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FACTORS INFLUENCING DISTRIBUTION OF MUSSELS IN THE BLANCO RIVER OF CENTRAL TEXAS

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ABSTRACT

Environmental parameters that influence the distribution of eight mussels in the Blanco River of Central Texas were studied. The effects of type of substrate, stream flow rate and physicochemical features on mussel distribution were evaluated, but emphasis was given to the role of organic enrichment of the river by a city sewage treatment plant. From tolerance tests to ammonia and low oxygen on five local mussels and from field studies, the following remarks can be made about mussel distribution in the Blanco River.

(1) Low dissolved oxygen levels (0 - 0.5 mg O_2 l^{-1}*) proved lethal to 47% of the mussels tested in seven days.*

(2) Levels of 5 mg NH^+_4 *-* $NH_3 l^{-1}$ *(pH 7.8 to 8.0;* NH_3 *- N = 0.26 mg -1) were lethal to 40% of the mussels tested in seven days.*

(3) Corbicula manilensis *was more tolerant, and* Amblema p. plicata *less tolerant than the other mussels tested to elevated ammonia and low oxygen concentrations associated with sewage enrichment.*

(4) Even though the physicochemical parameters did not indicate stressful conditions on the days sampled, mussels of the Blanco River seemed to have been adversely effected by enrichment from the secondary sewage treatment plant of San Marcos. Fewer mussels were found downstream from the sewage plant than upstream, even where the river bottom, depth, and flow rates were similar.

INTRODUCTION

The use of freshwater mussels (Bivalvia) as aquatic indicators of ecological changes brought about by agriculture, mining practices, effluents from industrial and/or domestic disposal plants has not been studied extensively. Freshwater mussels might be valuable indicators of both past and present ecological conditions of aquatic environments.

For the following reasons, mussels might be especially good as indicators of stream conditions.

(1) Unlike plankton or free swimming fauna, bivalves as benthic invertebrates usually remain in relatively fixed positions in streams (Weber 1973).

(2) Mussels can directly absorb nutrients, simple organic compounds (Churchill 1916) and various pollutants. Such pollutants might be pesticides, radioactive materials and heavy metals which often would show up in biologically magnified concentrations (Weber 1973; Butler 1965; Fuller 1974; Bedford *et al.* 1968; Mathis and Cummings 1973). Bivalves also indirectly reflect ecological conditions by taking up pollutants by feeding from the basic trophic levels or aquatic food chains (Fuller 1974).

(3) The freshwater mussels (Unionacea) have relatively long life cycles, up to 17 years and longer (Williams 1969; Bedford *et al.* 1968). Therefore, their community and population structures are accumulatively affected by environmental perturbations (Weber 1973).

(4) Unlike periodic chemical analyses, the benthic mussels are continuously exposed, except when buried, to the water conditions and might reflect variable or infrequent discharges of pollutants (Weber 1973).

Our present knowledge is insufficiently detailed, however, to define Unionaceae or Sphaeriidae (Pisidiidae) as pollutional indicators in chemical terms (Fuller 1974; Ingram 1967). A great deal of work needs to be done on identifying the reactions of bivalves to specific natural factors in the environment, and on the reactions of mollusks to pollutants (Butler 1965).

Recently, Neel and Allen (1964) noted the decimation of various mussel populations in the upper Cumberland Basin by coal mine acids, while Charles (1964) found that very heavy populations of mussels have been virtually destroyed by brine pollution from oil wells. Even potassium has been suggested to regulate the survival and distribution of freshwater mussels (Imlay 1973).

Because they concentrate certain pollutants otherwise not detectable in water or sediments, mussels have been utilized as indicators of pesticide and metal pollution (Bedford *et al.* 1968; Mathis and Cummings 1973). The bivalves concentrated both pesticides and metals in higher concentrations than was found in the surrounding water, but contained lower levels of most of these toxic compounds than occurred in sediments. Possibly the best utilization of freshwater mussels as indicators of stream conditions is as "indicators of the biological recovery zone" (Simons and Reed 1973).

The purpose of the current study was to explore the environmental parameters that influence distribution of mussels in the Blanco River. Special emphasis was given to the effects of organic enrichment of the river by a city sewage treatment plant. Tolerance tests to ammonia and low oxygen on five species of local mussels were conducted in the laboratory in an atempt to evaluate such enrichment.

STUDY AREA

The Blanco River is located at the headwaters of the Guadalupe River Basin in central Texas. The Blanco River flows over the Edwards Plateau and joins the San Marcos River approximately 4 km east of the perimeter of the plateau. The Edwards Plateau is composed of uplifted limestones that contribute to the natural calcareous hardness of the Blanco River. At the Kyle sampling station the mean annual flow for a 19 year sampling period was 4.3 m^3 sec^{-1} (U.S.G.S. 1976). At the Kyle gauging station, about 9 km upstream from the study area, no flow levels occurred in the summers of 1956, 1963, 1964 and twice in 1971 (U.S.G.S. 1976). Except during periods of flooding, the lower portion of the Blanco River is usually transparent enough for a visual analysis of the substrate.

The drainage basin of the Blanco River above the Kyle sampling station is 1,067 sq. km. and contains little arable land. Most of the basin is sparsely populated, and agriculture consists mainly of grazing with only limited crop farming on the rocky terrain. After the Blanco River leaves the plateau, the river traverses a more populated area where crop farming predominates.

