Effects of Land-use Activities in the Ayuquila River's Fauna

by

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Abstract

Land use change is an important human force causing modifications in the structure and quality of freshwater ecosystems worldwide. Multiple stressors have affected the Ayuquila-Armeria River in Jalisco, the third largest river and second in biodiversity in that Mexican state. Despite an on-going monitoring program focused on water quality, no evidence exists on how land use activities have affected its aquatic communities. I characterized the invertebrate and fish communities using the functional feeding group approach. Abundance, functional diversity, richness, and pollution tolerance were compared spatially and temporally using various multivariate metrics and were related to water chemical variables. Stable carbon and nitrogen analyses, and trace metal characterization were performed to biological and sediment samples from 17 sites in this River to determine sources of organic matter and to look for associations with surrounding land uses.

Filter and gatherer collector invertebrates and fish omnivores dominated the community composition, which reflected the amount of suspended sediments in the water column. The presence of exotic species, fecal coliforms and total nitrogen above Mexican guidelines, were additional evidence of disturbance that contrasts the quality given by biotic indices.

Spatial differences in diversity and composition were significant between agricultural and forested sites. Surrounding agricultural vegetation did not influence the δ13C values in river components which were influenced by either riparian vegetation or autochthonous carbon sources, while δ15N values in sediment and animal tissues confirmed the influence sewage and animal–derived organic matter has in the river's structure. Metal concentrations were site and season dependent; concentrations in invertebrates were higher than in sediment and fish, while those from agricultural sites were higher than those from forested sites. Concentrations of Cd, Cu, Pb and Mn were associated to urban and mine runoff.

Keywords: Ayuquila-Armeria River, Jalisco, Mexico; Macroinvertebrate Functional group structure; Fish trophic guilds; Land use; Carbon and Nitrogen isotopes; Trace metal concentrations.

Dedication

"Count your age by friends not years; count your life by smiles, not tears."
John Lennon

I consider myself so fortunate to have been backed through this arduous path by both old and new friends. This thesis is just the result of a long interesting journey in which I have been blessed to have had the support from a solid group of friends here in Vancouver, who is now my extended family: Jose, Gloria, Enrique, Perla, Dolly, Carlos, Virginia, Adrián, Juan José, Maribel, Roberto. Similarly, my friends in Mexico, who encouraged me to start the journey, and kept on cheering me up when despair was imminent: Arturo, Guadamena, Luis, Edith, Ramón, Nora, Martin, Judith, Chuy, Pita, Lety, Luis Guz, Pepita. Muchas gracias!

However, I cannot be grateful enough for the endless smiles, joy, love, and support given by my family: Lorena –my beautiful 'other half', Aurea, Juan and Sofia, who not only accepted moving thousands of miles to a strange and fascinating land, but also had to endure the rough and bitter moments this process had on my temper, particularly as the end approached. Both Yaya, my eternal inspiration, and Lucia, my lovely sister, were also there all the time, despite the distance... My love to you all, always!

This is to and for all of you… cheers!

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1. Land-use Impacts in the Ayuquila Watershed

1.1. Introduction

Human progress is linked to the use of aquatic ecosystems, as water is required for drinking, agriculture, fisheries, industrial activities, transportation, recreation and waste disposal. However, this interaction has negatively affected the quality, composition and functioning of these systems, making them among the most impacted in the world (Malmqvist and Rundle 2002). Human land use is known to be a major force that shapes the composition and structure of ecological communities (Dodson *et al*. 2005). In a watershed, elements like vegetation cover, geomorphology and lithology, among others, also influence the physicochemical characteristics and diversity of the freshwater systems found (Carral *et al*. 1995; Ometto *et al.* 2000; Hunsaker and Hughes 2002; Hughes and Hunsaker 2002).

Land use change is a term used to address all direct and indirect human modifications of Earth's surface, mostly to obtain food and other resources, but also those resulting from industrialization and urbanization (Ellis 2010). Land use change is considered as the single most important component of global change affecting the structure and composition of ecological systems (Vitousek 1992, 1994), with aquatic diversity facing greater risks and higher extinction rates than terrestrial species (Hughes and Hunsaker 2002). The effects of land use change at different levels became important as a research topic in the last decades of the 20th Century, as it was linked to global climate change (Houghton 1994), a concern that was, and still is, getting attention. Not only do land use and land cover change affect natural communities, they also reflect the history, economic development and population growth of human communities and, as such, are forces that drive social change (Ojima 1994).

Watersheds are ideal organizational units for natural resource management and decision-making as they delimit identifiable landscape characteristics and human communities. Thus, as wetlands reflect and integrate the biological, physical and social processes operating at a larger scale, the changes occurring at the catchment's landscape drastically affect their freshwater systems' composition and function. Consequently, wetlands in general and lotic systems in particular, are facing multiple threats that jeopardize their existence and the services they provide worldwide (Brinson and Malvarez 2002; Junk 2002; Malmqvist and Rundle 2002).

Aquatic biodiversity's composition and integrity are regulated by *periodic* or *pulse* short-term natural disturbances, that include floods and droughts, and continuous anthropogenic *press* disturbances (Yount and Niemi 1990). Ecosystems and species react and recover differently to these two stressors, which frequently occur simultaneously. However, the most common agents of change in lotic systems belong to the second category, all part of what is termed land use change, and include agriculture expansion, channelization, logging, urban development, water diversion and extraction, impoundment, and pollution, among many others (Harding *et al.* 1998).

Native forest and grassland cover have been reduced drastically worldwide to give way to cropland and urban development, two major forces of land change. A 400% increase in agricultural land and a 30% decrease in forest cover have been estimated to have occurred between 1700 and 1980 (Meyer and Turner 1992). These changes affect all ecosystem components and, if coupled with global climate change (Carpenter et al. 1992), the impacts will undoubtedly act synergistically to impoverish the quality, biological richness and function of a community.

Freshwater has mostly been used for irrigation purposes since the beginning of human history and, despite an increase in the demand for clean water to meet the requirements of population and industrial growth, water for agriculture still accounts for almost two thirds of the total withdrawals which have also increased around 40 times since 1700 (McNeill 2000). Water pollution is the most common and widespread outcome of this overexploitation and misuse.

In the United States, municipal and industrial wastewater plants and waste disposal sites are the most common point sources of water pollution. Diffuse or non-point leading sources, on the other hand, include agricultural activities, hydrological modification (i.e., dredging, channelization and dam construction), habitat modification, and runoff from urban areas (U.S. EPA 2000).

Although point-sources of water pollution have been identified and controlled in many countries (Berka *et al.* 2001), particularly in temperate industrialized ones, it is a major problem in subtropical and tropical areas due to the lack of infrastructure to treat domestic and industrial effluents (Ometto *et al.* 2000). Diffuse-source or non-point pollution is still an important and usually overlooked problem worldwide. The increase of impervious surfaces -associated with urbanized areas- increases the amount and diversity of contaminants in what has been called the "urban stream syndrome" (Walters *et al.* 2009). This "cocktail" gets mixed with agricultural-derived effluents drastically polluting aquatic systems with suspended sediments, nutrients, heavy metals, inorganic and organic wastes, pesticides and pathogens (Carpenter *et al.* 1998, Welch and Jacoby 2004). This affects not only the quality of water to be used by human and riparian communities located downstream but also has an effect on the structure and functioning of freshwater and, ultimately, marine ecosystems.

1.2. Tracking Environmental Changes in Aquatic Systems

Several techniques have been used to track past and present ecological changes. Remote sensing using satellite imagery, aerial photographs, and geographical information systems are useful in evaluating physical changes in the landscape and land cover (e.g. Mas 1999). Analysis of lake and marine sediments, ice cores, tree rings, fossil records, combined with dating techniques using radioactive isotopes helps assess long-term changes in the environment (e.g IASC 2010). Stable isotopes, like their radioactive counterparts, are also used in long-term monitoring but have proven very useful in many other areas of ecological research (Thompson *et al.* 2005; Dawson and Siegwolf 2007). Unlike other techniques, the application of stable isotopes rests on the principle of predictable changes in the isotope ratios that can be obtained from plants and animals as these isotopes are assimilated in their tissues (Peterson and Fry 1987). Many of the increasing uses stable isotope analysis have include identifying animal migration patterns (Hobson 1999, Bowen *et al.* 2005), energy and matter flow (Kidd 1998, Finlay 2001), land use and ecological change (Heaton 1986; Fry 1999, Martinelli *et al.* 1999), as well as the source of organic matter (Peterson *et al*. 1985, Wayland and Hobson 2001).

For instance, differences in the photosynthetic pathways in plants have helped differentiate the origin of carbon between C_3 (i.e., forest vegetation and temperate grasses) and C_4 plants (i.e., tropical and salt marshes grasses). The former have an isotopic mean value of -27‰ whereas C_4 plants have a mean value of -12‰ (O'Leary 1988). The relative differences between these groups of plants are transferred up the food chain and are also maintained as plant tissues are incorporated into the soil's organic matter, breathed as carbon dioxide, or transformed into carbonates during the soil formation processes (Stevenson et al. 2005). In a similar fashion, nitrogen isotope ratios are associated with trophic transfer (Minagawa and Wada 1984), habitat source (e.g. marine versus freshwater, or terrestrial versus aquatic; France 1994), or nutrient pollution sources (Heaton 1986; Lake et al. 2001).

Just like specific vegetation types or human activities can be traced using stable isotope analyses, certain pollutants are also associated with particular land uses. Pollution in rivers can be linked to the presence of fine sediment and sediment-bound pollutants that originated in soils located upland and that can be traced to different land use types (Papanicolau et al. 2003). Mining, land filling, deforestation, and agriculture, for instance, are the main sources of heavy metals into the environment (Chang and Cockerham 1994; Morrisey *et al.* 2005b), while certain metals, such as zinc, cadmium, lead and copper are associated to city streets and roadside dusts (Hopke 1980; Harrison 1981). Atmospheric deposition is considered as the main source of metals and other pollutants to aquatic systems (Lovett 1994), with higher concentrations being deposited in rainy seasons (Samontha et al. 2007). The most obvious end result of metal presence in aquatic systems is the change in species and community composition (e.g. Deniseger *et al.* 1986, Van Griethuysen *et al.* 2004). Benthic macroinvertebrates and fish assemblages have been widely used as indicators of stream quality as each group responds differently to disturbances, offering a contrasting snapshot of the water quality of a site (Karr and Dudley 1981; Barbour et al. 1999).

The assimilation of metals, the use of biological indicators, the modification in community structure, and the change in isotopic ratios in animal or plant tissues, are just a few examples of the various techniques nowadays implemented to track environmental change in aquatic ecosystems. The application of these techniques has helped in the management and conservation of resources at different organizational levels (Shanley *et al.* 1998, Fry 2006).

1.3. The Ayuquila-Armería Watershed as a Case Study

Located in western Mexico, the 321 km long Ayuquila-Armería River (18°51’05” - 20°28’03” N and 104°38’17” -103°34’41” W) originates at 2,560 meters above sea level crossing the south portion of the state of Jalisco, and flowing through the state of Colima into the Pacific Ocean (Meza 2006). With a total drainage area of almost 9,900 km^2, it is considered one of the 15 most important rivers in the Mexican Pacific region due to its biodiversity (Hudson *et al*., 2005). Dry shrub, tropical deciduous, oak, pine, and riparian forests are the dominant natural vegetation types, covering 60% of the basin, while 30% of the landscape is dedicated to agriculture, with various types of agricultural products farmed and technology applied on its route; the basin has an average population density of 56 people km^2 (Hudson *et al*. 2005). Although different vegetation types occur at the watershed level, the dominant forest cover associated with the Ayuquila River is tropical dry deciduous forest in the surrounding mountains below the 1800 meters above sea level. Agricultural fields are constricting the riparian vegetation along the valleys. In many cases, sugarcane and maize fields border the river against environmental regulations.

Three sub-basins make up this watershed (Figure 1.1): Ayuquila, Tuxcacuesco and, when these two merge, Armería, with a length of 204, 119 and 116 km, respectively (Meza 2006). The sources of both the Ayuquila River and the Tuxcacuesco River are in the Sierra de Quila, in the centre of the state of Jalisco, at approximately 2,500 (20°27'09N, 104°10'39W) and 2,250 (20°16'53N, 104°04'24W) masl, respectively. The basin's environmental complexity, because of variations in geomorphology and land use, is responsible for the regional biological richness, making the conservation of aquatic fauna and associated human activities an essential activity. The Ayuquila-Armería watershed's biological importance is highlighted when considering that 10% of its total area is within the boundaries of five national protected natural areas (Graf *et al.* 2006, Meza 2006). On the social context, the human landscape is mostly rural with only six cities larger than 20,000 people; however, the river provides water to more than 500,000 inhabitants in both states (Martinez-Rivera *et al.* 2000).

The most recent agents of land use change in the study area have been urban growth, cattle grazing and agave cultivation, the latter needed for tequila production. Hostettler (2007) mentioned that in the municipality of Autlán the extension of dry forest decreased 10% while grasslands increased by 18% from 1990 to 2000. Land allocated for agricultural activities and urban expansion grew slightly in that same period. The expansion of agriculture –and reduction of dry forest- has occurred mainly in the hillsides, where temporal maize fields are losing ground to both pastures and agave plantations, the latter grown with large amounts of fertilizers and pesticides. Similar scenarios are common in other municipalities in this basin (Cárdenas *et al.* 2010). Despite the fact that there has only been a 2% increase in agricultural area in the valley of Autlan – El Grullo (Hostettler 2007), cash crops such as sugarcane, tomato, chilli pepper, and legumes dominate the agricultural landscape. On the other hand, while neighbouring municipalities are experiencing population decline, the cities of El Grullo and Autlan are steadily growing (Lomelí *et al.* 2003), and the valley concentrates the higher industrial and most intensive agriculture in the whole upper basin (Lyons *et al.* 1998).

Pollution, water diversion, change in the water flow, impoundment, introduction of species and habitat loss are among the main problems affecting the ecological integrity and conditions for human communities of Ayuquila basin (Martínez-Rivera *et al.* 2000; Henne *et al.* 2002; Graf *et al.* 2006). Municipal and industrial (sugar mill and tequila distilleries) wastewater is discharged into the river without treatment, although there are plans for the creation of water treatment facilities in several communities (Graf *et al.* 2006). In addition, numerous pig, *Tilapia*, and cattle farms exist along the river, while cash crops (mainly sugar cane, agave for tequila production, tomato, maize and chilli peppers) dominate the valleys, resulting in unknown discharges of sewage, nutrients, and pesticides into the river. Municipal open garbage dumps are common sight, and leakage of chemicals to irrigation channels, streams, and the river is highly possible. Water quality monitoring in various parts of the watershed have shown fecal bacteria and the presence of heavy metals in levels above those permitted by the Mexican law, suggesting that the Ayuquila-Armería can be unsuitable for agricultural and human use (Martinez-Rivera *et al.* 2000; Henne *et al.* 2002).

Aside from the "typical" characteristics that differentiate distinct climatic seasons in a river's context (i.e., wet and dry events), such as precipitation, amount of runoff and sediment load, temperature, evaporation rates, flow, among many others, all of which have an impact on the biological communities, in the study area the river's water flow is "reversed". The dams drain off more water to help with the sugar-cane irrigation schemes, and in the rainy season, the dams' gates are closed to accumulate water. Thus, when the river's flow should be in its maximum it is usually at its lowest, and it is particularly notorious in some sites (see Tables 1.1 & 1.2).

This problem in flow regime alteration is not new nor is it exclusive to this watershed (e.g. Poff *et al.* 1997; Bunn and Arthington 2002). Although the effect on biodiversity of flow regime alteration is not the purpose of this work, the study of seasonal differences on biota's structure and composition in an altered flow scenario, can help give guidelines for a better management of the watershed.

Another feature that characterizes dry season is the application of pesticides in the fields, which are undoubtedly dispersed easily and far away due to the strong winds that blow from February to May, and during the rainy season (June to October), these pollutants are washed down.

All these cumulative and synergistic effects have already made an impact in the River's biotic composition. Seven fish species have been extirpated or their populations drastically reduced from the upper and middle sections of the Ayuquila. These include the highly appreciated bullhead catfish (*Ictalurus dugesi*) and the endemic mullet (*Agonostomus monticola*) favoured by local fishermen (Lyons *et al*. 1998). The abundance and composition of the remaining fish community is being further altered by the presence of five introduced species, including two species of Tilapia (*Oreochromis aureus* and *Tilapia rendalli*) and the largemouth bass (*Micropterus salmoides*) (Lyons *et al*. 1998). Little is known about the macroinvertebrate fauna, but a similar scenario would not be surprising, as the previously unregistered Asian clam (*Corbicula fluminea*) and Apple snail (*Pomacea flagellata*) have become abundant and most likely already have displaced species of the poorly known native mollusc community of this river.

1.4. Study`s Sample Design

Seventeen locations were selected in the middle portion of the Ayuquila-Armeria watershed (Figure 1.1): twelve in the Ayuquila River, four in the Tuxcacuesco River basin, and an additional site at the Armería River. Samples were collected in August 2007, February and August 2008. The two field seasons in August will be named "wet" (i.e. "wet07" and "wet08"), as these coincide with the rainy season, and "dry08" to the one done in February. In order to show the differences between these seasons, I present the average temperature (T^oC) and rain precipitation (mm) values obtained from Mexican national environmental databases (INEGI 2007) for the months of February and August (Table 1.1). Table 1.2 summarizes some of the sites' physical characteristics, such as wet width and average depth, plus the average river flow (m^3/s) per season

(University of Guadalajara, unpubl. data). I hope that this information will aid in contextualizing the differences between seasons in a “river context”, which undoubtedly affect its biological structure and composition.

The location of the sampling sites coincides with those where monitoring has been taking place for more than 15 years (Martinez *et al.* 2000). All these sites follow the river’s flow and were selected based on accessibility, flow patterns, surrounding land use, and known point (sewage derived, urban, and industrial) or diffuse (agricultural, forest, and urban) sources of pollution. Although a mixed array of land uses surrounds many sites, the dominant land activity was established using Google Earth and confirmed visually. Four sites were surrounded mostly by forest; seven were in agriculture dominated landscape with two being open urban sewage drains; two were in agricultural – urban settings, while the remaining four were in forest –agricultural landscape. The general characteristics of each site are presented in Table 1.3. The acronyms used for each site reflect both their position in the upstream–downstream gradient (number or lower case letter as presented in Figure 1.1) and the presumed dominant land use influence (see further explanation ahead). Thus, the first number or letter in this code corresponds to the site’s position in the river’s gradient (shown in Figure 1.1), while the following two letters correspond to the main land use. When another land use covered a large area (i.e., ≥30% of the total area), the acronym included the additional two letters from that land use name.

In a river’s context, disturbance is expected to be progressive and cumulative. Under this criterion, the first site would be the least impacted while the last one in the gradient would be worse off. A simple analysis of the potential influence this accumulated disturbance has in the water column was made. The possible graphical outcome (Figure 1.2) and an alternative coding using this accumulated land use influence is presented in Table 1.4. Three sites in the Ayuquila sub-basin (DrenA “5.UrIA”, DrnG “7.Ur”, and Arroyo Manantlan “10.Fo”), and one from the Tuxcacuesco sub-basin (Tonaya “l.Ur”) are not considered in the accumulation analysis as the others as they are independent sources to the

River’s flow: the water from the first two originates from untreated municipal sewage and receive water from agricultural fields along its way, the third one is a stream flowing from a mountainous area with scattered temporal agricultural fields, and Tonaya flows into the Tuxcacuesco River originating in the mountain range of Tapalpa; it crosses the tequila producing town of Tonaya. The difference assigned in the coding to both sewage sites results from the length each drain has before it reaches the river. DrenA (“5.UrIA”) is almost 20 km from its source to the mouth, while DrnG (“7.Ur”) is only 7 km long. In all cases, the sites located after these three sites merge into the river are included the land use additions. As a result, the sites considered to be the least impacted, i.e.: La Laja (site “1.FoTA”), Presa (“a.Fo”) and Manantlan (“10.Fo”) were considered as reference sites for comparison with the other sites. The coding for each site resulting from this analysis (last column Table 1.4) is used in the figures, tables and analyses presented in the following chapters.

1.5. Aim and Organization of This Research

Despite the fact that there is a general good understanding of how land use modifications influence water quality and freshwater communities, there is a need for evidence on how these changes are affecting the aquatic communities in the Ayuquila Armeria watershed in Mexico. Such information is crucial in identifying those activities that might be posing higher risks to suggest management guidelines that will help improve the watershed’s health.

In the present study I employ isotope analysis and flame spectrophotometry to measure the carbon and nitrogen isotope ratios and metal concentrations from the Ayuquila-Armeria River’s sediment and fauna community from sites adjacent to different land use activities: agricultural, urban, and forest in two distinct seasons: rainy versus dry. Changes in the isotopic ratios can be related to either changes in vegetation cover (e.g. a shift from C_3 trees to C_4 grasses due to deforestation and agricultural expansion), or increases in human-derived pollutants, such as the increase of animal enclosures and city sewages, which enrich the nitrogen isotope ratios. In a similar fashion, heavy metals are known to increase because of urbanization and of the intensification of agriculture. These and other pollutants are then mobilized either in dust airborne particles during the dry season, or washed down and carried by storm runoff and irrigation channels to rivers and wetlands after a rainstorm.

The overall objectives are:

a) to determine whether the isotopic signatures and metal concentrations in the sediment and animal tissues from the Ayuquila River are associated to the isotopic and metal signatures commonly associated to the run-off and discharges from agricultural, forested or urban origins; and,

b) to determine if seasonal differentiation exists both in the isotopic ratios and metal concentrations found

The general research questions that I try to answer are:

1) Do the changes in adjacent land use increase the amount of suspended sediments in the river's column that causes a change in the river's biological composition?

2) Does the shift from forested to agricultural or urban landscape result in a carbon and nitrogen enrichment in the isotope ratios of the faunal riverine components?

3) Can agricultural and urban runoff in this watershed be associated with the presence of particular metals in the river biological components analysed?

This thesis is organized as follows: in Chapter 2 I describe the macroinvertebrate and fish species composition for each site sampled, make spatial and temporal comparisons, and try to relate the functional feeding group assemblages to water chemical variables using different metrics. In chapter 3, using carbon and nitrogen isotope analysis I aim to identify the sources of organic matter and characterize the food web structure in each site, and try to relate these to adjacent land use activities. Additionally, with the food web characterization obtained from stable isotope analysis, I quantify the concentrations of six metals in sediment and each functional group, and also try to relate them to surrounding landscape. I describe these findings in chapter 4. Finally, in a final chapter I summarize the major findings and caveats of this study. The long-term goal is to incorporate isotope tracing and metal analyses to the on-going river's restoration and water monitoring programs that could result in an improvement of the river's quality and the overall welfare of this region in western Mexico.

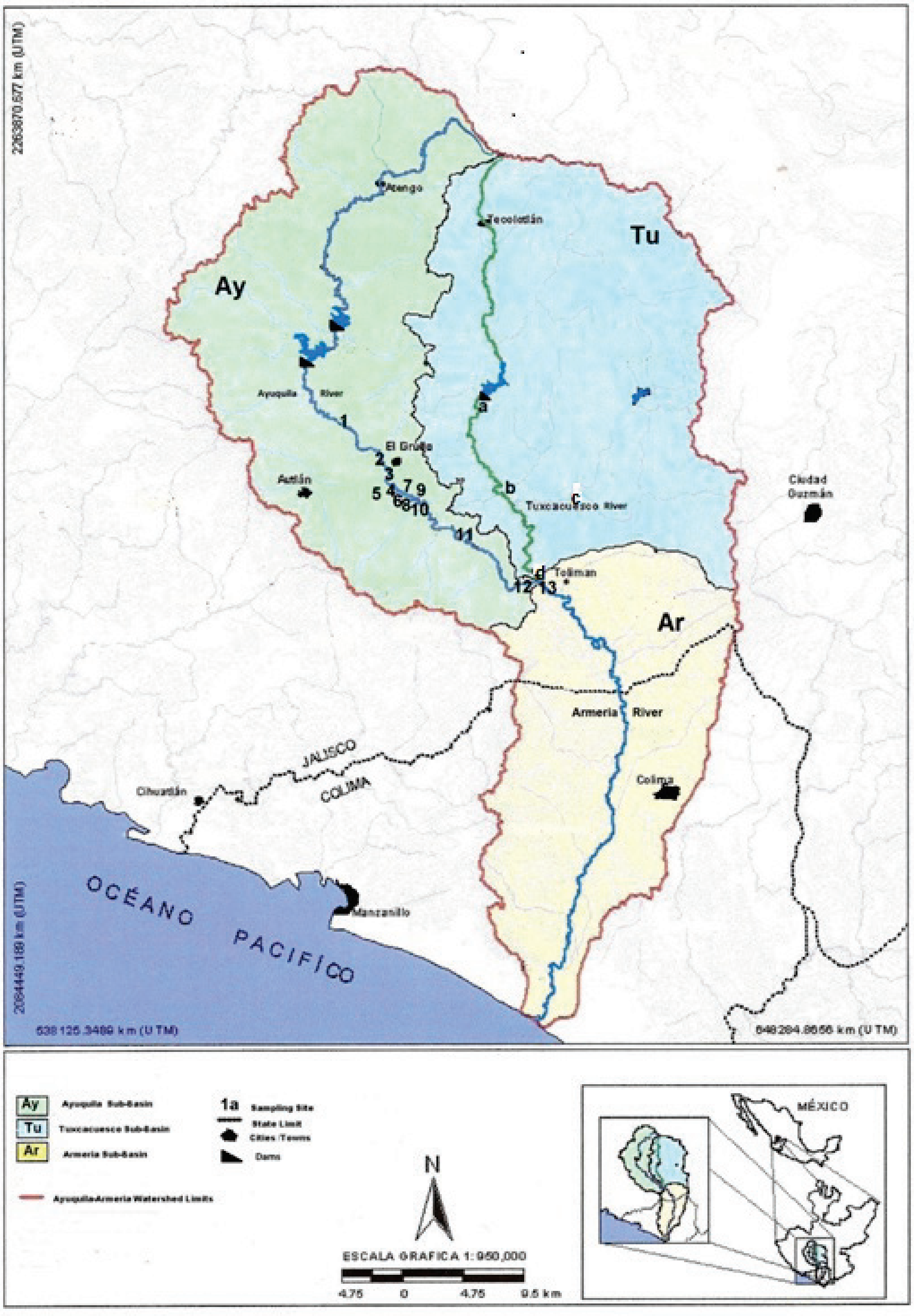


Figure 1.1. Map of the Ayuquila- Armería watershed in western Mexico (from Meza 2006). The coloured areas correspond to the three sub-basins that form the watershed. Numbers and letters show the location of the sites.

Table 1.1. Average temperatures and rain precipitation values from three localities in the state of Jalisco located in the study area. Data from El Grullo and Tuxcacuesco, in the Ayuquila-Armeria watershed, are from a 30-year period, while data from Tecomates, located 30 km south, in a more humid region, includes the average values from a 45 year period, as well as the average from 2006 (INEGI 2008).

	February (Dry Season)		August (Rainy Season)	
	T(°C)	Precipitation (mm)	T(°C)	Precipitation (mm
(1971-2000):				
El Grullo	21.2	8.5	25.6	181.4
Tuxcacuesco	21.7	8.3	26.7	145.1
Tecomates (1962-2006)	26.8	5.2	30.5	301.9
Tecomates (2006)	24	0	26.1	421.3

Table 1.2. Average river width, depth and flow in each of the study sites during the three sampling periods.

	Wet07			Dry08			Wet08		
Site	Avg wet width (m)	Avge depth (m)	River Flow (m^3/s)	Avge wet width (m)	Avge depth (m)	River Flow (m^3/s)	Avg Wet width (m)	Avge depth (m)	River Flow (m^3/s)
1.FoTA	33	0.42	2.65	33	0.66	11.46	30	0.29	0.72
2.IAFo	FL	FL	FL	5	0.35	--	8	0.3	-
3.IA	FL	FL	FL	8	0.29	11.7	7	0.22	2.42
4.IA	FL	FL	FL	18	0.26	1.91	18	0.26	1.72
5.UrIA	4	0.62	0.89	4	0.65	1.43	6	0.75	0.47
6.IA	8	1	11.19	8	0.41	4.05	7	0.4	3.92
7.Ur	10	0.79	2.62	9	0.49	1.99	8	0.3	0.41
8.IA	FL	FL	FL	27	0.15	6.36	30	1	--
9.IA	FL	FL	FL	21	0.95	5.65	24	1.2	--
10.Fo	13	0.27	4.05	11	0.24	1.17	12	0.48	4.53
11.IAFo	22	0.82	15.76	19	0.35	4.81	20	0.6	13.37
12.IAFo	FL	FL	FL	39	0.5	4.01	37	0.6	9.86
13.IAFo	FL	FL	FL	42	0.62	9.71	40	1.5	12.69
a.Fo	14	0.55	--	12	0.7	--	13	0.8	--
b.FoIA	FL	FL	FL	10	0.6	--	10	0.7	--
c.Ur	10	0.4	--	--	--	--	7	0.3	--
d.FoUr	FL	FL	FL	17	0.45	3.58	17	0.7	6.13

Table 1.3. General characteristics of the sampling sites with main surrounding land uses and observed human uses. (Acronyms used for each site).

Site	Long	Lat	Alt. masl	Site characteristics and surrounding landscape[1]	Observed human uses/Point sources[2]
Ayuquila River Sub-basin					
La Laja **(1.FoTA)**	19°51'23"	104°17'20"	915	River bank with > 20 m wide corridor; native trees. Abundant riffles & pools. Village on left bank. Fo 65%; TA. 30%; Urb 5%	Recreation, fishing, clothes washing, cattle. Heavy transited paved road ~200 m away. Untreated water from village directly to river.
Chacalito **(2.IAFo)**	19°48'45"	104°14'26"	871	River bank with grasses and scattered trees. One lane low bridge crosses the site. IA. 97% Urb 3%	Recreation, fishing, car washing, gravel grinding site 30 m away. Low bridge.
Puente El Grullo **(3.IA)**	19°47'27"	104°13'45"	870	Banks with grasses, abundant vegetation in the river. IA. 95% Urb 5%	Gravel and river sand extraction. Rain storm drain. Underneath a bridge with heavy traffic.
La Herradura **(4.IA)**	19°45'40"	104°12'56"	863	Left River bank >10 m wide corridor and grasses, right bank with trees and cattle. IA.95% Urb 5%	Fishing, recreation. Car washing. Low water crossing. Cattle corral
Dren Autlan **(5.UrIA)**	19°45'35"	104°13'03"	864	Banks with grasses. Open air sewage channel. Urb 70%, IA 30%	Water from untreated municipal sewage.
Palo Blanco **(6.IA)**	19°44'29"	104°10'41"	858	Village on left bank. Bridge over site. Grasses & scattered trees. IA. 95% Urb 5%	Untreated water from village
Dren Grullo **(7.Ur)**	19°44'40"	104°09'58"	861	Banks with grasses, . Open air sewage channel Urb 85%, IA 15%	Water from untreated municipal sewage .
Achacales **(8.IA)**	19°42'12"	104°08'38"	858	Banks > 20m wide corridors native trees. TA.75% IA15%, Urb 5%; Fo 5%	Cattle watering and crossing.
Antes Manantlan **(9.IA)**	19°41'26"	104°08'23"	856	Forested banks and forest. Fo 95% TA 5%	Cattle watering.
Arroyo Manantlan **(10.Fo)**	19°41'23"	104°08'17"	860	Forested banks > 20 m wide. Fo 95% TA 5%	Recreation. Water coming directly from mountain
Zenzontla **(11.IAFo)**	19°39'58"	104°05'08"	850	F 60%. TAgr 40% Abundant riffles and pools	Cattle, Crayfish fishing, fishing, recreation.
Paso Real **(12.IAFo)**	19°36'01	103°57'52"	689	F 70% IAgr.20%, TA 10% > 20 m wide tree corridor	Fishing, recreation. Cattle watering
Tuxcacuesco River Sub-Basin					
Presa LasPiedras **(a.Fo)**	19°54'43"	104°03'58"	815	F 75%, TA 15%. Dam curtain 10%	Recreation.
S Buenaventura **(b.FoIA)**	19°47'29	104°03'14"	762	Village on left bank. IA. 65% Urb 25%, F 10%. Grasses and trees. Sand islands, partly channelized.	Fishing, cattle. Untreated water from village directly to river
Rio Tonaya (**c.Ur)**	19°46'46"	103°58'46"	804	Urb 80% IA.20% Grasses in river banks. Trash common in both banks	No observed uses.
Tuxcacuesco **(d.FoUr)**	19°36'41"	103°57'57"	696	TA. 60% F 40% Native trees in bank..	Cattle, crayfish fishing
Armeria River Sub-Basin					
Rio Armeria **(13.IAFo)**	19°35'59"	103°57'29"	685	TA.75% Urb 25% bridge crosses area, Small town above	Clothes washing, low bridge, sand extraction. Crayfish fishing

1= Landscape surrounding the site considered ~10km area upstream with emphasis on vegetation types, human buildings; includes site's bank characteristics: **TA**= Temporal fields with no irrigation, also used as cattle grazing areas; **IA**= intensive agriculture: irrigated sugar cane and maize fields; agave plantations which, although not irrigated, use agrochemicals; Cattle= pig or cattle enclosures. **U**rban includes towns, roads or buildings; **Fo**r= Forest, mostly tropical deciduous or sub-deciduous vegetation.The bolded figure corresponds to the classification assigned. **2**= Recreation includes areas for picnics and swimming.

Table 1.4. Sites' arrangement and coding based on upstream landuse versus a coding based on potential effects from accumulated disturbances.

Site	Name	Surrounding Landuse (%)				Code based on	Cumulative average fractions upstream -downstream				Code used in this study based on accumulated
		Fo	TA	IA	Ur	Landscape	Fo	TA	IA	Ur	Influence
1	La Laja	65	30		5	FoTA	**0.65**	**0.30**	0.00	0.05	1.FoTA
2	Chacalito			97	3	IA	**0.33**	0.15	**0.49**	0.04	2.IAFo
3	Puente El Grullo			95	5	IA	0.22	0.10	**0.64**	0.04	3.IA
4	La Herradura			95	5	IA	0.16	0.08	**0.72**	0.05	4.IA
5	Dren Autlan			30	70	UrIA	0.00	0.00	**0.30**	**0.70**	5.UrIA
6	Palo Blanco			95	5	IA	0.11	0.05	**0.69**	0.16	6.IA
7	Dren Grullo			15	85	Ur	0.00	0.00	0.15	**0.85**	7.Ur
8	Achacales	5	75	15	5	TA	0.09	0.13	**0.55**	0.23	8.IA
9	Antes Manantlan	95	5			Fo	0.18	0.12	**0.49**	0.20	9.IA
10	Arroyo Manantlan	95	5			Fo	**0.95**	0.05	0.00	0.00	10.Fo
11	Zenzontla	60	40			FoTA	**0.30**	0.14	**0.39**	0.17	11.IAFo
12	Paso Real	70	10	20		Fo	**0.33**	0.14	**0.39**	0.15	12.IAFo
a.	Presa Las Piedras	75	15		10	Fo	**0.75**	0.15	0.00	0.10	a.Fo
b.	San Buenaventura	10		65	10	IA	**0.43**	0.08	**0.28**	0.23	b.FoIA
c.	Rio Tonaya			20	80	Ur	0.00	0.00	0.20	**0.80**	c.Ur
d.	Tuxcacuesco	40	60			FoTA	**0.31**	0.19	0.19	**0.31**	d.FoUr
13	Armeria		75		25	TA	**0.30**	0.19	**0.32**	0.20	13.IAFo

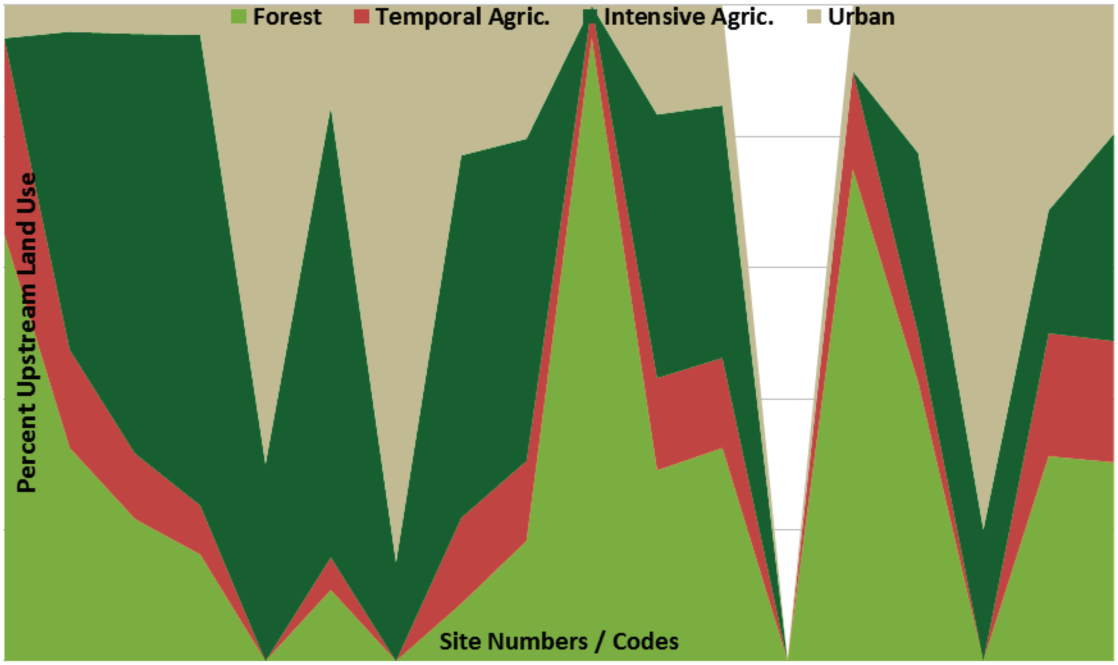


Figure 1.2. Accumulated landuse influence in each site in 17 locations in the Ayuquila-Armeria River. Site numbers are in a upstream – downstream gradient using the information in Tables 1.2 and 1.3. Sites 1 to 13 are from the Ayuquila River, a to c from the Tuxcacuesco River, and T from the Tonaya site. See text and Table 1.3 for more information.

2. Description of the Ayuquila River's Macro-invertebrate and Fish Composition

Abstract

The objectives of this study were to 1) characterize and compare the invertebrate and fish composition in different sites in the Ayuquila River, Mexico, and, 2) to relate each site functional feeding assemblages with each site's water physicochemical variables. Invertebrate were identified to family while fishes to species level, and grouped into functional groups. Community composition was compared spatially and between dry and rainy seasons. Non-Metric Multidimensional Scaling (NMDS) was used to look for spatial site arrangement based on species composition, while Canonical Correspondence Analysis (CCA) was performed to look for environmental relationships. Invertebrate composition and abundance were significantly different ($p<0.001$) among sites but not between seasons, with the rainy seasons showing higher diversity. Diversity was lower in agricultural areas and increased as the river flowed through forested sites. Fish species composition showed no statistical differences between seasons but among sites differences were significant in the rainy season ($p=0.043$). Filter and gatherer collector invertebrates and omnivore fishes were the most abundant functional groups throughout the sites. Invertebrate abundance was negatively correlated with turbidity ($p=0.033$) and total inorganic nitrogen ($p=0.046$) only in the dry season, while fish abundance was positively associated with hardness and conductivity ($p<0.05$) in both seasons. Although some water variables influenced species composition, weak and inconclusive relationships suggest the influence of other variables in determining community structure. Benthic invertebrate and fish indices of biotic integrity suggested sites' quality conditions ranging from fair to good. Turbidity, dissolved iron, inorganic nitrogen values, and concentrations of coliform bacteria suggested poorer conditions in many sites, with concentration limits above Mexican guidelines for irrigation and recreation purposes.

2.1. Introduction

Aquatic ecosystems are among the most threatened ecosystems worldwide; at the same time, water for human use is becoming a scarce resource and turning into a commodity in many places around the world (Postel 2000; Malmqvist and Rundle 2002). In addition to the problem of water scarcity, the increasing impacts human activities can have on this resource are reducing its quality and usability. Among the major human activities that are known to degrade water quality include deforestation, urbanization, runoff from agriculture and cities, flow modification and diversion, and untreated water discharge.

Many methods and techniques have been implemented for monitoring water quality. Initially, the goal of monitoring was, and still is, to identify organic pollution that could affect human health and spread disease. Subsequently, the main objective was the identification of various chemicals and their toxicity to organisms, to finally include the effects that landscape disturbances have in the structure and functioning of aquatic systems (Cairns and Dickson 1971, Karr and Chu 1997). Thus, disturbance is assessed by monitoring the impacts on the biological communities' structure and composition directly. Physical and chemical characteristics give a snapshot of the conditions of the river when the variables are taken, while the use of aquatic biota gives information of a longer period of time. In this regards, aquatic biota have become central in biological assessments, as it provides an integrative measure of water chemistry and physical conditions of its environment (Barbour *et al.* 1999). However, the response organisms have to anthropic disturbances depends on many variables (e.g., type of organism, feeding strategy, magnitude of the disturbance) and, as a result, a wide array of field and laboratory methods have been implemented worldwide to assess the effects on aquatic communities (de Zwart 1995), and in many instances, the approach taken requires the combination of different techniques.

Several of these measures generally use benthic macroinvertebrate (usually aquatic insects, crustaceans, molluscs, and annelids) and fish assemblages to assess impacts on streams, and include among their variables species diversity, community composition, similarity indices, pollution tolerance or biotic indices, and trophic or functional structure (Resh *et al.* 1996, Barbour *et al.* 1999). At the same time, the resulting assemblages represent the endpoint of the combined influences of various site attributes, such as channel morphology, hydrology, and water quality and quantity (Wang and Lyons 2003). In addition, the use of invertebrates and fish assemblages as indices for water quality offer different advantages and reflect different structural and condition attributes that complement the information sought for (Karr and Dudley 1981; Karr 1987; Lammert and Allan 1999). Invertebrates, which are relatively sedentary, provide a snapshot overview of prevailing water conditions in contrast to fish, which reflect longer term conditions of a site. Fish assemblages have also been amply used as indicators of river health (e.g. Karr 1991; Aguilar-Ibarra *et al.* 2003), human and natural disturbance (e.g. Fausch *et al.* 1990; Poff and Allan 1995), ecological restoration (Paller *et al.* 2000), making them key elements in numerous biological indices used worldwide (e.g. Lyons *et al.* 2000; Roset *et al.* 2007).

In most situations, analyses are done using the taxonomic and/or the functional approaches, in particular when the goal is to characterize an ecosystem's condition or where information on the taxonomic composition is scarce or poorly studied (Cummins *et al.* 2005). Functional feeding grouping is based on behavioural and morphological mechanisms of food acquisition. The benefit of using this approach is that one focuses on groups of organisms that obtain their food and process energy in a similar fashion instead of studying hundreds of different taxa (Merrit and Cummins 1996). It also allows for comparisons between geographic regions where communities are comprised by different taxa (Simberloff and Dayan 1991). Additionally, by classifying biota into functional feeding groups (FFGs), the assemblage structure provides information on the physical characteristics, quality of a system, the origin of energy sources, and its responses to environmental perturbations (Barbour *et al.* 1999). Based on the way food is assimilated, invertebrates have been generally classified as 1) *scrapers/grazers* which consume algae and associated material; 2) *shredders*, which consume leaf litter or other coarse particles of organic matter (CPOM); 3) *collector-gatherers*, which collect fine particles of organic matter (FPOM) from the stream bottom; 4) *collector-filterers*, which collect FPOM from the water column using a variety of filters; and, 5) *predators*, which feed on other consumers (Wallace and Webster 1996). Omnivores constitute a sixth category that, although initially considered rare or ignored in food web interactions (Pimm and Lawton 1977), has been accepted as a general feature of aquatic and terrestrial food webs (Cabana and Rasmussen 1994, Thompson *et al.* 2005) with different degrees of impacts in community structure and functioning (Pringle and Hamazaki 1998). In river ecosystems, crustaceans are considered the main invertebrate omnivores (e.g. Pringle *et al.* 1993; Parkyn *et al.* 2001). Based on their diet, fish are generally classified as 1) piscivores, 2) herbivores; 3) omnivores, 4) insectivores; 5) filter feeders; 6) invertivores; and, 7) generalists (Barbour *et al.* 1999). This trophic guild classification is based on the similarity in resource sharing, and is different from the term of functional group, where the similarity lies in the ecosystem function that each species perform in the environmentl (Blondel 2003).

The use of fish and invertebrates to assess water conditions and quality on a site are based on the presence of species with particular characteristics that combine and provide information on ecological organization, pollution tolerance, and natural life history, among others, and are known as integral biotic indices (Karr 1991, Karr and Chu 1997). The index values range from 0 to 10, where 10 is indicative of high nutrient concentrations. Therefore, the communities will be composed of species whose tolerance to organic pollution varies from very sensitive to tolerant. Once scored, the quality ratings range from excellent to very poor based on the likely amount of organic pollution in the site sampled. Coupled with the integral biotic indices (IBI), other metrics used include those that measure community richness (e.g. number of taxa), diversity (Shannon diversity H'), dominance (e.g. Simpson´s index D), evenness (J'), composition measurements (e.g., percentage of individuals of the orders Ephemeroptera, Plecoptera and Trichoptera, known as EPT index), and number of predators, among several others.

It has been widely accepted that a gradient exists in the macroinvertebrate functional composition in temperate rivers as these flow through lowland valleys towards its mouth from shredders to scrapers to filter and collector-gatherers with subtle changes in predator species numbers along the gradient (Vannote *et al.* 1980; Hawkins and Sedell 1981). However, it has also been noted that this generalized pattern varies as a result of basins characteristics or biome differences, with more drastic changes in those watersheds severely altered by human activities (see Delong and Brusven 1998). Additionally, inconsistencies in community functional changes, as well as differences in biological traits and stressors have been observed between neotropical and temperate rivers (Tomanova *et al.* 2007, 2008).

2.1.1. The Ayuquila River case study

As in many parts of the world, streams and rivers in west-central Mexico are severely degraded as result of human activities (Lyons *et al.* 1995; Mercado-Silva *et al.* 2006). Pollution, water diversion, introduction of species and habitat loss are among the main problems affecting the ecological integrity of the Ayuquila River in the states of Jalisco and Colima (Martinez-Rivera *et al.* 2000; Henne *et al.* 2002). A water quality monitoring program was implemented 15 years ago, and the application of fish and invertebrate biological indices followed a few years later. However, a systematic study of the structure of these assemblages or of the possible changes in their composition resulting from disturbances is lacking. In this part of the watershed, the change from adjoining forested slopes to agricultural flat valleys dominated by sugar cane, irrigated maize crops and, most recently, agave plantations would undoubtedly influence the trophic structure of existing communities. The removal of canopy cover and/or the reduction of riparian buffering vegetation, activities that are common in the study area, increase solar exposure and the susceptibility to bank erosion, which increase water temperatures and turbidity affecting the biotic composition.

Additionally, agricultural nutrient and pesticide runoff contributes to a differentiation in the composition and density of macroinvertebrate and fish communities, impacts that have been widely recorded worldwide (e.g. Lenat 1984; MacLeod 1998; Hooda *et al.* 2000; Parr and Mason 2003; Zimmerman *et al.* 2003; Scherr 2010), usually reducing the diversity found, and the functional group composition in relation to less impacted areas.

With these ideas in mind, this study is part of a larger project that aims to answer the question about the relationship between human activity and its ecological effects at a watershed level in western Mexico. It is also a first exercise to compare the composition of this neotropical river's macroinvertebrate and fish assemblages along a temporal and spatial gradient in the Ayuquila basin with a multimetric approach and a perspective of functional groups. Two previous works (Henne *et al.* 2002, Weigel *et al.* 2002) only identified the structure of macrofauna to validate a benthic biotic index, but despite the on-going monitoring program, an analysis of the long-term changes in its structure and composition through time and space has not been done so far.

The objectives of this study are a) to characterize and compare the invertebrate and fish community compositions in different sites in the Ayuquila River, and b) to relate the functional feeding group assemblages with water physicochemical variables. I expect that each site's community composition will be related to the amount of suspended sediments and organic content. That is, filter and gatherer collectors will dominate in those sites with higher turbidity In addition, the macrofauna assemblage and tolerance to organic pollution will reflect the water quality conditions of the sites monitored as suggested by the Indices of Biotic Integrity.

2.2. Methods

2.2.1. Sample collection

Site selection and sampling design are explained in the previous chapter. Similarly, the general characteristics of each site are summarized in Table 1.3.

Physical and chemical parameters taken for each site included average stream depth (m), stream wet width (m), temperature (^{O}C), dissolved oxygen (mg/l), conductivity (μohms/s), and turbidity (NTU). Water samples were collected in 1 L plastic bottles at the middle of the river for laboratory analysis of water hardness ($CaCO_3$), iron, nitrates (NO_3-N), ammonium (NH_4-N) sulphates (SO_4), phosphates (PO_4), and fecal coliforms. Nitrate and ammonium values were summed up and considered as Total Inorganic Nitrogen (TIN) (Table 2.1). Some of these parameters (i.e. fecal coliforms, PO_4, NO_3, turbidity and iron) were compared with Mexican water quality guidelines for human consumption and irrigation (DOF 1989; DOF 2000). In addition, a Benthic Invertebrate Index of Biotic Integrity (B-IBI) based on family presence *sensu* Henne *et al.* (2002), and a Fish Index of Biotic Integrity (F-IBI) *sensu* Lyons *et al.* (1995) were calculated to contrast our biological findings with each site's conditions pertaining recreational use, irrigation, or as source for potable water. These criteria are summarized in Table 2.2.

Macro-invertebrates were collected using a D-frame kick net with a 600-μm mesh perpendicular to the river flow and disturbing the area upstream the net for a total of 6 meters in different stretches along the river. Asian clams (*Corbicula fluminea*) and Apple snails (*Pomacea flagellata*) were caught by hand. Although apple snails cannot be considered benthic invertebrates, its presence indicates disturbance and, being an introduced species, constitutes a possible problem as an agricultural pest, vector of disease, and ecological competition (Cowie 2002), but have also proven to be good biological indicators of contamination (Fu *et al.* 2011). All samples were placed in labelled plastic whirl-pak or Ziploc bags in an icebox. In the laboratory, these were counted and identified to family or genus when possible. Invertebrates were grouped as scrapers, filter-collectors, gather-collectors, shredders, predators, and omnivores following (Merrit and Cummins 1996). Fish were caught using 10 m seine nets along the riverbanks for a period of 45 minutes. Each fish species was identified and categorized by feeding or trophic guild as herbivores, omnivores and carnivores *sensu* Mercado-Silva *et al.* (2002). Although the difference between trophic guild and functional group is acknowledged and have been used as synonyms (Blondel 2003), I use the term of functional feeding group to refer to the fish species trophic guilds in this study.

Benthic invertebrates and fish data were analyzed independently. I calculated community structure and composition for each site considering: total abundance (i.e., number of individual animals per sample), taxonomic richness (i.e., total number of fish species, and number of invertebrate families), Shannon diversity index (H'), number of different FFGs, and their tolerance to organic pollution to obtain each site's index of biotic integrity (Lyons *et al.* 1995, Henne *et al.* 2002, Weigel *et al.* 2002). Species diversity indices were calculated using PcOrd for Windows, version 5.10 (McCune and Mefford 2006).

2.2.2. Data analysis

Parametric and non-parametric analyses of variance were used to test for differences in water chemical characteristics, and invertebrate and fish composition data among sites and seasons. Pearson product moment correlations between invertebrate and fish abundances with individual water chemical variables were examined. These analyses were performed using SigmaPlot version 11.0 (Systat software 2008), with a *p* value significance level of 0.05.

Indirect and direct multivariate ordination methods were performed to analyze community composition and its relation with water variables using PcOrd for Windows, version 5.10 (McCune and Mefford 2006). Non-metric multidimensional scaling (NMDS) (Kruskal 1964, Mather 1976), an indirect method, based on macroinvertebrate family and fish species assemblages was used to identify similarity patterns in species data sets based on ranking distances (Beals 2006) Composition was assessed in this analysis using as input data each site's invertebrate family or fish species abundances. Initial runs were made in autopilot mode using 6 dimensions, Sørensen (Bray-Curtiss) distance as the index to measure dissimilarity, and 250 iterations to evaluate "stress" or instability. An outlier analysis was performed to detect those sites that would skew the results, and if found, data from the site was discarded. I ran the program several times to find the lowest number of axes or dimensions that showed the least stress possible. Low stress indicates that the ordination obtained is a good representation of the original data (McCune and Grace 2002). A final run was made with the number of axes suggested by the program, and the resulting graph was used.

Canonical correspondence analysis (CCA), a direct ordination method, constrains an ordination of one matrix (i.e. species composition and abundance) by a multiple linear regression on a matrix containing the environmental variables for the same samples (McCune and Grace 2002). Thus, CCA helps answer the question of how much variation in species presence and abundance is directly explained by the environmental variables. The options selected for the analysis were: axis score centered and standardized to unit variance, ordination scores to optimize sites, graphing sites to show linear combinations of environmental variables, and a MonteCarlo test of significance where null hypothesis was no linear relationship between matrices.

In this analysis, CCA was performed to address the answer if the community structure was related to a particular water variable. Invertebrate and fish abundances per site were transformed to presence absence data, and were related to nine environmental variables used: temperature (^{O}C), dissolved oxygen, turbidity, conductivity, total inorganic nitrogen (TIN), dissolved iron, sulphate, phosphate, and fecal coliform concentrations. Prior to the multivariate ordinations, Pearson product correlations were run to eliminate those variables that were significantly correlated.

2.3. Results

Seventeen sites in total were sampled in the three seasons. Sampling in 2007 was done in nine sites where the conditions were safe to sample invertebrates, as the river's current was stronger than normal due to flooded conditions; fish sampling was omitted for these reasons.

I collected 2,923 invertebrates from 24 families, 1,254 fishes from 15 species and 9 families. Leeches and planarian worms were abundant in one site, but were not considered in the diversity and biotic indices calculations. Only 120 fish individuals were sacrificed for laboratory analyses. These were classified into six invertebrate functional feeding groups and three fish trophic guilds.

2.3.1. Invertebrate community metrics and functional feeding groups

Taxa richness was higher in wet08 than the two other seasons with 24 families, followed by dry08 and wet07 with 20 and 14, respectively. However, no statistically significant differences in the number of families and total abundances between field seasons were observed (H_{df2}=0.549, *p*=0.760). Overall, three mayfly (Ephemeroptera: Leptophlebidae, Baetidae and Heptageniidae) and one caddisfly (Trichoptera: Hydropsychidae) families comprised more than 60% of the total macroinvertebrates caught, with riffle beetles (Coleoptera: Elmidae) and dragonflies (Odonata: Coenagrionidae) following in abundance. Crayfish (Palaemonidae), "burrower" Gomphidae dragonflies, long-toed water beetles (Dryopidae), and giant water bugs (Belastomatidae) were only caught during the wet08 season (Figure 2.1). Site by site composition generally followed the above pattern, i.e., caddisflies and mayflies were the dominant families. The relative and total invertebrate abundances arranged by family per site for each field season are listed on Appendices 2.1 to 2.3.

Total invertebrate abundance, diversity (H') and the number of families varied considerably between sites and seasons, and no pattern was discernible. A sharp decrease in the total abundance and number of families was seen from site "1.FoTA" up to site "8.IA" during both wet seasons, with an opposite trend in dry season; numbers increased afterwards (Figure 2.2a). It was in this last site (8.TA) where leeches, planarian and other freshwater worms were abundant, with the particularity that the rest of the invertebrates caught were particularly small. Taxa richness and abundance comparisons between sites in each field season were statistically significant ($p<0.001$); however, when compared among seasons, no statistical significance existed for taxa richness (F_{df2}=1.989, p=0.149), diversity (H') (F_{df2}=1.86, p=0.176), or total abundance (F_{df2}=2.848, p=0.075). Community metrics for each season by site are listed on appendix 2.4.

Regression analyses for each water quality variable against invertebrate abundances were applied only to dry08 and wet08 data (Figure 2.3). No statistically significant associations were seen between abundance and water variables for both seasons, except for turbidity (p=0.033) and total inorganic nitrogen (p=0.03), which presented negative and significant correlations only in dry08 (Figure 2.3. g-h). Most of the variables were positively related in one season but showed a negative association in the other.

Diversity (H') values ranged from 0.19 to 2.19 for the three field seasons. Diversity per site decreased as the Ayuquila river entered the Autlan - El Grullo valley (sites 1.FoTA to 8.IA), and increased in those sites where the river crossed a mountainous area (sites 10.Fo through 12.IAFo).

Two introduced mollusk species, Apple snails (*Pomacea flagellata*) and Asian clams (*Corbicula fluminea),* were quite common throughout the sampling sites. The latter is first reported for this watershed in this study, and was the only bivalve found. Empty shells of both species were seen along the banks, irrigation channels, and some sugar cane fields.

