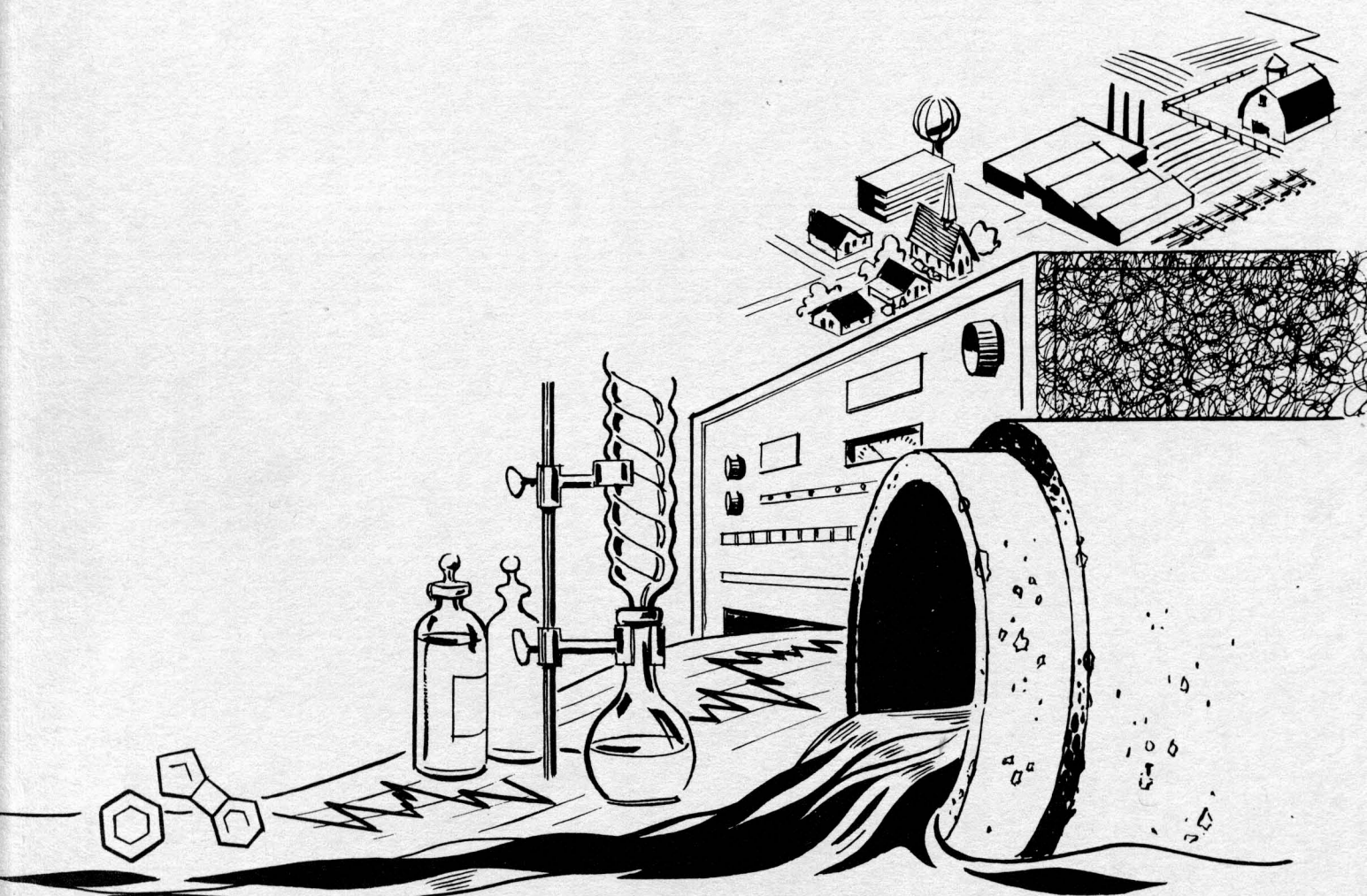CLEAN WATER

Michael Hubbard

Mayfly Distribution
as a
Water Quality Index

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MAYFLY DISTRIBUTION AS A WATER QUALITY INDEX

by

Winona State College
Winona, Minnesota 55987

for the

WATER QUALITY OFFICE

ENVIRONMENTAL PROTECTION AGENCY

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WQO Review Notice

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ABSTRACT

Three species of burrowing mayflies (Hexagenia bilineata, Hexagenia limbata, and Pentagenia vittigera) are sufficiently abundant to cause nuisance problems along portions of the Mississippi River. Mayfly distribution, as determined by collections made by ship captains and other cooperators over a 13-year period, has proven to be an excellent index of general water quality on a river which is so large that it cannot be monitored effectively or economically by standard methods. Pollutants have severely reduced the numbers of all three species for 30 miles below Minneapolis, Minnesota, and for over 300 miles below St. Louis, Missouri. P. vittigera is able to emerge only in early and late summer in the St. Louis area when cool water temperatures lessen toxic effects in the zone of degradation. Impoundment and enrichment of the Upper Mississippi River has temporarily increased the carrying capacity of the river for H. bilineata which now dominates areas formerly dominated by H. limbata. The total productivity of the Upper Mississippi is being reduced by pollution, man's encroachment into the flood plain and by the filling of navigation pools by sand.

Methods have been developed to rear large numbers of Hexagenia nymphs in the laboratory. Bioassay tests utilizing artificial, burrow-containing substrates reveal that H. bilineata nymphs can survive anaerobic conditions for as long as 11 hours. TLm values for hydrogen sulfide varied from 0.42 ppm at 48 hr to 0.17 ppm at 96 hr. Of several heavy metals (Cr, Ni, Zn, Cu) tested, copper was the most toxic to H. bilineata nymphs. TLm values for copper ranged from 0.54 ppm at 12 hr to 0.22 ppm at 48 hr.

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FIGURES

INTRODUCTION

The general life histories of Hexagenia mayflies are well known (Needham, Traver, and Hsu, 1935; Hunt, 1953; Fremling, 1960). The burrowing nymphs construct U-shaped respiratory tubes in the muddy bottoms of lakes and rivers where they ingest mud, organic detritus, algae and bacteria. Hexagenia nymphs require from three months to a year to mature in the Upper Mississippi River, whereupon they rise to the sur-face, usually at night, cast their nymphal exuviae and emerge as subimagoes. Subimagoes rest in the shade along the river bank until the following afternoon when a final molt occurs and the imagoes emerge. Mating occurs aerially along the shoreline at dusk and the females return to the river where each alights on the surface and deposits two egg packets, each of which contains about 4,000 eggs. The eggs sift downward to the river bottom where most of them hatch in 10-12 days, if conditions are favorable. Male and female imagoes die within hours after they have mated.

Hexagenia mayflies tend to emerge en masse, and river residents are ac-customed to nuisance problems caused by the insects during periods of maximum emergence. Tree limbs droop under their weight, and drifts of the insects form under street lights where they decay and create ob-jectionable odors. Shoppers desert downtown areas as the large, clumsy insects fly in their faces, cover windows, and blanket sidewalks. In extreme cases snowplows are called out to reopen highway bridges which have become impassable. Particles of cast mayfly cuticle cause allergic reactions in some people. Mayflies become a hazard to navigation when they are attracted by the powerful arc and mercury-vapor searchlights used by tow-boats to spot unlighted channel markers. Because mayflies cause severe nuisance problems, several river cities have tried unsuc-cessfully to control them.

The name "Green Bay fly" is often used for the mayfly because people still recall the hordes of Hexagenia mayflies which formerly arose from Green Bay of Lake Michigan and literally covered portions of the city of Green Bay, Wisconsin. Because of pollution, Green Bay flies are now rare on the lower reaches of the bay near the mouth of the Fox River (Lee, 1962). Pollution has decimated the Hexagenia mayfly population in the western end of Lake Erie (Britt, 1955; Beeton, 1961; Carr and Hiltunen, 1965). Hexagenia emergences were once common along the Illinois River, but pollution has virtually eliminated the insects from the upper 150 miles of the river (Richardson, 1921; Mills et al., 1966). Hexagenia mayflies, which were once common along the entire Upper Mis-sissippi River, are now rare for 30 miles below Minneapolis, Minnesota, and for almost 200 miles downstream from St. Louis, Missouri (Fremling, 1964). Hexagenia and Pentagenia mayflies still occur abundantly in the less polluted areas of the Upper Mississippi River. In Pool 19, for example, Carlander et al. (1967) estimated the June, 1962, nymphal Hexagenia population to be 23.6 billion.

Burrowing mayflies are excellent indicators of general water quality because their life cycles are relatively long. Nymphs are unable to swim long distances to escape toxic elements, hence their presence or

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FIGURE 1. Imago <u>Hexagenia</u> <u>limbata</u> mayflies rest upon a tree branch prior to forming evening mating swarms.

absence in otherwise suitable habitats reflects the quality of the water which flows over them.

Sampling of the benthos with dredges and other samplers is an extremely difficult and time consuming task, however. For results to be valid, large numbers of samples must be taken from many sampling stations. Collection of adult mayflies, on the other hand, is relatively simple, less time consuming and much more economical. It is virtually impossible for one investigator to make a significant number of mayfly collections along 2300 miles river. Therefore, a system has been developed whereby cooperators assist in the collection of adult insects.

