REPRODUCTIVE AND FEEDING ECOLOGY OF TWO SYMPATRIC *DIONDA*

(CYPRINIDAE) IN THE RIO GRANDE BASIN, TEXAS

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Sarah M. McMillan, B.S.

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Committee Members Approved:

_____

Timothy H. Bonner, Chair

_____

Weston H. Nowlin

_____

Joe N. Fries

Approved:

_____

J. Michael Willoughby
Dean of the Graduate College

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ABSTRACT

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Sarah Melissa McMillan, B. S.

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SUPERVISING PROFESSOR: TIMOTHY BONNER

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.

CHAPTER I

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. argenstosa* 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.

CHAPTER II

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 ($29^0$24.163N, $100^0$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

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($29^0$54.274 N,00.324 W) and the lower site was adjacent to Finnegan Springs (29^0

53.974 N, 00^0 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 course 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 course 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

gonodosomatic indices[(gonad weight/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

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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 ehemeropterans were left whole for analysis.

Epiphytic algae and lithophytic algae samples were transferred to pre-combusted

GFF filters. Macrophytes, filamentous algae and course 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}C) and Nitrogen (^{15}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 δ^{15}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 δ^{15}N between age groups. The primary consumer with the lowest average δ^{15}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:

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

where $\delta^{15}N_{Consumer}$ is the δ^{15}N value for an individual consumer sample, $\delta^{15}N_{Baseline}$ is the average δ^{15}N value for the selected baseline consumer, and f is estimated δ^{15}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 $\delta^{13}C$ 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 $\delta^{15}N$ 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 (Hildebrand 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):

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

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 $< 2\mu m$ in diamteter), 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:

$$IRI_{ij} = (\% \ abundance_i + \% \ biovolume_i) * \% \ occurence_i$$

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:

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

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:

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

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 range from 0 (minimum niche breadth, specialist) to 1 (maximum niche breadth, generalist).

CHAPTER III

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 (±66.4) at Pinto Creek and 147 oocytes (±97.8) at Devils River (Figure 7). Estimated mean clutch size for *D. argentosa* was 166 oocytes (±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

$\delta^{15}N$ values were significantly different between estimated age classes for *D. diaboli* 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 $\delta^{15}N$ value of 6.47 (±1.4) in Pinto Creek and 7.3 (±1.7) in the Devils River. Trophic position of age-1 *D. diaboli* was estimated as 4.1 in Pinto Creek and 3.5 in the Devils River. Trophic position of age-0 *D. diaboli* 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.

$\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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. diaboli* 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. diaboli* 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 course 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).

CHAPTER V

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 22⁰C and photoperiods ranging from 10 to 12.5 hours of light per day (Gibson

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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 (16-27⁰C;

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 jemezanus* (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 24.5⁰C in August, lower than the extreme warm temperatures found in the

Devils River (28.9⁰C) 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 3⁰C 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 (22-23⁰C) 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 Whitside 1977; Folb 2010; Perkin et al. 2012). Therefore, selection for synchronous batch spawning does not seem to be an adaption 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 (Williams 1959; Hubbs et al. 1968; Constantz 1979). 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 that 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 course 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 $\delta^{15}N$ values between age classes. At both sites, *D. diaboli* had significant differences in $\delta 15N$ 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 *Astyanyx mexicanus*, juvenile *Lepomis* and *Micropterus* species, and other *Cyprinid* species. Differences in $\delta^{15}N$ 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 δ^{15}N values of *Astyanyx mexicanus* in Pinto Creek as compared to the Devils River, but δ^{15}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.

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 Devils River in October 2009 (F), January 2010 (W), April 2010 (Sp), and July 2010 (S).

