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Relationships among spring flow, habitats, and fishes within evolutionary refugia of the Edwards Plateau

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Abstract. Aquifers and spring complexes within the arid and semi-arid karst Edwards Plateau region of Texas are evolutionary refugia supporting a large number of endemic flora and fauna. Spring fishes, many of which are endemic, are associated with the spring complexes, whereas river fishes generally avoid spring complexes. Purposes of this study were to experimentally test affinities of spring fishes and river fishes for spring complexes and rivers among six independent spring-river systems of the Edwards Plateau and to quantitate relationship among spring fishes, river fishes and spring flow magnitude within spring complexes. We found that spring fishes did not exclusively use spring complexes but were more abundant in spring complexes than rivers and that species richness, relative abundance, and density of spring fishes were directly related to spring flow magnitude. Patterns in affinities for river fishes were less distinct. Quantitation of fish–habitat relationships will assist efforts in conserving aquatic resources within the Edwards Plateau and provides predictive models to assess biological integrity of other spring complexes. In addition, consistency in distributional patterns of spring and river fishes provides a framework for testing underlying processes of species segregation and diversification, establishes boundaries of aquatic evolutionary refugia, and expands the value of evolutionary refugia from historical refugia to contemporary and future refugia for Edwards Plateau aquatic biota.

Key words: evolutionary refugia; fish endemism; Pleistocene glacial events; spring complexes.

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INTRODUCTION

Origins of endemic freshwater fishes and other aquatic biota within the semi-arid to arid regions of western gulf slope drainages of United States and Mexico are attributed to pre-Pleistocene vicariance and Pleistocene glacial dispersion and interglacial isolation events (Echelle and Echelle 1978, Kelsch and Hendricks 1990, Horne and Kahn 1997, Al-Rabab'ah and Williams 2004). Ancestral lineages from northern and eastern basins (e.g., Mississippi River basin) disperse south and west into western gulf slope drainages during cooler and wetter climates of glacial maxima because of interconnectivity of drainages (Conner and Suttkus 1986). Southwest climates transition toward more arid climates during interglacial periods (Conner and Suttkus 1986, Al-Rabab'ah and Williams 2004), fragmenting and isolating aquatic habitats (Van Devender 1976). Extirpations and extinctions of freshwater fishes are more common during the arid interglacial periods (Smith and Miller 1986), but those surviving in aquatic refugia are more likely to diverge and form endemic fauna (Near and Benard 2004, Reyjol et al. 2006). Subsequent glacial events allow additional dispersion of phylogenetically related fishes, often producing two distinct and abutted distributions of fishes (Baumsteiger et al. 2014): a community of fishes persisting within and near aquatic refugia and another community in downstream river systems (Conner and Suttkus 1986).

Aquatic refugia, such as artesian springs of the karst Edwards Plateau region of Texas, are evolutionary refugia with sufficient permanency to enable biota survival and diversification over millennia of climatic change (Tzedakis et al. 2002, Tribsch and Schönswetter 2003, Keppel et al. 2012). Characteristics of aquatic evolutionary refugia include longevity, water sources largely decoupled from local precipitation, and a large number of endemic and relic fauna (Davis et al. 2013). The Edwards Plateau is a large (3200 km^2; Longley 1981) karst-terrain ecoregion of central Texas and with its voluminous subterranean aquifers and numerous surface water discharges (e.g., artesian springs) provide perennial aquatic resources. Karstification of the Edwards Plateau began at least 6 MYA within faulted and downdipping Cretaceous limestone along the margin of the Gulf of Mexico sedimentary basin (White et al. 2009). As aquifers evolve downward within Cretaceous-age limestone (Deike 1990, White et al. 2009), surface streams and rivers continuously erode and intercept confining layers of the aquifer, forming and later bypassing artesian springs (Woodruff and Abbott 1979, Grimshaw and Woodruff 1986). Consequently, artesian springs in arid and semi-arid regions of the Edwards Plateau provide perennial flows that are moderately decoupled from local precipitation and recharge events but not completely. Prolonged dry periods within the Edwards Plateau region reduce spring discharge rates, and precipitation recharges aquifers with noticeable increases in spring discharges (Abbott and Woodruff 1986). Occurrence of over 90 endemic aquatic invertebrates, fishes, amphibians, and reptiles (Bowles and Arsuffi 2006, Maxwell 2012) inhabit numerous artesian springs and spring runs (hereafter "spring complexes") before merging with larger river systems (hereafter "rivers").

