APPENDIX D

Eastern Mosquitofish Studies

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1.0 Eastern Mosquitofish

A predator protection criteria of 0.1 mg/kg THg for prey species has been proposed by the USFWS (Eisner et al. 1987). The eastern mosquitofish, *Gambusia holbrooki* whole body THg concentrations were presented in the proceeding section. About 15% of the canal miles and almost 70% of the marsh area have mosquitofish with mercury concentrations exceeding the predator protection criteria of 0.1 mg/kg. Because the mosquitofish is a prey species for piscivorous fish and birds and is an excellent indicator of Hg bioaccumulation, additional analyses were conducted on the mosquitofish populations in the canals and marsh. The purpose of these analyses were to determine if differences in population attributes or feeding habits among subareas or among latitudes might contribute to mercury bioaccumulation. The results are presented in the following section.

1.1 Descriptive Statistics

The descriptive statistics of the eastern mosquitofish were examined to determine the nature of the sample population and the possible relationships with Hg bioaccumulation, size, and condition factors. An additional test sample of mosquitofish was collected from near the marsh Hg hotspot and the Everglades ENR in July 1997.

1.1.1 Canal Fish

A length frequency histogram of the mosquitofish in the combined canal sample showed a normal distribution (Figure D.1). A total of 1,074 mosquitofish was analyzed with a median total length of 24.0 mm (0.95 inches), ranging in size from 10 mm (0.4 inches) to 38 mm (1.5 inches). Females made up 70.3% of the population sample and males made up 28.5% with the remainder made up by juveniles. The median length for males was 22.9 mm (0.9 inches), 1.2 mm (0.05 inches) smaller than the females at 24.2 mm (0.95 inches). A box and whisker analysis (Figure D.2) of the fish lengths by cycle indicated fish sizes in cycles 1 and 2 were significantly larger and smaller, respectively, than cycles 0 and 3. A box plot analysis of fish length by geographic subarea showed significantly larger fish occurred in ENP, followed by EAA, WCA, and BCNP. Length/weight distributions and growth curves for each cycle show similar condition for each subpopulation (Figure D.3). A condition factor (CF) was calculated with the formula CF $= w/l^3$, where w = individual weight and l = individual length (Tesch 1968). The data combined by cycles showed significantly higher condition factors in fish from the EAA and ENP and lower condition factors for fish in the WCA canals and BCNP (Figure D.4).

1.1.2 Transect Fish

A length frequency histogram of the mosquitofish in the combined transect sample population is shown in Figure D.5. A total of 225 fish was analyzed, with 73.8% females, 25.3% males, and the remainder juveniles. The median total length was 25.6 mm (1.0 inch), and the sample population ranged from 14.2 mm (0.56 inch) to 34.4 mm. The median total length for males was 24.7 mm (0.74 inch), which was 1.9 mm (0.07 inch) less than females at 26.6 mm (1.05 inch). A box and whisker analysis (Figure D.6) of fish length found a significant difference between the LNWR and WCA3 in the fish sizes among the transects, however, all other distributions were not significantly different. Length/weight distributions showed similar condition among subareas (Figure D.7). The condition factors were significantly higher for mosquitofish from WCA2 and ENP transects than those on LNWR and WCA3 transects (Figure D.8).

1.1.3 Marsh Fish

A length frequency distribution of marsh fish included 2,158 individuals with a median size of 23.0 mm (0.04 inch), ranging from 9 mm (0.35 inch) to 39 mm (1.54 inches) (Figure D.9). The sample was normally distributed with 65.5% females, 25.1% males and 9.5% juveniles. Males had a median length of 22.4 mm (0.88 inch), which was 1.2 mm (0.05 inch) less than the females at 23.6 mm (0.93 inch). A box and whisker analysis (Figure D.10) of the data by cycle indicated the sample population had significantly larger fish in cycle 0 (April 1995) and significantly smaller fish in cycle 3 (September 1996) than the similar distributions in cycles 1 and 2. The September 1996 sample followed the driest dry period in spring 1996, which apparently killed a large number of these small fish due to dry down and predation, followed by recruitment of young individuals during the following wet season. Length/weight distributions were plotted by cycle with associated growth curves(Figure D.11). There were no apparent differences in condition; however, in cycle 3 was somewhat less due to preponderance smaller individuals. The condition factor plotted by subarea for the combined data, indicated the highest condition factor occurred in fish from LNWR and BCNP and decreased in WCA2 and WCA3 with the lowest condition factor associated with fish in the ENP (Figure D.12).

1.1.4 Fish in 1997 Test Sample

A mosquitofish sample was collected from two marsh sites in 1997, the ENR and WCA3 near where the Hg concentrations in mosquitofish were previously found to be the highest in the marsh system. The ENR is an old agricultural field that has been converted into a prototype wetland stormwater treatment area designed to remove TP from stormwater. Agricultural fields were not sampled as a routine part of the REMAP study. A total of 153 fish was analyzed (Figure D.13). The median fish size at the ENR and WCA3 sites was 19.7 mm (0.78 inch) and 17.2 mm (0.68 inch), respectively. The fish ranged in size from 9.0 mm (0.35 inch) to 35.6 mm (1.40 inches) at the ENR site and from 7.6 mm (0.3 inch) to 34.0 mm (1.34 inches) at the WCA3 site. A box and whisker plot shows the ENR fish were significantly larger than the WCA3 fish (Figure D.14). The condition factor was higher for ENR fish (Figure D.15). The THg concentrations in fish from the ENR were found to be the lowest measured in fish during this study (Figure D.16). Samples analyzed by three different laboratories found that THg concentrations in fish from the ENR were less than 10 μg/kg while the THg in fish from the WCA3 site averaged about 150 μg/kg (Figure D.16).

It is apparent from these data that a consistent sample of mosquitofish was collected from both canal and marsh habitats throughout the course of this study. The sample collected is representative of the naturally occurring mosquitofish population in the system. The average size of the mosquitofish populations observed in the Everglades ecosystem are very small for the species found anywhere in their range (Trexler personal communication). The Everglades ecosystem is a food limited, oligotrophic system (Loftus personal communication) that reduces the size of this species. The usefulness of this species as an important indicator for monitoring the bioaccumulation of Hg in the Everglades ecosystem is evident from the changes in THg concentrations in this species across the TP, TSO_4 and TOC gradients in this system. The consistent future sampling and analysis of mosquitofish can provide the information needed to assess changes and trends in Hg contamination in the South Florida Everglades ecosystem.

1.2. Mosquitofish Gut Contents

Bioaccumulation and biomagnification of MeHg through the food web is influenced by both the quantity and quality of the ingesta. An additional study was conducted to determine the gut contents of mosquitofish collected during the September 1996 sampling cycle.

Many species are known to switch diets both during development and based on food availability in their environment. Omnivorous species have the potential to yield important influences on their prey by switching their diet choice with regard to its changing abundance, targeting it in times of plenty, and ignoring it in times of scarcity. Furthermore, the ability to switch foods, even going from herbivore to carnivore, may buffer a species from fluctuations in food supply and permit it to sustain larger population densities. Ontogenetic changes in diet may also reduce competition among age classes (Werner and Gilliam 1984). One possible outcome in such a case is that individuals may begin to specialize in subsets of their potential prey (Magurran 1993). Also, local populations of omnivores may diverge substantially in their feeding biology, and role in local ecological communities, as a result of local environmental conditions and food availability.

In spite of these possibilities, few studies have characterized the diet of an omnivorous species over a large spatial scale, probably because of the inherent difficulties of collecting adequate specimens to do so. The eastern mosquitofish (*Gambusia holbrooki*), and its western congener, *Gambusia affinis*, are known to be aggressive omnivores (reviewed in Meffe and Snelson 1989) with the potential to yield greater effects on the ecosystems where they live than might be predicted based on their small size (Courtney and Meffe 1989). They have been widely introduced world-wide for mosquito control and have been responsible for the extinction of native fishes in some cases through consumption of larvae (Meffe et al. 1983). Experimental ecological studies have documented their potential to regulate the dynamics of other community members through direct consumption and competition (Hurlburt et al. 1972, Hurlbert and Mulla 1981, Harris 1995, Belk and Lydeard 1994, Schaefer et al. 1994). Mosquitofish are also cannibalistic (Krumhotz 1948, Meffe and Crump 1987). Mosquitofish are known to feed on plant matter and detritus, in addition to these predatory predilections.

1.2.1 Methods

1.2.1.1 Data Gathering

Mosquitofish were collected by dipnet from 101 locations scattered across the Florida Everglades (Figure D.18) from September 18 to 23, 1996. The sites were selected by a stratified-random procedure described earlier. Immediately following capture the fish were placed in 10% formalin in the field to rapidly stop the digestion of the stomach contents. Twenty fish were collected at each site of which 12 to 14 specimens were analyzed for stomach contents. Duplicate samples were taken at 10 sites.

Individual mosquitofish were dissected and their gut contents removed and separated into six categories: (1) plant matter (pooling algae, vascular plant, and detritus), (2) cladocera, (3) aquatic mites, (4) chironomid larvae (midge larvae), (5) adult midges, and (6) other (primarily spiders, ants, aquatic beetles, and fish). Counts of the number of items in all animal categories were recorded for each mosquitofish, along with their sex and standard length. Males could be identified readily by the presence of a gonopodium, and females were identified by presence of mature ovaries or by standard length exceeding 18 mm (0.71 inch). Juveniles were all fish below 18 mm (0.71 inch) standard length lacking a gonopodium. The presence or absence of plant matter was recorded for each specimen, and if no food was present this was also noted. All food items for the fish from a single population sample were pooled and the mass of each food category was determined. The sum of these masses provided an estimate of the total mass of food consumed by that sample of fish.

1.2.1.2 Statistical Analyses

The pattern of food choices by individual fishes were examined, followed by analyses of the population samples. No mass estimates were available for the food items of individual specimens, particularly for plant food, so the presence or absence of food types was examined. Principle components analysis was conducted to investigate patterns in the food choices. The covariance matrix was factored because all data were scored as present or absent rendering all variables on a similar scale (Stevens 1986); varimax factor rotation was used. The effect of fish size and sex on food choices were examined by logistic regression of presence/absence of food types in individual fish gut contents (Trexler and Travis 1992). Fish with empty stomachs were excluded from these analyses. The presence/absence of food in the gut of individual fish was coded as a dependent variable and this was examined separately by logistic regression with fish sex and standard length as independent variables.

The percentage of each food category in the diet of fishes from each population sample was calculated from the mass data. These percentages were analyzed in analyses of covariance by grouping populations into geographic regions of the study area using two schemes. First, populations were grouped according to the water management region where they were found: LNWR, WCA2, WCA3, ENP, and BCNP. There are general north to south gradients in productivity across the Everglades following patterns of nutrient enrichment from agricultural runoff (Davis 1994, Stober et al. 1996). The effects of this pattern by grouping the populations into 6 regions by latitude from north to south were examined: (1) > 26.4ºN; (2) 26.4ºN to 26.2ºN; (3) 26.2ºN to 25.9ºN; (4) 25.9ºN to 25.7ºN; (5) 25.7ºN to 25.5ºN; and (6) <25.5°N. The average standard length of fish from each collection was retained as a covariate in these analyses. In all cases, data were examined for consistency with the assumptions of standard statistical procedures such as normality, and transformations were applied as needed to fulfill the assumptions of analyses (Zar 1984).

