Development, Growth and Reproduction of Plains Topminnow, *Fundulus sciadicus*, in a Broodstock Pond Located at Sacramento-Wilcox Management Area

Final Performance Report

Project No. T-57

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Introduction

The plains topminnow, *Fundulus sciadicus*, was once distributed in the United States from the Mississippi River to the Rocky Mountains, north to South Dakota and as far south as Oklahoma (Baxter and Stone 1995). Two disjunct populations are recognized; one is centered in Nebraska and extends to northeastern Colorado and southwestern Wyoming (Baxter and Stone 1995; Rahel and Thel 2004). The second is centered in Missouri and extends from Kansas south to Oklahoma (Weitzel 2002).

The geographic range of plains topminnow appears to be experiencing significant declines. In Iowa, the plains topminnow is believed extirpated (Harlan et al. 1987). South Dakota and Kansas list plains topminnow as threatened and in Minnesota, Missouri, Colorado, Wyoming and Nebraska, this topminnow is listed as a species of special concern (Bailey and Allum 1962; Everhart and Seaman 1971; Brinkman 1994; Weitzel 2002). Recent research of Nebraska distribution found the plains topminnow to be absent from more than 75% of the 643 historic Nebraska sites (Haas 2005; Pasbrig unpublished data).

The decline in plains topminnow populations has been linked to the de-watering of critical backwater habitats from irrigation drawdown and drought. Nearly 10% of historic sites were dry when sampled in 2004 and 2005 (Haas 2005). In 2009, this study found 5% of the 643 historic plains topminnow sites to be dry (Pasbrig unpublished data).

In addition, the introduction of western mosquitofish (*Gambusia affinis*) has been implicated in the reduction of plains topminnow populations. The introduction and spread of the western mosquitofish has caused shifts in species assemblages through predation, competition, and stress. This has led to the reduction and elimination of fish species, aquatic invertebrates, and amphibian communities around the world (Moyle 1976; Meffe 1985; Bence 1988; Mills et al. 2004; Becker et al. 2005; Zeiber et al. 2008).

Mosquitofish from Utah were first imported to Nebraska in 1972. Between 1972 and 1985, mosquitofish where introduced into 19 counties. Presently, the western mosquitofish is common throughout the Republican and Platte River drainages (Lynch 1988). In these rivers it has been spreading an average of 10 km/year upstream and downstream (Lynch 1988). Concerns by municipalities and citizens over West Nile Virus and other mosquito-vectored diseases in Nebraska may perpetuate the use of western mosquitofish for biological control of mosquitoes.

With natural populations of plains topminnow depleted from a large portion of their historic range and continuing threats from changes in water levels and competition from exotic species, reintroductions of fish may be necessary to re-establish populations at historic sites. Extensive culture can be a useful tool; as habitat becomes available either through increases in natural precipitation, local management of water resources, or through public and private habitat projects designated to increase backwater habitat. A need exists for a reliable source of plains

topminnow for future re-introductions. The intent of this project was to accomplish the following objectives:

1) Establish a population of plains topminnow that can be used for re-introduction into historic and renovated sites (public or private) that are now dry or unoccupied.
2) Describe reproductive biology and production capabilities of plains topminnow in an extensive culture situation.
3) Describe habitat preferences and growth rates of plains topminnow adults and juveniles in a small pond.

Procedures

Objective 1

A pond designated to hold a broodstock plains topminnow population was constructed at the Sacramento-Wilcox Wildlife Management Area in Phelps County, Nebraska (40.38403, -99.21732) in September 2007. Pond construction was completed by Nebraska Game and Parks Commission (NGPC) staff and equipment as qualifying match for an internal state wildlife grant.

Surface area of the constructed pond is approximately 0.3 ha with a maximum depth of 3 m. Soils dug to create the pond were banked to add pond depth without breaching the clay barrier that naturally occurs in this area. A total of 8,709 kg of casing seal (bentonite) was added to the pond substrate in October 2007. Additionally, a soil composition probe employed by the Natural Resource Conservation Service identified the maximum percent clayey material available was 28% and located at 1.0-1.5 m depth. Soil from these depths were set aside and used to cover the added casing seal.

A t-connection pipe was welded into an existing irrigation line in March 2008. Irrigation pipe was laid to the pond and water was added in April 2008 until the pond was approximately half full. Sediment below the terminal end of the irrigation pipe was eroding with the addition of water, so a rock apron and plastic tarp was placed over this area in May 2008. The pond was completely filled immediately after the addition of the rock apron.

Plains topminnow were introduced into the pond in April 2008 after the pond was filled half way. A total of 68 plains topminnow were transported from Dry Creek in Loup County, Nebraska (41.88433, -99.24311) and stocked in the broodstock pond on 30 April 2008. Average total length of plains topminnow initially stocked was 52.2 ± 0.52 mm. Total holding time was less than 24 hours and a turbine agitator was employed to provide oxygenation in the 378.5 L hauling tank. The pond temperature at the time of stocking was 14.5 °C and tank temperature was 15.0 °C. In October 2008, an additional 55 fish were collected from Dry Creek in Loup County, Nebraska (41.88433, -99.24311) and stocked in the broodstock pond.

Objective 2

Habitat additions to the pond began in October 2007 and continued through the 2009 sampling season. Wheat seed (*Triticum spp.*) was planted around the pond to assist in establishing

vegetative cover to reduce sediment run-off. Cattail (*Typha spp.*) and pondweed (*Potamogeton spp.*) were added in small quantities in May 2008 to initiate the establishment of vegetation. Additional vegetation was added in June 2008 in the form of cattails, duckweed (*Lemna spp.*), and top moss (*Physcomitrium spp.*). A woody structure was added to the northwest side of the pond in June 2008. In July 2008, bulrush (*Scirpus spp.*) and duckweed were added. In 2009, additional cattails, duckweed, and six logs were added. Logs were tied and anchored to cinder blocks on the southeast side of the pond (Figure 1).

