Photo: Rio San Juan (tributary to Rio Sierpe) by D. Arscott

A Preliminary Water Quality Study of the Rio Sierpe and its tributaries (Costa Rica)

Prepared for the Blue Moon Foundation

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I. Introduction

This report documents the results from a preliminary field study conducted by the Stroud Water Research Center in April 2009 in the Rio Sierpe watershed in southwest Costa Rica (Fig. 1). The field study was commissioned by the Blue Moon Foundation (Charlottesville, VA, USA) as part of their effort to help understand and protect valuable ecosystems. We conducted an initial scoping study that will guide the development of a more rigorous project to measure both the current state and future changes in the health of the stream network within the Rio Sierpe and nearby regions.

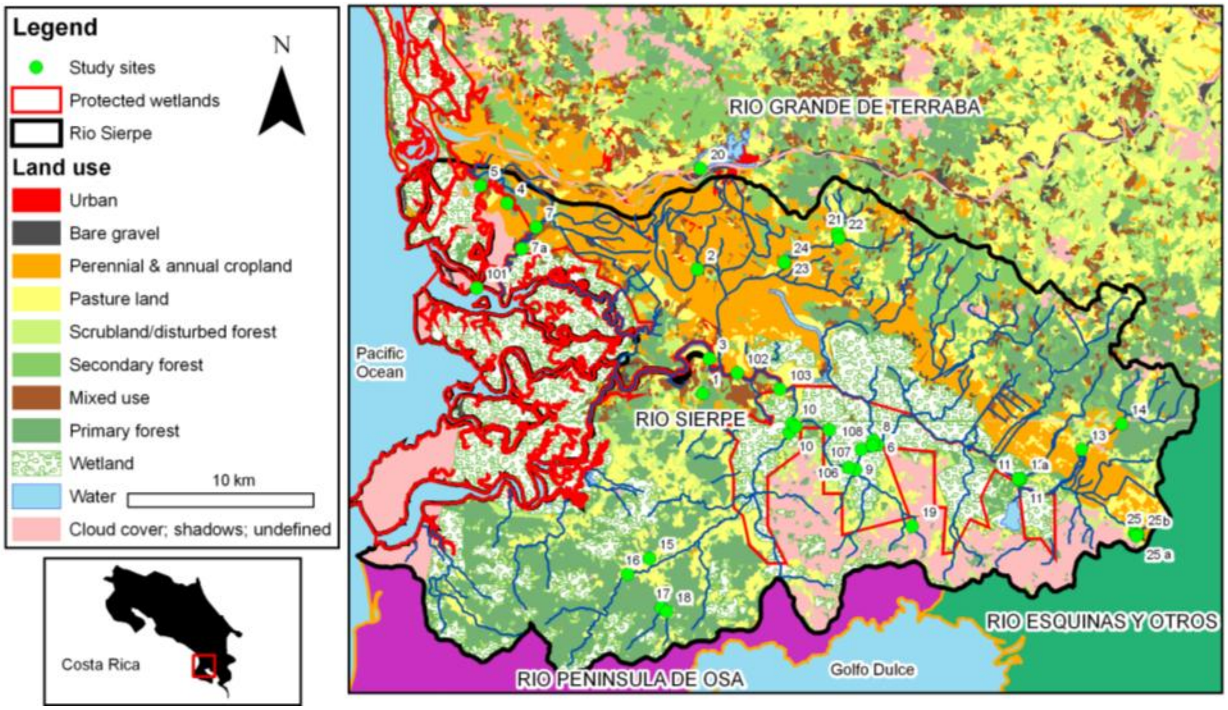

Figure 1: Land use and study sites in the Rio Sierpe watershed, northwest Costa Rica.

The Rio Sierpe is a medium sized river located in southwestern Costa Rica with mixed land uses throughout (Fig. 1) including native forest, agriculture (primarily palm oil plantations, rice production, banana plantations, shrimp farming, and livestock grazing). The lower reaches and estuary support one of the largest mangrove swamps (~270 km^2) along the Pacific coastline of Latin America, the Térraba Sierpe National Wetlands (est. March 1994). This mangrove, in turn, supports an important recreational fishery and is economically important to the region. The mangrove swamp is a primary factor responsible for a large effort to establish a scalable and replicable framework for the integration of *Technical, Institutional and Conceptual Solutions* (TICOS) to promote its sustainable development (Azur Moulaert, University of Vermont, - personal communication).

The Rio Sierpe watershed has gradually undergone human development resulting in significant portions of the original old growth tropical forest being eliminated and replaced by agriculture and other human activities. Moreover, significant changes are expected in the near future due to increased interest in aquaculture and the changing nature of agriculture in the region and urbanization associated with the possible expansion to service international flights at the airport at Palmar Sur, a small town whose suburbs extend into the Rio Sierpe watershed (Dan Janzen personal communication). These expected changes in the local economy and potentially in the environment result in a need to: (i) quantify current environmental conditions in streams and rivers of the Rio Sierpe watershed and mangrove swamp; (ii) determine relationships between stream water quality and land use; (iii) develop environmental monitoring tools and baseline physical, chemical, and biological data to track degradation and improvement in water quality over time; and (iv) engage and train local residents in the use of monitoring tools. The objective of this study was to survey stream chemical and biological (aquatic macroinvertebrates and fish) conditions throughout the Rio Sierpe watershed to determine the feasibility of conducting a larger scale effort to quantify water quality and develop protocols and community involvement in monitoring the health and integrity of the watershed. In April 2009, a one-week expedition was conducted to: (i) rapidly assess chemical and biological quality of small, medium, and large streams/rivers in the watershed that represented the range of current landuses; (ii) assess the feasibility of establishing a 2-3 year monitoring program to provide a quantitative baseline to firmly and accurately establish the current spatial and temporal status of water quality; and (iii) establish working relationships with individuals/institutions/ organizations/decision makers who are interested in, working with, or affected by water quality and who can help implement a water quality monitoring program and/or use it for purposes of local conservation, education, or economic well being of the community.

Understanding the local and regional variability in stream water chemistry is a prerequisite for the determination of the impact of human activities on water quality. Human activities can result in subtle, but important, increases in the concentrations of ions and nutrients dissolved in stream water. For example, some human activities result in small increases in stream nitrate-nitrogen and inorganic phosphorus concentrations that can result in increased standing stock and growth rates of algae. Increased standing stock of algae can alter aquatic macro-invertebrate communities and change food type and availability for certain fish species. These subtle changes may ultimately result in altered fish community structure and a change in the type of fish available for human consumption or for recreational opportunities. Other types of human activities can result in the release of toxic chemicals into streams and more directly impact both fish and human health. Human activities can also result in the degradation and/or alteration of a streams physical habitat. For example, deforestation without re-vegetation often results in increased delivery of fine sediments to a stream that can smother stream bed sediments thereby reducing hydrological exchange between surface and groundwater and affecting the interstitial habitat for both invertebrates and fish. For these reasons, a comprehensive stream monitoring program typically involves the quantification of chemical, physical, and biological attributes within a stream.

Aquatic macroinvertebrate and fish communities are useful for assessing water and habitat quality and ecosystem structure and function in streams and have been used for this purpose for more than 100 years (Cairns and Pratt 1993). Benthic (i.e., bottom-dwelling) macroinvertebrates, in particular, are one of the most common groups of aquatic organisms included in these programs (Hellawell 1986). Aquatic macroinvertebrates include insects, aquatic worms, flatworms, leaches, shrimps, crabs, crayfish, clams and mussels among others. Aquatic macroinvertebrates are a widely accepted group for assessing the quality and functionality of streams and their ecosystems for a number of reasons (Weber 1973, Rosenberg and Resh 1993). First, most river and stream ecosystems have relatively diverse macroinvertebrate assemblages (100-200 species in natural undisturbed temperate streams), with many species representing each of the major taxonomic orders (e.g., Ephemeroptera [mayflies], Trichoptera [caddisflies], Plecoptera [stoneflies], Coleoptera [beetles], Diptera [true flies] among others). Each species is to some degree unique; as a result, each potentially possesses different habitat and feeding preferences, tolerances to changes in environmental conditions, etc. Thus, together, the aquatic macroinvertebrates are a sensitive measure of current environmental conditions as well as a good measure of ecosystem change and stress over both space and time. Second, the relatively long life spans (a few months to a year or more) of macroinvertebrates make the presence or conspicuous absence of macroinvertebrate species at a site a meaningful record of environmental quality during the recent past. Third, their abundance lends itself to statistical analyses, which play an integral role in assessing the quality of habitat and water as well as ecosystem integrity.

Fish provide additional information on water quality (Karr 1981). Fish provide information on connectivity of streams and rivers, and fish can be surveyed in fresh and saline waters. In addition, fish may be more sensitive to flow variability than macroinvertebrates (Lammert and Allan 1999), whereas macroinvertebrates may be more sensitive to riparian habitat (Sweeney et al. 2004), and may recover from disturbance more quickly. Fish also represent an important endpoint in water quality monitoring because providing "fishable" waters is often a goal of water protection regulations. There are no good surrogates for directly monitoring fish communities (Karr 1991), therefore to provide a more complete picture of biotic integrity both fish and macroinvertebrates should be surveyed.

II. Study Sites and Methods

In April 2009, we visited 39 locations in the Rio Sierpe catchment and one location near the mouth of the Térraba River (Table 1, Fig. 2) to collect water samples for chemical analyses, fluvially deposited sediments (for future analysis of pesticide content), aquatic macroinvertebrates, and fish communities. Six people were involved in sampling (Drs. D.B. Arscott, W. Eldridge, and B.W. Sweeney from SWRC; R. Morales from ACIS & SWRC; Dr. R.W. Flowers from Florida A & M; and S. Avila A. from Instituto Nacional de Biodiversidad [INBIO], Costa Rica). All tasks were not conducted at every site (see Table 1). Water samples for chemical analyses were collected from 25 sites. Aquatic macroinvertebrates and fish were sampled at 18 and 15 sites respectively, and sediment grab samples were collected from 6 sites. At all sites water temperature and specific conductivity was measured at the surface of the stream/river. At 11 sites along the mainstem of the river, water temperature and specific conductivity were measured at either 2 or 3 different depths from a boat during high tide to determine the upstream extent of saltwater encroachment. Finally, at a site located along the mainstem of the Rio Sierpe at the village of Sierpe, a data logging conductivity and temperature probe was deployed from a floating dock at a depth of ~3 m below the surface for ~ 12 hours to quantify the tidal influence on salinity concentrations.

Sites were selected based on the surrounding riparian and upstream land use characteristics in an effort to sample streams draining a range land cover types (Table 1). Five sites were located within the mangrove wetland above and below a shrimp farm (sediments near the edge of a dry rice paddy field were collected at one of the sites). The shrimp farm was not active during our visit and no water withdrawals or discharges were visible.

At each site a Garmin 60csx GPS was used to record latitude and longitude. The physical stream habitat at each site was also visually assessed and several variables were estimated and recorded by D.B. Arscott including: riparian land use on each bank, % riparian canopy shading the stream, approximate wetted channel width, average and maximum channel depth, the presence of large wood debris in the channel, and the relative percentage of substrate in 5 size categories (boulder/rubble > 256 mm, cobble 64 – 256 mm, large gravel 16 – 64 mm, small gravel 2 – 16 mm, sand 0.0625 – 2 mm, and silt/clay < 0.0625 mm).

A. Water Chemistry

At most sites, specific conductivity, temperature, dissolved oxygen, and pH were measured in the field using portable hand-held meters (YSI® model 600XL [Yellow Springs, Inc., Yellow Springs, Ohio] or WTW Multi 3500i [WTW GmbH, Weilheim, Germany]). Stream water from 25 sites was analyzed for major cations (Mg^{+2}, Ca^{+2}, K^{+}, Na^{+}, NH^{+}_4) and anions (Br^{-}, Cl^{-}, NO^{-}_3, NO^{-}_2, PO^{-3}_4, SO^{-2}_4), dissolved organic carbon (DOC), and total suspended solids (TSS). At these sites, stream water was collected in a pre-cleaned 1-L wide-mouthed plastic bottle. Water for analysis of DOC, TSS, and major ions was sub-sampled from the 1-L bottle using a 60-ml syringe and various filters. Samples for DOC and TSS analyses were filtered through pre-combusted (and weighed) ~0.7 µm glass fiber filters (Whatman GF/F). Approximately 40 ml of the filtrate was dispensed into borosilicate vials (pre-combusted) pre-loaded with 35 µl of 3.6 M Sodium Azide (to inhibit microbial activity) for analysis of DOC. Depending on the turbidity of the stream water, an additional 200-860 ml was pushed through the filter to collect particles for analysis of TSS. DOC samples were analyzed at SWRC with an Inonics Sievers 800 or 900 TOC analyzer equipped with an inorganic carbon removal module. Filters for analysis of TSS were dried at 60°C to a consistent weight at SWRC and the final weight was recorded to determine TSS (pre-sample weight minus sample weight divided by volume filtered). Two 60 ml plastic vials were filled with water samples for anion and cation determinations after filtration through 0.22 µm syringe type filters (Millipore MillexGP). Major ions were analyzed by ion chromatography with conductivity detection (Dionex ICS 3000) at SWRC. Samples were either frozen (major ions), held at room temperature (DOC), or air dried (TSS) at our hotel in Sierpe village and then shipped to SWRC for sample analysis.

Fluvially deposited sediments were collected from five sites located in the lower portion of the Sierpe watershed. Wet sediments at the stream edge were collected using a pre-combusted metal spatula to scoop sediments into a 500 ml pre-combusted glass wide-mouth jar. Sediment samples were frozen and are retained at the SWRC for future analysis of pesticide content.

B. Aquatic Macroinvertebrate Methods

Aquatic macroinvertebrates were sampled using a semi-quantitative method. Four people each equipped with pond dip nets (500 um mesh) each spent approximately 1 hour kicking stream bottom substrate, wood debris, and aquatic vegetation (when present) to flush aquatic invertebrates from their substrate into the pond dip net. Nets were regularly emptied into a white sorting tray (~ 20 x 30 cm). Invertebrates were picked from sorting trays using forceps and placed in glass collecting vials containing 70% ethanol (ETOH) and returned to the lab for identification. Invertebrates from all samples were sorted under a dissecting microscope (10X magnification) and identified to the lowest practical level by S. Avila (INBIO) and verified by R.W. Flowers (Florida A & M).

As part of this preliminary study of aquatic macroinvertebrates in the Rio Sierpe, taxonomic richness at each site was used to assess differences among streams across the watershed and to begin to infer biological degradation. The presence or absence of certain species or taxonomic groups at a site is often related to that groups' tolerance or sensitivity to various types of pollution (i.e., toxic metals, organic material, sediments, or loss of food source preference). It is well known that collectively most Ephemeroptera (mayflies) and Plecoptera (stoneflies) and several Trichoptera (caddisflies), a.k.a. EPT taxa, are sensitive to environmental degradation and their absence can be used as an indicator of degradation. It is also known that many species of chironomid midges, oligochaete worms, and certain snails are more tolerant of some forms of degradation. The richness of these taxon groups were summarized and used to begin to infer high quality and poor quality stream habitat throughout the Sierpe watershed.

<u>Total Richness</u> summarizes responses (as presence/absence but not abundance) of all taxa, including pollution-sensitive and pollution-tolerant taxa. It is reported as the mean number of aquatic macroinvertebrate taxa found in each subsample. Total Richness generally decreases in response to moderate to severe pollution. Richness was also expressed as EPT richness, chironomid plus other non-insect richness, and other insect richness.

<u>EPT Richness (EPT)</u> is often calculated in addition to Total Richness and reported as the mean number of Ephemeroptera, Plecoptera, and Trichoptera taxa found in each subsample. These three insect orders contain many pollution-sensitive taxa; thus, this metric summarizes responses of mostly pollution-sensitive taxa. EPT Richness generally decreases in response to moderate to severe pollution.

C. Fish Methods

Contacts

One objective of the preliminary study was to develop contacts (landowners, regional biologist, local watershed/educational groups, outside conservation groups active in the watershed, etc) to coordinate future research and to initiate a local monitoring effort. We established a valuable contact with William Bussing of the University of Costa Rica. He is the foremost authority on the freshwater fishes of Costa Rica and has firsthand experience with the Rio Sierpe.

Preliminary list of fish species

Another objective of the preliminary study was to identify and collect relevant literature and background information. We created a list of studies focused on the conservation of tropical freshwater fishes with an emphasis on Central America. We found a few studies which examined community structure and impacts of human land use. One of the more relevant studies was for an index of biotic integrity based on fish distributions for a region of central Mexico (Lyons et al. 1995). Only a few species were in common with the Rio Sierpe, however the study provided useful guidance on methods to collect and monitor tropical stream fish health.

We also developed a preliminary list of the fish species in and around the Rio Sierpe and recorded what is known about their biology. We used Google Scholar to search for published surveys of fish in the Térraba physiographic region to create a list of freshwater fish species for the Rio Sierpe. The Térraba region includes the Pacific slope of Costa Rica from the Rio Térraba to the Osa Peninsula and Golfo Aqua Dulce north of the Rio Coto (Bussing 2002). From these studies we developed a list of primary, secondary and peripheral freshwater fish species that have been previously observed in the Térraba physiographic region. Primary freshwater fish species are derived from families that have very low salt tolerance and were confined to South America prior to the exposure of the Isthmus of Panama (Myers 1966). Primary species naturally occur as far north as Costa Rica or Nicaragua (Miller 1966, Myers 1966). Secondary species have a marine ancestry and can tolerate some salinity (Myers 1966). Peripheral marine species are species with a marine ancestor that now spend the majority of their time in freshwater (Miller 1966, Winemiller 1983).

