Abstract

BUTTERMORE, ELISSA NICOLE. Contaminant and Trophic Dynamics of Tropical Stream Ecosystems. (Under of the direction of Thomas J. Kwak and W. Gregory Cope).

The fate and effects of pollution are complex processes, and many contaminants present in low levels in the environment may increase in concentration from one trophic level to the next, reaching concentrations that are harmful to wildlife and human consumers. Puerto Rico has a history of anthropogenic chemical usage, and its human population density is among the highest globally, providing a model environment to study human impacts on tropical island stream ecosystems. The objective of our study was to quantify occurrences and patterns of aquatic contaminants [polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), pesticides, and metals] as related to watershed land-use characteristics and trophic relationships. We used stable isotope analyses of carbon, nitrogen, and sulfur to elucidate contaminant and trophic dynamics. We sampled reaches within 13 rivers spanning broad riparian and watershed land-use patterns (e.g., urban, agricultural, industrial, and forested). Additional intensive studies were conducted at four of these sites, involving stable isotope analyses of all food web components and contaminant analyses of native fish and shrimp species. Overall, stream ecosystems in Puerto Rico were not severely polluted, with the exception of elevated concentrations of nickel in sediment at sites associated with agricultural watersheds and PCBs and mercury in some fish species. Trophic level and contaminant concentrations were poorly correlated in these dynamic ecosystems that are characterized by frequent hydrologic disturbances, nutrient pulses, and

marine influences. Calculation of food web magnification factors was complicated by low levels of contaminants, biased estimates of trophic level due to $\delta^{15}N$ enrichment from nutrient pollution, and short food chains. Lipid content of consumers was a better predictor of contaminant concentration than trophic level. These findings enhance understanding of contaminant dynamics in tropical stream ecosystems and provide natural resource managers and public health agencies scientific information to guide ecosystem and fisheries management and human health risk assessment.

Contaminant and Trophic Dynamics of Tropical Stream Ecosystems

by
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Dedication

For my parents

Biography

I have lived somewhat of a nomadic lifestyle. I was born in Wichita, Kansas, but I grew up in Carlsbad, California, Jupiter, Florida, and South Windsor, Connecticut. My brothers and I would play in our neighborhood ditches, ponds, and woods catching crawdads, bugs, turtles, minnows, snakes, and any other creatures we could get our hands on. I maintained a keen interest in animals and the outdoors as I grew older. In preparation for high school, I decided to apply to magnet schools. I was torn between two magnet programs—the environmental field studies academy or the pre-med program. It was not until after graduating from college and reading the book *Our Stolen Future* that I realized I did not have to decide and could combine these interests into a field called ecotoxicology. So, I chose the environmental path and then attended and graduated from University of Florida with a bachelor's degree in zoology. After graduation, I became a true nomad—waking up in a different place each morning on the Appalachian Trail, a footpath that stretches from Georgia to Maine. I completed the AT and briefly worked as a sales associate in the fashion industry, which was a little strange because I had worn the same outfit every day for the previous five months, and people often confused me for a homeless person. Then I returned once again to Florida, where I worked as a fisheries technician on a multitude of projects quickly gaining experience with all sorts of fishery techniques and gear. After a few years, I moved to North Carolina to study contaminants in the stream ecosystems of Puerto Rico (explained in detail in the following pages) with a goal to protect the health of wildlife and people…and lived happily-ever-after.

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Table of Contents

List of Tables…….. ix

List of Figures……. x

Chapter I—Water, Sediment, and Biota Contamination of Tropical Island Stream Ecosystems in Relation to Land Use

Abstract…….. 2

Introduction…… 3

Methods…….. 7

uPSDs……. 8

Sediment……. 9

Fish and Shrimp…….. 10

Laboratory Analyses and Quality Control……..…...……. 12

Contaminant Criteria and Guideline Exceedance…….. 14

Results……. 15

Water Quality…….. 15

Water Contaminants……..... 15

Sediment…….. 16

Fish and Shrimp…….. 17

Contaminant Criteria and Guideline Exceedance……... 19

Discussion……... 21

Contaminants and Hydrology……. 22

General Conclusions……... 25

References…… 25

Appendix 1……... 65

uPSDs…….. 65

Sample Processing……... 67

Organic Toxicant Analysis of uPSDs……. 67

Organic Toxicant Analysis of Sediment……. 68

Biota Extraction of Organic Toxicants…… 70

Composite Samples……. 71

Chapter II—Pollution of Tropical Island Stream Ecosystems: Relation of Biotic Accumulation to Land Use and Trophic Dynamics

Abstract…… 74

Introduction……. 75

Methods……... 77

Sample Collection…… 79

Water Quality…….. 80

Contaminant Analyses…… 80

Stable Isotope Analyses……...……... 81

δ^{13}C Lipid Correction Techniques…….. 82

Estimation of Trophic Level……... 83

Quality Control…… 84

Results……. 85

Water Quality…….. 85

Stable Isotopes ……..…….. 85

Contaminant Patterns…….. 87

Discussion…… 88

Stable Isotope Variation Among Sites…… 88

Food Web Magnification……..…… 95

Contaminant and Other Stable Isotope Relationships……. 96

Future Studies…….. 98

Conclusions…….. 99

References…… 100

List of Tables

Chapter I

Table 1 Site characteristics including land use, location, distance to river mouth, elevation, watershed area, and gradient …… 35

Table 2 Water quality parameters for extensive sampling…… 36

Table 3 Water quality parameters for intensive sampling…….…… 37

Table 4 Estimated contaminant concentrations in fiber PSDs for extensive sampling…….. 38

Table 5 Mean estimated contaminant concentrations in cartridge PSDs for intensive sampling…….. 39

Table 6 Contaminant concentrations in sediment for each site during the extensive study…… 40

Table 7 Mean contaminant concentrations in sediment for during intensive sampling…….. 41

Table 8 Contaminant concentrations exceeding EPA consumption limit recommendations for extensive study sites……. 42

Table 9 Contaminant concentrations exceeding EPA consumption limit recommendations for intensive study sites…….. 43

Table 10 Contamination in Puerto Rico streams compared to the United States and other regions…… 44

Chapter II

Table 1 Site characteristics including land use, location, distance to river mouth, elevation, watershed area, and gradient……. 111

Table 2 Water quality parameters for each site…… 112

Table 3 Frequently detected mean contaminant concentrations

summarized by site and species…….. 113

Table 4 Correlation matrix of most frequently detected contaminant concentration versus stable isotope ratios and lipid content…….. 114

List of Figures

Chapter I

Fig. 1 Map of stream study sites…..……..……. 45

Fig. 2 Sediment normalization factors: total organic carbon, clay, and iron concentration for extensive sampling ……..…… 46

Fig. 3 Sediment normalization factors: total organic carbon, clay, and iron concentration……...……..…… 47

Fig. 4 PCB concentrations in fish and shrimp with EPA monthly consumption limits for extensive sampling…….….. 48

Fig. 5 PCB concentrations in fish and shrimp with EPA monthly consumption limits for intensive sampling…….…. 49

Fig. 6 Cadmium concentrations in fish and shrimp for extensive sampling with EPA monthly human consumption limits…….. 50

Fig. 7 Copper concentrations in fish and shrimp for extensive sampling..... 51

Fig. 8 Mercury concentrations in fish and shrimp with EPA monthly consumption limits for extensive sampling…… 52

Fig. 9 Mercury concentrations in fish for intensive sampling and EPA monthly fish consumption limits…….……. 53

Fig. 10 Lipid content for fish and shrimp for extensive sampling..... 54

Fig. 11 Lipid content for fish and shrimp samples for intensive sampling..... 55

Fig. 12 Length of fish and shrimp for extensive sampling……... 56

Fig. 13 Mean length of fish and shrimp for intensive sampling……..…. 57

Fig. 14 PCB concentrations normalized by lipid content for intensive sampling…… 58

Fig. 15 PCB concentrations normalized by total length for intensive sampling…… 59

Fig. 16 Mercury concentrations normalized by total length for intensive sampling……..… 60

Fig. 17 Copper concentrations in sediment with guideline exceedances for extensive sampling…….…… 61

Fig. 18 Copper concentrations in sediment for intensive sampling with guidelines…….……. 62

Fig. 19 Nickel concentrations in sediment with guideline exceedances for extensive sampling……. 63

Fig. 20 Nickel concentrations in sediment with guideline exceedances for intensive sampling……... 64

Chapter II

Fig. 1 Map of stream study sites …… 115

Fig. 2 A food web diagram of a stream ecosystem in Puerto Rico…..... 116

Fig. 3 $\delta^{13}C$ and $\delta^{34}S$ biplots for the stream sites……..... 117

Fig. 4 $\delta^{15}N$ values of food web components and trophic levels…..…... 118

Fig. 5 Lipid content of fish and shrimp…….. 119

Fig. 6 Lipid content of fish and shrimp and its relationship with C:N... 120

Fig. 7 Lipid content of fish and shrimp and its relationship to the difference between lipid extracted $\delta^{13}C$ and untreated $\delta^{13}C$…… 121

Fig. 8 Our relationship between C:N and difference between lipid extracted $\delta^{13}C$ and untreated $\delta^{13}C$, in comparison other studies.. 122

Fig. 9 Relationship of C:N and species differences in the difference of $\delta^{13}C$ between lipid extracted and non-lipid extracted samples……. 123

Fig. 10 Relationship between $\log_e$(concentration of PCB ng/g-lipid) and trophic level……. 124

Fig. 11 Relationship between PCB concentration and lipid content…... 125

Fig. 12 Relationship between chlordane concentration and lipid content……. 126

Fig. 13 Relationship between DDT concentration and lipid content….. 127

Chapter I–Water, Sediment, and Biota Contamination of Tropical Island Stream Ecosystems in Relation to Land Use

Abstract

Manufactured chemicals are continuously released into the environment with a variety of adverse ecological and human health effects. Puerto Rico has a history of anthropogenic chemical usage, and its human population density is among the highest globally, providing a model environment to study human impacts on tropical island stream ecosystems. Our objectives were to quantify occurrences of Polycyclic Aromatic Hydrocarbons (PAHs), historic-use chlorinated pesticides, current-use pesticides, Polychlorinated Biphenyls (PCBs), and metals (mercury, cadmium, copper, lead, nickel, zinc, and selenium) in the habitat and biota of Puerto Rico streams and associate those findings with land-use patterns. We sampled water, sediment, and native fish and shrimp species at 13 sites spanning broad riparian and watershed land-use patterns (e.g., urban, agricultural, industrial, and forested) and conducted intensive sampling at four of these sites. Overall, our findings indicated that stream ecosystems in Puerto Rico were not severely polluted, with the exception of nickel in sediment at sites associated with agricultural watersheds. While nickel concentrations were greatest at agricultural sites, a site with a highly urbanized watershed generally had the greatest concentrations of most classes of contaminants. PCBs may pose human health hazards with some fish concentrations exceeding the EPA consumption limit for 1 meal/month; greatest concentrations were in mountain mullet (*Agonostomus monticola*) and American eel (*Anguilla rostrata*). Bigmouth sleepers (*Gobiomorous dormitor*) may be the most suitable fish for human consumption with low levels of organic contaminants, but mercury accumulation exceeded EPA's consumption

limit for 3 meals/month at 1 of the 13 sites. These results provide public health and natural resource agencies the scientific information required to guide ecosystem and fisheries management and human health risk assessment.

Keywords Ecosystem · Fish · Stream · Pollution · Puerto Rico · Water

Introduction

Over 100,000 anthropogenic chemicals are released into the environment annually (Shea 2010). Some aquatic organisms may accumulate contaminants that are present in low concentrations in the environment to levels that are harmful to the organism and also exceed human health guidelines (Thrower and Eustace 1973). These contaminants exert a variety of adverse effects on organisms, including alterations in behavior (Chura and Stewart 1967) and morphology (Park et al. 2001), reproductive abnormalities (Guillette 1999), and mortality. Water pollution is especially problematic for tropical islands with dense human populations (Hunter and Arbona 1995). Few contaminant studies have been conducted in the Caribbean (Rodríguez and Pérez de González 1981; Neal et al. 2005), and no study has examined the effects of contamination in stream ecosystems there. More knowledge is needed on the occurrence and patterns of contaminants in Caribbean stream ecosystems to inform ecosystem and fisheries management and human health risk assessments.

Contaminant issues are of global importance because pollutants are known to demonstrate long-range, trans-continental, transport (Oehme 1991; Welch et al. 1991; Iwata et al. 1993; Burkow and Kallenborn 2000). Garrison et al. (2006) suggest that dust air

masses from the Sahara Desert, Africa, are likely a source of atmospheric persistent organic pollutants (POPs) at downwind Caribbean sites. In the Caribbean, surface currents move rapidly, transporting pollutants from other areas and spreading pollution from island to island (Ross and DeLorenzo 1997). Several deep ocean basins in the Caribbean receive very little renewal or flushing and coastal upwelling could potentially cause release of high contaminant concentrations (Rawlins et al. 1998). These oceanographic features in combination with locally high human population densities and associated activities make the Caribbean especially susceptible to the accumulation of contaminants (Hunter and Arbona 1995; Ross and DeLorenzo 1997).

Puerto Rico is a densely populated Caribbean island, supporting nearly 440 people per square kilometer, providing an appropriate model to study aquatic contaminant dynamics (Martinuzzi et al. 2007; Neal et al. 2009). During the past century, rapid industrialization and the subsequent human population growth have strained the limited natural resources of the island (Hunter and Arbona 1995). Water is a scarce resource in Puerto Rico because there are no natural lakes. Most of the rivers have been transformed by dam construction or other structures that are conducive for water collection (Cooney and Kwak 2010). However, the Puerto Rican people are becoming increasingly aware of the benefits associated with conserving stream ecosystems. González-Cabán and Loomis (1996) demonstrated that citizens would be willing to pay a total of $11.33 million to prevent dam construction on Río Mameyes, the last remaining pristine river in Puerto Rico.

The streams of Puerto Rico provide many services for local populations, including water for drinking, recreation, irrigation, and as a source of fish and crustaceans for consumption. Therefore, good water quality is necessary to protect human health as well as ecological integrity. However, the island has experienced an era of rapid human population growth leading to deteriorated water quality (Hunter and Arbona 1995; Fitzpatrick and Keegan 2007). The streams have a history of die-offs of fish, shellfish, shrimp, and domesticated animals, which were contaminated by industrial, agricultural, and municipal wastes (Hunter and Arbona 1995). Epidemiological evidence also suggests that water contamination has jeopardized human health (Colón et al. 2000). For example, Puerto Rico has the highest incidence of premature breast development (thelarche) in girls, with some affected patients younger than two years of age (Colón et al. 2000). High levels of endocrine-disrupting chemicals have been found in the serum of Puerto Rican girls with premature thelarche. Yet, there is a notable lack of research and available information on the degree and effects of water contamination in Puerto Rico.

Contaminants and land use

Rivers and streams are influenced by their surrounding landscapes (Hynes 1975; Vannote et al. 1980; Allen 1994). Direct correlations have been clearly demonstrated between land use and water quality (Lenat and Crawford 1994; Bolstad and Swank 1997; Fisher et al. 2000; Tong and Chen 2002). Surface runoff, especially after a drought, is a major contributor to non-point source pollution because it transports sediment and associated

chemicals into aquatic ecosystems. Runoff from varying types of land use is enriched with different contaminants; for example, runoff from urban areas may be enriched with rubber fragments and heavy metals, whereas runoff from agricultural lands may be enriched with fertilizers and pesticides (Lenat 1984; Osborne and Wiley 1988; Cooper 1993; Johnson et al. 1997; Tong and Chen 2002). Further, vegetation modifies land surface characteristics, water balance, and the hydrologic cycle through evapotranspiration, interception, infiltration, percolation, and absorption (Tong and Chen 2002). Human-altered land use also transforms the hydrological system by changing runoff dynamics and composition and quality of receiving water bodies (Changnon and Demissie 1996; Mander et al. 1998; Warne 2005).

Puerto Rico has undergone a number of anthropogenic alterations to its landscape as a result of agriculture, deforestation, stream channelization, industrial and municipal pollution, urbanization, and impoundment of rivers (Neal et al. 2009). Historically, Puerto Rico's economy was predominantly agricultural, but in the early 1900s, global markets changed and the economy shifted toward industry and tourism (Warne et al. 2005). While rapid industrialization of Puerto Rico most likely lead to an increase in contaminant volume and diversity, tourism relies upon oceans, beaches, and other minimally disturbed areas such as the El Yunque National Forest (Warne et al. 2005). Therefore, it is imperative that contaminants and water quality be quantified in Puerto Rico stream ecosystems to guide natural resource planning and economic development.

Objective

The purpose of this study was to quantify contaminants in water, sediment, and biota in the stream ecosystems of Puerto Rico across a spectrum of watershed land-use patterns. This was accomplished by first surveying habitat and biota extensively island-wide for contaminants and then conducting more intensive studies in selected areas. Associations among land use and contaminant occurrence in stream ecosystems may then suggest relationships for future investigations.

Methods

Field survey sites were selected based on presence of target species and watershed land use. Prior knowledge of target species distribution and abundance was provided by Kwak et al. (2007). Thirteen of the 46 major river drainages in Puerto Rico were sampled, and sites were categorized based on primary watershed land-use patterns or distinctive riparian features as one reference site within a primarily forested watershed [Río Mameyes (1R)], two industrial sites [Río Tallaboa (2I), Río Cañas (3I)], two urban sites [Río Piedras (4U), Río Bayamon (5U)], seven agricultural sites [Río Añasco (6A), Río Yauco (7A), Río La Plata (8A), Río Jacaguas (9A), Río Guanajibo (10A), Río Cartagena (11A), Río Arecibo (12A)], and one site with substantial recreational fishing effort [Río Fajardo (13A)] (Table1; Fig. 1).

Data from the initial extensive contaminant sampling (13 sites) were used to select sites and contaminants for additional intensive sampling, to include more species and replicate samples. Four sites, among those sampled in the extensive contaminant survey,

were selected for intensive contaminant investigation to represent specific water quality or watershed land-use effects. These sites are 1R (reference), 7A (agricultural), 3I (industrial), and 4U (urban). Water, sediment, and biota were collected at each sampling site.

Water chemistry is known to affect bioavailability and degradation of contaminants. For example, hardness influences bioavailability of metals as explained by the free ion activity model (Morel 1983). Thus, physicochemical water parameters were measured using a Yellow Springs Instrument (YSI) 556 multi-probe system and a Hach CEL/850 Portable Aquaculture Laboratory and included temperature, pH, alkalinity (mg/L $CaCO_3$), total hardness (mg/L $CaCO_3$), conductivity (µS/cm), nitrate concentration (µg/L NO_3^-), nitrite concentration (mg/L NO_2^-), and orthophosphorus concentration (mg/L PO_4). Water was collected using a 1-L container, rinsed repeatedly with site water, and then was submersed 0.25-0.50 m beneath the water surface, filled, and stored on ice in a cooler.

Universal passive sampling devices (uPSDs)

Time-integrated contaminant concentrations in water were sampled using Universal Passive Sampling Devices (uPSDs). Passive sampling devices are a less labor-intensive method for sampling and measuring water contaminants (Heltsley et al. 2005). They estimate ecologically relevant, chronic contaminant exposure (Hirons 2009) and bioconcentration for aquatic species (Heltsley et al. 2005). UPSDs offer advantages over traditional grab sampling because they represent exposure of the bioavailable portion and they collect transient contaminants at trace levels (Hirons 2009).

Two types of uPSDs were used in this study. Fiber passive sampling devices (fPSDs) were used for extensive sampling and cartridge passive sampling devices (cPSDs) were used for intensive sampling. FPSDs have a surface area of 5.8 cm^2, and cPSDs have an internal surface area of 6.2 cm^2 (Hirons 2009). The fPSDs are hollow, polyethersulfone fibers filled with Waters Oasis HLB® sorbent, with a diameter of 1 mm and pore size of 0.2 μm. CPSDs are incased in porous, stainless steel and filled with the same polymeric sorbent, Oasis HLB®. Three fPSDs were deployed at each site during the extensive survey, and 6 cPSDs were deployed at each site during the intensive study. They stayed submersed in the water for 3-4 weeks. Each uPSD was wrapped in aluminum foil immediately upon retrieval and placed inside a plastic bag with a label, indicating the retrieval date and time, sampling location, and condition of the uPSD. The uPSDs were kept on ice inside a cooler until they could be transferred to a -20˚C freezer.

Sediment

One composite sediment sample was collected from each site for the extensive study, and three per site for the intensive study, using a stainless steel scoop, rinsed with site water prior to use. Each sample consisted of 3 to 5 scoops from depositional areas, within the site area, totaling approximately 0.75 L. Only sediment from the biologically-active, surface layer (top 5 cm) was collected and any rocks, debris, or biota were removed. Each sample was sealed in a plastic bag, stored on ice in a cooler, and then transferred to a -20˚C freezer as soon as possible.

Measurements of sediment contaminant concentrations are influenced by a number of covariates and require careful interpretation (Hoffman et al. 2003; Luoma and Rainbow 2008). Thus, we measured total organic carbon, particle-size, and iron concentration of sediment samples in this study for normalization purposes. Aliquots from sediment samples were dried at 60°C and sent to the Environmental and Agricultural Testing Service Laboratory in the Department of Soil Science at North Carolina State University in Raleigh, North Carolina, for analysis of total carbon content of each sample and to the Soil Physical Properties Laboratory in the Department of Soil Science at North Carolina State University for particle size analysis, using the hydrometer method (Gee and Or 2002). If organic matter exceeded 2%, samples were treated with hydrogen peroxide. Freeze-dried sediment aliquots were analyzed for iron concentration by Environmental Conservation Laboratories in Cary, North Carolina, using EPA Method 6010C (www.epa.gov/sam).

Fish and shrimp

Few native fish inhabit the streams of Puerto Rico and other islands in the Caribbean and Greater Antilles because these volcanic islands are relatively newly formed and are isolated from potential sources of colonizing species (Neal et al. 2009). Only six freshwater native fish are commonly found in Puerto Rico and all share common specialized life history traits, specifically they are diadromous (Kwak et al. 2007; Neal et al. 2009; Cooney and Kwak 2010). Samples of all native freshwater fish species were analyzed for contaminants, with the exception of the fat sleeper (*Dormitator maculatus*), which was not collected at any

site. Native species sampled included bigmouth sleeper (*Gobiomorous dormitor*), smallscaled spinycheek sleeper (*Eleotris perniger*), American eel (*Anguilla rostrata*), mountain mullet (*Agonostomus monticola*), sirajo goby (*Sicydium* spp.), river goby (*Awaous banana*), and *Macrobrachium* shrimp. Exotic species, introduced by anglers, the aquaculture industry, and aquarium owners, are commonly found in Puerto Rico. Although, this study focused on the native species, because of their natural heritage value and because indigenous and exotic species have different distributions, some exotic species were collected including Nile tilapia (*Oreochromis mossambicus*), redbreast sunfish (*Lepomis auritus*), and channel catfish (*Ictalurus punctatus*). Target species represent different taxa and feeding strategies and are consumed by humans, except for the smallscaled spinycheek sleeper. Fish and shrimp were collected using backpack electrofishing (Kwak et al. 2007). Specimens were sorted by species into labeled plastic bags, cooled, and then transferred as soon as possible to a -20˚C freezer.

Fish and shrimp were analyzed as composite samples using whole body or muscle tissue. The whole body of sirajo gobies was analyzed because the local people consume the whole body of these fish, as do instream and avian predators. The whole body of river gobies and spinycheek sleepers was analyzed for contaminants, for similar reasons. The edible muscle, excluding skin or scales, of American eel, Nile tilapia, bigmouth sleeper, redbreast sunfish, and channel catfish, was analyzed. Abdominal muscle tissue was analyzed for *Macrobrachium* shrimp.

Laboratory analyses and quality control

Selected toxicants were analyzed in water, sediment, and biota to describe how they were compartmentalized within each part of the ecosystem. Only sediment and biota were analyzed for metals (mercury, selenium, copper, nickel, zinc, cadmium, and lead) because the passive sampling devices, used in this study, do not accumulate metals. Passive sampling devices and sediment samples were analyzed for 34 current-use pesticides, 26 chlorinated pesticides, 48 polycyclic aromatic hydrocarbons (PAHs), and 20 polychlorinated biphenyl congeners (PCBs). Current-use pesticides and PAHs were not tested in biota because they are rapidly metabolized (Eisler 1987; Cope et al. 2011).

Analysis of organic contaminants in uPSDs, sediment, and biota was performed at the North Carolina State University Department of Environmental and Molecular Toxicology Chemical Exposure Assessment Laboratory in Raleigh, North Carolina, using a gas chromatograph-mass spectrometer (Appendix 1). Sediment and biota samples were freeze dried and sent to Environmental Conservation Laboratories in Cary, North Carolina, for inorganic toxicant analyses. Cadmium, copper, lead, nickel, selenium, and zinc were analyzed using EPA Method 6010B for the extensive study and EPA Method 6010C for the intensive study (www.epa.gov/sam). Mercury was analyzed using EPA Method 7471A for the extensive study and EPA Method 7471B for the intensive study (www.epa.gov/sam).

A rigorous quality assurance protocol was followed during analyses. For metal analyses, quality assurance included blanks, laboratory control samples (LCS), matrix spike, matrix spike duplicates, post spikes, and surrogate internal standards. The blanks were clean

(i.e., no target analytes were detected), with the exception of one detection of iron (3.6 mg/kg) and a detection of copper (0.07 mg/kg) and 2 detections of lead (<0.13 mg/kg) below the method reporting limit (MRL). The relative percent difference (RPD) of duplicate samples averaged 15% and ranged from 0.4-64%. For the few RPD values that were out of range, the batch was accepted based on percent recoveries for these samples that were within range. Overall, percent recoveries averaged 95%. All LCS percent recoveries were within range (mean = 99%, range = 85-110%). Results were not corrected for recoveries, due to acceptable accuracy and precision revealed by this protocol.

Procedural blanks, uPSD blanks (for uPSD batches), matrix spikes, and surrogate internal standards (SIS) were used to assess organic contaminant data quality. Procedural blanks were clean with few exceptions. Five PAHs were detected during sediment analysis (<8 ng/g). PCB 138 was detected in a procedure blank during sediment analysis (2 ng/g) and during fish analysis (<2 ng/g). Mean RPD values were 6% (range, 0-17%). Average surrogate recoveries were 80% (range, 48-115%) for uPSDs, 61% (range, 13-137%) for sediment, and 75% (range, 25-178%) for fish. Results were not corrected for recoveries, due to acceptable accuracy and precision revealed by this protocol.

Duplicate samples were also analyzed for quality assurance of lipid content of fish and shrimp and organic carbon content and particle size composition of sediment samples. The mean RPD value for lipid data was 9% (range, 0-18%). RPD values for sediment total organic carbon averaged 3% (range, 0-5%) and percent clay averaged 3% (range, 0-7%).