Sand was placed on 10 m^2 at a depth of 25 mm on a location that was anticipated to be less than 0.5 m below the pond surface located at the southeast side of the broodstock pond. A sand-gravel substrate was added to the broodstock pond in an area of approximately 3 m^2. In 2009, additional sand substrate was added to the existing 10 m^2 plot to supplement eroded sand (Figure 1).

The observation blind that was proposed in the grant was not established because visually a single location could only view a few vantage points and plains topminnow could be viewed without the use of a blind. A seasonal technician was employed during both the 2008 and 2009 sampling seasons. Assessments of water depth, habitat, and substrate selection were made for all observed plains topminnow at twelve designated observation points twice each week from late April through October in 2008 and from late March through October in 2009. In addition, corresponding water temperature and dissolved oxygen level were recorded.

In 2009, macroinvertebrate and zooplankton samples were taken in order to determine availability of food. Monthly, horizontal zooplankton tows were taken at two fixed locations on opposite ends of the broodstock pond. Additionally, a 0.3 m x 0.3 m, 500 micron, D-frame net was used in a figure eight pattern in submerged vegetation in order to sample macroinvertebrate communities. Sampled zooplankton and macroinvertebrates were separated into general taxonomic groups.

In August 2009, mark and recapture techniques were used to obtain population estimates. Adult plains topminnows were marked by using red visible implant elastomer (VIE) tags, which were injected anterior to the dorsal fin. Plains topminnow shorter than 30 mm were not marked because of the likelihood of mortalities from being injected with the VIE tag and were not included in the population estimate. Captured minnows were examined for marks visually.

Marking and handling mortalities were estimated in a separate retention study. A total of 40 fish were collected and measured (total length) to the nearest millimeter and placed in a 1 m^3 holding crib anchored in the center of the pond. Twenty fish served as the control and were handled and measured only. The remaining twenty fish were handled, marked, and measured. Fish were examined for mark retention and mortality after 24 hours, 1 week, and 2 weeks, respectively.

This project derived estimates of the plains topminnow population by using the Lincoln-Peterson formula (Jensen 1989): $N=(M+1)(C+1)/(R+1)$, where N is the population estimate, C is the total sample taken at the second visit, M is the total number of captured and marked fish at the start, and R is the number of recaptures at the time of the second visit. Topminnow shorter than 30 mm captured during the second sampling visit were not included in the capture (C) total at the

time of the second sampling. Additionally, population estimates were adjusted by the estimated handling and marking mortality.

In late November, fifty plains topminnow were collected, measured, and weighed in order to determine a batch weight for plains topminnow. Biomass estimates were derived by enumerating the batch weight from the fifty collected fish and multiplying it by the population estimate divided by fifty.

Objective 3

Assessment of plains topminnow vegetation and substrate selection was recorded during a 5-minute observation period. These observations occurred twice a week at 12 fixed stations around the broodstock pond.

Water temperatures and turbidity were collected for the entirety of the 2008 and 2009 season with HOBO temperature units set at fixed, separate ends of the broodstock pond. Additionally dissolved oxygen and pH were monitored at two fixed stations twice weekly. During weekly observations water level, turbidity, and environmental conditions were noted.

In 2008, minnow traps were set monthly to collect juvenile plains topminnow, to help identify cohorts but were unsuccessful.

Beginning in late-May 2009, four light traps were deployed once a week, for a total of two separate one hour sets. Light traps were deployed for five weeks and were used to sample juvenile plains topminnow in order to determine approximate hatch dates and to determine if topminnow were successfully reproducing in the broodstock pond.

In October, at the end of the 2008 season, plains topminnows were captured using a (6.4 m x 1.2 m) net seine. Eighty-five plains topminnow were collected and each fish was measured (total length) to the nearest millimeter. Additional observations of approximately 100 plains topminnow were made.

In 2009, plains topminnow cohorts were sampled using a (6.4 m x 1.2 m) net seine once a month, with the exception of May and June due to lack of staff. Sites were seined approximately 20 m along the shoreline from the point of insertion. A total of five seine pulls were performed and fish were collected in a holding tank. The number of fish was recorded and each fish was measured (total length) to the nearest millimeter.

In October 2009, thirty-five plains topminnow where captured using a net seine and each fish was measured (total length) to the nearest millimeter. Scale samples were taken and read using a stereoscope. Ages were recorded in an age-frequency histogram.

Findings

Objective 1

Pond construction was completed in September 2007, which included the addition of bentonite casing seal and packed covering of 28% clayey material (Figure 2). The pond was completely filled with water in early May 2008. Despite efforts to seal the pond, water seepage was evident throughout the 2008 and 2009 seasons. Water levels dropped approximately 0.25 m/month, which is more than would occur from evaporation only. It is anticipated that a natural sealing process will occur over time, but until that happens groundwater is added to maintain water levels. Staff to operate and monitor the irrigation well and filling process were limited during the 2008 season, so water was added every 10-14 days. This resulted in large fluctuations of colder and un-oxygenated groundwater during the summer months. Maintaining water levels in the 2009 season was more closely monitored by staff and water levels were maintained weekly.