The invertebrates sampled were classified into six functional feeding groups (Table 2.3). As it was expected from the family assemblages described above, gatherer and filter collectors were the most abundant FFG and were also collected in most sites, followed by predators and scrapers. Omnivores and shredders comprised less than 1% of the total sample (Figure 2.4). There were statistically significant differences in functional composition in dry08 (H_{df5}=45.086, p<0.001) and wet08 seasons (H_{df5}=45.393, p<0.001). Although relative abundance varied drastically for some functional groups (e.g. range 2 to 257 individuals per site), statistically significant differences were only evident for gatherer collectors (F_{df2}=5.196, p=0.012).

2.3.2. Fish species and functional group composition

Fifteen fish species were captured during this study (Table 2.4), ten in the dry08 and four more in wet08 (Figure 2.5), with a total abundance of 552 and 671 individuals each. Number of individuals captured as well as the composition by site varied, with 30% of the 12 sites in dry08 and half of 14 sites in wet08 having more than two species (Figure 2.6). Fish species abundance and composition were dominated by two native (Goldbreast splitfin - *Ilyodon furcidens-,* and Pacific molly -*Poecilia butleri)* and one exotic (Blue tilapia *Oreochromis aureus*) species in both field seasons, with 89% and 87% of the total abundance in the dry and wet seasons respectively. Fish species composition and total abundance differences were not statistically significant between seasons (Mann-Whitney U 80.5, t=185.5, p=0.433), nor among sites in dry08 season (t_{df12}=2.091, p=0.057) but these were significant in wet08 (t_{df13}=2.238, p=0.043).

In general, fish abundance, species richness and diversity were higher in wet08 than in dry08; these values increased as the river left the valley and flowed through a mountainous area in both seasons. These three metrics showed statistically significant values ($p<0.03$) when compared among sites, both in dry08 and wet08 seasons. Similarly, when compared between seasons significant differences were obtained for richness (t_{df14}= -2.87, p=0.012) and diversity (H') (t_{df14}=2.679, p=0.018), but not for abundance (z=0.05972, p=0.583). These values by season and by site are summarized in Appendix 2.5.

Unlike invertebrates, fish abundances had positive associations with water variables except for dissolved oxygen, PO_4 and turbidity (Figure 2.7). However, hardness and conductivity were statistically significant ($p<0.05$) in both field seasons, while temperature (p=0.003), temperature (p=0.019), and SO_4 (p=0.013) were significant only in dry08 (Figure 2.7c-d).

Herbivores, omnivores and carnivores were all represented in both field seasons, with omnivores being the predominant group with more than 70% of the total composition for both seasons and sites (Figure 2.8). No statistically significant differences were observed for fish functional group composition neither between seasons, nor among sites in each season.

2.3.3. Water quality based on the invertebrate and fish assemblages

The fish (F-IBI) and invertebrate (B-IBI) biotic integrity indices obtained suggested that the overall water conditions ranged from excellent to fair (B-IBI average for three seasons= 3.2±1.5; F-IBI average for two seasons= 42.8±16.4; see Table 2.2). More than 80% of the invertebrate assemblages suggested good or better water quality conditions (i.e., value ≤ 4.1) in most sites (Figure 2.10a) compared to fish assemblages (Figure 2.9b), in which only 16% could be considered as optimal (values ≥ 60). In general, water quality conditions using both biotic indices were better in the wet08 season, with only one site (Prsa) indicating “very poor” conditions (Figure 2.10a). There were no statistical significant differences in the B-IBI values among the three seasons (F_{df2}=1.472, p=0.247), in contrast to the F-IBI value differences between two seasons (t_{df14}=2.942, p=0.011).

Finally, the presence of fecal coliforms suggest that the quality of the water in all sites but three (10.Fo, 12.IAFo, and 13.IAFo) were above the Mexican maximum permissible limits (1000 fecal coliform colonies/ 100 ml) for irrigation and as source of potable water (Figure 2.10), the main uses the river’s water is intended for by people in the surrounding communities. Recreation and wildlife protection limits of fecal coliforms were all surpassed. Other parameters measured, such as turbidity, iron and nitrates (see Table 2.2) were also above those established by the law.

2.3.4. Sites arrangement using multivariate ordination analysis

Wet07

Presence-absence data from 14 invertebrate families were analyzed using NMDS, which resulted in a 3-dimensional arrangement. Two axes that explained 88% of the total variance with a final stress of 0.0003 after 120 iterations is presented in Figure 2.11a. In contrast, the three resulting axes from the CCA analysis explained a total variance of 70.2%. The tolerance level for each axis was achieved after 39, 21, and 30 iterations, respectively. SO_4 (r=0.584), conductivity (r=-0.683) and dissolved Fe (r=-0.683) were strongly correlated with axis 1. Summary statistics of this analysis are shown in Table 2.5.

In both NMDS and CCA outputs, river sites were arranged in a similar way, although NMDS clearly separated three groups. Sites with similar accumulated landuse effects grouped closely, as well as the three reference sites, although an urban influenced site was also in that cluster (Figure 2.11a). Those sites closer to axis 1 in the CCA graph (Figure 2.11b) are more strongly influenced by sulphate, dissolved iron and conductivity, whereas those to the right of axis 2 are influenced both by high dissolved oxygen and turbidity concentrations. However, overall relationships between the species data and the environmental data were not statistically significant (p=0.1822).

Dry08

NMDS spatial arrangement for this season (Figure 2.12a) using invertebrate and fish functional group presence absence data from 12 sites yielded a 2-dimensional solution. These dimensions explained 97.2% of the total variance with a final stress of 4.2479 after 200 iterations. In this spatial arrangement, NMDS did not clearly separate the 12 sites in groups, although two sites, with known sewage derived influence (6IA and 8TA) were isolated. Furthermore, those sites with a mixed landuse intensive agriculture-forest were more closely grouped than those with intensive agriculture, which were scattered (Figure 2.12a).

Three dimensions were obtained from the CCA analysis which explained 74.8% of the total variance. Tolerance levels for each axis were reached after 21, 15 and 12 iterations, respectively. The graph with the two axes that explain 64% of this variance is shown in Figure 2.12b. Similarly to NMDS, the CCA biplot showed sites loosely arranged in the ordination space with five sites to the right of axis 2 being more influenced by higher values in five environmental variables. However, the only water variables strongly correlated with axis 1 were dissolved Fe (r=0.714) and fecal coliform concentrations (r=0.841). Species data and environmental data correlations in this season were not statistically significant (p=0.1451) (Table 2.6).

Wet08

The NMDS ordination with functional group data from 14 sites yielded a 3-dimensional plot in which 97% of the variance was explained, with a stress of 1.0248 after 200 iterations. The graph depicting the two axes which represent 92.4% of the cumulative variance is presented in Figure 2.13.

The CCA analysis resulted in a 3-dimensional ordination explaining 51% of the total variance in species data, in which conductivity (r=0.691) and dissolved oxygen (r=-0.666) were correlated to axes 1 and 2, respectively (Table 2.7). Thus, these two axes which accounted for 45.4% of the total variance, were graphically represented (Figure 2.13). Those sites to the right of axis 2 had higher coliform and conductivity concentrations. The relationship between the functional group data and the water variables in the ordination space was statistically significant (p=0.02).

2.4. Discussion

2.4.1. Invertebrate Community structure and Functional Group Composition

As mentioned before, this study is the first exercise to seasonally and spatially characterize and contrast the macroinvertebrate structure and composition in the middle section of the Ayuquila River, and to relate the resulting assemblages to several environmental parameters in a multimetric approach. Variations in abundance were important at the site level. Those sites with surrounding forest had higher diversity than those surrounded by agriculture.

Although the invertebrate samples obtained from the first field season were skewed as a result of the flooding conditions in most sites, fact that could be noted by a reduced taxa richness and overall abundance, the differences were not significant with respect to the other field seasons. Weigel *et al.* (2002)

reported a similar family composition pattern from the sites they sampled in the Ayuquila River to validate the B-IBI, without mentioning the abundance or presence of particular taxa, such as dragonflies and damselflies, which are abundant all over. However, these changes in structural diversity were negligible when considering the functional composition

The presence of apple snails in the Ayuquila River was reported in the early 2000s (Palomera-García *et al.* 2006), but other published reports on the mollusk fauna are not known for either the Ayuquila or Tuxcacuesco Rivers, so the presence of *Pomacea flagellata* is confirmed while this is *Corbicula fluminea*'s first report. The presence of these two mollusk species constitutes a call of attention for the need of a more thorough study in the region, in particular when the knowledge of the biodiversity is scarce, and when the presence of aggressive introduced species may pose a threat to the native biodiversity.

Invertebrate Functional Feeding Group Composition

The idea that the invertebrate FFG composition would be related to the amount of suspended sediment in the water column was confirmed in this study. Gatherers and filter collectors were by far the most abundant and ubiquitous groups, which reflect the amount of organic and suspended material flowing in the river, as well as the algal mats covering the river's substrate. The FFG assemblages observed in the study sites are distinctive of disturbed areas. Moreover, they are common in large order rivers at low altitudes areas, in which collectors and gatherers or grazers conform up to 75% of the total composition replacing shredders (Vannote *et al.* 1980) These two functional groups, which include the filterer *Corbicula* clams, whose shells were abundant in many sites, usually increase in numbers as disturbance increases (Barbour *et al.* 1999; Tomanova *et al.* 2008.).

The functional substitution from shredders to grazers results from the decreasing amounts of coarse particulate organic matter (CPOM), obtained from tree litter, as the river flows into more un-shaded areas where fine particulate organic matter (FPOM) becomes the main energy source (Cummins *et al.* 1989, Rosi-Marshall and Wallace 2002). The lack of riparian cover reduces the abundance of shredders while gather collectors and filter feeders become more abundant exploiting the increasing FPOM, reasons why these groups are considered indicators of disturbance (Barbour *et al.* 1999, Cummins *et al.* 05). In fact, the few shredders (Lepidoptera: Pyralidae) I obtained in the last two field seasons came from the three reference sites, which have notable wooded banks compared to the rest. This resulting assemblage can also be the consequence of increased organic matter and total dissolved solids from the agricultural and urban areas in the valley. Turbidity values and total inorganic nitrogen, two measures related to nutrient and sediment loads in the water, were inversely and significantly correlated with invertebrate abundance, during the dry season. Furthermore, Canonical Correspondence Analyses showed conductivity, fecal coliform, and sulphate concentrations to be correlated with the sites ordination arrangements.

Therefore, invertebrate assemblages are partially related to the amount of sediment in the water coming in from adjacent agricultural areas, but are also related to flow interruptions, such as those created by dams (Vinson 2001) and other disturbances. Cooper and collaborators (2006) suggested that invertebrate composition was more strongly affected by water quality than by vegetation. These facts are also supported by the amount of fecal coliforms found in the water samples. It is known that human impacts in a watershed can be traced using functional group composition, as well as the amount of coliform bacteria. Townsend and Hildrew (1994) suggested that a functional approach better detects impacts than traditional diversity and richness indices, while Lepori and Malmqvist (2007) found out that there is a differential response by invertebrates to disturbance: predators and scrapers were strongly associated to disturbance, collector-gatherers showed seasonal responses, and filter collectors appeared unrelated to it. The response invertebrates have to disturbance is not completely clear. There are contrasting results in literature, where in some cases disturbance causes an increase in numbers of certain groups (e.g. Spellberg

2005), while others report the opposite trend (e.g. Townsend and Riley 1999; Tomanova *et al.* 2008).

Thus, it is clear that land use change affects invertebrate composition, but other confounding factors, like water flow and municipal wastewater, and other environmental variables not measured in this study might also being influencing the functional group composition and abundance in these river sites.

Although the results presented here do not test the idea that freshwater invertebrate diversity and functional composition in the Ayuquila River can be determined only by the water variables measured, as disturbance is known to be a two-edged knife with regards to aquatic diversity (Lepori and Hjerdt 2006), suspended sediment loads play an important role. I suggest that the monthly monitoring program should not only include the family composition of the invertebrates sampled, but to organize the data into functional groups with the aim of increasing the amount of information on the impacts human activities are having in the River's biota that can be comparable to findings in other sites, and thus suggest management activities to improve the river's conditions.

2.4.2. Fish species composition

Fish communities' structure and diversity were different between the two field seasons of this study. Besides the seasonal variation that it is known to occur in many rivers worldwide as a result of flow variability, channel morphology, riparian vegetation, land use, resource availability, among many other variables (e.g. Jones III *et al.* 1999; Ostrand and Wilde 2002; Aguilar-Ibarra *et al.* 2003), other factors might have played a role in the differences obtained in this study. However, the water physicochemical variables and hydrology patterns have been suggested to have a bigger impact in conforming fish assemblages (Helms 2008).

Diversity and composition in the agricultural valley sites were similar, but increased as the river flowed into mountainous sites. It has been argued that fish assemblages along agricultural settings tend to be more homogeneous and dominated by omnivore and benthic invertivores species (Richter *et al.* 1997;

Rahel 2000). At the same time, it has been suggested that diversity, richness and functional composition increases with respect to the position the river has within a watershed augmenting as it flows away from the headwaters (Smith and Kraft 2005). Species richness is also related to channel morphology, resource availability, flow regulation and land use (Ostram and Wilde 2002; Aguilar-Ibarra *et al.* 2003). However, the presence of Tilapia and largemouth bass, two aggressive exotics, in addition to the dominance of omnivorous species throughout the river, suggest that the overall conditions of the fish community are less than ideal. Freshwater fish fauna is among the most threatened group, mostly as a result of the combination of factors, with introduction of species and change in the adjacent land use being the most common causes (Richter *et al.* 1997).

Our fish species list includes range distribution changes from previously known records. For instance, 21 species were reported for the Ayuquila River, in which Pacific molly (*Poecilia butleri*) and blue tilapia (*Oreochromis aureus*) were considered as uncommon, while largemouth bass (*Micropterus salmoides*) was listed as absent (Lyons *et al.* 1998). During the length of the study, and also as part of the bi-monthly Fish IBI sampling, these three species were present and quite common in most sites, both in Autlan –El Grullo valley and below it, in the mountainous sites of the river. The latter two have become quite appreciated by local fishermen. Three Tilapia farms were established in the valley in the last 8 years, and it is known that during flooding events fish have escaped from at least one of these locations; the bass, in contrast was purposefully introduced as fishing game in the region's reservoirs, from where it has undoubtedly dispersed. Moreover, while Lyons and collaborators (1998) also mention that the West Mexican Redhorse (*Moxostoma austrinus*) was extirpated; two large specimens were caught at the first site after the valley, and a medium size fish on the site close to the reservoir in the Tuxcacuesco River. Thus, the importance of continuous monitoring is highlighted, in particular when considering that many fish species are migratory, are released, or changing their distributions due to climate change.

2.4.3. Water quality using biotic indices

The species assemblages obtained in this study do not necessarily reflect the water quality conditions that the indices of biotic integrity suggest. For instance, the benthic invertebrate indices of biotic integrity indicated good to excellent water quality conditions for most sites, while the fish IBI indicated conditions ranging from very poor to fair, with only four sites having a good condition. In general, poorer conditions were evident for the dry season. This was unexpected as the river flow is higher during this time of the year, although the differences between seasons were not statistically significant.

However, a good trend is seen in the results. The B-IBI values for this study differ from those published ten years ago (Henne *et al.* 2002) which suggested poor to very poor conditions. The invertebrate richness and diversity obtained from this samples also contrast those of low taxa diversity that characterized the invertebrate macrofauna in the 30-km long river transect beyond the sugar mill's drain (Henne 1997). These conditions were the norm before the year 2000 when restrictions and fines were imposed to the sugar mill, and the first water treatment facility was built in the city of Autlan (Graf *et al.* 2006). So far, the monitoring program results so far indicate that the present conditions of better B-IBI values are more frequent. However, the presence of exotic species, such as *C. fluminea* and *P. flagellata* show that the community structure is still being modified as a result of human disturbances. The Asian clam thrives in disturbed areas, and their populations will most likely tend to increase with the amount of disturbance seen in the watershed. When the present biotic indices started being implemented almost three decades ago, these two exotics were absent, so further studies on the spread of exotics in this watershed are needed, as well as one on looking how their presence is affecting both the river's community structure and, as a result the tolerance values these indices portray. Additionally, the inclusion of groups not presently considered in the IBI calculations should be considered, like leeches and other freshwater worms.

Seasonal variation in invertebrate biotic indices has been shown in European rivers (Leunda *et al.* 2009). It would be interesting to see in the Ayuquila River, with the data obtained in almost 20 years of continuous sampling, if there are inter-seasonal variations in the indices, and if these reflect the prevailing conditions.

In the case of the Ayuquila River, the results obtained from the ongoing monitoring program have helped in the setting of management goals of the basin. More detailed work to see the changes in the structure and composition of these communities in space and time are needed to evaluate if the implemented strategies and actions are working to enhance this river´s condition. Additionally, a revision of the type of information both indices are giving is probably needed, as it is sometimes contrasting.

Fish assemblages in a basin reveal long-term trends in comparison to invertebrate composition, which is more representative of local conditions. The change in flow has had without doubt an impact in the River´s biota. Up to 75% less water flows on the second site located seven km away from the entrance of the valley, volume which recovers gradually as it flows along the agricultural area (Martinez *et al.* 2000). In addition, the presence of water impoundments facilitates the dispersion and settlement of exotic species and an increase in omnivores which usually have a heavy toll on native fauna, drastically affecting the structure and functioning of this basin, as it has been assessed in other Mexican watersheds (Mercado-Silva *et al.* 2009). The fish IBI takes into account the presence of native versus exotic species and, in this study, the second most common and abundant species in many sites was the non-native Blue Tilapia.

Although both IBI indices applied in this watershed show that conditions have improved since they were first implemented in 1985, other indicators such as fecal coliforms, nitrate, sulphate and iron concentrations suggest that those indices just give a snapshot of the conditions. Biotic indices oversimplify a site’s community structure (Spellberg 2005), so the integration of other metrics and a revision of the tolerance values of the invertebrate families and fish composition considered in the Ayuquila River monitoring program should be revised. The present change in community composition demands more attention, as well as the assessment of the pollution tolerance of some groups.

The use of benthic macroinvertebrate and fish compositions to assess the quality of a river has been used worldwide. Its application in Mexican rivers is in its infancy (Mathuriau *et al.* 2011), and government authorities have not yet accepted them as tools to monitor water quality. However, its increased use by other institution, and as the results obtained in this study suggest, might help convince authorities of their value in monitoring and assessing water conditions, as it is already being done in other countries.

2.5. Conclusions

Almost 3,000 macroinvertebrates from 24 families, and 1250 fishes from 15 species were classified into six functional feeding groups and three trophic guilds, respectively, and composition and abundance were compared spatially and temporally. Statistical significant differences existed between sites' composition but not between seasons.

Disturbance influenced the biological composition and species abundance throughout the study area. Agricultural areas were less diverse than forested sites. Functional group composition, conformed mostly by invertebrate filter collectors, gatherer collectors, and fish omnivores, reflected the signs of disturbance, mostly seen from suspended materials in the water column.

There were significant negative correlations between turbidity and total inorganic nitrogen with invertebrate abundance whereas hardness and conductivity were the variables positively correlated with fish abundance. NMDS ordination arrangements helped identify those sites that were more similar in their functional group composition, and most sites with similar accumulated landuse influence were grouped closely, but CCA analyses were not conclusive in determining the relationships between community composition and water environmental variables.

Benthic invertebrate and fish indices of biotic integrity suggest water quality conditions ranging from fair to good. However, the presence of pollution tolerant exotic species, and the concentrations of other variables such as turbidity, dissolved iron, inorganic nitrogen and fecal coliforms, usually higher than those approved by Mexican guidelines suggest otherwise.

This is the first report of *Corbicula fluminea* for this river, as well as the extension in distribution of other exotic invertebrate and fish species. I suggest that further monitoring studies should include other variables to give a more realistic assessment of the river's conditions. This would result in management guidelines that improve the overall biological and physical qualities of this important Mexican river.

Table 2.1abc. Water physical and chemical parameters

a) for **wet07** arranged by sites

	Temp (°C)	Dissolved Oxygen (mg/l)	Turbidity (NTU)[1]	Cond (μohms/s)	TIN (mg/dL)[2]	SO_4 (ppm)	PO_4 (ppm)	Iron (ppm)	H_2CO_3 (ppm)	($CaCO_3$) ppm	Fecal Coliforms (FC)[3]
1.FoTA	23.9	3.9	36.4	284	1.16	13	0.02	0.22	144	6	1000
2.IAFo	-	-	-	-	-	-	-	-	-	-	-
3.IA	22.6	3.8	132	186	2.46	22	0.04	0.11	106	20	2800
4.IA	24.2	3.55	72.2	236	1	72	0.05	0.17	166	8	2100
5.UrIA	23.9	1.7	140	1082	6	61	0.06	1.09	221	104	22800
6.IA	24.4	2.9	109	493	0.9	69	0.07	*0.17*	221	30	20000
7.Ur	25.1	2.8	34	1785	2.35	212	0.06	0.09	541	92	6800
8.IA	-	-	-	-	-	-	-	-	-	-	-
9.IA	24.6	3.5	93	684	2	69	0.08	32	231	70	7700
10.Fo	20.2	4.29	35.4	85	0.2	9	0.02	0.2	89.28	18	200
11.IAFo	24.3	6.66	794	415	1.5	42	0.06	0.24	198.4	28	14800
12.IAFo	24.2	4.09	152	470	1.56	72	0.06	0.27	171	46	4100
a.Fo	-	-	-	-	-	-	-	-	-	-	-
b.FoIA	-	-	-	-	-	-	-	-	-	-	-
c.Ur	-	-	-	-	-	-	-	-	-	-	-
d. FoUr	22.7	3.48	1200	237	3	54	0.01	0.71	168	20	20400
13.IAFo	22.2	4.13	501	333	2.72	48	0.02	0.61	169	24	11200
Ayuquila Basin Mean (SD)	**23.6 (1.4)**	**3.8 (1.2)**	**190.8 (239)**	**550.3 (492)**	**1.99 (1.5)**	**62.6 (55)**	**0.05 (0.02)**	**3.2 (9.6)**	**205.2 (120)**	**40.6 (33.6)**	**8500 (7779)**
Tuxca Basin Mean (SD)	**21.9**	**3.6**	**266.3**	**573.7**	**2.03**	**61.5**	**0.04**	**3.5**	**196.5**	**38.6**	**9300**

[1]NTU= Nephelometric turbidity units. [2]TIN= Total Inorganic Nitrogen: NH_4-N + NO_3-N. [3]FC= Fecal coliform colonies per 100 ml.

b) for **dry08** arranged by sites.

	Temp (°C)	Dissolved Oxygen (mg/l)	Turbidity (NTU)[1]	Cond (μohms/s)	TIN (mg/dL)[2]	SO_4 (ppm)	PO_4 (ppm)	Iron (ppm)	H_2CO_3 (ppm)	($CaCO_3$) ppm	Fecal Coliforms (FC)[3]
1.FoTA	18.7	6.03	3.09	185	1.2	26	1.6	0.05	944.8	192	14000
2.IAFo	19	-	-	-	-	-	-	-	-	-	-
3.IA	19.4	4.56	30.5	376	1.6	65	1.9	0.19	580	180	6800
4.IA	20.9	4.19	18.6	554	1.9	136	0.9	0.24	640	228	6800
5.UrIA	19.6	1.7	38.7	641	4.9	135	5.8	0.39	677	714	18800
6.IA	20.6	3.3	19.3	668	3.6	148	2.2	0.28	722	258	17700
7.Ur	16.6	3.2	69.5	826	6.8	150	4	0.47	677	180	23600
8.IA	20.6	3.9	22.3	661	2.1	144	1.5	0.06	722	228	14400
9.IA	19.4	5.3	628	14	1.3	126	4.9	0.18	387	174	500
10.Fo	16.5	6.58	1.33	135	0.6	14	0.2	0.05	640	84	84
11.IAFo	19	4.45	9.8	592	1.6	141	3	0.03	565	156	2800
12.IAFo	21.9	4.12	4	684	1.2	171	2.4	0.08	543	384	600
a.Fo	17	-	-	-	-	-	-	-	-	-	-
b.FoIA	-	-	-	-	-	-	-	-	-	-	-
c.Ur											
d. FoUr	20.2	4.43	15.3	605	0.9	144	0.5	0.09	618	234	4800
13.IAFo	21.4	4.67	6.04	672	1	168	2.2	0.06	506	180	2300
Ayuquila Basin Mean (SD)	**19.6 (1.7)**	**4.33 (1.3)**	**70.9 (176.5)**	**500.7 (259)**	**2.32 (1.86)**	**118.7 (53.3)**	**2.55 (1.63)**	**0.17 (0.15)**	**612.7 (112)**	**270 (185)**	**9032 (8281)**
Tuxca Basin Mean (SD)	**18.3 (2.3)**	**4.14**	**74.3**	**491.5**	**2.2**	**116**	**2.35**	**0.17**	**571.4**	**260**	**8700**

[1]NTU= Nephelometric turbidity units. [2]TIN= Total Inorganic Nitrogen: NH_4-N + NO_3-N. [3]FC= Fecal coliform colonies per 100 ml.

c) for wet08 arranged by sites.

	Temp (°C)	Diss. Oxygen (mg/l)	Turbidity (NTU)[1]	Cond (μohms/s)	TIN (mg/dL)[2]	SO_4 (ppm)	PO_4 (ppm)	Iron (ppm)	H_2CO_3 (ppm)	($CaCO_3$) ppm	Fecal Coliforms (FC)[3]
1.FoTA	25	2.75	13.2	379	2	9	0.01	0.38	278	142	2300
2.IAFo	25.8	4.1	36.6	378	-	-	-	-	-	-	-
3.IA	24.4	2.1	15.8	447	1.6	18	0.08	0.14	429	184	1200
4.IA	25.7	2.4	32.3	632	1.4	43	0.01	0.04	496	224	3800
5.UrIA	28.4	1.4	185	802	3.2	63	0.03	0.43	704	242	27200
6.IA	25.9	1.3	69.2	700	2.2	84	0.06	0.08	637	196	12300
7.Ur	29	3.2	30.8	1609	2.3	272	0.03	0.07	682	342	12500
8.IA	25.2	1.9	51.8	648	2.6	162	0.03	0.32	520	226	11000
9.IA	23	2.7	168	396	1.9	93	0.08	0.12	154	158	6100
10.Fo	20.8	2.9	68.7	110	0.5	5	0.01	0.12	278	44	500
11.IAFo	24.8	2.6	286	323	1.6	118	0.02	0.04	325	114	5700
12.IAFo	27.1	2.2	50.7	540	0.6	18	0.01	0.38	404	206	300
a.Fo	22	7.2	17.5	409	-	-	-	-	-	-	-
b.FoIA	22.5	5.9	53.2	463	-	-	-	-	-	-	-
c.Ur	24.6	6.4	30.5	499							
d. FoUr	27.5	2.4	1098	460	4.6	93	0.08	0.12	471	182	13600
13.IAFo	27.8	1.9	62	539	4.7	24	0.01	2.4	377	222	500
Ayuquila Basin Mean (SD)	**25.6 (2.3)**	**2.3 (0.6)**	**86.1 (83.3)**	**594 (370.5)**	**2.1 (1.1)**	**75.8 (78.7)**	**0.03 (0.03)**	**0.38 (0.65)**	**440.3 (173)**	**192 (74)**	**6950 (7864)**
Tuxca Basin Mean (SD)	**24.8 (3.9)**	**4.8 (3.4)**	**558 (764)**	**435 (36)**	**4.6**	**93**	**0.08**	**0.12**	**471**	**182**	**13600**

[1]NTU= Nephelometric turbidity units. [2]TIN= Total Inorganic Nitrogen: NH_4-N + NO_3-N. [3]FC= Fecal coliform colonies per 100 ml.

Table 2.2. Criteria used as reference for water pollution levels based on Mexican guidelines.

Mexican guidelines (NOM-127-SSA-1-1994; CE-CCA-001/89) for maximum permissible limits in water.					
Water parameter	**Potable water**	**Source of Potable water**	**Recreational**	**Irrigation**	**Wildlife Protection**
Fecal coliforms[1] (FC/100ml)	0	1000	200	1000	200
Total Phosphates (mg/l)[1]		0.1			0.025 (lakes) 0.1 (rivers)
Total Nitrates (mg/l)[1]	5				
Turbidity (NTU)[2]	5				
Iron (mg/l)[2]	0.3				

Benthic Invertebrate Index of Biotic Integrity parameters

Biotic Index	Quality	Degree of pollution
-- 3.75	Excellent	No apparent organic pollution
3.76 – 4.25	Very good	Possible organic pollution
4.26 – 5.00	Good	Some organic pollution
5.01 -- 5.75	Fair	Fairly significant organic pollution
5.76 – 6.50	Fairly poor	Significant organic pollution
6.51 – 7.25	Poor	Very significant organic pollution
7.26 –10.00	Very poor	Severe organic pollution

Fish Index of Biotic Integrity parameters

Biotic Index	Quality	Fish community attributes*
70-100	Good	Best situation. Species richness and abundance at or near its maximum expected. Full array of size and age classes. Sensitive species are present. Herbivores and carnivores are common.
45-65	Fair	Some environmental degradation. Richness and abundance below expected. Benthic and sensitive species uncommon or absent. Tolerant, exotic and omnivore species dominate
0-40	Poor	Greatly modified fish community. Fish abundance is low and most fish are small. Almost all species are exotic, livebearing, tolerant and omnivores.
No score	Very Poor	Few or no fish after thorough sampling. Index cannot be calculated.

From Mexican Official water quality guidelines:
Mexican guidelines [1] DOF (1989) and [2] DOF (2000).
[3] Benthic Invertebrate Biotic Index,Henne et al. (2002);
[4] Fish Biotic Index, Lyons et al. 1995.
* Fish community attributes are summarized.

Table 2.3. List of invertebrate families sampled in this study. Known species, functional feeding group (FFG) and tolerance value (B-IBI) are included as considered in the analyses. Bold letters in family or species name correspond to the abbreviation used in the text, figures and tables.

Class / Order	Family	Species	FFG[1]	IBI value[2]
INVERTEBRATES				
		Gastropoda		
Bivalvia	***Corb**iculidae*	*Corbicula fluminea*	*Filter Collector*	*6*
Gastropoda	***Ampu**llaridae*	*Pomacea flagellata*	*Scraper*	*8*
		Arthropoda / Insecta		
Ephemeroptera	***Baet**idae*			*4*
	***Hept**ageniidae*		*Gather Collector*	*4*
	***Lept**ophlebidae*			*2*
	***Tryc**orythidae*			*4*
Trichoptera	***Hydr**opsychidae*		*Filter Collector*	*4*
Plecoptera	***Perl**idae*	*Anacroneuria aff. quadriloba*	*Predator*	*1*
Odonata	***Gomp**hidae*			*1*
	***Libe**llulidae*		*Predator*	*9*
	***Calo**pterygidae*			*5*
	***Coen**agrionidae*			*9*
Diptera	***Taba**nidae*		*Predator*	*6*
	***Chir**onomidae*		*Gather Collector*	*6*
	***Simu**lidae*		*Filter Collector*	*6*
Megaloptera	***Cory**dalidae*	*Corydalus bidenticulatus*	*Predator*	*0*
Lepidoptera	***Pyra**lidae*		*Shredder*	*5*
Coleoptera	***Elmi**dae*			*4*
	***Psep**henidae*		*Scraper*	*4*
	***Dryo**pidae*			*5*
Heteroptera	***Nauc**oridae*		*Predator*	*5*
	***Bela**stomatidae*			*10*
		Arthropoda / Malacostraca		
Decapoda	***Pseu**dothelphusidae*	*Pseudothelphusa dilatata*	*Omnivore*	*6*
	***Pala**emonidae*	*Macrobrachium occidentale*		*6*

[1]FFG= Functional Feeding Group; [2] IBI= Index of Biotic Integrity value sensu Henne et al. (2001) and Weigel et al. (2002).

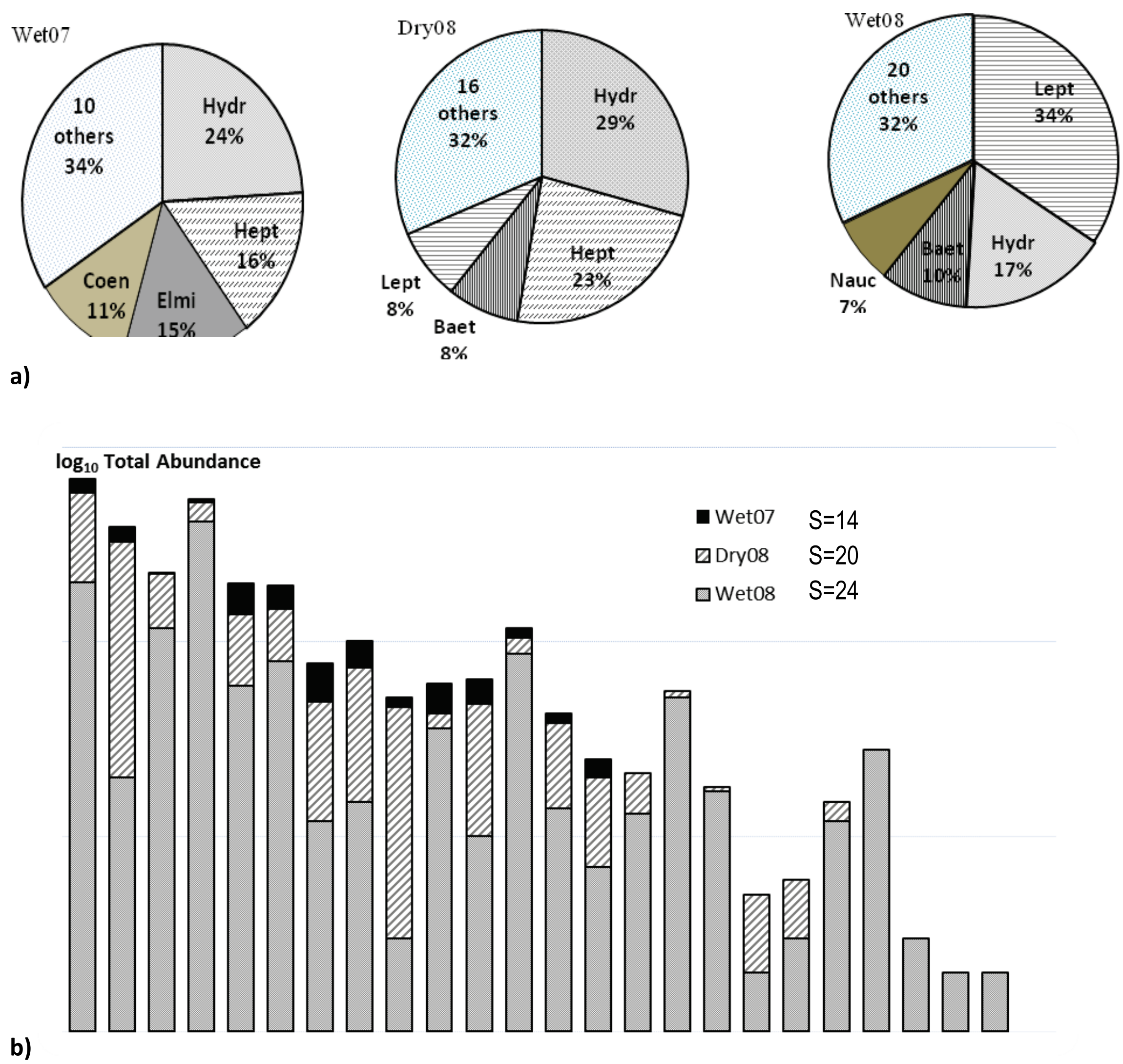
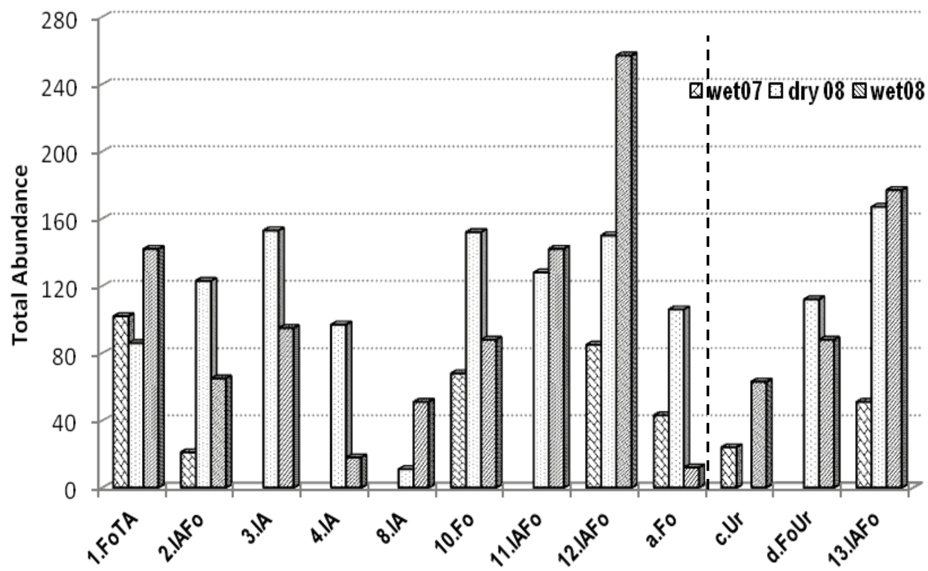


Figure 2.1. Invertebrate composition and abundance (log10) by field season. a) Invertebrate families that dominated in each sampling season (Coen: Coenagrionidae; Elmi: Elmidae; Hept: Heptageniidae; Hydropsychidae; Lept: Leptophlebidae; Baet: Baetidae). S: Taxa or family richness.



a)

b)

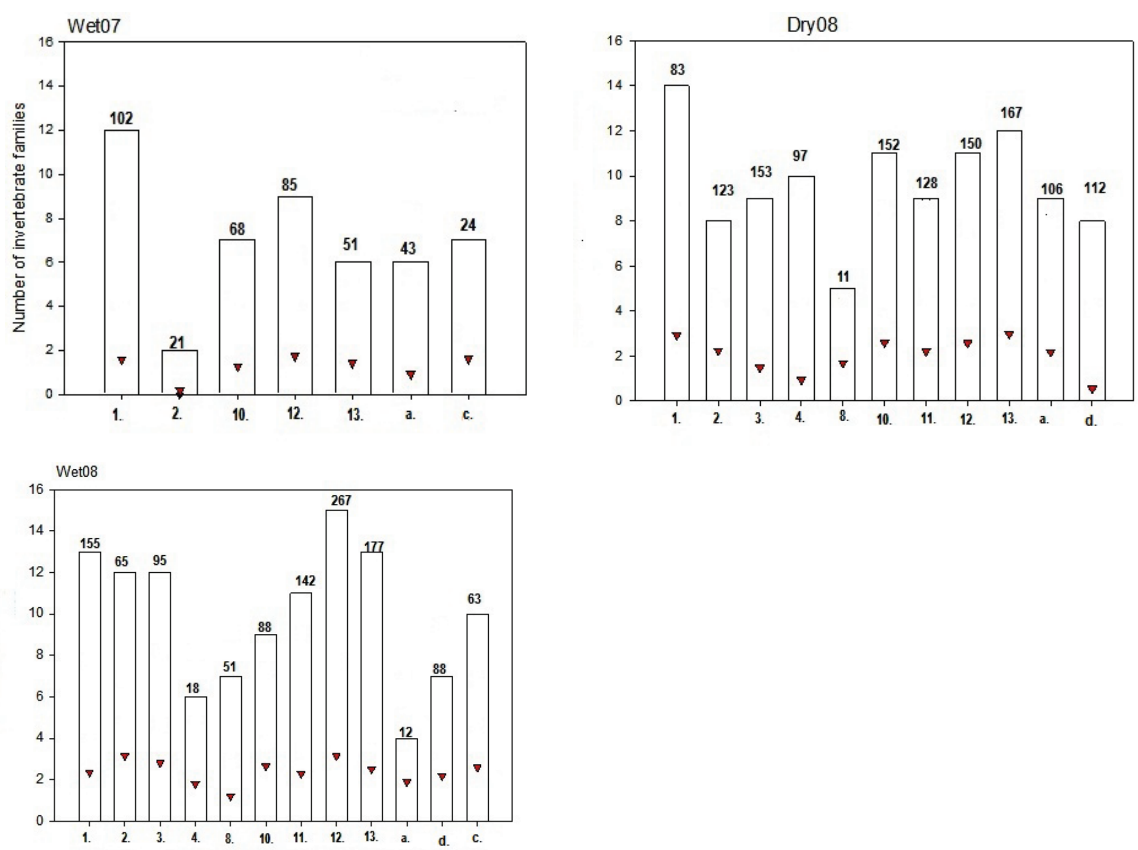


Figure 2.2. **a)** Total invertebrate abundance per site and per season. Dotted line separates sub-basins. **b)** Number of invertebrate families. The numbers above the bars indicate the total numbers in each site. ∇= Shannon (H') Diversity value. There were no statistically significant differences for these metrics between seasons.

T

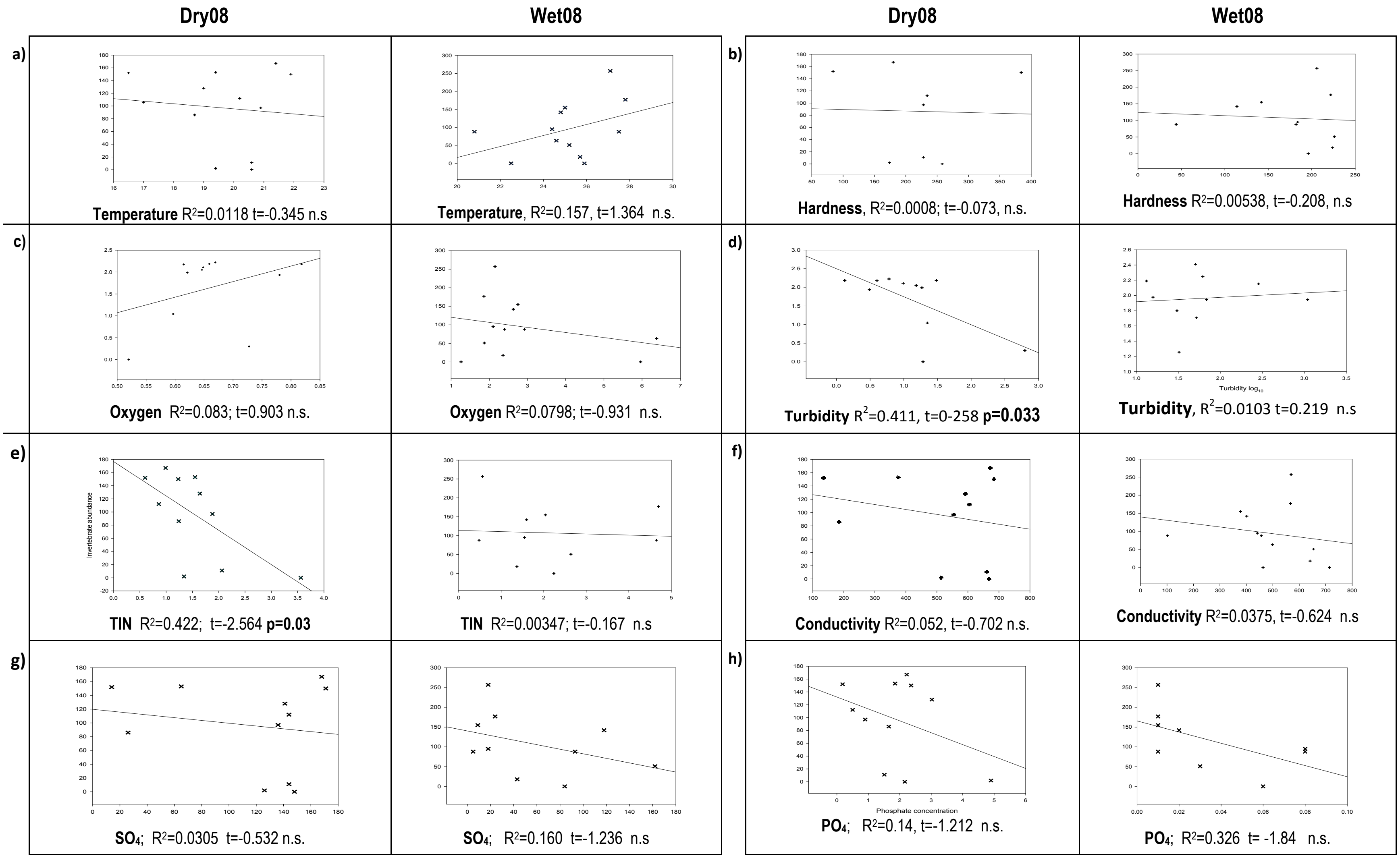


Figure 2.3. Invertebrate abundance plots contrasted with water physical-chemical variables arranged by season. n.s.: not statistically significant.

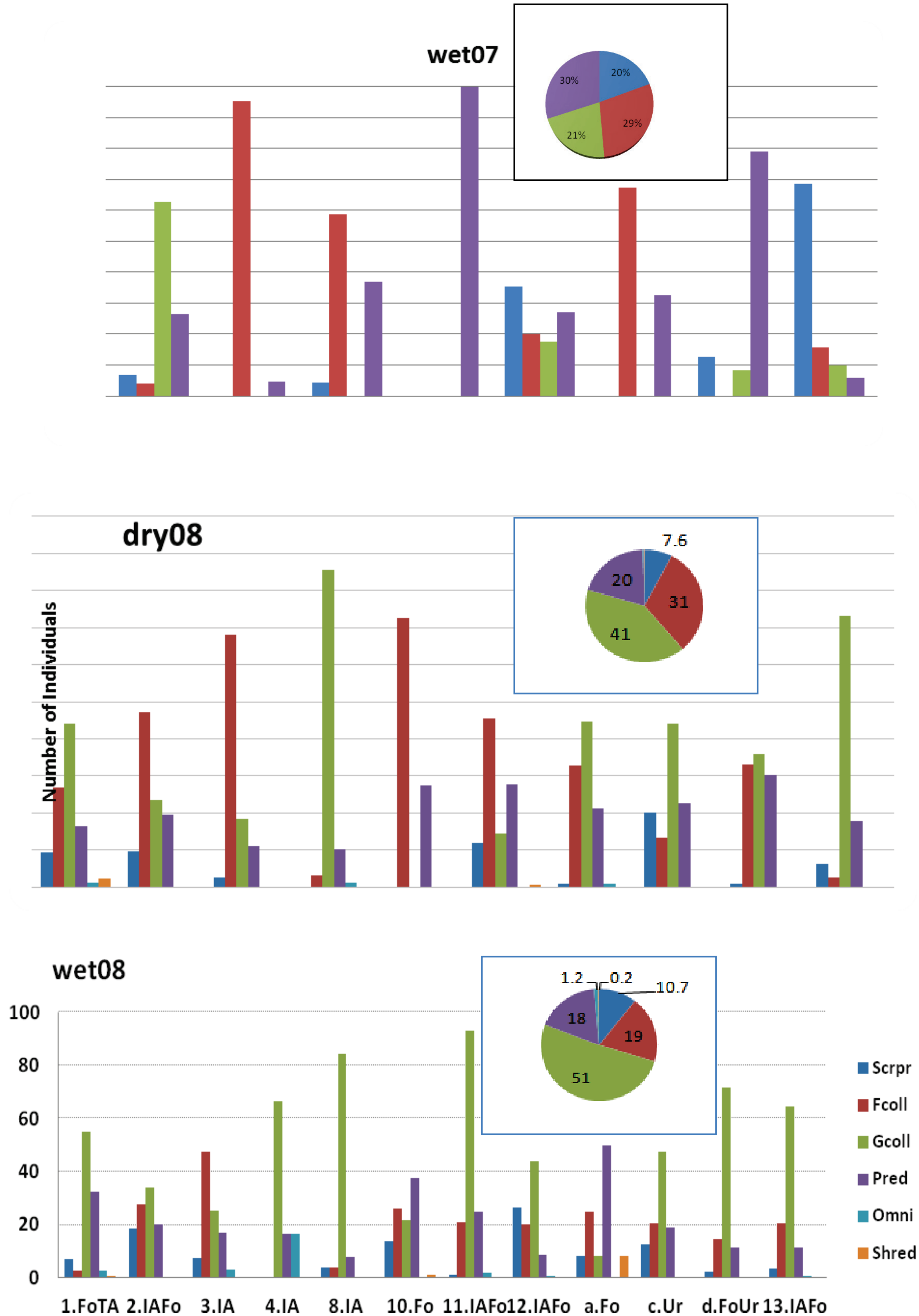


Figure 2.4. Invertebrate Functional Feeding Group (FFG) composition per site arranged per season. The circular graphs show the overall proportion of each FFG for that field season

Table 2.4. List of fish species sampled in this study. Their trophic guild, and tolerance value (B-IBI) are included as considered in the analyses. Bold letters in family or species name correspond to the abbreviation used in the text, figures and tables.

FISHES

Family	Common Name	Species[1]	Trophic guild[1]
Characidae	Banded Tetra	*Astianax aeneus* **(Asae)**	Omnivore
Catostomidae	West Mexican Redhorse	*Moxostoma austrinum* **(Moxa)**	Carnivore
Poecilidae	Green Swordtail	*Xiphophorus helleri* **(Xihe)**	Herbivore
	Pacific Molly	*Poecilia butleri* **(Pobu)**	Herbivore
	Golden Livebearer	*Poeciliopsis baenschi* **(Poba)**	Omnivore
Goodeidae	Goldbreast Splitfin	*Ilyodon furcidens* **(Ilfu)**	Omnivore
	Bandfin Splitfin	*Allodontichtys zonistius* **(Alzo)**	Carnivore
	Black Splitfin	*Xenotoca melanosoma* **(Xeme)**	Omnivore
Mugilidae	Mountain Mullet	*Agonostomus monticola* **(Agmo)**	Omnivore
Centrarchidae	Largemouth Bass	*Micropterus salmoides* **(Misa)**	Carnivore
Cichlidae	Redside Cichlid	*Nandopsis istlanum* **(Nais)**	Carnivore
	Blue Tilapia	*Oreochromis aureus* **(Orau)**	Omnivore
	Redbreast Tilapia	*Tilapia rendallii* **(Tire)**	Omnivore
Gobiidae	Multi-spotted Goby	*Sicydium multipunctatum* **(Simu)**	Herbivore
Haemulidae	Purple-mouth grunt	*Pomadasys bayanus* **(Pomb)**	Carnivore

[3]Species names and trophic guild *sensu* Mercado et al. (2002).

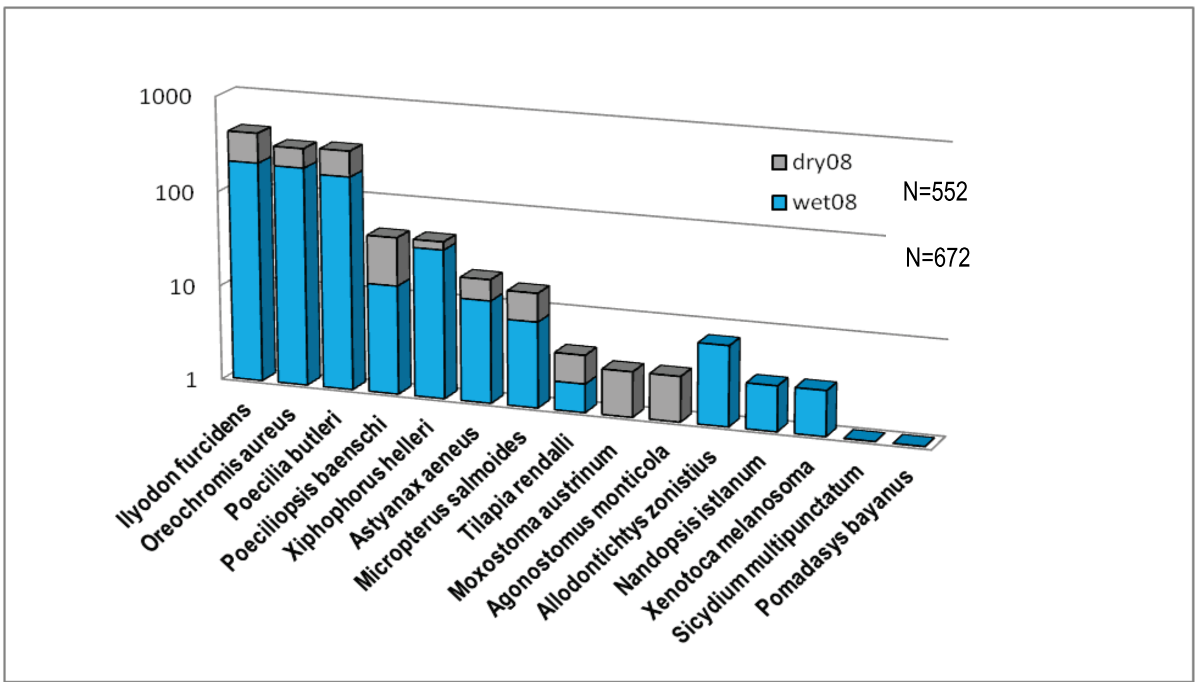


Figure 2.5. Fish species $\log_{10}$ abundance captured during dry08 and wet08 field seasons in the middle portion of the Ayuquila River.

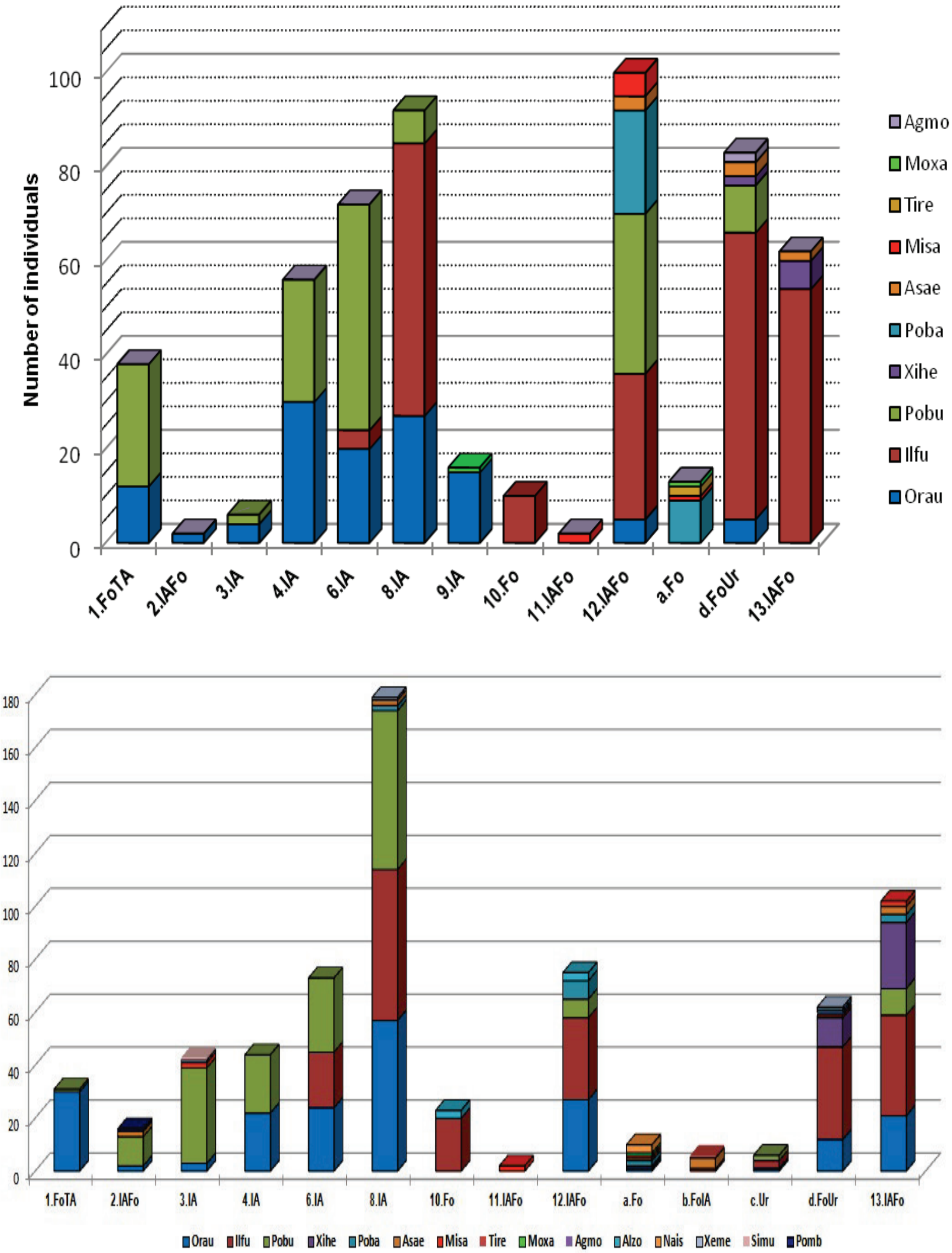


Figure 2.6. Fish community composition and abundance by site in two field seasons. Upper graph corresponds to Dry08, lower graph to Wet08. Species acronyms on the right are defined in Table 2.5

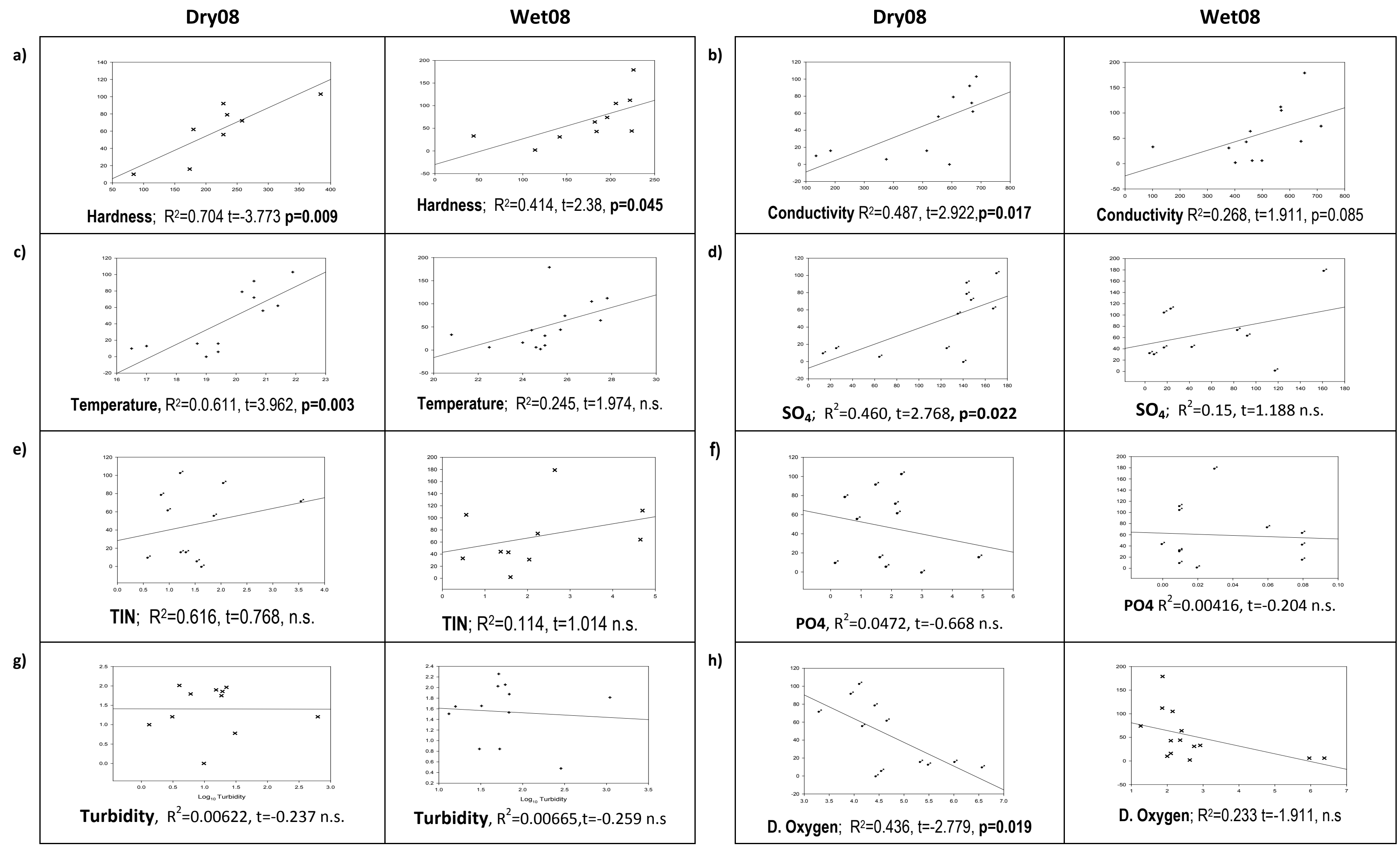


Figure 2.7. Fish abundance correlations to water physico-chemical variables compared by season. n.s.= not statistically significant.