We made a preliminary summary of each species life history and habitat preferences. An important focus of future research would be on the physical, chemical or biological attributes of the stream ecosystem that limit fish presence. For the primary, secondary and peripheral species we recorded their origin; if there was a prior record in the Rio Sierpe; their typical diet; range of stream size, current, substrate, elevation and temperature observations; body morphology and locomotor structure; typical position in the water column; reproductive behavior; saltwater tolerance; tolerance to habitat degradation; abundance; and distribution. This information will be necessary in the future to develop an index of biotic integrity (Karr 1981, Lyons et al. 1995). We determined a species origin and saltwater tolerance using the FishBase database (Froese and Pauly 2009). Diet was based on Bussing (2002) and Winemiller and Morales (Winemiller and Morales 1989). Stream size, current, substrate, elevation, and temperature range, and reproductive behavior was recorded from Bussing (2002). We described body morphology by a shape factor which was the ratio of a species total length to its maximum body depth (Webb and Weihs 1986). We described locomotor structure by a swimming factor which was the ratio of the species minimum depth at the caudal peduncle to the maximum depth of the caudal fin (Webb and Weihs 1986). We measured both factors on pictures in Bussing's book (2002) and we measured only the male when there were differences between the sexes. Position in the water column was taken from Bussing (2002) or based on our observations of their usual position in relation to the bottom. Tolerance to habitat degradation was based on the observed distributions in the Rio Sierpe. Fish were considered sensitive if they occurred only in sites with good water and habitat quality; tolerant if they occurred in good numbers at a wide range of sites including some with low dissolved oxygen, high levels of sedimentation and turbidity, and extensively degraded habitat, or if they were known to be tolerant of poor water quality; and moderate if they were not restricted to the best sites but were infrequently found at the worst. We did not differentiate if fish were tolerant to one type of degradation but sensitive to another (e.g. Lyons et al. 1995). Abundance and distribution were based on our observations, described below. A species was considered abundant at a site if more than 5 individuals were captured, moderate if 2-4 individuals were captured, and rare if only 1 individual was captured. A species was considered abundant in the Rio Sierpe if it was abundant at one or more sites and moderate at the rest, moderate if was moderate or rare at all sites, and rare if it was rare at all sites. A species was widely distributed if we caught it in 3 more regions, moderately distributed if it was caught in only 2 regions and limited if it was caught in only 1 region.

We also developed a list of marine fish species that have been observed within the fresh, brackish and estuarine waters of the Térraba physiographic region using the same published surveys as above. We did not record their life history information as these species generally are restricted to saline waters or the mainstem and enter freshwaters only temporarily.

Fish distribution

We conducted surveys in the Rio Sierpe to catalog the fish species that are present and to make a preliminary description of their distribution. We conducted fish surveys at the same locations as the macroinvertebrate surveys. We sampled for fish at 15 sites in 5 regions within the Rio Sierpe. At each location we made a number of attempts to capture fish using a variety of techniques. Active techniques included a polyester umbrella net (70 cm x 70 cm with 5mm square mesh), a monofilament cast net (75 cm radius with 12 mm mesh), and a 10 cm aquarium dip net. We also made one unsuccessful attempt to capture fish from the mainstem Rio Sierpe using a 30 meter gill net with three 10 meter panels of 5, 7.5 and 10cm mesh, respectively. One passive technique was tested, a minnow trap made from a 2 liter soda bottle by cutting off the top and reattaching it in the inverted position so that the mouth pointed into the bottle and piercing the bottle to create a number of drain holes. The minnow trap was baited with bread and was fished for up to 30 minutes. Fish caught during the macroinvertebrate surveys were also included. Within a site we sampled all habitat types present in order to obtain a representative sample of the fish assemblage. We sampled until no new species were captured (or until there was no change in the proportional abundance of species), although at some sites sampling was limited due to a lack of appropriate equipment and manpower (site 2, site 15). All sampling was qualitative, not quantitative and the most abundant species were culled so that only a few individuals were retained for data analysis.

Fish were photographed for later species identification and standard length was recorded (nearest mm) before returning fish to the stream alive (sites 1, 1a, 2, 11, 22, 23 and 25), or fish were preserved in isopropanol for later photographing and measuring (sites 11, 12, 13, 14, 16, 17, 18, 19, and 21). Species identification was conducted using William Bussing's 2002 book

(Bussing 2002). Family, genus and species names were taken from FishBase (Froese and Pauly 2009) when there was disagreement with Bussing (2002) and the name given by Bussing was retained in parentheses. Pictures of individuals that could not be identified to species were shared with Dr. William Bussing of the University of Costa Rica in San Jose for species confirmation (still awaiting confirmation). In addition, air dried fin clips brought back to the SWRC were used to confirm the identity of some species. DNA was purified from one individual of the unknown species using the DNeasy kit (Qiagen), and a 500-600 base pair fragment of the cytochrome oxidase subunit 1 (COI) gene was PCR amplified using existing primers and protocols (Ward et al. 2005). Sequencing was performed at the University of Pennsylvania Core Sequencing Facility. We used the Species Identification tool within the Barcode of Life Database (BOLD, www.boldsystems.org) to compared the COI sequence of the unknown species against all available barcode records. The unknown individual was assigned to the species with greater than 99% sequence agreement.

We used the fish capture data to analyze the distribution of species among sites, and to measure the richness within a site and region. We qualitatively analyzed the data to look for trends in distribution with habitat characteristics such as stream barriers, substrate, riparian habitat and forest cover, and dissolved oxygen. In future work, it would be informative to also check for disease, deformities, eroded fins, lesions, and tumors.

Diet, Contaminants and Tolerance Indicator Values

We proposed to explore the possibility of using diet to provide insight into the effects of land use activities on ecosystem integrity in the Rio Sierpe (Winemiller and Layman 2005, de Ruiter et al. 2005). Habitat modification can lead to reductions in important energy sources, for example terrestrial arthropods make up a smaller proportion of fish diets in tropical streams with less riparian cover (Chan et al. 2008). Altering the aquatic food web has the potential to affect the population dynamics of individual species and community stability (Layman et al. 2005). Thus, this initial effort was intended as a pilot study of this novel but promising approach to monitor ecosystem health by focusing on fish and their energy sources. To evaluate this approach, we examined the fish distribution data to determine if the same species occurred in a number of habitats. We noted the differences in habitat to determine if differences in diet should exist.

Contaminants from industry, agriculture or municipal sources pose an additional threat to fish and people. The impact of a contaminant will depend upon the amount and timing of its presence, solubility in water, and half-life or residence time, as well as the mechanism by which the contaminant affects fish. Contaminants such as fertilizers can impact fish by eutrophying the water. Pesticides can reduce prey that make up their diet, and other chemicals can enter the food chain and accumulate in fish tissues. We made a note of the possible sources of contaminants during our surveys of the area. The Rio Sierpe watershed is dominated by agriculture, particularly to the north. The dominant crops are palm oil, rice, banana, teak, and there is some cattle grazing. Unfortunately, there is presently no master list of agricultural pesticides being used in the watershed which could help guide future work intended on focusing on likely candidates that may be contaminating the Rio Sierpe.

We also proposed to investigate the feasibility of identifying attributes of the environment that affect species distributions. We proposed to explore the use of tolerance indicator values (TIV's) for certain chemical and physical characteristics to identify abiotic factors to which a species is most sensitive (Meador and Carlisle 2007). The procedure involves testing for correlations between a species natural distribution or abundance and attributes of the habitat such as chemical concentrations, riparian habitat, or species abundance. In order to develop TIV's it is necessary to sample a range of habitats, and a number of attributes within each habitat (REF). For TIV's to be valid we must be certain that the species would be present if not for the abiotic factor. Therefore we must identify sites that are not isolated by an impassible barrier or poor habitat downstream.

Table 1: Sites visited in the Rio Sierpe (and Terraba River) to survey water chemistry, aquatic macroinvertebrates, and fish during April 2009. Sites are organized by their "region" within the Sierpe watershed. Percent tree canopy shading the river and channel width at baseflow were visually approximated in the field. Latitude and longitude were determined using a Garmin 60csx GPS.

Region	Site	Site description	Latitude	Longitude	Approx. elevation (m a.s.l.)	Canopy %	Approx. channel width (m) at baseflow	Riparian land use	Field chem.	Lab chem.	Invertebrates	Fish	Sediment
Terraba	20	T. Terraba	8.95533	-83.4779	8.0	0	45	Village, pasture, open land	y	y	y	y	
Mangrove	4	Above Shrimp Farm outlet	8.93864	-83.5715	1.5	1	30	Shrimp farm on TLB, Palm on TRB	y	y			
Mangrove	5	Below Shrimp Farm outlet	8.9472	-83.5844	1.0	1	30	Shrimp farm on TLB, Palm on TRB	y	y			
Mangrove	101	Sierpe Mouth	8.89727	-83.5863	1.0	1	300	Mangrove	y				
Mangrove	7	Above Shrimp Farm intake	8.92788	-83.5598	1.5	20	20	Shrimp farm on TRB, Rice paddys on TLB	y	y			
Mangrove	7A	Bank Sediment Grab near Rice Field	8.91736	-83.5655	6.0	.	.	Dry rice paddy					y
EstuaryInfluence	2	Estero Azul	8.90762	-83.4803	5.0	0	3	Palm plantation	y	y	y	y	y
EstuaryInfluence	2B	Trib. Estero Azul	8.90859	-83.4799	4.0	0	3	Palm plantation			y		
SierpeMainstem	3	R. Sierpe below Sierpe Village	8.86284	-83.4737	2.5	0	150	Village, palm, native forest	y	y			
SierpeMainstem	6	R. Sierpe	8.82078	-83.3923	6.8	10	40	Pasture, palm plantation	y	y			
SierpeMainstem	8	Espero Ollia	8.82278	-83.3943	6.8	5	30	Pasture, ranch, palm plantation	y	y			
SierpeMainstem	9	R. Taboga downstream	8.80862	-83.4027	6.0	40	10	Pasture, ranch	y	y			
SierpeMainstem	10	R. Chacuaco	8.82714	-83.435	3.3	40	20	Native forest, pasture	y	y			
SierpeMainstem	10A	Sierpe Mainstem	8.83121	-83.4336	4.5	.	.	Native forest, pasture					y
SierpeMainstem	102	Sierpe Mainstem	8.85576	-83.4601	3.0	1	60	Pasture, palm plantation, ranch	y				
SierpeMainstem	103	Sierpe Mainstem	8.84781	-83.4394	3.2	1	60	Pasture, palm plantation, ranch	y				
SierpeMainstem	104	Sierpe Mainstem	8.83048	-83.4332	3.3	1	60	Pasture, palm plantation, ranch	y				
SierpeMainstem	105	Sierpe Mainstem	8.82818	-83.4157	3.4	1	60	Pasture, palm plantation, ranch	y				
SierpeMainstem	106	Sierpe Mainstem	8.80966	-83.4062	4.9	1	60	Pasture, palm plantation, ranch	y				
SierpeMainstem	107	Sierpe Mainstem	8.81856	-83.3998	6.2	1	60	Pasture, palm plantation, ranch	y				
SierpeMainstem	108	Sierpe Mainstem	8.82129	-83.394	6.8	1	60	Pasture, palm plantation, ranch	y				
Southern Tributaries	1	Quebrada Tomo Agua -drinking water for Sierpe	8.84568	-83.4769	64.6	65	0.4	Native forest	y	y	y	y	
Southern Tributaries	15	Trib Quebrada Sabalo	8.7643	-83.5017	20.0	50	2	Village, pasture	y	y			
Southern Tributaries	16	R. San Juan	8.75918	-83.5132	28.0	20	5	Pasture on TLB, native forest on TRB	y	y	y	y	
Southern Tributaries	16A	R. San Juan downstream	8.75997	-83.511	27.0	20	5	Pasture on TLB, native forest on TRB			y		
Southern Tributaries	17	Trib to Quebrada Machaca	8.74324	-83.4975	37.0	60	1	Palm and teak on TRB, native forest on TLB	y	y	y	y	
Southern Tributaries	18	Quebrada Machaca	8.74121	-83.4963	41.0	80	1.5	Palm plantation	y	y	y	y	
Southern Tributaries	19	R. Taboga upstream	8.78098	-83.3755	39.0	90	4	Native forest with pasture on TLB next to 10 m buffer	y	y	y	y	
SierpeHeadwaters-South	11	R. Sierpe u/s R. S. Nuevo	8.80396	-83.3225	7.5	10	3	Bannana, pasture	y	y	y	y	
SierpeHeadwaters-South	11A	R. Sierpe d/s R. S. Nuevo	8.80433	-83.3232	7.5	10	3	Bannana, pasture	y		y		y
SierpeHeadwaters-South	25	R. Sierpe headwater	8.78035	-83.2686	21.0	40	2	Palm plantation	y	y	y	y	
SierpeHeadwaters-South	25A	Trib1 to R. Sierpe headwater	8.77986	-83.2685	9.0	50	2	palm and emergent wetland vegetation	y				
SierpeHeadwaters-South	25B	Trib2 to R. Sierpe headwater	8.77986	-83.2685	9.0	100	1	Palm plantation	y				
SierpeHeadwaters-North	12	R. Salama Nuevo	8.80437	-83.3228	7.6	5	7	Palm and bannana plantation	y	y	y	y	
SierpeHeadwaters-North	13	R. Salama Viejo downstream	8.81838	-83.2933	19.0	5	5	Pasture, palm plantation	y	y	y	y	
SierpeHeadwaters-North	14	R. Salama Viejo upstream	8.83091	-83.274	61.6	75	3	Native forest	y	y	y	y	y
Northern Tributaries	21	Trib to R. Culebra	8.92377	-83.4121	22.0	5	2	Palm plantation on TRB, grass on TLB	y	y	y	y	
Northern Tributaries	22	R. Culebra upstream	8.92177	-83.4112	21.0	5	2	Pasture	y	y	y	y	
Northern Tributaries	23	R. Culebra downstream	8.90945	-83.4377	10.0	100	4	Bamboo, rice paddy, palm and bannana	y	y	y	y	y
Northern Tributaries	24	Field Drain Outlet to R. Culebra downstream	8.90969	-83.4377	10.0	50	0.2	Drainage ditch from rice paddies	y	y			

III. Results

A. Sites by Geographic Relationships

Thirty-nine sites in the Sierpe region were visited in April 2009. Sites could be descriptively separated into eight different regions: *mangrove channels, Sierpe mainstem, estuary-influenced tributaries, northern tributaries, Sierpe headwaters north, Sierpe headwaters south, southern tributaries, and a site on the mainstem of the Térraba River* (Table 1). *Mangrove channel sites* were located above and below a shrimp farm operation (not active during visit) and were only sampled for water chemistry (with a sediment sample collected from one site). Thirteen sites located along the *Sierpe mainstem* (or on a tributary ~100 m upstream from the confluence) were influenced by tidal fluctuations and were only sampled for water chemistry (again with a sediment sample collected from one site). The two *estuary-influenced tributary sites* (2 &2B) were located within a palm plantation and were obviously tidal since water hyacinth was observed floating upstream and downstream during tide cycles. Site 2 was sampled for water chemistry, invertebrates, and fish and site 2B only for invertebrates. Of the four *northern tributaries*, two sites (21 &22) were located upstream from the Inter-American Highway (Hwy. 2) and two sites below (23 & 24). All of these sites drained an upper watershed that has been partially deforested. Site 24 was a drainage ditch that serviced primarily rice fields. The rice fields were not flooded/saturated during our visit but the drain was delivering a small amount of highly colored water (i.e., higher dissolved organic carbon load than the stream). *Sierpe headwaters* were divided into north and south regions with three and five sites in each region, respectively. One of the *northern headwaters*, site 14 – Rio Salama Viejo, drained a steep watershed with primarily native forest and also served as a water supply for a nearby community. The other two northern headwater sites were below the Inter-American Hwy. The five *southern headwater sites* all drained a low gradient, agriculturally influenced area dominated by palm and banana plantations and wetland. The seven *Southern tributaries* had the greatest geographic range among regions and were all located on the Osa Peninsula side of the Sierpe watershed. Two of these sites drained predominantly forested watersheds with the remaining sites having variable levels of pasture, palm, and teak plantations in their watersheds. The single site on the *mainstem of the Térraba River* was only sampled for water chemistry in order to characterize this nearby water source. Some Térraba River water is transferred into the lower Sierpe watershed presumably for agricultural purposes. Most of those transfers appear to deliver water either to the Estero Azul or directly to the mangrove channels.

B. Water Chemistry

Mangrove channels

Water chemistry samples were collected from three locations in the Térraba-Sierpe National Wetlands to assess the impact of a local shrimp farm on water quality. At the time of our visit the shrimp farm was inactive. We used this opportunity to collect samples to develop a baseline understanding of water quality from locations above the intake facility (site 7), above the

Table 2: Temperature and chemical conditions at 3 sites within the Terraba-Sierpe National Wetlands (mangrove). Samples were taken during low tide. Note: shrimp farm was not active during sampling. Units are mg/l unless otherwise noted.

	Mangrove			[a]Typical Seawater
	7 - Above Shrimp Farm intake	4 - Above Shrimp Farm outlet	5 - Below Shrimp Farm outlet	
Temperature (^{o}C)	30.6	30.7	31.0	
Sp. conductivity (mS/cm)	44.7	42.1	37.0	
pH (pH units)	7.2	7.4	7.4	
Dissolved oxygen (%)	65.9	78.0	77.1	
Dissolved oxygen	4.2	5.0	5.1	
Ammonium-NH^{+}_{4}	0.055	0.480	0.233	
Calcium-Ca^{+2}	474.8	311.8	263.0	410
Magnesium-Mg^{+2}	1308.0	805.0	583.8	1290
Potassium-K^{+}	346.8	204.9	151.7	390
Sodium-Na^{+}	9541.3	5765.7	4320.1	10,760
Bromide-Br^{-}	61.7	55.9	39.9	67
Chloride-Cl^{-}	16403.8	15263.8	8719.7	19,350
Nitrate-NO^{-}_{3} –N	0.854	0.928	0.911	
Nitrite-NO^{-}_{2} –N	6.22	5.11	1.84	
Sulfate-SO^{-2}_{4}	2263.9	2085.0	1432.5	2710
Total suspended solids	129.3	139.5	125.1	

[a] – Duxbury and Duxbury. 1997. An introduction to the World's Ocean. 5th edition. WCB Publishers, Dubuque, IA. p 150. And http://www.seafriends.org.nz/oceano/seawater.htm

outflow (site 4) and below the outflow (site 5). Water temperature, specific conductivity, pH, dissolved oxygen, major ions, and total suspended solids were similar at all three sites (Table 2). The site above the shrimp intake was similar in ionic composition to seawater (Table 2), with chloride and sodium dominant. The two sites to the northwest of the shrimp farm were both brackish with sodium chloride concentrations at approximately half of that found in seawater. Dissolved organic carbon was not measured.

