Reproductive Ecology of a Relict Population of Ironcolor Shiner (*Notropis chalybaeus*) in the Headwaters of the San Marcos River, Texas

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North American Distribution and Ecology:

Ten to twenty thousand years ago the climate of North America differed substantially from present-day conditions. The Wisconsin stage of the Laurentide Ice Sheet was nearing its end and the Mankato glacial advance formed massive ice sheets over much of northern North America. Glaciers over two miles thick spanned from present-day Canada to portions of Illinois, Pennsylvania, Minnesota, and New York. Advancement and retreat of these glaciers would form the architecture of present-day North American drainage basins and create the Great Lakes. As a consequence of formation of such great glaciers, sea levels fell over 65 fathoms (~390 feet) below present levels, allowing coastal streams of the Gulf of Mexico to flow together and form master streams. Since this period, glaciers have continued to retreat, causing sea level to rise and many areas of North America to become drier. Drying was most severe 4,000-8,000 years ago during the Altithermal period when North America experienced an exceptionally dry and warm climate. The Altithermal period was particularly harsh throughout central North America and caused the drying of many river basins. Combined, these glacial advances and subsequent drying and warming periods had profound effects on the aquatic biota of North America. Retreat of melting glaciers formed new rivers that flowed into lowland seas, allowing interconnectivity of river basins and dispersion of freshwater fishes. As the warming trend continued and drying emerged as the prevailing pattern, sea levels rose and glacial streams desiccated, resulting in fish extirpations and isolation of populations. In Western Gulf Slope drainages, several relict populations of freshwater fishes, such as the Ironcolor Shiner (*Notropis chalybaeus*, Cope 1869) (Figure 1), persist in relatively small water bodies, long disconnected from eastern drainages. This article details the reproductive ecology of a relict population of Ironcolor Shiner in the headwaters of the San Marcos River (Guadalupe River drainage of Texas) and illustrates insights about fish conservation that were gained from a study funded by the North American Native Fishes Association (NANFA) in 2009.

The Ironcolor Shiner is a small-bodied cyprinid characterized by a dark mid-lateral stripe, which extends through the snout and into interior portions of the mouth. The term "ironcolor" is derived from this dark band that resembles a deep iron color when lighting is appropriate (Figure 2, p. 14). The known range of Ironcolor Shiner includes streams in the eastern half of the United States, encompassing Atlantic Coast drainages from New York to Florida and tributaries to the Mississippi River from the southern Great Lakes region to the Gulf of Mexico. Nearly a dozen isolated or disjunct populations of Ironcolor Shiner are reported toward the peripheries of the species' range, owing to natural and anthropogenic processes. Naturally occurring factors that have influenced the disjunct nature of isolated populations include alterations to river basins associated with glacial retreats at the end of the last ice age (as discussed above and by Gerking 1947) and have driven the emergence of isolated populations in the Northern portion of Ironcolor Shiner range. Human-mediated mechanisms for isolation include extirpations (localized extinctions) caused by the inundation of wetlands and impoundment of streams (Hubbs and Pigg 1976), and which have caused isolated populations in states like Oklahoma to become rare and endangered (Williams and Echelle 1998). Despite the frequent occurrence of isolated populations, relatively little is known about their ecology or the