The purposes of this study were to: (1) determine the degree to which mayfly distribution on the Mississippi River is limited by water quality; (2) refine laboratory procedures whereby Hexagenia nymphs can be reared in large quantities; (3) determine the tolerance limits of Hexagenia nymphs to various limiting factors; (4) learn more about the biology and ecological importance of burrowing mayflies; (5) to determine the present distribution patterns of burrowing mayflies along the Mississippi River so that future habitat changes can be accurately assessed.

DESCRIPTION OF STUDY AREA

The Mississippi is the largest river in the United States. From its source at Lake Itasca, in Northern Minnesota, it winds 2,319 miles to its mouth in the Gulf of Mexico, 95 miles downstream from New Orleans. The Mississippi and its tributaries drain about 41% (about 1,244,000 square miles) of the total area of the United States. By definition, the segment upstream from the mouth of the Ohio River, at Cairo, Illinois, is called the Upper Mississippi River (U.S. Army Corps of Engineers, 1958). The segment from the Gulf of Mexico to Cairo is termed the Lower Mississippi River (U.S. Army Corps of Engineers, 1965). The river is presently navigated by 9-foot draft vessels as far upstream as Minneapolis.

About 10,000 years ago, the last epicontinental glacier covered most of Minnesota, extending southward as far as Des Moines, Iowa (Bray, 1962). The southeastern corner of Minnesota and a portion of western Wisconsin, however, were left virtually unglaciated. As the glacier melted northward into Canada, it produced a large volume of melt water which could not flow northward into Hudson Bay because the glacier blocked the Red River drainage system. Glacial melt waters collected behind the ice dam to form Glacial Lake Agassiz which covered northwestern Minnesota, extreme eastern North Dakota, the southern half of Manitoba, southeastern Ontario and a narrow strip in east central Saskatchewan. Finally, when Lake Agassiz became overfull, a major portion of its overflow rushed down the Minnesota River Valley, to enter the Mississippi River at Minneapolis, Minnesota. This southern outlet stream was named the Glacial River Warren by Upham in 1884. The flow of the River Warren was augmented by the Glacial River St. Croix, which drained Glacial Lake Duluth - the ancestor of Lake Superior. Other smaller glacial rivers, the Mississippi proper, and the Chippewa added more water to the Glacial Mississippi River which cut a deep valley through limestone and sandstone strata as far south as Dubuque, Iowa. Consequently, along the southeastern border of Minnesota, the Mississippi River flows through a valley which is as much as 650 feet deep and 3 miles wide. By the time the river reaches Winona, Minnesota, it has dropped over halfway to sea level. The elevation of the valley floor at Winona is only 550 feet above sea level, but precipitous bluffs tower 650 feet above the city.

One hundred and forty-seven years ago, in 1823, the first steamboat probed its way up the Mississippi River as far as the present site of St. Paul. The next year, government-owned and operated boats began to improve the river for navigation by removing snags, boulders and other obstructions. In 1829, Captain Henry Shreve was commissioned to construct and operate a special twin-hulled snag boat on the upper river. It was imperative to the growth of the U.S. that the river be improved to provide a water highway to the sea because the interior of the continent was relatively inaccessible to overland freight haulers. Early channel improvements, however modest, enabled the United States to quickly exploit the interior of the entire North American continent. By the 1870's hundreds of shallow-draft steamboats routinely navigated the Upper Mississippi River.

Loggers used the river, too (Russel, 1928). By means of the Chippewa, the Black, the Wisconsin and smaller Wisconsin rivers, they quickly exploited

the pineries of northwest Wisconsin, floating huge rafts of saw logs down
to the Mississippi and then down to the sawmills of Winona, LaCrosse,
Clinton and Rock Island. At one time there were over 80 sawmills on the
upper river and at least 120 more on tributary streams.

In 1878, the U.S. Army Corps of Engineers was authorized by a Congres-
sional Appropriation Act to deepen the navigable channel of the Mississip-
pi River to four and one-half feet so that larger boats with deeper draft
could operate on the river. This was done by constructing rock closing
dams on side channels so that water which ordinarily went down side chutes
was conducted into the river proper. Obstructive rapids were by-passed
by constructing short lateral canals which contained navigation locks.
Hundreds of rock and brush structures called wing dams were also con-
structed (U.S. Army Corps of Engineers, 1962). The wing dams, often at
intervals of about ¼ mile, extended outward like rock piers from the
shore, at right angles to the main channel of the river. They diverted
the river into a single narrow channel, during low flow, so that the
river scoured its channel deeper. Troublesome sandbars were removed by
a dipper-type dredge. By 1905, the four and one-half foot channel was a
reality between St. Louis and the Washington Avenue Bridge at Minneapolis.

Meanwhile, larger, more powerful riverboats had evolved and they needed a
deeper channel to carry greater pay loads. Additional funds were appro-
priated by Congress in 1907 to deepen the navigable channel to 6 feet.
This was accomplished by building additional wing dams, closing dams, and
by dredging. Usually, on the opposite side of the river from the wing
dams, the shore was fortified with rock so that water which rushed
around the ends of the wing dams did not erode away the opposite shore.
Thus, the extreme channelization begun in 1878 was finally completed in
1912.

The short-lived logging boom which began in 1875 hit its peak in 1892;
and in 1915 the Ottumwa Belle snaked the last remnants of Wisconsin
lumber down the Mississippi River. Six-foot draft steamers also began
to disappear from the upper river because they could not compete with
the railroads.

The Rivers and Harbors Act of 1930 authorized the Corps of Engineers to
modify the obsolete 6-foot channel to provide a minimum depth of 9 feet
and a minimum width of 400 feet (U.S. Army Corps of Engineers, 1962).
This was achieved by the construction of a system of locks and dams,
supplemented by dredging. Most of the resultant 29 locks and dams were
constructed during the 1930's. A notable exception is Lock and Dam 19
at Keokuk, Iowa, which was constructed as part of a hydroelectric facil-
ity in 1914. The navigation locks are operated and maintained by the
Corps of Engineers, but the U.S. Coast Guard is responsible for the
maintenance of the elaborate system of navigation aids which guide
modern towboats as they navigate the river around the clock from early
spring until early winter.

The huge navigation dams of the Upper Mississippi have transformed the
river into a series of impoundments which occupy most of the flood plain
of the river. Consequently, the river is much wider at LaCrosse,
Wisconsin, than it is at New Orleans. Each impoundment consists of
three distinct ecological areas. The tailwater areas just downstream

from the dams show the river in relatively unmodified form. The areas are typified by deep sloughs and wooded islands. The middle portions of the pools are principally flooded hay meadows. They now provide the best marsh habitat. The downstream ends of the pools are deeper, however. They consist mainly of open water and their bottoms are heavily silted. Marsh vegetation is presently creeping downstream as the pools silt in. Marsh vegetation in the middle pool areas is being replaced, in turn, by trees and other terrestrial vegetation.

The old wing dams and closing dams, still partially functional, now lie beneath the water. The wing dams provide rocky corrugations on the river floor, so that they, in effect, have increased the total surface area of the river bottom - thus increasing its carrying capacity for inverte-brates such as hydropsychid caddisflies and periphyton. Impoundment has also increased the surface area of the river, thereby increasing the area of the trophogenic zone.

The seven-county area which contains metropolitan Minneapolis and St. Paul contains about 1/3 of Minnesota's population and the population in the seven-county area is expected to double in the next 30 years. The people of the seven-county area exert a profound influence on the Mississippi River.

The river has been severely polluted for many years for about 60 miles through and downstream from metropolitan Minneapolis and St. Paul (Metro-politan Drainage Commission of Minneapolis and St. Paul, 1928). In the metropolitan area about 1,768,000,000 gallons of industrial and municipal waste water enter the river each day. About 85% of this amount is cooling water from steam-electric generating plants (Federal Water Pol-lution Control Administration, 1966). Although they were not constructed for that purpose, the navigation pools serve as sewage lagoons so that with each subsequent impoundment and aeration in the tailwaters, the putrescible portion of the metropolitan pollutant load is decreased. Downstream cities add more pollutants but their additions are very small compared to those of Minneapolis and St. Paul. Also, large tributary rivers such as the St. Croix, Chippewa and Wisconsin add relatively clean water to the Mississippi thus increasing its ability to assimilate its pollutant load. Biologically, the Upper Mississippi River is comparatively clean from Wabasha, Minnesota (mile 760 Upper Mississippi River), to the mouth of the Illinois River just above St. Louis.

At the mouth of the Illinois, however, the Mississippi receives pol-lutants from Chicago, other large cities, industries and farms (Mills, et al., 1966). Prior to 1968, St. Louis added an average of 330,000,000 gallons of raw municipal sewage to the Mississippi every day. This was supplemented by additional wastes from surrounding municipalities and from many industries. Just upstream from St. Louis, the Missouri River, adds an extremely heavy load of silt.

The southernmost dam on the Mississippi River is the newly constructed Chain of Rocks facility at St. Louis. Downstream 185 miles from St. Louis, the Ohio River enters the Mississippi at Cairo, Illinois. Here, the Lower Mississippi begins a 954-mile path through its own immense, flat, alluvial delta to the Gulf of Mexico. The lower river, at one time,

constantly changed its course as it meandered about its flood plain. During the past two hundred years, however, the entire lower river has been channelized with earthen dikes to prevent flooding of the fertile delta through which the river flows. Furthermore, the U.S. Army Corps of Engineers has insured the channelization of most of the lower river by armoring its banks with rock to prevent the river from changing its course. The river has also been shortened by cutting off many meanders. Because there are no dams on the Lower Mississippi River, it is in essence a deep, narrow (200 feet deep, one-half mile wide at New Orleans), sinuous ditch which conducts most of the effluents of the United States very rapidly (average flow 611,000 cubic feet per second) to the Gulf of Mexico.

