FISH COMMUNITY AND HABITAT ASSESSMENTS WITHIN AN URBANIZED SPRING COMPLEX OF THE EDWARDS PLATEAU

by

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FAIR USE AND AUTHOR'S PERMISSION STATEMENT

DEDICATION

This work is dedicated to my wife, Dana, who selflessly inspired me to seek knowledge and realize my dreams and to my parents who always encouraged me to achieve my full potential.

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ABSTRACT

Spring complexes within the arid region of the Edwards Plateau are diversity hotspots and evolutionary refugia for numerous aquatic fauna. Within the last 100 years, anthropogenic modifications and uses of spring complexes are associated with imperilment of aquatic fauna. Purposes of this study were to assess biotic integrity (i.e., regional and natural species composition and diversity; Karr and Dudley 1981) of the fish community and to quantify current community structure and habitat associations within the Comal springs complex, the greatest discharge spring within the Edwards Plateau region of central Texas and located within the urban landscape. Fishes and habitats were quantified among wadeable and non-wadeable areas and among six reaches of the Comal springs complex seasonally for one year. Twenty-five species and 23,318 fishes were observed. Spring-associated fish richness (S) was six, comprising 77% of the total catch per unit effort. Compared to reference conditions and to historical records, species richness and relative abundances suggest that the Comal spring complex has high biotic integrity despite extremely low flow conditions and rotenone treatment in the 1950s and habitat modifications (e.g., low head dams, land use conversion, bank stabilization) and high recreation use since the 1950s. However, the fish community was not homogenous among all reaches. Within two reaches of high recreational use, spring-associated fish richness and relative abundances were lower than other four reaches but still maintained high relative abundances of the federally-listed Fountain Darter. Fish-habitat associations were similar to reported habitat associations for most fishes. A notable

exception was observed for the Fountain Darter, which had a more ubiquitous distribution and was not strongly associated with vegetation as previously found. Into the future, this study can be used as a baseline to monitor and assess threats to the Comal spring complex.

I. FISH COMMUNITY AND HABITAT ASSESSMENTS WITHIN AN URBANIZED SPRING COMPLEX OF THE EDWARDS PLATEAU

Introduction

Spring complexes (i.e., spring outflows and spring runs) in arid to semi-arid regions are often unique aquatic environments because their availability is decoupled from the local climate and voluminous outflows of groundwater sources provide stenoecious water quality (e.g., temperature, specific conductance, pH) and quantity (Davis 2013). As spring discharges become surface water, stenoecious water quality parameters persist until equilibrium with ambient conditions or mixing at a confluence with a larger, non-spring influenced stream occurs (Hubbs 1995, Groeger et al. 1997). Within the semi-arid and karst Edwards Plateau region of southcentral USA, spring complexes are evolutionary refugia for many endemic flora and fauna, referred to as spring-associated species (Craig et al. 2016). Craig et al. (2016) defines spring-associated fishes as those with densities greater within spring complexes than in riverine habitats (i.e., outside of spring complexes). Hubbs (1995) suspected and others (Kollaus and Bonner 2012, Craig et al. 2016) quantified that spring-associated fishes have affinities for stenoecious spring complexes, comprising >80% of the fish community, whereas riverine-associated fishes (i.e., those more common in non-spring influenced streams) tend to avoid stenoecious spring complexes but do maintain a small proportion of the fish community. Mechanisms underlying affinities for spring or riverine habitats are not well understood at this time but are possibly related to species fitness mediated by water temperature (DiMichele and Powers 1982).

Stream flow (e.g., magnitude, timing, duration) is considered the master variable in structuring and maintaining biotic integrity of aquatic communities (Poff et al. 1997). Biotic integrity is the regional and natural species composition, diversity, and functionality of aquatic communities, and is quantified for a region based upon minimally-disturbed (sensu Craig et al. 2016) reference sites or is quantified for a site or river reach by comparing changes in species composition, diversity, and functionality between historical observations, when available, and current observations (Karr and Dudley 1981). Like stream flow, spring flow is positively related to biotic integrity of spring fish communities, as defined by species composition (richness, relative abundance, and density of spring fishes; Craig et al. 2016). Among six minimally-disturbed spring complexes, spring fish richness ranged from two to seven, relative abundances ranged from 20% to 85%, and densities ranged from 0.2 fish/m^2 to >1.6 fish/m^2 among spring median flow magnitudes ranging from 0.07 to 4.47 cms. Among anthropogenically-altered spring complexes, excessive groundwater withdrawals are associated with reductions or elimination of spring flow and spring fishes within Comanche Springs (Echelle and Miller 1974) and San Antonio spring complex (Craig and Bonner, In review). Urbanization effects, which encompass a wide range of anthropogenic modifications within urban streams (Walsh et al. 2005), are associated with changes in spring fish communities within San Felipe spring complex (Del Rio, Texas; Garrett et al. 1992) and San Marcos spring complex (San Marcos, Texas; Kollaus et al. 2015). Despite some changes in the fish community (e.g., increases in introduced fishes), San Felipe and San Marcos spring complexes maintain high levels of biotic integrity with increased urbanization, support >65% relative abundances of spring fishes, and are designated as critical habitat for several federally-listed threatened and endangered species.

Comal spring complex (Comal County, Texas) generates 8.0 cms of surface flows and is the greatest discharge spring complex within the Edwards Plateau (Brune 1981). The complex supports four spring-associated and federally-listed species (i.e., Comal Springs Riffle Beetle *Heterelmis comalensis*, Comal Springs Dryopid Beetle *Stygoparnus comalensis*, Peck’s Cave Amphipod *Stygobromus pecki*, and Fountain Darter *Etheostoma fonticola*). Along a gradient of human disturbances, Comal spring complex, like San Felipe and San Marcos spring complexes, is located within an urbanized watershed (City of New Braunfels) and modified by numerous low-head dams, retaining walls, and flow diversions. Portions of the complex are used extensively for recreational uses (e.g., wading, swimming, and tubing; Edwards Aquifer Habitat Conservation Plan 2012).

Comal spring complex, however, differs notably from San Felipe and San Marcos complexes. In December 1951, multiple applications of the piscicide rotenone were added to the Comal spring complex for the purpose of eradicating non-native fishes and improving recreational fishing for Largemouth Bass *Micropterus salmoides* (Ball et al. 1952). Estimates of longitudinal extent of fish eradication are unknown, but 18 dump truck loads of targeted non-native Rio Grande Cichlid *Herichthys cyanoguttatus* and native centrarchids and ictalurids were removed from the river. “Desirable species” (i.e., minnows, other species, and specifically Fountain Darters; C. Hubbs cited in Schenck and Whiteside 1976) were removed prior to rotenone applications, held in a protective area, and re-released into the Comal River. In April 1952, six desirable species (i.e., Fountain Darter, three poeciliids, and two cyprinids) and five targeted species (i.e., *H.*

cyanoguttatus, three centrarchids, and one ictalurid) were recorded from the Comal River (Hendrickson and Cohen 2015), suggesting targeted and desirable fishes persisted after the rotenone treatment. In 1956, daily flows associated with the drought of record in Texas (McGregor 2015) ranged between 0.16 to 0.85 cms for 213 days between May and December (USGS Station 8169000). In the spring of 1972, a 410-cms high flow pulse (magnitude average for a 24-h period) inundated the Comal River. In 1973, Schenck and Whiteside (1976) reported the likely extirpation of Fountain Darter in the Comal River, citing the rotenone treatment in 1951, low flows in 1956, and the flood in 1972 as possible reasons underlying the extirpation event. Within a two-year period (1975 – 1976), about 450 Fountain Darters from the San Marcos River were introduced into the Comal River (Schenck and Whiteside 1976, USFWS 1996). Post repatriation efforts, the Comal River population of Fountain Darters was estimated to be 170,000 (95% confidence interval limits of 115,000 and 255,000; Linam et al. 1993). Only minor genetic divergence is detected between the two existing populations of Fountain Darters (i.e., San Marcos River population and Comal River population), and genetic diversities within both populations are currently considered secured (Olsen et al. 2016).

Purposes of this study were to assess the fish community and habitats of the Comal springs complex. In part, this study was designed to assess biotic integrity of the system, given that the system was stressed leading to possibly one (i.e., Fountain Darter) or more extirpations of spring-associated fishes. Biotic integrity was assessed by comparing spring-associated fish richness, abundances, and densities of the Comal spring complex to references conditions provided by Craig et al. (2016). Assessing biotic integrity by comparing historical records (Hendrickson and Cohen 2015) to contemporary fish community observations was attempted, similar to published studies in Rinne et al. (2005) and by Kollaus et al. (2015), but museum records and the number of published and unpublished reports were too sparse to estimate historical community fish community structure other than species richness within the Comal spring complex. Also this study was designed to quantify fishes and fish-habitat relationships for the entire fish community and among deep water habitats. In the past (Linam et al. 1993) and currently (Edwards Aquifer Habitat Conservation Plan 2012), Fountain Darter estimates and habitat associations are targeted for quantification at depths suitable for dip nets or drop nets (i.e., wadeable habitats). However, Fountain Darters occupy depths of non-wadeable habitats (Behen 2013), and several other spring-associated and regionally endemic fishes inhabit the Comal River, with one, Guadalupe Roundnose Minnow *Dionda nigrotaeniata*, listed as species of greatest conservation need (Texas Parks and Wildlife Department 2012).

