

Reproductive Ecology of the Plains Minnow *Hybognathus placitus*
in the Brazos River, Texas

by

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CHAPTER I

INTRODUCTION

The plains minnow *Hybognathus placitus* is a silvery minnow with a small head and small subterminal mouth (Miller and Robinson 1973). It reaches a maximum total length of 127 mm, or about five inches (Rees et al. 2005). The plains minnow is a member of the family Cyprinidae, which is characterized by having soft-rayed fins, abdominally-located pelvic fins, and one to three rows of pharyngeal teeth. The plains minnow is a sexually dimorphic species; the males have a relatively longer first dorsal fin ray, a larger head, shorter trunk, and longer caudal peduncle than females (Ostrand et al. 2001). The plains minnow is widely distributed and occurs in most major river systems of the Great Plains. It is distributed from Saskatchewan, Canada, and Montana south into Texas (Miller and Robison 1973; Lehtinen and Layzer 1988; Taylor and Miller 1990; Sylvester et al. 2005). Outside the Great Plains, plains minnow can be found in Tennessee, Kentucky, Illinois, Missouri, and Iowa (Lehtinen and Layzer 1988). In Texas, the plains minnow is distributed from the southern Brazos and Colorado river basins northward to the Red and Canadian river basins (Hubbs et al. 1991). Plains minnow is commonly found in large streams and shallow rivers with a braided channel, shifting sand or silt bottoms, and moderate current (Cross 1967; Miller and

Robison 1973; Pflieger 1975; Cross and Moss 1987). The plains minnow is suited to survive in the variable environment commonly found in Great Plains Rivers. It has been reported to thrive in harsh environments in which few other competing species are present (Lehtinen and Layzer 1988; Kelsch 1994). Plains minnow is an algavore and can be found feeding in areas with large amounts of aquatic vegetation, detritus, and algae (Cross et al. 1985; and Winston et al. 1991) in either turbid (Kelsch 1994) or clear waters (Wenke et al. 1993). It has been found congregating in backwaters, eddies, and shallow flowing waters (Matthews and Hill 1980; Lehtinen and Layzer 1988; Peters et al. 1988). These habitat associations predominately depend on hydrological and chemical conditions within the river.

The plains minnow supports an economically important bait industry in New Mexico, Oklahoma, Colorado, Kansas, and Texas (Sliger 1967). In the 1960's bait dealers collected thousands of plains minnows from the Red, Cimarron, and Arkansas river systems in Oklahoma during years of high reproduction (Sliger 1967). Due to its economic importance and functionality within complete species assemblages, fish biologists have started researching the causes for the species decline. Several studies have shown this species to be decreasing in abundance in its native distribution. Plains minnow is considered a species of conservation concern in Montana, North Dakota, and Kansas (Cross and Moss 1987;

Taylor and Miller 1990; Wenke et al. 1993; Bonner and Wilde 2000), and is listed as endangered in the state of Colorado.

The purpose of this research is to characterize the reproductive ecology of the plains minnow in the Brazos River, Texas, and how it relates to previous reproductive ecology studies of the plains minnow and other cyprinids in the Great Plains. Chapter II of this thesis is presented as a manuscript that will be submitted for publication in an appropriate scientific journal.

CHAPTER II

REPRODUCTIVE ECOLOGY OF THE PLAINS MINNOW IN THE BRAZOS RIVER, TEXAS

Abstract

The reproductive ecology of plains minnow, *Hybognathus placitus*, at two localities in the upper Brazos River was studied over a two-year period, January 2008 to December 2009. Using a combination of gonadosomatic indices, measurements of vitellogenic oocyte diameter, and histological analyses, I found that plains minnow spawning season lasts six months from, April through September. Spawning occurs multiple times throughout the spawning season and is often related to increases in stream discharge. The plains minnow population in the Brazos River spawns in synchrony, in relation to increases in discharge. However, these synchronized spawning events, within the population, are not only triggered by flood pulses, but also can be triggered by small increases in base flow. The histological analyses indicate that individual fish spawn asynchronously on a daily basis regardless of discharge. This indicates that the plains minnow is the third genus of fishes in the Great Plains that exhibit this type of spawning behavior (e.g. Arkansas River shiner *Notropis girardi*, smalleye shiner *Notropis buccula*, sharpnose shiner *Notropis oxyrhynchus*, and peppered chub *Macrhybopsis tetranema*).

These species represent significant proportions of the ichthyofauna of the Brazos and Canadian rivers. Since the plains minnow is widely distributed throughout the Great Plains, this suggests that synchronous and asynchronous reproductive strategies may represent the predominate spawning regime for pelagic broadcast-spawning cyprinids of the Great Plains.

Introduction

Cyprinidae is the most speciose family of freshwater fishes in the world with representatives distributed throughout North America, Africa, and Eurasia (Nelson 1994). Cyprinids also have been introduced into Australia and South America (Garcia et al. 2004; Arthington 2011). The family comprises over 2400 species and includes carps and true minnows. In many parts of the world, cyprinids are commercially valuable (Peter and Tan 1997; De Silva et al. 2006), and in Africa and Eurasia various carps, as well as many minnows, are an important source of protein (Penman et al. 2005). In North America, cyprinids are economically unimportant except for the bait trade possibly because most cyprinids are small, nondescript fish that serve little purpose for most individuals. Many of these species are becoming scarce or have become extirpated from their natural ranges.

One of the most notable causes for the decline of many of the world's fishes is decreased reproductive success. One cause for this is construction of dams (Bestgen and Platania 1990; Winston et al. 1991; Anderson et al. 1995; Platania and Altenbach 1998; Luttrell et al. 1999; Wilde and Ostrand 1999; Bonner and Wilde 2000; Perkin and Gido 2011). Dams and impoundments change chemical and physical conditions in river systems, most notably in their downstream reaches (Baxter 1977; Stanford and

Ward 1979). Baxter (1977), Holden (1979), and Bain et al. (1988) suggested that dams directly affect stream fish populations because of changes in substrate, water temperature, timing and volume of discharge, and presence of backwater areas for refuge. Not only do dams alter physical and chemical habitats of rivers but they also decrease river-fragment length. Decreases in distance between headwaters and dams, as well as shorter distances between dams, make reproduction difficult for pelagic-spawning fishes and prevent riverine spawning migrations (Platania and Altenbach 1998; Braaten and Guy 1999; Dieterman and Galat 2004; Perkin and Gido 2011). All of these effects are important when considering persistence of obligate riverine species.

Streams and rivers that are primarily supplied by precipitation events are often dewatered, which has an effect on reproductive success, in regions known to have large fluctuations in annual precipitation. Extirpation and species decline are most notable in the semi-arid regions of the world, such as Australia, parts of Spain, Portugal, and the United States, where water availability is variable (Cadwaller 1978; Cross and Moss 1987; Pfeieger and Grace 1987; Pigg 1991; Pittenger and Schiffmiller 1997; Luttrell et al. 1999; Aparicio et al. 2000; Bonner and Wilde 2000). Graf (1988) found that annual precipitation varied as much as 200% over a 20-year period in western portions of the United States. Dams and annual

precipitation shifts have negative impacts on stream discharge, and have negative consequences for downstream reaches of river systems. Bonner and Wilde (2000) showed that after construction of Ute Reservoir on the Canadian River, New Mexico, discharge below the dam decreased by about 38%. Bonner and Wilde also reported that Ute Reservoir and Sanford Dam on Lake Meredith, Canadian River, Texas both had a compounded decreased discharge below Sanford dam of about 76%.

Alterations in flow regimes have many effects on physical characteristics of the river as well as on the aquatic organisms that inhabit them. Because reduction in stream flow, due to construction of dams and precipitation shifts, has had such an impact on many stream fish species, a closer look at the reproductive ecology of these species could help fishery managers understand the cause of these impacts. With this information, it may be possible to mitigate negative impacts that dams seem to have on obligate riverine species by devising management plans that can take reproduction into consideration.

Understanding the reproductive ecology of a species is important when trying to understand changes in abundance, because the persistence of any species is measured by its ability to successfully produce offspring (Wootton 1990; Moyle and Cech 2004). To date, only four studies have examined the reproductive biology of the plains minnow, *Hybognathus*

placitus. The plains minnow spawns semi-buoyant nonadhesive eggs that float in the water column (Sliger 1967; Platania and Altenbach 1998).

From this, Miller and Robison (1973) hypothesized that eggs develop and hatch as they are carried down river during periods of high flow.

Lehtinen and Layzer (1988) and Taylor and Miller (1990) studied the reproductive ecology of the plains minnow using gonadosomatic indices (GSI) and vitellogenic oocyte diameters. They showed that the plains minnow has a protracted reproductive season that lasts from April through late August, as suggested by Cross (1967). Lehtinen and Layzer (1988) and Taylor and Miller (1990) observed that the plains minnow appeared to spawn in conjunction with flood pulses and their subsequent receding flows. Taylor and Miller (1990) observed that the plains minnow is capable of spawning multiple times during the season in association with flood pulse events. Lehtinen and Layzer (1988) attributed multiple peaks in young of year length-frequency distributions to possible periods of flood pulses. Taylor and Miller (1990), on the other hand, observed that the plains minnow was reproductively active throughout the spawning season and that spawning activity was more pronounced during periods of flood pulses. Durham and Wilde (2008a) observed that small numbers of smalleye shiner, *Notropis buccula*, spawn on a daily basis regardless of stream discharge. They also observed that spawning intensified and in

greater proportion within the smalleye shiner population in relation to increases in discharge. They described this behavior as asynchronous and synchronous spawning, depending on the proportion of the population that spawned at a given time. This suggests the plains minnow may use two different reproductive strategies, synchronous and asynchronous spawning, depending on flow conditions. While these reproductive studies provide information on synchronous population spawning and hypothesize asynchronous individual spawning, a more detailed investigation on the reproductive ecology of individual plains minnow is needed.

For years, studies of reproduction in fishes have been limited to investigations on either population-level spawning or spawning of the individual, but rarely both. Currently, most studies use GSI and vitellogenic oocyte measurements to estimate population-level information, (e.g., Lehtinen and Layzer 1988; Taylor and Miller 1990), whereas a few have used histological methods to infer individual-level information on spawning behavior (e.g., Bonner 2000; Durham and Wilde 2008a). Durham and Wilde (2008a) argued that in order to understand the reproductive ecology of a pelagic, broadcast-spawning fish multiple approaches are necessary. These approaches include using GSI, vitellogenic oocyte diameters, and histological methods. Durham and Wilde (2008a) used several approaches to determine the spawning habits

of smalleye shiner, *Notropis buccula*, in the Brazos River, Texas. By using GSI, vitellogenic oocyte diameters, and histological methods they were able to get a more complete picture of population level as well as individual-level spawning habits for smalleye shiner. In order to more fully understand the reproductive ecology of the plains minnow, exploration of the spawning habits of the individual by means of histological assessment, as well as habits of the population need to be assessed in conjunction with one another.

Methods

Study Area

The Brazos River is the largest drainage system in Texas. It is approximately 2,060 km in length, starting at Blackwater Draw in New Mexico, flowing southeast through the state of Texas until it releases into the Gulf of Mexico at Freeport, Texas. The headwaters of the Brazos River are composed of two tributaries, the North Fork of the Double Mountain Fork which rises in Lubbock County, and the South Fork of the Double Mountain Fork which rises in Garza County. These tributaries combine in eastern Kent County to form the Double Mountain Fork Brazos River. In northeast Stonewall County the main stem of the Brazos River is formed by the confluence of the Salt Fork of the Brazos River, which rises in south central Crosby County, and the Double Mountain Fork. This study was

conducted on the upper portion of the Brazos River, which is defined as the main stem of the Brazos River upstream from Possum Kingdom Reservoir, and its tributaries.

Two sites on the upper Brazos River were chosen for study (Figure 1). Site 1 is located on the Double Mountain Fork Brazos River at State Highway 70, north of Rotan, Texas, in Fisher County (32° 55.823'N; 100° 29.291'W). Site 2 is located on the Brazos River mainstem at State Highway 6 north of Knox City, Texas in Knox County (33° 30.034'N; 99° 48.176'W). I obtained stream flow data for Site 1 from USGS water information gauge 08080500. This gauge is located on the Double Mountain Fork of the Brazos River, south of Aspermont, Texas, on U.S. Highway 83, which is approximately 30-km downstream from the sampling site. Stream flow data for Site 2 were obtained from the USGS water information gauge 08082500, which is located on the Brazos River mainstem at Seymour, Texas, approximately 75-km downstream.

Fish Collections

I collected fish at Site 1 and Site 2, from January 2008 through December 2009, monthly except during the spawning season, April through September, when samples were collected twice each month. In 2008, only one sample was collected at Site 1 in April, and no samples were collected in October due to flooding. In December 2009, no samples were

collected at Site 2. My sampling protocol used a 3.7 x 1.8-m seine with 5-mm mesh to sample all available habitats in an attempt to collect at least 50 reproductively mature plains minnow at each site, on each sampling date. However, on several sampling dates the target number was not reached either because of high discharge or low fish abundance. Collected fish were preserved in 10% buffered formalin for two weeks and were then transferred into 70% ethanol for storage and analysis.

Population Size Structure

Each individual was approximately measured to the nearest 0.05-mm standard length (SL) using dial calipers. Length-frequency histograms were constructed to determine population size structure. Size structure was determined by visual observation of peaks in length-frequency histograms.

Ovarian Development

Up to fifty reproductively mature plains minnow (> 40 mm, SL), at each site on each sampling date, were weighed to the nearest 0.1 mg, dissected, and their gonads removed. Sex of each fish was determined by visual observation of the gonads. Male testes were weighed to the nearest 0.1 mg and discarded. Female ovaries were weighed to the nearest 0.1 mg and were classified into groups, based on maturity, for determination of reproductive status. Ovaries were categorized into one of four

developmental stages described by Phillip (1993), Bonner (2000), and Durham and Wilde (2008a). Stages I and II represent immature and developing ovaries, respectively, whereas stages III and IV correspond to mature and spent ovaries, respectively. In this system, a stage I ovary was small, flaccid, and contained no developing or mature oocytes. Stage II ovaries had begun to increase in size, and contained few developing and no mature oocytes. Stage III ovaries were considered to be mature and had the potential for spawning. These ovaries were larger than ovaries in other stages, yellowish in color, and contained mature vitellogenic oocytes. Stage IV, spent, ovaries started to recede in size, number of mature, and developing oocytes.

For graphical output, total body weight for both male and female plains minnow, was used along with gonad weight to determine gonadosomatic index ($GSI = [\text{gonad weight} / \text{total body weight}] * 100$), or percent body weight that was dedicated to reproduction. For statistical analysis body weight was calculated by ($\text{body weight} = [\text{body weight} - \text{gonad weight}]$) and log mean GSI was determined by ($\log GSI = [\log \text{gonad weight}] - [\log \text{body weight}]$).

Oocyte Classification

On each sampling date, ovaries of up to 15 mature fish were used for oocyte counts, oocyte size distributions, and histological assessment. For

histological analysis, one half of each ovary from mature female plains minnow from Site 1 was sent to the Texas Veterinary Medical Diagnostic Laboratory in Amarillo, Texas. There it was embedded in paraffin wax, sectioned longitudinally at a thickness of 5-9 μm , and stained in Weigert's hematoxylin and eosin stain following the methods used by Crim and Glebe (1990), Hinton (1990), Patiño and Takashima (1995), Bonner (2000), and Durham and Wilde (2008a). Histological sections from each slide were observed using a compound microscope at 40x magnification. One section from each ovary was observed and all oocytes were classified into one of seven groups: perinucleolar (PN), cortical alveoli (CA), early vitellogenic (EV), late vitellogenic (LV), germinal vesicle breakdown (GB), post-ovulatory follicle (PO), and atretic follicle (AT). Perinucleolar oocytes were the most abundant stage of development, but were left out of all graphs and analysis because they may or may not develop into mature oocytes.

Vitellogenic Oocyte Size Distribution and Fecundity

Half of each stage III paired ovary from Site 1 and 2 were used to create vitellogenic oocyte size distributions and determine fecundity. Each of the halved ovaries were placed on a counting wheel mounted on a dissecting microscope, and submerged in water. Mature vitellogenic oocytes then were separated from each other and from ovarian connective tissues by means of mechanical disruption using forceps. Mature oocytes

were counted and diameters of 100 randomly selected mature oocytes were measured to the nearest 0.1-mm using a calibrated ocular micrometer.

Statistical Analysis

Standard length and log mean GSI for plains minnow were compared among years, sites, and sampling dates by fitting a generalized linear model to the data. I used SAS statistical software and the GENMOD procedure to conduct this statistical analysis. Because samples were collected at the same sites on each sampling date, I treated site as a repeated measure in all models. I fit models to the data using a normal error distribution with an identity link function. Because generalized linear models are fit to data using maximum likelihood methods, significance was based on the X^2 distribution. In cases where year was a significant predictor of GSI ($\alpha = 0.05$), I recoded year as a class variable to evaluate differences between sites and sampling dates within each year. If year was not a significant predictor of length or GSI, I evaluated differences among sites and sampling dates across years.

Relationships between discharge and mean female GSI between years and sites, within the reproductive seasons, were modeled using separate linear regression. I used SAS statistical software and the GENMOD procedure to conduct these regression analyses. The regression models compared the running seven-day mean of daily mean discharge

previous to sampling dates and mean female GSI between sampling dates. If a general relationship existed between discharge and mean female GSI, I would expect the regression slope to be negative.

Discharge and mean vitellogenic oocyte diameter relationships were modeled using separate linear regression as well. The GENMOD procedure in SAS statistical software was used to conduct these regression analyses. The regression model compared the running seven-day mean of daily mean discharge previous to the sampling dates and mean vitellogenic oocyte diameter between sampling dates. If a general relationship existed between discharge and mean vitellogenic oocyte diameter, I would expect the regression slope to be negative.

Results

Population Size Structure

A total of 1,847 plains minnow was collected at Site 1 and Site 2 during 2008 and 2009. Length-frequency histograms indicate that from January through July of each year, at both sites, the population consisted of primarily age-1 individuals. Standard length of adult plains minnow collected at Site 1 ranged from 40.0 mm to 79.4 mm (Figures 2 - 4). At Site 1, recruitment of age-0 individuals started in late July 2008 and 2009.