Contaminant criteria and guideline exceedance

Established criteria and guidelines are useful to assess the hazard of chemicals measured in water, sediment, and fish. We consulted EPA national recommended water quality criteria, EPA Office of Pesticide Program's aquatic life benchmarks, consensus based sediment guidelines, and EPA consumption limit tables (http://water.epa.gov/scitech/swguidance/waterquality/standards/current/index.cfm; http://www.epa.gov/oppefed1/ecorisk_ders/aquatic_life_benchmark.htm; MacDonald et al. 2000; U.S. Environmental Protection Agency 2000). EPA aquatic life benchmarks are estimates of concentrations below which chemicals are not expected to harm aquatic life and are based on the most sensitive toxicity endpoint for taxa. The consensus-based Threshold Effect Concentration (TEC) reflects sediment concentrations below which harmful effects to benthic organisms are unlikely to be observed, and the consensus-based Probable Effect Concentration (PEC) represents a threshold that if exceeded, harmful effects are likely to be observed. The Severe Effect Level (SEL) represents a threshold where adverse effects of the majority of sediment-dwelling organisms are expected if exceeded (McDonald et al. 2000). Consumption limit tables (U.S. Environmental Protection Agency 2000) are useful to infer human risk associated with consumption of fish and shrimp. They list contaminant concentration ranges and the associated limited numbers of meals per month and are based on an adult body weight of 70 kg and a meal of size of 0.227 kg. Some contaminant consumption limits are based only on noncancer endpoints, or chronic, systemic effects, but others include both noncancer and cancer endpoints.

Results

Water quality

Water quality measurements varied widely among sampling sites. Value ranges for water quality variables were, temperature, 22.7-34.7°C; total dissolved solids, 0.08-0.90 g/L; conductivity, 106-1451 µS/cm; salinity, 0.05-0.69 ppt; nitrate as NO_3^-, 0.3-10.0 mg/L; nitrite as NO_2^-, 0.006-0.670 mg/L; ammonia, 0.00-0.69 mg/L; phosphorus as PO_4^-, 0.02-2.06 mg/L; alkalinity, 33-317 mg/L; hardness, 43-235 mg/L; turbidity, 1-22 FAU; pH, 7.18-8.90; dissolved oxygen, 4.34-12.36 mg/L (Tables 2 and 3). Stream water from the reference site, with a primarily forested watershed, was low in ionic and nutrient content. Measurements of total dissolved solids, conductivity, salinity, nitrogen, phosphorus, alkalinity, and hardness were generally low at the reference site, while the agricultural sites generally had greater measurements of total dissolved solids, conductivity, salinity, ammonia, phosphorus, alkalinity, and hardness.

Water contaminants

Low concentrations of contaminants were estimated in water by uPSDs at all sites (Tables 4 and 5). No PCBs were detected at any site. The only chlorinated pesticides detected were chlordane compounds. Current use pesticides (CUP) detected in water included butylate, carbaryl, trifluralin, simazine, prometon, atrazine, metolachlor, and malathion. Prometon was detected at the greatest concentrations at an urban site (4U), but was not detected at any other site. Trifluralin was the most frequently detected CUP and was

found at all tested sites, with the exception of an industrial site (2I). An urban site (4U) generally had the greatest water contaminant concentrations, including chlordanes, total CUP, and total PAHs.

Sediment

Total organic carbon was generally low among sites. It was less than 4% at all sampling sites, with the exception of an industrial sampling site (3I) that had a mean total organic carbon content of approximately 8%. Clay composition and iron concentration was variable among sites. Clay composition ranged from 3 to 20%, and iron concentration ranged from 23.9 to 60.3 g/kg dry (Figs. 2 and 3).

Organic contaminants were at low concentrations in sediment at all sites (Tables 6 and 7). Chlordanes (cis-chlordane, trans-chlordane, and transnonachlor), DDTs (4,4'-DDE and 4, 4'-DDD), and hexachlorobenzene were the only chlorinated pesticides detected in sediment. DDTs were detected at greatest concentrations at agricultural sites. Chlordanes had greatest concentrations at an industrial (3I) and an agricultural (4U) site. Tebuthiuron, carbaryl-1, carbofuran-1, cyhalothrin (lambda), and bifenthrin were the only CUPs found in sediment. Overall, PCBs in sediment were greatest at urban sites. During extensive sampling, the greatest concentrations of total PAHs (235.1 ng/g dry) were also found at the urban site (4U), but the greatest total PAHs were found at the industrial site (3I) (mean = 493.0 ng/g dry) during intensive sampling. The reference site (1R) appeared to be the least

contaminated site, with no detections of OCs or CUPs and the lowest level of PAHs. However, low levels PCBs were detected at site 1R.

Cadmium, lead, mercury, selenium, and zinc were found at low concentrations in sediment samples (Tables 6 and 7). Copper concentrations were at moderately high levels (38.3-103 mg/kg dry) in sediment and was at greatest concentrations at agricultural sites. Nickel concentrations in sediment were variable among sites ranging from 4.63 to 336 mg/kg dry weight, and also had elevated concentrations at agricultural sites (10A, 9A, and 7A).

Fish and shrimp

Relatively high concentrations of PCBs and low levels of chlorinated pesticides, with the exception of dieldrin, were detected in fish tissue (Tables 8 and 9; Figs. 4 and 5). Mountain mullet and American eels were generally the most contaminated species and an urban site (4U) was the most contaminated site in terms of organic pollution. Chlordane, DDT, hexachlorobenzene, dieldrin, and gamma-BHC were the only chlorinated pesticides detected in fish tissue. High concentrations of dieldrin were found in American eels at the urban site (4U). PCBs were in greatest concentrations in mountain mullet, American eels, and river gobies from an urban site (4U). DDT was greatest in river gobies and American eels from an agricultural site (7A). Hexachlorobenzene was greatest in mountain mullet samples from an urban site (4U).

Fish tissue generally contained low concentrations of metals. Cadmium, nickel, mercury, and lead concentrations were generally below the method reporting limit (MRL)

and method detection limit (MDL) for fish samples (Cd: <MDL = 59%, <MRL = 90%; Ni: <MDL = 57%, <MRL = 74%; Hg: <MDL = 75%, <MRL = 87%; Pb: <MDL = 77%, <MRL = 97%; N = 115). Copper and selenium concentrations of several samples were below the MRL and MDL (Cu: <MDL = 35%, <MRL = 48%; Se: <MDL = 33%, <MRL = 43%; N = 115), while zinc was above the MRL for most samples (Zn: <MDL = 0%, <MRL = 6%; N = 115) and varied among species and sites. Selenium concentrations were similar among all biota, and observed concentrations were low, ranging from 0.298 to 0.934 mg/kg wet weight. Cadmium and copper were found at greatest concentrations in *Macrobrachium* spp. (Fig. 6 and 7). Mercury was greatest in channel catfish from site 8A, but bigmouth sleepers from site 4U had the greatest concentrations of mercury among native fish species (Fig. 8 and 9).

Mountain mullet and American eel had the greatest concentrations of organic contaminants, explained by their greater lipid content. Fish and shrimp lipid content and size within species varied significantly among sites ($P < 0.05$), with the exception of lipid content in sirajo gobies ($P = 0.34$, $N = 6$) and total length of river gobies ($P = 0.079$, $N = 9$) and spinycheek sleepers ($P = 0.230$, $N = 6$) (Figs. 10-13). Thus, PCB and mercury fish and shrimp concentrations were normalized by fish and shrimp lipid content and size (total length) to provide a comparative basis among sites (Figs. 14-16). An urban site (4U) had the greatest mercury and PCB concentrations after size and lipid normalization.

Contaminant criteria and guideline exceedance

All estimated contaminant concentrations that we measured in Puerto Rico streams were below the available EPA national recommended water quality criteria and the Office of Pesticide Programs' aquatic life benchmarks for the protection of plants, invertebrates, and fish. All organic contaminants, mercury, lead, selenium, cadmium, and zinc that we measured in sediment were below available consensus-based guidelines (MacDonald et al. 2000). Sediment analyzed from all sites exceeded the consensus-based TEC for copper, but were below the consensus-based PEC (MacDonald et al. 2000; Figs. 17 and 18). Nickel sediment concentrations exceeded the consensus based-TEC at five agricultural and both industrial sites, with four of these sites also exceeding the consensus-based PEC (MacDonald et al. 2000) (Fig. 19). Two agricultural sites exceeded the consensus-based SEL by 4 orders of magnitude, and intensive sampling at another agricultural site revealed a SEL exceedance (Persaud et al.1993; MacDonald 2000; Fig. 20).

Some mercury, cadmium, chlordane, DDT, dieldrin, and PCB concentrations that we measured in fish and shrimp in Puerto Rico rivers exceeded EPA consumption limits (Tables 8 and 9). EPA human consumption limits are not available for zinc, nickel, lead, or copper. Mercury had the most consumption exceedances of all contaminants, and all are based on noncancer endpoints with most at the 16 meals per month limit (noncancer). Bigmouth sleepers from an urban site had the greatest mercury concentrations among native species and exceeded the EPA consumption limit recommended for 3 meals per month. However, the greatest mercury fish tissue concentration was a channel catfish from an agricultural site, also

exceeding the 3 meals per month limit. Chlorinated pesticides were generally detected at low levels in fish tissue, however, dieldrin concentrations in American eels from the urban site (4U) were above all human consumption limits for cancer endpoints (i.e., 0 meals per month). There were few threshold exceedances for DDT; river gobies from an agricultural site (7A) and American eels from an urban site (4U), both exceeded the EPA consumption limits recommended for 16 meals per month for cancer endpoints. Mountain mullet from site 4U had the greatest concentrations of PCBs, exceeding the recommended consumption limit for 1 meal per month for cancer endpoints (4 meals for noncancer endpoints). No bigmouth sleepers, sirajo gobies, or *Macrobrachium* spp. exceeded human consumption limits for PCBs.

Overall, Puerto Rico streams are relatively less polluted than water bodies of other tropical regions and the United States (Table 10). The maximum advisable concentration for mercury (300 ppb wet) was never exceeded in our study; a study of mercury in a Cuba river revealed only 4% of the samples exceeding the criterion for mercury (Rosa et al. 2009). Other researchers found that 27% of streams and 49% of predatory fish in lakes in the United States exceeded this criterion (Stahl et al. 2009; Scudder et al. 2009). PCBs were lower in Puerto Rico streams relative to other regions with 8% of all samples exceeding 12 ppb wet; in comparison, 50-75% benthic-feeding fish from lakes in the United States exceeded the same benchmark (Stahl et al. 2009). Only 5% of our samples exceeded the NAWQA benchmark$_{low}$ (6 ppb wet) for DDT; however, 63% and 76% of all samples for Hawaii and U.S. streams exceeded this concentration, respectively.

Discussion

Our results indicate that pollution in the stream ecosystems of Puerto Rico is not severe or widespread with several notable exceptions. Nickel concentrations in sediment at three agricultural sites exceeded the severe effect level. An urban site generally had the greatest concentrations of contaminants, including the greatest concentrations of PAHs and PCBs in sediment and the greatest mercury, PCB, and dieldrin concentrations in native fishes. An urban site (4U) had the greatest concentrations of current use and chlordane pesticides and PAHs in water and the greatest concentrations of PAHs, chlordanes, and PCBs in sediment. Site 5U, the other urban site, also seemed to be relatively contaminated. The sediment was relatively low in organic carbon (Table 6), but it had the third greatest concentration of PAHs and chlordane in water and second greatest concentrations of PCBs and PAHs in sediment. Native fish species were not available for comparison with the other urban site (5U) because they were not present. In urban areas, there may be a greater input of pesticides for mosquito control, maintenance of right-of-ways, golf courses, and domestic lawns (Miles and Pfeuffer 1997). A greater amount of contaminants may also be released into urban streams as a result of impervious surfaces (Klein 1979; Holland et al. 2003).

Nickel and copper were the only contaminants to exceed sediment quality guidelines. Nickel was highly elevated at three agricultural sites, even after normalization by iron concentration, total organic carbon, and percent clay. These high levels could be due to applications of nickel-based fungicides or from illegal disposal of various electroplated items (Rowell 1968; Tandon et al. 1977; Hunter and Arbona 1995; Eisler 1998). Elevated levels of

copper could possibly be due to the natural geology of the island, pesticide applications, or they may be associated with vehicle brake pads. Copper is a major component of automobile brake pads, and all of our sampling sites were near road stream crossings (Gasser et al. 2009).

Our findings at three sites were contrary to expectations considering their land use and potential pollution sources. The reference site (1R) was relatively contaminated with metals, which was unexpected because it was located within a predominately forested watershed of the El Yunque National Forest. This unexpected finding may be attributed to persistent legacy effects of past land use or management practices (Harding et al. 1998; Kwak and Freeman 2010). This area was historically mined for gold, copper, and chalcopyrite and was also farmed as a coffee plantation (Cardona 1984). We also revealed unexpected findings at two industrial sampling sites. Site 2I was downstream of a nonoperational oil refinery and was expected to contain greater concentrations of PAHs in sediment and water, but levels of both media were low. Site 3I was located near a cement production facility and was expected to be a significant source of mercury, yet low mercury levels were detected there. The unexpected findings at industrial stream sites may be related to hydrology.

Contaminants and hydrology

Although streams of Puerto Rico receive substantial amounts of pollution from a variety of sources (Hazen 1988; Hunter and Arbona 1995; Stallard 2001; Neal et al 2008),

our results indicate that these stream ecosystems are not severely polluted, with the sporatic exception of nickel in sediment and PCBs and dieldrin in fish tissue. Hydrology may be an important factor limiting contaminant bioaccumulation in the stream ecosystems of Puerto Rico. If so, we would expect lentic ecosystems in Puerto Rico to show better evidence of pollution. Lentic and lotic environments differ in chemistry, hydrology, and ecology, which consequently can affect bioaccumulation. For example, fish from lentic systems have been shown to bioaccumulate selenium at a rate 10 times greater than fish from lotic environments exposed to similar concentrations (Adams et al. 2000). Lentic systems form sediment from organic matter that is constantly being recycled within the system along with associated contaminants due to long hydraulic retention times (Jefferies and Mills 1990; Simmons and Wallschläger 2005). In contrast, lotic systems create high flushing rates that prevent sedimentation of contaminated organic matter and exposure of benthos and detritral components of the ecosystem, thus reducing bioaccumulation (Lillebo et al. 1988; Van Derveer and Canton 1997; Adams et al. 2000; Simmons and Wallschläger 2005). In addition, lotic systems have a greater redox potential than lentic systems, due to constant aeration from flowing water (Simmons and Wallschläger 2005). Reducing conditions can form metal species that are less bioavailable than those more oxidized metal species (Lenz and Lens 2009). Additionally, the streams that we studied were shallow, facilitating chemical degradation by photolysis.

Streams in Puerto Rico tend to be well incised and narrow and may have less of a potential for bioaccumulation because they lack connections to environments similar to lentic

systems, such as floodplains. Most of the sites that we sampled also lack a connection to reservoirs because the native species that we targeted for sampling are only found downstream of reservoirs due to their diadromous life history. Conversely, non-native channel catfish from site 8A collected upstream of Dos Bocas reservoir had the greatest concentrations of mercury in our study. It is likely that channel catfish from this site migrated upstream from the reservoir where they had been exposed to more contaminants. Relationships with benthic habitats, as illustrated by this channel catfish example, and other microhabitat affinities likely play a minimal role in these streams because they are shallow and the substrate appears low in organic content and thus, organic pollutants.

Similarly, bioaccumulation in coastal areas is also influenced by habitat type because sheltered intertidal shores and creeks allow for accumulation of fine sediments, providing sinks for contaminants (Rawlins et al. 1998). Although, most Caribbean islands have little continental shelf with effective mixing and dispersal of terrestrial-based pollution (Rawlins et al. 1998), several deep oceanic basins in the Caribbean receive little flushing, making them vulnerable to contaminant accumulation (Ross and DeLorenzo 1997). Impoundments and estuaries of Puerto Rico may be more polluted than the stream habitats that we sampled, as suggested by other studies. For example, a study of coastal sediments revealed that Guanica Bay, Puerto Rico, had elevated levels of PCBs and DDT (Pait et al. 2008). In contrast, another study generally found low concentrations of mercury in biota at three estuaries in Puerto Rico (Burger et al. 1992). High levels of contaminants in fish tissue were found in marine and some reservoir fish of Puerto Rico (Rodríguez and González 1981). Although, a

contaminant survey of redear sunfish and sediment in the Dos Bocas Reservoir, Puerto Rico, showed little evidence of contamination problems (Neal et al. 2005).

General conclusions

Stream ecosystems in Puerto Rico were not severely polluted especially when compared with other water bodies in tropical ecosystems and the United States; several exceptions were nickel in sediment at agricultural sites and PCBs and dieldrin at an urban site. All fish species contained variable concentrations of contaminants, but among those sampled, bigmouth sleepers may be the most suitable fish for human consumption. They have low levels of organic contaminants and rare occurrences of mercury. This is the first study examining contaminants and resulting trends in the freshwater ecosystems of Puerto Rico. Results of this project will assist water and natural resource agencies in identifying areas of concern, planning to improve ecological and human health, and development of freshwater fisheries.

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Table 1 Puerto Rico stream sampling site characteristics, including location, distance from river mouth, elevation, gradient, and watershed attributes

Site	Designated Land Use	River Name	Latitude	Longitude	Distance to River Mouth (km)	Site Elevation (m)	Watershed Area (km^2)	Gradient (%)	Watershed Land Use: Agriculture (%)	Forest (%)	Shrub and Woodland (%)	Urban (%)
1R	Reference	Mameyes	N18°21'23.7"	W65°46'06.9"	4.4	14.0	21.3	1.4	2.7	95.6	1.0	0.1
2I	Industrial	Tallaboa	N18°00'17.3"	W66°43'53.0"	1.8	6.1	82.7	0.1	22.0	47.1	25.4	5.3
3I	Industrial	Cañas	N18°01'23.2"	W66°38'26.4"	5.0	30.2	20.6	0.4	30.0	27.4	32.3	9.8
4U	Urban	Piedras	N18°23'02.8"	W66°03'31.2"	8.6	13.4	23.2	0.2	24.8	26.3	8.8	39.4
5U	Urban	Bayamon	N18°19'51.7"	W66°06'04.2"	19.1	58.8	22.1	1.5	25.0	50.0	13.4	11.6
6A	Agricultural	Añasco	N18°14'14.9"	W67°02'42.6"	25.7	201.6	9.2	1.3	77.9	7.9	13.4	0.8
7A	Agricultural	Yauco	N17°59'12.9"	W66°50'25.7"	5.7	10.1	115.9	0.1	24.9	31.5	36.6	6.1
8A	Agricultural	La Plata	N18°13'28.4"	W65°12'58.9"	43.4	188.4	146.5	0.4	44.2	21.6	25.5	8.1
9A	Agricultural	Jacaguas	N18°04'11.1"	W66°30'33.5"	19.2	52.4	124.1	1.0	36.9	39.4	26.3	4.8
10A	Agricultural	Guanajibo	N18°09'29.8"	W67°05'06.7"	23.6	48.8	48.2	2.0	53.5	34.4	10.2	1.8
11A	Agricultural	Cartagena	N18°01'42.5"	W67°06'48.6"	7.1	20.1	10.6	0.3	40.4	41.8	13.6	4.2
12A	Agricultural	Arecibo	N18°15'34.9"	W66°43'20.4"	40.8	146.3	112.2	0.5	13.5	25.0	58.7	2.4
13A	Agricultural	Fajardo	N18°19'20.5"	W65°38'59.3"	3.58	4.0	58.5	0.1	34.5	53.8	7.4	2.8

Table 2 Water quality parameter values measured at each site during the extensive study

	Site												
Parameter	1R	2I	3I	4U	5U	6A	7A	8A	9A	10A	11A	12A	13A
Temperature (°C)	29.0	27.5	29.8	28.3	28.5	24.9	27.4	22.7	30.0	29.3	25.6	27.7	30.3
Total dissolved solids (g/L)	0.09	0.18	0.27	0.20	0.25	0.10	0.70	0.13	0.17	0.17	0.28	0.17	0.12
Conductivity (µS/cm)	140	298	434	442	411	152	1125	106	289	265	430	280	211
Salinity (ppt)	0.06	0.13	0.20	0.20	0.18	0.07	0.53	0.05	0.12	0.12	0.20	0.13	0.09
Nitrate (mg/L as NO_3-)	2.7	0.9	6.2	2.7	0.8	0.3	4.6	0.3	0.3	0.8	10.0	3.9	3.9
Nitrite (mg/L as NO_2-)	0.01	0.02	0.01	0.01	0.06	0.05	0.01	0.01	0.01	0.04	0.06	0.07	0.04
Ammonia/nitrogen (mg/L as NH_3)	0.00	0.00	0.00	0.14	0.01	0.01	0.01	0.00	0.01	0.00	0.00	0.11	0.01
Phosphorus (mg/L as PO_4-)	2.06	0.31	0.17	2.06	1.21	0.40	0.30	0.33	0.50	2.26	0.69	2.06	2.06
Alkalinity (mg/L as $CaCO_3$)	46	314	121	123	123	70	139	39	97	123	317	98	38
Hardness (mg/L as $CaCO_3$)	49	208	153	145	149	72	140	44	103	129	191	113	43
Turbidity (FAU)	3	1	1	14	22	4	2	3	2	1	4	10	3
pH	7.64	7.81	8.47	8.04	8.43	7.79	7.76	7.62	8.38	8.64	7.43	8.43	8.90
Dissolved oxygen (mg/L)	7.80	9.08	8.70	7.92	8.09	7.30	4.34	6.79	9.76	7.60	4.75	8.03	10.90

Table 3 Water quality parameter values measured at each site during the intensive study

	Site			
Parameter	1R	3I	4U	7A
Water temperature (°C)	34.7	31.7	27.8	27.6
Total dissolved solids (g/L)	0.079	0.489	0.386	0.899
Conductivity (µS/cm)	121	844	628	1451
Salinity (ppt)	0.06	0.36	0.28	0.69
Nitrate (mg/L as NO_3-)	1.8	1.5	4.4	3.5
Nitrite (mg/L as NO_2-)	0.006	0.006	0.046	0.015
Ammonia/nitrogen (mg/L as NH_3)	<0.01	0.08	0.02	0.69
Phosphorus (mg/L as PO_4-)	0.02	0.95	0.59	0.83
Alkalinity (mg/L as $CaCO_3$)	33	131	118	248
Hardness (mg/L as $CaCO_3$)	38	185	182	235
Turbidity (FAU)	4	1	3	4
pH	7.27	8.10	7.29	7.18
Dissolved oxygen (mg/L)	8.26	12.36	8.23	5.43

Table 4 Estimated contaminant concentrations (ng/L) in fiber PSDs for each extensive study site. A dash symbolizes concentrations that were below detection limits. Concentrations were estimated using methods described in Appendix 1

	Site											
Analyte and Duration	1R	2I	3I	4U	5U	6A	7A	8A	10A	11A	12A	13A
Butylate	–	–	–	–	–	–	–	–	–	–	–	2.0
Carbaryl	–	1.3	–	–	–	–	–	–	–	–	–	–
Trifluralin	0.3	–	0.3	0.5	0.6	0.4	0.4	0.5	0.3	0.3	0.2	0.4
Simazine	–	–	–	5.6	–	–	–	–	–	–	–	–
Prometon	–	–	–	42.9	–	–	–	–	–	–	–	–
Atrazine	–	–	4.2	10.1	–	–	1.0	2.8	–	–	–	–
Metolachlor	–	–	–	–	–	1.1	–	–	–	–	–	–
Chlordanes	0.1	–	–	0.3	0.2	0.2	–	0.2	–	–	–	0.2
Total PAHs	101.9	41.7	148.9	293.2	216.0	83.4	94.4	226.0	79.8	67.3	108.1	148.1
Deployment duration (days)	25.9	28.9	27.9	22.2	22.1	26.9	29.2	21.2	29.9	30.3	24.9	26.0

Table 5 Mean estimated contaminant concentrations in cartridge PSDs for intensive sites. A dash symbolizes concentrations that were below detection limits

	Site			
Analyte (ng/L)	1R	3I	4U	7A
Atrazine	–	0.1	0.9	–
Malathion	–	0.8	–	–
Total PAHs	7.0	12.1	2.1	12.4
Deployment duration (days)	26.8	27.7	34.8	28.9

Table 6 Contaminant concentrations in sediment for each site during the extensive study. A dash symbolizes concentrations that were below detection limits

	Site												
Analyte	1R	2I	3I	4U	5U	6A	7A	8A	9A	10A	11A	12A	13A
Organics (ng/g dry)													
Hexachlorobenzene	–	0.072	–	0.073	–	–	–	–	–	–	–	–	–
Chlordanes	–	–	0.962	0.621	–	–	–	0.061	–	–	–	–	–
DDTs	–	–	–	–	0.145	–	0.423	0.623	–	–	–	–	–
PCBs	0.156	–	–	1.840	1.156	–	–	–	–	–	–	–	0.259
Total PAH	2.4	38.1	170.0	235.1	204.7	10.1	42.1	88.7	20.8	17.0	13.4	16.6	26.7
Tebuthiuron	–	–	–	–	8.7	–	–	–	–	–	–	–	–
Metals (mg/kg dry)													
Cadmium	–	0.14	0.09	–	0.04	0.14	0.05	0.23	0.12	0.08	0.22	0.26	–
Copper	53.2	59.4	38.3	51.9	94.1	76.4	52.5	103	50.9	49.8	44.5	84.7	65.3
Lead	4.36	10.20	5.79	9.55	10.90	7.97	9.45	9.91	7.19	6.58	5.44	11.8	5.21
Mercury	0.020	0.030	0.050	0.060	0.010	0.050	0.040	0.070	0.030	0.008	0.010	0.009	0.030
Nickel	19.5	33.2	31.9	22.1	10.2	38.9	50.1	52.1	327	336	19.9	6.3	4.6
Zinc	61.8	65.6	87.6	94.8	82.1	99.7	60.3	79.1	57.7	56.1	70.7	102.0	65.3

Table 7 Mean contaminant concentrations in sediment for each site during the intensive study. A dash symbolizes concentrations that were below detection limits. An asterisk indicates contaminants that were detected, but could not be quantified

	Site			
Analyte	1R	3I	4U	7A
Organics (ng/g dry)				
Hexachlorobenzene	–	–	0.092	–
Chlordanes	–	0.236	1.17	–
DDTs	–	–	0.170	0.877
PCBs	–	0.244	0.834	0.539
Bifenthrin	–	3.34	1.93	0.280
Carbaryl-1	–	*	–	–
Carbofuran-1	–	*	–	–
Cyhalothrin (lambda)	–	–	–	0.29
Total PAHs	2.9	493.0	171.4	87.7
Metals (mg/kg dry)				
Cadmium	0.184	0.426	0.144	0.290
Copper	46.3	43.3	48.3	63.9
Lead	6.2	10.6	13.4	15.3
Mercury	0.005	0.043	0.049	0.051
Nickel	13.8	30.7	12.2	148
Zinc	55.1	97.7	100.3	88.9

Table 8 Contaminant concentrations exceeding EPA consumption limit recommendations for extensive study sites. 'NA' represents contaminant's number of meals per month that are not applicable because consumption limits for cancer endpoints have not been established. 'UR' indicates an unrestricted number of meals per month (U.S. Environmental Protection Agency 2000)