Sampling areas on the Blanco River were located between 29°55′–29°51′ latitude and 97°55′–97°54′ longitude. The study area consisted of a 6 km stretch of the Blanco River located just upstream of the confluence of the San Marcos River (Fig. 1). A secondary sewage treatment effluent enters the Blanco River approximately 2 km downstream from the headwaters of the study area. The sewage effluent, except under very low flow conditions, is diluted naturally by a side channel of the river before it reaches the main stream. In the faster moving waters of the

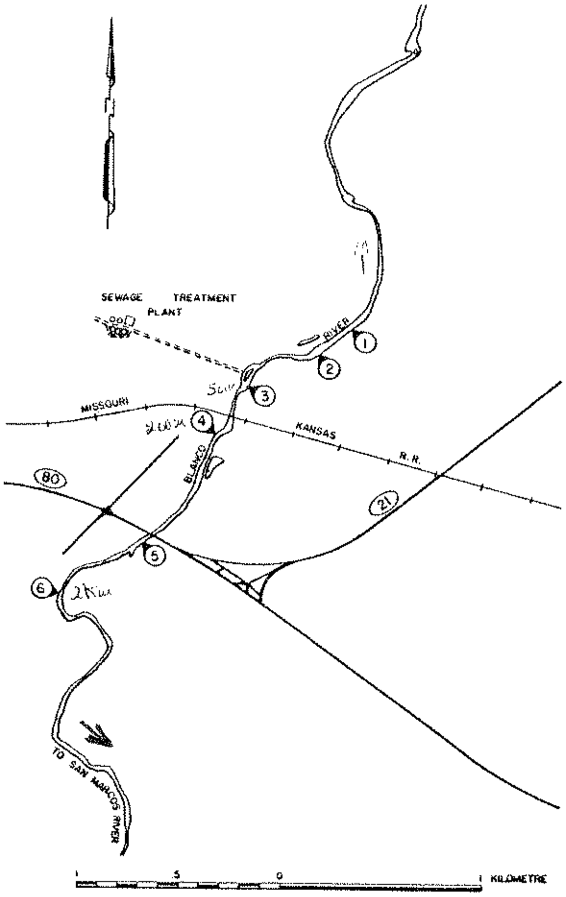

FIG. 1. *Location of sampling sites on the Blanco River, Texas.*

diluted side channel, toxicity study areas were established about 50 m before and after the point of entry of the sewage. Three sampling stations for physicochemical analyses of the river were located (1) about 50 m above (Station 3), (2) 200 meters (Station 4) and (3) 2 kilometers below (Station 6) the site where the sewage effluent enters the river (Fig. 1).

METHODS AND MATERIALS

Qualitative and Quantitative Determinations

Qualitative and quantitative mussel counts were performed at mid-day by visual inspection while wading or scuba diving in the deeper pools. The entire study area was quantitatively sampled for freshwater mussels. Three quantitative counts were made at stations above, and two quantitative counts below the sewage effluent (Fig. 1). Quantitative counts were made in 10 m^2 areas utilizing ten one meter transects. After identifying and recording the specimens, they were lifted from the substrate to prevent duplications. Only those individuals exposed or actively siphoning in the substrate were utilized in the quantitative determinations.

Mussel specimen identifications were made utilizing the taxonomic keys of Burch (1973), Murray (1962; 1968), Simpson (1913) and Strecker (1931). Identifications were checked and verified by Dr. H. D. Murray of Trinity University in San Antonio, Texas, and Dr. David Stansbery of Ohio State University in Columbus, Ohio. Specimen were deposited at Ohio State University (Collection Numbers OSUM: 1976: 352-361).

Tolerance Tests

Specimens utilized in the tolerance tests were collected in central Texas near the study area. The specimens were then placed in aerated aquaria for a minimum of five days for acclimation to laboratory conditions. During the period of acclimation and testing, the experimental and control specimens were not fed. Before being utilized in the toxicity tests each laboratory specimen was washed and scrubbed to remove adhering organisms. The ventral margins of the bivalves were notched with a triangular file so that the bivalves would be constantly exposed to the stressors. At least eight specimens of each species were utilized in the toxicity tests.

The chlorine content of the tap water used in the experiments ranged from 0.2 - 0.4 mg l^{-1} and was removed from the test waters by aeration or bubbling nitrogen. The total alkalinity of the water varied from 200 to 250 mg l^{-1} and depended upon the stressor(s) utilized. The total alkalinity of water from which the organisms were collected varied from 150 to 200 mg l^{-1}. The temperature of the experiments was ambient room temperature which was usually between 24°–26° C. The hydrogen ion concentration (pH) ranged between 7.8 and 8.2 during the laboratory experiments. At an average pH of 8.0 a solution containing ammonia ($NH+_4$ -NH_3) would consist of 94.7% ammonium ions and 5.3% ammonia gas (NH_3). In this manuscript the term ammonia refers to both the ionic and gaseous form, even though the gaseous ammonia is the toxic form.

At no time were more than four larger mussels used in a single experiment. The bivalves

Table 1. Substrate related distribution of freshwater mussels in the Blanco River.

