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Distribution, Habitat Preference and Population Size Estimate of *Etheostoma fonticola*

JOHN R. SCHENCK AND B. G. WHITESIDE

Etheostoma fonticola is an endangered species of fish living only in the San Marcos and Comal rivers, Texas, and the Dexter National Fish Hatchery, New Mexico. This species was reintroduced into the Comal River after extensive sampling revealed its absence, and introduced into the Dexter fish hatchery for protection and propagation.

E. fonticola was collected only in vegetated habitats. The habitats perferred by most of the fish had vegetation that grew close to the substrate; specifically, _Rhyzoclonium_ sp.

A conservative rough estimate of the number of _E. fonticola_ living in the San Marcos River watershed was 103,000.

ETHEOSTOMA fonticola is endemic to the spring-fed San Marcos and Comal rivers and is an endangered species of fish about which little is known. This fish was officially listed on the Federal Register for endangered species in 1970 (Fish and Wildlife Service, 1974). It is also listed as endangered by the Texas Parks and Wildlife Department (Floyd Potter, Texas Parks and Wildlife Department, Austin, pers. comm.), threatened by the Texas Organization for Endangered Species (1975) and rare by Miller (1972). *E. fonticola* has been reported from the San Marcos River, Texas (Jordan and Gilbert, 1886; Jurgens, 1951; Hubbs and Strawn, 1957; Texas Parks and Wildlife Department, 1959; Young, et al., 1973); the Comal River, Texas (Evermann and Kendall, 1894; Kuehne, 1955; and Hubbs and Strawn, 1957); the lower Nueces River, Texas (Texas Parks and Wildlife Department, 1965); Dickinson Bayou, Texas (Evermann and Kendall, 1894); and the Washita River, Arkansas (Jordan and Gilbert, 1886). Records from the Nueces River, Dickinson Bayou and the Washita River are discounted because of lack of verification and/or unsuitability of habitats in these areas. Jordan and Gilbert (1886) reported collecting the type specimens of *E. fonticola* in the San Marcos River immediately below the confluence of the Blanco River in 1884.

There is a paucity of literature concerning the ecology of *E. fonticola,* probably because of its localized distribution. While most of the literature involves general comments on habitat and distribution, Hubbs and Strawn (1957) used *E. fonticola* as experimental animals in hybridization studies, Strawn (1955, 1956) described breeding and raising this darter, and Strawn and Hubbs (1956) described a method of stripping ova and sperm from *E. fonticola.* We studied present distribution, habitat preference and population numbers of this species to provide a data base for protecting and monitoring the population.

MATERIALS AND METHODS

Study area.—The study area was the upper 8.4 km of the San Marcos River, Hays County, Texas (Fig. 1), and the entire Comal River, Comal County, Texas. Both rivers emerge as springs from the Edwards Aquifer in south-central Texas. Mean water discharge of the

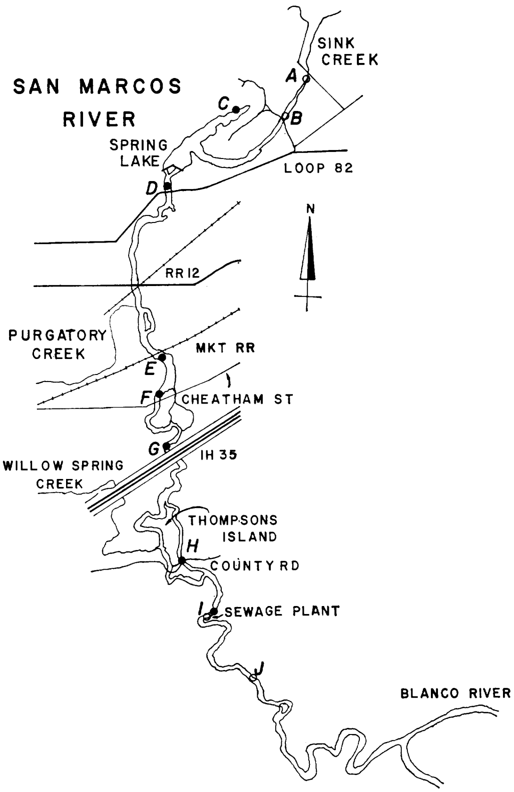

Fig. 1. Map of San Marcos River study area, Hays County, Texas. Open circles represent stations where no *Etheostoma fonticola* were collected and closed circles represent stations where *E. fonticola* were collected.