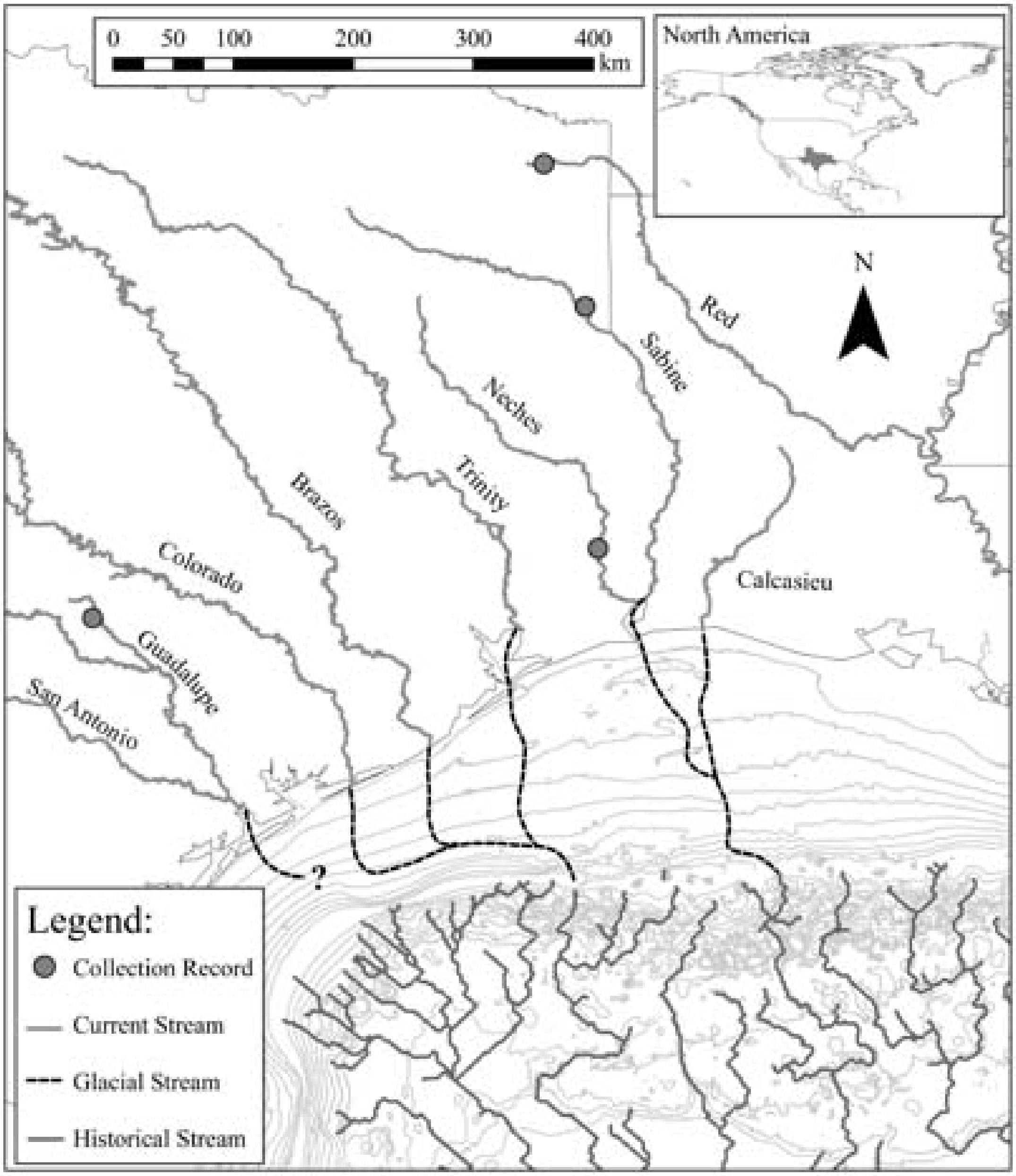

Figure 1. Distribution of Ironcolor Shiner in Western Gulf Slope drainages. The disjunct population in the Guadalupe River Basin is relict, arising because of historical stream connections during the last period of glaciation (11,000 years before present; black dashed lines) or earlier (>11,000 years before present; gray solid lines). Connectivity of the San Antonio Bay drainage is unresolved but likely connected to the Colorado-Trinity network during the last period of glaciation.

mechanisms that allow for their persistence in the face of little opportunity for dispersal from larger populations.