Plains topminnow stocked into the broodstock pond have consisted solely of topminnow collected from Dry Creek, Loup County, Nebraska (41.88433, -99.24311). A total of 123 fish in two separate stocking events have been added to the broodstock pond. The first stocking consisted of 68 fish averaging 52 ± 0.52 mm total length, which occurred in April 2008 (Figure 3). At the end of the 2008 season, plains topminnow were captured using a seine. Eighty-five plains topminnow, averaging 49.8 ± 0.96 mm total length, were collected and total lengths were recorded (Figure 4). During the 2008 season, recruitment was apparent and a moderate population of plains topminnow had become established. By the end of the 2009 sampling season, it was apparent that a strong population of plains topminnow was established.

A concern to the development of plains topminnow at this location was the abundance of natural predators using the broodstock pond. A dense population of bullfrogs (*Rana catesbeiana*) and northern leopard frogs (*Rana pipiens*), as well as, a few unknown species of turtles also existed in the pond. In the summer of 2009, efforts towards reducing the number of potential predators within the pond took place. Amphibian egg masses were removed, as well as, tadpoles and adults removed via trapping. Stomach samples were not collected; therefore whether or not such predation occurred is unknown. However, large enough numbers of bullfrogs and leopard frogs were present to potentially reduce topminnow numbers.

On 21 October 2009, the first reintroduction of broodstock plains topminnow was completed. A total of 2,300 plains topminnow were seined and transported to the Anderson Bridge State Wildlife Management Area, Cherry County, Nebraska (42.78667, -100.93658). This area is a tributary to the Niobrara River and offers introduced topminnow abundant aquatic vegetation and backwater areas.

Objective 2

Habitat additions to the pond began in October 2007 and continued through the 2009 season. The development of the vegetative community was slow during the 2008 season but a strong vegetative community of cattails, bulrush, and top moss was established by the end of the 2009 sampling season. Small quantities of pondweed have become established. Despite multiple

attempts to establish, a vegetative community of duckweed; duckweed has yet to be established. Observations reveal that vegetative communities have become well established and appear to have needed a full growing season before a strong vegetative stand was formed.

Few observations of plains topminnow were made during the 2008 sampling season. Plains topminnows observed during 2008 were primarily seen at the southeast and northwest ends of the pond in water less than 15 cm deep (Figure 1). These areas remain sheltered from the wind on windy days. During the 2008 season, vegetation was not associated with plains topminnow sightings, but they were frequently seen on the southeast shore where sand-silt substrate was prominent (Figure 5). During the 2009 sampling season, plains topminnow were observed more frequently and in greater numbers than 2008. Substrate and vegetation type did not seem to influence topminnow observations. Topminnow were observed throughout the pond, however, highest concentrations were at the southeast and northwest ends of the pond. In these areas, wind is less of an influence and water is shallow with gradual slopes.

Water temperatures when plains topminnow were observed ranged from 6.5 ºC to 31.9 ºC, with the average water temperature at 22.5 ± 0.63 ºC. Observations have revealed that topminnows primarily prefer areas sheltered from the wind and areas with shallow water and gradual slopes. A comparison of water temperatures between sites selected by observed topminnow and temperatures recorded by data loggers revealed that shallow areas have the potential to be up to 2 ^{o}C warmer than deeper waters (Figure 6).

Chemical and physical characteristics of the broodstock pond were measured twice weekly throughout the 2008 and 2009 season. Sampling during both years occurred from March to October. Water levels fluctuated up to 0.5 m throughout the sampling season. HOBO temperature units were set at opposite fixed locations and measured water temperature and light intensity every 12 hours. Mean water temperature was 21.4 ± 0.57 ^{o}C, with daily changes in water temperature averaging 4.8 ± 0.22 ^{o}C during a 12 hour period. Fluctuations in water temperature during a 12 hour period were as much as 12.5 ^{o}C. Light intensity readings were compromised because of sediment and algae covering the HOBO unit. Mean turbidity levels were 45.5 ± 2.56; dissolved oxygen, 10.7 ± 0.17 mg/L; and pH, 8.6 ± 0.06 (Table 1). Dominant invertebrates included Coleoptera, Odonata, Corixidae, Ephemeroptera, and Chironomidae. The density (number per liter) of zooplankton in the broodstock pond at the time of sampling (June 2009) was configured. Cyclopoids and Nauplii constituted majority of the zooplankton community at 214 and 58 per liter, respectively. Rotifers and Calanoids constituted a small portion of the zooplankton community at 5.7 and 0.51 per liter, respectively. Food was readily available to broodstock plains topminnow.

The population estimate with a 95% confidence interval for adult plains topminnow was 9,844 ± 1,698 in August 2009. During the population estimate, 993 adult plains topminnows were marked and used in the population estimate assuming 5% handling and marking mortality, and there were 102 recaptures. Juvenile topminnow less than 30 mm total length comprised 3% of the recaptured sample and were excluded from the population estimate. The mark retention and mortality study found mark retention to be 100% and marking and handling mortality to be 5% (Figure 7). Mortalities both from handling and marking, did not occur within the first 24 hours but rather after two weeks.

The 50 plains topminnow seined in November had a batch weight of 42.2 g and averaged 42.2 ± 0.59 mm total length. Total biomass of the plains topminnow population in the Sacramento/Wilcox broodstock pond during the 2009 sampling season was enumerated and estimated to be 27.7 kg/ha. The biomass and population estimates of this study should be viewed as low due to juvenile topminnow under 30 mm total length not being included in the population estimate.

Objective 3

Few observations of plains topminnow were made during the 2008 sampling season. No vegetation was associated with plains topminnow locations during 2008, but 56% of plains topminnow observations during 2008 took place on the southeast end where sand-silt substrate is prominent (Figure 1).