1.2.2 Results

1.2.2.1 Overview

Over 1200 fish averaging 15.9 mm (0.63 inch) standard length, most of which were juveniles were examined (Table D.1). Males were typically smaller than females by over 2 mm (0.08 inch). A conversion between standard and total length for males, females, and juveniles with an r^2 over 0.9 is : Standard length = -1.337 + 0.886 (total length). Periphyton comprised 36% of the diet of mosquitofish based on biomass in gut contents, with insect, crustacean, arachnid, and piscine prey accounting for the remaining 64%. Adult midges, undoubtedly gleaned from the water surface, accounted for 34% of the biomass of the diet, and midge larvae, probably taken from floating, epiphytic and benthic periphyton mats, accounted for an additional 9.6%. Two fish (both *Heterandria formosa*) and an assortment of spiders, ants, and beetles account for 15% of the diet by biomass. About 50% of the individual fish had plant matter present in their guts, and about 45% had adult midges (Table D.2). Chironomid larvae and "other" prey were both found in about 10% of the fish, while mites were present in around 8.0% and cladocerans in only 3.0% of the fish examined. Very few of the fish had empty stomachs (53 out of 1,265 fish examined).

1.2.2.2 Individual Fish

Both the size and sex of fish influenced the likelihood that they had empty stomachs. Larger specimens were more likely to have empty stomachs than smaller ones, though even the largest juveniles had less than a 5% chance to have empty stomachs because of their relatively small size (always less than 18 mm (0.71 inch); Figure D.19). Females and juveniles did not differ in the probability of having food present in their gut once size differences were accounted for; however, males were more likely to have empty guts than females or juveniles at the same size (Figure D.20). Still, the likelihood of not having fed prior to collection was low in the sample; the model estimated that even the largest specimens had only a 20% likelihood of no gut contents.

Principal components analysis indicated that patterns of consumption of the six food categories did not overlap. No factor component loaded heavily on more than one food type, although the first two components explained over 60% of the total variance. A component loading heavily on the incidence of plant matter explained 31.4% of the variance in gut contents, one loading on adult dipterans explained a similar amount (30.9%), and two other components loading on chironomid larvae and "other" each explained an additional 11.8%. The lack of structure in the data revealed by this analysis led to the consideration of each food class individually in subsequent analyses.

Size was a significant factor in the likelihood that mosquitofish had consumed plant matter immediately preceding collection. The likelihood of finding periphyton in the gut decreased with size (Table D.3) from approximately 60% for 10 mm (0.4 inch) juveniles to about 35% for a 25 mm (0.99 inch) adult female (Figure D.21). Males were less likely to have consumed periphyton than females (adult fish only, model: Pr [periphyton in gut] = constant + sex + size, t_{212} = 7.47, P = 0.032, size t_{515} = 2.28, P = 0.015, sex t_{515} = 2.14, P = 0.012; Figure D.22).

Some categories of animal prey decreased in frequency in the diets of mosquitofish as they got larger, others increased, and some were unaffected by size. The incidence of cladocerans decreased with size, while adult dipterans and "other" prey increased in frequency as fish got larger. Chironomids and mites were equally likely to appear in the diets of all size of fish (Table D.4). In general, the diets of males and females did not differ regarding the incidence of animal prey, with the exception of "other" prey. However, this difference was explained by the size difference between the sexes (adult fish only, model: Pr("other" in gut) = constant + sex + size +sex by size , t_{213} = 12.01, P = 0.009, size t_{515} = 2.26, P = 0.015, sex t_{515} = 1.63, P = 0.11, sex by size t_{515} = 1.7, P = 0.09).

1.2.2.3 Geographic Variation

The average size of fishes examined differed among the five water management units of the Everglades. Population samples of mosquitofish from LNWR and WCA3 contained significantly smaller fish than average, and those from WCA2 and BCNP tended to be larger (Table D.1). In light of the findings of diet changes with mosquitofish size, analyses comparing these water management areas must adjust for the size of specimens in samples. And, as expected, the mass of food found in the guts of fishes is related to the average size of specimens in the population samples (Table D.3). However, differences among management areas persist after size variation is accounted for: WCA2 and WCA3 fishes tended to have more food in their guts than average, while those from LNWR and BCNP had less than average (Table D.4). When the data were sorted into six latitudinal categories, similar patterns were revealed.

Some food items appeared to vary across the water management units in their prevalence in the diet of mosquitofish. The relative amount of plant matter in the diets displayed a tendency to vary among regions (Table D.3; P=0.088, dropping to P=0.056 when length was excluded from the model) with BCNP fishes displaying notably less plant matter than the fish from the other four regions examined; this result was obscured when regions were created along a north to south gradient (Table D.4). Chironomid larvae displayed a more marked variation among regions in their inclusion in diets. They accounted for over 20% of the diets in LNWR and WCA2, but dropped to below 10% in all other regions (Table D.4). This is also seen as a north to south gradient when the data are grouped by latitude (Table D.4). "Other" prey items appeared most frequently in the diet of fishes from BCNP, and least in data gathered from fish from LNWR, though this result was obscured when samples were compared along the latitudinal gradient (Table D.4). Adult midges and mites displayed no regional patterns in their incidence in mosquitofish diets (Table D.3).

Table D.1 Descriptive statistics of size of the fish examined. Standard length (mm) and 95% confidence intervals are reported, with sample sizes below.

Data Grouping	All Fish	Males	Females	Juveniles
All Populations				
	15.93 ± 0.20 1270	17.12 ± 0.34 108	19.66 ± 0.28 407	13.73 ± 0.07 748
By Region				
WCA1	15.04 ± 1.16 11	—	—	—
WCA2	17.20 ± 1.70 8	—	—	—
WCA3	15.20 ± 0.26 40	—	—	—
ENP	16.18 ± 0.75 33	—	—	—
BCNP	17.83 ± 1.54 9	—	—	—

Table D.2 Relationship of diet to size in juvenile and female eastern mosquitofish. Results from logistic regression of incidence (presence/absence) of food item in gut contents on female size measured as standard length. In one case, adult dipterans, a significantly better model fit was obtained by use of ln-transformed length. Average Size, n-columns indicate the average size (mm) and sample size of fish with each item absent or present. The Pr (Present) is the probability estimated at the grand mean size. Slope is the probability that each food item will be present in gut contents with a 1-mm increase in standard length, except for adult dipterans which were best fit on a log scale.

Food Type	χ^2	P	Item Absent Average Size, n	Item Present Average Size , n	Pr (Present) Observed	Pr (Present) Predicted	Slope Pr(Present) vs Standard Length
Periphyton	11.6	0.001	16.2, 580	15.5, 581	0.500	0.500	-0.014
Cladoceran	8.1	0.006	15.9, 1117	14.3, 39	0.034	0.031	-0.004
Adult Dipteran	3.9	0.05	15.7, 645	16.0, 511	0.442	0.442	0.127 *
Chironomid Larvae	0.5	>0.4	15.8, 1042	15.6. 114	0.099	0.098	
Mite	1.8	0.185	15.9, 1058	15.3, 98	0.085	0.084	
Other	17.9	<0.001	15.7,1040	17.2, 116	0.100	0.094	0.090

* indicates slope on a ln (standard length) scale

Table D.3 Geographical analysis of the percentage of total mass attributable to each food type from the gut contents of mosquitofish. Two geographical groupings were used, Water Management Units refers to comparisons of population means among management units, while Latitude refers to grouping populations by latitude. No interactions were significant.

		Water Management Units			Latitude		
Food Item	Effect	F	DF	P	F	DF	P
Plant Food	Length	0.004	1,95	0.843	0.060	1,94	0.807
	Region	2.088	4,95	0.088	1.410	5,94	0.228
Cladocera	Length	0.115	1,95	0.736	0.541	1,94	0.464
	Region	0.727	4,95	0.576	0.785	5,94	0.563
Adult Midges	Length	0.430	1,95	0.514	0.373	1,94	0.543
	Region	1.033	4,95	0.395	1.558	5,94	0.180
Midge Larvae	Length	8.379	1,95	0.015	9.034	1,94	0.003
	Region	4.130	4,95	0.014	5.042	5,94	0.001
Mites	Length	0.227	1,95	0.635	0.191	1,94	0.663
	Region	1.175	4,95	0.327	0.725	5,94	0.606
Other Prey	Length	1.974	1,95	0.163	4.046	1,94	0.047
	Region	1.900	4,95	0.117	1.224	5,94	0.304
All Food Mass	Length	7.373	1,95	0.008	6.85	1,95	0.010
	Region	2.878	4,95	0.027	2.580	5,95	0.031

Table D.4 Adjusted means from analyses of food items by geographical groupings. All means are adjusted to the grand mean fish size of 15.93 mm standard length.

Grouping	Plant Food (%)	Midge Larvae (%)	Other (%)	All Food (mg)
Water Management Units				
WCA1	27.3	25.1	6.1	0.973
WCA2	30.1	21.1	13.1	2.328
WCA3	42.7	7.9	16.5	2.352
ENP	36.5	6.0	13.1	1.348
BCNP	8.6	4.7	32.4	1.096
Latitude				
> 26.4	26.7	25.1	6.7	0.968
26.4 - 26.2	35.4	20.7	10.0	2.636
26.2 - 25.9	34.6	5.4	22.4	1.678
25.9 - 25.7	34.6	2.9	20.8	2.033
25.7 - 25.5	50.3	5.7	14.2	1.863
< 25.5	21.6	6.6	13.2	0.942

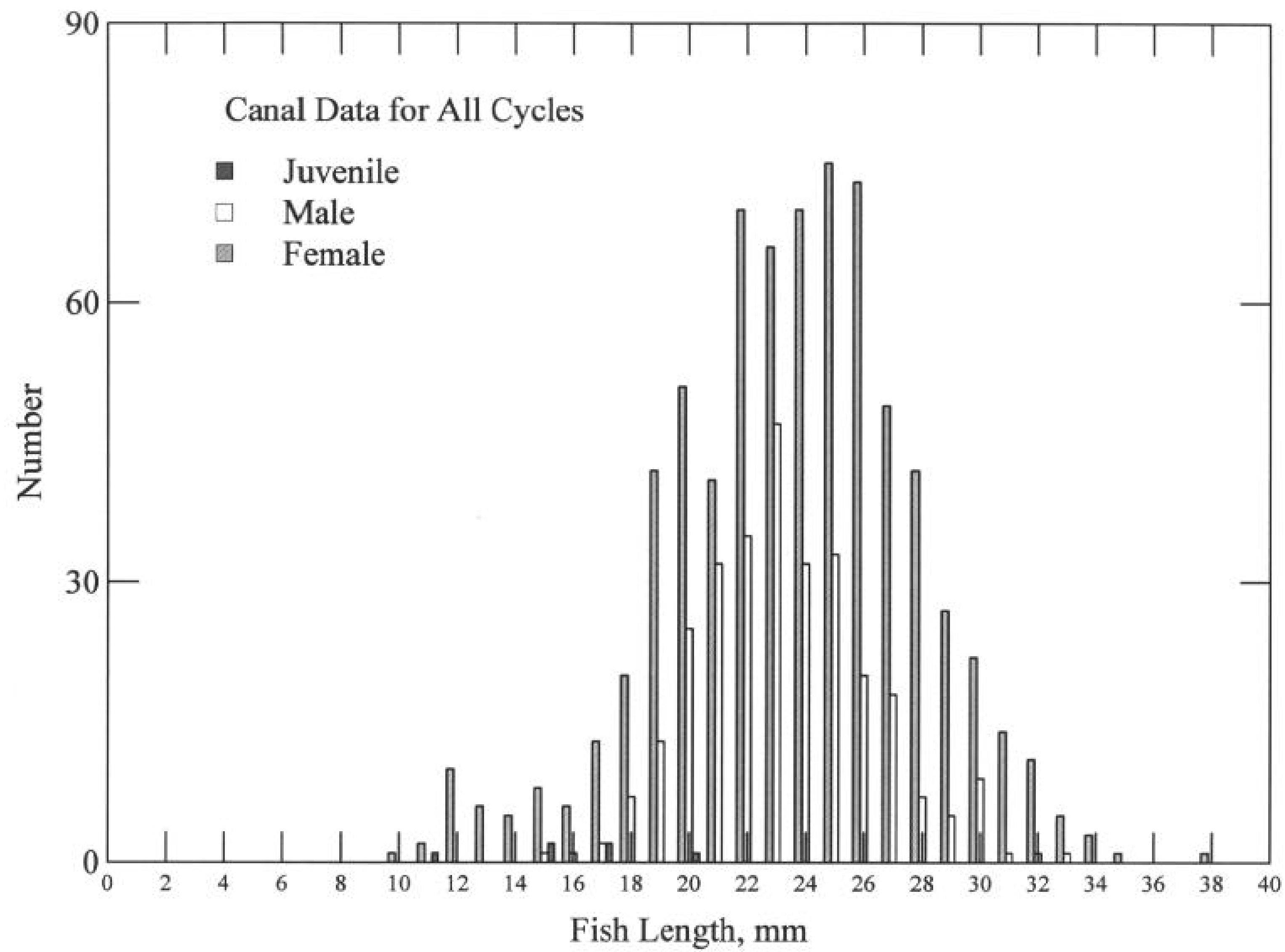


Figure D.1 Length frequency histogram of mosquitofish collected in the canals.