|	Pinto: *Dionda diaboli*						Devils: *Dionda diaboli*						Devils: *Dionda argentosa*					
	F	W	Sp	Su	Annual		F	W	Sp	Su	Annual		F	W	Sp	Su	Annual	
	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
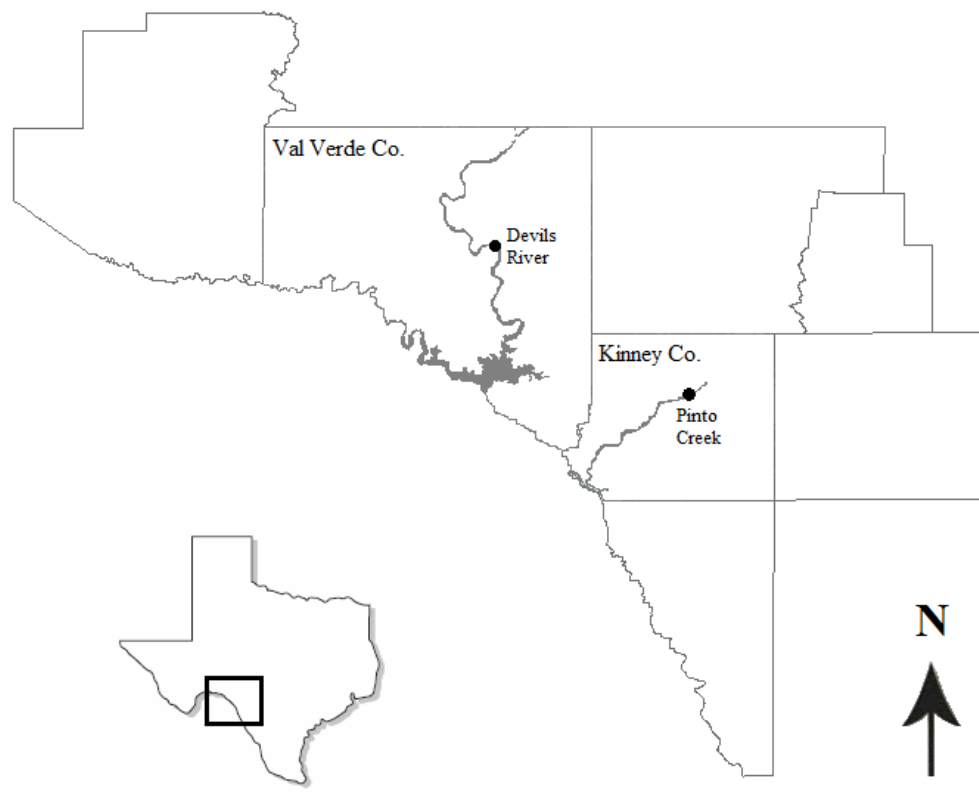
Figure 1. Map of study sites: Pinto Creek at private land, Kinney County, Texas and the Devils River at the Devils River State Natural Area, Val Verde County, Texas.
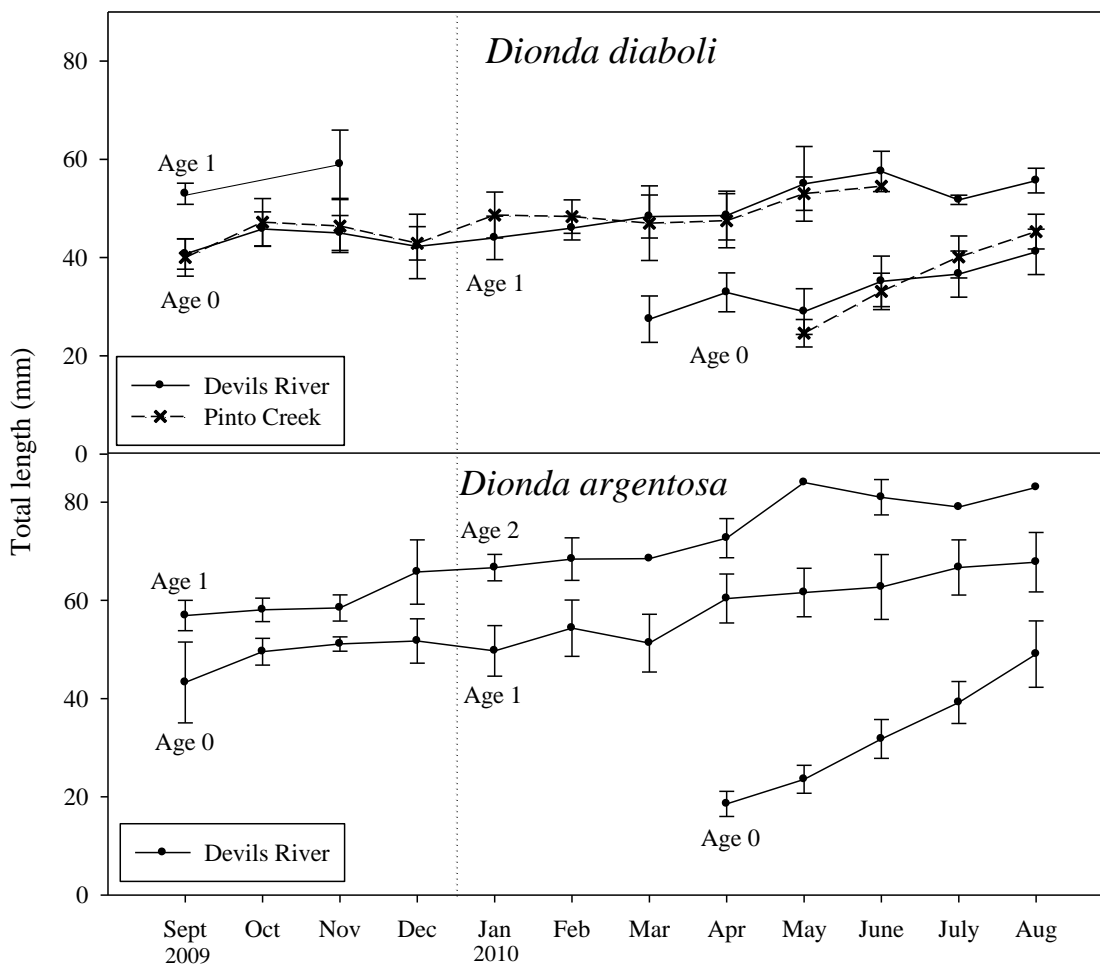
Figure 2. Monthly mean total lengths (±SD) for *D. diaboli* collected from Pinto Creek (N= 644) and the