Study objectives were to quantify wadeable and non-wadeable habitats, fish communities, and fish-habitat associations among six distinct reaches of the Comal spring complex from the uppermost reach to near the Comal spring complex confluence with the Guadalupe River. Using the linear and non-linear regression models of Craig et al. (2016), Comal spring complex fish community with high biotic integrity and a flow of 8 cms would consist of >6 spring-associated fishes with relative abundances of >65%, and densities >1 per m^2. However, fish community could reflect legacy effects of low flows, the rotenone treatment, and flood effects on the fish community and have low biotic integrity. Comal spring complex fish community with low biotic integrity would consist of fewer spring fishes ($N \leq 4$), less relative abundance (<50%), and lower density

(<0.4 fish per m^2), which are the predicted estimates for spring fish communities at <0.65 cms. Two additional observations can be made from this study: 1) Are fish communities within reaches of high recreation use noticeably different than from those with low recreation use, an explicit concern listed by USFWS (1996) and by the Edwards Aquifer Habitat Conservation Plan (2012), and 2) Are habitat associations of the Fountain Darter among wadeable and non-wadeable habitats similar to those reported in wadeable habitats? Among wadeable habitats, Fountain Darters are found only in vegetation (Schenck and Whiteside 1976), associated primarily with associated with filamentous algae (Linam et al. 1993), require undisturbed run, riffle, and pool habitats with a mix of submergent vegetation for cover (USFWS 1996), have strong preference for aquatic vegetation (Edwards Aquifer Habitat Conservation Plan 2012), and associate with vegetation for feeding, reproduction, and refugia (Alexander and Phillips 2012).

Methods

Groundwater within limestone-dominated, Cretaceous-aged Edwards Formation (i.e., Edwards Aquifer) provides the water source for the Comal spring complex (Guyton and Associates 1979). Multiple springs emerge along a 1.3-km linear distance of a former tributary (Blieders Creek) or former main stem channel of the Guadalupe River (Woodruff and Abbott 1979; Grimshaw and Woodruff 1986). Spring outflows form spring runs that flow into or emerge within the artificially impounded Landa Lake (Brune 1981). From Landa Lake, about 1.4 cms is diverted into the Old Channel with the remaining discharge diverted into the New Channel (formerly Dry Comal Creek; Edwards Aquifer Habitat Conservation Plan 2012). Old Channel (2.4 km in length) and New Channel (0.8 km) merge together to form the Lower River and flow an additional 2 km before merging with the Guadalupe River. The upper Comal River watershed is rural but the Comal spring complex is located with the City of New Braunfels. Within a 100 m radius of the spring complex, dominant land use is 42% residential, 25% open, and 15% commercial (City of New Braunfels, unpublished data). Riparian vegetation ranges from none (e.g., sidewalk) to dense understory and large trees. A large portion of the instream habitats are modified and fragmented by several low head dams and retaining walls. Instream habitats, with few exceptions, are designated as aquatic recreational areas; however, recreational activities are not equal among all areas (Edwards Aquifer Habitat Conservation Plan 2012). Wading, lounging, playing, kayaking, and tubing are more common within the New Channel and Lower River. Peak use is Memorial Day through Labor Day, on the weekends, and in the afternoon. Estimates of 3,000 to 5,000 people per weekend participate in some type of recreational activity during peak use.

Six reaches were selected fish and habitat quantification from Fall 2014 through Summer 2015: Blieders Creek, Upper Spring Run, Landa Lake, Old Channel, New Channel, and Lower River. Blieders Creek (29°43'15.37"N, 98° 7'38.23"W) is tributary to one of the spring runs (Upper Spring Run), dominated by surface runoff flows, and upstream from spring influence. Upper Spring Run (29°43'15.46"N, 98° 7'41.12"W; also referred to as Spring Run 4 in USFWS 1996) is the longest spring run within the Comal spring complex and flows about 0.8 km before entering Landa Lake. However, water level with Upper Spring Run is influenced by the dam that impounds Landa Lake and maintains water depths even when spring flow discharges are low (<0.01 cms; Nichols 2015). The remaining reaches were Landa Lake (29°42'43.03"N, 98° 8'6.32"W), Old Channel (29°42'39.22"N, 98° 7'22.30"W), New Channel (29°42'29.47"N, 98° 7'43.09"W), and Lower River (29°42'5.74"N, 98° 6'58.44"W). During the period of observation, median daily flow was 5.1 m^3/s with a minimum flow of 1.7 m^3/s (August 30, 2014) from USGS Station 08169000 located within the Lower Reach (Figure 1). Historical (1928 – 2015) median daily flow is 8.0 m^3/s.

Field methodologies followed protocols established by Behen (2013), which were developed for the nearby San Marcos River and a similar spring complex emanating from the Edwards Aquifer. Field methodologies were designed to quantify fish composition and habitat characteristics among reaches from wadeable and non-wadeable habitats and for pelagic and small benthic fishes. Each reach was sampled once per season (Fall, Winter, Spring, and Summer) using multiple gear types.

Wadeable habitats were sampled using a downstream 5-m seine (3 m x 1.8 m; mesh size = 3.2 mm; 15 m^2 each) haul or a 5-m downstream substrate kick into a seine

(Kollaus and Bonner 2012) within all reaches except in Landa Lake, which lacked suitable wadeable habitats because depth of water and benthic silt. A transect perpendicular to reach was established at the downstream most area of the wadeable habitat. Discrete habitats (i.e., run, riffle, pool, backwater, eddy) with homogenous depths and current velocities were sampled as encountered along the transect. A new transect was located 20 m upstream from the first, and seine hauls were repeated until 20 seine hauls or kicks were taken. After each seine haul or kick, fishes were identified to species and enumerated. Fishes were released except for vouchers, which were anesthetized with tricane methane-sulfonate (MS-222) and preserved in 10% buffered formalin. Water temperature (°C), dissolved oxygen (mg/l), conductivity (μS/cm), and pH were taken at each seine haul or kick with a YSI Model 556 multi-probe meter. Current velocity was taken with a Marsh-McBirney Flowmate Model 2000 meter. In addition, water depth, percent substrate type, percent vegetation, vegetation type, percent woody debris, and percent detritus were recorded.

Non-wadeable habitats were sampled with SCUBA. A deep pool or run mesohabitat was selected within each reach. Mesohabitats were sampled with four divers spaced equidistance apart (3 to 5 meters, depending on water clarity) and swimming from shoreline to the opposite shoreline. Fishes observed by divers were identified to species and enumerated to the lowest practical resolution; two *Gambusia* species, *G. affinis* and *G. geiseri* were identified as *Gambusia*. *Lepomis* was used when identification of *Lepomis* species was uncertain. Coordination and communication among divers minimized double-counting of fishes. Length and width of the mesohabitat was recorded. After each mesohabitat quantification, four 5-meter plastic pipes were dropped within the mesohabitat, visually spaced equidistance from one another shoreline to shoreline, to assess microhabitats. Microhabitat quantification consisted of two divers located on either side of a plastic pipe (1 m on each site; total area = 10 m^2). Both divers advanced slowly in a downstream to upstream direction, carefully moving substrates and vegetation to identify and enumerate fishes within the area. Benthic-associated Fountain Darter and Greenthroat Darter *Etheostoma lepidum* potentially coexist in all reaches of the Comal River. Darters were identified as *Etheostoma*, if identification to species was uncertain. After each microhabitat survey, current velocities (bottom, 60% of depth), water depth, percent substrate type, percent vegetation, vegetation type, percent woody debris, and percent detritus were recorded. Water temperature (°C), dissolved oxygen (mg/l), conductivity (µS/cm), and pH were recorded for each mesohabitat.

Statistical Analyses

Seasonal and spatial variation among wadeable and non-wadeable (microhabitat only) habitat parameters (e.g., depth, current velocity, substrate, conductivity) were assessed using Principal Component Analysis (PCA; SAS 9.1). Habitat parameters were z-scored transformed (Krebs 1999). Analysis of variance was performed on Principal Component axis (PC) I and PC II with a Fisher's Least Significant Difference ($\alpha = 0.05$) to detect habitat differences, as PC gradients, among seasons and reaches.

Fish community was quantified by total number of individual fish observations (N) and the number of species (species richness [S]) by reach and overall among wadeable and non-wadeable habitats. Fishes were then assigned to a gear type (i.e., wadeable-seine, SCUBA-mesohabitat, SCUBA-microhabitat) based on which gear type was most efficient at quantifying a species. Most efficient gear type was qualitatively defined as the gear type most appropriate to capture a species of fish (e.g., seines tend to underestimate densities of large body fishes, such as *Lepomis*; Bayley and Herendeen 2000) and quantitatively defined as the gear type that captured >50% of the species. For example, 173 (64%) of 271 Mimic Shiners *Notropis volucellus* were taken with seines and therefore assigned to the wadeable-seine gear type, whereas as 1,735 (85%) of the 2,045 Fountain Darters were quantified with SCUBA-microhabitat and therefore assigned non-wadeable-microhabitat gear type. Assigning species to a gear type was necessary in order to calculate total relative abundances by catch per unit effort (CPUE; number of fish per m^2) while excluding inefficient gear type densities. Small benthic darters are sometimes observed in the mesohabitat survey but observations underestimate their density because of their benthic association. Some *Lepomis* were observed in microhabitats but species, like Redbreast Sunfish *Lepomis auritus,* are more abundant in the pelagic zone. Redspotted Sunfish *Lepomis miniatus* were typically taken in swifter currents, assessed only by seines and not SCUBA-mesohabitat or SCUBA-microhabitat. After assigning a species to their respective most efficient gear type, CPUE was calculated for each species and relative abundance by CPUE (Percent of Total CPUE) was calculated across and within reaches. Percent of Total CPUE was also calculated for spring-associated fishes and non-native fishes across all reaches. Also by gear type, Bray-Curtis similarity matrices were generated in Primer 6 using log (N+1) transformed species counts to test differences among reaches with one-way analysis of similarities (ANOSIM; $\alpha = 0.05$, 10,000 permutations). Similarity percentage option (SIMPER) was used to identify fishes that contributed to the most dissimilarity among reaches.

