

# Relating Fish Abundance and Condition to Environmental Factors in Desert Sinkholes

Kristin M. Swaim and Wiebke J. Boeing  
Department of Fish, Wildlife and Conservation Ecology  
New Mexico State University  
2980 S. Espina; 132 Knox  
Las Cruces, NM, 88011

2008 Annual Progress Report  
New Mexico Department of Game and Fish  
Share with Wildlife

## **Abstract**

The relationships between fish assemblages and environmental factors have frequently been investigated. Often the focus has been on increasing or predicting yield or production for sport fisheries. Fish assemblages have also been studied to see if they show evidence of non-random patterns and, if so, what processes regulate those patterns. When relating fish communities to environmental variables, numerous studies have utilized a multiple-lake approach. These studies have largely been performed in north-temperate locations and little information is available about warmwater fish communities, like desert species. We studied native fishes in a sinkhole complex in New Mexico in the summers of 2006 and 2007 to determine if biotic, abiotic, or a combination of these factors influenced fish abundance and body condition. We investigated abundance using mark-recapture techniques and calculated population and catch-per-unit-effort estimates. Body condition was studied using the relative weight index. Abundance and condition were correlated with physical, chemical, and biological factors using regression analyses. Results showed that Pecos pupfish abundance was significantly lower in sinkholes where it occurred with other species. This was unexpected because all species were native and have potentially existed together for thousands of years. Pupfish body condition was also negatively influenced by the presence of other species. We suggest that low pupfish abundance and body condition is due to the presence of Pecos gambusia because it occurred in much greater numbers. Both predation and competition could be the means by which pupfish and gambusia interact. While pupfish and gambusia

do not compete for food or reproductive space, it appears likely that gambusia is in some way affecting pupfish reproduction. Abiotic factors like temperature, chlorophyll a, and salinity were significantly correlated with abundance and body condition, but the relationships varied between years, suggesting that either more data is necessary or that abiotic conditions in deserts are so variable that detecting patterns is difficult. This research contributed to multi-lake studies by providing information about desert systems and has serious management implications for the species studied since both Pecos pupfish and Pecos gambusia are species of concern.

## Introduction

The relationships between fish assemblages and environmental factors have frequently been investigated. Often the focus has been on increasing or predicting yield or production for sport fisheries (see Hinch 1991). Fish assemblages have also been studied to see if they show evidence of non-random patterns and, if so, what processes regulate those patterns (see Jackson et al. 2001). When relating fish communities to environmental variables, numerous studies have utilized a multiple-lake approach. That is, several lakes are used as experimental units and environmental variables are correlated to fish assemblage structure (which can include species richness and presence/absence), biomass, production, yield, or abundance. For example, fish biomass has been correlated with total phosphorus (Moyle 1956), lake depth (Prepas 1983), and a macrobenthos biomass-mean depth ratio (Hanson and Leggett 1982). Fish production has been associated with lake depth (Rawson 1952) and total phosphorus and primary productivity (Downing et al. 1990). Hanson and Leggett (1982) further related their macrobenthos biomass-mean depth ratio to fish yield and also found a relationship with total phosphorus. Matuszek (1978) found individual correlations with yield and macrobenthos biomass and lake depth. Oglesby (1977) related yield to phytoplankton biomass and Ryder (1965) developed the morphoedaphic index (MEI), which related total dissolved solids-mean lake depth ratios to fish yield. The MEI was both commended and criticized for its simplicity (Ryder 1982) and its use of ratios has been deemed inappropriate due to spurious self-correlations (Jackson et al. 1990).

Perhaps most commonly studied are the associations between environmental variables and fish assemblage structure. Assemblage structure has been linked to pH (Rahel and Magnuson 1983, Rahel 1984, Rago and Weiner 1986, Persson 1997, Jackson et al. 2001), dissolved oxygen (Jackson et al. 2001), conductivity (Persson 1997), lake depth (Robinson and Tonn 1989, Rodriguez and Lewis 1997, Tejerina-Garro et al. 1998), lake area (Eadie and Keast 1984, Robinson and Tonn 1989, Rodriguez and Lewis 1997), Secchi depth (Rodriguez and Lewis 1997, Tejerina-Garro et al. 1998), lake connectedness (Tonn and Magnuson 1982, Olden et al. 2001), and winter oxygen concentration (Tonn and Magnuson 1982, Rahel 1984).

Abundance is possibly the least studied of the fish "indices" that are related to environmental factors, due to the difficulty in obtaining accurate estimates. Instead, relative abundance has been used and has been correlated with lake depth (Marshall and Ryan 1987), lake size (Hinch et al. 1991), and macrophyte cover and primary productivity (Hinch and Collins 1993).

The previous examples may cause one to conclude that abiotic variables are more important in structuring fish communities and abundances than biotic factors like competition and predation. However, in several of these same studies predation was also considered highly influential (see Tonn and Magnuson 1982, Rodriguez and Lewis 1997, Jackson et al. 2001, among others). Competition likely plays a role, although it is less understood, particularly in structuring fish assemblages (Jackson et al. 2001).

Can one set of environmental variables override another, e.g., can the effects of predation be negated by favorable temperature regimes? Quist et al. (2003) hypothesized that favorable abiotic conditions will be superseded by biotic variables when predator/competitor densities are high. Persson (1997) reanalyzed data from Sumari (1971 as cited in Persson 1997), finding that while his analysis agreed with Sumari's conclusion that biotic factors influence fish community structure, abiotic factors were also important (also discussed in Quist and Hubert 2005). It is likely environmental factors interact to some degree (Hinch 1991, Rodriguez and Lewis 1997, Jackson et al. 2001) and the importance of biotic variables may be habitat-specific (Hinch 1991).

Further complicating this issue is the matter of scale. Hinch's (1991) review of fish ecology research on small lakes stated that most studies are conducted on either a large or small scale. Small-scale studies have small taxonomic and environmental scales, that is, fewer species are present (median number of species was 2.5) and there is little variation in abiotic variables among lakes. Small-scale studies are often conducted for less than one year and frequently estimate species abundance. Large-scale studies are conducted on lakes with more species (median number of species was 24) and the magnitude of variation of abiotic variables among lakes is much larger. Large-scale studies occur over longer periods and usually focus on fish assemblage structure, as obtaining abundance estimates can be quite difficult. Since, by their design, small-scale studies cannot assess how abiotic variables affect abundance, biotic interactions are usually proposed to drive abundance patterns. By

contrast, large-scale studies typically conclude that abiotic variables influence assemblage types (Hinch 1991 and further discussion by Jackson et al. 2001). Clearly, the scale of the study can determine what questions are asked and researchers may unwittingly infer patterns that are more a product of the study design than of the data.

Multi-lake studies have largely been performed in north-temperate locations such as Wisconsin, Ontario, and Alberta. Consequently, studies have focused on cold- and coolwater fish communities and little information is available about warmwater fish communities (Hinch 1991). Studies have been conducted on species in tropical lakes (Rodriguez and Lewis 1997, Tejerina-Garro et al. 1998) and solution holes in Florida (Kobza et al. 2004) but information on desert species is limited by scarce water resources and the rarity of multiple lakes within a relatively confined geographic region. Yet, some groupings of water bodies in deserts exist, such as springs and sinkholes, and they support fish communities (see Kodric-Brown and Brown 1993 for a study of desert springs). Desert fish communities tend to be depauperate; springs and small streams may contain a single species (Soltz and Naiman 1981). What is lacking is more data regarding fish abundance as related to environmental factors in desert systems. While desert fishes do not have the same level of economic importance that sport fishes do, studying their patterns of abundance is important because they are some of our most endangered species.

Desert lentic habitats can be considerably varied, from the relative constancy of thermal, chemical, and discharge characteristics of springs to more wide-ranging

conditions of temperature, turbidity, and salinity in lakes (Deacon and Minckley 1974). Perhaps not surprisingly, desert fishes tend to have wide physiological tolerances to abiotic factors: they can withstand high temperatures, low dissolved oxygen levels, and high salinities (Barlow 1958, Lowe et al. 1967, Lowe and Heath 1969, Brown and Feldmeth 1971). Alternately, low species diversity would seem to indicate that biotic interactions are minimized as there are fewer species competing for resources. There are few predaceous fishes in desert habitats (Meffe 1985) and segregation into different niches is apparent (Deacon and Minckley 1974). How abiotic and biotic factors might interact in desert systems is unclear, but the generalist nature of species with respect to their tolerances for extreme conditions may make it difficult to discern competitive interactions (Deacon and Minckley 1974). It would seem likely that highly tolerant species could easily move into different niches, camouflaging evidence of competition.

We studied a sinkhole complex in New Mexico, in which sinkholes inhabited by fish primarily contain a single native species, but several contain two to three native species; no non-native fish species are present. Abiotic conditions vary greatly among sinkholes (unpublished data). Obviously, biotic interactions are minimal in sinkholes with just a single species (although intraspecific competition should be considered). In these sinkholes we investigated which abiotic variables were most associated with abundance and body condition. In sinkholes containing multiple species both abiotic and biotic factors were assessed. Because this research could be

considered both small- and large-scale, results should not be limited by study design and should add to the body of knowledge that multi-lake studies provide.

## **Materials and Methods**

### *Study Area*

Bitter Lake National Wildlife Refuge (BLNWR, Fig. 1) comprises approximately 23,350 acres (36 square miles) in southeastern New Mexico (Brooks and Wood 1988). The geology of this region is limestone—formed by layers of marine organisms, sand, and mud from the ancient Permian Sea—overlain by gypsum (Land 2003). Within the limestone and underneath the city of Roswell and BLNWR lies an aquifer, part of the Roswell Artesian Basin (Barroll and Shomaker 2003). Groundwater from the aquifer slowly dissolves the overlying layers of gypsum and forms caverns and sinkholes. The middle tract of BLNWR contains several dozen sinkholes. Most are circular and often steep-sided with a small littoral zone, although others are more lake-like. The substrate is silt/limestone bedrock and vegetation consists mostly of *Chara* and *Potamogeton* (Hoagstrom and Brooks 1999). Rolling tumbleweed (*Salsola*) can line the shore in large numbers and in smaller sinkholes nearly fills them.

### *Fish Community*

Twenty-three sinkholes on the middle tract of BLNWR support native fishes. The Pecos pupfish (*Cyprinodon pecosensis*) occurs in twenty sinkholes. The Pecos

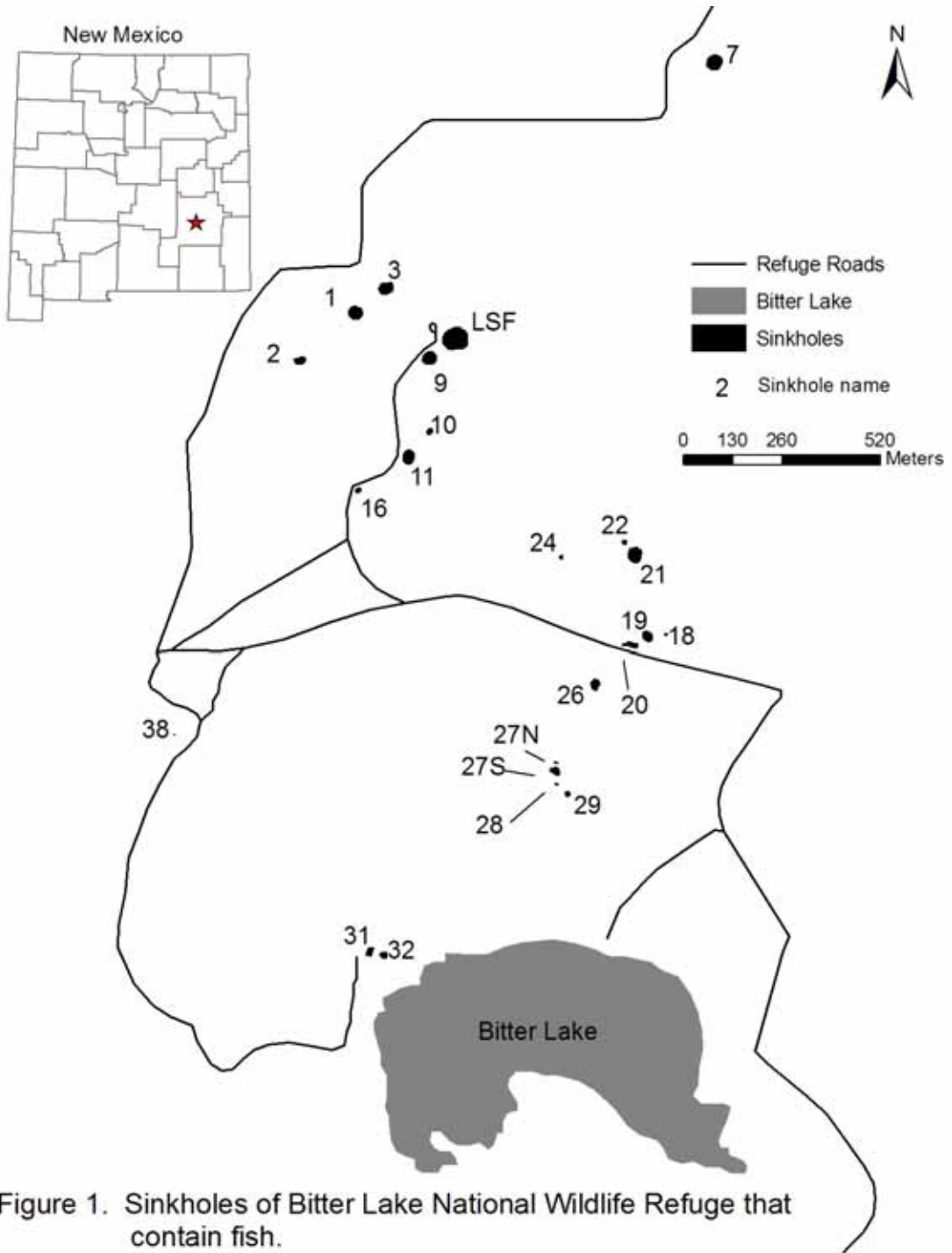


Figure 1. Sinkholes of Bitter Lake National Wildlife Refuge that contain fish.

*gambusia* (*Gambusia nobilis*) occurs in six sinkholes. The plains killifish (*Fundulus zebrinus*) occurs in five sinkholes and the red shiner (*Cyprinella lutrensis*) is present in one sinkhole. For a list of species per sinkhole see Table 1.

The establishment of the native fish community is unknown. The nearby Pecos River occasionally inundated the refuge (Brooks and Wood 1988) and could have been a potential source of fish, as could have Bitter Creek (Hoagstrom and Brooks 1999). In the 1930s many sinkholes were connected via a system of canals dug to harness water for the creation of waterfowl areas (Brooks and Wood 1988) and fish could have moved amongst the sinkholes (the canals have since been filled in). Several sinkholes were at one time stocked with game fish and native species could have accidentally been introduced (Brooks and Wood 1988). Finally, native species have been intentionally introduced to supplement populations but records are incomplete (Gordon Warrick, personal communication). These uncertainties greatly limit hypotheses concerning how the fish community may have evolved. One final note: although several sinkholes are in close proximity to each other, it is believed that subterranean movement of fishes among sinkholes is not likely (Gordon Warrick, personal communication).