Surface water among spring complexes of the Edwards Plateau is considered of high quality, exceptional water clarity, and hydrologically stable (Slade et al. 1986, Hubbs 1995, Groeger et al. 1997). Contributing watersheds are relatively small; hence hydrology of the first order streams is dominated by spring flows with few high flow events (Kollaus et al. 2015). With hydrological stability, spring complexes are stenoecious environments within a xeric landscape (Hubbs 1995). Hubbs (1995) observed spring fishes, many of which are endemic or relict species, were associated with spring complexes and rarely downstream in rivers and speculated that spring fishes are adapted to the environmental stability of spring complexes, in particular stenothermal conditions, and physiologically restricted in distributions. Furthermore, Hubbs (1995) noted that river fishes (i.e., fishes with distributions not typically associated with spring complexes) were more abundant in the downstream, eurythermal rivers. His observations, however, were anecdotal and not quantitated.

Kollaus and Bonner (2012) quantitated affinities of spring and river fishes for spring and river habitats in a large, spring-dominated river of the Edwards Plateau, finding that spring fishes are more strongly associated with spring complexes during the Winter and Summer than in the Spring and Fall and that river fishes are more strongly associated with river habitats independent of season. Among phylogenetically related fishes, *Dionda argentosa* were more associated with spring complexes, whereas *Dionda diaboli* were more common in the river, providing quantitative support for Hubbs (1995) observations but only in one spring-river system. The strength and ubiquity of associations by spring and river fishes within spring-river systems have not been assessed across a natural gradient of spring discharges and could provide insight into how spring and river fishes segregate between a spring complex and rivers at different spring flow magnitudes. Municipal and agriculture water demands drive groundwater extractions, decreasing the amounts of spring flow magnitude. Pumping of groundwater has real (Winemiller and Anderson 1997) and perceived (Kollaus et al. 2015) threats to spring complexes and spring fishes. Quantitation of observed patterns, specifically spring flow magnitude relationship with spring fishes, is necessary to formulate a balance between water for aquatic biota and water for agriculture and municipal use. Establishing a consistency in distributional patterns of spring and river fishes can advance our understanding of underlying processes of regional historical zoogeography and the conservation value and boundaries of evolutionary refugia in maintaining extant endemic fauna.

The purpose of this study was to characterize affinities of spring fishes for spring complexes and affinities of river fishes for rivers, thus assessing community segregation among six, minimally altered, independent spring-river systems within the Edwards Plateau. Objectives were: (1) to quantitate species richness (total N of species), relative abundances (percent of the total fish community), and densities (number of individuals per m^2) of spring and river fishes among multiple sites along a longitudinal gradient from spring complex to river for each spring-river system; and (2) to test for differences in species richness, relative abundances, and densities of spring and river fishes between spring complexes and rivers using each spring-river system as an independent observation. On the basis of Hubbs (1995) observations, we predicted that species richness, relative abundances, and densities of spring fishes would be greater in spring complexes than rivers and species richness, relative abundances, and densities of river fishes would be greater in rivers than spring complexes. The third objective was to quantitate the relationship between spring flow magnitude ($m^3 s^{-1}$, cms) and species richness, relative abundances, and densities of spring and river fishes. Reductions in base flow magnitude (i.e., portion of the hydrograph attributed to groundwater; Arnold et al. 1995) are associated with fish community shifts, specifically reductions in habitat specialist and increases in habitat generalist (Freeman and Marcinek 2006). Thus, we predicted that base flow magnitude (i.e., spring flow magnitude) would be directly related to species richness, relative abundances, and densities of spring fishes (i.e., habitat specialists) and inversely related to species richness, relative abundances, and densities of river fishes (i.e., habitat generalists) among spring complexes. In other words, naturally occurring spring complexes with less spring flow magnitude will have fewer spring fishes and more river fishes than spring complexes with greater spring flow magnitude.

METHODS

Species richness, relative abundances, and densities of spring and river fishes were obtained from published studies and theses. The six spring-river systems (Fig. 1) were Cypress Creek-Blanco River (Bean et al. 2007), Independence Creek-Pecos River (Watson 2006), headwater-downstream Devils River (Kollaus and Bonner 2012), headwater-downstream Pedernales River (Shattuck 2010), North Llano-Llano River (Curtis 2012), and the Upper San Marcos-San Marcos River (Behen 2013). These studies were selected because each contained multiple sampling sites in the spring complex and river along a longitudinal gradient, had multiple sampling events (≥4) across seasons for at least one year, had similar effort and techniques (i.e., seining only or seining and electroshocking), and provided area measurements sufficient to calculate densities. Some sites were excluded because of anthropogenic modification (i.e., low head dam at one site on the San Marcos River), existence of an additional spring-river confluence downstream (i.e., Devils and Blanco rivers), or sites located far downstream (>65 km) from spring complexes and only contained river fishes (i.e., Pedernales and Llano rivers). Decisions to exclude sites were made prior to analyses and for limiting the assessment to sites that are least-disturbed within spring complexes and river sites within close proximity to spring complexes. For the retained sites, 0 km was assigned to sites at or nearest artesian springs; longitudinal distances from 0 km were determined from areal maps for each downstream site. Flows (cms) were calculated from USGS stations, using the median discharge from the available period of record. For two of the studies, spring complexes (i.e., Pedernales River, North Llano River) were too remote from a USGS station to use median discharge as an appropriate estimate. In these two cases, median discharges were calculated from estimates taken from repeated measurements of flow at each site.