Fish-habitat associations were assessed using Canonical Correspondence Analysis (CCA; Canoco 4.55 2006) with a 1,000 permutation Monte Carlo simulation to test (α = 0.05) if observed pattern differed from a random pattern (ter Braak and Smilauer 2002). Three CCA models were developed for seine, mesohabitat, and microhabitat. For each model, species matrix was constrained by a multiple linear regression of variables within a habitat matrix (McCune and Grace 2002). Rows in seine species and habitat matrices corresponded with seine hauls. Rows in microhabitat species and habitat matrices corresponded with 10 m^2 plastic pipe area. Rows in mesohabitat species and habitat matrices were taken from the average of the four microhabitats taken within the mesohabitat. Fish counts were used instead of densities. Habitat matrices consisted of water temperature, dissolved oxygen, conductivity, pH, current velocity, water depth, percent substrate type, percent vegetation, vegetation type, percent woody debris, and percent detritus. All fish captured or observed in all three gear types were used. Species data was log (N+1) transformed, and rare species were downweighted. In resulting plots, species were censored, if not assigned to the gear type model. Censorship of species was done in order to negate habitat association of species based on minority individuals taken with a potentially inefficient gear type.

Results

Wadeable habitats among the five Comal River reaches and four seasons ranged in mean depth (± 1 SE) from 0.51 m (0.03) in Old Channel to 1.0 (0.03) in Lower River and in mean current velocity from 0.02 (0.00) in Upper Spring Run to 0.74 (0.08) in Old Channel (Table 1). Dominant substrate was silt (47 – 53%) in Blieders Creek, New Channel, and Lower River and gravel (42%) in Upper Spring Run and Old Channel. Mean percent vegetative cover ranged from 19% (2.97) in Old Channel to 56% in Blieders Creek. Dominant vegetation was *Chara* (46% ± 10.22) in Blieders Creek, filamentous algae in Upper Spring Run (31% ± 5.21) and Lower River (29% ± 5.92), *Ludwigia* (27% ± 4.31) in Old Channel, and Bryophytes (31% ± 4.61) in New Channel. Water temperature, dissolved oxygen, pH, and specific conductivity were not noticeably different among reaches. Principal component axes I and II explained 18% of the total variation in habitat and water quality parameters among reaches and seasons (Figure 2). Principal component (PC) axis I explained 11% of total variation and represented substrate, current velocity, and depth gradients. Strongest loadings along PC axis I were silt (0.36), depth (0.33), current velocity (-0.33), and sand (-0.33). Principal component axis II explained 7% of total variation and represented a gradient of water quality and substrate. Strongest loadings were temperature (0.45), pH (0.38), and silt (-0.38). Principal component scores differed among reaches along PC I ($F_{4,309} = 84.6$, $P < 0.01$) and PC II ($F_{4,309} = 9.2$, $P < 0.01$) (Figure 2A) and differed among seasons along PC I ($F_{3,310} = 6.0$, $P < 0.01$) and PC II ($F_{3,310} = 65.6$, $P < 0.01$) (Figure 2B). Lower River consisted of greater depths and silt substrates, and Old Channel consisted of shallower depths and swifter current velocity than Blieders Creek, Upper Spring Run, and New

Channel. Winter consisted of shallower depths and swifter current velocities than Spring, Summer, and Fall, and Winter and Summer consisted of cooler water temperatures and greater silt substrates than Spring and Fall.

Non-wadeable habitats among six Comal River reaches and four seasons ranged in mean depth (± 1 SE) from 0.78 m (0.04) in Blieders Creek to 1.88 m (0.09) in Lower River and in mean current velocity from 0.00 m/s (0.00) in Upper Spring Run to 0.03 m/s (0.01) in Lower River (Table 2). Dominant substrate was silt (51 - 90%) in Lower River, Landa Lake, New Channel, and Old Channel and gravel in Upper Spring Run (38%) and Blieders Creek (49%). Mean percent vegetative cover ranged from 38% (9.14) in Blieders Creek and Upper Spring Run to 83% in Old Channel. Dominant vegetation was filamentous algae in Blieders Creek (21% ± 9.64), Upper Spring Run (45% ± 6.62), and Lower River (29% ± 6.90), bryophytes (43% ± 5.01) and *Vallisneria* (40% ± 5.34) in Landa Lake, bryophytes (42% ± 3.71) and *Hygrophila* (42% ± 4.94) in Old Channel, and *Cabomba* (72% ± 4.06) in New Channel. Water temperature, dissolved oxygen, pH, and conductivity were not noticeably different reaches. Principal components axes I and II explained 23% of the total variation in habitat and water quality parameters among reaches and seasons (Figure 4). Principal component axis I explained 13% of total variation and represented a gradient of substrate and vegetation. Strongest loadings along PC axis I were gravel (0.29), cobble (0.26), silt (-0.49), and vegetative cover (-0.43). Principal component axis II explained 10% of total variation and represented gradient of water quality, substrate, and vegetation. Strongest loadings were conductivity (0.44), pH (0.44), gravel (-0.28), and filamentous algae (-0.20). Principal component scores differed among reaches along PC I ($F_{5,238} = 46.6$, $P < 0.001$) and PC II ($F_{5,238} = 32.2$, $P < 0.001$)

(Figure 4A) and differed among seasons along PC I ($F_{3,240} = 6.2$, $P < 0.001$) and PC II ($F_{3,240} = 44.5$, $P < 0.001$) (Figure 4B). Old Channel, Landa Lake, and New Channel consisted of greater silt substrates and vegetative cover, and Upper Spring Run, Lower River, and Blieders Creek consisted of greater gravel and cobble substrates. Lower River and New Channel consisted of greater depths than Blieders Creek, Upper Spring Run, Landa Lake, and Old Channel. Summer consisted of greater silt substrates, vegetative cover, and shallower depths than Winter, Fall, and Spring, and Spring consisted of deeper depths than Summer, Fall, and Winter.

Fish Community

A total of 25 species of fishes (94% native, 6% non-native) and 23,318 individuals were observed among wadeable and non-wadeable habitats within the Comal River (Table 3). Richness (S) ranged from six species (Landa Lake) to 17 (New Channel) among reaches. Overall CPUE was 1.9 fish/m^2 across all gear types and reaches. Spring-associated fish richness was six, comprising 77% of the total CPUE among all reaches and gear types with *Etheostoma fonticola* comprising >36% of total CPUE (0.7 fish/m^2), followed by *Gambusia geiseri* (16%), *Notropis amabilis* (4.9%), *Etheostoma lepidum* (4.5%), *Astyanax mexicanus* (2.3%), and *Dionda nigrotaeniata* (1.3%).

Among Wadeable Habitats

Fish assemblages differed among reaches (ANOSIM Global R = 0.26, $P < 0.01$) but not among seasons (Global R = -0.04, $P = 0.68$). Blieders Creek was dissimilar to Lower River (59%), Old Channel (50%), and New Channel (46%) with *G. geiseri*, *G.*

affinis, *N. amabilis*, and *L. miniatus* contributing to > 60% of the fish assemblage dissimilarity among the reaches. Old Channel was dissimilar to Lower River (38%) with *G. geiseri*, *G. affinis*, *N. amabilis*, *N. volucellus*, and *L. miniatus* contributing to 85% of the dissimilarity between reaches.

Canonical correspondence analysis explained 22% ($P = 0.02$) of the spatial and temporal variation in fish community structure of the Comal River based on physical parameters and site (Figure 3). Physical parameters and sites strongly associated with CC axis I were depth (0.61), Lower River (0.58), temperature (0.51), Old Channel (-0.57), sand (-0.50), and current velocity (-0.42). Physical parameters and sites strongly associated with CC axis II were bedrock (0.45), Lower River (0.44), vegetative cover (-0.53), and *Hygrophila* (-0.40). *Lepomis*, *L. gulosus*, and *L. macrochirus* were more abundant in deeper, warmer water at the lower sites with slower current velocities. *Cyprinella venusta* and *I. punctatus* were more abundant at sites with greater sand and higher current velocities. *Notropis volucellus*, *N. amabilis*, and *A. rupestris* were found at lower sites characterized by greater bedrock, whereas *A. melas* and *G. affinis* were more abundant in dense vegetative cover characterized by *Hygrophila*.

Among Non-wadeable Habitats: Microhabitat Scale

Fish assemblages differed among reaches in microhabitats (ANOSIM Global R = 0.368, $P < 0.01$) but not among seasons (Global R = -0.05, $P = 0.74$). Old Channel and Landa Lake were most similar (91%), whereas Old Channel was the most dissimilar from Lower River (98%), Upper Spring Run (96%), New Channel (63%), and Blieders Creek (44%) with *E. fonticola*, *E. lepidum*, and *Etheostoma* representing >50% of the dissimilarity among reaches. Landa Lake was most dissimilar from Lower River (79%), New Channel (48%), and Blieders Creek (44%) with *E. fonticola* and *Etheostoma* representing > 50% of the fish assemblage dissimilarities.

Canonical correspondence analysis explained 25% (P = <0.01) of the spatial and temporal variation in fish community structure based on physical parameters, site, and season (Figure 5). Physical parameters and sites strongly associated with CC axis I were *Cabomba* (0.50), Lower River (0.48), Landa Lake (-0.58), bryophytes (-0.56), vegetative cover (-0.54), and *Vallisneria* (-0.50). Physical parameters, sites, and season strongly associated with CC axis II were Upper Spring Run (0.34), Fall (0.33), *Ludwigia* (0.32), Lower River (-0.39), and bedrock (-0.30). *Etheostoma fonticola* were not strongly associated with axes I or II but was most abundant at Landa Lake, which is characterized by vegetative cover consisting of bryophytes and *Vallisneria*. *Etheostoma lepidum* was more abundant in upstream reaches with bryophytes.