### *Fish Sampling*

Jackson and Harvey (1997) stated that small-bodied fish species (which comprise all species in sinkholes at BLNWR) are most effectively captured with minnow traps. From this information, combined with the fact that the steep-sided

nature of the sinkholes eliminated the possibility of using seines, we concluded that minnow traps would most successfully capture fish. We conducted a pilot study in April 2006 to establish the appropriate number of minnow traps to set in each sinkhole. We decided to set vertical arrays of minnow traps so that a considerable portion of the water column could be sampled. One trap was set 0.5 meters from the bottom, one at mid water-column depth, and one 0-0.5 meters from the surface. Traps were attached to rope that was anchored to the bottom by a brick and secured at the top with an empty milk jug to ensure proper placement in the water column. In shallower sinkholes (those with perimeter depths of 2 meters or less) we removed the middle trap. Arrays were set approximately 2.5 meters from shore (nearshore) and further offshore. After an initial trial in which offshore traps caught few fish, we continued the pilot study with traps set only on the nearshore. We predicted that setting arrays in the nearshore every 5 meters around the perimeter of a sinkhole would allow me to capture a sufficient number of fish to effectively estimate population size. We tested this prediction on two sinkholes. Estimated population sizes were  $405 \pm 59$  and  $7486 \pm 1305$  (95% confidence interval) adult pupfish, which we thought were reasonable estimates. Because of the small number of fish captured in the middle traps in 2006, we eliminated them from my sampling protocol in 2007.

Full data collection began at the end of July and lasted approximately 2.5 weeks in both 2006 and 2007. This time period was selected because fish are most active in warmer months and it also coincided with the end of the pupfish spawning season. Aside from not wanting to hinder reproduction, male Pecos pupfish establish

and defend territories during the spawning season and there was concern that sampling during this time could result in lower catch rates because males may not move far from their territories. All sinkholes that contained fish ( $n = 23$ ) were sampled. Due to its connection to a small spring, sinkhole 31, while sampled for fish and environmental variables, was excluded from statistical analyses.

Mark-recapture methods were used to estimate abundance of adult ( $\geq 20$  mm standard length) pupfish. Baited minnow traps (0.32-0.64 cm mesh) were set in late afternoon / early evening, and retrieved the following morning. Traps were set from 13 to 20 hours. Captured fish were marked by clipping the upper portion of the caudal fin with scissors.

Due to the smaller caudal fin of Pecos gambusia, mark-recapture methods were not possible. Instead, catch-per-unit-effort (CPUE) was calculated to determine relative abundances of gambusia. In 2006, the mesh size of the minnow traps was too large to reliably capture gambusia and therefore CPUE was not calculated. In 2007, the traps were lined with window screening (0.1 cm mesh) that enabled juvenile and adult gambusia to be captured. Juvenile pupfish were also captured with the smaller mesh size. We kept separate counts of juvenile and adult pupfish so that comparisons could be made between years.

Standard lengths (tip of snout to posterior end of vertebral column, in mm) and weights (g) of up to fifty adult individuals per species were measured as an index of body condition. The diet of Pecos pupfish was also examined. Several unbaited traps were set for approximately 30 minutes and five captured adults were sacrificed

per sinkhole for the majority of sinkholes in which they occurred. Fish were preserved in 10% formalin until they could be processed in the lab. Once in the lab, fish were dissected under a microscope. Length was measured, sex was determined, and amount of fat (high or low) was recorded. Gut length was measured, as was relative fullness of the gut. Items in the gut were ranked according to abundance. Pecos gambusia diet was not analyzed.

#### *Environmental Factors – Abiotic*

Two morphometric variables, total depth and diameter, were measured with metered rope. To measure total depth, the rope was attached to a brick and dropped in the center of each sinkhole; depth was measured to the nearest tenth of a meter. The rope was then strung across the diameter of each sinkhole and measured to the nearest half-meter. For the few non-circular sinkholes, the long and short axes were measured and then averaged.

A Hydrolab (Hach Environmental) measured temperature, salinity, dissolved oxygen, pH, and turbidity at 1-m depth intervals and average values for each sinkhole were also calculated. A horizontal water sampler collected water samples at 1-m depth intervals. The samples from each meter were mixed in a bucket. Two 125-mL water samples were obtained in 2006: one for the analysis of total phosphorus and the second for calcium carbonate. Both samples were kept at 4°C until they were handed over to NMSU's Soil, Water, and Agricultural Testing (SWAT) Lab for analysis. Total phosphorus and calcium carbonate were not measured again in 2007.

Secchi depth, a measure of lake transparency, was measured with a Secchi disk.

Abiotic variables were collected from forty-one sinkholes (twenty-three with fish and eighteen fishless) in both years.

#### *Environmental Factors – Biotic*

Aside from competition and predation, there are a few other biotic variables to consider. From the water sample collected in the manner described above, chlorophyll a was measured, giving an estimate of phytoplankton biomass. 100-500 mL of the water sample was filtered with a hand pump onto a GF/C filter. The filters were wrapped in aluminum foil and kept frozen until they could be processed. Filters were ground in a foil-covered test tube with an aqueous acetone-magnesium carbonate solution and allowed to sit overnight. The following day the samples were centrifuged and the extract decanted. The extract was acidified with hydrochloric acid and chlorophyll a was measured with a spectrophotometer (Thermo Spectronic 20D+) according to APHA standards (Clesceri et al. 1998). Chlorophyll a was calculated as follows:

$$\text{Chlorophyll a, mg/m}^3 = \frac{26.7 (664_b - 665_a) \times V_1}{V_2 \times L}$$

where:  $V_1$  = volume of extract, liters

$V_2$  = volume of filtered sample, m<sup>3</sup>

$L$  = width of cuvette, cm

$664_b, 665_a$  = optical densities of extract before and after acidification, respectively.

Zooplankton samples were collected via vertical tows with a zooplankton net (Ø 20 cm, 110-µm mesh). Two samples were collected and pooled per sinkhole in 2006 and preserved in 95% ethanol. One sample per sinkhole was collected in 2007. Samples were processed in the lab. A subsample was taken and at least 100 individuals of the most abundant species were counted using a dissecting microscope.

A petite ponar was used to collect two benthic samples per sinkhole in 2006. Samples were taken from the nearshore where minnow traps were set. Approximately two cups from each sample were taken from the ponar, transferred to plastic containers, and preserved in 95% ethanol. Upon returning to the lab, samples were washed through a 4760-µm sieve to separate larger particles and then a 500-µm sieve. All invertebrates were sorted under a dissecting microscope, preserved in 70% ethanol, and then identified to family. Biotic variables were collected from forty-one sinkholes.

#### *Data Analyses*

*Fish abundance* – The modified Lincoln-Petersen method was used to estimate adult pupfish abundance (Ricker 1975, with Chapman’s modification that provides less bias).

$$\text{Population size, } N, = \left[ \frac{(M + 1) \times (C + 1)}{R + 1} \right] - 1$$

where:  $M$  = number of fish marked on first day

$C$  = number of fish captured on second day

$R$  = number of fish captured on second day that are recaptures.

Variance of  $N$ , with a modification from Seber (1970) for less bias, can be estimated as:

$$\text{Var}(N) = \frac{[(M+1) \times (C+1) \times (M-R) \times (C-R)]}{[(R+1)^2 \times (R+2)]}$$

For a few sinkholes, when capture rate was very low, traps were set for an extra night and the Schnabel method was then used to estimate abundance (Ricker 1975, with Chapman's modification).

$$\text{Population size, } N, = \frac{\sum (M_t \times C_t)}{(\sum R_t) + 1}$$

where:  $C_t$  = total number of individuals caught in sample  $t$

$M_t$  = number of marked animals in the population just before the  $t$ th sample is taken

$R_t$  = number of individuals already marked (recaptures) when caught in sample  $t$ .

The variance can be calculated by inverting the following:

$$\text{Var}(1/N) = \frac{\sum R_t}{(\sum (M_t \times C_t))^2}$$

CPUE was computed as fish per trap per day. For each trap, the number of fish captured was divided by the number of hours the trap was set (fish/hour). This was summed together for all traps in the sinkhole, and then divided by the number of traps set and multiplied by 24.

*Body condition* – I chose to look at body condition because it can indicate the general health of a fish. The weight of a fish relative to its length suggests its physiological condition and examination of condition may provide information about its habitat (Murphy et al. 1990, Blackwell et al. 2000). We used relative weight (Wege and Anderson 1978) as an index of body condition. Relative weight ( $W_r$ ) is:

$$W_r = W / W_s \times 100$$

where  $W$  = the observed weight of an individual fish

$W_s$  = a length-specific standard-weight value.

75<sup>th</sup> percentile weights are used to calculate standard weights because they represent fish in better-than-average condition (Wege and Anderson 1978). No standard optimal relative weight exists and some researchers contend that relative weight target ranges depend on the species and management objectives (Murphy et al. 1991). Nonetheless, ranges of 90-100, 95-100, and 95-105 have been suggested (see Murphy et al. 1990, Murphy et al. 1991). Anderson and Neumann (1996) stated that relative weight values well above 100 for a species may indicate that the species is not under enough predation pressure and that other species are not taking advantage of a surplus in this species and that this is undesirable from a management perspective. Since we

was not concerned with this scenario, we decided that a relative weight  $\geq 95$  indicates a fish in good condition.

To develop a standard weight equation we used the regression-line-percentile (RLP) technique (Murphy et al. 1990), which is a popular method for the development of standard weight equations (Blackwell et al. 2000). Equations are developed from several populations of the species of interest. The procedure is as follows (from Murphy et al. 1990): Mean fish weight in 1-cm length intervals is predicted for each population by regressing log weights against log lengths. The 75<sup>th</sup> percentile of the mean weights (from all populations) in each 1-cm interval is identified. The 75<sup>th</sup> percentile weights are then regressed against length and this determines the parameters for the standard weight equation. Because the species studied here were small (approximate adult size range 20-40 mm), we used 1-mm length intervals. Unlike Murphy et al. (1990), instead of calculating mean weights for each population (sinkhole), we decided to pool data from all sinkholes to develop a standard weight equation. We felt this was necessary because we wanted to compare relative weights among sinkholes. Equations were developed for both pupfish and gambusia; in 2007 a second equation was developed for pupfish to compare it to the equation from 2006. A concern with standard weight equations is that they may be length-biased, that is, relative weight increases or decreases with length (Murphy et al. 1990). The RLP technique removes any length-related biases (Neumann and Murphy 1991, although this is disputed by Gerow et al. (2004)). As long as length-related trends are not present in a large number of sinkholes, indicating a general

pattern of bias, then any trends within a sinkhole can be indicative of environmental conditions (Murphy et al. 1990, Neumann and Murphy 1991). We checked for length-related trends by regressing mean relative weights against length intervals as suggested by Murphy et al. (1990), which can also reveal other patterns across size classes. An average relative weight was then calculated for each sinkhole.

Standard weight equations are primarily used in fisheries management and have been mostly developed for centrarchid game species (Blackwell et al. 2000), though equations have recently been developed for some nongame species (Bister et al. 2000, Didenko et al. 2004). It may seem inappropriate to develop standard equations for such small species in which the range of size classes is narrow and may not be as ecologically significant as compared to larger fish. However, the objective was to develop a metric for body condition that could be regressed against environmental factors.

*Fish abundance and body condition vs. environmental factors* – Multiple linear regression analyses were used to discern associations between abundance and body condition and environmental factors. Separate regressions were done for both years and both dependent variables. Analyses were further divided into sinkholes containing just pupfish and sinkholes containing multiple species (herein referred to as “Pupfish-only Group” and “Multi-species Group”, respectively) because pupfish abundance was mainly influenced by the presence/absence of other species. To account for multiple species, a dummy variable was created where sinkholes containing only pupfish were given a value of 0 and those containing multiple species

given a value of 1. It was not possible to calculate pupfish population estimates in some sinkholes. As a result, CPUE was used because it gave a more accurate picture of pupfish abundance among sinkholes and allowed for all sinkholes to be used in the regression analyses. Data for gambusia was included in 2007. Sinkhole 27N, which contained only gambusia, was included in gambusia regression analyses to increase sample size, although close attention was paid to its role in results. This sinkhole was not included in the analyses that combined all sinkholes since it did not have pupfish. Fourteen total regression analyses were performed using SAS (SAS Institute 2003). Collinearity among variables, which can create misleading models, was first examined by calculating Pearson correlation coefficients, plotting variables, and examining variance inflation factors. Some variables were removed from further analysis if their correlations,  $|r|$ , were greater than or equal to 0.9, which indicates severe collinearity. Most often Secchi depth, diameter, and calcium carbonate were removed, but this depended on the analysis. The following model selection methods were applied: forward (entry level  $\alpha = 0.10$ ), backward (stop level  $\alpha = 0.10$ ), stepwise (entry level  $\alpha = 0.15$ , stop level  $\alpha = 0.10$ ), r-square, adjusted r-square, and Akaike's Information Criterion (AIC). Ideally, all techniques would result in the same model, but this was never the case. Instead, the best one-, two-, three-, and four-variable models provided by the selection techniques were evaluated based on overall model p-value, parameter p-values, and adjusted r-square value. Rather than specify a particular  $\alpha$  level for significance, the model with the lowest overall and parameter p-values was chosen and its p-values reported. Once a model was chosen,

residual diagnostics were performed, in which studentized residuals and potential influential observations and outliers were examined. The assumption of normality was tested with the Shapiro-Wilk test. Equal variances were checked by plotting residuals against predicted values. Any observations deemed to be outliers for several of the diagnostics were removed and the data were re-analyzed to see if a different model was more appropriate.

*Diet* – Once food items were ranked for each of the five fish sacrificed per sinkhole an average rank of food items per sinkhole was calculated. For example, diatoms were ranked 4, 4, 3, 5, and 6 in the five fish collected from Sinkhole 22, giving an average of 4.2. After each food item had been averaged as such, the averages were re-scored to provide rankings on a per-sinkhole scale so comparisons could be made among sinkholes. For example, if the diatom average score for sinkhole 22 was 4.2, detritus average score was 2.8, and dinoflagellate average score was 5.6, these would be re-scored as 2, 1, and 3, respectively. We used cluster analysis (SAS Institute 2003) to evaluate whether sinkholes could be meaningfully grouped based on pupfish diet. We standardized food items to zero mean and unit variance to limit those variables with larger variances from having more of an effect on cluster formation. Euclidean distance was used as the measure of dissimilarity and average linkage was the fusion strategy used to form clusters (McGarigal et al. 2000). Significant differences between cluster means for each food item were tested using Wilcoxon rank-sum tests.

## Results

### *Fish Abundance*

In 2006, pupfish CPUE was significantly higher in the Pupfish-only Group than the Multi-species Group (Wilcoxon rank-sum test,  $p = 0.0002$ , Fig. 2a). The same result was present in 2007 (Wilcoxon rank-sum test,  $p = 0.0001$ , Fig. 2b). *Gambusia* CPUE was not significantly different from pupfish CPUE where they co-occurred (Wilcoxon rank-sum test,  $p = 0.1320$ , Fig. 2b). This has largely to do with the very low catch rate for *Gambusia* in two sinkholes. Obviously, with the exception of those two sinkholes, catch rate was much greater for *Gambusia*. While *Gambusia* CPUE included both adult and juvenile stages, no juvenile pupfish were caught in the sinkholes where they occurred with *Gambusia* with the exception of two juveniles from Sinkhole 7; this allowed for direct comparisons to be made. Plains killifish CPUE (mean = 1.88) was not significantly different from pupfish CPUE (Wilcoxon rank-sum test,  $p = 0.2159$ , not shown). Significant differences in CPUE were not present between years in the Pupfish-only Group (Wilcoxon rank-sum test,  $p = 0.3064$ , Fig. 3a). The same was true for the Multi-species Group (t-test,  $p = 0.5969$ , Fig. 3b). Catch rate did vary among sinkholes. Within the Pupfish-only Group, sinkholes that had few pupfish in 2006 had higher catch rates in 2007 and vice versa (Figure 3a). Overall more pupfish were captured in 2007. Table 1 provides greater detail of CPUE for all species and population estimates for pupfish.