The ecology of Ironcolor Shiner has been studied among interconnected populations occurring in the eastern and southeastern United States. Early studies of the Ironcolor Shiner occurred during the 1940s in portions of Florida (Marshall 1947) and were followed by studies in the southeastern U.S. (Swift 1970) as well as Arkansas (Robison 1977), Wisconsin (Becker 1983), and Pennsylvania (Leckvarcik 2001). Based on these studies, we know that the Ironcolor Shiner is a short-lived species (up to three years) that inhabits smaller streams characterized by low turbidity, lower-than-neutral acidity (pH = ~6), and high abundance of aquatic plants. The species reaches reproductive maturity at one year, spawns during summer months, and dispenses sinking eggs that stick to sand particles in pool habitats. Ironcolor Shiner inhabit middle water column

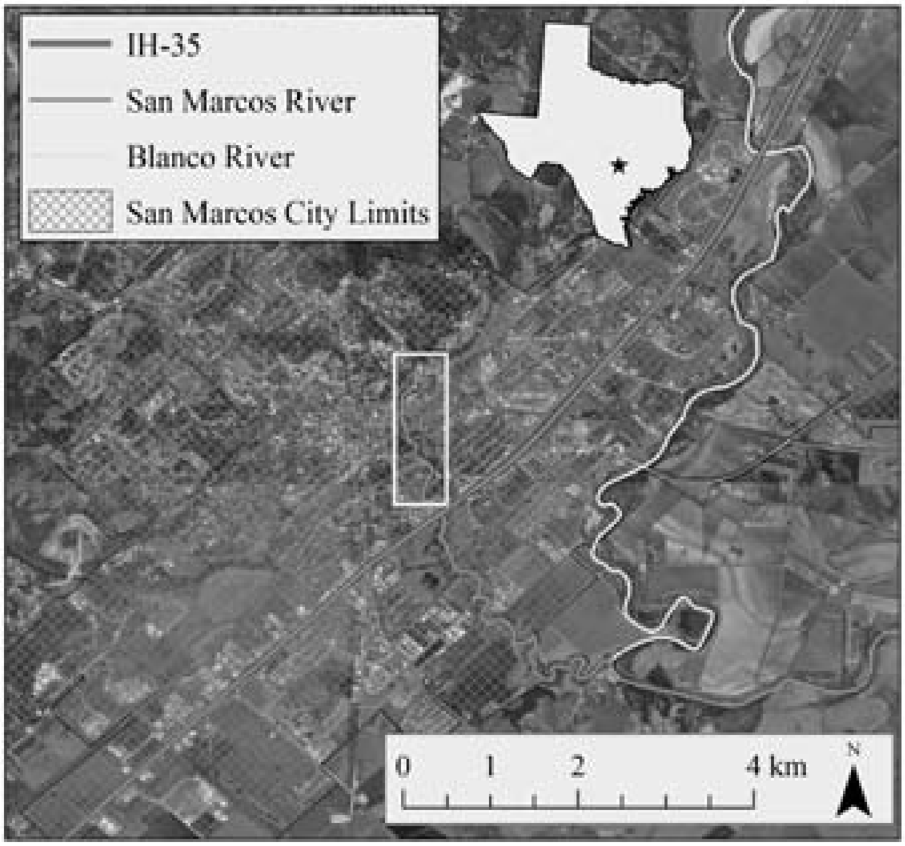

Figure 3. Range of Ironcolor Shiner in the upper San Marcos River. Rectangle represents a 2.2 km urbanized reach of the river where Ironcolor Shiner is restricted. Black star on insert of Texas represents city of San Marcos.

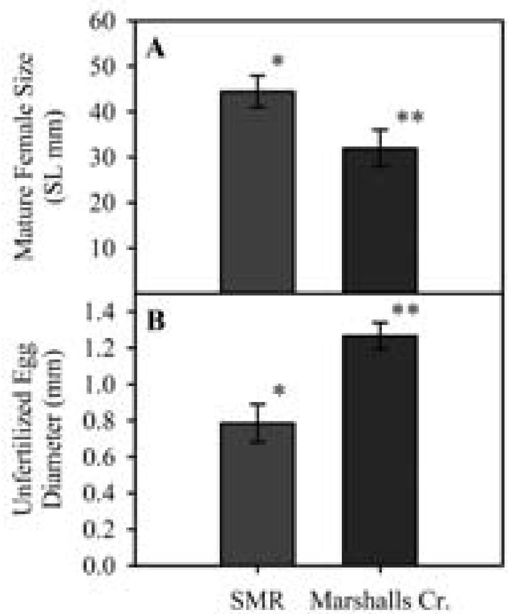

Figure 5. Comparisons of (A) mature female standard length (mm) and (B) unfertilized egg size (mm) between the upper San Marcos River, Texas (SMR) and Marshalls Creek, Pennsylvania (Marshalls Cr.). Asterisks indicate significant differences.

