light traps in the areas devoid of *Salvinia* spp. This is probably because the areas with dense growth of *Salvinia* are very dark, besides having low dissolved oxygen. September and October were the months with higher values for total number of macroinvertebrates, mainly explained by a bloom of microcrustaceans: cladocerans and copepods. Specifically for the month of October, there was a bloom of zoea, copepods and cladocerans in the areas devoid of *Salvinia* spp., but the Diversity Index values were at their lowest (H'=0 for station 2; H'=0.12 for station 4, and H'= 0.4 for station 5). Indeed, in same month, the Dominance Index achieved its highest value (D= 0.96 for station 4). This has been reported in several studies of coastal areas of the Caribbean, have shown that rainfall produce temporal and spatial variations creating high abundances of species of phytoplankton and zooplankton because of the availability of nutrients and light. (García and López, 1989; Corredor and Morell, 2001).

The order Diptera, with its 21represented families, dominated the fauna captured with the Malaise traps. The most abundant families were Culicidae (greatest abundance in September) and

Ephydridae (greatest abundance in July), both with their greatest abundances in the areas devoid of *Salvinia* spp. Dudley-Williams (2005) established that in habitats with higher salinity chironomids (which were among the dominant groups in stations with *Salvinia*) tend to be replaced by ceratopogonids and ephydrids. The presence of the large number of ephydrids collected in the areas devoid of *Salvinia* is important to stand out because Broche (2006), who studied the prey availability for the migratory birds in the saltern systems of Cabo Rojo, found that these dipterans were one of the three most abundant and possibly important preys. Therefore, as the BWR is also a bird refuge, these dipterans could also be important components of the food of the migratory birds therein. Culicidae, a family traditionally of public-health importance, occurred in areas with and devoid of *Salvinia* and was most abundant in September.

Escher and Lounibos (1993) studied the insects associated to another floating plant, *Pistia stratiotes*. They added emergence traps to make their survey more comprehensive, traps that have successfully used for monitoring the mosquito fauna of Florida macrophytes. This method greatly increased the numbers and taxa of identified insects. They established that many of the insects captured in emergence traps may only be incidentally associated with *P. stratiotes*. A distinctive phenomenon occurred in the emergence samples in our study; the insects collected with the Malaise traps contained unique individuals of dipterans families and odonates (Coenagrionidae) that were not collected with the other sampling methods.

For the D-net sweeps, the most abundant groups were Diptera and Acari in the areas with *Salvinia* spp.. Both groups had the greatest abundance in July. Reagan and Waide (1996) established that usually mites are numerically the dominant type of arthropod in litter. For the Diptera, the most abundant families were Culicidae (areas devoid of *Salvinia* spp.) and Chironomidae (areas with *Salvinia* spp.). This pattern is also shown in the PCA (Fig. 19). Other abundant groups in the areas with *Salvinia* spp. were the nymphs of Protoneuridae and snails (Physidae), which are mainly freshwater organisms. Escobar (2010) characterized the fauna and the water quality of four rivers of Puerto Rico and found Chironomidae and Physidae as the most abundant families in places with poor water quality. Giacometti and Bersosa (2006) studied the macroinvertebrates of rivers in Ecuador as bioindicators of water quality and recorded Chironomidae as the most abundant family in polluted areas. Batzer *et al*. (1999) performed a study of a wetland in Florida using D-net sweeps and their most predominant group was also Chironomidae, followed by the Odonata. Halse *et al*. (2002) collected aquatic invertebrates in wetlands and found that D-net sweeps was the most efficient method for collection a larger number of species.

Collembola was represented by five families collected only in the D-net sweeps (Entomobryidae, Isotomidae, Sminthuridae, Onychiuridae and Poduridae), all present in both areas with *Salvinia* spp. and devoid except for Entomobryidae (only in areas with *Salvinia* spp.) These hexapods were more abundant in the rainy months and, in general, more abundant in the areas devoid of *Salvinia* spp. Collembola are regarded as consumers of decaying plant material and fungal tissue (Reagan and Waide, 1996). Of the crustaceans collected in the D-net sweeps it is interesting to stand out that the Amphipods were collected only in the areas devoid of *Salvinia* spp., with the presence of a rare individual from Corophiidae, a tube building species (Appendix V, Fig. A88) which probably is a new record for Puerto Rico.

Each trap represented specific groups and “snap-shots” of the faunal diversity of the mangrove. As with any faunal survey, trapping methods bias collections in favor of certain taxonomic groups (Escher and Lounibos, 1993). Sweep sampling collected individuals more efficiently than the other two techniques but sampled a different community of organisms. In terms of diversity, the achieved values were not high (H'= 2.5-2.6) if compared to those determined for other types of forest. Deliz (2005) stated in her study of a subtropical marsh (Cartagena Lagoon) that wetlands insect diversity is typically lower than lakes, rivers and streams, possibly because of the stressful environments created in these ecosystems. This study probably is one of the first if not the first to calculate the Diversity, Dominance or Similarity indexes for non-marine invertebrates in mangrove forests.

Some of the aquatic insects families reported herein for the study area where also reported in the nearby Cartagena Lagoon reserve (Deliz, 2005), which supplies water to BWR when the dikes are open. However, Cartagena Lagoon is mostly made of marshy areas, while BWR is dominated by mangrove swamps; thus, differences in their respective aquatic fauna are not be expected. This project seems to be is the first to record of the macroinvertebrates associated to the mangrove forests of Puerto Rico, at least those animals pertaining to the inland, non-marine habitats.

As previously stated, Diptera was the overall most abundant group in the study (Table 4). Indeed, Diptera is the most diverse group at family level in other inland waters of Puerto Rico, followed by Coleoptera and Hemiptera (Gutierrez *et al*., 2013). Reagan and Waide (1996) established that Chironomidae was very abundant component of the dipteran community in the streams and other habitats of the Luquillo Experimental Forest (El Yunque).

In the current study, additional graphs presenting the temporal distribution of the Diptera, as determined with the three sampling methods, were prepared to show that these insects were always present and dominated specific months, being their distribution different for the areas with *Salvinia* spp. and areas without *Salvinia* spp. (Fig. 23). Despite being the most representative insects in many wetlands, this group has been poorly studied in most places, seldom included in the insect surveys. Batzer *et al.* (1999) presented a case of a created marsh in Florida were the abundances of Diptera were much higher in non-vegetated areas than in vegetated ones. Studies like the one by Parys *et al*. (2013), which centered on the insects associated to *Salvinia minima* in Louisiana, did not take Diptera into account. In Puerto Rico there has been a plethora of studies on *Aedes aegypti* and other important dipteran vectors of disease, but other dipteran groups associated to wetlands have been neglected. This study seems to be among the firsts to document the diversity of Diptera (other than Culicidae) associated to mangrove forest.

Even though an extensive study of the macroinvertebrates was intended, only a small portion of the wetlands in the BWR were covered. More studies are required to characterize all other microhabitats. Whether or not areas with lower salinities promote the establishment of a characteristic community, regardless and/or independently of the presence of *Salvinia*, is a topic that needs to be studied further. A manipulative experiment in freshwater areas are freed from *Salvinia* and allowed to repopulate with invertebrates could be conducted to test the hypothesis that the main driver of the invertebrate community composition is the salinity and *Salvinia* just responds like the rest of biota in stations 1,3 and 6, by preferring low salinity patches within BWR.

Aquatic plants, particularly floating plants, can cause hypoxia by reducing light availability and interfering with gas exchange. On the other hand, habitat diversity usually increases with increasing vegetation cover (Batzer *et al*., 1999). The niche theory predicts an increase in species richness with increasing habitat diversity. A predicted greater diversity in areas with *Salvinia* spp. will not necessarily hold true for those animal groups that are faced to live close to the anoxic sediments; however, overall, a greater richness (at family-level) was observed for the stations with *Salvinia*. Mangrove areas with and without *Salvinia* behave as two distinctive habitats; when the taxonomic composition of these sites were compared by trap-type, at the family-level, the similarity indexes (Jaccard-values) were J=0.52 (aquatic light traps), J=0.62 (Malaise traps) and J=0.52 (D-net sweeps); which indicates that they share only around the half of their taxa (families). Being such different at the family-level will certainly translate into a largest dissimilarity at species-level.

As previously mentioned, the majority of the works related to *Salvinia* spp. focus on management options to control their invasion in wetlands. Manual removal seems to only work for early stages of invasion. Studies have compared the use of chemical herbicides and insects as biocontrol (Julien *et al*., 2009). The use of herbicides has been discouraged because of the cage-like arrangement morphology of bristles found in the giant salvinia fronds that forms a water proof barrier to herbicides (Olivier, 1993). In order to breach this barrier with contact herbicides it is necessary to use a wetting agent. In Australia the use of the toxic herbicide paraquat has been successful (Miller and Pickering, 1980 in Oliver, 1993). A study in Zimbabwe found that the physical and chemical control of *Salvinia* was less effective and more expensive than biological control (Julien *et al*., 2009).

In terms of biocontrol, three insects stand out as possibilities: the salvinia weevil (*Cyrtobagus salviniae* Calder & Sands), an aquatic grasshopper (*Paulinia acuminata* De Geer), and a pyralid moth (*Samea multiplicalis* [Guenée, 1854]) (Forno and Bourne 1984; Miller and Wilson, 1989; Olvier, 1993, Julien *et al*., 2009). The one that has been most effective is the salvinia weevil (*Cyrtobagus salviniae*) because it feeds on buds and internal tissue and has been found on all the species of the *Salvinia auriculata* complex in South America (Julien *et al*., 2009). It has been used as biocontrol in India, South Africa, Botswana, Australia, where in some cases the plant coverage has been reduced to 1%.

Evaluating diversity and comparing with other studies associating invertebrates with *Salvinia* species has been difficult due to differences in native fauna between study locations, taxonomic groups considered, and sampling strategies (Parys *et al*., 2013). No investigations had been carried out to document diversity of invertebrates associated with either *Salvinia minima* or *Salvinia molesta* in the United States until Parys *et al.* (2013) conducted a survey in Louisiana, in a cypress-tupelo-blackgum freshwater swamp, where common salvinia colonized the open water. They used one hundred pitfall traps to collect a total of 7,933 insects (excluding dipterans and lepidopterans) and identified 70 families and seven orders. Coleoptera was the most species-rich order, followed by Hymenoptera. This has been the most similar study to the current one at BWR; therefore, the list of species, their counts and the Diversity, Dominance, and Similarity indexes derived from the respective data are compared in tables 5 and 6. The insect orders Blattaria and Orthoptera, taken into account by Parys and collaborators, were omitted from the comparison because they were not included in the present BWR study. Parys *et al*. (op.cit.) reported Coleoptera as the most abundant group. This is probably because they excluded dipterans from their study (Table 7).

