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Effects of an Ammonia-Rich Municipal Sewage Effluent on Iowa River Fauna Near Marshalltown, Iowa[1]

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The effect of the Marshalltown municipal sewage effluent on Iowa River water quality and fauna was evaluated from July 1976 through August 1977. The effluent contains high total ammonia and un-ionized ammonia concentrations due to ammonia-rich discharges from meat packinghouses. Dissolved oxygen, pH, temperature, total ammonia nitrogen, and un-ionized ammonia data were collected at 12 sampling stations extending 18 km downstream from the sewage effluent discharge. Wild fish collections were made by using electrofishing, seines, and hoopnets. Thirty-eight fish species were collected during the study. Channel catfish (*Ictalurus punctatus*) and smallmouth bass (*Micropterus dolomieui*) were the most common gamefish. No consistent depression in wild fish diversity was seen below the sewage discharge point. Eight hundred thirty caged channel catfish were used in conducting 13 4-day field toxicity tests at 5 different river stations. Only 2% mortality was observed. Macroinvertebrate diversity and density were determined by using artificial substrate samplers placed at 5 river stations during 2 3-week exposure periods in the summer of 1976. Macroinvertebrate diversity recovered 770-1550 m downstream from the sewage discharge point. The applicability of the EPA un-ionized ammonia criterion and the Iowa total ammonia nitrogen standard is evaluated in light of the findings from this study.

INDEX DESCRIPTORS: Iowa River, fish, macroinvertebrates, sewage, total ammonia, un-ionized ammonia.

Ammonia, long known to be toxic to aquatic organisms, is produced by decomposing organic matter. It is found in low concentrations in unpolluted waters and in high concentrations in sewage and sewage treatment plant effluents. Because most municipal sewage effluents discharge into streams and rivers, ammonia concentrations in receiving streams can have a significant impact on water quality.

When ammonia is added to water, the following reaction occurs: $NH_3 + H_20 \rightleftharpoons NH_3 \cdot H_2O \rightleftharpoons NH_4^+ + OH^-$ (U.S. Environmental Protection Agency, 1977). The proportion of total ammonia nitrogen in the un-ionized form, $NH_3 \cdot H_2O$, is determined largely by pH and temperature. Un-ionized ammonia is the fraction assumed to be toxic to fish. Computations using water temperature and pH allow estimates of un-ionized concentrations (Emerson et al., 1975); at higher pH and temperature values, more of the un-ionized form exists.

Laboratory Toxicity Tests

The toxicity of ammonia to fish has been extensively studied in the laboratory. Numerous acute toxicity tests have been conducted on salmonids (Ball, 1967; Buckley, 1978; Kemp et al., 1973; Martens and Servizi, 1976; Rice and Stokes, 1975; Smart, 1976, 1978; Thurston et al., 1978). Thurston et al. (1978) reported a 4-day LC_{50} value of 0.5-0.8 mg/1 un-ionized ammonia for cutthroat trout (*Salmo clarki*) while Buckley (1978) determined a 4-day LC_{50} value of .45 mg/1 for fingerling coho salmon (*Oncorhynchus kisutch*). For rainbow trout (*Salmo gairdneri*), the most common salmonid species used for toxicity testing, a 24-hour TL_m value of .41 mg/1 was determined by Ball (1967) while Rice and Stokes (1975) reported differential ammonia toxicity to various life stages of rainbow trout. After yolk absorption, alevins showed a 24-hour TL_m of .056 mg/1 un-ionized ammonia compared with the adult 24-hour TL_m of 0.079 mg/1 (Rice and Stokes, 1975). For periods of 1-4 days, the LC_{50} values reported for these salmonids fall within the range of 0.05-0.8 mg/1 un-ionized ammonia.