Rio Sierpe Lower Mainstem

A datalogging conductivity, salinity, and temperature probe was suspended for ~12 hours from a floating dock (at ~ 3m depth) at the village of Sierpe to determine the tidal influence on salt content in the main channel at that point along the rivers mainstem (Fig. 3). The 12 hour tidal pulse resulted in salinity concentrations ranging from close to freshwater (< 1 g/l salinity; <1.7 ms/cm) to brackish water (9.9 g/l salinity; 17.0 ms/cm) with salt and conductivity concentrations indicating about a 30% seawater influence (seawater is 35 g/l and 48 ms/cm).

On 6 April 2009, a boat was hired to access 12 sites along the Rio Sierpe mainstem to collect water samples and directly measure salinity and specific conductance of stream water during high tide. The purpose was to determine the upstream extent of saltwater influence along the mainstem (Fig. 4). Specific conductivity exceeded 4 mS/cm (3 mS/cm is generally considered the limit of "freshwater") for the first 27 river km from the mangrove mouth. At river-km 30 specific conductivity at depths -0.5, -5, and -10 m was 1.9 mS/cm with conductance continuing to decline to 0.372 mS/cm at river-km 38 (our upstream most site during this excursion). Thus, the

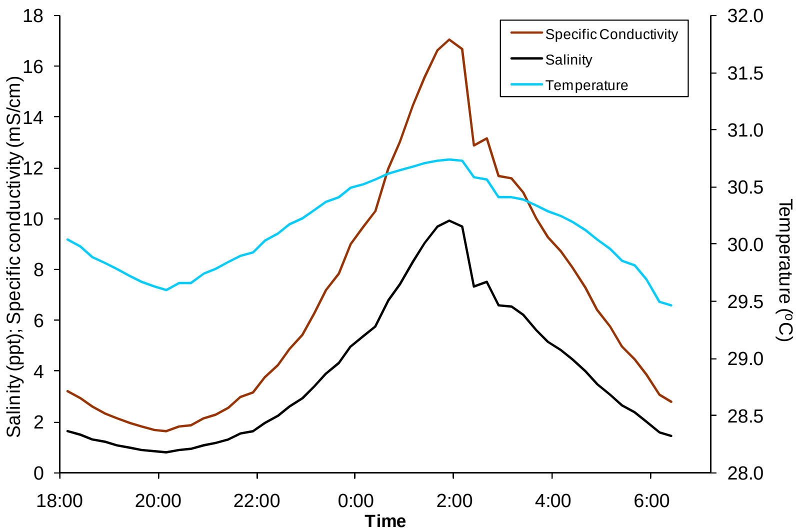

Figure 3: Salinity (black line; left y-axis), specific conductivity (brown line; left y-axis), and temperature (blue line; right y-axis) recorded at 5 minute intervals from 18:00 hrs on 5 April 2009 until 06:25 hrs on 6 April 2009 by a YSI datalogging water quality meter suspended from a dock (depth ~3m) in the Sierpe River at the Village of Sierpe (latitude 8.858278°, longitude -83.472072°). Fluctuations are caused by one tidal cycle.

upstream extent of saltwater influence was greatly diminished between river-kms 30-40 at points where 3 of the catchments larger tributaries meet the Rio Sierpe.

One water sample for analysis of major ions, dissolved organic carbon (DOC), and total suspended solids (TSS) was collected from the Rio Sierpe's lower mainstem during low tide (8 am on 6 April 2009 at a point just downstream from the village of Sierpe; Site 3; Table 3). Major ions (Ca, Mg, K, Na, Br, Cl, SO_4), like specific conductivity, indicated that the river water during low tide was close to freshwater. However, concentrations of sodium (Na) and chloride (Cl) were slightly elevated and indicated seawater influence compared to upland freshwaters. Ammonium concentrations at site 3 were the highest measured during this study. Although the measured concentration of ammonium (1.21 mg/l) was not "excessive", it was 2 times greater than for any other sample and it does indicate the probable influence of human waste water in the Sierpe mainstem.

Temperature, pH, specific conductivity, major ions, and total suspended solids tended to increase from the Rio Sierpe headwaters to near the mouth of the river. Seawater influence was, of course, a major cause increasing conductivity and ions at the site near the mouth. The lower section of the Sierpe mainstem was highly turbid due to a high suspend material load. We did not quantify either the mineral:organic ratio or the quantity of chlorophyll (an

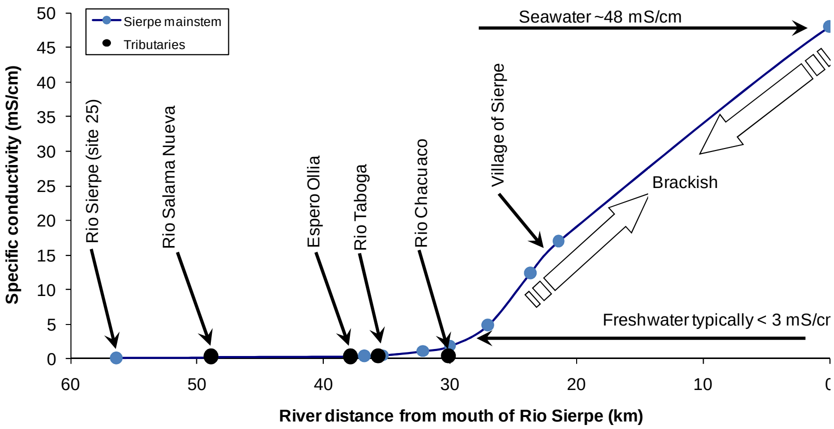

Figure 4: Specific conductivity measured at 0.5 m deep at 12 sites along the R. Sierpe mainstem (and major tributaries) during a boating expedition at or near high tide (10:00-15:00 hrs) on 6 April 2009. River distances were measured from near the mouth of the Sierpe mangrove using ARC GIS and Google Earth with field collected GPS locations delineating sampling sites. Specific conductivity of the water delivered by tributaries was measured ~ 100 m upstream from their confluences with Rio Sierpe.

Table 3: Temperature and chemical conditions along Rio Sierpe mainstem sites, in tributaries (sampled near their confluences with the Sierpe), at in the Rio Terraba in April 2009. River distances (km) were determined using GIS and are channel distances from the river mouth. Units are mg/l unless otherwise noted. U/S = upstream; D/S = downstream; NR = not recorded; BD = below instrument detection limit.

	Rio Sierpe mainstem				Tributaries (near Sierpe confluence)				
	25	11	6	3	12	8	9	10	20
	Head-water	U/S S. Nueva	U/S E. Ollia	D/S Village	Salama Nueva	Espero Ollia	Rio Taboga	Rio Chacuaco	Rio Terraba
	9 April	7 April	6 April	6 April	7 April	6 April	6 April	6 April	9 April
River distance (km)	56.4	48.9	38.0	21.4	48.9	37.9	35.7	30.1	20.0
Temperature (^{o}C)	27.4	28.6	29.1	29.4	29.0	29	29	29.5	30.3
Sp. conductivity (uS/cm)	192	227	372	1913	387	372	402	444	197
pH (pH units)	6.51	6.73	7.34	7.35	7.85	7.39	7.30	7.22	8.78
Dissolved oxygen (%)	2.05	0.55	NR	5.11	7.53	NR	NR	NR	11.51
Dissolved oxygen	26	7	NR	67	98	NR	NR	NR	153
Dissolved organic carbon	3.4	4.1	2.7	2.2	1.5	2.7	2.6	2.6	1.3
Ammonium-NH^{+}_{4}	0.08	BD	BD	1.21	BD	BD	BD	BD	BD
Calcium-Ca^{+2}	25.9	27.9	16.9	56.0	66.3	15.8	12.8	19.8	19.3
Magnesium-Mg^{+2}	2.5	4.4	7.0	45.0	1.2	6.5	4.1	9.1	1.8
Potassium-K^{+}	0.78	0.73	1.83	14.4	1.42	1.80	1.24	2.35	1.30
Sodium-Na^{+}	6.1	7.1	36.3	500.5	13.4	36.0	26.8	43.7	8.7
Bromide-Br^{-}	0.17	0.18	0.24	0.11	0.18	0.19	0.26	0.31	0.19
Chloride-Cl^{-}	0.8	3.1	46.6	523.2	1.7	48.5	56.6	65.4	1.9
Nitrate-NO^{-}_{3} –N	0.044	0.005	0.090	0.022	0.014	0.013	0.011	0.013	0.153
Nitrite-NO^{-}_{2} –N	0.285	0.431	0.273	0.952	0.681	0.394	0.275	0.337	0.261
Sulfate-SO^{-2}_{4}	16.4	4.6	8.3	57.7	19.8	10.0	10.6	11.2	9.1
Total suspended solids	6.4	4.4	14.6	45.5	4.0	16.4	22.6	17.2	19.3

indication of the amount of algae) in the suspended load. Dissolved organic carbon was highest in the headwaters (at Sites 11 and 25). Both of those sites also had very low concentrations of dissolved oxygen (DO). Low DO concentrations typically develop in response to high biological oxygen demand (BOD) usually driven by high carbon concentrations in slow flowing systems (i.e., re-aeration at the air-water interface is limited by laminar flow conditions). These conditions can occur naturally in lowland streams dominated by wetlands but they can also be an indication of human-derived materials being added to a stream. Low DO can also negatively affect aquatic macroinvertebrate and fish communities and select for organisms that are tolerant of these conditions. Further investigation is needed to resolve the cause of low DO at these sites.

Watershed-Scale Chemical Patterns

Specific conductivity, pH, dissolved oxygen (Fig. 5), major ions (Fig. 6), dissolved organic carbon, and total suspended solids (Fig. 7) are summarized for all freshwater sites in subsequent figures where sites are grouped by geographic regions. Stream water collected from the various geographic regions within the R. Sierpe watershed had distinct chemical differences. Northern tributaries and the northern Sierpe headwaters had high specific conductivity (Fig. 5) and higher concentration of major ions (Fig. 6) compared to the Sierpe southern headwaters and southern tributary sites. Ion chemistry differences were primarily due to higher concentrations $CaSO_4$

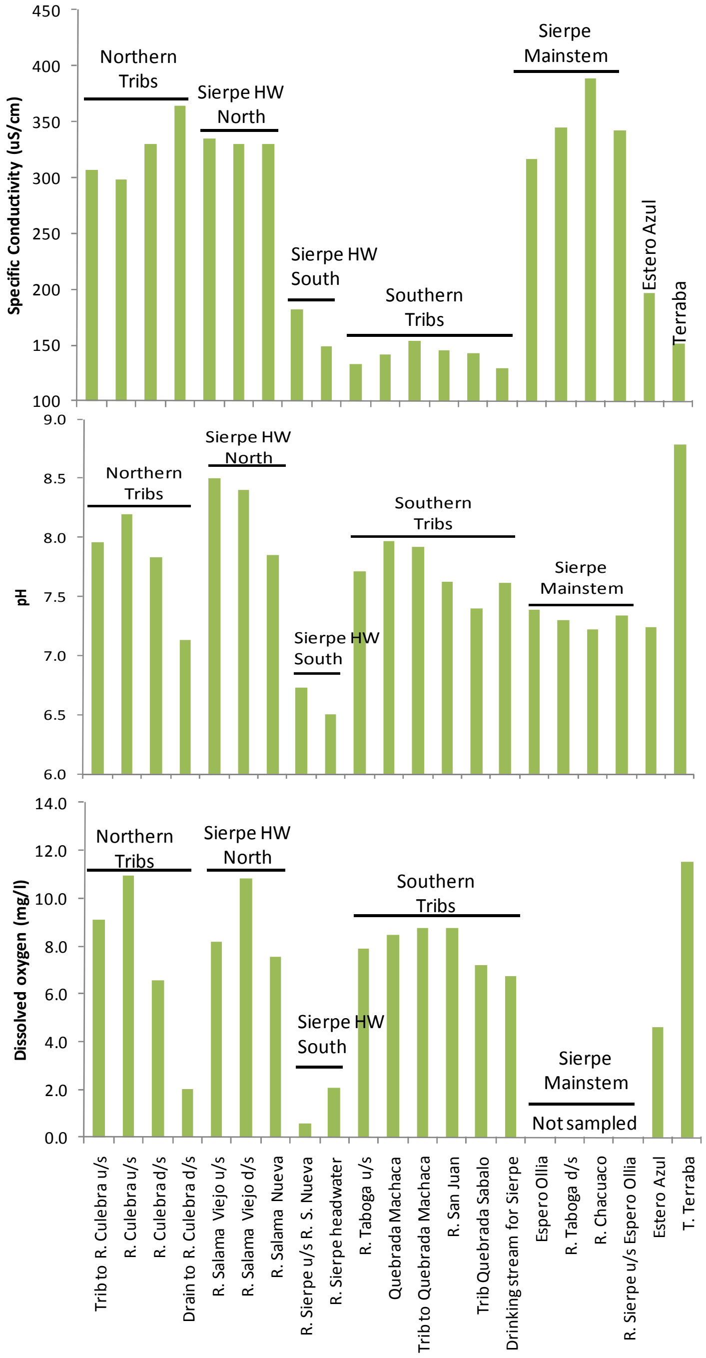

Figure 5: Specific conductivity, pH, and dissolved oxygen concentrations for all freshwater sites visited in the Sierpe watershed by geographic region and for the R. Terraba (adjacent river to the north).

(gypsum) at northern tributary sites. Ionic differences may be related to geological differences and or systematic differences in weathering rates or human land disturbance. Dissolved organic carbon (DOC) and total suspended solids (TSS; Fig. 7) were also consistently lowest at southern tributary sites. DOC was high in the southern headwater sites (previously mentioned) but was even higher in a small road ditch drain adjacent to rice fields (dry at the time of sampling) that delivered flow to Site 23 (Rio Culebra). DOC and TSS differences may be related to land cover differences such as percentage of wetland, agriculture, or other human land disturbances in the watershed or may be due to point source discharges. Further investigation is needed to resolve these potential causes.

As previously noted, Rio Sierpe lower mainstem sites as far inland as 35-40 river-km from the mouth were influenced by tidal motion but the influence of seawater was greatly reduced starting at about 30 river-km from the mouth. Despite this, Site 6 (R. Sierpe u/s Espero Ollia) on the R. Sierpe and sites 8, 9, 10 (Espero Ollia, R. Taboga d/s, and R. Chacuaco) sampled ~100 m from their confluences with the R. Sierpe all had ion chemistries dominated by NaCl (sodium chloride) and most likely related to seawater intrusion carried by spring tides and storm pulses. These preliminary analyses can be used to plan a more thorough investigation (involving sediment grab samples) into potential toxic contaminants such as pesticides and heavy metals.

Future Directions

A more detailed analysis of the relationships between nutrient and ion chemistry and land cover/use appears warranted. A more thorough sampling campaign, including dry and wet seasons would be necessary to better understand both the spatial and temporal patterns of stream chemistry and the potential chemical threats to the health and integrity of the ecological health of both the Sierpe River and the mangrove wetland.

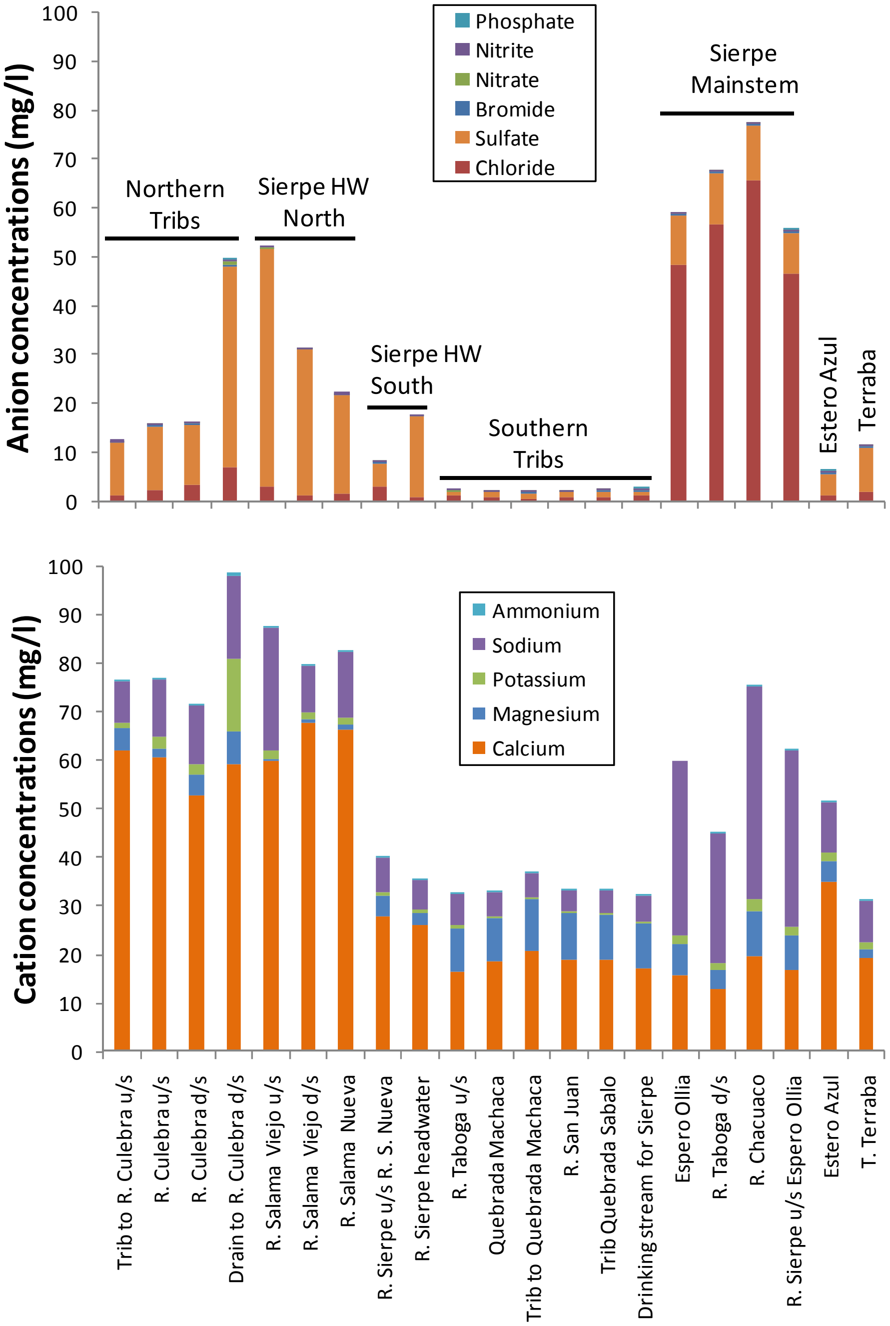

Figure 6: Anion and cation concentrations for all freshwater sites visited in the Sierpe watershed by geographic region and for the R. Terraba (adjacent river to the north).

