

## A Survey of Metazoan Parasites in Unionid Bivalves of Garza-Little Elm Reservoir, Denton County, Texas

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### ABSTRACT

A survey of the parasites of unionid clams of Garza-Little Elm Reservoir indicated the presence of the following trematode-host relationships: *Aspidogaster conchicola* in *Amblema plicata*, *Amblema costata*, and *Anodonta corpulenta*; *Cotylaspis insignis* in *Lampsilis anodontoides*, *L. radiata hydiana*, *Proptera purpurata*, *A. corpulenta*; and *Bucephalus elegans* in *P. purpurata*, *L. anodontoides*, and *L. r. hydiana*. A new species of rhopalocercous gorgoderid cercaria was found in association with *Amblema plicata*. Water mites, *Unionicola* spp., were taken from *A. plicata*, *A. costata*, *Leptodea laevisima*, *Arcidens confragosus*, *A. corpulenta*, *P. purpurata*, *L. anodontoides*, *L. r. hydiana*, and *Quadrula quadrula*.

Statistical treatment indicated that probability of parasitic infection increases significantly with size (age) of the clam in the following relationships: *C. insignis* in *L. r. hydiana*, *B. elegans* in *P. purpurata*, and gorgoderid sporocysts in *A. plicata*.

Evidence suggesting parasitic castration of the host was noted in *Bucephalus* infections of *P. purpurata* and *L. r. hydiana* and in gorgoderid infections of *A. plicata*.

### INTRODUCTION

In the summer and autumn of 1969 a series of collections was made in an attempt to determine the parasitic fauna associated with the unionid bivalves from Garza-Little Elm Reservoir, Denton County, Texas.

In a previous survey of the bivalves of this drainage system (Elm Fork of the Trinity River, north-central Texas) Read (1954) reported the occurrence of hydrachnid mites and leeches (*Placobdella* spp.).

The only other consideration of parasites of bivalves in Texas prior to the present study was a survey by Gentner and Hopkins (1966) of the trematode fauna of clams in the Little Brazos River.

### MATERIALS AND METHODS

Clams were collected at Sycamore Bend Park on the Hickory Creek arm of Garza-Little Elm Reservoir. Specimens were collected periodically from July through November at water depths ranging from 2 inches to 5 feet.

Clams were examined microscopically for both exo- and endoparasites. Each bivalve was measured to the nearest 0.5 cm and was sexed either by the general appearance of the gonad and the material expressed therefrom or by the presence of eggs or glochidia in the gills.

Determination of age for each clam proved difficult because of the indistinct delineation of growth lines in many specimens. Valve length was therefore chosen as an alternate index of age. An average size was determined for each successive growth period in each species of clam on the basis of several shells bearing relatively distinct growth lines.

Parasites were fixed in A.F.A. and stored in 70% alcohol. Mites were cleared in lactophenol and mounted in glycerine jelly for study. Trematode specimens were stained with Harris' or Ehrlich's hematoxylin or precipitated borax carmine slowly cleared in ascending concentrations of xylene and mounted in Piccolyte.

The t-statistic was applied to test the hypothesis that probability of infection with trematode species increased with size (age) of the host. The level of significance was set at  $P \leq 0.05$  for the one-tailed test. A 2-way contingency table was employed to test for a significant relationship between trematode sporocyst infection and atypical gonad structure and function observed in several bivalve specimens ( $\alpha = 0.05$ ).

#### RESULTS

Nine species of unionid clam were taken in collections from Garza-Little Elm Reservoir. The bivalve species and numbers of each collected were 146 *Amblema plicata* (Say), 4 *Amblema costata* Rafinesque<sup>1</sup>, 4 *Leptodea laevisissima* (Lea), 4 *Arcidens confragosus* (Say), 11 *Anodonta corpulenta* Cooper, 62 *Proptera purpurata* (Lamarck), 12 *Lampsilis anodontoides* (Lea), 14 *L. radiata hydiana* (Lea), and 51 *Quadrula quadrula* Rafinesque.

Parasites identified included adults of 2 aspidobothrean trematodes, *Aspidogaster conchicola* Baer and *Cotylaspis insignis* Leidy; sporocysts and cercariae of 2 digenetic trematodes, *Bucephalus elegans* Woodhead and a new species of rhopalocercous cercaria; and at least 2 species of water mite, *Unionicola* Haldeman.