Fewer acute toxicity tests have been conducted on warmwater fish (Ball, 1967; Flis, 1968; Gillette et al., 1952; Hazel et al., 1971; Summerfelt and Lewis, 1967). For green sunfish (*Lepomis cyanellus*), mortality occurred at 0.05 mg/1 un-ionized ammonia while concentrations of 0.04 mg/1 un-ionized ammonia and above repelled the sunfish (Summerfelt and Lewis, 1967). The striped bass (*Morone saxatilis*) and creek chub (*Semotilus atromaculatus*) seem more tolerant with un-ionized ammonia concentrations of 0.8 mg/1 (Hazel et al., 1971) and up to 0.97 mg/1 (Gillette et al., 1952), respectively, producing mortality. The channel catfish (*Ictalurus punctatus*) probably has received the most attention (Colt and Tchobanoglous, 1976, 1978; Knepp and Arkin, 1973; Robinette, 1976; Tomasso et al., 1980), of warmwater fish. Tomasso et al. (1980) reported a 24-hour LC_{50} value of 1.82 mg/1 un-ionized ammonia for channel catfish but noted that the LC_{50} value increased at higher pH. Robinette (1976) reported a 24-hour LC_{50} of 2.4 mg/1 un-ionized ammonia for the same species while a 96-hour TL_m of 3.8 mg/1 un-ionized ammonia was determined for 2-3 inch channel catfish (Colt and Tchobanoglous, 1976). For time periods of 1-4 days, the LC_{50} values and mortality values reported for warmwater fish vary between 0.05-3.8 mg/1 un-ionized ammonia. The highest value for warmwater fish is 4.5 times higher than the comparable value for salmonids.

Sublethal effects of ammonia include gill and kidney tissue damage (Smart, 1976; Thurston et al., 1978); increased oxygen consumption and increased heart rate (Smart 1978); increased urine output (Lloyd and Orr, 1969); and reduced growth rates (Robinette, 1976; Colt and Tchobanoglous, 1978). Woltering et al. (1978) found that 0.63 mg/1 and 0.86 mg/1 concentrations of un-ionized ammonia significantly affected bass prey consumption.

No ammonia toxocity tests have been performed on aquatic insects; however, Tarzwell (1965) has reviewed aquatic insect field surveys and reported a Trichopteran genus, *Hydropsyche*, existing where total ammonia nitrogen measured 10 ppm.

Field Studies

Very few field studies have examined the effects of high ammonia concentrations upon fish. A Montana sewage effluent (Avery, 1970) produced a river zone with less than .5 mg/1 un-ionized ammonia where brown trout (*Salmo trutta*), brook trout (*Salvelinus fontinalis*), and mountain whitefish (*Prosopium williamsoni*) were eliminated; yet rainbow trout (*Salmo gairdneri*) persisted. Allan et al. (1958) reported that 1.0 mg/1 un-ionized ammonia was negligibly toxic to chinook salmon (*Oncorhynchus tshawytscha*). Dugan and McGauhey (1977) reported rainbow trout flourishing in

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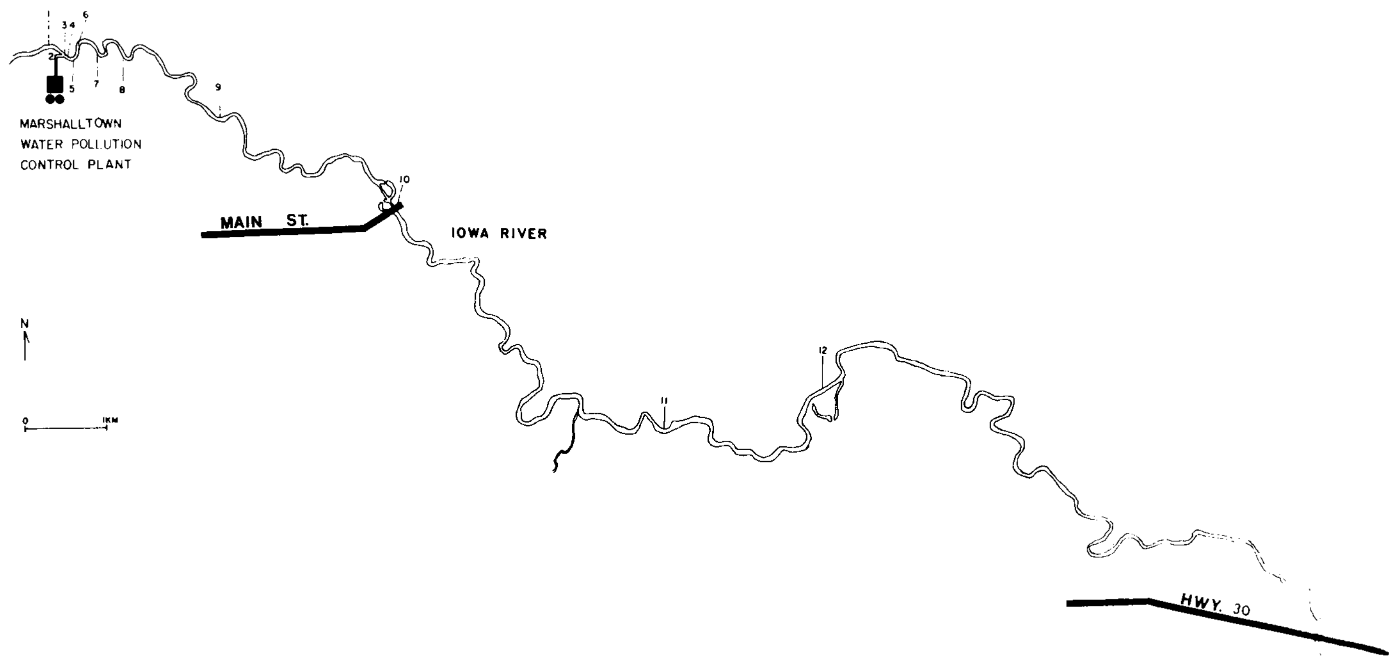