Among Non-wadeable Habitats: Mesohabitat Scale

Fish assemblages differed among reaches in mesohabitats (ANOSIM Global R = 0.699, P < 0.01) but not among seasons (Global R = -0.06, P = 0.73). Blieders Creek was dissimilar from all reaches (Landa Lake: 77%, Upper Spring Run: 73%, Lower Reach: 69%, New Channel: 69%, Old Channel: 57%) with *D. nigrotaeniata*, *Gambusia*, *L. auritus*, and *L. macrochirus* contributing to >80% of community differences among reaches. Landa Lake was dissimilar from Lower River (100%) and New Channel (100%) with *Gambusia*, *L. auritus*, and *L. macrochirus* representing > 95% of the dissimilarity among reaches. New Channel was dissimilar from Lower River (74%), Upper Spring

Run (100%), and Old Channel (80%) with *Gambusia, A. mexicanus, D. nigrotaeniata,* and *L. auritus* contributing to >80% of the dissimilarity among reaches.

Canonical correspondence analysis explained 72% (P = <0.01) of the spatial and temporal variation in fish community structure based on physical parameters and site (Figure 6). Physical parameters and sites strongly corresponding with CC axis I were *Cabomba* (0.70), New Channel (.60), Lower River (0.47), Landa Lake (-0.52), bryophytes (-0.42), and *Vallisneria* (-0.41). Physical parameters and sites strongly associated with CC axis II were Old Channel (0.64), woody debris (0.61), temperature (0.46), and Landa Lake (-0.42). *L. macrochirus* and *L. megalotis* were found in greater abundances at lower river sites associated with *Cabomba*. *Gambusia* and *O. aureus* were more abundant upstream and associated with bryophytes and *Vallisneria*. *Astyanax mexicanus* was associated with greater temperature at Old Channel.

Discussion

Spring fish richness (S = 6), relative abundance (77%), and CPUE (1.9 fish per m^2) among wadeable and non-wadeable habitats were similar to those among other minimally-altered, Edwards Plateau spring systems (Craig et al. 2016), and therefore do not support that the overall spring fish community had legacy effects from the low flows, the rotenone treatment, and flood effects. Based on these results, I tentatively conclude that the Comal springs complex is a biologically intact system with a high level of biotic integrity, although recognizing that spring fish richness, abundances, and CPUE in the Comal spring complex might be bolstered by the repatriation of Fountain Darters into the complex in the 1970s.

Although historical records are limited for Comal springs complex, available historical information could provide independent complementary or conflicting evidence to support my conclusion that the system is biologically intact with a high level of biotic integrity. Among museum holdings (Hendrickson and Cohen 2015), 31 single specimen records were taken in the late 1800s, 62 single and multiple specimen records were taken from 1933 through 1952 and prior to the 1956 dry period, and 32 single and multiple specimen records were taken from 1962 through 2003 (Appendix A). Combining with collections of this study, 35 species occur within the Comal springs complex. Thirty-four species were reported in the system from 1884 to 1952 and in this study. One species, the introduced Amazon Molly *Poecilia formosa* (Hubbs et al. 2008) taken from Landa Lake, was reported after 1956 and as recently as 2003 but not taken during this study. Among the 27 species recorded from 1884 through 1952 (i.e., historical) and 25 species reported in this study, 18 species were common to both time intervals, nine species were unique to historical, and seven species were unique to this study. Among the nine species unique to historical, all species except one currently exist in the main stem Guadalupe River (Perkin and Bonner 2013) and are likely transient within the Comal springs complex (Kollaus et al. 2015). The exception was the Brown Bullhead *Ameiurus nebulosus*. Brown Bullhead occurs in Texas only within the Red River drainage (Craig et al. 2016) and likely a misidentification of the Black Bullhead *Ameiurus melas*, which was taken during this study (i.e., 33 species reported, 19 species common, eight species unique historically, six species unique to this study, if accurate). Among the six species unique to this study, five species are non-native, one species is native, and all six are found in the Guadalupe River. Without any apparent extirpations (except reported for the Fountain Darter), contemporary fish community reported in this study is similar to historical collections and therefore complements my overall conclusion that the system is biologically intact with a high level of biotic integrity although introduced species might pose future threats to the system (Pound et al. 2011).

The reported extirpation of the Fountain Darter from the Comal springs complex is often cited as evidence of systems dewatering and the effects on endangered fishes (Schenck and Whiteside 1976, USFWS 1996, Hoagstrom et al. 2011, Dammeyer et al. 2013, Mora et al. 2013) although few (USFWS 1996, Dammeyer et al. 2013) mention other possible causes of Fountain Darter extirpations (i.e., rotenone treatment, flood) as reported by Schenck and Whiteside (1976). However, the extirpation event is enigmatic. Genetic comparisons between the Comal springs complex Fountain Darter population and the San Marcos River population indicate private alleles within the Comal springs complex, which might suggest that Fountain Darters were not extirpated when Fountain

Darters from San Marcos River were introduced 1974 – 1976 (Olsen et al. 2016). Olsen et al. (2016) offer an alternative perspective on why they believe the Fountain Darter was extirpated from the system before introductions from the San Marcos River. However, other spring associated fishes presumably survived the 1956 dry period, including the Guadalupe Roundnose Minnow *Dionda nigrotaeniata* and Greenthroat Darter *Etheostoma lepidum*. Guadalupe Roundnose Minnow is not reported in the lower Guadalupe River (Perkin and Bonner 2013), so their recolonization into the Comal spring complex from the Guadalupe River is unlikely. Greenthroat Darters are found in the lower Guadalupe River and could have recolonized the Comal spring complex, but Greenthroat Darters would need to navigate past several instream dams, which is unlikely, to recolonize Upper Spring Run. Alternatively, Guadalupe Roundnose Minnow and Greenthroat Darters persisted in the Comal spring complex during the 1956 dry period. If valid, then why was the Fountain Darter extirpated and the other two persisted, given that Greenthroat Darter is more of a riffle specialist (Hubbs and Echelle 1972) and Fountain Darter's thermal tolerances (Brandt et al. 1993, Bonner et al. 1998) are likely similar to that of the other two species and many other fishes within the Comal springs complex? Therefore, what were the mechanisms leading to extirpation of Fountain Darter (i.e., rotenone treatment, wide temperature fluctuations, decrease in habitat, decrease in water quality, increased predation; USFWS 1996) that was not experienced or resisted by other spring fishes? Likely, information does not exist to confidently conclude either if Fountain Darters were extirpated or not during the 1956 dry period.

Species and abundances of the spring-associated fish assemblage within the Comal spring complex were similar to other Edwards Plateau spring fish communities.

Within the Comal spring complex, greatest percent relative abundance by density was 53% for *Etheostoma* (*E. fonticola* and *E. lepidum*), followed by *Gambusia* (*G. geiseri* and *G. affinis*) (31%) and Texas Shiner *Notropis amabilis* (5%). In the nearby San Marcos River, Gambusia (*G. geiseri* and *G. affinis*; 51%), Texas Shiner (22%), and Fountain Darter (6%) are the most abundant fishes, estimated using the same techniques as this study (Behen 2013). Species with the greatest relative abundances assessed over 100 years of fish collections within the San Marcos River using seines and dip nets in wadeable waters are *Gambusia* (30%), Texas Shiner (8%), and Fountain Darter (7%). In the Edwards Plateau drainages of the Rio Grande and within the epicenter of *Dionda* radiation (Conner and Suttkus 1986), species with the greatest relative abundances are *Dionda* (*D. argentosa* and *D. diaboli*; 35%), Texas shiner (17%), and *Gambusia* (*G. speciosa*, *G. geiseri*, and *G. affinis*; 13%) in the Devils River (Kollaus and Bonner 2012) and *Dionda argentosa* (30%), *Gambusia* (*G. geiseri* and *G. affinis*; 29%), and Texas Shiner (19%) in Independence Creek, a spring complex tributary to the lower Pecos River (Bonner et al. 2005). The Rio Grande Darter, a swift-water specialist, is the only darter within the Edwards Plateau of the Rio Grande drainage and has a relative abundance of about 1% in Devils River and Independence Creek. Consequently, spring fish communities within spring complexes of the Edwards Plateau region include a mix of ubiquitously-distributed fishes (e.g., *N. amabilis*) and narrowly-distributed fishes (e.g., *E. fonticola*), which is consistent with evolutionary refugia serving as habitat for long-term divergent lineages (ubiquitously-distributed fishes within the Edwards Plateau) and short-term relicts (possibly narrowly-distributed fishes) (Byrne et al. 2008; Davis 2013).

Similar to fish community structure, fish-habitat associations among wadeable and non-wadeable habitats within Comal springs complex were typical among other spring systems (Kollaus and Bonner 2012, Behen 2013). Flowing water specialists Texas Shiner and Blacktail Shiner *Cyprinella venusta* were associated with swifter current velocities and shallower water depths and slackwater specialists Black Bullhead *Ameiurus melas* and *Lepomis* were associated with slower current velocities and deeper water depths in wadeable habitats. Fish associations among non-wadeable mesohabitats, *Gambusia* and *D. nigrotaeniata* were associated with greater amounts of vegetation in Landa Lake and Old Channel reaches, whereas *Lepomis* and Largemouth Bass *Micropterus salmoides* were associated with less vegetation and greater depths in the New Channel and Lower River reaches. Fish associations among non-wadeable microhabitats, Greenthroat Darter was associated with habitats within the Upper Spring Run and New Channel, whereas Fountain Darter PCA I and II scores were near zero, indicating a ubiquitous distribution among all reaches and habitats. Fountain Darters were often observed in dense vegetation of Old Channel (19% of the total number of Fountain Darters observed but not including those identified as *Etheostoma*) and Landa Lake (10%) but also in no to sparse vegetation among all reaches, especially in the sparsely vegetated Upper Spring Run reach (5%). Ubiquitous distribution within microhabitats of pool or run mesohabitats along with a few taken in wadeable swift water runs and riffles is similar to Fountain Darter habitats in the San Marcos River (Behen 2013) and inconsistent with Fountain Darters reported association in vegetation only (Schenck and Whiteside 1976) or a strong preference for aquatic vegetation (Edwards Aquifer Habitat Conservation Plan 2012).