Species	Stream Bottom Boulder	Cobble	Gravel	Pebbles	Sand	Silt-mud
Amblema p. plicata	+	+++	+++	+++	++	+
Anodonta imbecillis	-	-	-	-	-	+
Corbicula manilensis	-	+	++	++	++	+
Cyrtonaias tampicoensis	-	-	++	++	+	+
Lampsilis a. anodontoides	-	-	+	+	+	+
Lampsilis bracteata	-	-	+	+	+	+
Quadrula petrina	+	+	++	++	++	+
Toxolasma texasensis	-	-	-	-	+	+

+++ = More than 5/m^2
++ = 1-5/m^2
+ = Less than 1/m^2
- = Not Found

Amblema p. plicata (Say, 1817), *Anodonta imbecillis* (Say, 1829), *Corbicula manilensis* (Philippi, 1844), *Cyrtonaias tampicoensis* (Lea, 1838), *Toxolasma texasensis* (Lea, 1857) (=*Carunculina parva texasensis*) were subjected to laboratory tolerance tests for 168 hours. Specimen were removed from the testing apparatus after failure to respond to physical stimuli by closure of the valves. Death was established when the mussels did not respond by attempted closure when their valves were partially pried apart.

Low oxygen tolerance tests (0 - 0.5 mg 0_2 1^{-1}) were performed in a modified 8 liter desiccator with a regulated water flow of about 300 ml $hr.^{-1}$. A 16 liter reservoir of water was deoxygenated by bubbling prepurified nitrogen gas. The deoxygenated water was mixed with a magnetic stirrer and forced through the testing apparatus with low N_2 pressure. Water samples for chemical analyses were taken from the testing apparatus by removal and subsequent replacement of standard biochemical oxygen demand (BOD) bottles that were situated before and after the specimen container. By maintaining the flow at least 300 ml hr^{-1} the dissolved oxygen (DO) levels of the incoming and outflowing waters of the specimen container were similar.

In the combination high ammonia and low oxygen tolerance tests the water was deoxygenated first and ammonia then added to the above testing apparatus. In both the combination high ammonia-low oxygen and high ammonia tolerance tests, ammonia levels of 5 ± 0.5 mg 1^{-1} (NH^3 -N = 0.26 mg 1^{-1}) were obtained by the addition of ammonium bicabonate. Bunkhalter and Kaya (1977) estimated 0.25 mg NH^3-$N1^{-1}$ to be the incipient lethal threshold concentration for rainbow trout fry.

High ammonia tolerance tests were performed in 8 liters of aerated tap water in covered aquaria. Samples for chemical analyses were made by removal and subsequent replacement of similar amounts of water from the aquaria.

The tolerance tests in the Blanco River were performed in a side channel which contained diluted sewage. The bivalves *Amblema p. plicata, Anodonta imbecillis, Corbicula manilensis* and *Cyrtonaias tampicoensis* were put under stress. The mussels were placed in cages of 1/4 inch square mesh screen which were partially buried in the gravel substrate. The controls were located 50 m above the point of entry of the sewage and were placed in a similar substrate. The side channel toxicity tests were performed for one month periods.

Statistical analyses of the toxicity studies were performed by single factor analysis of variance and after hypothesis rejection analyses were followed by the Student Newman Kuels tests for differences in population ranges (Zar 1974). The survival times in hours were used as the observations in the statistical tests. Since the maximum utilizable value for survival time was sometimes limited by the length of the toxicity test, interpretations of the above statistical analyses were conservative.

Physicochemical Analyses

Water samples were taken with 1 liter polyethylene bottles 0.3 m below the surface on sunny mid-day periods. Water temperature was taken at the same time 0.3 m below the surface in shaded areas. Chlorine levels were determined in the field. Stream velocities were measured with a U.S.G.S. Pigmy current meter. Chlorine levels were determined in the field, whereas samples for pH, alkalinity, DO, $NH+_4$ -N, BOD_5 and total mercury were analyzed within forty-five minutes of collection in the laboratory. Samples for the determinations of Kjeldhal nitrogen, total dissolved phosphate-phosphorus, and potassium were stored at -20°C and analyses were conducted within three hours of collection. Chemical analyses of water were performed according to *Standard Methods for the Examination of Water and Wastewater* (A.P.H.A. 1975).

Sediment analyses were performed utilizing the modified Wentworth grade classification (Home and McIntyre, 1971 and Weber, 1973). The substrate was scooped into a container placed just downstream and analyzed using U.S. Standard sieves. Hydrogen ion concentrations were determined with a standardized Beckman Expandomatic pH meter. Total alkalinity analyses were performed by titration with 0.02N H_2SO_4 to a pH of 4.5. Dissolved oxygen determinations were performed utilizing the alkali-azide modification of the Winkler method. Ammonia nitrogen analyses were made by distillation of the ammonia into boric acid followed by nesslerization. Kjeldahl nitrogen determinations were made by sample digestion followed by distillation and nesslerization. Total dissolved phosphate-phosphorus samples were first filtered through 0.45 micron filters and then treated to persulfate digestion and the color developed by the ascorbic acid method. Chlorine levels were determined utilizing the orthotolidine colorimetric methods. Total mercury analyses were performed by the cold vapor technique using a mercury analyses system connected to an atomic absorption spectrophotometer. Total potassium ion determinations were made utilizing an atomic absorption spectrophotometer.