Fig. 1. Location of 12 sampling stations on the Iowa River near Marshalltown, Iowa during 1976-1977.

water containing .6 mg/l un-ionized ammonia. A field study using channel catfish was conducted in sewage lagoons where growth was shown even with un-ionized ammonia values greater than 0.20 mg/l (Huggins, 1969).

Many of these field studies are not compatible with the U.S. Environmental Protection Agency's water quality criterion (1977) of 0.02 mg/l un-ionized ammonia. This criterion is based mainly on laboratory toxicity and growth studies with salmonids (Colt and Tchobanoglous, 1978; Szumski et al., in press) and may be unduly restrictive for field situations, warmwater fisheries, and in some instances, even salmonids. Previously mentioned field and laboratory studies (Allan et al., 1950; Dugan and McGauhey, 1977; Huggins, 1969; Colt and Tchobanoglous, 1978) showed survival and/or growth for catfish, rainbow trout, and chinook salmon at un-ionized ammonia levels considerably greater than 0.02 mg/l. This discrepancy may be due in part to pH levels. Lloyd and Herbert (1960) and Tabata (1962) have shown that fish are more tolerant to un-ionized ammonia exposure at high pH. Other factors influencing un-ionized ammonia toxicity are temperature and alkalinity. Because these parameters, pH, temperature, and alkalinity, vary seasonally, un-ionized ammonia toxicity also varies seasonally. Hence, some investigators (Szumski et al., in press) believe that a seasonal standard varying as a function of pH, temperature, and alkalinity would be more realistic, with a more restrictive standard during periods of higher pH and water temperature.

In 1977, the State of Iowa changed its 2 mg/l total ammonia nitrogen standard to a seasonal standard recognizing that less un-ionized ammonia exists during the winter because of low temperatures and pH. The current Iowa standard for total ammonia nitrogen is 2 mg/l from 1 April to 31 October and 5 mg/l from 1 November to 31 March. This standard, however, is for total ammonia rather than un-ionized ammonia, which is the toxic fraction of total ammonia.

Although municipal sewage effluents may contain several potentially toxic substances, ammonia is considered the main potentially toxic compound in the Marshalltown sewage effluent. The objective of this study was to evaluate the effect of the sewage effluent upon fish and invertebrate fauna of the Iowa River. A secondary objective was to evaluate the applicability of the EPA un-ionized ammonia criterion and the Iowa total ammonia standard to a specific field situation.

STUDY AREA

The study area on the Iowa River is located in Marshall County extending .1 km above the Marshalltown Water Pollution Control Plant (WPCP) outfall to 18 km below the sewage outfall. The river near Marshalltown has a meandering channel 30-40 m wide, with a shifting-sand substrate. Although the river was approximately 0.5-2.0 m deep during the study, numerous brushpiles and logjams within the study area created holes 3-4 m deep. The Iowa River at Marshalltown has a 7-day, 10-year low flow of 0.65 m^3/s (Lara, (1979), with the average flow being 21.95 m^3/s (U.S. Geological Survey, 1980).

The Marshalltown WPCP is an activated-sludge, secondary-treatment plant, designed to treat an industrial-municipal waste. The Marshalltown plant has a design load of 20.8×10^6 l (5.5 MGD), 8172 kg (13,000 pounds) BOD_5/day, and 11,350 kg (25,000 pounds) suspended solids/day. Two packing-houses discharge a significant portion of the organic load received at the plant.

