

FINAL REPORT

As Required by

THE ENDANGERED SPECIES PROGRAM

TEXAS

Grant No. TX E-115-R

F09AP00241

Endangered and Threatened Species Conservation

**An evaluation of the relationship between stream flow and habitat availability  
for the Devils River minnow *Dionda diaboli*.**

Prepared by:

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27 May 2014

INTERIM REPORT

STATE: Texas GRANT NUMBER: TX E-115-R

GRANT TITLE: **An evaluation of the relationship between stream flow and habitat availability for the Devils River minnow *Dionda diaboli*.**

REPORTING PERIOD: 1 Sep 09 to 28 Nov 13

**OBJECTIVE(S):**

To assess the instream flow needs of the Devils River Minnow in one of the streams within the critical habitat designation over three years.

**Segment Objectives:**

The following tasks will be performed on representative reaches of Pinto Creek:

- a. Bathymetric mapping.
- b. Detailed, georeferenced maps of substrate, instream cover, and other relevant channel features will be developed.
- c. A two-dimensional hydraulic model will be developed.
- d. Habitat Utilization.
- e. Quantify patterns in life history and reproductive biology of the Devils River minnow.
- f. Data on existing stream habitat, habitat utilization, and hydraulic modeling results will be integrated and a range of alternatives presented.

**Significant Deviations:**

As described in the justification to extend Final Report deadline, and as mentioned in the 2013 Interim Report, unforeseen difficulties in completing a portion of the work had hampered completion of the Final Report, and continue to do so. We are submitting results on the completed task here: Task D was addressed in the attached manuscript, entitled, "Response of Devils River minnow and other fish in Pinto Creek, Kinney County, Texas during a severe drought," accepted for publication in Southwestern Naturalist; and, Task E which was addressed in the attached manuscript (being prepared for publication), entitled, "Reproductive and feeding ecology of two sympatric *Dionda* (Cyprinidae) in the Rio Grande Basin, Texas." Tasks A (bathymetric mapping), C (development of a two-dimensional hydraulic model) and F (data integration) are part of the hydraulic modeling which was subcontracted to Dr. Thomas Hardy who had suffered, at the time of the final report extension request, and as of this report, still suffers from debilitating medical problems related to back surgery. Dr. Hardy has made some progress on the remaining tasks for this project and we hope to have the full results as soon as possible which will then be forwarded to Region 2 to fully comply with satisfying the objective of this grant.

**Summary Of Progress:**

Please see Attachment A.

**Location:** Val Verde and Kinney Counties, Texas.

**Cost:** Costs were not available at time of this report, they will be available upon completion of the Final Report and conclusion of the project.

**Prepared by:** Craig Farquhar

**Date:** 27 May 2014

**Approved by:**  **Date:** 27 May 2014  
C. Craig Farquhar

**ATTACHMENT A**

**Response of Devils River Minnow and other fish in Pinto Creek, Kinney  
County, Texas during a severe drought  
(Addresses Task D)**

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ABSTRACT--We collected Devils River Minnow (*Dionda diaboli*) from an isolated population in the headwaters of Pinto Creek (Kinney County) during 2009-2011. We seined, and collected water quality and habitat data, at 19 sites to learn where *D. diaboli* occurred, its relative abundance, and its fish and habitat associations. We captured 8,619 fish of eleven species. The dominant species were *Gambusia speciosa* (54%), *Astyanax mexicanus* (28%), *Herichthys cyanoguttatus* (5%), and *Micropterus salmoides* (4%). *Lepomis* spp. made up 5% of the fish captured. Densities of *D. diaboli* were lower than historical. We captured 105 adult and 100 juvenile *D. diaboli*. The headwaters were a series of disjunct habitats for much of the period of study due to drought. We collected *D. diaboli* at seven locations: six pools and one run just upstream of one of the pools. All fish were found in locations with thick *Chara* sp. and low flow (<0.1 m/s). It is unclear whether our data reflect habitat preference or contraction of this population into the available habitat. By July 2011, because of continued severe drought conditions, the headwaters where our study sites were located were reduced to two pools. One pool had a surface area of about 30 m<sup>2</sup>; the other had a surface area of about 860 m<sup>2</sup>. These remaining pools were shallow (~0.5 m), turbid, and had no vegetation. We did not find *D. diaboli* within either pool. The current range contraction places *D. diaboli* at higher risk. It will be important to see if *D. diaboli* can persist downstream and naturally re-establish once conditions improve.

RESUMEN--Colectamos especímenes de Devils River Minnow (*Dionda diaboli*) de una población aislada en la cabecera de Pinto Creek (condado de Kinney) durante 2009-2011. Muestreamos con redes de cerca y recolectamos datos de calidad de agua y hábitat de 19 localidades para aprender donde ocurre *D. diaboli*, su abundancia relativa y sus asociaciones con peces y hábitat. Capturamos 8.619 peces de once especies. Las especies dominantes fueron *Gambusia speciosa* (54%), *Astyanax mexicanus* (28%), *Herichthys cyanoguttatus* (5%), y *Micropterus salmoides* (4%). *Lepomis* spp. compusieron 5% de los peces capturados. Densidades de *D. diaboli* fueron menores a los históricos. Capturamos 105 adultos y 100 juveniles de *D. diaboli*. Las cabeceras formaron una serie de hábitats desconectados durante la mayoría del período de estudio debido a las condiciones de sequía. Capturamos *D. diaboli* en siete localidades: seis remansos y un corrido justo arriba de una de los remansos. Todos los peces fueron encontrados en áreas de flujo bajo (<0.1 m/s) y alta densidades de *Chara* sp. No está claro si nuestros datos reflejan la preferencia de hábitat o una contracción de esta población en el hábitat disponible. Ya para julio del 2011, debido a las condiciones de sequía continuas, la cabecera donde se encontraban nuestras localidades de estudio fue reducida a dos remansos. Un remanso tenía una superficie de unos 30 m<sup>2</sup>; el otro tenía una superficie de aproximadamente 860 m<sup>2</sup>. Éstos remansos restantes eran poco profundos (~0.5 m), turbio, y sin vegetación. No encontramos *D. diaboli* dentro de estos remansos. La contracción actual de hábitat pone a *D. diaboli* en alto riesgo. Sera importante ver si *D. diaboli* logra persistir aguas-abajo y restablecerse naturalmente una vez las condiciones mejoren.

Spring systems throughout Texas are at risk and with them the unique endemic species that rely on them (Edwards et al., 1989; Bowles and Arsuffi, 1993). Of particular concern are the biota and spring ecosystems within the Edwards Plateau Region of Texas

where the majority of the state's threatened aquatic ecosystems occur (Edwards et al., 2004; Bowles and Arsuffi, 1993). Long-term climactic drying of the Edwards Plateau is evident (Toomey III et al., 1993). In addition, anthropogenic activities have caused many small springs and streams, as well as three of the largest springs in the Edwards Plateau, to become dry or experience significant decreases in flow (Bowles and Arsuffi, 1993). One system of particular concern is the Pinto Springs complex and the creek which they feed, Pinto Creek. The entire fish assemblage in Pinto Creek including a population of the endemic Devils River Minnow (*Dionda diaboli*, Cyprinidae) are at risk due to dewatering and reduced streamflow (Hubbs, 1995).

*Dionda diaboli* occupies limited geographic range and is imperiled throughout its range due to the threat of habitat loss (Garrett et al., 2004; Scharpf, 2005; USFWS, 2005). It is federally listed as threatened in the U.S (USFWS, 1999) and endangered in Mexico (CONABIO, 2002). Although a previous study (Garrett et al., 2004) suggested that Pinto Creek had a robust population of *D. diaboli*, a recent investigation indicates the population size is considerably lower than the historic average (Hanna 2011). In addition, genetic analyses suggest the Pinto Creek population is genetically distinct from all other known populations (Hanna 2011). Natural repopulation following extirpation would be unlikely because populations are fragmented by many uninhabited kilometers of river (Garrett et al., 1992; 2004). Further, were repopulation to occur, it would result in the loss of a genetically distinct form of this species.

Current knowledge of habitat requirements is insufficient to allow predictions of how the Pinto Creek population of *D. diaboli* might respond to changing water quantity and quality as reports of basic habitat requirements (Harrell, 1978; Cantu and Winemiller, 1997; Kollaus and Bonner, 2012) are somewhat conflicting. Until recently, the presumed habitat requirements of *D. diaboli* have been aquatic macrophytes (e.g., *Justicia americana*) associated with fast-flowing, spring-fed, gravel-cobble channels (Harrell, 1978; Cantu and Winemiller, 1997). Hubbs and Garrett (1990) suggested that *D. diaboli* most often occur where spring flow enters a stream, rather than in the spring outflow itself. These accounts suggest a requirement for flowing water in or near spring outflows. In contrast, Kollaus and Bonner (2012) found that in the Devils River, *D. diaboli* more frequently inhabited deeper pools rather than swift-flowing water. Further, *D. diaboli* seemed more eurythermic than previously reported. The study by Kollaus and Bonner (2012) indicates less dependence on flowing water, which may be critical for persistence during drought. Aside from temperature and flow, other water quality needs have been noted (Edwards, 2003; Robertson and Winemiller, 2003; Garrett et al., 2004; Phillips et al., 2011; Kollaus and Bonner, 2012), but poorly explored.

It is imperative that we understand how the species within this creek, particularly *D. diaboli*, respond to reduced stream flow and dewatering. Burka (2004) suggests that San Antonio, Texas and other metropolitan areas will seek additional water sources to support their growing populations. One potential source is the aquifer that supplies Pinto Creek (Anonymous, 2002; Mace and Anaya, 2004) and additional groundwater pumping may place added stress on this system. Currently the relationship between the quantity and quality of habitat in Pinto Creek and the response of *D. diaboli* and the associated fish community is unknown. The unusually dry conditions associated with the extreme drought of 2009-2011 allowed us to assess the population response to an extreme dewatering event.

We collected habitat association data within identified study reaches to assess habitat utilization and population abundance of *D. diaboli* and changes in fish community structure with increased drying of the upper portions of Pinto Creek. We anticipate that understanding habitat utilization, changes in population abundance, and changes in the fish community structure will allow for better management of Pinto Creek, as well as other *D. diaboli* populations.

**METHODS**--Pinto Creek is a spring-fed creek that was historically reported to originate about 15 (Bennett and Sayre, 1962) to 20 (Brune, 1981) km north of Brackettville in north-central Kinney County, TX. At the beginning of this study water began flowing considerably farther downstream, at the southwest and southeast Pinto Springs (as designated by Texas Water Development Board) complex, which are only about 11 km north of Brackettville, TX. From southeast Pinto Springs, Pinto Creek runs generally southwest for about 60 km where it joins the Rio Grande River. In Pinto Creek, *D. diaboli* only occurs in the upper 16 km above TX Hwy 90 (Garrett et al., 2004). Our study sites were located within the upper 4.5 km of Pinto Creek downstream from the Pinto Springs complex. This is within the area designated as critical habitat for *D. diaboli* (Federal Register, 12 August 2008: 50 CFR Part 17: Endangered and Threatened Wildlife and Plants; Designation of Critical Habitat for the Devils River Minnow, Final Rule).

Within this 4.5 km section we chose six sample reaches (Uppermost sample site: N 29°24'38", W 100°26'53"; lowermost sample site: N 29°24'04", W 100°29'00"). Within each reach we delineated three sites: one in each of three macrohabitat types (i.e., run, riffle, and pool). We sampled quarterly (January, April, July, and October) from October 2009 - August 2011 to measure the temporal stability of the system. We used multiple reaches to measure the spatial extent of the habitat and fish, and the segregation into macrohabitats to examine the habitat use. In addition, the spatial scale allowed for contrast in both the habitat and fish assemblage. We estimated the total area of each macrohabitat at each site using a range-finder to measure the overall dimensions from several spots. Within each macrohabitat, we collected a suite of habitat attributes (i.e., water quality and physical habitat measures) and fish. Water quality parameters were collected using a YSI® Model 556 and consisted of temperature, dissolved oxygen, conductivity, and pH. We also measured flow velocity (cfs; FlowMate® Model 2000), maximum depth (m), as well as visually estimated the percentage of substrate type and vegetation cover. Substrate categories followed a modified Wentworth scale (Wentworth, 1922): silt, sand, pebble, gravel, cobble, boulder, and bedrock. Vegetation cover was measured as cover class (Daubenmire, 1959).

We used 3-m and 5-m seines with 5-mm to 6-mm mesh to collect fish. Because Pinto Creek is extremely clear, we often saw and noted larger fish. Observed fish were not used for density estimation, but were used in noting the presence of species that escaped seine samples. We typically used three seine subsamples to characterize a site; except those sites that were too small to allow for non-overlapping subsamples. All *D. diaboli* were classified as either adult ( $\geq 30$  mm TL; Hulbert et al., 2007) or juvenile, counted, and released. We identified all other fish to species, with the exception of juvenile sunfish, which were classified as *Lepomis* spp. In addition to the six established reaches, we collected opportunistically at several other sites. For these, we were only interested in whether *D. diaboli* were present, and stopped sampling once they were collected. The area

seined was recorded for each seine haul. Fish collections were pooled by macrohabitat within each reach.

**METHODS--Data Analysis--**We standardized collections by dividing the number of individuals collected by the area seined in each seine haul. We used Primer-e (Version 6.1.15 PRIMER-E Ltd., Plymouth, UK) to examine several multivariate habitat and fish species composition questions. We used the SIMPER procedure to examine species differences and similarities among macrohabitats and BEST procedure to understand which environmental variables structured the biological assemblage. Environmental data were normalized before analysis. For species composition, we calculated Bray-Curtis similarity indices based on both fourth-root transformed standardized abundance and presence-absence to reduce the influence of high-abundance species or samples.

In addition to the multivariate community perspective, we also used various univariate techniques to answer specific questions associated with *D. diaboli*. A chi-square approach (Zar, 1984) was used to test whether *D. diaboli* were randomly distributed among the three macrohabitats. We used the Kolmogorov-Smirnov (Zar, 1984) approach to test whether there were significant differences in habitat attributes (i.e., water quality and physical habitat measures) where *D. diaboli* were observed compared with where they were not observed. The detection probability estimates the likelihood of capturing *D. diaboli* when they were present. Only those sites in which we observed *D. diaboli* in at least one of the seine subsamples were used. Detection probability was defined as the proportion of the seine subsamples in which *D. diaboli* were detected, given we knew they were present. Because the detection probability is essentially a ratio estimator, we estimated the detection probability as the slope of a weighted least-squares regression of the number of detections against the number of seine subsamples (Scheaffer et al., 1990). For all common species, we examined temporal changes in catch rates associated with changing conditions within the creek using locally-weighted scatterplot smoothing (Cleveland and Devlin, 1988). Statistical tests were declared significant at  $\alpha = 0.05$ .

**RESULTS--** Texas was in drought conditions for most of the two years of this project, and for much of 2011 Pinto Creek was in an area of extreme to exceptional drought (Brian Fuchs, Climatologist, National Drought Mitigation Center, pers. comm., 2011). Whereas the sparse historical record (Bennett and Sayre, 1961) suggests that headsprings would have existed within and above Reach 1 (i.e., northwest and northeast Pinto Springs), for most of the study spring flow originated just above Reach 2 (i.e., southwest and southeast Pinto Springs). Water levels were quite variable throughout the study period. The upper portion of Pinto Creek where this study occurred was discontinuous in both early and later parts of the study. Following rains in spring and summer of 2010, stream flow increased and the upper section of Pinto Creek was completely connected. Much of the creek was about 10 m in width, but ranged from runs <1 m to pools up to 43m wide. The creek was characterized by riffles with gravel, cobble, and bedrock bottoms, shallow runs with mixed bottoms, often followed by larger pools with silt bottoms (Table 1). Submergent and emergent aquatic vegetation was prevalent throughout Pinto Creek. The most common aquatic vegetation species encountered were *Justicia americana*, which was ubiquitous, and *Chara* sp., which was found predominantly in pools. Although vegetation was killed during dry periods, once the creek filled following spring and summer rain events in 2010, aquatic vegetation re-established very

rapidly, resulting in a significant increase in potentially useable habitat. We were able to sample all sites in July and October 2010 (Table 2). Because no significant rain occurred for the remainder of the project, the number of sites and types of macrohabitats sampled decreased as the creek dried. By January 2011 run and riffle habitat existed in only one of the upper reaches. By April 2011, none of the outlets of Pinto Springs were flowing, and only pool habitats remained. By the final sample (July 2011), all study sites were completely dry with the exception of two isolated pools. These remaining pools were shallow (~0.5 m), turbid, and contained no vegetation. The smaller pool had a surface area of about 30 m<sup>2</sup>; the larger pool had a surface area of about 860 m<sup>2</sup>.

Overall, 19 unique sites were sampled over the two-year period of study. We completed over 200 seine hauls, capturing 8,619 fish of 11 species. The dominant fish species were *Gambusia speciosa* (54%), *Astyanax mexicanus* (28%), *Herichthys cyanoguttatus* (5%; formerly *Cichlasoma cyanoguttatum*), *Micropterus salmoides* (4%), *D. diaboli* (2%), and *Notropis amabilis* (2%). *Lepomis* spp. made up 5% of the fish captured. Large *H. cyanoguttatus*, *M. salmoides*, and *Ictalurus punctatus* were routinely observed but not collected. Two primary temporal patterns emerged. *Dionda diaboli*, *M. salmoides*, *H. cyanoguttatus*, and *A. mexicanus* showed quite stable populations throughout the two years with a small increase in numbers during the middle portion of the sampling following rains that refilled the creek (Figure 1). *Gambusia speciosa*, *N. amabilis*, and *Lepomis* spp. were quite stable, but showed increasing populations towards the end of the two years (Figure 1) as the creek contracted and became predominantly stagnant pools (Table 2).