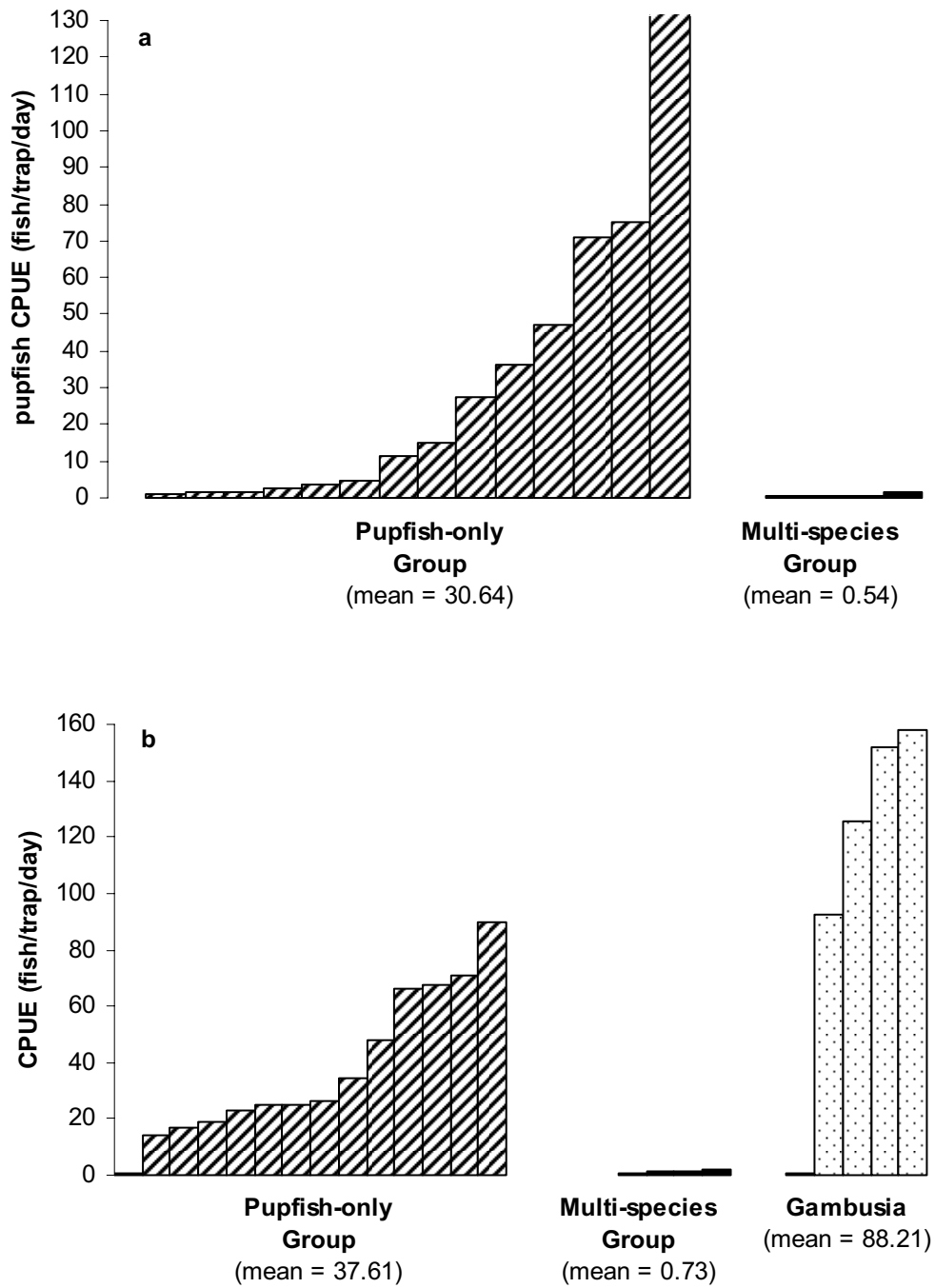
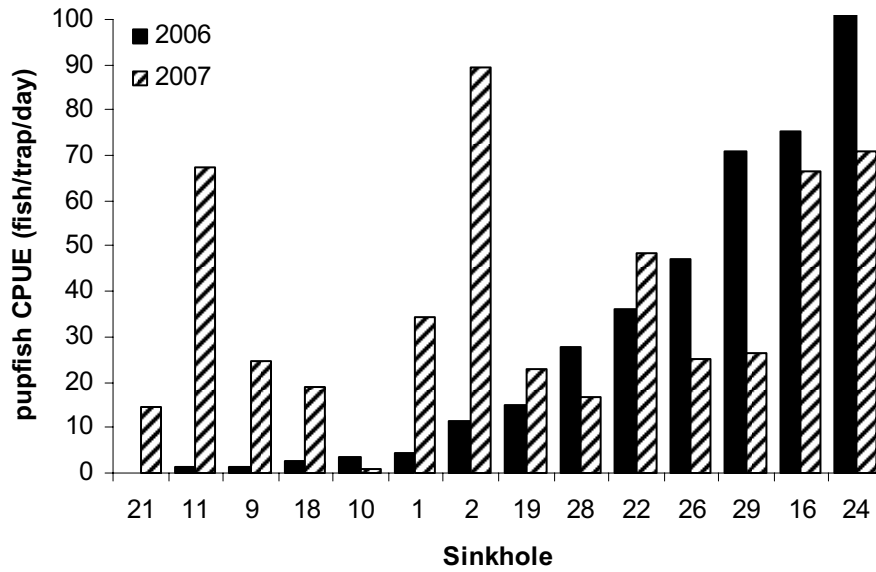


Figure 2. Pecos pupfish CPUE for 2006 (a) and 2007 (b), with data included for Pecos gambusia in 2007. Each bar represents a sinkhole.

**a. Pupfish-only Group**



**b. Multi-species Group**

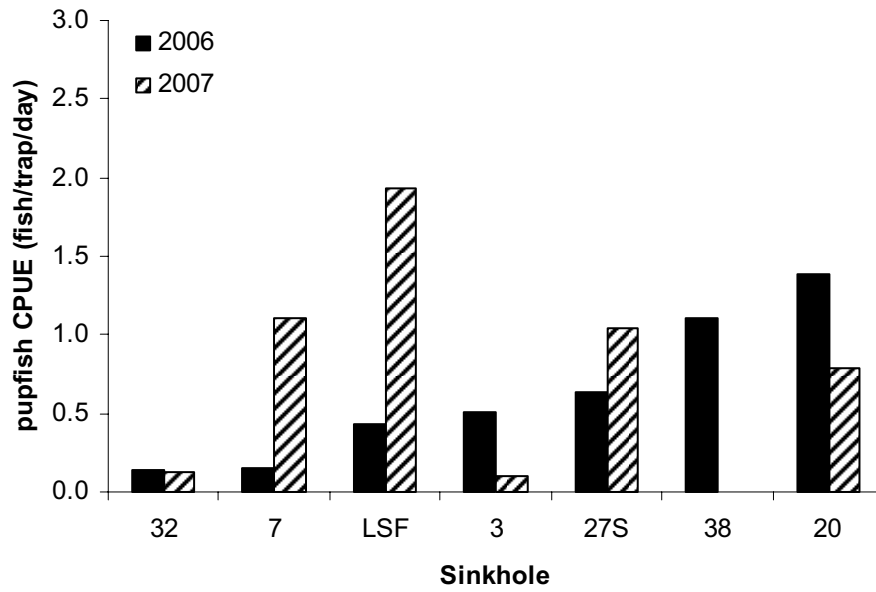


Figure 3. Yearly comparisons of Pecos pupfish CPUE for the Pupfish-only Group (a) and the Multi-species Group (b). Note the different scales on the Y-axes.

Table 1. Catch-per-unit-effort (CPUE), average relative weight, and population estimates for sinkholes of Bitter Lake NWR, 2006 and 2007. 95% confidence intervals for population estimates are given in parentheses. Pupfish CPUE is for adults only. An 'x' indicates the variable was not calculable or the estimate was poor; population estimates are for adult pupfish only. Pup = Pecos pupfish; Gam = Pecos gambusia; Killi = plains killifish; Shiner = red shiner.

Sinkhole	Species	CPUE (fish/trap/day)		Population Estimate		Avg. Relative Weight (%)	
		2006	2007	2006	2007	2006	2007
1	Pup	4.59	34.37	3524 (3265-3829)	5290 (4628-5951)	102	101
2	Pup	11.30	89.51	2656 (1786-3526)	7890 (7199-8581)	99	88
3	Pup	0.50	0.10	x	x	95	107
	Gam	x	92.82	x	x	x	84
7	Pup	0.15	1.11	x	116 (85-147)	82	94
	Gam	x	158.10	x	x	x	88
	Killi	0.66	0.28	x	x	x	x
9	Pup	1.49	24.68	3386 (3061-3787)	3454 (2980-3927)	83	85
10	Pup	3.37	0.84	664 (617-718)	x	101	88
11	Pup	1.32	67.22	x	5224 (4692-5755)	93	91
16	Pup	75.23	66.30	4657 (4076-5238)	4955 (3813-6096)	89	86
18	Pup	2.47	18.91	x	313 (236-391)	87	94
19	Pup	14.79	22.93	1850 (1537-2162)	1458 (1314-1601)	123	92
20	Pup	1.39	0.78	x	x	85	94
	Gam	x	0.74	x	x	x	111
	Shiner	7.84	1.44	x	x	x	x
21	Pup <sup>a</sup>	x	14.40	x	2721 (2275-3168)	x	101
22	Pup	36.06	48.26	x	991 (836-1146)	99	92

<sup>a</sup> Not captured in 2006.

Table 1 (continued)

Sinkhole	Species	CPUE (fish/trap/day)		Population Estimate		Avg. Relative Weight (%)	
		2006	2007	2006	2007	2006	2007
24	Pup	131.43	70.85	2462 (2235-2689)	1502 (1397-1608)	94	98
26	Pup	47.21	25.31	4871 (4321-5420)	2237 (2054-2421)	88	97
27N	Gam	x	185.61	x	x	x	91
27S	Pup	0.64	1.05	x	77 (69-86)	86	88
	Gam	x	151.75	x	x	x	80
	Killi	2.09	2.66	x	x	x	x
28	Pup	27.58	16.78	1458 (1168-1748)	826 (661-991)	98	89
29	Pup	71.02	26.22	4677 (3848-5507)	1278 (1103-1453)	93	86
32	Pup	0.14	0.12	x	x	92	x
	Gam	x	0.30	x	x	x	x
	Killi <sup>a</sup>	x	0.12	x	x	x	x
LSF	Pup	0.43	1.93	329 (36-622)	315 (299-333)	79	82
	Gam	x	125.57	x	x	x	88
	Killi	0.45	1.41	x	x	x	x
38	Pup <sup>b</sup>	1.11	0.00	x	x	90	x
	Killi <sup>a</sup>	x	4.92	x	x	x	x

<sup>a</sup> Not captured in 2006.

<sup>b</sup> Only one juvenile was captured in 2007

### *Body Condition*

In 2006, the standard weight equation for pupfish was:

$$\log(W_s) = 3.3599 * \log(\text{Length}) - 4.9423$$

where  $W_s$  = the length-specific standard weight and  $\text{Length}$  = standard length in mm.

In 2007, this equation was:

$$\log(W_s) = 3.0749 * \log(\text{Length}) - 4.5104$$

The standard weight equation developed for gambusia in 2007 was:

$$\log(W_s) = 3.0259 * \log(\text{Length}) - 4.6561$$

The equations were not length-biased. With the exception of a few sinkholes, only weak correlations existed between length and relative weight (Appendix A).

In 2006, pupfish relative weight was higher in the Pupfish-only Group than the Multi-species Group (Wilcoxon rank-sum test,  $p = 0.0242$ , Fig. 4a). Results are still significant if the sinkhole with the highest relative weight is removed. In 2007, there was no difference in relative weight (t-test,  $p = 0.7737$ , Fig. 4b). There was no difference in relative weight of pupfish and gambusia where they co-occurred (t-test,  $p = 0.6919$ , Fig 4b). For the Pupfish-only Group, a significant difference in relative weight between years was not present (Wilcoxon rank-sum test,  $p = 0.2689$ , Fig. 5a). The same was true for the Multi-species Group (t-test,  $p = 0.1936$ , Fig. 5b), although relative weight increased on a per-sinkhole basis. Table 1 gives exact average relative weights for each sinkhole.

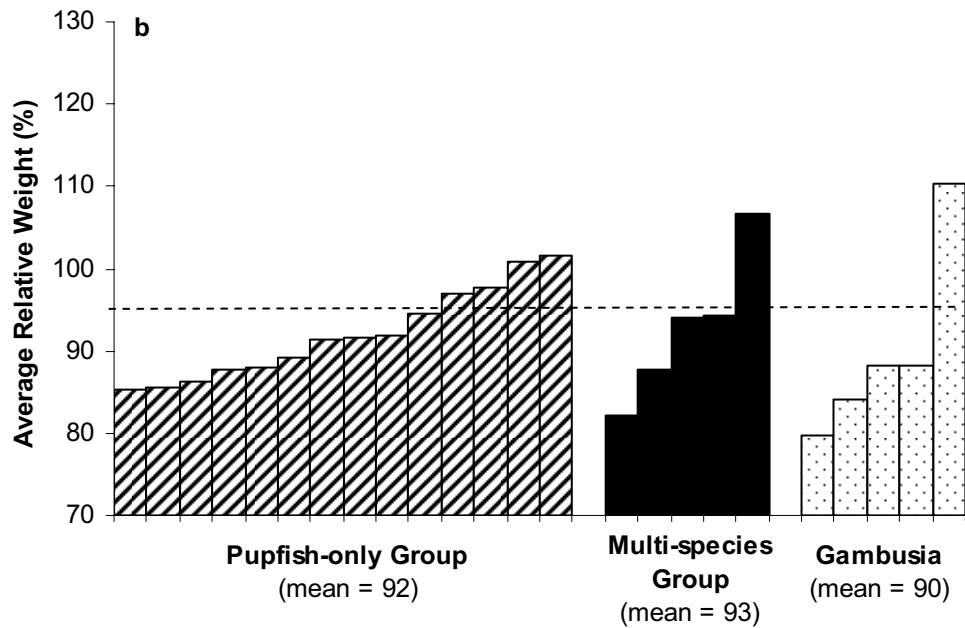
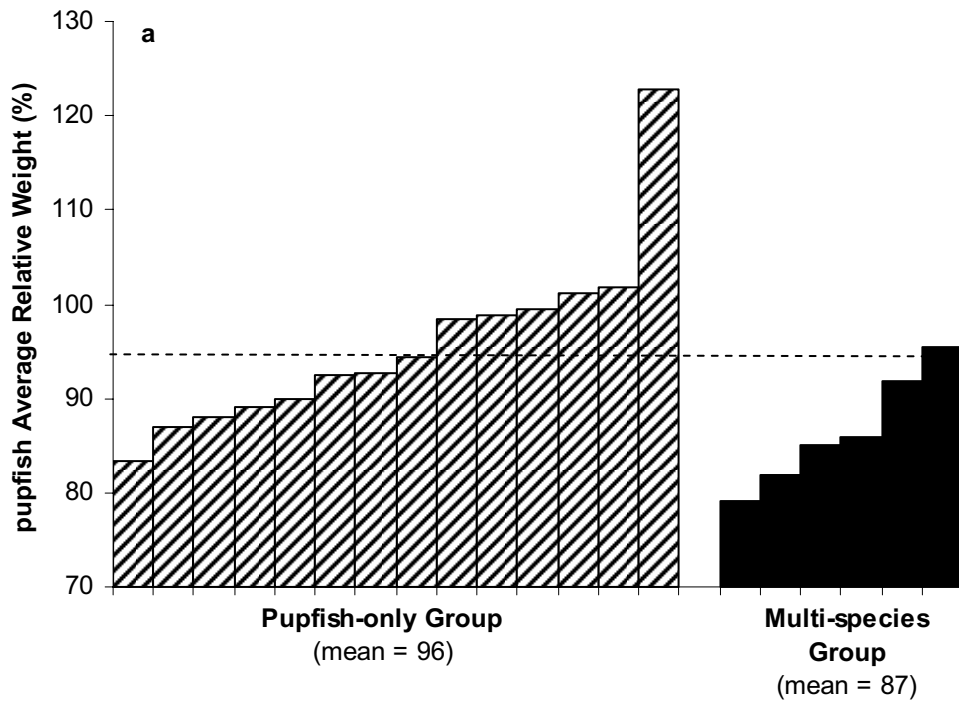
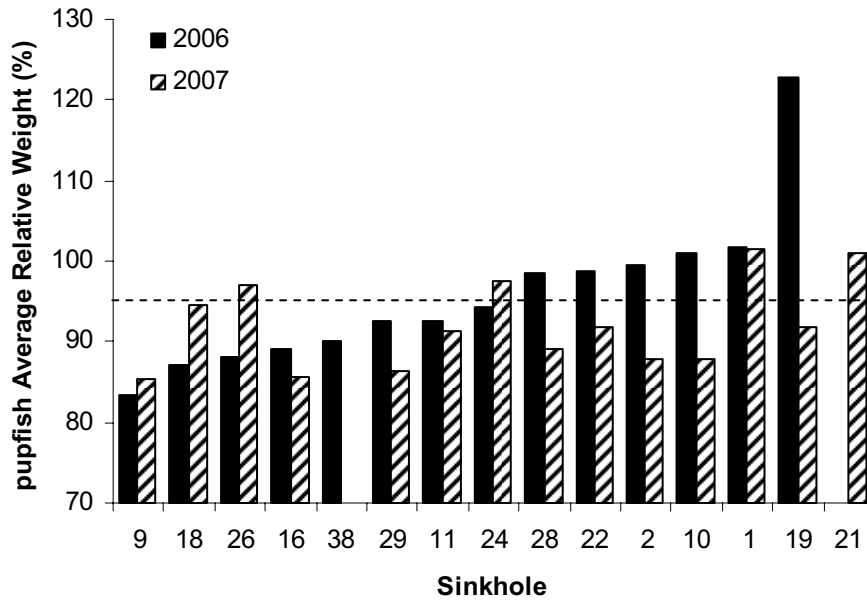


