Reproduction and age structure of the Plains Killifish
Fundulus zebrinus from two tributaries of the upper Red River, Texas

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ABSTRACT.—Plains Killifish Fundulus zebrinus (subgenus Plancterus) is native to river basins in Oklahoma, New Mexico, and Texas. Original descriptions of Plains Killifish life history information are now applicable to the sister taxon Northern Plains Killifish Fundulus kansae following recognition of the 2 species in 2001. Study objectives were to quantify (1) length of the reproductive season, using the gonadosomatic index and categories of gonadal maturation, (2) batch fecundity, and (3) number of age groups for Plains Killifish taken from 2 rivers within the upper Red River basin of Texas. Reproductive season was from March through September, with the production of multiple batches and a maximum batch of 131 mature oocytes. Plains Killifish were sexually mature at age 1 and had an estimated life span of 2–3 years. Reproductive season, production of multiple batches, and age of Plains Killifish were similar to those reported for Northern Plains Killifish and 2 other closely related subgenera, Wileyichthys and Zygonectes. These similarities suggest strong trait conservatism among a monophyletic group of fundulids that inhabit a diversity of freshwater and saltwater environments in western shallow bays and salt marshes, southwestern arid and prairie streams, and eastern low-gradient streams of North America.

RESUMEN.—Los Plain Killifish Fundulus zebrinus (subgénero Plancterus) son peces nativos de las cuencas de los ríos en Oklahoma, Nuevo México y Texas. Las descripciones originales de la información sobre la historia de vida de los Killifish ahora son aplicables al taxón hermano Northern Killifish Fundulus kansae después del reconocimiento de las dos especies en 2001. Los objetivos del estudio fueron cuantificar la duración de la temporada reproductiva, utilizando el índice gonadosomático (GSI) y las categorías de maduración gonadal, la fecundidad por lote y el número de grupos de acuerdo con la edad de los Plains Killifish tomados de dos ríos dentro de la parte superior de la cuenca del Río Rojo en Texas. La temporada reproductiva fue de marzo a septiembre con la producción de múltiples lotes y un lote máximo de 131 ovocitos maduros. Los Plain Killifish eran sexualmente maduros a la edad de 1 año y tenían una vida estimada de 2 a 3 años. La temporada reproductiva, la producción de lotes múltiples y la edad de los Plains Killifish fueron similares a los reportados para los de la especie Northern Plains Killifish y otros 2 subgéneros estrechamente relacionados, el Wileyichthys y Zygonectes. Estas similitudes sugieren un fuerte conservatismismo de rasgos entre un grupo monofilético de fundulídos que habitan en una diversidad de ambientes de agua dulce y salada en bahías poco profundas en el oeste y marismas salinas, arroyos áridos y praderas del sudoeste, además de los arroyos orientales de bajo gradiente ubicados en el este de América del Norte.

Topminnows and killifish (genus Fundulus, family Fundulidae) are broadly distributed among the fresh, brackish, and salt waters of North and Central America (Wiley 1986, Whitehead 2010). Northern Plains Killifish Fundulus kansae Garman, 1895 and Plains Killifish F. zebrinus Jordan and Gilbert, 1883, members of the subgenus Plancterus (Ghedotti and Davis 2013), persist among fresh and saline streams of the western Mississippi drainage and western gulf slope drainages of North America (Kreiser et al. 2001, Wurbs 2002). Their saline tolerance reflects marine ancestry (Whitehead 2010), and their inland populations are derived from an ancestral form that established inland through connections of Pleistocene and Pliocene rivers with the Gulf of Mexico (Cross et al. 1986).