Despite overall high biotic integrity, the Comal River fish community was not homogenous and spring fish relative abundances differed among reaches based on analyses of similarities. New Channel and Lower River reaches are the more popular areas for recreational activities with most of the activities occurring during warmer months (e.g., Spring through late Fall). Among parameters quantified in this study, water quality and habitat descriptions were similar among reaches, although Lower River had greater depths and more silt and bedrock substrates than other reaches of the Comal River. Fewer spring-associated fishes and lower spring-associated fish relative abundances observed in the New Channel and Lower River could be linked to the numbers and densities of spring fishes decreasing longitudinally in most spring systems (Craig et al. 2016). Spring-associated fish richness, abundances, and densities decrease along a longitudinal gradient from spring out flows to downstream of spring complex confluence with a larger river. Mechanisms explaining the longitudinal decrease is not understood at this time, but water temperature explains part of the spatial pattern with spring-associated fishes becoming more abundant downstream from spring outflows during the Fall and Spring months when river water temperatures are near the temperature of spring outflows (Kollaus and Bonner 2012).

Alternatively, fewer spring-associated fishes and lower spring-associated fish relative abundances observed in the New Channel and Lower River could indicate recreation-mediated effects. Non-consumptive recreation alters physical and biological environments of aquatic and terrestrial systems, depending on amount of use and type of use (Boyle and Samson 1985, Monz et al. 2010). Among freshwater systems, concerns are related primarily to vegetation cutting and shoreline erosion by motorboats (see review by Liddle and Scorgie 1980), but concerns of other water-related recreational activities (e.g., swimmers, SCUBA divers, wading, water play) are less documented. Decreases in water quality, in particular total coliform bacteria and phosphate, is associated with recreational use areas in a US National Forest in Minnesota, but activities associated with long-term camping near the water (e.g., pit toilet, clothes washing) were the likely sources of contamination (King and Mace 1974). Others report increases in turbidity and resuspension of nutrients when benthic sediments are disturbed by water users (Monz et al. 2010), and greater levels of turbidity negatively affect feeding behavior of the Fountain Darter (Swanbrow Becker et al. 2016), although the direct effect to aquatic flora and fauna populations are unknown except in oligotrophic freshwater systems. Within an oligotrophic Australian lake, increases in periphyton chlorophyll *a* were associated with nutrient additions of water users (Hadwen and Bunn 2005), but the exact sources of nutrient additions (e.g., resuspension of sediments, natural variation) are unknown (Hadwin et al. 2003). Direct effects on biota are reported for several terrestrial species, such as harassment and incidental encounters (Boyle and Samson 1985), and reported for nest-building fishes, specifically trampling of nests by waders (Roberts and White 1992). However, mechanisms that link water recreation to habitats and fish communities within the New Channel and Lower River are not known at this time, especially for the Fountain Darter which is abundant in the New Channel.

Ground water and spring complexes of the Edwards Plateau, like the Comal springs complex, provide unique and more permanent aquatic resources for numerous flora and fauna in addition to fish (Bowles and Arsuffi 1993). As the aquifer continues to erode the cretaceous strata layers, former wetted portions of the eroded limestone provides unique habitat even for terrestrial organisms (Reddell 1994, White et al. 2014). The uniqueness and permanency contributes to not only flora and fauna habitat today but also to past flora and fauna, enabling species radiation in the area, and into the future (Davis 2013), hence the designation of the Edwards Plateau regions as aquatic evolutionary refugium (Craig et al. 2016). However, the uniqueness and permanency of aquatic evolutionary refugium also are beneficial to society and provides aquatic resources for human populations as early as 12,000 years ago, indigenous people up to the 1800s, and European descendants from the 1700s until now (Kollaus et al. 2015). Conflicts between humans and spring flora and fauna have existed since the 1800s in some of the spring complexes (e.g., San Antonio spring complexes; Craig et al., In Review) and were increased with improvements in groundwater pumping by the early 1900s. Currently, natural threats (e.g., natural climate change during Holocene interglacial period) and anthropogenic threats (e.g., anthropogenic induced climate change, groundwater pumping, conflicts between water recreationists and biota) are possible, and often stated, stressors to the spring complexes (Bowles and Arsuffi 1994, USFWS 1996, Edwards Aquifer Habitat Conservation Plan 2012). At least for two of the spring complexes (i.e., Comal and San Marcos spring complexes), a habitat conservation plan was implemented, which identifies and mitigates perceived and realized threats to the groundwater resources and spring complexes (Edwards Aquifer Habitat Conservation Plan 2012). Into the future, species richness, abundances, and CPUE quantified in this study can be used as a baseline to monitor threats to the Comal spring complex, and the management strategies developed in the habitat conservation plan can be used in other Edwards Plateau spring complexes.

TABLE 1.— Mean (± SE) habitat and water quality parameters, Substrate (%), Vegetation (%) per reach for seine technique in the Comal River from Fall 2014 – Summer 2015.

	Blieders Creek	Upper Spring Run	Old Channel	New Channel	Lower River
Habitat Parameters					
CV 60% (m/s)	0.05 (0.01)	0.02 (0.00)	0.74 (0.08)	0.24 (0.05)	0.17 (0.02)
Depth (m)	0.77 (0.05)	0.75 (0.03)	0.51 (0.03)	0.74 (0.03)	1.00 (0.03)
Substrate (%)					
Silt	52.9	29.2	15.4	47.0	50.4
Sand	0.0	1.6	24.9	4.9	4.0
Gravel	33.4	41.9	41.5	28.3	12.5
Cobble	8.4	19.4	14.5	7.4	0.5
Bedrock	0.0	0.0	0.1	6.5	30.6
Detritus	0.0	5.4	1.2	1.3	1.4
Boulder	0.0	6.7	1.5	3.8	0.3
Vegetative Cover (%)	56.3	27.7	19.7	54.3	27.4
Bryophytes	0.0	0.3	13.0	31.1	8.9
Hygrophila	23.2	4.9	12.1	14.0	5.8
Ludwigia	0.0	0.3	27.8	11.9	3.7
Cabomba	13.7	3.2	1.5	7.6	19.3
Sagittaria	0.0	4.7	0.0	0.0	0.0
Valisneria	0.0	6.5	0.0	9.3	0.0
Potamogeton	0.0	0.0	0.0	2.7	0.0
Filamentous algae	5.3	31.4	0.1	3.4	28.9
Chara	46.8	9.6	0.0	0.0	0.0
Water Quality					
Temp (°C)	22.3 (0.32)	23.2 (0.10)	21.8 (0.16)	22.6 (0.09)	23.0 (0.16)
DO (mg/L)	6.7 (0.33)	9.2 (0.44)	7.8 (0.08)	8.7 (0.08)	10.2 (0.13)
pH	7.1 (0.09)	7.0 (0.05)	7.2 (0.06)	7.2 (0.06)	7.4 (0.07)
Sp cond (µS/cm)	560.0 (1.12)	562.6 (0.51)	572.1 (0.51)	565.6 (1.14)	564.6 (0.61)

TABLE 2.—Mean (± SE) habitat and water quality parameters, Substrate (%), Vegetation (%) per reach for microhabitat technique in the Comal River from Fall 2014 – Summer 2015.

	Blieders Creek	Upper Spring Run	Landa Lake	Old Channel	New Channel	Lower River
Habitat Parameters						
CV Bottom (m/s)	0.00	0.00	0.01 (0.00)	0.02 (0.00)	0.01 (0.00)	0.03 (0.01)
Depth (m)	0.78 (0.04)	1.37 (0.07)	1.26 (0.05)	1.59 (0.08)	1.62 (0.06)	1.88 (0.09)
Substrate (%)						
Silt	43.8	35.9	82.5	90.8	88.7	51.5
Sand	0.0	0.0	2.0	0.5	0.6	2.1
Gravel	49.1	38.0	14.0	4.7	1.5	12.1
Cobble	6.6	11.7	2.5	0.5	0.0	2.6
Bedrock	0.0	9.8	0.0	0.0	10.1	27.1
Detritus	1.9	6.7	4.3	12.0	0.0	1.1
Boulder	0.6	4.0	0.6	0.2	0.4	6.7
Vegetative Cover (%)	38.8	38.9	82.6	83.2	77.0	40.3
Bryophytes	0.0	6.7	43.3	42.9	18.1	3.1
Hygrophila	15.0	0.0	0.0	42.9	6.9	7.6
Ludwigia	4.4	0.0	0.0	0.0	0.0	0.0
Cabomba	19.4	6.0	0.0	1.0	72.7	25.7
Sagittaria	0.0	2.1	0.0	9.2	0.0	0.7
Valisneria	0.0	0.0	40.8	3.8	0.0	0.0
Filamentous algae	21.3	45.8	13.8	0.0	2.3	29.3
Chara	14.4	14.4	0.0	0.0	0.0	0.0
Myriophyllum	6.3	2.1	0.0	0.0	0.0	0.0
Water Quality						
Temp (°C)	23.0 (0.16)	23.2 (0.07)	22.8 (0.12)	22.8 (0.17)	22.8 (0.08)	22.7 (0.11)
DO (mg/L)	7.3 (0.67)	7.2 (0.29)	6.9 (0.26)	9.2 (0.13)	9.6 (0.17)	8.9 (0.09)
pH	6.9 (0.11)	6.9 (0.08)	6.9 (0.10)	6.9 (0.09)	7.1 (0.07)	7.7 (0.03)
Sp cond (µS/cm)	557.8 (0.45)	560.1 (0.63)	561.4 (2.04)	562.8 (1.29)	565.0 (1.31)	567.1 (0.41)

TABLE 3.—Total number of observations (N), Most appropriate gear type per species, Total number of observations per gear type, CPUE, and Relative abundance (%) per species per reach in the Comal River from Fall 2014 – Summer 2015.