Devils River (N= 706) and *D. argentosa* collected from the Devils River (N= 713) September 2009 through August 2010.
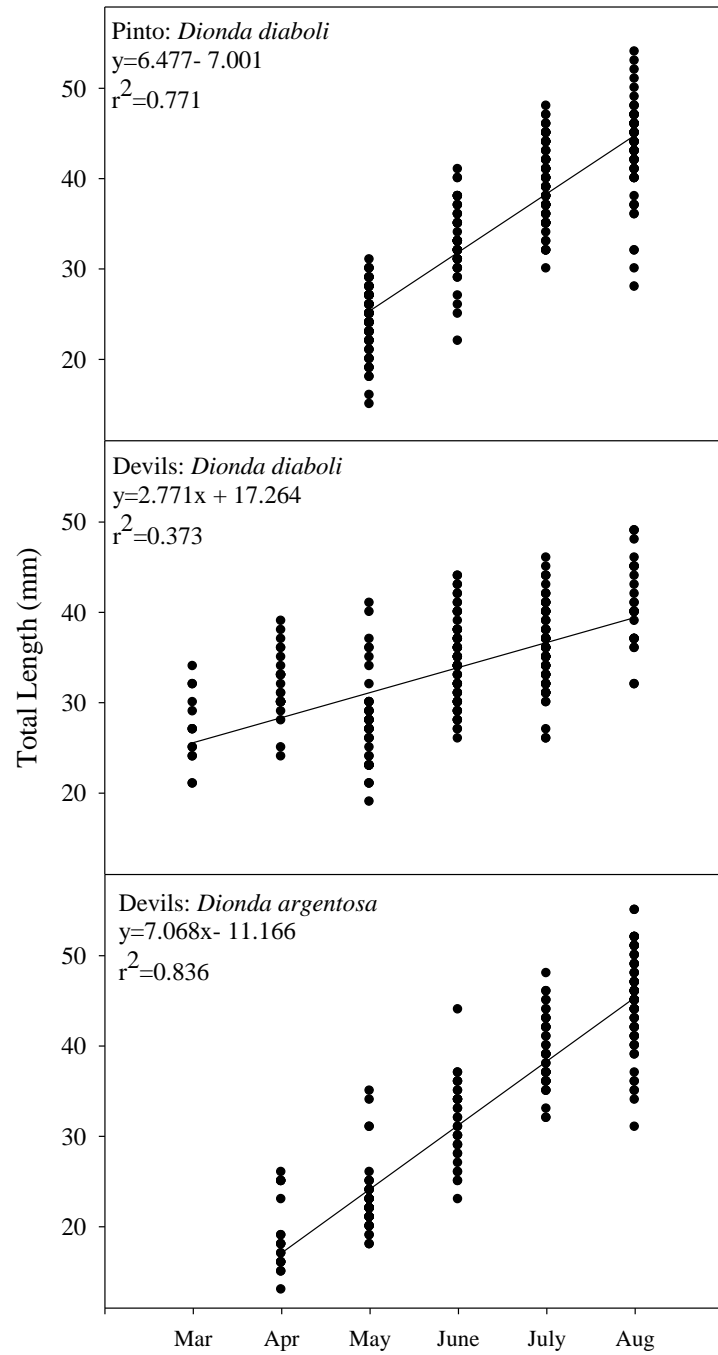
Figure 3. Linear regressions of fish total length versus time of *D. diaboli* and *D. argentosa* collected from Pinto Creek and the Devils River from March 2010 until August 2010.
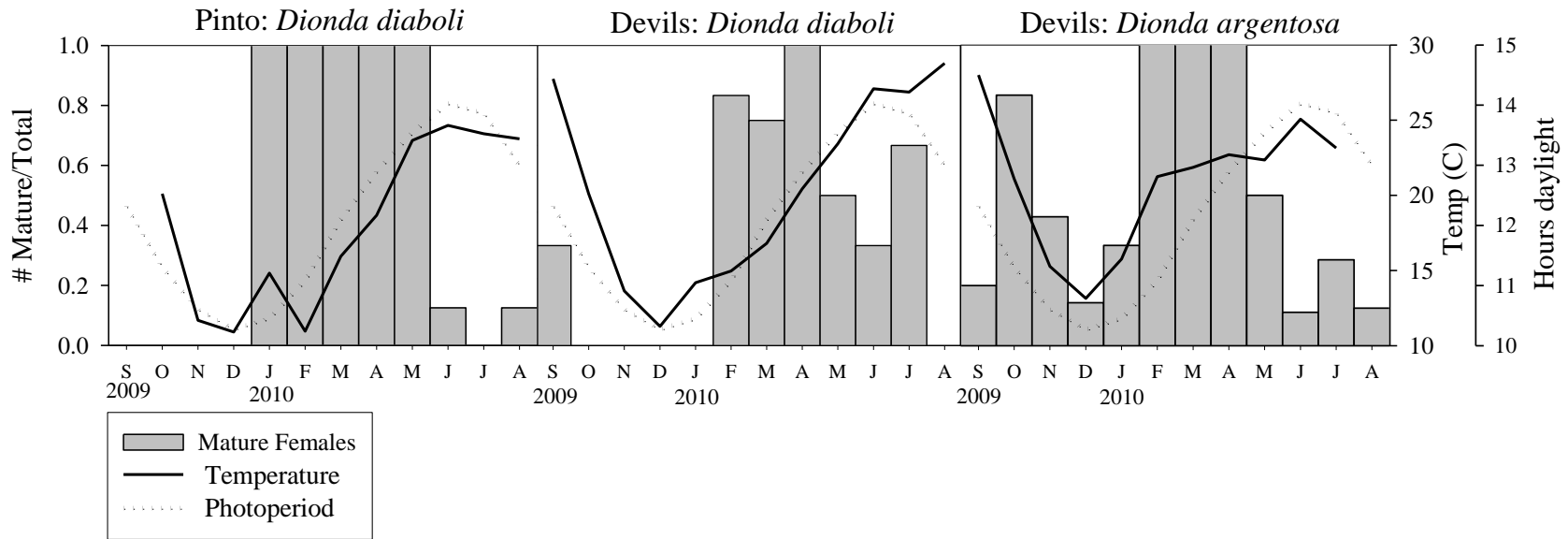
Figure 4. Bar graph representing the proportion of mature females out of the total number of females per month captured from the Devils River and Pinto Creek September 2009 