METHODS

A system was devised in 1958 whereby various river personnel were enlisted as cooperators to collect emergent mayflies. From 1958 to 1961 the project was limited to the navigable portion of the Upper Mississippi River but it was extended in 1961 to include the upper river to its source. In 1967, the sampling program was extended to include the entire Lower Mississippi River, its navigable tributaries and the Gulf Intracoastal Waterway.

Ship captains, lockmasters, harbor operators, resort owners, ferry operators, and other interested river residents were asked if they would collect mayflies whenever they encountered a mass emergence (Fig. 2). The cooperators were instructed not to collect isolated individuals but to collect only when mass emergences occurred. Self-addressed, stamped mailing tubes filled with instructions and alcohol-filled, plastic, specimen vials were mailed to about 150 cooperators each spring. Additional collecting materials were distributed to ship captains by the lockmasters at Lock and Dam 5a and Lock and Dam 19. The cooperators were asked to record on the specimen vial the name of the river, mile number, nearest city, time and date.

Shipping strikes, floods, recruitment of new cooperators, and other uncontrollable variables made it impossible to keep collecting effort constant from year to year. It was also impossible to maintain constant collecting effort over the entire river. There are no navigation locks on the lower river, consequently collecting was intensified on the upper river by the lockmasters there. This was particularly true at Keokuk, Iowa. Lock and Dam 19 is an extraordinarily large facility and it has more personnel on duty at any one time than any other lock (with the possible exception of Lock and Dam 27 at St. Louis). Also, the Mississippi River is not used equally by ship captains throughout its length. In 1962, for example, about 35 million tons of freight were transported past Memphis, Tennessee, while less than 10 million tons were transported as far upstream as Minneapolis. Even the lower reaches of the Illinois and the Ohio Rivers account for more annual freight than does the Mississippi River above Keokuk (U.S. Army Corps of Engineers, 1965).

A concerted effort was made to maintain contact with cooperators. Publications were sent to them upon request and a newsletter was distributed frequently. Each fall, the returned specimens were examined and the resulting data tabulated. Each specimen was examined to determine its species and sex and whether it was an imago or subimago. During the past year all of the data have been transferred to business machine cards. Programs have been written so that a computer will print distribution charts for any species, area, year or combination of years. Figures 5 and 6 are modified computer print outs. In addition to plotting distributions of the various species, the print outs are very useful in predicting when an emergence of a particular species is likely to occur in a given area.

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FIGURE 2. *Hexagenia bilineata* mayflies attracted to automobile headlights on Mississippi River bridge at Winona, Minnesota, 8 July 1966.

The river monitoring program was complemented by laboratory investigations. For this purpose, a 36 ft x 9 ft wet lab was constructed in the basement of Pasteur Hall at Winona State College. The laboratory was outfitted with running well water provided by a sand point well. Six fiberglas rearing tanks (24 in. wide, 22 in. deep, 72 in. long) were constructed. Each was fitted with running water, drain, screen canopy, lights and air bubblers. Rearing procedures were those described by Fremling (1967).

Bioassay experiments were conducted according to Standard Methods for the Examination of Water and Wastewater (American Public Health Association, 1965). A serial diluter, which was constructed according to the design of Mount and Brungs (1967), metered toxicants during bioassay experiments.

Attempts were made to study the behavior of Hexagenia nymphs within their burrows by utilizing thin aquaria filled with mud and water. Because the nymphs avoided light, however, they usually constructed their burrows so that little was exposed to the viewer. Hexagenia nymphs were also used in bioassay experiments to determine their tolerance to various toxicants. When test nymphs were contained in clean, water-filled vessels, however, they swam constantly as they attempted to burrow into the bottoms of the vessels. As a consequence of this activity they became fatigued and their susceptibility to toxicants was increased. If the bioassay vessels were provided with mud bottoms, the water in the vessels became turbid and it became difficult to observe the nymphs. Also, the mud made accurate toxicant monitoring virtually impossible. Consequently, it became necessary to fabricate inexpensive, inert, artificial substrates which would be suitable for nymphal behavior studies and for bioassay work (Fremling and Schoening, 1970).

The first substrate was made by imbedding 10 curled pretzels at uniform intervals around the interior of a 9 in. x 2 in. wooden mold which had been filled to a depth of 2 in. with epoxy resin and its hardening catalyst. The resin had been previously mixed with equal parts of dry sawdust to economize on resin. After the resin block had hardened it was removed from its mold and allowed to soak until the pretzels became soft enough to flush out. The edges of the burrows were then trimmed with a small chisel.

Epoxy resin substrates were quite expensive and it required considerable time to construct them. Therefore, a master mold was made of epoxy resin so that subsequent substrates could be easily manufactured from a resilient polyvinyl acetate plastic (Fig. 3).

Observation aquaria and bioassay vessels were constructed by building a tight-fitting glass aquarium around each substrate with glass glue. When used as bioassay vessels in conjunction with a Mount-Brungs proportional diluter, the aquaria were fitted with intermittent siphon drains and the vessels were arranged so that their sides touched, thus making the burrows dark. Blocks of wood were placed around the perim-
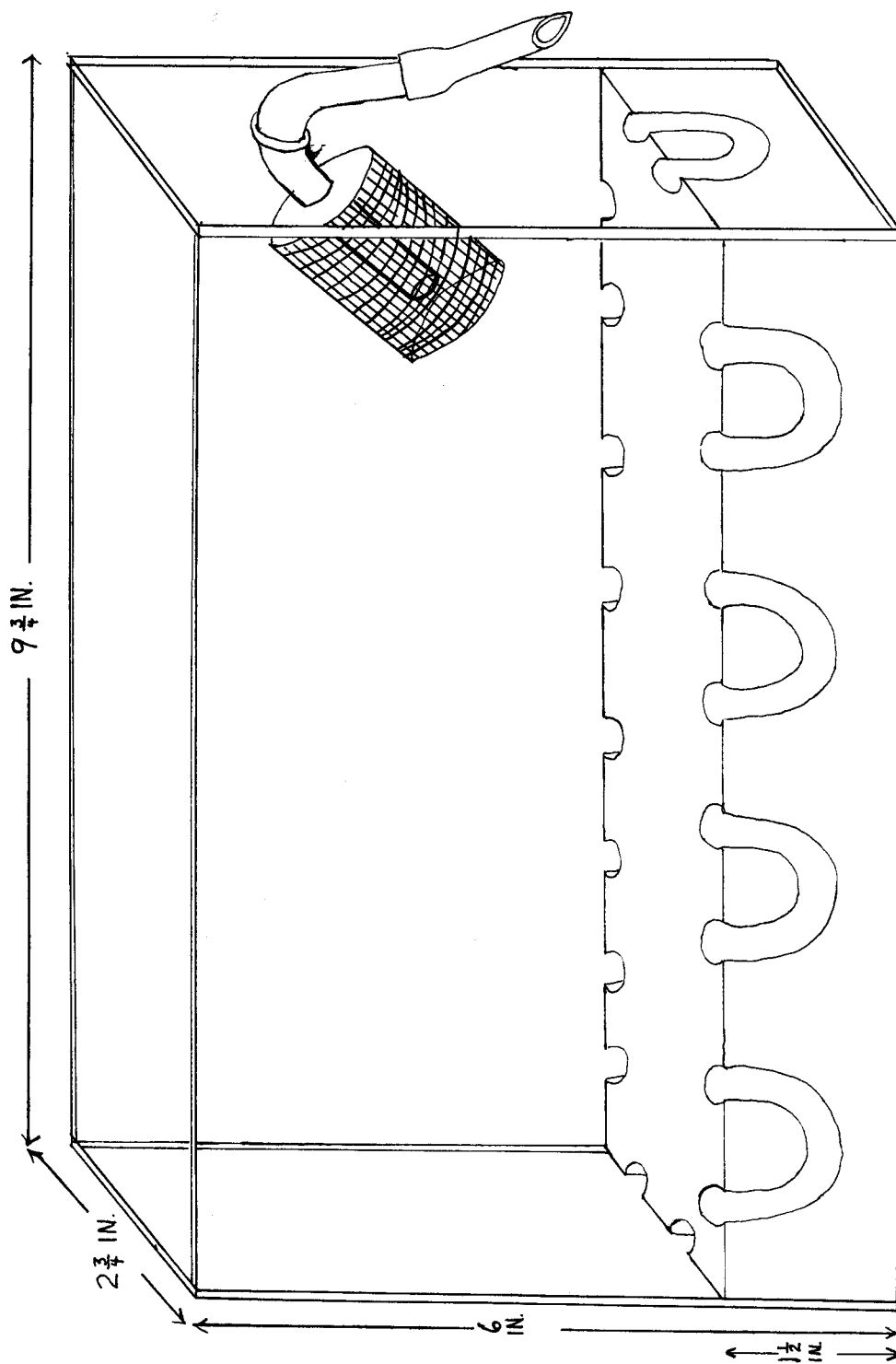
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FIGURE 3. Vessel used in bioassay experiments with Hexagenia nymphs. The burrow-containing substrate is molded from polyvinyl acetate plastic.

eter of the resultant block of vessels so as to darken the outer bur-
rows. At prescribed intervals during the bioassay, the wooden blocks
were removed and the vessels were separated so as to view the nymphs in
their burrows (Fig. 4). Nymphs almost always leave their burrows
before they die or are evicted by more vigorous nymphs. Thus, it was
possible not only to see which were dead but also those which were
"ecologically dead" (those which were still feebly alive but which had
abandoned their burrows and were thus exposed to predators). Behavior
studies of nymphs within their artificial burrows were best made under
red or yellow light because the nymphs are relatively insensitive to
long wave lengths of the visible spectrum.