Analyte	Site	Taxon	Concentration (ppm wet)	EPA noncancer consumption limit (meals/month)	EPA cancer consumption limit (meals/month)
DDTs	7A	River goby	0.0093	UR	16
PCBs	12A	Channel catfish	0.0029	UR	12
	13A	American eel	0.0046	UR	8
	9A	American eel	0.0037	UR	12
	2I	American eel	0.0086	16	4
	7A	American eel	0.0065	16	4
	4U	Mountain mullet	0.0189	8	2
	4U	River goby	0.0134	12	3
	7A	River goby	0.0049	UR	8
Cd	9A	*Macrobrachium*	0.1838	12	NA
	1R	*Macrobrachium*	0.2043	12	NA
	2I	*Macrobrachium*	0.1942	12	NA
Hg	3I	Bigmouth sleeper	0.0344	16	NA
	1R	Bigmouth sleeper	0.0363	16	NA
	4U	Bigmouth sleeper	0.0773	12	NA
	2I	Bigmouth sleeper	0.0390	16	NA
	8A	Channel catfish	0.2823	3	NA
	11A	American eel	0.0585	16	NA
	13A	American eel	0.0396	16	NA
	1R	American eel	0.0361	16	NA
	13A	*Macrobrachium*	0.0379	16	NA
	9A	Mountain mullet	0.0651	12	NA
	8A	Redbreast sunfish	0.0743	12	NA
	6A	River goby	0.0630	12	NA
	10A	River goby	0.0405	16	NA
	4U	River goby	0.0920	8	NA
	7A	River goby	0.0470	16	NA
	1R	Sirajo goby	0.0500	16	NA

Table 9 Contaminant concentrations exceeding EPA consumption limit recommendations for intensive study sites. 'NA' represents contaminant's number of meals per month that are not applicable because consumption limits for cancer endpoints have not been established. 'UR' indicates an unrestricted number of meals per month (U.S. Environmental Protection Agency 2000)

Contaminant	Site	Species	Concentration (ppm-wet)		EPA noncancer consumption limit (meals/month)		EPA cancer consumption limit (meals/month)	
			Mean	SD	Mean	Range	Mean	Range
PCBs	3I	American eel	0.0050	0.0027	UR	UR-16	8	4-12
		Mountain mullet	0.0044	0.0013	UR	UR-16	8	4-12
	4U	American eel	0.0250	0.0059	4	4-8	1	1-2
		Mountain mullet	0.0275	0.0032	4	4-4	1	1-1
		River goby	0.0062	0.0013	16	UR-16	4	4-8
		Spinycheek sleeper	0.0108	0.0033	16	12-16	4	3-4
	7A	American eel	0.0065	0.0024	16	UR-16	4	4-8
		Mountain mullet	0.0038	0.0020	UR	UR-16	12	4-16
		River goby	0.0020	0.0006	UR	UR-UR	16	16-16
		Spinycheek sleeper	0.0015	0.0013	UR	UR-UR	16	UR-16
Chlordanes	4U	American eel	0.0104	0.0037	UR	UR-UR	16	UR-16
		Mountain mullet	0.0135	0.0029	UR	UR-UR	16	16-16
DDTs	4U	American eel	0.0080	0.0050	UR	UR-UR	UR	UR-16
Dieldrin	4U	American eel	0.0142	0.0125	UR	UR-16	none	UR-none
Mercury	4U	Bigmouth sleeper	0.1679	0.0561	4	3-4	NA	NA
		American eel	0.0679	0.0651	12	UR-4	NA	NA
		Spinycheek sleeper	0.0775	0.0251	12	8-16	NA	NA
	7A	American eel	0.0391	0.0153	16	UR-16	NA	NA
		Mountain mullet	0.0281	0.0238	UR	UR-16	NA	NA
		Spinycheek sleeper	0.0406	0.0106	16	16-16	NA	NA
	3I	Bigmouth sleeper	0.0252	0.0239	UR	UR-16	NA	NA

Table 10 Comparison of aquatic contaminants in fish tissue among aquatic ecosystems, including U.S. lakes (Stahl et al. 2009), U.S. streams (Gilliom et al. 2007, Scudder et al. 2009), Puerto Rico streams (this study), one Cuba river (Rosa et al. 2009), and Hawaii streams (Gilliom et al. 2007). Benchmark exceedance concentrations are 300 ppb for mercury, 12 ppb for PCBs, 67 ppb for chlordane, and 69 ppb for DDT (U.S. Environmental Protection Agency 2000). A dash indicates data not available; 'ND' indicates that the concentration was below detection limits

	Mercury			PCBs			Chlordane			DDT		
Water body	Median (ppb)	Max (ppb)	Threshold Exceeded (%)	Median (ppb)	Max (ppb)	Threshold Exceeded (%)	Median (ppb)	Max (ppb)	Threshold Exceeded (%)	Median (ppb)	Max (ppb)	Threshold Exceeded (%)
U.S. lakes												
Predators	285	6,605	49	2	705	17	ND	100	<1	2	1,481	<2
Benthic feeders	69	596	<5	14	1,266	50-75	27	378	<5	13	1,761	10-25
U.S. streams												
Overall	169	1,950	27	–	–	–	ND	1,790	13	33	9,494	34
Urban	–	–	–	–	–	–	46	1,790	39	63	2,200	47
Agricultural	–	–	–	–	–	–	5	445	12	64	9,494	49
Undeveloped	–	–	–	–	–	–	<1	634	2	7	2,148	13
Mixed	–	–	–	–	–	–	13	586	17	48	7,200	40
Hawaii streams	–	–	–	–	–	–	17	1,790	30	10	361	15
PR streams												
Overall	13	282	0	<1	31	8	ND	15	0	0	12	0
Reference	13	50	0	<1	1	0	ND	ND	0	0	<1	0
Urban	19	233	0	7	31	8	<1	15	0	0.6	12	0
Industrial	13	53	0	<1	9	0	ND	2	0	0	2	0
Agricultural	18	282	0	<1	9	0	ND	3	0	0	10	0
Cuba river	–	375	4	–	–	–	–	–	–	–	–	–

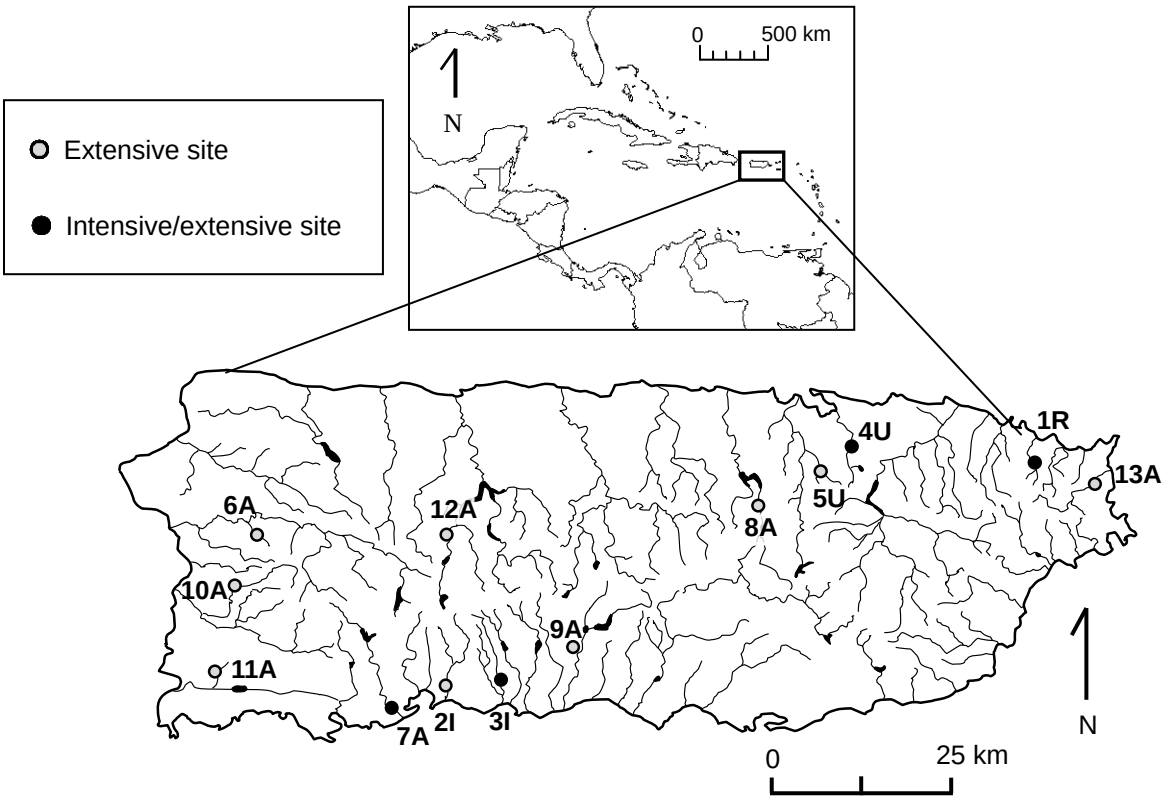

Fig. 1 Map of Puerto Rico indicating stream study sites

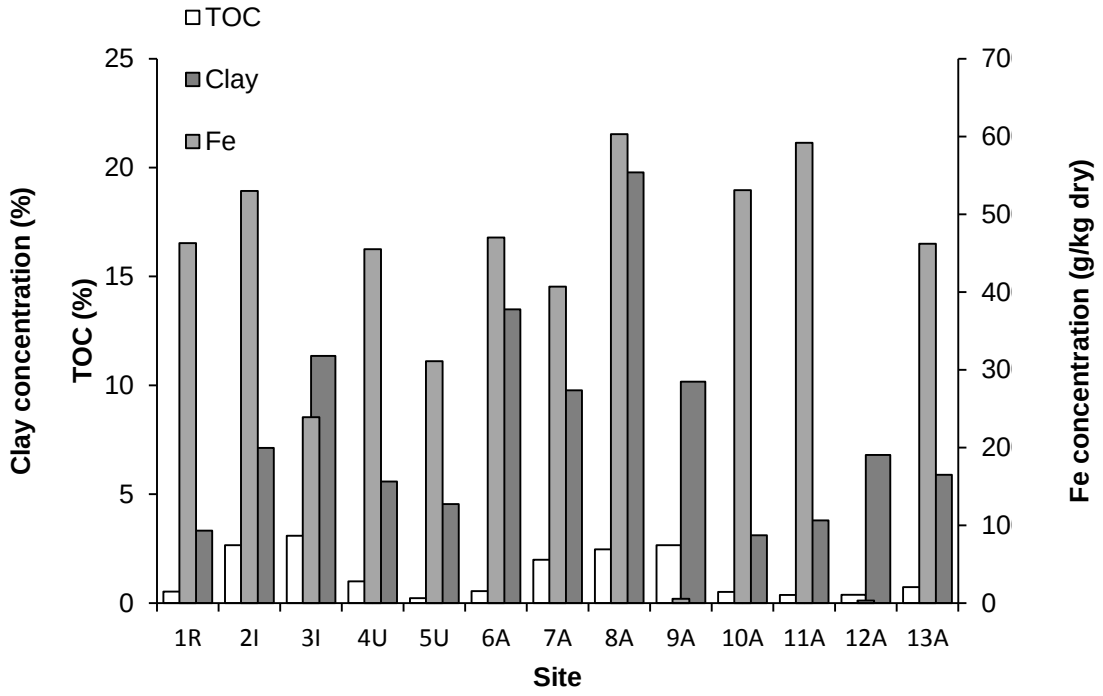

Fig. 2 Sediment normalization factors total organic carbon (TOC), clay, and iron (Fe) concentration for extensive study sites

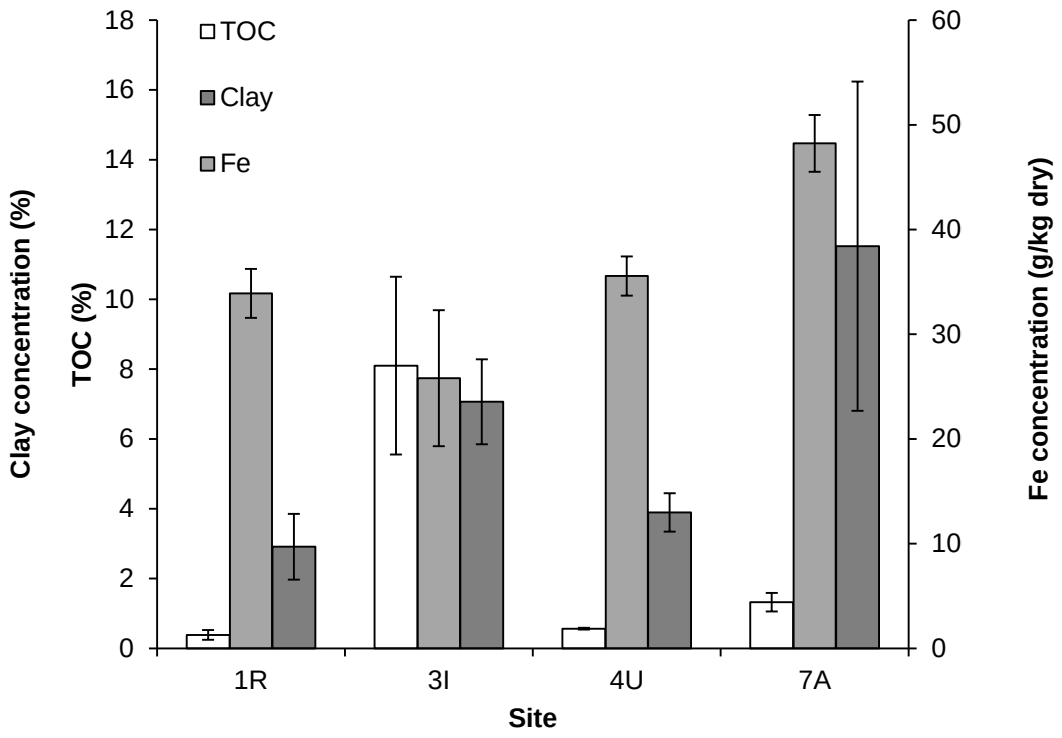

Fig. 3 Mean (± SD) sediment normalization factors total organic carbon (TOC), clay, and iron (Fe) concentration for intensive study sites

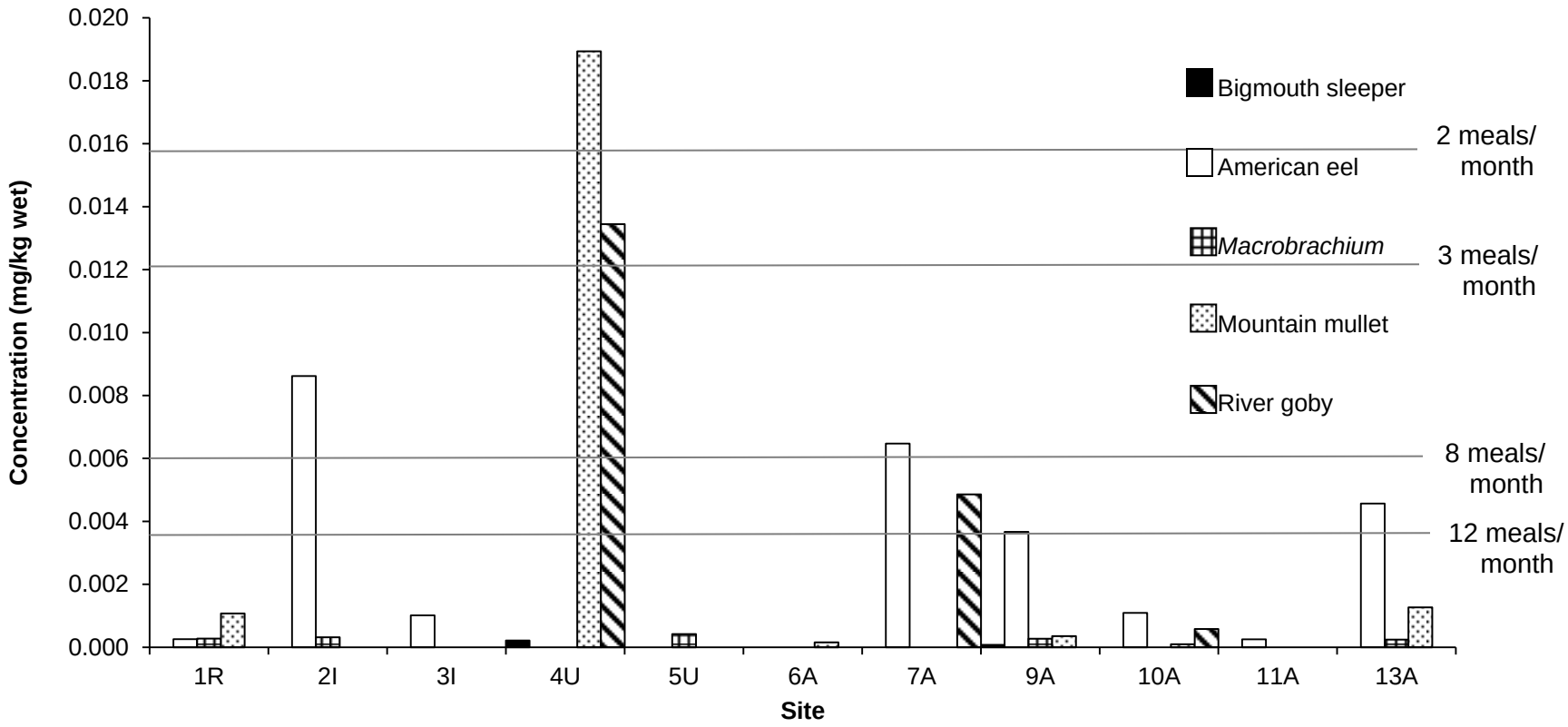

Fig. 4 PCB concentrations in fish and shrimp at extensive sampling sites with EPA monthly human consumption limits indicated (U.S. Environmental Protection Agency 2000)

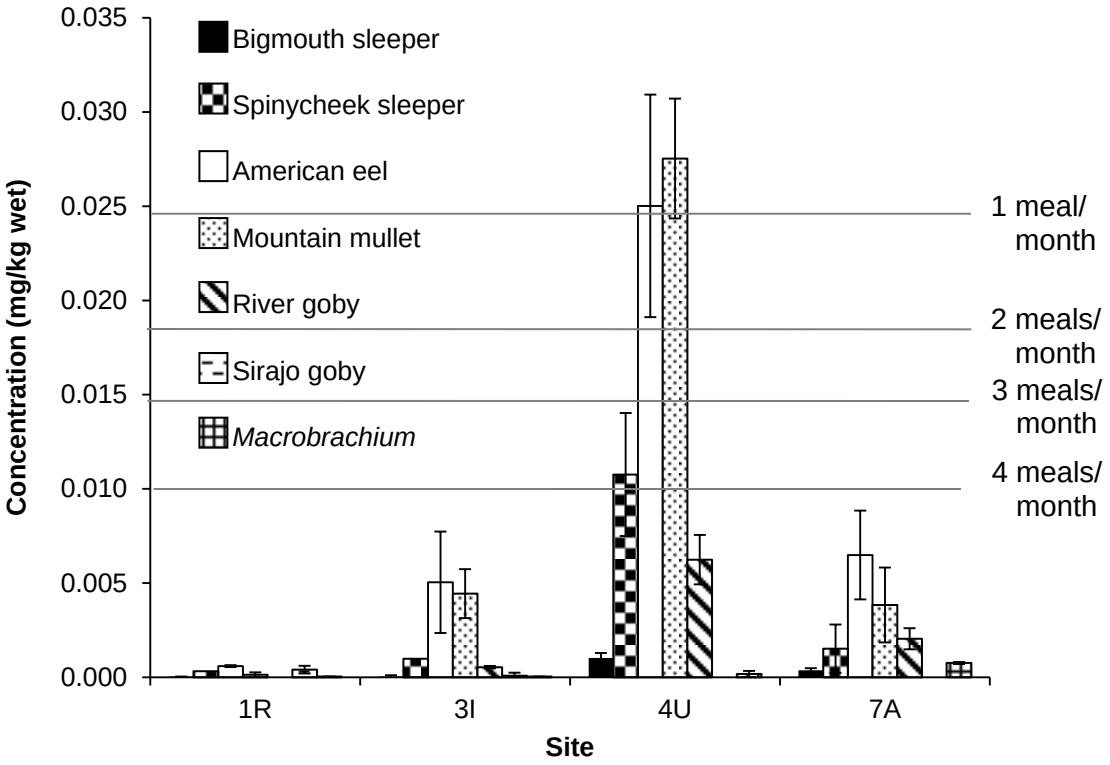

Fig. 5 Mean (± SD) PCB concentrations in fish and shrimp at intensive sampling sites with EPA monthly consumption limits indicated (U.S. Environmental Protection Agency 2000)

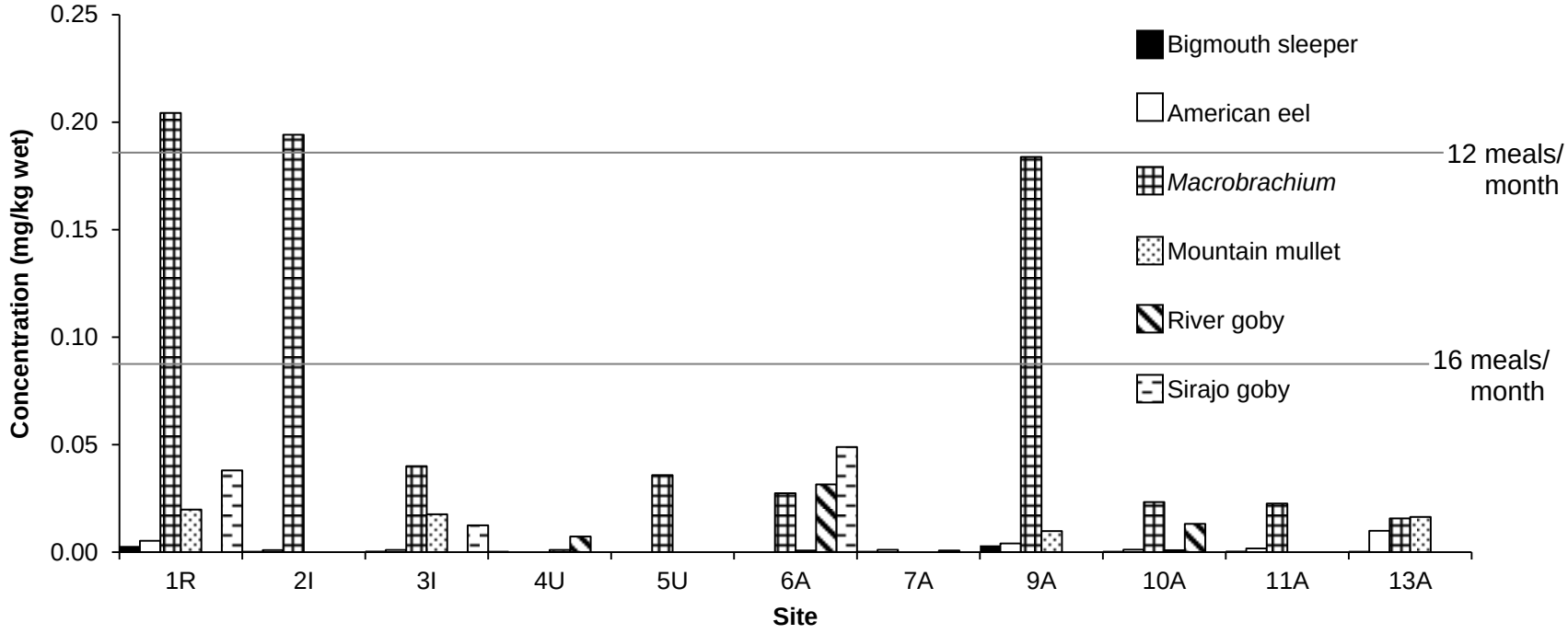

Fig. 6 Cadmium concentrations in fish and shrimp at extensive sampling sites with EPA monthly human consumption limits (U.S. Environmental Protection Agency 2000)

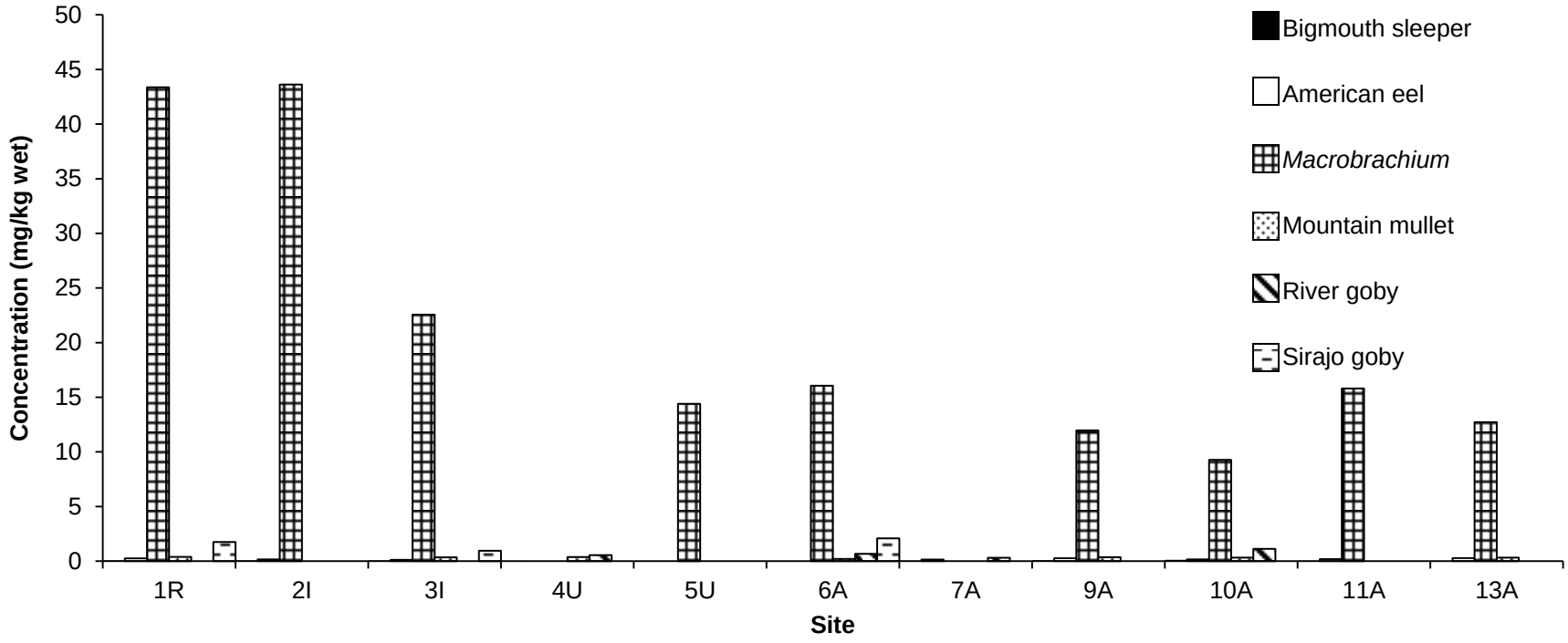

Fig. 7 Copper concentrations in fish and shrimp at extensive sampling sites

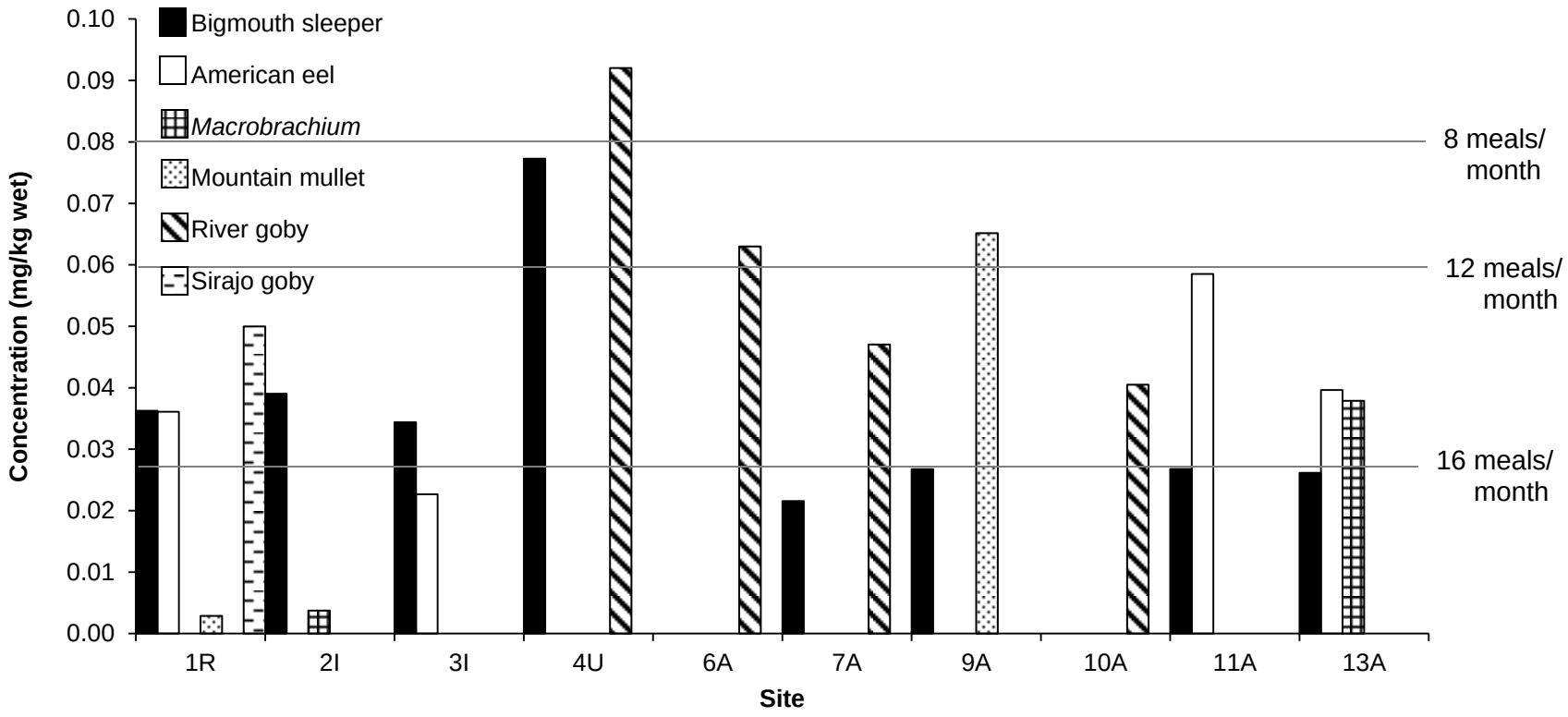

Fig. 8 Mercury concentrations in composite fish and shrimp samples at extensive sampling sites with EPA monthly consumption limits indicated (U.S. Environmental Protection Agency 2000)