At Site 2, age-1 individuals were most common in 2008 from January to early June, and then disappeared. In early August 2008, recruitment of age-0 individuals started, and was sporadic in abundance through the rest of the year. In 2009, at Site 2 age-1 individuals were present from January through December, and recruitment of age-0 plains minnow appeared in early July. Lengths at Site 2 ranged from 40.0 mm to 77.9 mm (Figures 5 - 7).

Standard length of plains minnow differed between years ($P \leq 0.001$), sites ($P \leq 0.001$), and between most sampling dates ($P \leq 0.001$). Samples collected on 5 August 2008 were not significantly different between sites. Even though differences in standard length of plains minnow were observed on different sampling dates, no consistent patterns were observed in length during 2008 and 2009.

Oocyte Classification

A total of 230 reproductively mature plains minnow ovaries were examined from fish at Site 1. All oocyte developmental stages were observed throughout the 2008 and 2009 spawning season (Figures 11 - 17) which indicates continuous recruitment towards late vitellogenic oocytes was occurring during both spawning seasons. However, late vitellogenic oocytes were the most abundant developmental stage in both years. Ovaries that contained oocytes undergoing germinal vesicle breakdown or

the presence of post-ovulatory follicles indicate that spawning was in progress or had recently occurred. The first evidence that spawning occurred, by the presence of germinal vesicle breakdown or post-ovulatory follicles, was on 21 April 2008 (Figure 11) and on 4 April 2009 (Figure 14). On 24 June 2008 there was an increase in post-ovulatory follicles observed in all fish from this sampling date (Figure 12). This also occurred on 22 July 2008, 5 August 2008 (Figure 13), 18 August 2009, and 17 September 2009 (Figures 17). Relative abundance of post-ovulatory follicles and decreased number of late vitellogenic oocytes on these dates suggest the population spawned in synchrony. The presence of oocytes undergoing germinal vesicle breakdown and the presence of post-ovulatory follicles occurred in at least one fish, on each sampling date, from April to September of both years. In 2008 germinal vesicle breakdown or post-ovulatory follicles were last seen on 1 September (Figure 13) and on 29 September in 2009 (Figure 17).

Ovarian Development

At Sites 1 and 2, ovarian development was similar in periodicity and duration during both years. In January 2008 and 2009 at Site 1, resting ovaries (Stage I) were observed in all females collected (Figure 8). Maturing ovaries (Stage 2) were first observed in February of both years, and remained present until April 2008 and May 2009. In April 2008 and

2009, Stage 3 (mature) ovaries started to appear within the population. By early May 2008 and late May 2009, all ovaries observed were classified as mature. Stage 4 (spent) ovaries first appeared in late July 2008 and early July in 2009. Stage 1 ovaries appeared in the population again starting in late August of both years.

In January of both years, at Site 2, stage 1 (resting) ovaries were observed in all mature female plains minnow. Stage 1 ovaries continued to be present until April of both years. Stage 2 ovaries first appeared in March of both years, and completely disappeared by May of both years. Stage 3 mature ovaries first appeared, at Site 2, in April 2008 and March 2009. In early May 2008 and 2009, stage 3 ovaries represented the dominant stage of reproductive development. Spent ovaries appeared in early August 2008 and late May 2009. Stage 1 ovaries started to appear again in early August 2008 and September 2009.

Female plains minnow GSI at Site 1 ranged from 0.11 to 31.40% in 2008, and from 0.33 to 37.21% in 2009 (Figure 9 A). Male GSI from this same locality ranged from 0.04 to 4.26% in 2008 and from 0.04 to 1.77% in 2009 (Figure 9 B).

Female plains minnow GSI in 2008 at Site 2 ranged from 0.30 to 25.15% and from 0.53 to 34.97% in 2009 (Figure 10 A). Male GSI at Site 2

ranged from 0.07 to 1.58% in 2008 and in 2009 ranged from 0.06 to 1.57% (Figure 10 B).

Statistical analysis indicate that that female plains minnow mean log-GSI was not significantly different between 2008 and 2009 ($P = 0.91$). In 2008 and 2009, mean female log-GSI was significantly different among sites ($P \leq 0.001$). Mean log-GSI of male plains minnow were not different among years ($P = 0.31$) but were different among sites in 2008 and 2009 ($P \leq 0.001$).

Slopes of regression variable estimates for female GSI were negative for three of the four site and year combinations (Table 1). This suggests the presence of a general negative relationship between female GSI and discharge.

Vitellogenic Oocyte Size Distribution and Fecundity

During the 2008 and 2009 reproductive season 350 reproductively mature females from both sites were examined to determine vitellogenic oocyte size and fish fecundity. Vitellogenic oocytes ranged from 0.4 mm to 1.5 mm in diameter (Figures 18 - 19). Mean diameter of vitellogenic oocytes during the reproductive season at Site 1 was 0.87 mm in 2008 and 0.92 mm in 2009. At Site 2 the mean size of vitellogenic oocytes were 1.01 mm and 0.99 mm in 2008 and 2009, respectively.

Mean vitellogenic oocyte diameter decreases can be seen repeatedly throughout the 2008 and 2009 spawning season at Site 1 and 2 (Figures 18 - 23). During the spawning seasons of both years at both sites, vitellogenic oocyte size and quantity increased within the population, which was followed by a size decrease toward smaller and more vitellogenic oocytes in the ovary. In 2008 at Site 1, this decrease took place between samples collected on 21 April and 6 May 2008 (Figure 20). On 21 April 2008, at Site 1, vitellogenic oocytes ranged from 0.6 mm to 1.3 mm with a mean size of 0.93 mm. When fish were sampled again on 6 May 2008, vitellogenic oocytes ranged from 0.4 mm to 1.3 mm with a mean size of 0.78 mm. This decrease in vitellogenic oocyte size coincided with an increased female mean GSI on 21 April 2008 and a subsequent decrease in mean female GSI on 6 May 2008 (Figure 9 A). Mean GSI decreases and oocyte diameter decreases are also present at Site 1 between 10 June and 24 June 2008 (Figure 20), 2 July and 22 July 2008 (Figure 20), 5 August and 20 August 2008 (Figure 20), 26 May and 20 June 2009 (Figure 21), 29 June and 13 July 2009 (Figure 21), 29 July and 10 August 2009 (Figure 21), 10 August and 18 August 2009 (Figure 21). This also occurred at Site 2 between 21 April and 6 May 2008 (Figure 22), 10 June and 24 June 2008 (Figure 22), 26 May and 20 June 2009 (Figure 23), 20 June and 29 June 2009 (Figure 23), and from 29 June to 13 July 2009 (Figure 23).

During the 2008 reproductive season, the mean number of vitellogenic oocytes in fish from Site 1 was 348.5 with a range of 35-1,474. In 2009, the mean number of mature oocytes increased to 408.1 and ranged from 24-2,999. At Site 2, the 2008 mean was 478.6 (range 52-1,304) and in 2009 was 437.8 (range 56-2,667).

Slopes of regression variable estimates for mean female vitellogenic oocyte diameter and discharge were negative for all four site and year combinations (Table 2). This suggests the presence of a general negative relationship between female oocyte diameter and discharge.

Discharge

Discharge in the upper Brazos River varied greatly throughout 2008 and 2009. Discharge at Site 1 fluctuated from 0.14 to 80.42 m³·s⁻¹ in 2008 and from 0.05 to 15.66 m³·s⁻¹ in 2009 with a mean daily discharge of 2.89 and 0.79 m³·s⁻¹, respectively (Figure 24). During the 2008 spawning season, April through September, discharge ranged from 0.14 to 80.42 m³·s⁻¹ with a mean daily discharge of 3.83 m³·s⁻¹. In 2009, discharge during the reproductive season ranged from 0.08 to 15.66 m³·s⁻¹ with a mean daily discharge of 1.18 m³·s⁻¹. Discharge at Site 2 fluctuated from 0.06 to 23.50 m³·s⁻¹ in 2008 and from 0.07 to 21.80 m³·s⁻¹ in 2009 (Figure 25). Mean daily discharge throughout 2008 and 2009 was 3.70 and 1.68 m³·s⁻¹, respectively.

Discharge fluctuated from 0.06 to 23.50 m³·s⁻¹ during the 2008 reproductive season and from 0.07 to 21.80 m³·s⁻¹ in the 2009 reproductive season. Mean daily discharge was 4.14 m³·s⁻¹ during the 2008 reproductive season and 2.57 m³·s⁻¹ in the 2009 reproductive season.

Discussion

Gonadosomatic indices, histological analysis, and oocyte size distributions show that the plains minnow, in the Brazos River, has an extended spawning season that lasts six months, from early April to late September. Throughout the spawning seasons, multiple peaks in mean GSI, and many decreases in oocyte size distributions show that the plains minnow exhibit the same reproductive patterns as other cyprinid species that are known to be multiple spawners (Lehtinen and Layzer 1988; Taylor and Miller 1990; Rinchard & Kestemont 1996; Bonner 2000; Durham and Wilde 2008a). Histological analyses of ovarian tissues showed that plains minnow oocytes were undergoing multiple stages of development, which is also consistent with multiple spawning species (Bonner 2000; and Durham and Wilde 2008a). The multiple techniques used to assess reproductive ecology show that the plains minnow exhibits two different methods of spawning dependent on flow conditions in the

river. Individual plains minnow spawn on a daily basis, but not in synchrony with other members of the population during times of low discharge, and during times of increased river discharge, the intensity and number of individuals spawning increased.

Beginning in February and March, female plains minnow begin to divert energy toward reproduction. Increases in adult mean GSI followed by decreases, in the population, indicate that a spawning event occurred. The first occurrence of this decrease was in May of both years at both sites. This continued until September, when mean GSI was at a minimum and did not increase again, at both sites during both years. Histological analyses showed the presence of oocytes undergoing germinal vesicle breakdown and the presence of post-ovulatory follicles within the ovaries indicating that spawning was in progress or recently completed (within the last 24 hours). Indications of spawning, as shown by these oocyte stages, started in late April and continued through early September in 2008 and from early April to late September in 2009. Histological analysis shows the plains minnow has a protracted spawning season that ranges from April to September, one month longer than Cross (1967), Lehtinen and Layzer (1988), and Taylor and Miller (1990) reported. Differences in duration of spawning season found in the Brazos River could be explained by differences in latitude. Cross (1967) observed the spawning ecology of

the plains minnow in Kansas, while Lehtinen and Layzer (1988) and Taylor and Miller (1990) observed plains minnow spawning in central Oklahoma. Farringer et al. (1979) found that the difference in latitude, from south central Oklahoma to central Texas, had an effect on reproductive duration in the red shiner, *Cyprinella lutrensis*.

Throughout the spawning season, multiple peaks in GSI were present that were followed by sharp decreases, and then followed by yet another increase in mean GSI. This is indicative of species that are multiple spawners (Gale 1986; Rinchard and Kestemont 1996; Bonner 2000; Durham 2007; Durham and Wilde 2008a). The presence of oocytes undergoing all four stages of development (perinucleolar, cortical alveoli, early vitellogenic, and late vitellogenic) on each sampling date shows that during the six month reproductive season, constant recruitment of mature oocytes was occurring, which is also consistent with species known to be multiple spawners (Heins and Rabito 1986; Ali and Kadir 1996; Wallace and Selman 1981). The idea that plains minnow are multiple spawners is further substantiated by the histological analysis, because on each sampling date at least one fish within the samples had either mature oocytes undergoing germinal vesicle breakdown or the ovary contained evidence of post-ovulatory follicles.

Mean GSI decreases, mean oocyte diameter decreases, increases in post-ovulatory follicles, and evidence of germinal vesicle breakdown can all be related to increases in stream discharge. When mean GSI is plotted with discharge it is evident that during both years, April through September, mean GSI gradually increased, and then decreased in relation to increased stream discharge (Figure 26 – 27). For example on 29 June 2009, at Site 1, a 15% decrease in mean GSI, indicating that the population spawned, occurred in relation to a $15 \text{ m}^3\text{-s}^{-1}$ increase in discharge (Figure 26 B). The histological analysis from this date (Figure 15) shows that 53% of the fish observed spawned in relation to this increase in discharge. However, mean GSI decreases, increased number of ovaries containing post-ovulatory follicles, and oocytes undergoing germinal vesicle breakdown, that are related to increases in discharge, not only occur with flood pulses. They also were observed when discharge increased marginally in relation to the pre-pulse base flow. Figure 26 B shows that between samples collected in early May and late May 2009, at Site 1, a spawning event occurred in relation to less than a $1 \text{ m}^3\text{-s}^{-1}$ increase in stream discharge. Histological data for these two dates confirm that 79% of the fish observed spawned in response to this increase in discharge. This relationship holds true for both sites during both reproductive seasons. These results are consistent with those of Lehtinen

and Layzer (1988) and Taylor and Miller (1990), except that plains minnow do not require a high flow pulse to spawn but just an overall increase in flow.

On all sampling dates, in both years, at least one fish in my samples had mature oocytes undergoing germinal vesicle breakdown or post-ovulatory follicles within the ovary. For example samples collected on 21 April 2008 (Figure 11) show that 27% of the fish showed signs of recent or ongoing spawning (i.e., post-ovulatory follicles or presence of germinal vesicle breakdown). Discharge on this date was $0.28 \text{ m}^3\text{s}^{-1}$ and had not increased for one week prior to this date to trigger a synchronized spawning event. The presence of relatively few individuals spawning during times of low discharge was also observed on 4 April, 8 May, and 20 June 2009 (Figures 14 and 15, respectively). Histological analyses show that individual plains minnow were spawning on a daily basis, but not in synchrony with other members of the population, regardless of discharge.

When spawning events occurred following increases in stream discharge, the proportion of individuals spawning and their spawning intensity increased. On 24 June 2008 at Site 1, plains minnow mean GSI had the largest decrease of the year, which was related to an increase in discharge (Figure 26). On this date, all but two individuals collected had an increase in the relative abundance of post-ovulatory follicles (Figure

12), and a decrease in oocyte diameter (Figures 28). These lines of evidence indicate that plains minnow spawned in response to this increase in discharge. Samples with decreases in mean GSI, ovaries containing post-ovulatory follicles, and mean oocyte diameter decreases were observed on several other dates (e.g., 6 May 2008, 22 July 2008, 1 September 2008, 13 July 2009, 18 August 2009, and 17 September 2009). At Site 2 mean GSI and mean oocyte diameter decreases on 24 June 2008, 20 June 2009, and 13 July 2009 were all in accordance with an increase in stream discharge (Figures 27 and 29). GSI, oocyte diameter, and histological analyses indicate that the reproductive ecology of the plains minnow is similar to other synchronized broadcast-spawning cyprinids that spawn in response to increases in stream discharge (Moore 1944; Sliger 1967; Lehtinen and Layzer 1988; Taylor and Miller 1990; Durham 2007; Platania and Altenbach 1998; Durham and Wilde 2008; Wilde and Durham 2008).

The reproductive biology of the plains minnow as documented here has potential management and conservation implications. If the decrease of the plains minnow in the Great Plains is, in fact, caused by a decrease in reproductive success, then other factors affecting reproduction might be involved. This study shows that plains minnow in the Brazos River spawned on a daily basis during the reproductive season, and during times

of increased discharge, a greater proportion of the population spawned in synchrony. It is known that the plains minnow spawns semi-buoyant nonadhesive eggs that develop and hatch as they float downstream (Sliger 1967; Platania and Altenbach 1998). Fragmentation of streams is likely to have a direct impact on reproductive success, given that the eggs and larval fish need a certain distance of unimpeded river to develop. Once the eggs hatch, the majority of juvenile plains minnow are located in downstream reaches making it necessary for them to migrate to repatriate upstream reaches (Cross et al 1985; Durham and Wilde 2008b). A pilot study I conducted in 2010 showed that plains minnow is capable of migrating over 80 km upstream in 55 days (unpublished data). Given that the reproductive ecology of the plains minnow is directly dependent on water availability it is essential that sufficient flow is maintained for migration, and periods of increased discharge are needed during the spawning season for successful synchronized spawning events.

Species that have been found to utilize synchronous and asynchronous spawning methods (e.g., Arkansas River shiner *Notropis girardi*, smalleye shiner *Notropis buccula*, sharpnose shiner *Notropis oxyrhynchus*, and peppered chub *Macrhybopsis tetranema*) are fish that inhabit mainstream swift flowing waters (Gilbert 1980; Moss and Mayes 1993; Eisenhour 2004) and are primarily insectivores (Cross 1967;

Goldstein and Simon 1999; Marks et al. 2001; Wilde et al. 2001). The plains minnow inhabits backwater and slack water pools (Miller and Robison 1973; Cross and Collins 1995; Goldstein and Simon 1999) and is mostly herbivorous. The membership of the plains minnow in this guild of fishes belies the notion that this spawning typology is related to diet or habitat preferences, rather it demonstrates this is an adaptation to specific river conditions. Therefore, I predict other species that are known or suspected to be broadcast spawners to use multiple spawning techniques regardless of trophic level and microhabitat uses.

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Table 1: Generalized linear regression describing the relationship between mean female GSI and discharge at the Double Mountain Fork Brazos River at State Highway 70 north of Rotan, Texas (Site 1) and the Brazos River at State Highway 6 north of Knox City, Texas (Site 2).

Year	Site	r	n	P
2008	1	-0.17	10	0.64
	2	-0.03	9	0.93
2009	1	0.20	12	0.54
	2	-0.91	12	< 0.0001

Table 2: Generalized linear regression describing the relationship between mean oocyte diameter and discharge at the Double Mountain Fork Brazos River at State Highway 70 north of Rotan, Texas (Site 1) and the Brazos River at State Highway 6 north of Knox City, Texas (Site 2).