RESULTS

Qualitative Sampling and Species Distribution

Living specimens of eight species of freshwater mussels, and shells of *Anodonta g. grandis* (Say, 1829) and *Lampsilis anodontoides fallaciosa* (Smith, 1899), were collected in the Blanco River study area (Table 1).

The type of stream bottom and the corresponding flow patterns seemed to limit the distribution and perhaps the abundance of some of the species (Table 1). Local geologic formations (recent alluvium) and periodically high stream velocities created a bottom composed of a gravel-cobble substratum in the faster flowing portions of the stream. Only on the periphery of the larger pools where the current was slow was a mud-silt bottom found (Fig. 1). All species collected in the river were present, although sometimes sparsely, in mud-silt substrates.

Individuals having relatively heavy shells, such as *Quadrula petrina* and *Amblema p. plicata*, were the only species found in waters with average stream velocities of 1 m sec^{-1} or greater. In these waters the bottom was typically composed of cobbles with some boulders and gravel present. Although *Corbicula manilensis* has a relatively thick shell its small size probably limits it from occurring in the swiftest waters (Table 1).

Freshwater mussels with shells of intermediate thickness such as *Cyrtonaias tampicoensis, Lampsilis a. anodontoides* (Lea, 1834) and *Lampsilis bracteata* (Gould, 1855) generally were found in regions of intermediate stream velocities (0.5 - 1 m sec^{-1}) where the usual bottom composition was coarse and/or medium sized gravel. The very thin shelled *Anodonta imbecillis* and the small sized *Toxolasma texasensis* were restricted to areas with the finest types of substrate material (Table 1), and both were rarely found in the river.

Physicochemical Parameters

During the sampling period from July, 1976, to June, 1977, the minimum flow was 1.5 $m^3 sec^{-1}$ and the maximum flow was about 112 $m^3 sec^{-1}$ at the Kyle gauging station. Unlike previous years very low or no flow periods did not occur during the sampling year. Water temperatures varied from 11.5°C in January, 1977, to 31.0°C in August, 1976. Secchi disc transparency varied from 0.8 m to 2.1 m in the Blanco River. Generally, transparency increased during low flow periods and decreased during high flow periods.

The secondary sewage plant adjacent to the Blanco River usually received less than 30% of the total sewage load of San Marcos. The mean flow through the sewage treatment plant for the twelve month sampling period was 0.84 million gallons/day (mgd) with extremes of about 0.05 to 1.1 mgd.

For June, 1977, average effluent values for the treatment plants and the diluted sewage of the side channel are given in Table 2. Also for June the sewage enriched side channel of the Blanco River (Fig. 1) had a total flow of approximately 0.1 $m^3 sec^{-1}$ of which about 20 - 50% was sewage effluent, depending upon the amount of effluent discharged. During the same period, the Blanco River received an average of 110 kg BOD_5 day^{-1}, 90 kg potassium day^{-1}, 50 kg of ammonia-N day^{-1} and 9 kg of total phosphate-phosphorus day^{-1}. The secondary treatment plant received waste from only the northeast portion of San Marcos.

Total mercury analyses for the sewage effluent and water samples from the Blanco River in March, 1977, were below detectable limits (less than one microgram l^{-1}). Chlorine also was not detectable (less than 0.1 mg l^{-1}) in the enriched side channel. Chemical parameters which were monitored at Stations 3, 4 and 6 (Fig. 1) are presented in Table 2. Where the sewage effluent entered the river, all parameters increased, except dissolved oxygen and pH. The largest increases occurred with ammonia and total dissolved phosphate which increased 300% and 100%, respectively. At the sampling station located 2 km downstream from the treatment plant (Site 6), all parameters were more similar to the uncontaminated waters above the sewage effluent. Only dissolved oxygen returned to its upstream (Site 3) concentration at the

Table 2. Water chemistry determinations for the sewage effluent and diluted sewage in Blanco River side channel.

Parameter (mg l^{-1})	Sewage effluent	Side channel (diluted sewage)
BOD_5	44.20	9.00
Chlorine	0.80	0.00 < 0.1
Dissolved oxygen	6.40	7.55
$NH_4^+ + NH_3$-N	18.40	6.80
pH (units)	7.65	7.85
Potassium	33.00	7.80
Total alkalinity	276.00	240.00
Total dissolved phosphate-phosphorus	3.30	1.70

Table 3. Water chemistry determinations for the Blanco River.

Parameter (mg l^{-1})	Twelve month average (range) Above (Site 3)	 Below (Sewage Plant)	 Downstream (Site 6)
BOD_5	1.2 (0.9 - 2.2)	1.8 (1.0 - 5.0)	1.6 (1.0 - 2.6)
Dissolved oxygen	8.97 (6.75 - 10.42)	8.79 (6.88 - 10.35)	8.97 (7.42 - 10.37)
$NH_4^+ + NH_3$-N	0.03 (0.01 - 0.05)	0.09 (0.01 - 0.27)	0.06 (0.01 - 0.14)
Organic - N	0.24 (0.09 - 0.36)	0.38 (0.09 - 0.63)	0.36 (0.08 - 0.49)
Potassium	2.06 (1.70 - 2.70)	2.27 (1.85 - 2.85)	2.48 (1.85 - 2.78)
pH (units)	7.88 (7.74 - 8.02)	7.87 (7.74 - 8.02)	7.89 (7.75 - 8.02)
Total alkalinity	193.00 (165 - 212)	197.00 (170 - 224)	196.00 (170 - 224)
Total dissolved phosphate-phosphorus	0.12 (0.04 - 0.26)	0.24 (0.05 - 0.49)	0.22 (0.04 - 0.72)

downstream station (Site 6). The nutrient levels of ammonia-N best indicated the enrichment of the sewage effluent upon the Blanco River (Table 3).