Table 5: Comparison of insects associated to *Salvinia* spp. in Louisiana and Puerto Rico. Numbers refer to counts (total number of individuals)

Orden	Family	Parys *et al*., 2013 (2009 +2010)	BWR, Puerto Rico 2013
Coleoptera			
	*Aderidae	1	0
	*Anthicidae	58	0
	*Buprestidae	1	0
	*Cantharidae	1	0
	*Carabidae	1211	0
	*Cerambycidae	3	0
	*Chrysomelidae	13	0
	*Ciidae	1	0
	*Cleridae	1	0

	*Coccineliidae	6	0
	*Corylophidae	6	0
	Curculionidae	267	4
	Dysticidae	19	60
	Elateridae	2	0
	Elmidae	0	67
	*Endomychidae	1	0
	*Erotylidae	3	0
	*Eucinetidae	4	0
	*Eucnemidae	2	0
	Haliplidae	1	1
	Helodidae	0	1
	Helophoridae	0	1
	*Heteroceridae	3	0
	*Histeridae	1	0
	Hydraenidae	4	0
	Hydrophilidae	926	19
	*Laemophloeidae	1	0
	*Latridiidae	44	0
	Limnichidae	33	0
	*Melandryidae	4	0
	*Mordellidae	7	0
	*Nitidulidae	5	0
	Noteridae	19	31
	Phalacridae	1	0
	Ptiliidae	4	0
	Ptilodactylidae	1	0
	Ptinidae	4	0
	*Scarabaeidae	73	0
	Scirtidae	1244	47
	*Sphindidae	1	0
	Staphylinidae	376	5
	*Tenebrionidae	5	0
	*Tetratomidae	1	0
	*Throscidae	1	0
	*Zopheridae	1	0
Hemiptera			
	*Achilidae	0	71
	*Aphididae	0	5
	Anthocoridae	1	0
	Belostomatidae	78	20
	*Cercopidae	1	0
	*Cicadellidae	76	5

	*Delphacidae	8	0
	Gelastocoridae	1	0
	Gerridae	23	2
	Hebridae	159	65
	Hydrometridae	557	0
	Macroveliidae	0	4
	Mesoveliidae	27	18
	Naucoridae	46	0
	Nepidae	12	0
	Pleidae	0	158
	Saldidae	34	0
	Veliidae	18	36
Hymenoptera	*Braconidae	110	0
	*Chalcidoidea	417	1
	*Formicidae	862	0
	*Icheumonidae	937	0
	*Platygastridae	1	0
	*Pompilidae	26	1
	Sphecidae	1	0
	*Vespidae	1	0
	*Eupelmidae	0	1
	*Pteromalidae	0	1
Odonata	Coenagrionidae	7	46
	Lestidae	0	1
	Libellulidae	3	31
	Protoneuridae	0	264
Psocoptera		1	2
Ephemeroptera	Caenidae	0	1
	Total	7767	967

(*) – Insect families that are not traditionally considered as aquatic or semi-aquatic.

Table 6: Comparison of the Diversity, Dominance, and Similarity indexes determined for the insects associated to *Salvinia* spp. in Louisiana and Puerto Rico

Index	Parys *et al*., 2013	Present work
Shannon	2.55	1.79
Simpson	0.099	0.047
Jaccard	0.22	

The indexes values were very different because the comparison was restricted to the groups Parys *et al*. (2013) reported. Diptera, the most abundant and diverse group in the BWR study, was eliminated from the comparison. Therefore, the species-lists can only be compared to a limited extent.

Forno and Bourne (1984) collected the arthropods associated to the *Salvinia auriculata* complex to study the insects that could potentially prey on *Salvinia molesta* in Trinidad, Venezuela, Guyana, Brazil, Uruguay, Paraguay, and Argentina. They found that only three species, *Cyrtobagou*s sp., *Samea multiplicalis* and *Paulina acuminata*, regularly visited and fed on all species in the *Salvinia auriculata* complex. However, they focused solely on herbivorous insects, eliminating some of the larger taxonomic groups. Samples of *Salvinia* spp. (500 cm^2) were collected with a bottomless bucket and, then, submerged in 20 cm of water for not less than 12 hours, so that the adults and immature stages could move to the surface. No counts were made by Forno and Bourne (op. cit.) of any larvae inside rhizomes nor of the pupae or other stages that did not come to the surface. This is a major difference from the present BWR study since a detailed examination was conducted of the rhizomes and associated sediments for *Salvinia* spp. (Table 7).

Table 7. Comparison of previous works and present study on insects in *Salvinia*.

Study	Forno and Bourne (1984)	Parys *et al*. (2013)	Present study
Where?	Trinidad, Venezuela, Guyana, Brazil, Uruguay, Paraguay, and Argentina *(Salvinia auriculata* complex in open water)	Lousiana (Cypress-tupelo-blackgum freshwater swamp)	Boquerón, PR (Mangrove forest)

Sampling gear	Bottomless bucket Sample was submerged in 20 cm of water for not less than 12 hours	Pitfall traps	Aquatic Light traps, Malaise traps, D-net sweeps
Sampling Process	Only detached organisms were counted	Excluded dipterans and lepidopterans	Whole samples were screened and all the organisms sorted and identified
Main Results	*Cyrtobagou*s sp., *Samea multiplicalis* and *Paulina acuminata*, regularly visited and fed on all species in the complex	7 orders /70 families Coleoptera most abundant	20 orders/ 81 families Diptera most abundant

Another study of the insects associated to an aquatic macrophyte was made by Escher and Lounibos (1993). They used emergence traps and plant quadrant samples to survey insects associated with monocultures of *Pistia stratiotes* in Florida. This work focused on the study on the population of *Mansonia* spp. (mosquitoes) whose larvae and pupae attach to the roots of the floating macrophyte. From 12-14 months of biweekly or monthly samplings, they collected a total of 47,251 specimens representing 13 orders and 90 families. Of the 20,221 individuals from emergence traps, 96.5% were Diptera, of which 87.1% belonged to the Chironomidae. Plant quadrats yielded the rest of the specimens of which 55.0% were aquatic dipterans, 22.3% odonates, 13.3% hemipterans, and 8.7% coleopterans. Mosquito larvae of the genus *Mansonia* accounted for 86.9% of the aquatic Diptera. Mean numbers of individuals were highest in the fall, the largest numbers of individuals were collected in October and November. As observed in the present BWR study, diversity at the family level was greater in the emergence traps. The taxa shared by both studies were: Diptera (Agromyzidae, Ceratopogonidae, Chaoboridae, Chironomidae, Culicidae, Dolichopodidae, Ephydridae, Psychodidae, Stratyomidae, Syrphidae, Tachinidae, Tipulidae), Hymenoptera (Chalcididae), Coleoptera (Carabidae, Chrysomelidae, Curculionidae, Dysticidae, Helodidae, Noteridae, Staphylinidae), Hemiptera (Hebridae, Pleidae, Mesoveliidae, Aphididae, Cicadellidae), Lepidoptera (Pyralidae), Collembola (Isotomidae, Entomobryidae, Sminthuridae), Ephemeroptera (Caenidae), Odonata (Libellulidae, Coenagrionidae), and Psocoptera.

Gutiérrez *et al*. (2013) published a list of aquatic insects of Puerto Rico. The most diverse groups of the list that they report at family level are Diptera, Coleoptera and Hemiptera, the same hierarchy found for the stations in BWR. They established that Puerto Rico has a high diversity of aquatic insects with some well-known groups (Ephemeroptera, Odonata and Trichoptera). Nevertheless, other groups require further research to understand their status or to complete inventories (Coleoptera, Hemiptera, and Diptera). At BWR no Trichoptera were found; these are typically inhabitants of streams and wet forest leaf-litter.

From the present study at BWR, some notable additions to the list of aquatic and semi-aquatic insect fauna of Puerto Rico are the following: Hemiptera (Macroveliidae,), Lepidoptera (Pyralidae), Coleoptera (Curculionidae, Helodidae, Helophoridae, Chrysomelidae); Diptera (Ephydridae, Anthomyiidae, Tachinidae, Trixoscelidae, Lauxaniidae, Mycetophilidae, Rhagionidae). Most of these taxa were known from non-aquatic habitats on the island.

Conclusions

- The study of the invertebrate fauna at the Boquerón Wildlife Refuge showed a highly diverse ecosystem, where many taxonomic groups are represented, and temporal and spatial scales are important in their appearance and distribution. The invertebrate community was alloted in a total of 20 orders and 81 families. A very diverse community was composed mainly of aquatic insects, crustaceans, and gastropods. This diversity has an important role in the feeding of fish and birds and in the ecosystem food web as a whole.
- Important processes were observed during the time of the study. The temporal and spatial variations influenced the abundance and the presence of these groups. The differences found in time and space point toward changes in the properties of the water column, favoring certain groups during the rainy months and others in response to light.
- Lack of relationship between the pH, salinity and precipitation is probably due to hydrologic changes in the mangrove channels.
- Mangrove areas with and without *Salvinia* behave as two distinctive habitats; the values for the Jaccard Similarity Index indicated that they share only around the half of their taxa (families). Being such different at the family-level will certainly translate into a largest dissimilarity at species-level.
- Dipterans are important inhabitants of wetlands and of this mangrove forest. Its family-level diversity (21 families) was the largest of all the groups.
- Difference among sampling methods showed that each trap rendered a distinctive fauna. Each trap is more effective for the collection of certain groups, and these differences are important when studies are focused in characterizing the whole community.

Recommendations

- There is little literature on the abundance and distribution of macroinvertebrates in wetlands of Puerto Rico. Therefore, there it is necessary to create biomonitoring programs and develop specific management ground plans for unique wetlands as mangrove forests.
- Future studies should examine the effects of other abiotic factors, for example nutrients, and examine the effect of hydrologic alterations in the refuge.
- More studies of the relationship between salinity and the presence of *Salvinia* spp. and the macroinvertebrates are needed. Differences in salinity at larger spatial and temporal scales may play important roles.
- For future studies of wetlands biodiversity, the inclusion of Diptera is imperative because it will provide a better understanding of the real community of a wetland.
- Promote more studies on the insect fauna in other mangrove forests of Puerto Rico and the Caribbean, and thus compare communities.
- Even though the objectives of this study were accomplished, a more extensive experimental design is needed to study the diverse microhabitats that BWR holds in these wetlands.

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Appendixes

Appendix 1: Physical-chemical parameters

Table A: pH and salinity values for the areas with *Salvinia* spp. in each sampling month. Numbers one to six represent the months from June to November.

	***Salvinia* spp.**																	
Stations	**Stat. 1**						**Stat. 3**						**Stat. 6**					
Months	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**
pH	6.3	7.1	8.1	7.1	7.4	6.9	0.6.3	7.2	8	7.2	6.8	7	7.1	7.5	7.3	7.5	7.1	7.2
salinity	0	0	0.4	0	0.4	0	0	0	0.9	50	0.3	0	0	0.1	0	0	0.1	0.1

Table B: pH and salinity values for the areas devoid *Salvinia* spp. in each sampling month. Numbers one to six represent the months from June to November.