San Marcos Springs, during the study period March 1973 through April 1974, was 6.2 m^3/sec while that of the Comal Springs, during the same period, was 11.6 m^3/sec (Edwards Underground Water District, 1974).

Three creeks, one river and the effluent of a secondary sewage treatment plant discharge into the San Marcos River study area (Fig. 1). Sink Creek, largest of the three creeks, discharges large quantities of runoff from the north into Spring Lake (impounded origin of the river) and the Spring Lake dam backs water approximately 1.6 km into Sink Creek. The other two creeks, Willow Springs and Purgatory creeks, are dry except during high rainfall. The Blanco River, a major tributary, empties into the San Marcos River 6.4 km downstream from the headsprings. The effluent of the new San Marcos secondary sewage treatment plant, built in 1969, empties into the river 4.8 km downstream from the headsprings.

The Comal River flows approximately 5 km before emptying into the Guadalupe River. The upper end of the river has been dammed and developed into a municipal recreational area.

Water temperatures in the upper San Marcos River and the entire Comal River are relatively constant. Twelve monthly water temperature determinations were made during the study period. Means and ranges were: headwaters of the San Marcos River 21.8 C (21.0–22.0 C); just above the confluence of the Blanco River 22.9 C (20.4–25.0 C); Comal Springs 24.0 C (23.3–24.5 C); Comal River at the confluence with the Guadalupe River 23.7 C (21.0–26.0 C).

Ten collecting stations (stations A–J) were established in the San Marcos River study area (Fig. 1). Stations D, E, F, G, H and I were each divided into habitats according to type and amount of vegetation, flow, depth and/or substrate. Stations A, B, C and J were not divided into habitats. In addition to these stations, numerous areas of the river were sampled between Station J and the Old San Marcos River crossing (Westfield Crossing) approximately 2 km downstream from the confluence of the Blanco River. For a complete description of collecting stations and habitats, see Schenck (1975).

Several collecting stations were established in the Comal River but were abandoned when no *E. fonticola* were collected. An attempt was then made to sample all possible areas of the river in hopes of collecting *E. fonticola*, but none were found.

Field collections.—*E. fonticola* was collected with a long-handled (1.8 m) retangular dip net (71 × 46 cm) with a 1.6 mm square mesh. The dip net was held at arms length and embedded in the substrate downstream from the investigator and the gravel, rubble and vegetation were kicked vigorously toward the net for about 30 sec. Each attempt using this procedure, herein termed a "standard dip net haul," covered approximately 1.3 m^2 of the substrate. Therefore, the calculation of the number of fish/m^2 of substrate was possible. Seines of various sizes were used to supplement dip net samples in the Comal River. Our experience indicates that, in these heavily vegetated habitats, the dip net method is more efficient than seines for collecting *E. fonticola*.

Habitat preference.—The number of *E. fonticola* collected from three standard dip net hauls each month in each of 17 habitats (6 at Station D, 6 at Station F and 5 at Station G) was used as an indicator of the habitat preference of the fish.

Population size estimate.—The estimated numbers of *E. fonticola* in the San Marcos River and in one small area of Spring Lake (Station C) were based on standard dip net hauls from the two areas.

The river from the Spring Lake dam to the outfall of the new San Marcos secondary sewage treatment plant was divided into six contiguous segments (Fig. 2). Within each of the six segments a representative sampling section was established (Station D in Section 1, Station E in Section 2, Station F in Section 3, Station G in Section 4, Station H in Section 5 and Station I in Section 6). The area of each segment, each sampling section and each habitat within each sampling section was measured in July 1974.

The mean number of fish/m^2 in each habitat within the sampling sections of river segments 1, 3 and 4 was determined by averaging the number of fish/m^2 from monthly collections in each habitat (see Habitat Preference) at stations D, F and G, respectively. Ten standard dip net hauls were used to collect fish in each habitat in stations E, H and I in August 1974. The mean number of fish/m^2 in each habitat within the sampling sections of river segments 2, 5 and 6 was determined by averaging the number of fish/m^2 based on these 10 standard dip net hauls in each habitat of stations E, H and I, respectively.