The Plains Killifish is endemic to the arid southwestern USA and is found in the Red River of Texas, the Oklahoma, Trinity, Brazos, and Colorado rivers of Texas, and the Pecos River of Texas and New Mexico (Poss and Miller 1983, Kreiser 2001, Hendrickson...
As a result of bait bucket release, populations of the Plains Killifish were introduced into the Big Bend region of the Rio Grande of USA and Mexico (Hubbs and Wauer 1973) and into the Colorado River basin of Arizona, Nevada, Utah, Colorado, and New Mexico (Miller 1952, Hughes 1981, Deacon and Williams 1984, Kreiser et al. 2000). Plains Killifish are associated with edge, backwater, and channel habitats of shallow, sandy rivers and creeks (Minckley et al. 1991). Field observations of reproductive behavior suggest that reproductive season begins in late March and extends into October (Echelle 1970). Previously, Plains Killifish were reported to be early maturing (age-1; Minckley and Klaassen 1969) and short lived (2.5 years) and to have a 3-month (June–August) spawning season with a mean batch fecundity that ranges from 20 to 25 vitellogenic oocytes and a maximum batch fecundity of 106 vitellogenic oocytes (Bonham 1962). However, these life history assessments were estimated from individuals taken from Kansas (Minckley and Klaassen 1969) and Missouri (Bonham 1962). Currently, the Arkansas River and Missouri River basins are now within the geographic range of the Northern Plains Killifish, which is recognized as a separate species from the Plains Killifish (Kreiser 2001).

The purpose of this study was to provide life history information for the Plains Killifish from the Red River drainage of Texas. Study objectives were to quantify (1) length of the reproductive season, using the gonadosomatic index and categories of gonadal maturation, (2) batch fecundity, and (3) number of age groups for Plains Killifish taken from 2 rivers within the upper Red River basin of Texas. Quantification of Plains Killifish life history contributes to a greater understanding of the species’ ecology and conservation, especially in areas where population declines are noted (Cheek and Taylor 2016), and it informs life history comparisons among members of Fundulidae.

**METHODS**

Plains Killifish were collected monthly between February 2016 and January 2017 from 3 sites on the Pease River (i.e., FM 104, Hwy. 6, and Hwy. 283) and 2 sites on the North Wichita–Wichita River (i.e., Hwy. 6 and FM 1919; Fig. 1). At each collection site and on each sampling date, a minimum of 10 seine hauls were made to capture Plains Killifish ≥31 mm TL (total length). Bonham (1962) reported 35 mm TL as the approximate minimum length for sexual maturity in Northern Plains Killifish. Smaller individuals were taken in this study in order to ensure accurate estimation of minimum length for sexual maturity. Total lengths (nearest mm) were measured for all Plains Killifish captured and were used to assess length frequency. Up to 10 Plains Killifish were retained for life history assessment, anesthetized with a lethal dose of tricane methanesulfonate (MS222), and fixed in 10% formalin.

For each site and month, up to 5 of the females fixed in formalin were randomly selected (to prevent bias in fecundity attributed to size of individuals), measured to the nearest millimeter, and weighed to the nearest milligram. An incision was made from the urogenital opening to the isthmus. The esophagus was severed, and the stomach, intestine, and fused ovaries (common in Cyprinodontiformes [Kobelkowsky 2012]) were removed. Ovaries were weighed and the gonadosomatic index (GSI = [mass of ovaries/mass of fish] × 100) was calculated for each fish. Ovary sizes were macroscopically categorized as (1) immature or resting ovaries with small, translucent oocytes, (2) developing ovaries with small (<0.5 mm in diameter) translucent oocytes and small (<2.0 mm in diameter) opaque oocytes indicating early stages of yolk deposition, (3) mature ovaries with small translucent oocytes, small opaque oocytes, and large (>2.0 mm in diameter) vitellogenic oocytes, and (4) spent ovaries with small translucent oocytes and a few large vitellogenic oocytes (Williams and Bonner 2006). Mature ovaries from up to 3 females were selected, and individual oocytes were removed by teasing the oocyte mass apart and redistributing the oocytes on a petri dish with a gentle swirling. For the first 100 oocytes in the field of view, we used a dissection microscope fitted with an ocular micrometer to measure oocyte diameters to the nearest 0.01 mm for oocytes that were >0.2 mm. Oocyte diameters were plotted by percent frequency of occurrence to estimate modality in clutch production (i.e., single spawning or multiple batch spawning), maximum oocyte diameter size, and range of oocyte diameters for the final batch of oocytes. Minimum oocyte diameter for the final batch...
was 2.0 mm, and this measurement was used to estimate batch fecundity in all other mature females. We reported only the range of batch fecundity to indicate the reproductive potential of Plains Killifish, because batch fecundity estimates of multiple spawning fishes are underestimations of spawning season fecundity (Hunter et al. 1985).