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Figure 2. Adult Ironcolor Shiner (51 mm total length) collected from the upper San Marcos River in the city of San Marcos, Hays County, Texas. Photo by Chad Thomas (Texas State University-San Marcos), used with permission. See page 11.

Figure 4. (A) San Marcos River in Sewell Park, San Marcos, Texas; (B) Ironcolor Shiner and Mexican Tetra *(Astyanax mexicanus*) as viewed in the clear waters of the upper San Marcos River; (C) Seining fishes from the San Marcos River near its headwaters (Gaby Timmins, left; Tom Heard, right); (D) San Marcos River near headwaters at Spring Lake, San Marcos, Texas.

depths where they school with other minnow species and forage on drifting invertebrates that are swept downstream by currents or deposited from the terrestrial environment. Vegetative stands are used by young Ironcolor Shiner as predator refuges and may provide for additional foraging opportunities, as evidenced by seeds and vegetation in the gut tracts of dissected individuals taken from streams in Florida (Marshall 1947). These documented life history attributes are likely similar among disjunct populations of Ironcolor Shiner, given most ecological traits are evolutionarily constrained. However, local variation in environmental characteristics associated with disjunct populations might allow for plasticity (ability to change) in some ecological traits. For example, the reproductive ecology of Brown Trout populations is known to vary according to local habitat characteristics, including water temperature determining relative egg production and size (Lobon-Cervia et al. 1997). Certainly variation in reproductive output contributes to a population's ability to persist through time, especially in disjunct situations where there is no connection with larger populations.

A Unique Environment:

Occurrence of the disjunct, relict population of Ironcolor Shiner in central Texas is notable because it represents the southwestern most extent of the species' range and is characterized by landscape and geophysical characteristics that are unique among streams inhabited by Ironcolor Shiner. The 2.2 km reach of stream in which Ironcolor Shiner are found (Perkin et al. 2012) is entirely surrounded by urbanized landscape (Figure 3, p. 13). In this headwater reach, water temperature is a constant 23ºC year-round because of aquifer-derived flows that surface in the City of San Marcos after being underground for up to 20 years (Groeger et al. 1997). Stable discharge and water temperature in the upper San Marcos River provide a unique spring-run environment, characterized by clear water, abundant aquatic plants, and slightly basic pH levels because of high calcium carbonate deposits within the aquifer (Figure 4, p. 14). Many of these aspects reflect what is known about streams inhabited by eastern populations of the species, with the notable exception of water temperature. The upper San Marcos River lacks the dynamic intra-annual fluxes in water temperature characterized by streams inhabited by all other known populations of Ironcolor Shiner. Consequently, the relict population of Ironcolor Shiner in the upper San Marcos River allows the opportunity to evaluate the effects of stable water temperature on the ecology of the species. Because of the strong linkage between water temperature and reproductive ecology (for example, work by Lobon-Cervia et al. 1997) the goal of our study was to evaluate responses in aspects of Ironcolor Shiner reproduction that might be influenced by stenothermal water.

Study Design:

We conducted monthly seining collections of Ironcolor Shiner in the upper San Marcos River between January and December of 2007. Sampling sites varied by month, but were always within the 2.2 km urbanized reach of the San Marcos River. No more than 12 individuals were opportunistically retained each month, anesthetized, and stored in 10% formalin for laboratory inspection. In the laboratory, we assessed five fundamental aspects of reproductive ecology. We assessed age at maturation by determining the age in years of the youngest reproductively mature female individual. For our purposes, reproductive maturity was assessed according to whether large diameter unfertilized egg cells were present in ovaries. We estimated length of life by determining the age of the oldest individual captured, using length frequency histograms to estimate number of year classes. Length and timing of reproductive season was determined by considering the number of months during which reproductive females were captured, and the number of months during which male gonad weight constituted more than 0.5% of total body weight. We then estimated the number of large diameter unfertilized eggs present in 20 reproductively mature females and measured the diameter (in millimeters) of each egg.