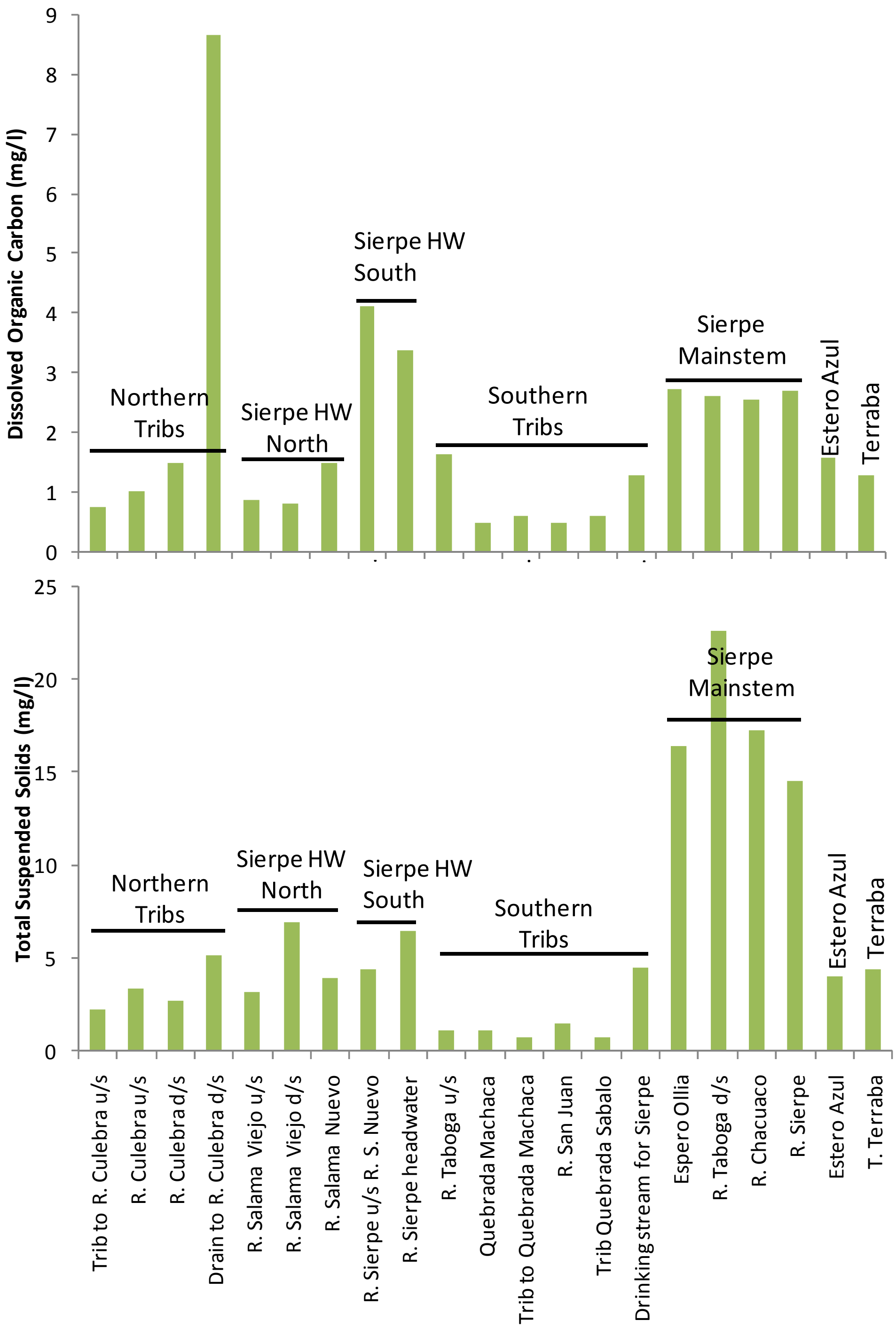

Figure 7: Dissolved organic carbon and total suspended solids concentrations for all freshwater sites visited in the Sierpe watershed by geographic region and for the R. Terraba (adjacent river to the north).

C. Aquatic Macroinvertebrates

The SWRC has collected macroinvertebrates from numerous sites located on the Osa Peninsula. SWRC has also organized and lead public workshops on the Osa Peninsula on the use of aquatic macroinvertebrates for monitoring stream ecosystem health. The past work lead to a short peer-reviewed publication describing mayfly communities on the Peninsula (Sweeney et al. 2009). The work describe here has increased our understanding of the distribution of aquatic invertebrates in the region even further.

Four thousand and seven macroinvertebrate individuals were collected from 17 sites using the kick net method. Individuals were identified into 106 different taxonomic groups (Table 4). Identifications of individuals were carried to the lowest practical level of resolution and resulted taxonomic groups at the following levels: 9 to species, 66 to genus, 26 to family, and 6 to order. For each taxonomic unit listed in Table 4 the number of individuals collected and their ubiquity (number of sites where present). Of the most ubiquitous taxa, chironomids (non-biting Diptera midges) were collected from 15 sites (88% of sites), freshwater palaemonid shrimp were collected from 14 sites, the small water strider (also known as a riffle bug) *Rhagovelia* spp. was collected from 13 sites, and three mayfly (*Americabaetis* spp., *Cloeodes* spp., *Tricorythodes* spp.) and one caddisfly taxon (Hydropsychidae) were collected from 12 sites.

Macroinvertebrate richness (i.e., number of taxa) associated with three groupings of data (EPT, Other Insects, Diptera & Non-Insects) is illustrated for each site in Fig. 8. EPT richness and Other Insect richness were significantly correlated ($p < 0.001$; $r = 0.88$). Diptera & Non-Insect richness was unrelated to either EPT or Other Insect Richness ($p = 0.45$). Greatest invertebrate richness was observed at a Northern Tributary site (Site 21, a tributary to the Rio Culebra upstream from Hwy 2) and a Sierpe Headwater North site (Site 13, Rio Salama Viejo downstream from Hwy 2). Interestingly, these two sites were not the “most pristine” or “least impacted” sites we visited. Site 21 had substantial riparian clearing, was adjacent to a Palm Plantation, drained a partially deforested watershed, and had visually obvious algal growth (filamentous green algae). Site 13 also had substantial riparian clearing, was below the Inter-American Hwy., but did have a predominantly forested upper watershed. Reasons for the apparent increase in richness at these sites were not entirely clear. However, in watersheds with a slight to moderate mix of open and forested land adjacent to the stream there is the potential to enhance richness by enhancing the area of stream habitat exposed to direct sunlight thereby increasing algal productivity and standing stock and thus increasing the availability of algal food resources for grazing invertebrates. If the increased algal production is not excessive (thus preventing invertebrate grazing simply due to its size and shape) and other changes to the stream habitat do not extirpate pre-existing species, then an overall enhancement in the number of species in a stream reach may be the result. Further investigations are necessary in the Sierpe region to provide a more complete inventory of the regional species pool and add additional quantitative information on local species richness (e.g., within a stream reach), watershed land use, and instream physical and chemical conditions.

Lowest richness was observed at Site 11 (R. Sierpe just upstream from R. Salama Viejo) at in a tributary to Estero Azul and Estero Azul (Sites 2B and 2, respectively). Both sites 2 and 2B were influence by tide water fluctuation and, as evidenced by the near absences of aquatic insects, were likely to be influenced by seawater resulting in variable saline conditions. Aquatic insects that respire primarily through gills are highly sensitive to saline conditions. Only three aquatic insect taxa were collected at Site 2B (two Hemiptera and one Coleoptera) and all three taxa were "air breathers", visiting the surface at regular intervals to re-oxygenate their air sacks. The very low richness at Site 11 was probably related to the observed low oxygen concentration (7 % of saturation; Table 3 and Fig. 5). Only 3 taxa were collected at Site 11, a bivalve mollusk (pea or fingernail clam) and two dipterans (a chironomid "bloodworm" and a ceratopogonid or biting midge). Bloodworms contain hemoglobin and are able to tolerate very low dissolved oxygen conditions.

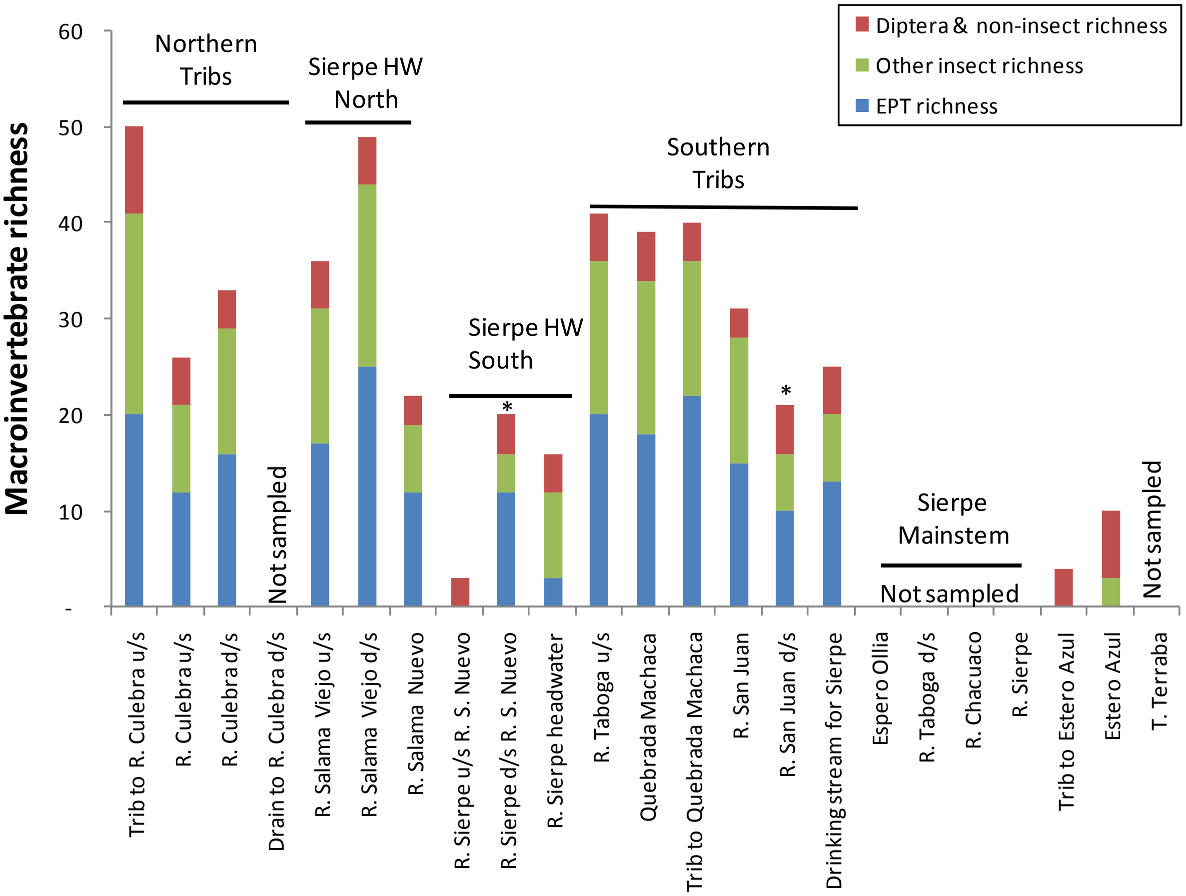

Figure 8: Richness of aquatic macroinvertebrate community collected at each site. Richness for EPT taxa (blue bars; Ephemeroptera=mayflies, Plecoptera=stoneflies, Trichoptera=caddis flies), other insects (green bars; Coleoptera, Odonata, Hemiptera, Megaloptera, Lepidoptera), and Diptera + non-insects (red bars; Gastropoda=snails, Bivalvia =clams, Acari=mites, Amphipoda and Isopoda=scuds, Hirudinea=leeches, Oligochaeta=worms, and Atyidae and Palaemonidae=freshwater shrimp). Asterisks denote sites sampled for macroinvertebrates but not water chemistry (i.e., not shown in water chemistry figures). Sites where macroinvertebrates were not sampled were included to maintain consistency with previous figures.

Future directions

This preliminary investigation of aquatic macroinvertebrates provides a foundation from which biomonitoring programs and macroinvertebrate based education programs [like Leaf Pack programs (http://www.stroudcenter.org/lpn/studies.htm)] can be planned. Developing robust metrics using aquatic macroinvertebrates requires an understanding of the sensitivity and tolerance of organisms within the region and an accurate quantification of the taxa present at sites ranging from natural or quasi-natural to severely degraded. It is now clear that the Rio Sierpe watershed contains streams spanning that gradient of environmental conditions and that a wide variety of macroinvertebrate taxa occur in the watershed and could, with more effort (especially quantification) be used as a basis for identifying key conservation issues to provide guidance for conservation priorities as well as a measure of its success. Future work should focus on developing quantitative approaches for both professional and lay monitoring of water quality using macroinvertebrates and for improving the taxonomy of key groups (like chironomids) which had to be left at family level in the present study due to limited resources.

Table 4: Aquatic macroinvertebrate taxon list, number of individuals collected and ubiquity.

Phylum **Class** **Order** **Family**	**Taxon**	**Individuals collected**	**Ubiquity (# of sites)**
Arthropoda			
Insecta			
Coleoptera			
Curculionidae	Undet.	2	**1**
Dryopidae	*Helichus* spp.	1	**1**
	Pelonomus spp.	3	2
Dytiscidae	Undet. (A & L)	3	**2**
Elmidae	*Disersus* spp.	1	**1**
	Heterelmis spp.	1	**1**
	Hexacylloepus spp.	6	6
	Macrelmis spp. (A & L)	11	4
	Neocylloepus spp. (L)	4	4
	Neoelmis spp. (L)	1	**1**
Gyrinidae	*Gyretes* spp. (A)	8	**1**
Hydrophilidae	Undet. (A)	5	5
Limnichidae	Undet.	3	3
Noteridae	Undet.	1	**1**
Psephenidae	Undet.	95	10
Ptilodactylidae	*Anchytarsus* spp.	16	4
Scirtidae	Undet.	1	**1**
Diptera			
Ceratopogonidae	Undet.	3	3
Chironomidae	Undet.	266	**15**
Culicidae	Undet.	8	3
Simulidae	Undet.	38	6
Stratiomyidae	Undet.	1	**1**
Tabanidae (P)	Undet.	1	**1**
Tipulidae	Undet.	29	11

Phylum Class Order Family	**Taxon**	**Individuals collected**	**Ubiquity (# of sites)**
Ephemeroptera			
Baetidae	*Americabaetis* spp.	147	**12**
	Apobaetis spp.	1	**1**
	Baetodes noventus	40	7
	Callibaetis spp.	50	3
	Camelobaetidius spp.	23	7
	Cloeodes spp.	74	**12**
	Fallceon spp.	90	10
	Guajirolus nanus	38	6
	Paracloeodes spp.	3	2
	Varipes spp.	1	**1**
Caenidae	*Caenis* spp.	39	9
Euthyplociidae	*Euthyplocia hecuba*	5	**1**
Leptohyphidae	*Allenhyphes* spp.	3	2
	Cabecar serratus	14	3
	Epiphrades undatus	32	9
	Hagenulopsis spp.	2	2
	Leptohyphes spp.	309	11
Arthropoda			
Insecta			
Leptohyphidae	*Tricorythodes* spp.	178	**12**
	Vacuperinus packeri	137	8
Leptophlebiidae	*Farrodes* spp.	102	10
	Terpides spp.	55	11
	Thraulodes spp.	222	9
	Tikuna atramentun	1	**1**
	Ulmeritoides acosa	38	3
Hemiptera			
Corixidae	Undet. (L)	2	**1**
Gerridae	Undet. (L)	3	3
Hebridae	*Hebrus* spp.	1	**1**
Mesoveliidae	*Mesovelia* spp.	1	**1**
	Mesoveloidea spp.	4	**1**
Naucoridae	*Ambrysus* spp. (L)	28	8
	Cryphocricos spp.	20	4
	Limnocoris spp.	34	6
Velidae	*Microvelia* spp.	3	**1**
Veliidae	*Rhagovelia* spp.	65	**13**
Lepidoptera			
Pyralidae	Undet. (L)	9	2
Megaloptera			
Corydalidae	Undet. (L)	35	7
Odonata			
Calopterygidae	*Hetaerina* spp.	45	9
Coenagrionidae	*Acanthagrion* spp.	1	**1**
	Argia spp.	209	11
	Enallagma spp.	60	9