By estimating the area of each habitat and the mean number of fish/m^2 in each habitat, it was possible to roughly estimate the number of fish in each habitat and thus the number of fish in each sampling section.

The number of fish in a river segment was estimated by dividing the total number of fish estimated for the sampling section of that river segment by the percentage of the area of the river segment composed of the sampling section. The estimates of the number of fish in each of the six river segments were summed which provided an estimate of the number of *E. fonticola* in the river.

The estimated number of *E. fonticola* at Station C was based on measurements and collections made in August 1974. The mean number of fish/m^2, based on 12 standard dip net hauls, was multiplied by the area of the station (36 $\times$ 3 m) to estimate the number of fish in this portion of Spring Lake.

In order for this population estimate to be accurate two criteria must be met: 1) that each sampling section was representative of the respective river segment and 2) that all the *E. fonticola* within the area of a standard dip net haul were collected. It is doubtful that these criteria were met entirely, especially (2). Thus, the population size was probably underestimated. However, the purpose of this estimate was to establish that there is a fairly large population of *E. fonticola* in the river system and not to establish a density estimate.

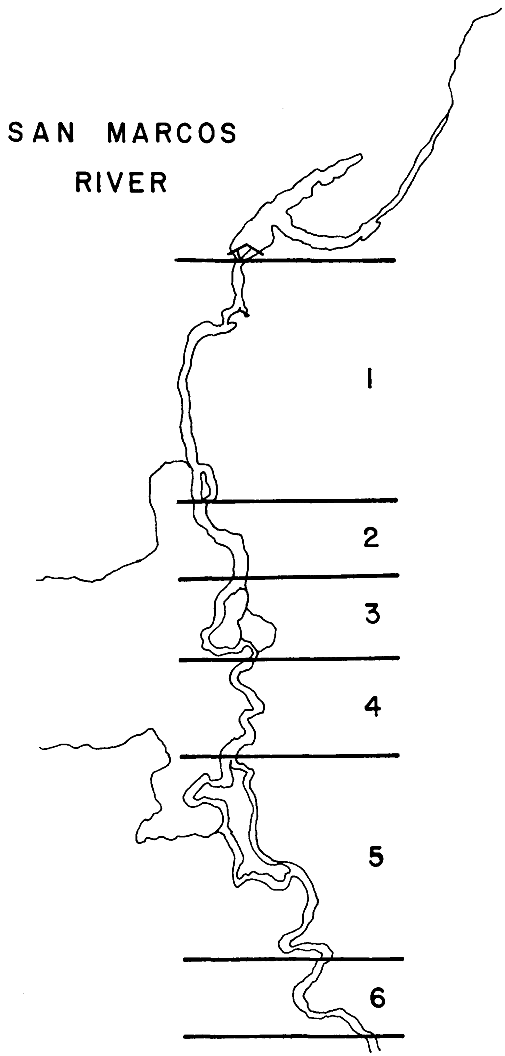

Fig. 2. Map of the San Marcos River study area showing six contiguous segments used in the estimate of population numbers of *Etheostoma fonticola*.

Results and Discussion

Distribution.—Our furthest downstream collection of this species was just above the outfall of the new San Marcos secondary sewage treatment plant. There are at least two possible reasons for the absence of *E. fonticola* below the sewage outfall. First, it is possible that the effluent from the sewage treatment plant caused the reduction in distribution. Second, in the early 1900's the river was dammed (Cumming's Dam) in the same area as Jordan and Gilbert's (1886) collection site which probably changed the habitat and could have eliminated the species from this area. Water in this segment is fairly deep and the river banks are sharply cut. These conditions restrict the growth of many types of vegetation which *E. fonticola* prefers in the upper reaches of the river.

Evermann and Kendall (1894) reported 43 specimens of *E. fonticola* from the Comal River in 1891, the first record for that locality. Hubbs and Strawn (1957) reported this species from the Comal River in 1954, the last record for that locality.