We compared our findings to other studies that have assessed the life history of Ironcolor Shiner. To do this, we used a literature review of existing knowledge of Ironcolor Shiner life history and we included studies that assessed similar ecological attributes as our study. One study in particular, a Master's thesis from Pennsylvania State University, contained a large amount of data pertaining to life history attributes of Ironcolor Shiner in Marshalls Creek, Pennsylvania (Leckvarcik 2001).

Findings and Conclusions:

We collected 118 Ironcolor Shiners from the upper San Marcos River during 12 months of sampling in 2007. Among these individuals, mean (±SD) standard lengths suggested four age groups were present, consisting of age 0 (33 ±1 mm), age 1 (39 ±3 mm), age 2 (44 ±3 mm), and age 3 (49 ±1 mm). Minimum size at maturation was 36 mm for a female collected during March, suggesting age at maturity was approximately one year. We observed reproductive activity during 10 of the 12 sampling months. Females containing mature ovaries were present March-December, when gonads constituted >3.0% of body weight and ranged from 3.4% to 8.0%. Similarly, gonads constituted >0.5% of male body mass during early April-December, when percent gonad weights ranged from 0.55% to 1.7%. Among 20 reproductively mature females (2 from each month of reproductive activity), mean (±SD) number of large diameter unfertilized eggs was 95 (± 43) and mean (±SD) diameter was 0.78 (± 0.1) mm.

Comparisons between Ironcolor Shiners collected from the San Marcos River and Marshalls Creek, Pennsylvania indicated differences in reproductive ecology. Standard length of mature females was notably larger in the San Marcos River (mean ± SD = 42 ± 3) compared to Marshalls Creek (mean ± SD = 32 ± 4; Figure 5a, p. 13). Inversely, mean diameter of unfertilized eggs was notably smaller in the San Marcos River (0.78 ± 0.1) compared to Marshalls Creek (1.26 ± 0.1; Figure 5b). Reproductive seasons also differed between populations, with that of the San Marcos River spanning ten months and the Marshalls Creek population only two. Interestingly, despite these differences in reproductive ecology, the number of eggs per female did not notably differ between San Marcos River (mean ± SD = 95 ± 43) and Marshalls Creek (mean ± SD = 121 ± 43) populations.

Comparison with Ironcolor Shiner populations from other stream systems revealed the upper San Marcos River population of central Texas spawned for much longer than other populations. Reproductive season length in the upper San Marcos River was greatly protracted relative to populations that