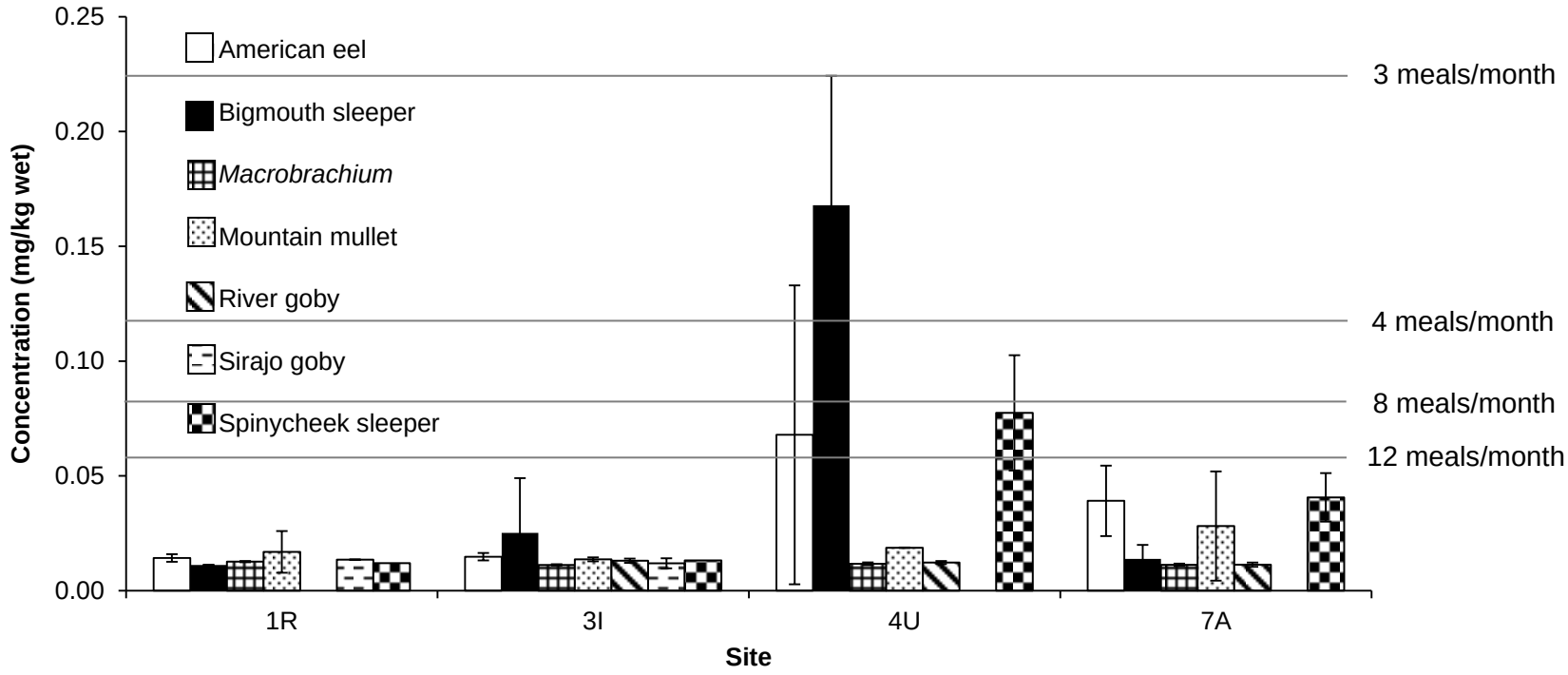

Fig. 9 Mean (±SD) mercury concentrations in fish and shrimp at intensive sampling sites and EPA monthly fish consumption limits indicated (U.S. Environmental Protection Agency 2000)

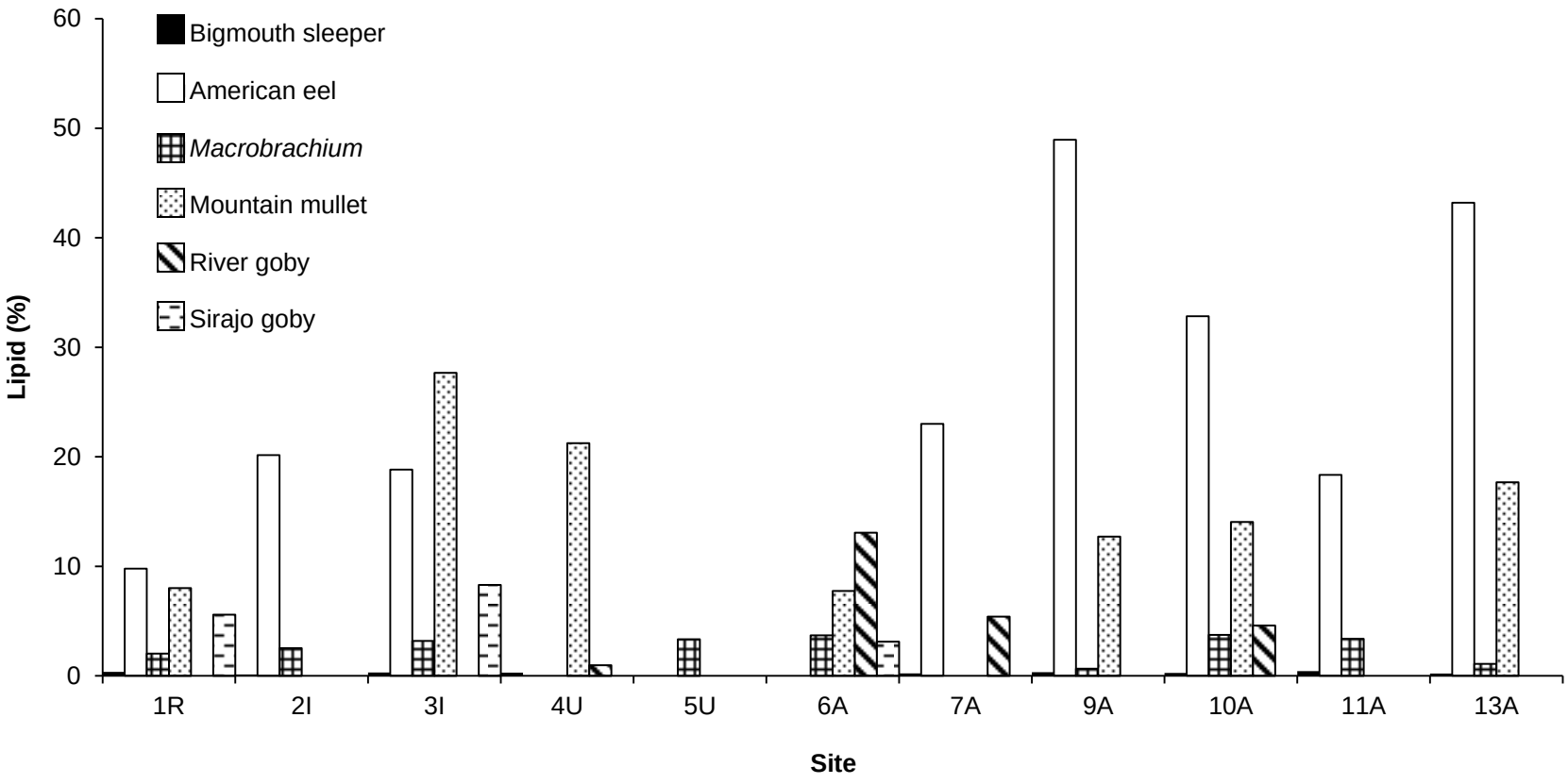

Fig. 10 Lipid content in fish and shrimp samples at extensive sampling sites

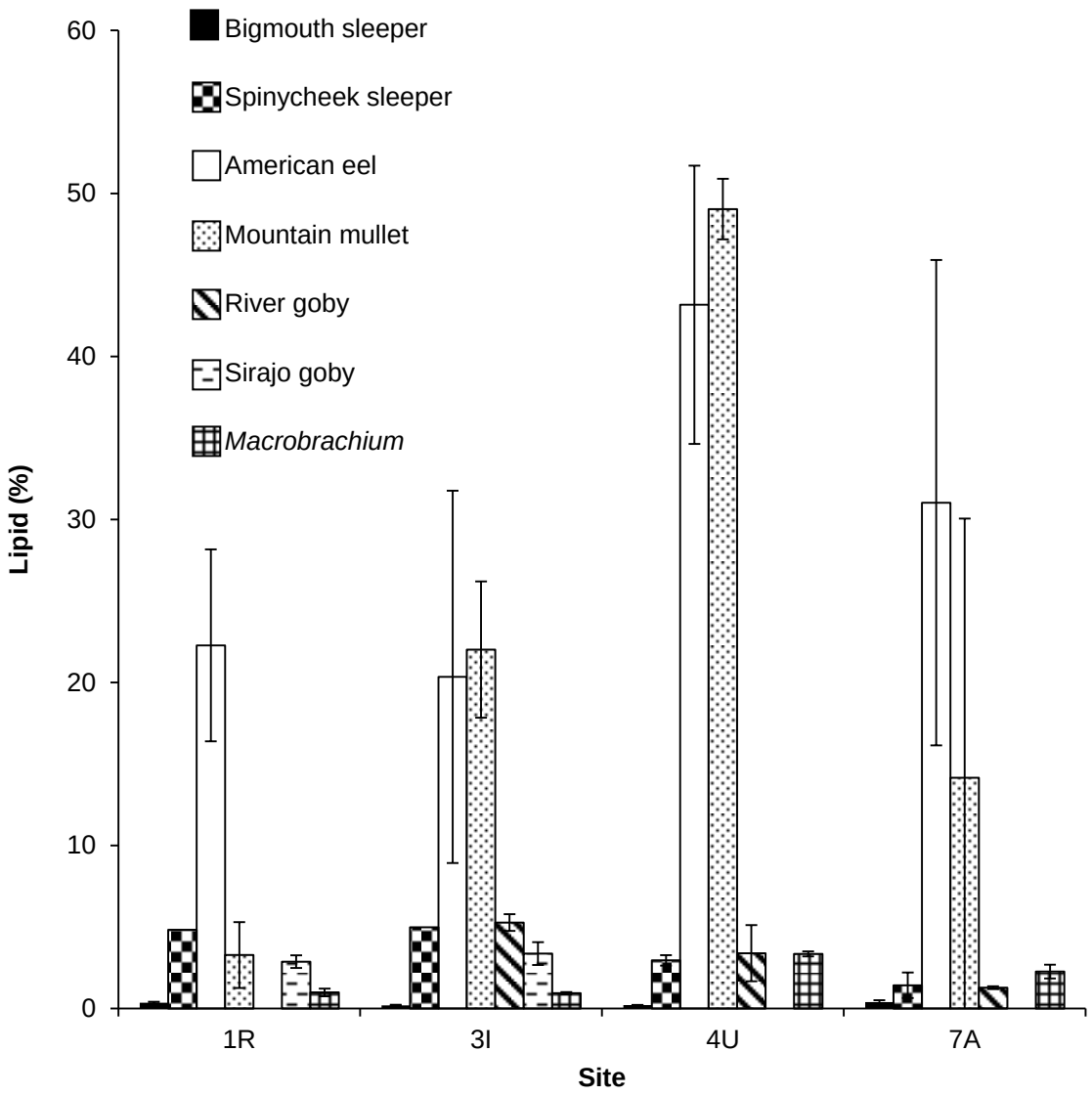

Fig. 11 Mean (± SD) lipid content for fish and shrimp at intensive sampling sites

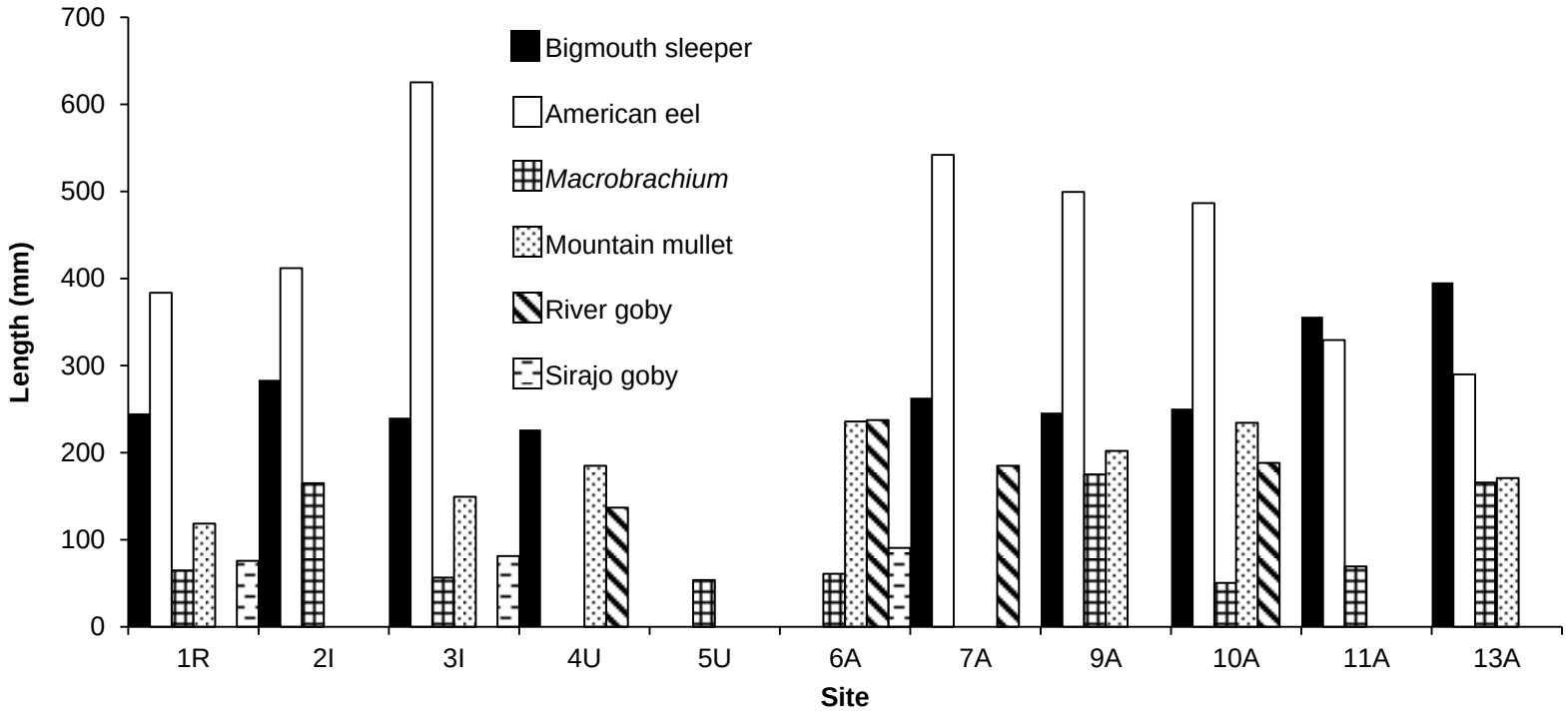

Fig. 12 Length of fish and shrimp sampled at extensive sites

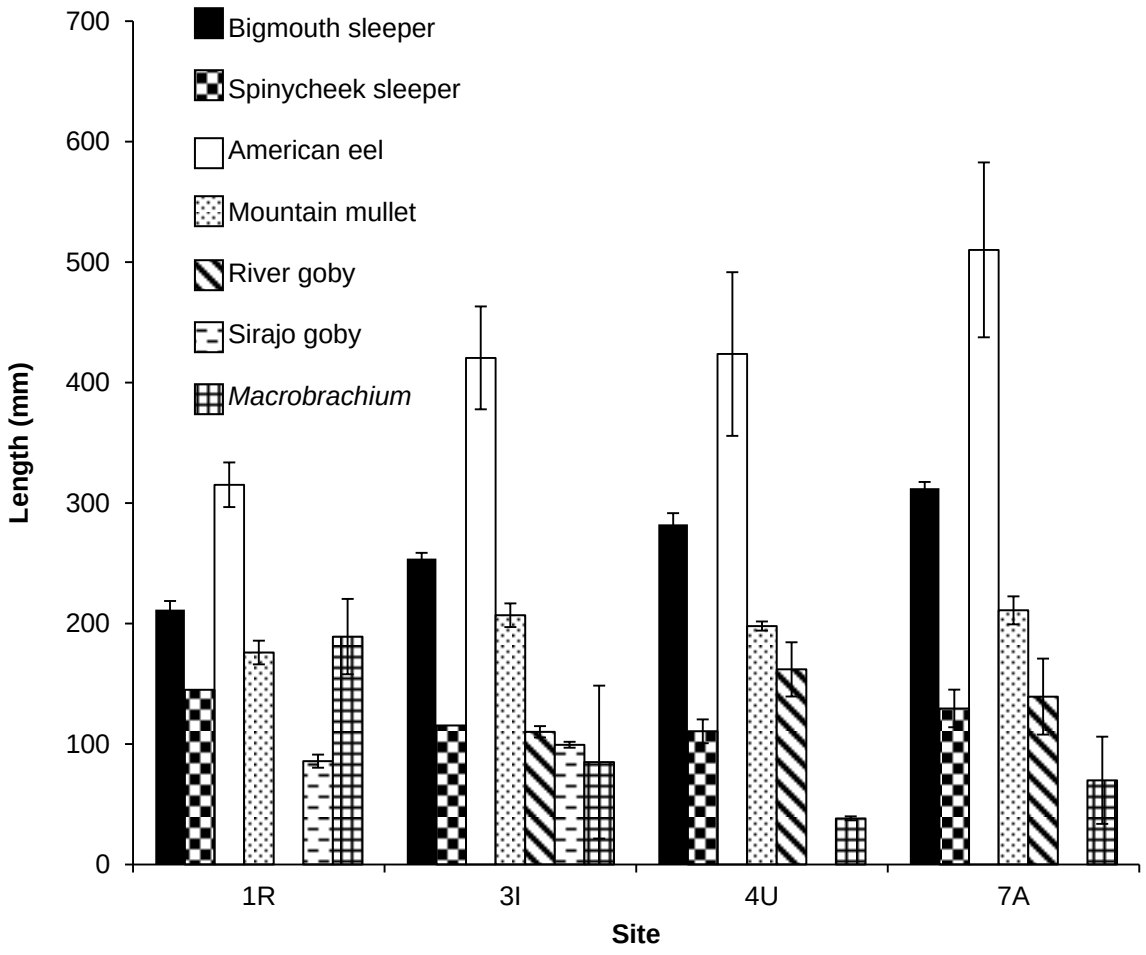

Fig. 13 Mean (± SD) length of fish and shrimp sampled at intensive sites

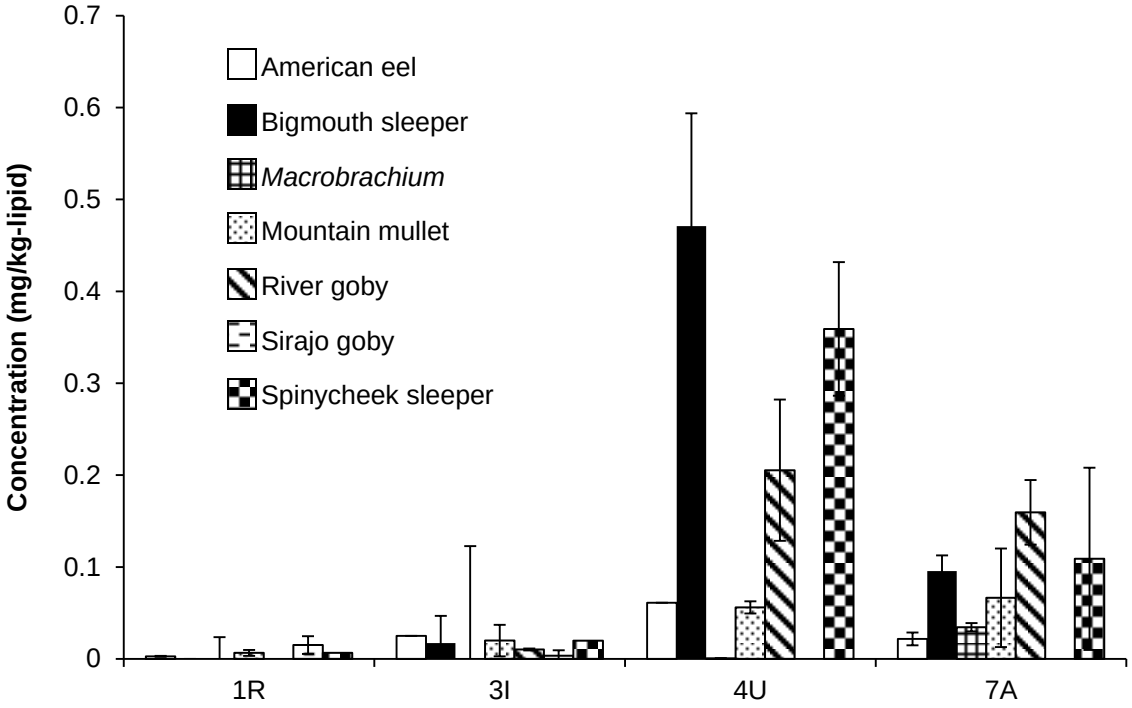

Fig. 14 Mean (± SD) PCB concentrations normalized by lipid content for fish and shrimp sampled at intensive sites

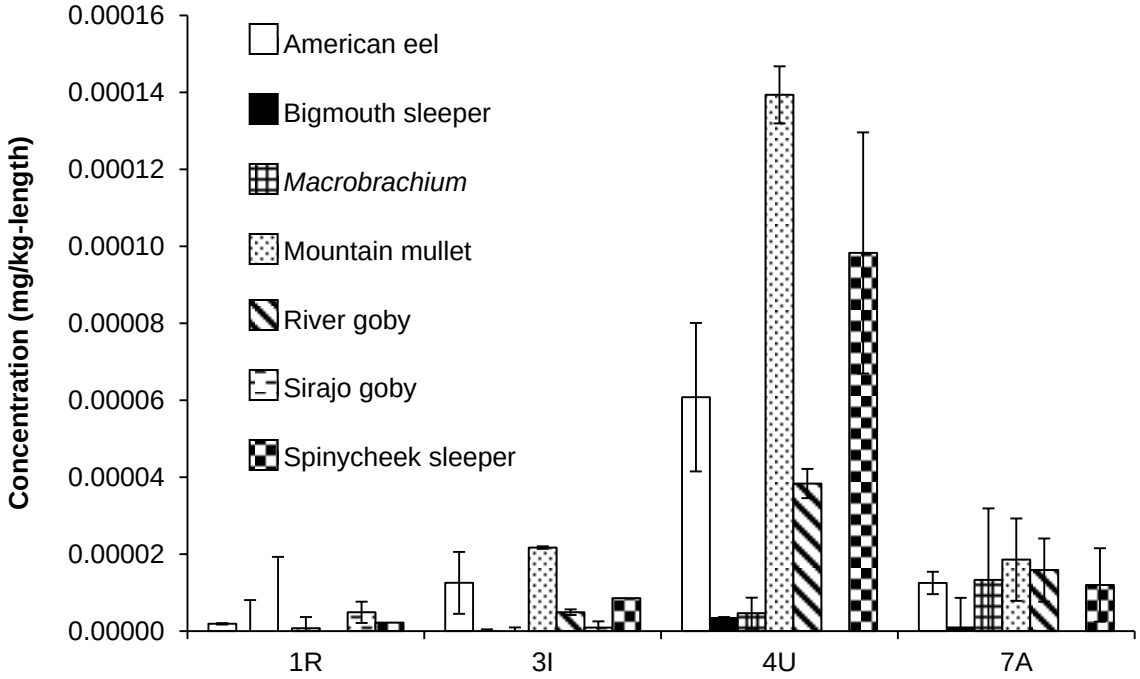

Fig. 15 Mean (± SD) PCB concentrations normalized by organism length for fish and shrimp sampled at intensive sites