through August 2010 overlaid with average monthly water temperature where *Dionda* were collected (solid line) and average hours of daylight (dotted line).
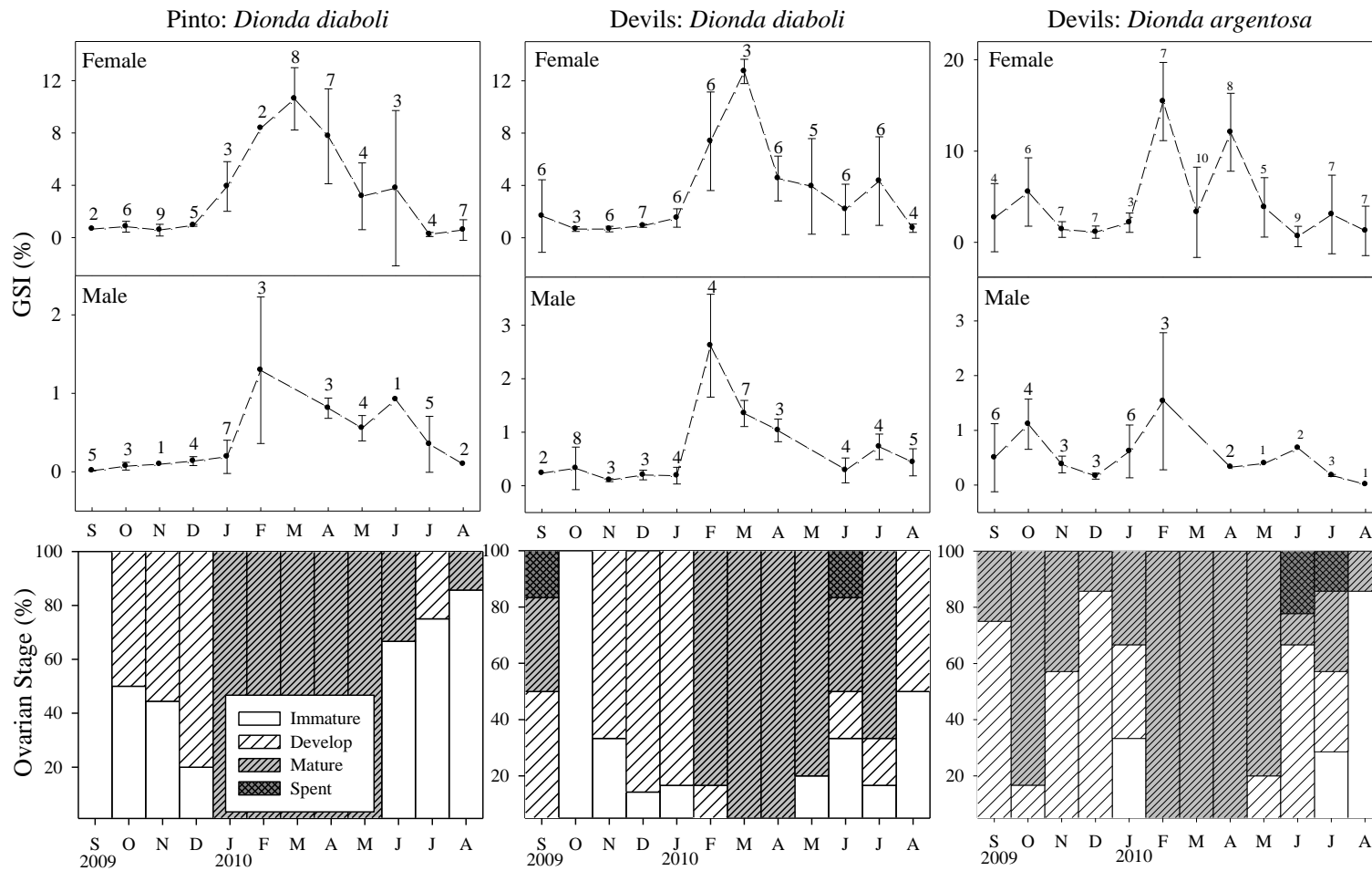
Figure 5. Scatter plots of mean monthly gonadosomatic index (GSI±SD) for female and male *D. diaboli* and *D. argentosa* collected from Pinto Creek and the Devils River September 2009 through August 2010. Numbers located above monthly means represent *n* values. Bar graphs represent monthly ovarian condition (% from total females examined).