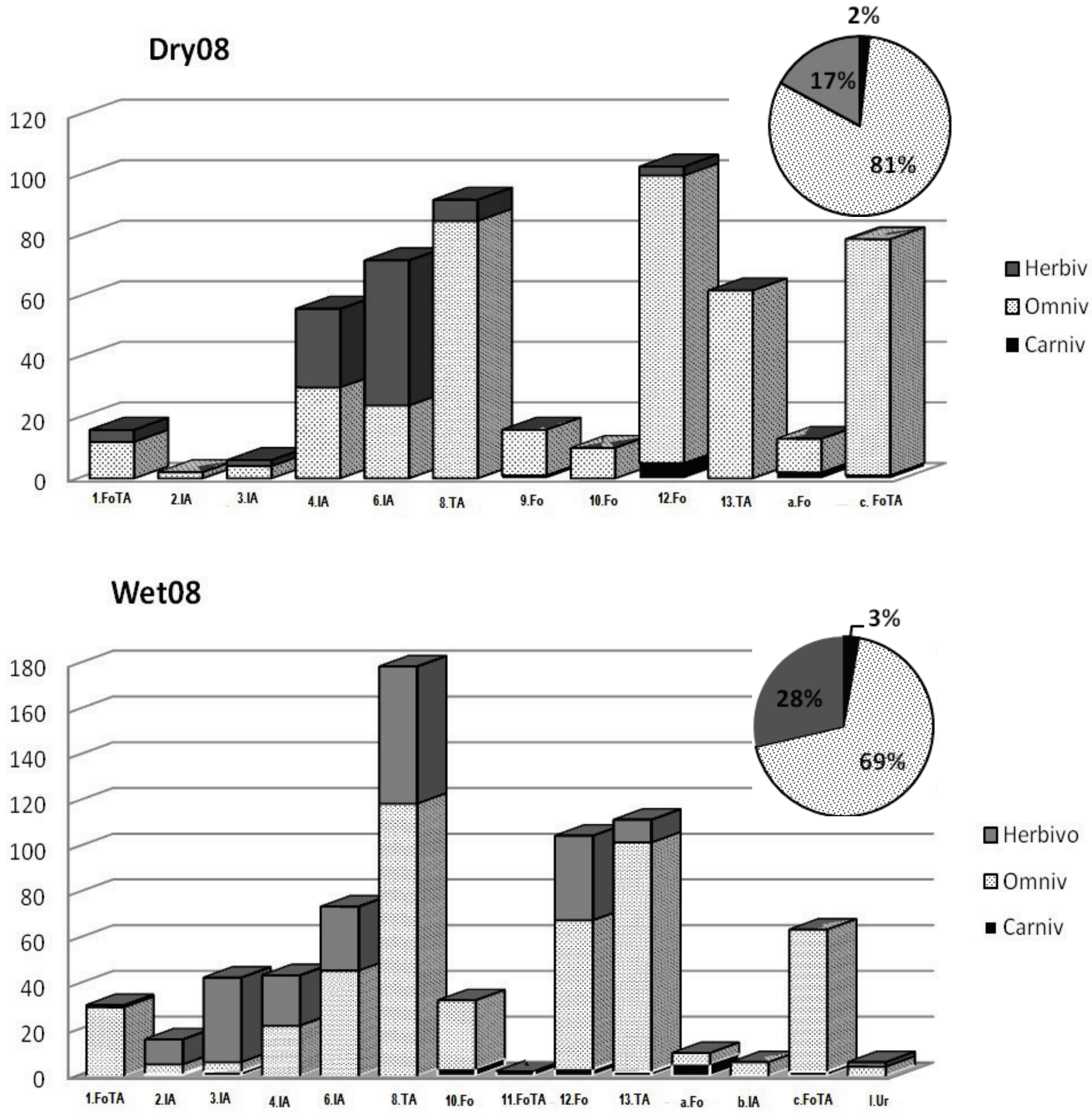


Figure 2.8. Fish trophic composition per site and sampling season.

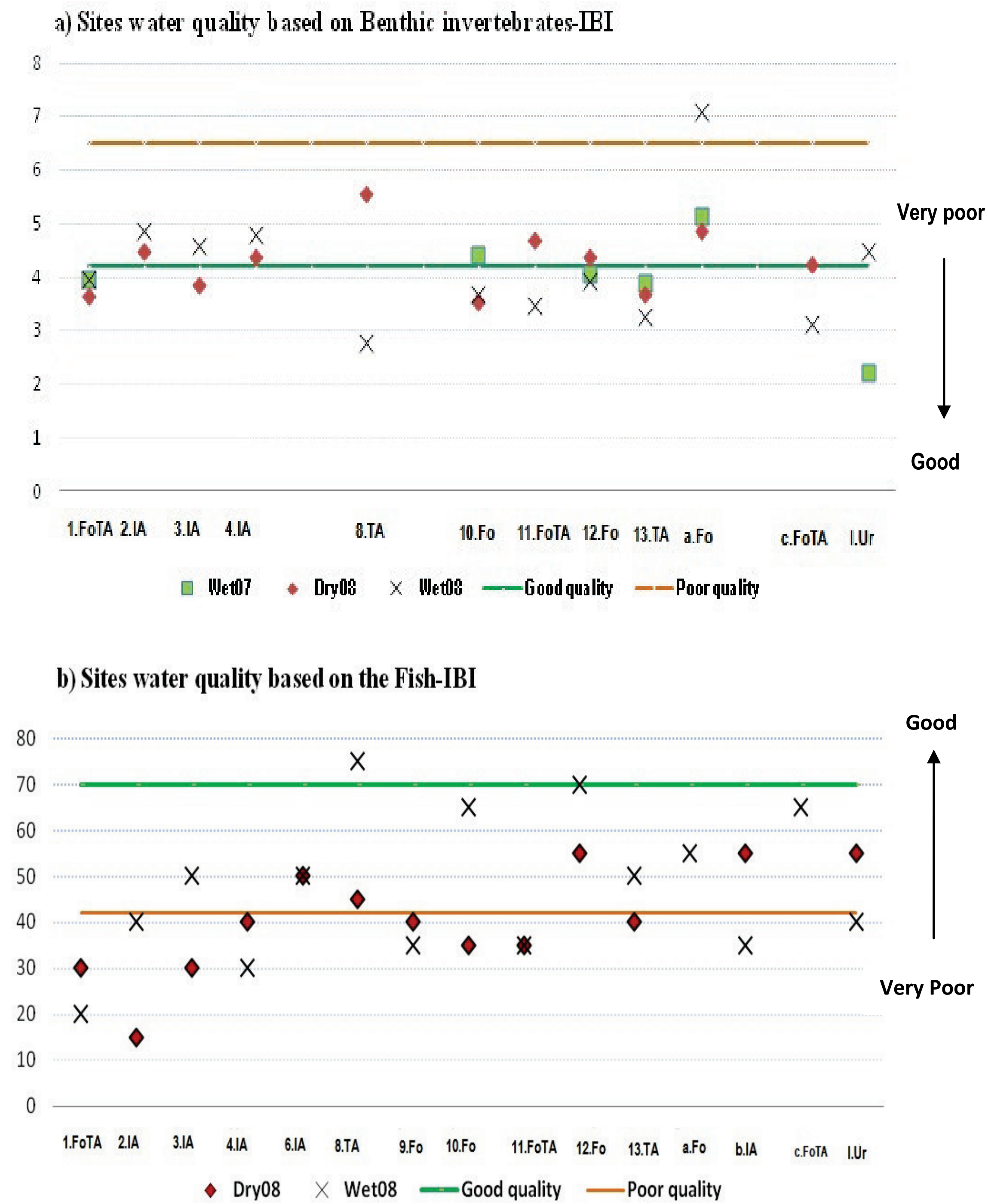


Figure 2.9. Water quality of each site based on: **a)** the Benthic Invertebrate and **b)** Fish Indices of Biotic Integrity (IBI) indices. Three sites(PBco, SnBn, and Amtl() were not sampled for invertebrates. The ochre line indicates the limit beyond which the conditions are poor, the green line is the limit where water conditions are considered of good quality. Notice that these lines are in inverse order in each graph. The higher the B- IBI value, the poorer the conditions; the opposite occurs with the F-IBI (see Table 2.3).

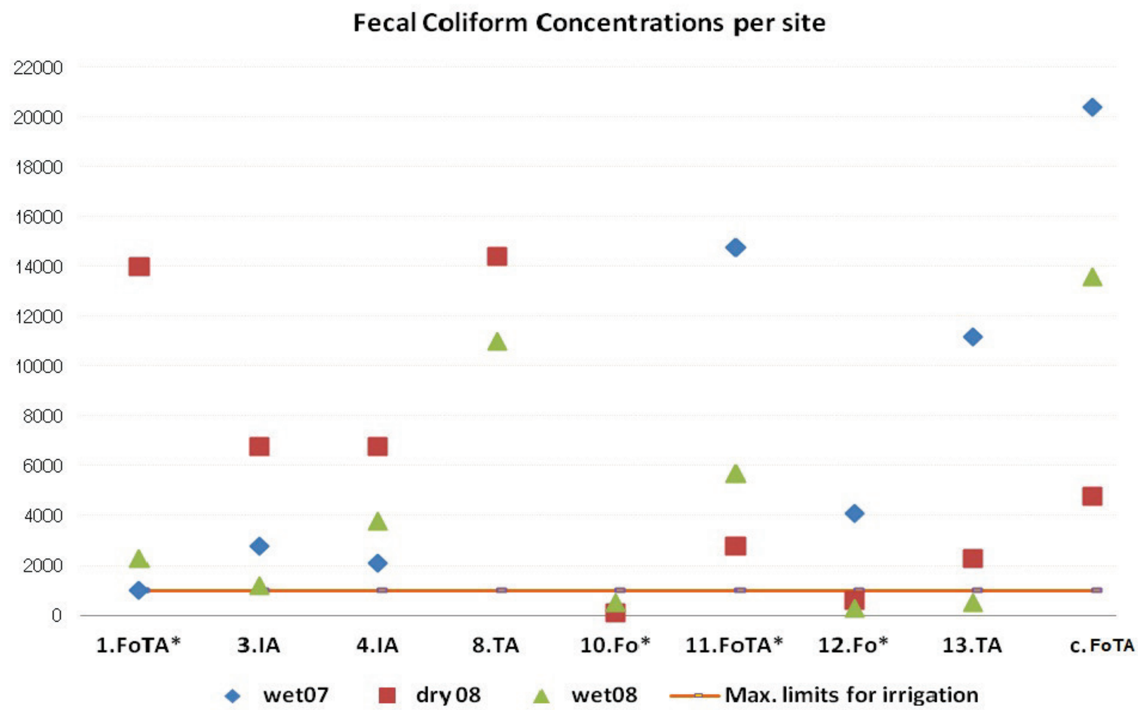


Figure 2.10. Fecal coliform (FC) concentrations per 100 ml measured for each site and field season. The ochre line establishes the maximul permissible limits established by Mexican guidelines for irrigation purposes and as a source for potable water. The sites with an asterisk are those where people usually swim and recreate. The maximum limits for this activity are 200 FC per 100 ml.

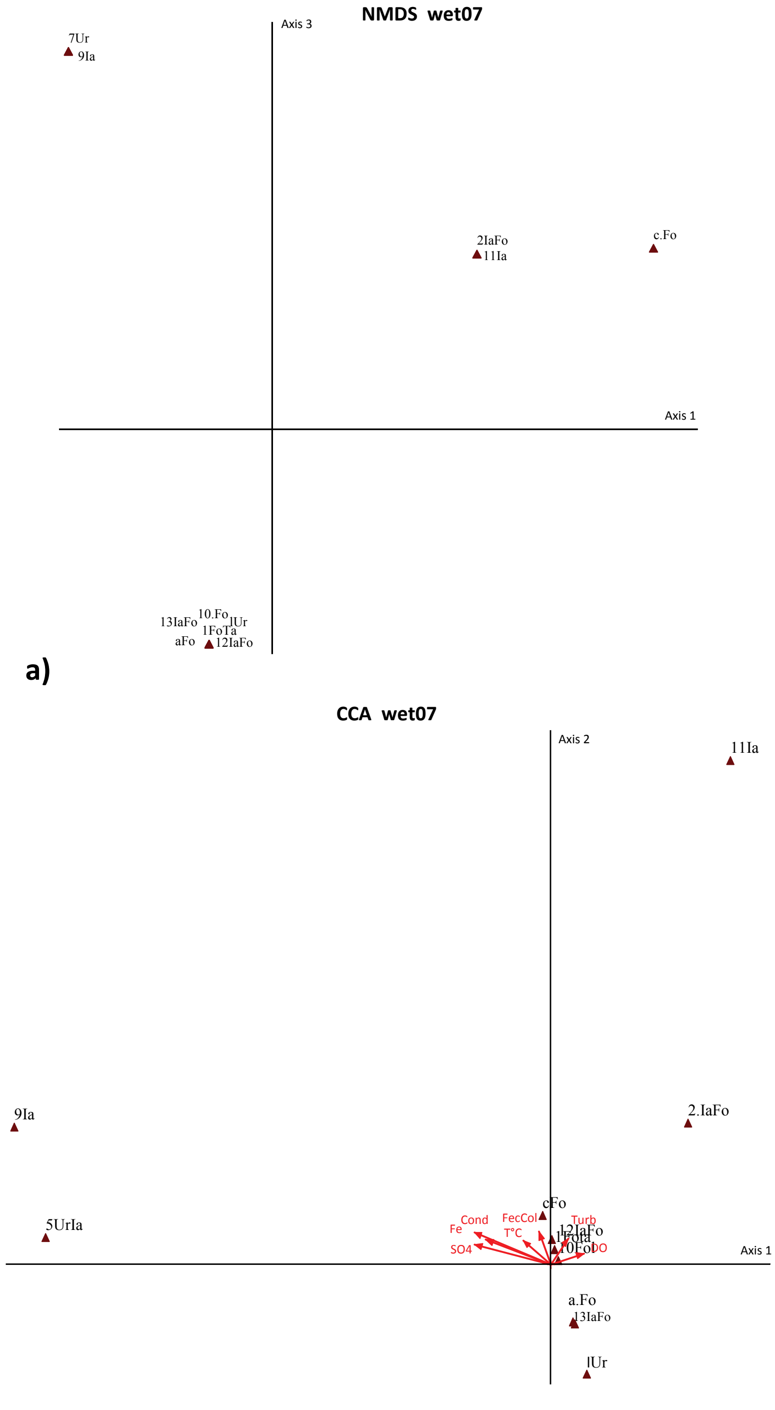


Figure 2.11. Wet07 a) NMDS ordination biplot showing the 11 Ayuquila River sites spatial arrangement based on macroinvertebrate composition. These two axes explain 88% of the total variance (stress 0.0003, p=0.0392); b) CCA ordination biplot showing the sites' arrangement for wet07 based on the relationships between the macro-invertebrate composition and nine water variables. These two axes explain 59.4% of the variance in species data (p=0.1822). Sites' acronyms follow the assumed accumulated land use effect (see Table 1.4). The first number or letter represents the site's position in the river's gradient, while the following letters the most influential landuse (i.e. Ia: intensive agriculture, Ta: temporal agriculture, Fo: forest, and Ur: urban.

Table 2.5. Summary of the results from the CCA of the Wet07 invertebrate family presence-absence data. Total variance ("inertia") in the species data is 2.5994, so statistics on variance explained are based on ratios against this number. The p-value (0.182) associated with the Monte Carlo results indicate no significant relationship between the species data matrix and the environmental data matrix. High correlations between raw-data distance and ordination distance indicate a good representation of the original data for axis 1. Values that are ideal for interpretation are bolded and show that only axis 1 should be considered for interpretation.

	Axis 1	Axis 2	Axis 3
Eigenvalue	0.968	0.577	0.281
Variance in species data			
% of variance explained	37.2	22.2	10.8
Cumulative % explained	37.2	59.4	70.2

CORRELATIONS AND BIPLOT SCORES for 9 environmental variables

	Correlations*			Biplot Scores		
Variable	Axis 1	Axis 2	Axis 3	Axis 1	Axis 2	Axis 3
1 T°C	-0.244	-0.251	-0.392	-0.240	-0.190	-0.208
2 DO	0.297	-0.116	0.338	0.292	-0.088	0.179
3 Turbidity	0.160	-0.281	-0.456	0.157	-0.214	-0.242
4 Conductivity	**-0.683**	-0.340	-0.014	-0.672	-0.258	-0.007
5 TIN	-0.168	-0.335	-0.190	-0.165	-0.254	-0.101
6 SO4	**-0.584**	-0.267	-0.064	-0.575	-0.203	-0.034
7 Fe	**-0.683**	-0.214	0.027	-0.672	-0.162	0.014
8 PO4	-0.067	-0.011	0.236	-0.066	-0.009	0.125
9 Fecal Coliforms	-0.105	-0.352	-0.354	-0.103	-0.268	-0.188

MONTE CARLO TEST RESULTS -- EIGENVALUES

	Real data	Randomized data Monte Carlo test, 998 runs			
Axis	**Eigenvalue**	Mean	Minimum	Maximum	p
1	0.968	0.804	0.337	1.000	0.0911
2	0.577	0.474	0.203	0.761	
3	0.281	0.228	0.166	0.288	
Axis	**Spp-Envt Corr.**	Mean	Minimum	Maximum	p
1	0.993	0.957	0.743	1.000	**0.1822**
2	0.893	0.854	0.632	0.999	
3	0.990	0.893	0.558	1.000	

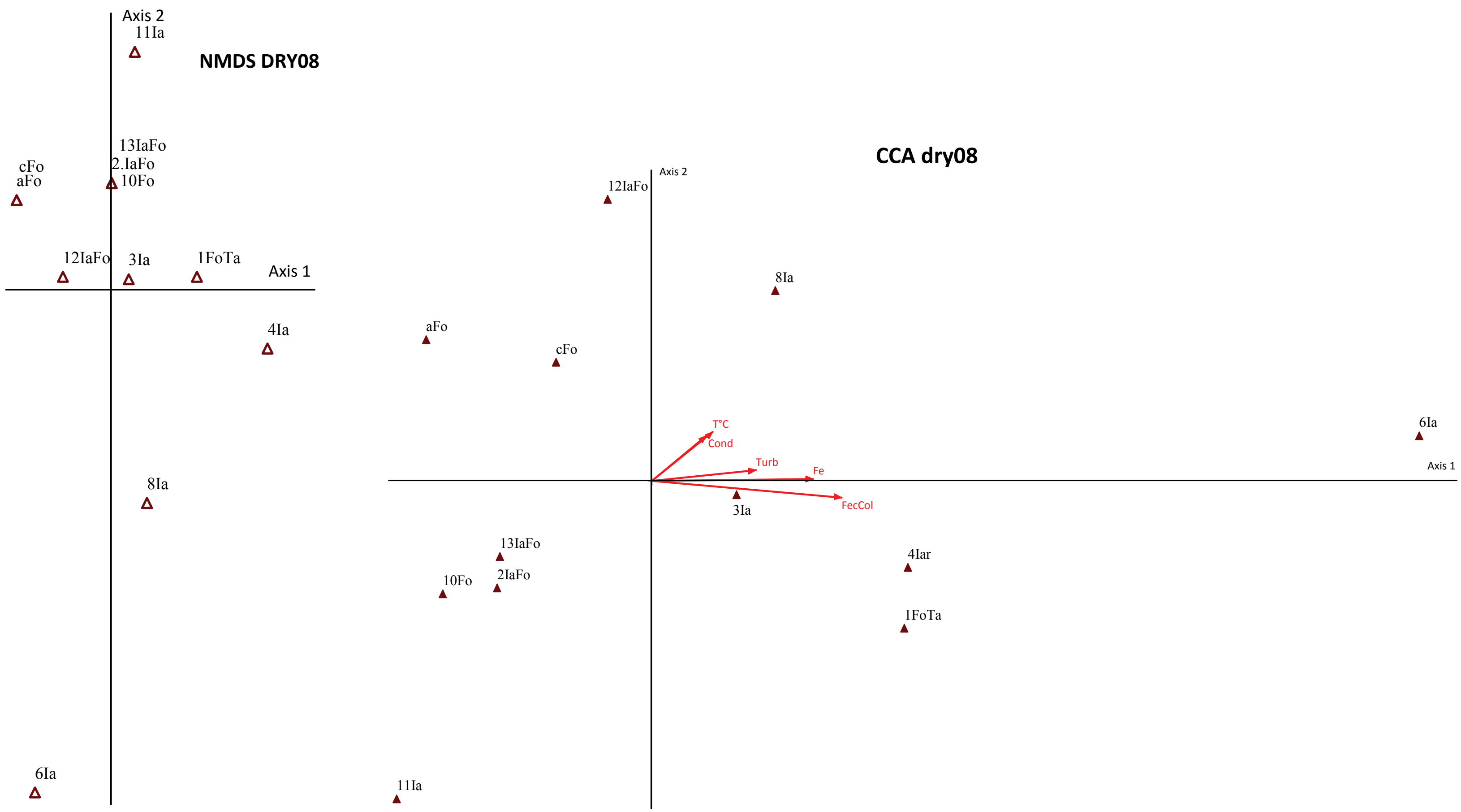


Figure 2.12. Dry08 **a)** NMDS ordination biplot showing the 12 Ayuquila River sites' spatial arrangement based on macroinvertebrate and fish functional groups. These two axes explain 97% of the total variance (stress 4.2479, p=0.0196); **b)** CCA ordination biplot showing the sites' arrangement based on the relationships between the functional group composition and eight water variables. These two axes explain 63.9% of the variance in species data (p=0.1451).

Sites' acronyms follow the assumed accumulated land use effect (see Table 1.4). The first number or letter represents the site's position in the river's gradient, while the following letters the most influential landuse (i.e. Ia: intensive agriculture, Ta: temporal agriculture, Fo: forest, and Ur: urban.

Table 2.6. Summary of the results from the CCA of the Dry08 invertebrate and fish functional group presence-absence data. Total variance ("inertia") in the species data is 0.4861, so statistics on variance explained are based on ratios against this number. The p-value (0.1451) associated with the Monte Carlo results indicate no significant relationship between the species data matrix and the environmental data matrix. High correlations between raw-data distance and ordination distance indicate a good representation of the original data for axis 1. Values that are ideal for interpretation are bolded and show that only axis 1 should be considered for interpretation.

	Axis 1	Axis 2	Axis 3
Eigenvalue	0.213	0.097	0.053
Variance in species data			
% of variance explained	43.9	20.0	10.9
Cumulative % explained	43.9	63.9	74.8

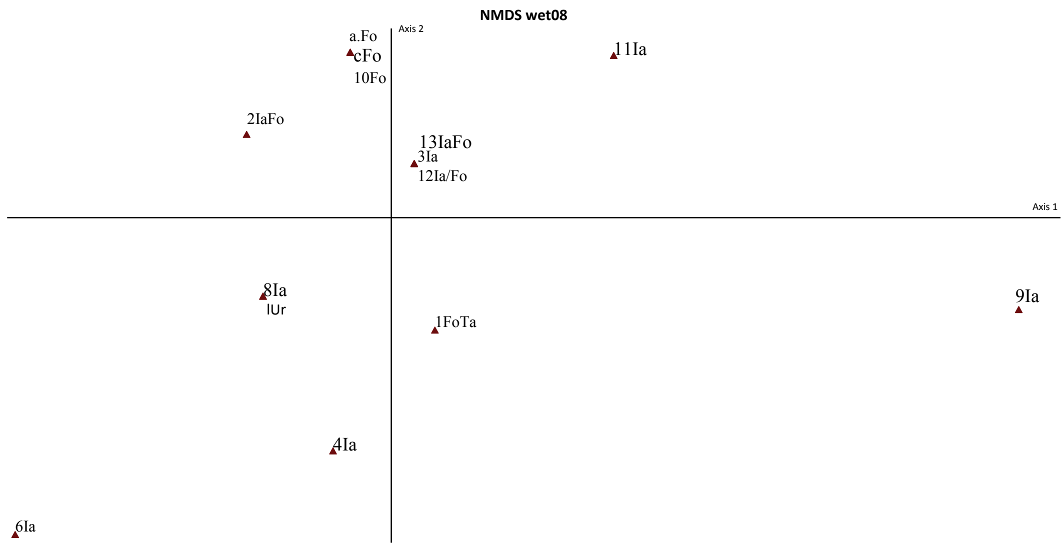
CORRELATIONS AND BIPLOT SCORES for 8 environmental variables

Variable	Correlations* Axis 1	Correlations* Axis 2	Correlations* Axis 3	Biplot Scores Axis 1	Biplot Scores Axis 2	Biplot Scores Axis 3
1 T°C	0.268	0.301	-0.194	0.124	0.094	-0.045
2 DO	-0.022	-0.014	-0.382	-0.010	-0.004	-0.088
3 Turb	0.460	0.066	0.398	0.213	0.021	0.091
4 Cond	0.242	0.279	0.220	0.112	0.087	0.050
5 SO4	0.171	0.303	0.134	0.079	0.094	0.031
6 Fe	**0.714**	0.015	0.031	0.330	0.005	0.007
7 PO4	0.244	-0.077	0.401	0.113	-0.024	0.092
8 FecCol	**0.841**	-0.105	0.107	0.389	-0.033	0.025

MONTE CARLO TEST RESULTS -- EIGENVALUES

Axis	Real data **Eigenvalue**	Randomized data Monte Carlo test, 998 runs Mean	Minimum	Maximum	p
1	0.213	0.175	0.079	0.231	0.1041
2	0.097	0.090	0.042	0.125	
3	0.053	0.051	0.017	0.083	

Axis	**Spp-Envt Corr.**	Mean	Minimum	Maximum	p
1	0.965	0.908	0.681	1.000	0.1451
2	0.895	0.881	0.466	0.998	
3	0.784	0.732	0.361	0.996	



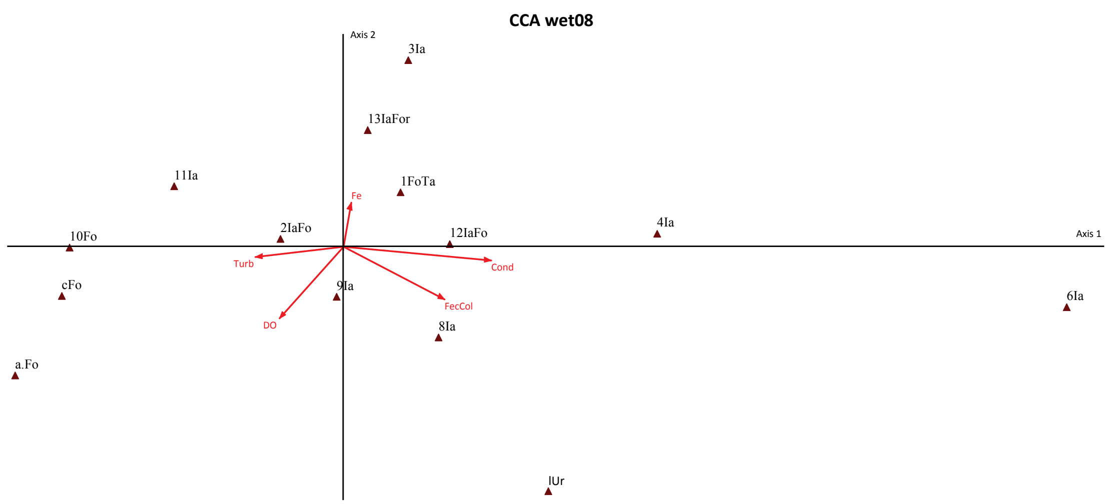


Figure 2.13. Wet08 **a)** NMDS ordination biplot showing the 14 Ayuquila River sites' spatial arrangement based on macroinvertebrate and fish composition. These two axes explain 92.4% of the total variance (stress 1.0248, p=0.0588); **b)** CCA ordination biplot showing the sites' arrangement based on the relationships between the functional group composition and seven water variables. These two axes explain 35.2% of the variance in species data (p=0.02).

Sites' acronyms follow the assumed accumulated land use effect (see Table 1.4). The first number or letter represents the site's position in the river's gradient, while the following letters the most influential landuse (i.e. Ia: intensive agriculture, Ta: temporal agriculture, Fo: forest, and Ur: urban

Table 2.7. Summary of the results from the CCA of the Wet08 invertebrate and fish functional group presence-absence data. Total variance ("inertia") in the species data is 0.3581, so statistics on variance explained are based on ratios against this number. The p-value (0.02) associated with the Monte Carlo results indicate statistically significant relationship between the species data and the environmental data matrices. High correlations between raw-data distance and ordination distance indicate a good representation of the original data for axis 1. Values that are ideal for interpretation are bolded and show that only axis 1 should be considered for interpretation.

	Axis 1	Axis 2	Axis 3
Eigenvalue	0.129	0.033	0.019
Variance in species data			
% of variance explained	36.1	9.2	5.4
Cumulative % explained	36.1	45.4	50.8

CORRELATIONS AND BIPLOT SCORES for 7 environmental variables

	Correlations*			Biplot Scores		
Variable	Axis 1	Axis 2	Axis 3	Axis 1	Axis 2	Axis 3
1 DO	-0.301	**-0.666**	-0.151	-0.108	-0.121	-0.021
2 Turb	-0.414	-0.098	-0.050	-0.149	-0.018	-0.007
3 Cond	**0.691**	-0.129	-0.159	0.249	-0.024	-0.022
4 Fe	0.037	0.407	0.074	0.013	0.074	0.010
5 PO4	-0.019	0.258	0.501	-0.007	0.047	0.070
6 SO4	0.192	-0.209	0.385	0.069	-0.038	0.054
7 FecCol	0.472	-0.490	0.481	0.170	-0.089	0.067

MONTE CARLO TEST RESULTS -- EIGENVALUES

	Real data	Randomized data Monte Carlo test, 998 runs			
Axis	**Eigenvalue**	Mean	Minimum	Maximum	p
1	0.129	0.090	0.029	0.141	0.0180
2	0.033	0.046	0.013	0.100	
3	0.019	0.016	0.003	0.031	

Axis	**Spp-Envt Corr.**	Mean	Minimum	Maximum	p
1	0.959	0.816	0.544	0.983	0.0200
2	0.584	0.630	0.330	0.936	
3	0.593	0.667	0.270	0.962	

3. Carbon and Nitrogen Isotope Characterization of the Ayuquila River's Sediment and Macrofauna

Abstract

The objectives of this study were threefold: through the use of stable isotope analysis 1) to identify sources of organic matter, 2) to characterize the food webs on each site based on the isotopic signatures, and then 3) to relate the sources of organic matter and resulting food webs to adjacent land use activities. I characterized the sediment and macrofauna isotopic signatures from the middle portion of the Ayuquila River watershed. Benthic macroinvertebrates, fishes, and sediment were sampled from sites located in the Ayuquila and Tuxcacuesco rivers in this basin along a gradient of agriculture, forest, and agriculture land uses. Sampling was done during two rainy and one dry field season in 2007 and 2008, in sites that form part of a program to monitor the river's water quality. Temporal and spatial differences were noted in the isotopic values obtained, with a higher isotope range variation in the dry season. Between-season comparisons were statistically significant for both isotopes in faunal tissues. Differences between basins were statistically significant only in the wet 2007 season. Stable $\delta^{13}C$ isotope analysis indicated that organic matter was influenced by forest C_3 and/or autochthonous sources rather than from surrounding agricultural vegetation. However, $\delta^{15}N$ were around the ratios known from sewage and animal waste throughout the sampling area, but were more pronounced in the Ayuquila basin. NMDS ordination grouped the sites based on $\delta^{15}N$ suggesting that organic matter from human and animal sewage has more influence than specific land use activities. $\delta^{15}N$ values indicated two to three trophic level food webs at most sites: exceptions occurred on one location, which was highly anoxic. At this site, gather collectors were on top of the food web instead of predators. This study confirmed known point sources of nitrogen pollution and

helped identify other sources, all of which require management practices to improve the water quality and reduce the impact to the biota.

3.1. Introduction

Stable isotope ecology has become increasingly used in various areas of ecological science to track movement of energy and matter through ecosystems. It is based on measuring the ratios between the heavy and light isotopes of an element. These isotope ratios are reported in “delta” (δ) notations which represent the parts per thousand (‰) deviation of the sample from international recognized standard materials (Howard et al. 2005). Stable isotopes remain constant over time and their abundance and variation rates are known (Fry 2006), making stable isotope analysis ideal to follow changes in ecosystems' composition and structure (Boutton *et al.* 1999).

Carbon and nitrogen are two of the most common elements used in isotope ecology, and by measuring and analyzing their isotope ratios ($^{13}C/^{12}C$, $^{15}N/^{14}N$), ecologists have inferred trophic links (Layman *et al.* 2007), traced ecological change in time (Dawson and Siegwolf 2007), or determined the sources and origins of matter (e.g. marine versus freshwater, forest versus savanna, detritus or planktonic, nutrient pollution or anthropic derived (Hamilton et al. 1992; McClelland and Valiela 1998; Martinelli *et al.* 1999a, 1999b, 2007), among many other increasing uses.

Isotope values have widely been used to identify and elucidate trophic positioning in food webs and, by using an isotope baseline, the ecological relationships in a community can be deduced (Gustafson *et al.* 2007). Stable isotope tracing offers two potential advantages in terms of food-web analyses; first, the stable carbon ($\delta^{13}C$) and stable nitrogen ($\delta^{15}N$) isotope values of animal tissue represent the integration of carbon and nitrogen over a prolonged period, and second, they are based on assimilation rather than ingestion (Peterson and Fry 1987). For nitrogen, $\delta^{15}N$ exhibits stepwise enrichment with trophic transfers between 2.5 and 3.4‰ per each trophic position (Minagawa and Wada 1984, Peterson and Fry 1987, Post 2002; Vanderklift and Ponsard 2003); while $\delta^{13}C$

varies substantially among primary producers with different photosynthetic pathways (e.g., C_3 versus C_4 plants), but changes little (~1‰) with trophic transfers (DeNiro and Epstein 1981).

Another important application N isotope analysis has in aquatic ecosystem functioning is the identification of sources of nitrogen pollution derived from human activities (Lake *et al.* 2001, Vizzini and Mazzola 2004). Many are the human sources of N to water bodies, but the most important include animal or sewage wastes, atmospheric deposition, and run-off from agricultural fields, waste dumps and burned forests (Heaton 1986; Camargo and Alonso 2007). The excess N found as ammonia and nitrate can be tracked as it is incorporated in a system's biological components in different environments (Lindau *et al.* 1997; Vander Zanden *et al.* 2005). As such, stable isotope ecology has been proposed and used as a tool in aquatic biological assessments (Diebel and Vander Zanden 2009).

Although not part of the biological trophic structure, sediment is pivotal in sustaining and structuring benthic community composition through the type of substrate found (Davis and Lathrop 1992), or as sinks and sources of pollutants to aquatic biota (e.g. Brown *et al.* 2000), and has been frequently used in detecting human impacts in aquatic systems in conjunction with the biological components it sustains (Schorer and Eisele 1997; Birch *et al.* 2001; Lake *et al.* 2001).

In sum, several models have been accepted in isotope ecology using these two isotopes (Newsome *et al.* 2007). For instance, in freshwater ecosystems high or enriched $\delta^{13}C$ values will most likely indicate C_4 plants as sources of energy while low or depleted ratios suggest C_3 plants (i.e., grassland/agriculture versus forest, or benthic versus pelagic). In the tropics, C_3 plants have $\delta^{13}C$ values ranging from -34 to -27‰ and C_4 plants -14 to -11‰ (Martinelli et al. 1999a). Similarly, enriched $\delta^{15}N$ and $\delta^{13}C$ ratios are associated to xeric conditions, pollution, and higher trophic levels (Finlay and Kendall 2007).

Therefore, the overall goal of this study was to relate sources of organic matter and food web composition to surrounding land use activities. Specific objectives were threefold: through the use of stable isotope analysis 1) to identify sources of organic matter, 2) to characterize the food webs on each site based on the isotopic signatures, and then 3) to relate the sources of organic matter and resulting food webs to adjacent land use activities. That is, I would expect that the C isotope ratios in those sites within the agricultural valley be more enriched and characteristic of sugar cane or maize organic matter (C_4 plant) and the N ratios reflecting manure or agricultural run-off, versus those adjacent to forested sites possessing lighter carbon and nitrogen ratios characteristic of C_3 plants and cleaner sites.

The study took place in the middle portion of the Ayuquila River in western Mexico which has been monitored for water quality in the last 15 years. While both the point and diffuse sources of pollution have been identified, little is known about the effects of contaminants on the river’s biological structure and composition. By identifying or corroborating sources of organic matter (e.g., agriculture versus urban, or forest versus agriculture), remedial or preventive actions can be proposed to improve watershed conditions as part of the undergoing monitoring program. The use of stable isotope analysis will incorporate novel information in this regards and will help identify the sources of energy (carbon), and the movement of matter (nitrogen) in the river´s biotic components and sediment.

3.2. Methods

3.2.1. Sample collection

Seventeen sites were sampled along a land use and pollution gradient in the Ayuquila River and Tuxcacuesco River, both part of the Ayuquila-Armeria watershed. The code assigned to each site which will be used in this chapter is found in Table 1.3. At each site, three sediment core samples were obtained using a 10-cm long 5 cm diameter PVC tube and placed in whirl-pak sampling bags inside a cooler. As contaminants in sediments concentrate in stream margins and pools in contrast to riffles, where fast water impedes sediment to accumulate, samples were obtained mostly from the river´s margins and pools, and were mixed before lab processing.

Invertebrates were collected using a D-frame 500 μm mesh kick-net from different sections along the river, mostly from riffles, for a total length of 6 meters. Fish were caught with a 10-m long seine net along the river for a total of 45 minutes.

Sediment and animal samples were placed in plastic Ziploc bags inside an icebox, and once in the laboratory placed in a refrigerator for further analysis. More details about the collection of macrofauna are explained in chapter 2.

3.2.2. Laboratory preparation for stable isotope analysis

Once invertebrates were sorted out into functional groups, and fish identified to species, these were oven-dried along with sediment samples for 36 hours at 70^{o}C. Invertebrates were dried completely, except for crabs in which muscle was extracted from the hard shell. Similarly, small fish were dried whole, while pieces of dorsal muscle from fish specimens larger than 10 cm were placed in the oven. Once the samples were dry, sediment was sieved with a 60μ-mesh to obtain the fine sediment, while animal tissues were ground to fine powder using a mortar and pestle.

Four 30 mg sediment sub-samples and three to four 1 mg sub-samples from invertebrate and fish tissues from each site were placed in 9 x 5 mm tin capsules. In some cases, due to the macroinvertebrates size or the small number of specimens collected, I could not bring together enough tissue for more than one sub-sample. Samples were sent to the UC Davis Stable Isotope Facility for carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) isotope ratio analyses. These were performed with a PDZ Europa 20/20 isotope ratio spectrometer. δ (delta) notations were measured against the reference standards as:

$$\delta^{13}C \text{ or } \delta^{15}N = \{(R_{sample} - R_{standard}) / (R_{standard})\} \times 1000,$$

where R_{sample} is $^{13}C/^{12}C$ or $^{15}N/^{14}N$ of the samples, and $R_{standard}$ are the Pee Dee Belemnite (PDB) for Carbon, and atmospheric nitrogen for Nitrogen reference standards. The standard deviation (± 1 S.D.) for the analytical standards was ±0.1‰ for C isotopes and ±0.2‰ for N isotopes.

3.2.3. Estimation of trophic levels

Several studies (e.g. DeNiro and Epstein 1981; Minagawa and Wada 1984; Vander Zanden and Rasmussen 1999) have shown that $\delta^{15}N$ values are good predictors of trophic position in aquatic systems due to a predictable ^{15}N enrichment of consumers relative to their diets. This has permitted to calculate the trophic level (TL) of consumers and construct food webs based on the $\delta^{15}N$ values that have been assimilated in the tissues of organisms. TL was estimated using the following formula (Post 2002):

$$TL = \lambda + (\delta^{15}N_c - \delta 15N_b) / \Delta$$

where $\lambda =$ is the trophic level of the base, $\delta^{15}N_c$= is the nitrogen isotope value of the consumer, $\delta 15N_b$ = the nitrogen value of the food base, and Δ= the enrichment factor or average increase in $\delta^{15}N$ values per trophic level. I considered the sediment's $\delta^{15}N$ values as the food base (sensu Jepsen and Winemiller 2002), so $\lambda = 1$ as sediment values are "equalled" to those of primary producers, and a fractionation or enrichment factor of 2.54, based on Vanderklift and Ponsard (2003) who proposed this factor based on a meta-analysis that included the variation in the N sources.

3.2.4. Data analysis

Analyses of variance were used to compare sediment, fish, and invertebrate FFG's $\delta^{13}C$ and $\delta^{15}N$ values between sites and seasons. These analyses were performed using SigmaPlot version 11.0 (Systat software 2008), with a significance level of $p<0.05$.

Additionally, in order to graphically compare the sampling sites based on the functional feeding groups' isotope ratios similarities, I applied a Non-Metric

Multidimensional Scaling (NMDS) (Kruskal 1964; Mather 1976) using PcOrd 5.10 for Windows (McCune and Mefford 2006). NMDS is an indirect multivariate ordination method used to score and identify units that are more similar plotting those that are closer together (Beals 2006). This technique helps summarize and represent in a graphical way complex non-normal or discontinuous ecological data, revealing spatial and temporal variation in community composition and relationships (McCune and Grace 2002). The analyses were performed initially for carbon and nitrogen independently, and then considering both variables for each season. I used the functional group and sediment's mean $\delta^{13}C$ and $\delta^{15}N$ values from each site as variables. Initial runs were made in autopilot mode using 6 dimensions, Sorensen distance measure (Bray-Curtis), and 500 maximum iterations with a random starting configuration each time. For carbon isotope ordinations, the isotope ratios were multiplied by -1 to eliminate negative numbers. Plots of stress versus iteration were examined after several runs to find homogeneity in the stress values and to look for those with less stress, as low stress indicates that the ordinations produced are a good representation of the original data and values >20 stress should be interpreted with caution (McCune and Grace 2002). A final run was made with the number of axes suggested by the program. In some cases, when the ordination output showed high stress values, or the resulting ordination plot suggested a short gradient in its values, and thus a linear response, a Reciprocal Analysis was applied to justify the use of a linear method. In those cases, Principal Component Analysis (PCA) was performed. PCA works well with data that show a linear response to the variables that are being measured (Jolliffe 2002; McCune and Grace 2002).

3.3. Results and Discussion

A total of 192 sediment samples and 255 invertebrate and fish samples from three field seasons were analyzed for carbon and nitrogen isotopes. A summary of the mean isotope values obtained in this study, arranged by season and functional groups, is presented in Table 3.1.

3.3.1. Temporal Isotope Variability

Seasonal variation in $\delta^{13}C$ values

The $\delta^{13}C$ values of all sediment samples from the three field seasons ranged from -29.92‰ to -8.47‰ with a mean of -21.04‰. This wide range (~22‰) corresponded to samples obtained in wet07, and was almost twice the range obtained in each of the two other field seasons (Table 3.1.; Figure 3.1a). Inter-seasonal comparisons for sediment's $\delta^{13}C$ values were not statistically significant ($H=0.900$, $p=0.638$).

The amplitude in the sediment's $\delta^{13}C$ values from wet07 is probably due to the incorporation into the water column of soil and sediment from adjacent fields and forests as a result of excessive rainfall and flooding conditions. Soil organic matter $\delta^{13}C$ values reflect the composition of the plants that grow on them (Boutton 1996; Martinelli 1999a). Moreover, there is a substantial overlap in the isotopic values of the major organic sources that flow into river systems (Finlay and Kendall 2007). Martinelli *et al.* (1999a) reports that tropical areas with savannas and C_4 grasses have a 9‰ range in the $\delta^{13}C$ values from particulate organic matter. However, in areas with sugar cane and other C_4 grasses, the origin of the particulate carbon is not from the decomposition or ingestion of these plants by herbivores, but of the soil that is ran off to rivers in rainy events (Bunn et al. 1997; Martinelli et al. 1999a; Clapcott and Bunn 2003).

Mean $\delta^{13}C$ values for all invertebrate and fish samples was -24.5‰ with ratios ranging from -37.1‰ to -12.8‰. The most depleted $\delta^{13}C$ value (-37.1‰) belonged to a filter collector in wet07, while the most enriched value (-12.8‰), and widest range were obtained from filter collectors in dry08 (Table 3.1.; Figure 3.1b). Differences in faunal isotope values among the three seasons were statistically significant ($F=5.151$, $p=0.007$). However, when running pairwise comparisons only the differences between wet07 and dry08 seasons were statistically significant ($t=3.205$, unadjusted p value=0.002).

Invertebrate and fish functional group $\delta^{13}C$ values presented a larger variation in the dry08 than in the two wet seasons, and on average more depleted ratios than that of sediment (Figure 3.2.). The $\delta^{13}C$ values in lotic invertebrates that consume periphyton tend to be more depleted than those from terrestrial sources (Lancaster and Waldron 2001). It is in the dry season that the river's water is, on average, less turbid; in addition, solar exposure is on its height allowing for the abundance of periphyton in the substrate. Periphyton and phytoplankton have isotope ranges of almost 40‰ and 20‰, respectively (Finlay and Kendall 2007). MacLeod and Barton (1998), on the other hand, found that periphyton's $\delta^{13}C$ values were more enriched as a result of light intensity. Additionally, sewage also impacts the isotopic value range of coarse and fine particulate organic material. A ~12‰ variation in $\delta^{13}C$ values has been reported in places under sewage influence (DeBruyn and Rasmussen 2002).

Seasonal variation in $\delta^{15}N$ values

The $\delta^{15}N$ values for all sediment samples (N=192) averaged 5.17‰ with a range from 0.6‰ to 11.3‰. As with $\delta^{13}C$ values, the widest range in N ratios was from wet07 samples. However, unlike $\delta^{13}C$ values, inter-seasonal differences in sediment's $\delta^{15}N$ values were statistically significant ($H=12.507$, $p=0.002$).

Mean $\delta^{15}N$ values in invertebrate and fish samples (N=255) were of 10.36‰, with ratios ranging from 0.4‰ to 19.6‰. The most enriched $\delta^{15}N$ values corresponded to macrofauna and fish tissues obtained in dry08 (Table 3.1.; Figure 3.1b). As it was expected, the most enriched mean nitrogen ratios correspond to fish predators. Differences in $\delta^{15}N$ values among the three seasons were statistically significant ($F_{df2}=3.889$ $p=0.023$).

Similarly to what happens in carbon fractionation, $\delta^{15}N$ values are affected by both landscape (e.g., geomorphology, tree cover, and climate (Minshall *et al.* 1985)), and local variables (e.g., light intensity, water temperature, pH, water velocity: Hecky and Hesslein 1998; MacLeod and Barton 1998; Vuorio *et al.* 2006). Thus, many are the confounding factors and sources of variation that may blur a precise interpretation (Jennings *et al.* 1997, Maier *et al.* 2011), in particular in areas with a diverse land-use mosaic. However, seasonal variation in the nitrogen ratios are less pronounced than those of carbon (Kendall *et al.* 2001; DeBruyn *et al.* 2003).

3.3.2. Spatial variation in sediment and FFG's $\delta^{13}C$ and $\delta^{15}N$ values

Just like seasonal variation in isotope ratios is an accepted fact, so is spatial variation. An analysis by sub-basin showed that when the mean FFG isotope ratios were considered for all sites, a ~8‰ N trophic enrichment, and a ~6‰ range in the carbon ratios were apparent in wet07 (Figure 3.3a). However, the breadth in isotopic values differed when analyzed separately: the Ayuquila sub-basin's $\delta^{13}C$ value range was narrower (~4‰) and its $\delta^{15}N$ value range increased to ~12‰ (Figure 3.3b). In contrast, the Tuxcacuesco sub-basin had a 3‰ nitrogen enrichment and a ~21‰ carbon range, with the faunal components having a lighter (more negative) value with respect to the sediment's mean ratio (Figure 3.3c). These inter-basin differences were statistically significant ($p<0.001$) for both isotope values from sediment in this season only.

A similar scenario between basins was seen in dry08 where $\delta^{13}C$ values had a wider range (20‰), particularly in the Tuxcacuesco basin, with gather collectors presenting the highest variation in both isotopes (Figure 3.4). Contrastingly, the $\delta^{15}N$ value range (~12‰) was wider in the wet08 than the $\delta^{13}C$ values. However, when isolated, the Tuxcacuesco's ratios were the opposite (Figure 3.5). Nonetheless, these inter-basin isotopic differences were only statistically significant for $\delta^{15}N$ values ($p=0.045$) from dry08.

The sites in the Ayuquila sub-basin had, on average, a higher $\delta^{15}N$ signature than those in the Tuxcacuesco. This difference in range could be explained by the presence of more human settlements, a higher population density, larger area and longer history of irrigated sugar cane and maize fields, and more cattle, pig and fish production areas, among other probable causes. This wide-ranging nutrient supply creates a synergy with a final increased $\delta^{15}N$

seen in the Ayuquila River's biological components; thus the resulting complex picture of nitrogen sources and effects (Diebel and VanderZanden 2009)

Similarly, the heavier $\delta^{13}C$ values in the sediment samples from the Tuxcacuesco sub-basin's, above -20‰ in the range of C_4 grasses, might be due to the incorporation of C_4-derived soil and sediment into the water, as explained above. However, sugar cane cultivation in this sub-basin is relatively recent. Vitorello and collaborators (1989) found that it took around 50 years for 40% of C_3 carbon in former forested soils to be replaced by C_4 carbon from sugar cane debris. Thus, the probable source of C_4 organic carbon in this sub-basin comes from the xeric vegetation's soil, which is known to present, on average, more enriched carbon ratios than mesic or moister vegetation (Newsome *et al.* 2007). Although not exclusive to this basins' sites, C_3 riparian forest corridors with a >20m width, except in one site (d.FoUr), that could stop or reduce erosion from nearby agricultural landscapes (Pires *et al*. 2009) are practical inexistent. The absence or scarce wooded margins also increase light intensity and temperature, factors that are known to produce enriched $\delta^{13}C$ values in freshwater and marine algae (MacLeod and Barton 1998; Staal *et al.* 2007).

It has also been postulated that a pattern exists from deleted to enriched $\delta^{13}C$ along a river gradient as a river flows from forested headwaters through savannas and agricultural land (e.g. Bird *et al.* 1994; Martinelli *et al.* 1999b). Sousa and collaborators (2008) found a similar gradient in the $\delta^{13}C$ of methane originated from organic matter's decomposition. Although not the norm, this is apparently happening in the Tuxcacuesco River before it flows into the Ayuquila to form the Armeria River. This gradient of depleted towards enriched isotopic ratios is also reflected in the consumers' isotopic signature (e.g. Benedito-Cecilio and Araujo-Lima 2002).

In our study sites, variability in the isotopic signatures between similar functional groups was frequent. The causes, as explained before, are many. Each site $\delta^{15}N$ /$\delta^{13}C$ biplot graph arranged following the river's flow for each season are presented in the appendices 3.1 to 3.3.

3.3.3. Influence of surrounding landscape

Sediment and FFG δ^{15}N and δ^{13}C values from this study revealed spatial and temporal variations which were partially influenced by surrounding landscape. This was confirmed graphically using NMDS ordinations. Although carbon and nitrogen stable isotopes respond differently to the same variable (Chang *et al.* 2009), in this case NMDS isolated those sites known to receive high nutrient-derived effluents (i.e., untreated urban sewage and cattle enclosure runoffs) when including either one of the isotopes, or both isotopes as variables in the analyses. This suggests that the nutrient load in the river is strong enough to make such distinction, masking the importance terrestrial and exogenous energy sources have in the carbon isotope characterization of those sites. In marine environments, algae δ^{13}C signatures seemed to be affected by salinity and amount of nitrogen in the environment (Brutemark et al 2007; Levy *et al.* 2010), and heavier δ^{13}C ratios in plant tissues have also been associated with increased nutrient availability (Chang *et al.* 2009).

For example, in the NMDS ordination graph from wet07 with δ^{13}C values as variables (Figure 3.6), three groups were clearly separated, one which was composed of four closely packed sites (Amtl, Herr, DrnG, Txca); while two were loosely grouped in two subgroups. All these sites had different dominant surrounding land uses. On the other hand, the δ^{15}N NMDS arrangement suggested two large groups, one of which could be divided in three smaller groups (Figure 3.7). The group consisting of the sites Herr, Amtl, DrnG and Txca (also singled-out in the δ^{13}C arrangement) are known to correspond to sites with high nutrient load, located in or close to sewage drains or cattle enclosures. All but Amtl are immersed in an agricultural landscape. However, when both isotopes were included in the ordination analysis (Figure 3.8), these four sites were also grouped together.

Similar graphical arrangements resulted when using the data from dry08 (Figures 3.9 to 3.11) and wet08 (Figures 3.12 to 3.14). In these cases, the sites that were usually singled out (e.g., DrnA, Pbco, DrnG, Amtl and Snbn site numbers 5, 6, 7, 9, and b, respectively) are strongly influenced by sewage derived point sources, versus the others which have mixed diffuse sources. The opposite was seen with Mntl (site 10), a site that was completely isolated in both dry08 and wet 08 $\delta^{15}N$ NMDS ordination plots (Figures 3.10 & 3.13). In addition, trophic webs from this forested site were usually placed in the lower levels (Figure 3.15). Thus, these results suggest that the N loads do have a higher influence in determining the similarities between sites.