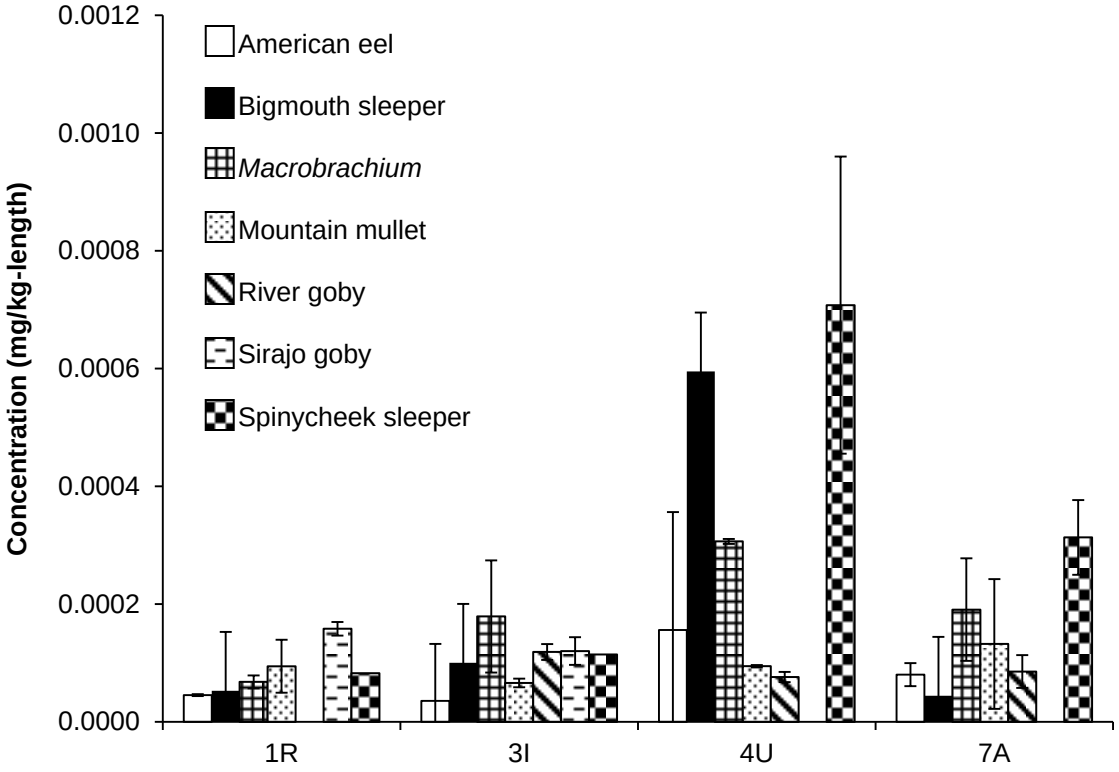

Fig. 16 Mean (± SD) mercury concentrations normalized by organism length for fish and shrimp sampled at intensive sites

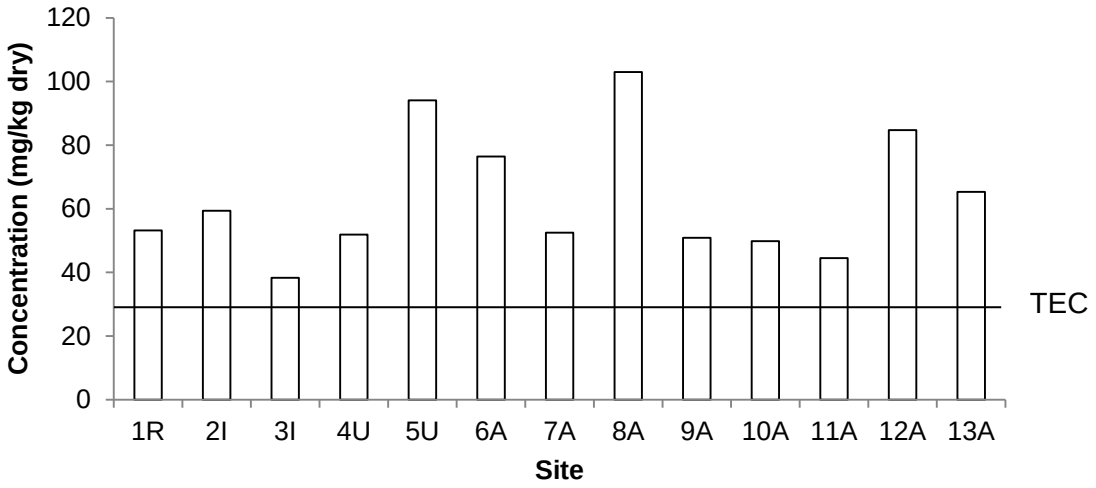

Fig. 17 Copper concentrations in sediment with guideline exceedances for extensive sampling sites. TEC indicates the consensus based-threshold effect concentration (MacDonald et al. 2000)

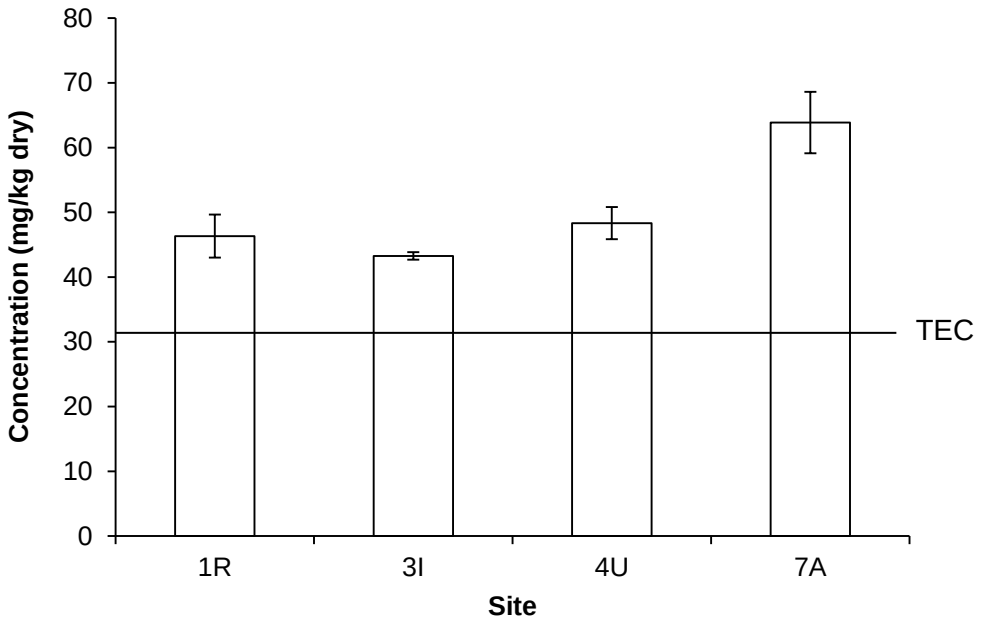

Fig. 18 Mean (± SD) copper concentrations in sediment for intensive sampling sites. TEC indicates the consensus based-threshold effect concentration (MacDonald et al. 2000)

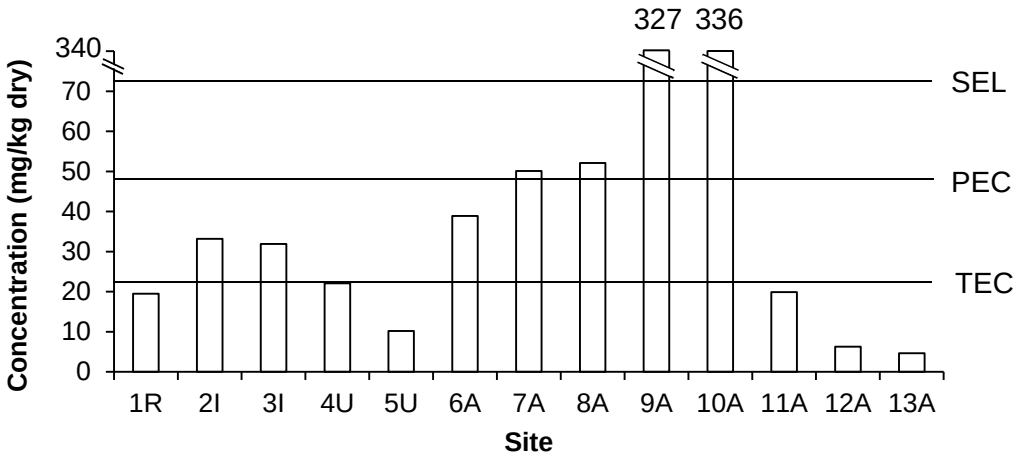

Fig. 19 Nickel concentrations in sediment for extensive sampling sites. TEC indicates the consensus based-threshold effect concentration; PEC is the consensus based-probable effect concentration; and SEL is the severe effect level (MacDonald et al. 2000)

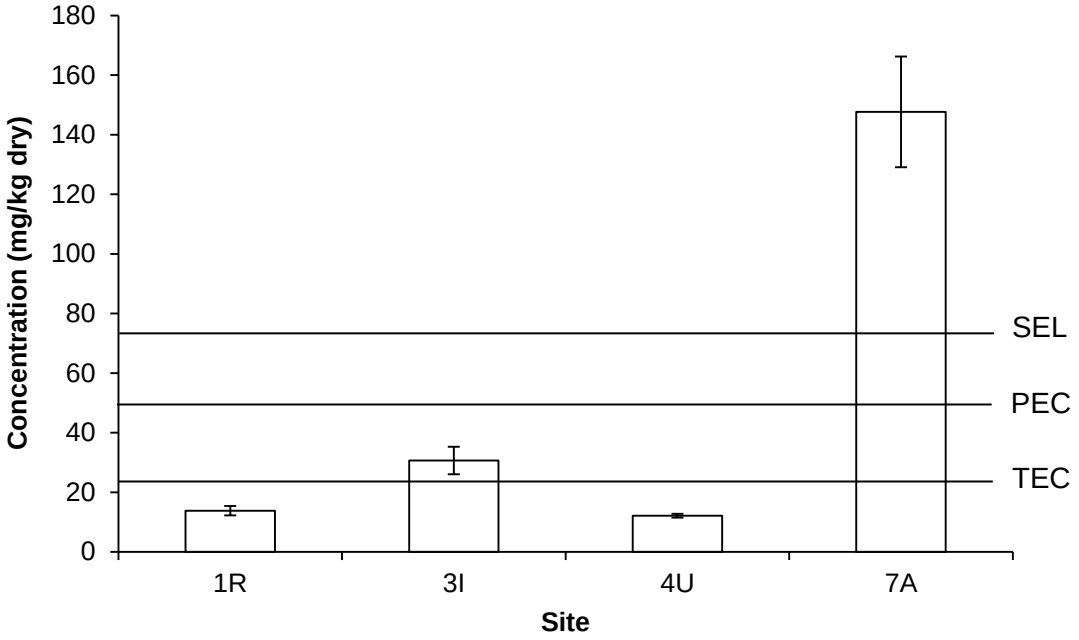

Fig. 20 Mean (± SD) nickel concentrations in sediment for intensive sampling sites. TEC indicates the consensus based-threshold effect concentration; PEC is the consensus based-probable effect concentration; and SEL is the severe effect level (MacDonald et al. 2000)

Appendix 1

This section describes sample preparation and organic contaminant analytical methods for uPSDs, sediment, and fish in further detail.

uPSDs

Universal Passive Sampling Devices (uPSDs) were used to estimate organic contaminant concentrations in stream water. Environmental conditions, such as temperature, flow rate, and biofouling can affect the uptake rates. Performance reference compounds (PRCs), analytically non-interfering compounds with chemical properties similar to analytes of interest, can be used to correct for changes in uptake rate and to develop environmental adjustment factors (Huckins et al. 2002, Hirons 2009). The changes in uptake rate are proportional to the changes in the elimination rate of PRCs (Booij et al. 1998, Booij et al. 2002, Huckins et al. 2002, Hirons 2009). However, our estimates were not PRC-corrected because the PRCs do not release linearly from the Oasis HLB® sorbent (Alvarez et al. 2008, K. O'Neal and P. Lazaro personal communication August 24, 2010).

The uPSDs were washed with methanol. Then performance reference standards were added because this was prior to the discovery that the PRCs are not compatible with the sorbent. fPSDs were enveloped in a protective, stainless steel cage wiring and cPSDs were enveloped in an aluminum cage wiring. uPSDs were then wrapped in aluminum foil that was baked approximately 12 hours, and placed in a plastic bag.

Prior to deployment, an anchor (50-ml centrifuge tube filled with pebbles from the site) was attached to one end of the uPSD wire envelope with a zip tie, and a float (approximately a 1.3-cm section of pipe insulator, dimensions: 2.5-cm x 1-cm x 1.8-m) was attached to the opposite end with 22.7-kg test monofilament fishing line. Monofilament was also attached to the same end of the wire envelop as the anchor and then attached to a stable structure to ensure that the uPSD remained stationary. The aluminum foil was removed immediately before deployment. Date, time, and location were recorded for each uPSD deployment.

Rate constants developed at North Carolina State University Department of Environmental and Molecular Toxicology Chemical Exposure Assessment Laboratory were used to obtain chemical concentrations in the water for each site. A rate constant of 0.15 L/day was used for the fiber PSDs, and 0.22 L/day was used for the cartridge samplers (D. Shea, personal communication 2009).

Contaminant concentrations (C_w) in water were estimated using the following equation:

$$C_w = N_t/R_s t,$$

where N_t is the mass (in nanograms) in the uPSD, R_s is the sampling rate (L/d), and t is the deployment duration (days) (Luellen and Shea 2002, Hirons 2009).

Sample processing

Processing equipment was thoroughly cleaned to avoid sample contamination. Tissue excision was performed on a clean, heavy duty aluminum foil sheet using a titanium-bonded boning knife. All utensils were washed with lab detergent, tap water, acetone, and distilled water. Work surfaces were cleaned with acetone, followed by a deionized water rinse, and then they were allowed to dry completely. Samples were inspected for condition and wet weight was recorded. Fish were thawed partially and then placed in a weighing tray that was lined with clean, heavy duty aluminum foil. Total length was measured for fish and body length was measured for shrimp.

Hands and then gloves were washed with lab detergent and tap water followed by a deionized water rinse. Specimens never came into contact with contaminating surfaces. Only light pressure was applied while excising fish tissue, to avoid contaminating tissue with materials from the internal organs. If tissue was contaminated with gut contents, it was noted and either discarded or rinsed with deionized water. Any remaining bones were removed from the tissue sample. Tissue was excised and wet weights were recorded. Tissue samples were then wrapped in clean, heavy duty aluminum foil, labeled, and then placed in a plastic bag, and in a -20˚C freezer. The tissue was homogenized in a food processor.

Organic toxicant analysis of uPSDs

uPSD extraction of organic contaminants was performed at the North Carolina State University Department of Environmental and Molecular Toxicology Chemical Exposure

Assessment Laboratory in Raleigh, North Carolina. Polypropylene tubing adaptors were used to attach a 2-ml glass pipette to a SPE manifold. The pipette, adaptor, valve, and manifold port were rinsed with acetone and then ethyl acetate. The instrument was inspected for leaks by closing the valve and filling the pipette with 2-ml of ethyl acetate. The sampler was removed from its wire envelope, inspected for damage, and then rinsed with deionized water, in order to remove any debris or biofouling. The sampler was then dried, using a Kimwipe®, and inserted into the pipette, which was filled with 2 ml of ethyl acetate and allowed to extract for 1 hour. The extract was then drained into a test tube. The samplers soaked for another hour in ethyl acetate. The extract was combined with the first extract and then was concentrated to 1 ml by using a gentle stream of nitrogen gas. Sodium sulfate was used to dry the extract if necessary. The extract was filtered through a 0.45-µm PTFE filter and transferred to an autosampler vial. It was stored in a -20˚C freezer and then analyzed using a gas chromatograph-mass spectrometer.

Organic toxicant analysis of sediment

Organic toxicant analysis was performed at North Carolina State University Department of Environmental and Molecular Toxicology Chemical Exposure Assessment Laboratory. All of the glassware used in the extraction and analysis was baked. The samples were thawed and the excess water was decanted. Samples were homogenized with a clean spatula, which was also used to weigh 10-30 g of sample and then used to transfer the sample into a clean, labeled, Teflon® jar. The wet weight was recorded and the sample surplus was

archived in a -20˚C freezer. Syringes were rinsed with dichloromethane, acetone, and hexane (5 times with each solvent). Each sample was spiked with a surrogate internal standard solution containing (Naphthalene-d8, Acenaphthene-d10, Chrysene-d12, Perylene-d12, Dibromooctafluorobiphenyl, PCB 112 (a pentachlorobiphenyl), PCB 197 (an octachlorobiphenyl). The matrix spike sample, matrix duplicate spike sample, and the blank spike sample were also spiked with the same internal standard mixture. The meniscus of the internal standard solution was then marked with a permanent marker as a way of assessing if any solution evaporates, which would alter the concentrations in the solution. The internal standard solution was then resealed by wrapping a strip of Teflon® tape around the cap and bottle neck in the direction that tightens the cap. The internal standard was returned to a -20˚C freezer where it was stored. Acetone (30 ml) and 30 ml of DCM was added to the sediment sample jar. Then enough anhydrous sodium sulfate was added to each sample jar, so that there were not any clumps. The lids were tightened onto the jar and manually shook until the samples were well mixed. Then samples were placed on an orbital shaker table for 12 hours. Samples were centrifuged at 3,000 rpm for 10 minutes. The extract was poured through funnel, lined with a baked out glass fiber filter, holding anhydrous sodium sulfate. The filter and sodium sulfate was rinsed with DCM. Meanwhile, an appropriately labeled Erlenmeyer flask collected the extract.

The extraction process was repeated an additional two times. DCM (30 ml) and 30 ml of acetone were added to each sample jar. Clumps were broken up with a spatula and sodium sulfate was added. The jars were manually shaken and then placed on a shaker table

for 2-4 hours. The samples were centrifuged for 10 minutes. The extracts were filtered through sodium sulfate and glass filters, followed by a DCM rinse, which drained into the appropriate Erlenmeyer flasks. This was repeated once more. The Erlenmeyer flasks were stored in refrigerator, until ready for further processing. When removed from refrigerator, lids were immediately removed from the flasks, in order to prevent the flasks from breaking. In between samples, the inside and outside of the ground glass connection of the roto-evaporation unit was rinsed with DCM. Three Teflon® boiling chips were added to each flask. The extract was concentrated to approximately 30 ml. The concentrated extract was then transferred to a 50-ml graduated cylinder using a Pasteur pipette and DCM. Acetone was used to clean the metal tips of the nitrogen evaporation apparatus. The samples were positioned in the nitrogen evaporation apparatus and allowed a gentle stream of nitrogen gas that further concentrated the extracts to 3 ml. The extract and extract rinses were transferred to 4-ml vial and capped. It was run through the GC-MS and then stored in a -20˚C freezer.

Biota extraction of organic toxicants

Biota extraction of organic toxicants was performed at North Carolina State University Department of Environmental and Molecular Toxicology Chemical Exposure Assessment Laboratory. Samples were freeze-dried and dry sample masses were obtained. All glassware and utensils were either baked or solvent rinsed. A spatula was used to transfer 4 g of dried sample to a 50-ml Teflon® centrifuge tube. If the sample was not free-flowing it was broken up with a spatula, while being careful not to abrade the inside of

centrifuge tube. Sample surplus was archived in a -20°C freezer. Then samples, matrix spike, and matrix spike duplicate were spiked with a surrogate internal standard solution. 20 ml of dichloromethane were added to each sample. The samples were placed on a shaker table for 18-24 hours. The extract was then filtered through a baked glass fiber filter into a 250-ml boiling flask. The filters were rinsed with DCM and allowed to drain into the boiling flasks. The extraction process was repeated an additional two times but were only shaken for 2-4 hours. The samples were concentrated to 5-10 ml using rotary evaporation and then transferred to a 15-ml test tube. A gentle stream of nitrogen was used to further concentrate the samples down to 1-2 ml. The extract was filtered through a 0.45-µm PTFE filter. The extract was further concentrated to 1 ml using a gentle stream of nitrogen. The extracts were transferred to an autosampler vial, capped, and stored in a -20°C freezer until it could be processed by the GC-MS.

Composite samples

Composite samples were used for the contaminant analyses. Composite samples consisted of individuals of an array of sizes, and the number of individuals in each sample varied. Composite samples are prepared from two or more individual organisms of the same species, collected at a particular site, and analyzed as a single sample (U.S. Environmental Protection Agency 2000). They are most cost-effective for estimating average tissue concentrations of target analytes in target species populations (U.S. Environmental Protection Agency 2000). They also ensure an adequate sample mass to perform analyses for all

recommended target analytes; however, variation among individual organisms is not quantified in these integrated samples (U.S. Environmental Protection Agency 2000).

Chapter 2—Pollution of Tropical Island Stream Ecosystems:

Relation of Biotic Accumulation to Land Use and Trophic Dynamics

Abstract

Fate and effects of pollution are complex processes and many contaminants present in low levels in the environment may increase in concentration from one trophic level to the next, reaching concentrations that are harmful to wildlife and human consumers. Puerto Rico has a history of anthropogenic chemical usage, and its human population density is among the highest globally, providing a model environment to study human impacts on tropical island stream ecosystems. The objective of our research was to quantify occurrences and patterns of aquatic contaminants [polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), pesticides, and metals] as related to riparian and watershed land-use characteristics and trophic relationships. We used stable isotope analyses of carbon, nitrogen, and sulfur to elucidate contaminant and trophic dynamics within four rivers with differing riparian and watershed land-use patterns (e.g., urban, agricultural, industrial, and forested). Overall, stream ecosystems in Puerto Rico were not severely polluted, with the exception of elevated concentrations of PCBs and mercury in some fish species. Trophic level and contaminant concentrations were poorly correlated in these dynamic systems that are characterized by frequent hydrologic disturbances, nutrient pulses, and marine influences. Calculation of food web magnification factors was complicated by low levels of contaminants, distorted estimates of trophic level due to $\delta^{15}N$ enrichment from nutrient pollution, and short food chains. Lipid content of consumers was a better predictor of contaminant concentration than trophic level. These findings enhance understanding of contaminant dynamics in tropical stream ecosystems and provide natural resource managers

and public health agencies scientific information to guide ecosystem and fisheries management and human health risk assessment.

Introduction

Fate and effects of pollution are complex processes and many contaminants present in low levels in the environment may increase in concentration from one trophic level to the next (i.e., biomagnify), reaching concentrations that are harmful to wildlife and human consumers (Thrower and Eustace 1973; Rasmussen et al. 1990). Puerto Rico stream ecosystems may serve as an optimal model to study impacts and processes of pollution because of the island's dense human population and history of anthropogenic chemical usage. During the past century, rapid industrialization and human population growth have strained the limited natural resources of Puerto Rico (Hunter and Arbona 1995). Freshwater is an especially scarce resource, because no natural lakes occur on the island; consequently, most of the rivers have been transformed for water collection (e.g., construction of impoundments; Cooney and Kwak 2010). Streams in Puerto Rico provide many services for local populations, including water for drinking, recreation, irrigation, and as a source of fish and crustaceans for consumption (Neal et al. 2009). Therefore, a common conundrum exists where good water quality is necessary to protect human health and ecological integrity, but rapid human population growth has led to deteriorated water quality (Fitzpatrick and Keegan 2007). The streams have a history of die-offs of fish, shellfish, shrimp, and domesticated animals, caused by contaminated industrial, agricultural, and municipal wastes (Hunter and Arbona 1995). Yet, research is lacking on the degree and effects of contamination, and to

our knowledge, no studies have applied multiple stable isotope analyses to elucidate trophic processes associated with contaminants in tropical island streams.

Stable isotope ratios of carbon ($^{13}C/^{12}C$ or $\delta^{13}C$), nitrogen ($^{15}N/^{14}N$ or $\delta^{15}N$) and sulfur ($^{34}S/^{32}S$ or $\delta^{34}S$) are often used to trace organic matter pathways in aquatic ecosystems (Peterson and Fry 1987; Kwak and Zedler 1997). Generally, nitrogen isotopic signatures may be used to estimate trophic position, carbon isotopes determine trophic pathways, and sulfur isotopes can identify migrant populations (Jardine et al. 2006). $\delta^{13}C$ signatures can determine the relative importance of different diet items by measuring the amount of carbon assimilated from each food source (Peterson and Fry 1987). Nitrogen indicates trophic position because consumers are enriched in $\delta^{15}N$ compared to their diet (Fry 1991). Sulfur stable isotope analysis is useful as a source tracer because amino acids containing sulfur are essential to animals, and it eliminates fractionation in the food chain, even revealing feeding relationships in complex estuarine systems that are inhabited by diadromous fish populations (Hesslein et al 1991).

An approach that integrates stable isotope and contaminant analyses can elucidate dietary exposure and biomagnification of contaminants, because diet is the major route of exposure for many persistent pollutants (Thomann et al. 1984; Hall et al. 1997). Stable isotope studies are a more powerful approach than assigning organisms to discrete trophic level classifications to predict contaminant concentrations of top predators because they account for large $\delta^{15}N$ variations, sometimes exhibited in omnivores or consumers of complex food webs, by representing a continuous, integrative measure of trophic level

(Rasmussen et al. 1990; Cabana et al. 1994; Cabana and Rasmussen 1994,1996; Jardine et al. 2006). Estimating the food web magnification factor of contaminants is important, because contaminants with a high potential to biomagnify pose a greater threat to the health of humans and wildlife that rely on aquatic biota as a food source (Jardine et al. 2006). The biomagnification potential of contaminants in aquatic ecosystems is also useful information for guiding environmental policy and regulations, such as the maximum total release of industrial pollutants or other chemicals into the environment (Mackay and Fraser 2000).

The objectives of this study were to (1) measure stable isotope compositions of aquatic food web components, (2) examine patterns in isotopic composition of organic matter sources and consumers to identify trophic pathways, (3) estimate trophic position of consumers using $\delta^{15}N$, and (4) describe relationships among isotope ratios, trophic level, lipid content, and contaminant concentrations to enhance understanding trophic dynamics of contaminants in tropical island rivers.

Methods

To examine anthropogenic effects on native fauna in stream ecosystems, sites were selected based on presence of target species and predominant riparian and watershed land uses. Prior knowledge of target species distribution and abundance was found in Kwak et al. (2007). Four stream sites were selected to represent varying predominant riparian and watershed land uses including a reference site (1R) downstream of a protected forest area within the El Yunque National Forest on Río Mameyes; an urban site (2U) within a densely populated region of San Juan on Río Piedras; an industrial site (3I) downstream of a cement

plant on Río Cañas; and an agricultural site (4A) on Río Yauco, surrounded by banana plantations (Table 1; Fig 1).

Only four native freshwater fish families (consisting of less than 10 species) and three decapod families are found in Puerto Rico streams, and most of these species require a connection to the ocean to complete their life cycle (Kwak et al. 2007; Neal et al. 2009). *Xiphocaris elongata*, Atyid shrimp, and *Macrobrachium* spp. feed on algae, microbes, plant matter, and small insects (Pringle et al. 1993; Covich and McDowell 1996). Additionally, *Macrobrachium* shrimp consume mollusks, small fish, and other shrimp (Covich and McDowell 1996). *Neritina punctulata* and *Thiara granifera* are algae-grazing snails that are also known to consume terrestrial material (March et al. 2001, 2002). Mountain mullet (*Agonostomus monticola*) consume insects, shrimp, fruit, and algae (Phillip 1993; Aiken 1998; March and Pringle 2003). River gobies (*Awaous banana*) consume mostly periphyton and algae (Debrot 2003; Coat et al. 2009). Sirajo gobies (*Sicydium* spp.) rely mostly on algal sources and have been described as strict herbivores (Erdman 1961; Erdman 1986; Watson 2000), but others suggest that they may also consume insects (Parenti and Maciolek 1993; March and Pringle 2003). The diet of American eels (*Anguilla rostrata*) is dominated by the most abundant macroinvertebrates available (Tesch 1977). Smallscaled spinycheek sleeper (*Eleotris perniger*) diets are comprised mostly of biofilm, macroinvertebrates, and fish (Coat et al. 2009). Bigmouth sleepers (*Gobiomorous dormitor*) are generalist predators including a wide range of diets items (Hildebrand 1938; Winemiller and Ponwith 1998). In rivers, they

consume fish, shrimp, crabs, snails, ostracods, insects, arachnids, terrestrial invertebrates, and reptiles (Bachelor et al. 2004) (Fig. 2).