The parasites associated with each bivalve species and their frequency of occurrence are presented in Table 1. For more comprehensive treatment each parasitic species and its hosts are discussed individually.

Only a few specimens were found to be free of any of these parasites.

<sup>1</sup> Despite the conclusion of Clarke and Clench (1966) that *A. costata* is merely an ecophenotype of *A. plicata*, these forms are treated here as distinct species. All specimens of both types observed were completely sympatric and morphologically distinct; no intergrades were found.

The species of unparasitized specimens and the ratio of these specimens to total sample for that species are: 2/146 *Amblema plicata*, 1/4 *Amblema costata*, 1/4 *Leptodea laevissima*, 1/4 *Arcidens confragosus*, and 2/14 *Lampsilis r. hydiana*.

TABLE 1

Frequency occurrence of parasites from host species

Host	Unparasitized	<i>Aspidogaster conchicola</i> (Adult)	<i>Cotylaspis insignis</i> (Adult)	<i>Bucephalus elegans</i> (Sporocyst)**	<i>Cercaria</i> sp. (Sporocyst)	<i>Unionicola</i> spp.	Concurrent Trematode Infections
<i>Amblema plicata</i>	1.37% (2/146) <sup>a</sup>	2.74% (4/146)	---	---	5.48% (8/146)	98.63% (144/146)	---
<i>Amblema costata</i>	25.00% (1/4)	50.00% (2/4)	---	---	---	75.00% (3/4)	---
<i>Leptodea laevissima</i>	25.00% (1/4)	---	---	---	---	75.00% (3/4)	---
<i>Arcidens confragosus</i>	25.00% (1/4)	---	---	---	---	75.00% (3/4)	---
<i>Anodonta corpulenta</i>	---	9.09% (1/11)	72.73% (8/11)	---	---	100% (11/11)	<i>C. insignis</i> - <i>A. conchicola</i> 9.09% (1/11)
<i>Properia purpurata</i>	---	---	*20.97% (13/62)	*35.48% (22/62)	---	100% (62/62)	<i>C. insignis</i> - <i>B. elegans</i> 6.45% (4/62)
<i>Lampsilis anodontoides</i>	---	---	*33.33% (4/13)	*58.33% (7/12)	---	100% (12/12)	<i>C. insignis</i> - <i>B. elegans</i> 75.00% (3/12)
<i>Quadrula quadrula</i>	---	---	---	---	---	100% (5/5)	---
<i>Actinonais carinata</i>	14.29% (2/14)	---	*35.71% (5/14)	*35.71% (5/14)	---	85.71% (12/14)	<i>C. insignis</i> - <i>B. elegans</i> 21.43% (3/14)

<sup>a</sup> Denominator of fraction indicates total size of mollusk sample in that row; numerator indicates number of mollusks in the category of that column

\* New host record

\*\* First locality record for Texas

Erratum: For *Actinonais carinata* read *Lampsilis radiata hydiana*.

#### *Aspidogaster conchicola* Baer, 1827

Infections of *Aspidogaster conchicola* Baer, 1827, were observed in the following clams: *Amblema plicata* (Say) (frequency=4 infected/146 clams collected), *Amblema costata* Raf. (f=2/4), and *Anodonta corpulenta* Cooper (f=1/11).

This aspidobothrean was always found within the pericardial cavity and kidneys of its host. Only adult stages were noted, although the larvae are known to develop in the same host species as the adult.

No relationship between clam size (age) and *Aspidogaster* infection was statistically demonstrable.

The modal burden for all host species was 1 *Aspidogaster*; however, occasional specimens harbored surprising numbers of this fluke, the heaviest infection noted being 22 worms in a single *Amblema plicata*.

*Cotylaspis insignis* Leidy, 1857

Specimens of *Cotylaspis insignis* Leidy, 1857, were taken from the following unionids: *Anodonta corpulenta* Cooper (frequency=8 infected/11 clams collected), *Proptera purpurata* (Lamarck) (f=13/62), *Lampsilis anodontoides* (Lea) (f=4/12), and *L. r. hydiana* (Lea) (f=5/14). *P. purpurata*, *L. anodontoides*, and *L. r. hydiana* are new host records.