When the polyvinyl acetate substrates were newly-constructed they ap-
parently contained excess plasticizer and volatiles which were toxic.
Therefore, they were routinely stored in a well-aerated area until
their smell was gone. They were also washed thoroughly in detergent
water prior to use. Polyvinyl acetate mayfly substrates can now be
obtained from the NASCO Company, Fort Atkinson, Wisconsin, 53538.

Routine water chemistry work was done with a variety of Hach chemical
kits. A colorimeter was used when greater precision was desired.
Toxicant levels of heavy metals were determined with a Beckman model
DB-G spectrophotometer fitted with a Beckman model 1301 atomic absorp-
tion unit and a Beckman laminar flow burner.

Nymphs for most bioassay experiments were raised in the laboratory.
Care was taken to use nymphs which were not in their last instar and
which were fairly uniform in size. Laboratory-reared specimens were
preferred because it was possible to know their species with certainty.
There is no way to positively distinguish medium-sized nymphs of H.
bilineata from those of H. limbata.

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FIGURE 4. Hexagenia bilineata nymph residing in an artificial burrow.

Hexagenia bilineata

The biology of H. bilineata has been reviewed by Needham, et al.,
(1935) and by Fremling (1960). The species is very abundant in most of
the navigation pools of the Upper Mississippi River. Most of the
nuisance problems created along the river may be attributable to this
species. The species does well in silted impoundments and it is truly
a "big river" mayfly. Although I once collected the species 8½ miles
from the river I have found that the species usually confines its
swarming activities to the river's edge and its upstream oviposition
flights to the river proper. During a mass emergence, subimagoes may
congregate on either shore if the night is virtually windless. Usually,
however, they are concentrated on one shore or the other by the wind.
Most nuisance problems are caused by imagoes which are attracted to the
lights of boats, bridges and cities during their upstream oviposition
flight (Fremling, 1968). Like the subimagoes, imagoes are usually
wafted by breezes toward one shore or the other. Consequently, a city
on one side of the river may be deluged with mayflies while its sister
city on the opposite side of the river may be free of mayflies. In
general, cities on north or east banks of the river are most prone to
be consistently troubled with mayflies. I have no evidence that this
species seeks out small tributary streams for oviposition.

The silted navigation pools of the Upper Mississippi River provide
excellent habitat for this species. Smaller impoundments upstream
from Minneapolis also provide H. bilineata habitat and the species has
been collected at St. Cloud (mile 927 U.M.R.), Sartell (mile 935 U.M.R.)
and Brainerd (mile 1001 U.M.R.).

H. bilineata is conspicuously rare in the river for 30 miles down-
stream from Minneapolis (Fig. 5). An extremely heavy pollutant load
in that area precludes the existence of the species because the river
bottom is anaerobic for much of the year. Conversations with long-
time river residents have revealed that H. bilineata was formerly
abundant in Lake Pepin (mile 766 - 786 U.M.R.) and that it often caused
nuisance problems in Lake City (mile 773 U.M.R.). Its numbers have
been severely reduced in Lake Pepin in recent years, however. Lake
Pepin evidently serves as a settling basin for pollutants from the
Minneapolis-St. Paul area and also for algae whose proliferation is
caused by upstream fertilization. Chironomid midges have replaced bur-
rowing mayflies throughout most of Lake Pepin. Emergence records
indicate that H. bilineata reaches maximum concentrations in the area
from Dubuque, Iowa (mile 580 U.M.R.), to Keokuk. The species is seldom
reported below St. Louis (Fig. 5). It is unlikely that Vicksburg,
Mississippi, is merely the southern limit of the range of H. bilineata
in the Mississippi River because the species has been collected as far
south as Florida (Berner, 1950). The scarcity of H. bilineata below
St. Louis is due primarily to two factors. The river changes character
at St. Louis (the sight of the last navigation dam) and the swift,
channelized river below St. Louis provides meager habitat. The pol-
lutant load downstream from St. Louis is extreme because of sewage from
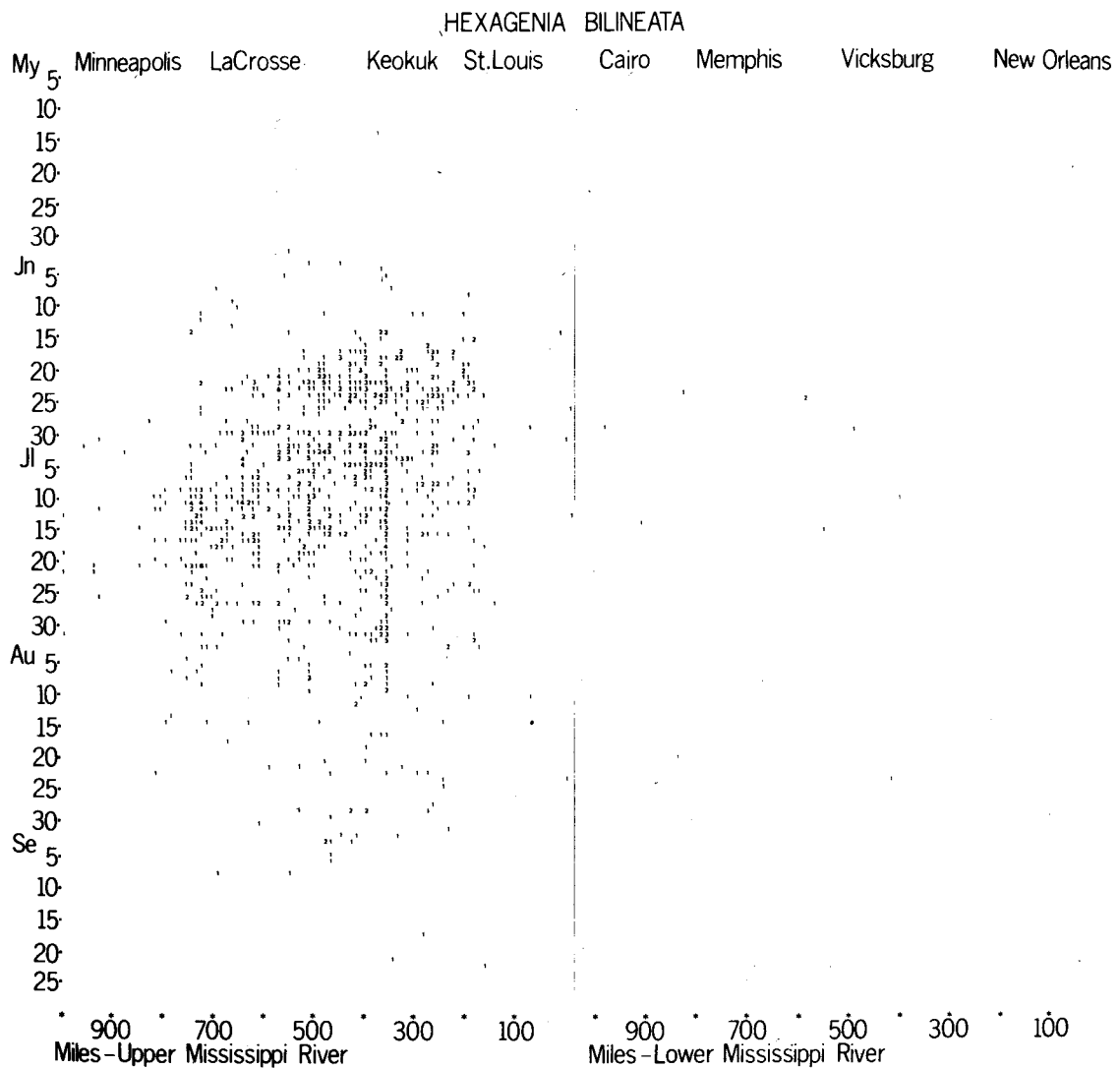
HEXAGENIA BILINEATA

FIGURE 5. Seasonal and geographical distribution of Hexagenia bilineata on the Mississippi River from Brainerd, Minnesota, to the Gulf of Mexico as indicated by collections of imagoes and subimagoes during mass emergences. Each number indicates the total mass emergences thus reported at that point during the interval 1957-1969.

metropolitan St. Louis itself, but also from the Illinois River. The
Mississippi River at St. Louis becomes an open sewer.

Hexagenia limbata

The biology of H. limbata has been reviewed by Needham, et al. (1935)
and by Hunt (1953). In general, the distribution of H. limbata follows
that of H. bilineata on the Mississippi River (Fig. 6). H. limbata,
however, also inhabits tributary rivers, streams and lakes in which
there is sufficient respiratory oxygen the year around. H. limbata is
a versatile mayfly and it is able to occupy a variety of silted habitats
from south-central Canada to central Texas (Hamilton, 1959). On the
Upper Mississippi River, H. limbata occurs farther north than H.
bilineata. Adults of H. limbata have been collected at Little Falls
(mile 965), Brainerd (mile 1000), Aitkin (mile 1055), Grand Rapids
(mile 1180), Bemidji (mile 1304) and Lake Itasca (mile 1365 - the
source of the river).