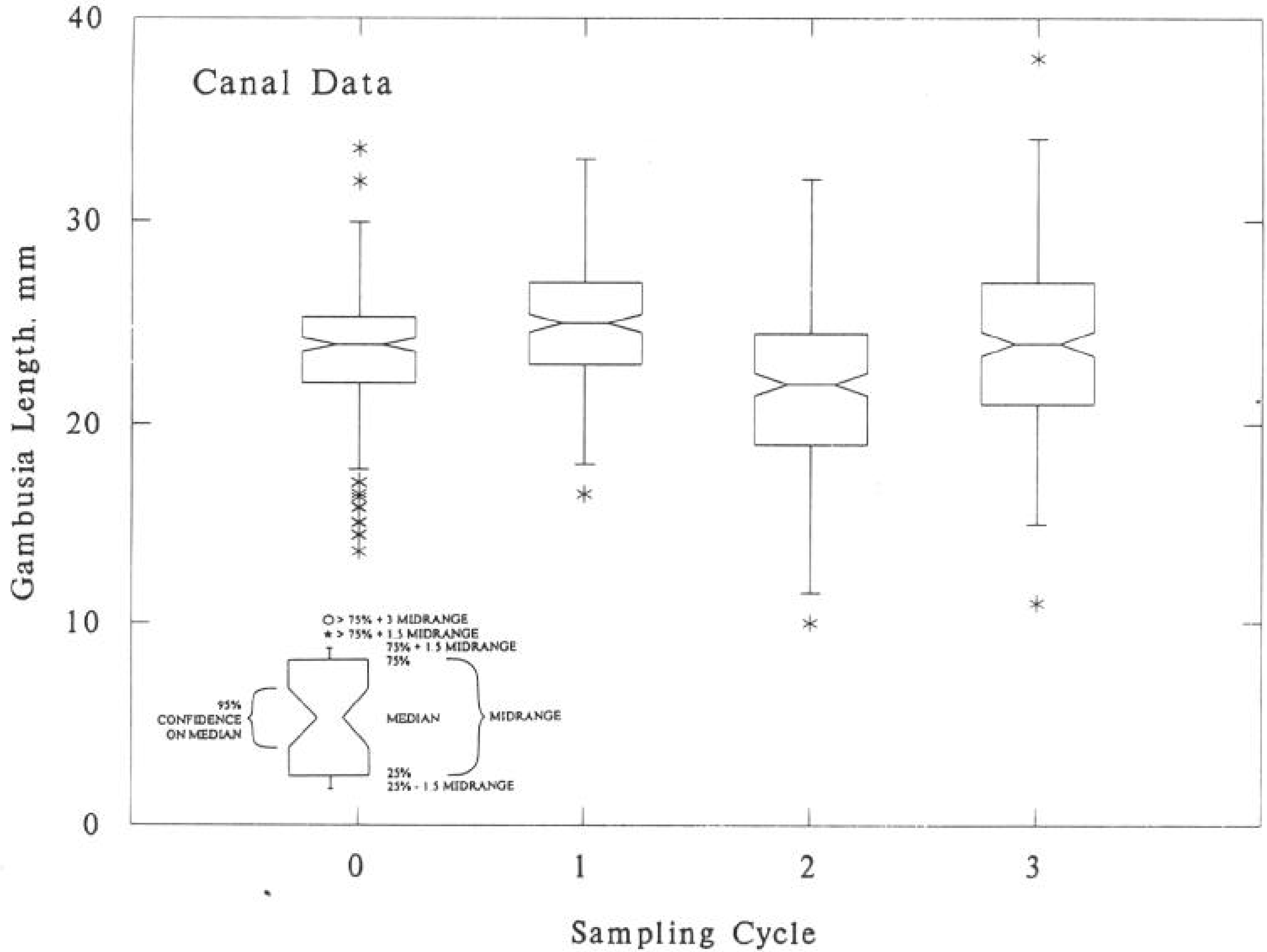


Figure D.2 Notched box and whisker plot comparing mosquitofish length for each sampling cycle.

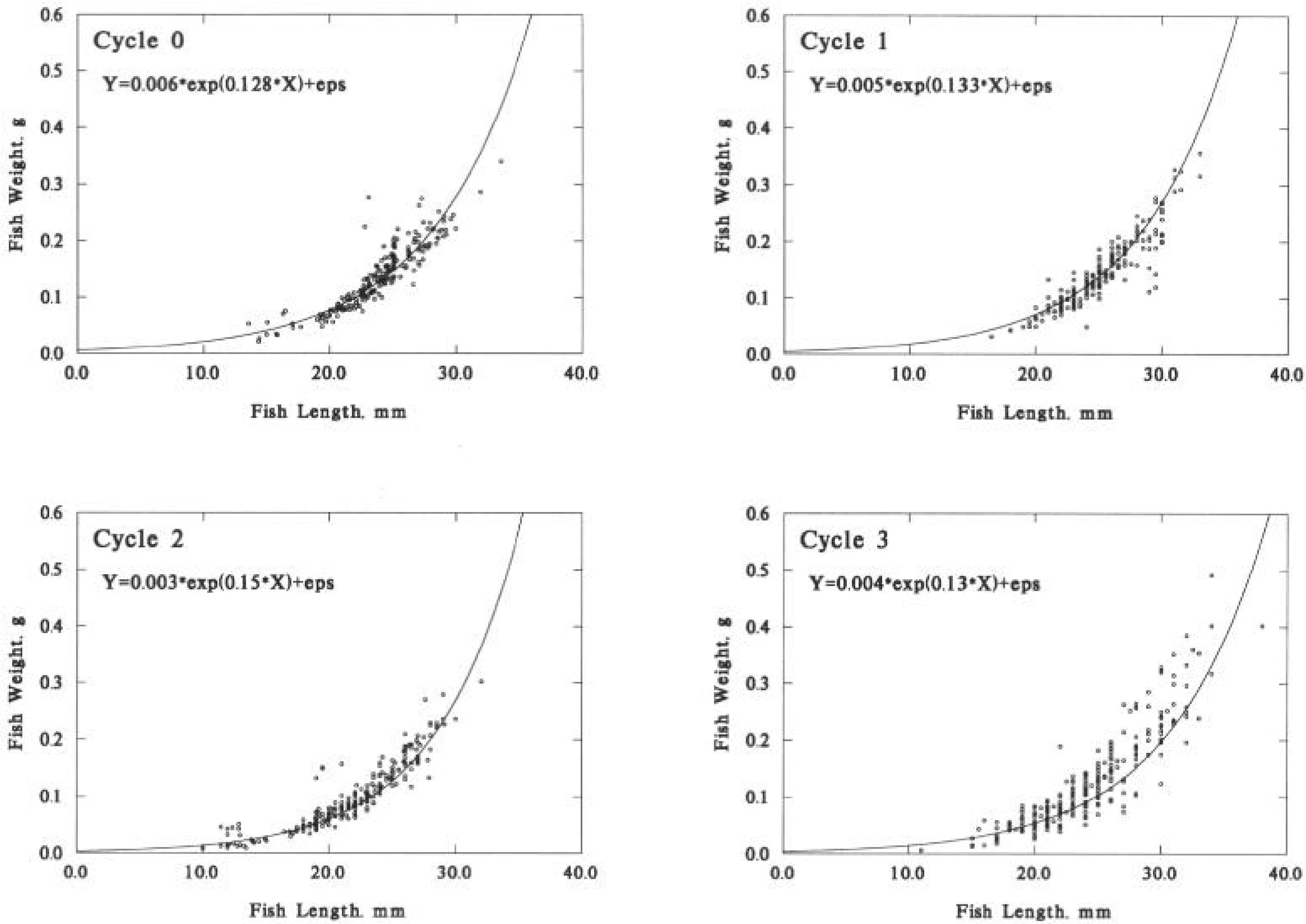


Figure D.3 Canal mosquitofish length versus weight with derived growth curves for each sampling cycle.

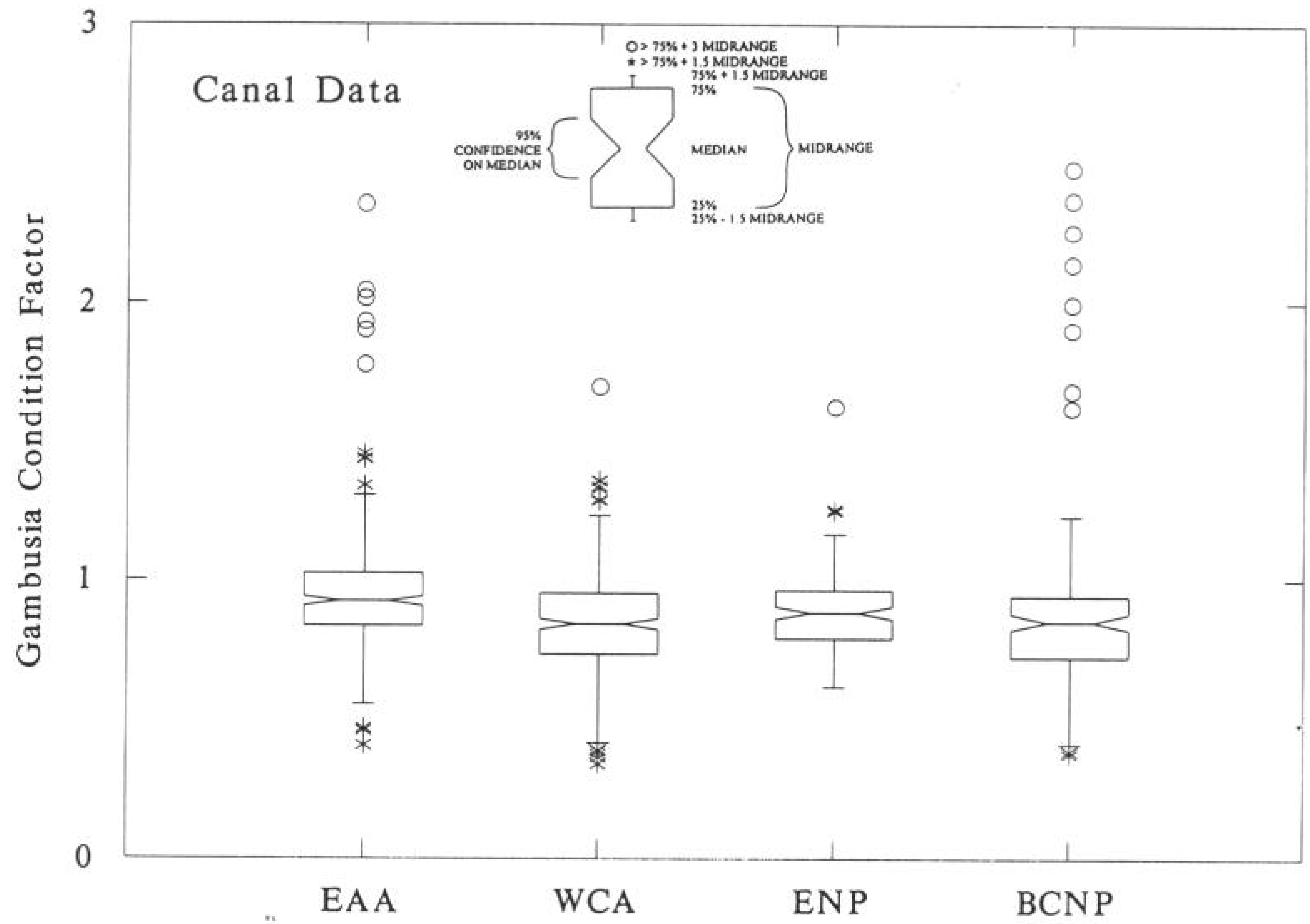


Figure D.4 Notched box and whisker plot comparing condition factors for canal mosquitofish in subareas.