Year	Site	r	n	P
2008	1	-0.37	9	0.33
	2	-0.73	7	0.06
2009	1	-0.31	12	0.33
	2	-0.13	9	0.74

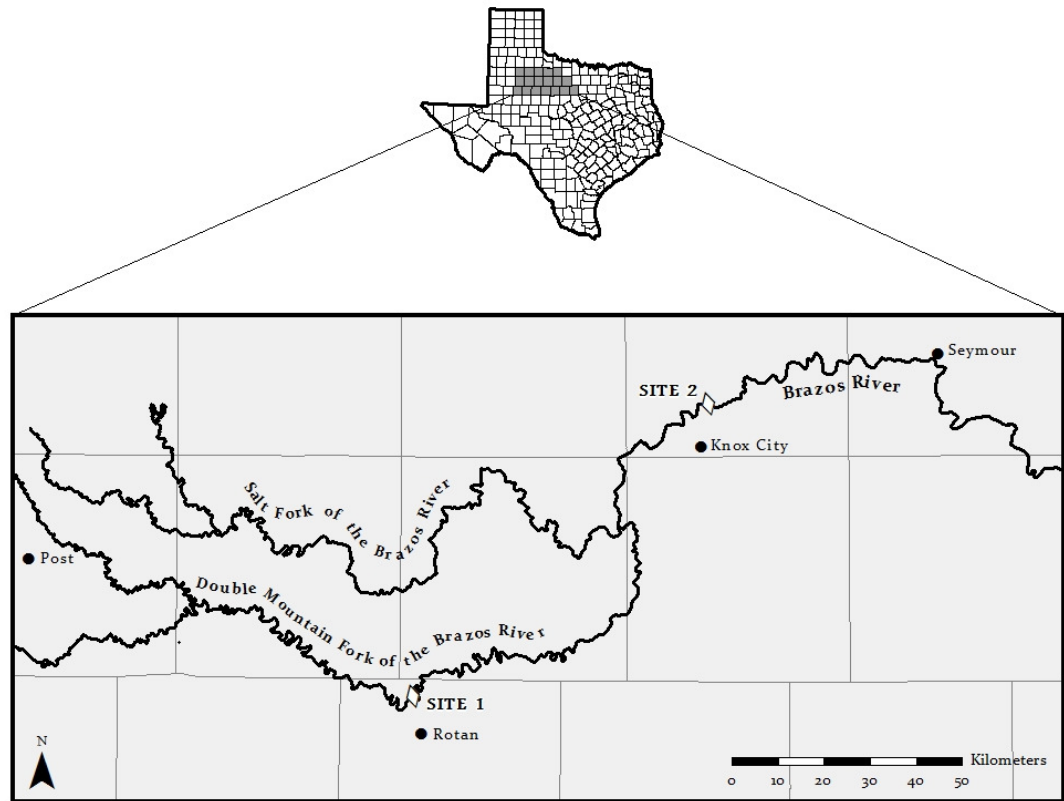


Figure 1: Study area and location of sampling sites on the upper Brazos River, Texas.

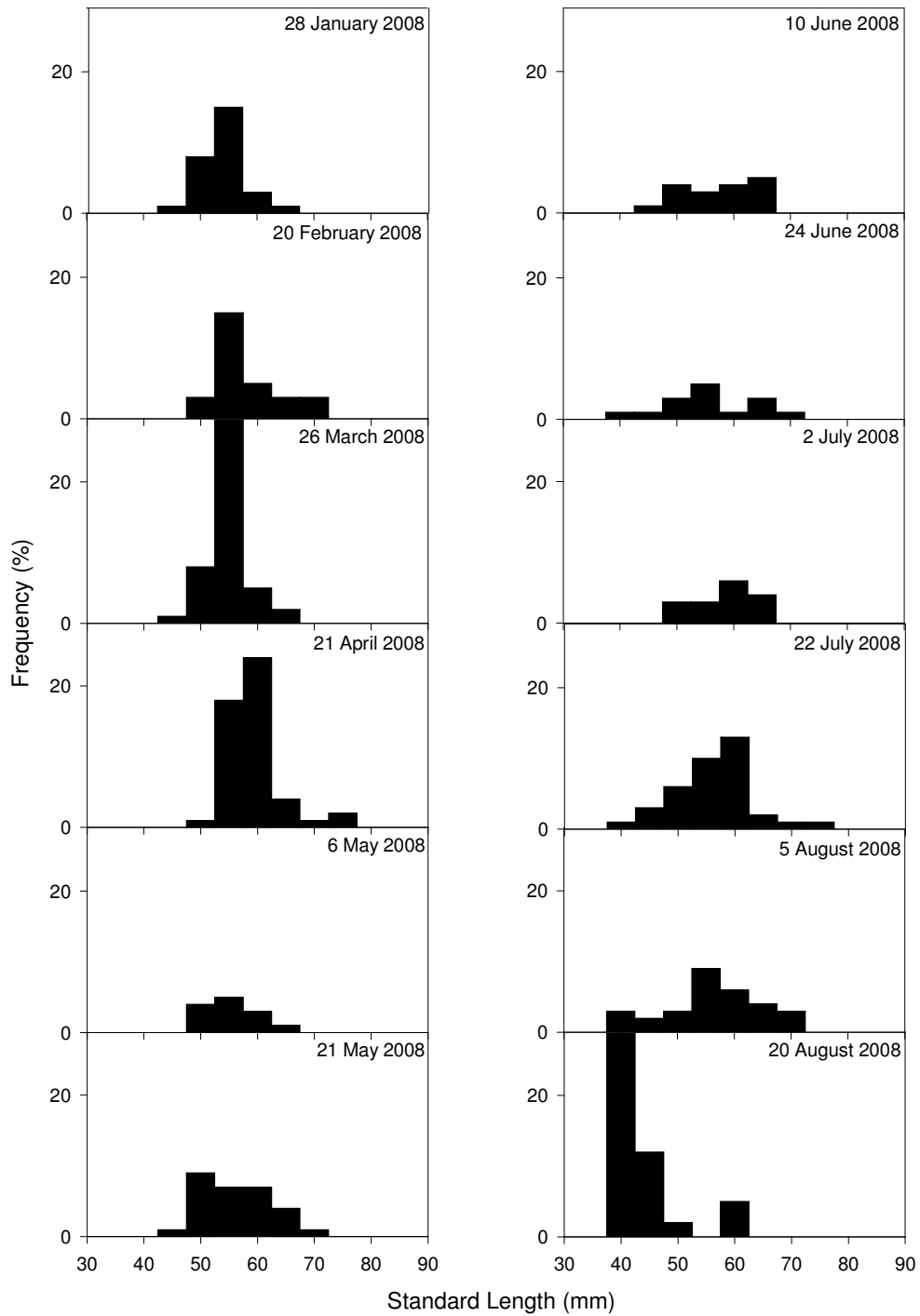


Figure 2: Length-frequency histograms for plains minnow collected from the Double Mountain Fork Brazos River at State Highway 70 north of Rotan, Texas from January through August 2008.

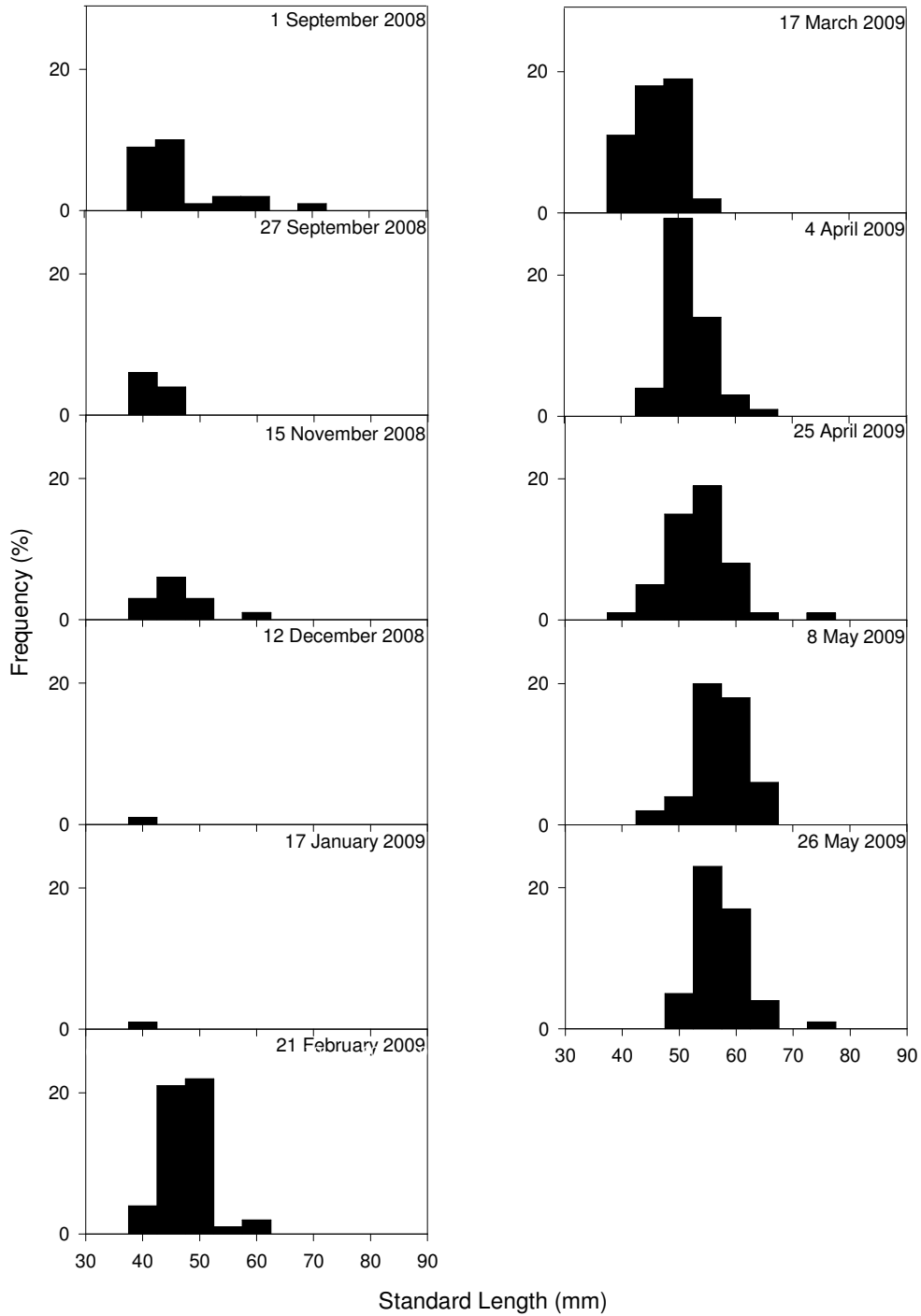


Figure 3: Length-frequency histograms for plains minnow collected from the Double Mountain Fork Brazos River at State Highway 70 north of Rotan, Texas from September 2008 through May 2009.

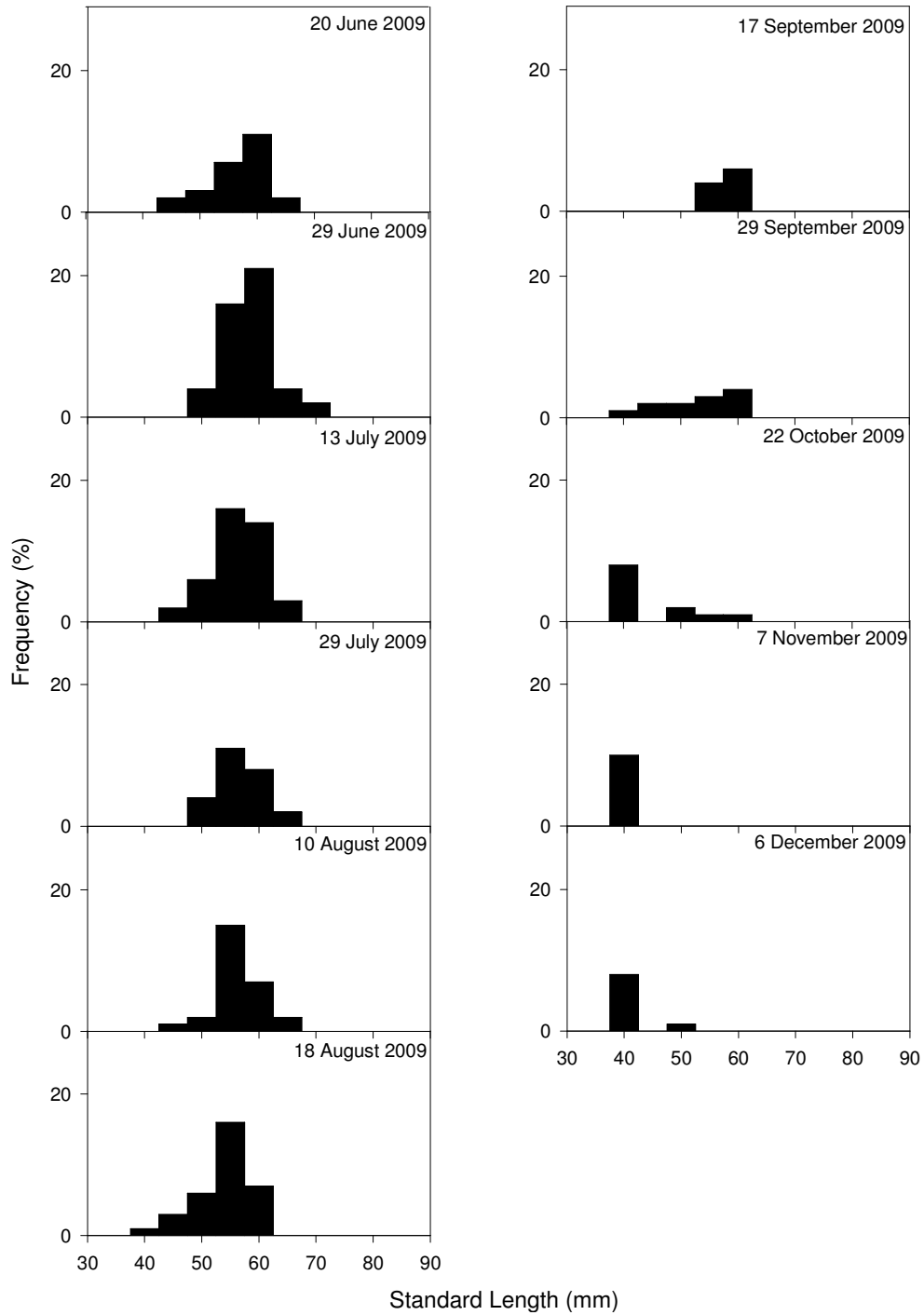


Figure 4: Length-frequency histograms for plains minnow collected from the Double Mountain Fork Brazos River at State Highway 70 north of Rotan, Texas from June through December 2009.

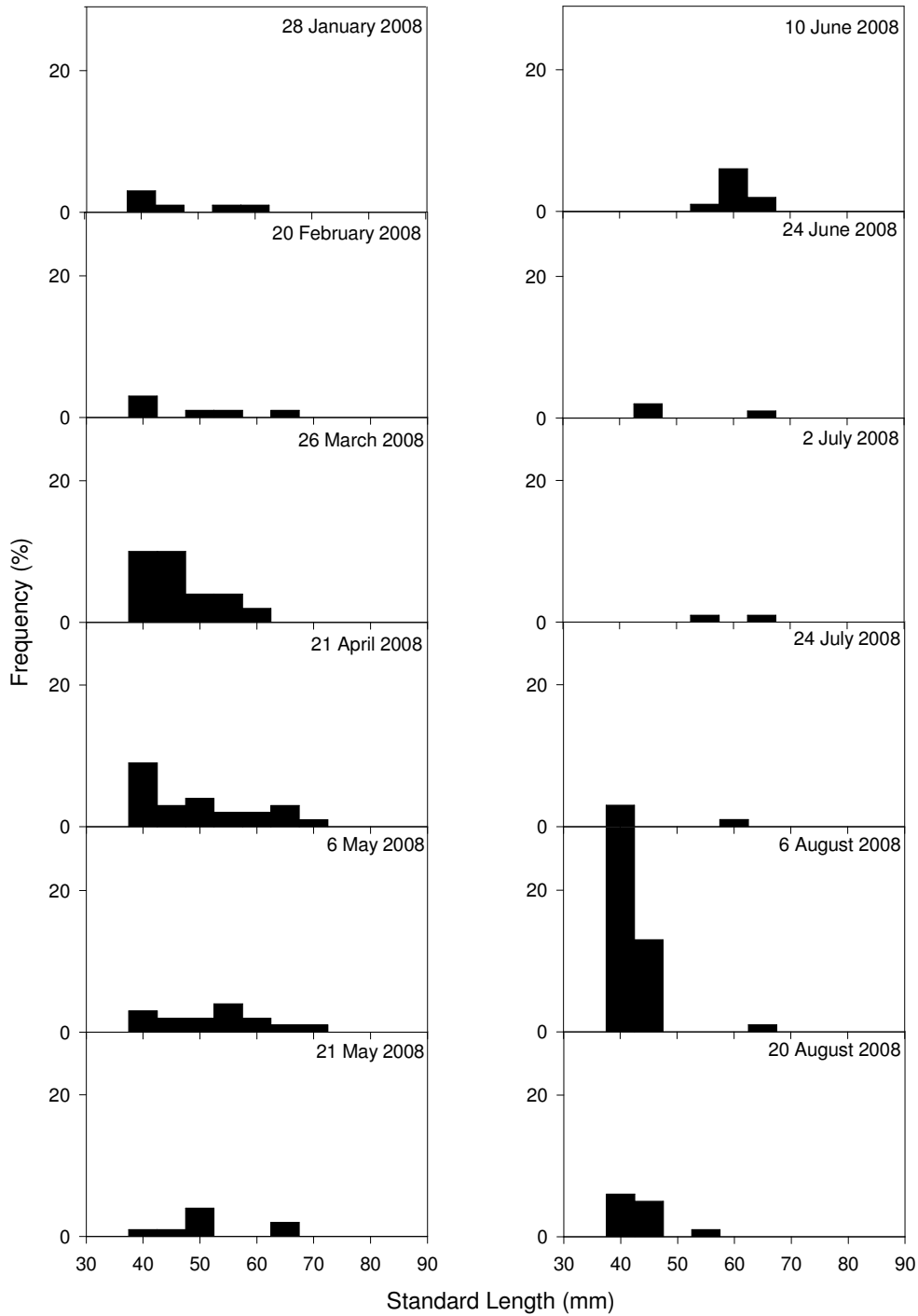


Figure 5: Length-frequency histograms for plains minnow collected from the Brazos River at State Highway 6 north of Knox City, Texas from January through August 2008.