H. limbata, unlike H. bilineata, does not confine its mating and ovi-
position activities to the river proper. Whereas the mating swarms of
H. bilineata are usually large and along the river's edge, H. limbata
swarms may consist of a few individuals, often just above the tree tops
and often several hundred yards from the water's edge. The oviposition
flights of H. limbata extend far up small tributary streams and even
overland. H. limbata is consistently found farther from the river than
H. bilineata. Mayfly nuisance problems created in the interior areas
of river cities are usually attributable to H. limbata.

H. limbata attains maximum concentrations in the area from Winona,
Minnesota (mile 726 U.M.R.), to Prairie du Chien, Wisconsin (mile 635
U.M.R.). Here a large, early-summer population is apparently due, in
part, to downstream drift of H. limbata nymphs from tributary rivers
such as the Chippewa, Zumbro, Whitewater, Trempealeau, Black and Bad
Axe as well as many smaller streams populated by H. limbata. Prelim-
inary studies conducted at Winona, Minnesota, indicate that large
numbers of Hexagenia nymphs drift down the Mississippi River. Swanson
(1967) reports that mass drifting of Hexagenia nymphs also occurs in
the Missouri River.

It is evident from Figures 6 and 7 that the Keokuk Pool, which produces
many H. limbata in early summer, becomes marginal habitat for H. limbata
in mid-summer. Here, H. bilineata obviously becomes the dominant form
as the summer progresses. It may also be observed that the early,
upstream portion of H. bilineata's seasonal emergence distribution is
vacant and that the early, upstream portion of H. limbata's distrib-
ution fits neatly into the space. H. limbata emergences usually occur
prior to those of H. bilineata because H. limbata is able to emerge at
lower water temperatures. Under laboratory conditions, H. limbata has
emerged and flown vigorously at temperatures as low as 14.5°C. H.

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HEXAGENIA LIMBATA

My
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Minneapolis LaCrosse   Keokuk St.Louis   Cairo   Memphis   Vicksburg   New Orleans
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900 · 700 · 500 · 300 · 100   · 900 · 700 · 500 · 300 · 100
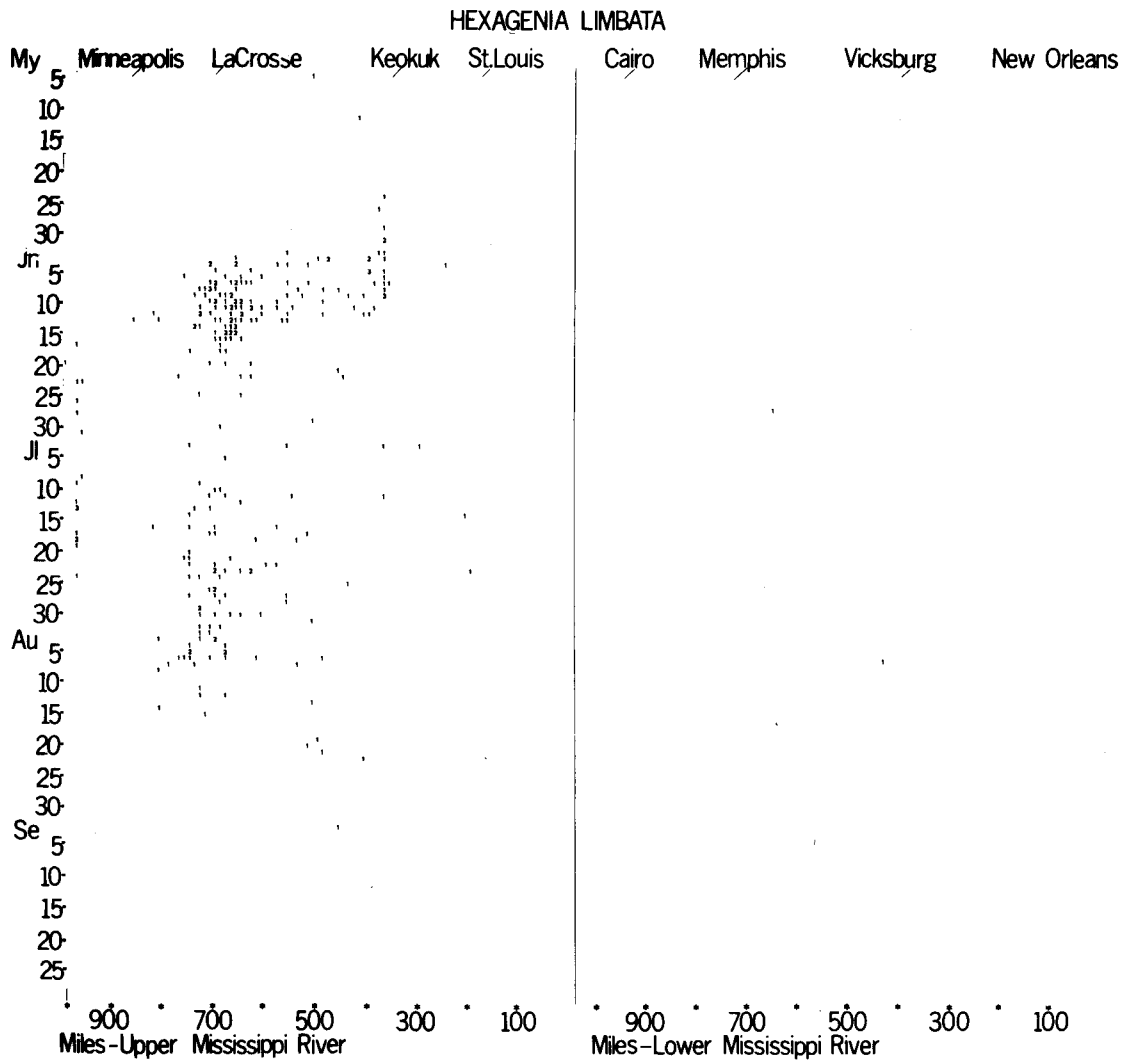Miles-Upper Mississippi River   Miles-Lower Mississippi River

FIGURE 6. Seasonal and geographical distribution of Hexagenia limbata
on the Mississippi River from Brainerd, Minnesota, to the Gulf of Mexico
as indicated by collections of imagoes and subimagoes during mass
emergences. Each number indicates the total mass emergences thus
reported at that point during the interval 1957-1969.

HEXAGENIA BILINEATA HEXAGENIA LIMBATA

Minneapolis Dubuque Keokuk St. Louis Minneapolis Dubuque Keokuk St. Louis

1000 900 800 700 600 500 400 300 200 100 1000 900 800 700 600 500 400 300 200
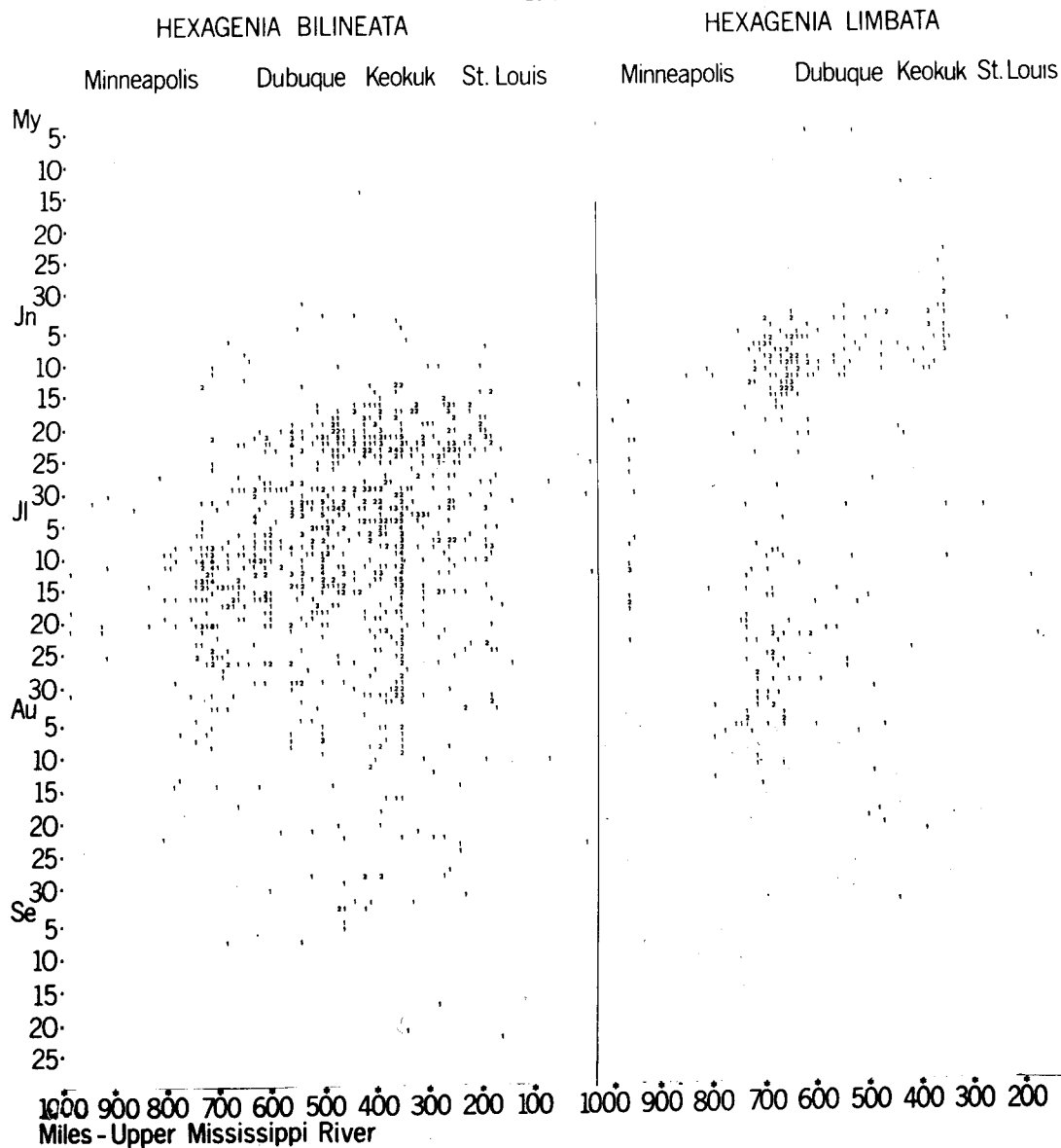Miles - Upper Mississippi River