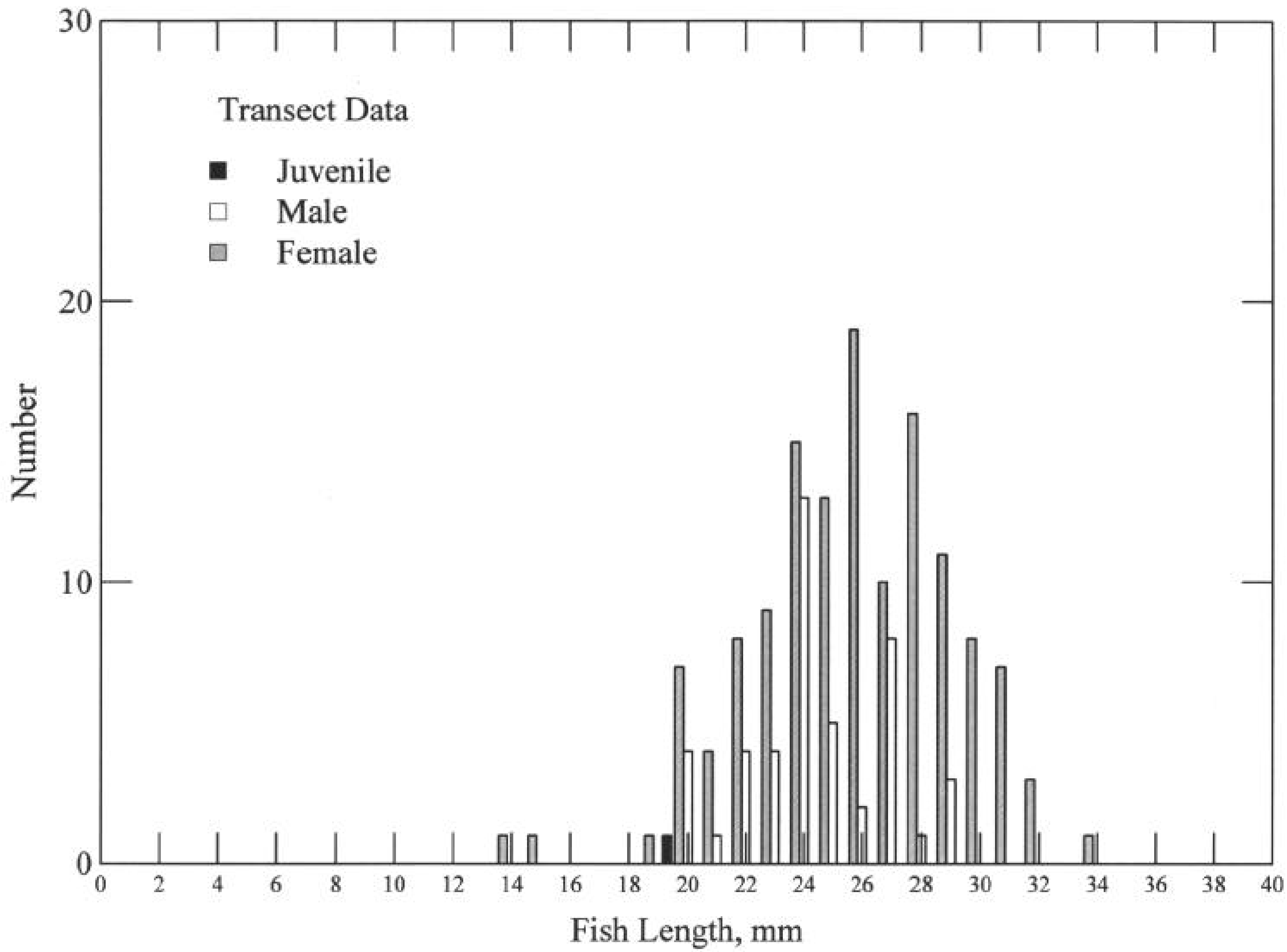


Figure D.5 Length frequency histogram of mosquitofish collected along the marsh transects.

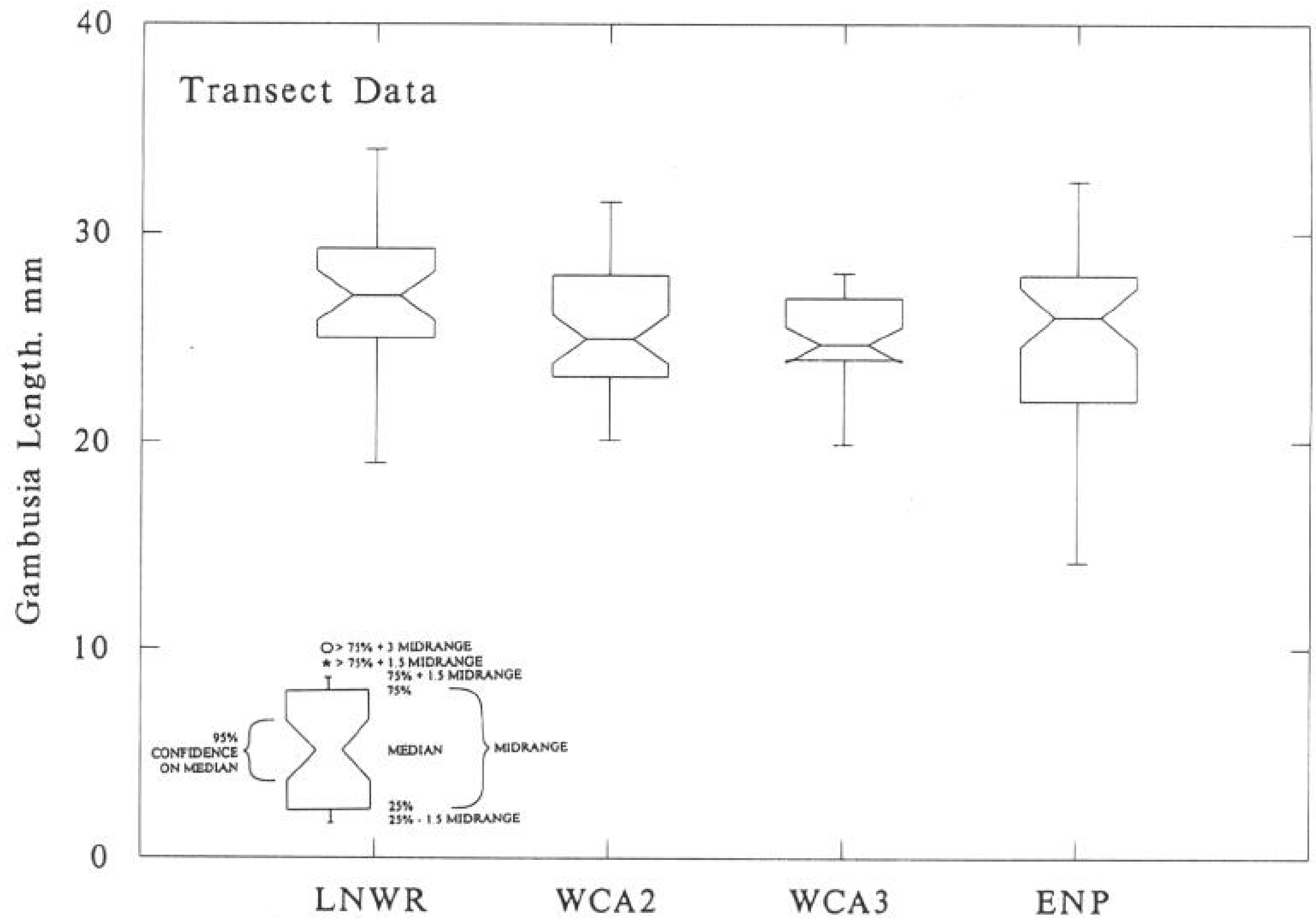


Figure D.6 Notched box and whisker plot comparing mosquitofish lengths in each of the marsh transects.

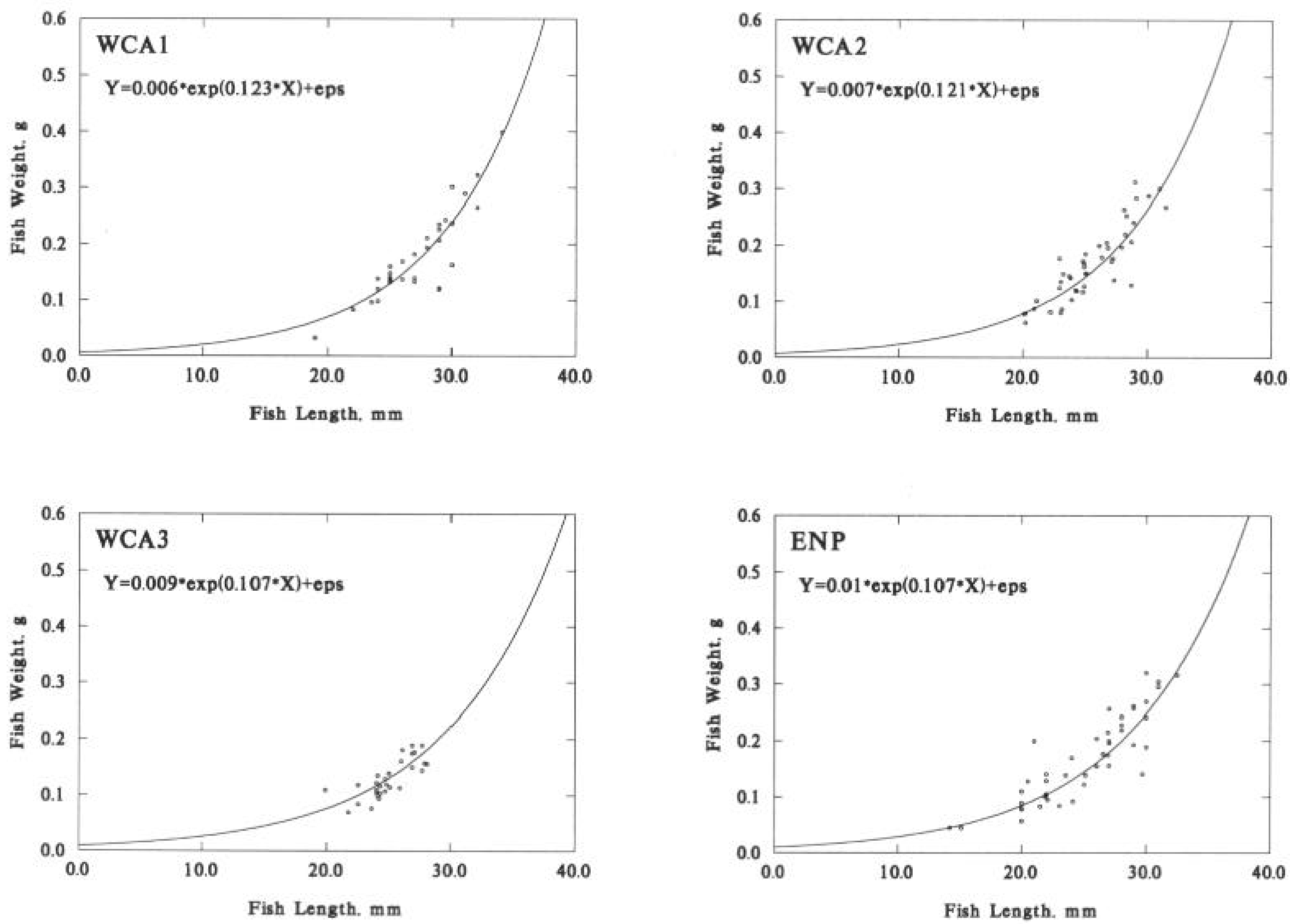


Figure D.7 Mosquito fish length versus weight with derived growth curves for each marsh transects.