Since March 1973, we have spent 300 man-hours sampling the Comal River and no *E. fonticola* have been collected. There are at least three possible reasons why *E. fonticola* is absent from the Comal River. First, the Comal River was treated with rotenone in December 1951. Many specimens of desirable fishes, including *E. fonticola,* (Clark Hubbs, Univ. Texas, Austin, pers. comm.), were seined and held in a protected area until the rotenone dissipated (Ball, et al., 1952). This reduced the number of *E. fonticola* but apparently did not cause its immediate elimination, since the last collection of this species in the Comal River was in 1954. Second, Comal Springs ceased to flow for a six month period in 1956 which probably caused drastic temperature fluctuations in the remaining pools of water. Since *E. fonticola* occupies areas with constant water temperatures, it is likely that the temperature fluctuations resulted in the elimination of this species. Third, a flood from Blieders Creek inundated the entire Comal River in the spring of 1971 and may have removed any *E. fonticola* still present.

We are currently reintroducing *E. fonticola* into the headwaters of the Comal River. One hundred specimens from the San Marcos River were introduced in March 1975 and another 150 individuals in May and June 1975.

E. fonticola has also been introduced into a refugium for endangered species at the Dexter National Fish Hatchery in New Mexico. According to Clark Hubbs (pers. comm.), 50 fish were introduced in November 1974 and were found to be reproducing in March and September 1975.

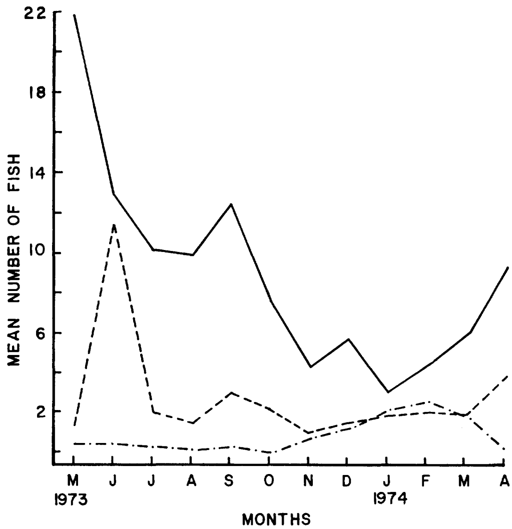

Fig. 3. Mean numbers of *Etheostoma fonticola* in three types of habitat: with vegetation growing close to the substrate (–); with vegetation with long leaves floating well above the substrate (– – –); and originally lacking vegetation (– • –). (Mean numbers of fish are based on three standard dip net hauls each month in each habitat.)

Habitat preference.—*E. fonticola* were collected only in vegetated habitats. A fish was occasionally collected in habitats originally described as lacking vegetation but these probably resulted from the growth of vegetation in the habitat during the study period. For example, fish were collected in habitat D_1 from December 1973 through the remainder of the study period after *Rhyzoclonium* sp. (filamentous algae) had become established in that habitat. No fish were ever collected in habitats D_6 and F_3 which supported no vegetation throughout the study period.

The habitats preferred by most *E. fonticola* had vegetation such as *Rhyzoclonium* sp., *Hydrilla* sp. (Florida elodea) and *Ludwigia* sp. (water primrose) which grew close to the substrate. To illustrate this further, the 17 habitats were separated into three groups. One group (seven habitats) contained habitats with vegetation which grew close to the substrate. A second group (four habitats) had plants such as *Potamogeton* sp. (pondweed), *Vallisneria* sp. (eel-

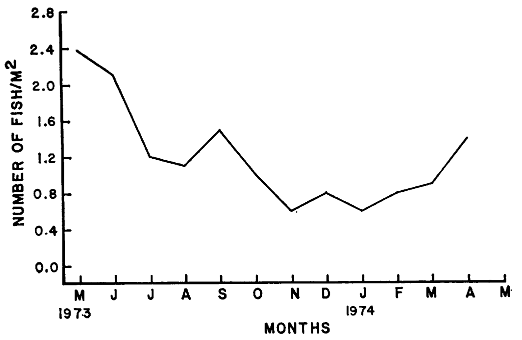

Fig. 4. Seasonal fluctuations in the mean number of *Etheostoma fonticola*/m² collected each month from all 17 habitats (stations, D, F and G) in the San Marcos River, Hays County, Texas.