Overall, species segregation was significantly related to environmental variables ( $Rho = 0.225$ ,  $P = 0.01$ ). The variables which were most influential were temperature, DO, flow, and cover class. The SIMPER analysis on the presence-absence data showed we most frequently found centrarchids (*M. salmoides* and *Lepomis* spp.) in pools, sometimes found in runs, but never found in riffles (Table 3). Likewise, we never captured *D. diaboli* or *N. amabilis* within riffle habitat. We frequently found *H. cyanoguttatus* in pools and runs, but rarely in riffles, whereas *G. speciosa* and *A. mexicanus* were ubiquitous throughout. The SIMPER analysis on the fourth-root transformed data had very similar conclusions. *Micropterus salmoides* and *Lepomis* spp. were most abundant in pools, were less abundant in runs, but were never found in riffles. *Herichthys cyanoguttatus* were equally abundant in pools and runs, but were rare in riffles. *Gambusia speciosa* and *A. mexicanus* were abundant throughout, though least abundant in riffles.

There was strong evidence ( $P(\chi^2 > 26.99) < 0.001$ ,  $df = 2$ ) to suggest that *D. diaboli* were disproportionately found in pool macrohabitats (Tables 1 and 2), rather than riffles or runs. Physical habitat data showed that *D. diaboli* were most frequently found in water about 1m deep, with thick vegetation, and no flow (Figure 2). The K-S tests suggested there were significant differences between where *D. diaboli* were and were not found for these three variables (Figure 2): depth ( $D_{max} \geq 0.51$ ;  $P < 0.007$ ), vegetative cover ( $D_{max} \geq 0.46$ ;  $P < 0.003$ ), and flow ( $D_{max} \geq 0.39$ ;  $P < 0.02$ ). There was no significant difference found for temperature ( $D_{max} \geq 0.25$ ;  $P = 0.33$ ). However, the distributional plot of temperature (Figure 2) shows that the range of temperatures where *D. diaboli* were located was substantially broader (i.e., 15.1–27.9°C) than the temperature range just below the spring outfall (i.e., 20.8–25.0°C).

*Dionda diaboli* were found in less than 20% of the available reaches and macrohabitats (Tables 1 and 2) but there were no obvious patterns in DO or conductivity that discriminated presence from absence (Table 3). Adult and juvenile *D. diaboli* were captured in similar numbers: 105 adults and 100 juveniles. We observed juvenile *D. diaboli* only during the first spring, most frequently in pool habitats with silt bottoms. We observed range expansion and contraction of *D. diaboli* associated with changes in connectivity and inundation (Table 2) of the creek. It appeared that at least a few *D. diaboli* used increasing connections to move into new areas; however, we saw no evidence that these populations were able to increase in abundance or persist. We estimated detection probability at 64% (SE=6%).

DISCUSSION—The extreme drought conditions and a single significant rain event that occurred during the course of this study allowed us to document the expansion and contraction of water and habitat and the response of the fish community in Pinto Creek. Flow is the primary driver in many riverine ecosystems and changes in flow can affect all aspects of a species life history (Poff et al., 1997; Bunn and Arthington, 2002; Gido et al. 2013). Most species in Pinto Creek had increased densities in response to increasing water levels following the rains midway through the study; a common response for desert fish (Balcombe and Arthington, 2009; Gido et al. 2013) to exploit new habitat. Because species increased simultaneously, species ranks varied little throughout the study. This is a routinely documented phenomenon in streams undergoing natural fluctuations (Ross et al., 1985; Matthews et al., 1988, Anderson et al., 1995; Hargrave and Taylor 2010). However, as flow declined and the creek was reduced to only stagnant pools, three species (*Gambusia speciosa*, *Notropis amabilis*, and *Lepomis* spp.) benefited, whereas the rest declined. These changes in relative and absolute abundance as the creek became dewatered were likely associated with changes in habitat, food resources (Bunn et al., 2006; Balcombe et al. 2005; Sternberg et al. 2008) and water quality (Magoulick and Kobza, 2003; Arthington et al., 2005). These findings suggest that the species within Pinto Creek are quite resilient and are able to persist through routine droughts. However, as water quantity and quality decline, only the most tolerant species remain.

Our study showed quite different absolute and relative fish abundances than did a previous study where flows were considerably higher (Garrett et al. 2004). In contrast to Garrett et al. (2004), adult *D. diaboli* densities in Pinto Creek were quite low, suggesting declines in relative and absolute abundance. By the end of the study, most of our sample sites were dry and no *D. diaboli* could be found in the remaining pools despite quite high detection probability. Garrett et al. (2004) found that *D. diaboli* were the third-most abundant species (10%) in the section of Pinto Creek upstream of the Texas State Highway 90 bridge, after Mexican Tetra (*A. mexicanus*) and Tex-Mex Gambusia (*G. speciosa*). In contrast, we found that *D. diaboli* adults were only 1% of the sample. Both Rio Grande Cichlid (*H. cyanoguttatus*) and Largemouth Bass (*M. salmoides*) were found in low numbers in 2004, yet were quite abundant throughout our sampling. In a single collection, Garrett et al. (2004) collected 457 *D. diaboli*, with single sites yielding as many as 200 individuals. They captured approximately 4,300 individual fish in the section of Pinto Creek above the Texas State Highway 90 bridge. In actual abundance, we collected only 105 adult *D. diaboli* across the entire study while capturing 8,619 individual fish. Although it is unclear whether the conditions in 2004 could be classified as typical, there

should be little doubt that densities of *D. diaboli* were substantially lower in 2009-2011 than previously documented in 2004.

Current ideas about the habitat needs of *D. diaboli* should be expanded. Whereas it has been common to suggest that these minnows are associated with channels of fast-flowing, spring-fed water over gravel substrates (Harrell, 1978; Cantu and Winemiller, 1997), work in the Devils River (Kollaus and Bonner, 2012) suggested *D. diaboli* more frequently inhabited deeper pools and were frequently found in backwater habitats with extreme temperatures. Our data confirmed that *D. diaboli* can use still-water habitats with heavy vegetation, silt bottoms, and fluctuating temperatures, at least temporarily in times of low to no stream flow. In the current study, the majority of *D. diaboli* were collected in pool habitats that exhibited these conditions. Garrett et al. (1992) also documented use of drying, stagnant pools in Sycamore Creek in 1988-89 during a period without spring flow. Within the pools where we collected *D. diaboli*, they appeared to show an affinity to *Chara* sp. rather than *Justicia americana* as reported by Garrett et al. (2004), a common emergent ubiquitous throughout Pinto creek. We suspect *D. diaboli* utilized this alga both for cover from predators and as a substrate for foraging. These findings suggest *D. diaboli* are well adapted for protracted periods without any observable spring flow.

We found evidence of reproduction (i.e., presence of juveniles) in pool habitats, suggesting the possibility of the population persisting without flow across generations. However, the lack of documented reproduction in the second year of this study (spring 2011) is cause for concern. Gibson et al. (2004) found, in a hatchery setting, that *D. diaboli* could successfully reproduce under a variety of situations; however, gravel seemed to be a preferred substrate. Each of the pools where we found *D. diaboli* had predominantly silty bottoms, but significant levels of submerged vegetation. Reproduction that was observed in pools with silt bottoms might have occurred in isolated patches of gravel or within the heavy vegetation. Phillips et al. (2011) suggested that *D. diaboli* can use the discarded nests of largemouth bass when appropriate substrate is limited. This would be possible, as pools where *D. diaboli* were found contained largemouth bass as well as other centrarchids. Further, although some portion of the abiotic niche necessary for reproduction has been defined (Gibson et al., 2004; McMillan, 2011), because we saw evidence of reproduction in only one of the two years, we think future research needs to determine the suite of abiotic conditions (e.g., conductivity, pH, flow) necessary for reproduction in Pinto Creek and other systems.

*Dionda diaboli* are quite tolerant to a range of water quality conditions, which gives them the ability to sustain periods of drought. Water quality, especially temperature and dissolved solids, can affect individuals, populations, and fish communities (Baltz et al., 1982; Linam and Kleinsasser, 1996; Dunson and Travis, 1991; Arthington et al., 2005). Sub-optimal conditions can reduce overall performance (Fry 1967), including swimming and spawning (Reynolds 1977). Previous studies (Lopez-Fernandez and Winemiller, 2005; Fries and Gibson, 2010; Kollaus and Bonner, 2012) showed *D. diaboli* were found in water temperatures from 18-31°C, and having thermal maxima of 32.6–37.8°C. These thermal maxima are quite similar to *Lepomis* spp., *Gambusia affinis*, *Cyprinella lutrensis*, and *M. salmoides* (Carveth et al., 2006), fish typically considered to be generalists. Whereas thermal regimes seem quite well defined, conductivity is less so. Information from Edwards (2003) and Phillips et al. (2011) suggests previous studies have found *D.*

*diaboli* at conductivities between 0.40–0.48 mS/cm, values in the middle of the range (i.e., 0.30–0.53 mS/cm) where we found *D. diaboli*. It should be noted that both reports erroneously used  $\mu\text{S}/\text{cm}$  for the conductivity units when the units should have been mS/cm (R. Edwards, pers. comm.; R. Gibson, pers. comm.). Garrett et al. (2004) suggest that *D. diaboli* might not be found downstream of State Highway 90 because of changes in water quality. This study and others suggest that neither temperature nor conductivity is limiting expansion. We suggest lab-based studies be conducted to define acute and chronic limits to various water quality metrics for *D. diaboli*.

The loss of water in the upper 4.5 km of Pinto Creek has resulted in a range contraction of *D. diaboli*, which leaves this population more at risk. When the amount of water in the creek increased, we observed *D. diaboli* in areas where we had not previously seen them. As water levels dropped, individuals persisted in isolated pools. However, it is not possible to say if these pools were preferred habitats or areas of subsistence during extreme conditions. Fish routinely move from preferred habitat to refugia during drought conditions (Magoulick and Kobza, 2003; Rayner et al., 2009). It remains unknown whether *D. diaboli*, which have a life span of 1–2 years (USFWS, 2005), can endure long-term reliance upon pools. By the end of this study, the entire study area was reduced to two small pools with 900 m<sup>2</sup> of surface area with no *D. diaboli* observed. In addition to drought, pumping for irrigation can cause both seasonal and long-term reductions in spring flow (Brune, 1981; Hutchison et al., 2011; Shi et al. 2012). Hence, decreases in water quantity caused by prolonged drought conditions (several years) or pumping could lead to significant decreases in abundance and genetic variability (Hanna 2011), and increase the extinction risk (Taylor and Warren, 2001) of *D. diaboli* in Pinto Creek. Following completion of this study, in September 2011 and July 2012 we sampled several isolated pools approximately 7.5 km downstream from our study area (which by then was completely dry) and captured over 200 *D. diaboli*. Significant physical barriers exist between these remaining populations of *D. diaboli* and the upper reaches of Pinto Creek. These barriers may curtail the natural dispersion and recolonization abilities that this species has demonstrated in other systems (Kollaus and Bonner, 2012). It will be important to see if *D. diaboli* can persist downstream and naturally re-establish once conditions improve.

Available habitat is not the only potential issue facing *D. diaboli*. During our surveys, we found *Melanoides tuberculatus*, an exotic snail, in Pinto Creek. *Melanoides tuberculatus* is a vector for a trematode (*Centrocestus formosanus*) known to infest the gills of cyprinids, including *D. diaboli* (McDermott, 2000; Fleming, 2002; Mitchell et al., 2005; Fleming et al., 2011). This snail has been known to be present in several other west Texas spring systems; however, we know of no previous reports within Pinto Creek. We examined several Texas Shiners and found heavy infestations of the fish by the parasite. This finding is cause for concern because this trematode has the potential to infect *D. diaboli* and might further reduce the viability of this population. Fortunately, as of September 2011, all areas where *M. tuberculatus* had been documented were dry. At this time, we are unsure if a source population of *M. tuberculatus* persists.

During this study it became evident that the surface water hydrology of Pinto Creek was poorly understood. The creek was routinely discontinuous. It was unclear whether persisting large pools within the creek were being fed by undocumented springs or by

subsurface flow from documented springs. The stream is ungaged and the information on historic spring inputs is poorly documented or anecdotal. Groundwater models produced by the Texas Water Development Board suggest that persistent flows within Pinto Creek are unlikely without intervention (Hutchison et al., 2011). These models also show that groundwater extraction to supply cities outside Kinney County has a high potential to end outflows from Pinto Springs (Hutchison et al., 2011; Shi et al., 2012), and thus threaten the future of the *D. diaboli* in Pinto Creek. Climate projections (Nielsen-Gammon, 2011; Jiang and Yang, 2012) suggest warmer temperatures. Whereas projections of precipitation vary (Nielsen-Gammon, 2011; Jiang and Yang, 2012), the overall prediction is for a drier climate due to increased evaporation and decreased recharge (Uddameri and Parvathinathan, 2007). The combined effects of increased water demand and decreased water supply are likely to further stress Pinto Creek (Hubbs 1995). Given the high likelihood of unstable surface flow and the apparent importance of persistent pools in maintaining the Pinto Creek population of *D. diaboli*, we suggest an evaluation of subsurface flow and connectivity among disconnected pools within the channel.

We thank K. Reeves, D. Buckmeier, and D. Mosier for creating the proposal that funded this project. We thank the landowners along Pinto Creek for granting us access. G. Garrett provided introductions to landowners and to U. S. Fish and Wildlife Service personnel. He and several scientists at Texas Parks and Wildlife Department Heart of the Hills Fisheries Science Center reviewed the design, analysis, and writing, and provided constructive comments on previous drafts of this manuscript. We appreciate their help, along with T. Bonner and K. Kollaus, in improving this manuscript. The paper was further improved through comments from two anonymous reviews and the editorial staff. Thanks go to M. de Jesus for helping us with the Spanish translation of the abstract. Partial funding was provided by the US Fish and Wildlife Service through Section 6 of the Endangered Species Act.

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TABLE 1-- The distribution of samples within the various macrohabitats (i.e., run, riffle, pool) that did or did not contain *Dionda diaboli*, the median values of some of the physical characteristics, and the average substrate composition for macrohabitats within Pinto Creek.

	Macrohabitat		
	Pool	Riffle	Run
<i>Samples with D. diaboli</i>			
<i>Present or Absent</i>			
<i>D. diaboli</i> Absent	96	31	56
<i>D. diaboli</i> Present	41	0	2
<i>Physical Characteristics<sup>1</sup></i>			
Depth (m)	0.60	0.15	0.30
Velocity (ft <sup>3</sup> /s)	0.00	1.15	0.39
Cover class	5	3	6
<i>Type of Substrate</i>			
<i>(Average Percent)<sup>1</sup></i>			

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Silt	85	1	34
Sand	1	0	6
Pebble	1	2	5
Gravel	5	33	17
Cobble	2	55	27
Boulder	1	2	1
Bedrock	5	7	10

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<sup>1</sup> Within the pool macrohabitats, these characteristics reflect the areas that we sampled, and may not reflect the overall habitat.

TABLE 2—Availability of various macrohabitats in Pinto Creek throughout this study. An asterisk within a cell indicates that we sampled that macrohabitat, whereas empty cells indicate that a specific macrohabitat was absent on that date. Cells with darker shading indicate we collected at least one *Dionda diaboli* in that macrohabitat during that collection.

Reach	Macrohabitat	Date of Collection							
		Oct '09	Jan '10	Apr '10	July '10	Oct '10	Jan '11	Apr '11	July '11
1	Run				*	*			
	Riffle								
	Pool	*	*	*	*	*	*		
2	Run	*	*	*	*	*	*		
	Riffle	*	*	*	*	*	*		
	Pool	*	*	*	*	*	*	*	
3	Run	*	*	*	*	*	*		
	Riffle	*	*	*	*	*	*		
	Pool	*	*	*	*	*	*	*	
4	Run	*		*	*	*			
	Riffle								
	Pool	*	*	*	*	*	*	*	*
5	Run				*	*	*		
	Riffle				*				
	Pool	*	*			*	*	*	
6	Run				*	*			
	Riffle				*	*			
	Pool				*	*	*		
Total Surface Area of Sites Sampled (x 1,000m <sup>2</sup> )		9	6	9	21	17	9	3	0.9

TABLE 3--Average percent occurrence and density (fish per square meter) in run, riffle, and pool macrohabitats for fish within Pinto Creek. Fish species are *Micropterus salmoides* (Ms), *Lepomis* spp. (Ls), *Dionda diaboli* (Dd), *Notropis amabilis* (Na), *Herichthys cyanoguttatus* (Hc), *Gambusia speciosa* (Gs), and *Astyanax mexicanus* (Am).