Figure 4. Pecos pupfish relative weight for 2006 (a) and 2007 (b), with data included for Pecos gambusia in 2007. Each bar represents a sinkhole. Values above the dashed line indicate fish in good condition.

**a. Pupfish-only Group**



**b. Multi-species Group**

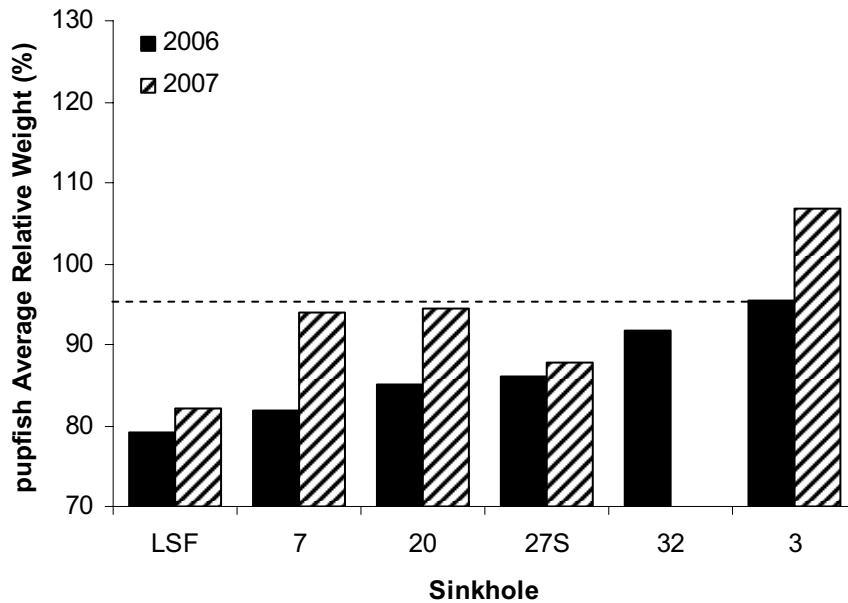


Figure 5. Yearly comparisons of Pecos pupfish relative weight for the Pupfish-only Group (a) and the Multi-species Group (b). Values above the dashed line indicate fish in good condition.

*Environmental Factors – Abiotic and Biotic*

Appendix B lists average values of the environmental variables for each sinkhole. Calcium carbonate was so highly correlated with salinity ( $r = 0.92$ ) that it was not measured in 2007. Total phosphorus resolution was too poor to be useful for the regression analyses and also was not measured in 2007 (we used total phosphorus data collected by W. Boeing from 2005 but it was ultimately not significant in any regression models). Zooplankton and benthic samples are still being processed and thus were not part of the analysis. Averaged from all forty-one sinkholes, there were significant increases from 2006 to 2007 for total depth ( $p = 0.0471$ ) and turbidity ( $p = 0.0005$ ) and chlorophyll a was nearly significant ( $p = 0.0531$ ). Salinity significantly decreased from 2006 to 2007 ( $p = 0.0220$ ; all tests were Wilcoxon rank-sum tests). Between the two groups of sinkholes, salinity was significantly higher in the Pupfish-only Group (Wilcoxon rank-sum test,  $p = 0.0101$ ) and oxygen was significantly lower (t-test,  $p = 0.0311$ ) in 2006. In 2007, salinity was again significantly higher in the Pupfish-only Group (Wilcoxon rank-sum test,  $p = 0.0013$ ), as was temperature (t-test,  $p = 0.0052$ ) and chlorophyll a (Wilcoxon rank-sum test,  $p = 0.0285$ ). Of note was sinkhole 21: in 2006 this sinkhole had almost no dissolved oxygen and a salinity of 122 ppt, nearly four times saltier than the ocean. Yet this sinkhole contained pupfish. Although no pupfish were captured in the traps, we observed a few swimming at the surface, likely obtaining oxygen from the air. We were able to catch one fish with a small dip net and found it to be emaciated. In 2007, salinity had decreased to 87 ppt and we estimated the pupfish population to be at least 2500.

### *Abundance and Body Condition vs. Environmental Factors*

Figures 6-9 show the results of the fourteen regression analyses. Figure 6 illustrates the results for CPUE in 2006. When all sinkholes were included, the negative effect of other species on pupfish abundance was confirmed (Fig. 6a). In the Pupfish-only Group, CPUE was associated positively with oxygen and chlorophyll a and negatively with total depth (Fig. 6b). In the Multi-species Group, abundance was positively associated with temperature and negatively associated with chlorophyll a, but this model was not significant (Fig. 6c). Results were different for pupfish abundance in 2007. The effect of other species on pupfish abundance was still apparent (Fig. 7a). However, in both groups of sinkholes, CPUE was associated with temperature: positively in the Pupfish-only Group (Fig. 7b; note that the model was not significant), but negatively in the Multi-species Group (Fig. 7c). Temperature was highly correlated with total depth ( $r = -0.86$ ) in the Multi-species Group. *Gambusia* abundance was related positively to salinity and negatively to oxygen and chlorophyll a, but the model was not significant (Fig. 7d).

Figure 8 shows the results for relative weight in 2006. When all sinkholes were included, the negative effect of other species on pupfish relative weight was recognized (Fig. 8a), yet it appeared to be driven by the same high relative weight as in Figure 4a. If this sinkhole is removed then pupfish relative weight was negatively associated with total depth (Fig. 8b). Again, this model appeared to be driven by a single point. If this sinkhole is also removed, then pupfish relative weight was once

again negatively associated with the presence of other species (not shown). There was a positive relationship between relative weight and temperature in the Pupfish-only Group, but the relationship is poor and may be due to a single observation (Fig. 8c). If this sinkhole is removed then no factors correlate with relative weight and this may be a more appropriate conclusion. In the Multi-species Group relative weight was positively associated with salinity and chlorophyll a (Fig. 8d). In 2007, the presence of other species did affect pupfish relative weight when all sinkholes were analyzed together, as did temperature (Fig. 9a). In this case, species presence is positively related to relative weight, yet there was no difference in relative weight among the two groups of sinkholes (Figure 4b) and Fig. 9a shows little difference between the two groups. When sinkholes are split into their respective groups both are positively associated with temperature (Figs. 9b and 9c). In the Pupfish-only Group, temperature was highly correlated with salinity ( $r = 0.87$ ). The strength of this model appears to be influenced by one observation, as does the correlation with salinity. When this sinkhole is removed the best model is still one with temperature as a predictor but the p-value becomes much larger ( $p = 0.3985$ ) and there is no longer a strong correlation with salinity. Again, it may be more appropriate to conclude that no variables explain relative weight for this group. In the Multi-species Group temperature was highly correlated with total depth ( $r = -0.85$ ). *Gambusia* relative weight was negatively associated with total depth and salinity (Fig. 9d) but the model was not significant. Results are summarized in Table 2.

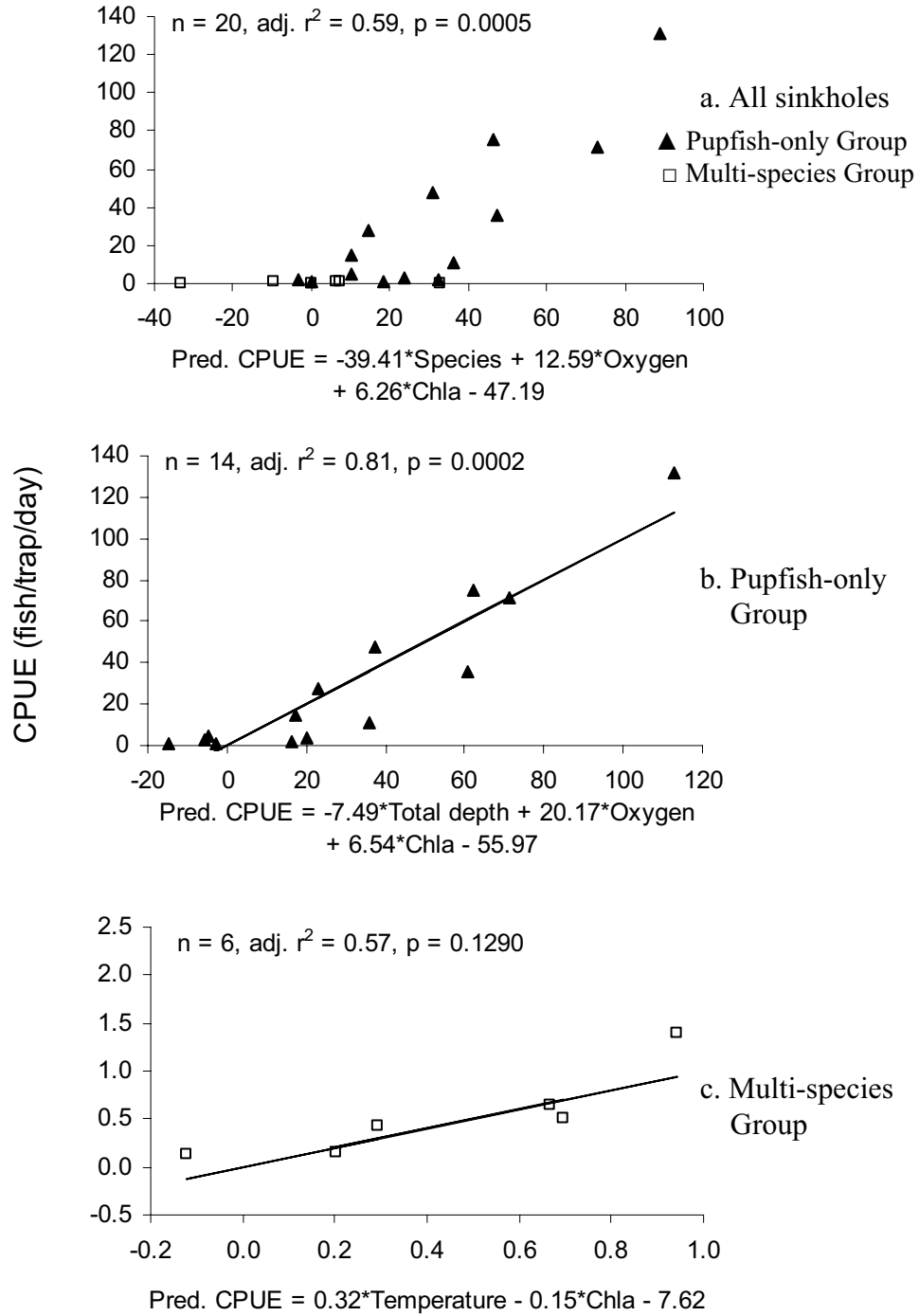


Figure 6. Predicted regression models for pupfish CPUE, 2006. Where there are two or more predictors, the x axis becomes a combination of the predictors so that results can be displayed graphically.

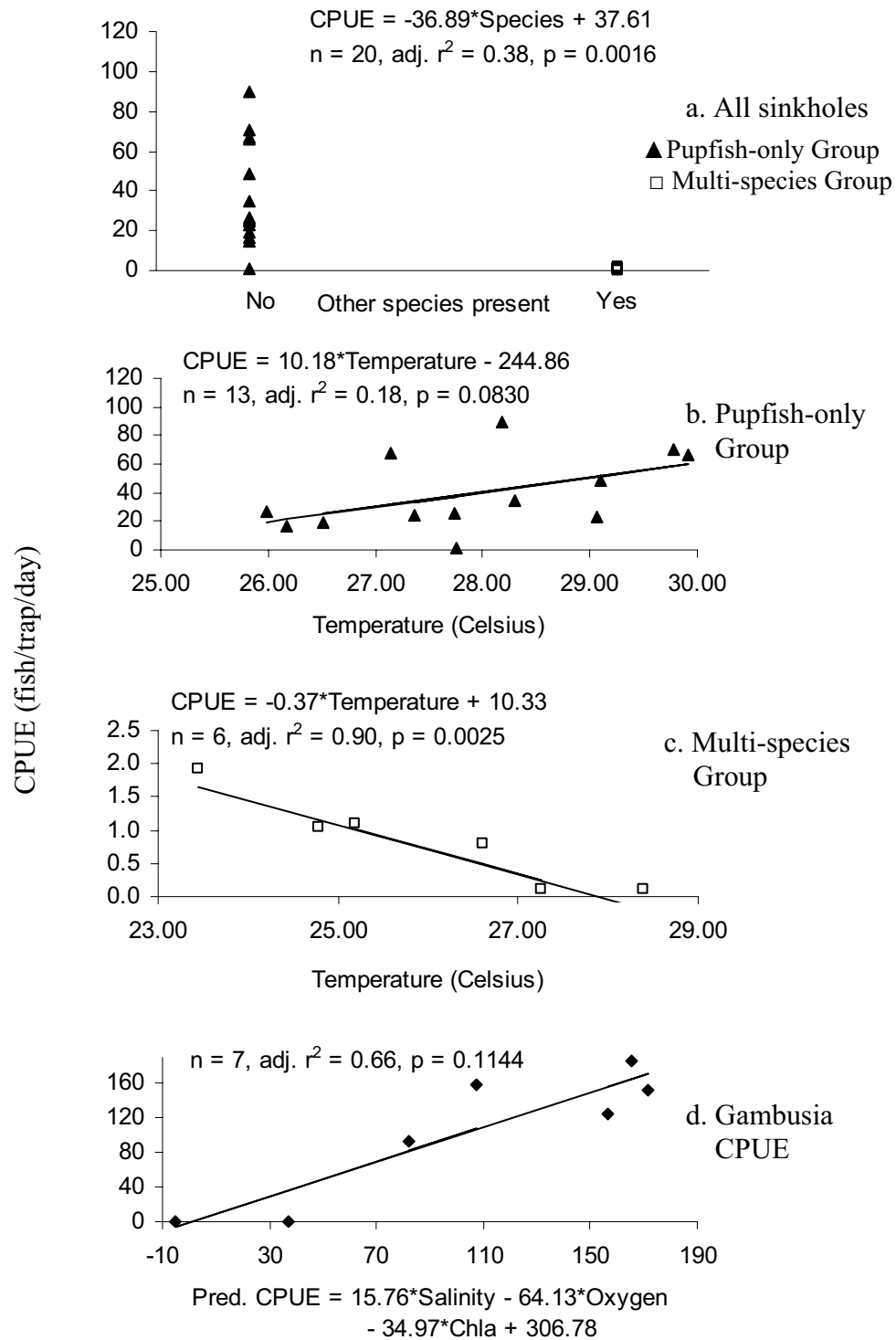


Figure 7. Predicted regression models for pupfish CPUE, 2007. Where there are two or more predictors, the x axis becomes a combination of the predictors so that results can be displayed graphically. Gambusia CPUE is also included.