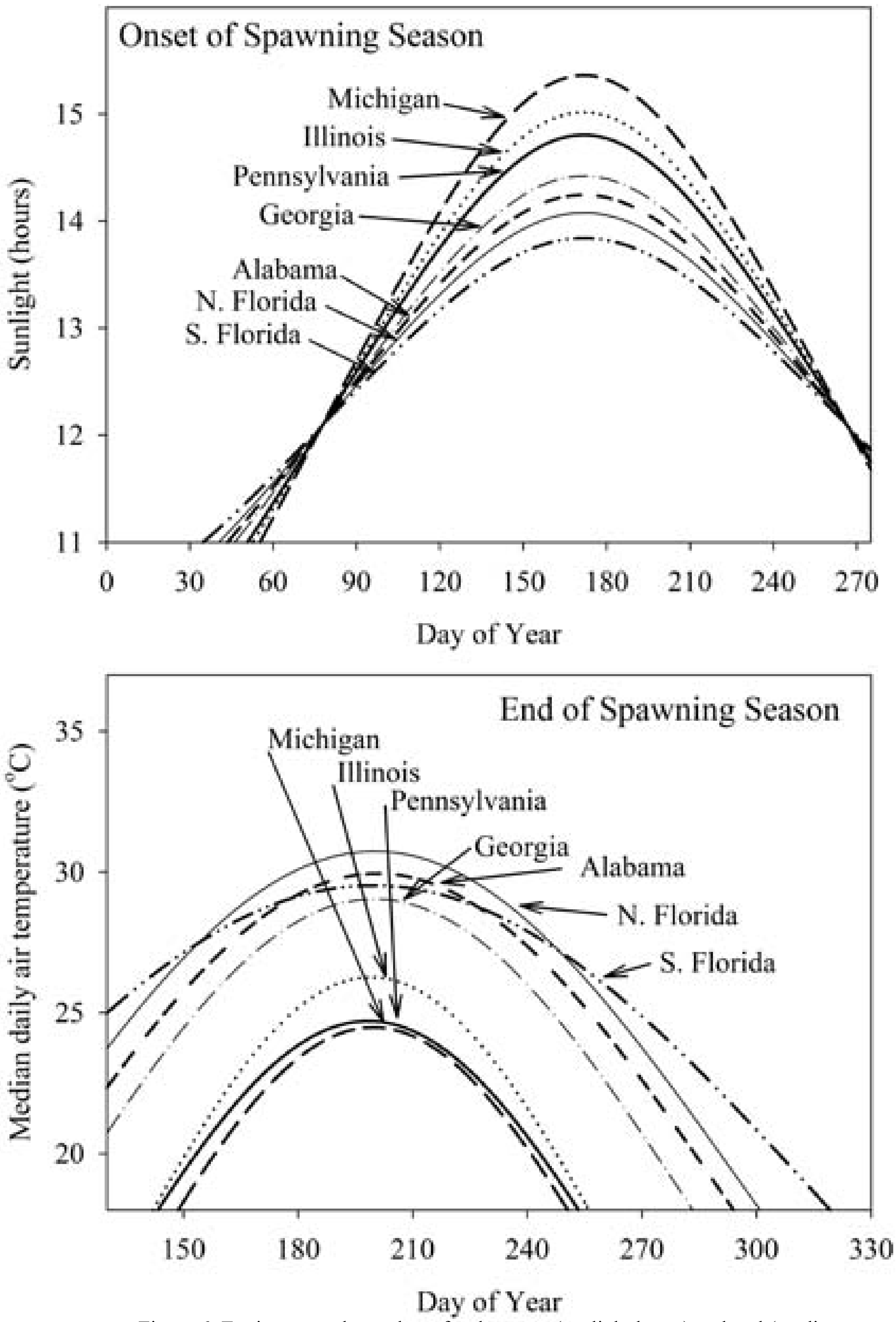

Figure 6. Environmental correlates for the onset (sunlight hours) and end (median daily air temperature, ºC; used as a proxy for water temperature for non-spring influenced streams) of Ironcolor Shiner reproductive seasons estimated from seven locations reported in literature accounts. Sunlight hour and median daily air temperature data were obtained from the National Oceanic and Atmospheric Administration (NOAA; www.noaa.gov; <access July 1, 2011>).

spanned a latitudinal gradient. In northern portions of their range (Illinois and Pennsylvania), Ironcolor Shiner are known to spawn for approximately 2-3 months (Becker 1983, Leckvarcik 2001). As populations move southward, spawning seasons increase to approximately four months in Alabama, four months in northern Florida, and five months in portions of southern Florida (Swift 1970, Boschung and Mayden, 2004). Furthermore, although the San Marcos River is among the middle-lying latitudes, reproductive season length was more than double the length of populations at even the most southern latitudes (i.e., 10 months versus 4-5 months). This apparent latitudinal trend is consistent with seasonal changes in water temperature, where length of warmer (summer) water temperatures increase in a southerly direction (Gotelli and Pyron 1991). The lack of conformity to this pattern by the San Marcos River population is likely related to the unique spring-run environment in the upper San Marcos River and lack of reproductive terminating cue triggered by declining stream temperatures (Perkin et al. 2012).