FIGURE 7. Seasonal and geographical distribution of Hexagenia bilineata
and Hexagenia limbata on the Upper Mississippi River from Brainerd,
Minnesota, to Cairo, Illinois. Note that the seasonal distribution of
H. limbata complements that of H. bilineata.

bilineata, on the other hand, does not emerge under laboratory conditions or from the river until water temperatures reach 18°C (Fremling, 1964; Thomforde and Fremling, 1968). It seems likely that H. limbata produces a summer generation in the Mississippi River. Late summer emergences are not as abrupt as those of early-summer, however, because the latter have not been coordinated by winter.

Like H. bilineata, H. limbata finds the zones of degradation below Minneapolis and St. Louis unsuitable for habitation. While both H. bilineata and H. limbata are excellent indicators of good general water quality, H. limbata is the better indicator of the two on the Upper Mississippi River.

Pentagenia vittigera

The biology of Pentagenia vittigera is poorly known. The nymphs, which apparently live in faster water areas than H. bilineata or H. limbata, are difficult to collect. Like H. limbata, however, the insect is versatile and it has been collected from a wide latitudinal area. Ide (1955) has collected adult P. vittigera along the bank of the Assiniboine River near its junction with the Red River at Winnipeg, Manitoba. P. vittigera has also been collected as far south as the Apalachicola River in Florida (Berner, 1950).

Although Daggy reported in 1941 that the species occurred in the Mississippi River at Red Wing, Lake City and Minneapolis, Minnesota, the species is rarely collected in that area now. The seasonal distribution of P. vittigera is very unusual in the 400-mile segment of river below St. Louis (Fig. 8). There the insect emerges only during the very early summer and very late summer, even though it occurs all summer above St. Louis and from mile 600 L.M.R. almost to New Orleans. The pollutant load below St. Louis is apparently sufficient to render about 400 miles of the river uninhabitable during the time when river temperatures are highest. It seems likely, in that area, that organic enrichment causes low dissolved oxygen levels during the heat of the summer even though the river is not impounded. Certainly, summer water temperatures and low dissolved oxygen levels also combine to make Pentagenia nymphs more vulnerable to the complex of agricultural and industrial pollutants which enter the river in the St. Louis area. Early summer and late summer emergences from the 400-mile zone below St. Louis are apparently caused by nymphs which have drifted into the area when low water temperatures and elevated dissolved oxygen levels have made the zone of degradation less deadly.

Discussion

Because of its impounded state, the Mississippi River provides excellent habitat for H. bilineata throughout much of the area from Hastings,
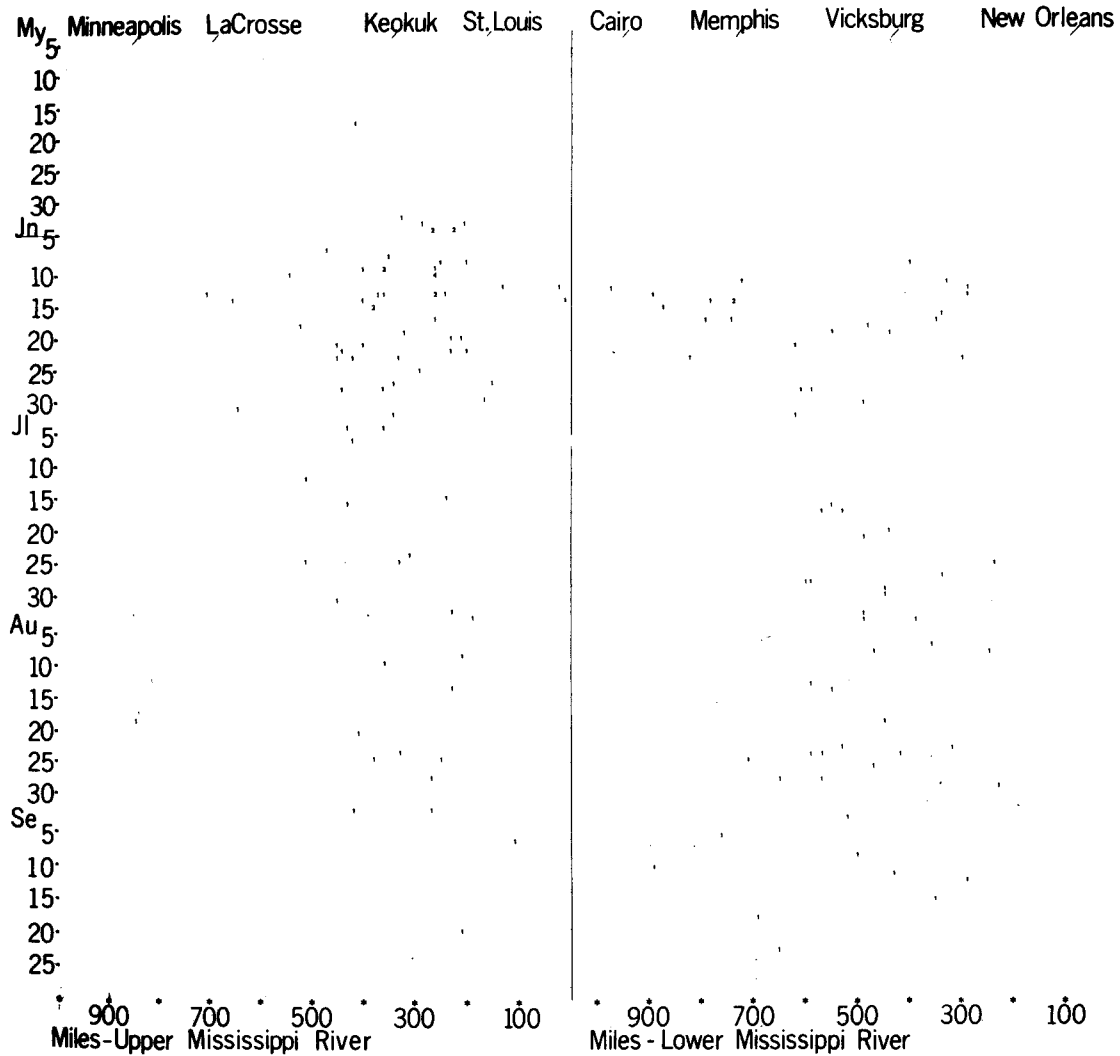
20

PENTAGENIA VITTIGERA

FIGURE 8. Seasonal and geographical distribution of Pentagenia
vittigera on the Mississippi River from Brainerd, Minnesota, to the
Gulf of Mexico as indicated by collections of imagoes and subimagoes
during mass emergences. Each number indicates the total mass emer-
gences thus reported at that point during the interval 1957-1969.

Minnesota, to St. Louis, Missouri. The navigation dams which were constructed during the 1930's have undoubtedly increased the carrying capacity of the Upper Mississippi River for H. bilineata mayflies. It is likely that this very specialized species now dominates many areas which were formerly dominated by H. limbata.

Water pollutants are severe limiting factors to mayflies. To a degree, however, nutrients from metropolitan Minneapolis and St. Paul have increased the carrying capacity of portions of the Upper Mississippi River for Hexagenia mayflies. In areas where dissolved oxygen is not a limiting factor, the enrichment has caused algae to proliferate, thus causing an increased food supply for burrowing mayflies.

The compact emergence patterns of H. bilineata during late June and of H. limbata during early June are due to a "stockpiling" of large nymphs during the winter months. The nymphs are able to grow slowly during the winter but they are unable to complete last instar development until early summer temperatures are attained. Thus, early summer emergence is coordinated by cold winter temperatures. Hexagenia emergences which occur later in the summer are obviously less well coordinated.

In early summer, the Upper Mississippi River provides good H. limbata habitat as far south as Keokuk. With increasing summer temperatures, however, H. limbata becomes less common in the Keokuk area. Increasing summer temperatures cause the apparent displacement of H. limbata by H. bilineata in the impoundments near Keokuk.

Water pollution obviously poses a severe threat to the burrowing may-flies of the Mississippi River. Even if sewage treatment plants effectively remove most putrescible wastes and fertilizer elements, other contaminants may eventually erradicate them. Non-biodegradable insecticides, heavy metals and other toxicants pose serious threats. Unless sewage treatment keeps pace with population growth and increased industrialization, burrowing mayflies may be eliminated from the Mississippi River as they have been in many other areas of the U.S. (Fremling, 1968).