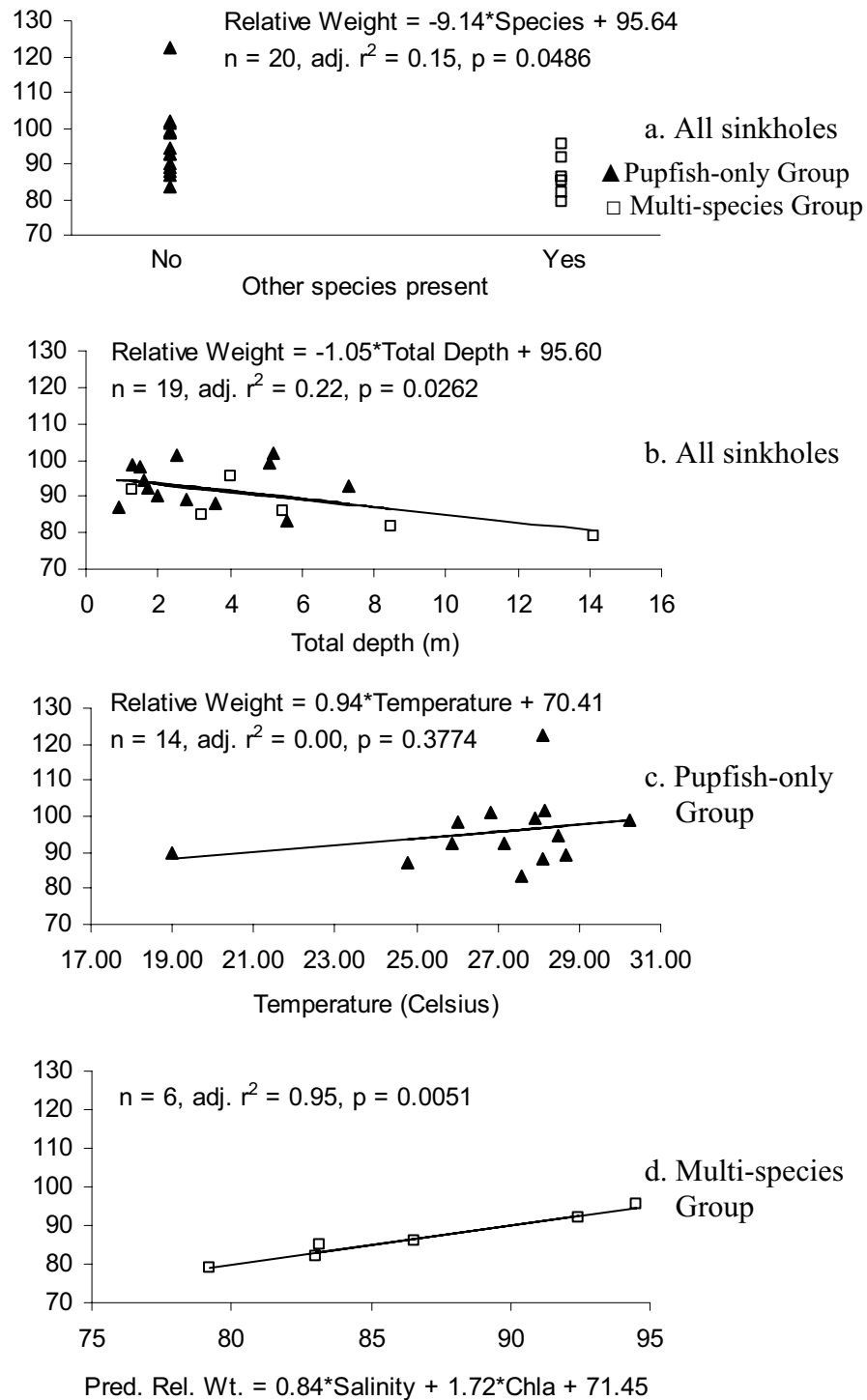


Figure 8. Predicted regression models for pupfish relative weight, 2006. Where there are two or more predictors, the x axis becomes a combination of the predictors so that results can be displayed graphically.

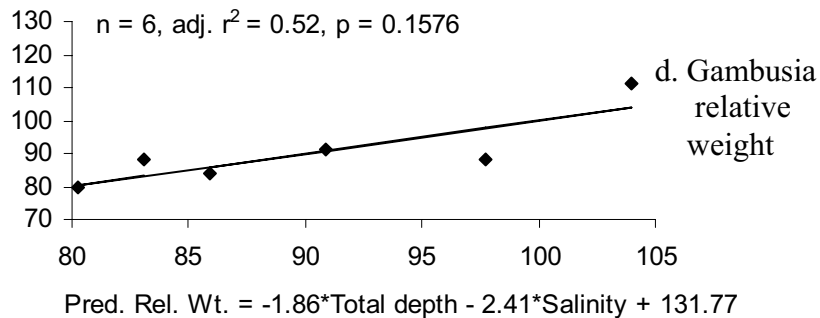
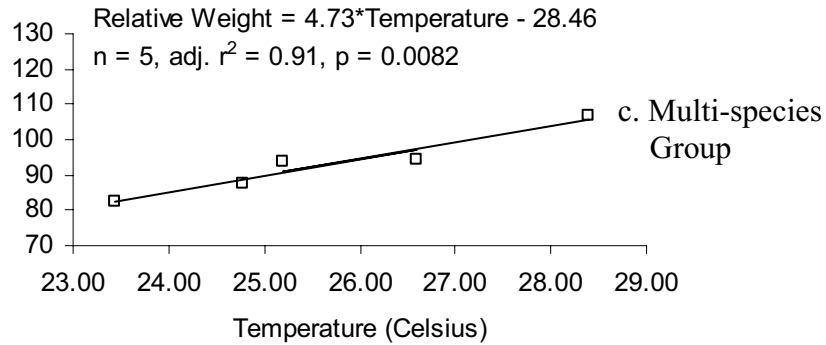
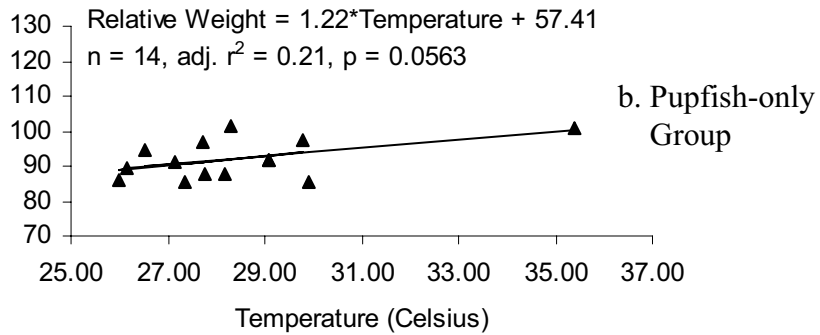
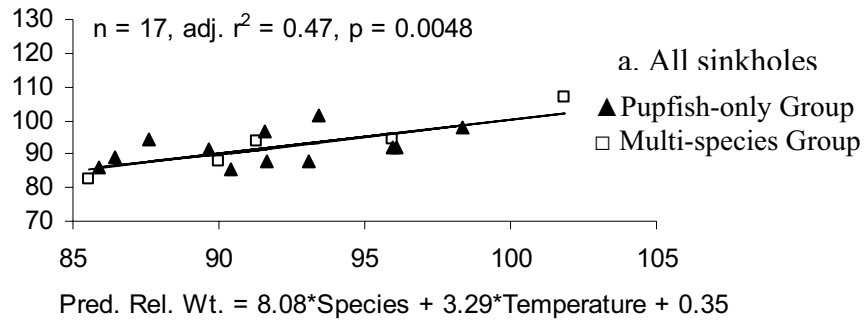


Figure 9. Predicted regression models for pupfish relative weight, 2007. Where there are two or more predictors, the x axis becomes a combination of the predictors so that results can be displayed graphically. Gambusia relative weight is also included.

Table 2. Summary of regression results.

	CPUE		Relative Weight	
	2006	2007	2006	2007
All sinkholes	Species (-) Oxygen (+) Chl a (+)	Species (-)	Species (-) and/or Total depth (-)	Species (+) Temperature (+)
Pupfish-only Group	Total depth (-) Oxygen (+) Chl a (+)	Temperature (+)	Temperature (+)	Temperature (+)
Multi-species Group	Temperature (+) Chl a (-)	Temperature (-)	Salinity (+) Chl a (+)	Temperature (+)
Gambusia	No data	Salinity (+) Oxygen (-) Chl a (-)	No data	Total depth (-) Salinity (-)

*Diet*

Table 3 provides the results from the cluster analysis. Pupfish from all sinkholes had large amounts of detritus and diatoms in their gut. Cluster 1, which contained sinkholes 16, 19, 22, 24, and 26, was comprised of pupfish that had larger amounts of detritus and algae in their gut. Cluster 2, which contained sinkholes 1, 2, 9, 10, 11, 20, 28, and 29, included pupfish that had larger amounts of diatoms and dinoflagellates in their gut. Lake St. Francis (LSF) stood out as a separate cluster because pupfish had larger amounts of gypsum and pollen in their gut. Significant

Table 3. Results from the cluster analysis on food items in the guts of Pecos pupfish. P-values are from tests comparing means of food items from Clusters 1 and 2.

<b>Gut Item</b>	<b>Cluster 1</b>		<b>Cluster 2</b>		<b>Cluster 3</b>		<b>P-Value</b>
	<b>Mean Rank</b>	<b>Std. Dev</b>	<b>Mean Rank</b>	<b>Std. Dev</b>	<b>Mean Rank</b>	<b>Std. Dev</b>	
			Sinkholes 1, 2, 9, 10, 11, 20, 28, 29		Sinkhole: Lake St. Francis		
Detritus	1.0	0.00	2.0	0.53	1.0	x	0.0047
Diatoms	2.4	0.55	1.3	0.46	2.0	x	0.0124
Green algae	2.8	0.84	4.9	1.73	9.0	x	0.0202
Dinoflagellates	7.9	1.08	3.8	0.89	4.5	x	0.0008
Insects	6.4	2.68	7.7	1.25	6.0	x	0.5773
Copepods	4.8	0.84	4.3	1.75	9.0	x	0.7133
Gypsum	7.5	1.97	8.1	0.94	4.5	x	1.0000
Fish scales	5.9	1.52	7.1	1.27	7.0	x	0.1880
Pollen	8.4	0.22	8.4	1.13	3.0	x	0.7941
Woody plant material	7.9	1.08	7.6	2.50	9.0	x	0.6301

differences were present between clusters 1 and 2 for detritus, diatoms, algae, and dinoflagellates.

Additionally, fish from Sinkhole 20 and LSF had parasites. All five fish within each sinkhole were infected with numerous cysts embedded in the liver.

## **Discussion**

### *Influence of Species Presence on Abundance*

Pecos pupfish abundance was primarily influenced by biotic factors. That pupfish abundance was considerably affected by the presence of other species was surprising, as all species are native to the Pecos River and presumably sinkholes on BLNWR and have possibly coexisted for thousands of years. We would have expected abundance to be somewhat higher in sinkholes where no other species were present since pupfish could exploit all available habitats (Deacon and Minckley 1974). But where pupfish occurred with other species, abundance was one or more orders of magnitude lower. As an example, sinkholes 2 and 3 were of similar size, had comparable environmental conditions, and were set with the same number of traps. Sinkhole 2 had just pupfish and we captured over 2000 individuals. Sinkhole 3 also contained gambusia and we captured only three pupfish. Clearly, interspecific interactions must be playing a role in pupfish abundance. The lack of juvenile pupfish captured in sinkholes with multiple species was also troubling.

We suggest that Pecos gambusia was the principal species affecting pupfish abundance, as it most commonly occurred with pupfish and with the exception of two sinkholes was present in much higher numbers (plains killifish will be discussed subsequently). Both predation and competition should be considered as possibilities. While we have not found any information suggesting that Pecos gambusia exhibits piscivory, another species of gambusia, *Gambusia affinis*, is a known predator in systems in which it is introduced (Meffe 1985). Predation could also help to explain why almost no juvenile pupfish were captured in the Multi-species Group.

Although the specific mechanisms were not investigated, we will attempt to narrow down possible sources of competition. Species tend to separate along three dimensions: food, temporal, and habitat (Schoener 1974). Species that overlap in habitat tend to eat different foods or may eat at different times of the day. Both vertical and horizontal habitat segregation can occur. Overall, habitat is more important than food type, which is more important than temporal segregation (Schoener 1974), which has also been shown for fish (Werner et al. 1977).

Pupfish and gambusia do not appear to be competing for food. We found that Pecos pupfish diet consisted primarily of detritus and diatoms and the same result was found by Davis (1981) in Pecos pupfish collected from the Pecos River. The presence of items such as gypsum and sand indicate that pupfish are bottom foragers (Davis 1981 and Table 3). In contrast, Pecos gambusia is a carnivorous surface feeder, primarily ingesting dipterans and hemipterans; feeding occurs mostly at night (Bednarz 1979). Both species have dietary flexibility (Davis 1981, U.S. Fish and

Wildlife Service 1983) and therefore food should not be a source of competition. It is not known if there are ontogenetic shifts in diet.

The reproductive behaviors of Pecos pupfish and Pecos gambusia are substantially different. Pupfish have a territorial breeding system, in which males establish and defend territories that function as sites for courtship, mating, and oviposition (Kodric-Brown 1977, 1978). Females primarily oviposit on bare rock rather than algae or sand (Kodric-Brown 1977). By contrast, Pecos gambusia males use gonopodial thrusting, which does not entail courtship. Dominance hierarchies and territoriality are not present (Farr 1989). As livebearers, gambusia females do not need substrate on which to release eggs. The reproductive period apparently overlaps. Pecos pupfish reproduction peaks in late June/mid July (Kodric-Brown 1977). Bednarz (1979) collected gravid Pecos gambusia in late July from Blue Spring, Eddy County, NM. Although reproduction may coincide, it appears unlikely that pupfish and gambusia are competing for reproductive space.