During 2009, plains topminnow were frequently observed in high abundances throughout the pond. Majority, (31%) of plains topminnow observations were again observed on the south-southeast end of pond, where 6 logs were added as additional habitat in 2009. Eighteen percent of observations were made near the irrigation pipe and rock apron on the north end of the pond. A difference observed between the 2008 and 2009 sampling season, was that plains topminnow were observed in and around vegetation and habitat structures. Water temperatures where plains topminnow were observed ranged from 6.5ºC to 31.9 ºC, with the average temperature at 22.5 ± 0.63ºC. Observations have revealed that topminnows primarily prefer areas sheltered from the wind and areas with shallow water and gradual slopes. A comparison of water temperatures between sites selected by observed topminnow and temperatures recorded by data loggers revealed that shallow areas have the potential to be up to 2 ^{o}C warmer than deeper waters.

Observations of spawning behaviors and hatched young were not documented or observed during the 2008 sampling season. Minnow traps that were set to collect juvenile plains topminnow, to help identify cohorts were unsuccessful. Larval production during 2008, may have been compromised due to several factors. First, the vegetative community developed slowly and there was limited vegetation and algal mats available during the spawning season. Secondly, topminnow were stocked in late April, immediately prior to the spawning season. The added stress from movement may have impeded natural reproductive development.

During 2009, light traps were deployed as an alternative method for capturing juvenile plains topminnow (Figure 8). Optimal spawning temperature, 18 to 24° C (Kaufman and Lynch 1991), was reach on 28 May. Larval topminnows collected had a CPUE of 1/hr in light traps beginning on 17 June and continuing through 24 June. Larval topminnows collected averaged 7.6 ± 1.86 mm total length. Light traps began collecting larval topminnows when water temperatures had reached 28.1 ^{o}C and continued through water temperatures reaching 31.8^{o}C. During the last week of light trapping, one of the larval fish collected measured 15 mm total length. Free-swimming fry were first observed on 1 July 2009.

Plains topminnow numbers seined with the same amount of effort differed throughout the 2009 sampling season (Figure 9). As the sampling season progressed, visual observations increased,

as well as numbers seined. Topminnow observations increased as water temperatures increased. Average total length remained constant throughout the year but decreased from 2008 to 2009 (Table 2, Figure 10). Scale samples taken from 35 topminnow displayed two age classes (Figure 11). One hundred percent of fish less than 49 mm total length were age 0. Fifty percent of fish within the 50 to 54 mm length category were age 0. One hundred percent of fish greater than 60 mm were age 1. Observations and scale samples suggest production by young of the year topminnows to constitute majority of the total production by plains topminnow in Sacramento-Wilcox Wildlife Management broodstock pond. Observations showed rapid growth of young of the year topminnow, this is similar to other short-lived fish species. Payer and Scalet (1978) estimated production by young of the year fathead minnows (*Pimephales promelas*) at 99% total production in a South Dakota Slough. Payer and Scalet (1978) found fathead minnow production to consist of two age classes and estimated biomass at 17.5 kg/ha. By the end of the 2009 sampling season, it was apparent that a strong population of broodstock plains topminnow had become established.

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Deviations from proposal

The only noted deviation was that no observation blind was built. It was determined that visual assessment of plains topminnow was limited from a blind and it would not assist us in observing specific substrate and vegetation habitats that were being selected for use by plains topminnow.

Program Plan

We are pleased with the establishment of this broodstock facility. It took two years to establish enough vegetation to provide adequate spawning conditions. As it currently sits we anticipate this facility to be a valuable component of our recovery efforts for plains topminnow in the state of Nebraska.