In many areas of the Upper Mississippi River, roads, dikes and railroads have dissected the flood plain, thus cutting off the flow of water into old river channels. Such occluded channels and oxbow lakes stagnate in the summer and exhibit marked thermal stratification. The hypo-limnia in such bodies of water usually become deficient in dissolved oxygen during the summer because of the high biochemical oxygen demand of bottom sediments. The same lakes often become deficient in oxygen during the winter when heavy ice and snow cover prevents sufficient light penetration to produce oxygen by photosynthesis. Such lakes usually produce no burrowing mayflies. Furthermore, they are death traps for nymphs which drift into them during spring floods.

The most insidious threat to burrowing mayfly populations on the Upper Mississippi River is sand. Ever since the Wisconsin glacial period the bed of the river from the mouth of the Chippewa River (mile 763 U.M.R.) to Keokuk has been slowly rising. Here, sand washed down from precipitous sandstone-limestone bluffs has entered the river at a rate faster than the river can remove it. The activities of man (agriculture, construction, etc.) have greatly accelerated the rate of sand deposition in recent years. The navigation dams, in turn, have provided places where the sand can accumulate. As a consequence, the pools are rapidly filling with sand. The U.S. Army Corps of Engineers dredges constantly to remove sand from the navigable channel of the river, but the dredged sand continues to accumulate outside the main channel in the navigation pools. As a consequence, silted river bottoms are rapidly being replaced by sand bars and islands. Unless corrective measures are initiated very soon, the Upper Mississippi River will be transformed into a single, narrow, unproductive channel as is the Lower Mississippi River.

Burrowing mayflies are good indicators of general water quality because their life cycles are relatively long. Although nymphs may drift for considerable distances, they are unable to swim directively for long distances to escape toxic conditions. It is obvious that even downstream drift of nymphs cannot compensate for nymphal deaths in the most severely polluted segments of the river. Unlike chemical tests which describe pollutant levels only at the time the tests were taken, mayfly distribution indicates what water conditions have been like for a prolonged period. Moreover, while chemical tests only test for specific pollutants, mayfly distribution indicates the subtle synergistic effects of combinations of many pollutants. This is especially evident below St. Louis where P. vittigera is only able to emerge in early and late summer. The mayfly distribution data resulting from this study should also provide valuable baseline data so that future changes in general water quality can be objectively assessed along the entire Mississippi River.

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TOLERANCE OF HEXAGENIA NYMPHS TO VARIOUS
ENVIRONMENTAL STRESSES

Low Levels of Dissolved Oxygen

Experiments conducted at low levels of dissolved oxygen reveal that
H. bilineata nymphs are very hardy. Bioassay vessels containing
artificial substrates were used as test containers. A siphon with
pinch clamp was fitted so that water could be drawn conveniently
from the vessel at the burrow level for testing. Test vessels were
covered to prevent air from entering. Thus, nymphs were unable to
swim to the water's surface to obtain oxygen. Observations were
made under yellow light so that nymphal activity would not be in-
creased. Control animals in well-oxygenated aquaria were utilized
in all instances.

Four large nymphs were observed as the oxygen level in their test
vessel was gradually lowered, at 25°C, by effervescing the water
with nitrogen. At an initial dissolved concentration of 7 ppm the
nymphs remained quiescent in their burrows with a slow, regular
gill beat (1 beat every 5 sec). The dissolved oxygen level was
gradually decreased to 0.6 ppm over a period of 3 hr and 40 min.
Swimming movements and a resultant competition for burrows increased
as the oxygen level fell below 1 ppm. After the test period, air
was bubbled into the test vessel and all test nymphs were alive the
following day.

The same 4 nymphs were used in another experiment 2 days later.
The oxygen level, at 24°C, was decreased from 7.2 ppm to zero over
a 4-hr period. As the oxygen level fell below 1 ppm, the nymphs
left their burrows with increasing frequency. At 0.1 ppm the
nymphs lost equilibrium, walked about on the substrate, and swam
occasionally (always toward the surface). Gill movements, which
had increased initially, became erratic at 0.1 ppm and almost
ceased as the D.O. approached 0 ppm. At 0 ppm all 4 nymphs had
abandoned their burrows. Nymphal respiratory movements increased
quickly to 2 beats per sec when nitrogen washing was discontinued
and aeration was begun.

In another experiment, test water was washed with nitrogen for 2 hr
to deplete the dissolved oxygen at 23°C. Ten nymphs of various
sizes were placed in the test vessel at an initial D.O. of 0.3 ppm.
After 41 min, the nymphs exhibited very little swimming activity,
but their gills moved rapidly (5-10 beats per sec). During the
last 2 hr of the experiment no dissolved oxygen could be detected.
After a total time of 4 hr and 7 min, aeration was begun and only a
last instar nymph failed to recover quickly. The nymph was thought
to be dead, but it had molted to the subimaginal state by the fol-
lowing morning.

In a subsequent experiment, 6 nymphs were placed in water which con-
tained 1 ppm D.O. at 24°C. The D.O. was then lowered by supple-
mental nitrogen effervescence for 10 hr. After 4 hr at zero D.O.,

the nymphs appeared to be dead. At intervals of about 1½ hr, however, they swam feebly and then returned to their "dead" position. The nymphs recovered quickly with oxygenation at the termination of the 10-hr period, having lived through a 9-hr period during which no dissolved oxygen could be detected. Further testing revealed that about one out of 6 nymphs could recover after being treated anaerobically for 11 hr.

Low D.O. in combination with elevated carbon dioxide levels commonly occurs in nature. Carbon dioxide was bubbled into a test vessel (not covered) for 1 hr and 40 min. During that time the D.O. fell from 7 ppm to 2.8 ppm while the carbon dioxide level rose from 5 ppm to 575 ppm. At 50 ppm carbon dioxide, swimming activities increased markedly. At 100 ppm, equilibrium problems developed. After 1 hr and 40 min, carbon dioxide bubbling was discontinued and the vessel was allowed to stand with no aeration. All the nymphs were dead 12 hr later.

In another test at 31°C, the initial D.O. was 6.8 ppm and the initial carbon dioxide concentration was 3 ppm. Carbon dioxide was slowly bubbled into the water. Seven min after bubbling began, most nymphs left their burrows and swam actively. At 17 min (190 ppm carbon dioxide) the nymphs began to lose their equilibrium, ceased swimming, and lay upside down upon the substrate with gills barely moving. At 40 min (carbon dioxide 750 ppm, pH 6.1, D.O. 0.8 ppm) all nymphs (10) appeared dead. Bubbling was continued, however, and two nymphs were removed at hourly intervals and placed in oxygenated water. Nymphs recovered after being in the test situation for as long as 3½ hr.

From the previous experiments it is evident that H. bilineata nymphs can tolerate extremely low dissolved oxygen levels and extraordinarily high carbon dioxide levels for several hours. The nymph's first reaction to these conditions is to abandon its burrow and to swim to the surface. In rearing chambers which were caused to go anaerobic because of the addition of substances which had a high biochemical oxygen demand, the nymphs came to the surface and crawled partially out of the water so as to expose their gills to the atmosphere. The same phenomenon has been observed in late winter on the Mississippi River where oxygen levels in sloughs have dropped. There the nymphs congregate in muskrat runways and in holes cut by fishermen. After prolonged low oxygen levels, the nymphs become torpid and lie on the bottom seemingly dead, but are able to recover in oxygenated water. In nature, nymphs undoubtedly swim actively or drift long distances with the current to escape low oxygen conditions. It is obvious that nymphs could drift passively for over 20 miles through a zone of degradation if the current speed approached 2 mph.

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Hydrogen Sulfide

The effects of hydrogen sulfide have been determined on certain fish and aquatic organisms (McKee and Wolf, 1963; Bonn and Follis, 1967; Jones, 1948; Van Horn, Anderson, and Katz, 1949). The studies present conflicting results in several instances, however. This is probably because the early studies did not account for varying pH. Bonn and Follis (1967) state that the toxicity of hydrogen sulfide to channel catfish varies with changes in pH of the solution. It is known that hydrogen sulfide dissociates primarily according to pH, with temperature and other factors playing a minor role (McKee and Wolf, 1963). In general, the lower the pH, the greater the degree of dissociation.

In the present study, 10 laboratory-reared H. bilineata nymphs (20-25 mm in length) were placed in each of five bioassay vessels (previously described). The volumes of the mixing chambers of the serial diluter were adjusted by placing marbles in the chambers to give final dilutions of 20 ppm, 10 ppm, 5 ppm, and 2.5 ppm of total hydrogen sulfide. The fifth bioassay vessel received no toxicant, thus serving as a control. A stock solution of hydrogen sulfide was prepared by saturating distilled water with sodium sulfide. The stock solution of hydrogen sulfide was placed in a Mariotte bottle and the serial diluter allowed a small quantity of this solution to enter each mixing chamber at the start of each cycle.

The pH of the water in the test vessels was determined to be 7.9. The dissolved total sulfide content was tested with a Hach colorimeter. The un-ionized hydrogen sulfide was calculated from the dissolved total hydrogen sulfide and the pH of the sample (American Public Health Association et al., 1965). The bioassay vessels were aerated by the discharge from the serial diluter, and the D.O. varied from 5.5 ppm to 6.2 ppm. The test was conducted for 96 hr at 22°C.