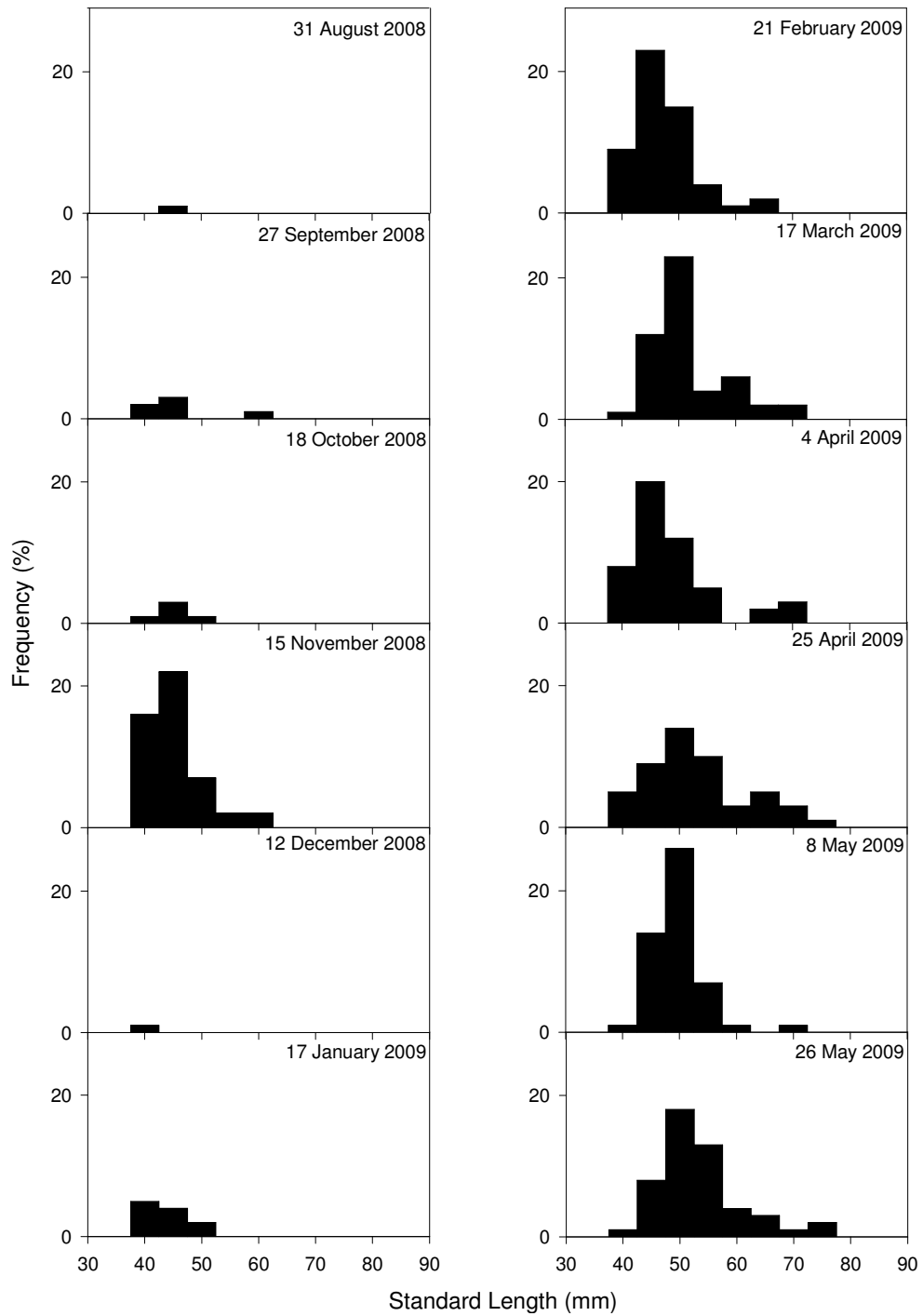


Figure 6: Length-frequency histograms for plains minnow collected from the Brazos River at State Highway 6 north of Knox City, Texas from September 2008 through May 2009

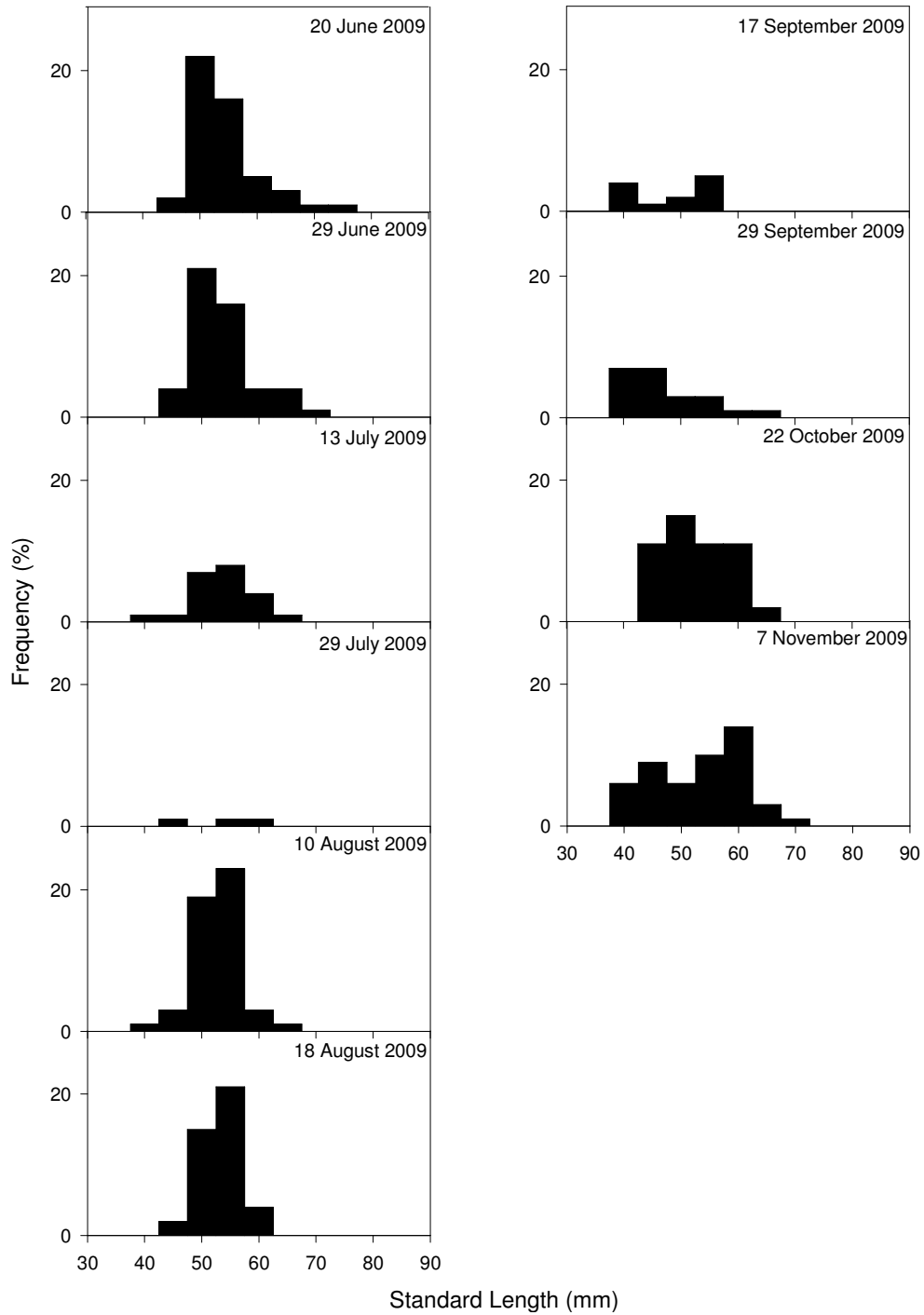


Figure 7: Length-frequency histograms for plains minnow collected from the Brazos River at State Highway 6 north of Knox City, Texas from June through December 2009.

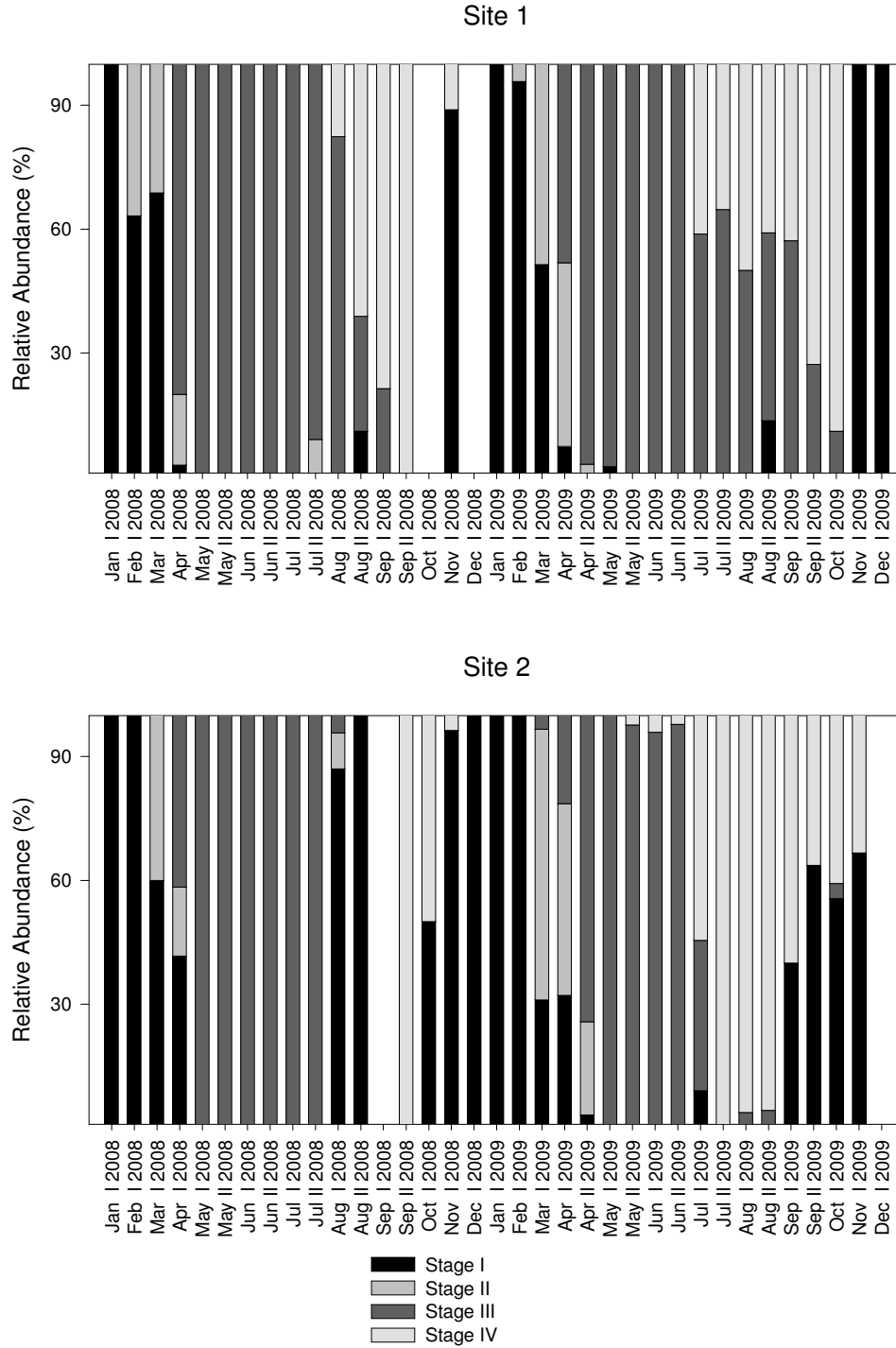


Figure 8: Relative abundance of female plains minnow ovarian stages I (resting), II (developing), III (mature), and IV (spent) from two sites on the Brazos River, Texas from January 2008 through December 2009.

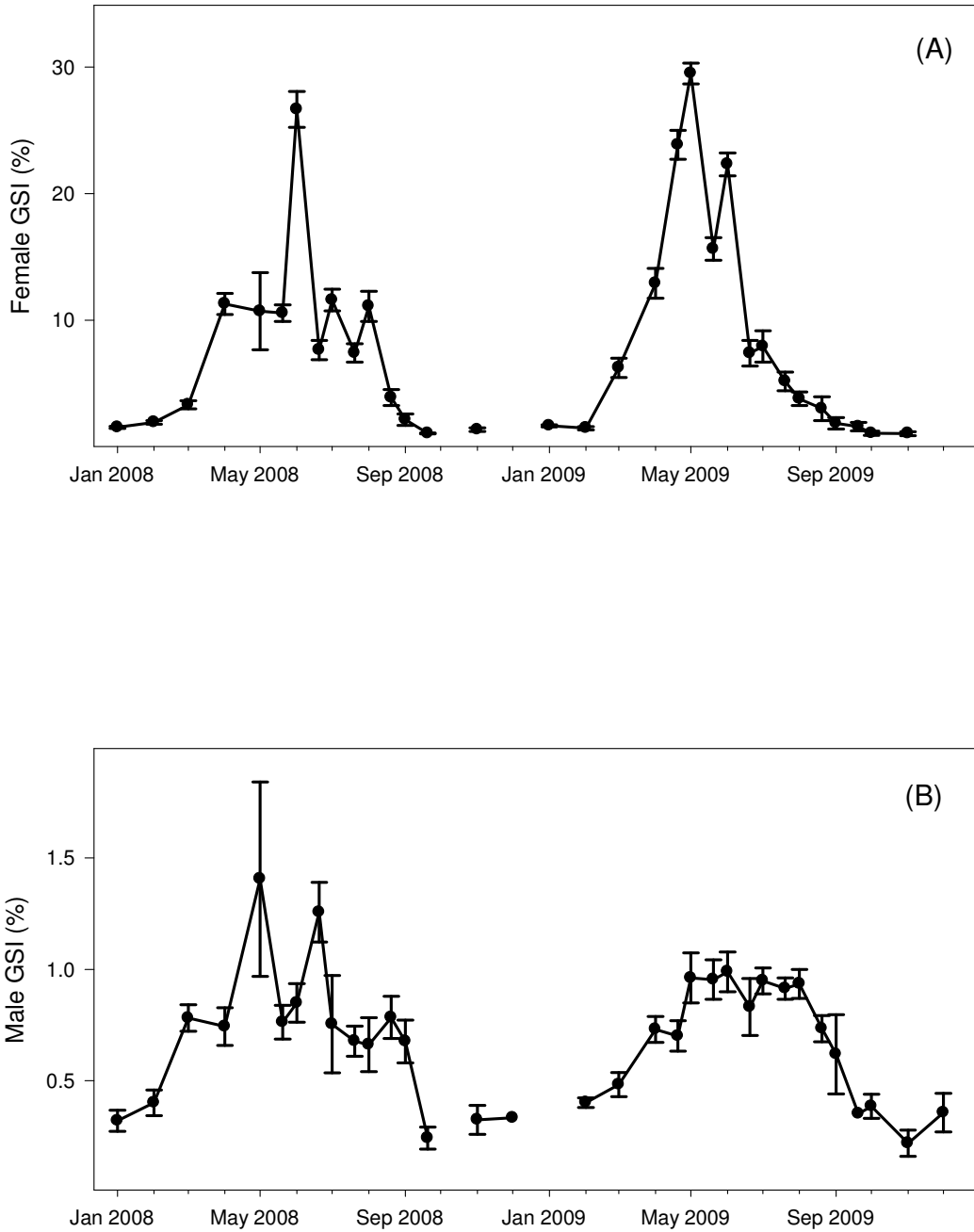


Figure 9: Mean gonadosomatic index \pm SE for female plains minnow (A) and male plains minnow (B) from the Double Mountain Fork Brazos River at State Highway 70 north of Rotan, Texas. Dates without data indicate that no mature individuals were present in samples or samples were not collected due to high discharge.

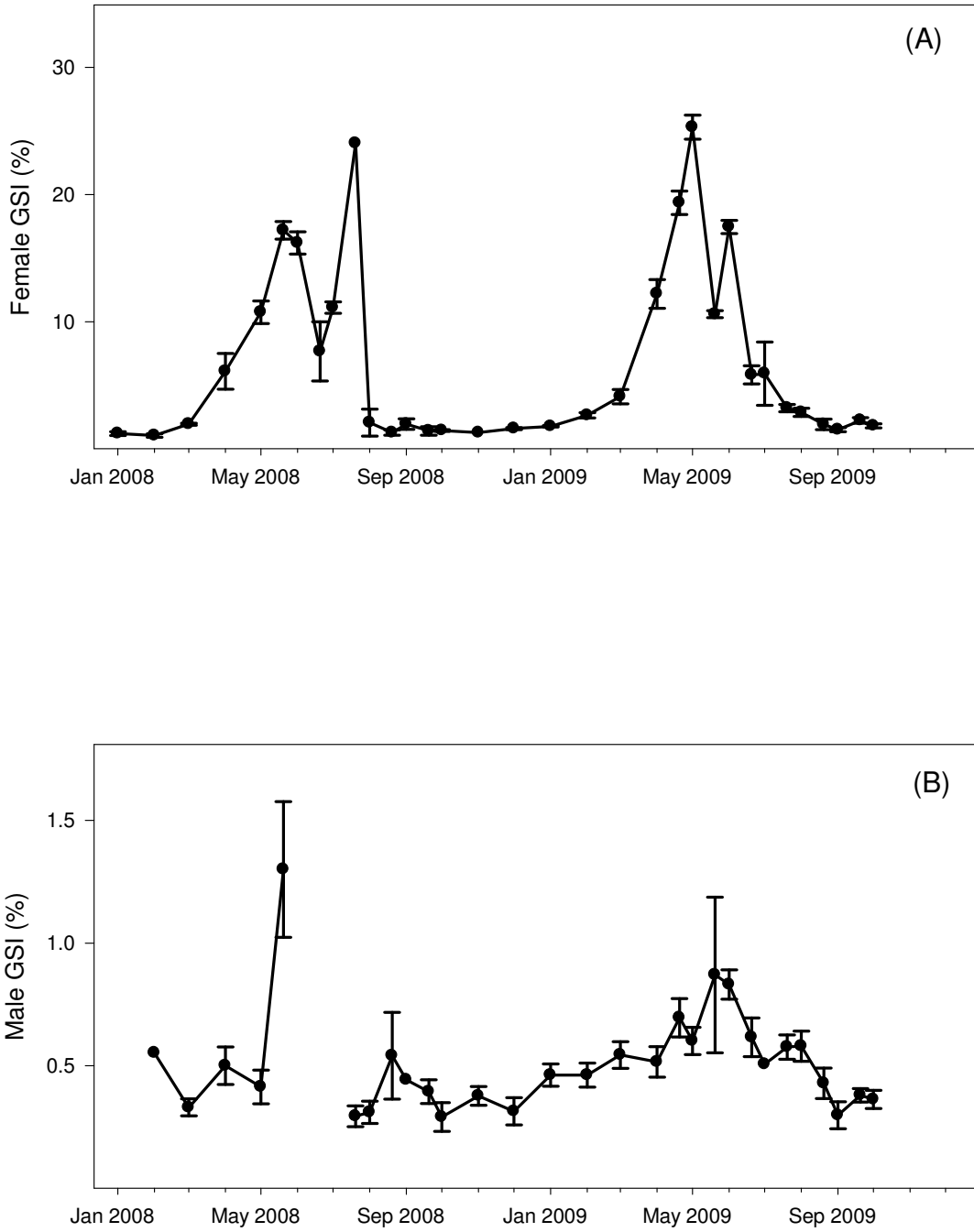


Figure 10: Mean gonadosomatic index \pm SE for female plains minnow (A) and male plains minnow (B) from the Brazos River at State Highway 6 north of Knox City, Texas. Dates without data indicate that no mature individuals were present in samples or samples were not collected due to high discharge.