Sample Collection

Fish were sampled during the summers of 2009-2010 and analyzed for contaminants and stable isotope ratios of C, N, and S. Native species sampled included bigmouth sleeper, smallscaled spinycheek sleeper, American eel, mountain mullet, sirajo goby, river goby, and *Macrobrachium* shrimp. Exotic species, introduced by the aquaculture industry, aquarium owners, anglers, and by agencies for fishing opportunities, are common throughout Puerto Rico (Neal et al. 2009). Our study focused on native species, but a reduced number of exotic species were sampled for stable isotope analyses to further describe the stream food web. Exotic fishes sampled included Nile tilapia (*Oreochromis mossambicus*), Amazon sailfin catfish (*Pterygoplichthys pardalis*), green swordtail (*Xiphophorus hellerii*), and guppy (*Poecilia reticulata*). The coastal river sites are inhabited by marine fish species that were also collected, including fat snook (*Centropomus parallelus)* and burro grunt (*Pomadasys crocro*). Fish, shrimp, and crabs were collected using backpack electrofishing following methods described by Kwak et al. (2007). Other macroinvertebrates were collected from instream cobble, bedrock, leaf packs, and macrophytes using a sweep net. Leaves and macrophytes were thoroughly rinsed of all invertebrates and fine particulate organic matter and then placed in a labeled plastic bag. Algae were collected using a scalpel to remove them from their substrate. Invertebrates and detritus were removed from algae and sorted

under a dissecting scope. Aquatic snails were collected by hand. All samples were stored in a -20˚C freezer until they could be further processed.

Water Quality

A suite of water quality parameters was measured at each site during organism sampling procedures. Water was collected using a 1-L container that was submersed 0.25-0.50 m beneath the water surface, in laminar flow, and stored on ice in a cooler. Physicochemical water variables were measured using a calibrated Yellow Springs Instrument (YSI) 556 multi-probe system and a Hach CEL/850 Portable Aquaculture Laboratory and include temperature, pH, alkalinity (mg/L $CaCO_3$), total hardness (mg/L $CaCO_3$), conductivity (µS/cm), nitrate concentration (µg/L NO_3^-), nitrite concentration (mg/L NO_2^-), and reactive phosphorus concentration (mg/L PO_4).

Contaminant Analyses

Fish and shrimp tissue was analyzed for contaminant concentrations as composite samples using whole body or muscle tissue. The whole body of sirajo gobies was analyzed because humans consume the whole body of these fish, as do instream and avian predators. The whole body of river gobies and spinycheek sleepers was analyzed for contaminants, for similar reasons. The edible muscle, excluding skin or scales, of American eel and bigmouth sleeper was analyzed. Abdominal muscle tissue was analyzed for *Macrobrachium* shrimp.

Organic contaminant analyses of 26 chlorinated pesticides and 20 polychlorinated biphenyl congeners (PCBs) were performed at the North Carolina State University Department of Environmental and Molecular Toxicology Chemical Exposure Assessment Laboratory in Raleigh, North Carolina, using a gas chromatograph-mass spectrometer (Appendix 1). For metal analyses, biota samples were freeze dried and transported to Environmental Conservation Laboratories in Cary, North Carolina, for analyses of mercury, selenium, copper, nickel, zinc, cadmium, and lead. Cadmium, copper, lead, nickel, selenium, and zinc were analyzed using EPA Method 6010C and mercury was analyzed using EPA Method 7471B (www.epa.gov/sam).

Stable Isotope Analyses

Freeze-dried aliquots of fish and shrimp samples analyzed for contaminants were also used for stable isotope analyses. Stable isotope samples were dried at 60˚C to a constant weight. All samples were comprised of multiple individuals, and insect samples may have consisted of more than one species, but only one family. Only the muscle tissue of crustaceans, mollusks, and fish was analyzed for stable isotopes. Dried samples were sent to the Colorado Plateau Stable Isotope Laboratory at Northern Arizona University in Flagstaff, Arizona, and were combusted to gases (CO_2, N_2, and SO_2), which were analyzed with an isotope mass spectrometer for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$. Snail samples were acid fumigated to remove any shell fragments, which consist of non-dietary derived carbonates that would bias results.

Isotopic ratios were calculated as $\delta X = (R_{sample}/R_{standard} - 1) \times 1000$; where X is the heavier isotope, R_{sample} is the ratio of the heavy to light isotope in the sample, and $R_{standard}$ is the ratio of the heavy to light isotope in the standard (Peterson and Fry 1987, Fry 1991). Analytical standards included atmospheric air for nitrogen, Vienna Pee Dee Belenite for carbon, and Canyon Diablo Triolite for sulfur (Fry 2006).

$\delta^{13}C$ Lipid Correction Techniques

Plant and animal species vary in lipid content, which is related to differences in life history constraints and foraging behavior (Schultz and Conover 1997; Post et al. 2007). Studies have shown lipid content is negatively correlated with their $\delta^{13}C$ values (Tieszenel et al. 1983; Focken and Becker 1998; Doucett et al. 1999) because lipids consist of a greater amount of the lighter isotopes as a result of oxidation of pyruvate to acetyl-CoA, during lipid formation (DeNiro and Epstein 1977). Two methods are used to correct for variation in $\delta^{13}C$ caused by lipid content—chemical extraction and mathematical normalization. Lipid normalization involves an adjustment of $\delta^{13}C$ based on the relationship between lipid content and C:N in tissues (McConnaughy and McRoy 1979). It offers some advantages over lipid extraction because it requires less sample preparation and preserves the integrity of $\delta^{15}N$, but its accuracy is questionable (Post et al. 2007). It is most important to correct for lipids in species that have highly variable lipid content to correctly estimate food source (Post et al. 2007). Therefore, subsamples of fish and shrimp were analyzed for $\delta^{13}C$ and $\delta^{15}N$ both before and after lipid extraction treatment to evaluate the degree that lipids affect stable

isotope signatures of these samples and to better understand how to account for $\delta^{13}C$ variation introduced by lipids.

Estimation of Trophic Level

Natural and anthropogenic inputs of nutrients are common in many aquatic food webs (Polis et al. 1997; Carpenter et al. 1998), which influence stable isotope signatures (Wayland and Hobson 2001). Thus, we estimated the base of the food web using primary consumers instead of primary producers because variation in $\delta^{15}N$ of primary producers can produce variation in $\delta^{15}N$ within consumers, leading to erroneous interpretations of trophic levels and food chain length (Kline et al. 1993; Cabana and Rasmussen 1996). Trophic level (TL) was estimated as: $TL_{organism} = (\delta^{15}N_{organism} - \delta^{15}N_{baseline})/\Delta 15N + 2$, where $\delta^{15}N_{baseline}$ is the measured $\delta^{15}N$ of a long-lived primary consumer (TL = 2). $\Delta 15N$ is a selected constant that represents the increase in $\delta^{15}N$ from one trophic level to the next (i.e., trophic enrichment) and is considered to be 3 to 4‰ (Minagawa and Wada 1984; Fry 1988). For this study, we set $\Delta 15N$ at 3‰, because ammonia-excreting organisms, as in stream ecosystems, have lower $\Delta 15N$ (Vanderklift and Ponsard 2003).

Bivalves (Gustafson et al. 2007), gastropods (Kidd et al. 1998; Post 2002), copepods (Fisk et al. 2001; Moisey et al. 2001; Fisk et al. 2003; Campbell et al. 2005) and other invertebrates (Vander Zanden and Rasmussen 1999) have been used to standardize the base of food webs. However, small organisms show greater temporal variability in $\delta^{15}N$ signatures than larger organisms (Cabana and Rasmussen 1996). Therefore, large primary

consumers are optimal for site comparisons of isotopic signatures because of their slower nitrogen turnover rate; thus, we used snails as the baseline organism for this study.

Quality Control

A rigorous quality assurance protocol was followed during analyses. For metal analyses quality assurance included blanks, laboratory control samples (LCS), matrix spike, matrix spike duplicates, post spikes, and surrogate internal standards. The blanks were clean, with the exception of a few detections below the method reporting limit (MRL) including one detection of copper (0.07 mg/kg) and two detections of lead (<0.13 mg/kg). The relative percent difference (RPD) averaged 15% and ranged from 0.4-64%. For the few RPD values that were out of range, the batch was accepted based on percent recoveries for these samples that were within range. Overall, percent recoveries averaged 95% and ranged 2-185%. Very few matrix spike samples had percent recoveries that were out of range and all LCS percent recoveries were within range (mean = 99%, range = 85-110%), so range-exceeding samples were accepted based on the corresponding LCS percent recoveries. Procedural blanks, matrix spikes, and surrogate internal standards were used to assess organic contaminant data quality. Procedure blanks were clean with a few exceptions of PCB 138 in procedure blanks (<2 ng/g). Mean RPD values were 2% (range 0-4%) and average surrogate recoveries were 79% (range 28-176%). Results were not corrected for recoveries. Duplicate samples were also analyzed for quality assurance of lipid content of fish and shrimp and for stable isotope data. The mean RPD value for lipid data was 2% (range 0-4%).

Results

Water Quality

Water quality measurements varied widely among sampling sites, with notable differences between the reference site and other sites. Study site value ranges for water quality variables were, temperature, 27.6-34.7°C; total dissolved solids, 0.079-0.899 g/L; conductivity, 121-1,451 µS cm^{-1}; salinity, 0.06-0.69 ppt; nitrate as NO_3^-, 1.5-4.4 mg/L; nitrite as NO_2^-, 0.006-0.046 mg/L; ammonia, 0.00-0.69 mg/L; phosphorus as PO_4^-, 0.02-0.95 mg/L; alkalinity, 33-248 mg/L; hardness, 38-235; turbidity, 1-4 FAU; pH, 7.18-8.10; dissolved oxygen, 5.43-12.36 mg/L (Table 2). Stream water from the reference site, with a primarily forested watershed, was the lowest in ionic and nutrient content. Measurements of total dissolved solids, conductivity, salinity, nitrogen, phosphorus, alkalinity, and hardness were lowest at the reference site, while the agricultural site had the greatest measurements of total dissolved solids, conductivity, salinity, ammonia, phosphorus, alkalinity, and hardness. The agricultural site also had the lowest dissolved oxygen.

Stable Isotopes

One-way ANOVA for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$, and pairwise comparisons between sites revealed that the baseline values of each site were significantly different from all other sites for all isotope ratios ($P < 0.05$; Figs 3 and 4). The reference site had the greatest baseline $\delta^{13}C$ lipid uncorrected values (mean = -23.3‰, SD = 0.36) and lowest baseline $\delta^{15}N$ values (mean = 4.6‰, SD = 0.22), while the agricultural site had the lowest baseline $\delta^{13}C$ (lipid

corrected mean = -26.5‰, SD = 0.29; uncorrected mean = -24.7‰, SD = 0.26) and the greatest baseline $\delta^{15}N$ values (mean = 19.5‰, SD = 0.37). The urban site had the greatest baseline $\delta^{34}S$ values (mean = 6.3‰, SD = 0.30) and the lowest baseline $\delta^{34}S$ values were found at the industrial site (mean = 0.2‰, SD = 0.56). Samples collected from the agricultural site were approximately 15‰ enriched in ^{15}N compared to the reference site.

Studies have shown lipid content affects $\delta^{13}C$ values (Tieszenel et al. 1983; Focken and Becker 1998; Doucett et al. 1999) and that it is most important to correct for lipids in species that have highly variable lipid content (Post et al. 2007), which are mountain mullet and American eels in our study (Fig 5). Lipid extraction prior to stable isotope analyses resulted in a $\delta^{15}N$ average difference of 0.31‰ that ranged from 0.03‰ to 0.72‰ with a significant difference between extracted and unextracted samples ($N = 39$; $P < 0.0001$). We also detected a significant difference in $\delta^{13}C$ between lipid-extracted and unextracted samples averaging 0.82‰ with a range of -0.05‰ to 3.60‰ ($N = 39$; $P < 0.0001$). There was also a strong relationship between lipid content and C:N ($P < 0.01$, $R^2 = 0.82$; Fig 6) and change in $\delta^{13}C$ and C:N ($P < 0.01$, $R^2 = 0.63$; Fig. 7), demonstrating that mathematical normalization is appropriate. McConnaughy and McRoy (1979) and Post et al. (2007) both developed equations for lipid normalization that were compared to this study (Fig 8). McConnaughy and McRoy (1979) correction technique underestimated most of the data while Post et al. (2007) was a better fit. Our results demonstrate species-specific differences in the relationship between change in $\delta^{13}C$, due to lipids, and C:N (Fig 9).

Stable isotope ratios did not consistently reveal food web interactions and structure, likely due to the complex and dynamic temporal variation of lotic systems. For example, lipid corrected and uncorrected $\delta^{13}C$ and $\delta^{13}S$ results did not indicate dietary sources. Secondly, predicted trophic levels of the agriculture site's food web were clearly inaccurate (e.g., algae were most enriched in ^{15}N), although, at the other sites, $\delta^{15}N$ exhibited similar trends, with top predators having more enriched ^{15}N values and producers being the most depleted in ^{15}N. In addition, microbial processes were revealed by significant negative correlations found between $\delta^{34}S$ and $\delta^{15}N$ at all sites ($P < 0.05$), except for at the reference site where there was a significant positive correlation ($P < 0.001$), possibly indicating trophic fractionation.

Contaminant Patterns

We generally found low contaminant concentrations at our sample sites (Table 3). Few organochlorine pesticides and few metals were consistently detected in fish samples. Although, bigmouth sleeper samples from the urban site had high levels of mercury (average = 0.17 mg/kg wet, range = 0.13-0.23 mg/kg wet), most samples (78%) contained mercury concentrations that were below detection limits. Food Web Magnification Factors were not calculated because no correlation was detected between lipid corrected contaminant concentrations and trophic level ($P > 0.05$; Table 3; Fig 10), with the exception of DDT concentration that was negatively correlated ($P < 0.001$). Trophic level, derived by $\delta^{15}N$ values, did not explain variance in lipid corrected concentrations of PCBs, chlordane, and

mercury ($P > 0.05$) (Table 3). Contaminant concentrations were highly correlated with lipid content for all contaminants ($P < 0.001$), with the exception of mercury ($P > 0.05$; Figs 11-13).

Trophic level, $\delta^{15}N$, and $\delta^{13}C$ were not correlated with contaminant concentrations, but $\delta^{34}S$ was correlated with PCB concentration, chlordane concentration, and mercury concentration when all sites were included in a single regression analyses ($P < 0.01$). However, when stratified by site, these variables were not significantly correlated ($P > 0.05$). No significant correlation of $\delta^{13}C$ with any contaminant concentration (PCBs, chlordane, DDT, and Hg) was detected after adjusting for lipid content ($P > 0.05$).

Discussion

Stable Isotope Variation Among Sites

Many factors influence isotopic composition of organic matter resulting in variation both among and within species and sites; such influences may include lipid content, salinity, hydrology, climate, and anthropogenic nutrient inputs (DeNiro and Epstein 1977; Gustafson et al. 1997; Finlay and Kendall 2007). Our results indicated significantly different baseline $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values among sites and a significant lipid extraction effect on $\delta^{15}N$ and $\delta^{13}C$. After adjusting for lipid content, our results indicated that lipid content is not a primary cause for $\delta^{13}C$ variation among sites, because baseline $\delta^{13}C$ values adjusted for lipids remained significantly different among sites. Our results confirm the need to account for lipid-related variation in ^{13}C, using techniques such as chemical lipid extraction or

mathematical normalization, for biota with variable and high lipid content. The magnitude of the lipid-extraction effect on isotopic composition (i.e., difference between corresponding extracted and unextracted samples) was significantly explained by lipid content of organisms for $\delta^{13}C$, indicating potential for misinterpretation of dietary source if not lipid corrected. One disadvantage of chemical lipid extraction is that it is known to affect ^{15}N (Post 2007). We found a significant difference between the relative percent difference of quality control duplicates and the relative percent difference of the difference between ^{15}N lipid extracted and unextracted samples with the lipid extraction having a larger RPD than that associated with analytical error (i.e., RPD of duplicate samples) ($P < 0.001$). However, we found a maximum ^{15}N enrichment of 0.72‰, which would only result in an erroneous increase of 0.24 of a trophic level. We compared our results on lipid effects to the findings of other researchers, McConnaughy and McRoy (1979) and Post et al. (2007) and found that their equations did not fit our data. In addition, our data demonstrates that species-specific differences exist in the relationship between enrichment in ^{13}C due to lipids and C:N. Therefore, one generalized lipid correction equation cannot predict lipid correction of $\delta^{13}C$ as accurately as one developed for a single species.

Another factor that may influence isotopic composition is water salinity. Coastal ecosystems are typically enriched in ^{13}C, because C_4 saltmarsh grasses form the base of the food web (Kwak and Zedler 1997; Garcia et al. 2007; Winemiller et al. 2010). Our baseline $\delta^{13}C$ data conform to this trend, with baseline values decreasing with increasing salinity. Coastal ecosystems with higher salinities are also more enriched in ^{15}N than forested

ecosystems (Ambrose and DeNiro 1987; Heaton 1987; Ambrose 1991; Hebert and Wassenaar 2001). Indeed, our agricultural site, with the most enriched $\delta^{15}N$ values, had the highest salinity, but it is unlikely that this variation accounted for the 15‰ difference observed between the reference and agricultural sites.

Stable isotope ratios of ^{13}C and $\delta^{34}S$ did not reveal diet sources of food web components, likely due to the complex and dynamic temporal variation of lotic systems associated with hydrology. We expected to observe a marine influence on these food webs as an explanation for the missing food web end members, due to the amphidromous nature of these shrimp and fish, and as suggested by stable isotope studies in other tropical island streams (Coat et al. 2009). However, all food web components $\delta^{34}S$ values ranged from -1.6 to 8.4‰, which is consistent with a range typical in freshwater ecosystems (Fry 2006). In contrast, marine $\delta^{34}S$ producers typically range from 17- 21‰ (Fry 2006). Animals that migrate between ecosystems with isotopically distinct food webs may retain isotope signatures from other feeding areas (Hobson 1999), but this probably does not explain our difficulty predicting dietary sources, because we sampled non-migratory adult consumers. Another possibility is that we did not sample the true organic matter sources, but other researchers indicated algae as a primary dietary source supporting a Puerto Rico stream food web (March and Pringle 2003).

Stable isotope ratios did not consistently indicate food web structure, which may also be attributed to the complex temporal variation of lotic systems associated with hydrology. $\delta^{15}N$ trends exhibited similar patterns for each site, with top predators having more enriched

^{15}N values and producers being the most depleted in ^{15}N, with the exception of the predicted trophic levels of the agriculture site's food web, which were clearly inaccurate (e.g., algae were most enriched in ^{15}N). Riverine food webs have high spatial and temporal complexity and variability relative to other ecosystems. Streams typically contain a complex mixture of allochthonous and autochthonous organic matter, as a result, food web interactions are difficult to elucidate by traditional approaches (Finlay and Kendall 2007). Isotopic conditions of aquatic plants are more variable and consequently less predictable than terrestrial plants, mainly because of the large variation in the concentrations stable isotope ratios of dissolved inorganic carbon, nitrogen, and sulfur in freshwater ecosystems and the physiological diversity of aquatic producers (Finlay 2004; Finlay and Kendall 2007). Physical and biogeochemical processes, such as assimilation/uptake, nitrification-denitrification, carbon dissolution, degassing exchange, photosynthesis, respiration, methane oxidation, may cause large variations in isotopic compositions of dissolved inorganic species and lead to variability in isotopic compositions of aquatic plants (Finlay and Kendall 2007).

Stream hydrology strongly influences the $\delta^{13}C$ signature of dissolved inorganic carbon ($\delta^{13}C_{DIC}$) with fluctuations in $\delta^{13}C$ during high-flow events, because of shifts in the relative proportion of flow from surface water versus groundwater contributing to discharge (Finlay and Kendall 2007). It is especially problematic for the use of stable isotope techniques when stochastic processes are involved, such as floods, which render stable isotope values unpredictable (Finlay and Kendall 2007). Flood events may mobilize new and distinctly different sources of carbon, nitrogen, and sulfur than those during baseflow

conditions sources, resulting in isotope values within food webs that vary temporally and $\delta^{13}C$ values that constantly change making it difficult to determine carbon source (Finlay and Kendall 2007; Buda and DeWalle 2009). Other researchers demonstrated a negative correlation between discharge and $\delta^{13}C$ and $\delta^{13}C_{DIC}$, with most of the temporal variation in algal $\delta^{13}C$ explained by discharge (Finlay 2004; Finlay and Kendall 2007).

Hot, arid environments tend to be associated with higher nitrogen isotope ratios than cool, wet environments (Heaton 1987; Ambrose 1991), because denitrification rates are related to temperature (Kendall 1998), and precipitation can affect riverine N export (Hebert and Wassenaar 2001; Showers et al. 2006). A mountain range in central Puerto Rico forms a barrier to northeast tradewinds causing a rainshadow effect over much of the southern coast, which receives less than 1,140 mm of rain annually, whereas northern Puerto Rico averages about 2,030 mm (Hunter and Arbona 1995). However, this climatic trend does not explain the differences in $\delta^{15}N$ that we observed among sites. As expected, the reference site located in the northern, rainforest region of the island had the lowest $\delta^{15}N$ values and those of the agricultural site in the southern, dry forest region were the greatest, but the industrial and urban sites had similar $\delta^{15}N$ values even though the industrial site is located on the southern mountain slope and the urban site is on the northern mountain slope. Thus, factors other than water availability must influence spatial variation in ^{15}N enrichment.

Pollution and anthropogenic nutrient enrichment can affect trophic state and alter aquatic food webs in watersheds exposed to urbanization or agriculture (Fogg et al. 1998; Clements et al. 2000; deBruyn and Rasmussen 2002). $\delta^{15}N$ differences in the base of the

food web among ecosystems may indicate anthropogenic nitrogen inputs from sewage or agriculture (Anderson and Cabana 2005; Cabana and Rasmussen 1996). $\delta^{15}N$ nitrate values of commercial fertilizers generally range from -2.5 to 2.0‰; organic soil nitrate ranges from -2 to 9‰; and human and animal waste range from 10 to 20‰ (Kreitler and Jones 1975; McClelland et al. 1997; Kwak and Zedler 1997), but may vary further due to fractionation (Harrington et al. 1998). For instance, tracing nitrate sources with $\delta^{15}N$ values can be complicated by various sources of fractionation, such as volatile loss of ammonia from animal wastes (Heaton 1986; Macko and Ostrom 1994; Wayland and Hobson 2001), denitrification in soil and ground water (Bottcher et al. 1990; Aravena et al. 1993), and uptake of nitrate by microbes and algae (Estep and Vigg 1985). Our $\delta^{15}N$ values were elevated at the agricultural site, likely indicating nitrogen enrichment from animal waste applied to the land as fertilizer or from confined animal operations. An animal production facility is located approximately 7.2 km upstream of this site. Our finding is similar to that of Winemiller et al. (in press), who also found a site near a banana plantation with most enriched $\delta^{15}N$ values of their stream sites at about 10‰ greater than their reference site.

Nutrient and fecal pollution in river water are threats to the integrity of aquatic ecosystems in Puerto Rico (Hunter and Arbona 1995; Warne et al. 2005). Large-scale land use changes of the island have caused aqueous nitrogen and phosphorus concentrations to increase 10-fold (McDowell and Ashbury 1994; Warne et al. 2005), a pattern consistent with our results, with greatest nutrient loads at the agriculture and urban site. Urbanization has increased rapidly in Puerto Rico, creating a lag in infrastructural services and a supply of

human waste that exceeds treatment capacity, and consequently, raw sewage is sporadically discharged into streams (Hunter and Arbona 1995). In 1984, U.S. Geological Survey reported that 81 percent of their sampling stations exceeded maximum microbiological contaminant levels for recreational water, suggesting widespread fecal contamination, but tropical temperatures and humidity may promote high coliform counts, even in pristine forested sites (Lopez et al. 1987; Hazen 1988). Bacterial densities are positively correlated with water nutrient levels, particularly nitrates and phosphates, with high bacterial densities found at sewage outfalls (Carrillo et al. 1985). Wet, anoxic, and carbon-rich hydric soils, are associated with enriched groundwater nitrate ^{15}N, most likely due to denitrification (Showers et al. 2006). For example, $\delta^{15}N$ and $\delta^{18}O$ studies indicated that denitrification occurs in groundwater under biosolid and poultry litter application fields, but only in hydric soils (Showers et al. 2006). $\delta^{15}N$ of NO_3 and $\delta^{18}O$ could be measured in future research of these Puerto Rico streams to better identify NO_3 sources entering the food web (Fogg et al 1998).

Variation in $\delta^{34}S$ measurements in streams can be attributed to ocean proximity, bedrock geology, redox conditions, and pollution (Finlay and Kendall 2007). An unexpected finding was that the agricultural and industrial sites located closest to the coast were relatively depleted in ^{34}S. Competing microbial processes likely influence $\delta^{34}S$ variation among streams. We found negative correlations between $\delta^{34}S$ and $\delta^{15}N$ values at all sites, except for the reference site, where there was a significant positive correlation. The negative relationship may be explained by sulfate reduction inhibition of denitrification. Elevated levels of ^{15}N are created by denitrification, and suggest that high levels of sulfide (produced

by sulfate-reducing bacteria) are not widely distributed because denitrification inhibits sulfate-reduction (Joye and Hollibaugh 1995). Negative $\delta^{34}S$ values are indicative of sulfate reduction (Evans and Crumley 2005) and were not observed in most of our samples; however, nitrogen isotope enrichment was observed at the agricultural, industrial, and urban sites. The positive correlation of $\delta^{34}S$ with $\delta^{15}N$ at the reference site may be attributed to trophic fractionation. Sulfur isotopes are generally considered to remain unaltered with trophic transfers (Peterson 1999); however, McCutchan et al. (2003) demonstrated that trophic fractionation in $\delta^{34}S$ may reach 2‰.