		Average Percent Occurrence					
Macrohabitat	Ms	Ls	Dd	Na	Hc	Gs	Am
Pool	51	57	39	9	59	71	76
Riffle	0	0	0	0	8	62	92
Run	32	1	5	11	63	84	95
		Average Density (fish/m <sup>2</sup> )					
Macrohabitat	Ms	Ls	Dd	Na	Hc	Gs	Am
Pool	0.223	0.224	0.171	0.124	0.273	2.134	1.187
Riffle	0.000	0.000	0.000	0.000	0.013	0.561	0.535
Run	0.017	0.025	0.010	0.015	0.100	1.816	1.278

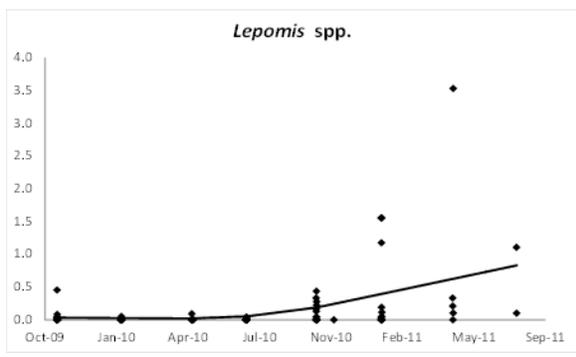
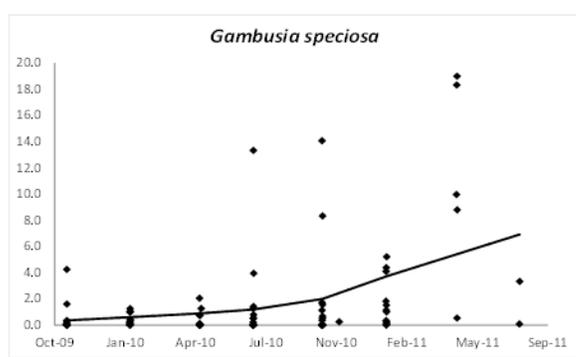
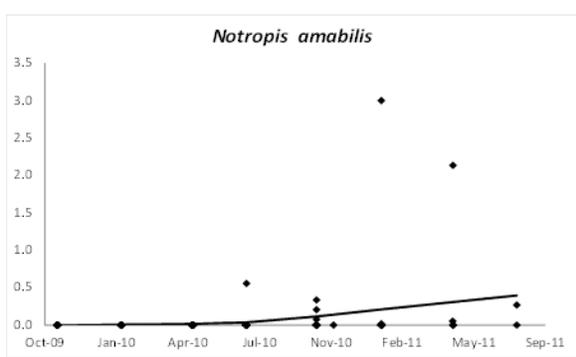
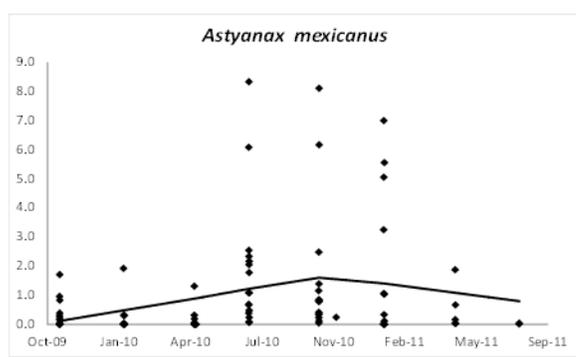
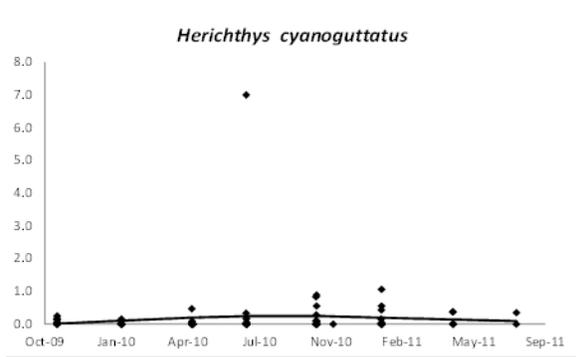
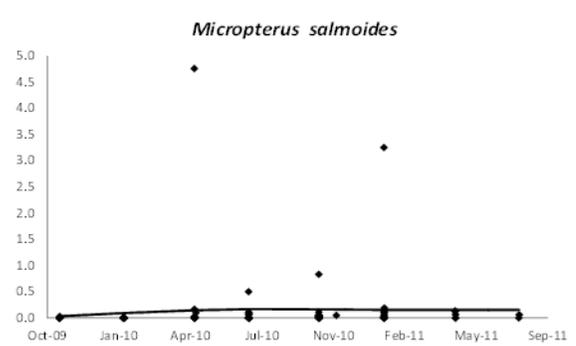
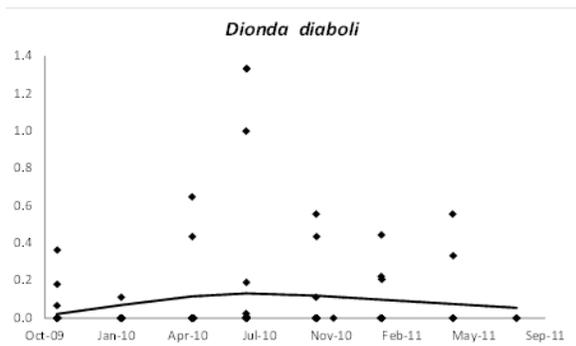
TABLE 4--Values for water quality variables, indicating the overall range as well as where *Dionda diaboli* were encountered. For these variables, statistical tests found no differences between where *D. diaboli* were and were not found.

Variable	Overall Range	Range Where Found
DO	(0.7 - 22.0)	(0.7 - 19.1)
Temp (C)	(10.9 - 29.6)	(15.1 - 27.9)
Conductivity (mS/cm)	(0.250 - 0.878)	(0.296 - 0.532)

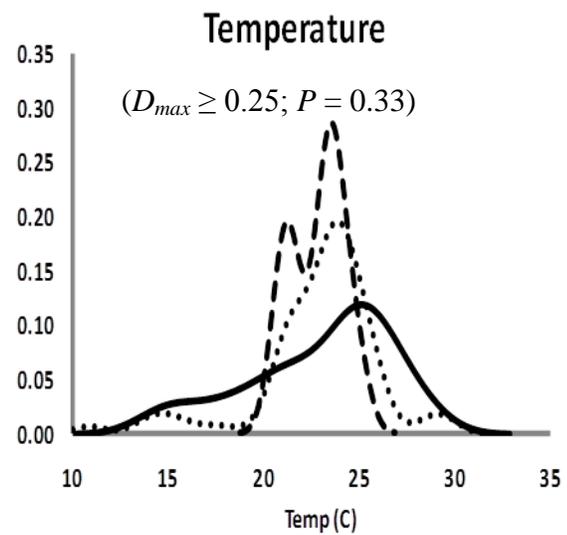
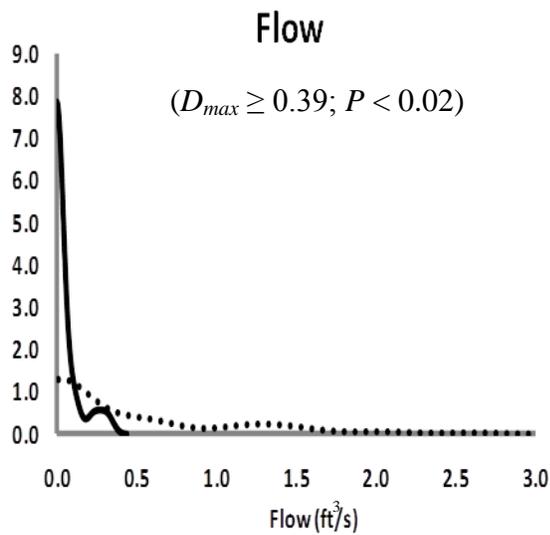
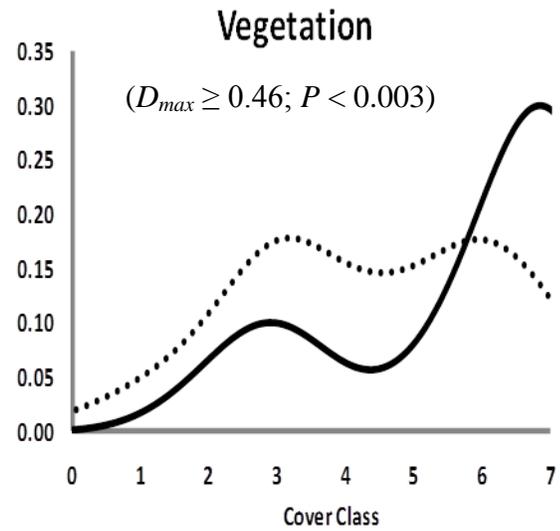
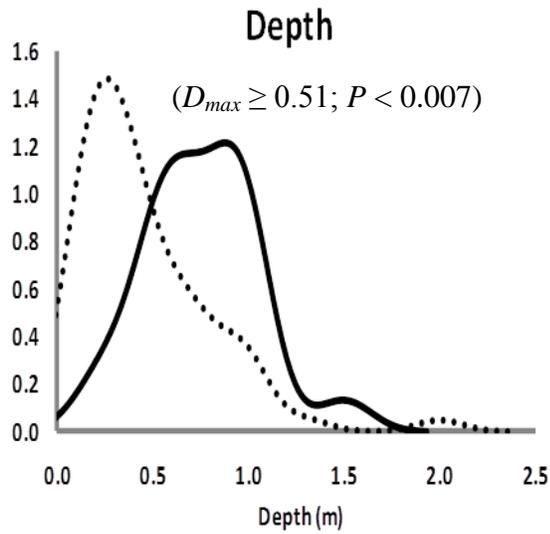
FIGURE 1--Changes in catch per unit effort for selected species throughout the period of study. Points show collections and smoothed lines show trends. Smoothed lines were produced using locally-weighted scatterplot smoothing.

FIGURE 2--Kernel density plots for depth (m), the proportion of the habitat covered by vegetation, measured as cover class, flow (ft<sup>3</sup>/s), and temperature (C). The solid line designates where *Dionda diaboli* were found, whereas the dotted lines designates where they were not found. Inserted within the figure are the results from the Kolmogorov-Smirnov tests of each environmental factor. The dashed line in the temperature graph indicates the temperature measured at the spring outflow. The y-axis for all plots is density.

CPUE (#/sq. m)



Date



## Final Report (addresses Task E)

### Reproductive and feeding ecology of two sympatric *Dionda* (Cyprinidae) in the Rio Grande Basin, Texas

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February 13, 2012

*Abstract:* The genus *Dionda* consists of at least 12 species, all of which inhabit groundwater-dominated streams within the western Gulf slope drainages of North America but with some slight differences in habitat preferences. The purpose of this study was to assess the influence of spring or river habitat selection on reproductive characters and feeding of two sympatric *Dionda* in the Devils River (TX) and between two *Dionda diaboli* populations taken from riverine habitats in the Devils River and from spring habitats in Pinto Creek (TX). The two species and three populations of *Dionda* were short lived (<3 years of age), produced multiple batches of oocytes in a spawning season, and primarily consumed algae and amorphous detritus. *Dionda diaboli*, which were taken from riverine habitats in the Devils River but were constrained to spring habitats in Pinto Creek, had a life span of 1 to 2 years and a six month spawning season. Individuals taken from Pinto Creek generally had narrower diet breadths, consumed more amorphous detritus, had a higher trophic position, and spawned earlier than those taken from

river habitats in the Devils River. *Dionda argentosa*, which were taken from spring habitats in the Devils River, had a longer life span, higher growth rates, a more protracted spawning season (8 to 12 months), and intermediate diet breadths when compared to the two populations of *D. dionda*. Differences in reproductive characters between the spring and river *Dionda* species were consistent with theory that stenothermal waters of springs lack terminating cues to induce gonadal quiescence in fishes. However, a protracted spawning season was not observed in *D. diaboli* taken from springs of Pinto Creek. This suggests that protracted spawning is an adaptive trait, but findings were inconclusive in that low spring outflows in Pinto Creek produced more riverine-type environments rather than the more stenothermal environments normally associated with spring habitats. Likewise, differences in diets between the two populations of *D. diaboli* were attributed to low spring outflows and declining environmental conditions within Pinto Creek.

## INTRODUCTION

Fishes inhabiting karst landscapes are classified as either spring-adapted or stream-adapted forms (Hubbs 1995, 2001). Spring-adapted fishes are located within or in close proximity to spring outflows of karst aquifers, whereas stream-adapted fishes inhabit areas of rivers outside the direct influence of the relatively constant physical and chemical environments of spring outflows. Segregation of the two forms occurs during Winter and Summer but becomes less distinct during Fall and Spring when water temperatures in streams are similar to those of spring outflows (22 - 24°C; Kollaus and Bonner 2011). Preferences for spring-influenced environments by several lineages of fishes are likely genetically-based and represent

convergently derived characters, but studies (Brown and Feldmeth 1971; Gotelli and Pyron 1991; Brandt et al., 1993, Bonner et al. 1998; Folb 2010) do not support Hubbs (1995, 2001) initial predictions about spring-adapted fishes. Specifically, Hubbs (1995, 2001) predicted that spring-adapted fishes will have stenothermal optima that allow them to produce gametes year round, have increased growth rates, and increased probability of survival. In contrast, Brown and Feldmeth (1971) found that thermal tolerances of cyprinodonts in thermal springs remained unmodified despite sufficient time for evolutionary changes to occur, and Brandt et al. (1993) and Bonner et al. (1998) demonstrated under laboratory conditions that thermal tolerances for survival and reproduction were similar between a spring-adapted *Etheostoma* and stream-adapted fishes. Gotelli and Pyron (1991) and Folb (2010) offer a more parsimonious theory about production of gametes year round in spring-adapted fishes. Stenothermal waters lack the necessary terminating cues to initiate gonadal quiescence. Lacking support for predicted adaptations, spring-associated is a more accurate descriptor of fishes with preferences for spring outflows than spring-adapted.

Species within the genus *Dionda* (Cyprinidae) predominately inhabit karst landscapes within western Gulf Slope drainages of North America and are associated with spring systems. When *Dionda* co-occur in a stream, they often have parapatric habitat associations, with some species utilizing more riverine habitats. When spring outflows and stream habitats are concurrently accessible, *Dionda diaboli*, the Devils River minnow, selects stream habitats, whereas the sympatrically-occurring *Dionda argentosa*, the manantial roundnose minnow, selects spring habitats in the Devils River (Val Verde County, Texas; Kollaus and Bonner 2011). However, two extant populations of *D. diaboli* are limited to regions in close proximity with

spring outflows in upper reaches of the Pinto Creek (Kinney County, Texas) and San Felipe Creek (Val Verde County, Texas). Consequently, distributions of *D. diaboli* among stream habitats in Devils River and spring habitats in Pinto Creek and of *D. argentosa* in spring habitats in Devils River offer a unique opportunity to elucidate annual life history characters among fishes inhabiting two distinct aquatic habitats within karst landscapes.

*Dionda* are small-bodied fishes, reaching maximum total length of 90 mm and having an estimated life span from 2 to 5 years (Wayne 1979; U. S. Fish and Wildlife Service 2005). *Dionda* are associated generally with riffle, run, and pool mesohabitats and sand or gravel substrates near stenothermal spring outflows (Hubbs and Brown 1956; Hubbs and Miller 1977; Hubbs et al. 1991; Watson 2006; Schönhuth et al. 2008). *Dionda* are herbivorous, consuming detritus, algae, diatoms, and plant material (Wayne 1979; Cohen 2008). Reproduction occurs from Winter through Summer (Hubbs and Miller 1977; Wayne and Whiteside 1985) with adult fish broadcasting demersal eggs into gravel substrates (Johnston and Page 1992; Johnston 1999; Philips et al. 2009). Among the 13 named and undescribed species, only two species (*D. diaboli* and *D. argentosa*) share sympatric distributions among tributaries of the Rio Grande. *Dionda diaboli* is listed by USFWS as threatened (U. S. Fish and Wildlife Service 1999) and all other *Dionda* occurring in Texas, including *Dionda argentosa* are listed as imperiled (Bender et al. 2005; Hubbs et al. 2008).

Purposes of this study are to quantify various aspects of population structure, reproductive biology, diet, and trophic ecology of two populations of *D. diaboli* and one population of *D. argentosa*. *D. diaboli* were taken from Pinto Creek and the Devils River to assess the role of spring influence and abiotic factors on reproduction, diet, and trophic ecology

of the Devils River minnow. *D. argentosa* were taken from the Devils River to assess if reproduction, diet and trophic ecology differ between two sympatric *Dionda* species and if differences relate to their respective habitat associations. Study objectives are to determine age structure, growth rates, longevity, monthly gonadosomatic indices, fecundity, oocyte diameters, gut contents and trophic position of *D. diaboli* in Pinto Creek and the Devils River and *D. argentosa* in the Devils River.

## METHODS

### *Study Sites*

Pinto Creek is a spring-fed creek in the Rio Grande drainage and is approximately 55 km in length. The upper reaches of Pinto Creek are fed by multiple spring outflows originating from the Edwards limestone formation (Brune 1981). These springs provide a source of water annually consistent in water quality and temperature. The lower half of Pinto Creek is supplied by a different aquifer and receives water with high total dissolved solids. *D. diaboli* are restricted to the Edwards aquifer spring-influenced waters of the upper Pinto Creek (Garrett et al. 2004). This stream was chosen because of immediate threats of dewatering and because it is defined as critical habitat of *D. diaboli* (U.S. Fish and Wildlife Service 2008). The study site on Pinto Creek was located on private land (29<sup>o</sup>24.163N, 100<sup>o</sup>27.622W) which contained the headwaters of Pinto Creek (Figure 1).