Phylum Class Order Family	Taxon	Individuals collected	Ubiquity (# of sites)
	Leptobasis vacilans	3	3
	Nehalenia spp.	5	2
Gomphidae	*Epigomphus* spp.	1	**1**
	Erpetogomphus spp.	6	3
	Phyllocycla spp.	2	**1**
	Phyllogomphoides spp.	4	4
	Progomphus spp.	2	**1**
Libellulidae	*Brachimesia* spp.	2	**1**
	Brechmorhoga spp.	21	6
	Dythemis spp.	9	2
	Macrothemis spp.	1	**1**
	Palthotemis spp.	12	**1**
	Perithemis spp.	25	4
Megapodagrionidae	*Heteragrion* spp.	45	6
Perilestidae	*Perrisolestes* spp.	1	**1**
Platysticitidae	*Palaemnema* spp.	21	6
Polythoridae	*Cora* spp.	1	**1**
Arthropoda			
Insecta			
Plecoptera			
Perlidae	*Anacroneuria* spp.	90	7
Trichoptera			
Calamoceratidae	*Phylloicus* spp.	22	5
Glossosomatidae	Undet. (L)	77	6
Helicopsychidae	*Helicopsyche* spp.	15	5
Hydropsychidae	*Macronema* spp.	21	4
	Undet. (L)	574	**12**
Hydroptila	*Hydroptila* spp.	2	2
Leptoceridae	*Nectopsyche* spp.	28	7
Neotrichia	*Neotrichia* spp.	1	1
Philopotamidae	*Chimarra* spp.	183	11
Polycentropodidae	*Polycentropus* spp.	4	2
Xiphocentronidae	*Xiphocentron* spp.	1	**1**
Arachnida			
Acari			
Undet.		1	**1**
Malacostraca			
Amphipoda			
Undet.		18	2
Decopoda			
Atyidae		23	6
Palaemonidae		55	**14**
Isopoda		3	**1**
Annelida			
Clitellata			
Hirudinea		3	**1**
Oligochaeta		10	3

Phylum Class Order Family	**Taxon**	**Individuals collected**	**Ubiquity (# of sites)**
Mollusca			
Bivalvia			
Veneroida			
Sphaeriidae		8	3
Gastropoda			
Neritimorpha			
Pulmonata			
Neritidae	*Neritina* spp.	16	2
Ancylidae		2	2
Sorbeoconcha			
Planorbidae		21	2
Thiaridae		35	3

D. Fish

Fish of the Térraba Physiographic Region
A handful of fish surveys have been undertaken in the Térraba physiographic region. Bussing (2002) examined two sites in the Rio Sierpe and a number of sites in the Térraba physiographic region. Chicas (2001) examined fish in the Sierpe estuary, Winemiller (1983) and Winemiller and Morales (1989) examined fish in Cocovado National Park on the Osa Peninsula, and Rojas and Rodríguez (2008) examined four sites in the Rio Térraba . An earlier summary of fish of Central American did not differentiate rivers of Costa Rica (Miller 1966) and so was not included.

The Rio Sierpe is very near the southern extreme of the Chiapas-Nicaraguense fish province (Miller 1966). This province is defined by fish species with a similar geographic distribution and includes the Pacific side of Central America from the Osa Peninsula and Golfo Dulce in Costa Rica north to Chiapas, Mexico. Many species in this region are endemic, that is they are found nowhere else in the world. There are 41 primary, secondary, and peripheral fish species representing 14 families in the Térraba physiographic region, of which 22 species have been previously observed in the Rio Sierpe (Table 5). During our surveys we found an additional 9 species in the Rio Sierpe, 8 of which had been previously observed in the Térraba region. At this point there are 31 freshwater fish species in the Rio Sierpe and 10 additional species in the Térraba region that have not been found in the Rio Sierpe. These 10 species may have limited distributions in which case they do not occur or are rare in the Rio Sierpe. We did not include in our list three species identified by Rojas and Rodriguez (2008) in the Rio Térraba because these species were not observed by Bussing in this region, although similar appearing congeners are known to be present in the Rio Térraba. The species that were excluded are *Poecilia mexicana, Poeciliopsis elongata* and *Amphilophus (Astatheros) altifrons*.

Almost all of the fish species in the Rio Sierpe are native to Costa Rica and 10 species are endemic to Costa Rica. The species *Leptophilypnus panamensis* is considered native by FishBase and an earlier survey (Miller 1966), although it was not identified by the recent surveys in the Térraba region. The poeciliid *Priapichthys panamensis* is native to Panama and of questionable origin in Costa Rica. The tilapia *Oreochromis niloticus* was introduced from Africa for the aquaculture industry, and has been collected in the Rio Térraba (Rojas and Rodríguez 2008), but not the Rio Sierpe.

There are an additional 71 marine species in 34 families that have been collected in fresh and brackish waters of the Térraba physiographic region (Table 7).

Species distribution and Site summaries
The survey sites were divided into 5 regions. The tributaries entering the Rio Sierpe from the north were medium-large, low-gradient streams running through agriculture with almost no forest cover (sites 21, 22, 23). The substrate was rocky but the streams had a high suspended sediment load making them appear cloudy. The northern headwater streams were also of medium-large width, and low-medium gradient (Sites 12, 13, 14). The upper site was

predominantly forested, with a substrate of large cobble, and the middle and lower sites were low gradient in agricultural settings with a pebble or sand bottom. The southern headwater stream was small-medium width and low gradient (Sites 11, 25). The upper site was narrow and deep with poor visibility and low dissolved oxygen. The lower site was wider but was marked by extremely low dissolved oxygen. Both sites had some forest cover. The tributaries entering the Rio Sierpe from the south were small-to-large streams flowing through mostly forested settings (Sites 1, 15, 16, 17, 18, 19). The streams were low gradient except for Site 19 which had a medium gradient. Site 15 was a small pebbly stream less than 20 centimeters deep. Site 1 was above a large water fall which probably acts as a barrier to fish movement. The estuary site was a low gradient stream with forest on one side and a rocky substrate that was subject to large tidal fluctuations in flow, salinity and depth (Site 2).

We collected 303 fish at the 15 sites representing 18 freshwater fish species in 6 families and 11 marine species in 6 families (Table 8). The combination of photographs and COI gene sequences was sufficient to identify most individuals to species. The sequence of the sleeper *Leptophilypnus panamensis* matched only to genus with the BOLD database, so a second comparison was performed with the GenBank database (http://www.ncbi.nlm.nih.gov/Genbank/), which provided a match to species. One individual that we judged too small to non-lethally sample for DNA was tentatively identified as a juvenile of the haemulidae *Pomadasys bayanus* based solely on its photograph. Three species that we sequenced were new contributions to the COI database: *Centropomus nigrescens*, and the poeciliids *Poeceliopsis retropina* and *Priapichthys panamensis.*

Following are some preliminary assessments of the species distributions. Fish sampling was qualitative, not quantitative, and the most abundant species were culled so that only a few individuals were documented at each site. A number of species were rare in our samples, and

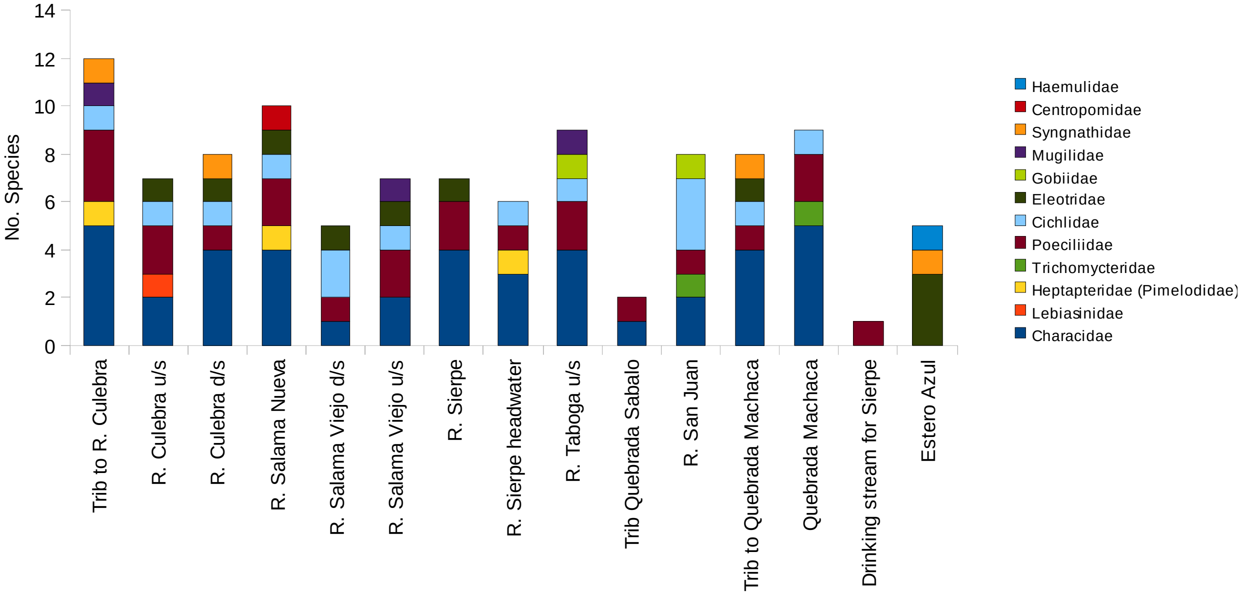

Figure 9: Number of fish species captured at 15 sites in the Rio Sierpe, Costa Rica in April 2009. Species are grouped by family.

we cannot be certain that the distribution we observed reflects the true distribution or a failure to detect these rare species in more places.

Our preliminary observations suggest that fish species were evenly distributed among the four freshwater regions of the Rio Sierpe, but not among sites within regions (Table 8, Figs. 9, 10, 11, 12, 13). Of the 29 species we observed, 5 species were found in two regions, and 12 species were found in three or four regions (Fig. 12). No species was found in all five regions. Differences in species communities among regions appeared to be affected by the proximity to salt water, dissolved oxygen, and visibility/turbidity. The average number of species within a region was 13 with a minimum of 5 and maximum of 18 (Fig. 10). Species were unevenly distributed among sites within a region, most species were observed at only one of the sites within a region (Fig. 11). Stream size and presence of a barrier appeared to affect distributions within a region, although other abiotic factors likely play an important role, as well. On average each site had 7 species, with the minimum being 1 species and the maximum being 12 species (Fig. 9). Additional sampling will be necessary to confirm the role that abiotic factors such as gradient, substrate, forest cover, and terrestrial habitat play in affecting species distributions.

The region with the most species was the northern tributaries (Sites 21, 22, 23), although only one species was unique to this region (Fig. 12). A total of 17 species were observed here but only 6 species were observed at more than one site within the region (Fig. 11). A low gradient, medium sized stream with moderate turbidity that had no forest cover and ran through a cow pasture (Site 21) had 12 species from 6 families (Fig. 9), the most of any site in the Rio Sierpe. Fish density was very high in the pools at this site, and in the riffles schools of poeciliids were seen feeding on algae.

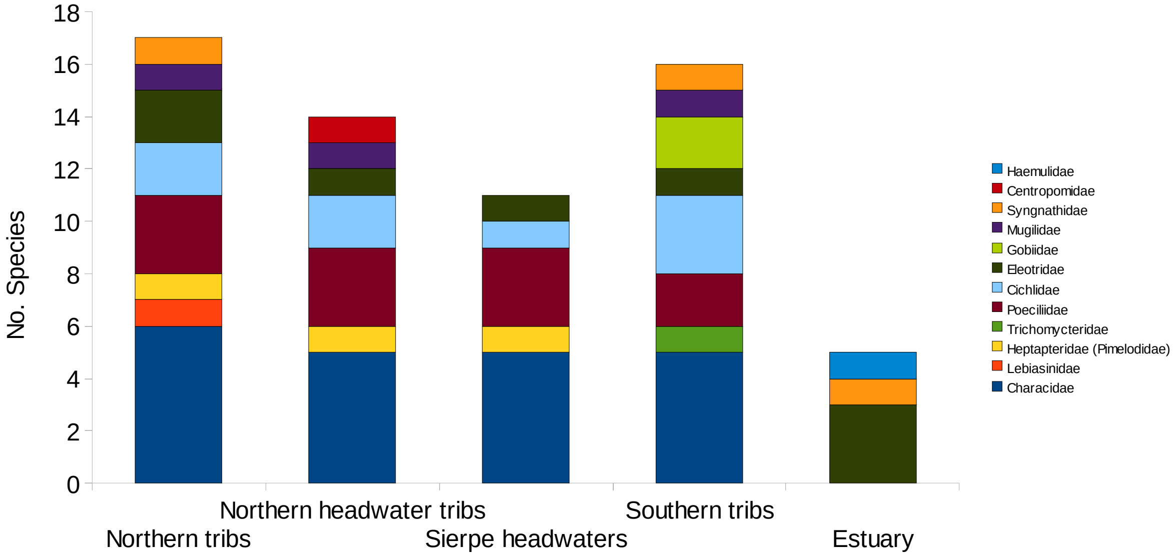

Figure 10: Number of fish species captured at 5 regions of the Rio Sierpe, Costa Rica in April 2009. Species are grouped by family.

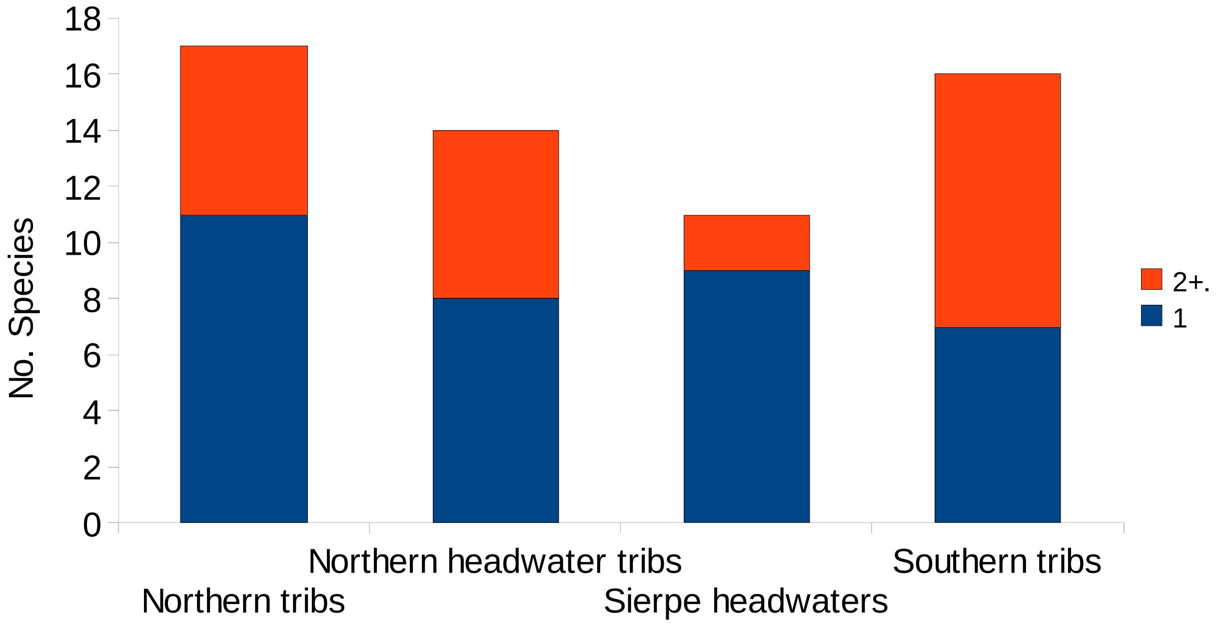

Figure 11: Number of fish species caught at 1 or 2+ sites within a region of the Rio Sierpe, Costa Rica. Four regions of the Rio Sierpe were sampled at 2 or more sites in April 2009.

The northern Siepre headwaters (Sites 12, 13, and 14) had an ichthyofauna much like the other freshwater regions. Two species were unique to this region, one species of barbeled catfish, and a species of snook. Both of these species were probably present in other regions, as well, although we did not sample them. The catfish is a mostly nocturnal species (Bussing 2002), which we could have missed elsewhere by focusing our sampling during the day time. The snook is a marine species that regularly enters freshwater to feed (Bussing 2002).

The southern Sierpe headwaters (Sites 11 and 25) had dissolved oxygen levels that were the lowest we observed, and poor visibility because of high turbidity. We considered the fish species we observed here to be tolerant of human disturbance, particularly hypoxia. Eleven fish species were observed here despite these poor conditions: five species of characin, three species of poeciliid, one recently dead barbeled catfish, one sleeper, and one cichlid. Two species were unique to this region, *Bryconamericus terrabensis* and *Priapichthys panamensis*.

The region with the second highest species total was the southern tributaries (Sites 1, 15, 16, 17, 18, 19). Sixteen species were observed here including three species that were unique to the region: two species of goby and one species of pencil catfish. Within this region, fish species diversity increased in a downstream direction, which has been observed previously in Costa Rica (Winemiller and Leslie 1992). Only one species of poeciliid was found above a barrier waterfall (Site 1), where it was very abundant, and another small stream had only this poeciliid species and one charcin species (Site 15). The remaining sites were in medium sized streams containing 8 or 9 species representing 4 or 5 different families (Fig. 9).

The Sierpe estuary (Site 2) was the closest to salt water and had a fish community unlike anywhere else in the watershed. The Sierpe estuary had low diversity, only 5 species representing 3 families, but 4 of those species were not found elsewhere in the Rio Sierpe.