The 12 sampling stations and their locations are shown in Figure 1. Station 1, the upstream control, was located 100 m upstream from the sewage outfall. Station 2 was the sewage effluent, and the remaining stations were located in the river at the following distances downstream from the effluent outfall: station 3 (10 m), station 4 (70 m), station 5 (170 m), station 6 (300 m), station 7

Table 1. Average dilution factors and stream water temperatures during the 7 periods of the study.

Period	Date	Dilution Factor	Temperature °C
1	7-30 Jul 1976	43:1	24.3
2	2-30 Aug 1976	15:1	22.4
3	1 Sep-1 Oct 1976	8:1	18.6
4	8 Oct-22 Nov. 1976	9:1	4.7
5	3 Dec-14 Feb 1977	3.1:1	0.0
6	21 Feb-31 May 1977	20:1	13.1
7	7 June-4 Aug 1977	4.7:1	27.2

(770 m), station 8 (1550 m), station 9 (3000 m), station 10 (7200 m), station 11 (15,000 m), and station 12 (18,000 m). During July through October, 1976, only the 1st 9 stations were sampled for water quality and fisheries data. As river flows dropped, additional stations were added to monitor potential changes further downstream. Macroinvertebrate sampling was conducted at stations 1, 4, 7, 8, and 9.

METHODS

Water quality and fisheries data were gathered from July 1976 through August 1977 during 7 different sampling periods (see Table 1). Each sampling period exhibited similar daily dilution factors, the ratio of stream flow to sewage effluent flow rates. The dilution factor was determined daily by using the Iowa River flow measurements from the USGS Iowa River gauging station at Marshalltown (#05451500) and the sewage flow measurements from the Marshalltown WPCP.

Water Quality

Water samples for ammonia nitrogen and pH measurements were collected in 300 ml plastic bottles and taken to the laboratory at the Marshalltown WPCP within 1 h after sample collection. The pH analyses were completed by using a Fischer Acument Model 200 pH meter, and ammonia nitrogen was determined by using the direct Nesslerization method, with color development measured on a Bausch and Lomb Spectronic 20. Conversion of total ammonia nitrogen to un-ionized ammonia nitrogen was made by using the equation derived by Emerson et al. (1975). Temperature and dissolved oxygen measurements were obtained in the field with a Yellow Springs Instrument Model 57 dissolved oxygen-temperature meter.

Fishery Methods

The goal of the wild fish collections was to sample all available habitats and communities with some reproducible effort. Wild fish collections were routinely made by using electrofishing, seines, and hoopnets. Two electrofishing units were used during the study because of variable stremflow and water depth. A 220 volt boat-mounted unit was used during most periods, but during low flows, a 110-volt paddle rig was used. Four hoopnets 0.8 m in diameter and 1.3-2.4 m in length were anchored to snags or tree roots at each station. Of the seines used, a 6.35 mm mesh, 10 by 4 m bag seine proved most effective and was used for most of the study. During the 1st 3 periods hoopnetting was used on a continuous basis while seining was conducted once a week. Electroshocking was performed on an irregular basis throughout the study. Fish collected by all 3 methods were identified in the field or preserved for later identification. During periods 1-3, larger fish were tagged with numbered floy tags before being released.

Caged fish toxicity tests were patterned after Olson (1975) and conducted from July 1976 through January 1977. Cages were wooden boxes approximately 40 1 in volume and with screened openings to allow circulation. The boxes were anchored in the Iowa River on available snags and logjams at stations 1, 2, 4, 5, 6, 7, and 8 during 9 toxicity tests in summer and late fall, 1976. During the winter of 1976-77, 4 toxicity tests were conducted at stations, 1, 4, 6, 7, and 9. Ten channel catfish fingerlings (5-10 cm) were placed in each cage, and the cages were placed in the river for a 96-h toxicity test. Mortality was checked at 48 and 96 h.

Macroinvertebrates

Hester Dendy artificial substrate samplers with a total of 8 plates each 7.62 cm square were suspended from a No. 20 gauge wire stretched horizontally between 2 iron fence posts driven into the river bottom. Three samplers were suspended 20-30 cm above the river bottom at each station for 2 3-week exposure periods during 1976. The midsummer sampling period began on 21 July and ended 9 August, and the late summer sampling period began 24 August and ended 14 September. Invertebrate identification was made to the lowest possible taxon with chironomids left at family.