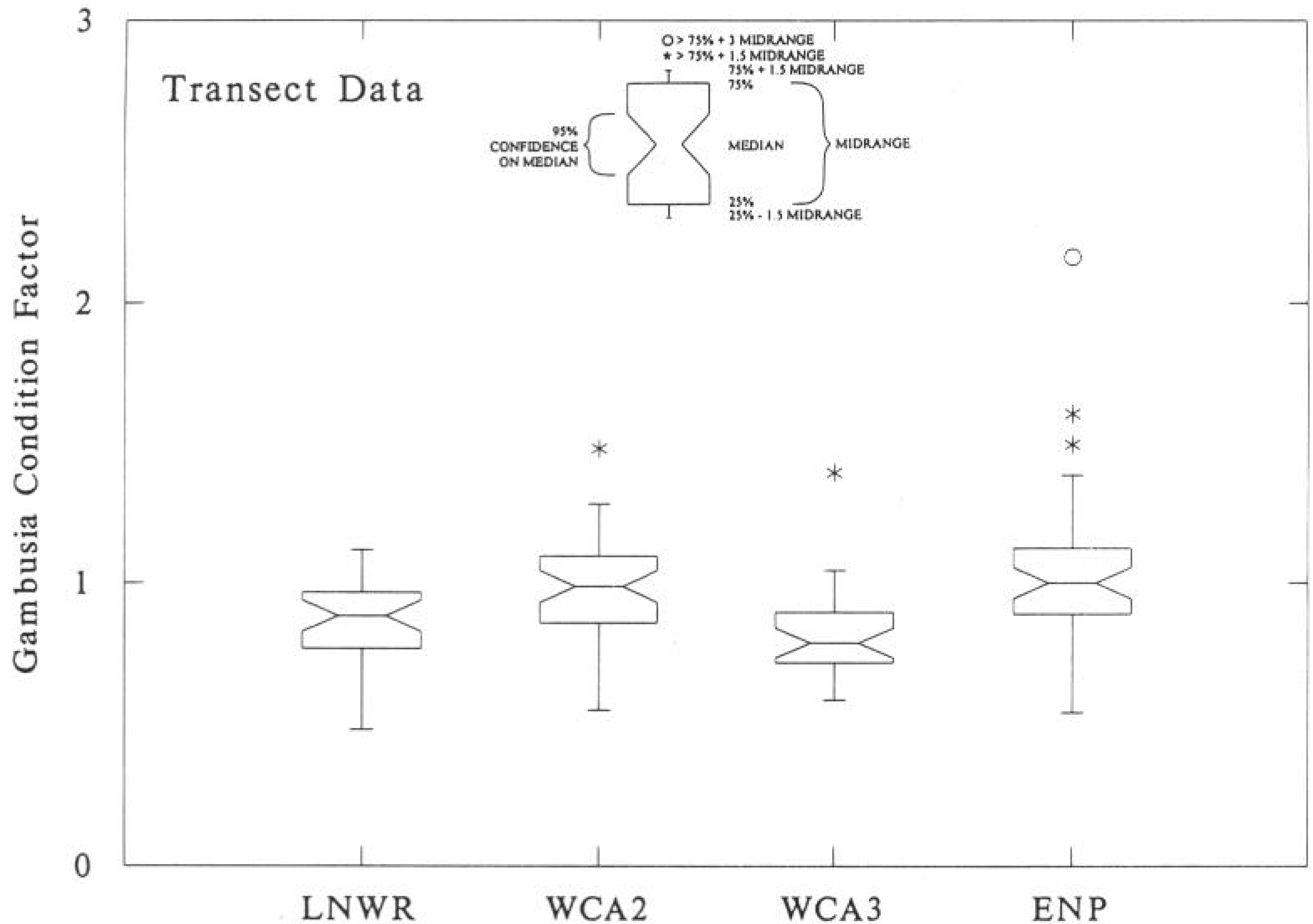


Figure D.8 Notched box and whisker plot comparing mosquitofish condition factors for transects.

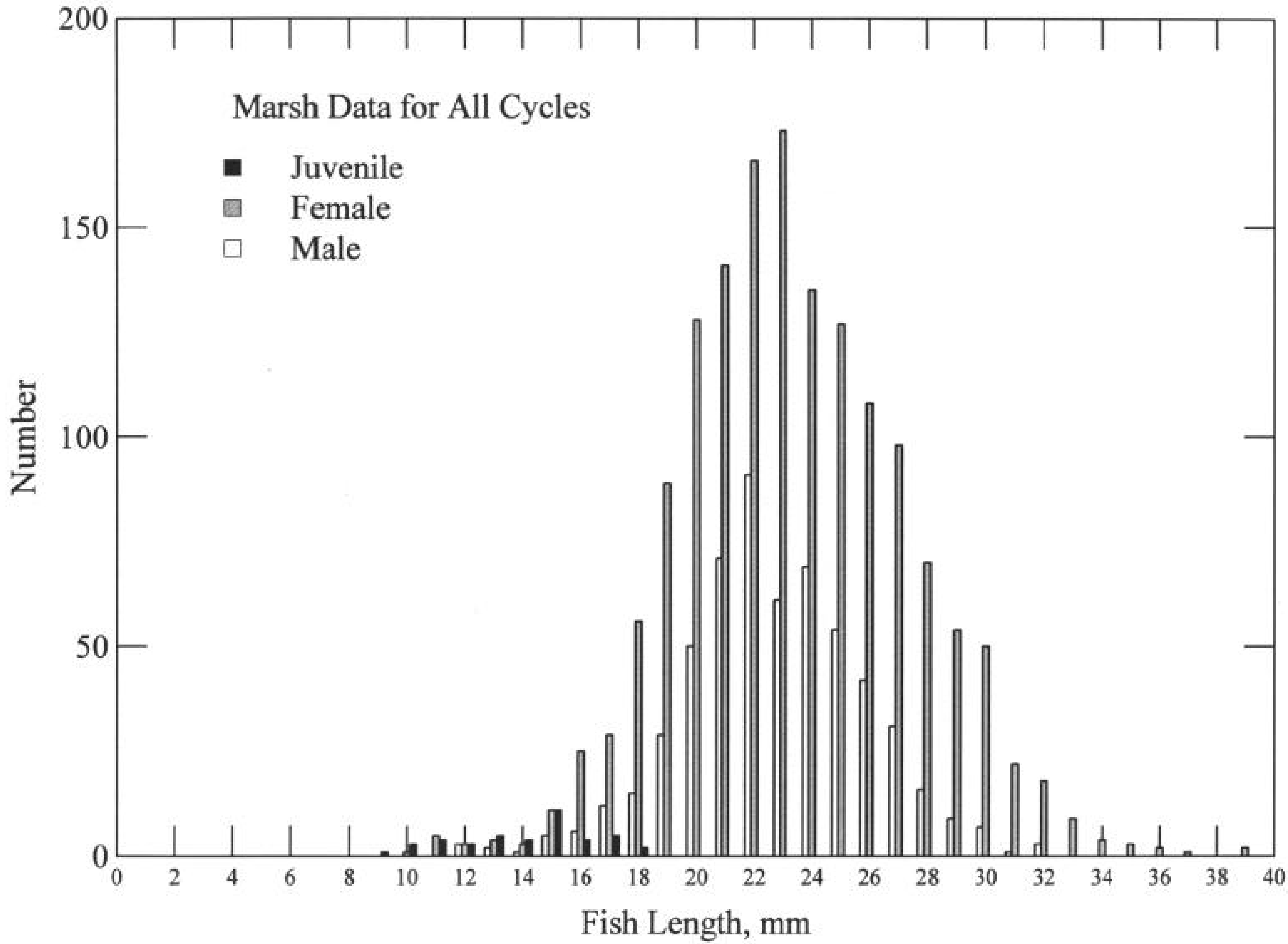


Figure D.9 Length frequency distribution for mosquitofish collected in the marsh.

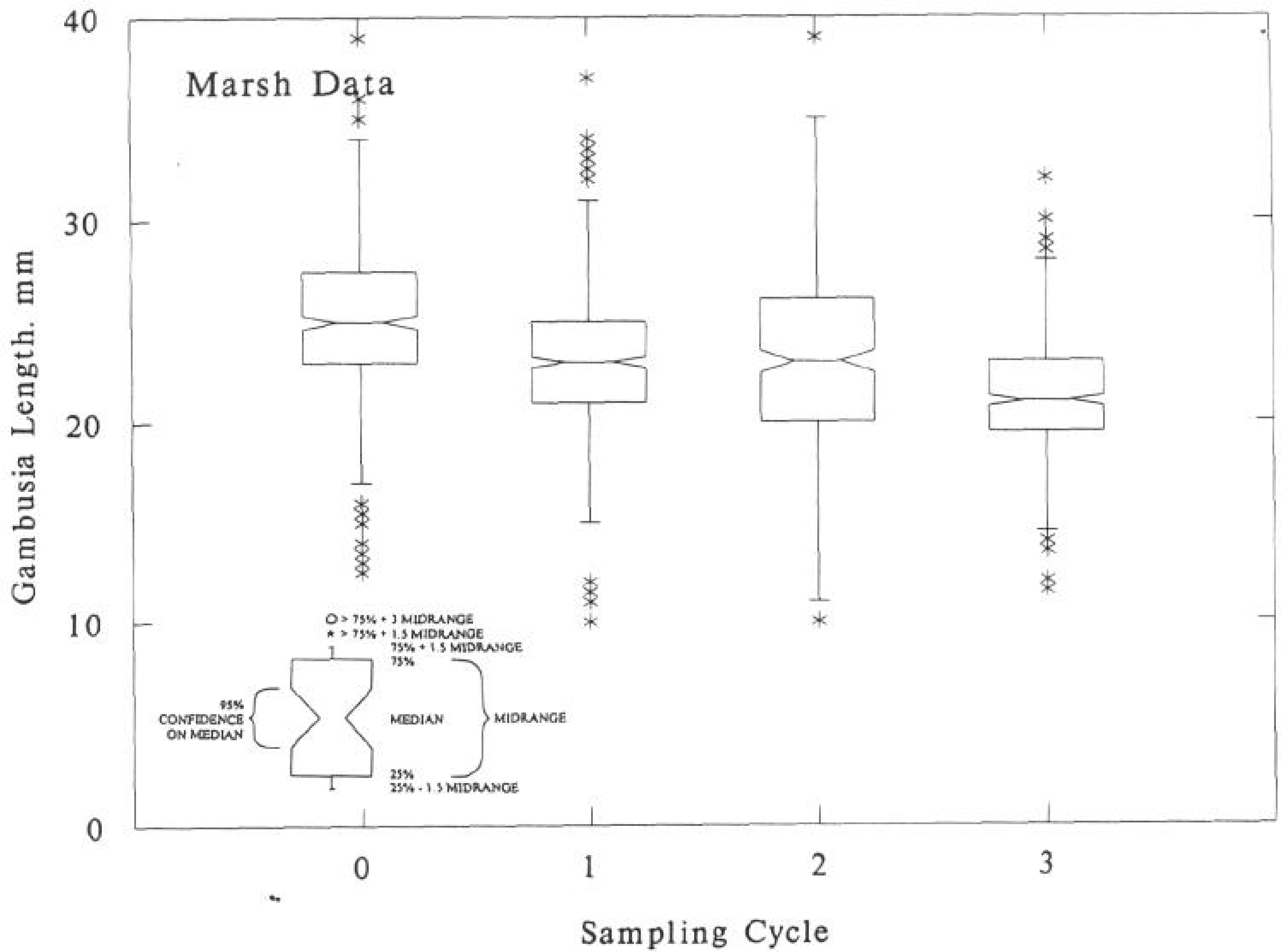


Figure D.10 Notched box and whisker plot comparing lengths of mosquitofish collected in the marsh for each sampling cycle.