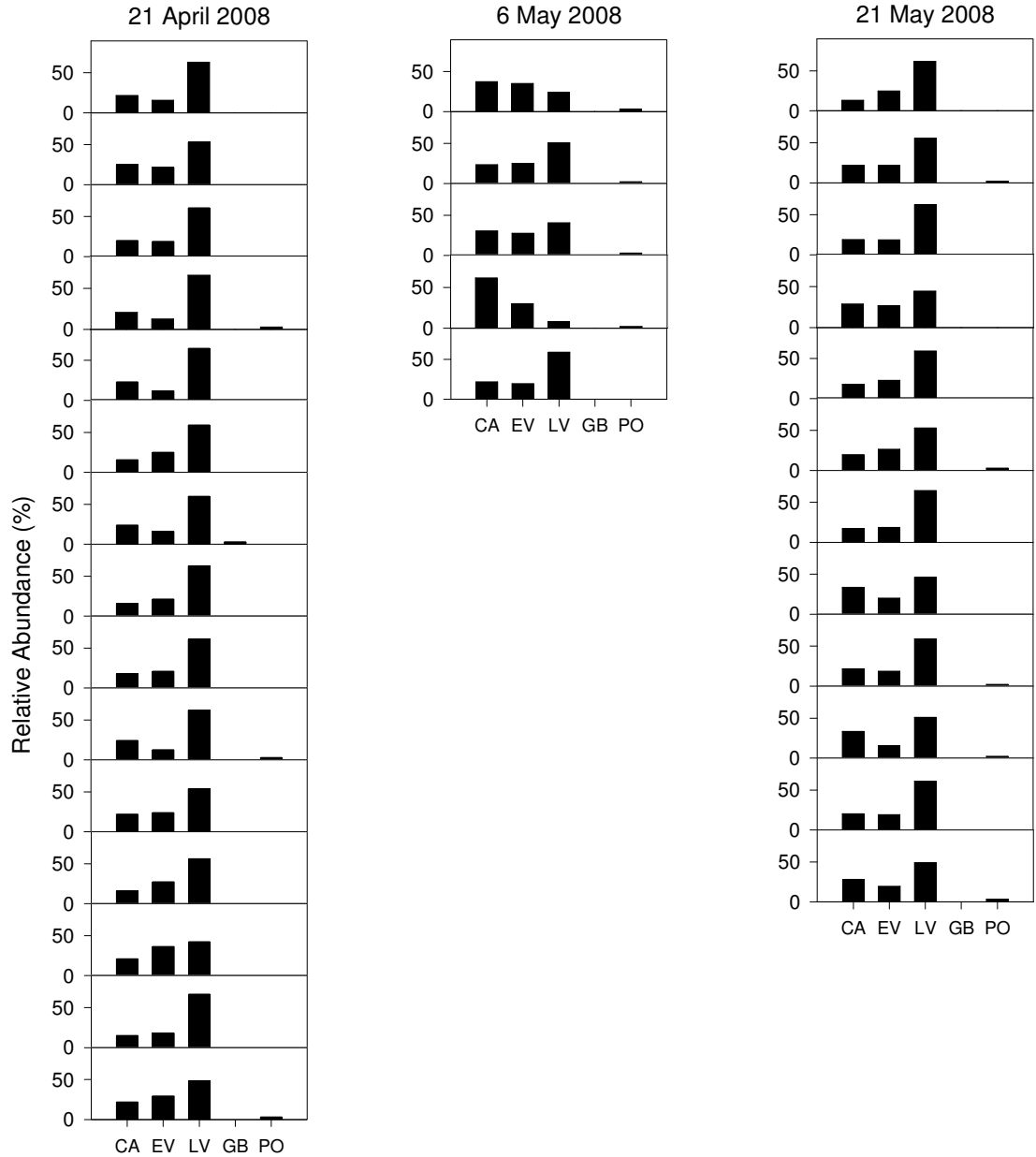


Figure 11: Relative abundance of oocyte classification as cortical alveoli (CA), early vitellogenic (EV), late vitellogenic (LV), germinal vesicle breakdown (GB), or post-ovulatory follicle (PO) from female plains minnow collected from the Double Mountain Fork Brazos River at State Highway 70 north of Rotan, Texas on each sampling date during the reproductive season April through May 2008.

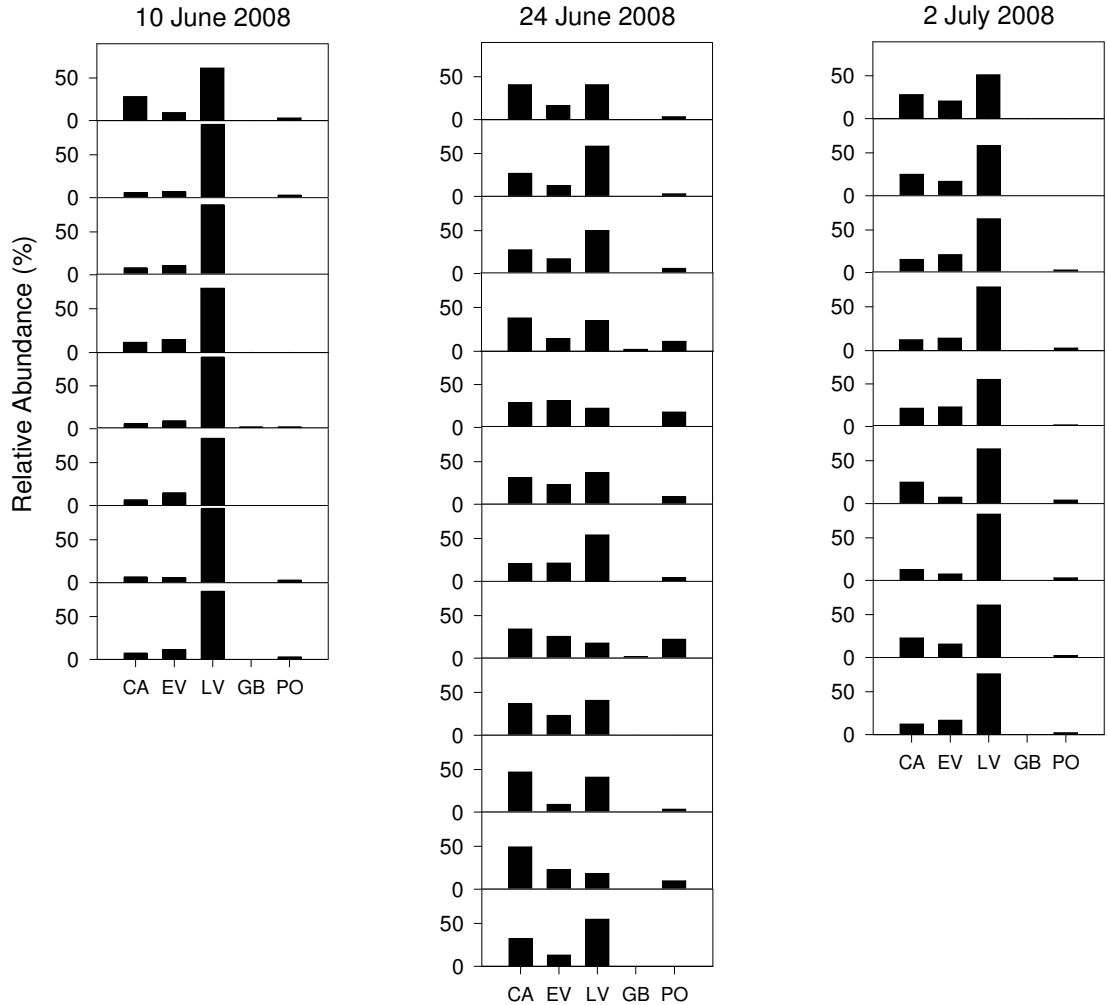


Figure 12: Relative abundance of oocyte classification as cortical alveoli (CA), early vitellogenic (EV), late vitellogenic (LV), germinal vesicle breakdown (GB), or post-ovulatory follicle (PO) from female plains minnow collected from the Double Mountain Fork Brazos River at State Highway 70 north of Rotan, Texas on each sampling date during the reproductive season June through July 2008.

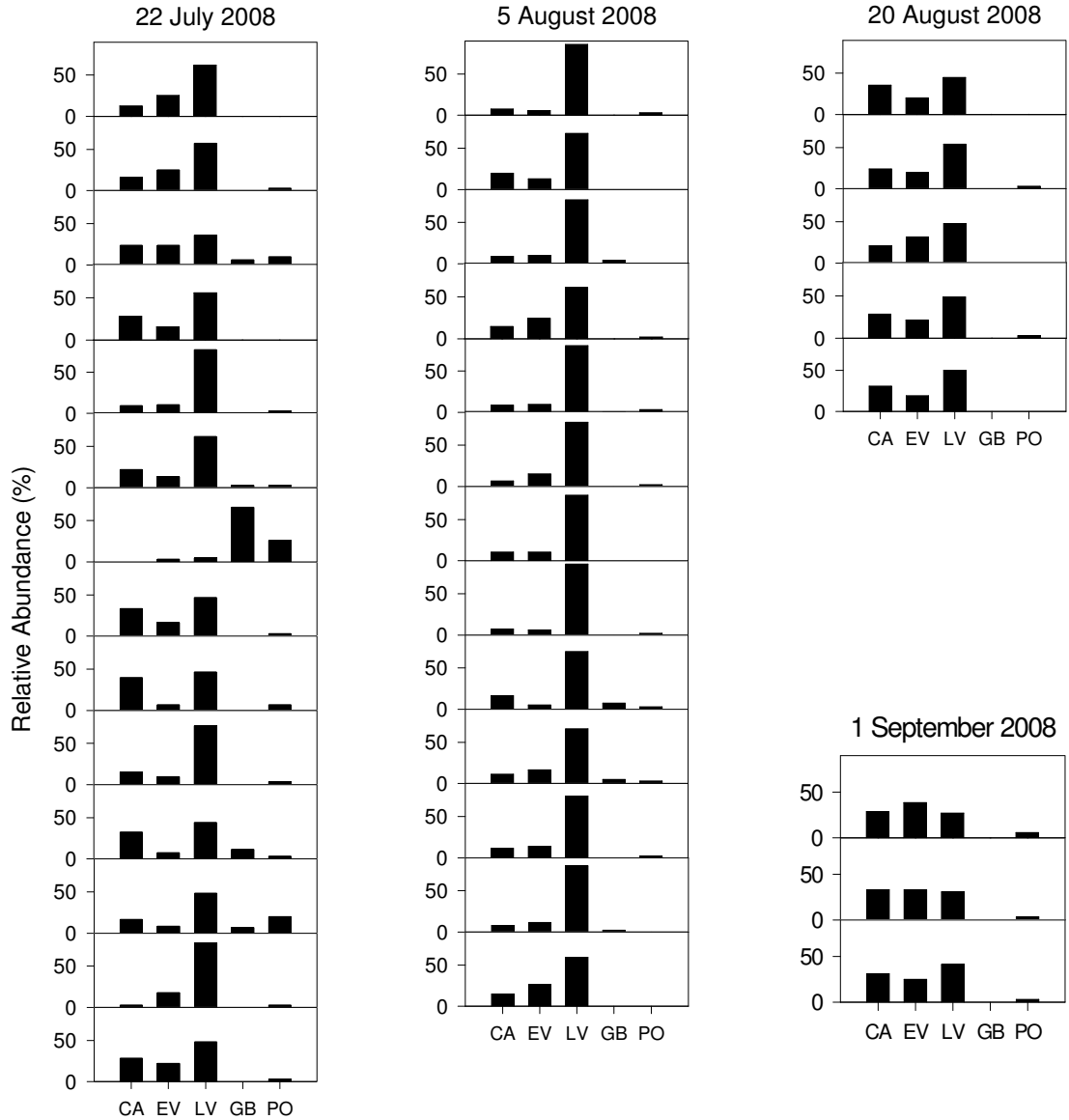


Figure 13: Relative abundance of oocyte classification as cortical alveoli (CA), early vitellogenic (EV), late vitellogenic (LV), germinal vesicle breakdown (GB), or post-ovulatory follicle (PO) from female plains minnow collected from the Double Mountain Fork Brazos River at State Highway 70 north of Rotan, Texas on each sampling date during the reproductive season July through September 2008.

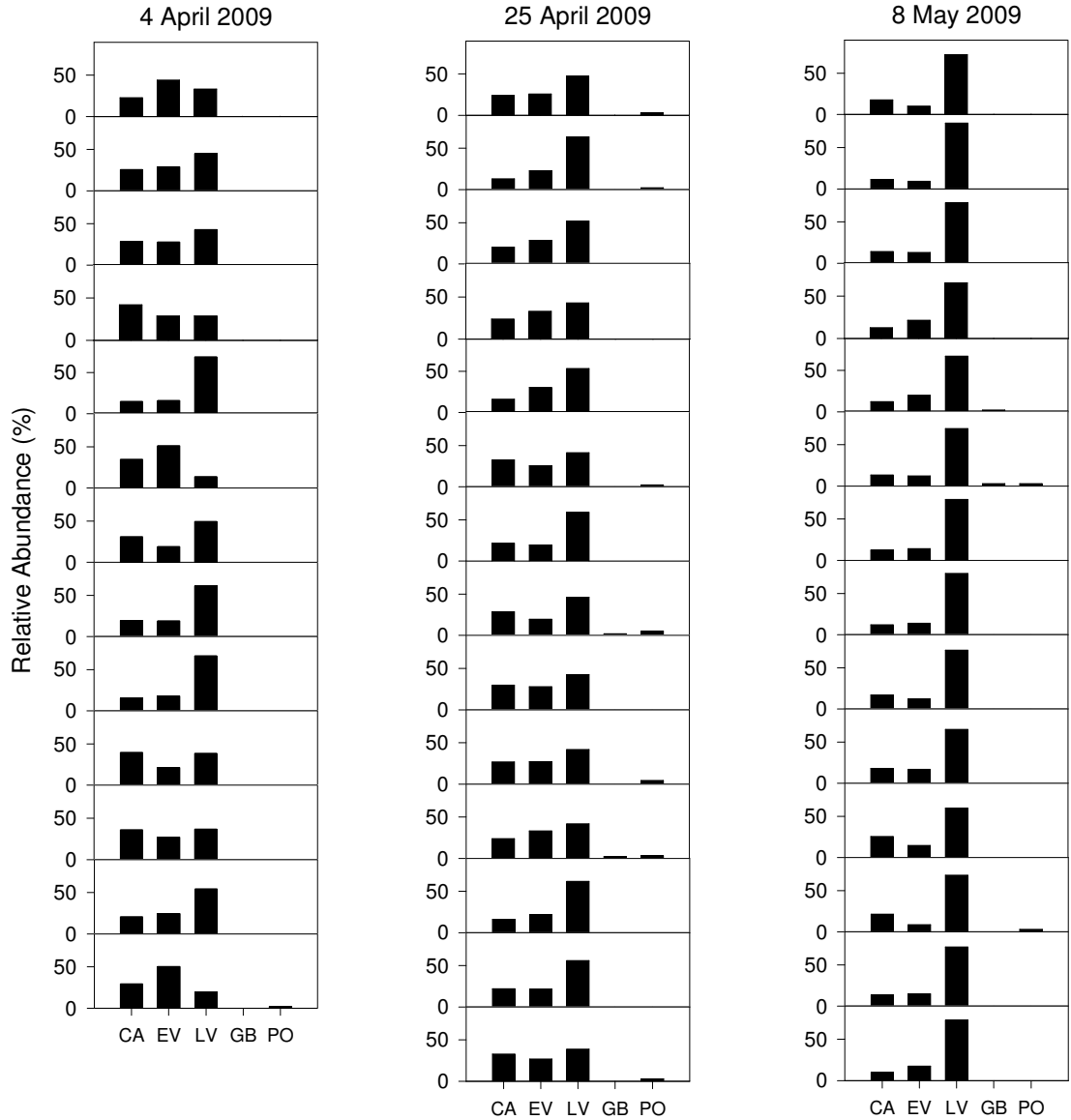


Figure 14: Relative abundance of oocyte classification as cortical alveoli (CA), early vitellogenic (EV), late vitellogenic (LV), germinal vesicle breakdown (GB), or post-ovulatory follicle (PO) from female plains minnow collected from the Double Mountain Fork Brazos River at State Highway 70 north of Rotan, Texas on each sampling date during the reproductive season April through May 2009.