Recorded fishes were classified as a spring or river fish. Spring fishes are those that are quantitatively (Watson 2006, Bean et al. 2007, Shattuck 2010, Curtis 2012, Kollaus and Bonner 2012, Behen 2013) or qualitatively associated with spring

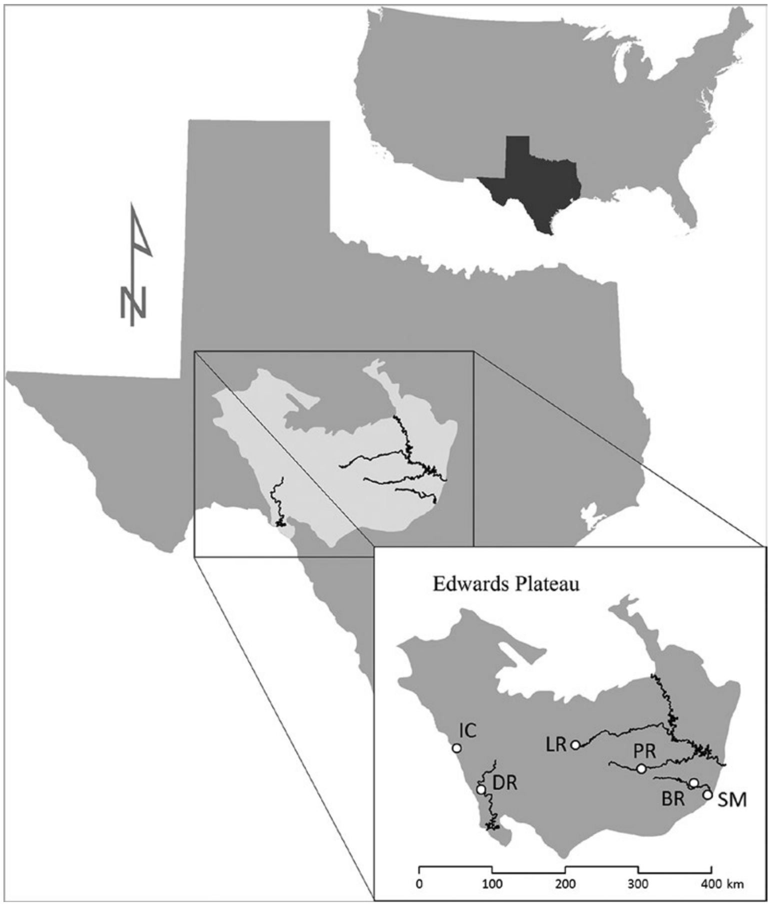

Fig. 1. Locations of selected spring complexes sampled within the Edwards Plateau of Texas, United States. Sampling locations are represented by white dots and the abbreviations for spring complexes sampled are Cypress Creek-Blanco River (BR), Independence Creek-Pecos River (IC), headwater-downstream Devils River (DR), headwater-downstream Pedernales River (PR), North Llano-Llano River (LR), and the Upper San Marcos-San Marcos River (SM).

systems (Table 1; list of spring fishes and references provided). Among listed spring fishes, all are endemic to one or more drainages of the Edwards Plateau, except *Notropis chalybaeus* (Pleistocene relict, Perkin et al. 2012) and *Astyanax mexicanus*. *Astyanax mexicanus* is a neotropical species with northernmost native distribution in the Nueces River and Rio Grande basins of south Texas, but introduced populations exist throughout the Edwards Plateau (Edwards 1977). We included native and non-native populations of *A. mexicanus* in our analyses because of their reported affinities for spring complexes and their native status in some of our drainages (Edwards 1977). Other non-native species with affinities for spring complexes (e.g., Suckermouth Catfish *Hypostoma*) were not included.

Diagnostic plots and statistical analyses were used to assess patterns of spring and river fishes within spring complexes and rivers. Relative abundances of spring fishes were calculated as the percent total of spring fishes among all fishes and were plotted by site and distance from artesian spring or the site nearest the artesian spring. Two clusters were apparent and informative: ranges of relative abundances (49% to 98%) were greater among the three greater discharge spring complexes (≥0.65 cms; Independence Creek, San Marcos River, and Devils River) than ranges of relative abundances (22% to 43%) in three lesser

Table 1. List of spring fishes and occurrences (X) among spring complexes within spring-river systems of the Edwards Plateau. References provided to support association of each fish with spring complexes.