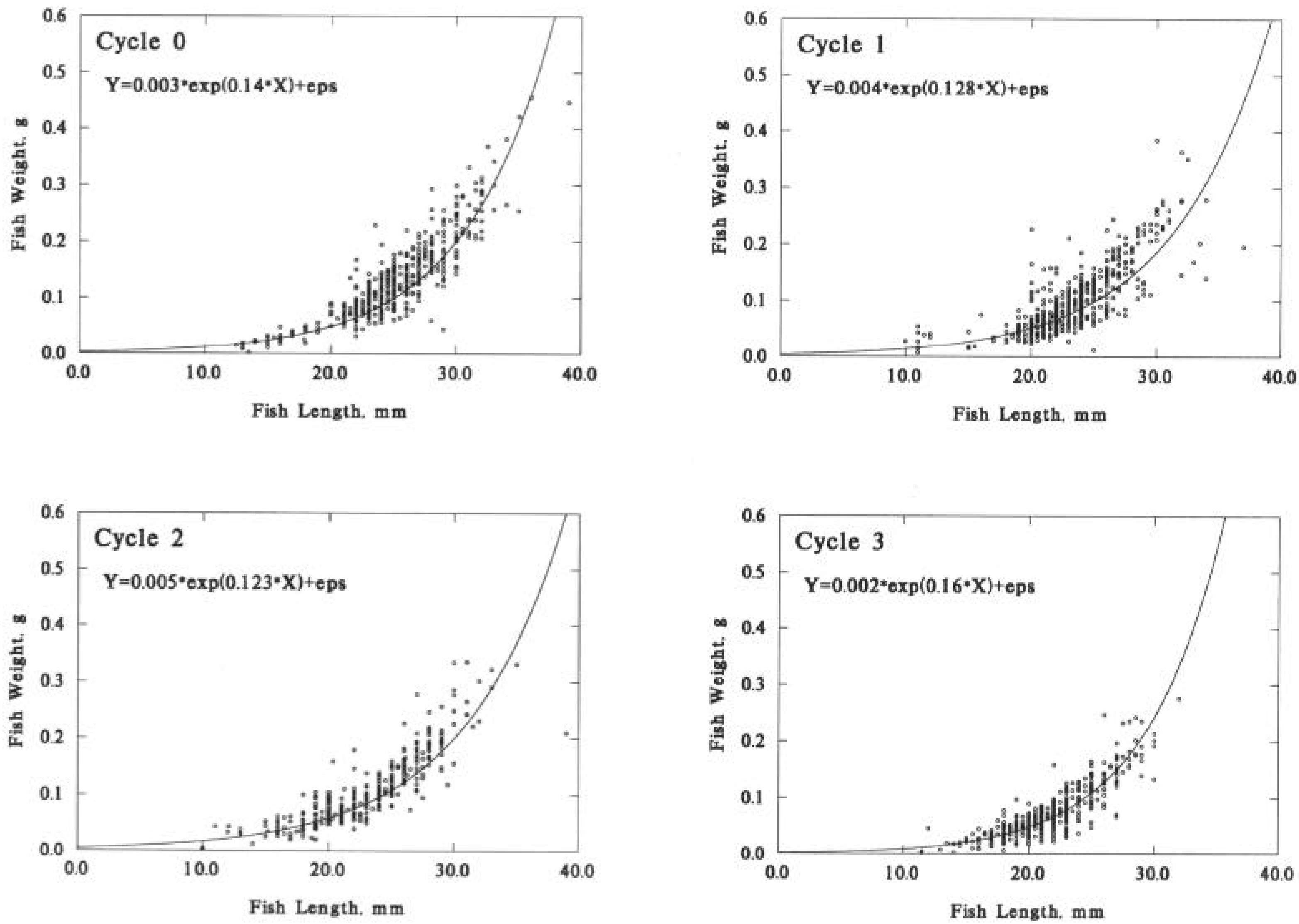


Figure D.11 Length versus weight of mosquitofish collected in the marsh during each sampling cycle with derived growth curves.

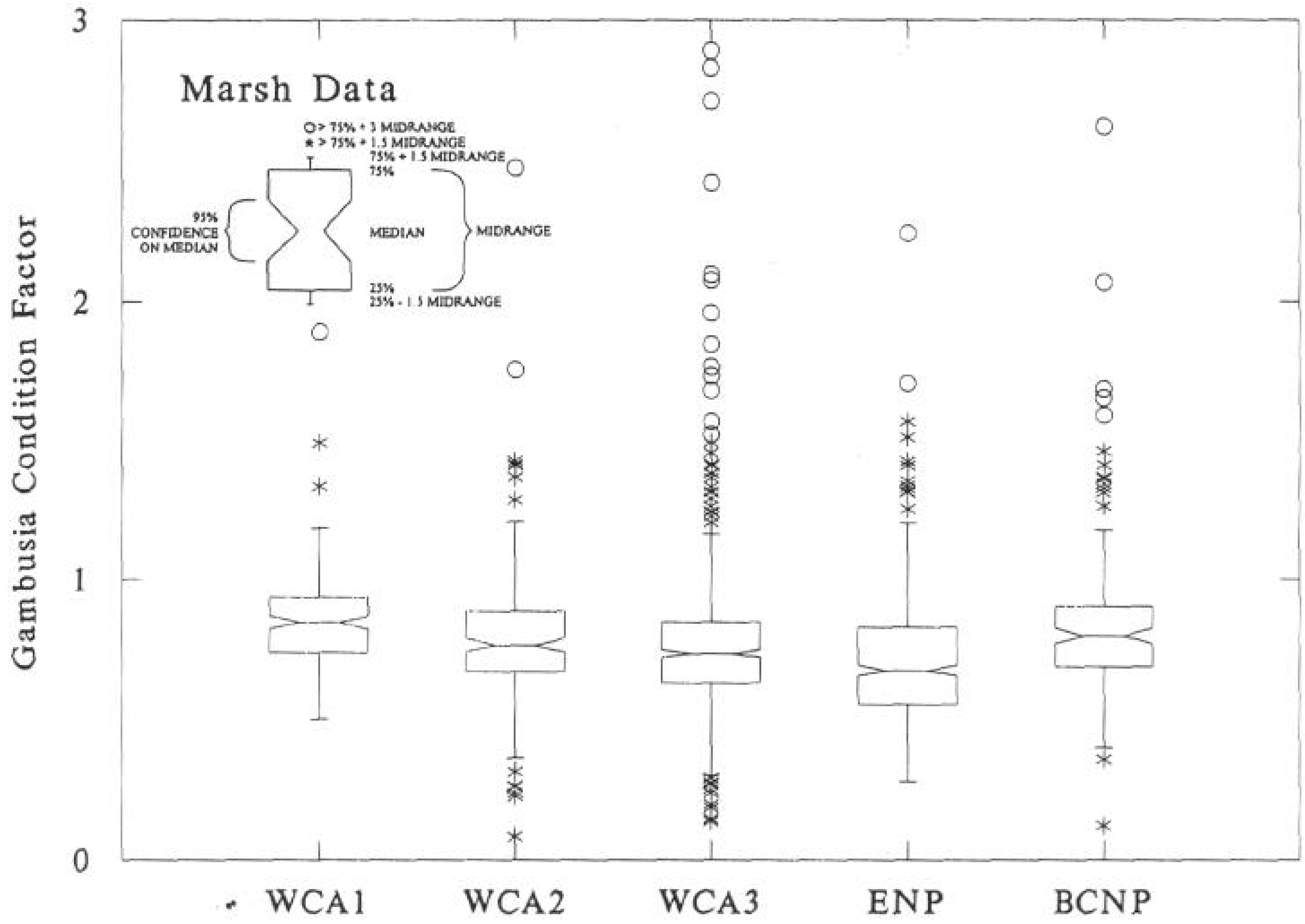


Figure D.12 Notched box and whisker plot comparing condition factors for marsh mosquitofish collected in subareas.

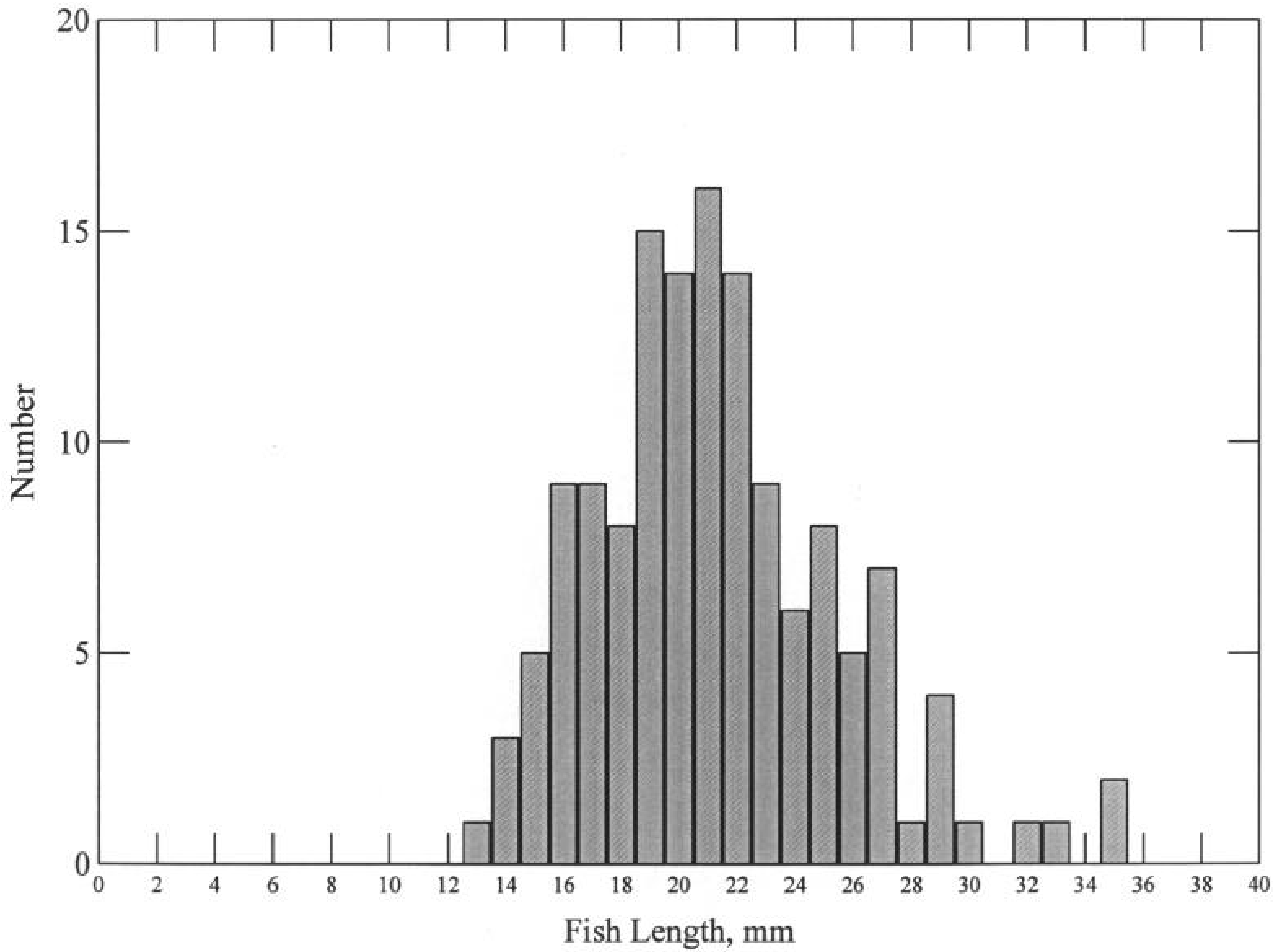


Figure D.13 Length frequency distribution of mosquitofish in the 1997 test sample.