Nonetheless, when the all FFG and sediment isotope values from forested sites were statistically compared with those from agricultural dominated landscapes, differences in the $\delta^{15}N$ values were not significant in the three seasons, whereas the $\delta^{13}C$ values were (wet07: H=16.429, p=<0.001; dry08: H=5.917, p=0.015; and wet08: H=8.1857, p=0.004). Additionally, if the FFG isotope values were analyzed independently from those of sediment, comparisons were not statistically significant for FFG's C and N isotope values nor for sediment's $\delta^{15}N$ values, while differences in the sediment's $\delta^{13}C$ values from these two land uses were significant (p<0.001) for the three seasons. These findings suggest that the carbon isotope signature from a site is strongly influenced by the $\delta^{13}C$ values in the organic layers in sediment, which contradict the NMDS graphical displays explained above, and remind us of the multiple sources that influence isotope values in a system. This calls for further research.

3.3.4. Trophic arrangement

A trophic web characterization for each site was obtained based on its FFG's $\delta^{15}N$ values. An enrichment factor of +2.54‰ relative to the diet was considered for each trophic level (Vanderklift and Ponsard 2003). The trophic levels obtained for each FFG are summarized on Tables 3.2 to 3.7. On average, the different functional groups' trophic position corresponded to what would be expected, although there were a several exceptions. For instance, filter and gatherer collectors in several sites were at the same or upper trophic level than invertebrate predators or fish omnivores, which based on the assumption of stepwise trophic enrichment, should be in a higher position. At the same time, the food web in the site with water flowing in from a forested protected area (Mntl site 10), had the lowest nitrogen imprint throughout the study, with the opposite trend in Zenz (site 11), where both invertebrates and fish had the highest nitrogen levels on average.

There are several reasons that can explain the fact that filter-collectors and gatherer invertebrates had higher $\delta^{15}N$ values than those of predators. First, the ingestion of nitrogen enriched suspended material coming from sewage or animal enclosures. This was more evident in the dry08 season (Table 3.2), where these functional groups were in a higher position in five sites. Nitrogen isotopic signatures tend to be more variable among invertebrates than in fish, and in herbivores versus carnivores, as a result of the variability of sources that affect nitrogen fractionation (DeBruyn *et al.* 2003; Vander Zanden and Rasmussen 2001). Various studies have reported that aquatic plants and animals that live in areas receiving effluents from agricultural and urban areas assimilate the inorganic nitrogen, reflecting an enriched $\delta^{15}N$ value in their tissues (e.g., Cabana and Rasmussen 1996; McClelland and Valiela 1998; Vander Zanden *et al.* 2005; Kohzu et al 2008; Bucci *et al.* 2011). A literature review done to compare the $\delta^{15}N$ values reported for filter collectors, scrapers, gather-collectors and omnivore species from tropical and temperate regions (Table 3.2), some in similar landscape scenarios, showed higher nitrogen ratios in the samples from this study region. The differences between the mean values of the four groups compared were statistically significant for filter-collectors ($p=0.023$) and omnivores ($p=0.008$).

An incorrect classification can be a second reason. This is a mistake that can easily happen when the ecology and natural history of a taxonomic group is not thoroughly known, or when using information from temperate reasons to apply it in tropical scenarios where knowledge gaps still exist in this area (Tomanova *et al.* 2006). Variations in the $\delta^{15}N$ values of primary producers in the base of a food web, change with fractionation rates, fluctuating resource availability, and even human population density within a watershed (Cabana and Rasmussen 1996; Jardine et al 2005). Finally, species from different taxa are many times arbitrarily assigned to the same functional feeding group, masking physiological and ecological differences that are not necessarily recognized using nitrogen isotope analysis. Even individuals from a same species with similar diets can have different $\delta^{15}N$ values (Jardine et al 2005), which explains in part the difficulty in assigning a trophic level.

As many authors repeatedly have suggested, more research in this area of trophic ecology is still necessary, as questions like the impacts land use change or invasive species are having in the structure and functioning, and overall health, of this and other rivers in Mexican scenarios remain unanswered.

3.4. Conclusions

This is the first exercise that uses isotope analyses to characterize the carbon and nitrogen isotope values from sediment, macroinvertebrates and fish samples from two rivers in the middle portion of the Ayuquila-Armeria watershed. The aim was to test the idea that sources of organic matter and resulting food webs could be related to adjacent land uses.

This was true for nitrogen isotope ratios of most samples. $\delta^{15}N$ values suggested sewage and animal waste as main sources of organic matter, rather than from specific land use activities. This was more evident in the more densely populated Ayuquila sub-basin. No significant differences between forested and agricultural scenarios were found in the nitrogen signatures, suggesting a strong influence from urban dwellings. NMDS ordination arrangements confirmed these findings, grouping those sites which had a higher impact from sewage derived organic matter.

The inverse happened with the carbon isotope signatures; $\delta^{13}C$ values from the samples did not correspond to C_4 agricultural vegetation as I had expected, but organic matter carbon ratios were influenced by forest C_3 vegetation and/or authochthonous sources; the C isotope ratios in sediment apparently determined a site's $\delta^{13}C$ signature. Differences between forested and agricultural sites $\delta^{13}C$ values were statistically significant during the three seasons sampled.

Spatial and temporal comparisons in the isotope values were divergent. Seasonal differences in sediment $\delta^{13}C$ were not statistically significant but faunal ratios were, whereas $\delta^{15}N$ ratios were significant for both types of samples. Similarly, seasonal differences between the two sub-basins were only significant for the isotope values in one wet season.

$\delta^{15}N$ values indicated two or three trophic levels in the food webs at most sites. Exceptions in many sites occurred where trophic enrichment did not correspond to what was expected or in situations where anoxic conditions prevailed. Food webs receiving water from a mountainous forested site had the lowest nitrogen enrichment factors in the three seasons, reinforcing the idea that human and animal waste are main determinants in the isotopic signature in the rest of the sites.

The use of this technique helped confirm known sources of pollution while identifying other sources. The organic matter isotope values from these were reflected in the animal tissues and sediment layers analyzed. Thus, it has an important potential in providing other polluting sources that can eventually help in the restoration and rehabilitation programs being implemented in this watershed.

Table 3.1. Summary of $\delta^{13}C$ and $\delta^{15}N$ values in sediment and functional feeding groups from all sites and seasons sampled (n= number of samples, SD= standard deviation).

FFG	n	Mean $\delta^{15}N$ (0/00) ±SD	Range	Max -Min	Mean $\delta^{13}C$ (0/00) ±SD	Range	Max -Min
Wet 07							
Filter collectors	12	7.6 ±2.6	7.75	10.5 - 2.7	-25.5 ± 5.4	15.84	-21.3 -37.1
Gatherers	5	7.9 ± 0.5	1.17	8.4 - 7.2	-24.5 ± 1.2	2.77	-23.4 -26.2
Scrapers	7	9.2 ± 1.9	5.75	11.3 - 5.6	-25.0 ± 2.5	7.14	-20.9 -28.0
Omnivores[1]	4	16.4 ± 0.3	0.63	16.6 -16.0	-24.8 ± 1.9	4.05	-23.4 -27.4
Predators	23	9.4 ± 3.2	12.25	16.9 - 4.6	-24.9 ± 2.7	9.69	-20.5 -30.2
Sediment	100	4.8 ± 2.0	10.65	11.3 - 0.6	-20.7 ± 4.5	21.46	-8.5 -29.9
Dry 08							
Filter collectors	15	8.5 ±4.1	16.6	18.5 - 2.5	-24.8 ± 4.3	18.66	-12.8 -31.4
Gatherers	10	10.9 ± 3.0	11.42	18.1 - 6.7	-27.6 ± 3.2	8.67	-25.2 -33.8
Scrapers	16	8.1 ± 2.9	10.88	11.3 - 0.4	-24.4 ± 2.5	9.4	-17.3 -26.7
Omnivores[1]	4	13.7 ± 3.4	7.25	18.7 - 11.4	-22.4 ± 1.6	3.12	-21.0 -24.1
Predators	21	10.7 ± 3.8	16.12	19.6 - 3.5	-25.6 ± 2.1	8.24	-20.5 -28.8
Fish omnivores	18	11.1 ± 2.8	9.15	15.0 - 5.9	-24.6 ± 2.4	8.94	-20.4 -29.4
Fish herbivore	5	10.8 ± 4.4	11.37	14.6 -3.2	-27.2 ± 3.7	8.53	-23.1 -31.7
Fish carnivore	1	11.84	--	---	-23.9	--	---
Sediment	43	5.5 ± 1.3	6.74	8.0 - 1.3	-21.7 ± 2.9	11.53	-14.1 -25.6
Wet 08							
Filter collectors	17	8.9 ± 2.4	8.2	11.6 - 3.4	-23.7 ± 2.3	8.5	-21.5 -29.5
Gatherers	12	7.9 ± 2.9	9.2	11.8 - 2.6	-24.6 ± 2.4	7.2	-20.4 -27.6
Scrapers	19	8.4 ± 2.1	8.9	11.8 - 2.9	-24.9 ± 1.9	7.8	-20.6 -28.4
Omnivores[1]	15	11.3 ± 2.0	6.6	14.9 - 7.6	-21.8 ± 1.7	5.2	-18.7 -23.9
Predators	39	9.8 ± 3.0	11.1	14.2 - 3.1	-24.4 ± 2.3	9.4	-19.1 -28.5
Fish Herbivores	9	8.7 ± 2.7	6.7	12.8 - 6.1	-24.5 ± 1.6	4.8	-21.8 -26.6
Fish Omnivores	63	11.0 ± 3.2	13.8	15.6 - 1.8	-23.2 ± 2.4	11.3	-18.2 -29.5
Fish Predators	17	14.8 ± 1.2	3.7	16.6 - 12.9	-24.1 ± 1.9	6.5	-21.0 -27.5
Sediment	49	5.5 ± 1.7	9.8	10.5 - 0.7	-21.4 ± 3.2	13.3	-13.5 -26.8

[1]Crabs and crayfish were considered as invertebrate omnivores.

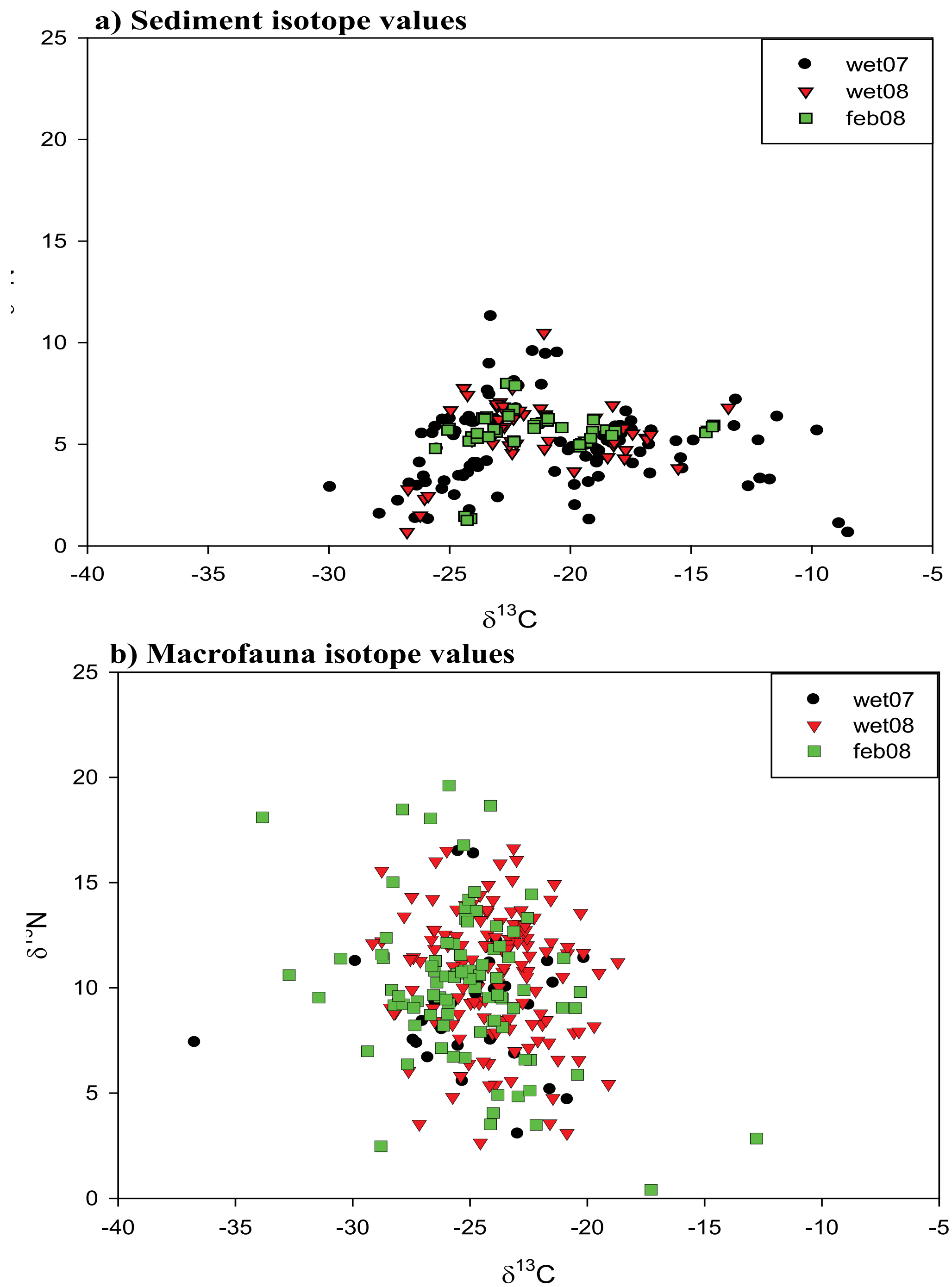


Figure 3.1. Dual $\delta^{13}C$ and $\delta^{15}N$ bi-plots from a) sediment (N=192) and, b)| macrofauna samples (N=255) from the three sampling seasons.

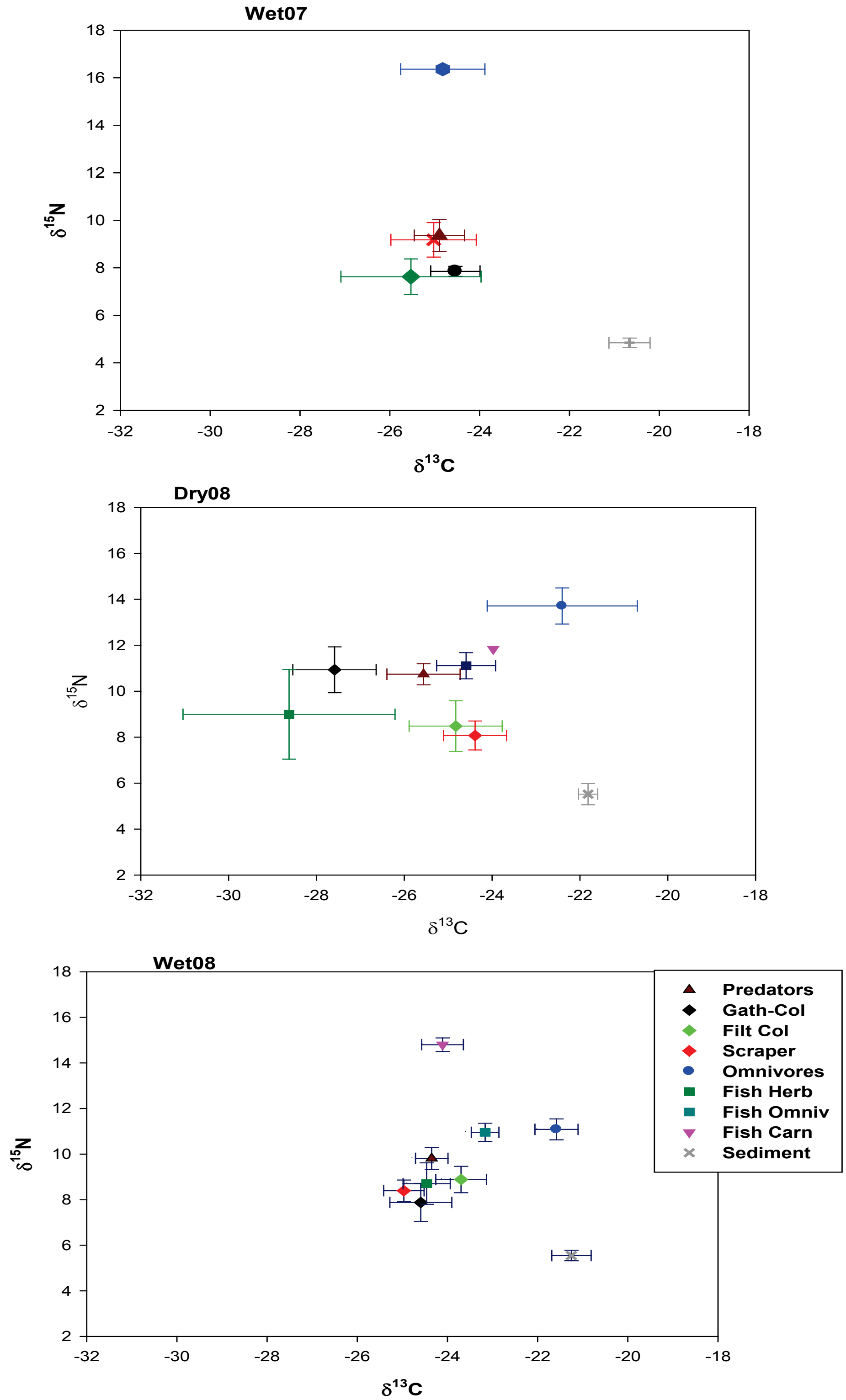


Figure 3.2. Mean (± 1 s.e) FFG and sediment's $\delta^{15}N$ / $\delta^{13}C$ biplots grouped by field season.

Wet07

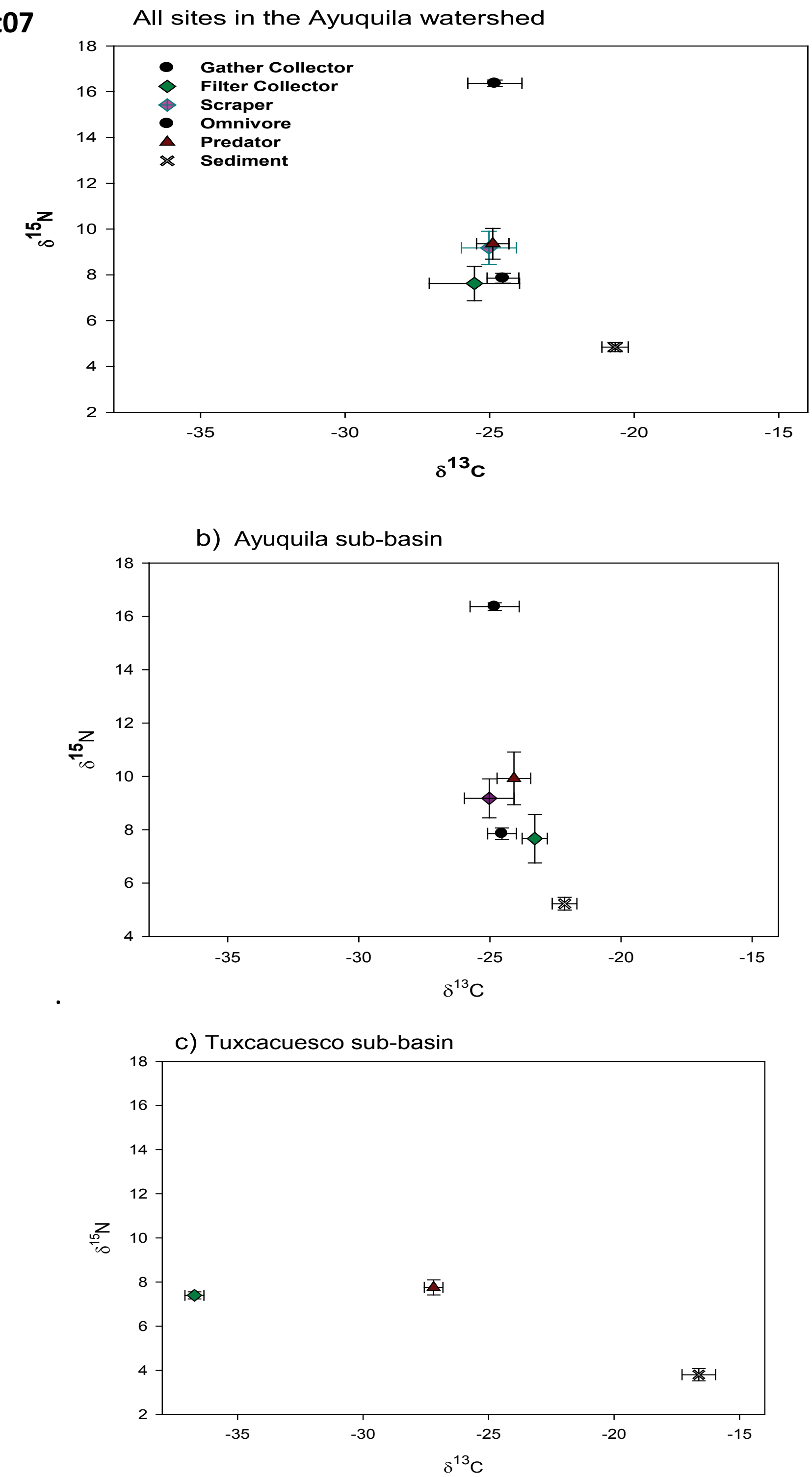


Figure 3.3. Mean (± s.e.) $\delta^{13}C$ and $\delta^{15}N$ biplot arrangement of sediment and faunal samples for wet07 by sub-basin. Differences were statistically significant ($p<0.001$) for both isotopes; a) all sites considered; b) Ayuquila sub-basin; and, c) Tuxcacuesco sub-basin.

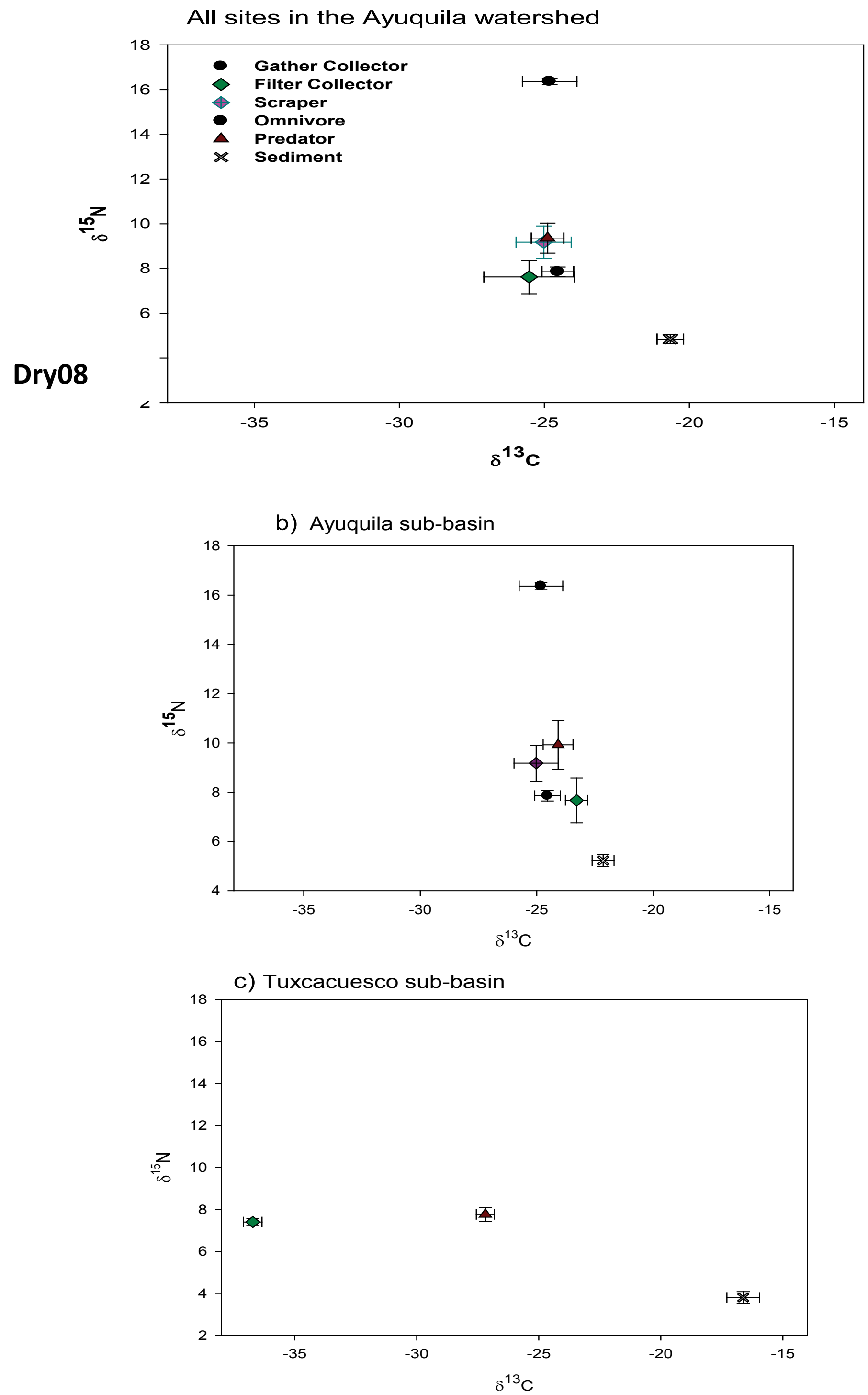


Figure 3.4. Mean (± s.e.) $\delta^{13}C$ and $\delta^{15}N$ biplot arrangement of sediment and faunal samples for dry08 by sub-basin. Differences were not statistically significant; a) all sites considered; b) Ayuquila sub-basin; and, c) Tuxcacuesco sub-basin.

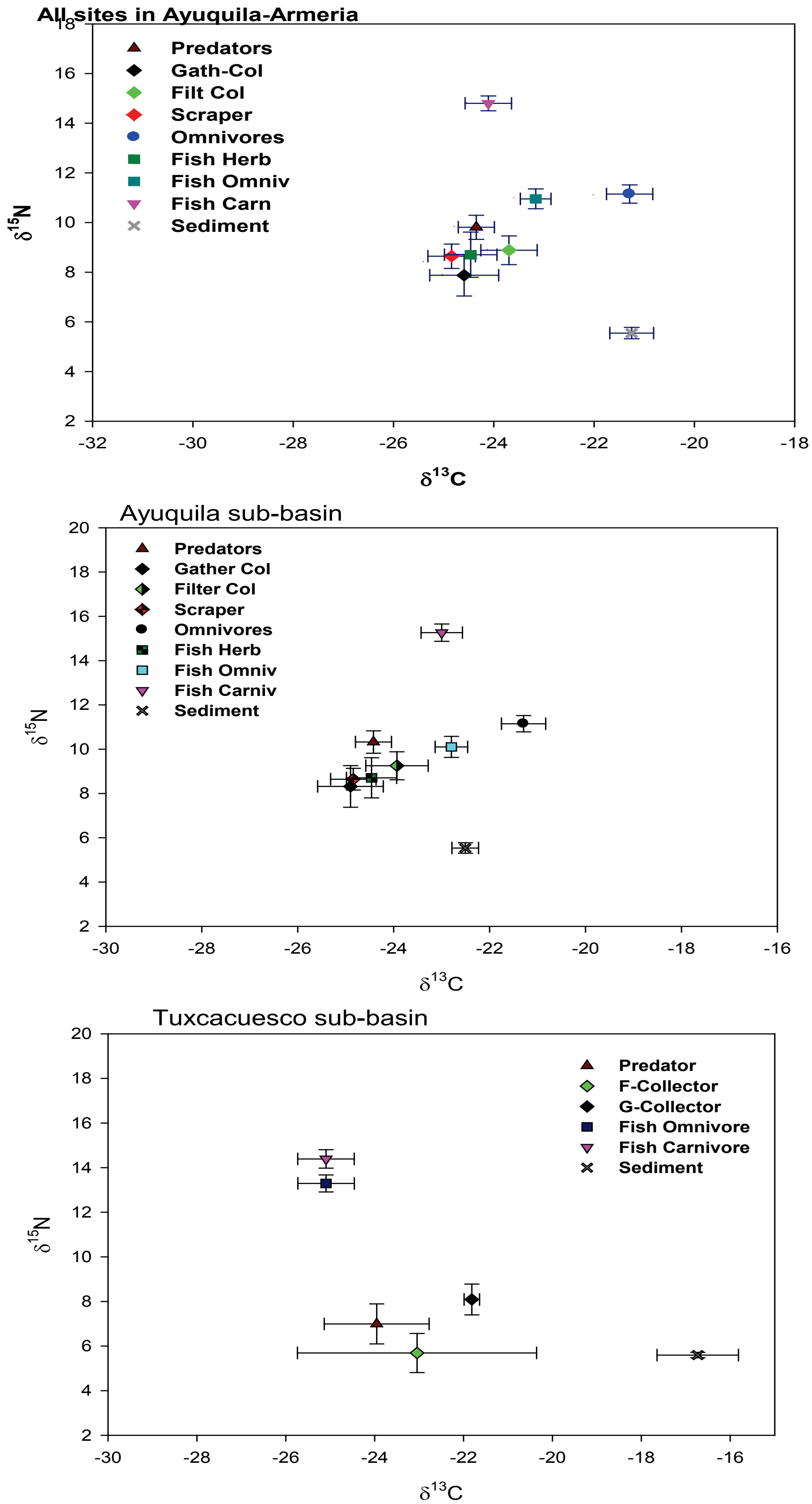


Figure 3.5. Mean (± s.e.) $\delta^{13}C$ and $\delta^{15}N$ biplot arrangement of sediment and faunal samples for wet08 by sub-basin. Differences were statistically significant ($p=0.045$) for faunal N values. a) all sites considered; b) Ayuquila sub-basin; and, c) Tuxcacuesco sub-basin.

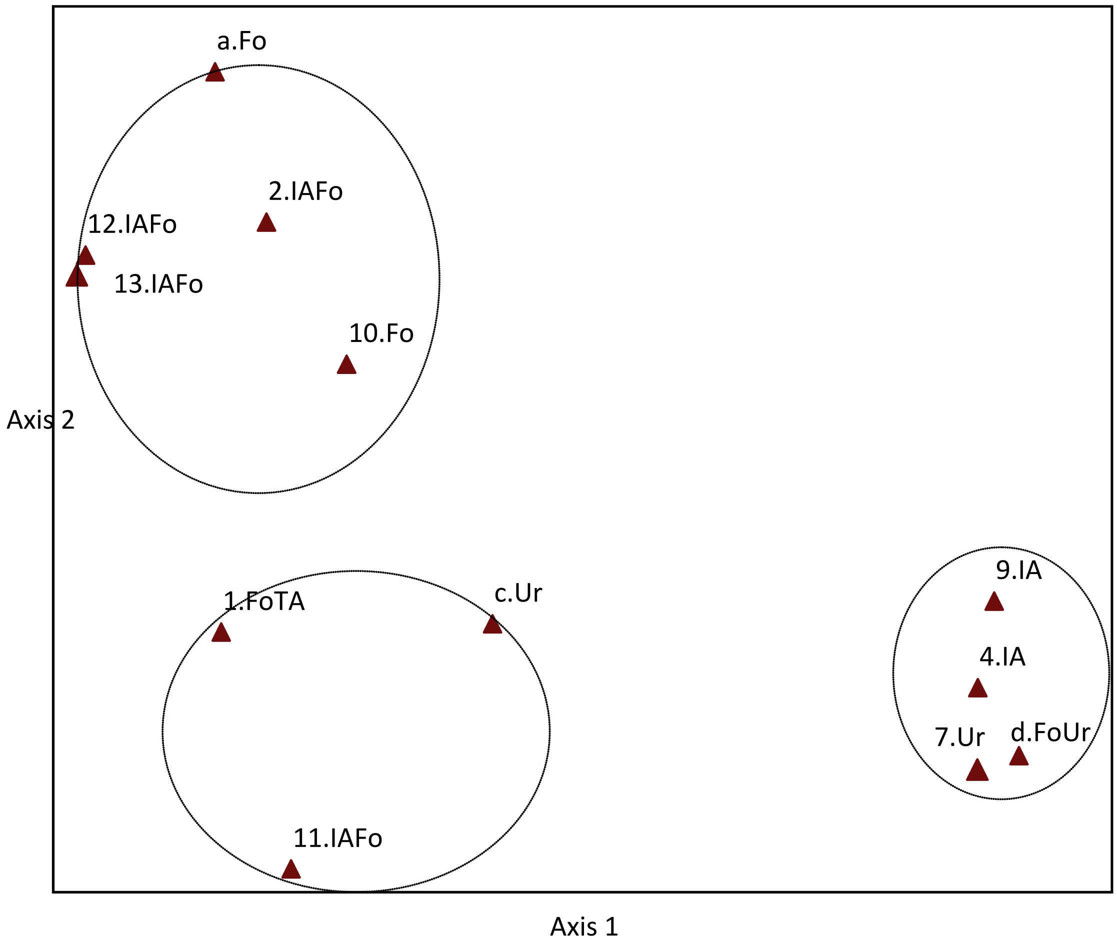


Figure 3.6. NMDS plot showing 2-dimensional distances among sites according to their sediment and biota's $\delta^{13}C$ values for wet07. Final stress of 6.3307 after 103 iterations.

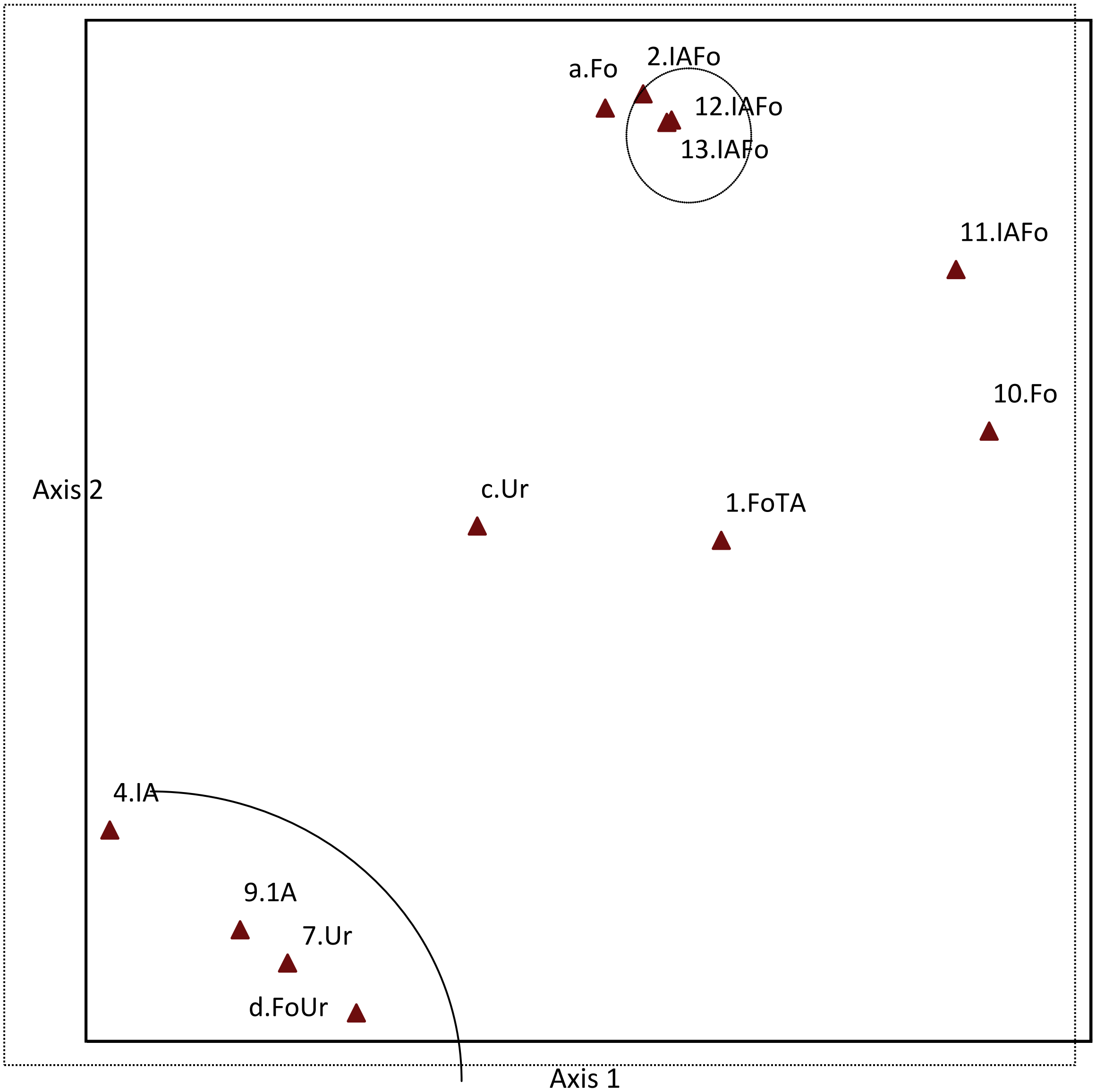


Figure 3.7. NMDS plot showing 2-dimensional distances among sites according to their sediment and biota`s $\delta^{15}N$ values for wet 07.

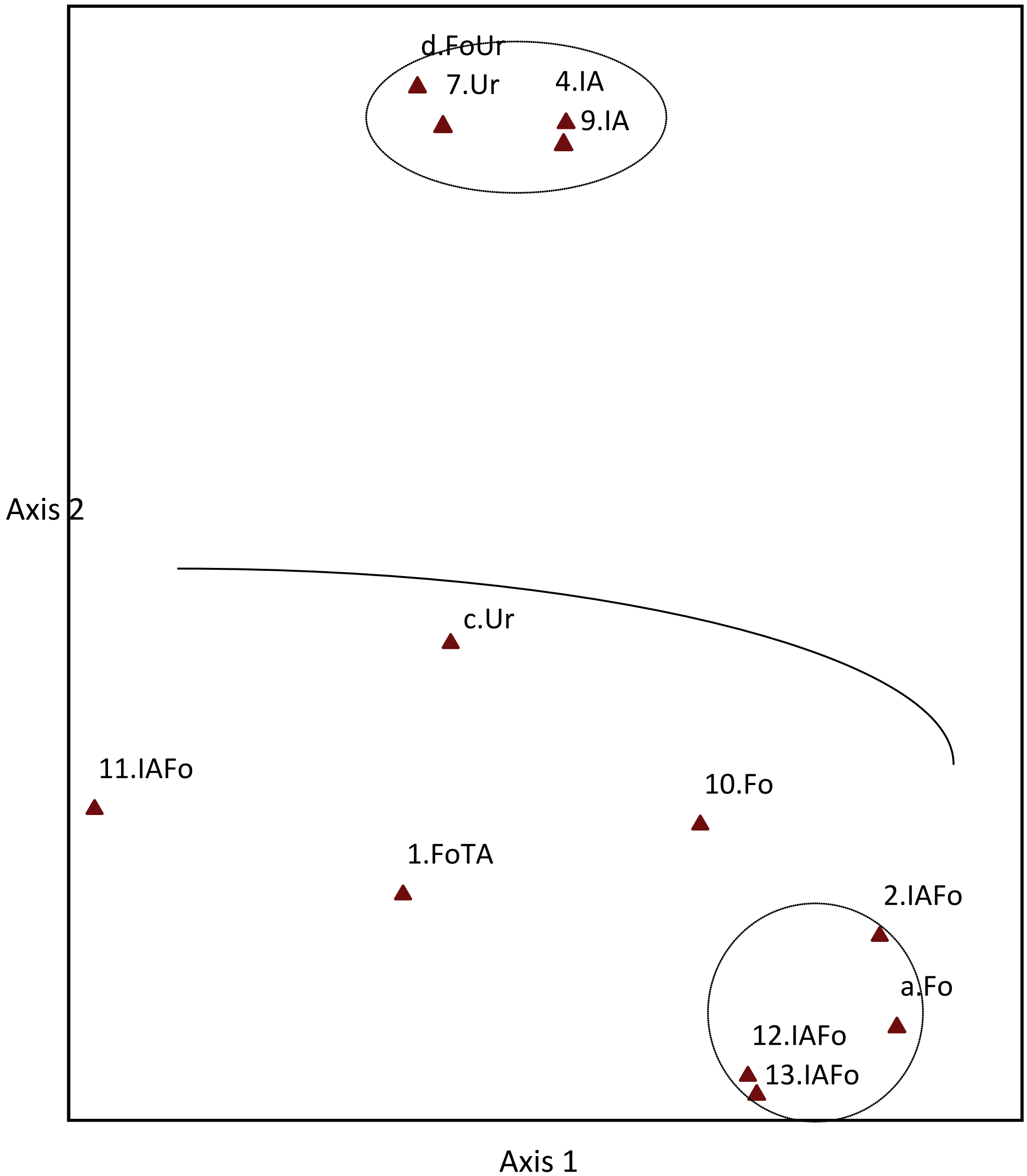


Figure 3.8. NMDS plot showing 2-dimensional distances among sites according to their sediment and biota's $\delta^{15}N$ and $\delta^{13}C$ values for wet07.

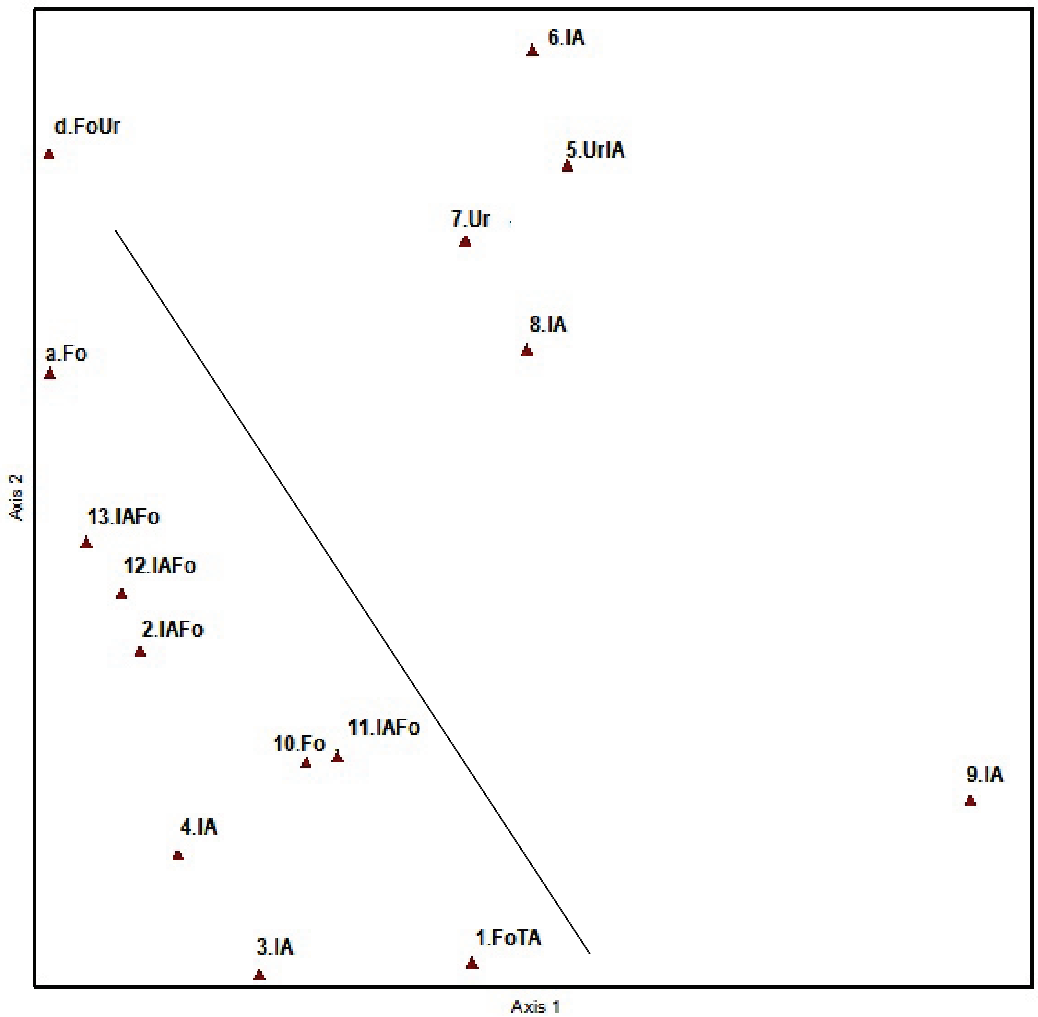


Figure 3.9. PCA plot of the sites according to their FFG and sediment's δ13C values for dry08. The two axes that accounted for 76.2% of the total variation are presented.

Note: A 1-dimensional NMDS ordination graph resulted for sites' arrangement based on δ13C, with a stress value of 25.44 after 37 iterations suggesting that the test performance was poor (McCune and Grace 2002). Thus, a Reciprocal Averaging (RA) test was performed, resulting in a 0.76 total variance. When variance is less than 1, the use of linear ordination methods, such as PCA, is justified; therefore, a PCA analysis was run.

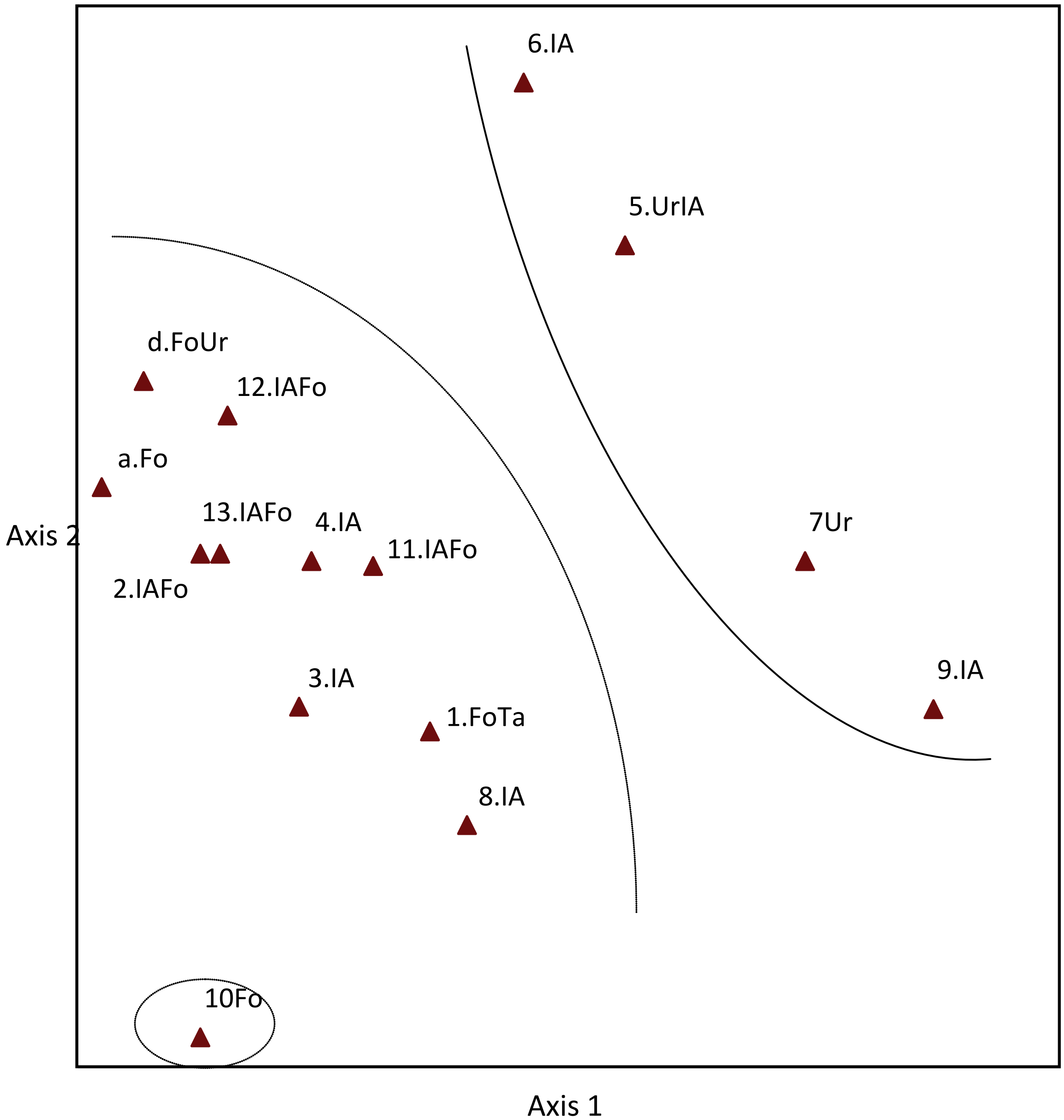


Figure 3.10. NMDS ordination plot showing 2-dimensional distances among sites according to their $\delta^{15}N$ values for dry08.

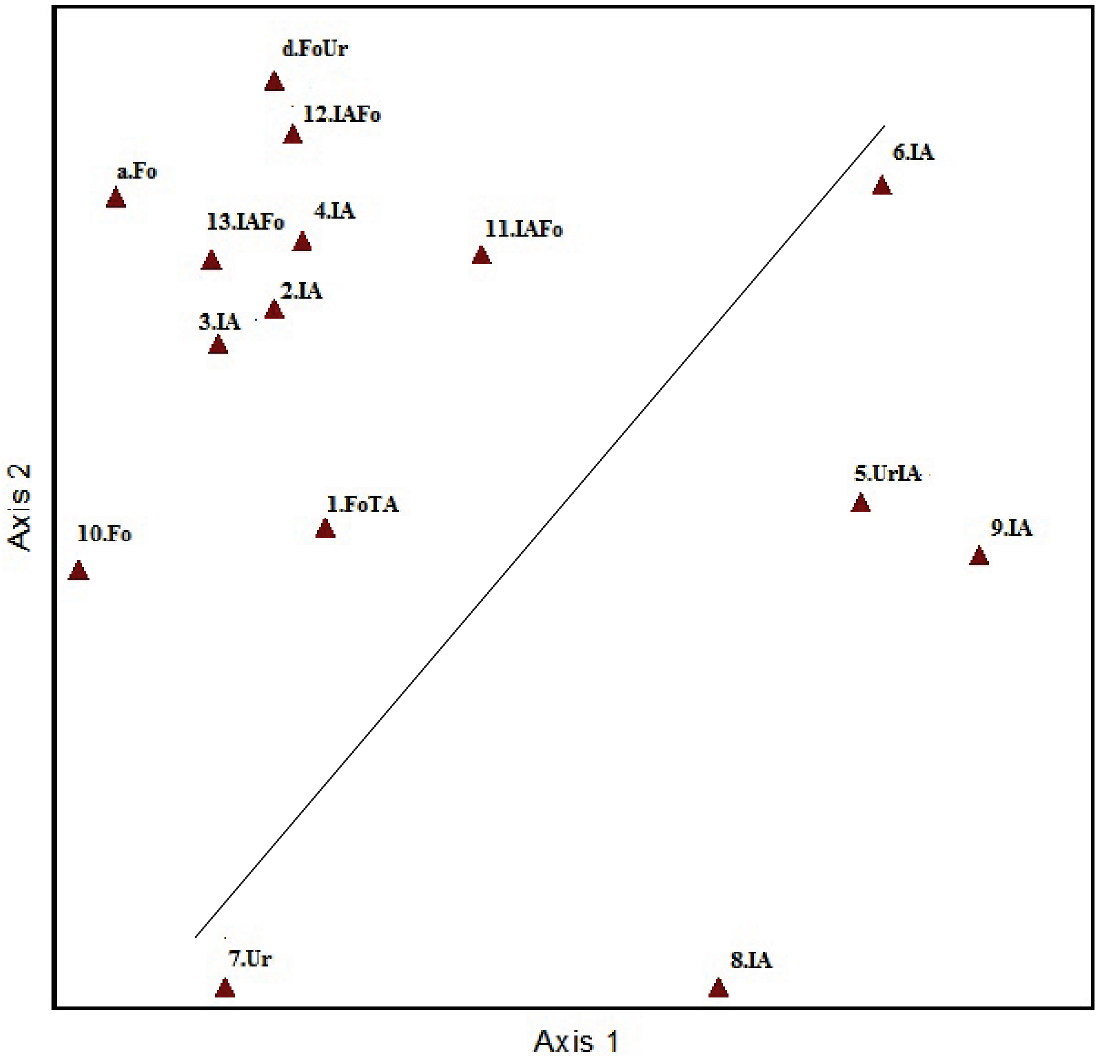


Figure 3.11. NMDS arrangement of the sites according to their sediment and biota's $\delta^{13}C$ and $\delta^{15}N$ values for dry08. Those sites to the right of the dotted line have a higher influence of sewage waste.