The Devils River, also in the Rio Grande drainage, is approximately 95 km in length and fed through multiple spring sources originating from the Edwards limestone formations (Brune 1981). Due to the river's larger size, the Devils River contains more heterogeneous habitats and areas that are less spring dominated than habitats found in Pinto Creek. There were two study

sites on the Devils River, both located within the Devils River State Natural Area. The upper site was located away from spring outflows (29°54.274 N, 00.324 W) and the lower site was adjacent to Finnegan Springs (29° 53.974 N, 00° 59.865 W) (Figure 1).

### *Collections*

*Dionda diaboli* were collected from the Devils River and Pinto Creek and *Dionda argentosa* were collected from the Devils River monthly for 1 year. At each site, short seine hauls were conducted until between 50 and 100 individuals of each species were captured (when available). Among fish collected, 10 fish of estimated reproductive size (> 25 mm SL) of each species were retained (Harrell 1980). All other fish were measured for total length in millimeters and released. Fish that were retained were euthanized using MS-222 and transferred to 10% formalin for fixation. At each location of fish capture, water temperature was taken and used as a surrogate for spring influence, with increasing spring influence related to a decrease in the range of annual temperatures.

Seasonally, 10 additional *D. diaboli* were collected from Pinto Creek and the Devils River and 10 additional *D. argentosa* were collected from the Devils River for gut content and stable isotope analyses. These additional *Dionda* were measured, pithed, and eviscerated in the field. Entire gut tracts and gonads were removed, transferred to 10% formalin, and stored in the dark for dietary analysis. Epaxial muscle tissue was stored in 95% ethanol for stable isotope analysis. Representative samples of other consumers (dominant fish and invertebrate taxa) and sources (lithophytic algae, epiphytic algae, filamentous algae, and coarse particulate organic matter) were taken for stable isotope analysis. All epiphytic algae, lithophytic algae, and

filamentous algae were taken from different macrohabitats of varying flows when available to account for effects of flow on isotope fractionation (Finlay 2001). Samples of macrophytes, epiphytic algae, filamentous algae, and coarse particulate organic matter were stored in stream water, put on ice, and transported to the lab. Lithophytic algae samples were obtained using a nylon bristle brush to gently scrape algae from randomly selected rocks. Milli-Q water was used to rinse loosened lithophytic algae from each rock into a test tube. Lithophytic algae samples were put on ice and transferred back to the lab. Fish collected other than *Dionda* were preserved in 95% ethanol. Invertebrates were stored in stream water and packed in ice until returned to the lab. This gave invertebrates time to excrete waste before being preserved in 95% ethanol.

#### *Population Structure and Reproductive Ecology*

In the laboratory, all *Dionda* retained for reproductive analysis were measured to the nearest millimeter, weighed to the nearest milligram, and eviscerated. Gonads were separated, removed of adipose and peritoneal tissue, and weighed to the nearest milligram. A dissecting scope was used to determine sex and ovarian condition of females. Ovarian categories were defined as immature, developing, mature, and spent (Williams and Bonner 2006). Total and gonadal weights were used to calculate gonadosomatic indices  $[(\text{gonad weight}/\text{total weight}) * 100]$  for each fish. GSIs were pooled by species, site, sex, and month to assess reproductive trends. A Pearson product moment correlation ( $\alpha = 0.05$ ) was used to determine if average monthly male and female GSIs for each species and site combination were correlated and the nature of that

correlation. Chi squared tests were performed to determine if observed female:male ratios deviated from the expected 1:1 sex ratio for each species and site combination.

To assess fecundity and oocyte development, up to three mature females of each species were randomly selected from each site during each month when reproductively mature females were present. The left ovary was selected from each female when intact and teased apart in a petri dish to separate oocytes. If the left ovary was not intact or appeared reduced the right ovary was used. The ovary used for each fish was weighed to get proportion of total gonadal weight in milligrams. Using a dissecting microscope with a digital micrometer, the first 100 oocytes in view were measured for the largest and smallest diameters. Each pair of diameters was averaged to obtain mean oocyte diameters. Mean oocyte diameters were grouped into 0.05 mm bins. Individual size frequency histograms were constructed using the bin groupings of mean oocyte diameters for each fish to demonstrate clutch distributions. The largest clutch or size grouping present in histograms for each species was separated out and assigned a minimum size. These ovaries were then reevaluated and all vitellogenic oocytes greater than the determined minimum size were counted. This count was then extrapolated to the total weight of both ovaries in each fish to give an estimate of clutch fecundity for the most mature clutch. Linear regressions and analysis of variance tests (ANOVAs) were with run with  $\alpha = 0.05$  to assess correlation between total fish length and clutch fecundity estimates for each species and site combination.

Length frequency histograms were constructed by pooling total length data from all fish captured by species, site, and month. Lengths were put into 2 mm bins and plotted in histograms. Modal class progression analysis, Bhattacharya method (FiSAT II, Version 1.2, Food and Agriculture Organization 2005), was used to determine population structure and mean

monthly lengths for each age classification of each species at each site. Fish were assigned age groupings for the 2009 season with the smallest grouping being age-0 and the largest being age-1. After January 1, 2010 one year is added to all existing age groups and any new fish spawned that year are assigned age-0 (2010) (Murphy and Willis 1996).

### *Trophic Ecology*

Once in the lab, epaxial muscle of fish greater than approximately 25 mm was removed and stored in individual glass vials. Smaller fish that did not yield enough epaxial muscle were eviscerated and stored in vials whole. All samples were placed in a drying oven set at 60°F for 48 - 72 h. Once removed from the oven, each sample was homogenized with a clean mortar and pestle. Invertebrates were first sorted to the lowest taxonomic group possible (usually family) and stored in glass vials. Larger invertebrates such as dragonfly larvae had digestive tracts removed prior to drying. Invertebrate samples were placed in a drying oven set at 60°F for 48 - 72 h. Larger invertebrates and muscle tissue were ground. Elytra from invertebrates that would not homogenize were removed. Smaller invertebrates such as ephemeropterans were left whole for analysis.

Epiphytic algae and lithophytic algae samples were transferred to pre-combusted GFF filters. Macrophytes, filamentous algae and coarse particulate organic matter samples were rinsed with milli-Q water to remove other particulates. All samples were then dried at 60°C for 48 to 72 hours. Lithophytic, epiphytic, and filamentous algae samples were transferred to a fuming HCl chamber for 24 h to gas off any inorganic carbon. Samples were then dried again

for 48 hours. Course particulate organic matter and macrophytes were chopped up into a homogeneous mixture.

All isotope samples were weighed out into 5x9 mm tin capsules and shipped to UC Davis Stable Isotope Facility for Carbon ( $^{13}\text{C}$ ) and Nitrogen ( $^{15}\text{N}$ ) analysis (Gaston and Suthers 2004). Nitrogen signatures served to determine the trophic position of *D. diaboli* and *D. argentosa* and set up a hypothetical trophic hierarchy for each stream (Peterson and Fry 1987). Carbon signatures were used to estimate sources of dietary carbon for *D. diaboli* and *D. argentosa* (Finlay 2001).

An analysis of variance was used to evaluate differences in  $\delta^{15}\text{N}$  values between age-1 and age-0 *D. diaboli* from Pinto Creek and the Devils River and between age-2, age-1, and age-0 *D. argentosa* from the Devils River. Trophic position estimates were pooled and averaged by age class if there were no significant differences in  $\delta^{15}\text{N}$  between age groups. The primary consumer with the lowest average  $\delta^{15}\text{N}$  value and presence across seasons and sites was used as the baseline consumer. The following formula from Anderson and Cabana (2007) was used to estimate trophic position of both *Dionda* species:

$$\text{Trophic position}_{\text{Consumer}} = ((\delta^{15}\text{N}_{\text{Consumer}} - \delta^{15}\text{N}_{\text{Baseline}})/f) + 2$$

where  $\delta^{15}\text{N}_{\text{Consumer}}$  is the  $\delta^{15}\text{N}$  value for an individual consumer sample,  $\delta^{15}\text{N}_{\text{Baseline}}$  is the average  $\delta^{15}\text{N}$  value for the selected baseline consumer, and  $f$  is estimated  $\delta^{15}\text{N}$  fractionation factor between each trophic level (3.4‰).

The Bayesian mixing model SIAR v4 (Parnell et al. 2010) was used to estimate the proportion of contribution of sources into the diets of both *Dionda* species using  $\delta^{13}\text{C}$  values

using methods from the Package 'siar' handbook (Parnell and Jackson 2011). Standard rates for fractionation of carbon (1‰) and nitrogen (3.4‰) have been supported in the literature and were used when analyzing data. Before analyses were run all source data samples for each species and site were grouped by type and tested for outliers using Grubb's test for outliers. Any significant outliers were omitted. Due to inaccuracy of source  $\delta^{15}\text{N}$  values these were omitted and replaced with values below than the lowest consumer group when running the model.

*Dionda* of each species from each season and site combination were selected for gut content analysis from the fish eviscerated in the field. In the laboratory, foreguts (defined here as the esophagus and stomach, consisting of the first 2 loops of the gut tract) were removed using a dissecting scope, emptied into individual vials containing 5% formalin, and stored in the dark. If a stomach was empty it was noted and another fish was selected for analysis. Each vial containing the gut contents from one fish was measured for total volume to the nearest tenth of a milliliter and then shaken until the contents were thoroughly suspended. Three 0.1 mL subsamples were taken from each vial using a micropipette and placed into a Palmer-Maloney counting cell (Palmer and Maloney 1954). Each subsample was viewed at 320X (40X objective) and assessed to determine if at least 10 algal cells were present in each field of view (Cohen 2008). If fewer than 10 algal cells were present the sample was allowed to settle for 24 hours and formalin was decanted off. If too many algal cells were present to accurately count each field of view more formalin was added. A Whipple ocular micrometer with a 100 square grid of 0.5 mm x 0.5 mm was used as the field view with a known area when enumerating samples. For each 0.1 mL subsample 30 fields of view were selected and all items present were enumerated. All algal and invertebrate taxa were identified to the lowest feasible taxonomic level (usually

genus). Complexes of material with no distinguishable algal cells were categorized as amorphous detritus.

To estimate biovolume of diet items, a Whipple micrometer was used to take 30 sets of linear measurements of individual algal cells in each taxonomic category. Once all measurements were taken for a taxonomic category, the median measurement for each dimension was selected and used in the appropriate volume formula (Hillebrand et al. 1999) to estimate the volume for an individual algal cell or colony. Taxa biovolumes were estimated for each site and season to account for any seasonal, spatial, and life cycle size differences. Linear measurements were also taken for amorphous detritus, invertebrates, and macrophyte fragments. These categories were assigned the most representative volume formula (Hillebrand et al. 1999) and biovolumes were calculated using the median measurement or by calculating biovolume for each individual if abundances were < 30.

Abundances of each diet category were summed from the three 0.1mL subsamples taken from each gut. These abundances were extrapolated out to the expected number of diet items per milliliter for each fish using the following formula from Wetzel and Likens (2000):

$$\frac{\text{Count}}{\text{ml}} = \frac{C \times 1000 \text{ mm}^3}{A \times D \times F}$$

In this equation  $C$  is the sum of the number of times an item was counted in three 0.1 mL subsamples,  $A$  is the area of the field of view (area of the Whipple grid measured at 40X objective),  $D$  is the depth of the field (0.4 mm), and  $F$  is the total number of fields counted for three subsamples (90). The count per milliliter for each diet item was then extrapolated out to

the total volume of the gut content by multiplying the count/mL by the total volume of the gut content sample. To get a total biovolume estimate for a diet category within each gut sample the biovolume estimate for a taxonomic category was multiplied by its total estimated count for that sample.

Diet categories were condensed into the following classifications: blue-green algae or cyanobacteria (colonial), filamentous blue-green algae, green algae (colonial and individual), filamentous green algae, picoplankton (algae < 2µm in diameter), diatoms, amorphous detritus, invertebrates, and macrophyte fragments. Gut content items were expressed as percentage of total abundance and percentage of total volume for each individual fish. Pinkas et al. (1971) Index of Relative Importance (IRI) was calculated for each prey category consumed by each species at each site using following equation:

$$IRI_{ij} = \% abundance_i + \% biovolume_i * \% occurrence_i$$

Percent abundance and percent biovolume are the average abundance and biovolume estimates of diet item *i* for all consumer species *j* individuals. Percent occurrence is the percentage of times diet item *i* occurred in all consumer species *j* individuals analyzed. IRI scores were standardized to make them comparable between species and sites by transforming them into percent IRI. This index combines abundance, biovolume, and occurrence of a diet category thus giving a standardized measurement of importance of each diet category in the overall diet.

Principle component analysis was used to weight the proportional gut content biovolumes for each sample to assess seasonal differences for both species at both sites (Canoco v. 4.55 2006). Analysis of variance was run on seasonal categories for *D. diaboli* from Pinto Creek, *D. diaboli* from the Devils River, and *D. argentosa* from the Devils River to determine if there were significant differences between seasons. Sample scores were grouped by season, site, and species, averaged, and plotted to assess seasonal shifts in gut content composition. Principle component analysis also was run on proportional gut content biovolumes for each *D. diaboli* samples to assess ontogenetic shifts in diet. Analysis of variance was run on sample scores of age-1 and age-0 *D. diaboli* from Pinto Creek and the Devils River to determine gut content differences between age classes at each site.

Diet overlap was estimated using Pianka's (1974) formula for measurement of niche overlap:

$$O_{jk} = \frac{\sum p_{ij} * p_{ik}}{\sum p^2_{ij} * \sum p^2_{ik}}$$

Where  $p_{ij}$  and  $p_{ik}$  are the volumetric proportions of diet category  $i$  in the gut contents of consumers  $j$  and  $k$  respectively. This estimate of dietary overlap was made between the Devils River populations of *D. diaboli* and *D. argentosa* for each season and the annual average. Values of  $O_{jk}$  range from 0 or no overlap to 1 complete overlap in the diets of the two consumers,  $j$  and  $k$ .

Diet breadth was estimated using Hurlbert's (1978) formula for standardized niche breadth:

$$B_A = \frac{((\sum p_{ij}^2)^{-1}) - 1}{n - 1}$$

where  $p_{ij}$  is the volumetric proportion of diet item  $i$  in the total diet of consumer  $j$  and  $n$  is the total number of prey categories. Values of  $B_A$  ranged from 0 (minimum niche breadth, specialist) to 1 (maximum niche breadth, generalist).

## RESULTS

### *Population Structure*

From September 2009 through August 2010, total lengths were taken from 1,350 *Dionda diaboli* (Pinto Creek: 644, Devils River: 706) and 713 *Dionda argentosa*. Populations of *D. diaboli* and *D. argentosa* consisted of two to three ages groups (ages 0 – 2) (Figure 2). Age-0 *D. diaboli* were taken from May through December in Pinto Creek, from March through December in the Devils River and age-0 *D. argentosa* were taken from April through December in the Devils River. Growth rates of age-0 *D. diaboli* were 0.216 mm/d (range in lengths: 15 to 54 mm) in Pinto Creek and 0.092 mm/d (19 to 49 mm) in the Devils River during the first summer (Figure 3). Growth rates of age-0 *D. argentosa* were 0.236 mm/d (18 to 55 mm) in the Devils River during the first summer. Age 1 *D. diaboli* were taken from January through June in Pinto Creek, January through November in the Devils River and age-1 *D. argentosa* were taken from January through December in the Devils River. Age-2 *D. argentosa* were taken from January through August in the Devils River.

### *Reproductive Characteristics*

Gonads were extracted from 234 *D. diaboli* (Pinto Creek: 113, Devils River: 121) and 122 *D. argentosa*. Sex ratios did not differ from 1:1 (female:male) in Devils River *D. diaboli* population ( $X^2 = 1.9$ ,  $P = 0.17$ ), but was biased towards females in Pinto Creek *D. diaboli* (1:0.69;  $X^2 = 3.9$ ,  $P = 0.05$ ) and in *D. argentosa* (1:0.44;  $X^2 = 18.9$ ,  $P < 0.05$ ). Reproductive seasons, as defined by elevated GSIs and the presence of mature ovaries, occurred primarily from Winter through Summer among *D. diaboli* and year round among *D. argentosa*. The onset of the reproductive season coincided with increased photoperiod and temperature in Winter and reproductive season termination coincided with extreme warm temperatures in Summer for both *D. diaboli* populations (Figure 4). *D. argentosa* reproductive season did not terminate during the period of this study but peaks in GSI and proportion of mature females occurred when fish were collected at or near spring temperatures. Age-1 *D. diaboli* females had elevated GSIs (>2%) and mature ovaries from January through June 2010 in Pinto Creek and from February through July 2010 in the Devils River (Figure 5). Reproduction extended into Fall the previous year with elevated GSIs and mature ovaries occurring as late as September 2009 among age-1 *D. diaboli* in the Devils River. Developing or mature ovaries occurred as late as August and September in Pinto Creek and Devils River among *D. diaboli* tentatively identified as age-0 with total lengths of 45 mm in Pinto Creek and 42 to 45 mm in Devils River. Between both age groups, smallest lengths of mature *D. diaboli* were 39 mm in Pinto Creek and 42 mm in the Devils River. Male *D. diaboli* GSIs were positively correlated with female GSIs in Pinto Creek ( $r = 0.86$ ,  $df = 9$ ,  $P < 0.01$ ) and in the Devils River ( $r = 0.74$ ,  $df = 9$ ,  $P < 0.01$ ). Proportions of age-1 and age-2 *D. argentosa* collected had elevated GSIs and mature ovaries January through December in the

Devils River. Smallest length of mature *D. argentosa* was 45 mm. Male *D. argentosa* GSIs were positively correlated ( $r = 0.62$ ,  $df = 9$ ,  $P = 0.04$ ) with female GSIs.