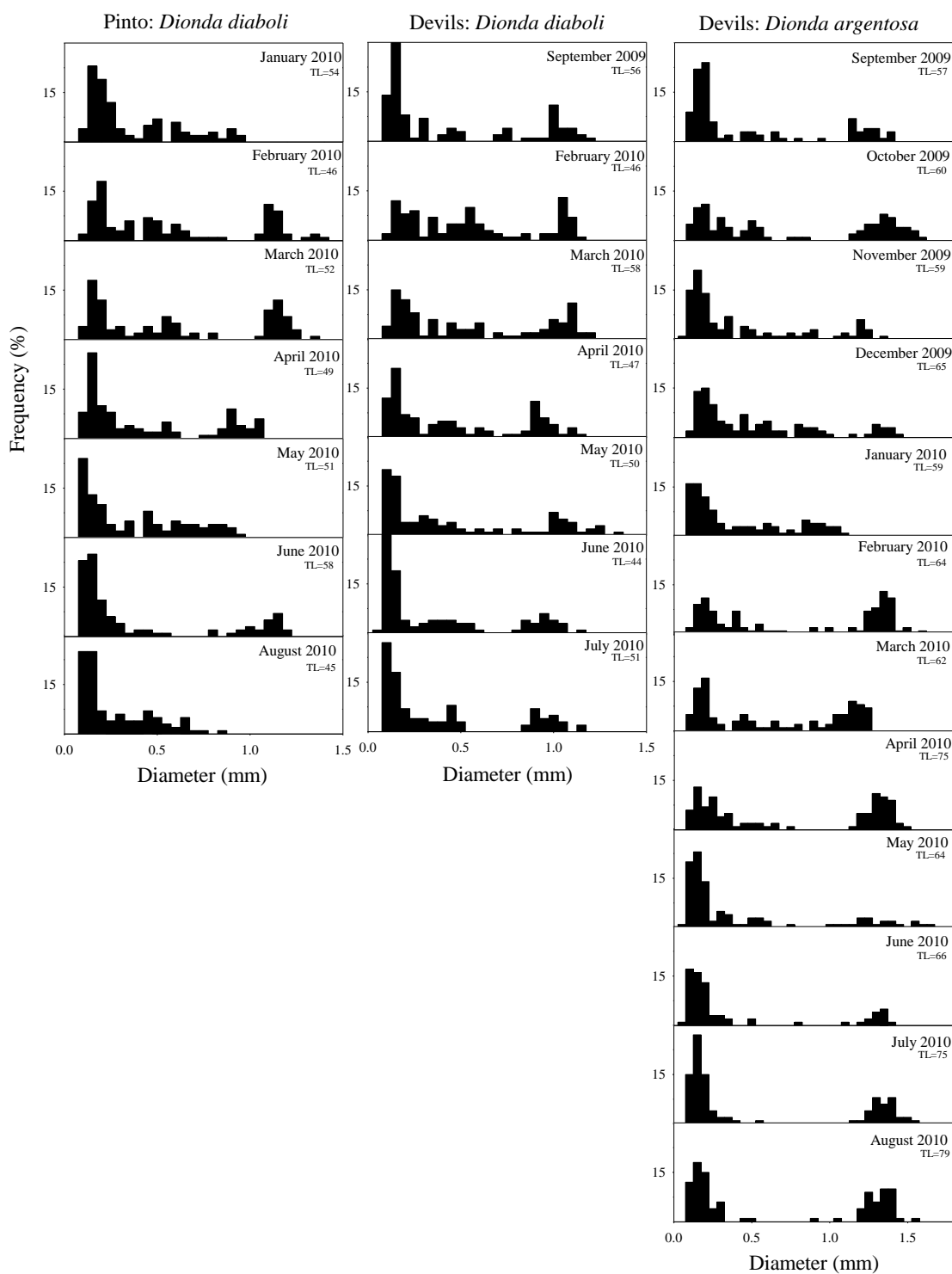
Figure 6. Size frequency histograms of oocyte diameters measured from one *D. diaboli* from each site and one *D. argentosa* displaying mature ova per month. Fish were collected from the Devils River and Pinto Creek September 2009 through August 2010.
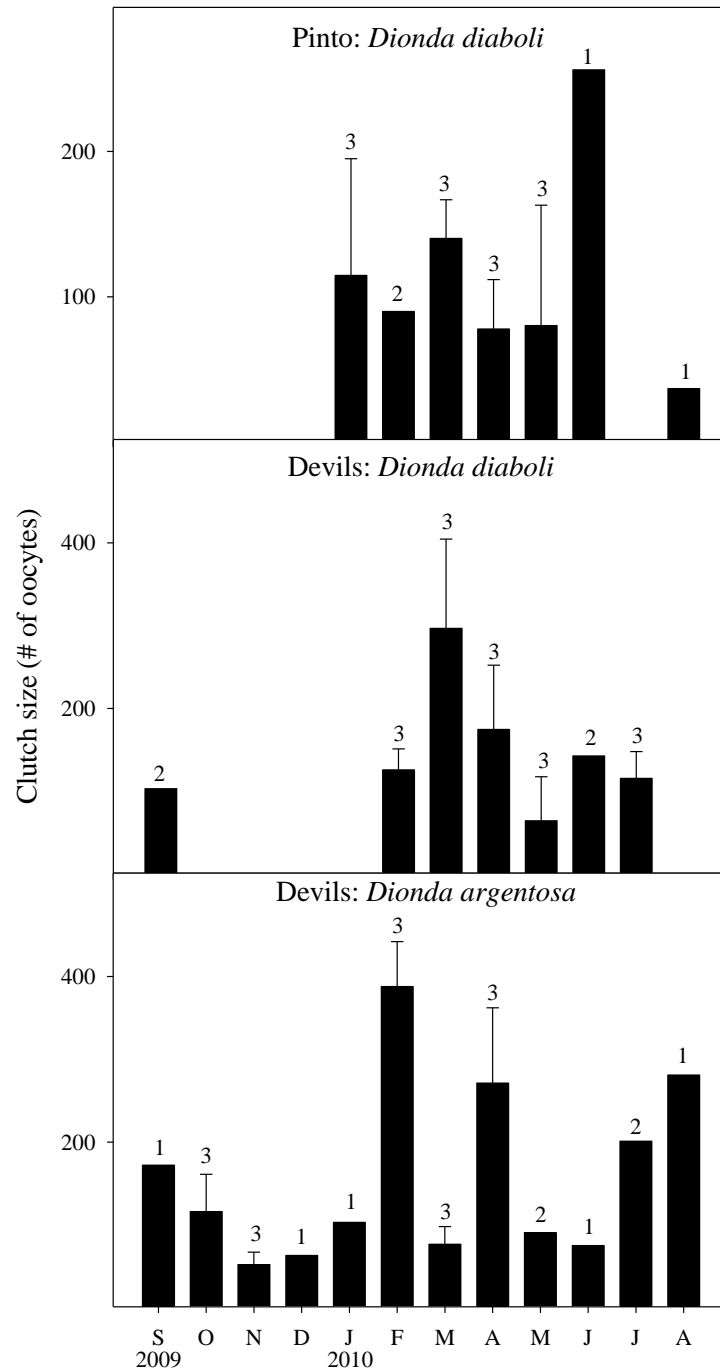
Figure 7. Monthly mean (±SD) clutch abundance estimates for *D. diaboli* and *D. argentosa* collected from Pinto Creek and the Devils River September 2009 through August 2010. Numbers above error bars represent *n* values.