FIGURES

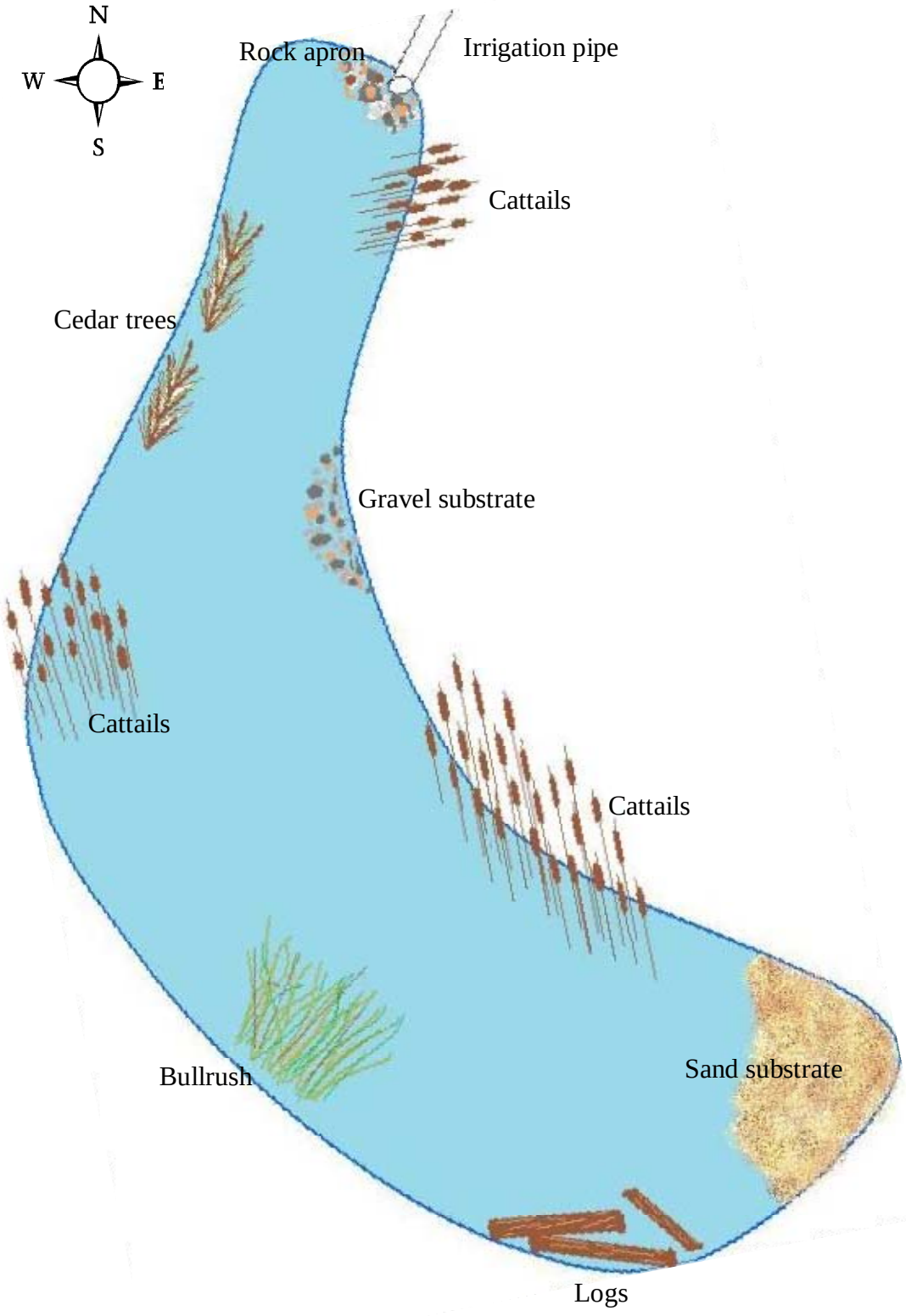

Figure 1 Diagram of plains topminnow broodstock pond located at Sacramento-Wilcox Wildlife Management Area (note diagram is not to scale).

Figure 2 Plains topminnow broodstock pond located at Sacramento-Wilcox Wildlife Management Area during construction (September 2007).

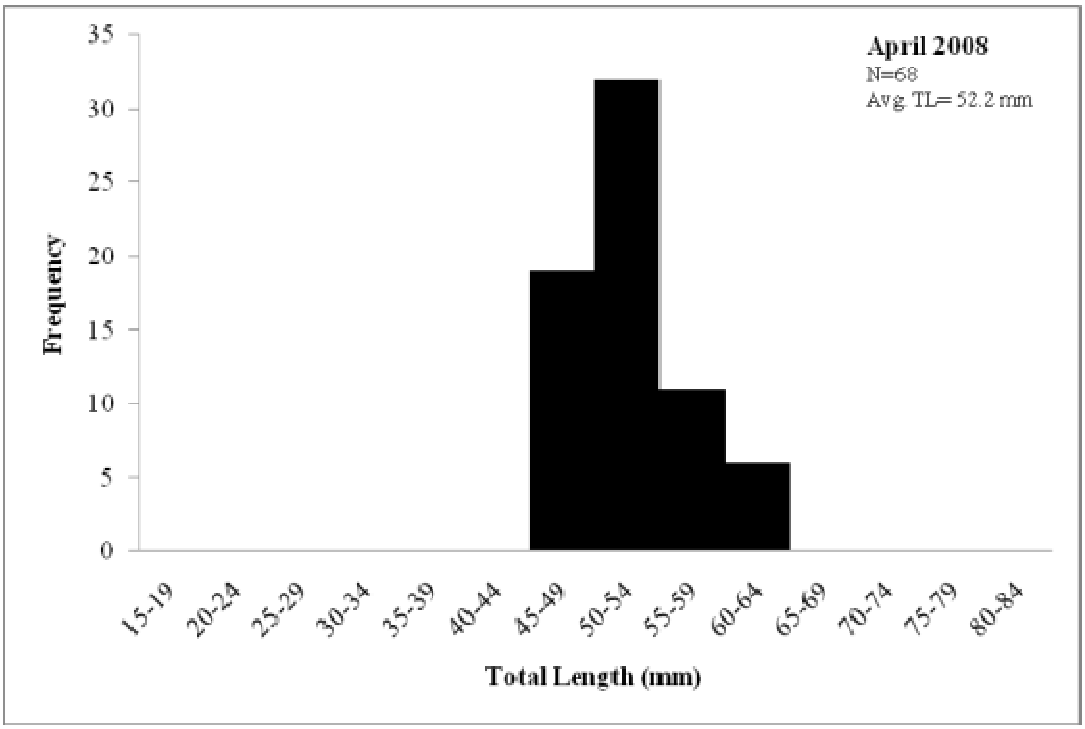

Figure 3 Length frequency histogram of plains topminnow initially stocked at Sacramento-Wilcox Wildlife Management broodstock pond (April 2008).