Tolerance Tests

The laboratory tolerance tests were chosen because they measured parameters that were potentially toxic to freshwater mussels and which may result from organic enrichment. Since the laboratory tests lasted only seven days, highly stressful conditions were needed for definitive results. Nevertheless, the concentrations chosen were environmentally realistic. During the laboratory tolerance tests, it was frequently observed that the more tolerant species had their shells tightly shut, while the least tolerant species usually continued siphoning or had their mantles exposed.

The exotic asiatic clam *(Corbicula manilensis)* demonstrated greatest survival to low oxygen conditions (Table 4). The native mussels, *Anodonta imbecillis* and *Toxolasma texasensis*, also had relatively high survival capacities to low dissolved oxygen, whereas *Cyrtonaias tampicoensis* and *Amblema p. plicata* had the lowest survival tolerances. *Amblema p. plicata* had a significantly lower survival tolerance to low dissolved oxygen (Table 5). Although the four other species exhibited large differences in their survival capacities (Table 4), they were not significantly different at 95% confidence limits (Table 5).

Toxolasma texasensis demonstrated the highest survival capacity during the aerated high ammonia tests (Table 6), whereas *Anodonta imbecillis* and *Amblema p. plicata* had the lowest tolerance to high ammonia concentrations. Due to their frequent gaping, snapping of valves and extrusion of glochidia when gravid, *Anodonta imbecillis* appeared to be the most stressed species. All species frequently secreted mucous at the beginning of the aerated ammonia experiments. The interspecific survival capacities were not statistically different at 95% confidence limits for the mussels in high ammonia (Table 5).

In the combination high ammonia—low oxygen tolerance tests, *Corbicula manilensis* again demonstrated the highest survivorship (Table 7). No apparent synergistic effects were detected in the combination high ammonia—low dissolved oxygen tests. Interspecifically, *Amblema p. plicata* had significantly lower survival tolerance and *Corbicula manilensis* had significantly higher tolerance as compared to most of the other species (Table 5).

Survival of the mussels, except *Corbicula manilensis* in the combination low oxygen—high ammonia tests, appeared to be related to the mussel's tolerance to one of the two most stressful parameters. Intraspecfic survival capacities in the laboratory tolerance tests were not significantly different at the 95% confidence limit.

The tolerance tests in diluted sewage again demonstrated that *Corbicula manilensis* had significantly higher survival capacities (Table 5 and 8). *Amblema p. plicata* had significantly lower tolerance to the diluted sewage, while *Crytonaias tampicoensis* and *Anodonta imbecillis* exhibited intermediate survival capacities that were not significantly different from each other.

The levels of potential toxicants in the sewage side channel are given in Table 2. Ammonia and possible potassium were found at potentially lethal concentrations. However, the measured daylight and nocturnal oxygen levels did not appear near the lethal range nor potentially stressful during the tolerance tests. Although low oxygen levels may not have been present in the sewage side channel (Table 2), the relative tolerance of the mussels was comparable to their survival capacities in the combination low oxygen—high ammonia tolerance tests (Tables 5, 7 and 8).

Quantitative Samples

Quantitative sampling sites were chosen in areas with similar substrates at mid-stream locations in both the slow and fast moving waters. The substrate composition shown in Table 9 is from faster moving waters (0.3 - 1.5 m deep), but even at the sampling stations in the slower moving waters (1 - 4 m deep) more than 90% of the substrate was composed of fine gravel or larger. All quantitative sampling sites were located in areas containing relatively high populations of freshwater mussels. Therefore, data presented in Table 10 is representative of the more dense mussel populations of the Blanco River. Marked differences were noted in the number of species and the populations of mussels above and below the point of entry of the sewage effluent (Table 10). Initially during the study period large numbers of mussels were found in the river immediately below the sewage effluent (Fig. 1). At the end of the study period in July, 1977, very few mussels were found alive at this site. The large numbers of mussels initially found in the uppermost portion of the enriched study area may have been transported by floods from a large bed of mussels found just upstream at Site 3 (Fig. 1).

Of the native species, *Amblema p. plicata* and *Quadrula petrina* were the most abundant. The asiatic clam *(Corbicula manilensis)* was not

Table 4. Percentage survival in low oxygen concentrations (0-0.5 mg O_2 l^{-1}).

Species (Number used)		55 hr	110 hr	165 hr
Amblema p. plicata	(8)	88	0	0
Anodonta imbecillis	(8)	100	88	75
Corbicula manilensis	(8)	100	89	89
Cyrtonaias tampicoensis	(8)	88	62	38
Toxolasma texasensis	(8)	100	88	62

Table 5. Statistical analyses of tolerance tests (SNK) for interspecific mean survival times.