Ovaries consisted of oocytes with multiple size classes in *D. diaboli* and *D. argentosa* throughout their reproductive season (Figure 6). Estimated mean clutch size for *D. diaboli* was 107 oocytes ( $\pm 66.4$ ) at Pinto Creek and 147 oocytes ( $\pm 97.8$ ) at Devils River (Figure 7). Estimated mean clutch size for *D. argentosa* was 166 oocytes ( $\pm 119.9$ ). Estimated clutch sizes did not differ between the three populations of *Dionda* ( $F = 1.68$ ,  $P = 0.2$ ). Total length was positively related to clutch size for *D. diaboli* ( $F = 11.2$ ,  $P < 0.01$ ) and *D. argentosa* ( $F = 20.8$ ,  $P < 0.01$ ) at Devils River (Figure 8). Relationship between total length and clutch size was not detected for *D. diaboli* at Pinto Creek ( $F = 3.08$ ,  $P = 0.1$ ).

### *Gut Contents*

Gut contents were extracted from 40 *D. diaboli* from Pinto Creek (18% empty), 35 *D. diaboli* from the Devils River (3% empty), and 40 *D. argentosa* (25% empty). Among fish with gut contents, quantification of food items were obtained from 20 *D. diaboli* from Pinto Creek, and 20 *D. diaboli* and 17 *D. argentosa* from Devils River. Across seasons, gut contents consisted primarily of detritus (53% in biovolume), blue-green algae (21%), and filamentous green algae (7%) in *D. diaboli* from Pinto creek, detritus (30%), blue-green algae (26%), and diatoms (14%) in *D. diaboli* from Devils River, and detritus (39%), filamentous green algae (27%), and blue-green algae (19%) in *D. argentosa* from Devils River. Collectively, highest IRI scores were detritus (33 – 55%) and blue-green algae (19 – 29%), followed by green algae (9%)

and picoplankton (6%) in *D. diaboli* from Pinto Creek, diatoms (18%) and green algae (12%) in *D. diaboli* in Devils River, and filamentous green algae (15%) and picoplankton (12%) in *D. argentosa* in Devils River (Table 1). All *Dionda* examined exclusively consumed algae and amorphous detritus with the exception of four individuals that consumed macrophytes and five individuals that consumed aquatic invertebrates (dipterans, trichopterans, and cladocerns).

Gut contents and diet breadth differed among seasons for *D. diaboli* and *D. argentosa*. The first two axes of PCA explained 48% of the variation. Macrophytes and aquatic invertebrates were omitted from this analysis due to low occurrences and high percent biovolumes that skewed the data. Principle component axis I explained 27% of the variation and described a gradient from filamentous green algae (-1.49) and diatoms (-0.33) to amorphous detritus (1.58), picoplankton (1.08), filamentous blue-green algae (0.91), and blue-green algae (0.42). Principle component axis II explained 21% of the variation and described a gradient from green algae (-1.44), diatoms (-1.44), and blue-green algae (-0.79) to filamentous green algae (1.09), filamentous blue-green algae (0.94), and detritus (0.42). Seasonal differences in gut content compositions were observed for *D. diaboli* from Pinto Creek ( $F= 5.05, P=0.01$ ) and the Devils River ( $F= 6.97, P < 0.01$ ) and *D. argentosa* from the Devils River ( $F=13.64, P < 0.001$ ) (Figure 9). *D. diaboli* from Pinto Creek generally consumed filamentous algae and detritus in Fall and Spring and shifted to a more heterogeneous diet during Winter and Summer. *D. diaboli* from the Devils River consumed diatoms, green algae, and blue-green algae during Fall and Winter, but shifted to filamentous algae in the spring and a heterogeneous diet in Summer. *D. argentosa* from the Devils River primarily consumed detritus and blue-green algae in Fall and Winter and shifted to consuming filamentous algae and detritus in Spring and Summer. Diet

breadth varied temporally, between sites, and between species (Figure 10). Annually, *D. diaboli* at the Devils River had the widest diet breadth and *D. diaboli* at Pinto Creek had the narrowest. While all three populations of fish consumed items from similar diet categories annually, gut contents from *D. diaboli* from the Devils River had a more even distribution of biovolumes from all categories giving them a higher adjusted diet breadth (0.24 – 0.50) as compared to *D. diaboli* from Pinto Creek (0.12 – 0.27) and *D. argentosa* from the Devils River (0.19 – 0.31). *D. diaboli* and *D. argentosa* gut contents from the Devils River showed a high overall diet overlap of 92% which varied seasonally ranging from 64% to 90%.

*Dionda diaboli* from both sites were separated by age groups and a PCA was used to assess on the percent biovolume of gut content items. One outlier was omitted due to an exceptionally high biovolume percentage of macrophytes. The first two axes of this PCA explained 51% of the variation and principle component axis I described 28% of the variation and a gradient from individual and colonial green algae (-0.93), diatoms (-0.83), and filamentous green algae (-0.62) to detritus (1.66), picoplankton (1.53), and filamentous blue-green algae (0.87). Principle component axis II described 17% of the variation and a gradient from filamentous green algae (-1.17), aquatic invertebrates (-0.99), and diatoms (-0.50) to colonial blue-green algae (2.25). PCA results indicated differences between diets of age-1 and age-0 *D. diaboli* from the Devils River ( $F= 10.25, P < 0.01$ ) and Pinto Creek ( $F= 4.24, P= 0.06$ ) (Figure 11). In both populations of *D. diaboli* age-0 fish consumed more blue-green algae and age-1 fish consumed more invertebrates, filamentous green algae, and diatoms.

### *Stable Isotope Analysis*

$\delta^{15}\text{N}$  values were significantly different between estimated age classes for *D. diabolis* at Pinto Creek ( $F=49.63$ ,  $P<0.01$ ) and the Devils River ( $F=28.20$ ,  $P<0.01$ ) but not for *D. argentosa* ( $F=0.70$ ,  $P=0.51$ ). Ephemeroptera were selected as the baseline consumer to calculate trophic position and had an average  $\delta^{15}\text{N}$  value of  $6.47 (\pm 1.4)$  in Pinto Creek and  $7.3 (\pm 1.7)$  in the Devils River. Trophic position of age-1 *D. diabolis* was estimated as 4.1 in Pinto Creek and 3.5 in the Devils River. Trophic position of age-0 *D. diabolis* was estimated as 3.9 in Pinto Creek and 2.9 in the Devils River. Trophic position of *D. argentosa* in the Devils River was estimated as 3.2.

$\delta^{13}\text{C}$  values were highly variable within most source types at Pinto Creek and the Devils River leading to high overlap among sources (Figure 12). This made it difficult to partition sources contributing to the diets of species. In Pinto Creek, all consumers had  $\delta^{13}\text{C}$  values aligned closest to the mean value for pool lithophytic algae but encompassing values of course particulate organic matter and pool epiphytic algae. The Devils River site encompasses run and riffle habitats and therefore more potential carbon sources. Consumer  $\delta^{13}\text{C}$  values spanned all source carbon signatures but were more concentrated near course particulate organic matter, riffle lithophytic algae, and run algae signatures. The Bayesian model partitioned the annual diet of *D. diabolis* from Pinto Creek into pool lithophytic algae with a mode of 58% (95% confidence interval: 36 – 83%), course particulate organic matter 40% (13 - 61%), and pool epiphytic algae 0.8% (0 - 8.9%). The annual diet of *D. diabolis* from the Devils River was partitioned into 29% course particulate organic matter (14 - 69%), run lithophytic algae 17% (6.5 - 27%), riffle epiphytic algae 13% (0.1 - 32%), riffle lithophytic algae 11% (0.2 - 23%), run epiphytic algae

5.6% (0.1 - 15%), and filamentous algae 2.1% (0 - 22%). The annual diet of *D. argentosa* from the Devils River was partitioned into coarse particulate organic matter 24% (5.6 - 50%), run lithophytic algae 22% (9.4 - 33%), riffle epiphytic algae 18% (0 - 29%), riffle lithophytic algae 17% (0.5 - 29%), run epiphytic algae 13% (1.0 - 22%), and filamentous algae 2.5% (0 - 22%) (Figure 13).

## DISCUSSION

The two species and three populations of minnow, *Dionda diaboli* and *D. argentosa*, were short lived, produced multiple batches of oocytes in one spawning season, and had diets consisting of algae and amorphous detritus. *Dionda diaboli*, a species that is not directly associated with spring outflows, had a life span of 1 to 2 years and a 6 month spawning season during the period of study. Differences were observed between the two populations of *D. diaboli*. The population from Pinto Creek had narrower diet breadths, consumed a larger proportion of amorphous detritus, fed at higher trophic position, and spawned earlier than individuals from the Devils River. *Dionda argentosa*, the species most closely associated with spring outflows, had a life span of 2 to 3 years, rapid growth rates, an 8 to 12 month spawning season, a lower trophic position, and intermediate diet breadths when compared to the two populations of *D. diaboli*. Reproduction and diet of *D. diaboli* support previous qualitative assessments of this species in the wild (Garret et al. 2002; Lopez-Fernandez and Winemiller 2005) but differ from laboratory results. In captivity, *D. diaboli* spawned at temperature ranging from 18 to 22°C and photoperiods ranging from 10 to 12.5 hours of light per day (Gibson et al. 2004; Gibson and Fries 2005); however, in the wild we found evidence of reproductive activity

at a much wider range of temperatures and photoperiods (16-27°C; 10.5-14 hrs/day). Diets and reproductive seasons found during this study are generally consistent with those of congeners. Specifically, predominant food items are detritus filamentous green algae in *Dionda nigrotaeniata* and *Dionda episcopa* and picoplankton, diatoms, and plant material in *Dionda serena* (Cohen 2008), and reproductive season starts in January and extends into August for *Dionda nigrotaeniata* with photoperiod as the likely initiating cue and no relationship between temperature and gonadal quiescence (Wayne and Whiteside 1985).

Reproductive season of both species were consistent with general patterns reported for riverine-associated and spring-associated species. *D. diaboli* had a defined reproductive period from Winter through Summer with reproductive timing correlated to photoperiod and water temperature. For this latitude, reproductive season length is expected to be 20 to 30 weeks, which encompasses the lengths of season for both populations of *D. diaboli* (Gotelli and Pyron 1991). Reproductive season beginning in Winter at this latitude is likely associated with avoidance of extreme warm temperatures that reduce the survivability of young (Hubbs 1985; Houde 1989; Green and Fisher 2004). However, other studies have found that some species restricted to this latitude such as *Notropis braytoni* (Heard 2008) and *Notropis jemezianus* (Sublette et al. 1990; Hoagstrom and Brooks 2005) spawn into August, times of potentially harsh warm temperatures. Evidence of reproductive activity in *D. diaboli* was present from Pinto Creek during August; however, due to the spring influence of this system, temperatures only reached 24.5°C in August, lower than the extreme warm temperatures found in the Devils River (28.9°C) where mature females were absent. In contrast, *D. argentosa* had a prolonged

spawning season that did not correlate with photoperiod or water temperature. *D. argentosa* inhabits stenothermal environments typically deviating less than 3°C from the spring temperature (Kollaus and Bonner 2011) and therefore does not encounter thermal reproductive termination cues (Hubbs and Strawn 1957; Hubbs 1985; Folb 2010; Perkin et al. 2012). Although no distinct reproductive season occurred in *D. argentosa*, reproductive peaks coincided time when individuals were collected around spring temperatures (22-23°C) suggesting the possibility of an optimal reproductive temperature. Similar results have been seen with another spring associate, *Etheostoma fonticola* (Schenck and Whiteside 1977). The prolonged spawning season of *D. argentosa* was 2 to 32 weeks longer than predicted for cyprinids residing within this latitude (Gotelli and Pyron 1991), similar to trends found in other spring-associated fishes but not congruent with most freshwater fish. Continuous or near-continuous spawning as seen in *D. argentosa* is a rare reproductive strategy, seen in only 4% of temperate and subtropical North American freshwater fishes (Folb 2010). Seasonal spawning (< 8 months) is thought to coincide with periods of abiotic stability, optimal conditions to lessen interspecific competitions, and synchronization of spawning aggregates (Bye 1984; Folb 2010). If reproduction truly coincides with these factors, stenothermal environments, such as spring systems, would negate the need for seasonal spawning.

Multiple modal histograms of oocyte diameter distributions of mature *Dionda* indicate synchronous batch spawning. Synchronous batch or group spawning is characterized by females who spawn multiple clutches of eggs during a reproductive season, have a heterogeneous pool of developing oocytes, and a distinctly larger batch of synchronously developing mature oocytes

(Heins and Baker 1988, 1989; Taylor and Miller 1990). Synchronous batch spawning is common among fishes found in environments with fluctuating hydrographs and eurythermal conditions and is attributed to an increased probability that at least a proportion of the total spawn will survive to maturity (Weddle and Burr 1991; Rinchard and Kestemont 1996; Durham and Wilde 2008). However, synchronous batch spawning also is found in stenothermal waters such as spring outflows (Schenck and Whiteside 1977; Folb 2010; Perkin et al. 2012). Therefore, selection for synchronous batch spawning does not seem to be an adaptation to a specific hydrology or temperature regime. Other possible mechanisms for the development of group synchronous spawning are competition avoidance for favorable reproductive sites (Gale and Gale 1977) or an attempt to maximize reproductive output in a small-bodied, space limited species (Hubbs et al. 1968; Schenck and Whiteside 1977). Clutch fecundity estimates were not different between the two species of *Dionda*; however, *D. argentosa* produced larger oocytes which accounts for higher GSIs. Due to the protracted spawning season of *D. argentosa* and similar batch fecundities between the species, overall reproductive output is likely higher in *D. argentosa* than in *D. diaboli*, although this is dependent on spawning frequency which is often constrained by food availability (Constantz 1979; Williams 1959; Hubbs et al. 1968). This claim is difficult to test because of the continual development of oocytes in the ovaries of synchronous batch spawning species which makes estimating total annual fecundity or spawning frequency extremely difficult (Heins and Rabito 1986; Heins and Baker 1989).

*D. diaboli* and *D. argentosa* demonstrated life history strategies that are consistent with spring-associated or stream-associated strategies. *D. argentosa* is more widely distributed than *D. diaboli* which is a possible result of inhabiting permanent areas of spring outflow in an arid

environment full of semi-permanent stream systems. Spring outflows often provide an area of refugia during times of stream dewatering, allowing spring associated species to persist. The protracted spawning season of *D. argentosa* gives the potential for an increased annual fecundity and therefore increased chances for sustainability in differing environments. Parapatry of habitat use coupled with different life history strategies has been noted in another sympatric pair of *Dionda* occurring in Mexico: spring associate *Dionda dichroma* and riverine associate *Dionda mandibularis* (proposed for listing in a new genus: *Tampichthys* (Schnönhuth et al. 2008)) (Hubbs and Miller 1977). In this species pair, the spring associated species was more widely distributed than the riverine species providing evidence for the theory of springs as areas of habitat refugia. It is hypothesized that *Dionda* species north of the Rio Grande are derived from one common widespread ancestor that was permitted to disperse between basins through connections of the headwaters of drainages due to northern glacial events (Conner and Sutkus 1986; Smith and Miller 1986; Schönhulth 2008). Genetic diversity suggests that *D. argentosa* is more closely related to other northern *Dionda* taxa while *D. diaboli* is more divergent and most likely a more recent colonization from the Nueces River drainage. *D. mandibularis*, like *D. diaboli* is the most divergent *Dionda* species within the southern Mexican grouping of the genus and has a more restricted range than its sympatric partner *D. dichroma*. These two pairs of sympatric species suggest that ancestral lineage is a more likely mechanism for different life history strategies as compared to the theory that they are due to adaptations to differing habitats.

Members of the genus *Dionda* are classified as herbivores based on the lack of a defined stomach and the presence of a long coiled intestine, however little quantification of their diets has been published to verify this. During the period of study *D. argentosa* exclusively consumed

detritus, algae, and macrophytes and had a trophic position of 3.2, falling within the range of previously published trophic position estimates for herbivorous cyprinids of 2.7-3.2 (Franssen and Gido 2006). *D. diaboli* had a similar diet with the addition of the consumption of aquatic invertebrates in a few individuals. This has been documented in other herbivorous fishes such as the closely related *Hybognathus* genus (Magana 2009). Trophic position of age-0 *D. diaboli* from the Devils River was estimated as 2.9, similar to other herbivorous cyprinids. Age-0 *D. diaboli* from the Devils and age-1 *D. diaboli* from both sites had trophic position estimates that ranged from 3.9 to 4.1, similar to omnivorous and insectivorous fishes (Franssen and Gido 2006). Bayesian mixing models estimated a high proportion of coarse particulate organic matter contributing to the diets of both *Dionda* species coinciding with high proportions of detrital matter found in gut contents. High carbon isotope variability within algal sources and high overlap among algal sources made it hard to partition diet into macrohabitat source or epiphytic versus lithophytic algae; however, due to the diversity of algal taxa present in the gut contents, it is likely that these species are opportunistic feeders utilizing primarily microscopic attached algae in nearby habitats. It is also likely that seasonal shifts present in the diets of these two species are a manifestation of seasonal or disturbance algae succession (Moore 1976; McCormick and Stevenson 1991). Diet overlap between the two species was high year-round, with the highest overlap estimates occurring in Fall and Spring. This coincides with times of the year in which spring-associated species, such as *D. argentosa*, move out of areas of thermal refugia to inhabit other areas of the river, potentially co-occurring with *D. diaboli* (Kollaus and Bonner 2011). Diet overlap between sister taxa was high, but these two species have seasonal parapatric distributions thus partitioning habitat resources for a portion of the year, a phenomena

that is common in other pairs of similar sympatric species (Fowler and Taber 1985; Wheeler and Allen 2003; Robertson et al. 2008). Previous qualitative assessments on habitat use of *Dionda* species coupled with the findings of this study and Kollaus and Bonner (2011) provide evidence that high diet overlap coupled with habitat segregation may be common for other sympatrically occurring *Dionda* species.