Spring fishes	Cypress Creek-Blanco	North Llano-Llano	Pedernales	Independence Creek-Pecos	Devils	Upper San Marcos-San Marcos	Literature support
Cyprinella proserpina				X	X		Hubbs (1995)
Dionda argentosa				X	X		Garrett et al. (2002)
Dionda nigrotaeniata	X	X	X			X	Hubbs et al. (1953)
Notropis amabilis	X	X	X	X	X	X	Gilbert (1980)
Notropis chalybaeus						X	Perkin et al. (2012)
Astyanax mexicanus	X			X	X	X	Birkhead (1980)
Ictalurus lupus				X			Sublette et al. (1990)
Gambusia geiseri				X	X	X	Stevens (1977)
Etheostoma fonticola						X	Bonner and McDonald (2005)
Etheostoma grahami				X	X		Hubbs et al. (2008)
Etheostoma lepidum		X	X				Hubbs (1985)
Percina apristis	X		X			X	Robins and Page (2007)
Percina carbonaria		X	X			X	Linam and Kleinsasser (1998)

discharge spring complexes (≤0.34 cms; North Llano River, Cypress Creek, and Pedernales River). Consequently, subsequent analyses were split between greater discharge and lesser discharge spring complexes. Richness was estimated by counting the total number of spring and river fishes reported within a spring complex or in a river and independent of site. Relative abundances and densities (number of individuals per m^2) were estimated by averaging site estimates within a spring complex or river across sites and species. Species richness, relative abundances, and densities were calculated for spring fishes and river fishes in spring complexes and rivers for spring complexes with greater (≥0.65 cms) and lesser (≤0.34 cms) discharges. Each spring-river system represented an independent observation. A series of one-tail Student's *t*-tests was used to assess differences in species richness, relative abundance, and density: (1) between spring complexes and rivers for spring fishes within greater discharge springs; (2) between spring complexes and rivers for river fishes within greater discharge springs; (3) between spring complexes and rivers for spring fishes within lesser discharge springs; and (4) between spring complexes and rivers for river fishes with lesser discharge springs. Relative abundances of spring fishes and river fishes are inversely related but were treated as separate hypotheses. Linear and non-linear models were used to assess relationships between median discharge and species richness, relative abundance, and density of spring and river fishes among spring complexes. Linear or non-linear regression models were applied after viewing diagnostic plots and determining an appropriate model. Regression models were simple linear regression ($y = a + bx$), exponential rise to maximum ($y = a(1-e^{(-bx)})$), and exponential decay ($y = ae^{(-bx)}$). Regression models were generated using Systat Software in SigmaPlot for Windows, Version 11.0. Significance level was set at $\alpha = 0.05$ for each pairwise contrast and regression model.

RESULTS

The six spring-river systems collectively contained 13 species of spring fishes and 54 species of river fishes. As a general characterization of sites, relative abundances of spring fishes ranged from 22% to 98% among 14 spring complex sites and 0% to 48% among 10 river sites as spring flows were diluted by confluences (Independence Creek-Pecos River, upper San Marcos River-San Marcos River, Cypress Creek-Blanco River) or influenced by ambient conditions (Devils River, North Llano River, Pedernales River; Fig. 2). Spring fishes dominated (>50% in relative abundance) the fish community up to 20 km downstream from a spring complex in the Devils River, and occurrence of a spring fish extended up to 60 km in the Llano River downstream from spring complex in the North Llano River. Relative abundances of spring fishes ranged from 49% to 98% in spring complexes with greater discharges (0.65 to 4.47 cms; Independence Creek, upper San Marcos River, and Devils River), whereas relative abundances ranged from 22% to 43% in spring complexes with lesser discharges (0.07 to 0.34 cms; North Llano River, Cypress Creek, and Pedernales River). Spring complexes with greater discharge also supported greater abundances of spring fishes (28%–48%) in river sites than lesser discharge spring complexes (0%–15%).

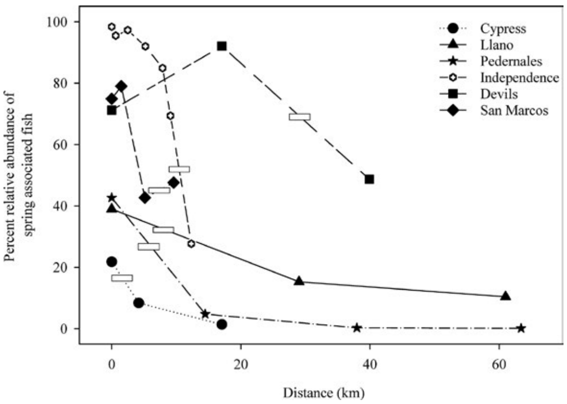

Fig. 2. Relative abundances (%) of spring fishes by site plotted at distance (km) downstream from artesian spring (or uppermost site). White horizontal bar represents loss of spring influence (i.e., confluence or due to ambient conditions downstream).