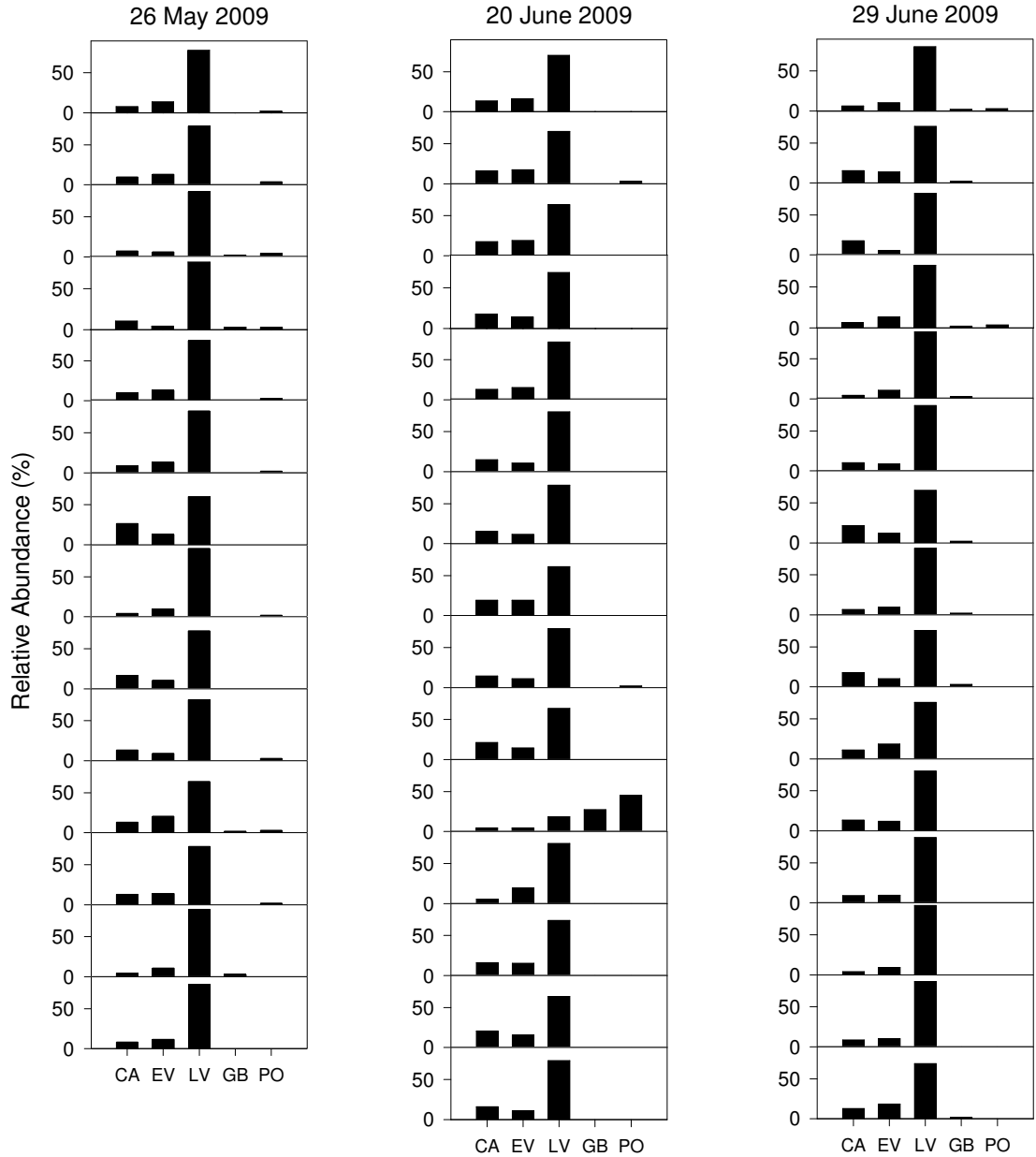


Figure 15: Relative abundance of oocyte classification as cortical alveoli (CA), early vitellogenic (EV), late vitellogenic (LV), germinal vesicle breakdown (GB), or spent post-ovulatory follicle (PO) from female plains minnow collected from the Double Mountain Fork Brazos River at State Highway 70 north of Rotan, Texas on each sampling date during the reproductive season May through June 2009.

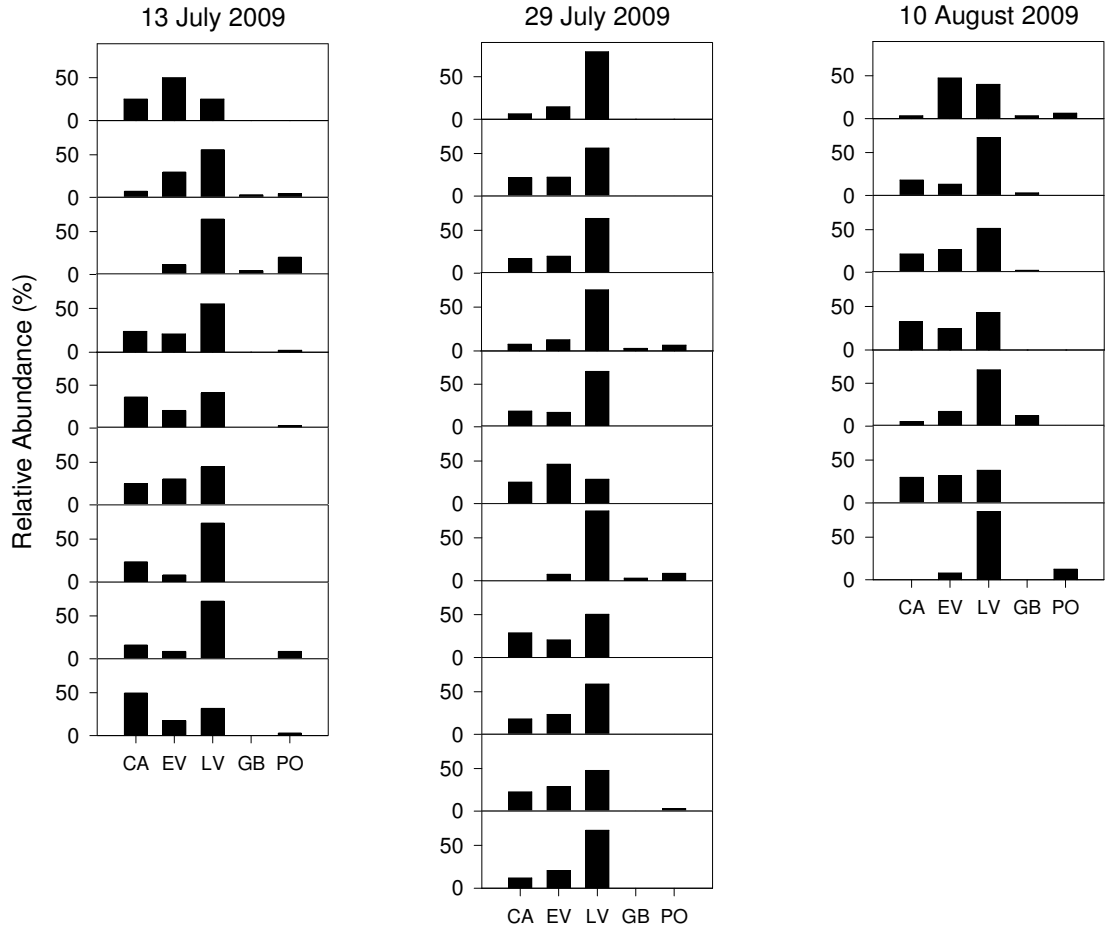


Figure 16: Relative abundance of oocyte classification as cortical alveoli (CA), early vitellogenic (EV), late vitellogenic (LV), germinal vesicle breakdown (GB), or post-ovulatory follicle (PO) from female plains minnow collected from the Double Mountain Fork Brazos River at State Highway 70 north of Rotan, Texas on each sampling date during the reproductive season July through August 2009.

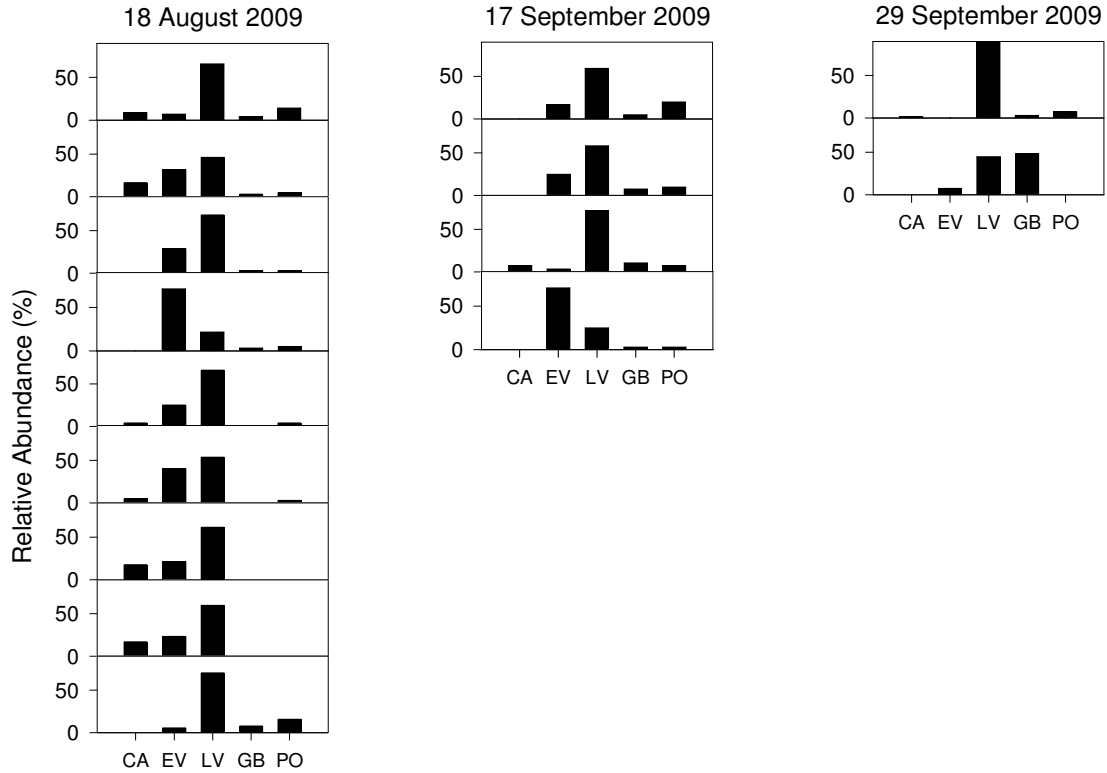


Figure 17: Relative abundance of oocyte classification as cortical alveoli (CA), early vitellogenic (EV), late vitellogenic (LV), germinal vesicle breakdown (GB), or post-ovulatory follicle (PO) from female plains minnow collected from the Double Mountain Fork Brazos River at State Highway 70 north of Rotan, Texas on each sampling date during the reproductive season August through September 2009.

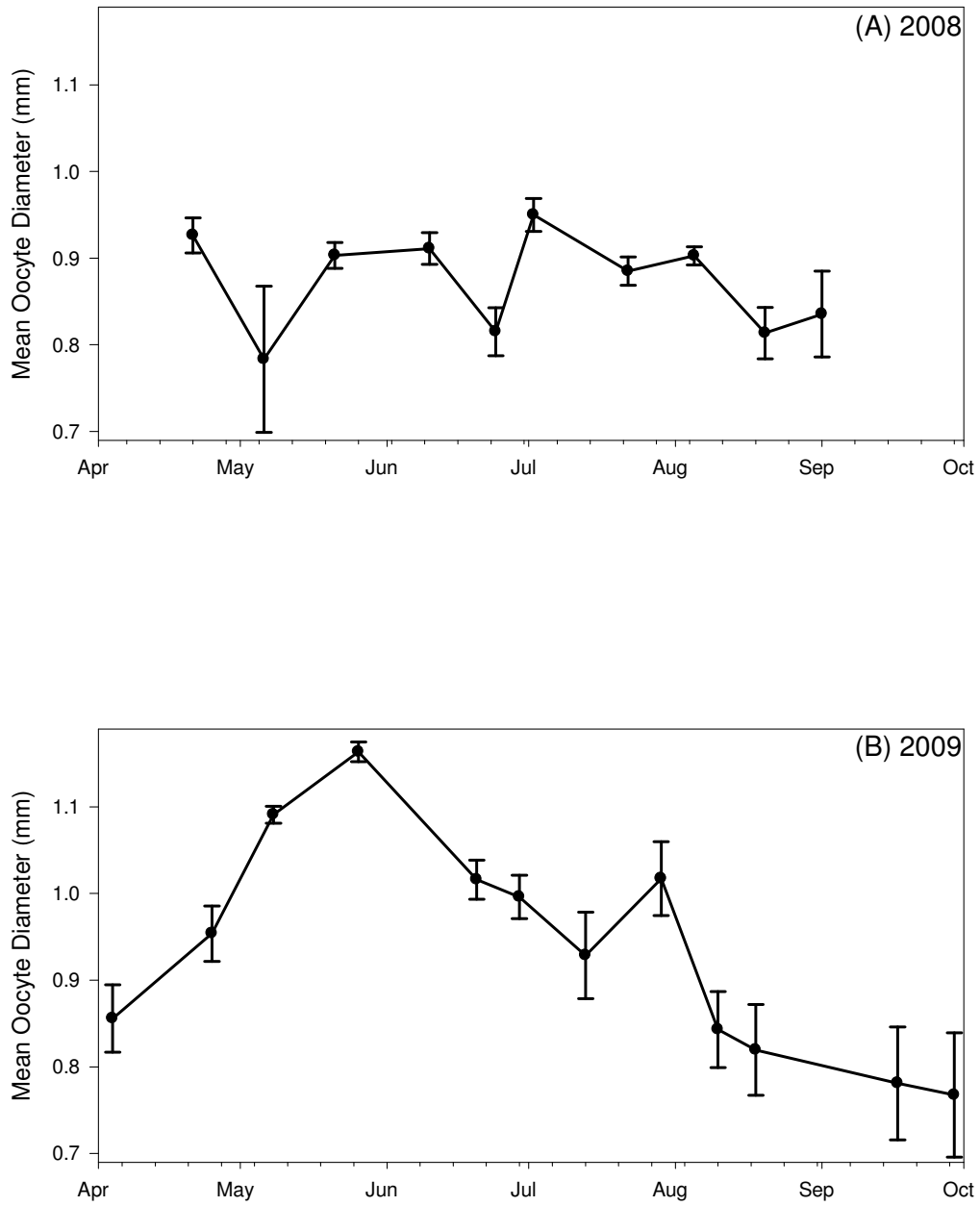


Figure 18: Mean vitellogenic oocyte diameter of mature female plains minnow collected from the Double Mountain Fork Brazos River at State Highway 70 north of Rotan, Texas during the 2008 (A) and 2009 (B) reproductive seasons.

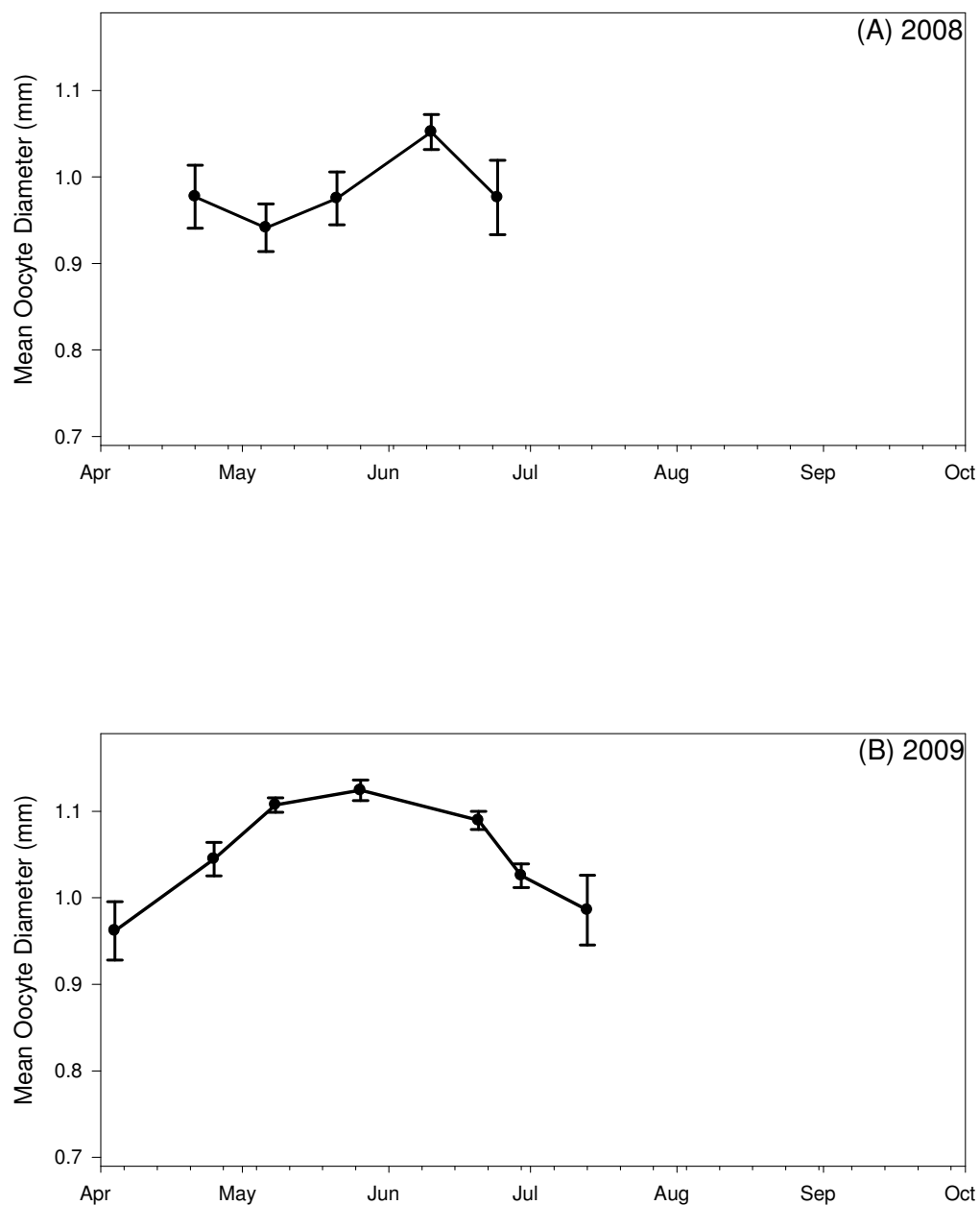


Figure 19: Mean vitellogenic oocyte diameter of mature female plains minnow collected from the Brazos River at State Highway 6 north of Knox City, Texas during the 2008 (A) and 2009 (B) reproductive seasons