Ontogenetic shifts have been documented in other herbivorous fishes and have been attributed to differences in habitat use or nutritional requirements (Watson et al. 2009); however the occurrence of ontogenetic shifts has never been suggested for any *Dionda*. *D. argentosa* had a trophic position similar to the closely related *Campostoma anomalum* (Evans-White et al. 2011) and had no significant differences in  $\delta^{15}\text{N}$  values between age classes. At both sites, *D. diaboli* had significant differences in  $\delta^{15}\text{N}$  values between age classes with age-0 individuals displaying trophic positions more closely related to herbivorous fishes and age-1 individuals displaying trophic positions similar to insectivorous and omnivorous species such as *Astyanax mexicanus*, juvenile *Lepomis* and *Micropterus* species, and other *Cyprinid* species. Differences in  $\delta^{15}\text{N}$  values between age classes is supporting evidence of the presence of an ontogenetic shift in diet in *D. diaboli*. Principle component analysis of gut content items separated by age class supports the hypothesis of ontogenetic shifts in *D. diaboli* by more heavily weighting invertebrates in the diets of age-1 individuals. Pinto Creek *D. diaboli* had higher trophic positions for both age classes than the Devils River fish. Individuals from Pinto Creek had narrower diet breadths and a larger proportional biovolume of amorphous detritus. It is possible that amorphous detritus consumed while foraging for algae contained animal parts that were enriched in nitrogen and therefore elevated the trophic position of *D. diaboli*. Nitrogen

enrichment has also been documented in organisms that are nutrient limited and must use fat reserves to persist (Hobson et al 1993; Gannes et al 1997). A larger scale possible source of nitrogen enrichment is that the Pinto Creek drainage is a nitrogen-enriched system as compared to the Devils River due to increased anthropogenic and agricultural influences (Gormly and Spalding 1979; Peterson and Fry 1987; Aravena et al. 1993; McClelland et al. 1997). The latter theory is supported by significantly higher  $\delta^{15}\text{N}$  values of *Astyanax mexicanus* in Pinto Creek as compared to the Devils River, but  $\delta^{15}\text{N}$  values of *Ephemeroptera* and *Gambusia* species showed no significant difference between sites.

The genus *Dionda* is unique in the fact that many of its species occur in sympatric species pairs that are allopatrically distributed with respect to other member of the genus. Life history, diet and trophic ecology of *Dionda diaboli* and *Dionda argentosa* are important not only to the conservation of both species, but to provide insight into the ecology of other members of the genus as well as other spring associated and river associated species. *D. diaboli* and *D. argentosa* currently are listed as threatened and a species of special concern respectively due to threats of habitat degradation, dewatering, and invasive species (Bender et al. 2005, Jelks et al. 2008). This study supports previous work that states maintenance of diverse habitats is important to the coexistence of two species (Wheeler and Allen 2003). Conservation of these two species must focus on a broad scale approach to protect consistent spring flows and maintain thermal stability for *D. argentosa* life history success as well as riverine habitats with more heterogeneous temperature regimes for *D. diaboli* life history success.

Work performed under Texas Parks and Wildlife Department Scientific Permit Number SPR-0601-159, Federal Fish and Wildlife Permit Number TE236730-0, and Texas State University IACUC Number 0932\_1019\_34. Partial funding was provided by the US Fish and Wildlife Service through Section 6 of the Endangered Species Act.

Table 1. Seasonal percent biovolume, annual average percent biovolume, and standardized index of relative importance (IRI) contributions to gut contents of *D. diaboli* and *D. argentosa* collected from Pinto Creek and the

	Pinto: <i>Dionda diaboli</i>						Devils: <i>Dionda diaboli</i>						Devils: <i>Dionda argentosa</i>					
	F	W	Sp	Su	Annual	IRI	F	W	Sp	Su	Annual	IRI	F	W	Sp	Su	Annual	IRI
	Biovolume					IRI	Biovolume					IRI	Biovolume					IRI
Blue-green	8.4	25.6	8.6	39.3	20.6	21.4	23.2	10.3	25.5	46.0	26.2	28.6	18.5	26.6	5.9	23.1	18.5	18.8
Fil. blue-green	7.0	>0.1	-	0.2	1.8	1.5	0.3	-	0.2	0.1	0.2	0.2	0.3	4.2	-	-	1.1	1.3
Green algae	6.5	9.4	1.3	5.4	4.9	8.7	27.0	10.6	7.8	7.8	13.3	11.7	11.6	7.0	5.2	1.0	6.2	10.4
Fil. green	7.6	2.6	16.3	1.5	7.0	4.0	5.1	10.9	38.3	-	13.6	6.3	3.7	2.4	59.9	42.5	27.1	15
Picoplankton	>0.1	>0.1	>0.1	>0.1	>0.1	5.6	>0.1	>0.1	>0.1	>0.1	0.01	2.9	0.1	0.1	>0.1	>0.1	0.1	12
Diatoms	1.1	7.1	1.1	15.5	4.8	3.7	17.3	28.1	4.1	7.0	14.1	17.6	15.5	1.4	6.0	5.9	7.2	6.6
Detritus	69.5	55.4	46.5	39.7	53.0	54.5	27.0	32.0	23.1	39.2	30.3	32.5	50.4	54.4	23.3	27.5	38.8	35.6
Invertebrates	-	-	24.7	5.7	7.6	0.6	-	8.2	1.0	-	2.3	0.1	-	-	-	-	-	-
Macrophytes	-	-	-	-	0.4	>0.1	-	-	-	-	-	-	-	3.9	-	-	1.0	0.3

0 (W), April 2010 (Sp), and July 2010 (S).

Devils River in October 2009 (F), January 201

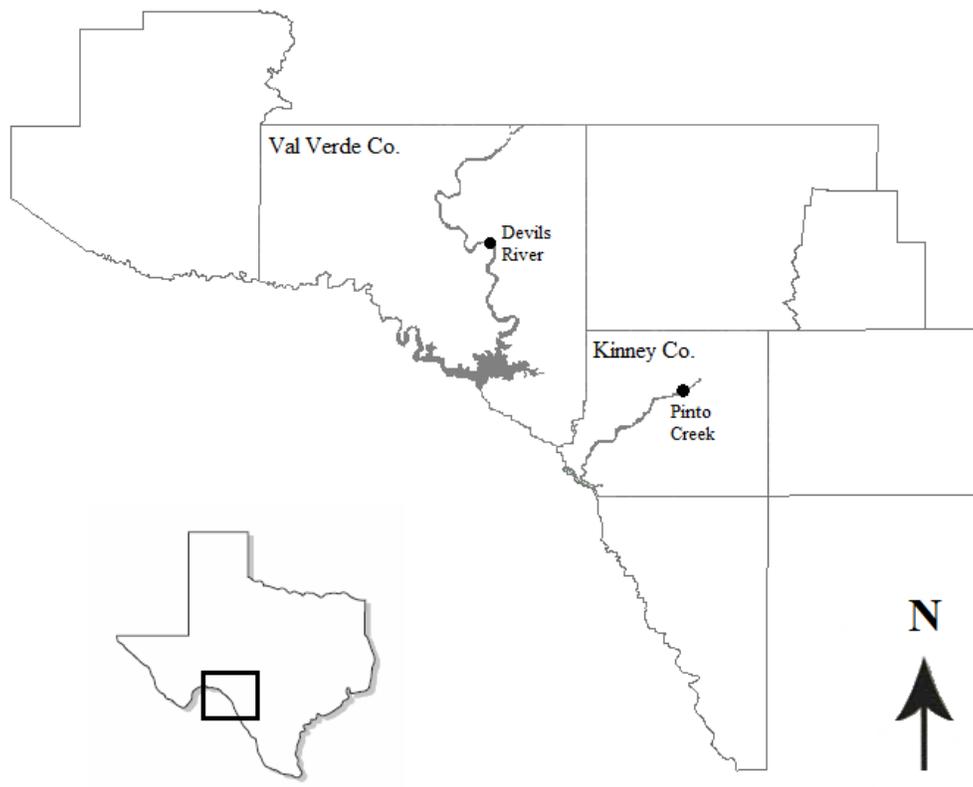


Figure 1. Map of study sites: Pinto Creek at private land, Kinney County, Texas and the Devils River at the Devils River State Natural Area, Val Verde County, Texas.

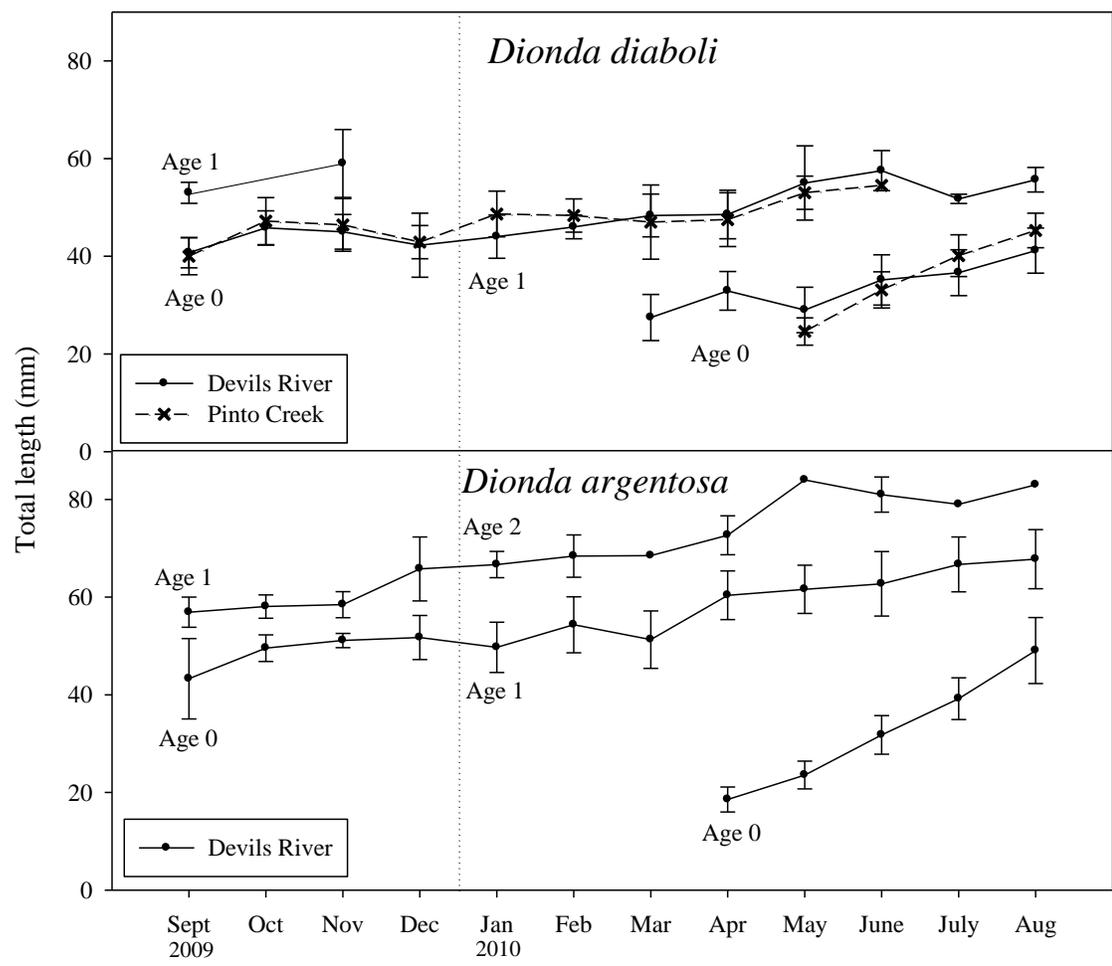


Figure 2. Monthly mean total lengths ( $\pm$ SD) for *D. diabolis* collected from Pinto Creek (N= 644) and the Devils River (N= 706) and *D. argentosa* collected from the Devils River (N= 713) September 2009 through August 2010.

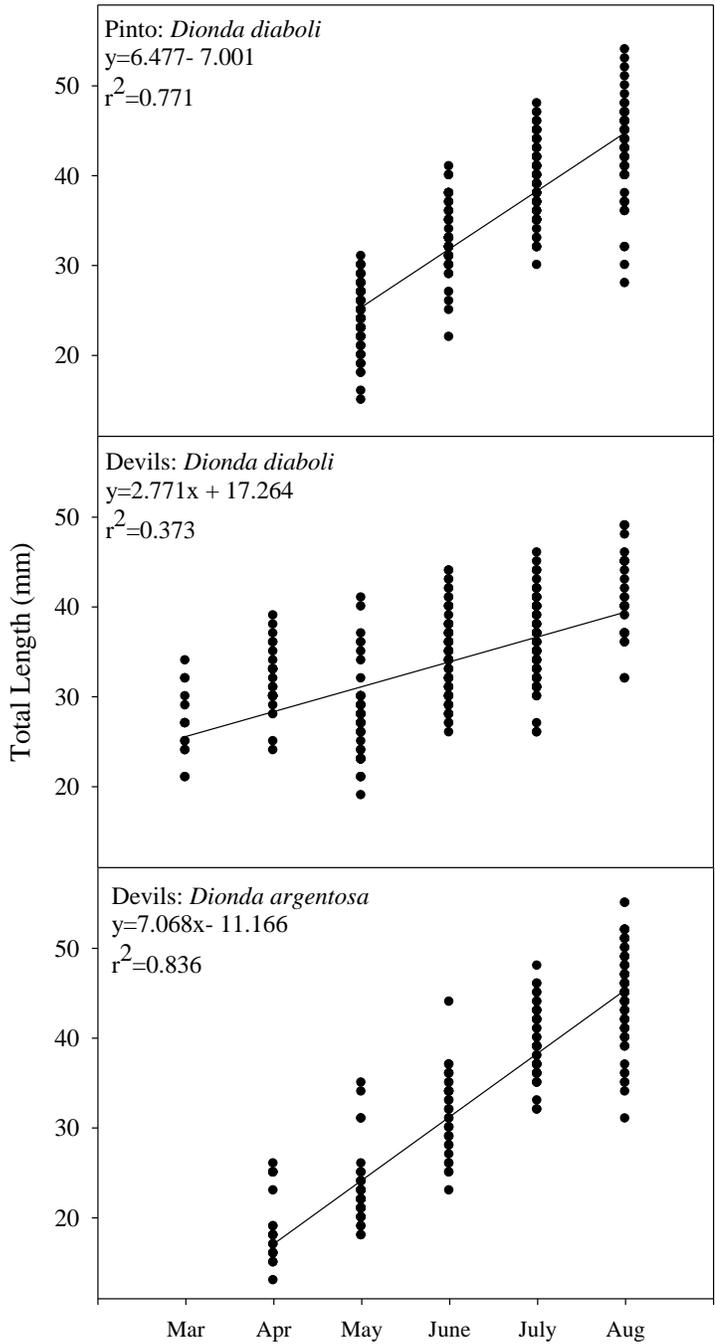


Figure 3. Linear regressions of fish total length versus time of *D. diaboli* and *D. argentosa* collected from Pinto Creek and the Devils River from March 2010 until August 2010.

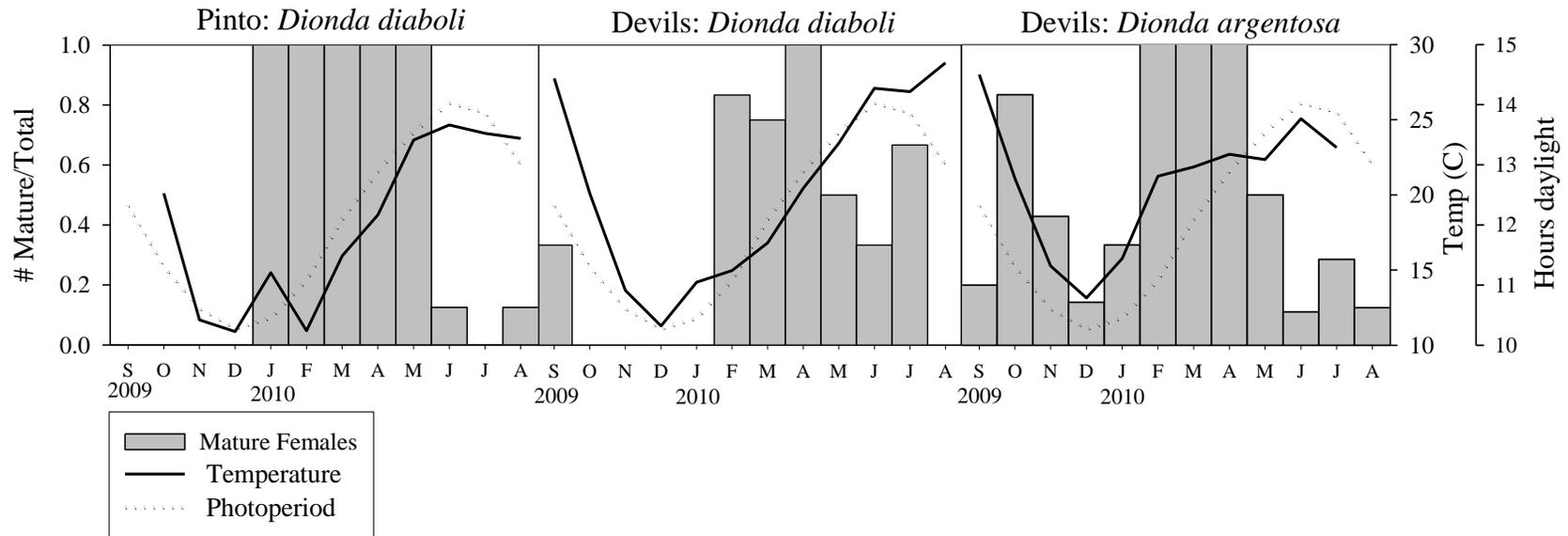


Figure 4. Bar graph representing the proportion of mature females out of the total number of females per month captured from the Devils River and Pinto Creek September 2009 through August 2010 overlaid with average monthly water temperature where *Dionda* were collected (solid line) and average hours of daylight (dotted line).