Among our testable hypotheses, spring complexes with greater discharges had greater relative abundances and densities of spring fishes and lesser relative abundances of river fishes within the spring complexes than the rivers (Fig. 3). Spring complexes with lesser discharges had greater relative abundances of spring fishes and lesser relative abundances of river fishes within the spring complexes than the rivers (Fig. 4). Species richness did not differ between spring complexes and rivers for spring or river fishes.

Across all spring complexes, richness, relative abundance, and density were related to spring flow magnitude for spring fishes but not for river fishes (Fig. 5). Positive relationships were detected between spring flow magnitude and richness of spring fishes (exponential rise to maximum: $a = 7.08$, $b = 3.13$, $r^2 = 0.83$, $P = 0.04$), relative abundance of spring fishes ($a = 82.53$, $b = 3.35$, $P = 0.03$, $r^2 = 0.87$), and density of spring fishes (simple linear regression: $a = 0.203$, $b = 0.352$, $P < 0.01$, $r^2 = 0.84$). Diagnostic plots suggest inverse relationships between spring flow magnitude and river fishes for species richness and relative abundance but relationships were not detected with statistical models.

DISCUSSION

Our initial predictions about affinities of spring fishes and river fishes for spring complexes and rivers were partially supported: relative abundances of spring fishes were greater in spring complexes than rivers and, correspondingly, relative abundances of river fishes were greater in rivers than in spring complexes among all spring-river systems, and densities of spring fishes were greater in spring complexes than rivers but only in greater spring complexes with greater discharges. In addition, richness, abundance, and density of spring fishes were dependent on spring flow magnitude. Collectively, we quantitatively demonstrated that spring fishes have affinities for, but not exclusive to, spring complexes, and complexes with greater spring discharges support greater numbers, abundances, and densities of spring fishes. Although we quantitated the affinity of spring

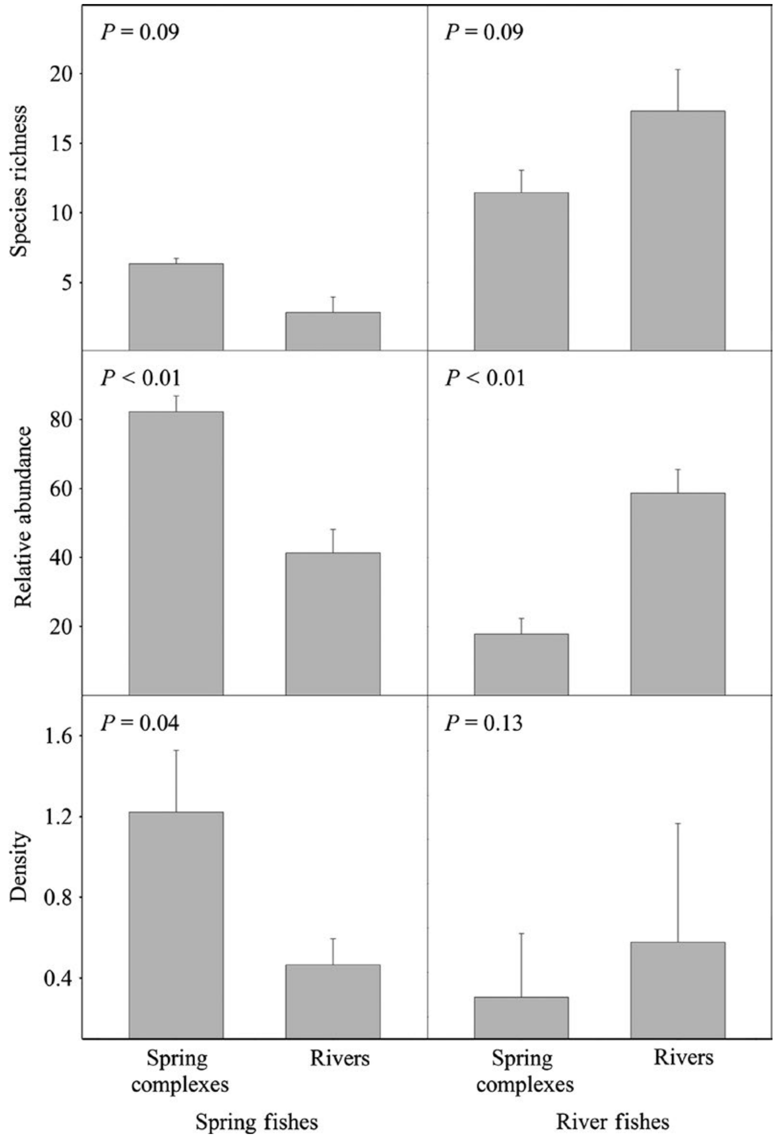

Fig. 3. Mean and standard error for species richness, relative abundance (%), and densities (fish per m^2), comparing spring fishes between spring complexes and rivers (left panel) and comparing river fishes between spring complexes and rivers (right panel), for spring complexes with discharges ≥0.65 cms ($n = 3$). *P*-value for a Student's *t*-test is provided for each comparison.

fish for spring complexes, we failed to detect complete segregation between spring fishes and river fishes in spring complexes.