These flukes were invariably found in the suprabranchial chamber of the bivalve on the surface of the body wall overlying the kidneys. No immature stages of *Cotylaspis* were observed, although the larvae of this aspidobothrean probably develop in the same host as the adult.

Only in *Lampsilis r. hydiana* could any relationship be statistically demonstrated between host size (age) and *Cotylaspis insignis* infections ( $t=2.878$ ,  $d.f.=10$ ,  $0.005 < p < 0.01$ ). The mean size of infected *L. r. hydiana* was 7.20 cm., representing approximately 10 growth periods (range: 6.7–7.5 cm., 10–11 growth periods). Uninfected *L. r. hydiana* averaged 5.79 cm., 9 growth periods (range: 4–7 cm., 6–10 growth periods).

The density of *Cotylaspis insignis* infections per host varied between 1 to 7 for each unionid species infected. The size of individual burden was not directly related to the size of the host.

*Bucephalus elegans* Woodhead, 1929

*Bucephalus elegans* Woodhead, 1929, was found to parasitize *Proptera purpurata* (Lamarck) (frequency=22 infected/62 clams in sample), *Lampsilis r. hydiana* (Lea) (f=5/14), and *Lampsilis anodontoides* (Lea) (f=7/12). This is the first documented report of *B. elegans* from Texas.

The ramified sporocyst was easily visible upon host dissection as a system of filamentous tubules pervading the gonad and digestive gland. In many mussels thick bundles of sporocyst rami completely filled the lumina of the branchial blood vessels—to such an extent, in fact, that it was difficult to understand how circulation could have been adequate to support life. Nevertheless, many specimens so infected were successfully maintained under the additional stress of laboratory conditions for as long as 6 months prior to sacrifice.

Large numbers of *B. elegans* cercariae were released daily from hosts maintained in the laboratory.

Only in *Proptera purpurata* was the size (age) of *Bucephalus*-infected specimens found to be significantly greater than that of the uninfected group ( $t=2.703$ ,  $d.f.=49$ ,  $P < 0.01$ ). The mean valve length of infected *Proptera* was 11.88 cm., representing an "age" of approxi-

mately 11 growth periods (range: 9–17 cm., 9–17 growth periods). Uninfected *Proptera* averaged 9.88 cm., 9 growth periods (range: 3.5–16 cm., 4–16 growth periods).

Although *Bucephalus*—infected unionids showed little other gross evidence of damage, it was noted that sex determination was impossible for many infected specimens. The gonads of these individuals appeared watery and lacked any evidence of sperm or egg production. Since there was an alternative possibility that these were simply individuals having spent their gametes, a test for statistically significant difference between infected and uninfected clams was performed. Significance was indicated in *Proptera purpurata* ( $\chi^2=33.642$ , d.f.=1,  $P<0.005$ ) and in *L. r. hydiana* ( $\chi^2=7.819$ , d.f.=1,  $0.005<P<0.010$ ). Although not statistically significant, 3 of the 4 infected *Lampsilis anodontoides* showed the condition, whereas none of the 4 uninfected exhibited this condition.

#### Rhopalocercous Cercariae

Sporocysts of the gorgoderid rhopalocercous cercariae were found in 5.48% (8/146) of *Amblema plicata* (Say). The sporocysts were present in large numbers in the lymph spaces of the gonad, digestive gland, and renal wall of the host. It was noted that viability could be maintained for as long as 3 weeks in sporocysts left in the dissected host and kept at temperatures near 0°C.

Numerous *A. plicata* bearing infections of these gorgoderid sporocysts displayed an abnormal condition of the gonadal tissue much like that noted in *Bucephalus* sporocyst infections in other host species. Since some uninfected specimens of *A. plicata* also had such an appearance, the chi-square statistic was applied to test for significant difference. The frequency of the atypical gonadal condition was shown to be significantly greater in the infected group ( $\chi^2 = 13.261$ , d.f. = 1,  $P<0.0005$ ).

Statistical analysis for a host size-infection relationship indicated that clams of the infected sample were significantly larger than those of the uninfected sample ( $t=1.8172$ , d.f.=134,  $0.025<P<0.05$ ). The average valve length of infected *Amblema plicata* was 10.58 cm., representing an "age" of approximately 8 growth periods (range: 9–15.5 cm., 7–9 growth periods). The mean valve length of uninfected *A. plicata* was 8.97 cm., representing approximately 7 growth periods (range: 4–12.5 cm., 4–9 growth periods).