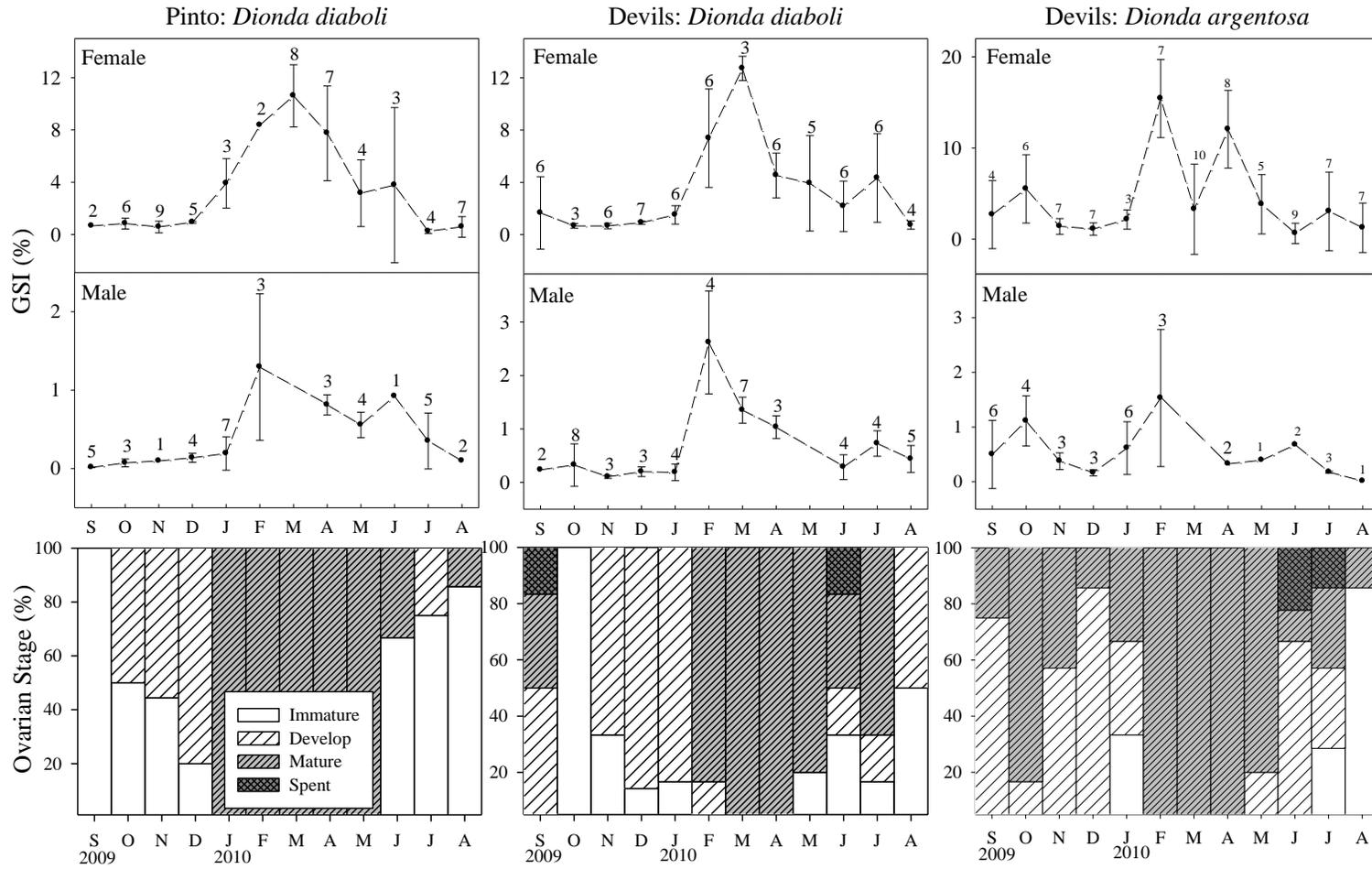


Figure 5. Scatter plots of mean monthly gonadosomatic index ( $GSI \pm SD$ ) for female and male *D. diaboli* and *D. argentosa* collected from Pinto Creek and the Devils River September 2009 through August 2010. Numbers located above monthly means represent  $n$  values. Bar graphs represent monthly ovarian condition (% from total females examined).

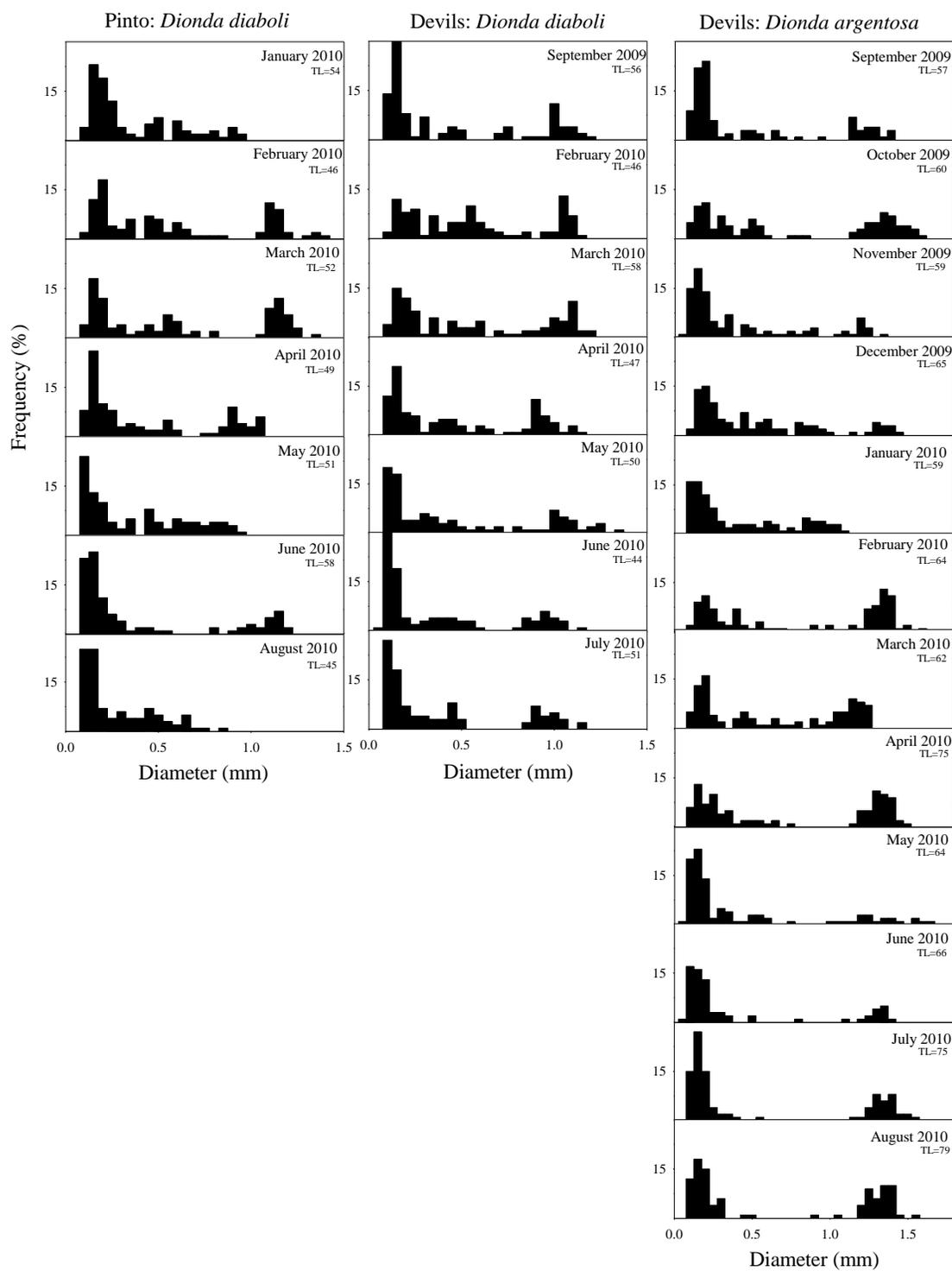


Figure 6. Size frequency histograms of oocyte diameters measured from one *D. diaboli* from each site and one *D. argentosa* displaying mature ova per month. Fish were collected from the Devils River and Pinto Creek September 2009 through August 2010.

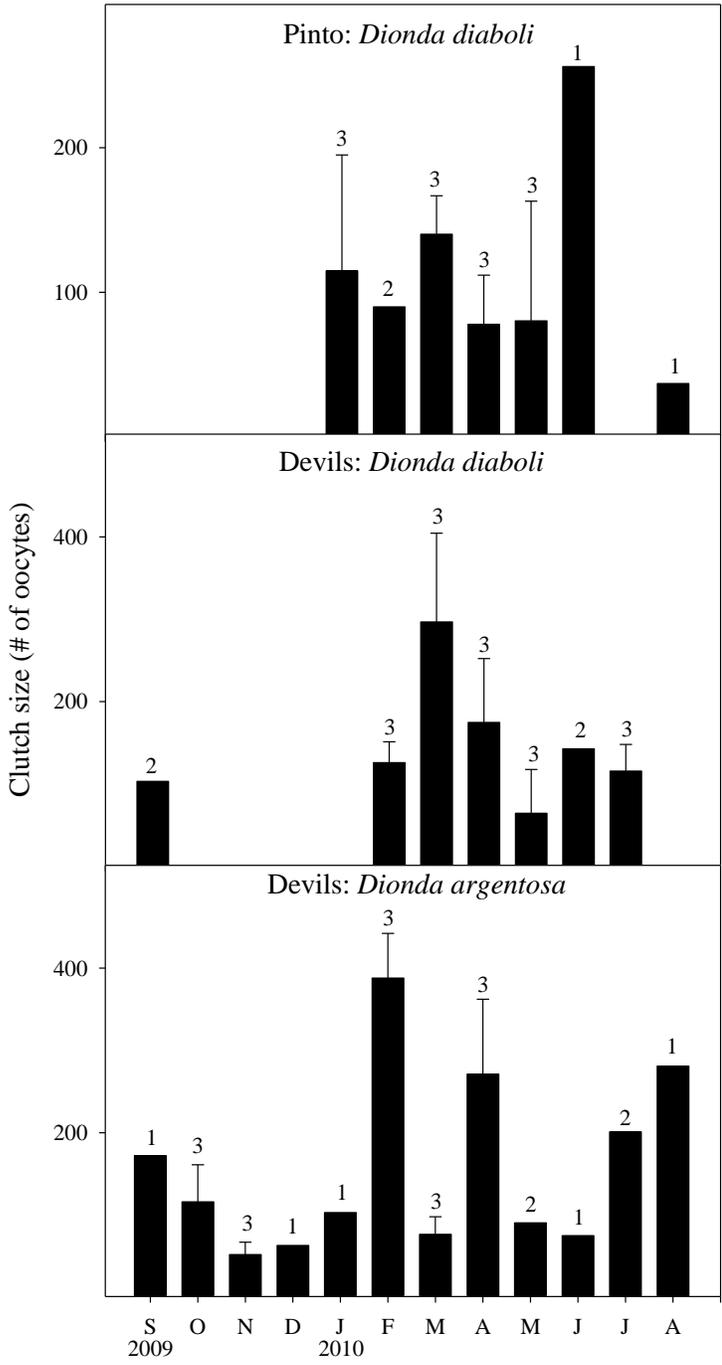


Figure 7. Monthly mean ( $\pm$ SD) clutch abundance estimates for *D. diaboli* and *D. argentosa* collected from Pinto Creek and the Devils River September 2009 through August 2010. Numbers above error bars represent *n* values.

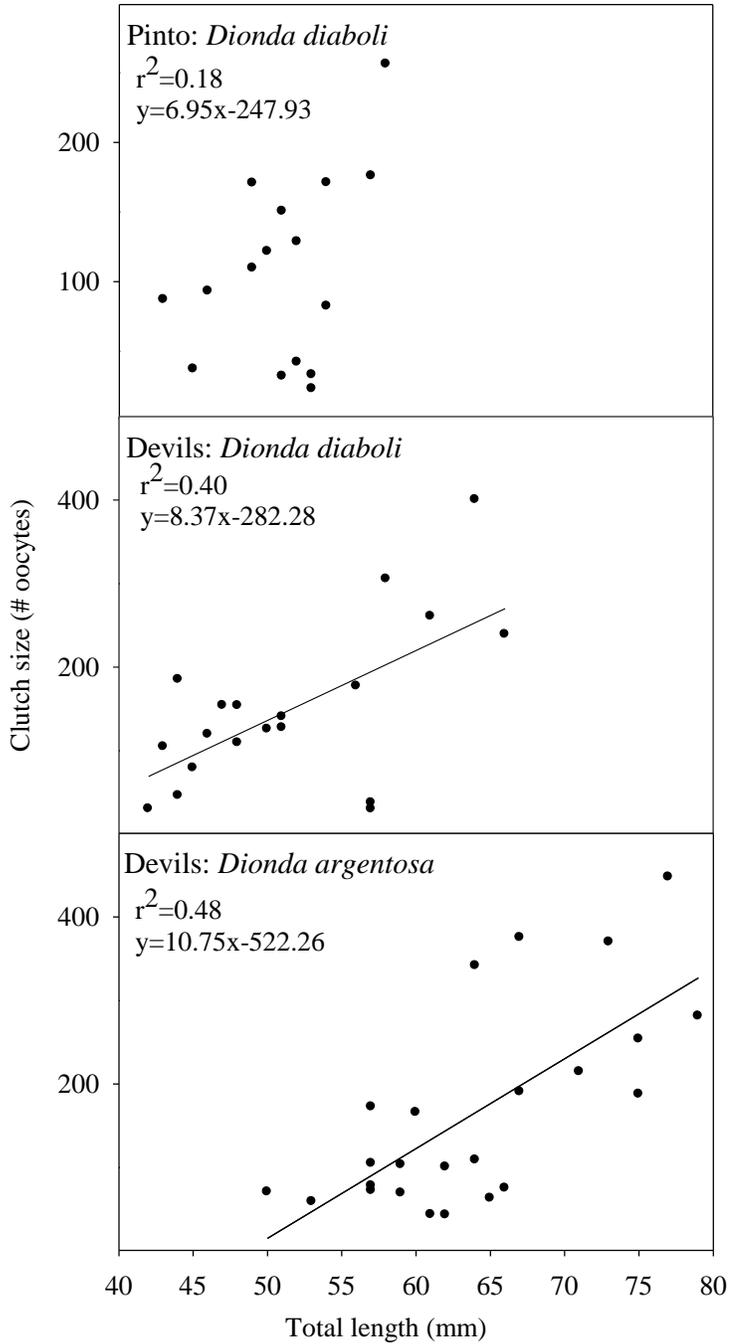


Figure 8. Linear regressions for total length versus estimated clutch size for *D. diaboli* and *D. argentosa* collected from Pinto Creek and the Devils River September 2009 through August 2010.