Pupfish reproduction has been shown to be affected by plains killifish. In a study by Kodric-Brown and Mazzolini (1992), the pupfish breeding season coincided with the killifish's and they spawned on similar substrate. Where killifish were numerically dominant and physically larger, male pupfish spent a considerable amount of time chasing killifish and frequently abandoned their territories. This resulted in lower reproductive success and pupfish eggs could have been preyed upon. Low pupfish density also meant that more males were able to establish breeding territories, which reduced intraspecific competition and lowered

reproductive success. Similar results have been observed for desert pupfish (*Cyprinodon macularius*) defending territories from tilapia (see Schoenherr 1981). In the current study, killifish had abundances similar to pupfish (Table 1). This suggests that the role of killifish in disturbing pupfish reproduction is limited, since there were not large numbers of killifish to chase. Also, sinkholes 3 and 20 did not contain killifish but pupfish abundance was still low, so some other factor must be at work in these two sinkholes. (*Gambusia* was present in these sinkholes and red shiner was also in Sinkhole 20.) Furthermore, Echelle et al. (1972) suggested that Red River pupfish (*Cyprinodon rubrofluviatilis*) is a better competitor than plains killifish.

Pecos gambusia may perform a similar role though. Both Pecos pupfish and Pecos gambusia inhabit the nearshore (personal observation and Bednarz (1979) for gambusia). *Gambusia* species typically inhabit shallow areas (Meffe and Snelson 1989) and Pecos pupfish spawn in water less than two meters deep (Kodric-Brown 1978). Although their body shape and morphology suggest they primarily reside near the surface, we captured a large percentage (up to fifty percent) of gambusia in bottom traps. If gambusia are spending a large amount of time near the substrate and occur in such high densities then they may be interfering with pupfish reproduction if male pupfish are spending time chasing individuals from their territories. This could partly explain the low number of juvenile pupfish captured and low body condition observed for adult pupfish (see “Influence of Species Presence on Body Condition”). But what would cause gambusia to shift to greater depths? A response to oxygen is unlikely since it was most abundant at the surface. In the lab, Gehlbach et al. (1978)

found that when kept separate, both Pecos gambusia and Comanche Springs pupfish (*Cyprinodon elegans*) preferred temperatures of 26-30°C. When placed together, pupfish shifted to temperatures between 21 and 25°C and gambusia stayed in the 26-30°C zone. Therefore, it seems unlikely that gambusia would move deeper to find cooler temperatures, especially since surface temperatures ranged from 26.69-29.30°C. Gambusia could be reacting to intense solar radiation though. Deacon and Minckley (1974) documented fishes with sloughing tissue on dorsolateral surfaces and damaged caudal and dorsal fins, which they attributed to sunburn. This could be highly likely in clear water and all of the sinkholes in the Multi-species Group were clear (Appendix B). It is possible that even a temporary shift of gambusia to greater depths could be sufficient to disrupt pupfish reproduction.

The physical nature of the sinkholes may contribute to species interactions. Compared to lakes in general, sinkholes were small and shallow. The aquatic macrophyte community was simple and overhanging vegetation was minimal (the occasional tamarisk tree notwithstanding). As such, fish habitat could be considered homogeneous. Small, simple lakes increase the likelihood of contact between species because patterns of habitat use overlap (Tonn and Paszkowski 1987). While pupfish and gambusia seem to have different ecological requirements, it is something to keep in mind.

Obviously, pupfish-gambusia-killifish interactions and pupfish-gambusia-shiner interactions should be given further consideration. Without observational or experimental data, however, the contribution of each species is difficult to tease apart.

Red shiner could quite possibly influence abundance because both pupfish and gambusia abundances were low in the sinkhole in which shiner was present. While red shiner are omnivorous, they are not known to be piscivorous (Sublette et al. 1990).

#### *Influence of Species Presence on Body Condition*

Pupfish body condition was negatively associated with the presence of other species in 2006. This could be due to males chasing individuals from territories, which would use sizeable amounts of energy and could correspond to lower body condition. Alternatively, if gambusia does prey upon pupfish, then pupfish could also be spending energy attempting to hide from gambusia. Interestingly, pupfish body condition was positively associated with the presence of other species in 2007, even though there were no significant differences in condition between the two groups of sinkholes and pupfish condition did not differ from gambusia condition. Since pupfish relative weight was also positively associated with temperature (Fig. 9a), perhaps there was an interaction of species presence and temperature.

#### *Additional Environmental Influences on Abundance and Body Condition*

*Temperature* – Pupfish abundance was positively associated with temperature in the Pupfish-only Group in 2007 and the Multi-species Group in 2006. A preference for warmer temperatures has been observed for pupfish (Heath et al. 1993, Rogowski et al. 2006), although it has also been suggested that optimum temperatures

exist, particularly for reproduction (Gerking et al. 1979). In 2007, the range of average temperatures in the Pupfish-only Group was 25.99-29.92°C (Sinkhole 21, which had temperatures as high as 39.5°C was deemed an outlier and was not part of the regression analysis). This range can be expanded to 25.50-33.40°C when values are not averaged, as temperatures tended to be warmer at the surface and cooler with depth. In the Multi-species Group, the range of average temperatures in 2006 was 24.73-27.99°C and can be expanded to 21.22-29.19°C when values are not averaged. Within a sinkhole, pupfish could have inhabited cooler or warmer temperatures than the average temperature. In 2007, pupfish abundance in the Multi-species Group was negatively associated with temperature. This result was unexpected: since sinkholes within the Pupfish-only Group had higher temperatures and more fish, one would expect abundance to increase with temperature. Again, the average temperatures given for each sinkhole do not take into account changes in temperature that occur with depth. Neither of the models in which temperature was positively associated with temperature were statistically significant. Ultimately, temperature may not influence abundance.

Temperature was positively associated with pupfish relative weight in the Pupfish-only Group in both 2006 and 2007 and the Multi-species Group in 2007. Growth increases with increasing temperature (Moyle and Cech 2004) and higher relative weight may indicate more growth. However, in the Pupfish-only Group both models were poor. This suggests that relative weight was not related to any environmental variables for this group.

*Chlorophyll a* — In the Pupfish-only Group in 2006, pupfish abundance was positively associated with chlorophyll a. In this group, quantities ranged from 0-15.13 mg/m<sup>3</sup>. Since chlorophyll a is an indicator of phytoplankton biomass, increases in chlorophyll a indicate greater productivity and suggest that more food (e.g., diatoms and detritus) is available. In the Multi-species Group, pupfish abundance was negatively associated with chlorophyll a in 2006. Quantities ranged from 0-5.34 mg/m<sup>3</sup>. The amount of chlorophyll a in this group implies that they are oligotrophic (Wetzel 2001) so it was surprising that abundance would increase; this also contradicts the results from the analysis of all sinkholes combined and from the Pupfish-only Group in which abundance increased with greater amounts of chlorophyll a (Figs. 6a and 6b). However, phytoplankton communities do exhibit seasonal patterns and successions (Wetzel 2001) and perhaps some sinkholes in the Multi-species Group were more productive earlier in the spring or summer prior to or during reproduction. It is also important to keep in mind that this model was not significant. Pecos gambusia abundance was also connected negatively to chlorophyll a, but again, the model was not significant.

Pupfish relative weight was positively associated with chlorophyll a in the Multi-species Group in 2006. Even though sinkholes within this group were considered to be oligotrophic and thus of low productivity, any increase in chlorophyll a could indicate greater productivity and more food production. A link between greater food availability and higher body condition is reasonable.

*Salinity* – For the Multi-species Group, pupfish relative weight was positively associated with salinity in 2006. Optimal growth and reproduction can occur in salinities as high as 35 ppt (Kinne 1960) and some amount of salinity may be necessary for pupfish survival (Gerking and Lee 1980). Since pupfish relative weight was higher in sinkholes with greater salinity in 2006 it is plausible that some amount of salinity is needed for greater growth and reproduction that is higher than the salinities in the Multi-species Group (range: 7.26 to 18.71 ppt) and this could explain why condition increases with salinity.

Salinity was positively associated with Pecos gambusia abundance and negatively with gambusia relative weight. Pecos gambusia does not tolerate the high salinities that Pecos pupfish does (Bednarz 1979) so an increase in abundance with salinity was not anticipated. It has been suggested that Pecos gambusia cannot tolerate salinities above 30 ppt (Echelle and Echelle 1980). The range of salinities in the Multi-species Group (6.95 to 16.42 ppt) was fairly narrow, so this effect may not be biologically meaningful. How salinity affects body condition and growth is not well understood: some poeciliid species grow more in higher salinities while for others the reverse is true (see Snelson 1989). Neither model regarding gambusia abundance and body condition was significant.

*Total depth and Oxygen* – In the Pupfish-only Group in 2006, pupfish abundance was positively associated with dissolved oxygen and negatively associated with total depth. Oxygen was also positively related to pupfish abundance when all sinkholes were analyzed together. Oxygen is essential to bodily functions like

metabolism (Moyle and Cech 2004) and increases in oxygen have been shown to increase the rate of embryo development in pupfish (Kinne and Kinne 1962), so this result is not unexpected. In general, deeper (>4 m) sinkholes were less turbid and had lower amounts of chlorophyll a, suggesting they were nutrient- or light-limited (Wetzel 2001). A potentially confounding factor is that deeper sinkholes also tended to be steeper-sided, meaning that the shoreline was not very shallow. Since pupfish spawn in fairly shallow water, this implies that pupfish have less area in which to spawn. Indeed, vertical substrate has been shown to be less-optimal for spawning (Kodric-Brown 1977). This seemed to be a plausible explanation for low pupfish abundance except that in 2007 the deeper sinkholes caught considerably more fish.

Pecos gambusia abundance was connected negatively to oxygen. A negative association with oxygen is unusual. However, this model was not significant and could not account for changes in oxygen levels with depth. Gambusia relative weight was negatively associated with total depth. Once more, deeper sinkholes tended to have less productivity, and this may have been reflected in lower body condition, but again, the model was not significant.

The effect of species presence on pupfish abundance was quite striking and it compelled me to divide sinkholes into two groups based on species presence. This resulted in a narrowing of the variation in environmental factors within the two groups. Sinkholes within the Pupfish-only Group had significantly higher temperatures and amounts of chlorophyll a in 2007 and higher salinities in both years than the Multi-species Group. Sinkholes within the Multi-species Group had

significantly more oxygen in 2006. This could cause one to conclude that pupfish have a preference for higher salinity, temperature, and chlorophyll a and lower amounts of oxygen but this would be misleading since it is not known how pupfish got into these sinkholes or how long they have been in them. Unfortunately, these factors are confounded with the presence of other species. If species presence had not been such an obvious factor then I could have analyzed all sinkholes together and environmental variation would have been much greater. I suggest that temperature, chlorophyll a, salinity, oxygen, and total depth may have ultimately not been useful indicators of abundance and body condition, especially since the influence of these factors differed between years and because the relationships (positive or negative) also varied. The non-significance of both models of gambusia abundance and relative weight provides supporting evidence.

Relative weight may have not been an appropriate condition index to use. Indeed, Blackwell et al. (2000) noted that published studies regarding relative weight and small fishes are few. Perhaps calculating an average relative weight per sinkhole was too simplistic and did not allow for length-related trends to be detected, but we did not notice any meaningful patterns (Appendix A). Obviously, this study occurred over a small period of time and condition may vary between seasons and years.

### *Effect of Parasites*

Parasites found on the liver of fish from Sinkhole 20 and LSF were likely trematode metacercaria. Fish serve as intermediate hosts for the parasite, whose life

cycle also involves fish-eating birds and snails as hosts (Hoffmann 1999). Rogowski and Stockwell (2006) found that trematode prevalence in White Sands pupfish increased with the presence of an endemic springsnail, which they presumed served as a host for the parasite. White Sands pupfish collected from sites that lacked snails also lacked trematodes. The presence of trematodes resulted in lower condition and lipid levels in pupfish and fewer older fish were collected, indicating a higher mortality rate (Rogowski and Stockwell 2006). Both Sinkhole 20 and LSF contained snails. Pupfish dissected from these two sinkholes had the lowest amounts of fat compared to other sinkholes in which fish were dissected. I think it is likely that the relationship observed between White Sands pupfish, springsnails, and trematodes is similar for Pecos pupfish where snails are also present. Parasites may have contributed to the low body condition seen in Sinkhole 20 and LSF (although relative weight was much higher for Sinkhole 20 in 2007). It is not known if parasites also infect Pecos gambusia, but this appears likely.

#### *Contributions to Multi-Lake Studies*

This research brought a new perspective for multi-lake studies by providing information about desert systems. Although this study would be considered to have a small taxonomic scale it had a large environmental scale and studies such as this one have not been common (Hinch 1991). Biotic interactions were so strong that sinkholes had to be grouped to account for multiple species. Even though abiotic variation was lessened by grouping sinkholes, it does not diminish the fact that

sinkholes had wide-ranging amounts of oxygen, salinity, etc. It was surprising that in a system with low species diversity, biotic interactions were strong. Our results agree with Quist et al.'s (2003) hypothesis that biotic processes dominate abiotic factors when competitor densities are high. However, due to the fact that different factors were significant between years we also emphasize that the importance of variables may be habitat-specific.

### *Management Implications*

The results presented here potentially have serious consequences for Pecos pupfish, a state-threatened species, and Pecos gambusia, a federally-endangered species. As is typical for many native fishes in the western United States, both species have declined due to habitat alteration and non-native species introductions. Pecos pupfish formerly occurred in the mainstem Pecos River from Roswell, NM to the mouth of Independence Creek, TX and in small tributaries, springs, and sinkholes (Echelle and Echelle 1978). The construction of several dams along the river has altered both the flow and thermal regimes. Spring snowmelt and rain events are attenuated, flooding is essentially non-existent, and river reaches are seasonally-desiccated (Propst 1999). Non-native sheepshead minnow (*Cyprinodon variegatus*), likely introduced in the early 1980s in Texas (Echelle and Connor 1989), hybridizes with Pecos pupfish (Echelle et al. 1987). Hybrids have rapidly moved upstream (Echelle and Connor 1989, Wilde and Echelle 1992, Echelle et al. 1997). The largest populations of Pecos pupfish are on BLNWR (Brooks and Wood 1988). Pecos

gambusia populations have primarily declined because of groundwater pumping that reduced or eliminated habitat and restricted gambusia to springs (U.S. Fish and Wildlife Service 1983). The species is now limited to four areas: BLNWR; Blue Spring, in southern NM; a series of springs near Toyahvale, TX; and Diamond Y Spring and Leon Creek near Fort Stockton, TX (Johnson and Hubbs 1989). Gambusia had been introduced into several sinkholes on BLNWR but most populations were extirpated; failure was attributed to high salinity (Bednarz 1979).

Undoubtedly, BLNWR serves as an important refuge for both Pecos pupfish and Pecos gambusia. Pecos pupfish numbers are fairly high when they are the only species present and they do occur in a number of sinkholes as well as nearby Bottomless Lakes State Park. While Pecos gambusia abundance is quite high they are limited to fewer sinkholes. Augmenting both species' populations would need to be done in a very careful manner. Until more observational or experimental data is available, we think it would be unwise to stock these species together. Fishless sinkholes that could be stocked with gambusia include sinkholes 4, 40, 42N, 42S, and 59. Pupfish could be introduced into sinkholes 5, 15, 17, and 25. However, I say this with great hesitation, since environmental conditions can vary greatly from year to year and because fish have previously been stocked into some of these sinkholes but have since been extirpated. The invertebrate communities within fishless sinkholes are of importance too, and the effects on them should be given consideration.

### *Limitations of This Study*

This study was conducted in the summer over two years. As such, seasonal variation could not be addressed and two years is not sufficient to encompass yearly differences. The fact that different factors influenced abundance and body condition between years suggests that either it is necessary to collect data for several years and over the course of a year or it may be that in desert systems conditions are so variable that finding predictable patterns is nearly impossible.

The results of some of these analyses may be limited by small sample sizes. We suggest that these results should be interpreted for general patterns but placing significance on them should be done with caution.