	No *Salvinia* spp.																	
Stations	**Stat. 2**						**Stat. 4**						**Stat. 5**					
Months	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**
pH	3.8	7.1	7.4	7.3	7.4	7.2	6.6	7.1	7.6	7.5	7.1	7.2	6.6	6.9	7.1	7.4	7	6.9
salinity	0	0.1	0	5	1	5	0.2	1.5	0	0	0.1	0	0.4	1.2	0.3	0	0	0

Appendix II: Diversity summary of each sampling months and stations grouped in the areas with *Salvinia* spp. and devoid *Salvinia* spp. classified by sampling method: Aquatic light trap (L), Malaise trap (M), and D-net sweeps (D).

	Salvinia spp.																		**No *Salvinia spp.***																	
Stations	Stat. 1						Stat. 3						Stat. 6						Stat. 2						Stat. 4						Stat. 5					
Months	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Order Odonata																																				
Protoneuridae	D	L D	L D	L D	D	L D	LD	L D	D	D	L D	D	L D	D	D	D		D		D			D												D	
Coenagrionidae	L	M	M	M				L M D					D	M D				M		M	M	M							M	M		M	M	M	M	M
Lestidae																		D																		
Libellulidae	D	D			L D	L	LD	L D			D	L D		D	D		L D	D				L	D						L				L	D	L D	
Order Hemiptera																																				
Pleidae	D	L D	D	L D	D	D		D	L D	D	D	L D	D	L D	D	D	D	L D	D		L D	D	D	D			L	L						D		
Belostomatidae					D	L D	D		L	L		L D			D							L		D												
Veliidae	L			D	D		D	D			D	D	D		L	D		L D				D						L D	L		L D		D	L D	L	
Macroveliidae		D					D		D																			D					D			
Mesoveliidae		L D	D				D	D					D			D	L D			D		D														
Hebridae	D	D	D		D	D	D	D	D	D	D	D				D		D		D																
Notonectidae																			L	D	D		D	L D										D	L	
Gerridae				D												D				D		D		D				D			D		D	D		
Aphididae			L						D			D																								
Achilidae		M	M	M	M	M	M	M	M	M				M	M		M	M		M	M			M	M		M		M						M	M
Cicadellidae	M																	M																		

Appendix II: (Cont.)

Order Diptera																																				
Chironomidae	D	D	LD	D	LD	MD	LMD	LD	D		MD	LD	LD	MD	D	MD	LD	LMD	L	D	D	D	D	MD				LD	LD						LD	
Culicidae	LMD	LM	M	M	D	M	LMD	LMD	M	M	M	D	LMD	MD	LM	MD	MD	LMD	LM		LMD	M	MD	M	MD	MD		M		M	LMD	LMD	LD	M	LMD	M
Dolichopodidae	M	M		M		M	M	M	M	MD			M		M		M	M	M					M	MD		M	M		M	M	M		M		M
Ephydridae	LM	MD	M	MD	D	L	MD	MD	M	D	MD	D	MD	MD	MD	MD	MD	MD	D		M	MD		MD	D	MD	M		M		M		D		M	M
Stratiomyidae	LD	MD		D	D		D	D	D	D	MD	D	LD			D	D	D							D											
Ceratopogonidae		MD		MD	D	D	D	D	D		D	LD	D	D	M	MD	M	MD	D	D			D	M	D											
Tabanidae	D			D		LD	D	M	D	D	D	D	D	D	D		D	D	M	M					M	M										
Tipulidae	M		M			M		M	MD					D																						M
Simuliidae																M	M												M							M
Anthomyiidae											M														M											
Tachinidae		M	M				M		M		M			M	M		M		M		M	M	M		M	M								M		
Chaoboridae																	M																			
Rhagionidae			M										M	M																						
Muscidae								M			M								M						M											
Trixoscelidae																															M					
Lauxaniidae														M							M				M											
Mycetophilidae						M		M													M															
Psychodidae						M									M			M						M												
Agromyzidae		M						M															M													
Sciomyzidae		M							M				M																							
Syrphidae																			M										M							
Pupa	D	D	D	D	D	LD	D	D	D		D	D	D	D	D	D	D	LD	D	D		D	D		LD							L	D			

Appendix II: (Cont.)

Undetermined (larva)													D			D																				
Undertermined (Adult)			M						M							M																				M
Order Coleoptera																																				
Dysticidae (larva)			L D		L D	L		D	L	L D	D	L D		L	D	D	D	L D		D	D		D	D												
Dysticidae (adult)					L	L		D			D				L		D																			
Noteridae (larva)						D		D									D																			
Noteridae (adult)		L	L D	D		L D	L	L		L D	D					D		L D										L D						L	L	
Hydrophilidae (larva)						D						D																					D			
Hydrophilidae (adult)					D							D																								
Elmidae		D	L		D	L D				D		D	D		L	D	D	L D		D	L D	D											D			
Curculionidae				M				M							M		M				M															
Haliplidae (larva)																																	D			
Carabidae																					D															
Helodidae																																	D			
Chrysomelidae																														D			D			
Staphylinidae											D																									
Helophoridae (larva)										D																										
Scirtidae (adult)		M	M	M	M	M	M	M			M			M	M	M	M	M				M			M			M	M		M					M
Scirtidae (larva)	D				D					D		L		D											D	D					D	D	D			L
Undetermined (larva)			D	D																										D			D			
Order Lepidoptera																																				
Pyralidae	D	M D	D		D	M D	D M	D	D	D	M	D	D	M D	D M	M	M			D						M D			M	M				M		

Appendix II: (Cont.)

Order Hymenoptera																																				
Pompilidae										M																										
Eupelmidae							M																													
Pteromalidae																												M								
Chalcididae		M																																		
Undetermined							M																													
Order Ephemeroptera																																				
Caenidae								D																												
Subclass Collembola																																				
Isotomidae			D	D						D																							D			
Sminthuridae								D																									D			
Onychiuridae																																	D			
Entomobryidae												D				D																				
Poduridae					D								D	D	D			D										D					D			
Class Gastropoda																																				
Lymnaeidae					D	D							D		D												D	D					D			
Hydrobiidae	L	L D				L						L	L D	L	L D			D		D			D	L D	L D	D		D	D	L D	L D	L D	L		D	L D
Physidae	D		D	D	D	L D			L	L D	D	L D	L D	D	L D			L D		L D		D			D	D			L							
Planorbidae	D		L D	D	D	L D	D		L D	D	D	D	D	L D	L D		D																			
Thiaridae																												D	D							
Juvenile mollusk							D	D		D																										
Subclass Acari	L D	L D	L D	D	D	L D	LD	L D	L D	L D	L D	D	L D	D	L D	D	D	L D	D			L D						L D	L	D	L D	D	D	L		L
Hydrachnidae								D	D						L	D		L																		

Appendix II: (Cont.)

Subphylum Crustacea																																				
Order Decapoda																																				
Paleamonidae																								L							L	LD			
Sesarmidae																										D									
Juvenile shrimp																										D	D								
Ocypodidae																		L																	
Zoea																		L		LD				L		L		L			D	D			
Order Amphipoda																									D										
Corophiidae																																D			
Order Isopodo																																D			
Class Ostracoda	LD	L	LD	D	LD	LD	LD	LD	LD	LD	LD	LD	LD	D					L			D		LD	D								L	L	
Suborder Cladocera	LD	D		D			LD	LD	D		D	LD	LD		D	D	D	LD	D	D		LD							L				L	L	
Subclass Copepoda	LD	LD	D	D	D	D	LD	LD	D	D	LD	LD	LD	D	D	D	D	L	LD			LD			L			D				L		L	
Others																																				
Phylum Platyhelminthes				D		D	D	D	D		D	D						D																		
Subclass Hirudinea				D					D																											
OrderPsocoptera																																D			
Order Geophilomorpha																																D			
Class Symphyla																																D			

Appendix III: Macroinvertebrates in areas with and devoid *Salvinia* spp. for each sampling method.

Light Traps

	Salvinia spp.	No *Salvinia* spp.
Protoneuridae	+	-
Coenagrionidae	+	-
Libellulidae	+	+
Pleidae	+	+
Belostomatiidae	+	+
Veliidae	+	+
Mesoveliidae	+	-
Notonectiidae	-	+
Chironomidae	+	+
Culicidae	+	+
Ephydridae	+	+
Stratiomyidae	+	+
Ceratopogonidae	+	+
Tabanidae	+	+
pupa of Diptera	+	+
Dysticidae-larvae	+	-
Dysticidae-Adult	+	-
Noteridae-Adult	+	+
Hydrophilidae-A	+	-
Elmidae	+	+
Scirtidae-Larvae	+	+
Hydrobiidae	+	+
Physidae	+	+
Planorbidae	+	-
Acari	+	+
Hydrachnidae	+	+
Palaemonidae	-	+
Ocypodidae	-	+
Cladocera	+	+
Ostracoda	+	+
Copepoda	+	+
Amphipoda	-	+
Zoea	-	+

Appendix III: (Cont.)

Malaise Traps

	Salvinia spp.	No *Salvinia* spp.
Coenagrionidae	+	+
Cicadellidae	+	-
Achillidae	+	+
Chironomidae	+	+
Culicidae	+	+
Dolichopodidae	+	+
Ephydridae	+	+
Stratyomidae	+	-
Ceratopogonidae	+	+
Tabanidae	+	+
Tipulidae	+	+
Simulidae	+	+
Mytocephalidae	+	+
Anthomyiidae	+	+
Tachinidae	+	+
Rhagionidae	+	-
Muscidae	+	+
Trixoscelidae	-	+
Lauxaniidae	+	+
Psychodidae	+	+
Sciomyzidae	+	-
Agromyzidae	+	+
Syrphidae	-	+
Curculionidae	+	+
Scirtidae	+	+
Pyralidae	+	+
Pompiilidae	+	-
Eupelmidae	+	-
Pteromalidae	-	+
Chalcididae	+	-

Appendix III: (Cont.)

D-net sweeps

	Salvinia spp.	No *Salvinia* spp.
Protoneuridae	+	+
Coenagrionidae	+	-
Lestidae	+	-
Libellulidae	+	+
Pleidae	+	+
Belostomatiidae	+	+
Veliidae	+	+
Macroveliidae	+	+
Mesoveliidae	+	+
Hebridae	+	+
Notonectiidae	-	+
Gerridae	+	+
Aphididae	+	-
Chironomidae	+	+
Culicidae	+	+
Dolichopodidae	+	+
Ephydridae	+	+
Stratyomidae	+	+
Ceratopogonidae	+	+
Tabanidae	+	-
Tipulidae	+	-
pupa of Diptera	+	+
Dysticidae- larvae	+	+
Dysticidae- Adult	+	-
Noteridae- Larvae	+	-
Noteridae-Adult	+	+
Hydrophilidae-Larvae	+	+
Hydrophilidae-Adult	+	-
Elmidae	+	+
Haliplidae	-	+
Carabidae	-	+
Helodidae	-	+
Staphylinidae	+	+
Chrysomelidae	-	+
Helophoridae	+	-
Scirtidae- Adult	-	+
Scirtidae-Larvae	+	+
Pyralidae	+	+
Isotomidae	+	+
Smirthuridae	+	+
Onychuridae	-	+
Entomobryonidae	+	-
Poduridae	+	+
Lymnaeidae	+	+
Hydrobiidae	+	+
Physidae	+	+
Planorbidae	+	-
Thiaridae	-	+
Juvenil Molusk	+	-

Appendix III: (Cont.)