RESULTS

Water Quality

During this study, the Iowa River at Marshalltown had extreme low flow conditions. Flows at the Marshalltown gauging station set a record low flow on 25 January, 1977, of 0.13 m^3/s, far below the 7-day 10-year low flow of 0.65 m^3/s for this gauging station. Stream flows of less than 0.65 m^3/s occurred on 51 days during the study period.

Dissolved oxygen concentrations in the Iowa River were not adversely affected by the Marshalltown sewage effluent during the study. Values at river stations ranged from 5.1 to 14.4 while the effluent averaged 6.0 mg/1 during the entire study. During period 5 in the winter, dissolved oxygen levels were critically low (1.5-4.1 mg/1) at station 1, above the effluent discharge point, and at stations 10, 11, and 12. The low dissolved oxygen levels at these stations probably were caused by low stream flow and thick ice cover rather than by an unusual organic load in the stream.

Tables 2 and 3 summarize total ammonia nitrogen and un-ionized ammonia nitrogen data, respectively, for the 7 periods of the study. Downstream total ammonia nitrogen concentrations generally were lower when water temperatures were high (periods 1, 2, 3). The opposite was true of un-ionized ammonia nitrogen concentrations because the lowest un-ionized ammonia concentrations were observed during periods when water temperatures were lowest (periods 4 and 5).

Total ammonia nitrogen concentrations at station 1 never exceeded the Iowa stream standard during the study. Mean values for all periods averaged less than .20 mg/1 except durings periods 5 and 6 when mean concentrations were 2.85 mg/1 and .66 mg/1, respectively. These 2 higher means for total ammonia nitrogen were found during periods of low stream flow with heavy ice cover on the river. The source of this high level of background ammonia nitrogen was not determined and is particularly puzzling since there is no significant discharge of municipal or industrial waste for a distance of 75 km upstream from Marshalltown.

The effluent from the Marshalltown WPCP contained relatively high concentrations of total ammonia nitrogen throughout the study period and significantly ($P<0.05$) raised total ammonia concentrations at stations 3, 4, 5, and 6 located immediately below the effluent discharge point (Moore, 1977). Total ammonia nitrogen con-

Table 2. Means and standard error for total NH_3–N (mg/1).

Station	Periods						
	1	2	3	4	5	6	7
	7-30 Jul	2-30 Aug	1 Sep-1 Oct	8 Oct-22 Nov	3 Dec-14 Feb	21 Feb-31 May	7 June-4 Aug
1	.13	.17	.16	.14	2.84	.66	.20
	(±.034)	(±.047)	(±.016)	(±.02)	(±.4)	(±.3)	(±.1)
2	16.83	20.98	10.86	16.26	18.36	14.67	8.71
	(±.622)	(±1.073)	(±.995)	(±1.4)	(±1.4)	(±1.3)	(±1.9)
3	12.46	20.72	10.90	—	—	—	—
	(±1.642)	(±1.055)	(±.975)	—	—	—	—
4	7.99	18.83	10.83	13.72	12.80	6.19	4.17
	(±1.059)	(±1.046)	(±1.005)	(±1.4)	(±1.2)	(±.9)	(±1.1)
5	1.22	3.15	3.06	—	—	—	—
	(±.729)	(±.435)	(±.240)	—	—	—	—
6	.61	2.52	2.67	3.63	9.42	2.72	1.52
	(±.090)	(±.443)	(±.229)	(±.4)	(±1.0)	(±.4)	(±.3)
7	.33	1.42	1.19	2.10	7.51	1.67	1.16
	(±.087)	(±.302)	(±.154)	(±.2)	(±.8)	(±.3)	(±.2)
8	.24	.87	1.06	—	—	—	—
	(±.051)	(±.258)	(±.156)	—	—	—	—
9	.3	.5	.3	1.66	5.12	1.29	.79
	(±.1)	(±.1)	(±.05)	(±.2)	(±.4)	(±.3)	(±.2)
10	—	—	—	.72	5.44	.90	.49
	—	—	—	(±.07)	(±.7)	(±.3)	(±.1)
11	—	—	—	—	6.4	.85	.36
	—	—	—	—	(±.8)	(±.3)	(±.1)
12	—	—	—	—	4.91	.78	.32
	—	—	—	—	(±.4)	(±.3)	(±.1)

Table 3. Means and standard error for un-ionized NH_3–N (mg/1).