#### CONCURRENT TREMATODE INFECTIONS

All 3 species of bivalves found to serve as hosts for *Bucephalus*

*elegans* were also found to harbor *Cotylaspis insignis*. Concurrent infections with both of these flukes were noted with following frequencies: 4/62 *Proptera purpurata*, 3/12 *Lampsilis anodontoides*, 3/14 *L. r. hydiana* (see Table 1). No trematodes other than *C. insignis* were found in the same host species as *B. elegans*.

*Aspidogaster conchicola* was found in the same host species as the rhopalocercous cercariae, however, no concurrent infections were noted. *Aspidogaster conchicola* was also found to share one host, *Anodonta corpulenta*, with *Cotylaspis insignis*. Only one of the 11 *A. corpulenta* examined bore an infection by both of these parasites concurrently.

In all host-parasite relationships where concurrent trematode infections were noted, the chi-square statistic showed the probability of infection with one of the parasites to be independent of the probability of infection with the other. Likewise the absence of any concurrent infections of *Aspidogaster conchicola* and the gorgoderid sporocyst in *Amblema plicata* was found to be statistically insignificant because of the low frequency of occurrence of each parasite and the relatively small size of the sample.

#### *Unionicola* spp.

All bivalve species examined bore infections of the water mite *Unionicola*. At least 2 species of genus *Unionicola*, subgenus *Pentatax* were involved in these infections as indicated by the arrangement of genital acetabula and conformation of coxal plates. Positive specific identifications were not, however, attempted.

*Unionicola* infections were nearly universal in individuals of each host species. The frequency of infections for each host species were as follows: *Amblema plicata*,  $f=144/146$ ; *Amblema costata*,  $f=3/4$ ; *Leptodea laevissima*,  $f=3/4$ ; *Arcidens confragosus*,  $f=3/4$ ; *Anodonta corpulenta*,  $f=11/11$ ; *Proptera purpurata*,  $f=62/62$ ; *Lampsilis anodontoides*,  $f=12/12$ ; *Quadrula quadrula*,  $f=51/51$ ; *Lampsilis r. hydiana*,  $f=12/14$ .

All principal developmental stages of unionicolids (egg, larva, nymph, and adult) were noted in each host species. Teleiochrysalids were seen in all but *Amblema costata*, *Leptodea laevissima*, *Arcidens confragosus*, and *Lampsilis r. hydiana*. The relative numbers of parasites and sites of various stages within the host varied markedly among host species.

Certain gross symptoms of pathogenicity were noted in hosts bearing especially heavy infections (particularly *Proptera purpurata*, *Leptodea laevissima*, *Anodonta corpulenta*, *Lampsilis anodontoides*,

and *L. r. hydiana*). These symptoms included moderate hypertrophy of mantle and visceral mass tissues surrounding embedded eggs and chrysalids. In *Proptera purpurata* scattered clusters of mite eggs appeared to be dead and degenerating, the eggs having become densely opaque and brown in color. The surrounding host tissue showed a yellowish to brownish pigmentation.

#### DISCUSSION

##### *Probability of Trematode Infection as a Function of Host Size*

Inspection of data compiled in this survey suggested that there was some relationship between probability of infection by some trematode species and the size of the host mollusk. That is, the incidence of parasitism increased in frequency with the size of the hosts. Each trematode-mollusk relationship was therefore analyzed statistically to test this hypothesis. The t-test confirmed such a relationship only for *Cotylaspis insignis* in *Lampsilis r. hydiana*, *Bucephalus elegans* in *Proptera purpurata*, and the gorgoderid sporocyst in *Amblema plicata*.

Assuming that a relationship exists between size of the mollusk and the incidence of parasitism, the exact nature of the relationship remains to be explained. Since growth rate did not appear to differ markedly between infected and uninfected age-mates of the same species, the explanation could not be one of parasite-induced gigantism. Menzel and Hopkins (1955), in a study of *Bucephalus cuculus* in *Crassostrea virginica*, suggested that bivalve host growth was only slightly accelerated during the first season after infection and was highly retarded in following seasons.