About 24 hr after the test began, the nymphs showed some effects of the hydrogen sulfide. In the bioassay vessels with the greater concentrations, nymphs deserted their burrows and began to move their gills very rapidly. It was several hours after they left their burrows that the nymphs actually died. The nymphs were considered to be dead when all visible gill movements had ceased and they failed to respond to mechanical stimuli.

TL_m values varied from .42 ppm at 48 hours; to .21 ppm at 72 hours; to .17 ppm at 96 hours. All TL_ms are given in ppm of un-ionized hydrogen sulfide rather than total dissolved hydrogen sulfide.

From the previous experiments with dissolved oxygen, carbon dioxide and hydrogen sulfide it is apparent that Hexagenia nymphs abandon their burrows long before they succumb to toxic concentrations of the three gases. In nature, depressed D.O., and elevated carbon dioxide and hydrogen sulfide levels occur commonly in areas where high B.O.D. materials accumulate at the mud-water interface. These conditions force nymphs from their burrows, thus causing their "ecological death" in many instances. Those nymphs which are not killed by

predators must either swim to better habitat or enter the current so that they can drift downstream. In areas where no current exists, nymphs swim upward to escape toxic conditions at the mud-water interface. Although the water at higher levels may not be toxic, the nymphs are not adapted for life there. Their specific gravity is such that they begin to sink as soon as they stop swimming. Thus, when exhausted from swimming, they sink to their deaths on the surface of the mud. The latter situation has been observed several times in laboratory rearing tanks.

Heavy Metals

The acute toxicities of several heavy metals to Hexagenia mayfly nymphs were determined by flowing water bioassay procedures. As indicated previously, a Mount Brungs diluter was used to meter out the various toxicants. Ten nymphs were placed in each of five bioassay vessels which contained artificial substrates. Four of the vessels contained various dilutions of toxicant. The fifth vessel served as a control. Whenever possible, laboratory-reared nymphs were used so that their species was known with certainty. Occasionally, however, it was necessary to dredge nymphs from the river. Laboratory-reared nymphs were preferred because they were priorly acclimated to laboratory conditions. Nymphs of relatively uniform size were used. Nymphal length was determined by measuring from the anterior tip of the pronotum to the posterior end of the abdomen.

The well water used in the bioassay tests was monitored regularly. Total hardness during a one-year test period varied from 357 to 448 ppm, calcium hardness varied from 187 to 238 ppm, and total alkalinity varied from 289 to 391 ppm. Nitrates, nitrites and phosphates were not present in detectable concentrations. With minor exceptions, temperature varied from 24 to 28°C and pH varied from 7.0 to 7.3. Oxygen concentrations were relatively constant in the bioassay vessels, varying from 35 to 50% of saturation. Toxicant concentrations for all metals (with the exception of mercury) were determined by standard flame emission analyses with an atomic absorption spectrophotometer. Numerous runs were made with each toxicant in an effort to obtain TL_m values for 12, 24, 48, 72 and 96 hr. Results of these tests are summarized in Table I.

Zinc

The toxicity of zinc to various species of fish has been summarized by Skidmore (1964), Doudoroff and Katz (1953), McKee and Wolf (1963), Pickering (1968), and Brungs (1969). Mount (1966), in his work with the acute toxicity of zinc to fathead minnows (Pimephales promelas), reports that toxicity increased at high pH levels and that precipitated zinc may have accounted, in part, for the increased toxicity.

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In my tests, zinc precipitated out (presumably as zinc carbonate) at
concentrations in excess of 12.5 ppm. The zinc precipitate had no
apparent detrimental effect on test nymphs even though it accumulated
visibly in their burrows. It was noted, however, that nymphs seemed
to moult with unusual frequency during the tests. At the pH and
hardness levels utilized in these experiments, zinc at 12.5 ppm ex-
hibited no apparent toxicity, even during a 96-hr period. Galvanized
stock watering tanks have been used with excellent results for 10
years for rearing Hexagenia nymphs in my laboratory. Thousands of
insects have been reared through complete life cycles in galvanized
containers.

Copper

In flow-through bioassay experiments to determine the toxicity of
copper to adult fathead minnows, Mount (1968) obtained a 96-hr TLm
value of 0.47 ppm in water which ranged in pH from 7.9 to 8.5
(mean hardness 198 ppm as $CaCO_3$). In soft water (ph 7.7 - 8.1,
total $CaCO_3$ hardness 35 - 50 ppm), Arthur and Leonard (1970) deter-
mined average 96-hr TLm values for copper to be 1.7 ppm for
Campeloma decisum snails, 0.039 ppm for Physa integra snails and
0.02 ppm for Gammarus pseudolimnaeus amphipods. Warnick and Bell
(1969) tested the effects of copper on the mayfly Ephemerella
subvaria and the stonefly Acroneuria lycorias. In water which had a
pH of 7.25 and total hardness of 44.0 ppm, they obtained a 48-hr
TLm of 0.32 ppm for E. subvaria and a 96-hr TLm of 8.3 ppm for A.
lycorias. In my experiments, copper was extremely toxic to H.
bilineata nymphs. TLm values were 0.54 ppm for 12 hr, 0.34 ppm for
24 hr and 0.22 ppm for 48 hr.

Nickel

Nickel, even at concentrations of 27.2 ppm, caused no mortality among
H. bilineata nymphs for periods as long as 96 hr. Warnick and Bell
(1969) in experiments with softer water (alkalinity, 40 ppm; hard-
ness, 44 ppm), report 96-hr TLm values of 4 for the mayfly Ephemerella
subvaria and 33.5 for the stonefly Acroneuria lycorias.

Chromium

Chromium was relatively toxic to Hexagenia nymphs. TLm values ranged
from 12.8 ppm at 48 hr to 8.6 at 96 hr. Warnick and Bell (1969)
report a 96-hr TLm value of 2 for E. subvaria.

Mercury

During the summer of 1970, many bioassays were conducted with mercury.
I was unable, however, to determine precise toxicant levels because

Table I

Results of bioassays to determine TLm values for various heavy metals.

ION	SOURCE	TLm (ppm)					TEMP	SPECIES	SIZE
		12 hr	24 hr	48 hr	72 hr	96 hr			
Cr	$K_2Cr_2O_7$	-----	-----	12.8	12.1	8.6	26-27C	_Hexagenia_ sp.	8-19 mm
Ni	$NiSO_4$	-----	-----	-----	-----	greater than 27.2	26-27C	_H. bilineata_	20-28 mm
Zn	$ZnSO_4$	-----	-----	-----	-----	greater than 12.5	24-25C	_H. bilineata_	15-25 mm
Cu	$CuSO_4$	0.54	0.34	0.22	-----	------	24-25C	_H. bilineata_	15-25 mm
Cd	$CdSO_4$	Tests run, but final toxicant levels not determined							
Hg	$HgCl_3$	Tests run, but final toxicant levels not determined							

of the lack of sensitivity of our atomic absorption unit for this
element.   Toxicant concentrations were estimated emperically and
water samples were taken from the test vessels at regular intervals.
The samples were preserved in sealed glass bottles for analysis
when improved atomic absorption procedures could be instituted.   At
present, we are constructing the necessary apparatus to do the
analyses according to methods described by Fishman (1970).   It was
possible, by the method previously described, to make determina-
tions of the 12, 24, 48 and 96 TLm for mercury.   Although precise
determinations cannot be yet reported, mercury is apparently very
toxic with a 24-hr TLm level of less than 1 ppm.   In their work with
the mayfly Ephemerella subvaria, Warnick and Bell (1969) report
96-hr TLm values of 2 ppm for mercury.

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1 Accession Number	2 Subject Field & Group	SELECTED WATER RESOURCES ABSTRACTS INPUT TRANSACTION FORM

5 Organization
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6 Title
Mayfly Distribution as a Water Quality Index

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As above.

27 Abstract

Three species of burrowing mayflies (Hexagenia bilineata, Hexagenia limbata, and Pentagenia vittigera) are sufficiently abundant to cause nuisance problems along portions of the Mississippi River. Mayfly distribution, as determined by collections made by ship captains and other cooperators over a 13-year period, has proven to be an excellent index of general water quality on a river which is so large that it cannot be monitored effectively or economically by standard methods. Pollutants have severely reduced the numbers of all three species for 30 miles below Minneapolis, Minnesota, and for over 300 miles below St. Louis, Missouri. P. vittigera is able to emerge only in early and late summer in the St. Louis area when cool water temperatures lessen toxic effects in the zone of degradation. Impoundment and enrichment of the Upper Mississippi River has temporarily increased the carrying capacity of the river for H. bilineata which now dominates areas formerly dominated by H. limbata. The total productivity of the Upper Mississippi is being reduced by pollution, man's encroachment into the flood plain and by the filling of navigation pools by sand.

Methods have been developed to rear large numbers of Hexagenia nymphs in the laboratory. Bioassay tests utilizing artificial, burrow-containing substrates reveal that H. bilineata nymphs can survive anaerobic conditions for as long as 11 hours. TLm values for hydrogen sulfide varied from 0.42 ppm at 48 hr to 0.17 ppm at 96 hr. Of several heavy metals (Cr, Ni, Zn, Cu) tested, copper was the most toxic to H. bilineata nymphs. TLm values for copper ranged from 0.54 ppm at 12 hr to 0.22 ppm at 48 hr.

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