Species	Total N	Gear type	N for gear type	CPUE	% Total CPUE	Blieders	USR	Landa	OC	NC	Lower
Cyprinella venusta	3	Seine	3	0.001	0.03						
*Dionda nigrotaeniata**	621	Meso	545	0.025	1.27	24	1	3	6		
*Notropis amabilis**	911	Seine	458	0.097	4.91		1		3	5	30
Notropis volucellus	271	Seine	173	0.037	1.85				1		18
*Astyanax mexicanus**[i]	954	Meso	871	0.045	2.25		3	2	16	6	
Ameiurus melas	7	Seine	7	0.001	0.07					1	
Ictalurus punctatus	10	Seine	10	0.002	0.11				0.2	0.3	
Loricariidae[i]	17	Meso	12	0.001	0.04				0.2	0.2	0.4
Gambusia affinis	276	Seine	276	0.059	2.96	16	1		3	6	
*Gambusia geiseri**	307	Seine	307	0.065	3.29	20			4	9	
Gambusia	14,864	Meso	12,886	0.484	24.44		82	80	36		
Poecilia latipinna[i]	42	Seine	27	0.006	0.29					3	
Ambloplites rupestris[i]	7	Seine	4	0.001	0.04						0.5
Lepomis auritus[i]	512	Meso	325	0.015	0.77		1		1	8	16
Lepomis cyanellus	6	Micro	4	0.002	0.08				0.4		
Lepomis gulosus	14	Seine	12	0.003	0.13					1	1
Lepomis macrochirus	215	Meso	121	0.006	0.28	2				9	2
Lepomis megalotis	63	Meso	41	0.002	0.10					1	
Lepomis microlophus	2	Meso	1	<0.001	<0.01				0.1		
Lepomis miniatus	159	Seine	142	0.030	1.52	12				5	3
Lepomis	592	Meso	424	0.021	1.05	5	2		1	12	11
Micropterus dolomieu[i]	1	Seine	1	0.000	0.01						0.1
Micropterus salmoides	292	Meso	185	0.009	0.45	4	1		1	6	2
Micropterus	4	Meso	4	<0.001	<0.01			<0.1			
*Etheostoma fonticola**	2,045	Micro	1,735	0.711	35.88	4	5	10	19	18	8
*Etheostoma lepidum**	280	Micro	219	0.090	4.53	6	1	1	2	1	3
*Etheostoma **	615	Micro	611	0.250	12.63	5	2	3	5	7	5
Herichthys cyanoguttatum[i]	208	Meso	125	0.006	0.31	2	1		1	1	
Oreochromis aureus[i]	20	Meso	8	0.000	0.02		<0.1	<0.1		0.1	
				1.968							
N	23,318										
S	25					11	12	6	14	17	11

* = *Spring-associated*, [i] = *Non-native*

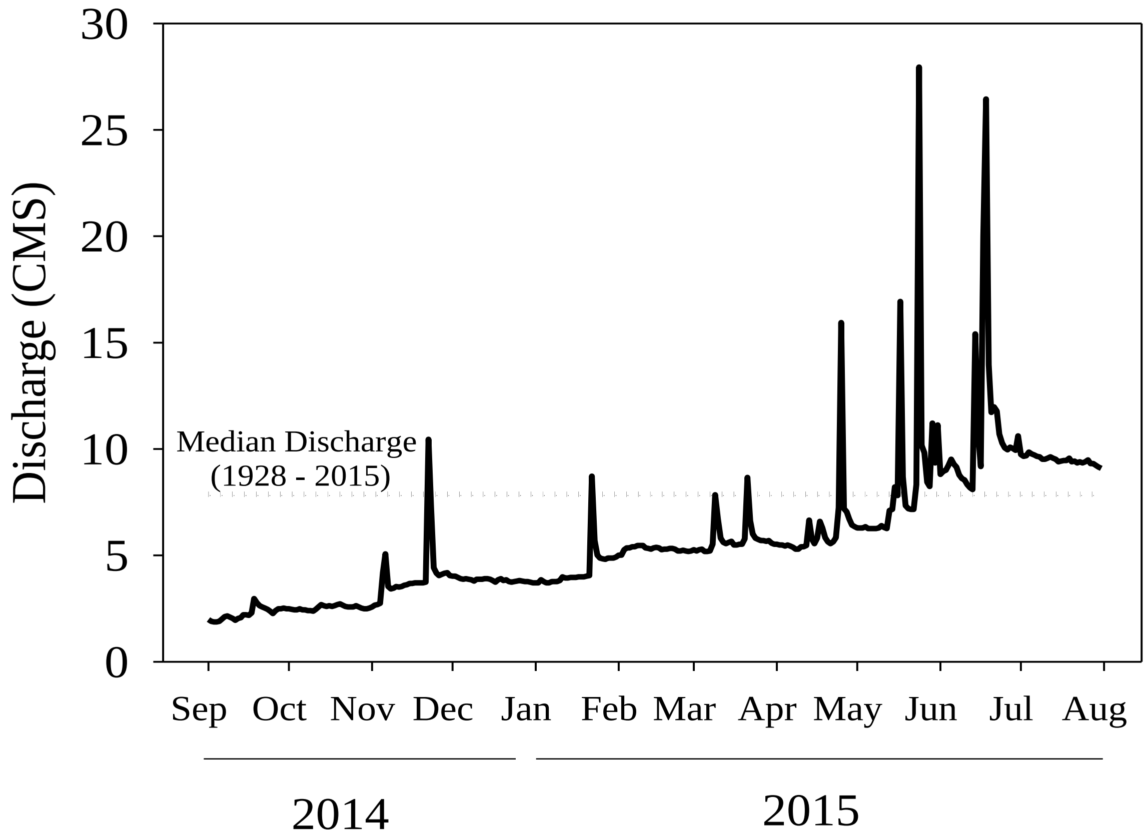

FIGURE 1.—Flow (m^3/s) of Comal River throughout the study period (Fall 2014 – Summer 2015) with depicted historical median discharge of 8.0 m^3/s (USGS Station 08169000).

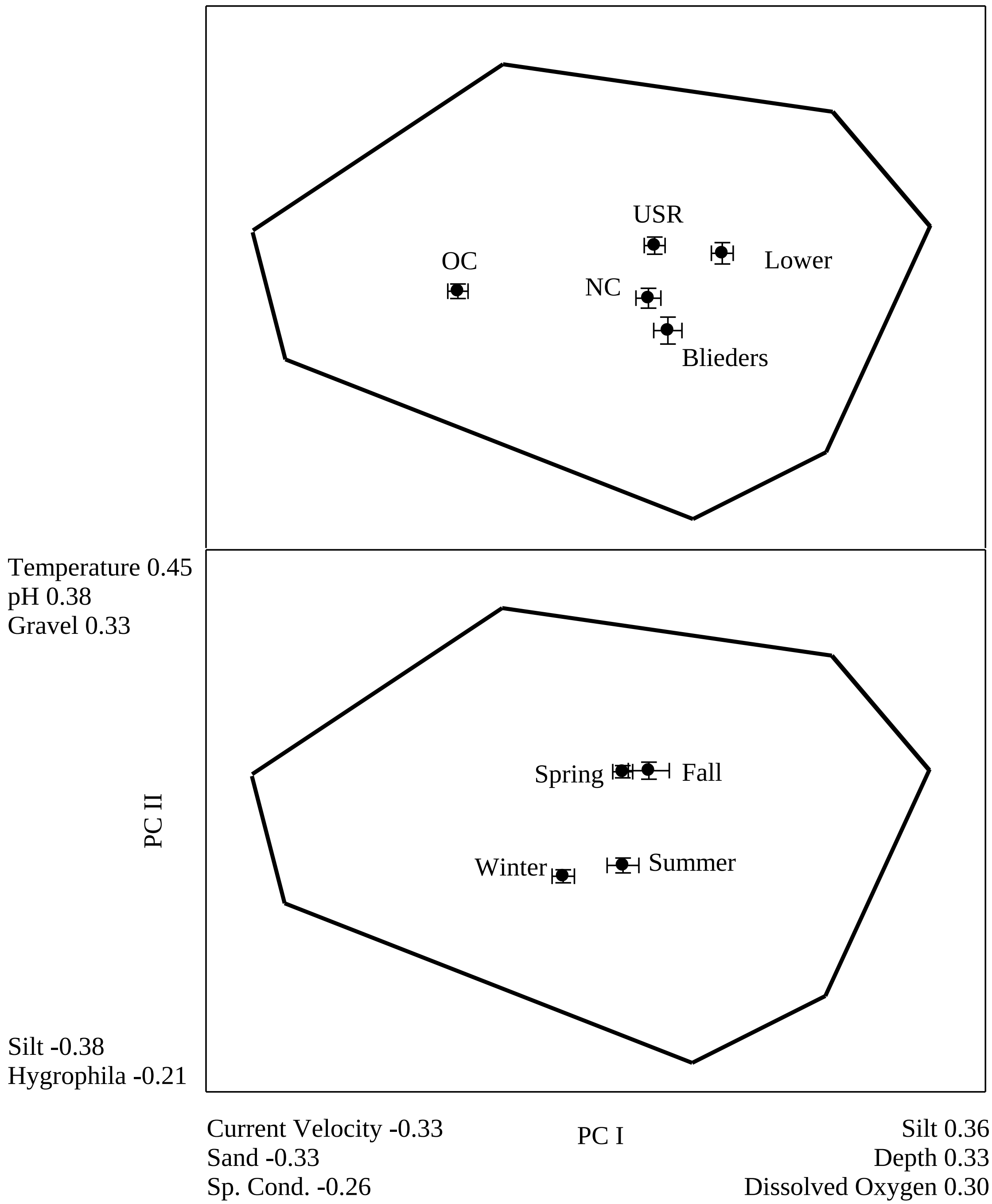

FIGURE 2.—Principal Components Analysis plot of mean ± SE and range of values (represented by outer shape) for habitat parameters within each reach for seine technique in the Comal River from Fall 2014 – Summer 2015. Reach name abbreviations are USR-Upper Spring Run, NC-New Channel, and OC-Old Channel.