TABLE 1. ESTIMATED NUMBER OF *Etheostoma fonticola* IN THE SAN MARCOS RIVER FROM THE SPRING LAKE DAM TO THE OUTFALL OF THE NEW SAN MARCOS SECONDARY SEWAGE TREATMENT PLANT. Area and estimated number of fish are also given for each of the six river segments.

River Segment	Area (m^2)	Estimated Number of Fish
1	32,147	37,027
2	18,384	37,500
3	10,437	14,961
4	14,605	12,665
5	21,472	813
6	6,588	30
San Marcos River	102,633	102,966

grass) and *Zizania* sp. (wild rice) with long leaves which floated well above the substrate. A third group (six habitats) contained habitats which originally lacked vegetation. The mean number of fish collected each month in each of these three habitat groups is shown in Fig. 3.

E. fonticola was most abundant in habitats with vegetation which grew close to the substrate, and was usually associated with certain species of plants. It seemed to prefer habitats with filamentous algae. Of 960 fish collected from the 17 habitats, 22.8% were collected in habitat F_1 which had a dense mat of *Rhyzoclonium* sp., 15.2% were collected in habitat G_5 which had *Rhyzoclonium* sp. and *Hydrilla* sp. and 21.2% of the fish were collected in habitats D_2 and G_4 containing only *Ludwigia* sp.

Strawn (1955) reported that fountain darters lived in *Vallisneria* beds in shallow, flowing water but he did not indicate if they were found in other types of vegetation. Only one small habitat, F_2, was sampled that contained *Vallisneria* sp. and this habitat contained only 3.4% of all the fish collected. Three other habitats with long-leaved plants were sampled. Habitat D_4 with only *Potamogeton* sp. contained 6.7% of all the fish collected while habitats F_6 and G_3 with only *Zizania* sp. contained 1.6%.

Even though *E. fonticola* was found predominantly in certain types of vegetation, its abundance fluctuated in response to seasonal fluctuations in the amount of vegetation. This was especially apparent in habitat F_1 which contained the dense mat of *Rhyzoclonium* sp. With the exception of the August and September 1973 samples, there was a progressive decrease in abundance of fish in this habitat from May 1973 through January 1974 which coincided with a descrease in the amount of *Rhyzoclonium* sp. This was followed by a progressive increase in abundance of fish as the amount of *Rhyzoclonium* sp. increased. Similar fluctuations in abundance of fish in response to changes in condition of the habitat were noted in other habitats to a lesser degree.

What happened to *E. fonticola* when its preferred type of habitat disappeared from an area is purely speculative. A general progressive decrease in density of fish at all 17 habitats occurred from May 1973 through January 1974 followed by a progressive increase until the study was terminated (Fig. 4). The general decrease in the number of fish present during the winter possibly resulted from a decrease in preferred habitats.

Winn (1958) determined habitat preferences of different age groups and sexes of 14 species of darters and concluded that the habitat type occupied depended upon the size and sex of the darters. He also indicated that the main differences in habitat preference between males and females were responses to spawning. We found that young *E. fonticola* preferred vegetated habitats in areas with little water flow but there was no indication whether males and females preferred different habitats or whether certain habitats were used more commonly as breeding grounds. However, Strawn (1955) indicated that aquarium-held fountain darters spawned almost exclusively on filamentous algae if it was present.

Population size estimate.—The total number of *E. fonticola* in the San Marcos River was estimated at approximately 103,000 (Tables 1 and

TABLE 2. ESTIMATED NUMBER OF *Etheostoma fonticola* BY HABITATS IN EACH SAMPLING SECTION OF EACH SAN MARCOS RIVER STATION (FIG. 1). The remaining area of each sampling section besides that given as habitats is designated as other.