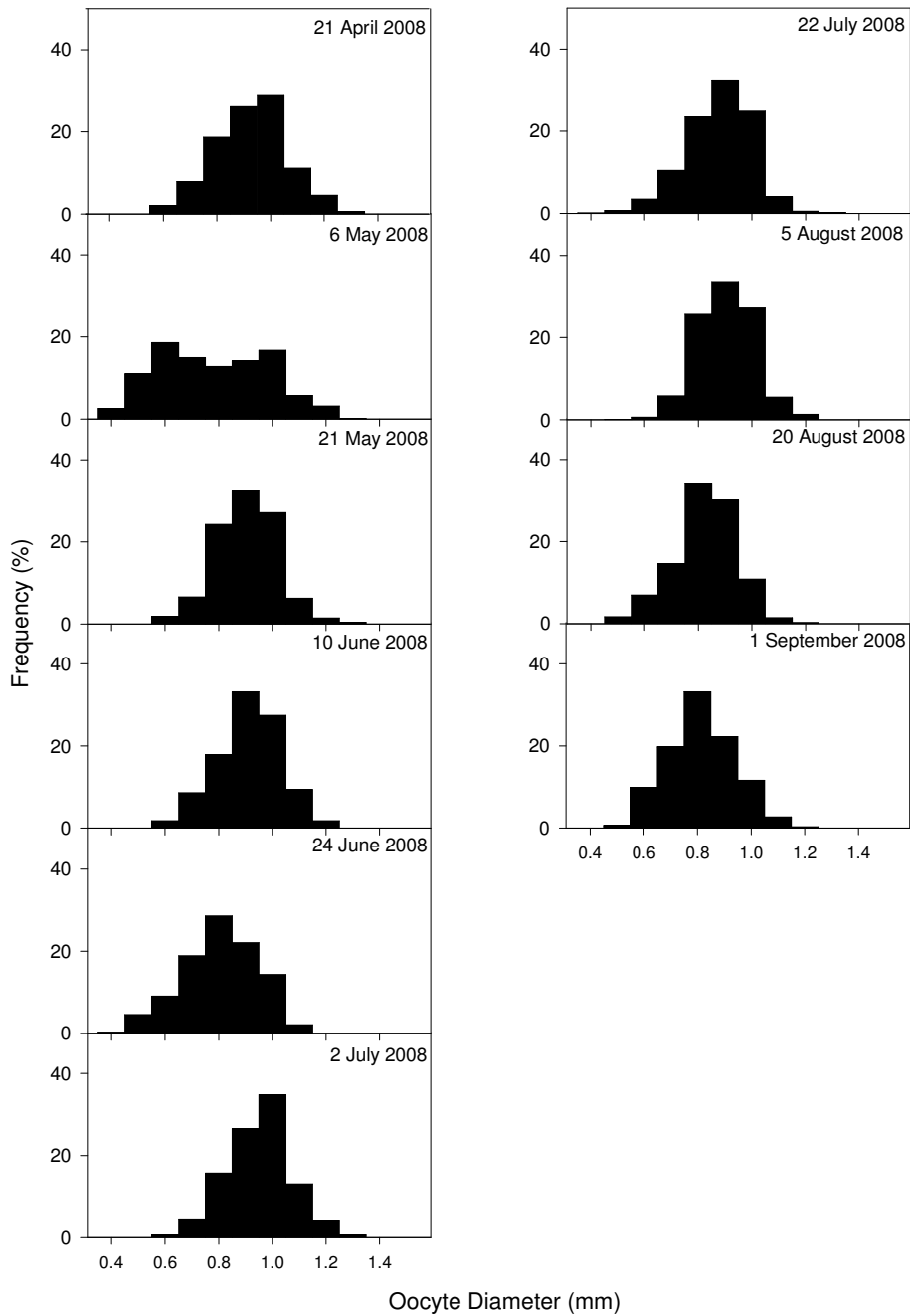


Figure 20: Size-frequency distributions of vitellogenic oocytes from ovaries of female plains minnow collected from the Double Mountain Fork Brazos River at State Highway 70 north of Rotan, Texas during the 2008 reproductive season.

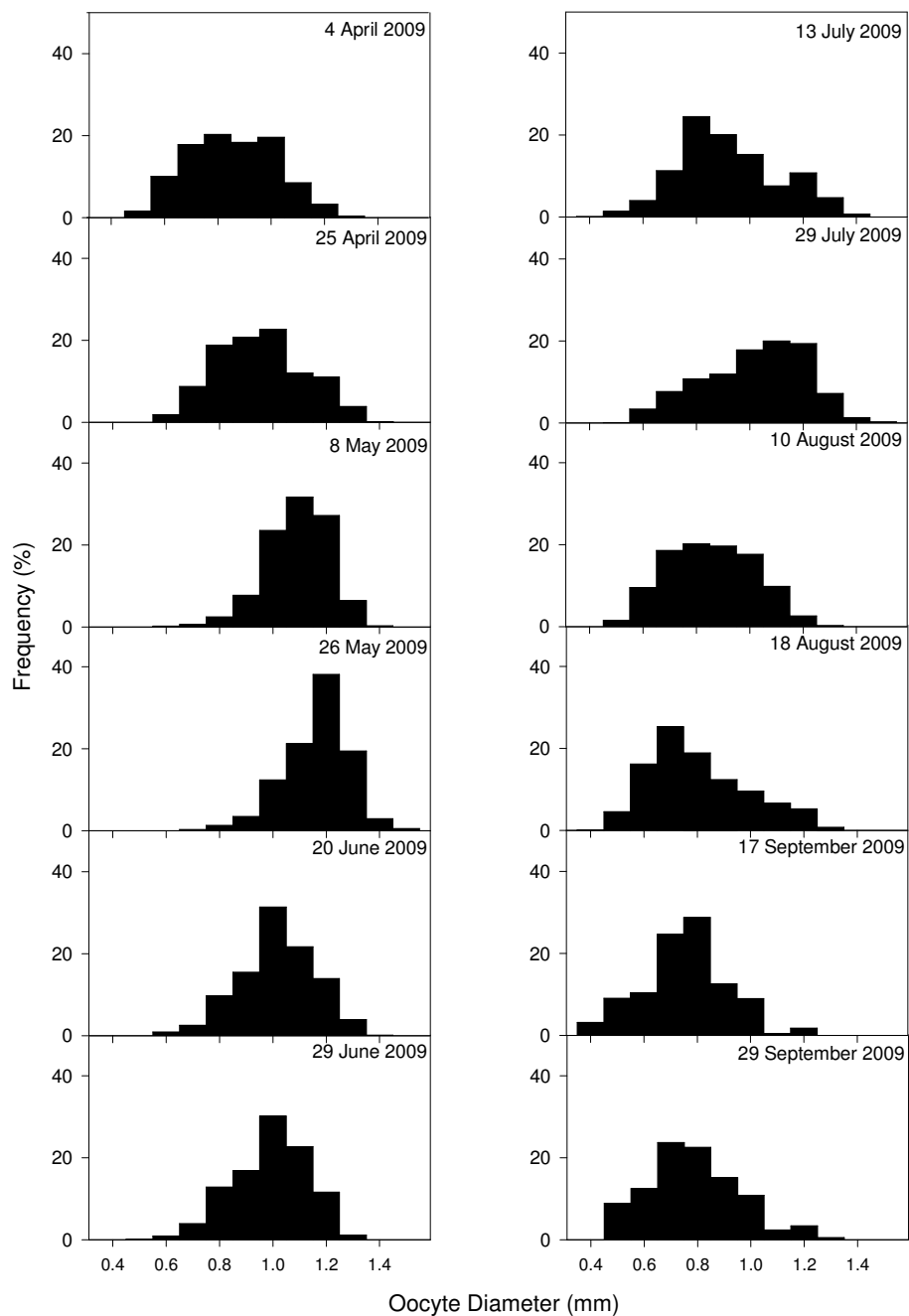


Figure 21: Size-frequency distributions of vitellogenic oocytes from ovaries of female plains minnow collected from the Double Mountain Fork Brazos River at State Highway 70 north of Rotan, Texas during the 2009 reproductive season.

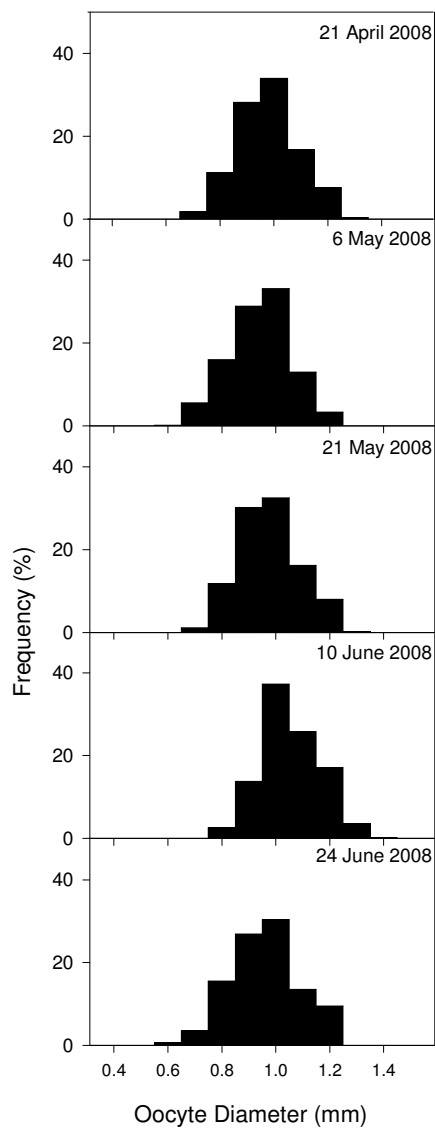


Figure 22: Size-frequency distributions of vitellogenic oocytes from ovaries of female plains minnow collected from the Brazos River at State Highway 6 north of Knox City, Texas during the 2008 reproductive season.

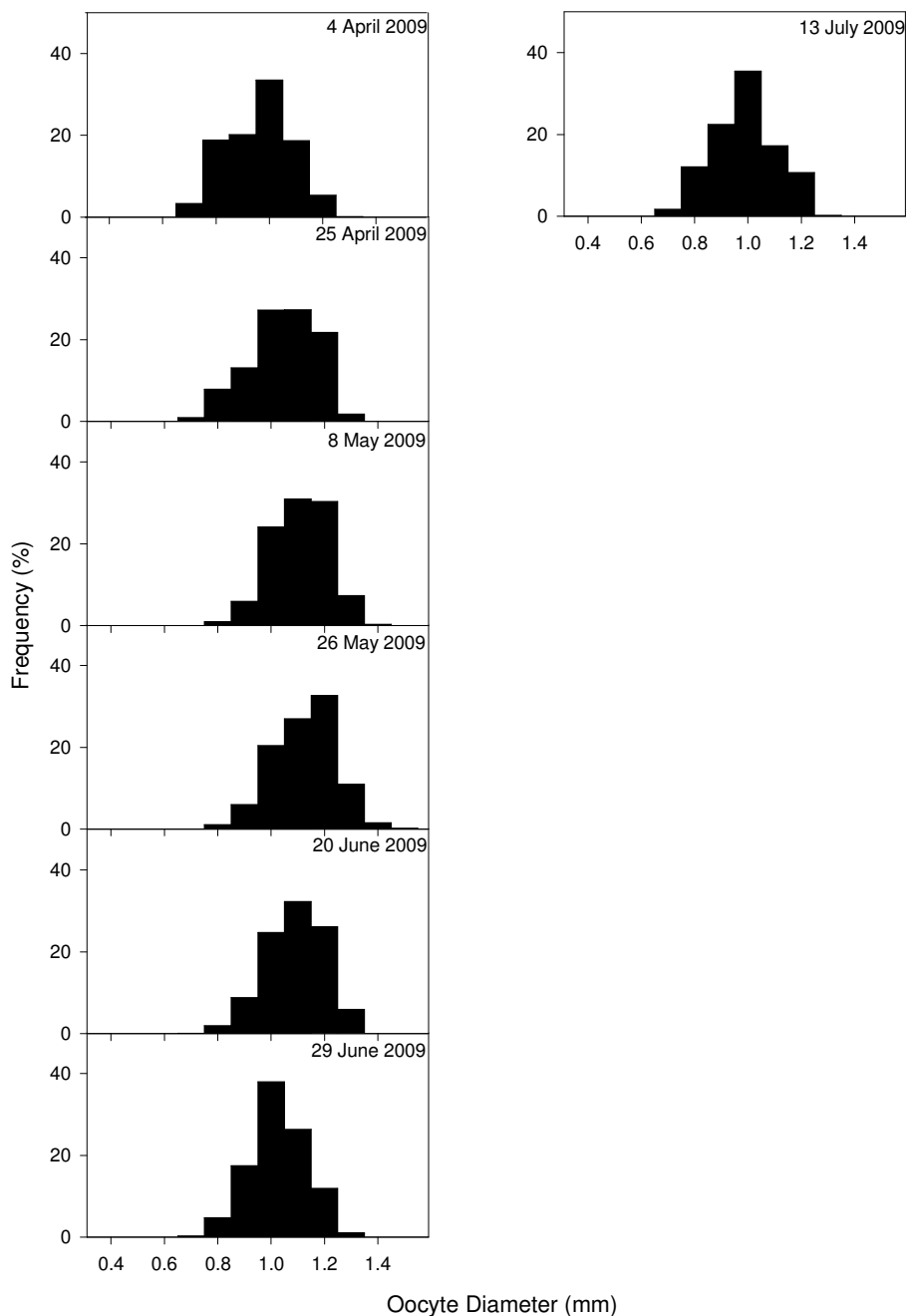


Figure 23: Size-frequency distributions of vitellogenic oocytes from ovaries of female plains minnow collected from the Brazos River at State Highway 6 north of Knox City, Texas during the 2009 reproductive season.

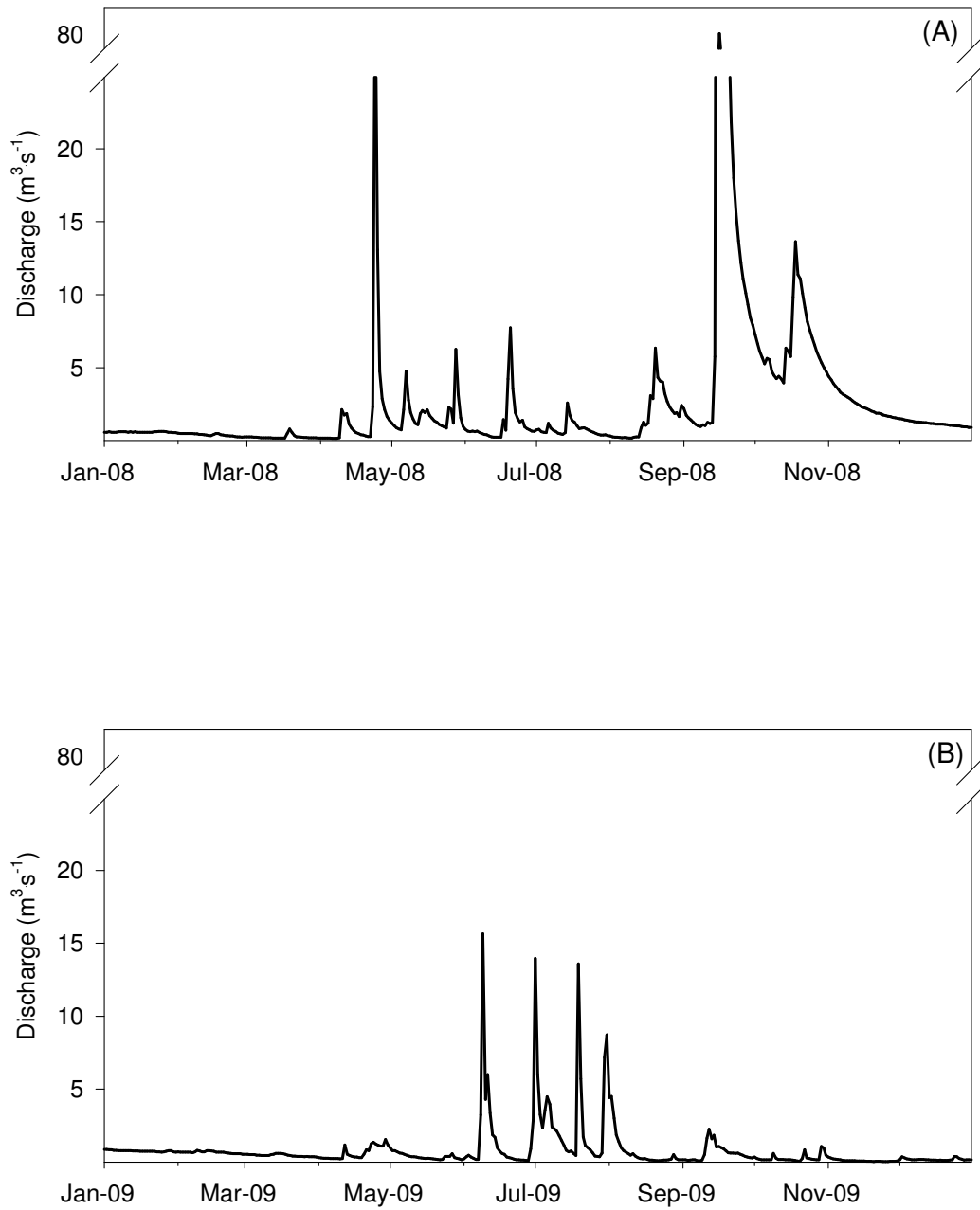


Figure 24: Mean daily discharge for the Double Mountain Fork Brazos River at State Highway 70 north of Rotan, Texas in 2008 (A) and 2009 (B). Data was collected from United States Geological Survey gauging station number 08080500.