Species	Tolerance Test			
	Low O_2	High NH_3	Low O_2 + High NH_3	Diluted Sewage
1) Amblema p. plicata	-S(all)	NS	-S(3,4,5)	-S(all)
2) Anodonta imbecillis	+S(1)	NS	-S(3)	+S(1) -S(3)
3) Corbicula manilensis	+S(1)	NS	+S(1,2,5)	+S(all)
4) Cyrtonaias tampicoensis	+S(1)	NS	+S(1) -S(3)	+S(1) -S(3)
5) Toxolasma texasensis	+S(1)	NS	+S(1) -S(3)	--

S = Significantly different at 95% confidence interval (-S = lower; +S = higher).
NS = Not significantly different at 95% confidence interval.

noticeably present in the study area in the spring or summer of 1976. However, immature *Corbicula manilensis* were found about 7 km upstream of the study area in the spring of 1976. Immature specimens were first evident in the study area in the spring of 1977. *Corbicula manilensis* was found in much higher concentrations above the sewage effluent than given in Table 9. Densities of up to 50 m^{-2} of small individuals were found in the uncontaminated headwaters of the Blanco River side channel (Fig. 1). The highest numbers of *Corbicula manilensis* occurred in sand-fine gravel substrates. No living specimens of *Corbicula manilensis* were found below the entrance of the sewage effluent in the Blanco River.

DISCUSSION

From the previous records of Strecker (1931) all of the species collected in the present study, except the exotic asiatic clam *(Corbicula manilensis)*, have been present in the Guadalupe River drainage for many years. Since *Lampsilis bracteata* is still present in the Guadalupe and San Antonio River drainages, and *Quadrula aurea* (Lea, 1859) is also present in the Guadalupe River drainage, the continued existence of these species may not be threatened. Athearn (1970) has considered both *Lampsilis bracteata* and *Quadrula aurea* as rare and endangered in central Texas.

Some species of mussels are limited in their distribution by the type of stream bottom. For example, mussels of the genus *Anodonta* and *Leptodea fragilis* were only rarely found in rocky substrates (Murray and Leonard, 1962). In the Blanco River *Anodonta imbecillis* and other species with relatively thin, light weight shells did not occur in swift waters with coarse substrates. This might be due, in part, to their physical displacement and/or destruction by the shearing forces in faster waters.

Considering the rapid dissemination and population growth of *Corbicula manilensis*, their abundance in the upper half of the study area in 1977 was not suprising even though none were noted in 1976. Gardner *et al.* (1976) observed that the population of *Corbicula manilensis* in the Altamaha River (Georgia) increased from a minimum of 0/m² in 1971 to a maximum of 10,000/m² in 1974. *Corbicula manilensis* maintains a distinctive reproductive advantage over the usually dioecious, slow growing, glochidial-producing native freshwater mussels. *Corbicula* is monoecious, incubates its free-living larvae and is sexually mature in less than one year (Gardner *et al.* 1976).

The physicochemical parameters measured from July, 1976, to June, 1977, for the Blanco River were similar to those found from other parts of the Guadalupe River drainage (Hannan *et al.* 1973; Young *et al.* 1972). The large increases of ammonia-N (300%) below the point of entrance of the sewage effluent into the river suggested organic enrichment of the stream. Ammonia values often are a good index of changes in trophic status of streams that have been influenced by excessive enrichment by organic wastes (Ellis 1937).

Although pronounced changes in the water chemistry were found below the point of entrance of sewage effluent into the river, none of the parameters measured were at concentrations known to be toxic or harmful to freshwater mussels. Upon consideration of the sources of waste entering the secondary treatment plant, excessive pollution by heavy metals or pesticides was not likely. The lack of measurable flow in the Blanco River as reported by the Kyle gauging station during previous dry periods (U.S.G.S. 1976), however, could increase the levels of potential toxicants to concentrations equal to or greater than the levels found in the diluted sewage side channel. When the Blanco River stops flowing, as it does every few years, the sewage is not diluted when it enters the river and is then the primary source of water below the sewage plant.

The levels of ammonia-N, potassium and nocturnal dissolved oxygen could be potential hazards for the mussels during such low flow periods. Imlay (1973) found potassium levels of 11 ppm to be toxic in 36-52 days to 90% of the freshwater mussels tested, and for long term survival. Imlay (1973) postulated that potassium levels should be no higher than 4 to 10 mg 1^{-1}. It is doubtful that potassium would be a problem in the Blanco River. In contrast, it is well known that nocturnal dissolved oxygen deficiencies also can be critical in determining stream distribution of organisms (Gaufin and Tarzwell 1952). Organically rich pools or slow moving waters in the Blanco River might experience extreme fluctuation in 0_2 concentration, especially at the mud-water interface. Considering the levels of potential toxicants (ammonia, low 0_2 and potassium) in the Blanco River, as demonstrated by their values in the diluted sewage side channel, ammonia is probably the most lethal stressor to mussels during the low flow periods.