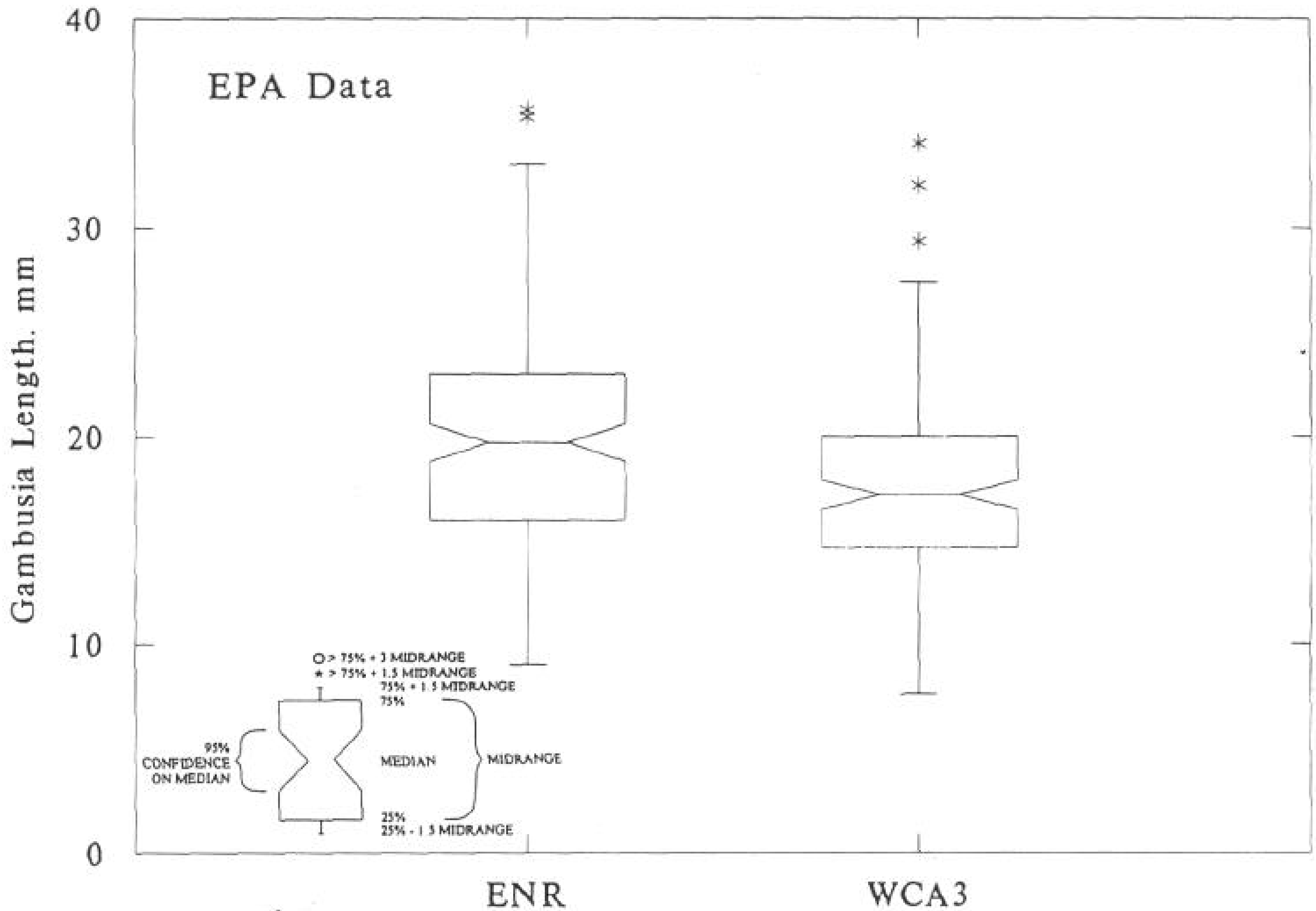
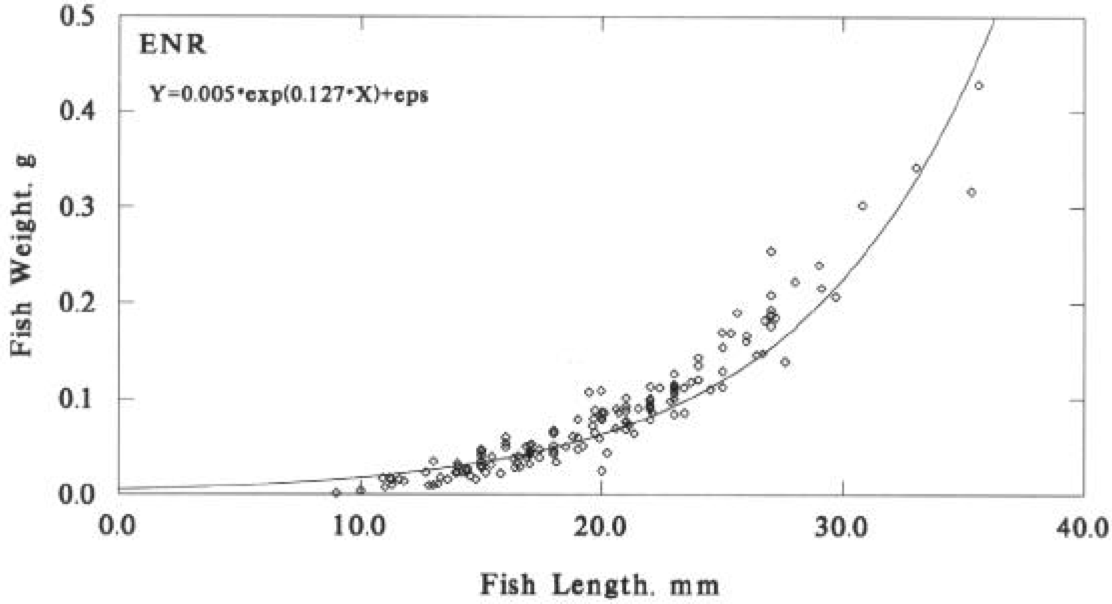
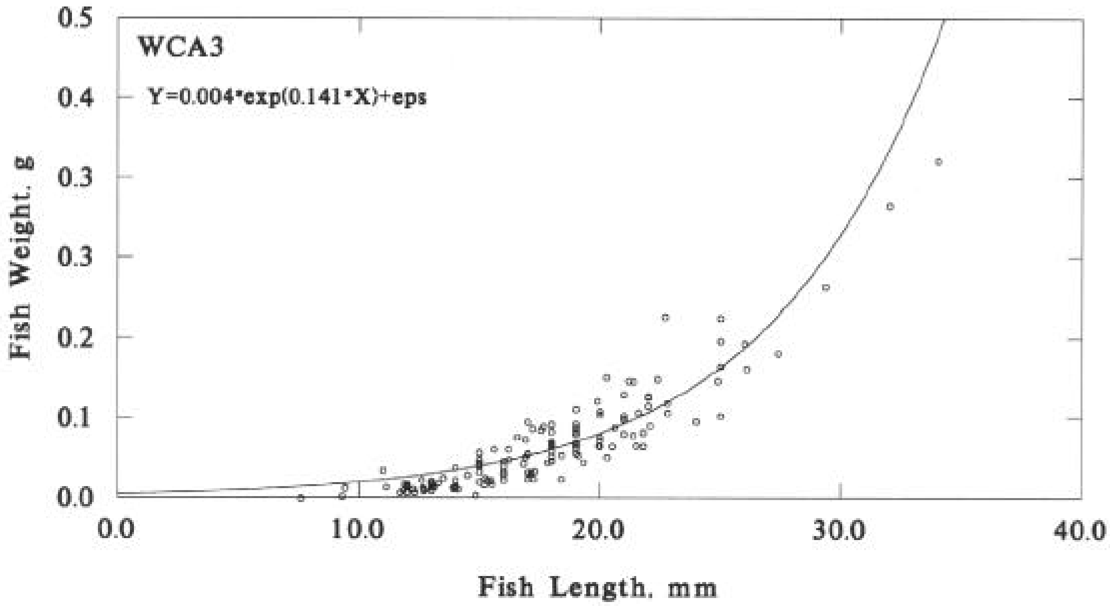
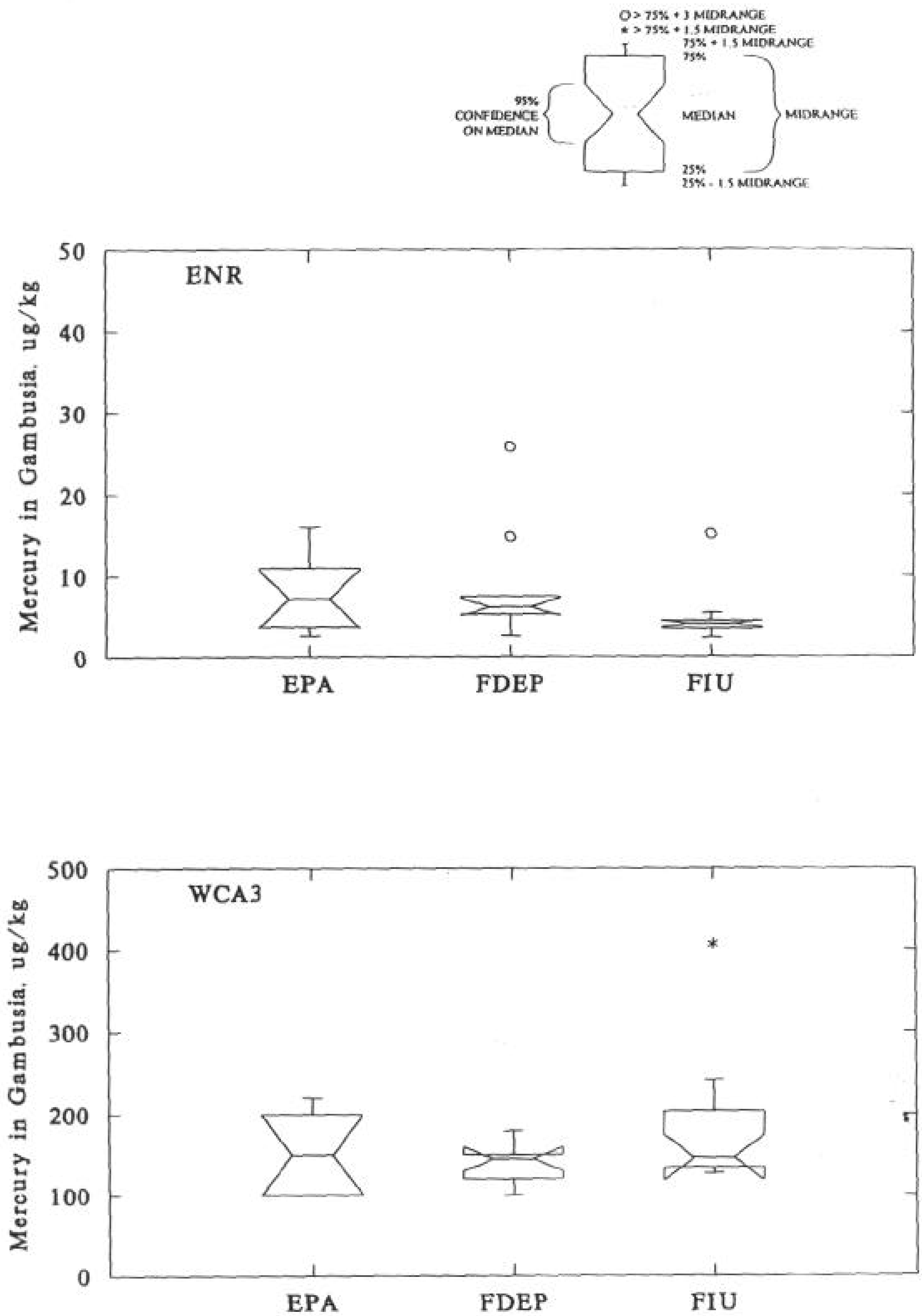