The most obvious conclusion is that the older, and therefore larger, clams have been exposed longer to the infective stages of the parasite, thereby increasing the probability of infection. However, Bowers and James (1967) suggested that a similar situation in *Cercaria bucephalopsis haimeana* infections of *Cardium edule* could be explained by the fact that larger bivalves create stronger inhalant currents, so that more infective stages are drawn into the mantle cavity. It cannot, of course, be discounted that certain physiological changes accompanying maturation and aging (as indicated by size) might in some instances facilitate infection. In fact, James (1968) has suggested that in the parasitism of the gastropod, *Littorina saxatilis*, by certain digenetic flukes, infection can be achieved only in reproductively spent mollusks. It is not known, however, to what extent such a pattern might apply to the relationship in question here.

Although this study indicated that in certain host-parasite relationships the probability of trematode infection increases with the size of

the clam, the cause of this phenomenon still remains to be explained.

#### *Parasitic Pathogenicity*

In determination of sex of the clams collected it was found that the gonads of certain parasitized individuals were so atypical in appearance that sex could not be established. This gonadal material differed from the normal primarily in the absence of any identifiable gamete production. Although most specimens were examined immediately before and during the regular breeding season, when it would be expected to find spent individuals having this appearance, the frequency of this condition was notably higher among those clams infected with *Bucephalus elegans* and with the gorgoderid sporocyst. The chi-square statistic confirmed a significantly greater probability of this condition in rhopalocercous gorgoderid infections of *Amblema plicata* and in *Bucephalus elegans* infections of *Proptera purpurata* and *Lampsilis r. hydiana*.

The possibility of parasitic castration of the host is suggested by these observations. Similar damage to marine bivalve species by bucephalid sporocysts is a well-established phenomenon (Cheng and Burton, 1965; Sanders, 1966). Effects of bucephalids in freshwater unionid hosts have been cited by Kelly (1899) and Kniskern (1952). Kelly stated that parasitic castration is commonly encountered in *Bucephalus*-infected clams. Castration of *Lampsilis siliquoidea* by *Rhipidocotyle septapillata*, another bucephalid, was noted by Kniskern.

According to Cheng and Burton (1965) the histopathology of *Bucephalus* infections in *Crassostrea virginica* involves disruption of the connective tissues of the gonad, degeneration of the germinal epithelium, and resorption of the ova.

Biochemical changes in tissues of *Bucephalus*-infected oysters have also been demonstrated. Cheng (1965) recorded a reduction in neutral fats and fatty acids in the Leydig cells between digestive gland diverticula and a concurrent increase in the amount of these 2 types of material in the diverticular cells themselves. Carbohydrates also undergo marked changes in kind and distribution. Cheng and Burton (1966) found reduced glycogen and glucose levels in Leydig cells, tissues of the palps, gills and mantle of hosts bearing *Bucephalus* sporocysts. Concurrent circulatory hyperglycemia was also evident, indicating that the parasites had disrupted the centers of glycogen synthesis in those tissues. In addition, parasitized bivalves showed a markedly reduced number of ova.

Pathological effects similar to these may be sources of the gonad alterations seen in *Bucephalus elegans* and rhopalocercous gorgoderid



infections. However, more detailed histological examination would be required to confirm a diagnosis of parasitic castration.

Gross inspection indicated that sporocysts of *B. elegans* and of the rhopalocercous gorgoderid were mainly confined to the lymph spaces of the gonad, digestive gland, renal walls, etc. This observation conforms to the pattern of sporocyst dispersal described for most other trematode infections in mollusks (Cheng and Snyder, 1962; Cheng and Burton, 1965; Wright, 1966). In gorgoderid infections sporocysts were noted to occlude completely the vessels which were easily visible in the walls of the kidneys. These vessels displayed a somewhat knotted appearance, each swelling marking a distention of the walls to accommodate a sporocyst within. Likewise, massive bundles of sporocyst branches of *B. elegans* filled the lumina of most of the vessels of the renal wall and of the gills. Such situations must undoubtedly be pathogenic, not only because of mechanical distortions of host tissues from sheer mass of the sporocysts, but also because of the extensive circulatory obstruction produced.