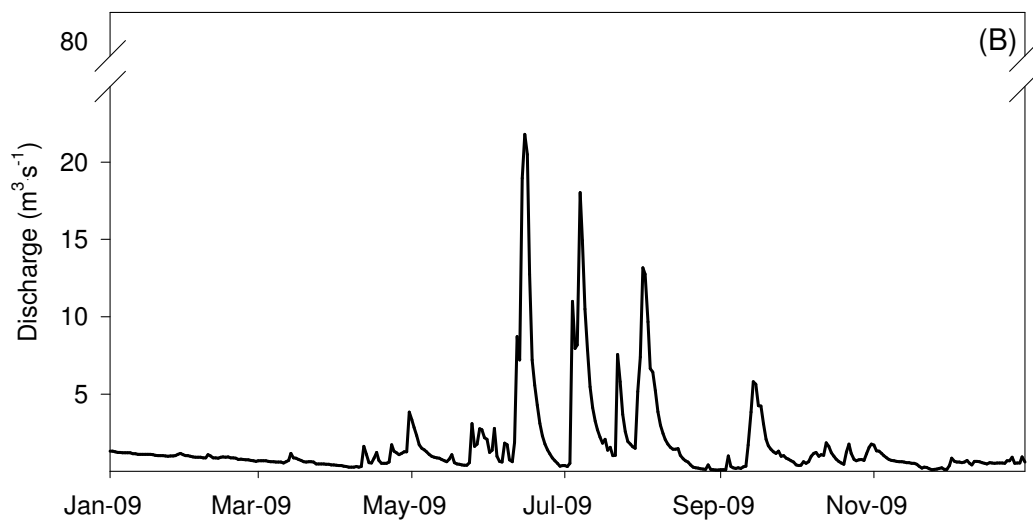
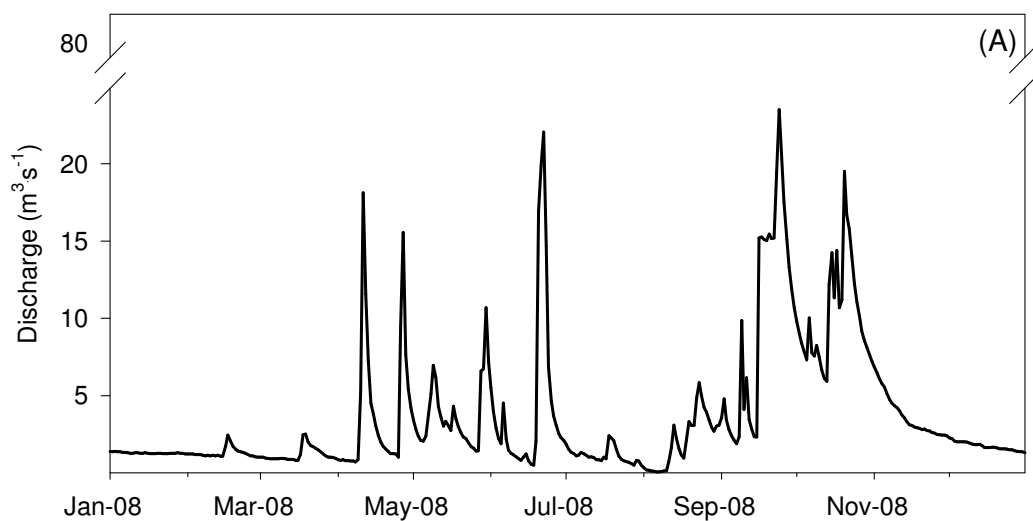


Figure 25: Mean daily discharge for the Brazos River at State Highway 6 north of Knox City, Texas in 2008 (A) and 2009 (B). Data was collected from United States Geological Survey gauging station number 08082500.

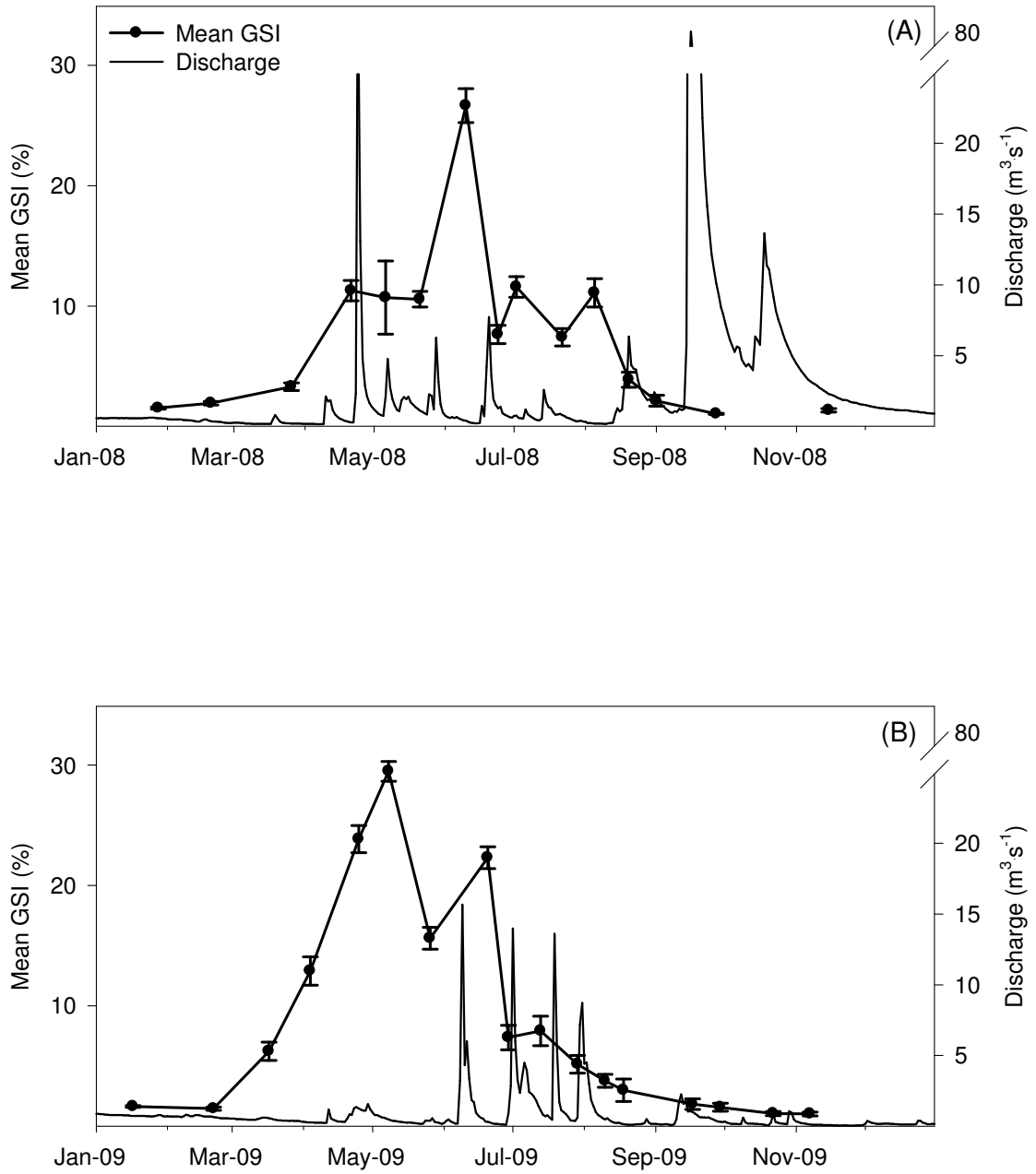


Figure 26: Mean gonadosomatic index \pm SE for female plains minnow and mean daily discharge at the Double Mountain Fork Brazos River at State Highway 70 north of Rotan, Texas during the 2008 (A) and 2009 (B) reproductive seasons.

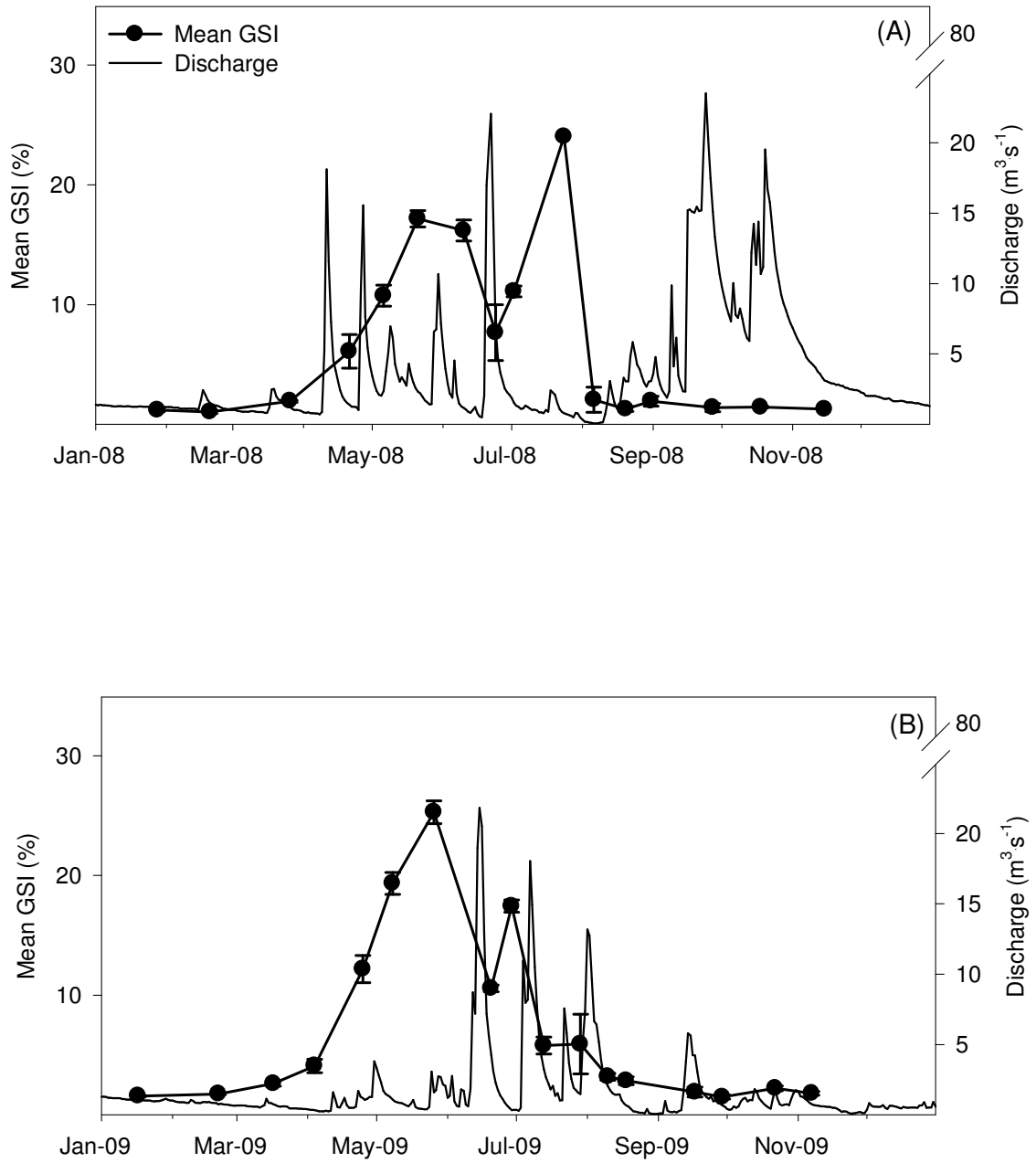


Figure 27: Mean gonadosomatic index \pm SE for female plains minnow and mean daily discharge at the Brazos River at State Highway 6 north of Knox City, Texas during the 2008 (A) and 2009 (B) reproductive seasons.

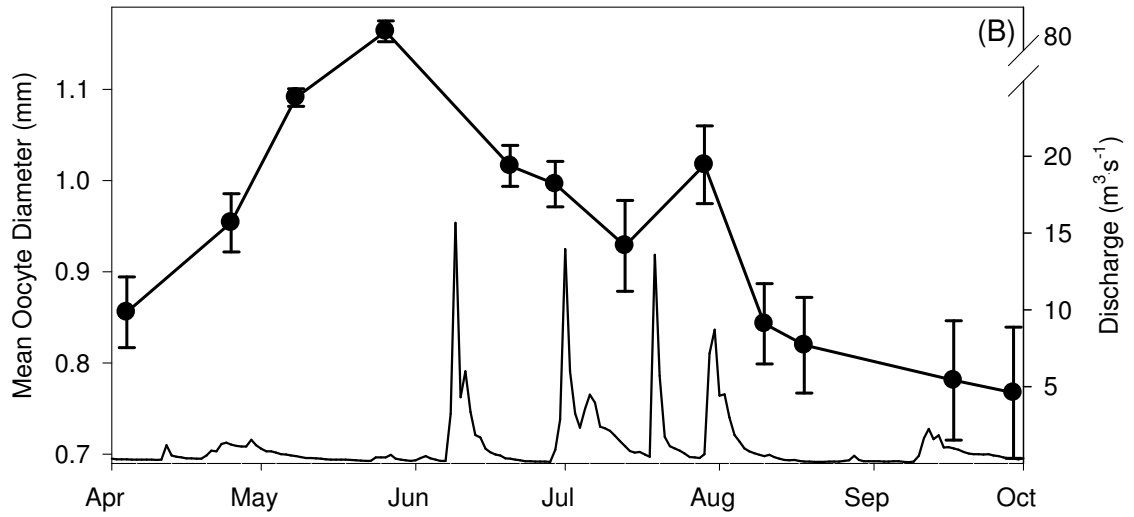
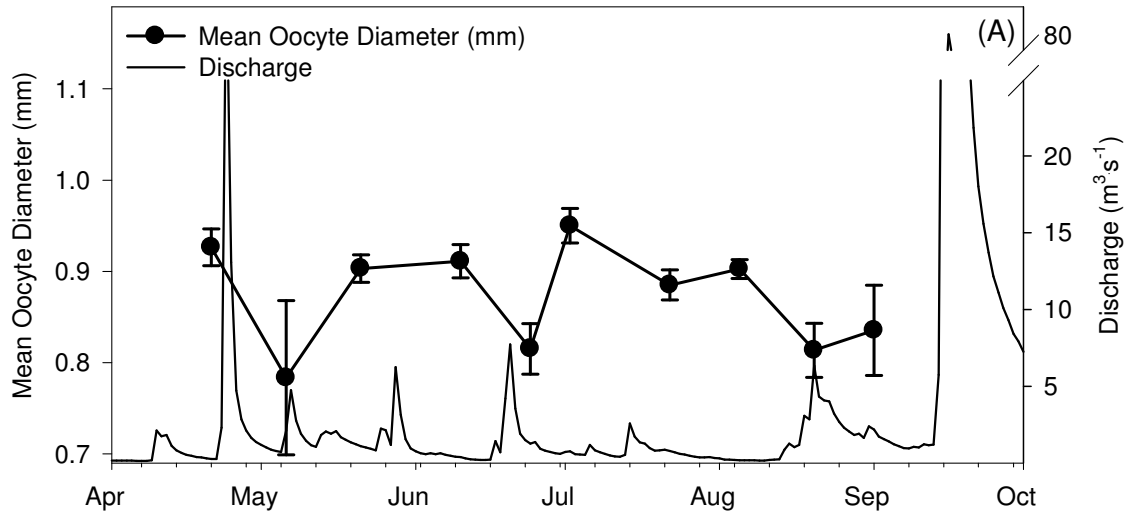


Figure 28: Mean vitellogenic oocyte diameter for female plains minnow and mean daily discharge at the Double Mountain Fork Brazos River at State Highway 70 north of Rotan, Texas during the 2008 (A) and 2009 (B) reproductive seasons.

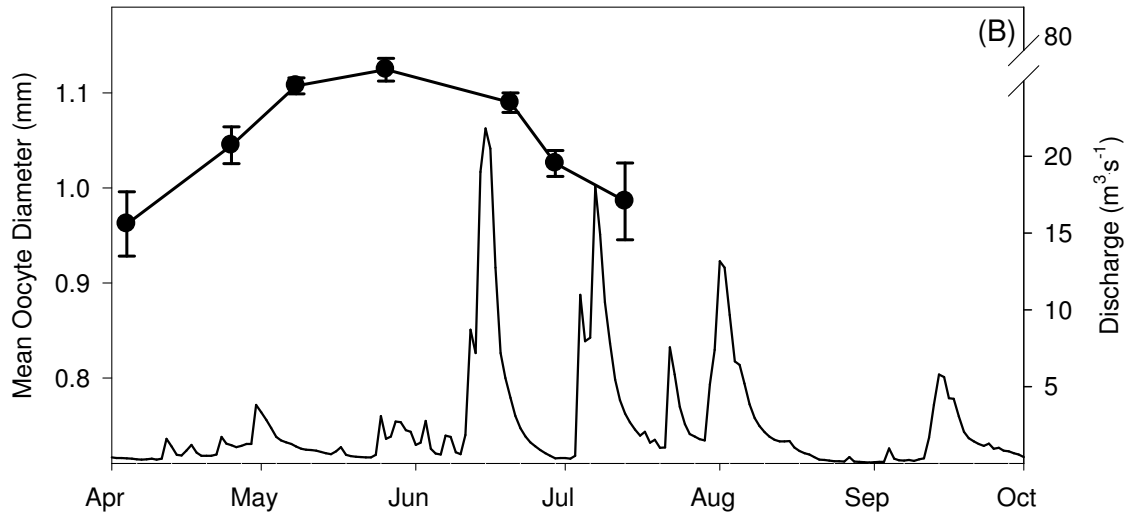
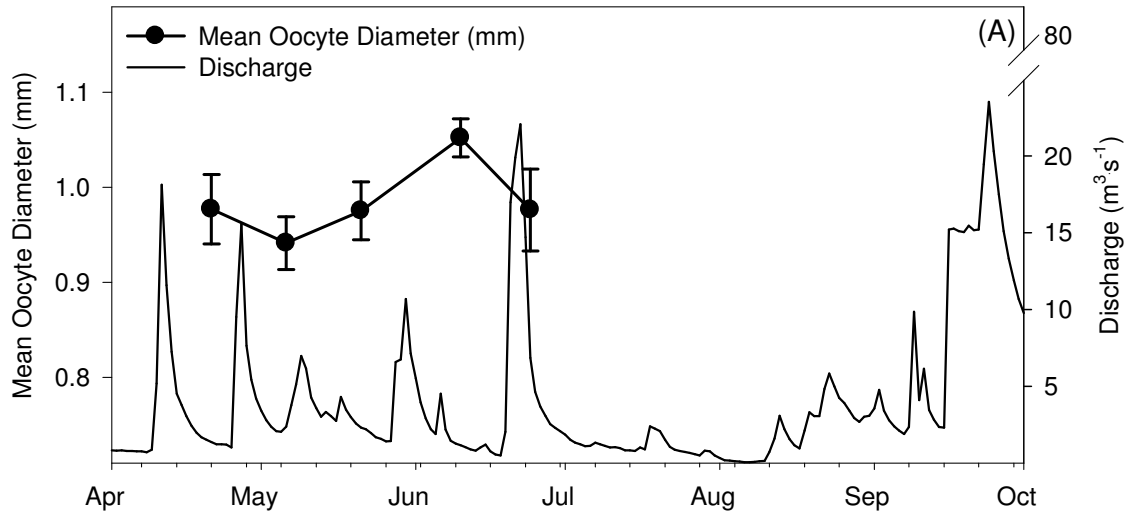


Figure 29: Mean vitellogenic oocyte diameter for female plains minnow and mean daily discharge at the Brazos River at State Highway 6 north of Knox City, Texas during the 2008 (A) and 2009 (B) reproductive seasons.