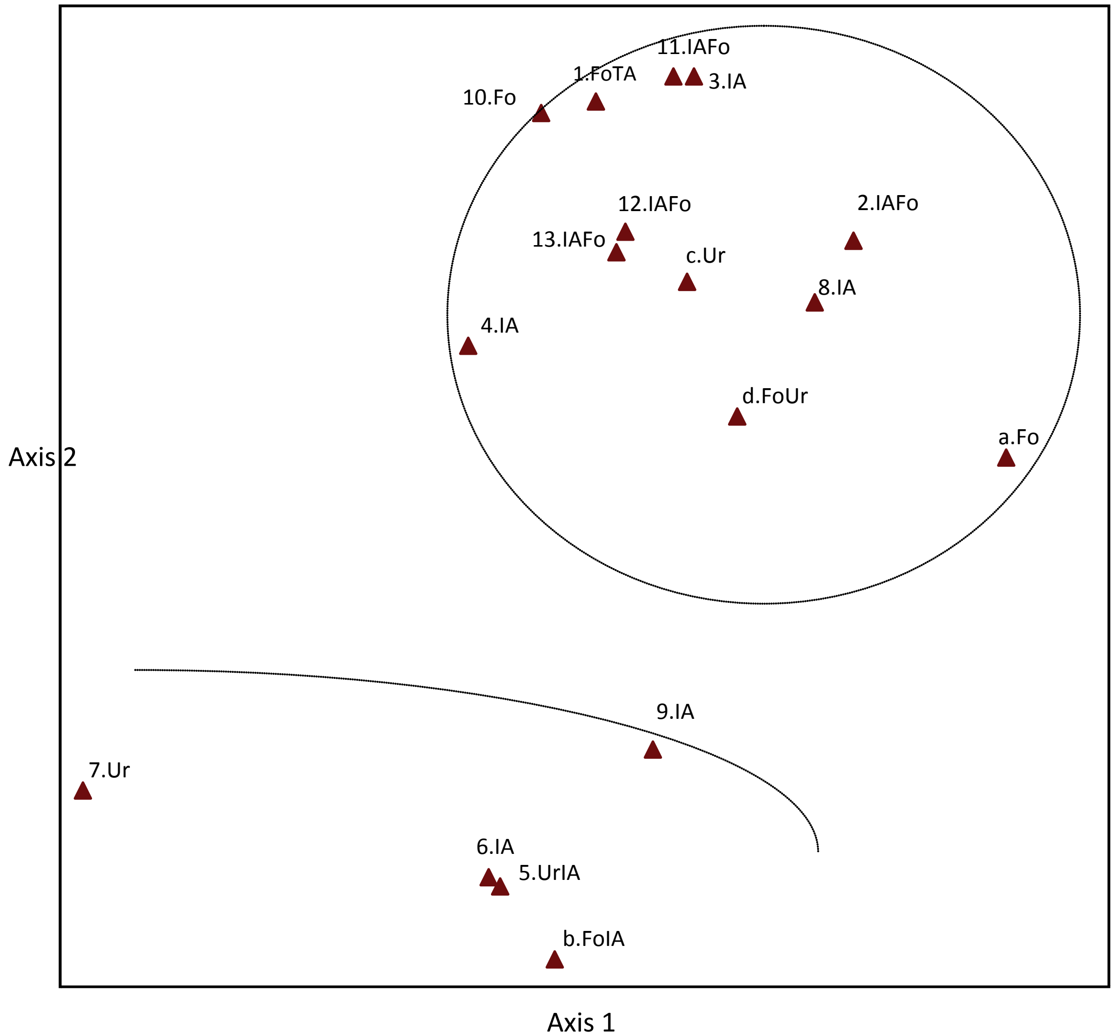


Figure 3.12. 2-dimensional NMDS ordination arrangement of the sites according to their sediment and biota's $\delta^{13}C$ in wet08. Final stress of 8.5355 after 129 iterations

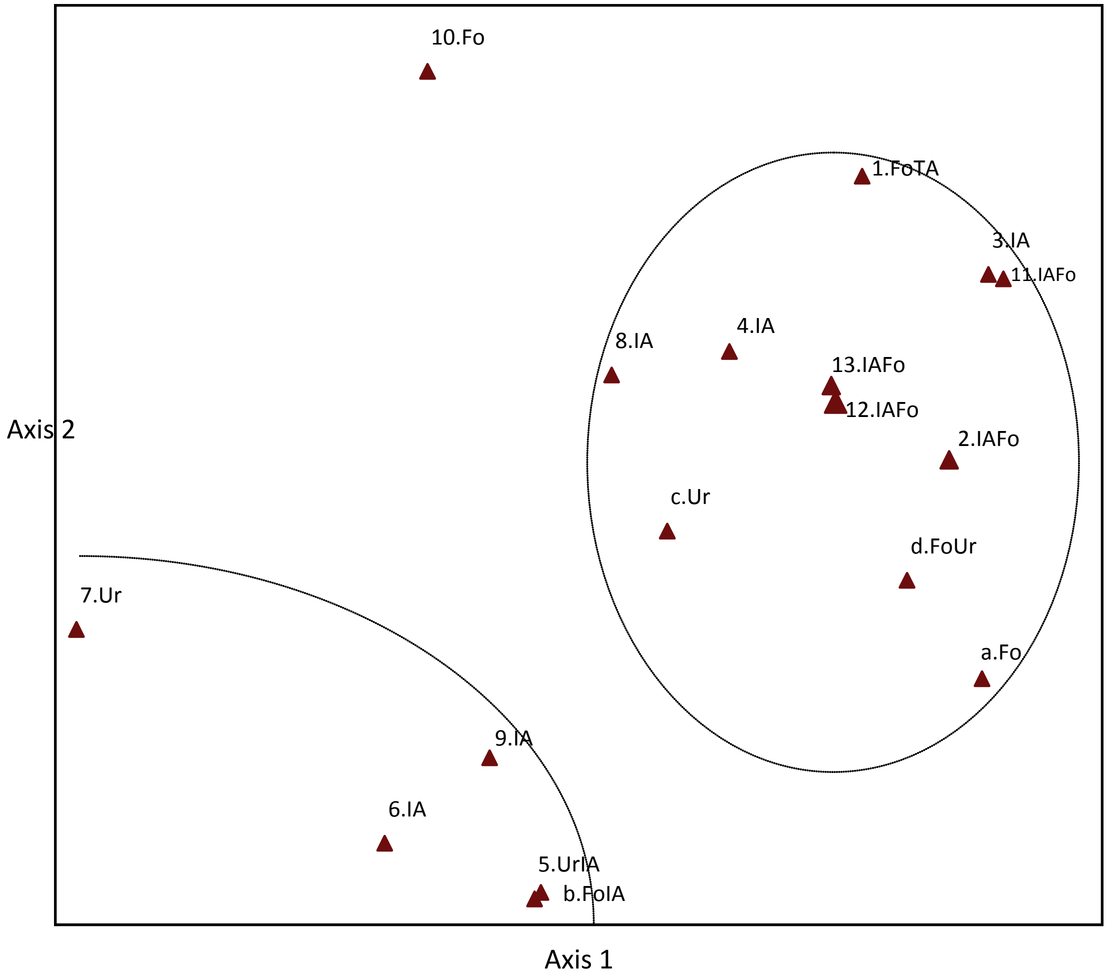


Figure 3.13. NMDS plot of the sites according to their sediment and biota's $\delta^{13}N$ values for wet08

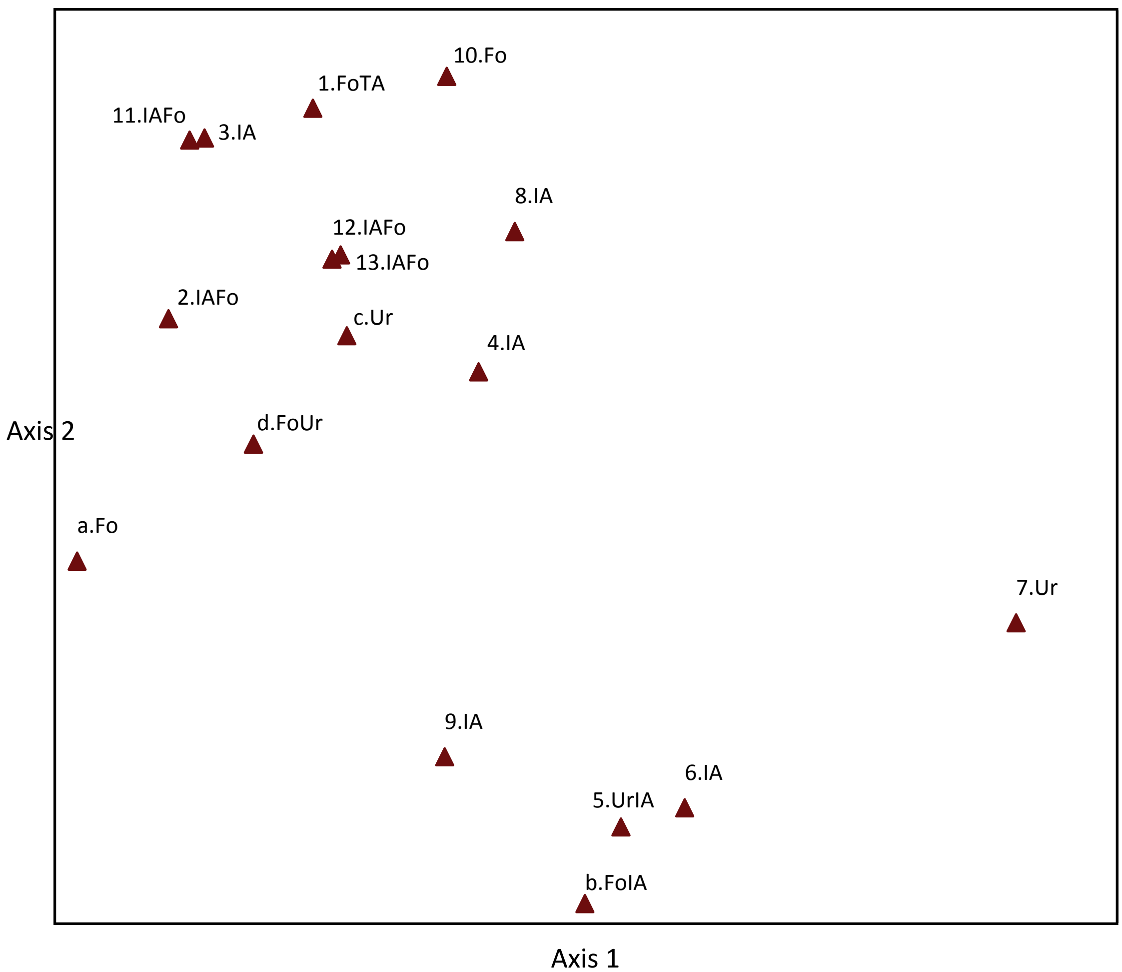


Figure 3.14. NMDS ordination graph of the sites according to their sediment and biota's $\delta^{13}C$ and $\delta^{15}N$ values for wet08

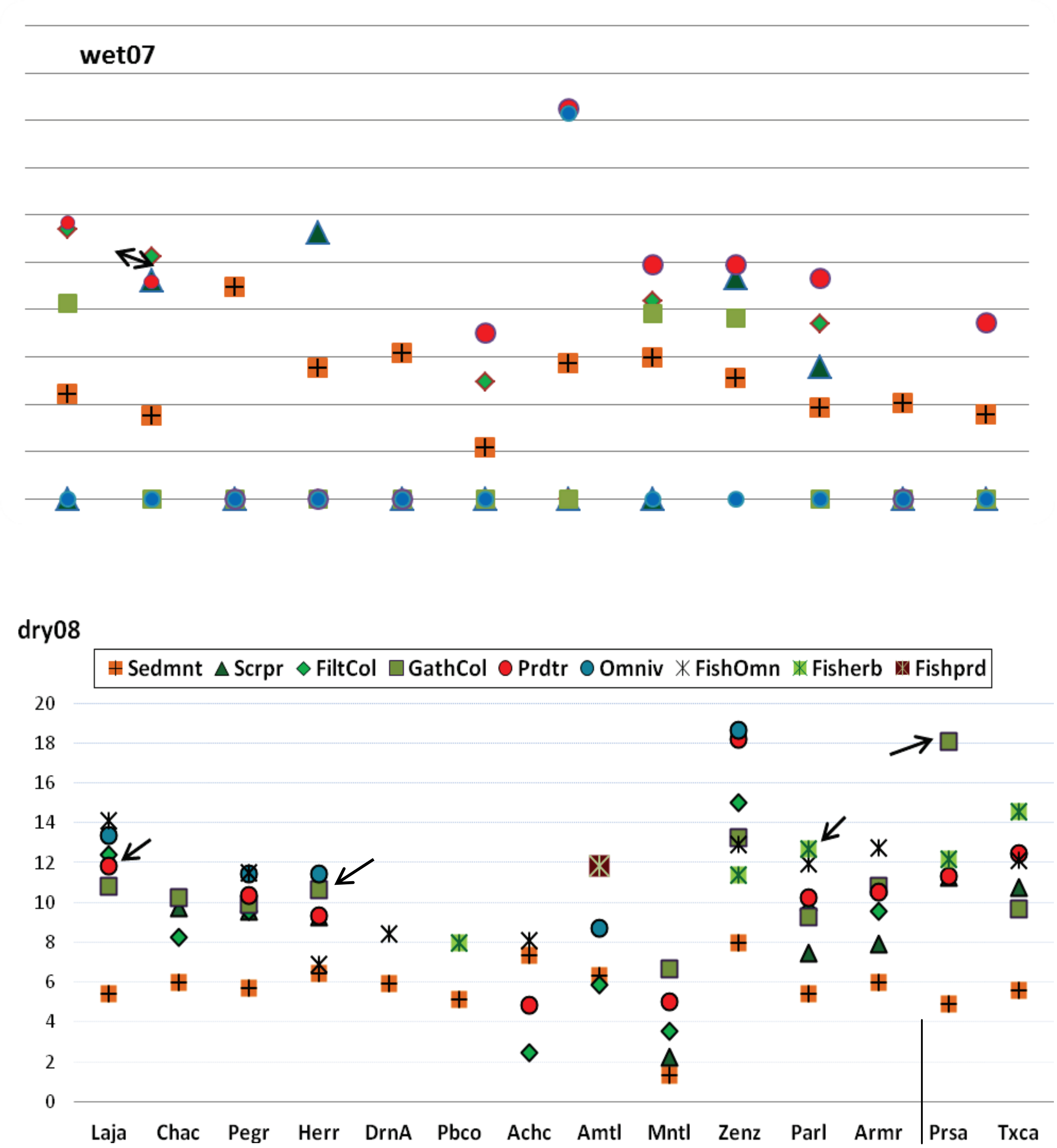


Figure 3.15. Trophic structure arrangement on each site following the Functional feeding group classification for wet07 and dry08. The arrows show those groups whose N values place them above a normal situation. The sites are arranged following the river's flow. The vertical line in the x- axis indicates the division between the Ayuquila sub-basins sites and those from the Tuxcacuesco sub-basin.

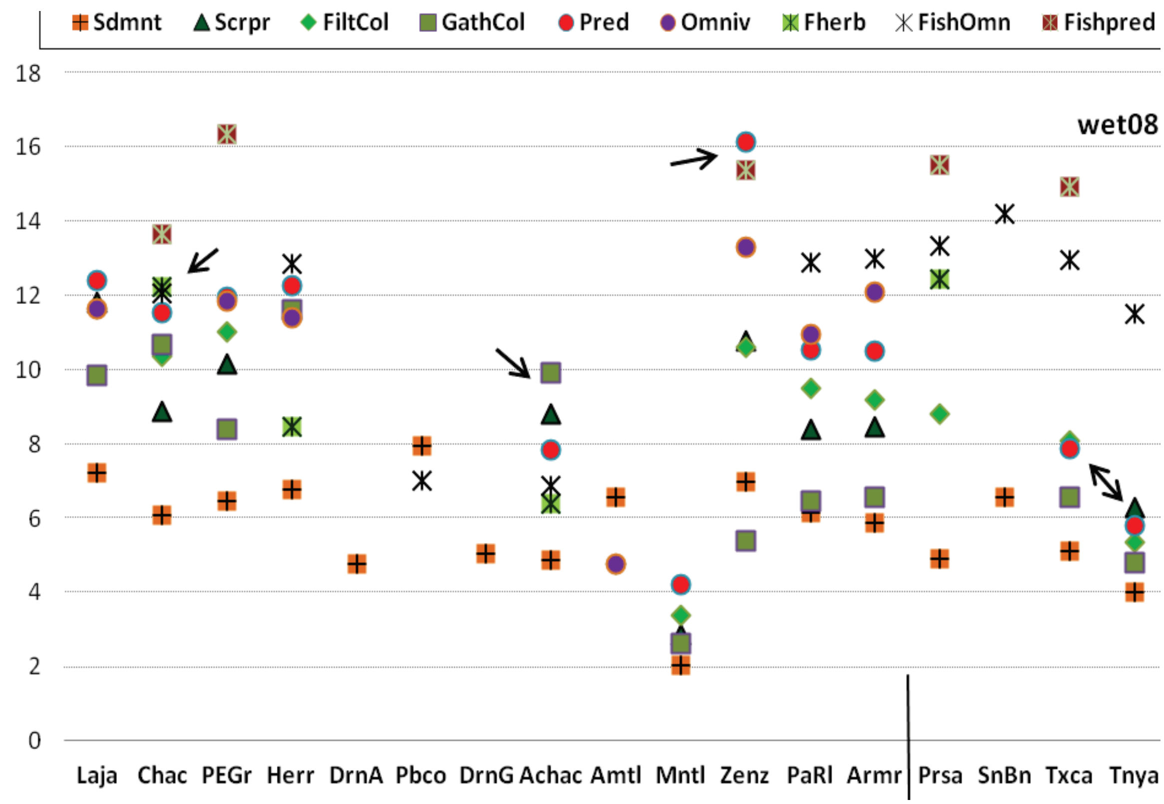


Figure 3.16. Trophic structure arrangement on each site following the Functional feeding group classification for wet08. The arrows show those groups whose N values place them above a normal situation. The sites are arranged following the river's flow. The vertical line in the x- axis indicates the division between the Ayuquila sub-basins sites and those from the Tuxcacuesco sub-basin.

Table 3.2. Mean trophic level estimated from invertebrate functional feeding groups' $\delta^{15}N$ values in different sites in the Ayuquila-Armeria River in wet07. Sediment's $\delta^{15}N$ was considered as the isotopic baseline.

FFG	1FoTA	2.IA	10.Fo	11.FoTA	12.Fo	13.Ta	a.Fo	I.Ur
Omnivore				**5.21**				
Predator	**3.9**	**3.2**	**2.9**	**5.2**	**2.6**	**2.9**	**3.1**	**2.5**
Gather	**2.5**				**1.6**	**2.0**		
Scraper		**3.3**			**2.0**	**2.7**	**1.7**	
Filter		**3.6**	**2.1**		**2.2**	**2.9**	**2.4**	
Sediment	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**

Table 3.3. Trophic level (TL) of each invertebrate functional group in each site sampled in the Ayuquila-Armeria River in wet07 according to its $\delta^{15}N$ value shown in Table 3.2. Bold figures show FFGs that would not correspond to that TL.

TL	1.FoTa	2.IAFo	10.Fo	11.IAFo	12.IAFo	a.Fo	c.Ur	13.IAFo
5-6				Omnv Pred				
4-5								
3-4	Pred	**FCol** Scrp Pred				Pred		
2-3	GCol		Pred FCol		Pred FCol Scrp	FCol	Pred	Pred FCol Scrp GCol
1-2					GCol	Scrp		
0	Sdmnt	Sdmnt	Sdmnt	Sdmnt	Sdmnt	Sdmnt	Sdmnt	Sdmnt

Table 3.4. Mean trophic level estimated from invertebrate and fish functional feeding groups' $\delta^{15}N$ values in different sites in the Ayuquila-Armeria River in dry08. Sediment's $\delta^{15}N$ was considered as the isotopic baseline.

FFG	1.FoTA (Laja)	2.IAFo (Chac)	3.IA (Pegr)	4.IA (Herr)	5.UrIA (DrnA)	6.IA (Pbco)	8.IA (Achc)	9.IA (Amtl)	10.Fo (Mntl)	11.IAFo (Zenz)	12.IAFo (Parl)	a.Fo (Prsa)	d.FoUr (Txca)	13.TA (Armr)
FsPrd								**3.18**						
FsOmn		**4.20**		**2.99**	**1.38**	**2.30**				**2.35**	**3.96**	**4.02**	**3.57**	**3.12**
FsHrb						**2.11**					**3.86**		**4.52**	
Omn	**4.12**		**3.25**	**2.97**				**1.71**		**5.21**				
Pred	**3.52**	**1.88**	**2.81**	**2.15**			**0.02**		**2.45**	**3.83**	**2.90**	**3.51**	**3.69**	**2.79**
Scrp	**3.12**	**2.68**	**2.65**	**2.64**					**3.10**	**3.09**	**2.51**	**6.18**	**2.60**	**2.89**
GCol		**2.09**	**2.52**	**2.12**					**1.35**		**1.81**	**3.50**	**3.02**	**1.76**
FCol	**3.74**	**1.90**	**2.51**	**2.65**			**-0.92**	**0.82**	**1.85**	**3.57**	**2.66**			**2.40**
Sdmnt	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**

Table 3.5. Trophic level (TL) of each invertebrate and fish functional group in each site sampled in the Ayuquila-Armeria River in dry08 according to its $\delta^{15}N$ value shown in Table 3.4.. Bold figures show FFGs that would not correspond to that TL.

TL	1.FoTA	2.IAFo	3.IA	4.IA	5.UrIA	6.IA	8.IA	9.IA	10.Fo	11.IAFo	12.IAFo	a.Fo	d.FoUr	13.IAFo
6												**GathCol**		
5-6										Omnv				
4-5	Omnv	FshOmn										FshOmn	Fsherb	
3-4	**FiltCol** Pred GathCol		Omnv						**GathCol**	Pred FiltCol Scrpr	FshOmn Fsherb	Predtr Scrpr	Predtr **FshOmn** Scrpr	FshOmn
2-3		**GathCol** **Scrpr**	Pred GathCol Scrpr FiltCol	FishOmn Omnv FiltCol GathCol **Pred** Scrpr		FishOmn FishHerb		FishPred	Pred	**FshOmn**	Pred FiltCol GathCol		GathCol	**GathCol** Pred FiltCol
1-2		FiltCol Pred			FishOmn		Pred FiltCol*	Omnv FiltCol	FiltCol Scrpr					Scrpr
0	Sedmnt	Sedmnt	Sedmnt	Sedmnt	Sedmnt	Sedmnt	Sedmnt	Sedmnt	Sedmnt	Sedmnt	Sedmnt	Sedmnt	Sedmnt	Sedmnt

Table 3.6. Mean trophic level estimated from invertebrate and fish functional groups' $\delta^{15}N$ values in different sites in the Ayuquila-Armeria River in **wet08**. Sediment's $\delta^{15}N$ was considered as the isotopic baseline.

FFG	1.FoTA (Laja)	2.IAFo (Chac)	3.IA (Pegr)	4.IA (Herr)	6.IA (Pbco)	8.IA (Achc)	9.IA (Amtl)	10.Fo (Mntl)	11.IAFo (Zenz)	12. IAFo (Parl)	a.Fo (Prsa)	b.IA (SnBn)	c. Ur (Tnya)	d.FoUr (Txca)	13. TA (Armr)
FsPrd		3.99	4.89						4.31		5.18			4.86	
FsOmn		3.36		3.39	0.64	1.79				3.65	4.33	4.01	3.95	4.09	3.82
FsHrb		3.43		1.66		1.59					3.97				
Omn	2.74		3.12	2.82			0.29		3.48	2.9					3.46
Pred	3.05	3.16	3.16	3.16		2.17		1.85	4.60	2.74			1.70	2.09	2.83
Scrp	2.82	2.11	2.46			2.55		1.32	2.49	1.90	6.18		1.89		2.03
GCol	2.04	2.81	1.76	2.89		2.98		1.23	0.37	1.13			1.31	1.57	1.28
FCol	2.74	2.69	2.80				0.29	1.53	2.42	2.33	2.53		1.53	2.17	2.31
Sdmnt	1	1	1	1	1	1	1	1	1	1	1			1	1

Table 3.7. Trophic level (TL) of each invertebrate and fish functional group in each site sampled in the Ayuquila-Armeria River in wet08 according to its $\delta^{15}N$ value shown in Table 3.6.. Bold figures show FFGs that would not correspond to that TL.

TL	1.FoTA	2.IAFo	3.IA	4.IA	6.IA	8.IA	9.IA	10.Fo	11.IAFo	12. IAFo	a.Fo	b.FoIA	c.Ur	d.FoUr	13.IAFo
5-6											FsPrd				
4-5			FsPrd						**Pred** FsPrd		FsOmn	FsOmn		FsPrd FsOmn	
3-4	Pred	FsPrd FishHerb FSOmn Pred	Pred Omn	FSOmn Pred					Omn	FsOmn	FsHrb		FsOmn		FsOmn Omn
2-3	Scrp Omn FCol GCol	**GCol** **FCol** **Scrp**	FCol Scrp GCol	GCol Omn		GCol Scrp Pred			Scrp FCol	Omn Pred FCol	FCol			**FCol** Pred Gcol	Pred FCol Scrp
1-2				FsHrb	FsOmn	FsOmn FsHrb	Omn FCol	Pred FCol Scrp GCol	GCol	Scrp GCol			Scrp Pred FCol GCol		GCol
0	**Sdmnt**	**Sdmnt**	**Sdmnt**	**Sdmnt**	**Sdmnt**	**Sdmnt**	**Sdmnt**	**Sdmnt**	**Sdmnt**	**Sdmnt**	**Sdmnt**	**Sdmnt**	**Sdmnt**	**Sdmnt**	**Sdmnt**

4. Trace Metal Concentrations in the Ayuquila Watershed

Abstract

The specific objectives of this study were to 1) determine the concentrations of the trace metals Cd, Pb, Cu, Zn, and macronutrients Mn and Fe in food webs (sediment, macroinvertebrate and fish) from 17 sites in the Ayuquila-Armeria River in both wet and dry seasons, and 2) to determine if patterns in metal concentrations within each site specific foodweb were season or site dependant where land use activities differed at each site. Metal concentrations in sediments were site and seasonal dependent: Cd, Cu, and Mn had higher concentrations in the dry season, while Zn concentrations were higher in the wet season ($H=126.292$, $p<0.001$) Temporal differences existed in Cd, Cu, Pb, Zn and Fe concentrations from different functional groups. Metal concentrations decreased as trophic level increased. Invertebrates and fish with higher metal concentrations were from the agricultural valley, Mean Cd, Pb, Cu and Zn concentrations in invertebrate and fish tissues were above the thresholds established as safe by the Mexican government for the protection of aquatic wildlife and human consumption. Concentrations of Cd, Cu, Pb, and Mn in sediment and functional groups were associated to urban and mine runoff. This study provides a baseline for metals in the Ayuquila-Armeria river ecosystem which will allow for an evaluation of different management activities and unregulated mine extraction now proliferating in the region.

4.1. Introduction

Metals are natural elements many of which are physiologically essential for life. At the same time, human history and social development are associated with the use of metals. The extraction of metals from their ores and transformation into tools is considered one of the oldest applied sciences and the catalyst of technological advancement (Nriagu 1996; Hong *et al.* 1996). Records from human-caused pollution resulting from the production of copper can be traced back to the Roman and Chinese empires (Hong *et al.* 1996; Rhind 2009). Metal pollution developed into a global environmental problem since the Industrial Revolution when the amounts of metals released have increased steadily and exponentially, making metal toxicity surpass the toxicity of radioactive and organic material pollution combined (Nriagu and Pacyna 1988; Nriagu 1996). There are 80 elements classified as metals of which 30 have been reported to be toxic to humans (Hollengberg 2010).

Trace metals is the term used to identify those elements present in living organisms in limited amounts, which include those known as essentials (e.g., Fe, Mg, Mn, Co, Cu, Cr(III) and Zn) in addition to those that have no known metabolic function, such as Cd, Hg, different forms of Cr, Pb, among others (Viarengo 1985). Source and fate of metals in the environment are well documented, all of which agree that, aside from natural deposition and rock weathering, metals derived from industrial processes, mining, urban runoff and agricultural practices constitute one of the five major types of toxic pollutants (Mason 2002); twenty-three of these metals, known as heavy metals, constitute a group with higher adverse potential. All metals are potentially toxic: “the dose makes the poison”. However, toxic thresholds between essential metals, like Mn and Fe, and those that are toxic at low concentrations have many levels of difference. For example, the concentration level in sediment below which no harmful effects to organisms are expected for Cd is 0.99 mg/kg dry weight (MacDonald *et al.* 2000), while that in Fe is 20,000 mg/kg dw (US-EPA 2012).

Unlike organic chemicals and other pollutants, metals do not degrade and tend to accumulate in sediments and soils, and can be transferred and magnified through food webs (Dudka and Adriano 1997; Croteau *et al.* 2005). Therefore, although metals are essential in biological -and industrial- processes, their present concentrations in the environment as a result of human activities constitute a constant hazard to an ecosystem's overall health (Dudka and Adriano 1997; Rhind 2009).

Freshwater and marine environments have been long studied in relation to fate and effect of anthropogenic metals within these aquatic ecosystems. These systems receive the direct and indirect depositions from natural and human surrounding activities, exceeding by far the amounts of trace metals released into the atmosphere (Nriagu and Pacyna 1988). Metal pollution in streams is related to mine extraction, industrial and domestic sewage, lixiviation of waste material, agricultural and urban runoff, deforestation, and atmospheric deposition (Richardson and Kiffney 2000; Adriano 2001). Metals behave differently once they enter an aqueous medium; some remain in the water column, but most of them are transported by colloids to be absorbed by periphyton and sediment, or bind directly to sediments, so the concentrations in the water column are usually lower than those in the sediment (Luoma 1983). It is here where metals are ingested by macroinvertebrates or fish and then transferred up in the food chain (Farag *et al.* 2007). Metal availability depends largely on the chemical and physical characteristics of the environment, such as temperature, pH, water hardness and salinity, among others (Breder 1982; Luoma 1983, van Griethuysen *et al.* 2004).

Metals affect individual organisms and the overall ecosystem in different ways with fluctuating toxic level thresholds (Goodyear and McNeill 1998; Adriano 2001). Some aquatic invertebrates are known to tolerate or even avoid metals better than others due to their capacity of producing metal-binding proteins (Rainbow 2002; Kiffney and Clements 2003; Lefcort *et al.* 2004), the food they prey upon (Reinfelder and Fisher 1991), or the organ in which the metal is stored (Hare *et al.* 2003). Despite the fact that some plant species have been used to remove toxic contaminants from the environment, known as phytoremediation (Lasat 2002), essential metals (e.g. Cu, Fe, Mn and Zn) and non-essential ones (e.g. As, Cd, and Pb) affect plant physiology if assimilated in high levels (Dudka and Miller 1999; He *et al.* 2005; Smical *et al.* 2008). In contrast, these same metals vary highly in invertebrates, posing contrasting toxic effects even in organisms from a same taxon (Adriano 2001; Rainbow 2002), or humans of growing age (Goyer 1995; Brewer 2010). Trace metals in terrestrial ecosystems usually accumulate in soils, where they are transferred to plants, microorganisms, animals and humans (Dudka and Miller 1999). In aquatic ecosystems, the transference usually starts in sediments, but metals in the water column and food items can also be transferred through food webs, and in some cases, tend to bioaccumulate in larger organisms (Hare *et al.* 2003; Croteau *et al.* 2005). In spite of the relevance sediment has for river macroinvertebrate communities, many biological indices focus on testing water quality disregarding the role sediment has as a source of metals and other pollutants (Adams et al. 1992). As all metals have applications nowadays, metal pollution is globally widespread and increasing with huge environmental costs despite the fact that many metal applications are being replaced by plastics (Dudka and Adriano 1997; Hare *et al.* 2003). However, more than the direct effect metal pollution has on individual organisms and species, which can be quite variable within and between sites, the consequences it has in disrupting ecosystem processes may be of greater importance (Kiffney and Clements 2003).

Distribution and concentration of metals in aquatic ecosystems varies greatly in time and space (e.g., Smith *et al.* 1996; Rainbow 2002, Tulonen *et al.* 2006; Kpee *et al.* 2009), as water's pH, alkalinity, temperature, and organic content, among many other environmental variables, as well as those caused by humans like wastewater discharge and irrigation (e.g. Luoma 1983; Iwashita and Shimamura 2003; Nicolau *et al.* 2006) influence the amount of trace metals in the water column and bed sediments. These concentration variations can even be daily (Brick and Moore 1996), and are strongly related to the sediment's grain size (Huang and Lin 2003; Cenci *et al.* 2004), the floodplain width and a river's flow hydraulics (Wyzga and Ciszewski 2010), as well as the presence of organic material (Charriau *et al.* 2011).

As with sediments metal bioavailability and uptake by organisms is influenced by several environmental factors, age, size, physiological strategies and seasonal conditions (Hinch and Stephenson 1987; Phillips and Rainbow 1989; Metcalfe-Smith *et al.* 1996). Nonetheless, the presence of metals in the environment does not mean that these are bio-available. Substantial differences were noted when comparing metal concentrations by functional feeding groups, as water chemistry, type of substrate, pH, hardness, and temperature, among other environmental variables (Luoma 1983; Goodyear and McNeill 1999; Anderson *et al.* 2004), while individual differences such as variation in the diet within a feeding guild, age and size of the specimens (Timmermans *et al.* 1989; Goodyear and McNeill 1999) also influence the uptake by organisms.

Despite the fact that literature abounds with information about the temporal and spatial variability of metal concentrations in aquatic systems, there is no consensus on the effects these have on organisms (Mayer-Pinto et al. 2010). However, there is still a need to continue looking for evidence in different geographical and temporal scenarios about the fate and effects metals have in the environment in order to aid in conservation and management actions (Mayer-Pinto et al. 2010).

4.1.1. Aim of this study

The Ayuquila-Armeria River is considered the second most important river due to its biodiversity and the third largest in drainage area in the state of Jalisco, Mexico. As such, it is important as a water source for agricultural activities and human consumption. However, the use of agrochemicals, the increasing number of animal enclosures, and the lack of water treatment facilities that could reduce the amount of urban and industrial pollutants, cause the point and diffuse runoff of several organic and inorganic pollutants into the river. For almost two decades, water quality monitoring in the watershed has focused in detecting sources of organic pollution and its impact in the fish and invertebrate communities' composition through the use of biotic indices. Even so, research on the presence of other contaminants is scarce and has focused mainly on detecting heavy metals in the water column (e.g. Medina-Pineda 2002; Martinez *et al.* 2007, 2008), without looking at the possible impacts these might have in the system's biological components.

The present study is part of a larger project that aims to assess the influence land use has in the river's biota in this part of western Mexico. As such, I aim to include in the watershed's monitoring program the analysis of metals in the sediment to have a better idea of the river's conditions and the possible effects these pollutants have in the river's biological structure. I wanted to test the hypothesis that metal concentrations within the river food webs were independent of surrounding land use activities. That is, I consider that those sites in areas surrounded by intensive agriculture will have higher concentrations of particular metals associated with agrochemicals, such as Cd, Zn or Cr, while those that receive urban-derived effluents will be higher in Cd and Pb. To test this hypothesis I determined concentrations of six metals from 17 locations along the river and related these concentrations to land use activities. The sites selected are part of the water quality program, and are known for particular point and diffuse pollution, in addition to being located adjacent to different land use activities.

Specific objectives were to 1) determine the concentrations of the trace metals Cd, Pb, Cu, Zn and macronutrients Mn and Fe in food webs (sediment, macroinvertebrate and fish); and 2) to determine if patterns in metal concentrations within each specific food web were season or site dependant where land use activities differed at each of the sites in the Ayuquila-Armeria River. That is, I would expect higher concentrations of non-essential metals (Cd, Pb) and Cu in areas adjacent to agriculture or urban runoff, in addition to higher concentrations of metals in those invertebrates closer to the sediment, such as the gatherer collectors. The goal is to incorporate these findings, as well as the methodology, into the river's on-going monitoring program. By indentifying those sites that require more attention, remediation activities could be proposed to better manage and improve the river system's conditions.

4.2. Methods

4.2.1. Sample Preparation and Metal Analyses

Samples were obtained from sites located in the middle region of the Ayuquila-Armeria watershed (Figure 1.1.) in the state of Jalisco, Mexico, in August 2007, February 2008, and August 2008. Twelve sites were chosen in the Ayuquila River, four in the Tuxcacuesco sub-basin, and one in the Armeria River, 1 km from where the Ayuquila and Tuxcacuesco Rivers confluence. Each site´s general characteristics are summarized in Table 2.1, while water parameters for each site at the moment of obtaining the biological samples are found in Table 2.2.

At each site three sediment core samples were taken using a 10-cm long and 5 cm radius PVC tube, for an approximate volume of 4700 cm^3. Samples were placed in an ice box and away from direct sunlight, and once in the lab, these were oven-dried at 70^oC for 36 hrs, sieved with a 60μ mesh and stored in plastic Ziploc bags for further analysis. 500 mg to 1 g of clay and silt were digested using 10 ml reverse aqua regia (HNO_3-HCl, 3:1), left overnight (13-15 hrs) to finally boil on a hotplate at 110^oC for 4 hrs. Once at room temperature, the samples were filtered and one ml was extracted and diluted to 10 ml with double distilled water. These 10ml sub-samples were later analyzed for metals.

Macroinvertebrates were collected using a D-frame kick net with a 500μ mesh placed perpendicular to the river flow in different stretches for a total of 6 meters. *Pomacea flagellata* apple snails and *Corbicula fluminea* mussels were picked up by hand. Fish were caught using seine nets along the river banks for a total period of 45 minutes. Animals caught were also placed in plastic bags inside a freezer. Once in the lab, invertebrates were classified into functional feeding groups while fish were separated by species. All samples were oven-dried for 36 hrs at 70^oC. Dorsal muscle fillets were cut off from large fish specimens and placed in Petri dishes to be dried for the same time length and temperature. Small fish were oven-dried whole. Macrofauna's tissues were finely pulverized using a mortar and pestle. (Sub-samples of 150 mg were digested with 10ml environmental grade nitric acid in 25 ml Erlenmeyer flasks placed on hotplates to boil to dryness (~0.5 ml). This residue was diluted with 10 ml distilled de-ionized water to be used for AAS metal analysis. All glassware and digestion tubes were acid washed in 10% nitric acid for at least 24 hours and then rinsed several times with distilled and double distilled water prior to use.

4.2.2. Flame Atomic Spectroscopy Metal Analysis

All flame atomic absorption spectroscopy measurements were performed with a Perkin-Palmer model 100 Atomic Spectrophotometer. Procedural blanks and reference material from the National Research Council Canada (NIST 1566b Oyster Tissue, TORT-2 lobster hepato-pancreas, and MESS-3 marine sediment) were run with the samples as individual samples. Flame-AAS metal analyses were performed for Cd, Pb, Mn, Zn, Fe, and Cu. All metal concentrations are expressed as µg/g dry weight (ppm) of sample.

4.2.3. Data Analysis

Data were compared among sites and seasons, and between basins using one-way ANOVA or Student's t-tests to test for statistical differences, with Tukey or Dunn's post hoc comparison procedures. Spearman rank order correlations were used to assess for significant relationships between metal concentrations in sediments, and between concentrations in macrofauna's tissues and sediment. Simple regressions were done between metal content in fauna's tissues and their nitrogen isotope ratio to verify if metals bioacumulated along the functional feeding groups. Statistical analyses were performed with SigmaPlot (version11.0), and differences between samples were considered statistically significant when $p \leq 0.05$.

Metal concentrations found at each site in each FFG were correlated with their respective $\delta^{15}N$ values, as described in the previous chapter, to relate the metal concentrations with trophic level. Using the food web composition of each site obtained and presented in a previous chapter, I compared the metal concentrations of each FFG within and between sites.

Finally, the metal concentrations obtained in each site's food web were compared with the maximum water metal concentrations permitted by the Mexican government to protect wetlands and aquatic wildlife (NOM-001-ECOL-1996), and the Sanitary Specifications and maximum permissible limits of contaminants for products obtained from Fisheries: for fresh and frozen fish (NOM-027-SSA1-1993), crustaceans (NOM-029-SSA1-1993), and shellfish (NOM-031-SSA1-1993) (DOF 1995a, 1995b, 1995c, 1996). In matter of heavy metals, these three guidelines establish the same maximum limits for Cd, Hg, and Pb, so I will only refer to one in the results. Cd, Cu, Pb and Zn concentrations obtained from sediment, invertebrate filter collectors and omnivorous fish were compared against Mexican guidelines for wildlife protection, while concentrations from *Corbicula* shellfish, crustaceans and fish species known to be eaten by local people were compared with the second set of guidelines.

4.3. Results

4.3.1. Metals in Sediments

Twelve sites were sampled in Wet07, 15 in Dry08, and 16 in Wet08. A total of 157 samples were analyzed for six metals. As mentioned in chapter 1, differences in wetness between dry and rainy seasons are notorious (see Table 1.1). Metals and organic pollutants are washed off from the fields during the rainy period – season in which farmers apply higher doses of herbicides to deter competition from weeds.

Metal concentrations in sediment were highly variable among sites and between seasons and sub-basins. Sediment concentrations per field season are summarized on Table 4.1. Average metal concentrations decreased in the order Fe>Mn>Cu>Pb>Zn>Cd in 2007, and Fe>Mn>Pb>Cu>Zn>Cd in 2008. Metal concentrations were site and season dependent, for the exception of Pb which had statistically significant differences ($H=16.604$ $p<0.001$) only between both wet seasons. A summary of all Anova results for metal comparisons in sediment are shown in Appendix 4.1.

Sediment Cd was the lowest concentration for all metals for the three samplings periods, with amounts ranging from 0.02 to 1.15μg/g dry weight (d.w.) (Table 4.1). On average, concentrations per site and between sub-basins were lower in both wet seasons (around 0.3 μg/g dw) than in dry08 (> 0.45 μg/g dw). One site in particular, the open sewage drain DrnG (site "7.Ur"), had a mean value almost twice the season's average (Figure 4.1a). Temporal and spatial analyses showed lower concentrations in wet08 and higher variability in wet07 (Figure 4.1b). There were statistical significant differences in Cd concentrations in sediments between sites in wet08 ($F_{df15}=1.974$, $p=0.044$), between basins in wet07 ($U_{df1}=128$ $p<0.001$), and between the three sampling seasons ($H_{df2}=32.662$, $p<0.001$), although these were not significant between both wet seasons.

Sediment Pb was on average the second lowest after Cd, with concentrations ranging from 0 to 19.4μg/g in the sampling seasons. One site, (Parl: "12.IAFo") stands out for having the highest mean values of the three seasons (Figure 4.2a). Wet07 had both the highest mean concentrations and variability. This variation was higher in the Ayuquila sub-basin (Figure 4.2b). Statistical significant differences were obtained only when comparing all data among sampling seasons ($H_{df2}=16.604$, $p<0.001$).

Sediment Cu was third in abundance of the six metals analyzed, except for wet08, when it ranked fourth. Likewise, it was in wet08 when mean values and variation were lower when compared with the previous two sampling periods (Table 4.1). Overall concentrations ranged from 0.25 to 14.77 μg g^{-1}, with higher concentrations during dry08; three sites had more than twice the mean amount registered in this field season (Figure 4.3a). The Ayuquila sub-basin showed a wider variation range in the three sampling seasons, but was more remarkable in dry08 (Figure 4.3b). Statistical significant differences were observed for between site comparisons in wet07 (H_{df11}=47.033 $p<0.001$) and wet08 (H_{df15}=37.752 $p<0.002$), as well as between basin comparisons in wet08 (U_{df2}=83 $p<0.001$).

Of the 6 metals, Zn was atypical with wet08 mean values having a 3 and 10-fold increase with respect to the first and second seasons, respectively, with overall concentrations ranging from 0.26 to 14.63µg/g (Table 4.1). Likewise, sediment's mean values from sites sampled in wet07 and dry08 were lower than 3µg/g, in contrast to almost two thirds of the samples in wet08, with values close to or above 6µg/g (Figure 4.4a). The same atypical pattern was observed when comparing sub-basins where the range in variation differed drastically in the third season (Figure 4.4b). Statistical significant differences were observed in between sites comparisons from wet07 (H_{df11}=46.434 $p<0.001$), and among sampling seasons (H_{df2}=126.292 $p<0.001$).

Sediment Mn, the second most abundant metal, ranged from 14.3 to 295.8 µg/g d.w. (Table 4.1). Concentrations were quite variable among seasons, with important amounts in some sites during dry08. The reference site, Laja ("1.FoTA"), presented more than twice the mean concentrations in dry08 and wet08 seasons (Figure 4.5a). Dry08 also had higher mean values and more variation in both sub-basins when compared with the other seasons (Figure 4.5b). Differences were statistically significant between sites (wet07: H_{df11}=62.001 $p<0.001$; dry08: H_{df14}=24.963 $p=0.035$; wet08: H_{df15}=35.179 $p=0.004$), among sampling seasons during the three seasons (H_{df2}=27.843 $p<0.001$), and between basins only in wet07 (U_{df1}=100, $p<0.001$).

Sediment Fe ranged from 212.4µg/g to 1 mg/g, with mean values around 500µg/g for the three field seasons (Table 4.1). Variation between concentrations was more evident in both wet seasons, while most sites in the dry08 had similar mean values and small dispersion ranges (Figure 4.6b). Differences in mean values were statistically significant between sites (H_{df11}=54.807 $p<0.001$) and between basins (Student's t_{df70}=4.512 $p<0.001$) only in wet07.

Associations between the concentrations of metals were evident but these differed on each season and were not conclusive or statistically significant. For example, Zn had significant positive correlations with all metals but Cd, while Pb was correlated with Cu and Mn in the dry08 but not in the other two field seasons (Table 4.2).

4.3.2. Metal Concentrations in Invertebrate and Fish Functional Feeding Groups

Only samples of faunal tissues from dry08 and wet08 were analyzed for metals. As it occurred with sediments, evidence of the six metals was found in both invertebrate and fish samples, with higher concentrations for most metals in wet08 (Figure 4.7). Average metal concentrations from all sites and FFG taken as a whole decreased as Fe>Mn>Zn>Cu>Pb>Cd. However, metal concentrations varied with the functional group, the site and the season, and each metal behaved differently. Between season comparisons were significant for Cd (t_{df81}=10.559 p<0.001), Pb (t_{df81}=6.29 p<0.001), Cu (t_{df73}=3.463 p<0.001), and Fe (Wilcoxon ranked test Z=2.666, p=0.004). Similarly, when metal concentrations were compared between basins for both sampling seasons, statistical significant differences were found only during the dry08 season for Fe (F=9.997, p=0.002) and Zn (F=8.999, p=0.004).

In general, metal concentrations decreased as trophic level increased (Figures 4.7). No strong correlations were seen in either field season between each FFG metal concentrations, nor between the metal concentrations and the trophic position, seen this as a higher $\delta^{15}N$ value. Moreover, these regressions were not statistically significant. Those correlations which were positive in the dry season (Figure 4.8) were negative in the wet season (Figure 4.9), except for Fe which was negative in both seasons.

In addition, regression analyses between metal concentrations in FFG against the corresponding metal concentrations in sediments presented very low r^2 values, suggesting no or little relationship between these variables.

Statistics presenting mean metal concentrations (±s.e.) and value ranges for each FFG are summarized for dry08 and wet08 seasons in Table 4.3. Appendices 4.2 and 4.3 summarize show these data for by site for both seasons.

Cadmium concentrations

In contrast to sediment Cd where higher concentrations were evident in dry08, Cd mean concentrations in FFG were 2 to 3 times higher in the wet08 season than those obtained from dry08 with scrapers, filter collectors and invertebrate predators having the highest concentrations in wet08 (Figure 4.7 Cd). Mean Cd concentrations in gatherer collectors, scrapers, omnivores, and predators were higher than those from fish. Differences in mean concentrations were statistically significant between both field seasons only for fish omnivores ($U=84$ $p=0.015$) and fish predators ($t_{df12}=-2.748$, $p=0.018$). During dry08, all these groups but predators surpassed the Mexican limits established to protect aquatic wildlife in six sites, with omnivore fishes joining the group in two additional sites. In wet08 mostly invertebrate predators, filter collectors and scrapers were above these limits (Figure 4.10). Seven sites in the wet08 season had concentrations above the 0.5 mg/l limit for animals that are consumed by humans, compared to two in dry08. In one of these sites, the forested reference Mntl ("10.Fo") site, scrapers and filter collector concentrations were higher even from those sites with high disturbance, such as Armr ("13.IAFo"). However, except for the fact that fish had lower concentrations than invertebrates, no consistent or significant patterns were discernible per site.

Lead concentrations

Pb mean values were also higher in wet08 in most FFG except for fish predators which had similar concentrations in both seasons (Figure 4.7 Pb). Predators had the widest variation (281μg/g) in wet08 while fish predators the smallest (0.60μg/g) in dry08. Significant differences in the mean values between seasons were obtained for filter collectors ($U=11$, $p=0.002$), predators ($U=132$, $p=0.01$), and fish omnivores ($U=34$, $p=<0.001$).

Pb concentrations were also not consistent among FFG or between sites. FFG concentrations in both seasons at all sites (Figure 4.11) were above the 1.0 mg/l maximum limit for species consumed by humans, with omnivores having the highest concentrations in dry08, in particular at sites 1.FoTA, 3.IA, and 11.IAFo.

Filter collectors and omnivore fishes in six additional sites ranked second. In wet08, filter collectors, invertebrate predators, and omnivorous fishes from three sites had the highest concentrations.

Copper concentrations

Cu concentrations were higher in scrapers, omnivores and predators in that order with no clear spatial or seasonal dependence (Figure 4.7 Cu). Median concentrations were statistically significant between seasons only in predators (U=180, p=0.007). Cu concentrations in food webs had a contrasting behavior (Figure 4.12). During the dry season, all FFG, except for omnivore and predator fishes in all sites, had concentrations above the 6 mg/l limit to protect aquatic wildlife, with omnivore invertebrates and filter collectors in four sites presenting the highest mean concentrations. In wet08, only omnivores, scrapers and filter collectors in seven sites were above this limit.

Zinc concentrations

Overall Zn concentrations in the functional groups were not dissimilar between seasons with higher concentrations in dry08; these were only statistically significant for fish herbivores (t_{df10}=2.82, p=0.018). Gatherer collectors, scrapers and predators had the highest concentrations in both seasons (Figure 4.7 Zn).

Zn presented a similar pattern than that of Cu (Figure 4.13). Only sediment concentrations were below the 20 mg/l limits for the protection of wildlife in all sites, while filter collectors and predators in three sites total were above this range in wet08. No distinct pattern could be established in the concentrations among trophic levels.

Manganese concentrations

Manganese concentrations among FFGs were not different between seasons. No statistically significant differences were observed among the FFG's Mn mean values. Gatherer collectors followed by filter collectors had the highest mean concentrations in the dry season, while predators followed by gather and filter collectors were the most manganese loaded in wet08 (Figure 4.7 Mn).

Manganese in trophic webs were higher in wet08 than in dry08 (Figure 4.14). Filter collectors ranked first in most sites in the dry season, in particular at two sites: 1.FoTA and 3.IA, while predators had higher concentrations in wet08 than most other groups, except for filter collectors, again in site 1.FoTA. Toxicity levels were not considered, as manganese is considered toxic at water hardness below 50 mg/l H_2CO_3 (Howe et al. 2004). The Ayuquila River had H_2CO_3 levels above that threshold (Table 2.2).

Iron concentrations

Mean Fe concentrations were the highest for both seasons taken all samples as a whole, with higher values shown in wet08. Filter and gatherer collectors' mean values doubled or tripled those of the other groups during the wet season (Figure 4.7 Fe). Statistically significant differences in the mean values between seasons were evident only for filter collectors ($U=33$, $p=0.006$) and fish omnivores ($U=100$, $p=0.032$).

Filter and gatherer collectors had the highest Fe concentrations on average for both seasons in their respective foodwebs (Figure 4.15). Sites 1.FoTA, 11.IAFo in dry08, and 9.IA and 12.IAFo during wet08 were the sites with higher Fe concentrations. Toxicity levels were not considered either as extreme high levels are required to reach a toxic threshold. The B.C. Ministry of the Environment establishes a maximum limit of 0.35 mg/l dissolved iron to protect aquatic wildlife (BC Ministry of the Environment 2008), levels which were not reached at our study sites.

4.4. Discussion

4.4.1. Metals in sediments

This is the first study in the region that measures metal concentrations in sediment, and biota, so there are no reference points to compare the present concentrations. The two published reports (Martinez *et al.* 2007, 2008) focused on measuring metals in the water column.

Metal concentrations in sediments from this study area differed spatially and temporally. Cd, Mn, and Cu showed higher concentrations in the dry season, while Zn was abundant in the wet08. A contrasting behavior was seen with zinc and iron which presented higher concentrations in the wet seasons, while lead did not present a distinctive pattern. Similar findings are commonly found in other areas in the world. For instance, Iwashita and Shimamura (2003) mention that Cu and Pb did not show drastic seasonal variation, while Cenci and collaborators (2004) found that Cu exhibited a divergent behaviour.

The variation in the sediment's metal concentrations between dry and wet seasons can probably be explained by the change in flow, turbidity, and water temperature. Dissolved organic matter increases the solubility of metals in pore water and sediment (Charriau et al 2011), while changes in flow increases the amount of small size grains in the sediment, particularly in drastic flood-related events , variables that are known to be related to trace metal concentrations (Ciszewski 2001; Wyzga and Ciszewski 2010).

4.4.2. Metals in trophic webs

Numerous studies support the fact that high metal concentrations in sediment coincide with elevated metal concentrations in invertebrates (e.g., Smith *et al.* 1996; Goodyear and McNeill 1999; Quinn *et al.* 2003), or that herbivores that consume periphyton have higher loads than those that consume sediment (Farag *et al.* 1998; Besser *et al.* 2001). In this study, this was not the case as no relationship between the concentrations in sediment could be associated with those from faunal components. Concentrations in both invertebrate and fish tissues were, however, several fold higher than those in sediment, while those in invertebrates were many times those of fish.

Cd, Pb and Cu concentrations in invertebrate functional groups were generally above the maximum limits established by the Mexican government in both seasons, in particular filter collectors, scraper snails, and omnivore decapods, throughout the study sites. Decapod and snail's high Cu concentrations are probably due to the physiological need of this metal as component of their haemolymph (Anderson 1977; Timmermans et al 1989). Cd levels were indicative of contamination but below those considered as hazardous to an organism (Schmitt 2004).

Fish metal concentrations were below the limit threshold in most metals, except for Pb. This metal was above the permissible limits for fish used for human consumption in most sites, while Cd levels were above the limits for protection of wildlife only in some sites in the dry08 season.

Zinc had a peculiar behavior (Figure 4.13) with no discernible pattern in the contrasting concentrations. Zinc bioavailability and toxicity is influenced by temperature, pH, alkalinity and dissolved oxygen (Eislen 1993). Thus, a probable cause of the seasonal differences in Zn concentrations may be the differences in seasonal water T°C (t_{df28}= -8.176, $p<0.001$), and dissolved oxygen (t_{df24}= 5.415, $p<0.001$).

Sediment, aquatic invertebrates and fish showed a vast range of metal concentrations that result from multiple variables. Adaptation and tolerance to polluted sites has been studied in periphyton and bacteria (Lehmann *et al.* 1999; Fechner *et al.* 2012), and in fish species (Widianarko *et al.* 2000) as a result of long-term exposure. Although metal concentrations in the functional groups studied here exceeded the maximum exposure limits in many sites, tolerance must have evolved as a result of low levels of exposure to a mixture of contaminants that may reduce a single contaminant toxic effect (Eisler 1993, 1998; Brinkman and Johnston 2008; Fechner *et al.* 2012). As Rainbow (2002) stated "aquatic invertebrates show a vast range of accumulated trace metal concentrations, the reasons for which can be interpreted in terms of the particular accumulation patterns used by particular invertebrates for particular trace metals".

Previous work in the area (Martinez *et al.* 2008) found that metal concentrations in water and sediment were below the limits allowed by the Mexican government. Sediment data obtained in this study supports their findings. However, concentrations in faunal tissues suggest that metal uptake by invertebrates and fish is mainly through diet. Metals in water are an important source for many species but diet seems to be an important pathway for metal uptake in the study sites. Many authors have agreed that prey is the main source for metals in aquatic fauna (e.g. Dallinger et al. 1987; Hare et al. 2003; Croisetiere et al. 2006).

Likewise, the metal concentration behavior in the different organisms studied in this area did not present a consistent pattern. Although this study did not pursue assessing risks due to metal presence in the watershed, future studies should include an analysis of the metals both in the water column and in sediment as well as the inclusion of other water variables (like pH determination which was omitted in this study). Iron and gold extraction permits in the state of Jalisco have tripled in the last five years, which do not include environmental risk assessments and follow-up. The impacts these activities will be having in the structure and function of the river's ecosystems in the region will undoubtedly increase. Thus, future studies wil be required to help integrate the present and potential effects metals and other contaminants may pose to the welfare of this river's biological components.

Three trace metals (Cd, Cu and Pb) and one macronutrient (Mn) could be associated to particular land use activities or point sources. Cd and Cu were present in sediment and invertebrate functional groups at higher concentrations than average in sites with influence from urban wastewater and stormwater drains, or cattle sewage runoff (i.e., 3.IA, 4.IA, 5.UrIA, 7.Ur, and 8. IA). Similarly, Pb levels were high in sites close to busy roads and urban stormwater drains, particularly in dry08 (e.g., 1.FoTA, 3.IA). Pb sources in 11.IAFo and a.Fo are less clear. The macronutrient Mn was present in high concentrations in sediment and macroinvertebrates in sites close to urban storm and wastewater drains, and in areas close to vehicle transit (i.e, 2.IAFo, 3.IA, 9.IA). The reference site 1FoTA, located in the area of influence of a mine facility, had high Mn levels in sediment and functional groups in both sampling seasons.

Despite the fact that metals are natural elements and are expected in all environments, the sites that presented the highest concentrations of most metals in this study, are mainly located within the intensive agricultural valley of Autlan-El Grullo in the Ayuquila River sub-basin.

Although this study did not pursue assessing risks due to metal presence in the Ayuquila River biological components, the data presented here is a reference point for future needed research. One aspect that merits more in depth study is the amount of Pb in fish species, many of which are sought after by local people, and could pose a health risk.

Further studies will be essential, in particular with both the federal and state government encouraging several gold, silver, and iron extraction operations in this and other areas in western Mexico that lack the appropriate environmental assessments.

4.5. Conclusions

Average concentrations in sediment decreased in the order Fe>Mn>Cu>Pb>Zn>Cd. Differences in mean concentration values between sites and between sub-basins were statistically significant for all metals except Pb in both wet seasons. Between-site comparisons were significant in the dry season only for Mn. Only Cd and Pb presented statistical significant differences among sampling seasons. Seasonal dependence was observed for Cd, Cu, and Mn, which had higher concentrations during the dry season, while Zn was dominant in the wet season.

Metal concentrations in invertebrate and fish functional groups were overall higher than those in sediment, and concentrations in invertebrate were higher than those in fish. As with sediment, temporal and spatial variation was the norm, with statistical significant differences observed in general for Cd, Cu, Pb and Fe.

Seasonal differences in metal concentrations in FFGs were statistically significant for: Cd in fish omnivores and fish predators; Pb and Fe in filter collectors and fish omnivores; Cu in predators; and Zn in fish herbivores.

Trace metal concentrations decreased as trophic level increased. No significant correlations were evident between FFG's metal concentrations and trophic level, nor between metal concentrations in sediment and concentrations in faunal components.

Cd, Pb, Cu and Zn concentrations in trophic webs were above the limits permitted by Mexican guidelines in many sites in both dry and wet08 seasons. Except for Pb in which all FFG' concentrations were above the guidelines, fish exceeded these threshold in their Cd concentrations in few sites and only in dry08. These concentrations in fish species need further research as it can be a health risk for local communities in the region

Cd, Pb, Cu, and Mn concentrations in sediment and FFGs could be associated to particular land use activities or point sources, such as wastewater drains, transit roads, or mine facilities.

Data presented here is a reference point for further research of uptake and concentration of trace metals in an economical and political scenario that promotes mining activities without appropriate risk assessments.

Table 4.1. Summary statistics of the metal concentrations (μg g-1) found in sediments during three sampling seasons.