Stenothermal conditions associated with spring complexes are the oft cited abiotic parameter associated with spring fish occurrences and abundances (Hubbs 1995, Kollaus and Bonner 2012). Organisms encounter a wide range of temperatures, which prompt physiological, behavioral, and morphological responses (Angilletta et al. 2006). Responses are driven by genotypic differences expressed through different phenotypes that result in varying performances across a temperature gradient (i.e., thermal reaction norms; Ellers et al. 2008). Thermal reaction norms are based on the assumptions of physiological tradeoffs influencing the course of species radiation, including specialist–generalist

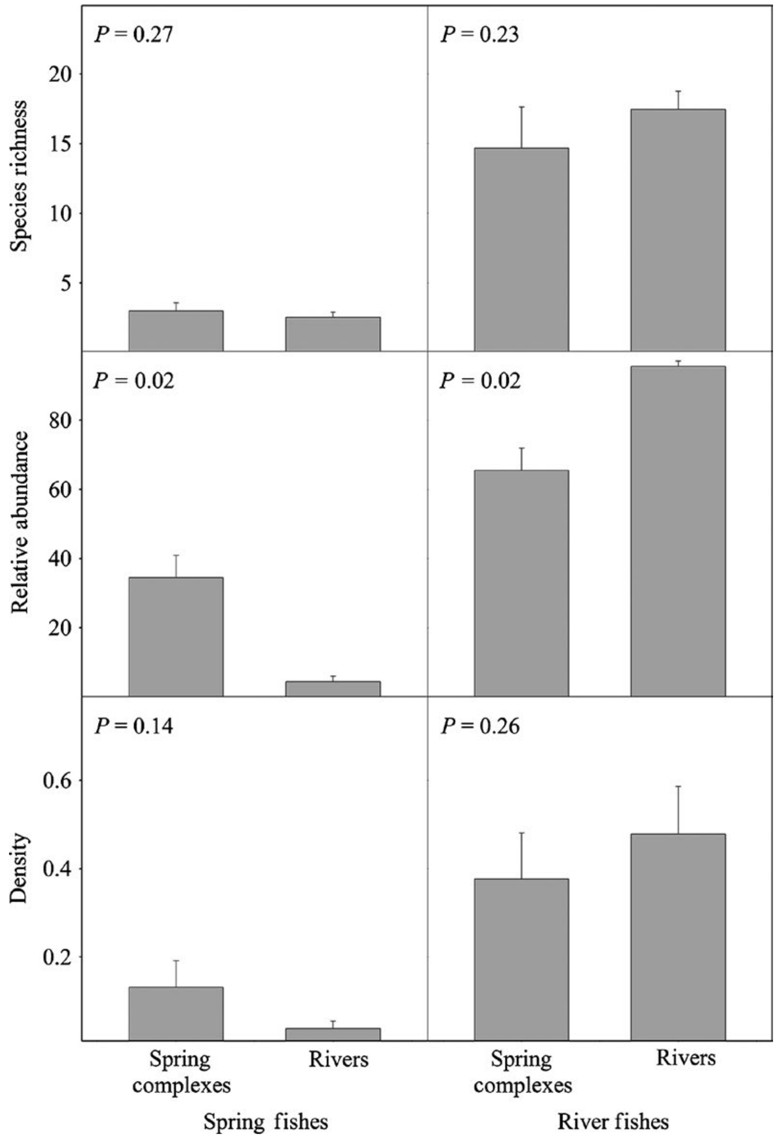

Fig. 4. Mean and standard error for species richness, relative abundance (%), and densities (fish per m^2), comparing spring fishes between spring complexes and rivers (left panel) and comparing river fishes between spring complexes and rivers (right panel), for spring complexes with discharges <0.65 cms (n = 3). P-value for a Student's t-test is provided for each comparison.