Food Web Magnification

The calculation of food web magnification factors (FWMF) for the streams we studied was not feasible because the studied stream ecosystems were not severely polluted, food chain length was short, and nutrient loads varied temporally. FWMF were not calculated because no correlation existed between most lipid corrected contaminant concentrations and trophic level ($P > 0.05$); this was the case for lipid corrected concentrations of PCBs, chlordane, and mercury. However, DDT concentrations were negatively correlated with trophic level, contrary to expectations. This trend is likely an artifact of erroneous trophic level estimates at the agricultural site. Stream food chains in Puerto Rico are short, consisting of less than 3 consumer trophic levels. Other studies have shown biomagnification increases with food chain length (Rasmussen et al. 1990). In the tropical streams that we studied, lipid content is a better predictor of organic contaminant

concentration than trophic level. Another stable isotope study examining an ecosystem with a short food chain showed that lipid and carbon dietary source were more dominant factors affecting biota contaminant concentrations than trophic level (Campbell et al. 2000). Our study predicted very strong positive correlations between contaminant concentrations and lipid content, with the exception of mercury. Because PCBs and chlorinated pesticides are lipophilic, the greater the lipid content of an organism, the greater the capacity for contaminant accumulation. Mercury has a different bioaccumulation mechanism than highly lipophilic organic contaminants. Mercury binds to sulphydryl groups in protein causing it to accumulate in muscle, gill, liver, and brain tissue (Spry and Wiener 1991), and thus, we detected variably low correlations between lipid content and mercury concentrations among sites.

Contaminant and Other Stable Isotope Relationships

Ecological characteristics that promote contaminant bioavailability are similar to those that support microbial fractionation processes of stable isotopes. $\delta^{34}S$ was correlated with PCB, chlordane, and mercury concentrations when all sites were included in the analyses ($P < 0.01$). However, when each site was examined separately, these variables were not correlated ($P > 0.05$). These variable results may simply be an artifact of sample size and test power. DDT was below detection limits for a majority of samples, and low sample sizes may partially explain the lack of significant correlations with $\delta^{34}S$ ($P > 0.05$). Sulfate

reduction and contaminant availability are both associated with organic matter, which varies temporally due to hydrological variation, a dynamic influence on our results.

Our finding that mercury concentrations were correlated with $\delta^{34}S$ ($P < 0.01$) support the hypothesis that there are significant biological linkages between bacterial sulfate reduction, methylmercury production, sulfur isotope fractionation, and methylmercury accumulation in fish, as also suggested by other studies (Evans and Crumley 2005; Ethier et al. 2008). A major source of mercury in fish muscle tissue is methylmercury produced by sulfur-reducing bacteria at near-anoxic conditions (Spry and Wiener 1991; Regnell et al. 2001). Increasing water sulfate concentrations, and thus $\delta^{34}S$ enrichment, can be associated with both increased sulfur-reducing bacterial activity and elevated methylmercury concentrations in fish (King et al. 2002). $\delta^{34}S$ may be correlated with fish mercury concentration, because sulfur is known to fractionate in the environment by sulfate-reducing bacteria (Ethier et al. 2008). Methylmercury production and subsequent food chain transfer is influenced by a number of site-specific factors, including sediment mercury concentrations, watershed area, water temperature, and water chemistry, in particular pH, alkalinity, concentrations of dissolved organic carbon, total nitrogen, and sulfate (Bodaly et al. 1993; Chen et al. 2005; Ethier et al. 2008). Other parameters that may influence fish mercury concentration are fish size, trophic level (as indicated by $\delta^{15}N$), and dietary carbon source (as indicated by $\delta^{13}C$) (Atwell et al. 1998; Weech et al. 2004).

Dietary carbon source ($\delta^{13}C$) may reflect an influence of feeding strategies (e.g., littoral, benthic, pelagic) on contaminant accumulation in some ecosystems (Kidd et al. 2001; Kidd et al. 2003; Jardine et al. 2006). However, we did not find significant correlations of $\delta^{13}C$ with contaminant concentrations (PCBs, chlordane, DDT, and Hg) after adjusting for lipid content. Our study streams are shallow systems that do not include disjunct macrohabitats like those in lentic systems where other researchers have shown relationships among pelagic, benthic, and littoral habitats and contaminant concentrations. Furthermore, considering that $\delta^{13}C$ did not indicate dietary source, it is expected that it would not predict contaminant accumulation, because diet is the major route of contaminant exposure.

Future Studies

Additional studies could improve understanding of contaminant trophic dynamics in tropical island streams. A one-time collection and analysis of food web components may not adequately represent food webs that are temporily variable due to periodic influxes in nutrients, organic matter, and contaminants from adjacent systems (Polis et al. 1997). Species-specific diet-tissue fractionation (i.e., trophic fractionation) and estimates of turnover rate would enhance mechanistic and ecological understanding (Post 2002; Gustafson et al. 2007). An approach using multiple tissue types would more precisely trace the flow of organic matter and contaminants through an ecosystem and could elucidate temporal dynamics (MacNeil et al. 2005), because different tissues have different tissue-isotopic ratio fractionation and different turnover rates (Hobson and Clark 1992). Stream flow data may be

used to account for temporal variation of stable isotopes because of the strong effect of discharge on parameters influencing stable isotope signatures of food web components in lotic systems (Finlay and Kendall 2007).

Conclusions

Pollution in Puerto Rico streams is not severe, with only a few localized exceptions, and consequently, it was challenging to elucidate contaminant trophic dynamics. PCBs were detected most frequently of all the contaminants tested, but results based on $\delta^{15}N$ did not indicate a correlation to trophic level, instead there was a stronger relationship with lipid content. Tropical island streams are complex systems with constant species movement and multiple food and energy sources. A better understanding of nutrient and organic matter exchange within these ecosystems may be important in assessing contaminant transfer. Identification of nitrogen inputs contributing to water quality degradation is crucial for management planning and regulation of water quality uses. Our results suggest that the $\delta^{15}N$ signature of primary consumers provides a useful tool for monitoring anthropogenic watershed impacts in Puerto Rico stream ecosystems. Landscape alteration for agriculture, urban development, and other uses can have important effects on the ecological integrity of rivers, including eutrophication and loss of biodiversity (Allan 2004; Kwak and Freeman 2010). These findings enhance understanding of contaminant dynamics in tropical stream ecosystems and provide natural resource managers and public health agencies scientific information to guide ecosystem and fisheries management and human health risk assessment.

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Table 1 Puerto Rico stream sampling site characteristics, including location, distance from river mouth, elevation, gradient, and watershed attributes

Site	Designated Land Use	River Name	Latitude	Longitude	Distance to River Mouth (km)	Site Elevation (m)	Watershed Area (km^2)	Gradient (%)	Watershed Land Use: Agriculture (%)	Forest (%)	Shrub and Woodland (%)	Urban (%)
1R	Reference	Mameyes	N18°21'23.7"	W65°46'06.9"	4.4	14.0	21.3	1.4	2.7	95.6	1.0	0.1
2I	Industrial	Cañas	N18°01'23.2"	W66°38'26.4"	5.0	30.2	20.6	0.4	30.0	27.4	32.3	9.8
3U	Urban	Piedras	N18°23'02.8"	W66°03'31.2"	8.6	13.4	23.2	0.2	24.8	26.3	8.8	39.4
4A	Agricultural	Yauco	N17°59'12.9"	W66°50'25.7"	5.7	10.1	115.9	0.1	24.9	31.5	36.6	6.1

Table 2 Water quality parameters measured at each Puerto Rico stream sampling site

	Site			
Parameter	1R	3I	4U	7A
Water temperature (°C)	34.7	31.7	27.8	27.6
Total dissolved solids (g/L)	0.079	0.489	0.386	0.899
Conductivity (µS/cm)	121	844	628	1451
Salinity (ppt)	0.06	0.36	0.28	0.69
Nitrate (mg/L as NO_3-)	1.8	1.5	4.4	3.5
Nitrite (mg/L as NO_2-)	0.006	0.006	0.046	0.015
Ammonia/nitrogen (mg/L as NH_3)	<0.01	0.08	0.02	0.69
Phosphorus (mg/L as PO_4-)	0.02	0.95	0.59	0.83
Alkalinity (mg/L as $CaCO_3$)	33	131	118	248
Hardness (mg/L as $CaCO_3$)	38	185	182	235
Turbidity (FAU)	4	1	3	4
pH	7.27	8.10	7.29	7.18
Dissolved oxygen (mg/L)	8.26	12.36	8.23	5.43

Table 3 Most frequently detected mean contaminant concentrations with standard deviation (SD), summarized by site and species. Organic contaminant concentrations are expressed as parts per billion wet weight and mercury (Hg) concentrations are expressed as parts per million wet weight. 'ND' indicates no detection, 'NA' indicates not applicable, an asterisk indicates that $N = 1$.

		PCBs		Chlordanes		DDT		Hg	
Site	Species	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1R	*Gobiomorus dormitor*	ND	NA	ND	NA	ND	NA	ND	NA
	Anguilla rostrata	0.594	0.051	ND	NA	0.244	0.231	ND	NA
	Macrobrachium spp.	ND	NA	ND	NA	ND	NA	ND	NA
	Agonostomus monticola	0.135	0.135	ND	NA	ND	NA	0.017	0.009
	Sicydium spp.	0.410	0.206	ND	NA	ND	NA	0.014	<0.001
	*Eleotris perniger**	0.321	NA	ND	NA	ND	NA	ND	NA
2U	*Gobiomorus dormitor*	1.027	0.268	ND	NA	ND	NA	0.168	0.056
	Anguilla rostrata	25.017	5.902	10.424	3.690	8.039	5.024	0.068	0.065
	Macrobrachium spp.	0.182	0.159	ND	NA	ND	NA	ND	NA
	Agonostomus monticola	27.529	3.182	13.467	0.274	4.866	0.228	ND	NA
	Awaous banana	6.247	1.308	0.399	0.084	ND	NA	ND	NA
	Eleotris perniger	10.762	3.263	2.205	1.394	0.274	0.475	0.077	0.025
3I	*Gobiomorus dormitor*	0.041	0.071	ND	NA	ND	NA	0.025	0.024
	Anguilla rostrata	5.047	2.693	0.547	0.323	0.721	0.378	ND	NA
	Macrobrachium spp.	ND	NA	ND	NA	ND	NA	ND	NA
	Agonostomus monticola	4.442	1.302	1.073	1.029	0.971	0.477	ND	NA
	Awaous banana	0.539	0.064	ND	NA	ND	NA	ND	NA
	Sicydium spp.	0.090	0.155	0.232	0.058	ND	NA	ND	NA
	*Eleotris perniger**	0.989	NA	0.222	NA	ND	NA	ND	NA
4A	*Gobiomorus dormitor*	0.375	0.108	ND	NA	0.130	0.028	0.014	0.015
	Anguilla rostrata	6.490	2.356	1.119	0.471	6.095	2.990	0.039	0.006
	Macrobrachium spp.	0.764	0.056	ND	NA	ND	NA	ND	NA
	Agonostomus monticola	3.841	1.988	0.373	0.470	2.954	1.552	0.028	0.024
	Awaous banana	2.050	0.562	ND	NA	0.793	0.252	ND	NA
	Eleotris perniger	1.525	1.281	0.082	0.071	0.657	0.584	0.041	0.011

Table 4 Correlation matrix (*R* values) of most commonly detected contaminants (PCBs, chlordane, DDT, and Hg) versus stable isotope ratios ($\delta^{13}C$, $\delta^{15}N$-derived trophic level (TL), $\delta^{34}S$) and lipid content, for all sites and within each site. Significant correlations are indicated by '*' ('*' = $P < 0.05$; '**' = $P < 0.01$; '***' = $P < 0.001$). $\delta^{13}C$ were lipid corrected, and contaminant concentrations were also lipid corrected, but only when examining correlations with $\delta^{13}C$ and trophic level. 'NA' indicates that the number of detections for that contaminant were inadequate to evaluate a correlation

Biotic Parameter	PCBs	Chlordane	DDT	Hg
		All Sites		
$\delta^{13}C$	0.149	0.081	0.190	0.154
$\delta^{34}S$	0.343**	0.290*	0.120	0.318**
Trophic level	0.007	0.033	-0.745***	0.029
Lipid content	0.713***	0.728***	0.717***	0.173
		Reference		
$\delta^{13}C$	0.136	NA	NA	NA
$\delta^{34}S$	0.329	NA	NA	NA
Trophic level	0.258	NA	NA	NA
Lipid content	0.705**	NA	NA	NA
		Industrial		
$\delta^{13}C$	0.432	0.439	0.493*	NA
$\delta^{34}S$	0.330	0.314	NA	NA
Trophic level	0.290	0.161	0.332	NA
Lipid content	0.979***	0.769***	0.936***	NA
		Urban		
$\delta^{13}C$	0.185	0.137	0.357	0.326
$\delta^{34}S$	0.268	0.196	NA	0.238
Trophic level	0.134	0.209	0.265	0.087
Lipid content	0.788***	0.868***	0.726***	0.374
		Agricultural		
$\delta^{13}C$	0.034	0.189	0.420	0.226
$\delta^{34}S$	0.476	0.395	0.400	0.056
Trophic level	0.149	0.384	-0.529*	0.415
Lipid content	0.666**	0.869***	0.875***	0.054

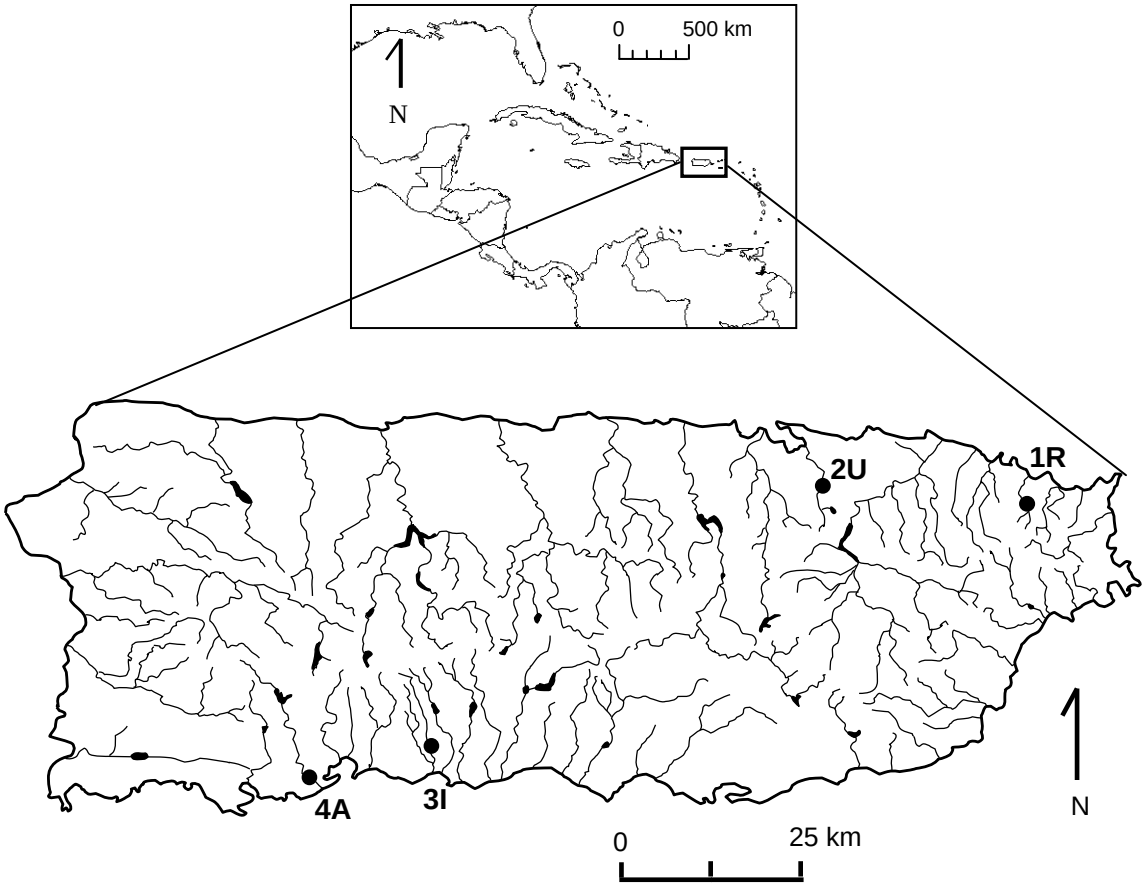

Fig. 1 Map of Puerto Rico indicating locations of study sites and streams

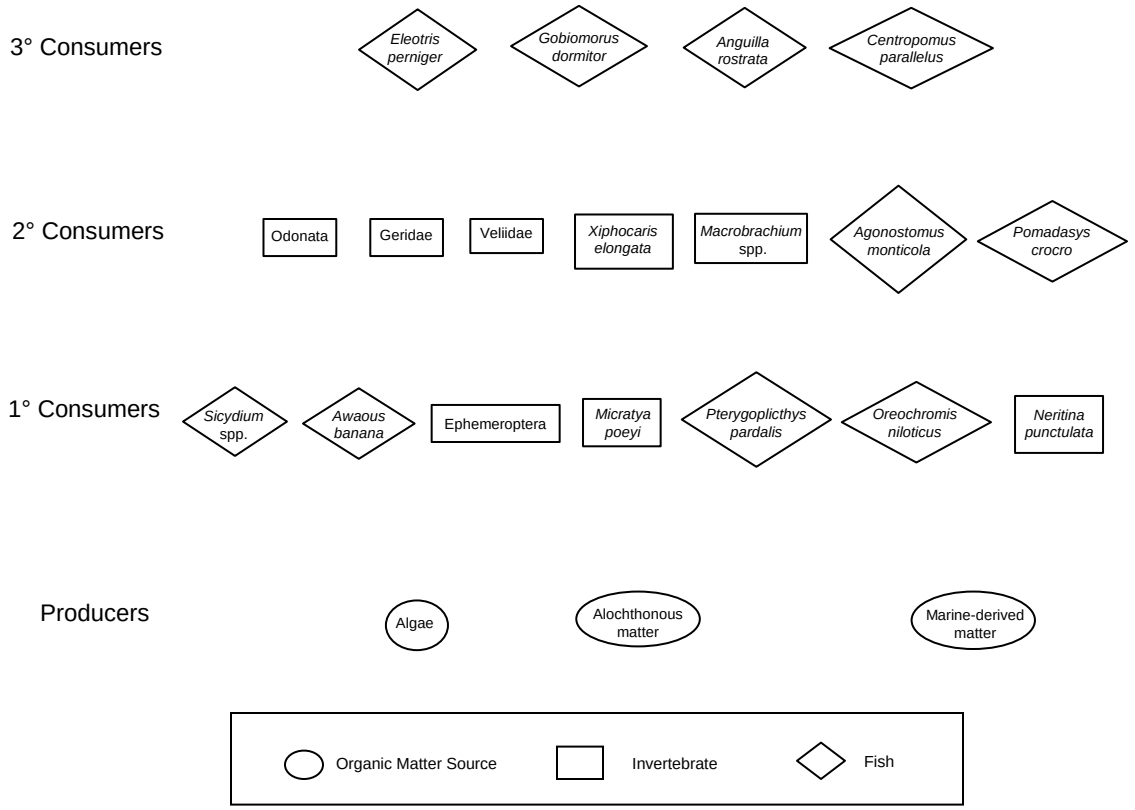

Fig. 2 A generalized food web diagram of a stream ecosystem in Puerto Rico

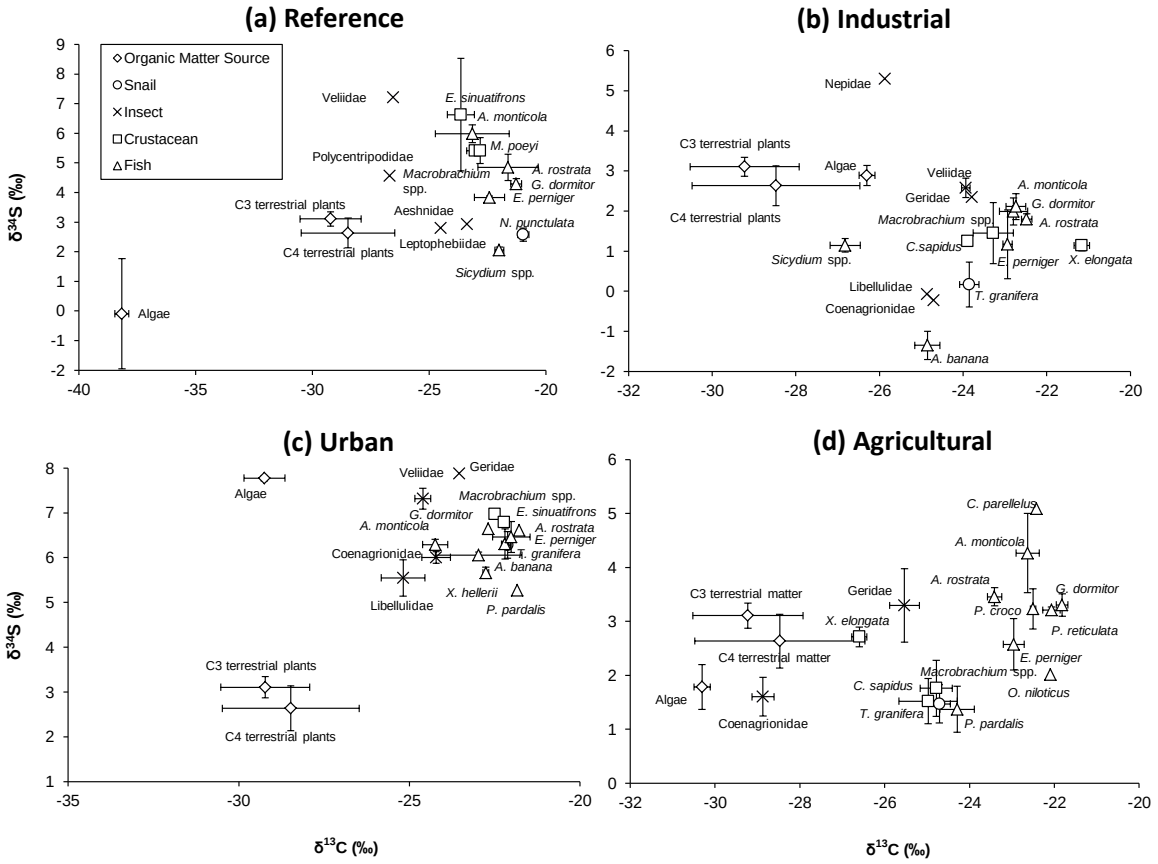

Fig. 3 Mean (± SD) lipid corrected $\delta^{13}C$ and $\delta^{34}S$ biplot for stream sites (a-d). General taxa categories of samples are indicated by symbols. C3 and C4 terrestrial-derived organic matter points represent a mean among all sites

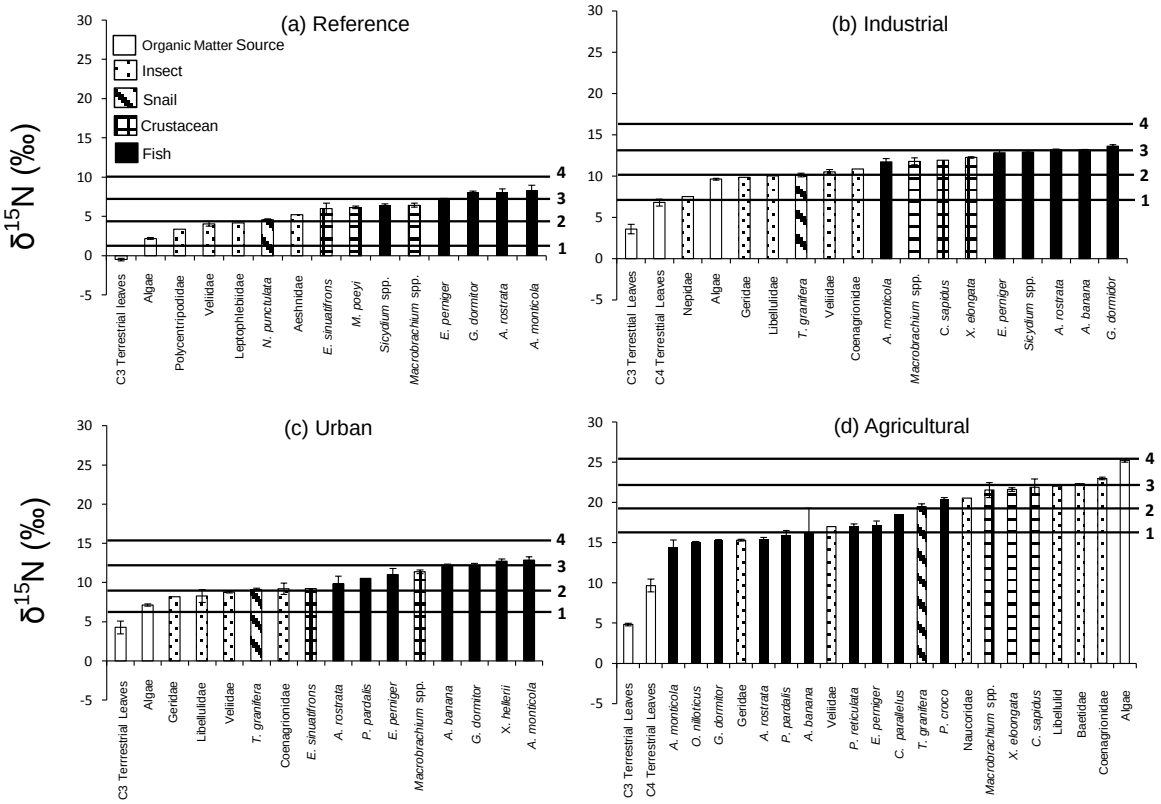

Fig. 4 Mean (± SD) $\delta^{15}N$ values of food web components, categorized into general taxa, for each stream site (a-d) with trophic levels indicated by horizontal black lines