	Cd μg/g	Pb μg/g	Cu μg /g	Zn μg /g	Mn μg/g	Fe μg/g
Mean	0.03	0.3	0.43	0.2	4.94	55.21
Std. Error	0.002	0.05	0.03	0.01	0.5	2.22
Max	0.08	1.93	1.32	0.44	15.62	99.97
Min	0.002	0.001	0.11	0.06	0.001	16.84
Mean	0.05	0.22	0.46	0.07	12.23	49.75
Std. Error	0.003	0.03	0.07	0.007	1.29	0.77
Max	0.12	0.57	1.48	0.21	29.58	59.97
Min	0.04	0.001	0.04	0.03	4.61	41.51
Mean	0.03	0.25	0.27	0.67	6.02	51.01
Std. Error	0.001	0.02	0.02	0.03	0.62	2.39
Max	0.06	0.75	0.65	1.46	27.75	84.63
Min	0.01	.001	0.03	0.3	1.43	21.24

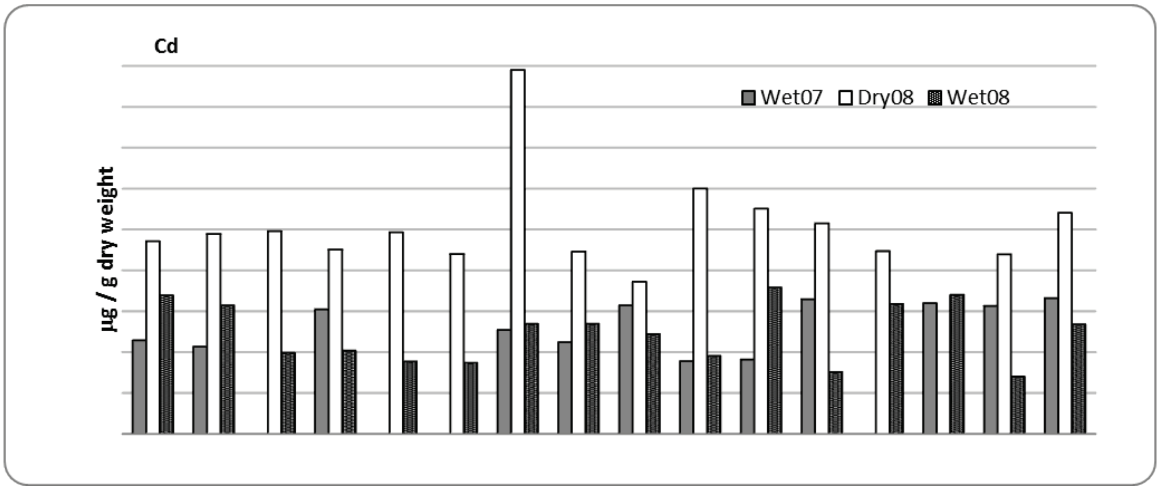
Table 4.2. Correlation values between metals found in sediment for each field season. **Bold** values are statistically significant (*p<0.05; **p<0.01; $^{+}$p<0.001).

	Pb	Cu	Mn	Fe	Zn
		WETO7 SEASON (n=70)			
Cd	-0.0927	-0.034	-0.0431	-0.0826	0.0302
Pb		0.0681	-0.0274	0.0664	0.0313
Cu			0.198	**0.35****	**0.395**$^{+}$
Mn				**0.627**$^{+}$	0.195
Fe					0.155
		DRYO8 SEASON (n=33)			
Cd	0.0824	0.104	0.346	**0.654***	0.187
Pb		**0.615***	**0.742****	-0.0385	**0.753****
Cu			0.61	0.0714	**0.885**$^{+}$
Mn				-0.104	**0.72****
Fe					0.033
		WETO8 SEASON (n=54)			
Cd	0.0558	-0.123	0.158	**0.484**$^{+}$	0.124
Pb		0.154	0.266	0.201	**0.454**$^{+}$
Cu			**0.408****	-0.152	**0.394****
Mn				**0.27***	**0.586**$^{+}$
Fe					**0.359****

Table 4.3. Summary statistics of metal concentrations (μg g^{-1}) in Functional Feeding Groups for dry08 and wet08 seasons. (FCol: Filter collectors; GCol: Gatherer collectors; Scrp: Scrapers; Prd: Predators; Omn: Omnivores; FHer: Fish herbivores; FOm: Fish omnivores; Fcar: Fish predators).

		Cd		Pb		Cu		Mn		Fe		Zn	
		Dry08	Wet08	Dry08	Wet08	Dry08	Wet08	Dry08	Wet08	Dry08	Wet08	Dry08	Wet08
FCol	Mean (±s.e.)	0.16±0.02	0.58±0.2	0.47±0.1	1.6±0.4	3.95±0.8	4.05±1.5	79.3±23.4	68.2±16	91.5±11.8	242.1±51.7	10.2±0.5	10.4±1.1
	Min	0.08	0.06	0.12	1.17	1.31	0.72	5.52	3.8	31.9	33.8	8.5	1.4
	Max	0.26	1.85	1.25	4.23	7.3	23.6	250.0	218.0	166.5	839.1	13.7	15.0
	n	10	14	11	10	9	15	12	15	12	15	9	15
GCol	Mean (±s.e.)	0.34±0.1	0.38±0.1	0.93±0.3	3.8+1.5	1.36±0.2	2.1±0.3	99.4±13.1	74.8±22.	137±32.8	284.4±61.3	14.7±2.5	14±1.3
	Min	0.08	0.07	0.4	0.3	0.7	0.6	34.4	13.9	39.8	55.5	10.2	8.5
	Max	0.82	0.93	2.23	14.34	1.73	3.82	116.2	271	279.6	767.8	19.2	21.5
	n	6	10	5	10	4	11	6	11	7	11	4	11
Scrp	Mean (±s.e.)	0.2±0.04	0.9±0.3	0.7±0.08	1.3±0.3	12.3±0.5	6.6±2.6	47±12.8	47.3±18.2	55.3±20.8	83±34.6	15.9±6.2	7.1±0.7
	Min	0.1	0.04	0.4	0.7	0.4	1.14	13.6	3.23	13.6	5.5	4.9	5.5
	Max	0.3	2.45	1.0	2.14	28.16	21.74	86.2	152.7	123.8	261.4	36.32	11.5
	n	6	8	6	5	6	8	6	8	6	8	6	8
Prd	Mean (±s.e.)	0.14±0.01	0.6±0.3	0.4±0.05	2.73±1.3	8.8±4.	2.4±0.2	68.2±0.1	90.7±1.7	82.04±6.6	90.15±11.1	11.2±0.8	12.7±1.0
	Min	0.06	0.04	0.07	0.07	0.6	0.25	1.02	5.5	24.9	26.0	1.84	1.1
	Max	0.25	6.6	0.84	28.16	81.1	5.7	130.6	370.4	157.1	272.7	23.33	25.2
	n	24	28	21	23	23	28	25	28	25	28	24	28
Omn	Mean (±s.e.)	0.4±0.02	0.3±0.04	3.3±0.5	4.04±0.6	4.5±0.5	11.8±1.9	19.2±6.4	62.2±1.5	21.4±4.7	32.6±7.1	8.3±0.03	7.7±0.6
	Min	0.3	0.8	1.6	1.3	3.34	4.8	8.3	4.4	12.9	8.7	7.8	4.6
	Max	0.5	0.5	4.5	8.5	5.4	27.1	33.8	139.7	38.7	81.5	9.14	11.5
	n	5	12	5	12	4	12	4	12	5	12	4	12
FHer	Mean (±s.e.)	0.2±0.03	0.2±0.04	3.1±1.0	2.68±0.8	0.75±0.3	0.8±0.2	8.1±1.4	21.2±5,3	26.8±24.4	90.8±44.3	3.74±1.4	8.34±0.9
	Min	0.14	0.09	0.55	0.73	0.11	0.13	2.3	3.56	1.51	1.82	0.07	5.76
	Max	0.34	0.36	5.58	6.04	1.55	1.37	12.95	34.41	148.8	261.3	8.22	10.54
	n	6	6	6	6	5	6	6	6	6	6	6	6
FOm	Mean (±s.e.)	0.17±0.05	0.3±0.05	0.8±0.3	3.28±0.7	4.73±0.3	0.65±0.1	2.48±0.9	7.5±2.0	15.1±7.8	49.2±21.0	4.84±0.9	6.52±1.0
	Min	0.03	0.07	0.08	0.51	0.07	0.09	0.07	0.2	0.13	0.82	0.05	1.84
	Max	0.93	1.09	4.22	12.07	24.12	1.95	13.24	26.42	108.8	323.7	13.74	15.51
	n	17	19	19	17	9	19	19	19	18	19	16	19
Fcar	Mean (±s.e.)	0.14±0.01	0.06±0.0	0.2±0.03	0.3±0.04	-	0.09±0.01	1.5±0.07	0.8±0.3	1.71±0.06	2.38±1.0	2.9±0.08	3.3±0.3
	Min	0.13	0.01	0.17	0.06	-	0.01	1.43	0.02	1.65	0.28	2.8	1.0
	Max	0.16	0.13	0.24	0.43	-	0.15	1.6	3.01	1.78	12.28	3.0	5.23
	n	2	12	2	8	-	12	2	12	2	12	2	12

a)



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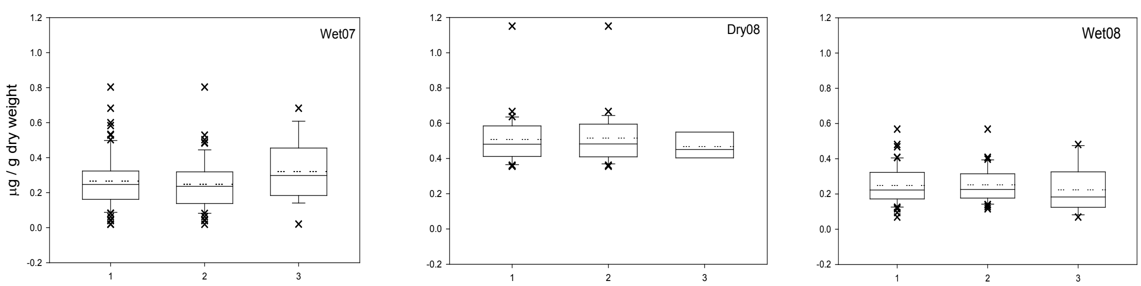
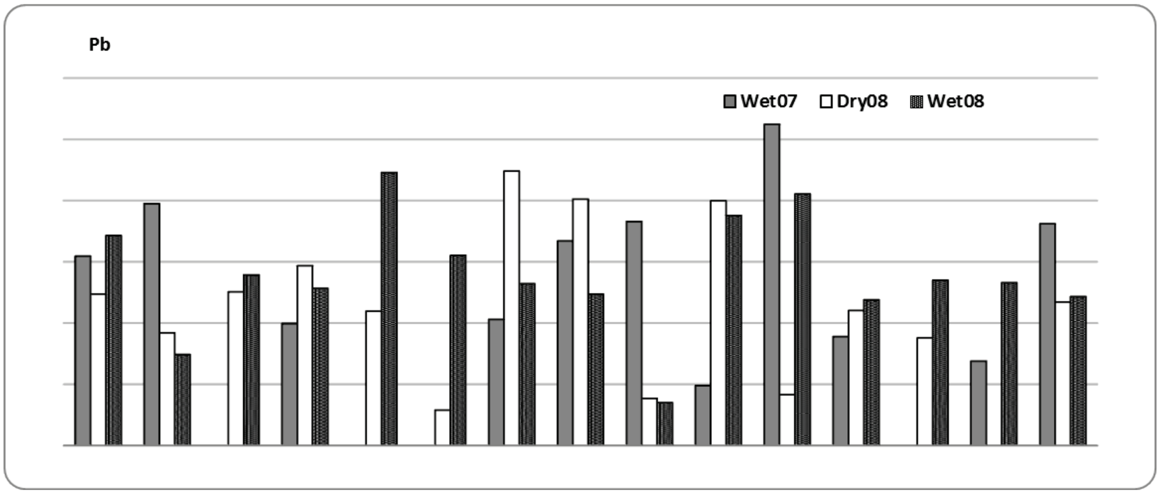


Figure 4.1. a). Cadmium (Cd) mean concentrations (μg/g dry weight) in sediment per site and season. b) Lower box graphs show concentrations variability among 1) all sites; 2) those in the Ayuquila; and 3) Tuxcacuesco sub-basins for each sampling season. Dotted lines are mean values.

a)



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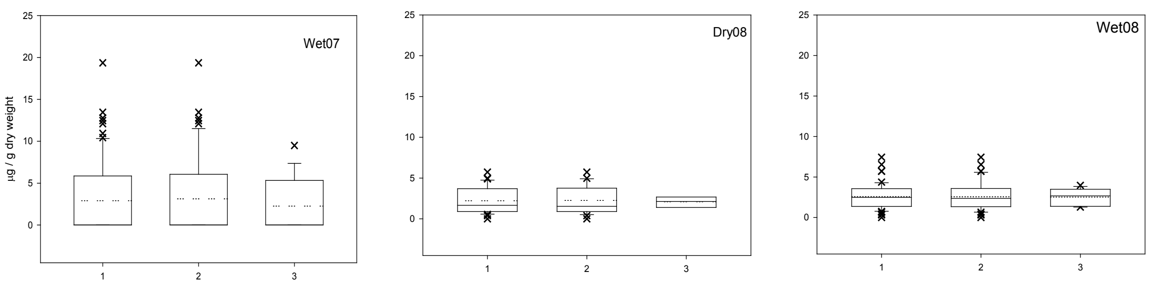
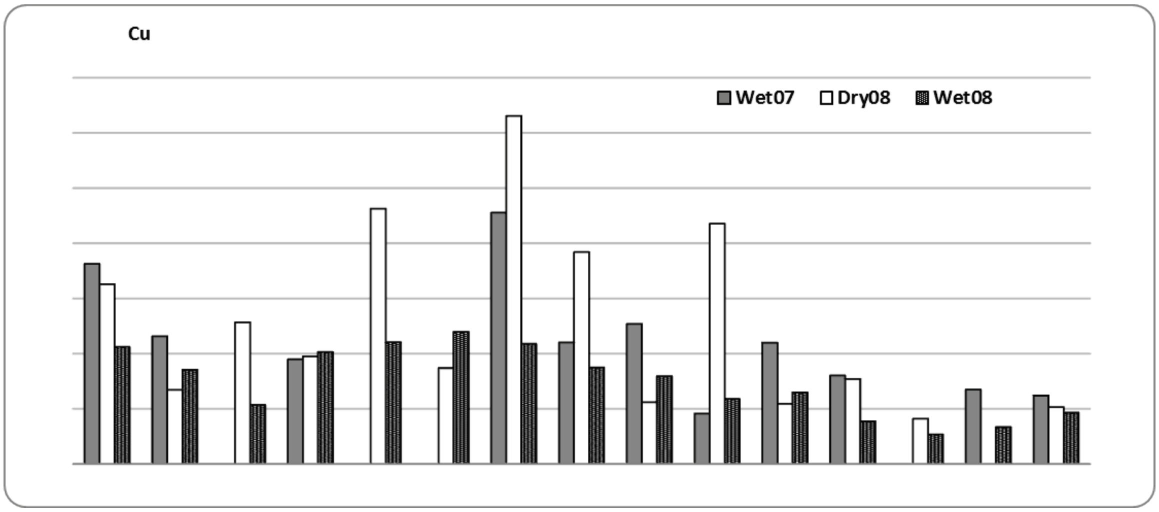


Figure 4.2. **a)** Lead (Pb) mean concentrations (µg/g dry weight) in sediment per site and season; **b)** Lower box graphs show concentrations variability among 1) all sites; 2) those in the Ayuquila; and, 3) Tuxcacuesco sub-basins for each sampling season. Dotted lines are mean values.

a)



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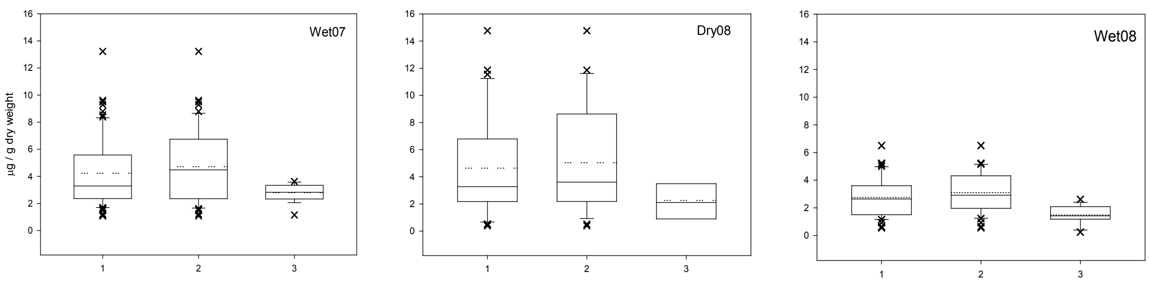
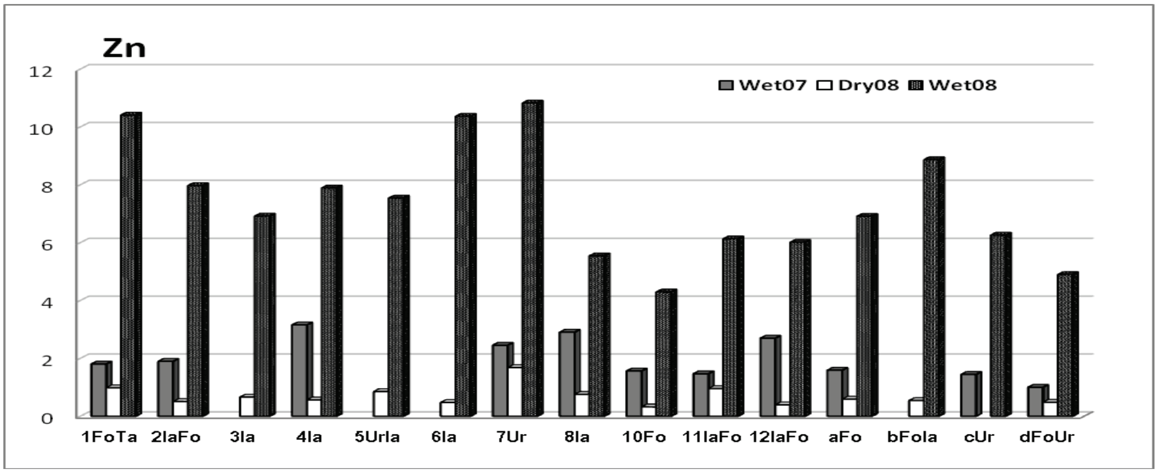


Figure 4.3. **a)** Copper (Cu) mean concentrations (µg/g dry weight) in sediment per site and season. **b**) Lower box graphs show concentrations variability among 1) all sites; 2) those in the Ayuquila; and, 3) Tuxcacuesco sub-basins for each sampling season. Dotted lines are mean values.

a)



b)

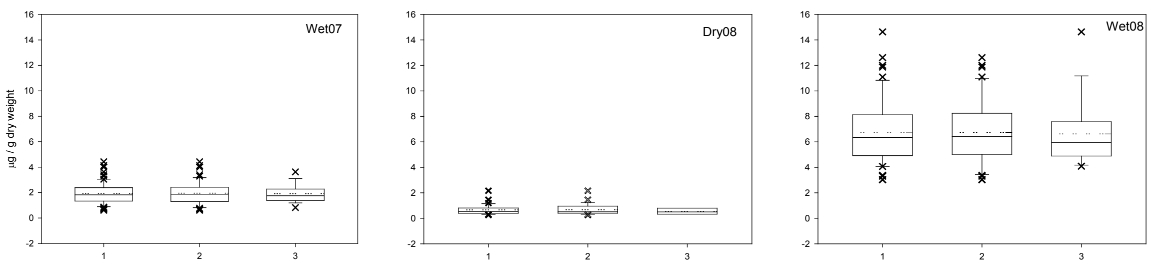
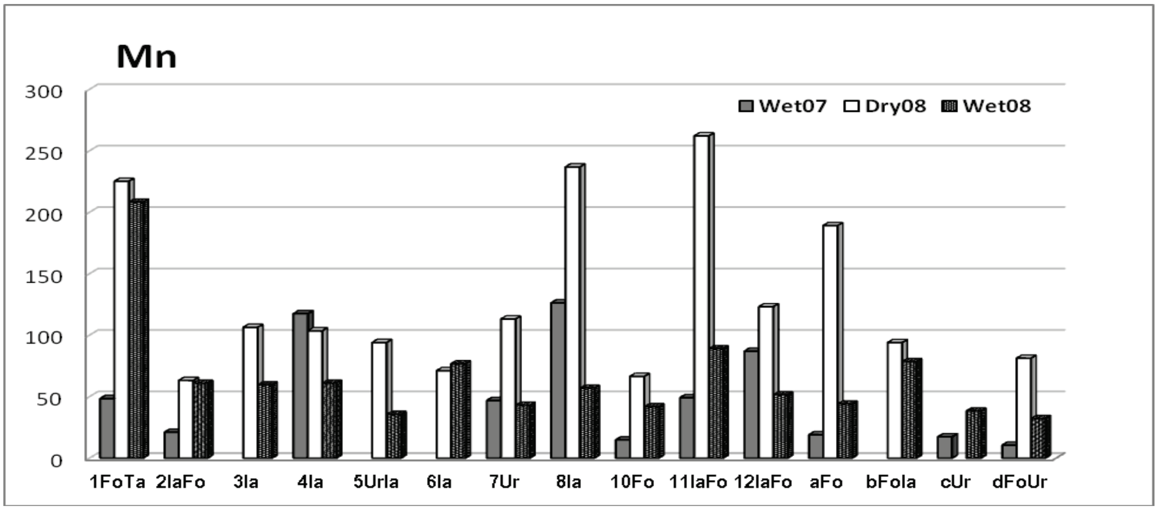


Figure 4.4. **a)** Zinc (Zn) mean concentrations (μg/g dry weight) in sediment per site and season; **b)** Lower box graphs show concentrations variability among 1) all sites; 2) those in the Ayuquila; and, 3) Tuxcacuesco sub-basins for each sampling season. Dotted lines are mean values.

a)



b)

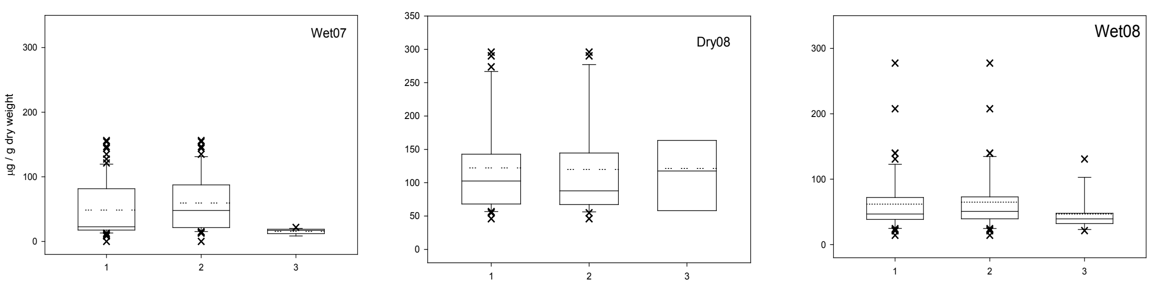
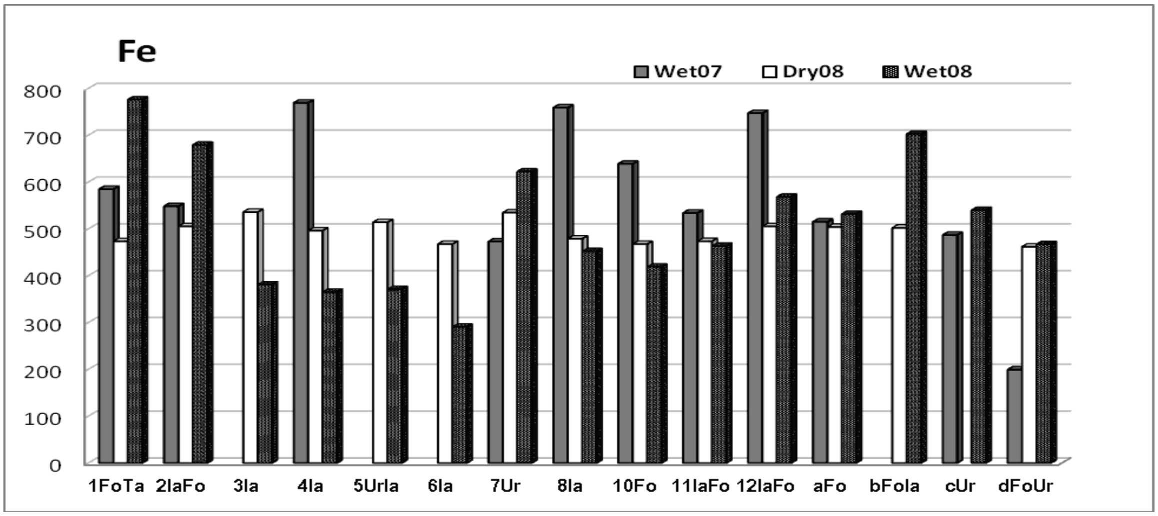


Figure 4.5. a) Manganese (Mn) mean concentrations (μg/g dry weight) in sediment per site and season. **b)** Lower box graphs show concentrations variability among **1**) all sites; **2**) those in the Ayuquila; and **3**) Tuxcacuesco sub-basins for each sampling season. Dotted lines are mean values.

a)



b)

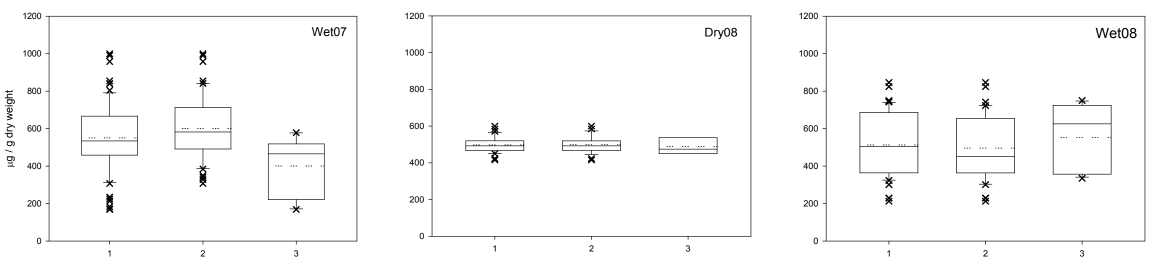


Figure 4.6. a) Iron (Fe) mean concentrations (μg/g dry weight) in sediment per site and season. **b)** Lower box graphs show concentrations variability among 1) all sites; 2) those in the Ayuquila; and, 3) Tuxcacuesco sub-basins for each sampling season. Dotted lines are mean values.

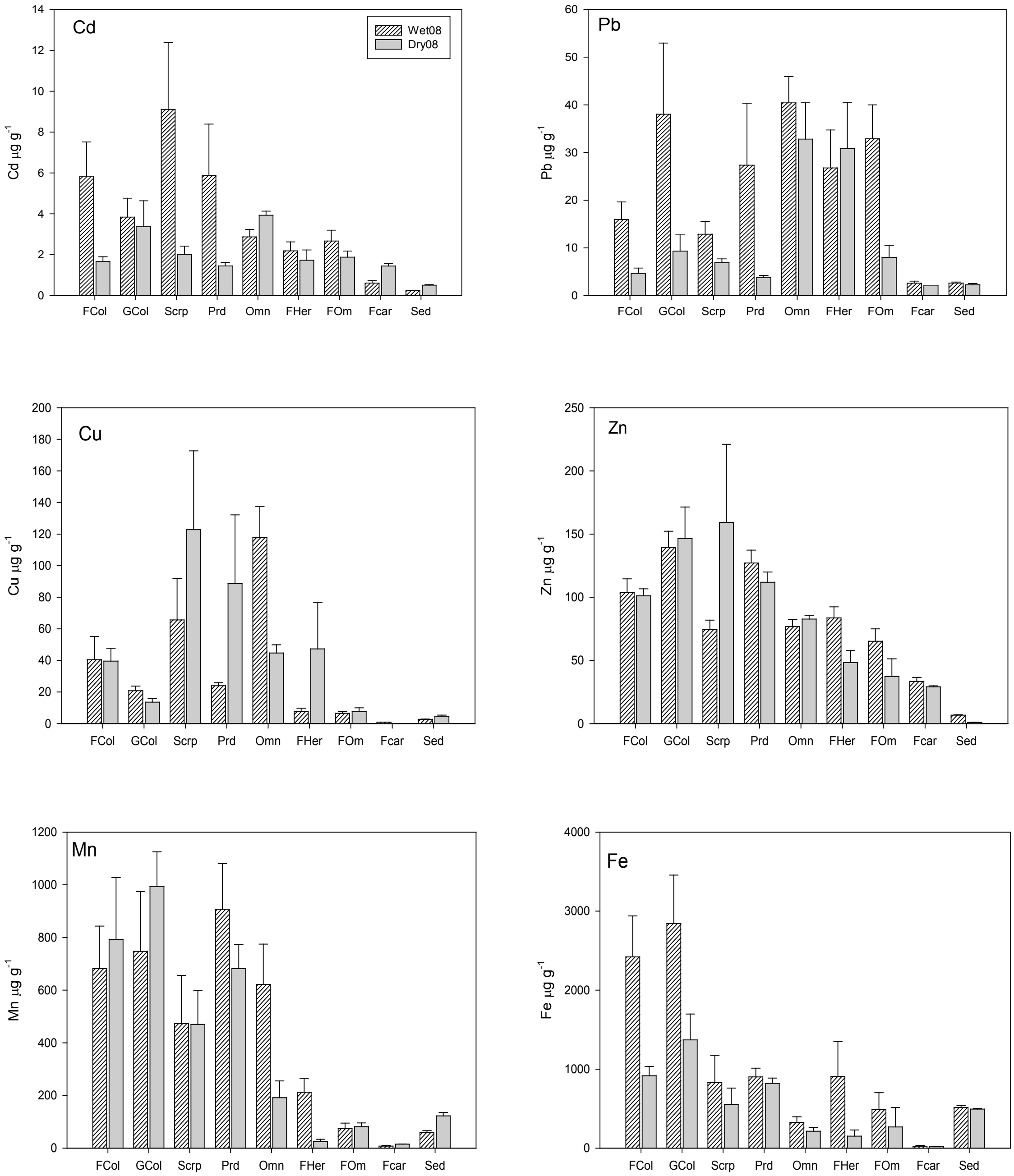


Figure 4.7. Mean (±s.e) metal concentrations in invertebrate and fish functional feeding groups for dry08 (gray) and wet08 (barred) field seasons in the middle part of the Ayuquila-Armeria Watershed. (FCol: Filter collectors; GCol: Gatherer collectors; Scrp: Scrapers; Prd: Predators; Omn: Omnivores; FHer: Fish herbivores; FOm: Fish omnivores; Fcar: Fish predators; Sed: Sediment

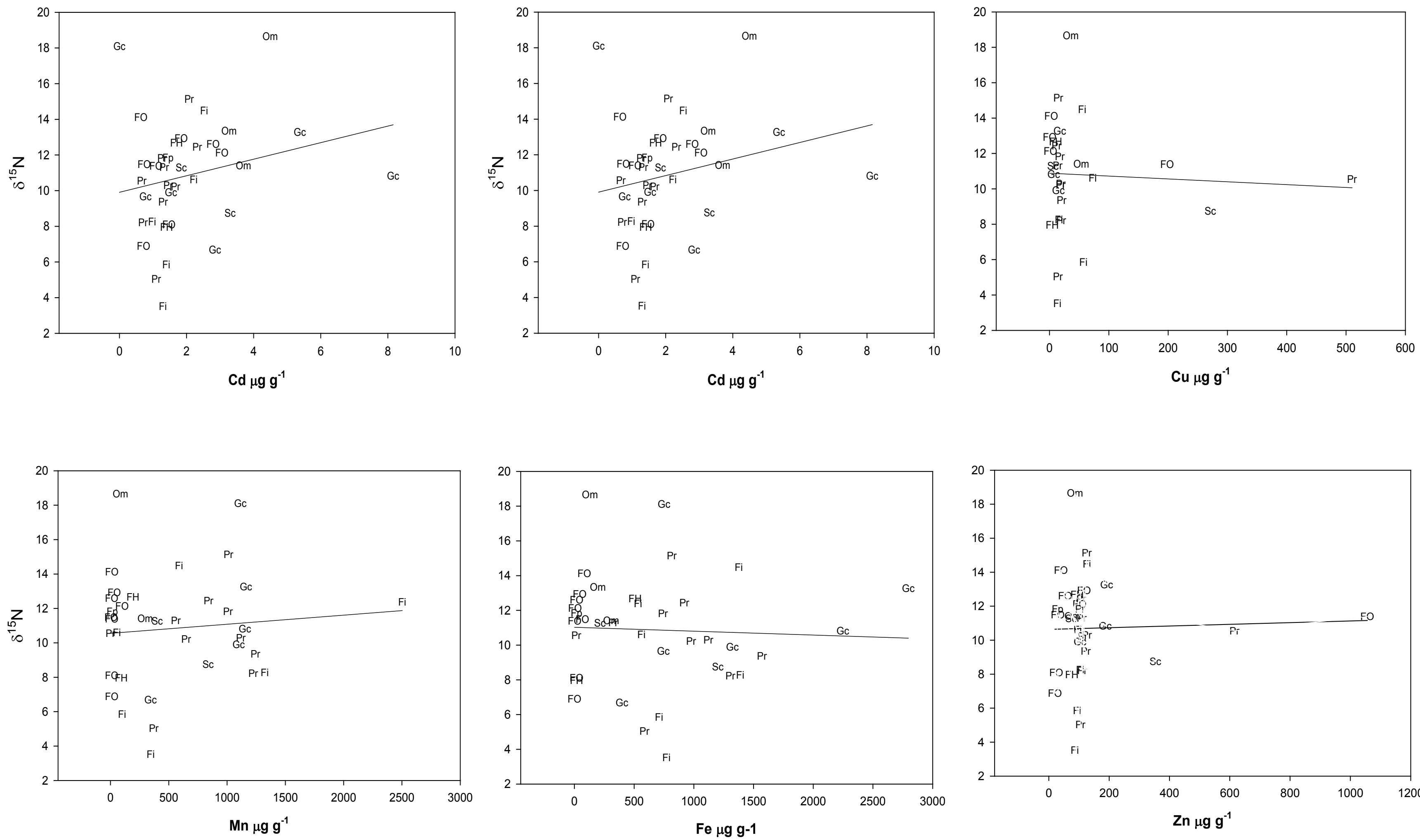


Figure 4.8. Mean $\delta^{15}N$ ratios (‰)/ mean metal correlation graphs for Dry08. Fi=Filter Collectors; Gc= Gather Collectors; Sc= Scrapers; Pr= Predators; Om= Omnivores; FH= Fish Herbivores; FO= Fish Omnivores; Fp= Fish Predators.

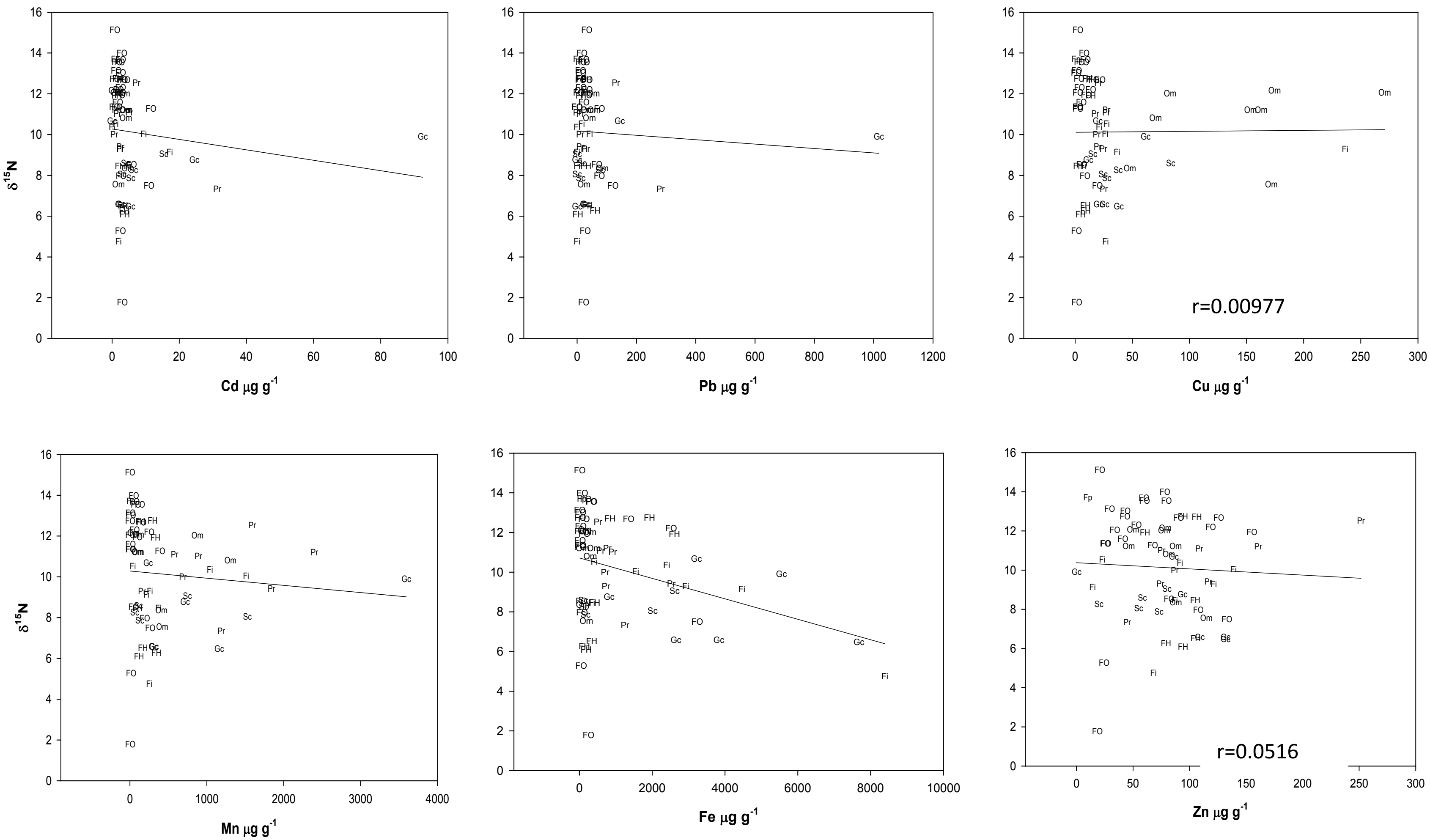
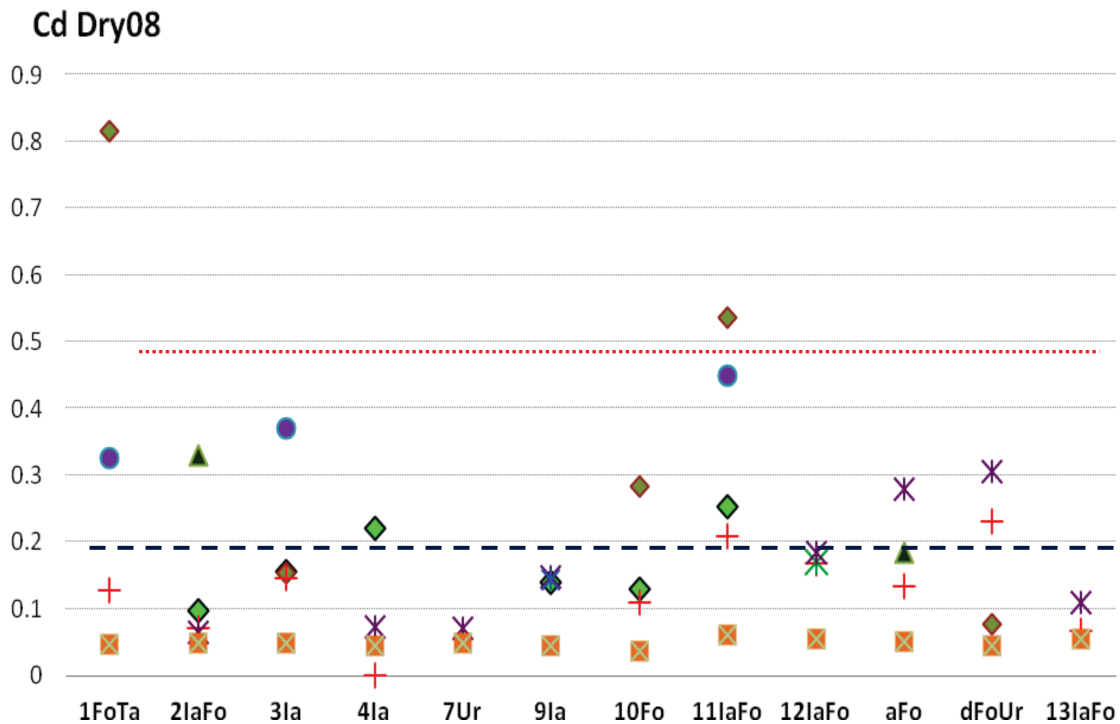


Figure 4.9. Mean $\delta^{15}N$ ratios (‰) /mean metal correlation graphs for Wet08. Fi= Filter collectors; Gc= Gather Collectors; Sc= Scrapers; Pr= Predators; Om= Omnivores; FH= Fish Herbivores; FO= Fish Omnivores; Fp= Fish Predators.



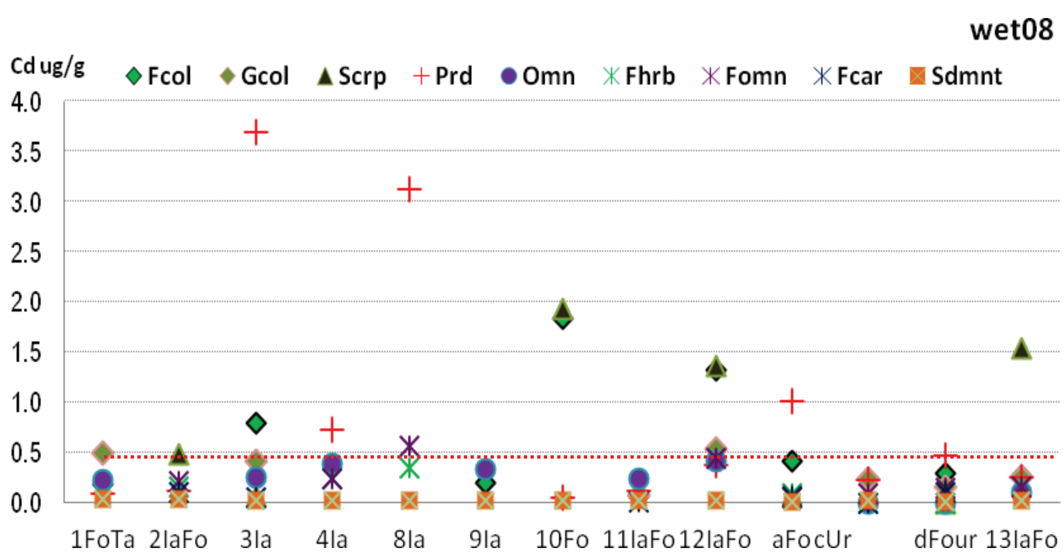


Figure 4.10. Cd mean concentrations in food web components per site for dry08 and wet08. Dotted blue line establishes the maximum admissible limits (0.2 ppm) established by the Mexican government to protect aquatic wildlife, while the red line establishes the concentration limits for animal species eaten by humans.(0.5 ppm) Notice the difference in scale between graphs.

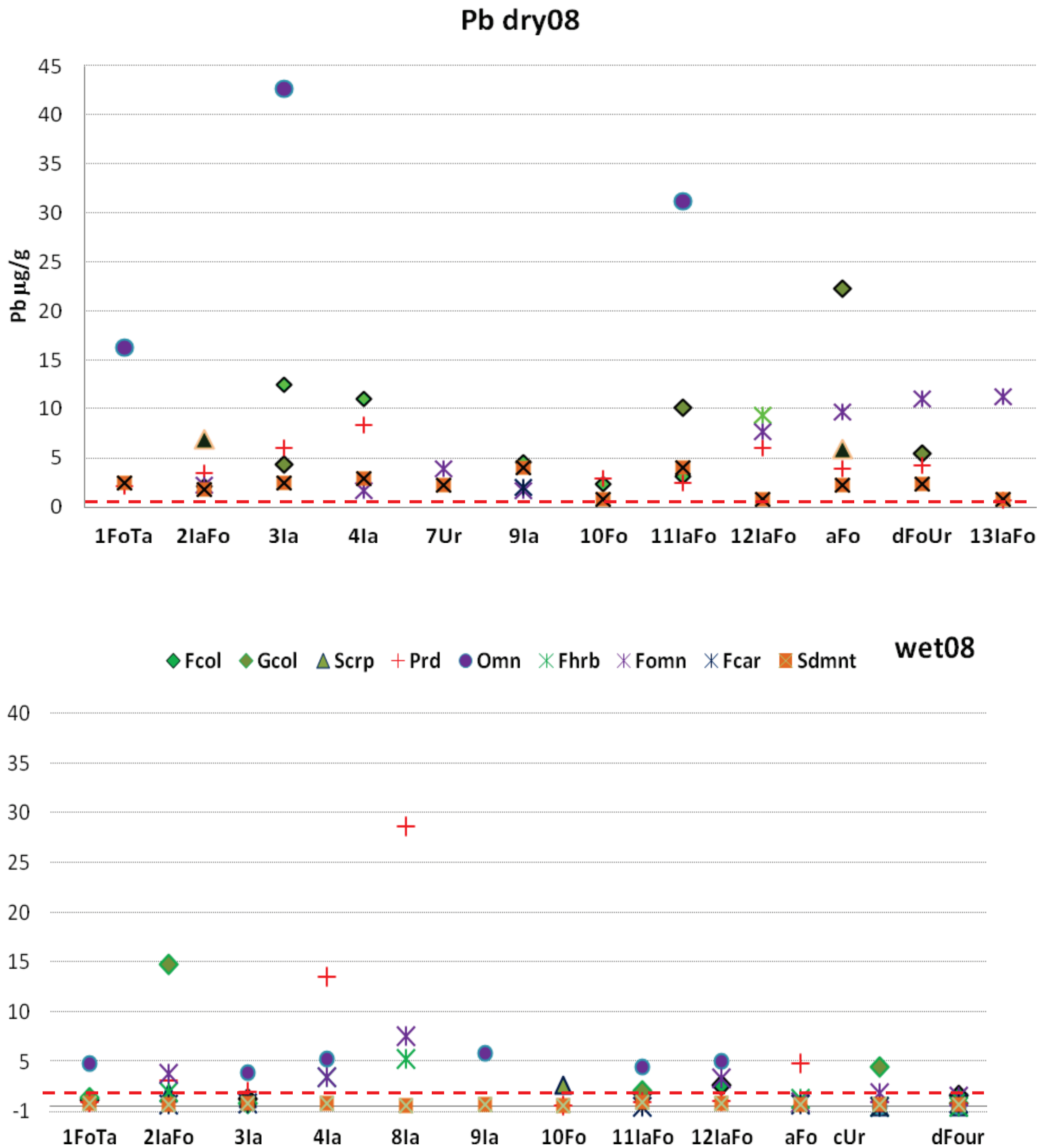


Figure 4.11. Pb concentrations in food web components per site for dry08 and wet08. Red dotted line establishes the maximum admissible limits (1.0 ppm) established by the Mexican government for animal species eaten by humans. Notice the difference in scale between graphs.

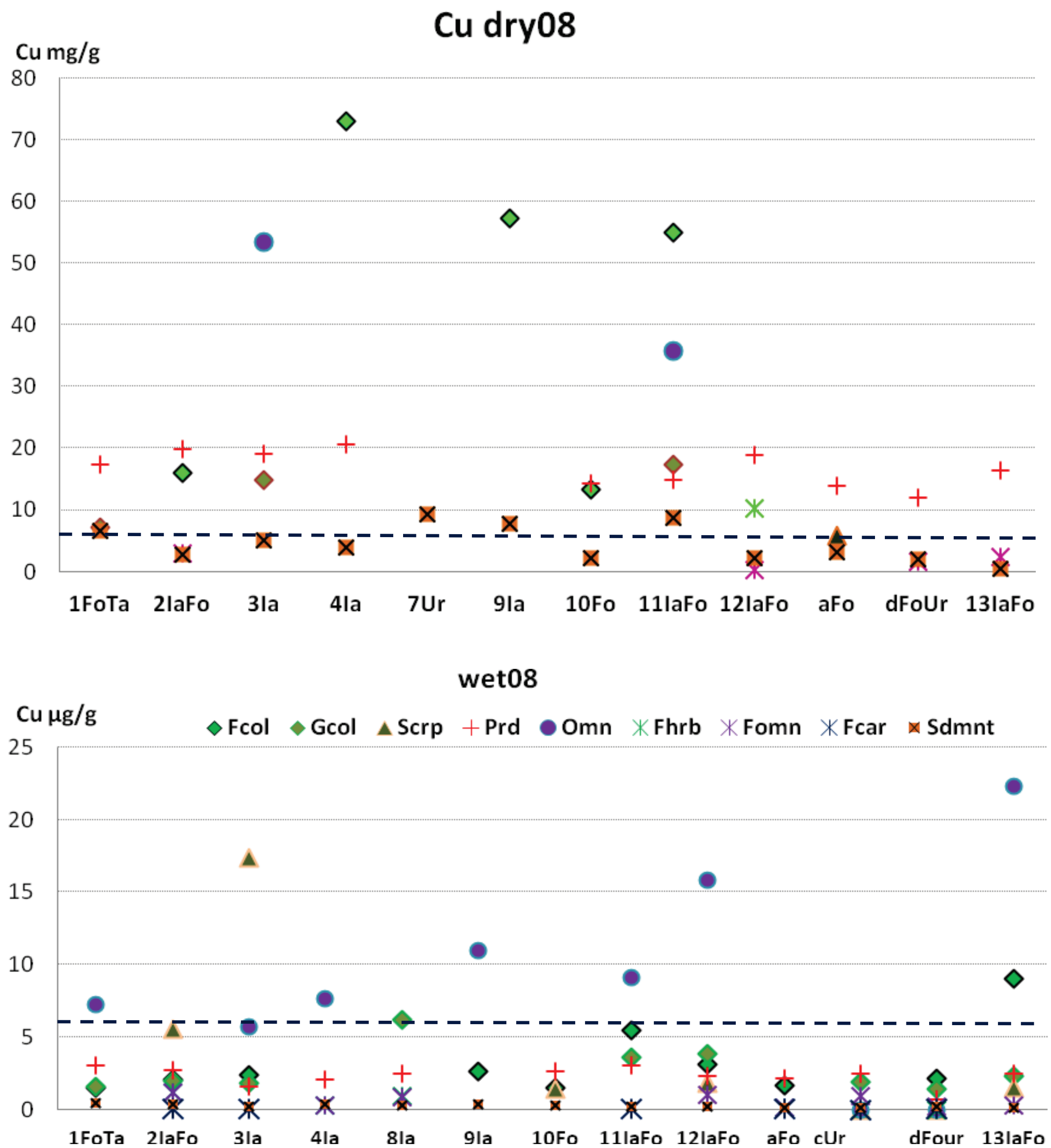


Figure 4.12. Cu concentrations in food web components per site for dry08 and wet08. Blue dotted line establishes the maximum admissible limits (6.0 ppm) established by the Mexican government for protection of aquatic wildlife. Notice the difference in scale between graphs.

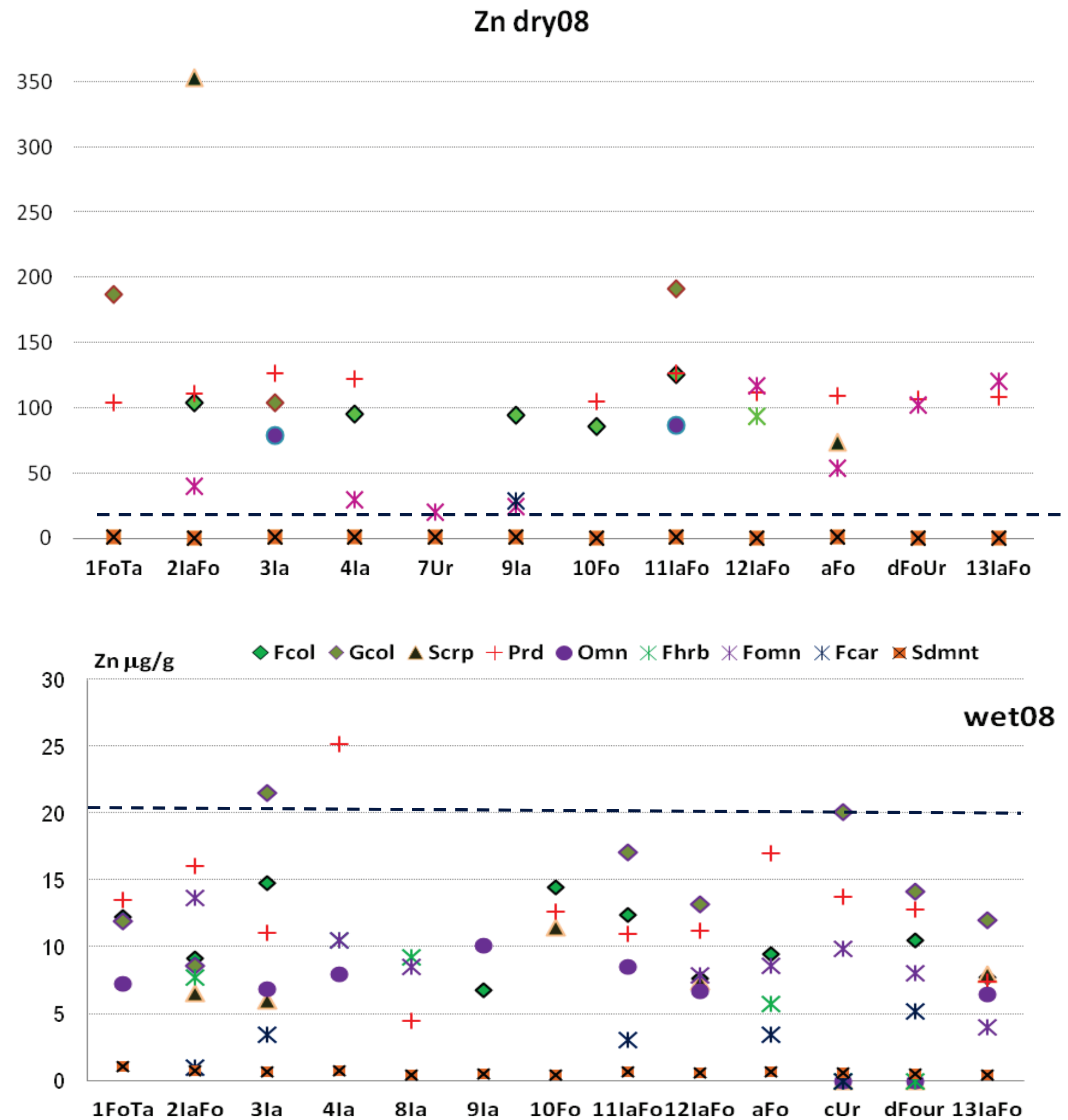
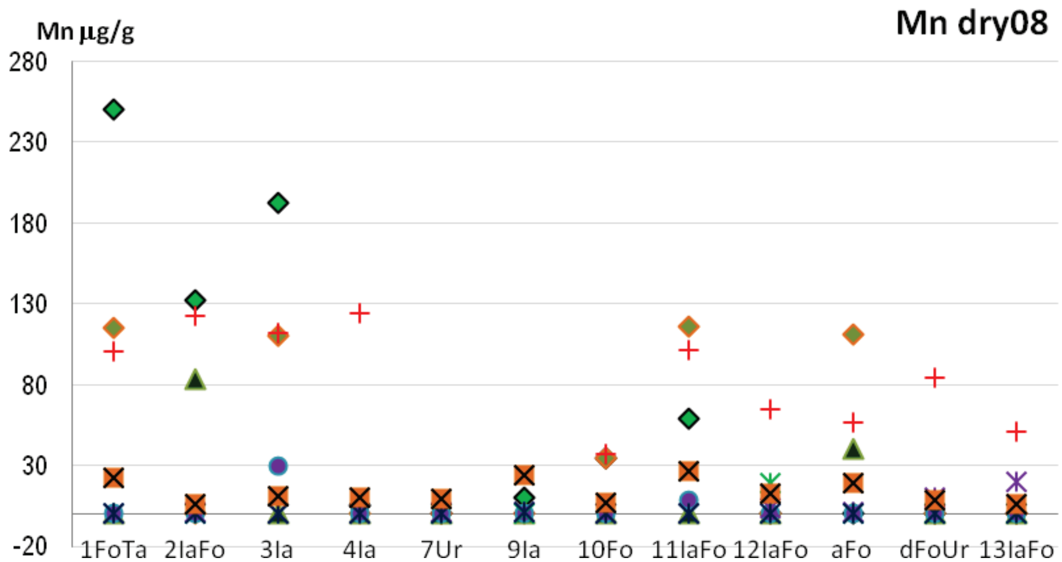


Figure 4.13. Zn concentrations in food web components per site for dry08 and wet08. Blue dotted line establishes the maximum admissible limits (20.0 ppm) established by the Mexican government for protection of aquatic wildlife. Notice the difference in scale between graphs.