D-net sweeps

Acari	+	+
Hydrachnidae	+	-
Palaemonidae	-	+
Sesarmidae	-	+
Cladocera	+	+
Ostracoda	+	+
Copepoda	+	+
Amphipoda	-	+
Zoea	-	+
Platyelminthes	+	-
Hirudinea	+	-
Ephemeroptera	+	-
Psocoptera	-	+
Geophilomorpha	-	+
Symphyla	+	+
Thysanoptera	-	+

Appendix IV: PCA Statistical Analysis values

A. Light Traps: Relationship of the 15 most abundant taxonomic groups in areas with *Salvinia* spp. and areas devoid *Salvinia* spp.

Nueva tabla: 7/26/2015 - 6:56:02 PM

Análisis de componentes principales

Datos estandarizados
Casos leidos 15
Casos omitidos 0

Variables de clasificación

Taxa

Autovalores

Lambda	Valor	Proporción	Prop Acum
1	1.10	0.55	0.55
2	0.90	0.45	1.00

Autovectores

Variables	e1	e2
Salvinia	0.71	-0.71
N. Salvinia	0.71	0.71

B. Light Traps: Relationship of the 15 most abundant taxonomic groups with the six sampling months.

Nueva tabla_2: 7/26/2015 - 9:01:11 PM

Análisis de componentes principales

Datos estandarizados
Casos leidos 15
Casos omitidos 0

Variables de clasificación

Taxa

Autovalores

Lambda	Valor	Proporción	Prop Acum
1	2.94	0.49	0.49
2	1.66	0.28	0.77
3	1.01	0.17	0.94
4	0.21	0.04	0.97
5	0.11	0.02	0.99
6	0.06	0.01	1.00

Autovectores

Variables	e1	e2
June	0.37	0.51
July	0.19	0.65
August	0.48	-0.33
September	0.43	-0.43
October	0.36	0.14
November	0.53	-0.04

Appendix IV: PCA Statistical Analysis values (cont.)

C. Malaise Traps: Relationship of the 15 most abundant taxonomic groups in areas with *Salvinia* spp. and areas devoid *Salvinia* spp.

Análisis de componentes principales

Datos estandarizados
Casos leidos 15
Casos omitidos 0

Variables de clasificación

Taxa

Autovalores

Lambda	Valor	Proporción	Prop Acum
1	1.84	0.92	0.92
2	0.16	0.08	1.00

Autovectores

Variables	e1	e2
Salvinia	0.71	0.71
N. Salvinia	0.71	-0.71

D. Malaise Traps: Relationship of the 15 most abundant taxonomic groups with the six sampling months.

Análisis de componentes principales

Datos estandarizados
Casos leidos 15
Casos omitidos 0

Variables de clasificación

Taxa

Autovalores

Lambda	Valor	Proporción	Prop Acum
1	3.42	0.57	0.57
2	1.50	0.25	0.82
3	0.59	0.10	0.92
4	0.36	0.06	0.98
5	0.08	0.01	0.99
6	0.05	0.01	1.00

Autovectores

Variables	e1	e2
June	0.45	-0.10
July	0.39	0.49
August	0.35	0.53
September	0.41	-0.49
October	0.45	0.11
November	0.40	-0.47

Appendix IV: PCA Statistical Analysis values (cont.)

E. D-net sweeps: Relationship of the 15 most abundant taxonomic groups in areas with *Salvinia* spp. and areas devoid *Salvinia* spp.

Nueva tabla: 7/26/2015 - 11:33:43 PM

Análisis de componentes principales

Datos estandarizados
Casos leidos 15
Casos omitidos 0

Variables de clasificación

Taxa

Autovalores

Lambda	Valor	Proporción	Prop Acum
1	1.11	0.56	0.56
2	0.89	0.44	1.00

Autovectores

Variables	e1	e2
Salvinia	-0.71	0.71
N. Salvinia	0.71	0.71

F. D-net sweeps: Relationship of the 15 most abundant taxonomic groups with the six sampling months.

Nueva tabla: 7/26/2015 - 11:45:13 PM

Análisis de componentes principales

Datos estandarizados
Casos leidos 15
Casos omitidos 0

Variables de clasificación

Taxa

Autovalores

Lambda	Valor	Proporción	Prop Acum
1	4.19	0.70	0.70
2	1.54	0.26	0.95
3	0.20	0.03	0.99
4	0.03	0.01	0.99
5	0.03	4.5E-03	1.00
6	0.01	2.1E-03	1.00

Autovectores

Variables	e1	e2
June	0.31	0.61
July	0.44	-0.23
August	0.48	-0.08
September	0.44	-0.33
October	0.47	-0.17
November	0.27	0.66

Appendix V: Pictorial Catalogue of Macroinvertebrates at Boquerón Wildlife Refuge (BWR)

Order Basommatophora

Figure A1: Hydrobiidae
Photo by Nahíra E. Arocho-Hernández
Station: 4B-June

Figure A2: Physidae
Photo by Nahíra E. Arocho-Hernández
Station: 1B-August

Figure A3: Planorbidae
Photo by Nahíra E. Arocho-Hernández
Station: 3A-June

Order Coleoptera

a. 

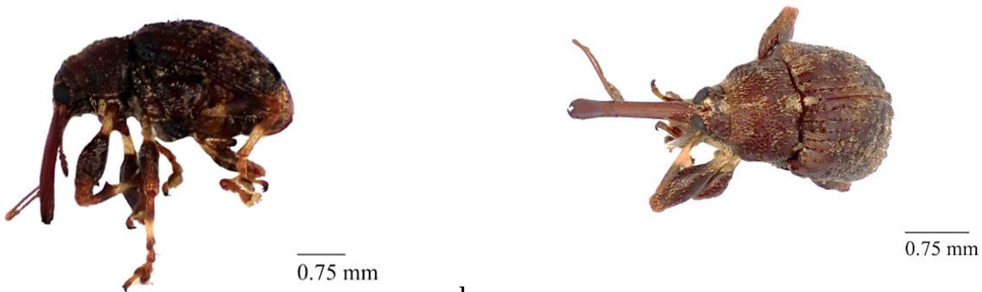
b. 

Figure A4: Carabidae. a. Dorsal View, b. Lateral View
Photo by Nahíra E. Arocho-Hernández
Station: 5 Light-October

a.  b. 

Figure A5: Chrysomelidae. a. Ventral View, b. Lateral View
Photo by Nahíra E. Arocho-Hernández
Station: 6A-November

Figure A6: Curculionidae.
Photo by Arlene Megill-Irizarry
Station: 3-July



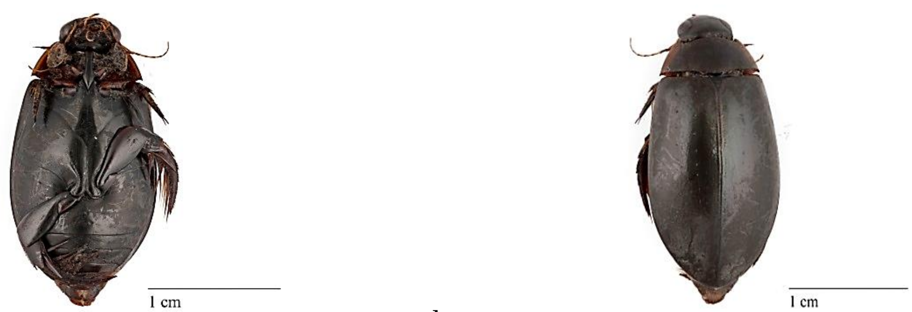
a. b.

Figure A7: Curculionidae. a. Lateral View, b. Dorsal View
Photo by Jeniffer Vega
Station: 2 August

Figure A8: Dysticidae.
Photo by Nahíra E. Arocho-Hernández
Station: 3 Light-November

Figure A9: Dysticidae.
Photo by Nahíra E. Arocho-Hernández
Station: 3A-July

Figure A10: Dysticidae.
Photo taken Nahíra E. Arocho-Hernández
Station: 1 Light-November



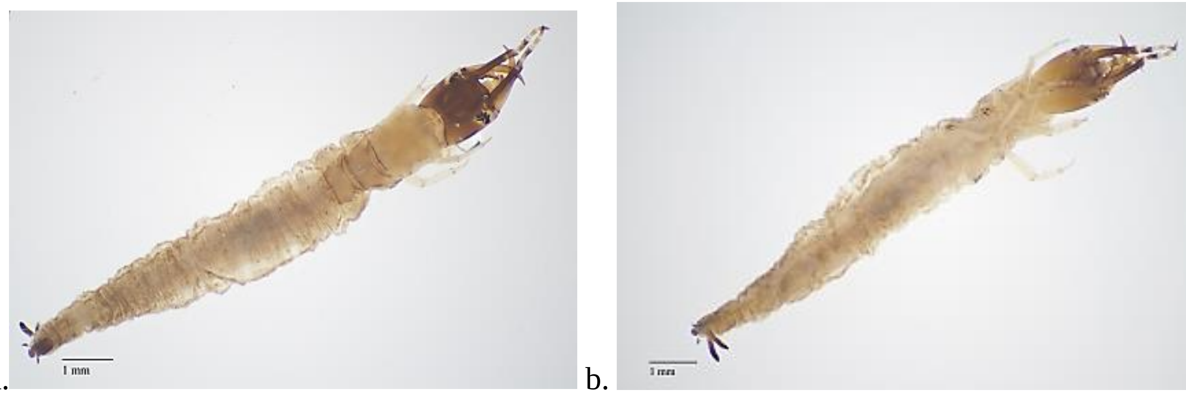
a. b.

Figure A11: Dysticidae. a. Ventral view, b. Dorsal view
Photo by Nahíra E. Arocho-Hernández
Station: 1 Light-October

a.  b. 