Most individuals were very small juveniles of marine species. Our observations probably underestimate the diversity at this site because our normal capture techniques were unsuccessful. Fish in the estuary were caught only by the macroinvertebrate surveys.

The most abundant and widespread families in the Rio Sierpe were the characins and poeciliids (Fig. 13). A poeciliid was found at 14 sites and a characin was found at 13 sites (Table 8). A characin or poeciliid was the most abundant species at all sites except for the estuary (Site 2) where neither family was collected. Of the 12 species that had a wide distribution (found in 3 or 4 regions), 5 species were characin and 3 species were poeciliid (Table 8). The characin *Astyanax aneneus* was found at 11 sites in 4 regions making it the most widespread fish species in the Sierpe. This species is also one of the most widespread charcins in Central America with a range extending from Mexico to Panama (Miller 2005).

Determining which species are tolerant to human impacts and which are sensitive will be important for measuring stream health (Lyons et al. 1995). Lyons *et al*. (1995) determined tolerance by the quality of the habitat each species occupied. Of the 12 widespread species we observed in the Rio Sierpe, 7 of them were found in the southern headwaters (Sites 11 and 25) and therefore we considered tolerant of human disturbance (Table 5). Poeciliids appeared to be particularly tolerant of handling stress. Lyons *et al*. (1995) considered *Astyanax. aeneus* to be tolerant of disturbance and *Agonostomus monticola* to be sensitive to habitat disturbance. Their fish communities included different species for the other families in common with our study, but in general they considered poeciliids tolerant of disturbance, native cichlids moderately tolerant, and gobies sensitive to disturbance, which appears to be the same in the Rio Sierpe.

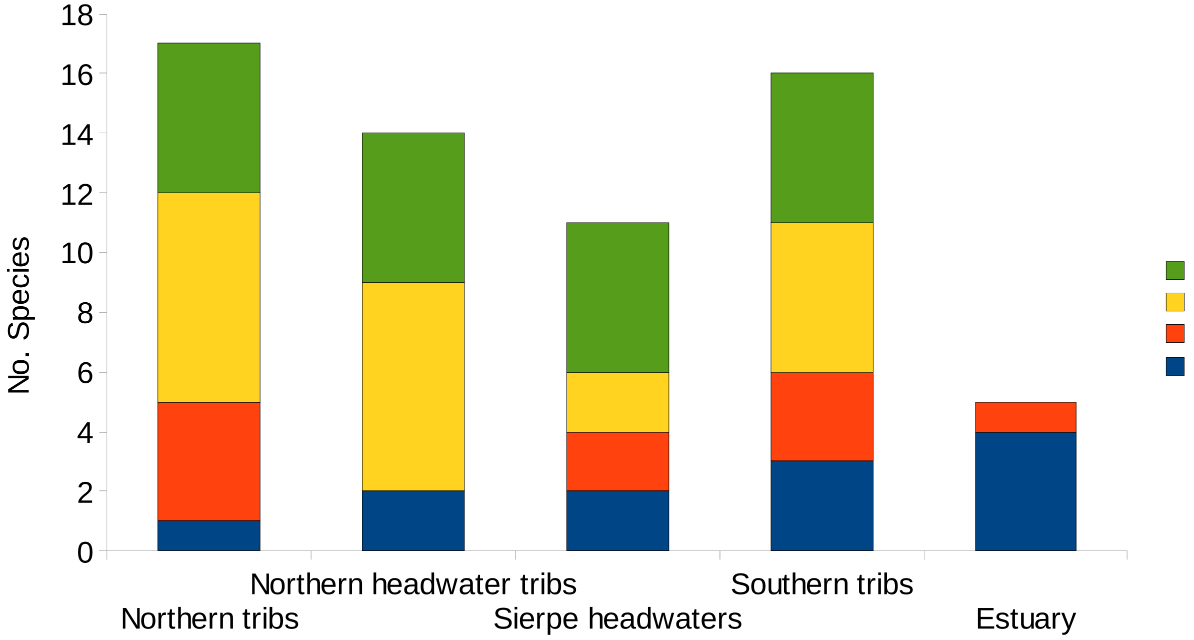

Figure 12: Number of fish species caught within a region of the Rio Sierpe, Costa Rica that were also caught at 0, 1, 2, or 3 other regions of the river. Sampling was conducted in April 2009.

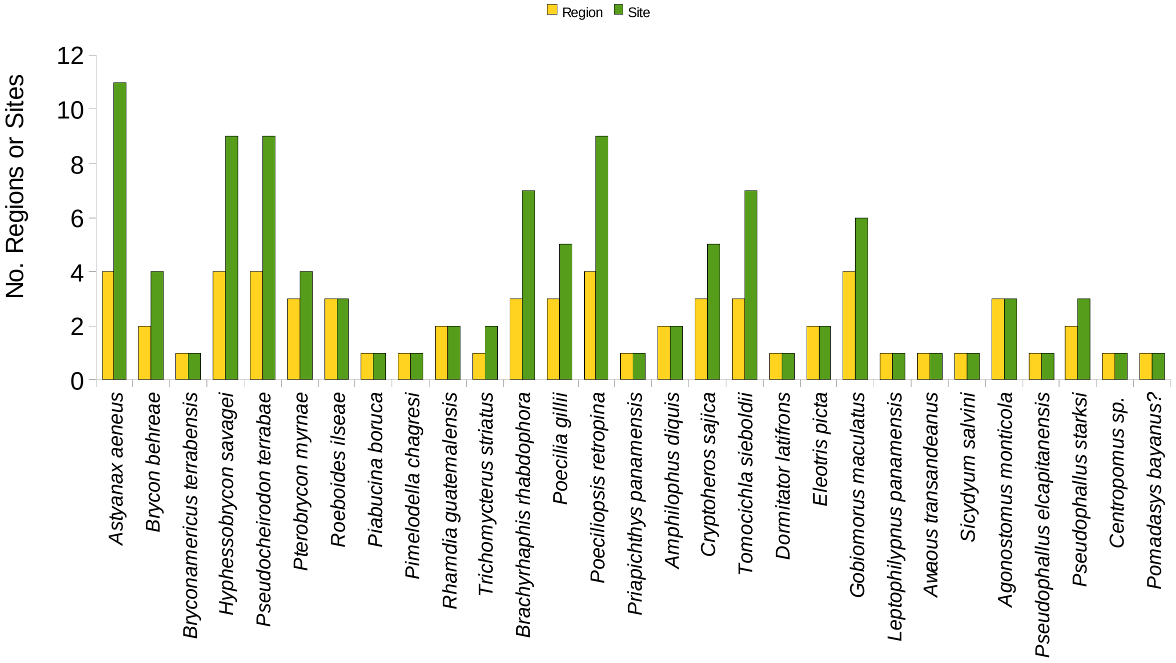

Figure 13: Distribution of fish species in the Rio Sierpe, Costa Rica. Sampling occurred at 15 sites in 5 regions in April 2009. The chart presents the number of regions and sites at which each species was observed.

Collection issues

We used a variety of nets and traps to capture fish, similar to what has been used by others in tropical streams (Winemiller 1983, Winemiller and Morales 1989, Lyons et al. 1995). Our gear was best suited to catching surface and midwater species. The umbrella net was most successful at catching mid-column and surface oriented fish in smaller streams of less than 0.5m depth. The umbrella net was most effective when dragged through the water. The cast net was most successful in streams less than 1m deep. The dip net was most successful when an individual fish could be targeted. The minnow trap was used in two locations (Sites 1 and 2) and did not capture any fish, but did capture a number of shrimp.

We failed to capture three species that were previously observed in the Rio Sierpe: *Rivulus uroflammeus, Hypostomus panamensis,* and *Gobiesox nudus* (Table 5). The Rivulidae is a surface oriented live-bearer species. The latter two species are benthic species with modifications for clinging to rocks. Bussing (2002) was able to capture these species using a combination of nets, hook and line, and ichthyicides. The ideal collection technique would capture surface-, mid-column- and bottom-oriented fish of all sizes in a non-lethal manner. We feel that electrofishing will be a more suitable capture technique for rare and benthic species (Lyons et al. 1995).

Future directions and conclusions

We also wanted to refine the rationale and questions that will be proposed in a future study. We initially proposed using either a candidate sites approach or a paired sites approach to compare aquatic communities in healthy and unhealthy streams in the Rio Sierpe watershed.

The objective of these preliminary surveys was to determine the information needs and protocols that can be used to monitor the health of the Rio Sierpe aquatic community. After exploring the Rio Sierpe during the end of the dry season in 2009 we decided that the best way to proceed with fish monitoring will be to develop an Index of Biotic Integrity (IBI) (Karr 1981) for the freshwater tributaries and to increase the temporal aspects of monitoring in the mainstem and estuary. The IBI is a measure of stream health based upon four major categories of fish observations: fish species composition and diversity, trophic composition, fish abundance, and fish condition. An IBI is developed by sampling the fish community in a variety of high and low quality habitats within the region of interest, and incorporating information on species life history and habitat preference to develop a scoring mechanism based upon the four major categories (Lyons et al. 1995). The IBI would allow interested parties to detect changes in the watershed affecting the fish communities. The IBI is flexible enough to be applied over a variety of sites within a region and is appropriate if some sites are already in a degraded state.

Some limitations of the IBI are that it is region specific and sensitive to natural variations. Developing an IBI will require a baseline that captures natural variation between seasons and between years (Karr et al. 1987). Many environmental variables not related to stream health can also influence fish distributions (Lyons and Schneider 1990). Therefore there is a need to sample a number of different types of habitat to better predict the impacts that degradation will have on a stream fish community.

Long term monitoring provides information on a specific site, and is useful when there are too few sites with comparable biological communities and habitats to develop a biological index. Long term monitoring captures the natural variability in a stream but also can be sensitive to gradual changes in fish communities (Hilborn et al. 2003, Eldridge and Naish 2007, Eldridge et al. 2009).

The predictive capabilities of biological monitoring can be improved by learning more about the life history of each fish species. The ability to survive in hypoxic or anoxic conditions is an important trait that may allow tropical fish species to tolerate human disturbance. Fish have evolved a number of interesting strategies that allow them to survive hypoxia or anoxia (Kramer 1987, Graham 1997). A number of tropical species, including characins and poeciliids, use aquatic surface respiration to breath at the well oxygenated micro-layer at the air-water interface (Kramer and Mcclure 1982, Kramer 1983), although this behavior may be energetically costly (Plath et al. 2007). Interestingly, this behavior has evolved quite a few times and often without a simultaneous evolution of jaw structure (Kramer and Mcclure 1982). It is unknown how the sleeper, catfish or chiclid species that we observed in the headwaters survive hypoxia, or if they essentially "hold their breath" during short ventures into hypoxic waters to feed or escape a predator. The family lebiasinidae contain both air breathing and non-air breathing species but it is unknown if *Piabucina boruca* is an air breather (Graham 1997). The closely related *P. panamensis* is a non-air breathing species, but *P. festae* is an air breather (Graham 1997). We observed *P. boruca* "walking on land" quite efficiently covering 0.5m in a matter of seconds. The fish moved in an upright position by undulating its body as if it were swimming.

Understanding the migratory behavior of certain species can be a useful indicator of habitat connectivity. A number of species in the Rio Sierpe exhibit daily or seasonal migrations. The mountiain mullet *Agonostomus monticola* apparently rears in streams and then migrates to the estuaries to spawn (Bussing 2002). The gobies may also move downstream to spawn closer to the mainstem Rio Sierpe (Bussing 2002). And there are a number of species that move from salt water into the river as juveniles to hide from predators or as adults to feed (Bussing 2002), such as the snook we observed near the Sierpe headwaters.

In conclusion*:*

I) There were 31 freshwater fish species in the Rio Sierpe, 10 of which were endemic to Costa Rica, and 71 marine fish species that were likely to enter the estuary and freshwater.

II) Fish species communities in the freshwaters were similar to the north, south and east, however within a region fish communities differed between sites, possibly because of differences in habitat degradation.

III) An Index of Biotic Integrity should be developed to monitor stream health in the freshwater tributaries

IV) More intense temporal monitoring should be initiated to better evaluate the health of the estuary and mainstem.

Table 5. Freshwater fish species of the Rio Sierpe and adjacent rivers in the Térraba physiographic region. Abbreviations are given in Table 6 (? refers to unknown).

Family - ***Common name***	**Species**	Origin	River	Diet	Stream	Current	Substr.	Elev. (m)	Temp (°C)	Shape	Swim.	Position	Repro.	Salt tol.	Toler.	Abund.	Distrib.
Primary																	
Characidae - *Characins*	*Astyanax aeneus*	N	S	H,I,F	G	G	G	0-1000	20-37	3.2	4.3	W	A	F	T	A	W
	Brycon behreae	N	S[a]	H,I,F	M,L	M,F	R,S	10-640	21-29	4.2	4.3	W	A	F	S	M	M
	Bryconamericus terrabensis	E	S[a]	H	M	M,F	?	60-940	22-27	3.8	3.2	?	?	F	T	R	L
	Hyphessobrycon savagei	E	S	I	G	G	?	0-70	24-30	3.0	3.6	W	?	F	T	A	W
	Pseudocheirodon terrabae	E	S	H	M,L	S,M	V	0-680	21-30	3.7	3.2	W	?	F	T	M	W
	Pterobrycon myrnae	E	S	I	M	M	?	10-80	25-30	6.4	3.1	W	L?	F	S	A	W
	Roeboides ilseae	E	S	L	M	S,M	?	10-660	28-30	2.9	3.6	W	?	F	T	M	W
Lebiasinidae - *Pencil fish*	*Piabucina boruca*	E	S	S	M	S,M	?	10-1000	23-29	4.5	2.0	S	?	F	S	M	L
Heptapteridae (Pimelodidae) - *Three-barbeled catfishes*	*Pimelodella chagresi*	N	S[a]	B	M	M	?	20-660	22-29	7.0	2.7	B	A	F	S	R	L
	Imparfinis (Nannorhamdia) lineata	E	T	B	M	M	?	80-980	22-27	6.6	2.0	?	?	F	?	?	?
	Rhamdia guatemalensis	N	S[a]	I,F	M	S	S,M	0-680	?	4.2	2.4	B	A	F	T	R	M
	Rhamdia laticauda (rogersi)	N	T	B	S,M	M,F	R,S	35-1350	20-33	7.6	2.0	?	?	F	?	?	?
Trichomycteridae - *Pencil catfishes*	*Trichomycterus striatus*	N	S	B	S,M	S,M	?	20-600	27-28	6.3	1.4	B	A?	F	S	R	L
Loricariidae - *Armored catfishes*	*Hypostomus panamensis*	N	S[b]	H	M,L	S,M	R	20-560	24-29	6.1	2.3	B	?	F	?	?	?
Secondary																	
Anablepidae - *Four-eyed fishes*	*Oxyzygonectes dovii*	N	T	H,S	M,L	S	?	0-15	27-30	5.6	1.4	S	L	F,B	?	?	?
Rivulidae - *Rivulines*	*Rivulus uroflammeus*	E	S[b]	I	G	S	?	20-1100	22-27	5.0	1.9		L	F	?	?	?
	Rivulus hildebrandi	N	T	I	G	S	?	10-90	25-29	5.9	1.6		L	F	?	?	?
	Rivulus glaucus	E	T	I	S,M	S,M	?	540-680	23-25	5.0	1.6		L	F	?	?	?
Poeciliidae - *Poeciliids*	*Brachyrhaphis rhabdophora*	E	S	I	S,M	S,M	?	3-540	23-32	3.7	1.7	S	L	F	S	A	W
	Brachyrhaphis terrabensis	N	T	S	S,M	M	?	40-1250	20-23	3.9	2.1		L	F	?	?	?
	Poecilia gillii	N	S	H	G	S,M	?	0-1220	19-37	3.8	1.9	D	L	F	T	M	W
	Poeciliopsis paucimaculata	E	T	H	G	M,F	R,S	20-940	21-28	5.4	2.0		L	F	?	?	?
	Poeciliopsis retropinna	N	S	H	G	S,M	R,S	0-940	21-29	4.9	1.9	S	L	F	T	A	W
	Poeciliopsis turrubarensis	N	T	H	G	?	S,M	0-120	23-37	4.7	1.8		L	F,B	?	?	?
	Priapichthys panamensis	Q	S[a]	B	S,M	S,M	?	0-15	26-30	5.3	1.3	S	L	F	T	M	L
Cichlidae - *Cichlids*	*Cryptoheros (Archocentrus) sajica*	E	S	H,B	M,L	G	R,S	10-680	25-30	2.8	2.2	D	G	F	?	M	W
	Amphilophus (Astatheros) diquis	E	S	I,H	M,L	S,M	R,S	16-700	23-33	3.2	2.3	D	G	F	T	R	M
	Tomocichla (Theraps) sieboldii	N	S	H	M,L	M	R,S	10-840	24-29	3.0	2.3	D	G	F	S	M	W
	Oreochromis niloticus	I	T	H,I	?	?	?	?	?	?	?	?	?	?	?	?	?
Peripheral																	
Eleotridae - *Sleepers*	*Dormitator latifrons*	N	S	H,F	M,L	S	S,M	0-30	25-33	3.9	1.3	B	E	F,B,M	S	A	L
	Eleotris picta	N	S	F	M,L	G	?	0-100	25-33	6.4	1.5	B	E	F,B	S	R	M
	Eleotris tecta	N	T	F	M,L	G	?	0-75	25-29	6.7	1.7		E	F,B	?	?	?
	Gobiomorus maculatus	N	S	F,B	M,L	?	?	0-115	24-33	5.8	2.4	B	E	F	T	M	W
	Hemieleotris latifasciatus	N	T	B	M,L	L,M	V	5-100	25-30	4.5	2.6		E	F	?	?	?
	Leptophilypnus panamensis	N	S[a]	?	?	?	?	?	?	?	?	?	?	F	?	R	L
Gobiidae - *Gobies*	*Awaous transandeanus*	N	S	H,B	M,L	S,M	S	0-120	25-31	6.6	1.8	B	?	F	S	M	L
	Sicydyum salvini	N	S	H	M,L	F	R	0-660	21-31	6.1	1.3	B	?	F	S	R	L
Mugilidae - *Mullets*	*Agonostomus monticola*	N	S	B,H	M,L	G	?	0-650	20-31	4.6	2.6	W	?	F,B	S	R	W
Syngnathidae - *Pipefishes*	*Pseudophallus elcapitanensis*	N	S[a]	B	M,L	S,M	?	0-20	26-31	24.7	1.5	W	L	F	S	R	L
	Pseudophallus starksi	N	S[a]	B	M,L	G	V	2-40	25-30	23.7	1.3		L	F	S	R	M
Gobiesocidae - *Clingfishes*	*Gobiesox nudus*	N	S[b]	S,L	M,L	M,F	?	0-140	25-30	5.4	1.3	B	?	F	?	?	?