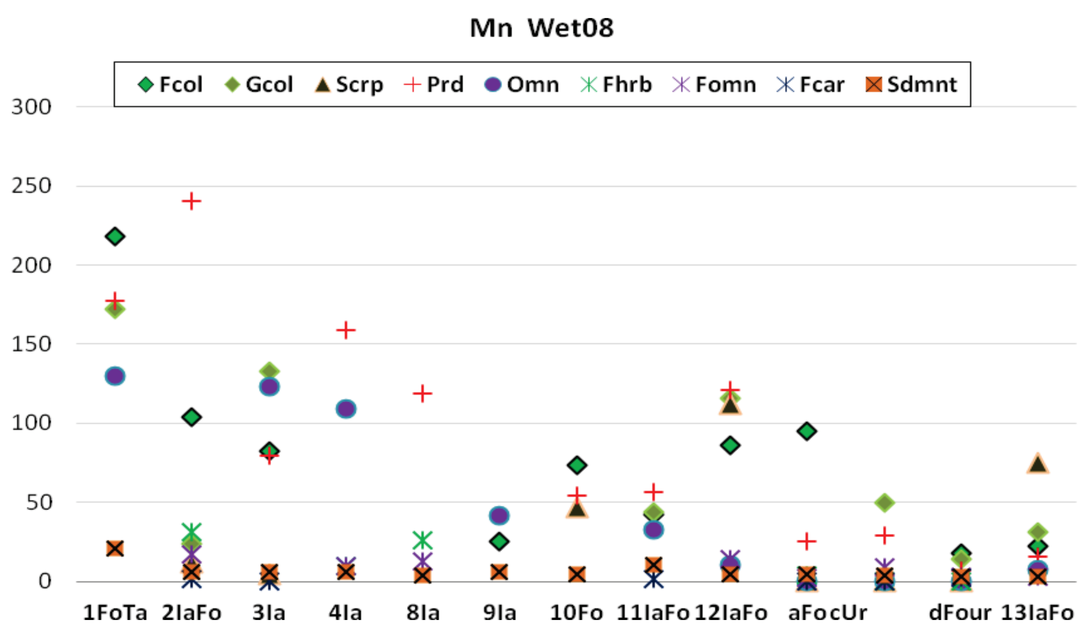


Figure 4.14. Mn concentrations in food web components per sites in both seasons. . Notice the difference in scale between graphs.

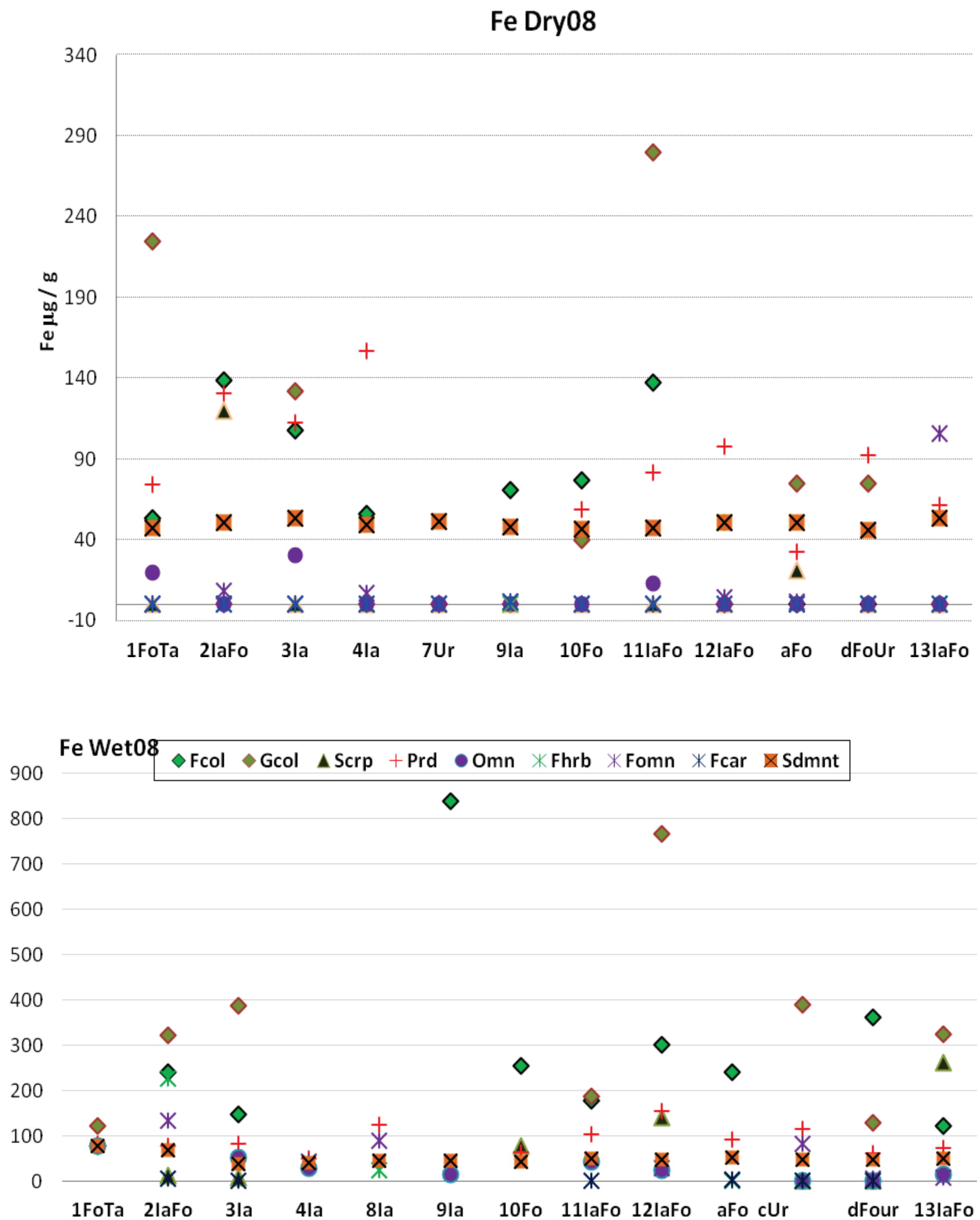


Figure 4.15. **Fe** concentrations in food web components per sites in both seasons. . Notice the difference in scale between graphs

5. General Conclusions and Final Remarks

It is estimated that freshwater constitutes 2.5% of the total water reserves present in the planet with almost 0.3% stored in lakes and rivers (Shiklomanov 1998). Rivers alone make up 0.006% of the total freshwater stock (Shiklomanov 1993). Despite this low figure, rivers have shaped social and economic processes through human history, and they are essential for the well-being of almost 80% of the world's population nowadays (Vörömarty *et al*. 2010). The interaction human societies have with rivers has made them among the most threatened ecosystems worldwide (Malmqvist and Rundle 2002).

Rivers are a product of their valleys (Hynes 1975), and as such, vegetation cover, geological morphology, and soil composition, in one side, and human activities and infrastructure, in the social one, play an important role in shaping the river's physical and biological characteristics. Due to the occurrence of both biological and human-derived phenomena, river catchments or watersheds are being used as the basic and integrative units of study in water resource management worldwide. Watersheds are considered useful study and organizational units as it is considered that water quantity and quality at one point on a stream reflect the cumulative characteristics of the up-gradient topographic area from that point (Omernik and Bailey 1997).

A monitoring program to assess the quality of the water in the middle portion of the Ayuquila-Armeria River in the state of Jalisco, Mexico, started in 1995 after several complaints from local communities. Their arguments were centered in the fact that fish would increasingly appear dead as a result of anoxia, and that skin problems were frequent after bathing in the river. Since then, the main sources of organic pollution have been identified and many actions to reduce some of the negative impacts have been put into practice. However, despite the long-term actions, there is still need for evidence on how the changes in the watershed's landscape have affected the river's ecosystem.

This study was implemented with the idea of filling in some of these information gaps, in particular in the area of inorganic pollution.

With this in mind and using data from sites in the Ayuquila River, presented in previous chapters, I wanted to determine if the River's biological composition could be related to changes in the adjacent landscape. I characterized the invertebrate and fish fauna of each of the sites sampled, and employed isotope analysis and flame spectrophotometry to identify sources of organic matter and trace metals in the faunal tissues, and to relate the findings with adjacent land use activities. The overall conclusions of this work and some recommendations are described in the following lines.

- This was the first research project that tried to assess the impact in organisms of land use change in this watershed.
- I described the Ayuquila River's macroinvertebrate and fish composition from 17 sites in one dry and two wet seasons with a functional feeding approach (Chapter 2). The fauna sampled was classified into 6 functional feeding groups and three fish trophic guilds. I compared the composition and quality of the faunal structure with the water's physicochemical variables using different metrics. The dominant functional groups corresponded to filter and gatherer collectors, and fish omnivores, which are more abundant in water rich in suspended material and disturbed areas. Water parameters were not conclusive in determining invertebrate abundance or composition, but conductivity and water hardness were positively associated with fish abundance.
- Biotic indices proved not to be useful indicators of water quality. The biological composition in most sites suggested regular to good water quality conditions, but the presence of pollution tolerant species, exotic fish and invertebrate species, and concentrations of some several variables above the limits proposed by environmental agencies suggest conditions less favorable. Although the use of indices of biotic integrity is helpful, the information provided should be complemented with the application of other variables. The functional feeding group approach is useful in this regards, but finer detail and further research is required.
- Although the presence of exotic invertebrate species are not a welcome scene in ecosystems, the presence of two, perhaps more, exotic mollusk species can serve as bioindicators of organic and inorganic pollution. These species can help identify critical levels of these and other compounds in the future.

- Stable isotope analyses suggested the influence of human activities on trace metals in sediment and organisms.
- Isotope analysis was done in sediment and faunal tissues from the samples obtained. The functional group composition characteristic of disturbed and polluted areas that dominated in most sites was confirmed with the application of nitrogen and carbon isotope analyses (Chapter 3). This technique permitted the identification of organic sources as well as the characterization of food webs based on the functional groups. Short trophic level food webs at most sites were the norm, another indication of disturbance. The presence of exotic species is probably an added ingredient to the resulting food web length and composition. Additionally, a shift in the trophic level occurred in some functional groups as a result of the incorporation of nitrogen rich sources.
- Nitrogen isotope ratios helped confirm the influence human and animal sewage have as sources of organic matter for the river's biological components, while carbon isotope ratios indicated that sources of energy were influenced by forest/riparian vegetation and/or autochthonous sources. No statistical significant differences were evident in the isotope ratios in biota from forest dominated sites and those from agriculture.
- Concentrations of four trace metals (Cd, Pb, Cu, and Zn) and two macronutrients (Fe and Mn) were determined in sediment and food web components, and the resulting levels were contrasted to land use activities surrounding the sites (Chapter 4). Metal concentrations in sediment were site and seasonal dependent, with levels of Cd, Cu, and Mn being dominant in the dry season, and Zn in the wet one. Seasonal differences in functional feeding groups were statistically significant for some groups and some metals. Mn concentrations did not differ seasonally. Trace metals decreased as trophic level increased.
- Cd, Pb, Cu and Zn concentrations in trophic webs were above the limits established by Mexican guidelines, with most of these thresholds surpassed by invertebrate FFGs. The exception was Pb that had concentrations above the limits in all FFGs. Cd, Pb, Cu, and Mn concentrations in sediment and FFGs could be associated to particular land use activities or point sources, such as wastewater drains, transit roads, or mine facilities.

Several information gaps still exist that require additional research. For instance, there is a need for more detailed taxonomic knowledge of the invertebrate species, as well as their functional feeding group position. A family level is too general for both pollution tolerance and functional position in an ecosystem.

Despite the presence of an important network of biological reserves, updates information on the changes in the landscape are needed, and most importantly, the effect these are having in the structure of what is left of the natural environments is an imperative. This study is a small step in filling these information gaps.

The inclusion of these (and other techniques) in the monitoring programs can provide a better idea of what is going, and the results obtained from this study can serve as a reference point for further research. The long-term goal of this research is to incorporate other techniques to the on-going monitoring program in this region, and to foster their implementation in other river systems in Mexico. By including other approaches to the analysis of land use change and its effects in the structure of lotic systems, management techniques can be implemented to improve the overall conditions of this River, while hopefully improving the conditions of its people.

Reference List

Adriano D.C. 2001. Trace elements in the terrestrial environment. Biogeochemistry, bioavailability and risks of metals. 2nd ed. Springer, New York.

Anderson C., and G. Cabana. 2007. Estimating the trophic position of aquatic consumers in river food webs using stable nitrogen isotopes. Journal of the North American Benthological Society 26:273-285.

Anderson B.C, T. Bell, P. Hodson, J. Marsalek, and W.E. Watt. 2004. Accumulation of trace metals in freshwater invertebrates in stormwater management facilities. Water Quality Research Journal 39:362-373

Anderson R.V. 1977. Concentration of cadmium, copper, lead, and zinc in thirty-five genera of freshwater macroinvertebrates from the Fox River, Illinois and Wisconsin. Bulletin of Environmental Contamination and Toxicology 18: 345-349.

Barwick M., and WA. Maher. 2003. Biotransference and biomagnification of selenium, copper, cadmium, zinc, arsenic, and lead in a temperate seagrass ecosystem from Lake Macquarie Estuary, NSW, Australia. Marine Environmental Research 56:471-502.

Barbour MT, J. Gerritsen, BD. Snyder, and JB. Stribling. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers. Periphyton, benthic macroinvertebrates, and fish. Washington, D.C.: U.S. Environmental Protection Agency. Office of Water; Report nr EPA 841-B-99-002.

Beals ML. 2006. Understanding community structure: a data-driven multivariate approach. Oecologia 150:484-495.

Bearhop S, DR Thompson, S Waldron, IC. Russell, G. Alexander, and RW Furness. 1999. Stable isotopes indicate extent freshwater feeding by cormorants *Phalacrocorax carbo* shot at inland fisheries in England. Journal of Applied Ecology 36:75-84.

Beasley G., and P.E. Kneale. 2003. Investigating the influence of heavy metals on macroinvertebrate assemblages using Partial Canonical Correspondence Analysis (pCCA). Hydrology and Earth System Sciences 7:221-233.

Benedito-Cecilio E., and C.A.R.M. Araujo-Lima. 2002. Variation in the carbon isotope composition of *Semaprochilodus insignis*, a detritovorous fish associated with oligotrophic and eutrophic Amazonian rivers. Jorunal of Fish Biology 60:1603-1607.

Berka C., H. Schreier, and K. Hall. 2001. Linking water quality with agricultural intensification in a rural watershed. Water, Air and Soil Pollution. 127:389-401.

Bernardes M.C., L.A. Martinelli, A.V. Krusche, J. Gudeman, M. Moreira, R.L. Victoria, J.P. H.B. Ometto, M.V.R. Ballester, A.K. Aufdenkampe, J.E. Richey, and J.I.

Hedges. 2004. Riverine organic matter composition as a function of land use changes, southwest Amazon. Ecological Applications 14:S263-S279.

Besser J.M., W.G. Brumbaugh, T.W. May, S.E. Church, and B.A. Kimball. 2001. Bioavailability of metals in stream food webs and hazards to brook trout (*Salvelinus fontinalis*) in the Upper Animas River Watershed, Colorado. Archives of Environmental Contamination and Toxicology 40:48-59.

Birch G.F., S.E. Taylor, and C. Mattkai. 2001. Small -scale spatial and temporal variance in the concentration of heavy metals in aquatic sediments: a review and some new concepts. Environmental Pollution 113:357-372.

Bird, M.I., P. Giresse, and A.R. Chivas. 1994. Effect of forest and savanna vegetation on the carbon-isotope composition of sediments from the Sanaga River, Cameroon. Limnology and Oceanography 39:1845-1854.

Boutton T.W., S.R. Archer, and A.J. Midwood. 1999. Stable isotopes in ecosystem science: structure, function and dynamics of a subtropical savanna. Rapid Communications in Mass Spectrometry 13:1263-1277.

Bowen G.J., L.I. Wassenaar, and K.A. Hobson. 2005. Global application of stable hydrogen and oxygen isotopes to wildlife forensics. Oecologia 143:337-348.

Bradac P., B. Wagner, D. Kistlar, J. Traber, R. Behra, and L. Sigg. 2010. Cadmium speciation and accumulation in periphyton in a small stream with dynamic concentration variations. Environmental Pollution 158:641-648.

Breder R. 1982. Optimization studies for reliable trace metal analysis in sediments by atomic absorption spectrometric methods. Fresenius Zeitschrift für Analytysche Chemie 313:395-402.

Brewer GJ. 2010. Risks of Copper and Iron toxicity during aging in humans. Chemical Research and Toxicology 23:319–326.

Brick CM, and J Moore. 1996. Diel variation of trace metals in the Upper Clark Fork River, Montana. Environmental Science and Technology 30:1953-1960.

Brinkman SF, and WD Johnston. 2008. Acute toxicity of aqueous copper, cadmium, and zinc to the mayfly *Rhitrogena hageni*. Archives of Environmental Contamination and Toxicology 54:466-472

Brown S.S., G.R. Gaston, C.F. Rakocinski, and R.W. Heard. 2000. Effects of sediment contaminants and environmental gradients on macrobenthic community trophic structure in Gulf of Mexico estuaries. Estuaries 23:411-424.

British Columbia Ministry of the Environment. 2008. Ambient aquatic life guidelines for iron [electronic resource]: overview report. Retrieved from the British Columbia Ministry of the Environment website: http://www.env.gov.bc.ca/wat/wq/BCguidelines/iron/iron_overview.pdf.

Brutemark A., E. Lindehoff, E. Graneli, and W. Graneli. 2009. Carbon isotope signature variability among cultured microalgae: influence of species, nutrients and growth. Journal of Experimental Marine Biology and Ecology 372:98-105.

Bucci J.P., J.F. Levine, and W.J. Showers. 2011. Spatial variability of the stable isotope (δ 15N) composition in two freshwater bivalves (*Corbicula fluminea* and *Elliptio complanata*). Journal of Freshwater Ecology 26:19-24.

Bunn S.E., P.E. Davies, and D.M. Kellaway 1997. Contributions of sugar cane and invasive pasture grass to the aquatic food web of a tropical lowland stream. Marine and Freshwater Research 48:173-179.

Cabana G., and J.B. Rasmussen. 1994. Modelling food chain structure and contaminant bioaccumulation using stable nitrogen isotopes. Nature 372:255-257.

Cairns J.J., and K.L. Dickson. 1971. A simple method for the biological assessment of the effects of waste discharges on aquatic bottom-dwelling organisms. Journal of Water Pollution Control Federation 43:755-772.

Camargo J.A., and A. Alonso. 2007. Contaminación por nitrógeno inorgánico en los ecosistemas acuáticos: problemas medioambientales, criterios de calidad del agua, e implicaciones del cambio climático. Ecosistemas 16: 98-110.

Cárdenas O.G., J. J. Zamora, and M.E. Flores-Preciado. 2010. Análisis de la expansión del cultivo de agave azul (Agave tequilana Weber) en cinco municipios de la cuenca baja del río Ayuquila Jalisco, y los patrones de cambio en la cobertura vegetal y uso de suelo asociado. Pages 111-126 In P. R. W. Gerritsen and L. M. Martínez-Rivera, eds. Agave azul, sociedad y medio ambiente, Universidad de Guadalajara, Autlán de Navarro, Jal., Mexico.

Carpenter S.R., N.F. Caraco, D.L. Correll, R.W. Howarth, A.N. Sharpley, and V.H. Smith. 1998. Nonpoint pollution of surface waters with phosphorous and nitrogen. Ecological Applications 8:559-568.

Carral E., R. Villares, X. Puente, and A. Carballeira. 1995. Influence of watershed lithology on heavy metal levels in estuarine sediments and organisms in Galicia (North-West Spain). Marine Pollution Bulletin 30:604-608.

Cenci RM, and JM. Martin. 2004. Concentration and fate of trace metals in Mekong River Delta. Science of the Total Environment 332:167-182.

Chang L.W., and L.G. Cockerham. 1994. Toxic metals in the environment. Pages 109-132 *In* L. G. Cockerham and B. S. Shane, eds. *Basic Environmental Toxicology*, CRC Press, Boca Raton, FL.

Chang C.C.Y., P.V. McCormick, S. Newman, and E.M. Elliott. 2009. Isotopic indicators of environmental change in subtropical wetland. Ecological indicators 9:825-836.

Chapin F.S.I., E.S. Zavaleta, V.T. Eviner, R.L. Naylor, P.M. Vitousek, H.L. Reynolds, D.U. Hooper, S. Lavorel, O.E. Sala, S.E. Hobbie, M.C. Mack, and S. Diaz. 2000. Consequences of changing biodiversity. Nature 405:234-242.

Charriau A, L Lesven, Y Gao, M Leermakers, W Baeyens, B Ouddane, and G Billon. 2011. Trace metal behaviour in riverine sediments: Role of organic matter and sulfides. Applied Geomechistry 26:80-90.

Ciszewski D. 2001. Flood-related changes in heavy metal concentrations within sediments of the Biala Przemsza River. Geomorphology 40:205-218.

Clapcott J.E., and S.E. Bunn. 2003. Can C4 plants contribute to aquatic food webs of subtropical streams? Freshwater Biology 48:1105-1116

Coat S., D. Monti, C. Bouchon, and G. Lepoint. 2009. Trophic relationships in a tropical stream food web assessed by stable isotope analysis. Freshwater Biology 54:1028–1041.

Coleman D.C., and B. Fry (eds.). 1991. Carbon isotope techniques. Academic Press.

Corbi J.J., S.S. Trivinho, A. doSantos, and M. del Grande. 2006. Diagnóstico ambiental de metais e organoclorados em córregos adjacentes a áreas de cultivo de cana-de-acúcar (Estado de Sao Paulo, Brasil). Quimica Nova 29:61-65.

Cowie RH. 2002. Apple snails (Ampullariidae) as agricultural pests: their biology, impacts and management. In: Barker GM, eds. Molluscs as crop pests. Wallingford (UK): CABI Publishing. p 145-192.

Croisetiere L, L Hare, and A Tessier. 2006. A field experiment to determine the relative importance of prey and water as sources of As, Cd, Co, Cu, Pb, and Zn for the aquatic invertebrate Sialis velata. Environmental Science and Technology 40:873-879

Croteau M.N., S.N. Luoma, and A.R. Stewart. 2005. Trophic transfer of metals along freshwater food webs: Evidence of cadmium biomagnification in nature. Limnology and Oceanography 50:1511-1519.

Cummins K.W., R.W. Merrit, and P.C.N. Andrade. 2005. The use of invertebrate functional groups to characterize ecosystem attributes in selected streams and rivers in south Brazil. Studies on Neotropical Fauna and Environment 40:69-89.

Cummins K.W., M.A. Wilzbach, D.M. Gates, J.B. Perry, and W.B. Taliaferro. 1989. Shredders and riparian vegetation. Bioscience 39:24-30

Dallinger R., F. Prosi, H. Segner, and H. Back. 1987. Contaminated food and uptake of heavy metals by fish: a review and a proposal for further research. Oecologia (Berlin) 73: 91-98

Davis W.S., and J.E. Lathrop. 1992. Freshwater benthic macroinvertebrate community structure and function. Chapter 8 in: Sediment Classification Methods Compendium. US-EPA Sediment Oversight Technical Committee, Washington, D.C.

Dawson T.E., and R.T.W. Siegwolf. 2007. Using stable isotopes as indicators, tracers, and recorders of ecological change: some context and background. Pages 3-18 *In* T. E. Dawson and R. T. W. Siegwolf, editors. Stable Isotopes as Indicators of Ecological Change, Academic Press, USA.

deBruyn A.M.H., D.J. Marcogliese, and J.B. Rasmussen. 2003. The role of sewage in a large river food web. Canadian Journal of Fisheries and Aquatic Sciences. 60: 1332–1344.

deBruyn A.M.H., and J.B. Rasmussen. 2002. Quantifying assimilation of sewage-derived organic matter by riverine benthos. Ecological applications 12:511-520.

DeNiro M.J., and S. Epstein. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochimica et Cosmochimica Acta 45:341-351.

Deniseger J., A. Austin, and W.P. Lucey. 1986. Periphyton communities in a pristine mountain stream above and below heavy metal mining operations. Freshwater Biology 16:209-218.

Deudero S., A. Box, S. Tejada, and J. Tintoré. 2009. Stable isotopes and metal contamination in caged marine mussel *Mytilus galloprovincialis*. Marine Pollution Bulletin 58:1025-1031.

De Zwart D. 1995. Biomonitoring. Ministry of VROM, The Hague, The Netherlands.

Diebel M., and M.J. Vander Zanden. 2009. Nitrogen stable isotopes in streams: effectsof agricultural sources and trasformations. Ecological Applications 19:1127-1134.

Dodson S. I., R.A. Lillie, and S. Will-Wolf. 2005. Land use, water chemistry, aquatic vegetation, and zooplankton community structure of shallow lakes. Ecological Applications 15:1191-1198.

DOF. (Diario Oficial de la Federación). 2000. Modificación a la Norma Oficial Mexicana NOM-127-SSA1-1994, Salud Ambiental. Agua para uso y consumo humano. Límites permisibles de calidad y tratamientos a que debe someterse el agua pasa su potabilización. México. (20 de junio, 2000).

DOF (Diario Oficial de la Federación). 1996. Norma Oficial Mexicana NOM-001-ECOL-1996 que establece los límites máximos permisibles de contaminantes en las descargas de aguas residuales en aguas y bienes nacionales. México (6 de enero, 1997).

DOF (Diario Oficial de la Federación). 1995a Norma Oficial Mexicana NOM-027-SSA1-1993, Bienes y servicios. Productos de la pesca. Pescados frescos-refrigerados y congelados. Especificaciones sanitarias. México (3 de marzo de 1995).

DOF (Diario Oficial de la Federación). 1995b. Norma Oficial Mexicana NOM-029-SSA1-1993, Bienes y servicios. Productos de la pesca. Crustáceos frescos-refrigerados y congelados. Especificaciones sanitarias. México (27 de febrero de 1995).

DOF (Diario Oficial de la Federación). 1995c. Norma Oficial Mexicana NOM-031-SSA1-1993, Bienes y servicios. Productos de la pesca. Moluscos y bivalvos frescos-refrigerados y congelados. Especificaciones sanitarias. México (6 de marzo de 1995).

DOF. 1989. Acuerdo por el que se establecen los criterios ecológicos de calidad del agua. CE-CCA-001/89. Diario Oficial de la Federación, México. (2 de diciembre, 1989).

Doucett R.R., G. Power, D.R. Barton, R.J. Drimmie, and R.A. Cunjak. 1996. Stable isotope analysis of nutrient pathways leading to Atlantic salmon. Canadian Journal of Fisheries and Aquatic Sciences 53:2058-2066.

Dudka S, and WP Miller. 1999. Accumulation of potentially toxic elements in plants and their transfer to human food chain, Journal of Environmental Science and Health, Part B 34:681-708.

Dudka S, and DC Adriano. 1997. Environmental impacts of metal ore mining and processing: a review. Journal of Environmental Quality 26:590-602.

Ehleringer J.R., and T.E. Cerling. 2002. Stable Isotopes. Pages 544-550 *In* H. A. Mooney and J. G. Canadell, eds. *The Earth System: biological and ecological dimensios of global environmental change*, John and Wiley Sons, Ltd.

Eisler R. 1998. Copper hazards to fish, wildlife and invertebrates: a synoptic review. US Geological Survey, Biological Resources Division, Biological Scientific Report USGS/BRD/BSR-1998-0002.

Eisler R. 1993. Zinc hazards to fish, wildlife and invertebrates: a synoptic review. US Fish and Wildlife Service Contaminant Hazards Reviews Report 26, Maryland, USA.

Farag AM, DA Nimick, BA Kimball, SE Church, DD Harper, and WG Brumbaugh. 2007. Concentrations of metals in water, sediment, biofilm, benthic macroinvertebrates, and fish in the Boulder River Watershed, Montana, and the role of colloids in the metal uptake. Archives of Environmental Contamination and Toxicology 52:397-409.

Farag AM, DF Woodward, JN Goldstein, W Brumbaugh, and JS Meyer. 1998. Concentrations of metals associated with mining waste in sediments, biofilm, benthic macroinvertebrates, and fish from the Coeur d'Alene River Basin, Idaho. Archives of Environmental Contamination and Toxicology 34:119-127.

Fechner LC, C Gourlay-Francé, A Bourgeault, and M-H Tusseau-Vuillemin. 2012. Diffuse urban pollution increases metal tolerance of natural heterotrophic biofilms. Environmental Pollution 162: 311–318.

Finlay J.C., and C. Kendall. 2007. Stable isotope tracing of temporal and spatial variability in organic matter sources to freshwater ecosystems. Pp. 283-333 in (R. Michener and K. Lajtha, eds) Stable isotopes in ecology and environmental science, 2nd. Ed. Blackwell Publishing Ltd, UKGoyer, R.A. 1997. Toxic and essential metal interactions. Annual Review of Nutrition 17:37-50.

Finlay J.C. 2001. Stable-carbon-isotope ratios of river biota: implications for energy flow in lotic food webs. Ecology 82:1052-1064.

Fry B. 2006. Stable Isotope Ecology. Springer Science, New York, USA.

Fry B. 1999. Using stable isotopes to monitor watershed influences on aquatic trophodynamics. Canadian Journal of Fisheries and Aquatic Sciences 56:2167-2171.

Fry B., and Y. C. Allen. 2003. Stable isotopes in zebra mussels as bioindicators of river-watershed linkages. River Research and Applications 19:683-696.

Fu,J., Y. Wang, A. Zhang, Q. Zhang, Z. Zhao, T. Wang, and G. Jiang. 2011. Spatial distribution of polychlorinated biphenyls (PCBs) and polybrominated biphenyl ethers (PBDEs) in an e-waste dismantling region in Southeast China: Use of apple snail (Ampullariidae) as a bioindicator. Chemosphere 82:648-655.

Garcia A.M., D.J. Hoeinghaus, J.P. Vieira, K.O. Winemiller. D.M.L. Motta Marques, M.A. Bemvenuti. 2006. Preliminary examination of food web structure of Nicola Lake (Taim Hydrological System, south Brazil) using dual C and N stable isotope analyses. Neotropical Icthiology 4:279-284.

Gerritsen P.W.R, AJ Lomelí, and C. Ortiz-Arrona. 2005. Urbanización y problemática socioambiental en la Costa Sur de Jalisco, Mexico. Una aproximación. Región y Sociedad 27:107-132.

Glanze,W.D. 1996. Mosby Medical Encyclopaedia. Revised edition. St. Louis, Mo.

Goodyear KL, and S McNeill. 1999. Bioaccumulation of heavy metals by aquatic macro-invertebrates of different feeding guilds: a review. The Science of the Total Environment 229:1-19.

Goyer RA. 1995. Factors influencing metal toxicity. Pp. 31-45 in RA Goyer, CD Klaassen, and MP Waalkes (eds.). Metal Toxicology. Academic Press Inc.

Graf M.S., E. Santana, L.M. Martínez-Rivera, S. García, and J.J. Llamas. 2006. Collaborative governance for sustainable water resources management: the experience of the inter-municipal initiative for the integrated management of the Ayuquila River Basin, Mexico. Environment and Urbanization 18:297-313.

Gustafson L., W. Showers, T. Kwak, J. Levine, and M. Stoskopf. 2007. Temporal and spatial variability in stable isotope compositions of a freshwater mussel: implications for biomonitoring and ecological studies. Oecologia 152: 140-150.

Hamilton S.K., W.M. Lewis, Jr., and S.J. Sippel. 1992. Energy sources for aquatic animals in the Orinoco River floodplain: evidence from stable isotopes. Oecologia 89: 324-330.

Harding J.S., E.F. Benfield, P.V. Bolstad, G.S. Helfman, and E.B.D. Jones. 1998. Stream biodiversity: The ghost of land use Past. Proceedings of the National Academy of Sciences of the United States of America 95:14843-14847.

Hare L, A Tessier, and U Borgmann. 2003. Metal Sources for Freshwater Invertebrates: Pertinence for Risk Assessment. Human and Ecological Risk Assessment: Vol. 9(4):779-793.

He ZL, XE Yang, and PJ Stoffella. 2005. Trace elements in agroecosystems and impacts on the environment. Journal of Trace Elements in Medicine and Biology 19:125–140.

Heaton T.H.E.1986. Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: a review. Chemical Geology (Isotope Geoscience Section), 59:87-102.

Hecky R.E., and R.H. Hesslein. 1998. Contributions of benthic algae to lake food webs as revealed by stable isotope analysis. Jourmal of the North American Benthological Society 14:631-653.

Henne, L. 1997. Development of a community-based biological monitoring program for the Ayuquila River, Jalisco, Mexico: a preliminary study. MsC Thesis, Urban and Regional Planning, University of Illinois Urbana-Champaign, USA.

Henne L.J., D.W. Schneider, and L.M. Martínez-Rivera. 2002. Rapid assessment of organic pollution in a west-central Mexican river using a family-level biotic index. Journal of Environmental Planning and Management 45:613-632.

Hesslein R.H., M.J. Capel, D.E. Fox, and K.A. Hallard. 1991. Stable isotopes of Sulfur, Carbon, and Nitrogen as Indicators of Trophic Level and Fish Migration in the Lower Mackenzie River Basin, Canada. Canadian Journal of Fisheries and Aquatic Sciences 48:2258-2261.

Hicks B.J. 1997. Food webs in forest and pasture streams in the Waikato region, New Zealand: a study based on analyses of stable isotopes of carbon and nitrogen, and fish gut contents. New Zealand Journal of Marine and Freshwater Research 31:651-664.

Hinch S.G., and L.A. Stephenson. 1983. Size- and age specific patterns of trace metal concentrations in freshwater clams from acid sensitive and circumneutral lake. Canadian Journal of Zoology 65: 2436-2442.

Hobson K.A. 1999. Tracing origins and migration of wildlife using stable isotopes: a review. Oecologia 120:314-326.

Hollengberg PF. 2010. Introduction: Mechanisms of metal toxicity special issue. Chemical Research and Toxicology 23(2):292-293.

Hong S, JP Candelone, CC Patterson, and CF Boutron. 1996. History of ancient copper smelting pollution during Roman and Medieval times recorded in Greenland Ice. Science 272 (5259): 246-249.

.Hostettler S. 2007. Land use changes and transnational migration: the impact of remittances in Western Mexico. PhD Thesis Lausanne, Switzerland: Ecole Polytechnique Federal de Lausanne. 200p.

Houghton RA. 1994. The worldwide extent of land-use change. BioScience 44:305-313.

Howard J.K, K.M. Cuffey, and M. Solomon. 2005. Toward using *Margaritifera falcata* as an indicator of base level nitrogen and carbon isotope ratios: insights from two California Coast Range rivers. Hydrobiologia 541:229–236.

Howe P, H Malcolm, and S Dobson. 2004. Manganese and its compounds: Environmental aspects. Consice International Chemical Assessment Document 63. UNEP, ILO and WHO. Geneva, Switzerland.

Huang KM, and S Lin. 2003. Consequences and implications of heavy metal spatial variations in sediments of the Keelung River drainage basin, Taiwan. Chemosphere 53:1113-1121.

Hudson P.F., D.A. Hendrickson, A.C. Benke, A. Varela-Romero, and R. Rodiles-Hernandez. 2005. Rivers of Mexico. Pages 1031-1084 In A.C. Benke and C.E. Cushing, eds. Rivers of North America, Elsevier Academic Press, USA

Hughes R.M., and C.T. Hunsaker. 2002. Effects of landscape change on aquatic biodiversity and biointegrity. Pages 309-329 In K. J. Gutzwiller, ed. Applying landscape ecology in biological conservation. Springer-Verlag, New York, USA.

Hunsaker C.T., and R.M. Hughes. 2002. Effects of landscape change on the physical and chemical components of aquatic ecosystems. Pages 286-308 In K. J. Gutzwiller, ed. Applying landscape ecology in biological conservation. Springer-Verlag, New York, USA.

Hynes H.B.M. 1975. The stream and its valley. Verh. Internat Verein Limnologie 19: 1-15

INEGI (Insituto Nacional de Estadistica y Geografia). 2008. Sistema para la consulta del Anuarios Estadistico del Estado de Jalisco. Ediciôn 2007. Consulted at: (http://www.inegi.org.mx/est/contenidos/espanol/sistemas/Aee07/estatal/jal/index.htm)

Iwashita M, and T Shimamura. 2003. Long-term variations in dissolved trace elements in the Sagami River and its tributaries (upstream area), Japan. Science of the Total Environment 312:167-179.

Jennings S., O. Reñones, B. Morales-Nin, N.V.C. Polunin, J. Moranta, and J. Coll. 1997. Spatial variation in the 15N and 13C stable isotope composition of plants, invertebrates and fishes on Mediterranean reefs: implications for the study of trophic pathways. Marine Ecology Progress Series 146:109-116.

Jepsen D. B., and K.O. Winemiller. Structure of tropical river food webs revealed by stable isotope ratios. Oikos 96: 46–55.

Jolliffe I.T. 2002. Principal Component Analysis. 2nd edition. Springer-Verlag, New York, USA.

Karr J.R. 1991. Assessment of biotic integrity using fish communities. Fisheries 6:21-27.

Karr J.R. 1987. Biological monitoring and environmental assessment: a conceptual framework. Environmental Management 11:249-256.

Karr, J.R., and E.W. Chu. 1997. Biological monitoring: essential foundation for ecological risk assessment. Human and ecological risk assessment 3:993-1004.

Karr J.R., and D.R. Dudley. 1981. Ecological perspective on water quality goals. Environmental Management 5:55-68.

Kendall C. 1998. Tracing nitrogen sources and cycling in catchments. Pp. 519-576 in C. Kendall and J.J. McDonnell (eds.). Isotope tracers in catchment hydrology. Elsevier, Amsterdam, The Netherlands.

Kendall C., Silva S.R., and Kelly V.J. 2001. Carbon and nitrogen isotopic compositions of particulate organic matter in four large rivers systems across the United States. Hydrological Processes 15:1301-1346.

Kidd K.A. 1998. Use of stable isotope ratios in freshwater and marine biomagnification studies. Pages 357-376 In J. Rose, ed. Environmental Toxicology: Current Developments, Overseas Publisher Association, Amsterdam.

Kiffney P.M, and W.H Clements. 2003. Ecological effects of metals on benthic invertebrates. Pp. 135-154 in (Simon TP ed.) Biological response signatures: indicator patterns using aquatic communities. CRC Press, Boca Raton, Fl, USA

Kilgour B.W., K.M. Somers, and D.R. Barton. 2004. A comparison of the sensitivity of stream benthic community indices to effects associated with mines, pulp and paper mills, and urbanization. Environmental Toxicology and Chemistry 23:212-221.

Kohzu A., T. Miyajima, I. Tayasu, C. Yoshimizu, F. Hyodo, K. Matsui, T. Nakano, E. Wada, N. Fujita, and T. Nagata. 2008. Use of stable nitrogen isotope signatures of riparian macrophytes as an indicator of anthropogenic N inputs to river ecosystems. Environmental Science and Technology 42:7837-7841.

Kpee F, O Ekpete, and I Loveday. 2009. Seasonal variation of Cd, Ni, Cu and Pb in catfish, sediment and water samples from Ipo Stream in Ikwerre District of Rivers State, Nigeria. Journal of Applied Science and Environmental Management 13:63-67.

Kruskal, J.B. 1964. Nonmetric multidimensional scaling: a numerical method. Psycometrika 29:115-129.

Lake J.L., R.A. McKinney, F.A. Osterman, R.J. Pruell, J. Kiddon, S.A. Ryba, and A.D. Libby. 2001. Stable nitrogen isotopes as indicators of anthropogenic activities in small freshwater systems. Canadian Journal of Fisheries and Aquatic Sciences 58:870-878.

Lammert M. and J.D. Allan. 1999. Assessing Biotic Integrity of Streams: Effects of Scale in Measuring the Influence of Land Use/Cover and Habitat Structure on Fish and Macroinvertebrates. Environmental Management **23**:257-270.

Lancaster J., and S. Waldron. 2001. Stable isotope values of lotic invertebrates: Sources of variation, experimental design, and statistical interpretation. Limnology and Oceanography 46(3): 723-730

Lasat MM. 2002. Phytoextraction of Toxic Metals. Journal of Environmental Quality 31:109-120.

Layman C.A., D.A. Arrington, C.G. Montaña, and D.M. Post. 2007. Can stable isotope ratios provide for community-wide measures of trophic structure? Ecology **88**:42-48.

Layman, C.A., K.O. Winemiller, and D.A. Arrington. 2005. Describing a Species-Rich River Food Web Using Stable Isotopes, Stomach Contents, and Functional Experiments. Pages 395-406 in Dynamic Food Webs: Multispecies Assemblages, Ecosystem Development and Environmental Change, P.C. de Ruiter, V. Wolters, and J.C. Moore, editors, Elsevier, Amsterdam.

Lefcort H, DP Abbott, DA Cleary, E Howell, NC Keller, and MM. Smith. 2004. Aquatic snails from mining sites have evolved to detect and avoid heavy metals. Archives of Environmental Contamination and Toxicology 46:478-484.

Lehmann V, GMJ. Tubbing, and W Admiral. 1999. Induced metal tolerance in microbenthic communities from three lowland rivers with different metal loads. Archives of Environmental Contamination and Toxicology 36:384-391

Levy O., M. Rosenfeld, Y. Loya, R. Yam, I. Mizrachi, and A. Shemesh. 2010. Anthropogenic stressors and eutrophication processes as recorded by stable isotopes compositions in coral skeletons. Biogeosciences Discussions 7: 7657–7672.

Likens G.E. 2001. Biogeochemistry, the watershed approach: some uses and limitations. Marine and Freshwater Research 52:5-12.

Lindau C.W., R.D. Delaine, and D.P. Alford. 1997. Monitoring nitrogen pollution from sugar runoff using ^{15}N analysis. Water, Air, and Soil Pollution 89: 389-399.

Lomelí J. A. J., P. W. Gerritsen, and C. Ortiz-Arrona. 2003. Diagnóstico integral de la Cuenca Baja del Río Ayuiquila en el Occidente de México. Informe Tecnico NCCR NS/IP5-AL4:1-73.

Luoma S.N. 1983. Bioavailability of trace metals to aquatic organisms – a Review. Science of the Total Environment 28:1-22.

Lyons J., and S. Navarro-Pérez. 1990. Fishes of the Sierra de Manantlan, West-Central Mexico. Southwest. Nat. 35:32-46.

Lyons, J., G. González-Hernández, E. Soto-Galera, and M. Guzmán-Arroyo. 1998. Decline of freshwater fishes and fiseries in selected drainages of west-central Mexico. Fisheries Management 23: 10-18.

Lyons J., S. Navarro-Perez, P.A. Cochran, E. Santana, and M. Guzmán-Arroyo. 1995. Index of biotic integrity based on fish assemblages for the conservation of streams and rivers in West-Central Mexico. Conservation Biology 9:569-584.

MacDonald D.D., C.G. Ingersoll, and T.A. Berger. 2000. Development and Evaluation of Consensus-Based Sediment Quality Guidelines for Freshwater Ecosystems Archives of Environmental Contamination and Toxicology 39:20–31.

MacLeod N.A. 1998. Trophic structure and the effects of agriculture in a headwater stream in southern Ontario using stable isotopes and secondary production. PhD dissertation in Biology, University of Waterloo, Waterloo, Ontario, Canada. 138 pp.

MacLeod N.A., and R.A. Barton. 1998. Effects of light intensity, water velocity, and species composition on carbon and nitrogen stable isotope ratios in periphyton. Canadian Journal of Fisheries and Aquatic Sciences 55: 1919-1925.

Maier G.O., J.D. Toft, and C.A. Simenstad. 2011. Variability in isotopic (δ13C, δ15N, δ34S) composition of organic matter contributing to detritus-based food webs of the Columbia River estuary. Northwest Science 85: 41-54.

Malmqvist B., and S. Rundle. 2002. Threats to the running water ecosystems of the world. Environmental Conservation 29:134-153.

Martinelli L.A., J.P.H.B. Ometto, F.Y. Ishida, T. Ferreira-Domingues, G.B. Nardoto, R. Silva, and J.R. Ehleringer. 2007. The use of carbon and nitrogen stable isotopes to track effects of land-use changes in the Brazilian Amazon region. Pages 301-317 *In* T. E. Dawson and R. T. W. Siegwolf, eds. Stable isotopes as indicators of ecological change, Academic Press, USA.

Martinelli L.A., M.V.R. Ballester, A.V. Krusche, R.L. Victoria, J.A. Camargo, M.C. Bernardes, and J.P.H.B. Ometto. 1999a. Landcover changes and δ13C composition of riverine particulate organic matter in the Piracicaba River Basin (Southeast Region of Brazil). Limnology and Oceanography 44:1826-1833.

Martinelli L.A., M.C. Piccolo, A.R.Townsend, P.M. Vitousek, E. Cuevas, W. McDowell, G.P Robertson, O.C, Santons, and K. Treseder. 1999b. Nitrogen stable isotopic composition of leaves and soil: Tropical versus temperate forests. Biogeochemistry 46:45-65.

Martinelli L.A., R.L.Victoria, B.R. Forsberg, and J.E. Richey. 1994. Isotopic composition of major carbon reservoirs in the Amazon Floodpalin. International Journal of Ecology

Martínez-Rivera L.M., A.M. Carranza, and M. García. 2000. Aquatic ecosystem pollution of the Ayuquila River, Sierra de Manantlan Biosphere Reserve, Mexico. Pages 165-181 *In* M. Munawar, S.G. Lawrence, I.F. Munawar, and D.F. Malley, eds. *Aquatic Ecosystems of Mexico: status and scope*. Backhuys Publishers, Leiden, The Netherlands.

Martínez RLM, D Meza, A Aguirre, and J Corza. 2008. Impacto de metales pesados en el ecosistema acuático del Río Ayuquila. Reporte Técnico. Universidad de Guadalajara. Departamento de Ecología y Recursos Naturales. Autlán de Navarro, Jalisco. Octubre. 24 p.

Martínez RLM, JL Olguín, D Meza, A Aguirre, B Castillo, and S. Garcia. 2007. Diagnóstico de la condiciones de abastecimiento de agua y saneamiento en los municipios de la Iniciativa Intermunicipal para la Gestión Integral de la cuenca del Río Ayuquila. Universidad de Guadalajara. Departamento de Ecología y Recursos Naturales. Autlán de Navarro, Jalisco.

Martínez-Rivera L.M., P. R. W. Gerritsen, J. J. Rosales, H. A. Moreno, S. Contreras, M. A. Solís, C. L. E. Rivera, O. G. Cárdenas, L. I. Iñiguez-Dávalos, R. Cuevas-Guzmán, C. Palomera-García, E. García-Real, G. A. Aguirre, and J. L. Olguín. 2007. Implicaciones socioambientales de la expansión del cultivo de agave azul (1995-2002) en el municipio de Tonaya, Jalisco, México. Pages 265-284 *In* P. Colunga-García, S. A. Larqué, L. E. Eguiarte, and D. Zizumbo-Villarreal, eds. *En lo ancestral hay futuro: del tequila, los mezcales y otros agaves*. CICY;CONACyT;CONABIO;Semarnat-INE, Mérida, Yuc.,Mexico

Mason CF 2002. Biology of freshwater pollution. 4th edition. Pearson Education Limited. Great Britain.Mather, P.M. 1976. Computational methods of multivariate analysis in physical geography. J. Wiley and Sons. London.

Mayer-Pinto M., A.J. Underwood, T. Tolhurst , and R.A. Coleman. 2010. Effects of metals on aquatic assemblages: What do we really know? Journal of Experimental Marine Biology and Ecology 391:1-9

McArthur, JV. and K.K. Moorhead. 1996. Characterization of riparian species and stream detritus using multiple stable isotopes. Oecologia 107:232-238.

McClelland J.W., and I. Valiela. 1998. Linking nitrogen in estuarine producers to land-derived sources. Limnology and Oceanography 43:577-585.

McCune B., and M.J. Mefford. 2006. PC-ORD for Windows: Multivariate analysis of ecological data, version 5.10. MjM Software, Gleneden Beach, Oregon.

McCune B., and J.B. Grace. 2002. Analysis of ecological communities. MjM Software Design, Gleneden Beach, Oregon.

McCutchan J.H. Jr., and W.M. Lewis Jr. 2001. Seasonal variation in stable isotope ratios of stream algae. Verhanlungen des Internationalen verein Limnologie. 27: 3304-3307.

Medina-Pineda N.A. 2002. Estudio hidrobiológico de la cuenca del Rio Armeria para predicciones de un desarrollo sustentable. MsC thesis in Medical Sciences. University of Colima. 120 pp.

Mercado-Silva N., M.R. Helmus, and M.J. Vander Zanden. 2009. The effects of impoundment and non-native species on a river food web in Mexico's central plateau. River Research and Applications 25:1090-1108.

Mercado-Silva N., J. Lyons, E. Díaz-Pardo, A. Gutiérrez-Hernández, C. P. Ornelas-García, C. Pedraza-Lara, and M.J. Vander Zanden. 2006. Long-term changes in the fish assemblage of the Laja River, Guanajuato, central Mexico. Aquatic Conservation: Marine and Freshwater Ecosystems 16:533-546.

Mercado-Silva N., J. Lyons, G. Salgado-Maldonado, and M. Medina-Nava. 2002. Validation of a fish-based index of biotic integrity for streams and rivers in central Mexico. Fish Biology and Fisheries 12:179-191.

Merrill J.C., J.J.P. Morton, and S.D. Soileau. 2001. Metals. Pages 649-698 In A. W.Hayes, ed. Principles and Methods of Toxicology, Taylor and Francis, Philadelphia.

Merrit R.W., and K.W. Cummins. 1996. An introduction to the aquatic insects of North America. Kendall/Hunt Publishing Company, Dubuque, IO.

Metcalfe-Smith JL, RH Green, and LC Grapentine. 1996. Influence of biological factors on concentrations of metals in the tissues of freshwater mussels (Elliptio complanata and Lampsilis radiata radiata) from the St. Lawrence River. Canadian Journal of Fisheries and Aquatic Sciences 53:205-219.

Meyer W.B., and Turner II, B.L. 1992. Human population growth and global landuse/cover change. Annual Review of Ecology and Systematics 23:39-61.

Meza D. 2006. Caracterización hidrográfica de la cuenca Ayuquila-Armeria mediante la aplicación de un sistema de informacion geográfica. Tesis Ingenieria en Recursos Naturales y Agropecuarios. Autlan de Navarro, Jal, Mexico: Universidad de Guadalajara, Centro Universitario de la Costa Sur.

Minagawa M., and E. Wada. 1984. Stepwise enrichment of 15N along food chains: Further evidence and the relation between δ15N and animal age. Geochimica et Cosmochimica Acta 48:1135-1140.

Minshall G.W., K.W. Cummins, R.C. Petersen, C.E. Cushing, D.A. Bruns, J.R. Sedell, and R.L. Vannote. 1985. Developments in stream ecosystem theory. Canadian Journal of Fisheries and Aquatic Sciences 42:1045-1055.

Morrisey C.A., L.I. Bendell-Young, and J.E. Elliott. 2005a. Identifying sources and biomagnification of persistent organic contaminants in biota from mountain streams of southwestern British Columbia, Canada. Environmental Science and Technology 39:8090-8098.

Morrisey C.A., L.I. Bendell-Young, and J.E. Elliot. 2005b. Assessing trace-metal exposure to American dippers in mountain streams of southwestern British Columbia, Canada. Environmental Toxicology and Chemistry 24:836-845.

Newman M. C., M. A. Unger. 2003. Fundamentals of Ecotoxicology. Lewis Publishers, Boca Raton, Fl.

Newsome S.D., C. Martinez del Rio, S. Bearhop, and D.L. Phillip. 2007. A niche for isotopic ecology. Frontiers in Ecology and the Environment 5:429-436.

Nicolau R, A Galera-Cunha, and Y Lucas. 2006. Transfer of nutrients and labile metals from the continent to the sea by a small Mediterranean river. Chemosphere 63:469-476.

Nriagu JO. 1996. A history of global metal pollution. Science 272:223-224.

Nriagu JO, and JM Pacyna. 1988. Quantitative assessment of worldwide contamination of air, water and soils by trace metals. Nature 333:134-139.

Ojima D.S., K.A. Galvin, and B.L. Turner II. 1994. The global impact of land-use change. Bioscience 44:300-304.

Omernik JM, and RG Bailey. 1997. Distinguishing between watersheds and ecoregions. Journal of the American Water Resources Association.33:935-349

Ometto J.P.H.B., L.A. Martinelli, M.V.R. Ballester, A. Gessner, A.V. Krusche, R.L. Victoria, and M. Williams. 2000. Effects of land use on water chemistry and macroinvertebrates on two streams of the Piracicaba river basin, southeast Brazil. Freshwater Biology 44:327-337.

O'Neill R. V., C.T. Hunsaker, K.B. Jones, K.H. Riiters, J.D. Wickham, P.M. Schwartz, I.A. Goodman, B.L. Jackson, and W.S. Baillargeon. 1997. Monitoring environmental quality at the landscape scale. Bioscience 47:513-519.

Parkyn S.M., K.J. Collier, and B.J. Hicks. 2001. New Zealand stream crayfish: functional omnivores but trophic predators? Freshwater Biology 46:641-652.

Peterson B. J., and B. Fry. 1987. Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics 18:293-320.

Peterson B.J., R.W. Howarth, and R.H. Garrit. 1985. Mutliple stable isotopes used to trace flow of organic matter in estuarine food webs. Science 227:1361-1363.

Phillips DJH, and PS Rainbow. 1989. Strategies of trace metal sequestration in aquatic organisms. Marine Enviromental Research 28:207-210.

Pimm S.L., and J.H. Lawton. 1977. Number of trophic levels in ecological communities. Nature 268:329-331.

Pires, L.F., Bacchi, O.O.S., V. Correchel , K Reichardt, and J. Filippe. 2009. Riparian forest potential to retain sediment and carbon evaluated by the ^{137}Cs fallout and carbon isotopic ratio techniques. Annais da Academia Brasileira de Ciencias 81:271-279.

Post D. M. 2002. Using stable isotopes to estimate trophic position: models, methods, and caveats. Ecology 83:703-718.

Postel S.L. 2000. Entering an era of water scarcity: the challenges ahead. Ecological Applications 10:941-948.

Pringle C.M., and T. Hamazaki. 1998. The role of omnivory in a Neotropical stream: separating diurnal and nocturnal effects. Ecology 79:269-280.

Pringle C. M., G.A. Blake, A. P. Covich, K.M. Buzby, and A. Finley. 1993. Effects of omnivorous shrimp in a montane tropical stream: sediment removal, disturbance of sessile invertebrates and enhancement of understory algal biomass. Oecologia 93:1-11.

Quinn MR, X Feng, CL Folt, and CP Chamberlain. 2003. Analyzing trophic transfer of metals in stream food webs using nitrogen isotopes. The Science of the Total Environment 317:73–89.

Rainbow PS. 2002. Trace metal concentrations in aquatic invertebrates: why and so what? Environmental Pollution 120:497-507.

Reinfelder JR and Fisher NS. 1991. The assimilation of elements ingested by marine copepods. Science 251:794-796.

Rhind SM. 2009. Anthropogenic pollutants: a threat to ecosystem sustainability? Philosophical Transactions of the Royal Society B. 364:3391-3401.

Richardson JS, and PM Kiffney. 2000. Responses of a macroinvertebrate community from a pristine southern British Columbia, Canada, stream to metals in experimental mesocosms. Environmental Toxicology and Chemistry 19(3):736–743.

Rosi-Marshall, E.J., and J.B. Wallace. 2002. Invertebrate food webs along a stream resource gradient. Freshwater Biology 47:129-141.

Roth N.E., J.D. Allan, and D.L. Erickson. 1996. Landscape influences on stream biotic integrity assessed at multiple scales. Landscape Ecology 11:141-156.

Rounick J.S., and M.J. Winterbourn. 1986. Stable carbon isotopes and carbon flow in ecosystems. Bioscience 36: 171-177.

Santana C.E., S. Navarro-Perez, L.M. Martinez-Rivera, A. Aguirre, P. Figueroa, and C. Aguilar. 1993. Contaminación, aprovechamiento y conservación de los recursos acuáticos del rio Ayuquila, Reserva de la Biosfera Sierra de Manantlan, Jalisco-Colima. Tiempo de Ciencias 30:29-38.

Schindler, D.W., and Lubetkin, S.C. 2004. Using stable isotopes to quantify material transport in food webs. In Food webs at the landscape level. Edited by G.A. Polis, M.E. Power and G.R. Huxel. The University of Chicago Press, Chicago. pp. 25-42.

Schmitt C. 2004. Concentrations of arsenic, cadmium, copper, lead, selenium, and zinc in fish from the Mississippi River Basin, 1995. Environmental Monitoring and Assessment 90: 289-321.

Schwartz, D., A. Mariotti, R. Lanfranchi, and B. Guillet. 1986. 13C/12C ratios of soil organic matter as indicators of vegetation changes in The Congo. Geoderma 39:97-103.

Schorer M., and M. Eisele. 1997. Accumulation of inorganic and organic pollutants by biofilm in the aquatic environments. Water, Air, and Soil Pollution 99:651-659.

Shanley J., E. Pendall, C. Kendall, L.R. Stevens, R.L. Michel, P.J. Phillips, R.M. Forester, D. L. Naftz, B. Liu, L. Stern, B.B. Wolfe, and C.P. Chamberlain. 1998. Isotopes as indicators of environmental change. Pages 761-816 In C. Kendall and J. J. McDonnell, eds. Isotope Tracers in Catchment Hydrology, Elsevier, Amsterdam.

Shiklomanov, I.A. 1998. A summary of the monograph World Water Resources. UNESCO.,

Shiklomanov, I.A. 1993. World freshwater resources. Pages 13-24 In PH. Gleick ed. Water in Crisis: a Guide to World's Freshwater Resources, Oxford University Press, New York, USA.