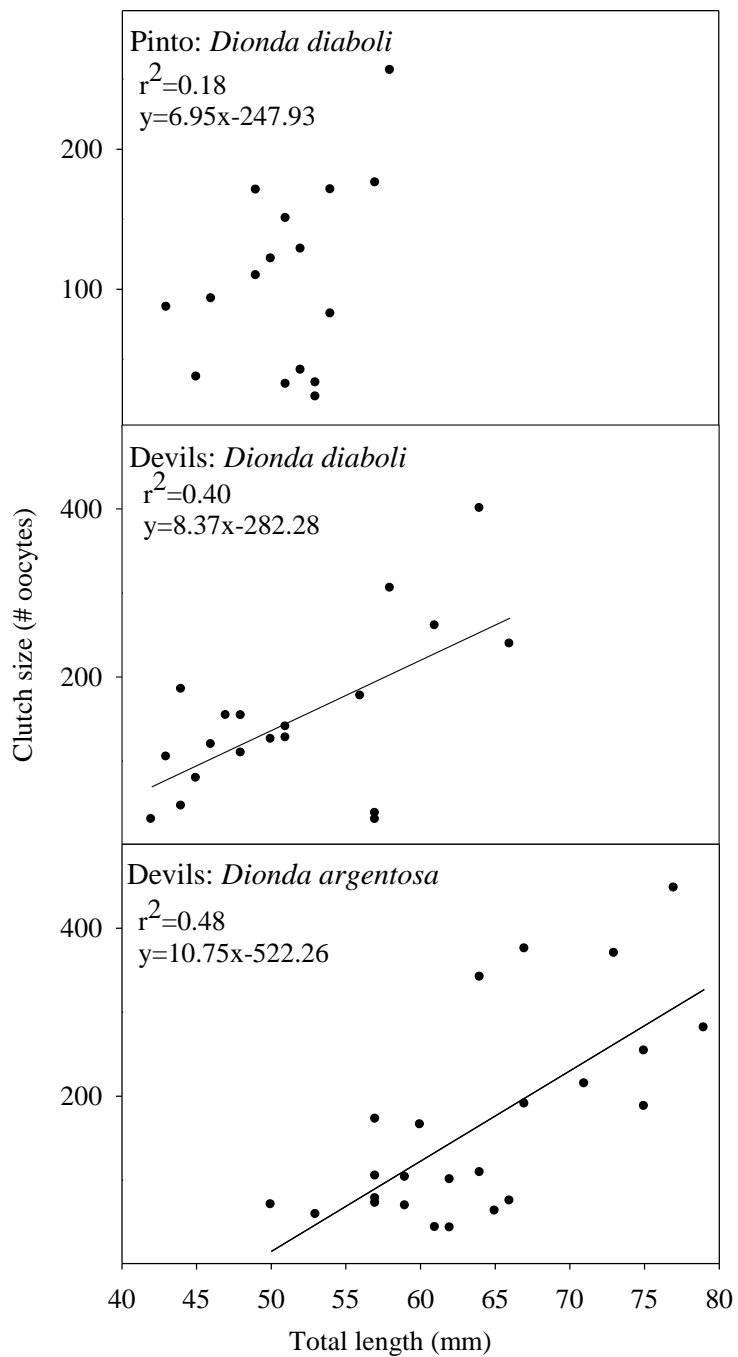
Figure 8. Linear regressions for total length versus estimated clutch size for *D. diaboli* and *D. argentosa* collected from Pinto Creek and the Devils River September 2009 through August 2010.
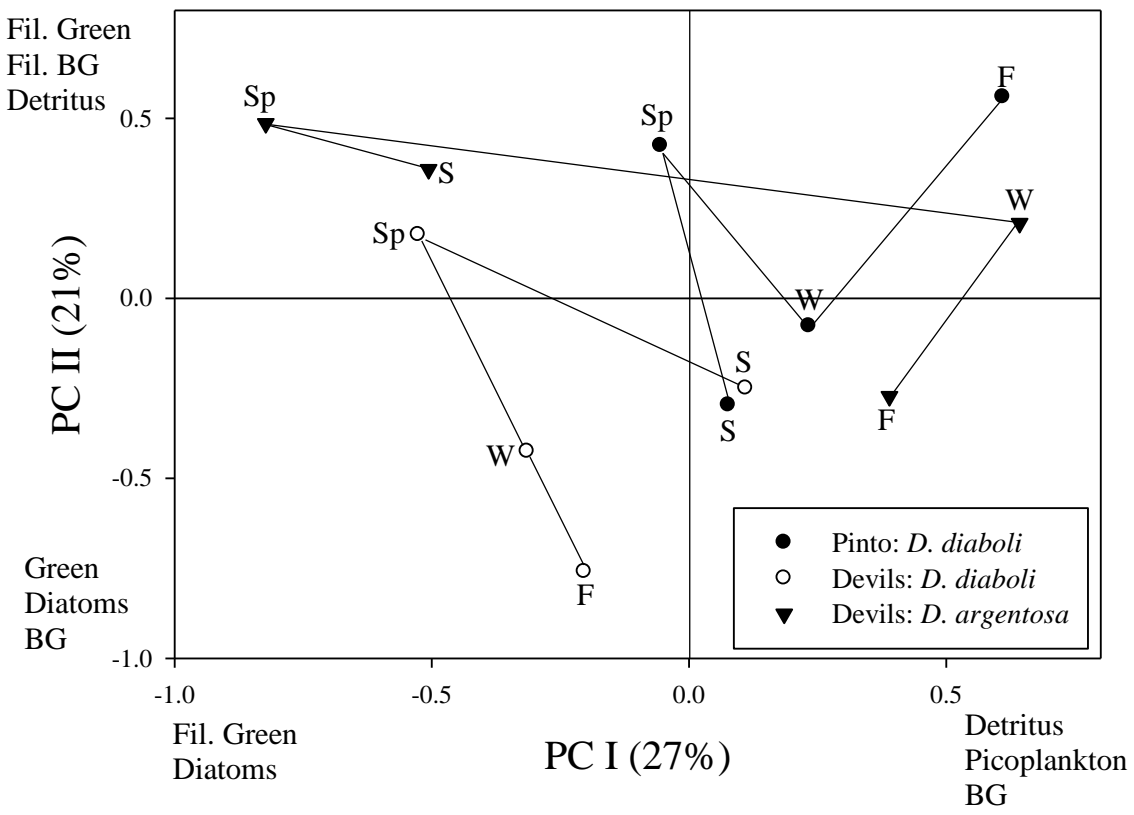
Figure 9. Principle component analysis (PCA) of average seasonal gut content items in percent biovolume for *D. diaboli* and *D. argentosa* collected from the Devils River and Pinto Creek September 2009 through August 2010. Abbreviations are as follows: Fil. (filamentous), BG (blue-green algae).