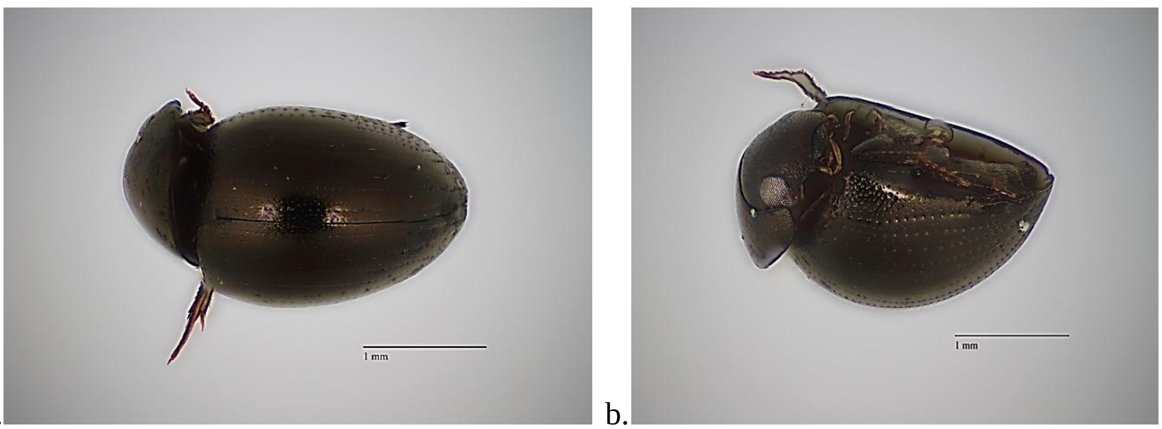
Figure A12: Elmidae. a. Dorsal View, b. Ventral View
Photo by Nahíra E. Arocho-Hernández
Station: 1B-August

Figure A13: Halipidae.
Photo by Nahíra E. Arocho-Hernández
Station: 5B-August



a. b.

Figure A14: Helophoridae. a. Dorsal View, b. Ventral View
Photo by Nahíra E. Arocho-Hernández
Station: 1A-August



a. b.

Figure A15: Hydrophilidae; Genus: *Derallu.s* a. Dorsal View, b. Lateral View
Photo by Nahíra E. Arocho-Hernández
Station: 3B-November

a. 

b. 

Figure A16: Hydrophilidae. a. Dorsal view, b. Ventral view
Photo by Nahíra E. Arocho-Hernández
Station: 3A-November

a. 

b. 

Figure A17: a. Noteridae. a. Dorsal View, b. Ventral View
Photo by Nahíra E. Arocho-Hernández
Station: 3B-July

a.


b.


Figure A18: Noteridae. a. Ventral View, b. Lateral View
Photo by Nahíra E. Arocho-Hernández
Station: 1 Light-August

a.


b.


Figure A19: Noteridae. a. Dorsal View, b. Lateral View
Photo by Nahíra E. Arocho-Hernández
Station: 5 Light-October

Figure A20: Scirtidae (larva).
Photo by Nahíra E. Arocho-Hernández
Station: 5B-June

Figure A21: Staphylinidae.
Photo by Nahíra E. Arocho-Hernández
Station: 3A-October

Figure A22: Staphylinidae.
Photo by Nahíra E. Arocho-Hernández
Station: 5A-August

Figure A23: Undetermined coleopteran larva.
Photo by Nahíra E. Arocho-Hernández
Station: 5A-August

Figure A24: Undetermined coleopteran larva.
Photo by Nahíra E. Arocho-Hernández
Station: 1A-August

Figure A25: Undetermined coleopteran larva.
Photo by Nahíra E. Arocho-Hernández
Station: 4B-November

Order Collembola

Figure A26: Entomobryidae.
Photo by Nahíra E. Arocho-Hernández
Station: 3A-November

Figure A27: Poduridae.
Photo by Nahíra E. Arocho-Hernández
Station: 4A-November

Figure A28: Isotomidae.
Photo by Nahíra E. Arocho-Hernández
Station: 6A-July

Order Diptera

a.

b.

Figure A29: Agromyzidae. a. Dorsal View, b. Lateral View
Photo by Dennis O. Pérez-López
Station: 2 October

a.


b.


c.


Figure A30: Anthomyiidae. a. Lateral View, b. Dorsal View, c. Ventral View
Photo by Jeniffer Vega
Station: 4 June

a. 

b. 

c. 

Figure A31: Anthomyiidae. a. Lateral View, b. Dorsal View, c. Ventral View
Photo by Jeniffer Vega
Station: 3 October

Figure A32: Ceratopogonidae (larva).
Photo by Nahíra E. Arocho-Hernández
Station: 2A-July

Figure A33: Ceratopogonidae (ventral view).
Photo by Nahíra E. Arocho-Hernández
Station: 2A-October

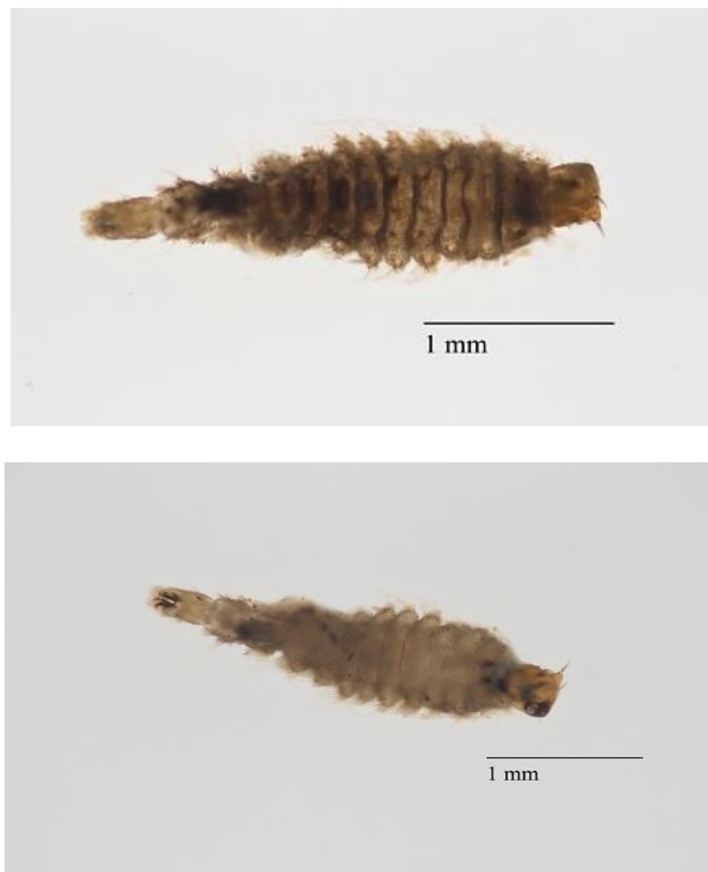


Figure A34: Ceratopogonidae larva.
Photo by Nahíra E. Arocho-Hernández
Station: 2A-October

Figure A35: Chironomidae (larva).
Photo by Nahíra E. Arocho-Hernández
Station: 2A-October

a. 

b. 

c. 

d. 

Figure A36: Culicidae.
Photo by Jeniffer Vega
Station: 2 October

Figure A37: Culicidae (larva).
Photo by Nahíra E. Arocho-Hernández
Station: 1 Light-June

Figure A38: Diptera larva.
Photo by Melanie Torres-Santana
Station: 6A-July

a. 

b. 

Figure A39: Dolichopodidae. a. Ventral View, b. Lateral View
Photo by Jeniffer Vega
Station: 6 June

a.

b.

Figure A40: Dolichopodidae. a. Lateral View, b. Dorsal View
Photo by Jeniffer Vega
Station: 3 August

Figure A41: Ephydridae.
Photo by Nahíra E. Arocho-Hernández
Station: 2B-June

a. 

b. 

c. 

Figure A42: Tachinidae. a. Dorsal View,
b. Ventral View, c. Lateral View
Photo by Jeniffer Vega
Station: 2 June

a. 

b. 

Figure A43: Rhagionidae. a. Dorsal View, b. Ventral View, c. Lateral View
Photo by Jeniffer Vega
Station: 6 June

a. 

b. 

c. 

Figure A44: Sciomyzidae. a. Dorsal View, b. Ventral View, c. Lateral View
Photo by Jeniffer Vega
Station: 1 July

a. 

b. 

c. 

Figure A45: Sciomyzidae. a. Dorsal View, b. Ventral View, c. Lateral View
Photo by Jeniffer Vega
Station: 3 August

Figure A46: Stratiomyidae (larva).
Photo by Nahíra E. Arocho-Hernández
Station: 1A-July

a.

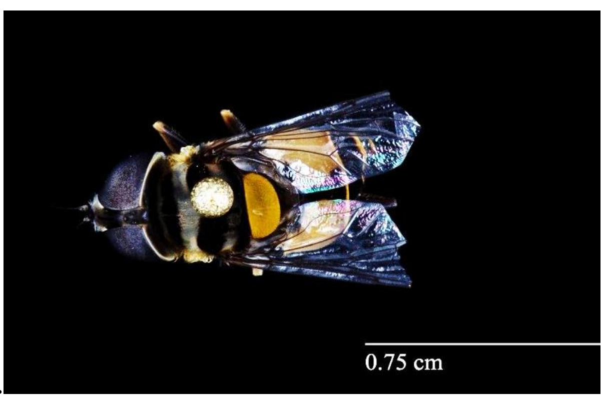
b.

c.

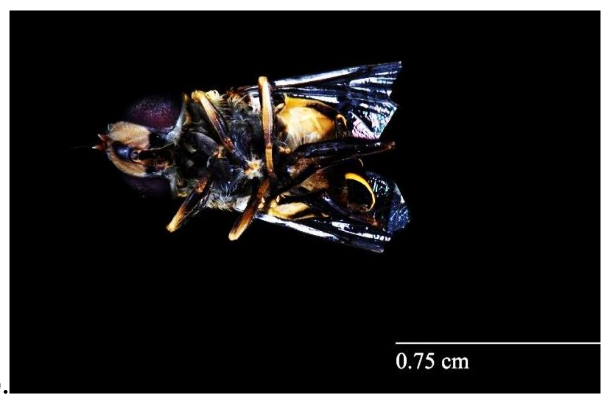
Figure A47: Stratiomyidae. a. Dorsal View, b. Ventral View, c. Lateral View

Photo by Jeniffer Vega

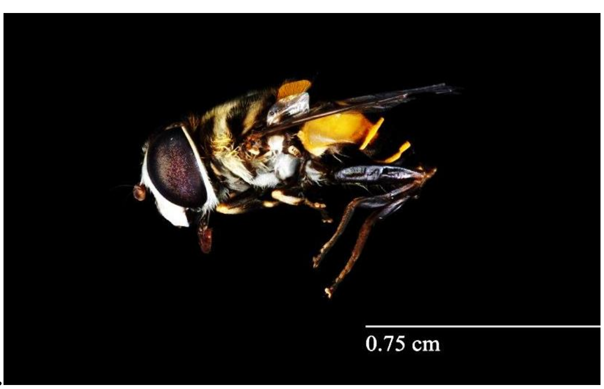
Station: 3 October



a.



b.



c.

Figure A48: Syrphidae; Genus: *Palpada.*
a. Dorsal View, b. Ventral View, c. Lateral View
Photo by Jeniffer Vega
Station: 4 October

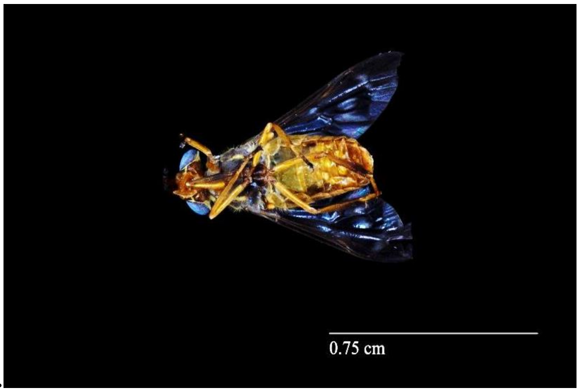
Figure A49: Tabanidae (larva).
Photo by Nahíra E. Arocho-Hernández
Station: 1A-June

Figure A50: Tabanidae (adult).
Photo by Arlene M. Megill-Irizarry
Station: 2-June

a.  b. 