[a] Species that had not been previously observed in the Rio Sierpe by Bussing (2002); [b] Species that had been observed in the Rio Sierpe by Bussing (2002) that we failed to collect.

Table 6. Abbreviations used in Table 5

Origin	*Native to Costa Rica*	N
	Endemic to Costa Rica	E
	Questionable origin	Q
	Introduced to Costa Rica	I
River	*Previously found in the Rio Sierpe*	S
	Previously found in the Térraba region but not the Rio Sierpe	T
Diet	*Herbivore – fruit, leaves, diatoms, awfuchs,*	H
	Surface or terrestrial invertivore	S
	Aquatic or benthic invertivore	B
	General invertivore	I
	Piscivore	F
	Scale eater (Lepidophagous)	L
Stream size preference	*Small*	S
	Medium	M
	Large	L
	Small-large	G
Current velocity preference	*Fast*	F
	Moderate	M
	Slow-none	S
	General	G
Substratum preference	*Rubble (rocky, gravel)*	R
	Sand	S
	Mud or silt	M
	Vegetated	V
	General	G
Elevation	*Range above sea level*	(Meters)
Temperature	*Range of water temperatures in which fish have been collected*	(°C)
Shape factor	*Shape factor – Total length divided by max. depth.*	(Continuous)
Swimming factor	*Swimming factor – Max. caudal fin divided by min. caudal peduncle*	(Continuous)
Position	*Surface oriented*	S
	Mid-water column	W
	Demersal- near bottom	D
	Benthic - on bottom	B
Reproduction	*Live-bearer (ovoviviparous, viviparous)*	L
	Nest guarded by parent	G
	Exposed embryos guarded by parent	E
	Brood hiders without further parental care	H
	Embryos attached to a substrate without further parental care	A
	No parental care, embyo not attached to a substrate	N
Salt tolerance	*Freshwater*	F
	Brackish water	B
	Marine salt water	M
Tolerance	*Tolerant – species found in Sierpe headwaters*	T
	Sensitive – species not found in Sierpe headwaters	S
Abundance	*Abundant – ≥5 individuals at 2 or more sites*	A
	Moderate – 2-4 individuals per site	M
	Rare – 1 individual per site	R
Distribution	*Widespread – ≥3 regions*	W
	Moderate – 2 regions	M
	Limited – 1 region	L

Table 7: Marine fish species observed in fresh, brackish and estuarine waters of the Térraba physiographic region.

Family - ***Common name***	**Species**	**References**
Achiridae - *Round sole*	*Achirus sp.*	5
	Trinectes fonsecensis	1,3
Albulidae - *Bonefishes*	*Sp. 1*	2
Ariidae – *Sea catfishes*	*Cathorops steindachnerii*	3
	Cathorops tuyra	3
Atherinopsidae (Atherinidae) – *Silversides*	*Atherinella argentea*	1
	Atherinella sp.	2
	Coleotropis sp.	5
Blenniidae - *Combtooth blennies*	*Hypsoblennius striatus*	4
Carangidae - *Jacks*	*Caranx caballus*	3
	Caranx caninus	3
	Caranx hippos	5
	Caranx latus	1,5
	Caranx sp.	4
	Caranx vinctus	2
	Oligoplites palometa	1
	Oligoplites sp.	5
	Oligoplites saurius	2
	Trachinotus sp.	5
Carcharhinidae - *Requiem sharks*	*Carcharhinus leucas*	1,4,5
Centropomidae - *Snooks*	*Centropomus armatus*	6
	Centropomus medius	1,5
	Centropomus nigrescens (undecimalis)	1,3,4,5,7
	Centropomus robalito	1
	Centropomus unionensis	6
	Centropomus viridis	1,3
Clupeidae - *Herrings*	*Clupeidae sp.*	2,5
Dasyatidae – *Stingrays*	*Dasyatidae sp.*	4,5
Eleotridae – *Sleepers*	*Erotelis armiger*	4,5
	Leptophilypnus panamensis	7
Elopidae – *Ten pounders*	*Elops affinis*	2
Engraulidae - *Anchovies*	*Engraulidae sp.*	2,5
Gerreidae - *Mojarras*	*Diapterus (Eugerres) sp.*	5
	Diapterus peruvianus	2
	Eucinostomus currani	1,2,3
	Eucinostomus gracilis	5
	Eucinostomus sp.	4
	Eugerres brevimanus	1,3
Gobiesocidae – *Clingfish*	*Gobiesox potamius*	1,5
	Gobiesox sp.	4
Gobiidae – *Gobies*	*Bathygobius andrei*	5
	Bathygobius ramosus	4
	Enypnias seminudus	4
	Gobionellus microdon	5
	Gobionellus sagittula	2,4,5
Haemulidae – *Drums*	*Pomadasys bayanus*	1,4,5,7
	Haemulopsis leuciscus	3
Labridae – *Wrasses*	*Halichoeres (Pseudojilus) notospilus*	4
Labrisomidae – *Labrisomids*	*Malacoctenus zonifer*	4

Family - *Common name*	**Species**	**References**
Lutjanidae - *Snappers*	*Lutjanus argentiventris*	1,2,4,5
	Lutjanus colorado	1,4,5
	Lutjanus jordani	3
	Lutjanus novemfasciatus	1,2,3,4,5
Microdesmidae – *Wormfishes*	*Microdesmus dipus*	2
	Microdesmus tabogensis	2
	Microdesmus sp.	4
Mugilidae - *Mullets*	*Mugil curema*	2,3,4,5
Mullidae - *Goat fish*	*Mullidae sp.*	5
Muraenidae - *Moray eels*	*Gymnothorax sp.*	4
Paralichthyidae - *Large-tooth flounders*	*Citharichthys gilberti*	1,2,5
Pleuronectidae - *Right-eye flounders*	*Pleuronectidae sp.*	4
Polynemidae - *Threadfins*	*Polyadictilus sp.*	5
Pomacentridae - *Damselfishes*	*Abudefduf analogus*	4
Pristidae - *Sawfishes*	*Pristis pectinatus*	5
	Pristis pristis	1
	Pristis sp.	4
Sciaenidae - *Drums*	*Sciaenidae sp.*	5
Scombridae - *Mackerals*	*Scombridae sp.*	4,5
Serranidae - *Groupers*	*Serranidae sp.*	4
Synbranchidae - *Swamp eels*	*Synbranchus marmoratus*	1
Characinodontidae - *Puffers*	*Sphoeroides annulatus*	2,4,5

1(Bussing 2002); 2(Chicas 2001); 3 (Rojas and Rodríguez 2008); 4(Winemiller 1983); 5(Winemiller and Morales 1989); 6 (Tringali et al. 1999); 7 this survey

Table 8: Fish species observed at 15 sites from 5 regions in the Rio Sierpe, Costa Rica in April 2009. The total number of fish caught (N), the abundance at each site (A >5 individuals, M 2-3 individuals, R 1 individual), the number of regions and sites at which the fish were observed, and the standard length (average(minimum, maximum)) are presented.

Family	**Species**	**N**	**21**	**22**	**23**	**12**	**13**	**14**	**11**	**25**	**19**	**15**	**16**	**17**	**18**	**1**	**2**	**Region**	**Site**	**SL (mm)**
Characidae	*Astyanax aeneus*	48	A	M	M		A	A	A	M	M		M	M	M			4	11	50.6 (35, 83)
	Brycon behreae	9	M								M			M	M			2	4	48.3 (24, 90)
	Bryconamericus terrabensis	1							R									1	1	65.0 (65, 65)
	Hyphessobrycon savagei	29	M	M	M	M		A	M		A	M			M			4	9	33.5 (22, 43)
	Pseudocheirodon terrabae	13	R		M	R			R	R	M		R	R	M			4	9	38.1 (25, 45)
	Pterobrycon myrnae	21			M	A								M	A			3	4	31.5 (24, 36)
	Roeboides ilseae	7	M			R				M								3	3	53.9 (17, 70)
Lebiasinidae	*Piabucina boruca*	2		M														1	1	87.0 (80, 94)
Heptapteridae	*Pimelodella chagresi*	1				R												1	1	58.0 (58, 58)
(Pimelodidae)	*Rhamdia guatemalensis*	2	R							R								2	2	111.5 (95, 128)
Trichomycteridae	*Trichomycterus striatus*	2											R		R			1	2	43.0 (43, 43)
Poeciliidae	*Brachyrhaphis rhabdophora*	47	R	M				M			M	M			R	A		3	7	24.7 (11, 42)
	Poecilia gillii	23	R	M	M	M			A									3	5	30.4 (13, 49)
	Poeciliopsis retropina	29	M			A	A	M		M	R		M	M	M			4	9	38.0 (10, 54)
	Priapichthys panamensis	3							M									1	1	24.3 (20, 28)
Cichlidae	*Amphilophus (Astatheros) diquis*	2								R			R					2	2	60.0 (54, 66)
	Cryptoheros (Archocentrus) sajica	11	M			R	M						M					3	5	41.9 (21, 53)
	Tomocichla (Theraps) sieboldii	16		M			M	R			R		M	M	M			3	7	60.5 (35, 145)
Eleotridae	*Dormitator latifrons*	5															A	1	1	20.0 (16, 22)
	Eleotris picta	2			R												R	2	2	46.0 (12, 80)
	Gobiomorus maculatus	16		R		M	M	M	M					A				4	6	95.7 (61, 170)
	Leptophilypnus panamensis	1															R	1	1	36.0 (36, 36)
Gobiidae	*Awaous transandeanus*	2											M					1	1	70.5 (69, 72)
	Sicydyum salvini	1									R							1	1	38.0 (38, 38)
Mugilidae	*Agonostomus monticola*	4	R					M			R							3	3	67.0 (59, 84)
Syngnathidae	*Pseudophallus elcapitanensis*	1															R	1	1	73.0 (73, 73)
	Pseudophallus starksi	3	R		R									R				2	3	110.0 (95, 121)
Centropomidae	*Centropomus sp.*[a]	1				R												1	1	940.0 (940, 940)
Haemulidae?	*Pomadasys bayanus?*	1															R	1	1	14.0 (14, 14)
Families			6	5	5	6	4	5	3	4	5	2	5	5	4	1	4			
Species			12	7	8	10	5	7	7	6	9	2	8	8	9	1	5			
Individuals			25	17	15	28	18	22	31	12	18	5	21	23	25	34	9			

[a] Either *C. nigrescens* or *C. virdis* caught be a fisherman below the confluence of the Salma Nueva and Sierpe Headwaters.

IV. Conclusions: Goals and Outcomes

We developed an understanding of the current conditions, habitat threats, and monitoring needs during our preliminary survey of the Rio Sierpe. There was a great deal of variability in water chemistry, aquatic macroinvertebrate and fish communities throughout the freshwater tributaries and estuary indicating that there were both high quality and degraded streams within the watershed. Several sites had low concentrations of dissolved oxygen (some with elevated dissolved organic carbon) and low invertebrate and fish diversity compared to other streams. There were also chemical differences among subregions that were related to geological differences and/or human impacts. The surveys of aquatic macroinvertebrate and fish communities reinforced our understanding of regional diversity. We identified a number of existing threats to the aquatic habitat including intensive agricultural efforts to the north and east of the watershed, however ion concentrations did not indicate a major source of pollution. These data will help us formulate a rigorous chemical and biological monitoring program. This monitoring program will ultimately be useful for local communities to track water quality and stream ecosystem health. The details of this program will be provided in a forthcoming proposal.

We had five goals for this preliminary survey. Those goals are listed and addressed here directly or by referencing information presented elsewhere within this report.

(i) Refine the rationale and questions that will be proposed in a future study, by on-the ground evaluation of landuses of primary concern and prioritization of program elements (physical, chemical, biological measurements). Specific questions and their rationale will be presented in the forthcoming project proposal, here we outline the knowledge gained during this preliminary study:

    a. Through field measurements and observations we have solid understanding of extent of seawater influence inland.
    b. We have a sound understanding of stream types and land uses throughout the watershed.
    c. A comprehensive project needs to include great spatial coverage (within subregions where fish and chemistry show similarities)
        i. Include more forested reaches, include pasture, palm, rice, wetlands sites
    d. A comprehensive project needs to include wet-season sampling.
    e. A comprehensive project needs to include a broader array of potential chemical pollutants (e.g., pesticides, herbicides).
    f. A comprehensive project needs to develop a more clear understand of factors influencing local fish distributions.
    g. A comprehensive project would benefit from exploring biological conditions in other coastal small and medium sized river systems north and south of Rio Sierpe.

(ii) identify and collect the relevant literature and background information;

  a. Geographic information that was provided by A. Moulert has been instrumental. We will continue to work with ECOTICOS as a large monitoring program develops and include a comprehensive statistical analysis of the relationships between geographic factors and chemical and biological data.
  b. The relevant literature and background information is reported herein.

(iii) We have made contact with organizations, landowners, biologists, and local conservation groups to coordinate future research and to initiate a local monitoring effort. Several of these contacts were instrumental in supporting our field effort in April 2009. The following is a list of contacts (in no particular order):

  a. Technical, Institutional, and Conceptual Solutions (TICOS), Azur Moulaert, University of Vermont
  b. Don Jorge Uribe, Owner of Las Vegas Restaurant, Village of Sierpe (506-2788-1082),
  c. Neotropica Foundation, Bernardo Aquilar–González (Executive Director), Adjunct Professor, Northern Arizona University (EEUU), Instructor at the Universidad Interamericana de Costa Rica (baguilar@neotropica.org),
  d. Área de Conservación de Osa (ACOSA), Etilma Morales (etilma.morales@gmail.com)
  e. Helena Molina-Ureña, Ph.D., Associate Professor of Biology, Universidad de Costa Rica, San Jose, Costa Rica 11501-2060
  f. Association of Friends of Nature of the Pacific Central and South (ASANA), Richard Margoluis (Director), Hacienda Barú, PO Box 215-8000, San Isidro, Pérez Zeledón, Costa Rica

(iv) Identify potential study sites along the Rio Sierpe mainstem and tributaries to refine future research of water quality;

We visited nearly 40 sites in the Rio Sierpe watershed (including one site on the Térraba River) at the end of the dry season in 2009, as described above. Water chemistry, macroinvertebrates, and fish were sampled or surveyed at many of these sites. Additional sites are necessary to develop an Index of Biological Integrity (IBI) based on fish and macroinvertebrates. Long term monitoring can be conducted at one or a few sites in the mainstem and estuary. Quantitative surveys can be conducted in many of the freshwater tributaries and some estuarine tributaries. In the mainstem, we may be able to perform only qualitative surveys of the biological communities. Additional surveys will be necessary to measure seasonal and annual variability in chemical and biological communities. Site access during the dry season was not problematic; however poor road conditions may limit access to some sites during the raining season.

(v) Evaluate sample collection protocols.

Macroinvertebrates. A qualitative sampling approach was used to collect aquatic macroinvertebrates in order to build a preliminary list of taxa and their distributions. The development of a biological monitoring protocol will require quantitative sampling, where possible, and the comparison of “put-and-take” leaf-pack sampling protocols with quantitative methods (i.e., known area sampling). The leaf pack protocols will be instrumental in providing a citizen-based monitoring tool.

Fish. We were limited to only qualitative surveys of species composition and rough estimates of relative abundance during this survey. We feel confident, however, that in the future we will be able to perform quantitative assessments in the shallow tributaries. In the mainstem we will be able to qualitatively assess species composition and abundance.