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.
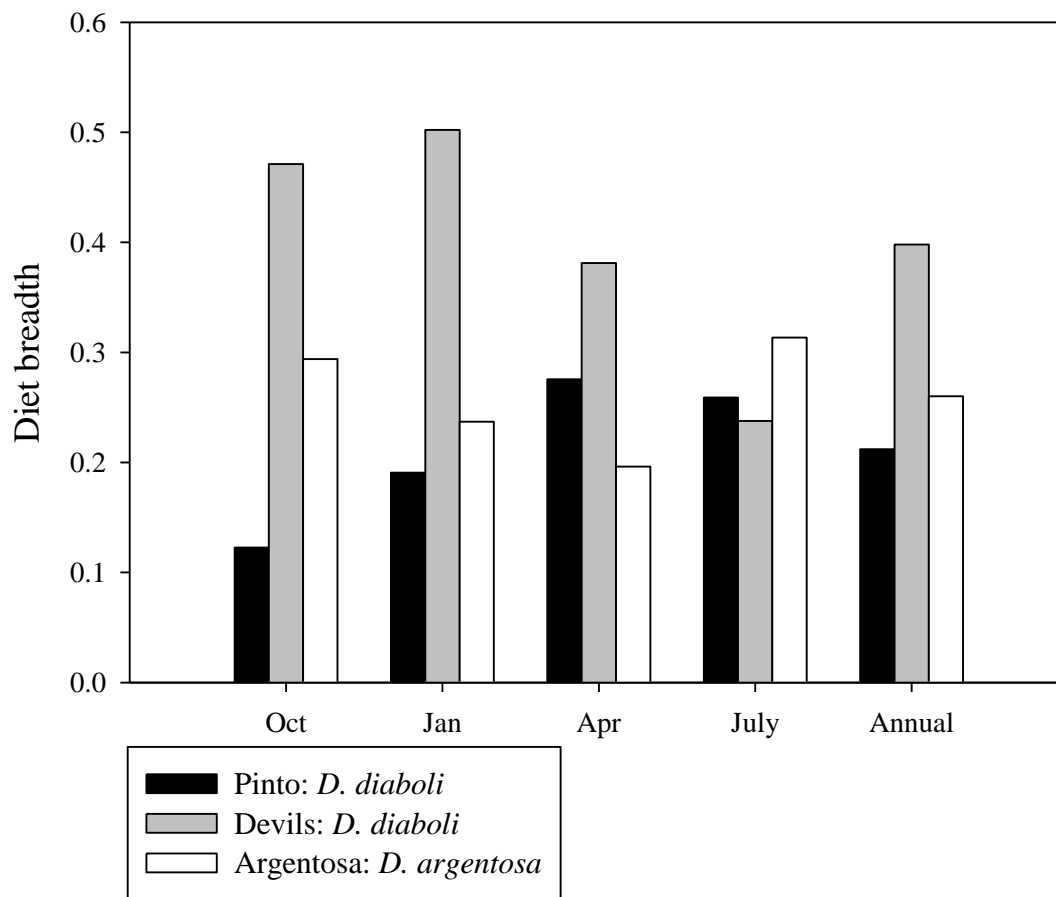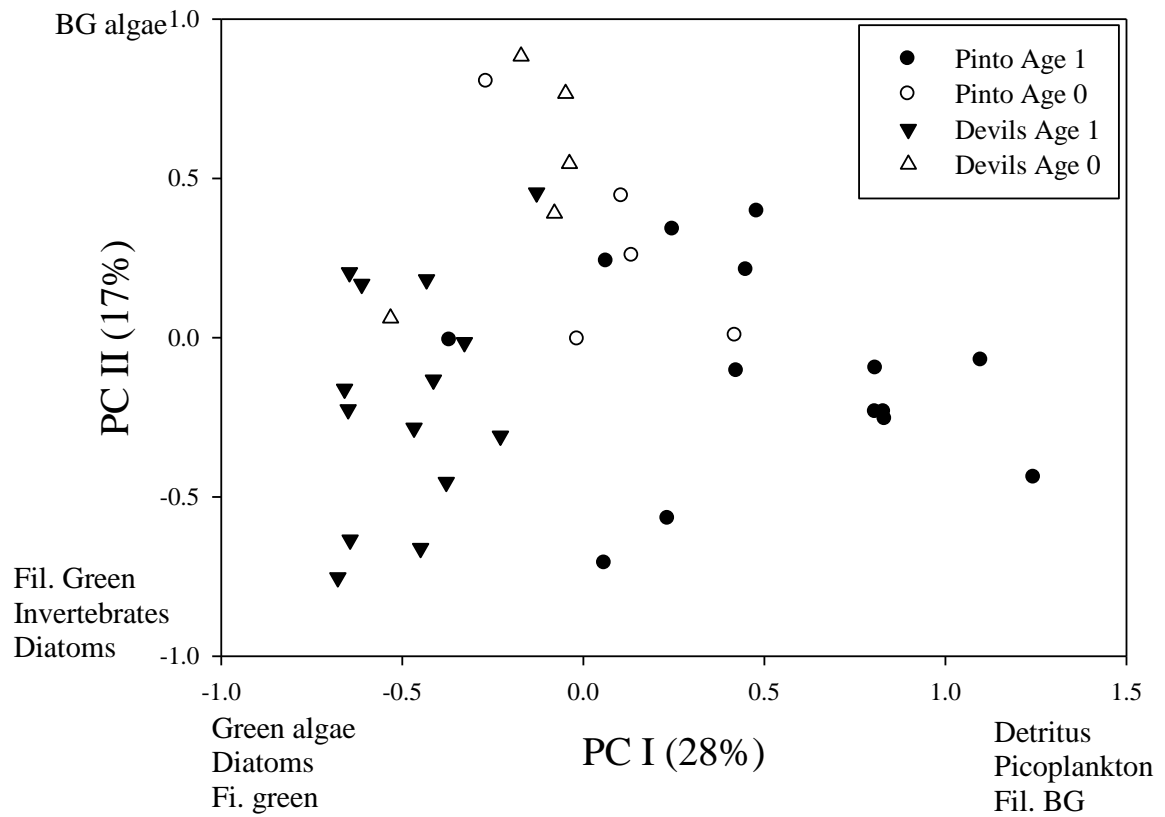
Figure 11. PCA of proportional biovolume of gut content items from age-1 and age-0 *D. diaboli* from Pinto Creek and the Devils River
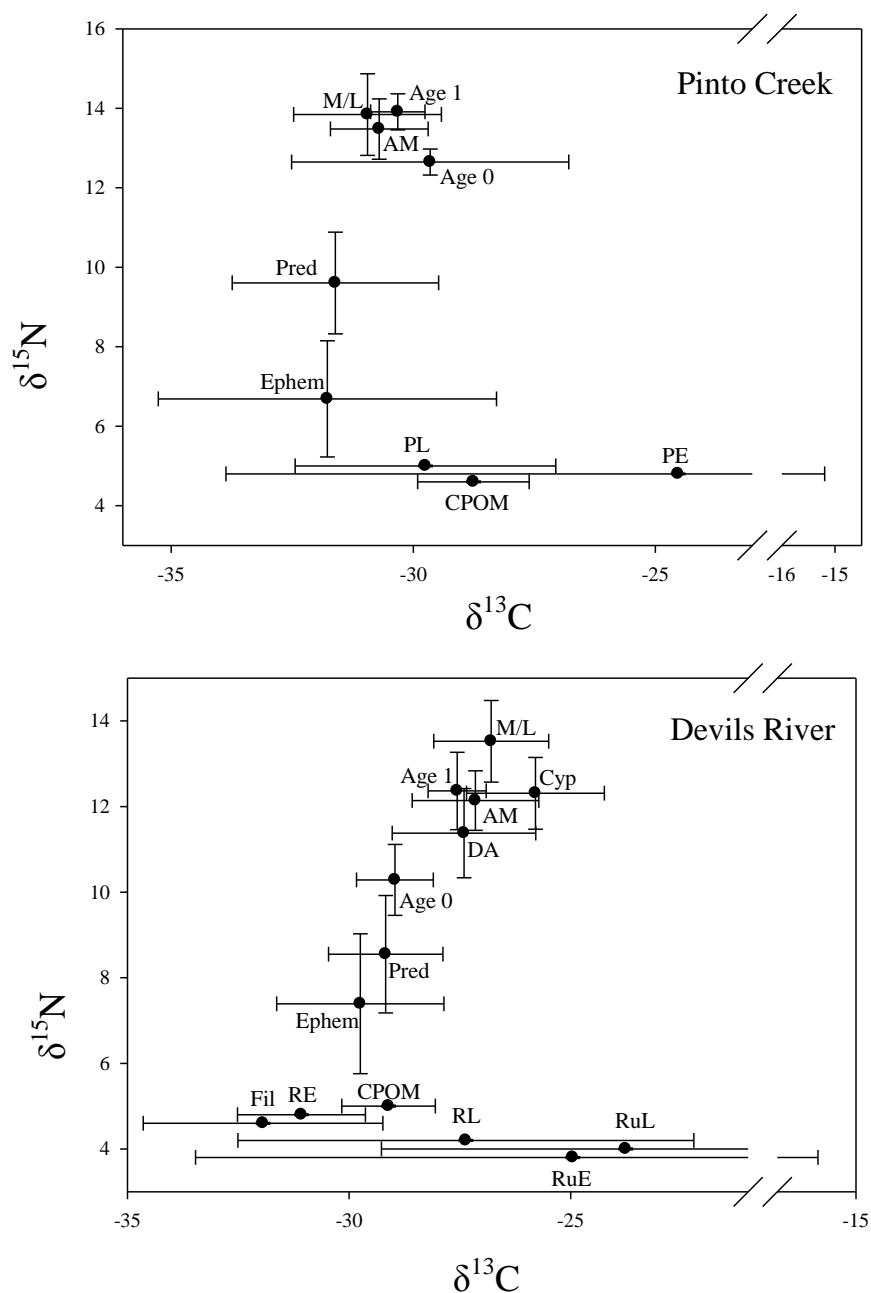
Figure 12. Biplot of average annual δ13C v. δ15N values (±SD) of fish, invertebrate, and source populations from Pinto Creek and the Devils River collected quarterly from October 2009 to July 2010. Abbreviations are as follows: pool lithophytic algae (PL), pool epiphytic algae (PE), course particulate 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 δ¹³C 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 course particulate organic matter.