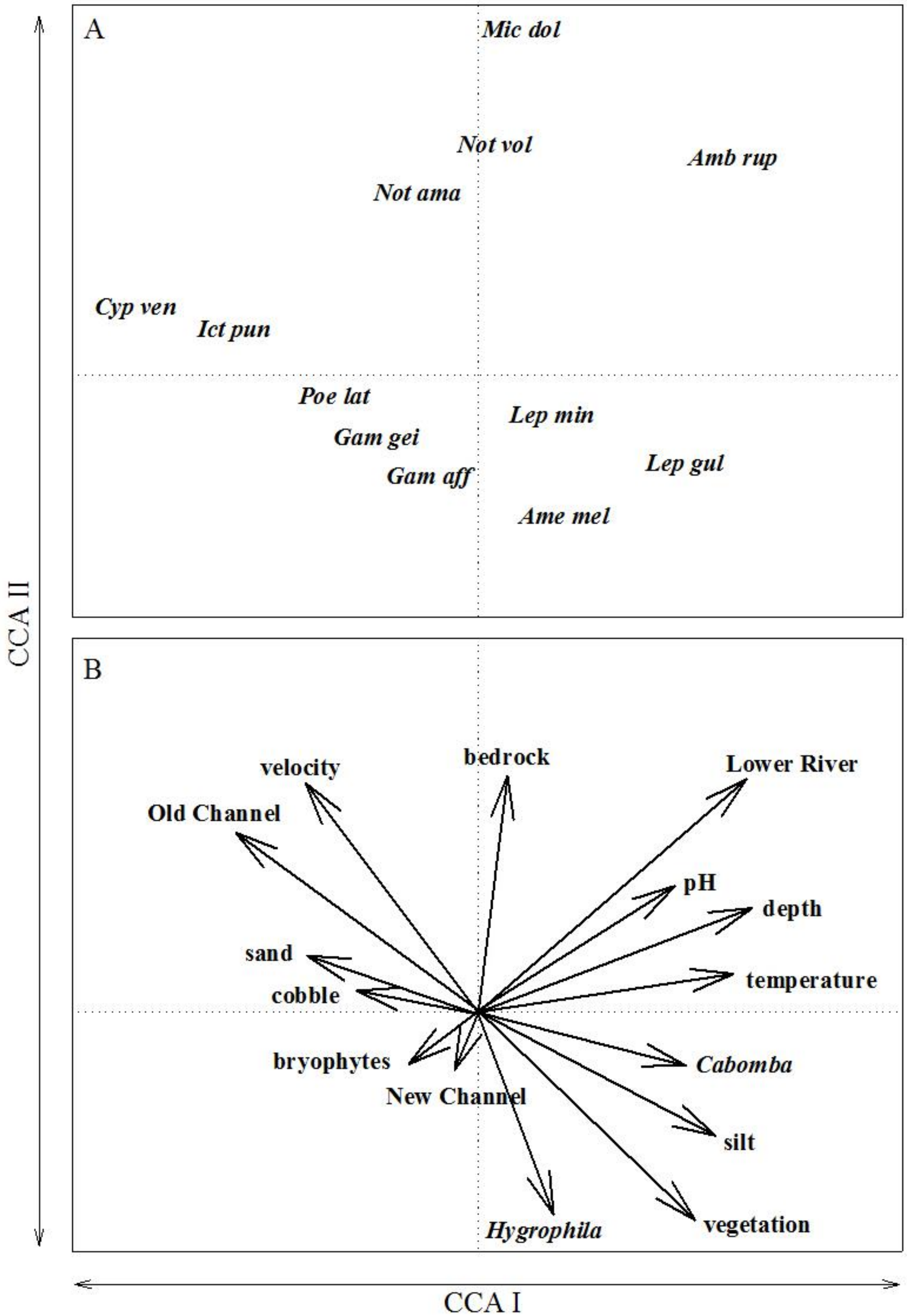

FIGURE 3.—Canonical Correspondence Analysis plot of fishes (A) and associated habitat parameters and reaches (B) for seine technique in the Comal River from Fall 2014 – Summer 2015. Species names are represented by the first three letters of genus and species epithets (See Table 3 for full species names).

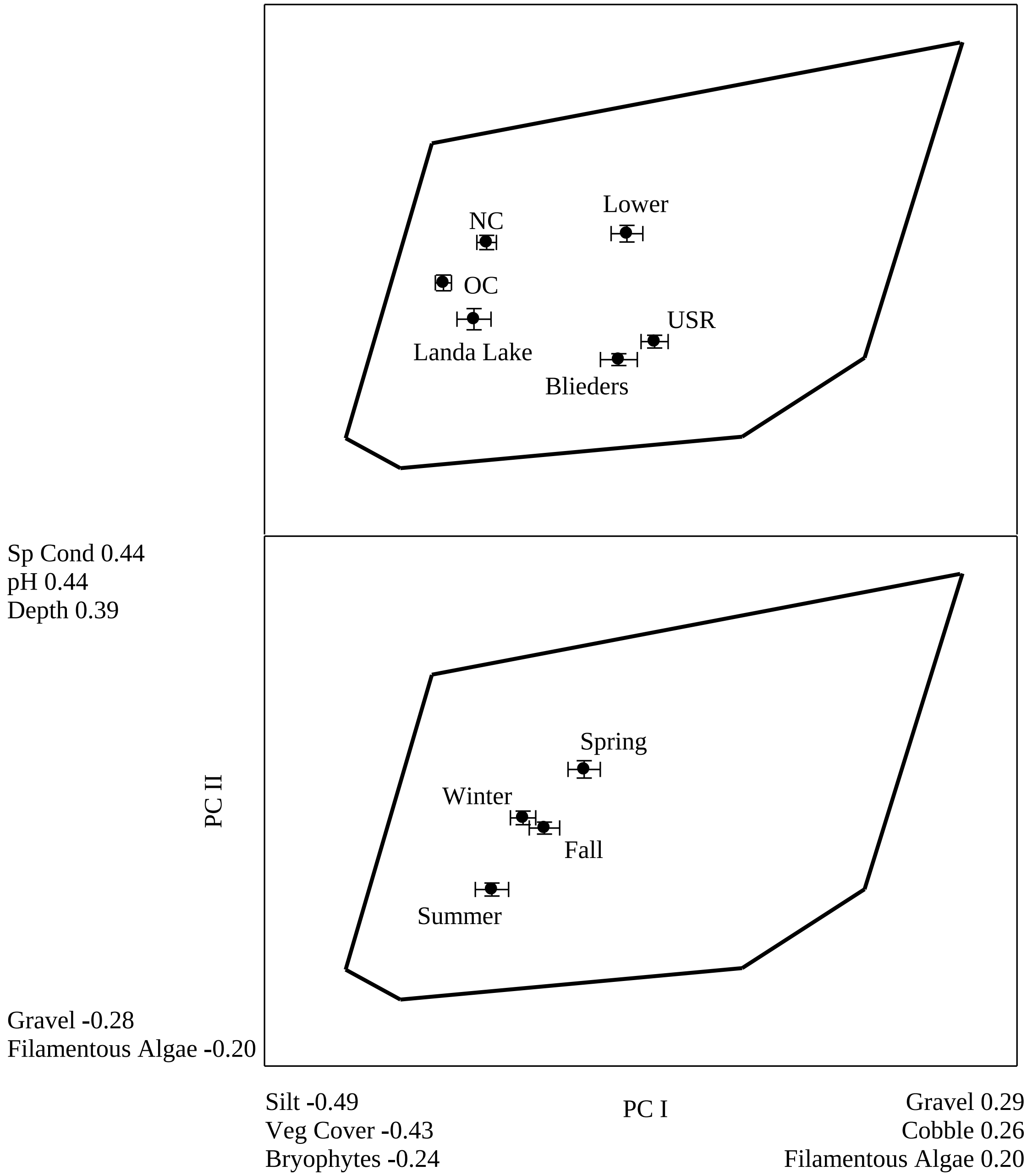

FIGURE 4.—Principal Components Analysis plot of mean ± SE and range of values (represented by outer shape) for habitat parameters within each reach for microhabitat technique in the Comal River from Fall 2014 – Summer 2015. Reach name abbreviations are USR-Upper Spring Run, NC-New Channel, and OC-Old Channel.