Figure D.14 Notched box and whisker plots comparing length of mosquitofish at the two marsh sites from the 1997 test sample.





D.15 Length versus weight of mosquitofish from the two sites in the 1997 test samples with derived growth curves.



D.16 Notched box and whisker plots comparing THg in mosquitofish analysis results from 3 labs.

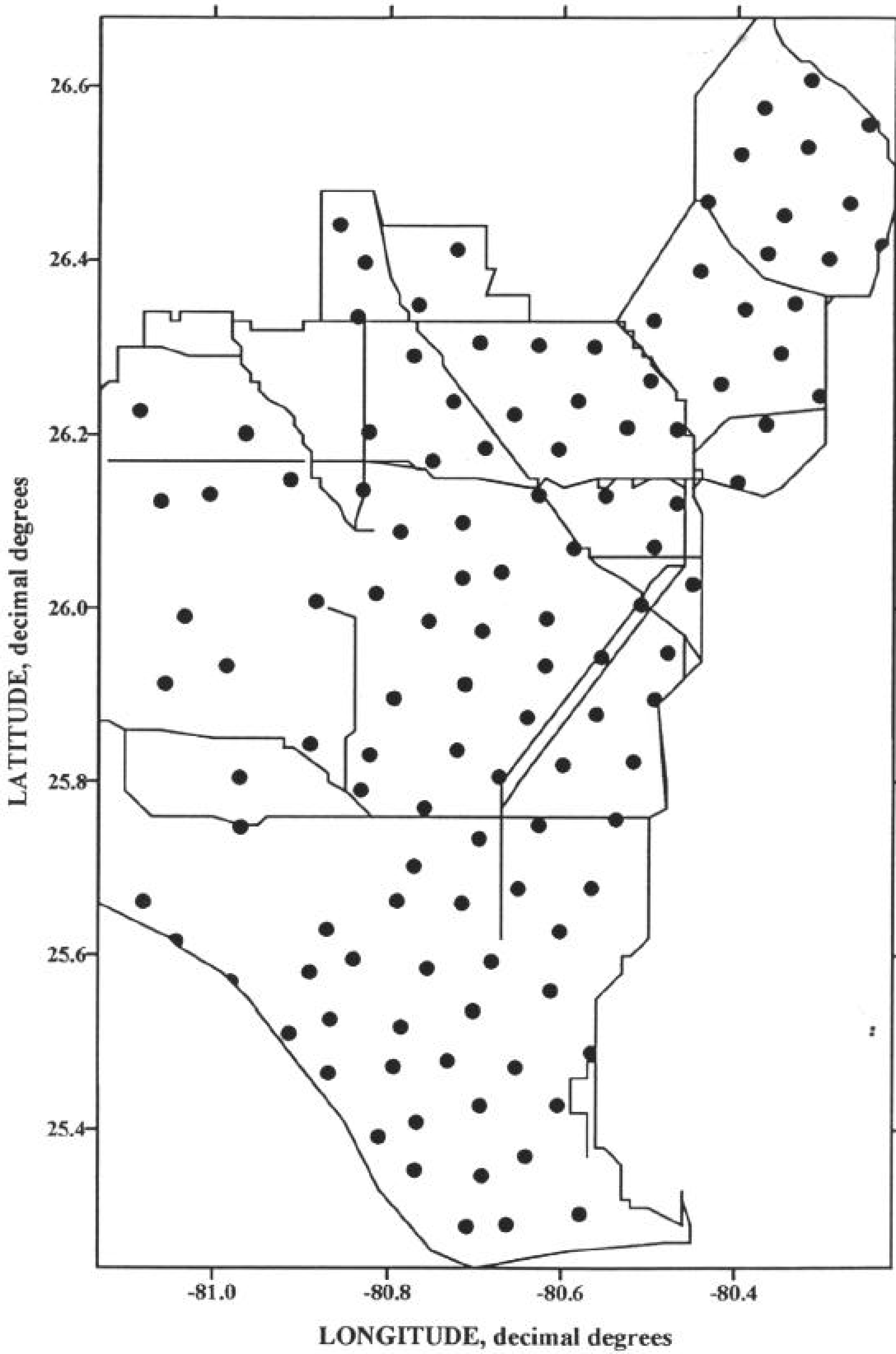


Figure D.17 Map of the study area indicating sites of sample collection.

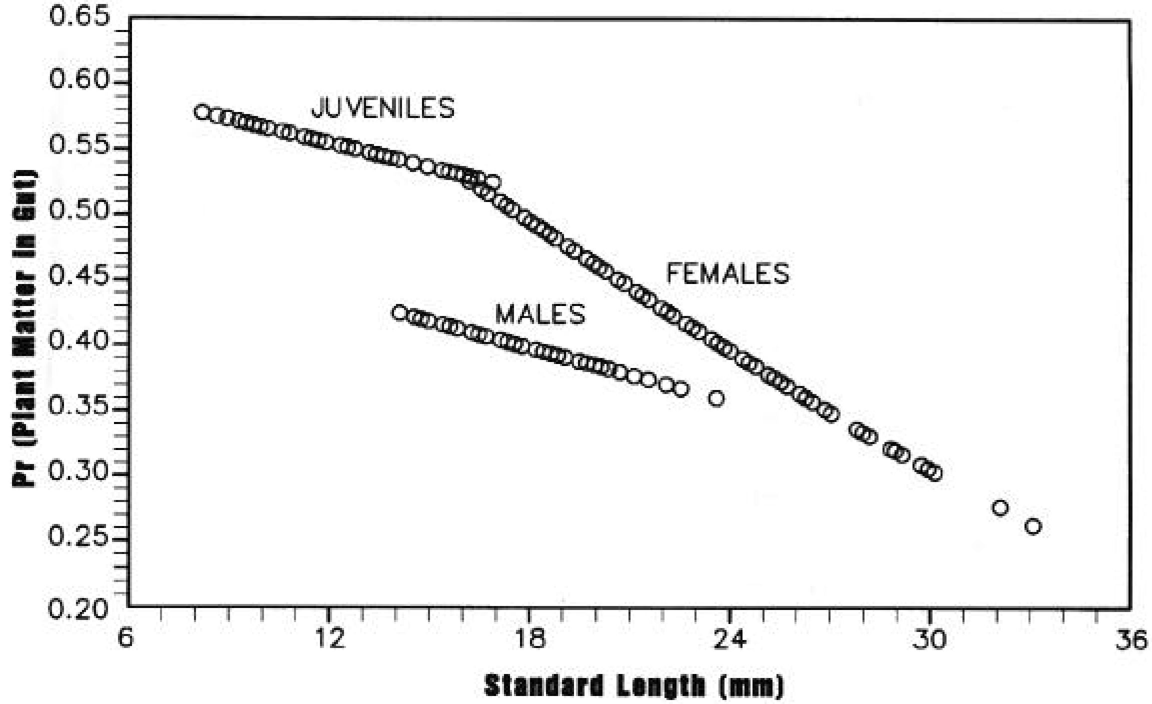


Figure D.18 Probability that an individual fish will have plant matter in its stomach relative to standard length. Based on logistic regression. Results for juveniles, males, and females are plotted separately.

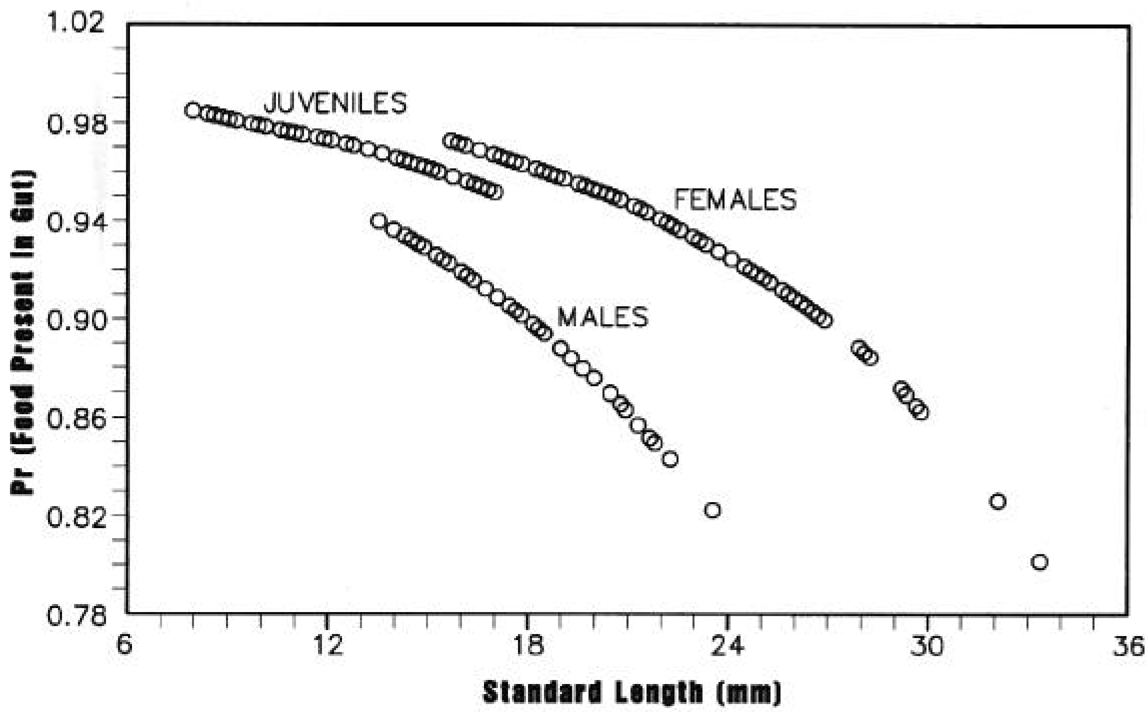


Figure D.19 Probability that an individual fish will have food in its stomach relative to standard length. Based on logistic regression. Results for juveniles, males, and females are plotted separately.

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