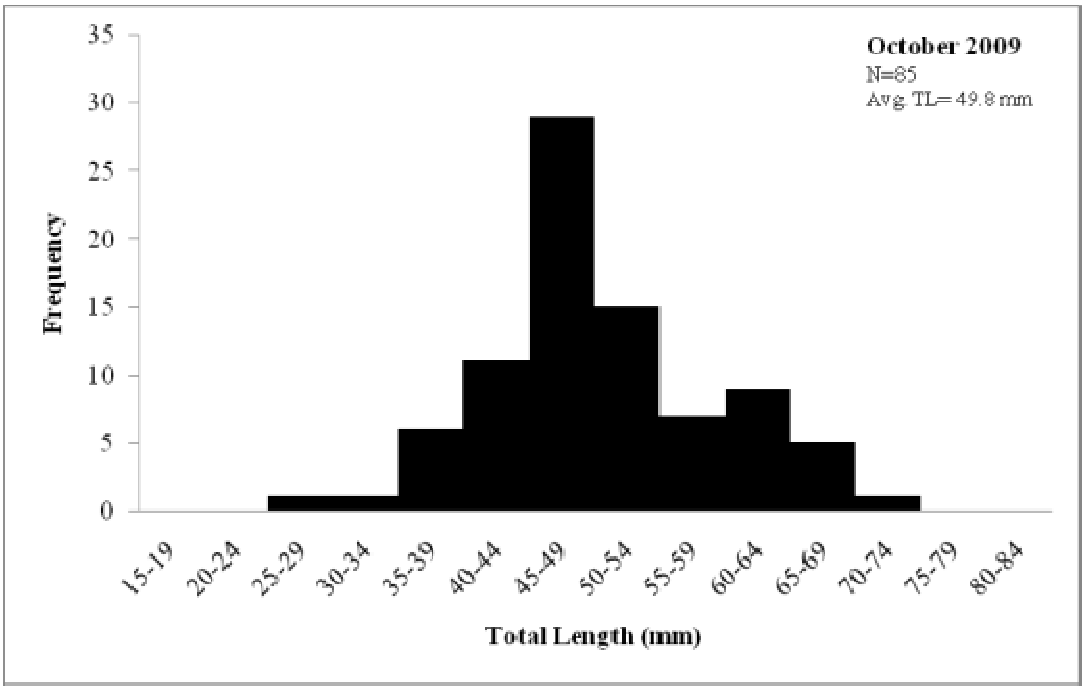

Figure 4 Length frequency histogram of plains topminnow seined at Sacramento-Wilcox Wildlife Management broodstock pond (October 2008).

Figure 5 Photo depicting common plains topminnow habitat selection at the Sacramento-Wilcox Wildlife Management broodstock pond.

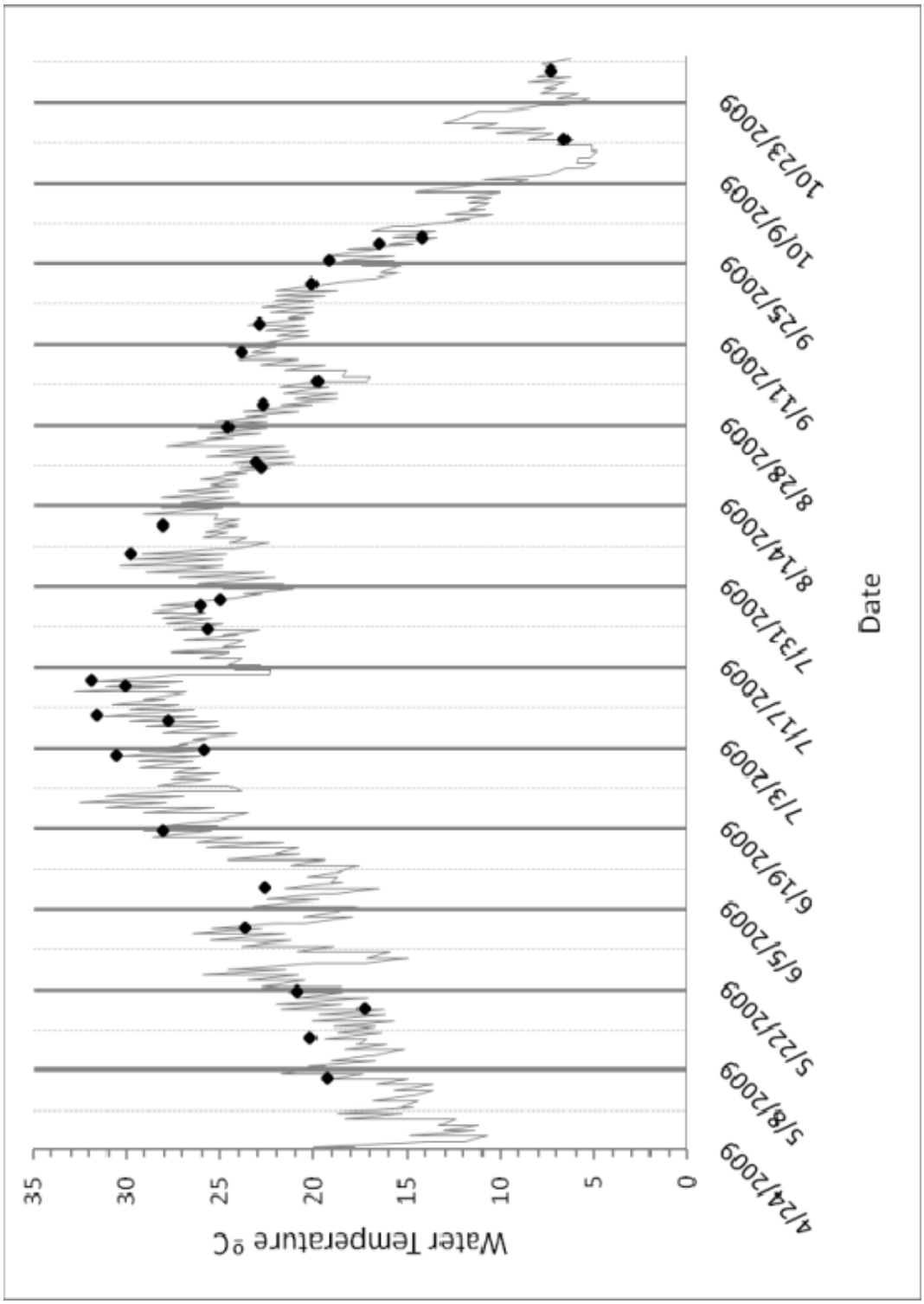

Figure 6 Recorded water temperatures of locations selected by observed plains topminnow (depicted by points) and temperatures recorded by data loggers.