Figure A51: Tabanidae (larva). a. Anterior end, b. Posterior end
Photo by Nahíra E. Arocho-Hernández
Station: 4A-October

a. 

b. 

c. 

Figure A52: Tabanidae. a. Dorsal View, b. Ventral View, c. Lateral View

Photo by Jeniffer Vega

Station: 4 June

Figure A53: Tipulidae .
Photo by Dennis O. Pérez-López
Station: 1 June

Figure A54: Trixoscelidae.
Photo by Nahíra E. Arocho-Hernández
Station: 5 June

a. 

b. 

Figure A55: Undetermined dipteran larva. a. Dorsal View, b. Ventral View
Photo by Nahíra E. Arocho-Hernández
Station: 1A-November

Figure A56: Undetermined dipteran larva.
Photo by Nahíra E. Arocho-Hernández
Station: 3B-June

Figure A57: Undetermined dipteran pupa.
Photo by Nahíra E. Arocho-Hernández
Station:3 Ligth

Figure A58: Undetermined dipteran pupa.
Photo by Nahíra E. Arocho-Hernández
Station: 1B-June

Figure A59: Undetermined dipteran pupa.
Photo by Nahíra E. Arocho-Hernández
Station: 3B-September

Figure A60: Undetermined dipteran pupa.
Photo Nahíra E. Arocho-Hernández
Station: 2A-October

Figure A61: Undetermined dipteran pupa.
Photo by Melanie Torres-Santana
Station: 2B-September

Figure A62: Undetermined pupa of Chironomidae
Photo by Melanie Torres-Santana
Station: 3B-June

Order Hemiptera

a. 

b. 

Figure A63: Achiilidae. a. Dorsal view, b. Ventral view
Photo by Jeniffer Vega
Station: 1A-November

a. b.

Figure A64: Belostomidae. a. Ventral view, b. Dorsal View
Photo by Nahíra E. Arocho-Hernández
Station: 2A-November

Figure A65: Gerridae
Photo by Nahíra E. Arocho-Hernández
Station: 2B-July

a.

b.

Figure A66: Hebridae. a. Dorsal View, b. Ventral View
Photo by Nahíra E. Arocho-Hernández
Station: 1A-July

Figure A67: Notonectidae.
Photo Nahíra E. Arocho-Hernández
Station: 2A-August

Figure A68: Pleidae.
Photo by Nahíra E. Arocho-Hernández
Station: 1B-June

Figure A69: Veliidae, Genus: *Rheumatobates.*
Photo by Nahíra E. Arocho-Hernández
Station: 5B-September

Figure A70: Veliidae.
Photo taken by Nahíra E. Arocho-Hernández
Station: 2B-September

Order Hymenoptera

Figure A71: Eupelmidae.
Photo by Nahíra E. Arocho-Hernández
Station: 3-June

Figure A72: Pompilidae.
Photo by Nahíra E. Arocho-Hernández
Station: 3-October

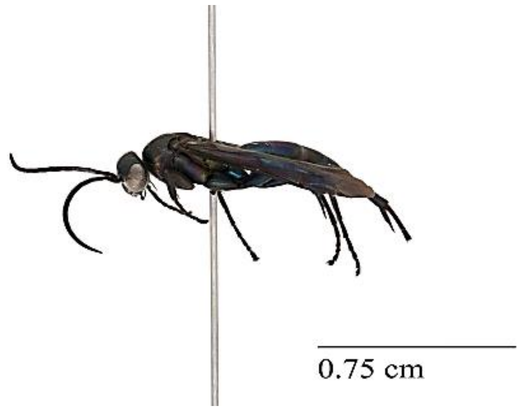


Figure A73: Pompilidae. Lateral View
Photo by Jeniffer Vega
Station: 3-October

a. 

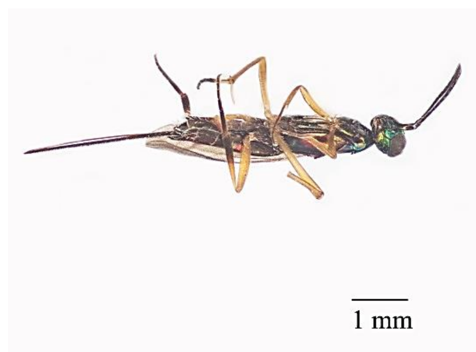
b. 

Figure A74: Pteromalidae. a. Dorsal View, b. Ventral View

Photo by Jeniffer Vega

Station: 4 October

Order Lepidoptera

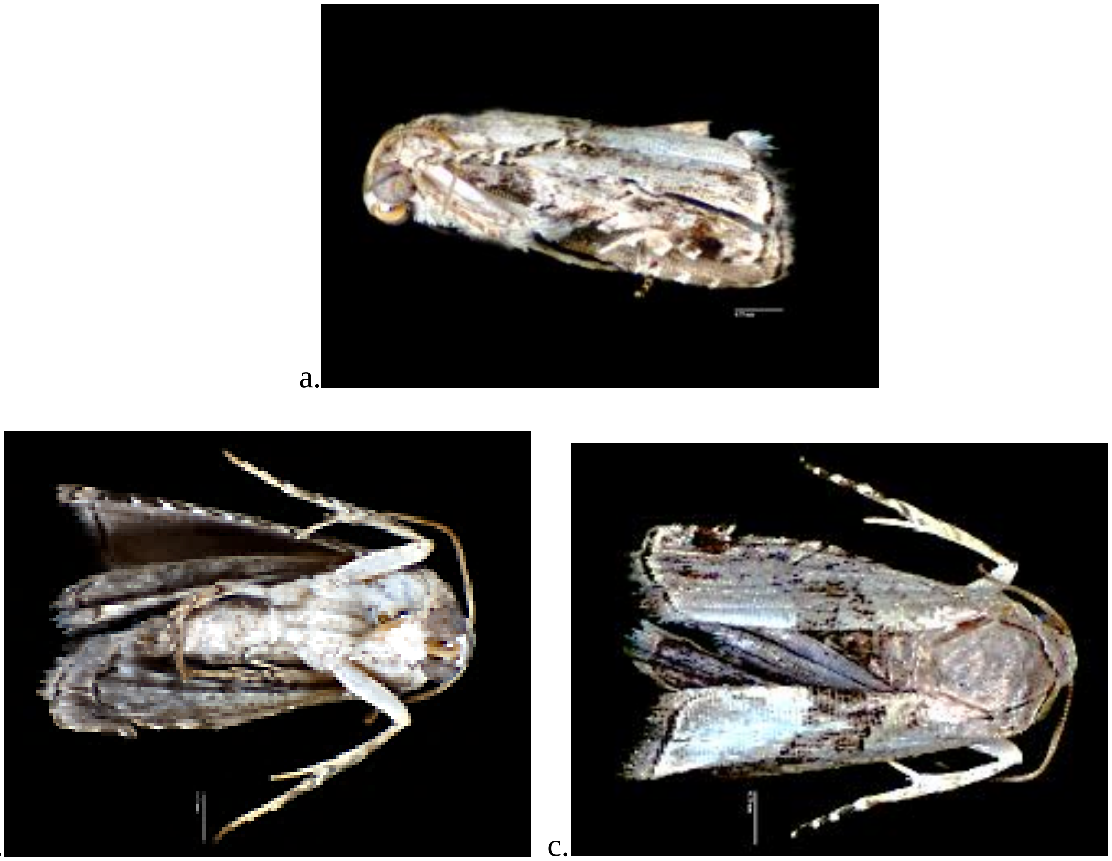
a.

b. c.

Figure A75: Pyralidae. a. Lateral View, b.Ventral View, c. Dorsal View
Photo by Jeniffer Vega
Station: 1A-November

Figure A76: Pyralidae.
Photo taken Nahíra E. Arocho-Hernández
Station: 5A-August

Figure A77: Pyralidae.
Photo by Nahíra E. Arocho-Hernández
Station: 1A-November

a.  b. 

Figure A78: Pyralidae. a. Dorsal View, b. Ventral View
Photo by Arlene M. Megill-Irizarry
Station: 3A-August

Figure A79: Pyralidae.
Photo by Nahíra E. Arocho-Hernández
Station: 1A-November

Order Odonata

a. 

b. 

Figure A80: Coenagrionidae. a. Nymph, b. Nymph with extended labium
Photo by Nahíra E. Arocho-Hernández
Station: 1Light-June

Figure A81: Coenagrionidae Nymph.
Photo by Nahíra E. Arocho-Hernández
Station: 6B-June

Figure A82: Libellulidae Nymph.
Photo by Nahíra E. Arocho-Hernández
Station: 6B-June

Figure A83: Libellulidae (labium).
Photo by Nahíra E. Arocho-Hernández
Station: 4A-October

a. 

b. 

Figure A84: Protoneuridae Nymph. a. Anterior end, b. Posterior end
Photo by Nahíra E. Arocho-Hernández
Station: 4A-October

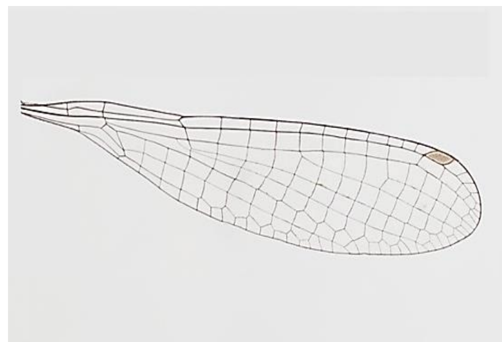
a. 
b. 

Figure A85: Coenagrionidae; *Enallagma civile.* a. adult, b. wing
Photo by Dennis O. Pérez
Station: 2-September

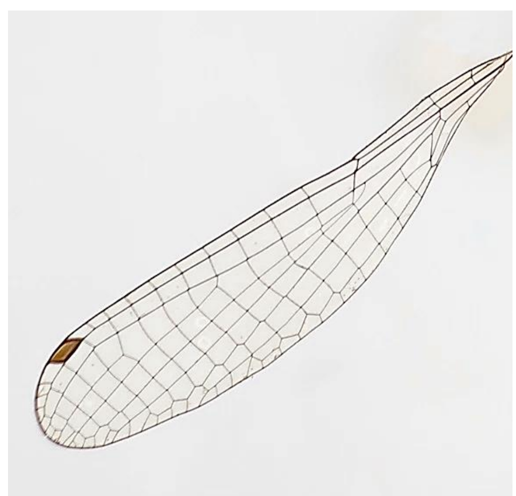
a. 
b. 