Environmental factors such as photoperiod and water temperature are known to influence the reproductive ecology of many species of fish, including the Ironcolor Shiner. For example, in Pennsylvania during the years 1999, 2000, and 2001, reproductive seasons of Ironcolor Shiner varied in length according to prolonged summer water temperatures. That is, when summer water temperature remained elevated for longer periods, the reproductive season of Ironcolor Shiner was prolonged, although the onset of the reproductive season remained consistent among years (Leckvarcik 2001). Given this pattern, Leckvarcik (2001) concluded that photoperiod was likely responsible for the onset of reproductive activity, and that declining water temperatures acted as a cue to cease reproductive activity. Our findings across seven populations of Ironcolor Shiner for which published accounts of reproductive season length were reported indicated a similar trend: reproductive activity corresponded with increasing day lengths and termination of the spawning season occurred as median daily air temperatures (a proxy for water temperature in non-spring influenced streams) declined (Figure 6, p. 19). In the case of the San Marcos River population, declining water temperatures do not occur because of stenothermal water and the cue for cessation of spawning is likely not detectable.

Perennially flowing springs and constant water temperatures infer multiple benefits to aquatic organisms and have contributed to enriching biodiversity on a global scale. Spring-run environments like the upper San Marcos River are generally associated with biodiversity "hot-spots," in which species richness within or surrounding a spring outflow is disproportionately greater than the surrounding ecosystem or biome (Dudgeon et al. 2006), because these water sources serve as refugia for aquatic taxa during periods of climatic extremes, especially during periods of extreme dryness like during the Altithermal period. Increased biodiversity associated with spring outflow is consistent with the large number of unique and narrowly distributed fauna and flora of the upper San Marcos River (Bowles and Arsuffi 1993). However, the mechanism by which spring systems allow for increased diversity is largely unknown. Isolation within a spring system with narrow environmental fluctuations might allow for adaptive radiation either through isolation alone (e.g., genetic drift contributing to new forms over long time periods) or because of environmental selection for genotypes with greater fitness (e.g., longer spawning seasons with greater reproductive output infer greater numbers of progeny). We've demonstrated here that reproductive biology differs between fishes in year-round constant temperatures and those in fluctuating temperatures, but cannot differentiate between genetic selection and simple plasticity. Brown and Feldmeth (1971) suggested isolation within thermally stable environments actually limits radiation among aquatic organisms because of the homeostatic nature of springs and reduced adaptation to ranging environmental conditions. However, Swift (1970) suggested continual groundwater flows during the Altithermal period allowed for persistence of ironcolor shiner by providing a continual source of stenothermal water while surrounding streams desiccated entirely. Combined, these two conclusions paint a enigmatic picture by which stable spring environments allow for persistence of organisms by providing refuge during periods of environmental harshness (Swift 1970) but impede the adaptive ability of the organisms they harbor by constraining the suite of environmental characteristics in which the organisms can then persist (Brown and Feldmeth 1971). Regardless of the mechanism, be it radiation by isolation, adaptive selection within a homeostatic environment, or some combination of the two, the population of Ironcolor Shiner in the upper San Marcos River was once considered a unique species in historical literature (Baughman 1950) and likely deserves a revaluation given the unique life history characteristics we report here.

The population of Ironcolor Shiner in the San Marcos River appears currently stable (Perkin 2009), though the species is listed as one of special concern in the state of Texas (Hubbs et al. 2008) and as vulnerable throughout its North American range (Jelks et al. 2008). The unique environment of the upper San Marcos River is rather specific, owing to the stenothermal character of its water and relative isolation of numerous disjunct populations of fishes (Perkin and Bonner 2011) as well as numerous spring-associated organisms that rely upon a continual supply of surfacing groundwater (Bowles and Arsuffi 1993, McDonald et al. 2007). The subsequent habitat and environmental variables, coupled with the uniqueness of the Ironcolor Shiner population in this system, warrants further research and possibly further protection. The upper San Marcos River is no exception to the worldwide threats to freshwater biodiversity, and organisms inhabiting the upper 2.2 km of stream face rising human pressures associated with species loss such as ever-encroaching urban development, increased water withdrawals, watershed alterations such as dam construction, introduction of alien species, and climate change (Strayer and Dudgeon 2010). Fortunately, there are federally listed species such as the Fountain Darter (*Etheostoma fonticola*) with overlapping distributions that have created an "umbrella" affect regarding conservation of habitat in the area. However, care should be taken with respect to preserving unique populations of organisms in the upper San Marcos River.

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