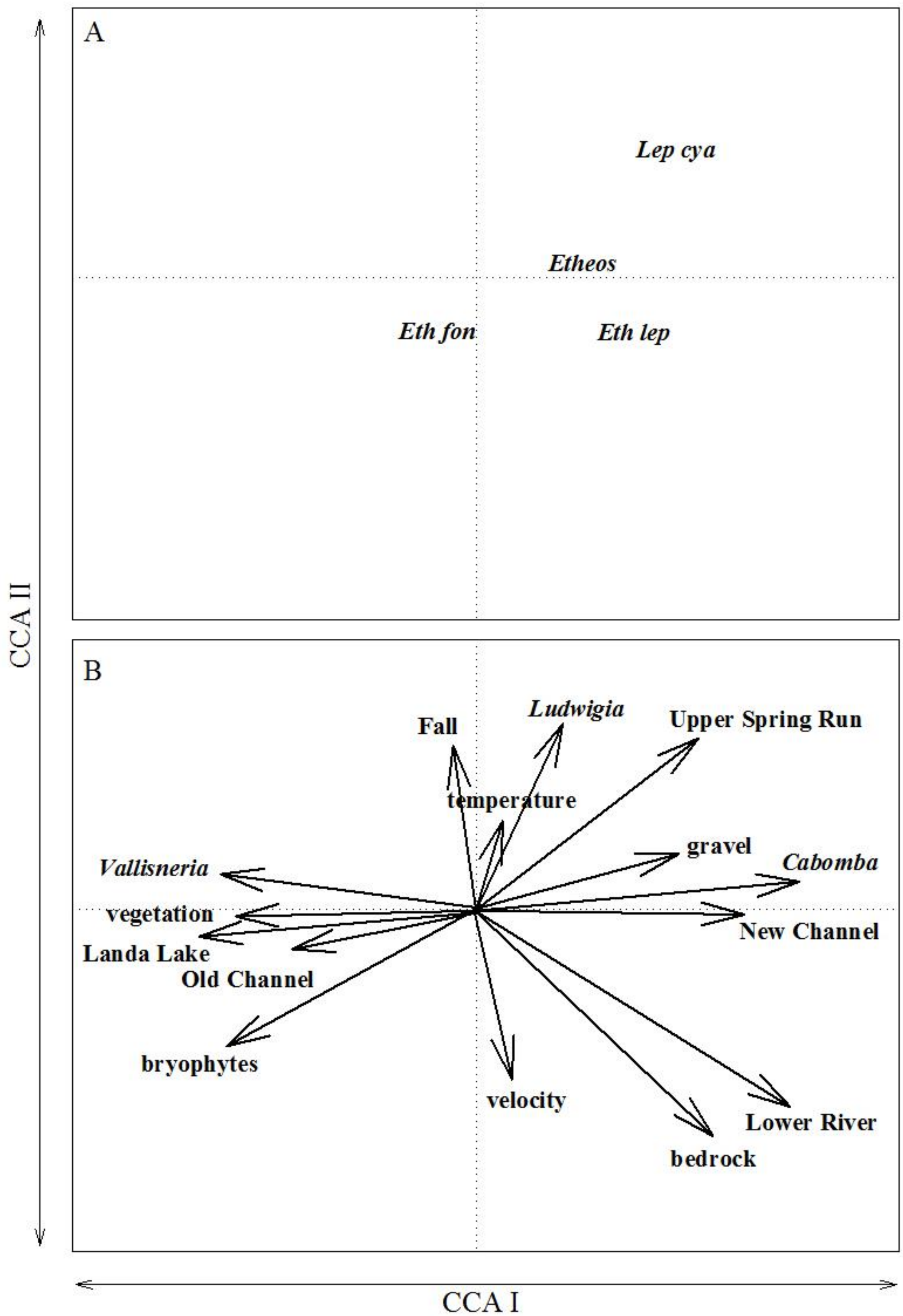

FIGURE 5.—Canonical Correspondence Analysis plot of fishes (A) and associated habitat parameters and reaches (B) for microhabitat technique in the Comal River from Fall 2014 – Summer 2015. Species names are represented by the first three letters of genus and species epithets (See Table 3 for full species names).

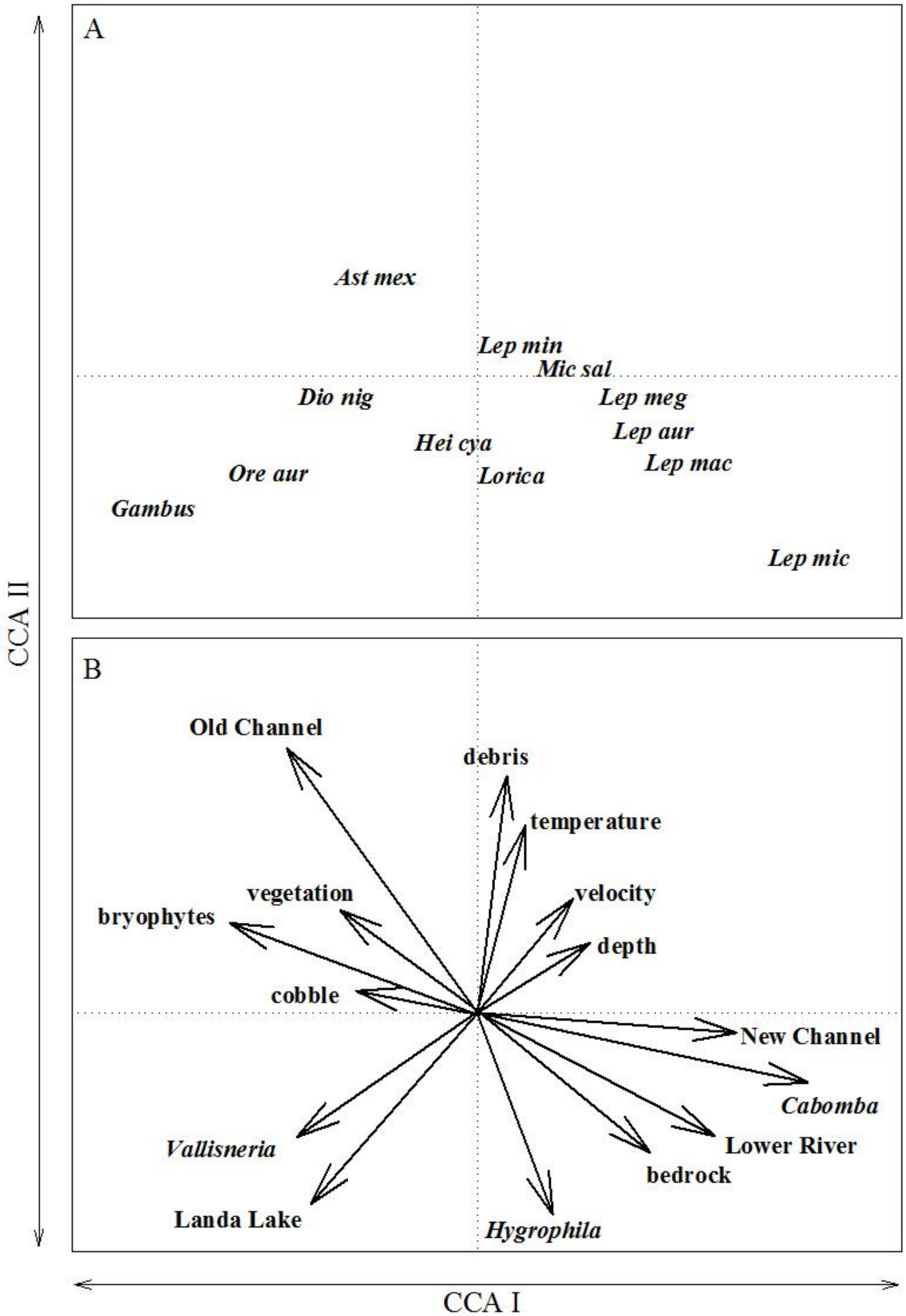

FIGURE 6.—Canonical Correspondence Analysis plot of fishes (A) and associated habitat parameters and reaches (B) for mesohabitat technique in the Comal River from Fall 2014 – Summer 2015. Species names are represented by the first three letters of genus and species epithets (See Table 3 for full species names).

APPENDIX SECTION

APPENDIX A

APPENDIX A. Occurrences of native and introduced fishes within Comal River, Comal County Texas, from 1884 through 2015. The three time periods represent historical collections taken before rotenone application and drought of record (1884 – 1952), after rotenone application and drought of record (1962 – 2003), and observations made during this study (2014 – 2015). Occurrence is denoted with an "X".

	1884 - 1952	1962-2003	2014 - 2015	Notes
Cyprinella lutrensis	X			Occurs historically and currently in Guadalupe River; Perkin and Bonner 2013
Cyprinella venusta			X	Occurs historically and currently in Guadalupe River; Perkin and Bonner 2013
Dionda nigrotaeniata *	X	X	X	
Macrhybopsis marconis	X			Occurs historically and currently in Guadalupe River; Perkin and Bonner 2013
Notropis amabilis *	X		X	
Notropis volucellus	X		X	
Pimephales vigilax	X			Occurs historically and currently in Guadalupe River; Perkin and Bonner 2013
Moxostoma congestum	X			Occurs historically and currently in Guadalupe River; Perkin and Bonner 2013
Astyanax mexicanus *[i]	X	X	X	First reported in 1955 within the Comal River; Hendrickson and Cohen 2015
Ameiurus melas	X		X	
Ameiurus natalis	X			Occurs historically and currently in Guadalupe River; Perkin and Bonner 2013
Ameiurus nebulosus	-			Unconfirmed and suspect record from 1891; Hendrickson and Cohen 2015, Craig et al. 2016
Ictalurus punctatus	X		X	
Noturus gyrinus	X			Occurs historically and currently in Guadalupe River; Perkin and Bonner 2013
Loricariidae [i]			X	First reported in 1990 within the Comal River; Whiteside and Berkhouse 1992

APPENDIX A.—Continued.

	1884 - 1952	1962-2003	2014 - 2015	Notes
Gambusia affinis	X	X	X	
Gambusia geiseri *	X	X	X	
Poecilia latipinna	X	X	X	
Poecilia formosa [i]		X		First reported in 1980 within the Comal River; Hendrickson and Cohen 2015
Ambloplites rupestris [i]			X	First reported in 1961 within the Guadalupe River; Perkin and Bonner 2013
Lepomis auritus [i]			X	First reported in 1939 within the Guadalupe River; Perkin and Bonner 2013
Lepomis cyanellus	X		X	
Lepomis gulosus	X		X	
Lepomis macrochirus	X	X	X	
Lepomis megalotis	X		X	
Lepomis microlophus	X		X	
Lepomis miniatus	X	X	X	
Micropterus dolomieu [i]			X	First reported in 1994 within the Guadalupe River; Perkin and Bonner 2013
Micropterus salmoides	X		X	
Etheostoma fonticola *	X	X	X	
Etheostoma lepidum *	X	X	X	
Etheostoma spectabile	X			Occurs historically and currently in Guadalupe River; Perkin and Bonner 2013

APPENDIX A.—Continued.

	1884 - 1952	1962-2003	2014 - 2015	Notes
Percina apristis	X			Occurs historically and currently in Guadalupe River; Perkin and Bonner 2013
Herichthys cyanoguttatus [i]	X	X	X	First reported in 1942 within the Comal River; Hendrickson and Cohen 2015
Oreochromis aureus [i]			X	First report within the Comal River; reported in 1977 within the Guadalupe and San Antonio River drainage; Hendrickson and Cohen 2015
Species Richness	27	11	25	

* Spring-associated fishes

[i] Introduced species

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