tradeoffs in which an increment in performance at certain temperatures results in a decrease in performance at other temperatures (Angilletta et al. 2003). Specialist–generalist temperature tradeoffs arise from differences in structure and function of enzymes (Fields 2001). The enzyme, lactate dehydrogenase (LDH), is identified in several fish species as an indicator of performance associated with water temperature (DiMichele and Powers 1982). The LDH enzyme is linked to the locus influencing ATP production. ATP production influences blood oxygen affinity, which in turn affects overall fitness in conspecifics and species (e.g., swimming capabilities and respiration rates). Quantitation of variability in LDH between spring and river fishes can be tested experimentally (i.e., competition between phylogenetically related species along a

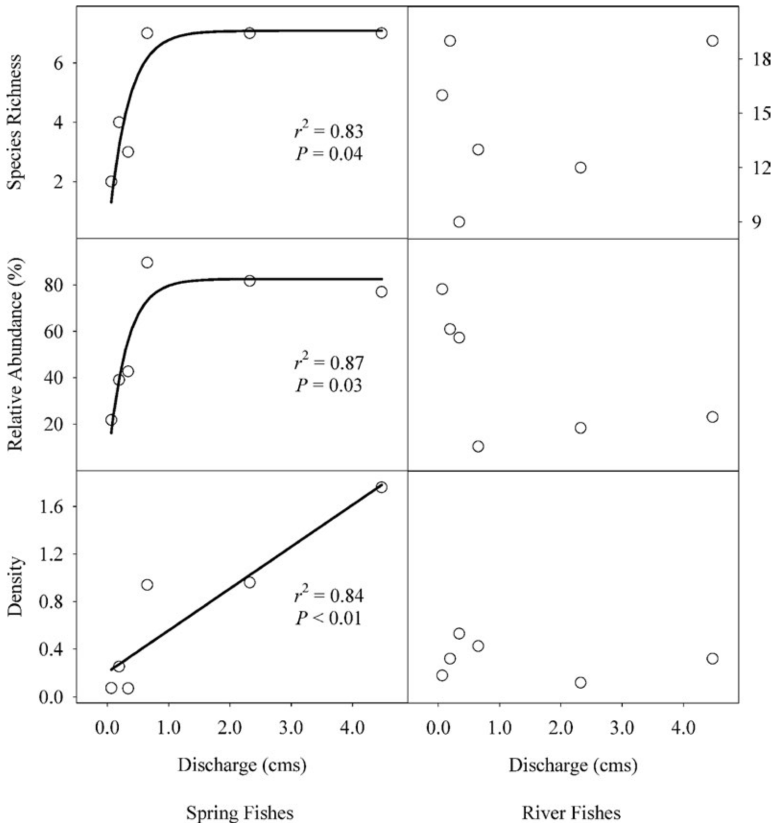

Fig. 5. Relationships between spring flow magnitude each spring-river system and species richness, relative abundance and density for spring fishes (left panel) and river fishes (right panel). See Methods for the selection of linear and non-linear models.

thermal gradient) and might yield genetically and evolutionarily linked insights into the mechanisms regulating fish distributions, radiation, endemism, and perhaps more accurately categorize fishes as "spring-adapted" or "spring-obligated". However, spring fish distributions, as demonstrated by our results, are not restricted by water temperature and include eurythermal waters downstream of spring complexes. Movement outside of spring complexes likely are natural dispersion tendencies, using river networks as conduits for dispersion unto other spring complexes (Dammeyer et al. 2013).

Richness, relative abundances, and densities of spring fishes were directly related to spring flow magnitude. Fewer spring fishes in low flowing spring complexes could be attributed to a combination of abiotic effects (e.g., reductions of size and volume of available habitat; Bunn and Arthington 2002) and biotic effects (e.g., exploitative competition; Peres-Neto 2004) with increases of river fishes (i.e., habitat generalists) into the spring complexes. The asymptotic relationship between spring flow magnitude and richness and abundances of spring fishes suggest spring fishes are resistant to spring flow reductions along a gradient from 4.48 cms (San Marcos River) to 0.65 cm (Independence Creek). One potential mechanism of the asymptotic relationships is that structurally diverse lotic systems (e.g., presumably spring complexes with greater spring flow) have greater buffering capacity against biotic shifts (Gorman and Karr 1978) than less structurally diverse systems. Another potential mechanism could be related to the thermal assimilative capacity of spring complexes. Thermal assimilative capacity is the ability of a water volume to retain temperature and buffer against external drivers of temperature (Poole and Berman 2001). If the spring fish physiologies are temperature-dependent (e.g., LDH; DiMichele and Powers 1982), then fluctuating water temperatures associated with low spring flows would be a less favorable environment for spring fishes. Regardless of the exact mechanism between spring fishes and spring flow, our models can be used to predict fish community integrity of other Edwards Plateau spring complexes and use to predict future changes in Edward Plateau spring complexes related to dewatering (e.g., groundwater pumping and surface water diversions).