Station and Habitat	Habitat Area (m^2)	Mean Number of Fish/m^2	Estimated Number of Fish
D_2	77	2.58	199
D_3	184	0.02	4
D_4	2,241	1.36	3,048
D_6	253	0.00	0
Other	67	0.00	0
Station total			3,241
E_1	2,176	3.15	6,854
E_2	1,749	0.69	1,207
E_3	210	4.46	937
E_4	210	0.08	17
Other	75	0.00	0
Station total			9,015
F_1	221	4.68	1,034
F_2	49	0.71	35
F_4	66	0.90	59
F_5	400	0.00	0
F_6	12	0.15	2
F_7	30	1.15	34
Other	34	0.00	0
Station total			1,164
G_1	96	0.19	18
G_2	32	2.29	73
G_3	200	0.00	0
G_4	186	1.77	329
G_5	234	3.12	730
G_6	400	0.00	0
Other	178	0.00	0
Station total			1,150
H_1	164	0.15	25
H_2	510	0.00	0
H_3	30	0.08	2
Other	10	0.00	0
Station total			27
I_1	20	0.08	2
I_2	392	0.00	0
Other	29	0.00	0
Station total			2

2) and the estimated number of fish at Station C was 339. The only population estimate of this species found in the literature was given in the book of rare and endangered wildlife of the United States (Office of Endangered Species and International Activities, 1973). The estimate was approximately 1,000 *E. fonticola* for the San Marcos River watershed and the method of estimation was not given.

We feel that this species is endangered because of its limited habitat. Because of the large San Marcos River population and because this species spawns year around (Schenck, 1975), we feel there is no immediate threat to the San Marcos River population with regard to scientific collecting. However, sampling the reintroduced Comal River population should be limited. Furthermore, the protection of *E. fonticola* requires control of the development along these rivers as well as limiting the pumping of water from the Edwards Aquifer.

ACKNOWLEDGMENTS

We gratefully acknowledge W. C. Young and D. G. Huffman for their constructive criticism of this manuscript. This research was supported in part by a faculty research grant from Southwest Texas State University.

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Karyotypes of Some Neotropical Turtles

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Analyses of meiotic and mitotic chromosomes are presented for eight species of Neotropical turtles. *Geochelone carbonaria* differs from *G. denticulata* by centromere position of one of the smaller macrochromosomes. *Geochelone carbonaria* is considered primitive in this respect because its karyotype is similar to that of the Old World batagurines. Three taxa of Greater Antillean *Chrysemys* (*C. terrapen, C. stejnegeri vicina, C. decorata*) possess a typical emydine karyotype and do not differ from other *Chrysemys* or other emydine genera that have been karyotyped. *Rhinoclemys punctularia* has two pairs of microchromosomes that are not found in *R. pulcherrima*. *Rhinoclemys punctularia* is considered to be karyotypically primitive to *R. pulcherrima* due to its closer similarity to the Old World batagurine karyotype. The karyotype of *Kinosternon scorpioides* from Trinidad is similar to previous reports of specimens from Brazil and of other species of *Kinosternon*.

NEOTROPICAL turtles have received little attention from cytogeneticists. This study presents karyological data from 27 specimens of eight taxa representing the families Kinosternidae, Testudinidae and Emydidae from the Carribbean region and Central America. The species include *Kinosternon scorpioides, Geochelone carbonaria, G. denticulata, Chrysemys* (= *Pseudemys*) *terrapen, C. stejnegeri vicina, C. decorata, Rhinoclemys pulcherrima,* and *R. punctularia*. Karyotypes of the first three species listed above were studied by other workers; (Barros et al., 1972; Sampaio et al., 1971; Stock, 1972) those of the last five taxa have not been reported previously. Analysis of both mitotic and meiotic chromosomes are presented here, and the phylogenetic implications are discussed.

MATERIALS AND METHODS

The family, species, number and sex of individuals and collecting localities are given in Table 1. Specimens were collected from natural populations (Table 1, localities 1–4, 6–8, 10) or purchased at food markets (Table 1, localities 5 and 9). The specimens from locality 10 (Table 1) are deposited in the U. S. National Museum; all others are deposited in The Museum, Texas Tech University.

The turtles were processed for chromosome preparations from spleen as described by Bickham (1975). Some individuals were also karyotyped from primary tissue cultures established from heart tissue and grown in Ham's F-10 medium fortified with 16% fetal calf serum.