Smical AI, V Hotea, V Oros, J Juhasz, and E Pop. 2008. Studies on transfer and bioaccumulation of heavy metals from soil into lettuce. Environmental Engineering and Management Journal 7:609-615.

Smith S, M-H Chen, RG Bailey, and WP Williams. 1996. Concentration and distribution of copper and cadmium in water, sediments, detritus, plants and animals in a hardwater lowland river. Hydrobiologia 341:71-80.

Sousa J.M., C.S. Martens, M.Z. Moreira, R.L. Lima, I.C. Goncalves, H.P. Mendlovitz, and M.C. Menton. 2008. Spatial and seasonal variations in the stable carbon isotopic composition of methane in stream sediments of eastern Amazonia. Tellus 60B:21-31.

Staal M., R. Thar, M.Kühl, M.C.M. van Loosdrecht, G. Wolf, J.F.C. de Brouwer, and J.W. Rijstenbil. 2007. Different carbon isotope fractionation patterns during the development of phototrophic freshwater and marine biofilms. Biogeosciences 4: 613-626.

Stevens, R.E., A.M. Lister, and R.E.M. Hedges. 2006. Predicting diet, trophic level and palaeoecology from bone stable isotope analysis: a comparative study of five red deer populations. Oecologia 149:12-21.

Thomas S.M., C. Neill, L.A. Deegan, A.V. Krusche, M.V.R. Ballester, and R.L. Victoria. 2004. Influences of land use and stream size on particulate and dissolved materials in a small Amazonian stream network. Biogeochemistry 68:135-151.

Thompson D.R., S.J. Bury, K.A. Hobson, L. I. Wassenaar, and J. P. Shannon. 2005. Stable isotopes in ecological studies. Oecologia 144:517-519.

Timmermans KR, B van Hattam, MHS Kaak, and C Davids. 1989. Trace metals in a littoral foodweb: Concentrations in organisms, sediment and water. The Science of the Total Environment 87/88:477-494.

Tomanova S., P.A. Tedesco, M. Campero, P.A. Van Damme, N. Moya, and T. Oberdorff 2007. Longitudinal and altitudinal changes of macroinvertebrate functional feeding groups in neotropical streams: a test of the River Continuum Concept. Fundamental and Applied Limnology. Archiv für Hydrobiologie. 170/3: 233–241.

Tomanova S., E. Goitia, and J. Helešic. 2006. Trophic levels and functional feeding groups of macroinvertebrates in neotropical streams. Hydrobiologia 556:251–264.

Trimble C.C., and C.A. Macko. 1997. Stable isotope analysis of human remains: a tool for cave archaelogy. Journal of Cave and Karst Studies 59:137-142.

Tulonen T, M Pihlström, L Arvola, and M Rask. 2006. Concentrations of heavy metals in food web components of small boreal lakes. Boreal Environment Research 11:185-194.Tomanova S. N. Moya,, and T. Oberdorff. 2008. Using macroinvertebrate biological traits for assessing biotic integrity of neotropical streams. River Resarch and Applications 24: 1230-1239.

US-EPA 2012. Ecological Risk Assessment. Freshwater Sediment Screening Benchmarks (http://www.epa.gov/reg3hwmd/risk/eco/btag/sbv/fwsed/screenbench.htm)

Vanderklift M.A, and Ponsard S. 2003. Sources of Variation in Consumer-Diet δ 15N Enrichment: A Meta-Analysis. Oecologia 136: 169-182

VanderZanden M.J., and J.B. Rasmussen. 2001. Variation in $\delta^{15}N$ and $\delta^{13}C$ trophic fractionation: implications for aquatic food webs. Limnology and Oceanography 46: 2061-2066.

Vander Zanden M. J. and J.B. Rasmussen. 1999. Primary consumer δ13C and δ15N and the trophic position of aquatic consumers. Ecology 80: 1395-1404.

Vander Zanden M.J., Y. Vadeboncoeur, M. Diebel, and E. Jeppesen. 2005. Primary consumer stable nitrogen isotopes as indicators of nutrient source. Environmental Science and Technology. 39:7509-7515.

Vander Zanden M. J., B. J. Shuter, N. Lester, and J. B. Rasmussen. 1999. Patterns of Food Chain Length in Lakes: A Stable Isotope Study. American Naturalist 154:406-416.

Van Griethuysen C., J. Van Baren, T.H.M. Peeters, and A.A. Koelmans. 2004. Trace metal availability and effects on benthic community structure in floodplain lakes. Environmental Toxicology and Chemistry 23:668-681.

Vannote R.L., G.W. Minshall, K.W. Cummins, J.R. Sedell, and C.E. Cushing. 1980. The river continuum concept. Canadian Journal of Fisheries and Aquatic Sciences 37:130-137.

Verburg P., S. S. Kilham, C. M. Pringle, K. R. Lips, and D. L. Drake. 2007. A stable isotope study of a neotropical stream food web prior to the extirpation of its large amphibian community. Journal of Tropical Ecology 23:643-651.

Viarengo A. 1985. Biochemical effects of trace metals. Marine Pollution Bulletin 16:153-158.

Vitorello V.A., C.G.Cerri, F. Andreux, and R. Victoria. 1989. Organic matter and natural C13 distribution in forest and cultivates soils. Soil Science Society of America Journal 53:773-778.

Vizzini S. and A. Mazzola. 2003. Seasonal variations in the stable carbon and nitrogen isotope ratios (13C/12C and 15N/14N) of primary producers and consumers in a western Mediterranean coastal lagoon. Marine Biology 142:1009-1018.

Vizzini S. and A. Mazzola. 2004. Stable isotope evidence for the environmental impact of a land-based fish farm in the western Mediterranean. Marine Pollution Bulletin 49:61-70.

Vörösmarty CJ, PB McIntyre, MO Gessner, D Dudgeon, A Prusevich, P Green, S Glidden, SE Bunn, CA Sullivan, C ReidyLiermann, and PM Davies. 2010. Global threats to human water security and river biodiversity. Nature 467:555-561

Vuori KM. 1995. Direct and indirect effects of iron on river ecosystems. Annales Zoologici Fennici 32:317-329.

Vuorio K., M. Meili, and J. Sarvala. 2006. Taxon-specific variaton in the stable isotopic signatures (δ13C and δ15N) of lake phytoplankton. Freshwater Biology 51: 807-722.

Wallace J.B., and J.R. Webster. 1996. The role of macroinvertebrates in stream ecosystem functioning. Annual Review of Entomology 41:115-139.

Walters D.M., A.H. Roy, and D.S. Leigh. 2009. Environmental indicators of macroinvertebrate and fish assemblage integrity in urbanizing watersheds. Ecological Indicators 9:1222-1233.

Wang L., and J. Lyons. 2003. Fish and benthic macroinvertebrate assemblages as indicators of stream degradationin urbanizing watersheds. Pages 227-249 In T. P. Simon, ed. Biological response signatures. Indicator patterns using aquatic communities, CRC Press, Boca Raton, Fl.

Wang L., J. Lyons, P. Kanehl, and R. Gatti. 1997. Influences of watershed land use on habitat quality and biotic integrity in Wisconsin streams. Fisheries 22:6-12.

Wayland M., and K.A. Hobson. 2001. Stable carbon, nitrogen, and sulfur isotope ratios in riparian food webs on rivers receiving sewage and pulp-mill effluents. Canadian Journal of Zoology 79:5-15

Weigel B.M., L.J. Henne, and L.M. Martínez-Rivera. 2002. Macroinvertebrate-based index of biotic integrity for protection of streams in west-central Mexico. Journal of the North American Benthological Society 21:686-700.

Widianarko B, CAM van Gestel, RA Verweij, and NM van Straalen. 2000. Associations between trace metals in sediment, water, and guppy Poecilia reticulata (Peters), from urban streams in Semarang, Indonesia. Ecotoxicology and Environmental Safety 46:101-107

Wyżga B, and D Ciszewski. 2010. Hydraulic controls on the entrapment of heavy metal-polluted sediments on a floodplain of variable width, the upper Vistula River, southern Poland. Geomorphology 117:272–286.

Yoshinaga, J., T. Suzuki, T. Hongo, M. Minagawa, R. Ohtsuka, R. Kawabe, T. Inaoka, and T. Akimichi. 1992. Mercury concentration correlates with the nitrogen stable isotope ratio in the animal food of Papuans. Ecotoxicology and Environmental Safety 24:37-45.

Zimmerman J.K.H., B. Vondracek, and J. Westra. 2003. Agricultural land use effects on sediment loading and fish assemblages in two Minnesota (USA) watersheds. Environmental Management 32:93-105.

Appendix 2.1. Relative invertebrate abundance (%) per family per site during Wet07 season, and the assigned Functional Feeding Group (FFG)

Family	FFG	1.FoTa	2.IaFo	4.Ia	8.Ia	10.Fo	11.IaFo	12.IaFo	a.Fo	c.Ur	13.IaFo	TOTAL
Mollusca												
Pomacea. flagellata	Scr			3	2							**5 (1.2)**
Corbicula. fluminea	Fc		20 (95.2)				8					**28 (6.9)**
Ephemeroptera												
Baetidae	Gc	1 (1.0)										**1 (0.3)**
Heptageniidae	Gc	57 (55.9)								2 (8.3)	5 (9.8)	**64 (15.7)**
Leptophlebidae	Gc	6 (5.9)						15 (17.7)				**21 (5.2)**
Trichoptera												
Hydropsychidae	Fc	4 (3.9)				40 (58.8)		17 (20.0)	29 (67.4)		8 (15.7)	**98 (24.1)**
Plecoptera												
Perlidae	Pr	4 (3.9)				10 (14.7)		2 (2.4)				**16 (3.9)**
Odonata												
Libellulidae	Pr					2 (2.9)		1 (1.2)	1	2 (8.3)		**6 (1.5)**
Coenagrionidae	Pr	11(10.8)				11 (16.2)		13 (15.3)	10 (23.3)	1 (4.2)		**46 (11.3)**
Diptera												
Tabanidae	Pr	2 (2.0)							1 (2.3)	1 (4.2)	1 (2)	**5 (1.2)**
Chironomidae	Gc											
Simulidae	Fc											
Megaloptera												
Corydalidae	Pr	4 (3.9)	1 (4.8)			2 (2.9)		5 (5.9)	2 (4.7)	11 (45.8)	2 (3.9)	**27 (6.6)**
Coleoptera												
Elmidae	Scr	7 (6.9)				1 (1.5)		29 (34.1)			23 (45.1)	**60 (14.7)**
Psephenidae	Scr					2 (2.9)		1 (1.2)		3 (12.5)	12 (23.5)	**18 (4.4)**
Heteroptera												
Naucoridae	Pr	6 (5.9)						2 (2.4)		4 (16.7)		**12 (2.9)**
Total		**102**	**21**	**3**	**2**	**68**	**8**	**85**	**43**	**24**	**51**	**407**

Appendix 2.2. Total and relative invertebrate abundance per family per site during Dry08 season. Functional Feeding Group (FFG): Sc=scraper; FC= Filter collector; Gc= Gather collector; Pr= predator; Sh= shredder; Om= omnivore.

Family	FFG	1.FoTa	2.IaFo	3.Ia	4.Ia	8.Ia	10.Fo	11.IaFo	12.IaFo	a.Fo	d.FoUr	13.IaFo	TOTAL
Mollusca													
Ampullaridae	Scr		10 (8.1)							1 (0.9)			**11 (0.84)**
Corbiculidae	FC		20 (95.2)			4 (22.2)						15 (9.0)	**37 (2.84)**
Ephemeroptera													
Baetidae	GC	1 (1.2)					12 (7.9)	10 (7.8)			81 (72.3)		**104 (7.98)**
Heptageniidae	GC	5 (5.8)	29 (23.6)	28 (18.3)	83 (85.6)		10 (6.6)	45 (35.2)	65 (43.3)			40 (24.0)	**305 (23.4)**
Leptophlebidae	GC	26 (30.2)								36 (34.0)		41 (24.6)	**103 (7.90)**
Trycorythidae	GC	4 (4.7)											**4 (0.31)**
Trichoptera													
Hydropsychidae	FC	23 (26.7)	58 (47.2)	104 (68.0)	3 (3.1)	6 (66.6)	69 (45.4)	42 (32.8)	20 (13.3)	34 (32.1)	3 (2.7)	21 (12.6)	**383 (29.4)**
Plecoptera													
Perlidae	Pr	2 (2.3)		1 (0.7)			18 (11.8)	1 (0.8)	6 (4.0)			10 (6.0)	**38 (2.92)**
Odonata													
Libellulidae	Pr		4 (3.3)	2 (1.3)	2 (2.1)	1 (11.1)	3 (2.0)		4 (2.7)	20 (18.9)	1 (0.9)	6 (3.6)	**43 (3.30)**
Calopterygidae	Pr	1 (1.2)								2 (1.9)			**3 (0.23)**
Coenagrionidae	Pr	3(3.5)	4 (3.3)		5 (5.3)		3 (2.0)	21 (16.4)	11 (7.3)	10 (9.4)	8 (7.1)	4 (2.4)	**69 (5.29)**
Diptera													
Tabanidae	Pr		7 (5.7)	4 (2.6)	1 (1.0)				5 (3.3)		4 (3.6)	3 (1.8)	**24 (1.84)**
Chironomidae	GC	2 (2.3)						2 (1.6)	1 (0.7)	2 (1.9)	1 (0.9)		**8 (0.61)**
Simulidae	FC									1 (0.9)			**1 (0.07)**
Megaloptera													
Corydalidae	Pr	1 (1.2)	9 (7.3)	10 (6.5)	1 (1.0)		13 (8.6)	5 (3.9)	5 (3.3)		7 (6.3)	8 (4.8)	**59 (4.53)**
Lepidoptera													
Pyralidae	Sh	2 (2.3)					1 (0.7)						**3 (0.23)**
Coleoptera													
Elmidae	Sc	8 (9.3)	2 (1.6)	4 (2.6)			16 (10.5)	1 (0.8)	26 (17.3)		7 (6.3)	16 (9.6)	**80 (6.14)**
Psephenidae	Sc						2 (1.3)		4 (2.7)			1 (0.6)	**7 (0.54)**
Heteroptera													
Naucoridae	Pr	7 (8.1)			1 (1.0)		5 (3.3)		3 (2.0)			2 (1.2)	**18 (1.38)**
Decapoda													**3 (0.23)**
Pseudothelphusidae	Om	1 (1.2)			1 (1.0)			1 (0.8)					
Total		**86**	**143**	**153**	**97**	**11**	**152**	**128**	**150**	**106**	**112**	**167**	**1305**

Appendix 2.3. Total and relative invertebrate abundance per family per site during Wet08 season. Functional Feeding Group (FFG): Sc=scraper; FC= Filter collector; Gc= Gather collector; Pr= predator; Sh= shredder; Om= omnivore.

Family	FFG	1.FoTa	2.IaFo	3.Ia	4.Ia	8.Ia	10.F0	11.IaFo	12.IaFo	a.Fo	c.Ur	d.FoUr	13.IaFo	TOTAL
Mollusca														
P. flagellata	Sc		3(4.6)	3(3.2)						1(8.3)				**7 (0.6)**
C. fluminea	Fc			6 (6.3)				2 (1.4)					4 (2.3)	**12 (1.0)**
Ephemeroptera														
Baetidae	Gc		17 (26.2)	4 (4.2)	11(61.1)			10 (7.0)	27 (10.5)		24 (38.1)	12 (13.6)	13 (7.3)	**118 (9.7)**
Heptageniidae	Gc								20 (7.8)					**20 (1.7)**
Leptophlebidae	Gc	80 (51.6)	2 (3.1)			41(80.4)	17 (19.3)	77 (54.2)	60 (23.4)		3 (4.8)	42 (47.7)	79 (44.6)	**416 (34.3)**
Trycorythidae	Gc	5 (3.2)		4 (4.2)	1 (5.6)		2 (2.3)	6 (4.2)			3 (4.8)	9 (10.2)	22 (12.4)	**52 (4.3)**
Trichoptera														
Hydropsychidae	Fi	4 (2.6)	13 (20.0)	39 (41.1)		2 (3.9)	23 (26.1)	19 (13.4)	52 (20.2)	3 (25.0)	1 (1.6)	13 (14.8)	32 (18.1)	**201 (16.6)**
Plecoptera														
Perlidae	Pr	6 (3.9)					4 (4.5)							**10 (0.8)**
Odonata														
Gomphidae	Pr										1 (1.6)		1 (0.6)	**2 (0.2)**
Libellulidae	Pr	1 (0.6)				1 (1.9)			1 (0.4)					**3 (0.2)**
Calopterygidae	Pr				1 (5.6)				1 (0.4)					**2 (0.2)**
Coenagrionidae	Pr	30 (19.4)	4 (6.2)	9 (9.5)	1 (5.6)	2 (3.9)		12 (8.4)	11 (4.3)	6 (50.0)			4 (2.3)	**79 (6.5)**
Diptera														
Tabanidae	Pr	1 (0.6)	2 (3.1)	6 (6.3)	1 (5.6)	2 (3.9)	1 (1.1)		3 (1.2)					**16 (1.2)**
Chironomidae	Gc		3 (4.6)	1 (1.1)					6 (2.3)	1 (8.3)				**13 (1.1)**
Simulidae	Fc		5 (7.7)								12 (19.1)			**17 (1.4)**
Megaloptera														
Corydalidae	Pr	2 (1.3)	1 (1.5)	1 (1.1)			3 (3.4)	3 (2.1)	1 (0.4)			1 (1.1)	3 (1.7)	**15 (1.2)**
Lepidoptera														
Pyralidae	Sh	1 (0.6)					1 (1.1)			1 (8.3)				**3 (0.2)**
Coleoptera														
Elmidae	Sc	11 (7.1)	9 (13.8)	4 (4.2)		2 (3.9)	11 (12.5)	1 (0.7)	38 (14.8)		7 (11.1)	2 (2.3)	2 (1.2)	**87 (7.2)**
Psephenidae	Sc						1 (1.1)		30 (11.7)		1 (1.6)		4 (2.3)	**36 (3.0)**
Heteroptera														
Naucoridae*	Pr	10 (6.4)	6 (9.2)			1 (1.9)	25 (28.4)	8	5 (1.9)		11 (17.5)	9 (10.2)	12 (6.8)	**87 (7.2)**
Belastomatidae	Pr							2 (1.4)						**2 (0.2)**
Decapoda	Om	4 (2.6)		3 (3.2)	3 (16.7)			2 (1.4)	2 (0.8)				1 (0.6)	**15 (1.2)**
TOTAL		**155**	**65**	**95**	**18**	**51**	**88**	**142**	**257**	**12**	**63**	**88**	**177**	**1211**

Appendix 2.4. Invertebrate Community metrics considered per site for each field season.

		Ayuquila Sub-basin								Tuxcacuesco Sub-basin			Armeria Sub-basin	TOTAL
		1.FoTa	2.IaFo	3.Ia	4.Ia	8.Ia	10.Fo	11.IaFo	12.Ia.Fo	a.Fo	c.Ur	d.FoUr	Armr	(avg±SD)*
Wet07[1]	**Total abundance**	102	21	-	3[2]	-	68	13[2]	85	43	24		51	**412**
	Invertebrate Richness (S)[2]	12	2	-	1	-	7	1	9	6	7		6	**14 (4.5)**
	Shannon Index H' (ln)	1.59	0.19	-	-	-	1.26	-	1.73	0.92	1.59		1.42	**(0.79)**
	Number of FFG	4	2	-	-	-	2	-	3	3	3		3	
	% EPT	70.6	-	-	-	-	73.5	-	40.0	67.4	**8.33**		25.5	**(47.5±27.1)**
	B-IBI	3.95	n.e.d.	-	n.e.d.	-	4.4	n.e.d.	4.07	5.14	2.21		3.88	**(3.4±1.7)**
Dry08	**Total abundance**	86	143	153	97	9	152	127	150	106		112	167	**1302**
	Invertebrate Richness (S)[2]	14	9	7	8	3	11	8	11	8		8	12	**20 (9)**
	Shannon Index H' (ln)	2.03	1.73	1.03	0.66	0.85	1.8	1.5	1.79	1.51		1.07	2.07	**(1.5)**
	Number of FFG	5	4	4	4	2	4	4	4	3		4	4	
	% EPT	70.9	70.7	86.9	88.7	54.5	71.7	76.5	60.7	66.0		75	67.1	**(71.7±10.1)**
	B-IBI	3.64	4.47	3.84	4.37	5.55	3.54	4.69	4.35	4.85		4.24	3.66	**(4.3±1.7)**
Wet08	**Total abundance**	155	65	95	18	51	88	142	257	12	63	88	177	**1211**
	Invertebrate Richness (S)[2]	13	12	12	6	7	11	11	15	4	10	7	13	**24 (10.2)**
	Shannon Index H' (ln)	1.64	2.19	1.94	1.24	0.84	1.84	1.59	2.18	1.31	1.8	1.51	1.75	**(1.65)**
	Number of FFG	6	4	5	3	4	5	5	5	5	4	4	5	
	% EPT	61.3	49.2	65.3	66.7	84.3	52.3	78.9	61.9	25.0	49.2	86.4	82.5	**(63.6±18.1)**
	B-IBI	3.95	4.86	4.58	4.78	2.78	3.68	3.46	3.91	7.08	4.48	3.1	3.27	**(4.2±1.1)**

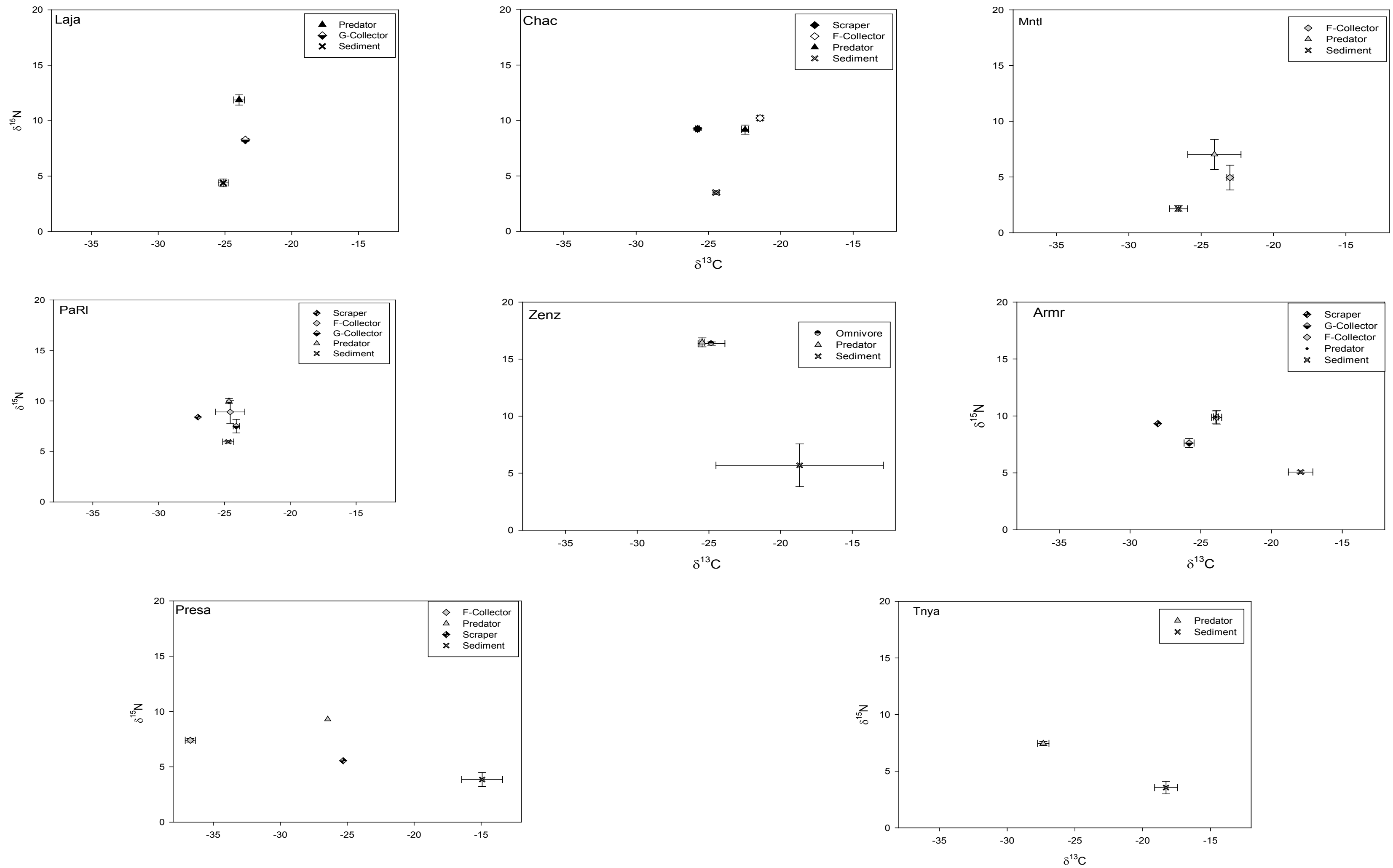
[1]Five sites (3.Ia, 4Ia, 8.Ia, 11.IaFo and d.FoUr) were not sampled due to flooded conditions in wet07. [2]Taxa richness (S) is at the family level. (n.e.d)= not enough data to calculate that index. [3]Site c.Ur was not sampled in dry08.

Appendix 2.5. Fish community metrics per site for two seasons.

	Ayuquila Sub-basin										Tuxcacuesco Sub-basin				Armeria Sub-basin	TOTAL
	1.FoTa	2.IaFo	3.Ia	4.Ia	6.Ia	8.Ia	9.Ia	10.Fo	11.IaFo	12.IaFo	a.Fo	b.FoIa	c.Ur	d.FoUr	Armr	(avg)
Dry 08																
Total abundance	38	2	6	56	72	92	16	10	2	100	13	NS	NS	83	62	**552 (42.5)**
Species richness	2	1	2	2	3	3	2	1	1	6	4	NS	NS	6	3	**10 (2.8)**
Shannon Index H'	0.62	n.e.d	0.64	0.69	0.79	0.85	0.23	n.e.d.	n.e.d.	1.47	0.94	NS	NS	0.95	0.46	**(0.59)**
Fish IBI	30	15	30	40	50	45	40	35	35	55	55	NS	NS	55	40	**(40)**
Trophic guilds	2	1	2	2	2	2	2	1	1	3	1	NS	NS	2	1	**3**
Wet 08																
Total abundance	31	15	43	44	73	179	1	23	2	75	10	6	6	62	102	**671 (44.5)**
Species richness	2	3	5	2	3	6	1	2	1	5	7	3	3	7	7	**14 (3.8)**
Shannon Index H'	0.14	0.77	0.65	0.69	1.1	1.23	n.e.d.	0.39	n.e.d.	1.31	1.83	0.87	1.01	1.21	1.55	**(0.85)**
Fish IBI	20	40	50	30	50	75	35	65	35	70	55	35	40	65	50	**(48)**
Trophic guilds	2	3	3	2	2	2	1	2	-	3	3	1	2	2	3	**3**

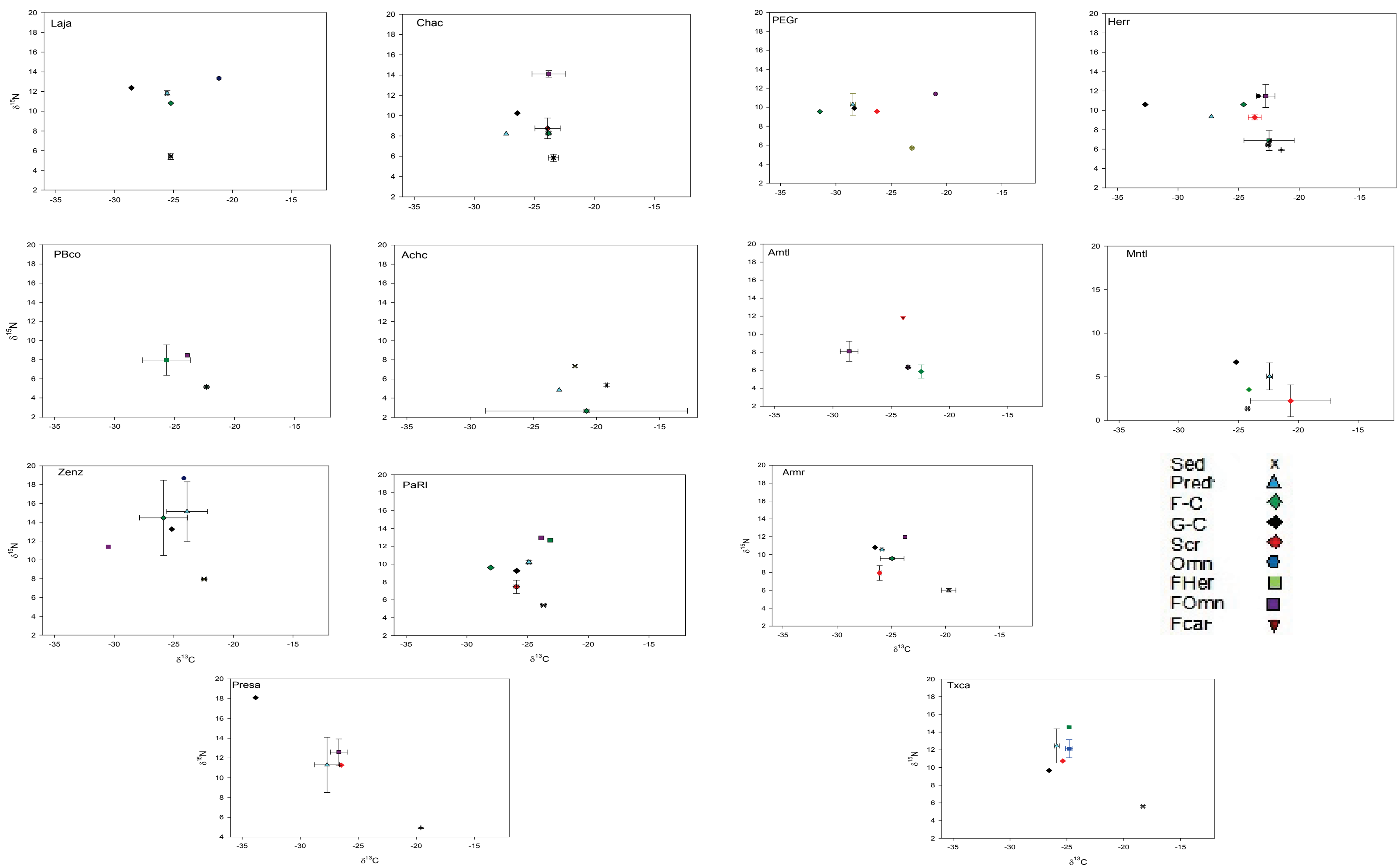
NS=Not sampled. (n.e.d)= not enough data to get that index. Numbers in parenthesis are average.

Appendix 3.1. Dual mean (± 1 s.e.) $\delta^{15}N$ /$\delta^{13}C$ biplots for each site in Wet07 in: a) Ayuquila River, and b) Tuxcacuesco River.



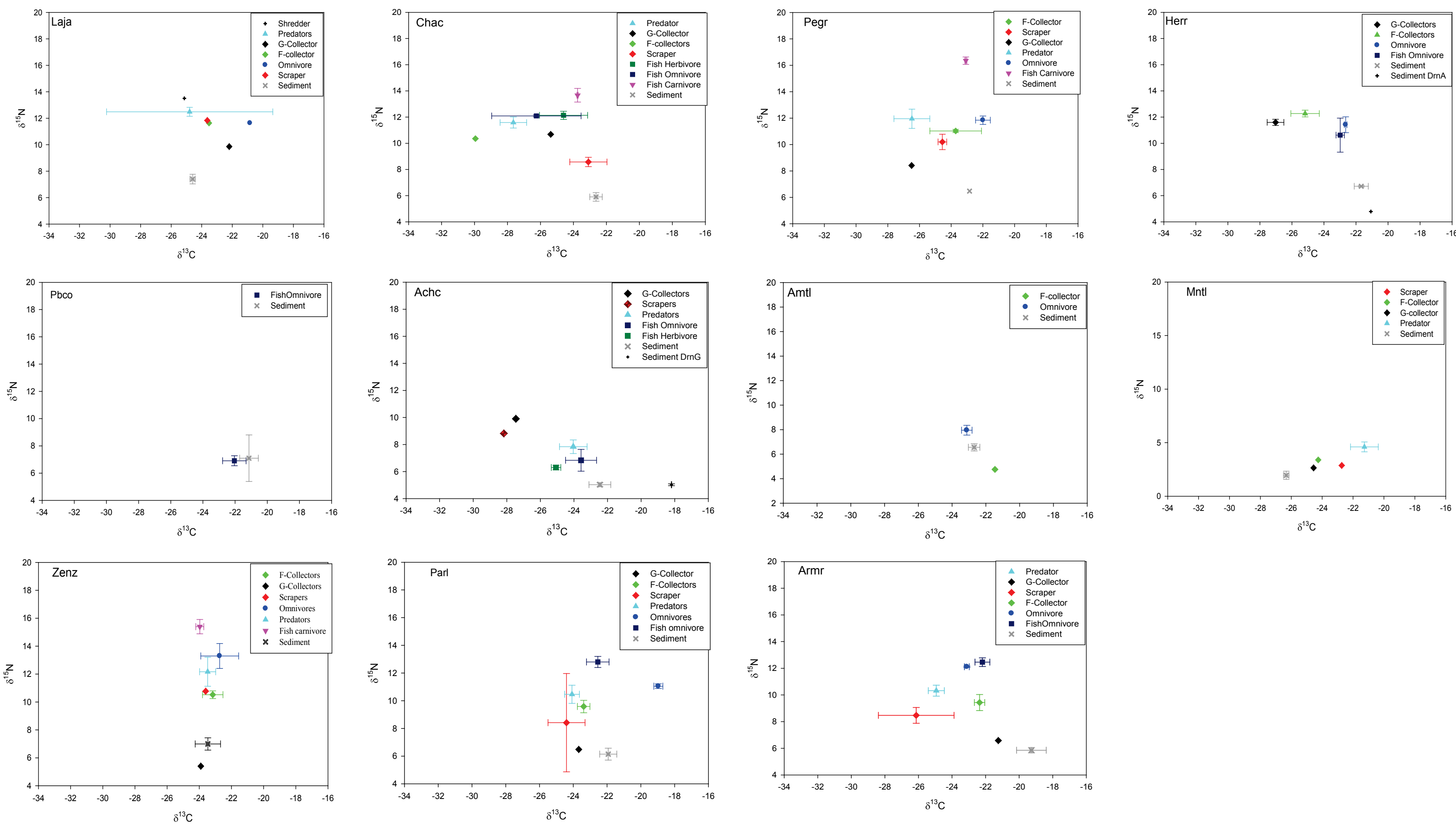
b) Tuxcacuesco sites

Appendix 3.2. Dual mean (± 1 s.e.) $\delta^{15}N$ /$\delta^{13}C$ biplots for each site in Dry08 in: a) Ayuquila River, and b) Tuxcacuesco River.



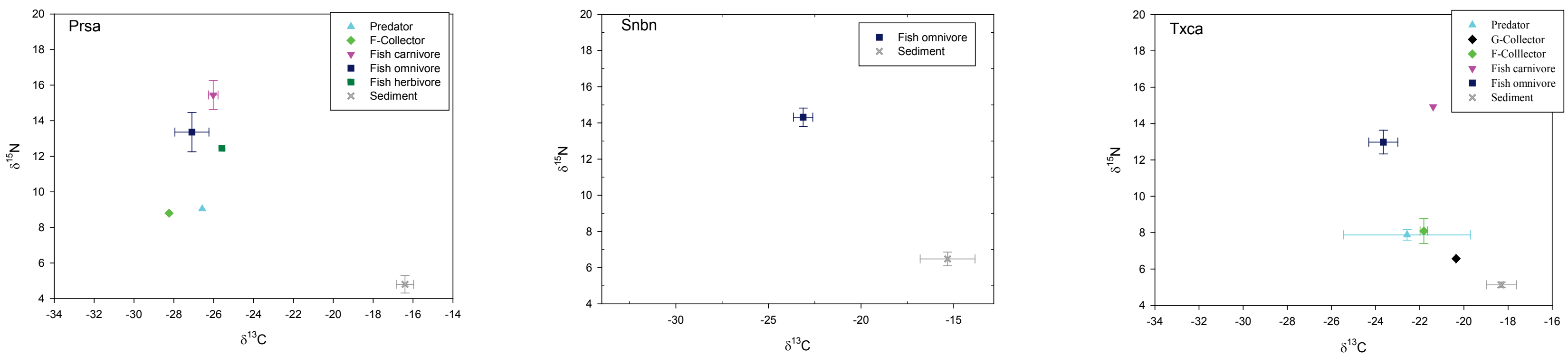
b) Tuxcacuesco sites

Appendix 3.3. Dual mean (± 1 s.e.) δ^{15}N vs δ^{13}C biplots for each site in Wet08 in a) Ayuquila River,. The plots are arranged in the direction of the river's flow.



a) Ayuquila river sites

Appendix 3.3T. Dual mean (± 1 s.e.) $\delta^{15}N$ vs $\delta^{13}C$ biplots for each site in Wet08 at Tuxcacuesco River. The plots are arranged in the direction of the river's flow



b) Tuxcacuesco River sites

Appendix 4.1. Summarized results of One-way ANOVA or t-tests for metal concentration comparisons in sediments between sites, among sampling seasons, and between sub-basins. Bold figures are statistically significant.

Metal	Wet07 (d.f. 11)	Dry08 (d.f. 14)	Wet08 (d.f. 15)
		Between sites comparisons	
Cd	H=9.473 p=0.578	H=14.352 p=0.424	F=1.974 **p=0.044***
Pb	H=2.584 p=0.995	H=18.565 p=0.182	F=1.710 p=0.089
Cu	H=47.033 **p=<0.001***	H=23.554 p=0.052	H=37.752 **p=0.002***
Zn	H=46.434 **p<0.001***	H=20.955 p=0.103	H=36.39 p=0.003*
Mn	H=62.001 **p<0.001***	H=24.963 **p=0.035***	H=35.179 **p=0.004***
Fe	H=54.807 **p<0.001***	F=0.562 p=0.859	F=1.914 p=0.052
		Between basins	
Cd	U=128 **p<0.001**	U=65.5 p=0.134	U=204 p=0.136
Pb	U=470 p=0.817	U=75.5 p=0.815	U=258 p=0.671
Cu	U=309 p=0.022	U=45.5 p=0.102	U=83 **p<0.001**
Zn	U=484 p=0.984	U=89.5 p=0.575	U=261 p=0.715
Mn	U=100 **p<0.001**	U=78.5 p=0.926	U=191 p=0.081
Fe	$t_{(d.f.=70)}$=-4.512 **p<0.001**	$t_{(d.f.=31)}$=-0.468 p=0.643	U=226 p=0.291
		Among sampling seasons (d.f. 2)	
Cd	H=32.662 **p <0.001** (except for wet08 vs wet07)		
Pb	H=16.604 **p= <0.001** (except for wet08 vs dry08, and wet07 vs dry08)		
Cu	H=4.182 p=0.124		
Zn	**H=126.292 p <0.001**		
Mn	**H.27.843 p <0.001**		
Fe	H=2.528 p=0.282		

*Although there were statistical significant differences among the sites' overall values, pairwise comparisons (Tukey, Dunn's or Holm-Sidak) indicated the opposite for some sites.

Appendix 4.2. Mean (±s.e.) metal concentrations (µg/g dw) of each FFG and sediment per site for Dry08.

Site	FFG	n	Cd	Pb	Cu	Mn	Fe	Zn
1FoTA	**Fcoll**	1	-	-	-	2502.45	533.57	-
(Laja)	**Gcoll**	1	8.16	0.00	7.09	1152.67	2248.59	187.26
	Pred	2	1.27±0.05	2.14±0.58	17.44±0.75	1008.44±49.15	742.13±39.41	103.86±4.42
	Omnv	1	3.26	16.32	-	-	195.87	-
	Sdmt	2	0.47±0.11	2.47±1.93	6.52±4.0	225.25±64.96	473.34±58.22	0.98±0.47
2.IAFo	**Fcoll**	2	0.97±0.22	2.17±0.98	15.97±1.43	1323.18±363.5	1388.75±276.48	103.99±6.39
(Chac)	**Scrpr**	4	3.3±0.2	6.95±1.03	271.6±14.29	837.36±24.57	1201.46±36.92	353.84±9.33
	Pred	2	0.7±0.05	3.49±0.16	19.82±0.61	1224.8±81.23	1306.6±69.52	110.96±8.14
	Fshom	4	0.64±0.17	2.28±0.48	2.92±1.12	11.48±0.97	83.37±42.03	40.26±4.82
	Sdmt	2	0.49±0.06	1.84±0.32	2.68±0.37	63.24±8.32	505.27±32.17	0.51±0.04
3.IA	**Fcoll**	1	1.56	12.47	-	1924.26	1075.26	-
(Pegr)	**Gcoll**	2	1.53±0.04	4.37±0.63	14.94±0.19	1099.74±18.36	1323.45±35.79	103.69±2.12
	Pred	2	1.46±0.1	5.98±0.79	19.17±0.26	1118.88±46.14	1121.93±±58.66	126.29±10.90
	Omnv	2	3.7±0.02	42.68±2.4	53.52±0.93	297.7±40.83	307.41±79.88	78.64±0.87
	Sdmnt	2	0.5±0.01	2.51±1.24	5.13±1.71	106.45±18.67	535.87±34.93	0.66±0.1
4.IA	**Fcoll**	1	2.21	11.05	72.92	55.25	561.29	95.46
(Herr)	**Pred**	1	1.30	8.42	20.52	1243.22	1570.87	122.64
	Fshom	2	0.74±12	1.68±0.31	-	5.37±0.10	67.22±6.64	29.60±3.34
	Sdmt	2	0.45±0.05	2.94±2.04	3.90±0.17	103.54±4.34	496.19±5.32	0.57±0.01
5.UrIA	**Fshomn**	2	0.72±0.05	3.93±0.10	-	10.74±2.67	1.31±0.03	20.35±2.26
(DrnA)	**Sdmt**	2	0.49±0.05	2.19±0.67	9.25±2.61	94.09±14.58	513.93±5.44	0.86±0.15
6.IA	**Fsherb**	2	1.40±0.04	6.16±0.70	4.61±3.51	91.99±10.79	18.12±0.31	76.05±6.10
(Pbco)	**Sdmt**	2	0.44±0.07	0.58±0.19	3.47±0.35	71.11±0.70	467.56±44.31	0.48±0.01
9.IA	**Fcoll**	2	1.40±0.48	4.63±1.51	57.21±2.16	100.06±9.10	709.40±59.42	94.24±4.00
(Amtl)	**Fshom**	4	1.47±0.02	1.64±0.39	-	10.67±4.52	17.63±4.80	24.52±0.26

Appendix 4.2. continued

Site	FFG	n	Cd	Pb	Cu	Mn	Fe	Zn
9.IA	**Fscrn**	2	1.45±0.13	2.04±0.32	-	15.03±0.72	17.14±0.61	29.10±0.82
(Amtl)	**Sdmt**	2	0.45±0.04	4.02±1.71	7,68±3.86	236.9±58.94	478.75±27.56	0.76±0.30
10.Fo	**Fcoll**	3	1.3±0.06	2.35±0.49	13.30±0.14	344.42±24.10	770.60±85.96	85.94±0.49
(Mntl)	**Gcoll**	1	2.85	-	-	343.71	398.34	-
	Pred	3	1.10±0.20	2.90±0.30	14.25±0.50	372.01±43.18	587.22±69.02	104.70±0.10
	Sdmt	2	0.37±0.02	0.77±0.12	2.24±0.06	66.47±0.81	467.43±7.57	0.32
11.IAFo	**Fcoll**	2	2.52±0.11	3.09±0.46	55.00±8.08	587.64±49.06	1376.67±85.54	126.01±10.99
(Zenz)	**Gcoll**	1	5.37	10.17	17.29	1162.05	2796.19	191.88
	Pred	4	2.08±0.04	2.47±0.72	14.86±2.27	1011.93±21.95	815.48±173.53	126.17±8.75
	Omnv	2	4.49±0.07	31.18±1.23	35.85±2.39	85.44±2.92	130.34±0.81	86.86±4.56
	Sdmt	2	0.60	4.00±0.23	8.71±0.09	262.16±11.54	473.65±7.70	0.96±0.01
12.IAFo	**Pred**	2	1.67±0.12	6.05±0.31	18.86±0.43	649.98±22.72	978.01±11.69	112.17±4.44
(Parl)	**Fshom**	2	1.84±0.18	7.71±1.08	0.26±0.01	32.35±9.83	44.76±25.55	116.77±15.62
	Fsherb	3	1.7±0.13	9.4±3.45	10.19±9.41	192.19±137.62	507.92±489.89	93.74±18.04
	Sdmt	2	0.55±0.07	0.83±0.08	2.18±0.08	123.17±9.7	505.25±18.1	0.4±0.01
a.Fo	**Gcoll**	1	-	22.26	-	1114.56	751.39	-
(Prsa)	**Scrp**	2	1.84±0.23	5.94±1.74	5.90±1.55	401.59±116.03	214.26±76.04	74.06±0.0710.49
	Pred	2	1.335±0.01	3.87±0.06	13.86	561.64±70.89	324.89±76.24	109.09
	Fshom	5	2.79±1.73	9.72±4.33	-	10.33±4.36	18.92±8.06	53.97±11.96
	Sdmt	2	0.52±0.07	2.2±1.32	3.08±1.62	189.19±51.48	503.7±26.93	0.59±0.22
d.FoUr	**Gcoll**	1	0.78	5.44	-	-	745.92	-
(Txca)	**Pred**	2	2.32±0.13	4.26±0.94	11.99	841.34±134.66	920.65±21.16	106.81±14.55
	Fshom	1	3.05	11.03	1.70	99.23	5.17	102.03
	Sdmt	2	0.44±0.02	2.34±0.05	2.06±1.03	81.20±24.60	461.62±10.47	0.48±0.15
13.IAFo	**Pred**	4	0.66±0.06	0.72±0.40	16.36±0.75	510.13±219.85	616.1±105.15	107.9±6.52
(Armr)	**Fshom**	2	1.09±0.14	11.27±1.78	2.4±0.21	198.37±42.88	1056.04±32.39	120.91±16.52
	Sdmt	2	0.54±0.1	0.74±0.01	0.46±0.06	63.39±8.61	534.79±15.09	0.28±0.02

Fcoll= Filter collectors; Gcoll=Gather collectors; Scrp= Scrapers; Pred= Predators; Omnv= Omnivores; Fsherb= Fish herbivores; Fshom= Fish omnivores; Fscrn= Fish carnivores; Sdmt= Sediment. Blank spaces indicate there was not enough sample to run the spectrometry

Appendix 4.3. Mean (±s.e.) metal concentrations (μg/g dw) of each FFG and sediment per site for wet08

Site	FFG	n	Cd	Pb	Cu	Mn	Fe	Zn
1.FoTa	**Fcoll**	1	1.85	6.16	15.41	2180.50	770.49	122.35
(Laja)	**Gcoll**	2	4.96±3.3	8.8±1.5	15.75±5.4	1720.6±982.2	1219.35±620.8	119.23±27.4
	Pred	5	1.07±0.3	3.79±1.7	30.44±4.2	1775.2±690.9	822.25±157.8	135.22±16.5
	Omnv	1	2.29	43.45	72.50	1295.63	789.04	72.73
	Sdmt	3	0.34±0.01	3.43±2.0	4.25±0.6	208.3±39.8	775.91±59.6	10.41±2
2.IAFo	**Fcoll**	1	-	-	21	1041	2400	91.5
(Chac)	**Gcoll**	1	-	143.376	19.776	235.83	3213.6	86.0256
	Scrp	2	4.77±0.8	13.94±2.04	55.66±27.9	119.26±8.3	135.22±31.4	65.61±7.4
	Pred	1	1.13	25.96	27.31	2400.75	767.52	160.73
	Fsherb	2	1.53±0.2	14.13±1.0	12.22±1.5	313.18±18.1	2269.15±344.1	77.39±16.8
	Fshom	2	2.10±0.2	33.26±0.9	11.73±2.0	173.57±76.8	1329.4±1190.5	136.91±18.2
	Fshcar	1	1.00	1.75	0.80	12.13	65.18	9.90
	Sdmt	3	0.31±0.1	1.49±0.3	3.41±0.6	61±9.6	679.16±30.0	7.97±0.5
3.IA	**Fcoll**	2	7.98±6.6	7.9±2.5	23.7±6.9	825.3±566.0	1472±119.6	148.3±1.4
Pegr	**Gcoll**	1	4.16	2.97	18.14	1330.47	3865.92	215.00
	Scrpr	2	0.63±0.3	7.48±0.3	174±43.4	42±9.7	70.2±15.4	60.16±0.5
	Pred	3	24.93±20.9	10.78±7.9	17.52±4.8	863.7±348.9	720.34±186.75	148.33±1.36
	Omnv	2	2.54±0.5	34.41±8.5	57.22±3.9	1231.3±165.7	529.88±285.1	68.95±16.5
	Fscrn	2	0.54±0.1	2.85±0.7	0.82±0.04	3.37±2.9	3.22±0.4	34.72±0.01
	Sdmt	3	0.2±0.04	2.79±0.2	2.14±0.3	59.67±5.7	380.8±33.2	6.92±0.2
4.IA	**Pred**	1	7.27	130.87	20.36	1590.80	508.94	251.56
(Herr)	**Omnv**	2	3.83±0.3	48.16±6.8	76.8±6.4	1093.64±214.3	289.77±7.7	79.69±2.1
	Fsherb	1	2.37	30.02	2.84	97.74	418.77	105.09
	Fshom	1	2.90	32.60	13.91	135.46	840.31	106.42
	Sdmt	2	0.22±0.03	3.90±1.1	3.7±0.8	54.2±11.8	435.6±69.8	7.44±0.8
5.UrIA	**Sdmt**	2	0.27±0.13	2.64±0.2	4.35±0.8	42.98±2.6	622.27±101.1	10.83±1.04

Appendix 4.3 continued

Site	FFG	n	Cd	Pb	Cu	Mn	Fe	Zn
6.IA	**Fshom**	5	0.27±0.1	1.94±1.0	2.87±0.5	71.1±26.0	61.50±21.2	72.6±16.3
(Pbco)	**Sdmt**	2	0.17±0.04	3.1±0.6	4.79±0.2	76.71±2.0	290.66±78.3	10.37±2.2
7.Ur	**Sdmt**	2	0.17±0.00	4.45±1.3	4.41±0.4	46.75±35.6	370.80±38.9	7.54±0.7
8.IA	**Gcoll**	1	92.48	1017.23	61.65	3594.20	5548.50	-
(Achc)	**Pred**	1	31.29	281.57	25.03	1187.81	1251.43	44.84
	Fshrb	2	3.42±0.2	47.53±12.8	9.03±0.1	256.74±87.3	237.04±101.9	92.29±13.1
	Fshom	5	4.99±1.6	62.29±17.9	7.36±3.3	105.08±52.3	733.3±627.0	73.12±22.6
	Sdmt	4	0.18±0.03	1.27±0.12	2.96±0.8	34.36±4.1	446.69±80.1	4.82±0.3
9.IA	**Fcoll**	1	1.95	-	26.67	251.15	8391.03	68.14
(Amtl)	**Omnv**	2	3.31±1.4	53.87±30.7	109.85±61.9	414.18±5.3	143.38±49.4	101.39±13.5
	Sdmt	2	0.27±0.02	2.47±0.12	3.49±0.5	56.91±15.2	452.13±64.8	5.54±0.012
10.Fo	**Fcoll**	1	18.45	-	15.03	736.59	2555.52	144.86
(Mntl)	**Scrpr**	1	19.29	21.43	14.29	470.12	785.91	115.03
	Pred	2	0.55±0.2	0.72±0.02	26.71±0.3	539.53±329.4	629.4±297.0	126.43±25.4
	Sdmt	5	0.24±0.03	0.7±0.3	3.18±0.9	41.77±5.8	418.91±95.5	4.3±0.5
11.IAFo	**Fcoll**	2	0.68±0.1	13.88±10.1	54.53±30.2	425.4±266.2	1767.2±240.1	123.83±6.7
(Zenz)	**Gcoll**	1	0.72	15.22	36.12	439.90	1863.56	170.66
	Pred	4	1.18±0.3	3.69±1.8	30.30±8.9	565.54±191.6	1041.2±229.04	110.1±47.2
	Omnv	1	2.42	39.25	91.49	326.62	417.43	85.67
	Fscrn	2	0.13±0.05	-	0.89±0.11	15.17±14.9	11.77±6.4	30.84±4.2
	Sdmt	3	0.22±0.03	3.82±1.4	2.41±0.3	105.45±17.3	488.24±74.3	6.54±1.5
12.IAFo	**Fcoll**	2	13.28±3.8	21.13±21.1	31.46±5.2	863.8±647	3006.9±1457	76.66±62.4
(Parl)	**Gcoll**	1	5.41	-	38.21	1160.84	7677.63	131.75
	Scrpr	2	13.7±10.8	-	17.96±6.5	1121.61±405.4	1412.67±597	74.41±19..4
	Pred	2	3.79±1.3	5.57±5.6	23.46±3.8	1210.7±630	1553.11±971.6	112.56±4.0
	Omnv	2	4.11±0.1	45.93±11.2	158.22±4.7	102.5±3.9	242.83±155.1	66.93±20.8
	Fshom	6	4.42±1.4	28.83±9.8	10.29±3.1	142.94±53.0	300.11±211.7	79.06±10.8
	Sdmt	4	0.36±0.1	0.7	2.59±0.5	51.52±12.9	568.3±70.0	6.01±0.3

Appendix 4.3 continued

Site	FFG	n	Cd	Pb	Cu	Mn	Fe	Zn
a.Fo	**Fcoll**	1	4.16	16.63	16.63	951.18	2414.68	94.54
(Prsa)	**Pred**	1	10.08	43.20	21.60	253.44	921.60	169.92
	Fshrb	1	0.87	7.27	1.31	35.56	18.18	57.66
	Fshom	4	0.46±0.1	3.85±1.6	1.66±0.4	21.21±4.8	27.32±6.5	86.31±27.9
	Fscrn	6	0.65±0.2	1.62±0.7	0.85±0.2	3.12±0.8	30.78±18.6	34.58±3.7
	Sdmt	4	0.15±0.3	2.38±0.6	1.54±0.4	43.98±10.9	531.46±104.9	6.91±0.5
b.FolA	**Fshom**	5	0.25±0.1	6.35±1.8	1.99±0.3	8.47±2.9	17.21±2.4	75.38±13.7
(Snbn)	**Sdmt**	3	0.32±0.1	2.70±0.7	1.07±0.3	78.54±26.7	702.51±36.5	8.87±3.1
c.Ur	**Gcoll**		2.36	39.34	18.88	498.04	3902.46	201.42
(Tnya)	**Pred**	3	2.3±1.5	3.94±1.1	25.12±1.7	292.66±79.1	1138.8±805.3	137.94±17.6
	Fshom	3	0.84±0.03	13.77±3.7	9.1±2.4	87.4±30.8	817.39±416.1	98.58±14.2
	Sdmt	3	0.34±0.1	2.7±0.8	1.34±0.1	38.36±1.9	539.9±103.3	6.26±0.64
d.FoUr	**Fcoll**	1	2.92	11.24	21.59	174.94	3627	104.78
(Txca)	**Gcoll**	1	1.57	3.13	14.10	139.42	1286.90	142.01
	Pred	2	4.71±1.6	2.88±2.9	7.28±4.8	64.47±9.8	610.2±183.6	127.82±14.8
	Fshom	4	1.41±0.5	9.99±4.6	1.91±0.7	26.99±13.3	61.01±24.5	80.82±21
	Fscrn	1	1.09	3.65	0.88	23.65	5.84	52.25
	Sdmt	4	0.14±0.03	2.43±0.5	1.86±0.3	32.23±4.3	466.9±92.7	4.9±0.3
13.IAFo	**Fcoll**	3	2.73±1.1	13.17±7.05	90.34±73.2	223.16±97.9	1223.6±849.6	77.05±28.9
(Armr)	**Gcoll**	2	2.49±0.2	28.49±2.0	22.99±2.6	308.61±5.0	3238.2±590.1	120.44±11.4
	Scrpr	1	15.375	0.5	15.375	747.225	2613.75	79.95
	Pred	3	1.57±0.5	16.97±6.4	20.38±2.2	578.4±219.6	784.06±66.6	78.79±4.1
	Omnv	2	1.07±0.3	18.81±5.5	222.77±48.2	76.89±32.6	148.56±0.3	64.36±14.2
	Fshom	10	1.55±0.2	13.67±3.4	3.25±0.6	26.84±13.5	82.39±40.8	40.24±5.7
	Sdmt	4	0.27±0.05	2.01±0.7	0.96±0.3	31.97±4.0	497.66±109.9	4.51±0.8

Fcoll= Filter collectors; Gcoll=Gather collectors; Scrp= Scrapers; Pred= Predators; Omnv= Omnivores; Fsherb= Fish herbivores; Fshom= Fish omnivores; Fscrn= Fish carnivores; Sdmt= Sediment. Blank spaces indicate there was not enough sample to run the spectrometry