Figure A86: Coenagrionidae; *Ischnura capreolus.* a. adult, b. wing
Photo by Dennis O. Pérez
Station: 2-September

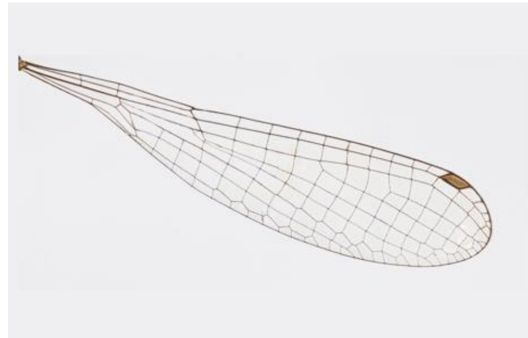
a.  b. 

Figure A87: Coenagrionidae; *Telebasis coralina*. a. adult, b. wing
Photo by Dennis O. Pérez
Station: 1-July

Subphylum Crustacea

Order Amphipoda

Figure A88: Amphipoda.
Photo by Nahíra E. Arocho-Hernández
Station: 5A-August

Figure A89: Amphipoda.
Photo by Nahíra E. Arocho-Hernández
Station: 4B- July

Suborder Cladocera

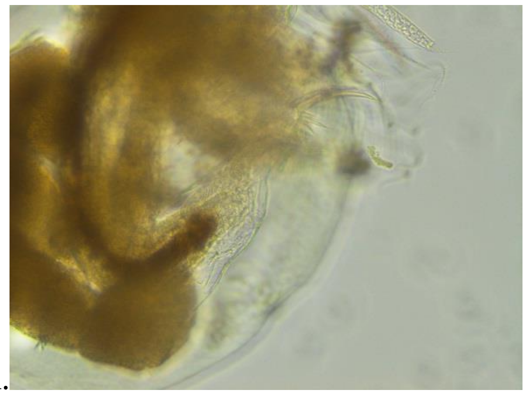
Figure A90: Cladocera.
Photo by Carlos J. Santos-Flores
Station: 4A-October

Figure A91: Cladocera.
Photo by Carlos J. Santos-Flores
Station: 4A-October

a. 

b. 

c. 

d. 

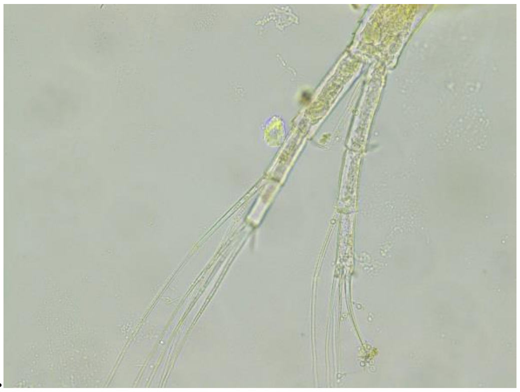
e. 

Figure A92: Genus *Moinodaphnia* (natatory antenna).
Photo taken by Carlos J. Santos-Flores
Station: 4A-November

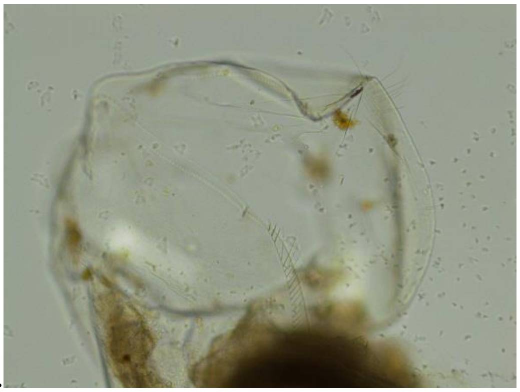
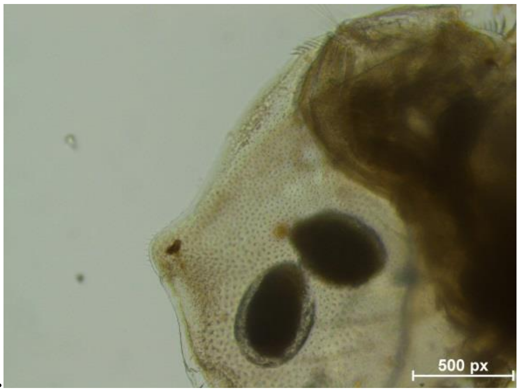
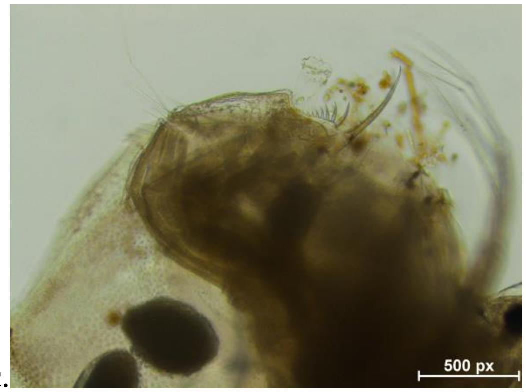
a.  b. 

Figure A93: Genus *Simocephalus*. a. Antennae and postabdomen, b. Posterior end
Photo taken by Carlos J. Santos-Flores
Station: 3B-November

a.  b. 

c. 

d. 

Figure A94: Genus *Simocephalus*. a. Habitus, b. Eggs on brood chamber, c. Postabdomen, d. Head and natatory antenna.
Photo by Carlos J. Santos-Flores
Station: 3B-November

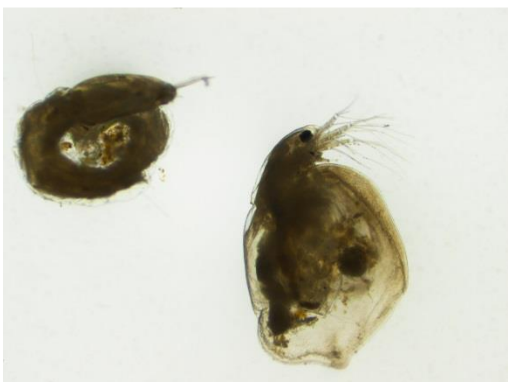
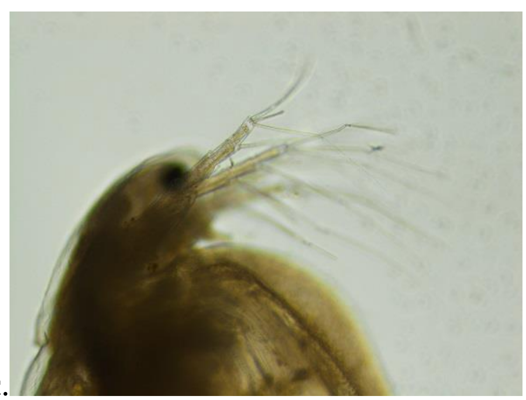
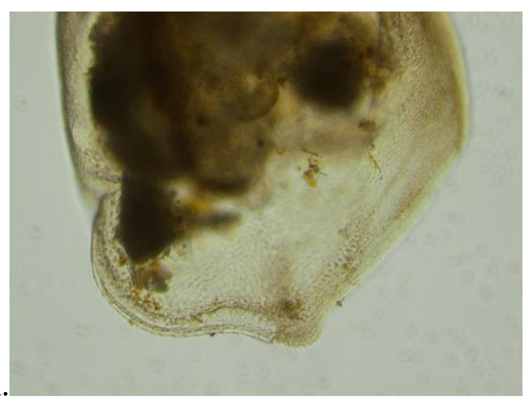
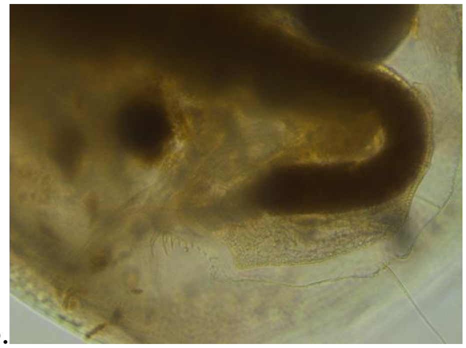
a. 
b. 
c. 
d. 

Figure A95: Genus *Simocephalus*. a. Habitus, b-c. Head and natatory antenna, d. Posterior end.
Photo by Carlos J. Santos-Flores
Station: 4B-November

a.  b. 

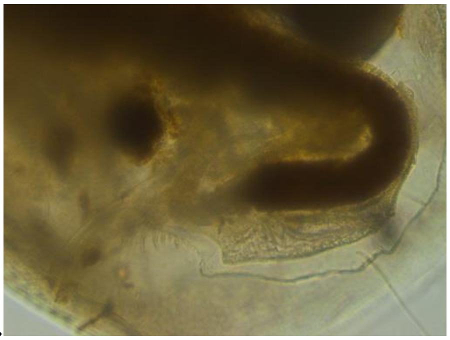
c. 

Figure A96: Genus *Simocephalu.*
a. Habitus, b-c. Postabdomen.
Photos by Carlos J. Santos-Flores
Station: 4B-November

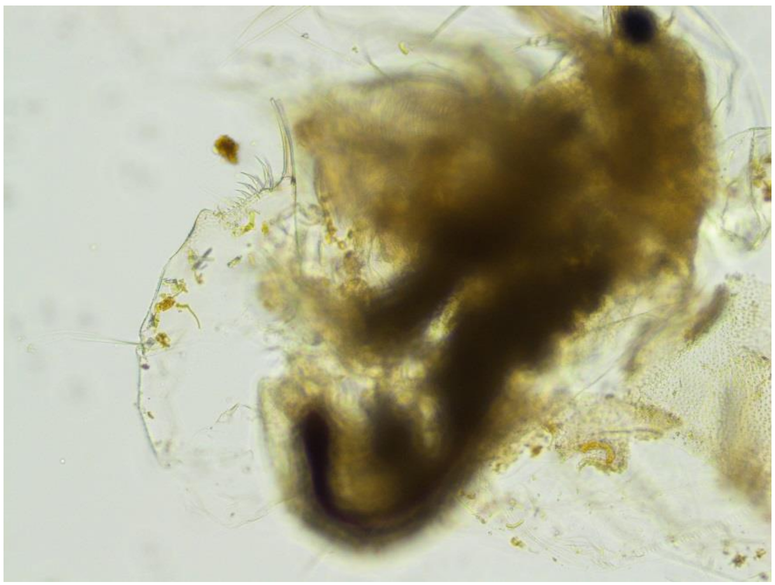


Figure A97: Genus *Simocephalus* (Habitus).
Photo by Carlos J. Santos-Flores
Station: 4B-November