The depletion of dissolved oxygen that results from sewage enrichment has been proposed as the principle stressor influencing molluscan survival (Ingram 1957). Ellis (1937) stated that juvenile mussels are very sensitive to low oxygen concentrations and that adults usually become quiescent

Table 6. Percentage survival in high ammonia (5 mg $NH_4^+ + NH_3$-N)

Species (Number used)		55 hr	110 hr	165 hr
Amblema p. plicata	(9)	78	56	33
Anodonta imbecillis	(9)	100	67	56
Corbicula manilensis	(14)	100	95	62
Cyrtonaias tampicoensis	(10)	100	100	70
Toxolasma texasensis	(10)	100	80	80

Table 7. Percentage survival in low oxygen and high ammonia (0-0.5 mg O_2 l^{-1} + 5 mg NH_4^++NH_3-N)

Species (Number Used)		55 hr	110 hr	165 hr
Amblema p. plicata	(8)	100	25	0
Anodonta imbecillis	(12)	80	60	40
Corbicula manilensis	(16)	100	93	93
Cyrtonaias tampicoensis	(10)	88	80	60
Toxolasma texasensis	(10)	90	80	20

when oxygen levels are at or below 20% saturation. However, mussels generally are more tolerant of low O_2 levels than freshwater fishes. One of the more tolerant of the freshwater fishes, the carp, survives only a short time in water containing 0.71 mg O_2 l^{-1}. In contrast, in the low O_2 tolerance tests (0 - 0.5 mg O_2 l^{-1}) about 53% of the mussels tested in this study survived for seven days (Table 4).

During the laboratory tolerance tests, the mussels that did not have their valves closed for extended periods were more senstive to stressors (NH_3 and/or low O_2). A similar conclusion was made by Ellis (1937), who reported that if mussels failed to respond by shell closure to low dissolved oxygen, then they were more vulnerable to destruction by pollution. Extended gaping of the valves usually precluded death. In the laboratory tolerance tests when a mussel began to gap its valves, death would usually follow within several hours.

Mussels that were stressed usually siphoned less and had their valves closed for longer periods than the non-stressed specimens. Badman (1975) noted that under hypoxic conditions, *Elliptio dilatatus* and *Pleurobema coccineum* increased periods of valve closure and reduced filtration rates, whereas in contrast, Allen (1923) reported widening of the siphons and mantles to pass more water through the mussel (increased respiration)

Table 8. Percentage survival to diluted sewage in the Blanco River side channel.

Species (Number Used)		7 days	14 days	21 days	28 days
Amblema p. plicata	(16)	12	0	0	0
Anodonta imbecillis	(10)	70	20	0	0
Corbicula manilensis	(20)	100	65	50	50
Cyrtonaias tampicoensis	(11)	64	27	0	0

Table 9. Substrate composition of two typical collecting areas.

Type	U.S. Series No.	Size (mm)	Percent Composition Upstream	Percent Composition Downstream
Boulder	-	256	-	-
Cobble	-	64-256	49.10	20.90
Coarse gravel	-	32-64	11.50	26.10
Medium gravel	-	8-32	24.60	36.50
Fine gravel	8	2-8	12.30	13.00
Very coarse sand	18	1-2	1.80	1.50
Coarse sand	40	0.5-1	0.62	1.54
Medium sand	60	0.25-0.5	0.02	0.32
Fine sand	120	0.125-0.25	0.04	0.10

as a result of low oxygen levels. The various species may respond differently to environmental stressors.

The mussels most tolerant of low dissolved oxygen were collected from standing or slow moving waters. For example, the more tolerant *Anodonta imbecillis* and *Toxolasma texasensis* were taken from ponds or reservoirs, while the more sensitive *Amblema p. plicata* was collected in the fast moving waters of the Blanco River. At least for some mussels, tolerance to low dissolved oxygen levels might be correlated with distribution.

Insufficient dissolved oxygen was suggested by Isom (1971) as a cause for the decline of the endemic mussel fauna in Fort Loudoun Reservoir, Tennessee. Organic enrichment of the reservoir was apparently the causative agent.

Perhaps even the rapid colonization of aquatic habitats by *Corbicula manilensis* is due to their tolerance to stressful physicochemical conditions as well as their reproductive capabilities. Hable (1970) found that *Corbicula* was resistant to low oxygen levels and that the presence of *Anodonta imbecillis* and *Corbicula manilensis* in Fort Loudoun Reservoir, when they had not been previously detected in the Tennessee River, may have been due not only to their fecundity, but also to their relative high tolerance to low dissolved oxygen. As with *Corbicula manilensis*, *Anodonta imbecillis* is monoecious and has glochidia that may develop to maturity without a living host (Murray and Leonard 1962).

The lack of significant differences in survival capacities to elevated ammonia levels demonstrated that of the mussels tested, all are relatively sensitive to ammonia. In aerated aquaria, where the pH of the testing waters varied from 7.8 to 8.2 and was similar to the pH of the Blanco River, concentrations of 5 mg ammonia-N 1^{-1} were lethal to 40% of the mussels tested in seven days (Table 6). The ammonium ion (NH_4^+) is not very toxic, but molecular NH_3 is highly toxic. The proportion of ammonia to ammonium ions greatly increases with decreasing hydrogen ion concentrations, and as reported by Ellis (1937), pH is an important factor in the toxicity of ammonium compounds to aquatic animals. For instance, Ellis (1937) found that for daphnia and gammarids, the toxicity of ammonium compounds increased 200% or more as pH increased from 7.4 to 8.0.