Length frequency histograms were constructed from collections of all Plains Killifish by using 2-mm bin increments that were combined across sites to estimate the number of age groups by month, total lengths per age group by month, and overall life span. Modal progression analysis (Bhattacharya’s Method in Fish Stock Assessment Tools II [FiSAT II]; Gayanilo et al. 2005) was used to estimate the number and total lengths of age groups (Perkin et al. 2012, 2013) between February 2016 and January 2017. Mean lengths (±1 SD) of age groups with <3 individuals per month were calculated independently of the modal progression analysis. Birth date follows the conventional standard of 1 January. Age-0 fish were spawned in 2016, age-1 fish were spawned in 2015, and age-2 fish were spawned in 2014.

RESULTS

A total of 153 female Plains Killifish were taken for reproductive assessments during the study period. Ovaries of adult fish (n = 50, range 33–62 mm TL) taken in February and October through January were classified as immature or resting, with the GSI ranging between <0.1% and 2.6%. Plains Killifish invested energy into reproduction between the months of March and September (Fig. 2). Developing ovaries (n = 14, 34–58 mm TL) were observed in March and April, with GSI ranging between 1.2% and 4.5%. Mature ovaries (n = 66, 36–71 mm TL) were observed from March through September, with GSI ranging between 2.1% and 19.8%. Mean monthly GSIs were greatest in May (7%) and August (10%). Size distribution of oocytes taken from one female with mature ovaries per month indicated continuous recruitment of oocytes (Fig. 3).
The maximum size of a vitellogenic oocyte was 2.66 mm. Batch fecundity ranged from 1 to 131 late vitellogenic oocytes (diameter, 2.0–2.66 mm) among 45 females with mature ovaries.

Length measurements were taken from 502 Plains Killifish \((n = 420 \text{ from Pease River; } n = 82 \text{ from North Wichita–Wichita River})\). The Plains Killifish population consisted of at least 3 estimated age groups: age-0, age-1, and age-2 (Fig. 4). Age-0 fish were first observed in May 2016 and reached a mean length of 37 mm TL \((±3 \text{ mm})\) by December 2016 (i.e., end of age-0). Age-1 fish represented 96% of the adult population and were observed year-round. Age-2 fish represented 4% of the adult population and were observed from March through June 2016. With a maximum total length of 71 mm and few larger individuals for analysis, it is possible that some of the age-2 fish were older.

**DISCUSSION**

Our findings suggest that Plains Killifish invest energy in reproduction from March through September, produce multiple batches
of oocytes (maximum batch fecundity, 131), become reproductively active at age-1, and have a life span of 2 or possibly 3 years. Variation in GSIs and in percent frequencies of ovarian stages within the reproductive season suggest that Plains Killifish reproduction could be influenced by local environmental factors (e.g., streamflow) or by shifts in age structure through time (i.e., lack of older fish in field collections after June) (Durham and Wilde 2008). Length of reproductive season reported herein is similar to that reported by Echelle (1970; March–October) for Plains Killifish. Length of spawning season is longer than reported by Bonham (1962) for the Northern Plains Killifish (i.e., June–August). However, differences in length of spawning season between Plains Killifish taken from the Red River at lower latitudes and Northern Plains Killifish taken from streams in Kansas and Missouri at higher latitudes could be environmental and attributed to latitudinal differences among study sites. Length of spawning seasons are shorter at higher latitudes among minnows (Cyprinidae; Gotelli and Pyron 1991), corresponding with latitudinal differences in photoperiod for gonadal recrudescence and temperature for reproductive termination (Hubbs 1985). Otherwise, age of sexual maturity, life span, and maximum batch fecundity are similar between Plains Killifish and Northern Plains Killifish.