The relative abundance model, likely the most useful model since the reporting of relative abundance is the most common method to quantitate fish communities, can be applied to other spring complexes in the Edwards Plateau region as an indicator of biotic integrity. Ichthyofaunal information from other spring complexes are available but not used herein, because the other spring complexes had a recorded history of degradation (e.g., Las Moras Creek, Upper San Antonio River) or the information did not have density measurements (e.g., San Felipe Springs). Using the relative abundance model, Las Moras Creek (Kinney County, Texas; Las Moras Springs discharge = 0.48 cms) is predicted to have 66% spring fishes. Reported relative abundance of spring fishes is 21%, likely a legacy effect of past chlorination events that were applied to maintain the spring complex for recreation activities (Garrett et al. 1992). Upper San Antonio River (Bexar County, Texas, historical spring discharge >5.7 cms; current discharge largely dependent on waste water return = 0.4 cms) is predicted to have 60% spring fishes. Reported relative abundance of spring fishes is 9% (CAC & THB, unpublished data). Low relative abundances of spring fishes in Upper San Antonio River, contained within an urbanized watershed, suggest that water quantity alone is not the only mechanism in maintaining spring fishes through time but also quality of the water. San Felipe Creek (Val Verde County Texas; San Felipe Springs = 2.5 cms) is predicted to have 83% spring fishes. Reported relative abundance of spring fishes is 93% (Garrett et al. 1992). Despite concerns of non-native fish introductions and urbanization, San Felipe Creek is considered a healthy spring fish community and is habitat for several endemic fishes, including the federally listed Devils River Minnow *Dionda diaboli* (Garrett et al. 1992, López-fernández and Winemiller 2005).

Prediction that river fishes would be less abundant in spring complexes was not supported. Lack of complete segregation in lesser discharge springs is understandable, given that spring fishes are fewer and thermal capacity of lesser discharges allow greater temperature fluctuations a short distance from the artesian spring. However, failure to detect differences was unexpected for densities of river fishes between spring complexes and rivers among greater discharge springs. Upon further inspection, densities of river fishes were less in the spring complexes than in rivers in the Devils River (spring complex: 0.12; river: 0.42) and Upper San Marcos-San Marcos River (spring complex: 0.32; river: 0.69), which supports our initial prediction on river fish affinities for rivers. In Independence Creek-Pecos River system, density of river fishes in the spring complex (0.43) was greater than in the river (0.38). Greater density of river fishes in the spring complex could be attributed to habitat degradation in the Pecos River. Pecos River near the Independence Creek confluence is a degraded system. Stream discharge regulation by upstream reservoirs (Hoagstrom 2003) and periodic fish kills attributed to frequent outbreaks of Golden Alga *Prymnesium parvum* (Rhodes and Hubbs 1992) have altered the Pecos River fish community. At times of duress, Pecos River fishes seek refuge (i.e., ecological refuge; Keppel et al. 2012) in spring-influenced tributaries (Rhodes and Hubbs 1992). As such, richness and densities of river fishes are lower than historical numbers and richness, and densities of river fishes in Independence Creek might be inflated because of conditions in the Pecos River. Consequently, densities of river fishes in two of three greater discharge springs were less in spring complexes than in the rivers. As more spring-river systems are quantitated, we anticipate that the prediction that densities of river fishes in spring complexes are less than those in river will be quantitatively supported for greater discharge springs. Until then, we can only tentatively conclude that spring fishes and river fishes exhibit a segregated distribution with a degree of overlap, dependent upon spring flow magnitude.

Despite failing to detect clear patterns in river fish affinities, our quantitative support of spring fish affinities for perennial spring complexes indicate a contemporary value of evolutionary refugia in addition to their historical value as aquatic resources for biota survival and diversification during Pleistocene climate change. With both contemporary and historical values, evolutionary refugia in arid regions are of higher conservation priority than ecological refuge (Davis et al. 2013) because of their value in providing habitat not only for contemporary biota, many of which are recognized as threatened and endangered species by various federal, state, and NGO groups, but also providing habitats for aquatic biota dispersion and radiation during future interglacial periods. Conservation and management strategies for the Edwards Plateau aquifers and spring complexes are ongoing and consistent with strategies elsewhere by reducing stressors to the systems, such as over extraction of aquifers and minimizing instream and watershed degradation (Davis 2014). This study advances our abilities to justify and conserve groundwater and spring complex water quantity and to set expectations for biotic integrity of spring fish communities within the Edwards Plateau region and in other arid interglacial refugia. However, the high value of evolutionary freshwater refugia in arid systems to past, current, and future biota is similarly valued by humans. Developing a sustainable balance between water resource extraction and protection includes a thorough understanding of patterns, processes, and boundaries of evolutionary refugia to quantitate ecosystems services value along with its societal (e.g., industry, agriculture, municipality, recreation) values.

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