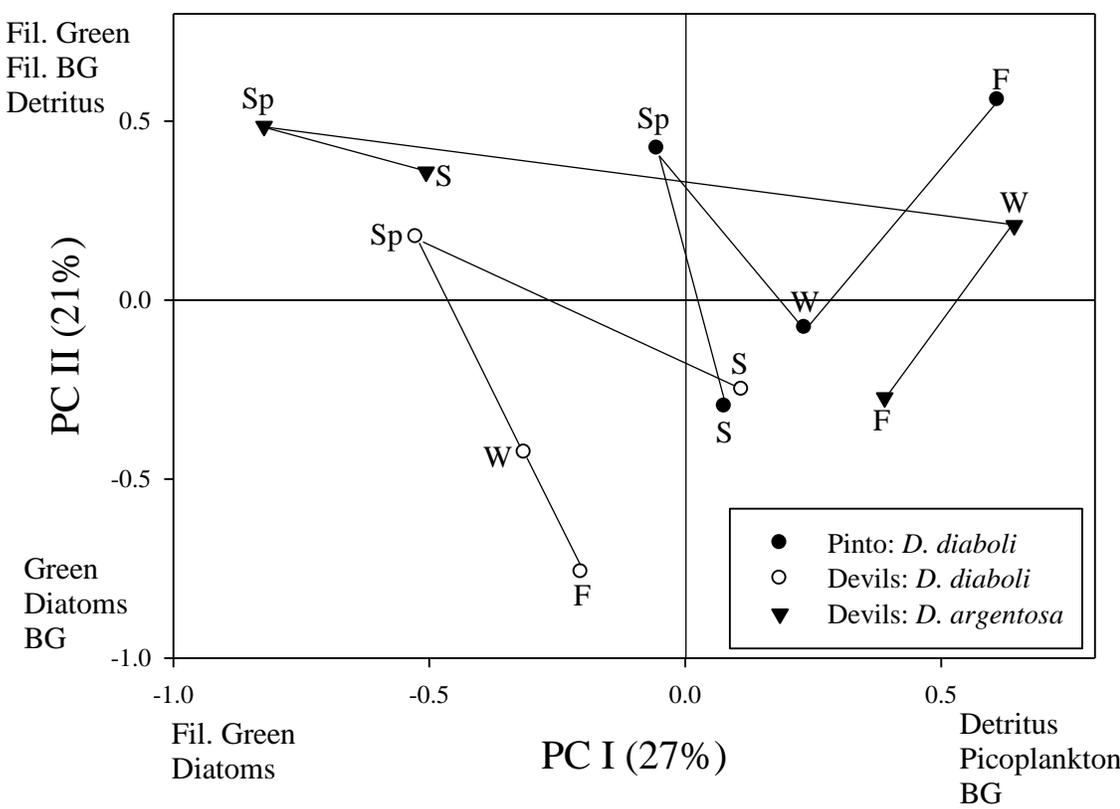


Figure 9. Principle component analysis (PCA) of average seasonal gut content items in percent biovolume for *D. diaboli* and *D. argentosa* collected from the Devils River and Pinto Creek September 2009 through August 2010. Abbreviations are as follows: Fil. (filamentous), BG (blue-green algae).

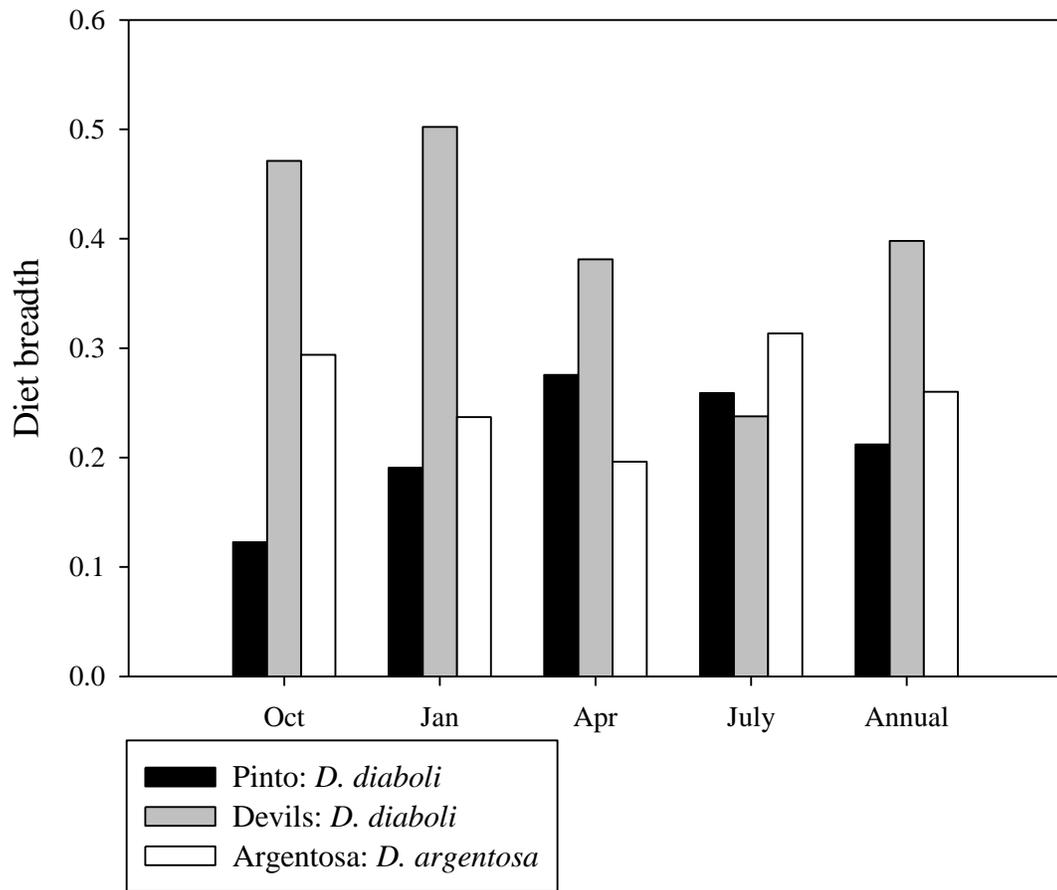


Figure 10. Standardized diet breadth for *D. diaboli* and *D. argentosa* collected from Pinto Creek and the Devils River collected quarterly from October 2009 through July 2010 and the annual average.

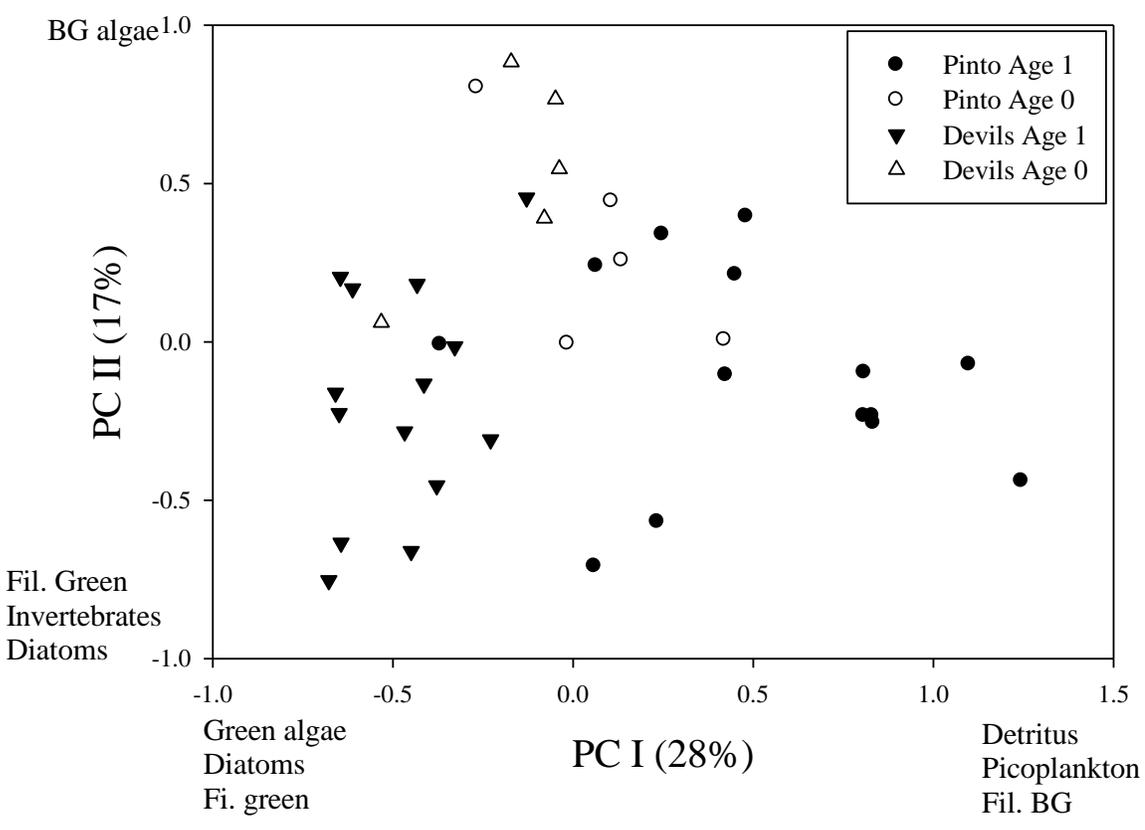


Figure 11. PCA of proportional biovolume of gut content items from Age 1 and Age 0 *D. diaboli* from Pinto Creek and the Devils River

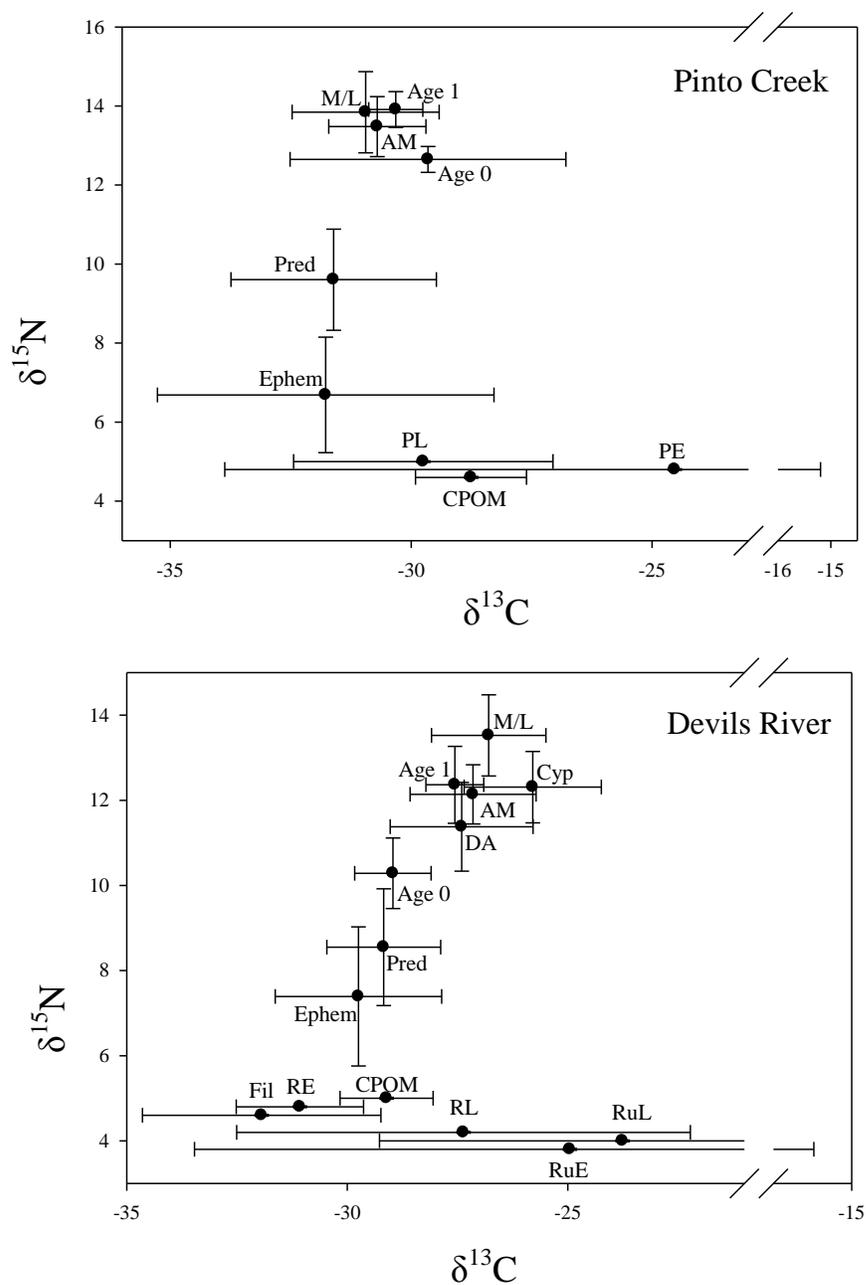
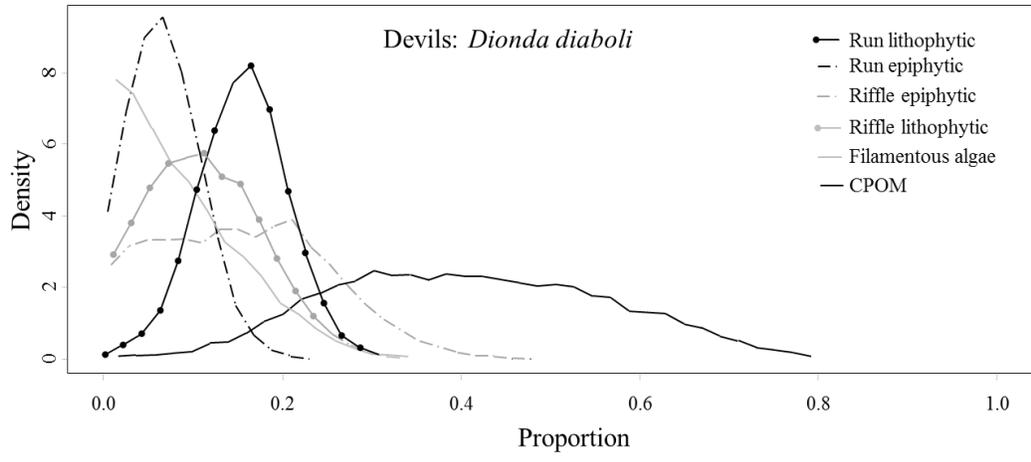
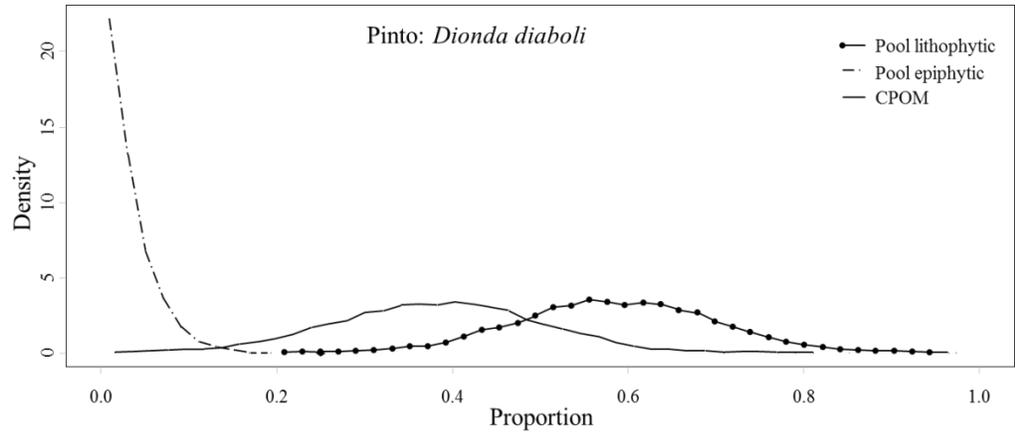


Figure 12. Biplot of average annual  $\delta^{13}\text{C}$  v.  $\delta^{15}\text{N}$  values ( $\pm\text{SD}$ ) of fish, invertebrate, and source populations from Pinto Creek and the Devils River collected quarterly from October 2009 to July 2010. Abbreviations are as follows: pool lithophytic algae (PL), pool epiphytic algae (PE), coarse particulate organic material (CPOM), run lithophytic algae (RuL), riffle

lithophytic algae (RL), run epiphytic algae (RuE), riffle epiphytic algae (RE), filamentous algae (Fil), Ephemeroptera (Ephem), predatory aquatic invertebrates (Pred), age 0 *D. diaboli* (Age0), age 1 *D. diaboli* (Age1), *Astyanx mexicanus* (AM), Juvenile *Micropterus* and *Lepomis* species (M/L), Cyprinid species (Cyp), and *Dionda argentosa* (DA).



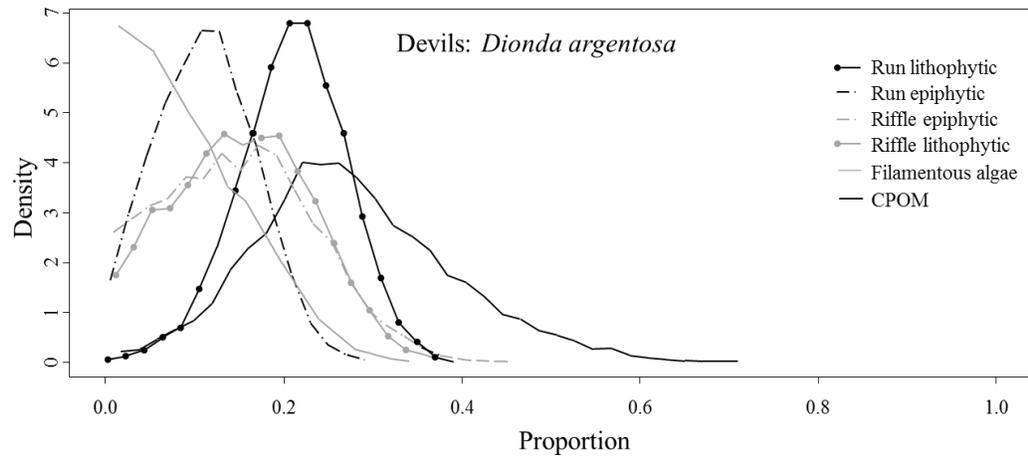


Figure 13. SIAR predictions of dietary composition by proportion based on  $\delta^{13}\text{C}$  values for *D. diaboli* and *D. argentosa* from Pinto Creek and the Devils River collected quarterly from October 2009 to July 2010. CPOM is an abbreviation for coarse particulate organic matter.

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