Extant members of the subgenus Plancterus (i.e., Plains Killifish and Northern Plains Killifish) are similar in reproductive and life history characteristics to members of the closely related subgenera Wileyichthys and Zygonectes. California Killifish *F. parvipinnis* in subgenus Wileyichthys, the sister lineage of Plancterus (Ghedotti and Davis 2013), are multiple batch spawners with a reproductive season spanning 6 months (i.e., April–September), maturity at

![Graph of oocyte size distribution](image-url)

**Fig. 3.** Percent frequency of oocyte size in mature ovaries of Plains Killifish (*Fundulus zebrinus*) taken from May 2016 through September 2016 (one female sampled per month).
Members of subgenus *Zygonectes*, specifically Golden Topminnow *F. chrysotus* (Foster 1967, Pflieger 1975), Blackstripe Topminnow *F. notatus* (Carranza and Winn 1954, Nieman and Wallace 1974), Blackspotted Topminnow *F. olivaceus* (Blanchard 1996, Moriarty and Winemiller 1997), and Starhead Topminnow *F. dispar* (Taylor and Burr 1997), are multiple batch spawners with spring to summer reproductive seasons and batch fecundity estimates of 10–30, maturity by age-1, and a life span of 2–3 years. Reproductive and life history trait conservatism appears strong among members of *Plancterus*, *Wileyichthys*, and *Zygonectes*, which is surprising given that lineages of the 3 subgenera diverged at least since the middle Pleistocene (Bernardi et al. 2007, Ghedotti and Davis 2013) and are widely distributed among diverse habitats within North America. Traits are similar among members that inhabit a wide range of chemical and physical habitats, including fresh and salt waters in shallow bays and salt marshes of California and Mexico (California Killifish; Fritz 1975), arid and prairie streams of the Great Plains (Plains Killifish and Northern Killifish), and low-gradient streams throughout the Mississippi River basin and coastal drainages of the Gulf of Mexico and the Atlantic Ocean (collectively, Golden Topminnow, Blackstripe Topminnow, Blackspotted Topminnow, and Starhead Topminnow; Ghedotti and Davis 2013).

Currently, the Plains Killifish is listed as a species of least concern (NatureServe 2013), with abundances ranging from rare (i.e., up to 4% in relative abundance) to frequent (i.e., up to 49% in relative abundance) within their native range (Ostrand and Wilde 2002, Faucheux et al. 2019, Pfaff 2019, Ruppel 2019). Population declines are reported in the Pecos River of Texas, possibly because of competition with nonnative Gulf Killifish *F. grandis* (Cheek and Taylor 2016), a member of subgenus *Fundulus*. Despite evidence of population declines in the Pecos River and in the Colorado River basin (Pfaff 2019), Plains Killifish are moderately ranked in estimates of redundancy, resiliency, and representation within their native and nonnative ranges of the Chihuahuan Desert and the Edwards Plateau ecoregions of Texas (Faucheux et al. 2019). Nevertheless, water quantity is predicted to decrease and salinization to increase, corresponding with decreases in surface water quantity within the southern Great Plains,

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**Fig. 4.** Estimated total lengths (black circles; mean ± SD) for age-0, age-1, and age-2 Plains Killifish (*Fundulus zebri-nus*) taken monthly from February 2016 through January 2017, calculated from a modal progression analysis. White circles represent total lengths (mean ± SD) taken from ≤3 individuals within an age group per month. Dashed line indicates the transition into the next age class.

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which are attributed to natural and anthropogenically accelerated climate change (Covich et al. 1997). Members of the southern Great Plains fish communities, specifically broadcast spawning cyprinids with life history and reproductive traits similar to those of the Plains Killifish (e.g., short lived, protracted spawning season, multiple batch spawner), might be negatively affected with decreases in water quantity (Wortthington et al. 2018) and increases in surface water salinity (Hoagstrom 2009), which could result in Plains Killifish being similarly affected. Alternatively, the substrate spawning reproductive strategy (Bonham 1962) and tolerance to salt water of Plains Killifish might allow them to persist longer in low-flow, fragmented, and saline waters; this persistence may drive diversification similar to the arid region diversification and increases in surface water salinity (Hoagstrom 2009), which could result in Plains Killifish being similarly affected. Alternatively, the substrate spawning reproductive strategy (Bonham 1962) and tolerance to salt water of Plains Killifish might allow them to persist longer in low-flow, fragmented, and saline waters; this persistence may drive diversification similar to the arid region diversification of another salt-tolerant and distantly related lineage, Cyprinodon (Echelle et al. 2005).

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