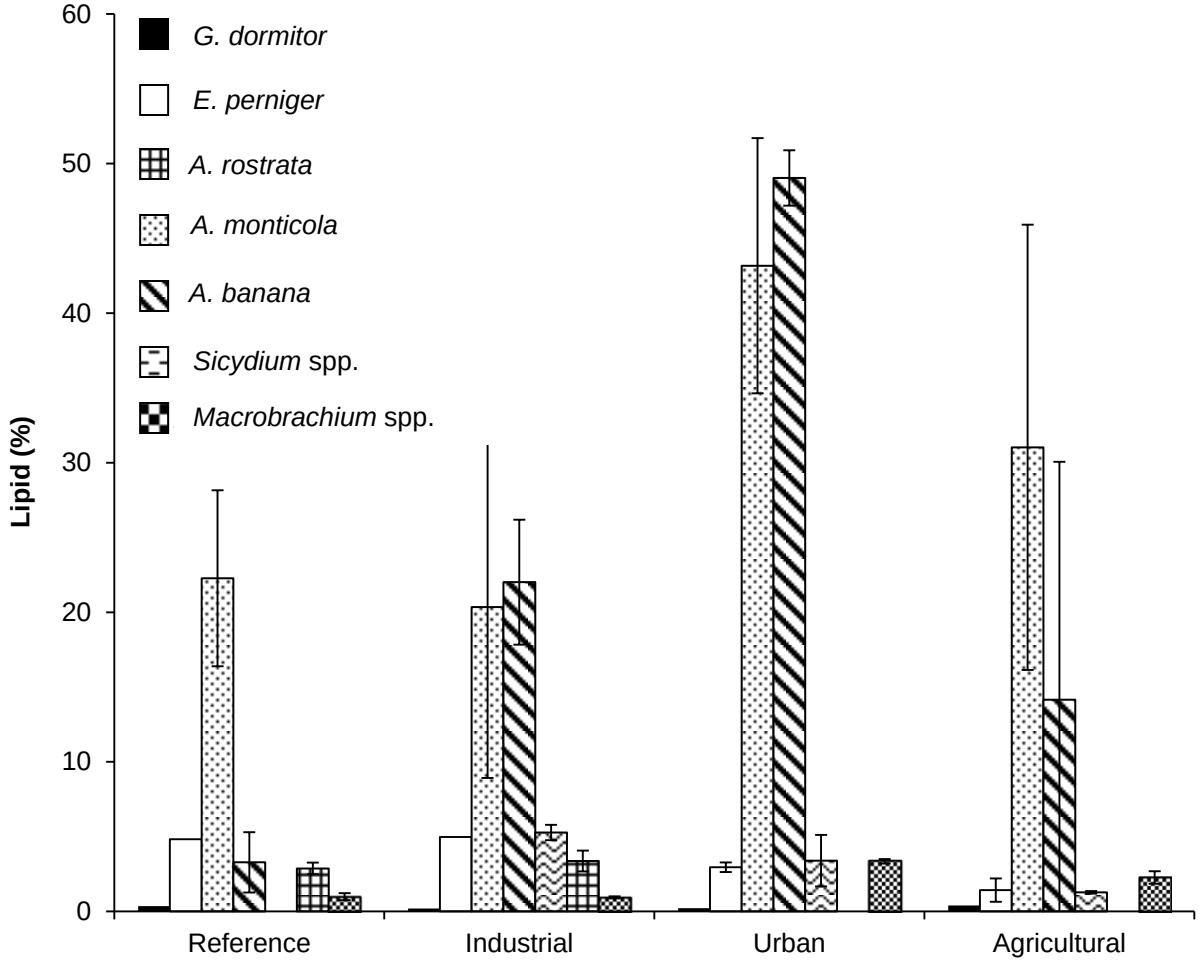

Fig. 5 Mean (± SD) lipid content for consumers sampled at each stream site

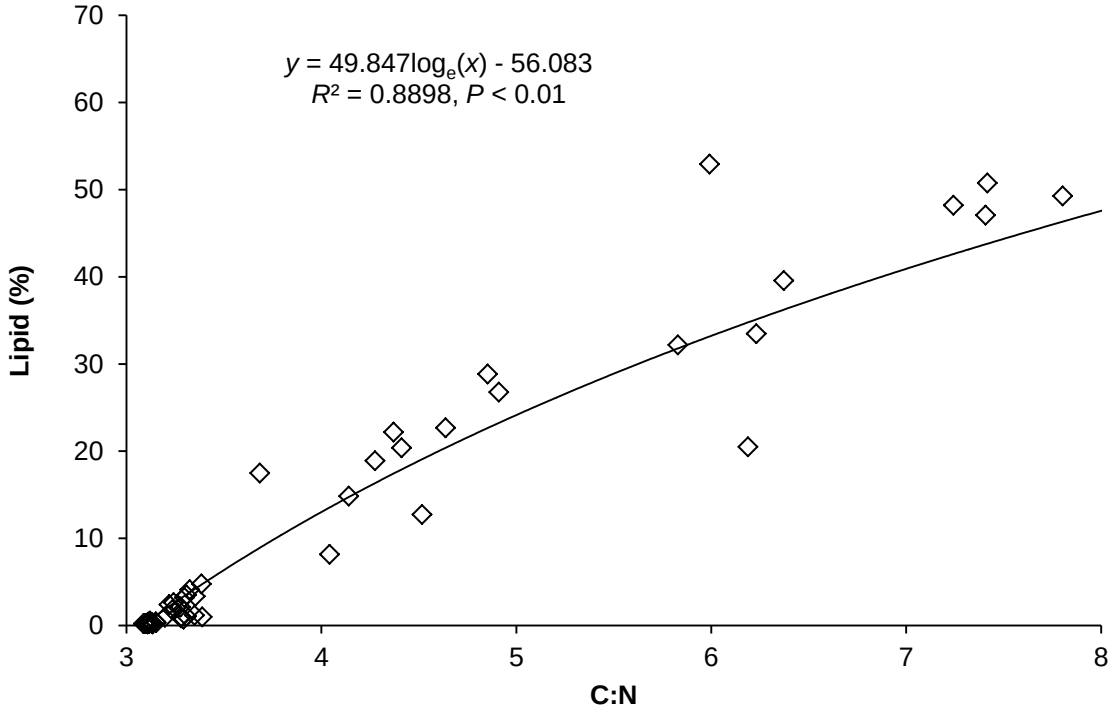

Fig. 6 Lipid content of fish and shrimp sampled from four stream sites related to C:N (without the lipid extraction treatment)

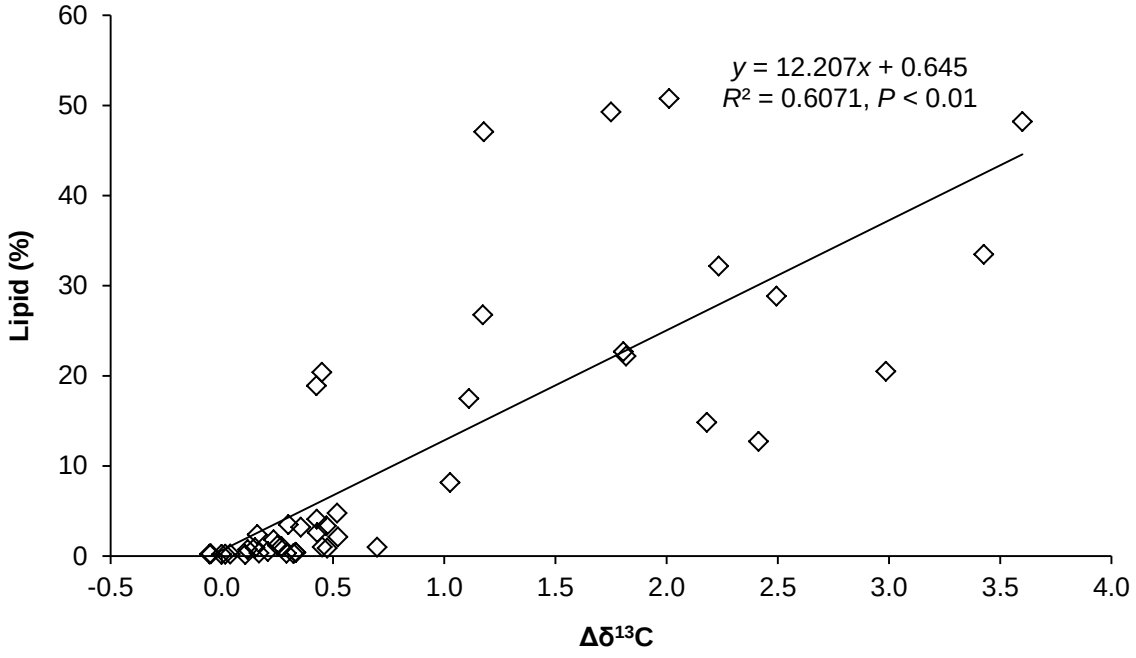

Fig. 7 Lipid content of fish and shrimp sampled from four stream sites related to the difference between lipid extracted $\delta^{13}C$ and unextracted $\delta^{13}C$ ($\Delta\delta^{13}C$ **)**

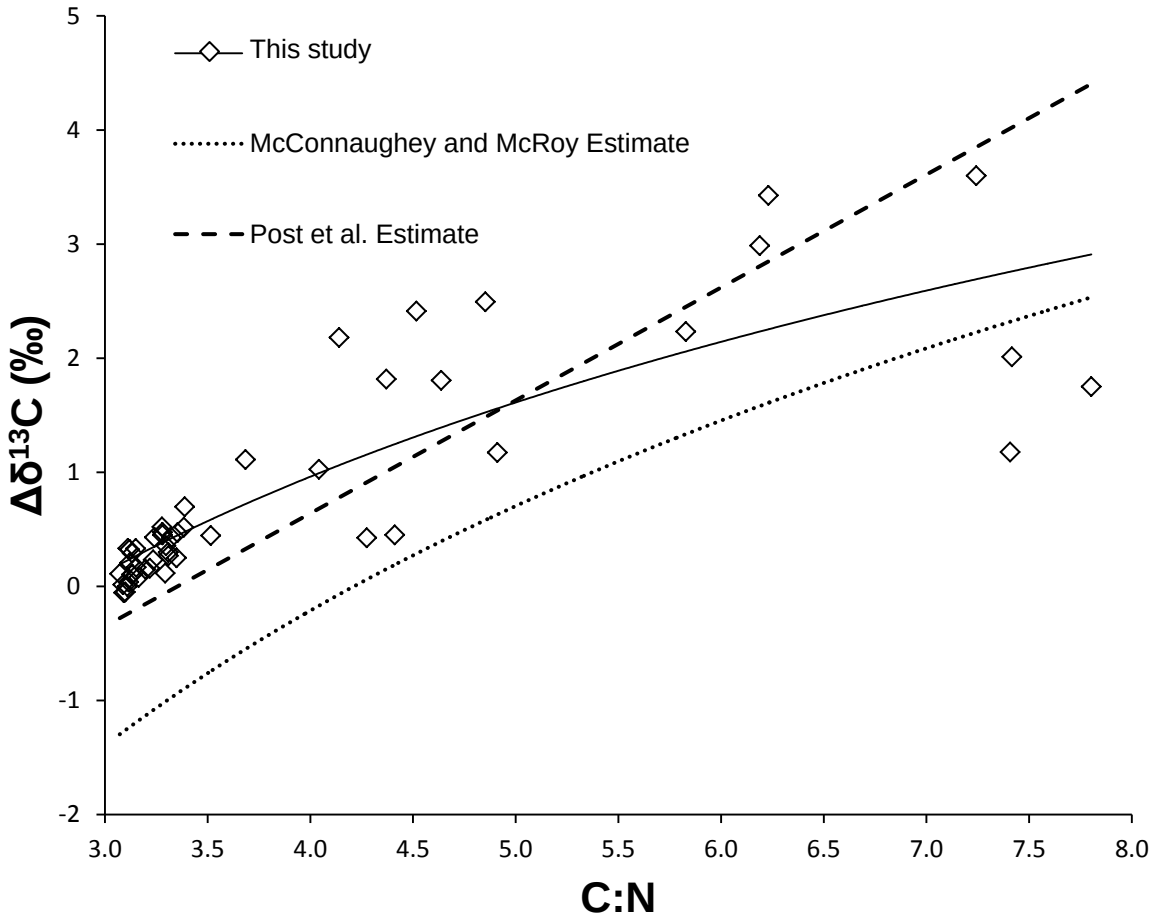

Fig. 8 The relationship between C:N and the difference between lipid extracted $\delta^{13}C$ and untreated $\delta^{13}C$ ($\Delta\delta^{13}C$), in comparison with McConnaughey and McRoy (1979) $\delta^{13}C$ correction estimate prediction and Post et al. (2007) $\delta^{13}C$ correction estimate prediction of our data from Puerto Rico streams

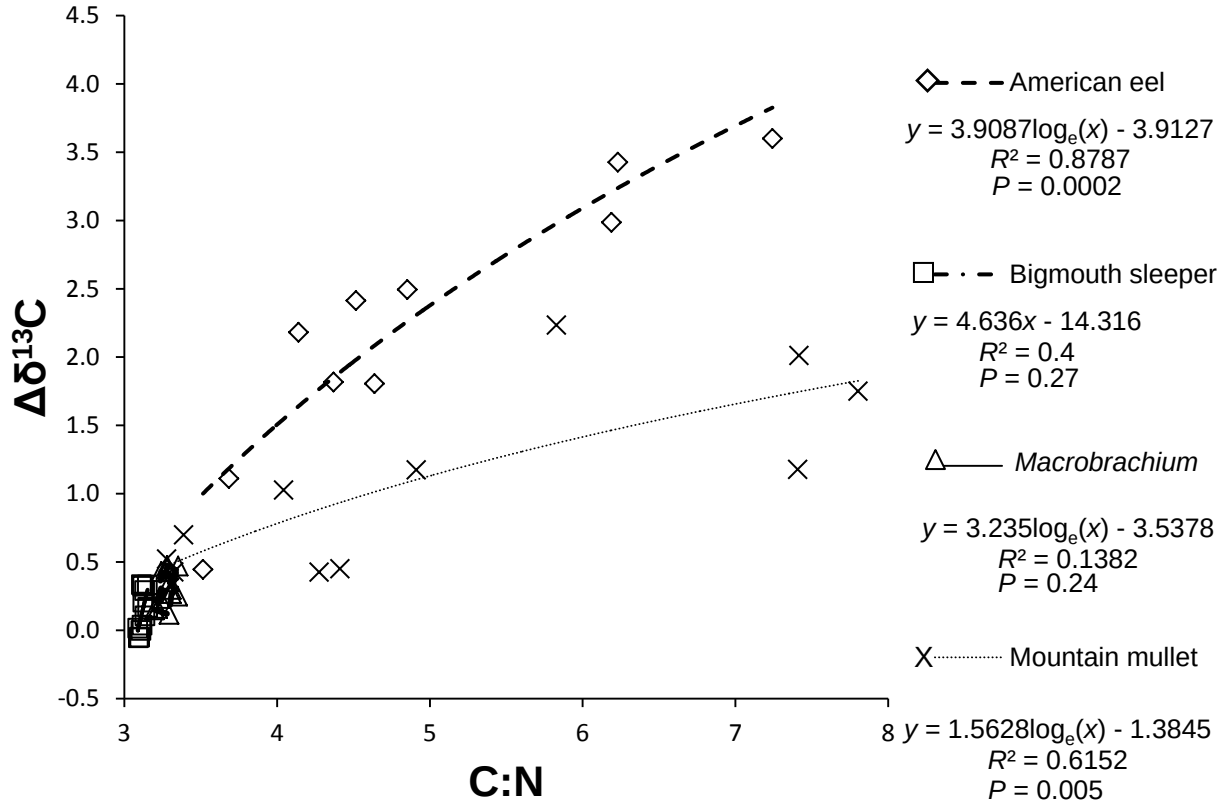

Fig. 9 The relationship of C:N and species differences in the difference of $\delta^{13}C$ between lipid extracted and non-lipid extracted samples ($\Delta\delta^{13}C$) of fish and shrimp from four stream sites

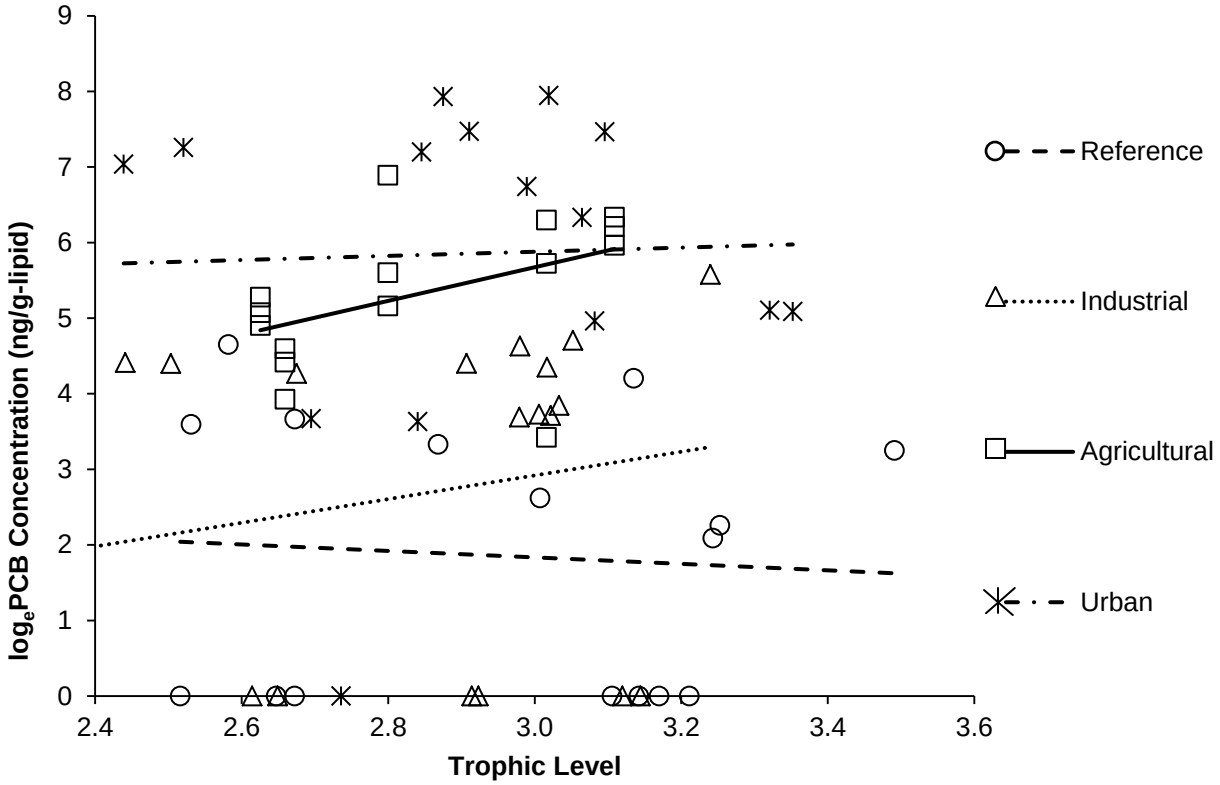

Fig. 10 PCB concentration as a function of trophic level of consumers at four stream sampling sites of varying dominant riparian and watershed land use (forested, industrial, agricultural, or urban). All linear relationships plotted were not statistically significant ($P > 0.05$)

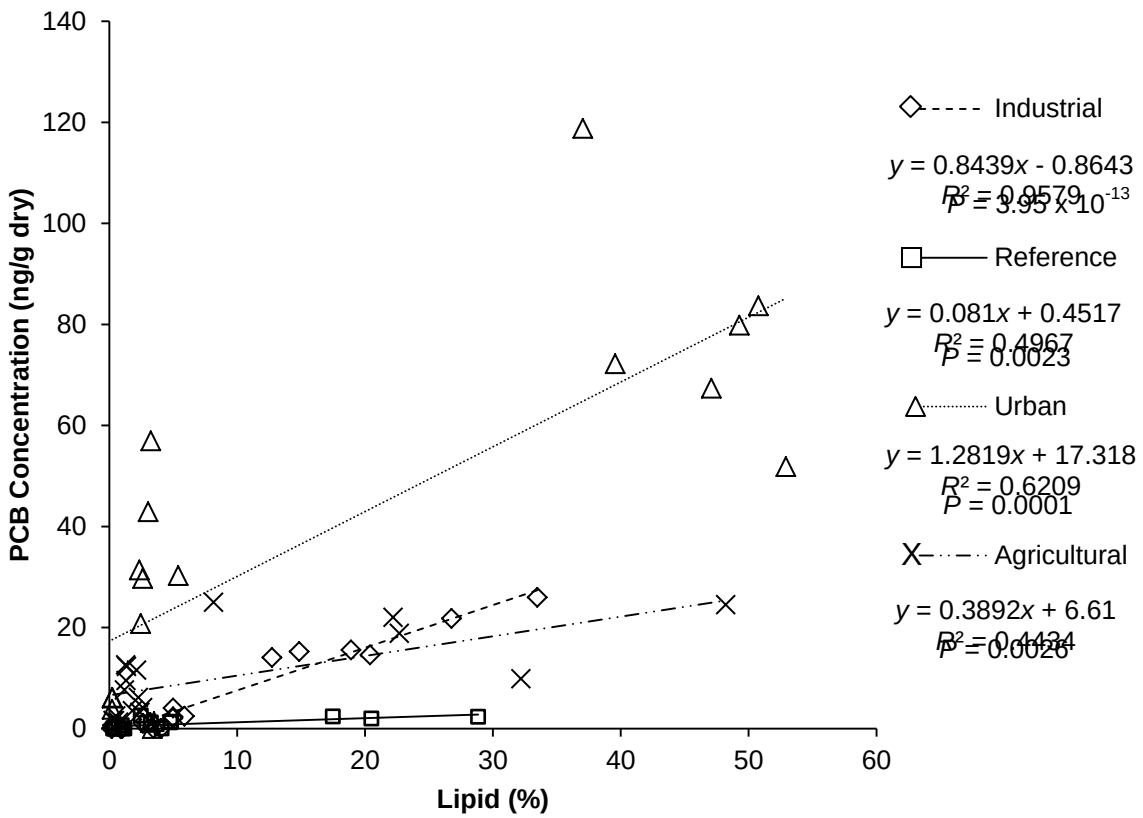

Fig. 11 PCB concentration as a function of lipid content of consumers at four stream sampling sites of varying dominant riparian and watershed land use (forested, industrial, agricultural, or urban)

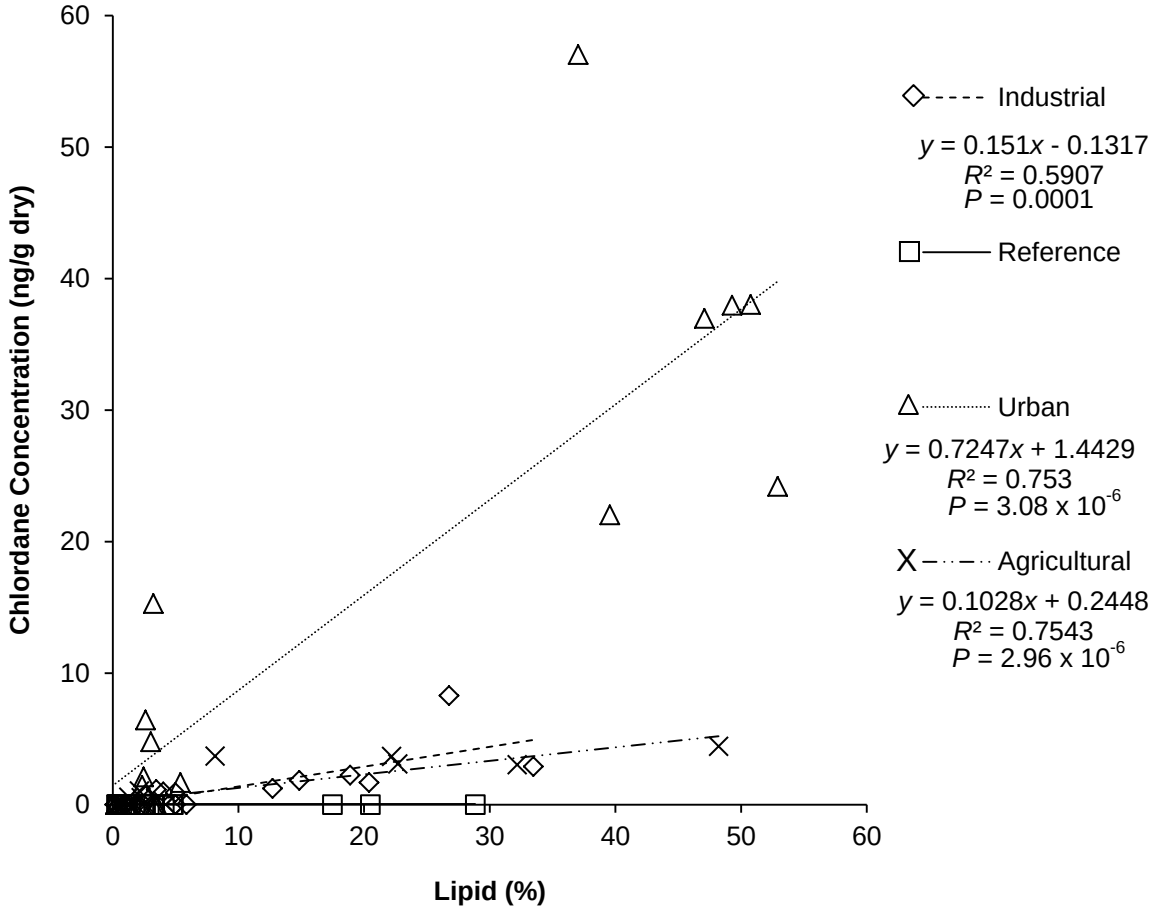

Fig. 12 Chlordane concentration and lipid content of each sample with a regression line representing the relationship for each site. Chlordane was not detected in any sample (N = 16) from the reference site

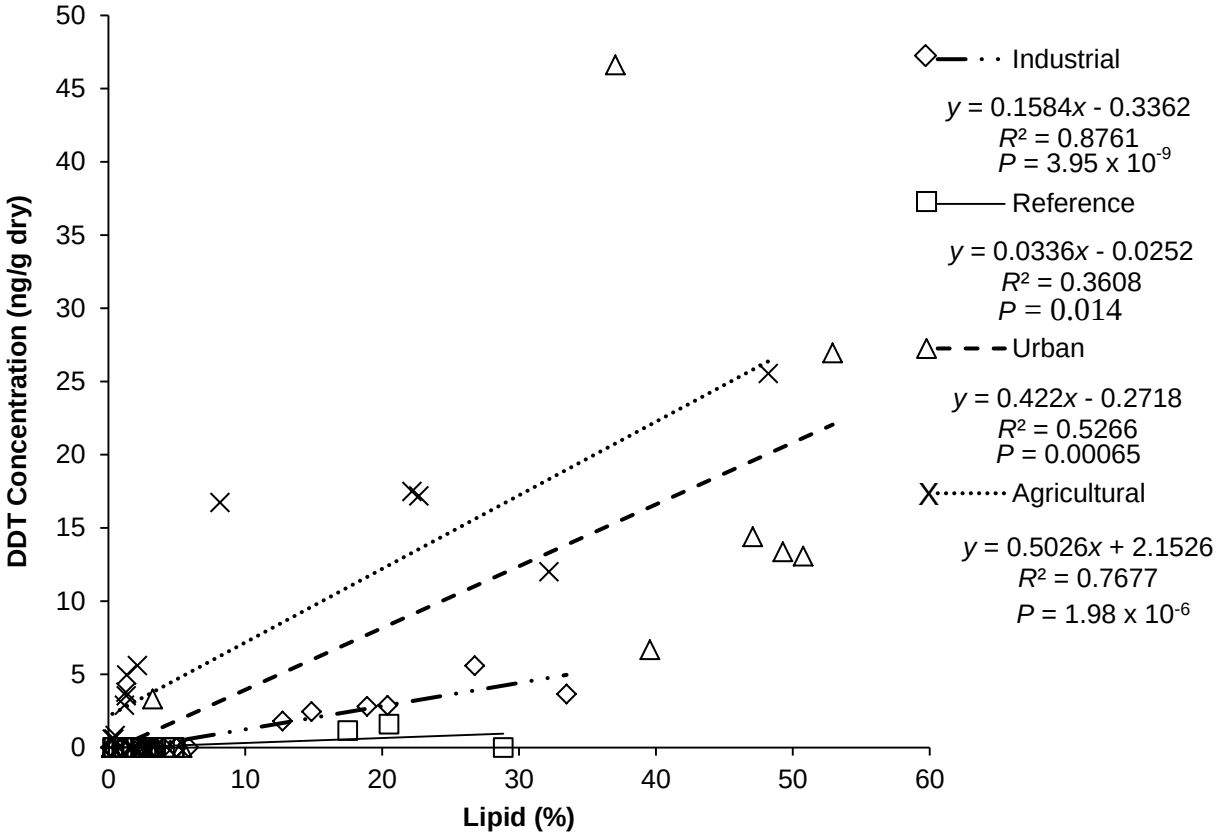

Fig. 13 DDT concentration and lipid content of each sample with a regression line representing the relationship for each site