Figure 7 Depiction of the VIE tagging procedure employed on plains topminnow at Sacramento-Wilcox Wildlife Management broodstock pond.

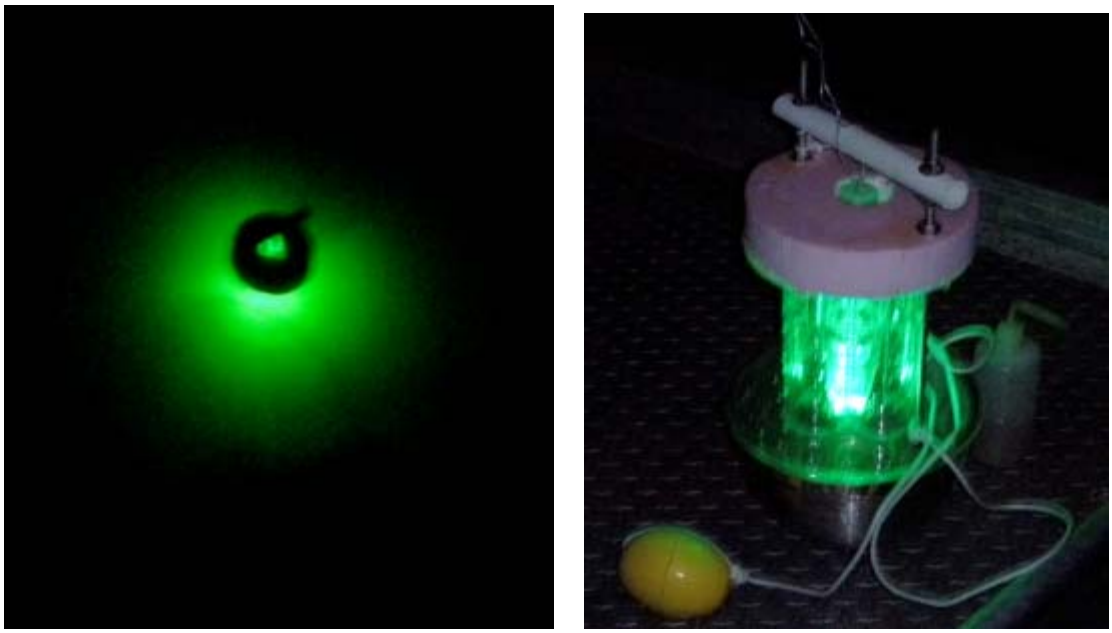

Figure 8 Depiction of light traps used to capture juvenile plains topminnow at the Sacramento-Wilcox Wildlife Management broodstock pond.

Figure 9 Depiction of seining method used to capture plains topminnow at the Sacramento-Wilcox Wildlife Management broodstock pond.