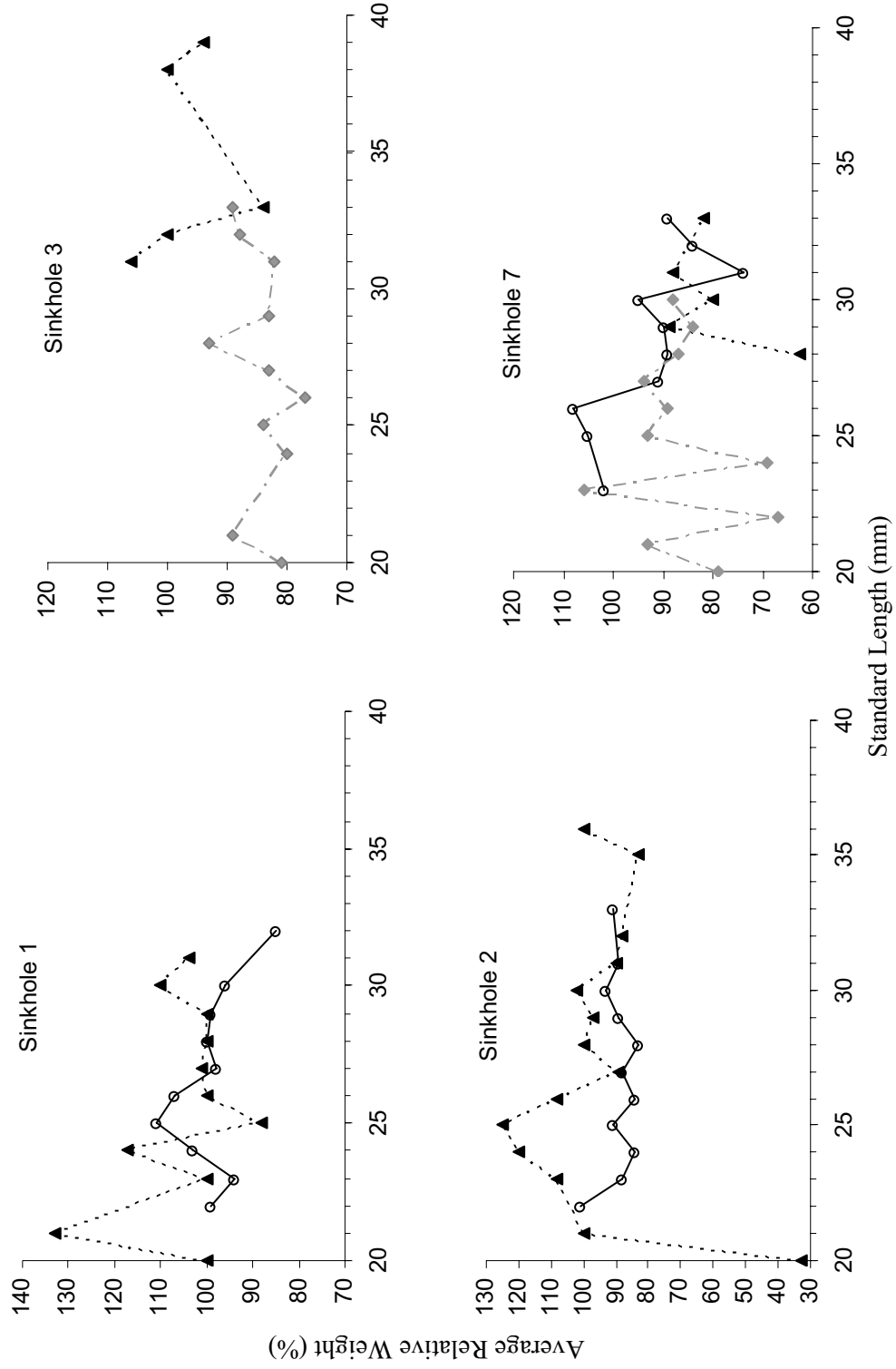
### *Future Research*

This study could benefit from future research in a couple of ways. First, direct observation (via snorkel surveys) could further illuminate interactions between pupfish and gambusia, as well as any role plains killifish and red shiner may play. Second, laboratory experiments could specifically evaluate where resources overlap or are limiting, allowing for the determination of the particular mechanisms of competition. Finally, examination of both juvenile and adult Pecos gambusia gut contents would provide a simple answer as to whether gambusia prey upon pupfish.

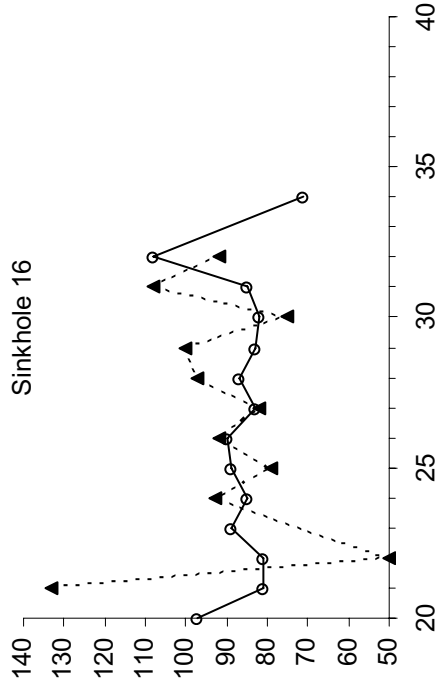
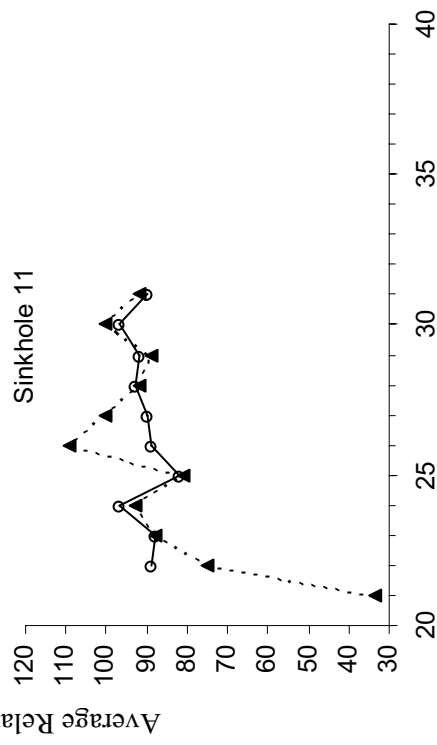
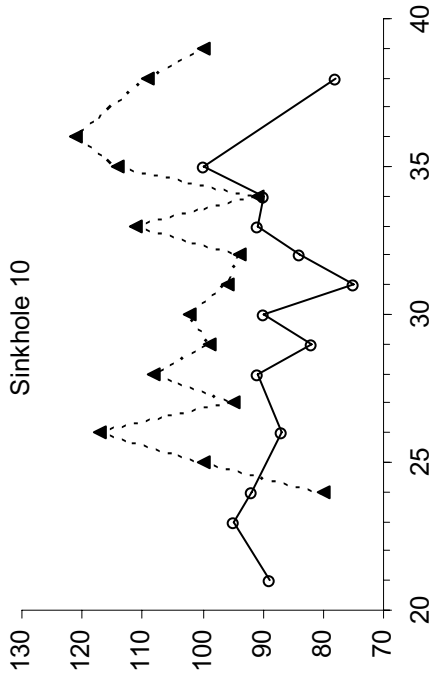
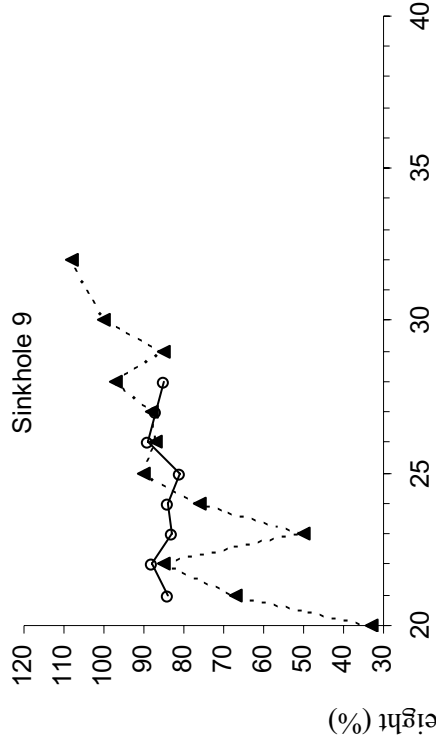
## *Conclusion*

It has been shown that biotic factors have an effect on pupfish abundance in a desert system, more so than abiotic factors. Biotic interactions were prominent enough that the survival of Pecos pupfish may seem uncertain where it coexists with other species. In small lakes, there can be great temporal variation in environmental factors like temperature and oxygen, such that periodic reductions in species abundance may occur (Jackson et al. 1992). Even though desert species can tolerate environmental extremes, conditions may occasionally go beyond what even they can withstand. Indeed, it has been suggested that poeciliid populations never reach carrying capacity because they are reduced by "seasonal or unpredictable abiotic events" (Meffe and Snelson 1989). The occasional reduction in gambusia populations could ultimately benefit pupfish where they co-occur, assuming pupfish are not similarly affected. That pupfish were seemingly on the verge of extirpation in a sinkhole where salinity was 122 ppt but then recovered the following year is a testament to how desert species can survive extremely harsh conditions and then thrive once conditions improve. It would be worthwhile to maintain monitoring programs for these species to see if indeed their populations fluctuate over time relative to one another. This could indicate that both biotic and abiotic factors affect abundance but that their importance varies on a periodic basis.

Appendix A. Trends in relative weight with respect to length for each sinkhole. ▲ = Pupfish 2006 ○ = Pupfish 2007  
 ◆ = Gambusia 2007

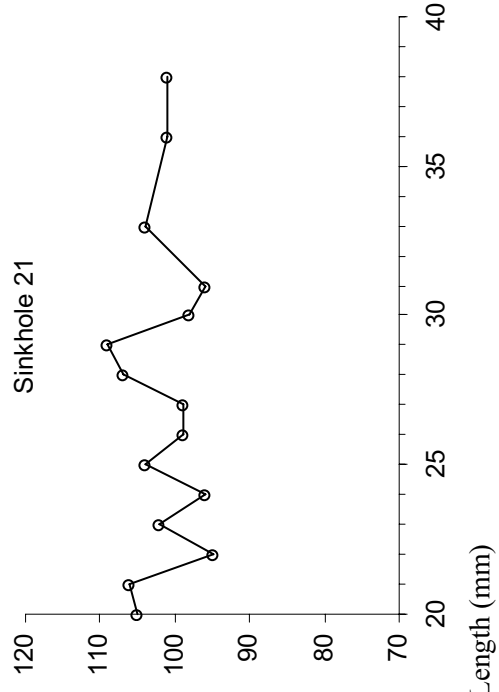
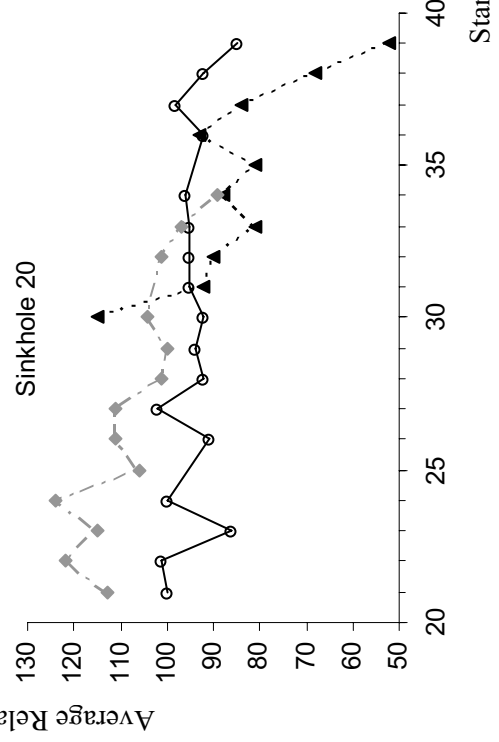
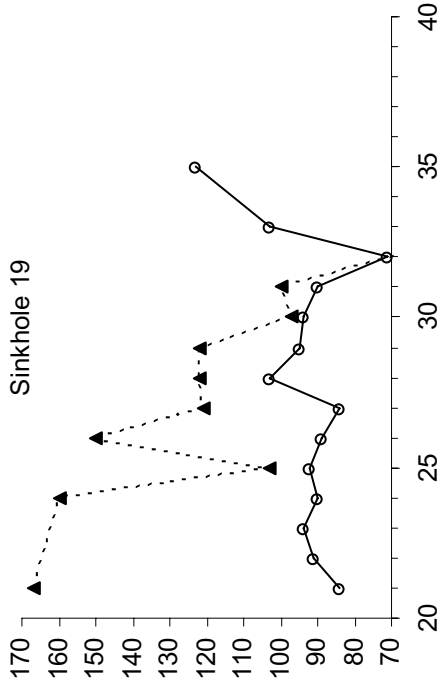
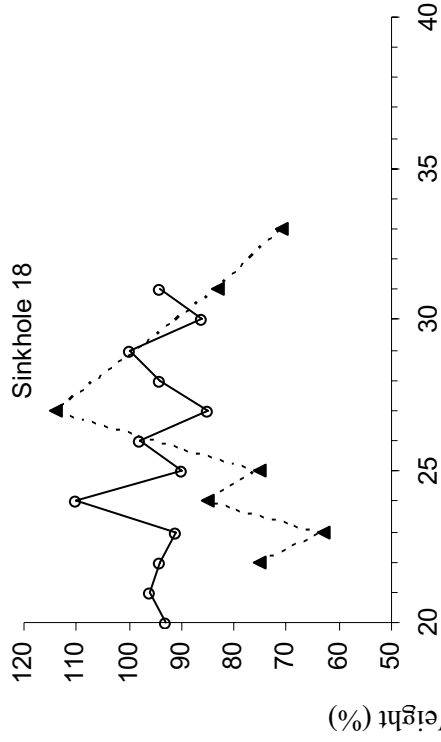


Appendix A (continued)