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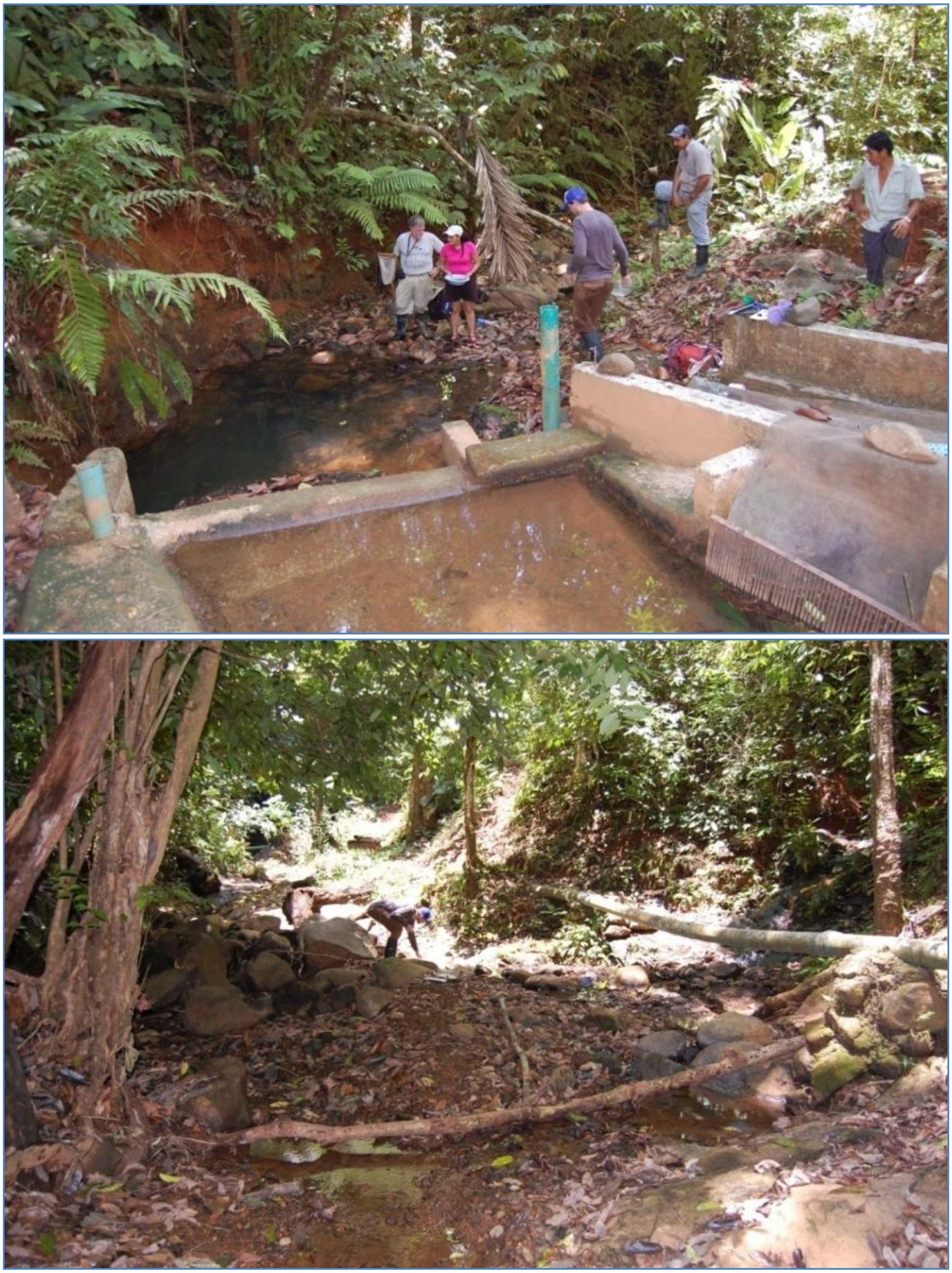
VI. Appendix 1: Site Photos



Site 1: Quebrada Tomo Agua - drinking water source for Sierpe. Upper photo looking at upstream reach (above water intake, lower photo looking at downstream reach (below water intake). Photo by D. Arscott.

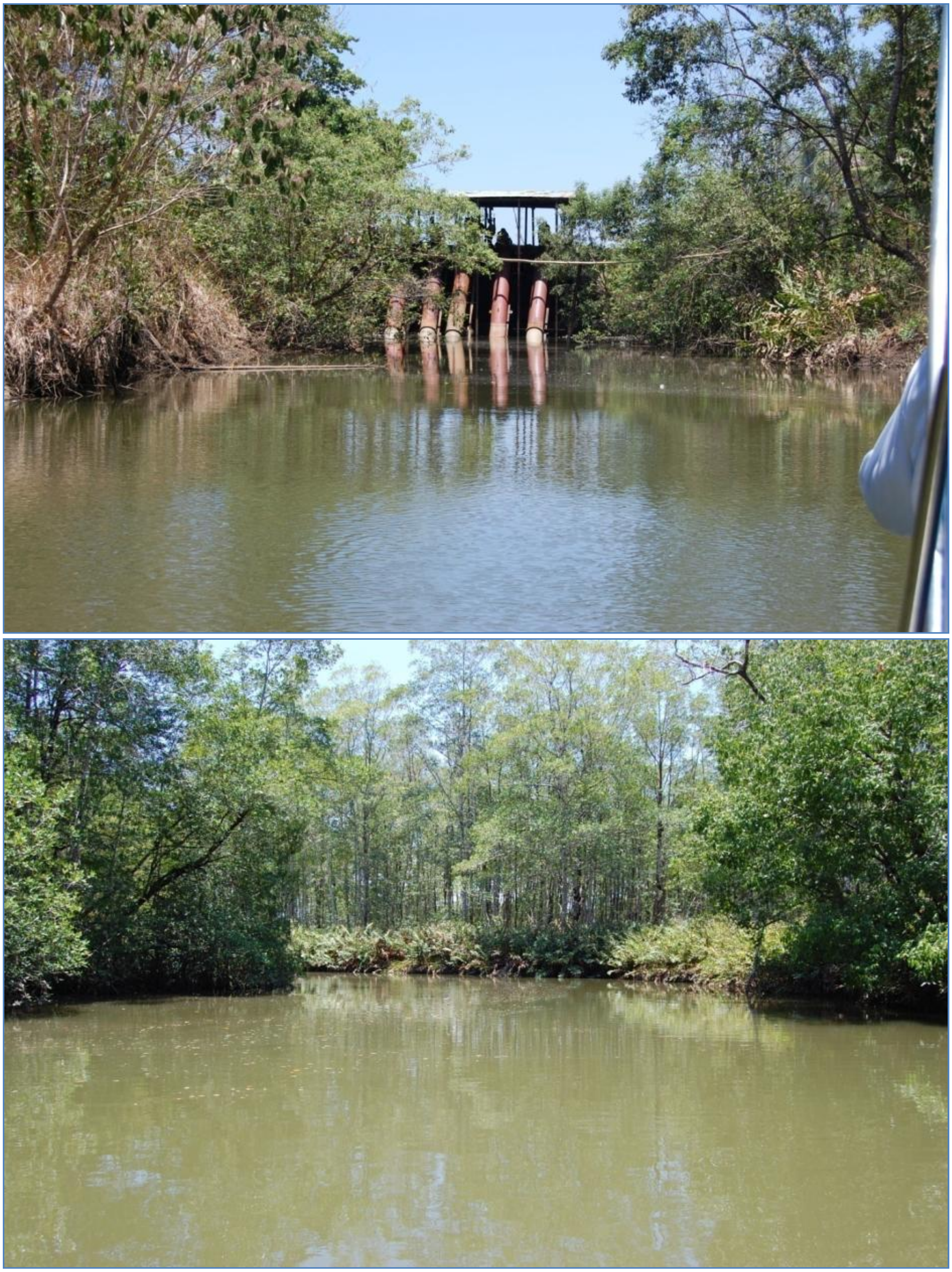
Site 2: Estero Azul. Upper photo looking upstream, lower photo looking downstream. Photo by D. Arscott.

Site 2B: Tributary to Estero Azul. Upper photo looking upstream, lower photo looking downstream. Photo by D. Arscott.

Site 3: Rio Sierpe maintstem just below the village of Sierpe. Upper photo looking towards true right bank (northern bank), lower photo looking downstream at true left bank (southern bank). Photo by D. Arscott.

Site 5: Below Shrimp Farm outlet in Térraba-Sierpe Mangrove (photo not taken at Site 4). Photo by D. Arscott.

Near Site 6: Rio Sierpe mainstem near confluence with Espero Ollia. Photo by D. Arscott.



Site 7: Above Shrimp Farm intake in Térraba-Sierpe Mangrove. Upper photo looking at true right bank (north bank) where intake pipes are located. Lower photo looking upstream. Photo by D. Arscott.

Site 8: Espero Ollia. Upper photo looking upstream, lower photo looking downstream. Photo by D. Arscott.

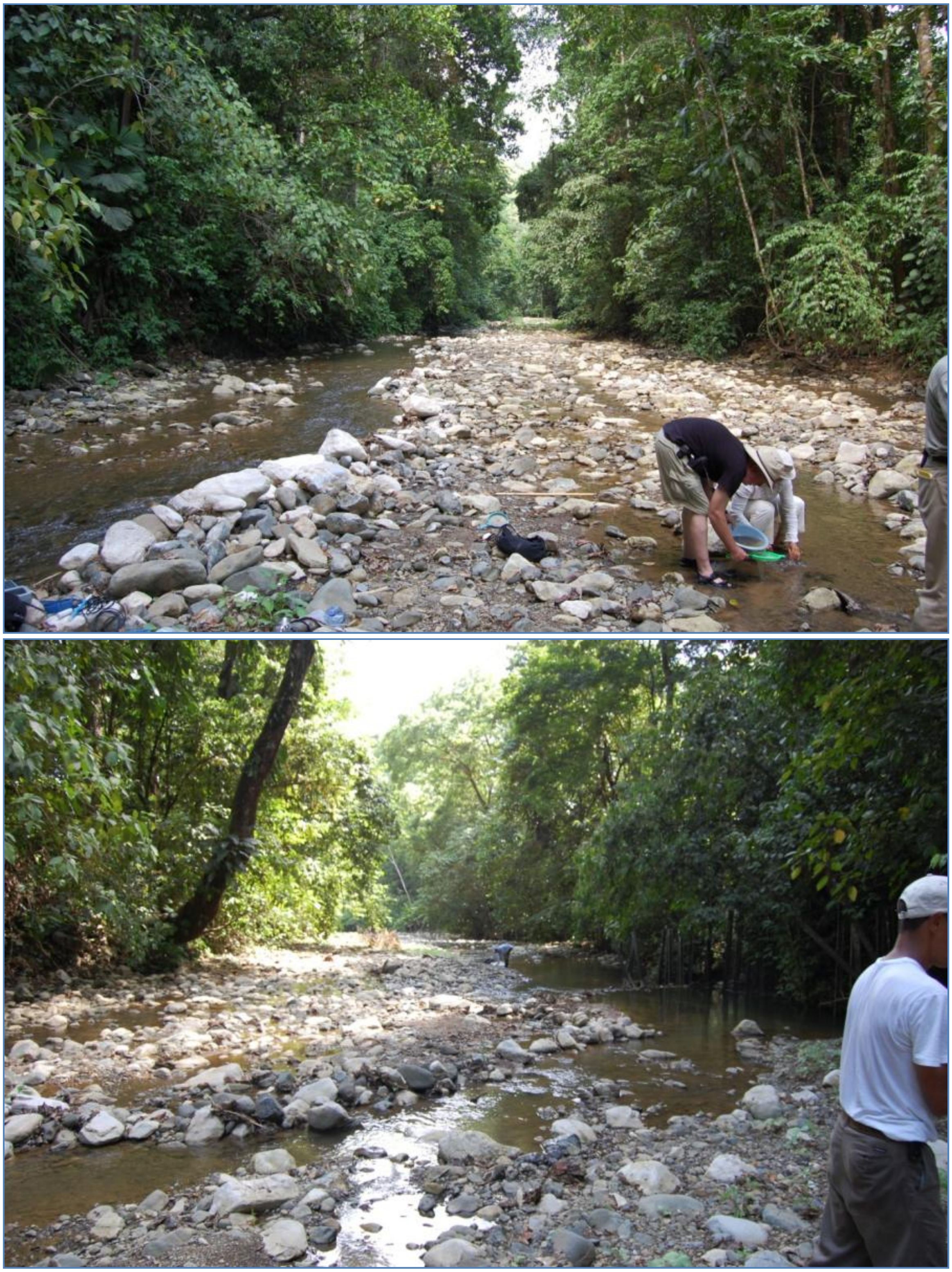
Site 10: Rio Chacuaco (near R. Sierpe confluence). Photo looking upstream. Photo by D. Arscott.

Site 11: Rio Sierpe (just upstream from R. Salama Nueva). Upper photo looking up- and lower photo looking downstream. Photo by D. Arscott.

Site 11A: Rio Sierpe (just downstream from R. Salama Nueva). Upper photo looking downstream from R. Sierpe and R. Salama Nueva confluence. Photo by D. Arscott.

Site 12: Rio Salama Nueva (just upstream from R. Sierpe). Upper photo looking upstream, lower photo taken from below the confluence with the R. Sierpe (to right side) showing R. S. Nueva entering from the left side. Photo by D. Arscott.

Site 13: Rio Salama Viejo downstream from Hwy 2. Upper photo looking upstream, lower photo looking down. Photo by D. Arscott.



Site 14: Rio Salama Viejo upstream from Hwy 2. Upper photo looking upstream, lower photo looking down. Photo by D. Arscott.

Site 15: Quebrada Sabalo. Upper photo looking upstream, lower photo looking down. Photo by D. Arscott.

Site 16: Rio San Juan. Upper photo looking upstream, lower photo looking down. Photo by D. Arscott.

Site 16A: Rio San Juan downstream below gravel extraction point. Upper photo looking upstream, lower photo looking down. Photo by D. Arscott.

Site 17: Tributary to Quebrada Machaca. Upper photo looking upstream, lower photo looking down. Photo by D. Arscott.

Site 18: Quebrada Machaca. Upper photo looking upstream, lower photo looking down. Photo by D. Arscott.

Site 19: Rio Taboga upstream. Upper photo looking upstream, lower photo looking down. Photo by D. Arscott.

Site 20: Térraba River. Upper photo looking upstream, lower photo looking down. Photo by D. Arscott.

Site 21: Tributary to Rio Culebra (northern tributary). Upper photo looking up and lower photo looking downstream. Photo by D. Arscott

Site 22: Rio Culebra (northern tributary). Upper photo looking up and lower photo looking downstream. Photo by D. Arscott

Site 23: Rio Culebra downstream from Hwy 2 (northern tributary). Upper photo looking up and lower photo looking downstream. Photo by D. Arscott

Site 24: Field drain outlet to Rio Culebra downstream from Hwy 2 (northern tributary). Both photos looking at the drain confluence with R. Culebra. Photo by D. Arscott.

Site 25: Rio Sierpe headwater. Upper photo looking up and lower photo looking downstream. Photo by D. Arscott.

Site 25A: Tributary 1 to Rio Sierpe headwater. Upper photo looking up and lower photo looking downstream where Trib 1 is on right and Trib 2 is on left with R. Sierpe Headwater below. Photo by D. Arscott.

Site 25B: Tributary 1 to Rio Sierpe headwater. Upper photo looking upstream. Photo by D. Arscott.

Site 101: Rio Sierpe Mouth. Upper photo looking out to the Pacific Ocean, lower photo looking into the mangrove wetland. Photo by D. Arscott.

Site 105: Rio Sierpe Mainstem. Upper photo looking upstream, lower photo downstream. Photo by D. Arscott.

VII. Appendix 2: Photographs of fish collected

Fish species collected in the Rio Sierpe in April 2009 and regions in which they were collected (estuary, northern tributaries, northern headwaters, southern headwaters, and southern tributaries). All photographs by W. Eldridge except *Centropomus* sp. By D. Arscott.

Centropomidae

Centropomus sp.
N. headwaters

Characidae

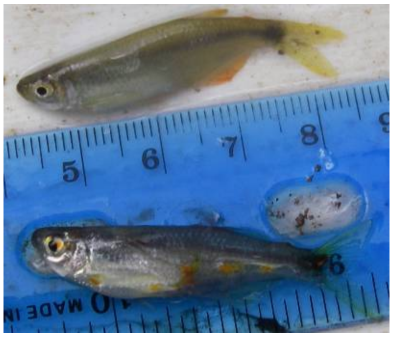
Astyanax aeneus
N. tribs, N. headwaters, S. headwaters, S. tribs

Brycon behreae
N. tribs, S. tribs.

Bryconamericus terrabensis
S. headwaters

Hyphessobrycon savagei
N. tribs, N. headwaters, S. headwaters, S. tribs

Pseudocheirodon terrabae
N. tribs, N. headwaters, S. headwaters, S. tribs



Pterobrycon myrnae
(female above, male below)
N. tribs, N. headwaters, S. tribs

Roeboides ilseae
N. tribs, N. headwaters, S. headwaters

Fish species collected in the Rio Sierpe in April 2009 Continued.

Cichlidae


Amphilophus (Astatheros) diquis,
S. headwaters, S. tribs


Cryptoheros (Archocentrus) sajica
N. tribs, N. headwaters, S. tribs


Tomocichla (Theraps) sieboldii
N. tribs, N. headwaters, S. headwaters, S. tribs

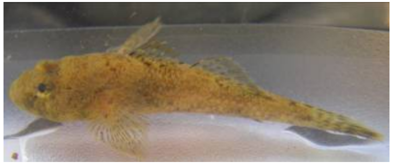

Tomocichla (Theraps) sieboldii
(female above, male below),
N. tribs, N. headwaters, S. headwaters, S. tribs

Eleotridae


Dormitator latifrons
Estuary


Eleotris picta
Estuary, N. Tribs


Gobiomorus maculates
N. tribs, N. headwaters, S. headwaters, S. tribs


Leptophilypnus panamensis
Estuary

Gobiidae


Awaous transandeanus
S. tribs


Sicydyum salvini
S. tribs

Haemulidae


Pomadasys bayanus
Estuary

Fish species collected in the Rio Sierpe in April 2009 Continued.

Heptapteridae

Pimilodella chargresi
N. headwaters

Rhamdia guatemalensis
N. tribs, S. headwaters

Lebiasinidae

Piabucina boruca
N. tribs

Mugilidae

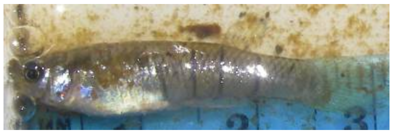
Agonostomus monticola
N. tribs, N. headwaters, S. tribs

Poecilidae

Brachyrhaphis rhabdophora
N. tribs, N. headwaters, S. tribs

Poecilia gillii
N. tribs, N. headwaters, S. headwaters

Poeciliopsis retropinna
N. tribs, N. headwaters, S. headwaters, S. tribs



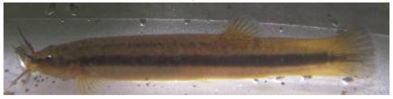
Priapichthys panamensis
S. headwaters

Sygnathidae

Pseudophallus elcapitanensis
Estuary

Pseudophallus starksi
N. tribs, S. tribs

Trichomycteridae



Trichomycterus striatus
S. tribs