Sub-Class Copepoda

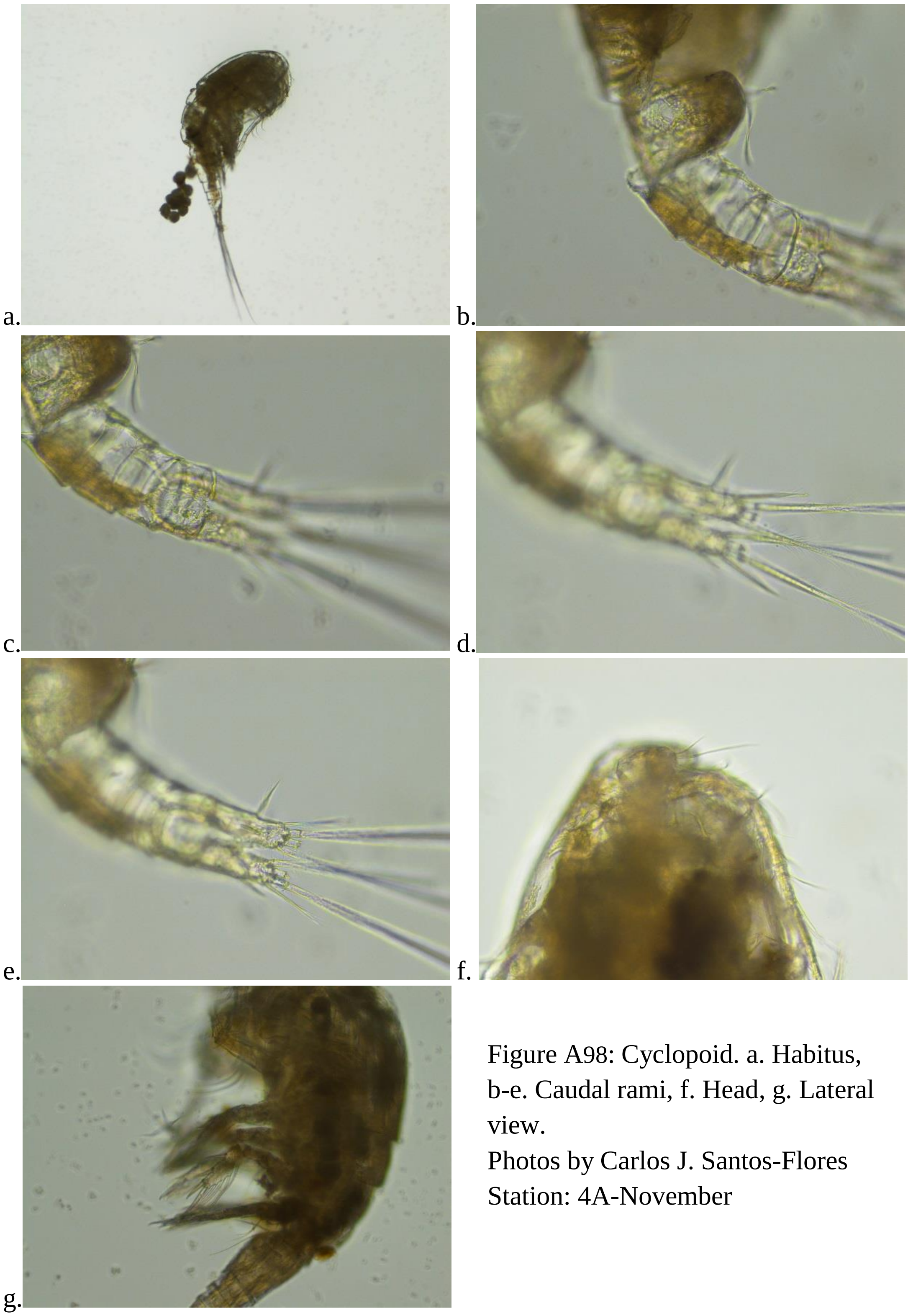
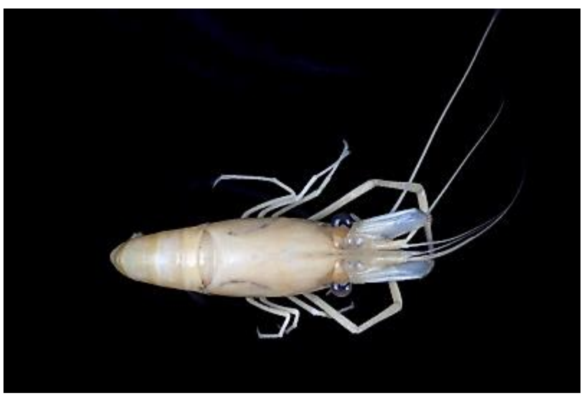
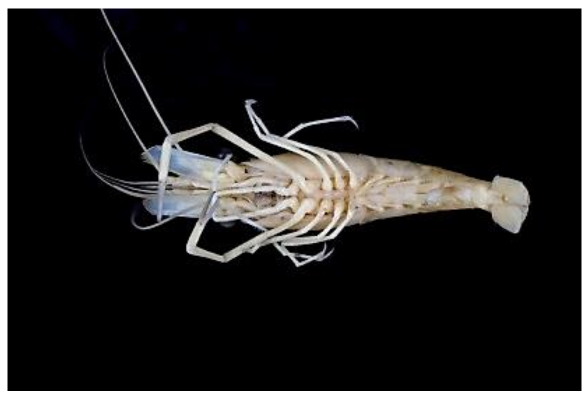


Figure A98: Cyclopoid. a. Habitus, b-e. Caudal rami, f. Head, g. Lateral view.
Photos by Carlos J. Santos-Flores
Station: 4A-November

Order Decapoda

Figure A99: Shrimp.
Photo by Nahíra E. Arocho-Hernández
Station: 4B-August

a.  b. 

c. 

Figure A100: *Palaemon pandaliformis*. a. Dorsal view, b. Ventral View, c. Lateral View
Photos by Carlos A. Negrón-Lugo
Station: 4Light-June

a. 

b. 

c. 

Figure A101: Ocypodidae. a. Dorsal View, b. Ventral View, c. Frontal View
Photos taken by Dennis O. Pérez-López
Station: 2 Light-June

Order Isopoda

a. 

b. 

Figure A102: Isopoda. a. Dorsal view, b. Ventral View
Photo by Nahíra E. Arocho-Hernández
Station: 1B-September

Order Ostracoda

Figure A103: Ostracoda.
Photo by Nahíra E. Arocho-Hernández
Station: 2B-September

Figure A104: Ostracoda.
Photo by Nahíra E. Arocho-Hernández
Station: 3B-October

Figure A105: Ostracoda.
Photo taken by Nahíra E. Arocho-Hernández
Station: 1 Light-November

Figure A106: Ostracoda.
Photo taken by Nahíra E. Arocho-Hernández
Station: 1 Light-November

Figure A107: Ostracoda.
Photo by Nahíra E. Arocho-Hernández
Station: 3A-November

Figure A108: Ostracoda.
Photo taken by Nahíra E. Arocho-Hernández
Station: 3A-Novmeber

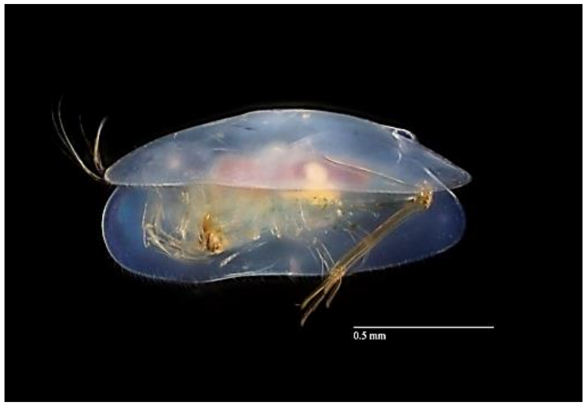


Figure A109: Ostracoda.
Photo by Nahíra E. Arocho-Hernández
Station: 2A-November

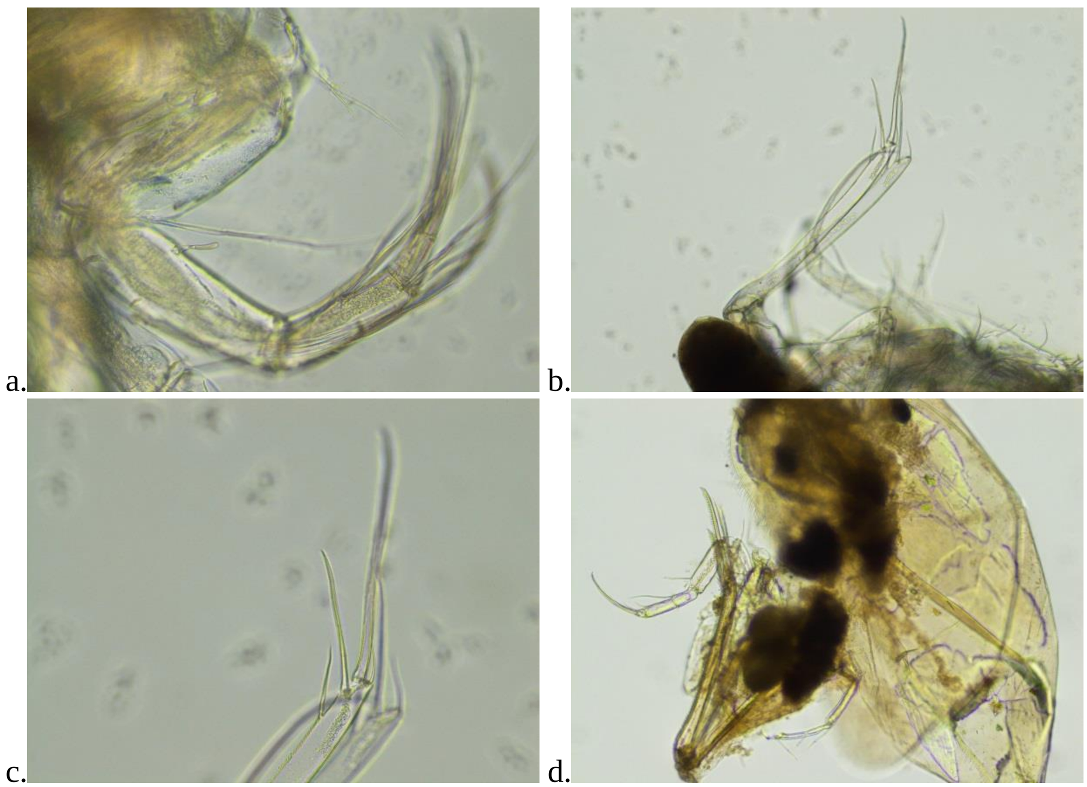


Figure A110: Ostracoda. a.Natatory antenna, b. Furca, c. Furcal claw, d. Habitus
Photos by Carlos J. Santos-Flores
Station: 1A-June

Others

Figure A111: Geophilomorpha.
Photo taken by Nahíra E. Arocho-Hernández
Station: 5A-August

Figure A112: Symphila.
Photo taken by Nahíra E. Arocho-Hernández
Station: 5A-August

Figure A113: Psocoptera.
Photo taken by Nahíra E. Arocho-Hernández
Station: 5A-August

Figure A114: Ephemeroptera. Genus *Caenidae*
Photo taken by Nahíra E. Arocho-Hernández
Station: 3B-July