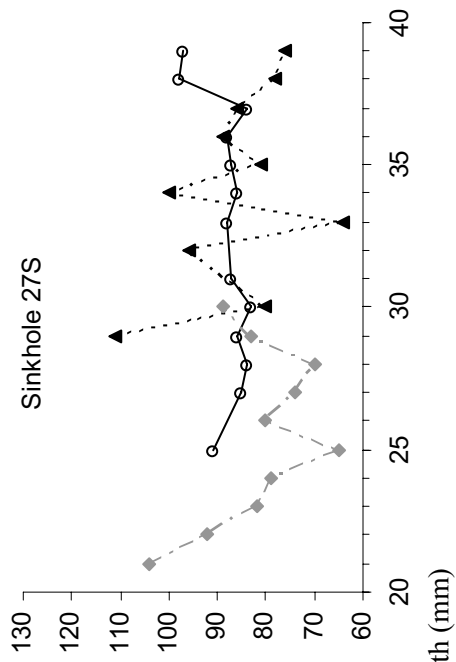
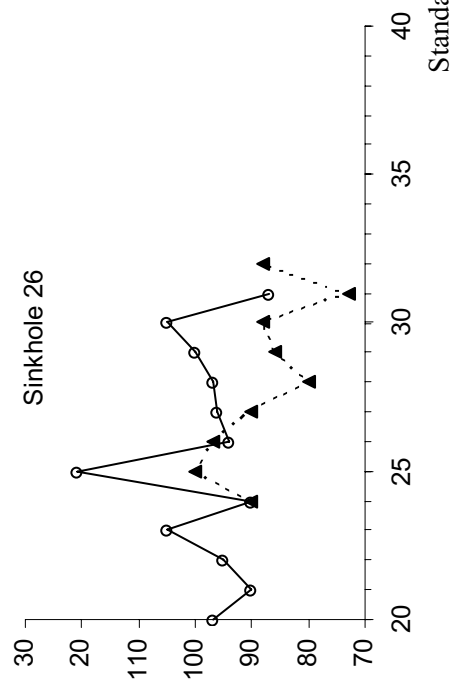
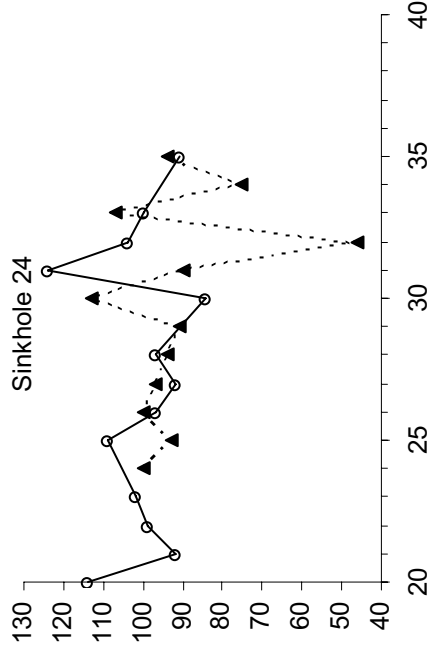
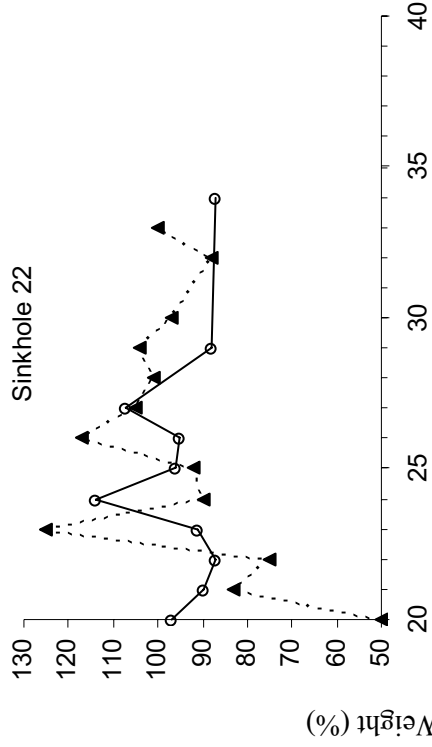


Standard Length (mm)

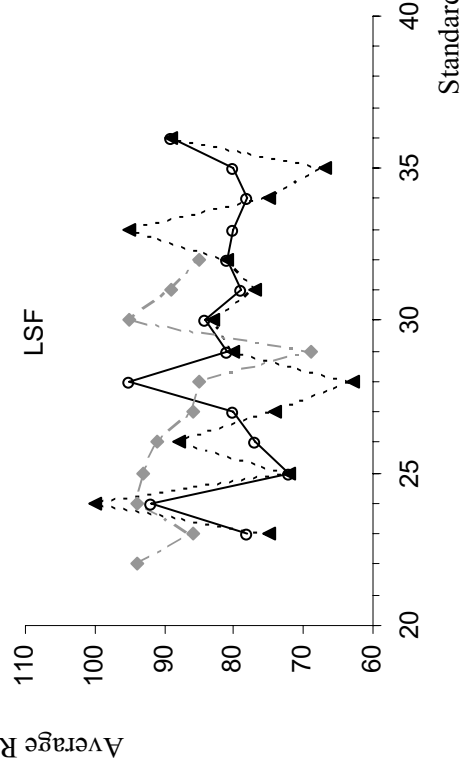
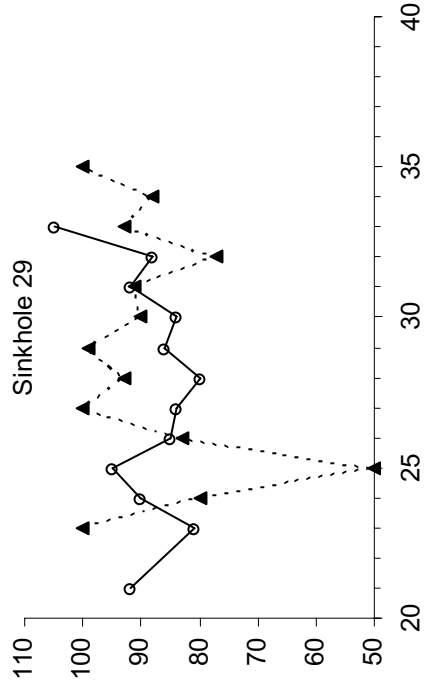
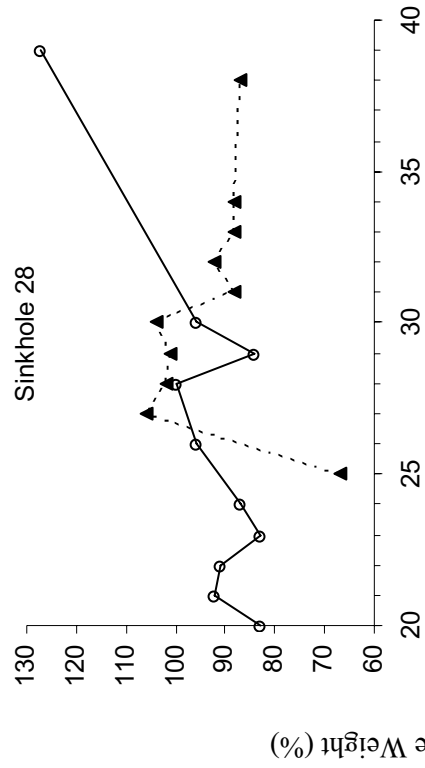
Appendix A (continued)



Appendix A (continued)



Appendix A (continued)



Appendix B. Environmental variables measured on sinkholes of Bitter Lake NWR, 2006 and 2007. Values are averaged from 1-meter depth interval measurements. An 'x' indicates the variable was not measured. An asterisk indicates a sinkhole that contains fish. Diameter was not measured in 2007.

Sinkhole	Total Depth (m)		Diameter (m)		Secchi Depth (m)		Temperature (°C)		Salinity (ppt)		Turbidity (NTU)	
	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007
1*	5.2	5.5	32.0	32.0	2.3	1.7	28.14	28.30	22.97	20.47	0.0	0.6
2*	5.1	5.6	28.5	28.5	3.0	2.3	27.91	28.18	16.80	15.05	0.0	0.2
3*	4.0	4.0	30.0	30.0	2.8	2.95	27.99	28.40	18.71	15.98	0.0	0.6
4	1.7	2	14.5	14.5	1.7	1.25	28.17	28.95	27.61	19.35	0.0	126.8
5	2.3	3	23.0	23.0	0.7	0.75	29.63	32.44	51.16	35.57	1.8	172.9
6	2.1	2.8	21.5	21.5	1.4	1.05	30.47	28.93	48.38	34.48	0.4	119.4
7*	8.5	9.3	41.0	41.0	3.8	2.25	25.95	25.19	7.26	6.95	0.2	0.8
8	0.8	1	10.0	10.0	0.4	0.8	28.20	28.35	67.23	27.78	12.6	10.4
9*	5.6	6.2	39.0	39.0	3.2	2.25	27.58	27.37	25.29	22.83	0.0	0.0
10*	2.5	2.9	17.0	17.0	1.3	1.1	26.81	27.75	17.41	14.60	0.0	0.6
11*	7.3	6.6	36.0	36.0	3.1	1.5	27.16	27.15	31.55	28.64	0.0	0.4
14	0.7	1.4	14.5	14.5	0.1	0.5	30.93	29.85	94.18	27.60	130.9	6.0
15	2.9	3.3	15.5	15.5	0.9	0.85	28.30	28.03	30.84	25.69	0.3	9.4
16*	2.8	3.4	17.0	17.0	1.1	1.3	28.68	29.92	49.44	36.49	0.6	45.0
17	2.0	3.2	15.0	15.0	0.7	0.3	27.91	28.26	90.28	53.38	8.7	19.6
18*	0.9	1.8	9.0	9.0	0.6	0.7	24.79	26.52	33.52	13.23	0.0	25.8
19*	2.5	3.3	32.5	32.5	1.8	1.85	28.12	29.07	36.00	25.41	0.0	6.4
20*	3.2	4.2	23.5	23.5	1.3	2.5	27.76	26.60	9.54	8.28	0.0	1.5
21*	3.6	4.2	41.0	41.0	0.3	0.6	26.93	35.37	121.60	86.62	29.2	44.9
22*	1.3	2	7.0	7.0	0.4	0.4	30.26	29.10	90.74	33.74	16.6	19.3
23	2.1	2.9	x	x	0.8	0.8	32.08	34.28	64.64	40.52	3.4	11.9
24*	1.6	2.6	11.0	11.0	0.5	0.75	28.50	29.78	42.88	25.91	15.1	4.9
25	1.4	2.3	20.0	20.0	1.4	1.7	29.31	26.42	24.61	16.75	0.0	2.7
26*	3.6	4	31.0	31.0	0.9	2.35	28.11	27.73	41.38	32.16	0.0	10.8
27N*	0.6	1.2	11.0	11.0	0.6	0.8	26.41	24.97	31.18	16.03	3.8	14.3

Appendix B (continued)

Sinkhole	Total Depth (m)		Diameter (m)		Secchi Depth (m)		Temperature (°C)		Salinity (ppt)		Turbidity (NTU)	
	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007
27S*	5.5	6.4	26.0	26.0	2.4	2.3	25.90	24.79	17.95	16.42	0.0	1.4
28*	1.5	2.2	10.5	10.5	1.1	0.9	26.03	26.17	33.13	25.07	0.0	5.9
29*	1.7	2.4	15.5	15.5	0.8	0.9	25.86	25.99	30.26	22.70	0.2	4.0
31*	0.9	1	19.0	19.0	0.9	0.95	24.31	20.45	5.92	6.34	0.0	12.2
32*	1.3	1.8	19.0	19.0	1.3	1.4	25.93	27.25	14.09	10.10	0.0	2.6
LSF*	14.1	14.5	59.0	59.0	4.7	4.25	24.73	23.44	9.25	9.00	0.0	0.0
38*	2.0	2.1	7.5	7.5	2.0	1.5	19.00	20.03	4.92	6.26	0.0	10.5
40	1.4	2.6	7.0	7.0	1.0	1.2	24.98	30.94	28.90	14.89	0.0	46.9
42N	0.4	0.5	x	x	0.4	0.5	27.15	30.52	12.47	7.27	3.4	4.5
42S	0.3	0.7	x	x	0.3	0.7	27.65	31.25	17.31	7.31	2.1	115.3
44	1.1	2.2	15.0	15.0	0.1	0.85	24.70	32.37	85.32	85.32	103.4	55.0
48	0.6	1.2	16.0	16.0	0.2	0.2	31.32	27.97	102.22	41.77	50.3	90.6
50	1.7	2.8	x	x	0.1	0.5	25.11	30.80	74.10	38.87	302.6	49.3
51	1.0	1.7	9.0	9.0	0.1	0.8	26.37	32.33	96.98	26.03	106.9	75.4
52	0.6	1.1	14.0	14.0	0.2	0.55	30.63	25.90	91.14	38.40	75.8	7.1
59	2.8	4.3	12.0	12.0	2.8	4.3	25.42	26.20	3.85	4.08	0.0	0.0

Appendix B (continued)

Sinkhole	Dissolved Oxygen (mg/L)		pH		CaCO <sub>3</sub> (mg/L)		Total Phosphorus (mg/L)		Chlorophyll a (mg/m <sup>3</sup> )	
	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007
1*	4.30	5.63	7.81	8.165	3545	X	<0.05	X	0.534	2.670
2*	6.11	6.86	8.07	8.40	6570	X	<0.05	X	1.068	2.670
3*	5.32	6.85	7.80	8.39	6430	X	<0.05	X	4.272	1.068
4	0.32	1.50	7.29	7.38	7285	X	0.07	X	9.612	118.370
5	0.84	4.65	7.64	7.79	11835	X	<0.05	X	16.020	142.400
6	2.23	1.43	7.94	7.44	8290	X	<0.05	X	6.230	205.590
7*	5.30	4.33	7.69	7.99	3485	X	<0.05	X	3.204	0.890
8	0.54	3.25	7.49	8.12	16230	X	<0.05	X	37.380	4.272
9*	4.45	3.89	7.99	7.93	8570	X	<0.05	X	3.738	1.602
10*	2.97	7.13	7.63	8.13	5940	X	<0.05	X	5.340	3.738
11*	3.89	6.45	7.81	8.25	5250	X	<0.05	X	2.670	1.068
14	2.64	2.66	7.60	7.88	16340	X	0.16	X	X	4.272
15	3.15	4.42	7.98	7.99	7710	X	<0.05	X	4.005	81.880
16*	5.86	6.57	8.03	8.26	13800	X	0.09	X	3.204	46.725
17	5.99	2.38	8.00	7.87	16275	X	<0.05	X	1.526	18.690
18*	1.61	2.29	7.83	7.61	10030	X	<0.05	X	3.738	5.340
19*	4.55	3.89	8.08	8.04	9440	X	<0.05	X	0.000	12.816
20*	6.31	3.91	8.02	7.90	3800	X	<0.05	X	2.136	4.272
21*	0.09	3.28	7.69	7.90	17100	X	0.1	X	6.408	2.670
22*	3.97	2.12	7.85	7.90	17920	X	0.09	X	7.120	23.140
23	0.91	2.10	7.93	7.79	11790	X	<0.05	X	10.680	10.680
24*	5.50	1.06	8.10	7.75	9715	X	<0.05	X	10.680	1.335
25	9.90	4.65	9.07	8.77	6250	X	<0.05	X	1.602	3.738
26*	5.54	3.88	8.02	8.03	9270	X	<0.05	X	1.335	16.554
27N*	4.01	2.07	8.06	8.23	8525	X	<0.05	X	0.534	7.476

Appendix B (continued)

Sinkhole	Dissolved Oxygen (mg/L)		pH		CaCO <sub>3</sub> (mg/L)		Total Phosphorus (mg/L)		Chlorophyll a (mg/m <sup>3</sup> )	
	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007
27S*	6.12	4.98	7.92	8.01	5050	X	<0.05	X	-0.534	2.136
28*	3.67	3.80	8.23	8.37	8000	X	<0.05	X	2.465	6.230
29*	2.04	1.20	8.02	7.80	10005	X	<0.05	X	15.130	5.874
31*	6.16	4.58	6.89	7.24	3120	X	<0.05	X	1.335	20.292
32*	6.83	6.77	8.66	8.75	5265	X	<0.05	X	5.340	1.068
LSF*	4.22	4.26	7.69	7.90	3295	X	<0.05	X	0.000	0.534
38*	2.68	1.61	6.87	7.15	2920	X	<0.05	X	2.136	2.136
40	0.17	4.27	7.18	7.75	6605	X	<0.05	X	16.554	X
42N	21.21	14.89	8.71	8.12	4440	X	<0.05	X	5.340	3.204
42S	15.93	15.47	8.83	9.46	5580	X	<0.05	X	1.526	3.204
44	1.15	1.97	7.59	7.61	15730	X	0.23	X	X	32.040
48	6.38	5.53	7.86	8.62	X	X	X	X	X	19.580
50	1.49	2.04	7.45	7.38	14840	X	0.59	X	X	82.770
51	2.14	2.15	7.59	7.67	17530	X	<0.05	X	X	13.350
52	3.76	3.45	7.65	8.51	17535	X	<0.05	X	X	1.602
59	11.70	7.10	8.18	8.02	2410	X	1.57	X	0.534	0.534