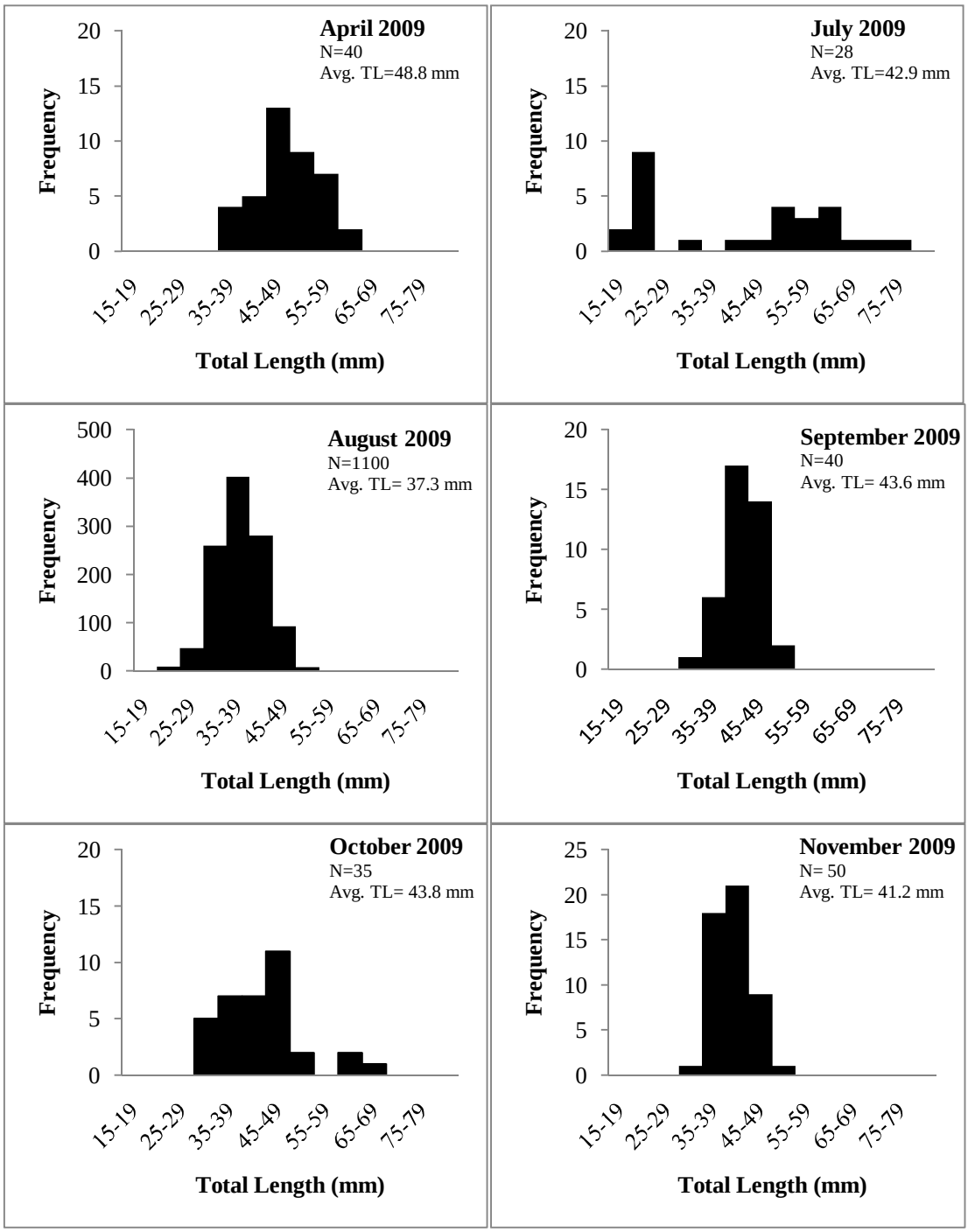

Figure 10 Length frequency histograms of seined plains topminnow during the 2009 sampling season from the Sacramento-Wilcox Wildlife Management broodstock pond.

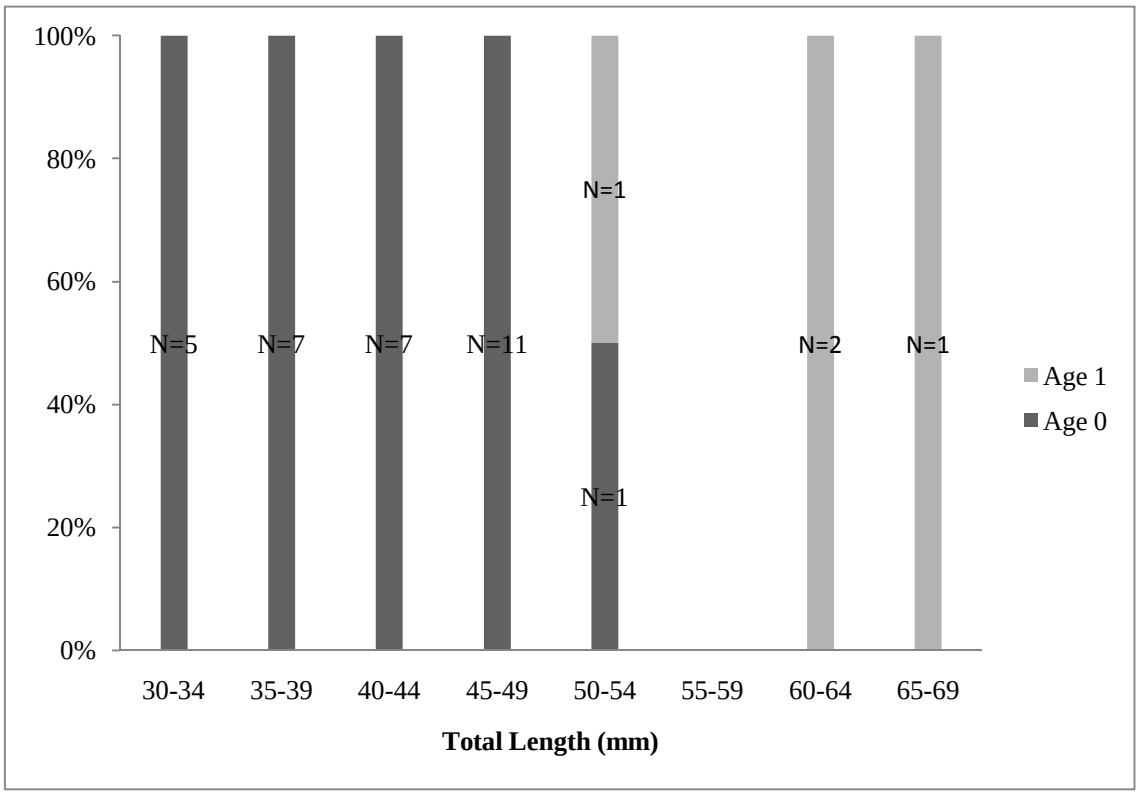

Figure 11 Age-frequency histogram of plains topminnow collected at Sacramento-Wilcox Wildlife Management broodstock pond (N=35).

TABLES

Table 1 Abiotic results comprised from 2008 and 2009 sampling data at Sacramento/Wilcox Wildlife Management Area.

	Water Temperature ºC	Change in Daily Water Temperature ºC	Dissolved Oxygen mg/L	Turbidity	pH
Minimum	5.5	0.2	6.32	5	7.5
Maximum	32.1	12.5	16.63	161	10.5
Average	21.4	4.8	10.74	45.5	8.6
Standard Error	0.57	0.22	0.17	2.56	0.06

Table 2 Cohort data from plains topminnow seined at Sacramento-Wilcox Wildlife Management broodstock pond, Phelps County, Nebraska, from March to November 2009.

Date	Number (N)	Mean Total Length (mm)	Min. Total Length (mm)	Max. Total Length (mm)	Biomass (g)
Apr-08	68*	52.1	45	62	--
Oct-08	85	49.8	29	72	--
Mar-09	9	50.9	43	67	--
Apr-09	40	48.8	35	64	--
May-09	--	--	--	--	--
Jun-09	--	--	--	--	--
Jul-09	28	42.9	18	79	--
Aug-09	1100	37.3	21	53	--
Sep-09	40	43.6	34	54	--
Oct-09	35	43.8	30	67	--
Nov-09	50	41.2	32	51	42.2

*Initial stocked fish