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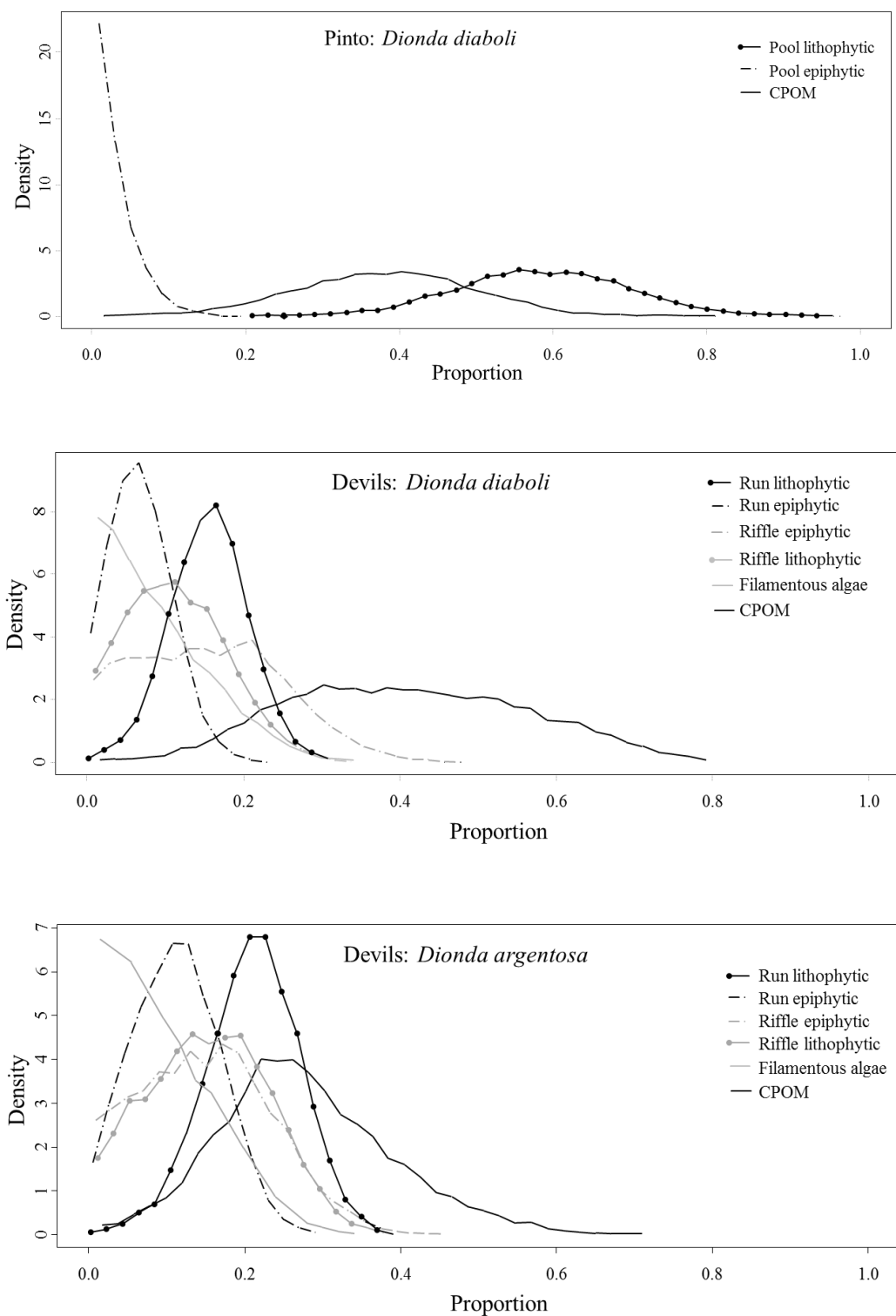Anderson, C., and G. Cabana. 2007. Estimating the trophic position of aquatic consumers in river food webs using stable nitrogen isotopes. Journal of the North American Benthological Society 26: 273-285.

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VITA

Sarah McMillan was born in Corpus Christi, Texas on August 26, 1985. After graduating from Lockhart High School, Lockhart, Texas, in 2003, Sarah entered college to pursue a degree in biology. She graduated with a B. S. in aquatic biology from Texas State University-San Marcos in December 2008. While pursuing her undergraduate degree, Sarah held an internship position with Texas Parks and Wildlife Department's River Studies Program where she decided she wanted to further her education. She entered the graduate program at Texas State in January 2009 to pursue a M. S. degree in aquatic resources with a focus on fisheries ecology.

Permanent Address:

     PO Box 1172

     Lockhart, Texas 78644

This thesis was typed by Sarah M. McMillan