No gross pathological symptoms were seen in hosts bearing aspidogastrid infections. *Cotylaspis insignis* was confined to the exterior surface of the renal cavities; *Aspidogaster conchicola* was found only within the renal and pericardial cavities. In no case were encysted *A. conchicola* found in other tissues of the host, as has been reported by Pauley and Becker (1968) for infections in *Gonidia angulata*. Pauley and Becker also noted marked distortion and distention of the renal cavities in *Anodonta* spp. infected with *A. conchicola*. This effect was not observed in any host examined in this study.

The most significant pathogenicity of *Unionicola* spp. was associated with the eggs, larvae, and chrysalids embedded in the mantle and surface of the visceral mass in certain hosts. Not only did the surrounding host tissues show some degree of hypertrophy, but in certain individuals there was evidence of response marked by a deposition of yellowish pigment around the embedded parasites. These eggs and larvae were often dead and degenerating and discolored a dark yellow or brown. These observations suggest an active defensive response by the host whereby the parasites are in some way walled off and destroyed.

#### *Concurrent Infections*

The probabilities of the concurrent trematode infections observed proved statistically to be equal to the product of the individual probabilities of the constituent infections; that is, the individual probabilities were shown to be independent. This fact therefore discounts any significant influence by the presence of one trematode species on the success of subsequent infection by the other trematodes observed for that

same host. This is not surprising since the trematode species found to employ the same host species occupy different niches within the host.

#### *Incidence of Parasites*

This study establishes for *Cotylaspis insignis* 3 new host records: *Proptera purpurata*, *Lampsilis anodontoides*, and *L. r. hydiana*. However, it should be mentioned that the account by Kelly (1899) of *Cotylaspis* sp. in numerous unionid hosts, including *Lampsilis anodontoides* and *Lampsilis luteolus* (= *L. radiata hydiana*?), is probably a reference to infections by *Cotylaspis insignis*. The only other record of *C. insignis* from Texas is that of Gentner and Hopkins (1966), who reported it from *Quadrula forsheyi* (= *Quadrula quadrula*) and *Anodonta corpulenta* of the Little Brazos River.

It is notable that neither in this study nor in that of Gentner and Hopkins was *Cotylaspis insignis* reported from *Amblema plicata* (= *Amblema perplicata* of Gentner and Hopkins), although Hendrix (1968) reported it from *Amblema peruviana* (= *A. plicata*) in the Tennessee River of Tennessee, Alabama, and Kentucky. It would be expected that the *A. plicata* of Texas would as likely host *Cotylaspis insignis* as would those of the Tennessee drainage since *C. insignis* is present in both regions. Mention has been made elsewhere in this paper that there is considerable confusion at present surrounding the taxonomy of the unionid genus *Amblema*—whether it is represented in North America by 2 distinct taxa or merely by 2 ecophenotypes of a single species, *A. plicata*. It is suggested that comparison of data on parasitic fauna could contribute much toward the clarification of the taxonomic positions of otherwise similar host forms. Such evidence in this particular case would seem to imply that the form of *Amblema* dealt with by Hendrix (1968) is not the same species mentioned in the 2 Texas studies, thereby supporting the case for the existence of at least 2 distinct species of *Amblema*.

*Cotylaspis insignis* burdens noted in this study were considerably smaller than those noted by Gentner and Hopkins (1966), at least in *Anodonta corpulenta*. The present study showed an average of 2.5 *C. insignis* per *A. corpulenta*, the maximum number observed being 6 per host. According to Gentner and Hopkins, each *A. corpulenta* of the Little Brazos River bore an average of 24.7 *C. insignis*. The discrepancy between burdens observed in these two studies may be evidence for a seasonal fluctuation in size of parasite populations. Collections for the present study were made during late summer and fall; those of Gentner and Hopkins were made in late winter and spring (February–May). This possibility merits further investigation.

This study establishes both new host and locality records for *Bu-*

*cephalus elegans*. Previously the only recorded intermediate host was *Micromya iris* (Lea). However, the *Bucephalus* sp. of Kelly (1899) probably was *B. elegans*. Kelly noted this species in *Anodonta corpulenta*, *Lampsilis luteolus* (= *L. radiata hydiana?*), and *Lampsilis anodontoides*. (In this study *A. corpulenta* was not found to be parasitized by *Bucephalus*.) No record could be found of *B. elegans* collected from hosts, intermediate or definitive, from Texas. However, adults have been taken from nearby Lake Texoma, just within the state of Oklahoma (Red River drainage system) by McDaniel (1963).

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