With concentrations of 6.8 mg ammonia-N l^{-1} (pH 7.85), the diluted sewage in the side channel contained ammonium levels which exceeded the experimental ammonium levels utilized in the laboratory. The concentrations of 18.4 mg ammonia-N l^{-1} in the sewage effluent would present potentially lethal levels if the effluent composed 20% or more of the total stream flow. Such conditions would exist in the Blanco River if the flow was reduced to about 0.1 m^3 sec^{-1} which would be 15 times lower than the minimum flow (1.5m^3 sec^{-1}) found for 1976-1977.

Over long periods, much lower concentrations of ammonia may be detrimental to mussels. Ellis (1937) found that 1.5 mg ammonia l^{-1} was the maximal concentration not indicative of organic pollution. In streams with pH values ranging from 7.4 to 8.5, ammonia levels of 2.5 mg l^{-1} would tend to be detrimental to many freshwater animals (Ellis 1937). Levels of ammonia-N probably should be kept below 1 ppm in all streams containing mussel populations.

Mussels are more sensitive to ammonia than the common goldfish, *Carassius auratus*, which Ellis (1937) listed as tolerant to 10 ppm ammonium carbonate (pH 7.7) for more than four days. Conversely, and as mentioned earlier, physiologically mussels are less sensitive to low dissolved oxygen levels than goldfish. However, a mussel's chances for survival when unfavorable conditions occur is reduced by their lack of mobility and confinement to the substratum. Maximum allowable ammonia-N levels in a fishery is 0.02 mg l^{-1} (Wellingham, 1973; NAS and NAE, 1972).

In general, laboratory tolerance tests demonstrated that *Corbicula manilensis* was the least, and *Amblema p. plicata* the most sensitive of the mussels (Table 5). However, not all *Amblema* can be called "sensitive". On the basis of their high densities in "conditionally polluted areas", Richardson (1928) postulated a species of *Amblema (A. rariplicata)* to be the least sensitive of the mussels sampled in the Illinois River.

Since specimens for this study were collected by handpicking, the youngest age classes of mussels were not observed. Mussels less than three years of age are commonly overlooked when handpicking (Van Cleave 1940). No information, therefore, was collected on mussel reproduction when exposed to the stressors or on larval tolerances. It is likely that individuals of the same species, but of different ages, have dissimilar tolerances to stream pollutants (Ellis

Table 10. Quantitative samples of the freshwater mussels of the Blanco River.

	Upstream ($\bar{x}/m^2$)			Downstream ($\bar{x}/m^2$)	
Species	Site 1	Site 2	Site 3	Site 5	Site 6
Amblema p. plicata	2.7	6.8	5.7	0.0	0.1
Corbicula manilensis	1.6	0.0	1.6	0.0	0.0
Cyrtonaias tampicoensis	0.0	0.6	0.0	0.0	0.0
Lampsilis a. anodontoides	0.1	0.0	0.0	0.0	0.0
Lampsilis bracteata	0.1	0.0	0.1	0.0	0.0
Quadrula petrina	0.2	0.5	1.3	0.0	0.1

1937). Pollution tolerance data, therefore, must be viewed with caution.

Based upon the results of this study the suggestion by Weber (1970) that *Corbicula* is less tolerant than *Anodonta imbecillis* to organic pollution may be incorrect.

Due to the intolerance of mussels to diluted sewage in the side channel and because the substrate and other physical factors below the effluent of the treatment plant of San Marcos are basically similar to those factors upstream, the decreased number of mussels downstream was probably due to organic enrichment (Fig. 10). The severity of sewage pollution would increase tremendously during low or no flow periods. No other explanation is available at present to account for the disproportionate lack of mussels below the entrance of the sewage effluent in the Blanco River.

Simons and Reed (1975) noted that the molluscan segment (mostly mussels) of the benthic community represented a more sensitive portion of the macrobenthos than did most insects in the North Anna River, Virginia. The point of full "biological recovery" of the North Anna River was assumed to have been where the mussel populations had been reestablished (Simons and Reed 1975).

As suggested by Ingram (1957) and from data presented here, mussels may have value as indicators of nonpolluted waters because their presence typically indicates high dissolved oxygen and associated chemical and physical conditions. For determination of the severity of water pollution reduced numbers of "clean water" species which were formerly present in the stream may be more important than an abundance of known pollution resistant forms (Richardson 1928).

The following concluding remarks can be made from the tolerance tests and field studies.

(1) Low dissolved oxygen levels (0 - 0.5 mg 0_2 1^{-1}) proved lethal to 47% of the mussels tested in seven days.

(2) Levels of 5 mg NH_4^+-NH_3 1^{-1} (pH 7.8 to 8.0 were lethal to 40% of the mussels tested in seven days.

(3) Even in waters with dissolved oxygen levels not indicative of pollution, ammonia levels can be lethal to mussels.

(4) *Corbicula manilensis* is generally more tolerant and *Amblema p. plicata* less tolerant than the other mussels tested to stressors associated with sewage enrichment.

(5) Even though the physicochemical parameters did not indicate stressful conditions on the days sampled, mussels of the Blanco River seemed to have been adversely affected by enrichment from the secondary sewage treatment plant of San Marcos. Fewer mussels were found downstream from the sewage plant than upstream.

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