Station	Periods						
	1	2	3	4	5	6	7
	7-30 Jul	2-30 Aug	1 Sep-1 Oct	8 Oct-22 Nov	3 Dec-14 Feb	21 Feb-31 May	7 June-4 Aug
1	.03	.02	.01	.003	.012	.023	.041
	(±.005)	(±.004)	(±.002)	(±.001)	(±.005)	(±.01)	(±.01)
2	.16	.15	.10	.090	.088	.104	.050
	(±.018)	(±.009)	(±.020)	(±.01)	(±.01)	(±.02)	(±.01)
3	.52	.15	.10	—	—	—	—
	(±.240)	(±.011)	(±.020)	—	—	—	—
4	.26	.16	.12	.085	.046	.143	.078
	(±.043)	(±.013)	(±.027)	(±.02)	(±.005)	(±.02)	(±.02)
5	.45	.17	.13	—	—	—	—
	(±.322)	(±.024)	(±.034)	—	—	—	—
6	.19	.15	.12	.058	.031	.127	.124
	(±.027)	(±.026)	(±.032)	(±.02)	(±.005)	(±.03)	(±.02)
7	.13	.11	.08	.040	.020	.080	.124
	(±.042)	(±.024)	(±.022)	(±.02)	(±.002)	(±.02)	(±.02)
8	.10	.07	.07	—	—	—	—
	(±.020)	(±.020)	(±.021)	—	—	—	—
9	.02	.03	.02	.039	.012	.064	.091
	(±.004)	(±.006)	(±.002)	(±.02)	(±.002)	(±.02)	(±.02)
10	—	—	—	.018	.012	.032	.074
	—	—	—	(±.01)	(±.002)	(±.005)	(±.01)
11	—	—	—	—	.02	.028	.056
	—	—	—	—	(±.007)	(±.01)	(±.01)
12	—	—	—	—	.012	.028	.062
	—	—	—	—	(±.002)	(±.003)	(±.01)

Table 4. Relative abundance of fish species collected in the Iowa River near Marshalltown, Iowa during 1976-77.

Species	Relative Abundance[a] Above Outfall	Below Outfall
Non-game fish		
Carp *(Cyprinus carpio)*	A	C
River Carpsucker *(Carpiodes carpio)*	C	C
Highfin Carpsucker *(Carpiodes velifer)*	C	O
Quillback Carpsucker *(Carpiodes cyprinus)*	O	R
Bigmouth Buffalo *(Ictiobus cyprinellus)*	R	O
Smallmouth Buffalo *(Ictiobus bubalus)*	—	R
White Sucker *(Catostomus commersoni)*	R	R
Northern Hogsucker *(Hypentelium nigricans)*	R	R
Northern Redhorse *(Moxostoma macrolepidotum)*	C	O
Golden Redhorse *(Moxostoma erythrurum)*	O	R
Silver Redhorse *(Moxostoma anisurum)*	R	R
Gizzard Shad *(Dorosoma cepedianum)*	R	R
Creek Chub *(Semotilus atromaculatus)*	R	R
Silver Chub *(Hybopis storeriana)*	R	R
Hornyhead Chub *(Nocomis biguttatus)*	—	R
Blacknose Dace *(Rhinichthys atratulus)*	—	R
Spotfin Shiner *(Notropis spilopterus)*	A	A
Common Shiner *(Notropis cornutus)*	A	A
Bigmouth Shiner *(Notropis dorsalis)*	O	O
Sand Shiner *(Notropis stramineus)*	R	O
Brassy Minnow *(Hybognathus hankinsoni)*	A	C-A
Bluntnose Minnow *(Pimephales notatus)*	R	O-C
Fathead Minnow *(Pimephales promelas)*	R	C-A
Suckermouth Minnow *(Phenacobius mirabilis)*	R	R
Johnny Darter *(Etheostoma nigrum)*	R	R
Stonecat *(Noturus flavus)*	R	R
Gamefish		
Bluegill *(Lepomis macrochirus)*	R	R
Green Sunfish *(Lepomis cyanellus)*	O	R-O
White Crappie *(Pomoxis annularis)*	R	R
Black Crappie *(Pomoxis nigromaculatus)*	R	R
Flathead Catfish *(Pylodictus olivaris)*	R	R
Channel Catfish *(Ictalurus punctatus)*	A	O-C
Black Bullhead *(Ictalurus melas)*	R	R
Yellow Bullhead *(Ictalurus natalis)*	R	R
Smallmouth Bass *(Micropterus dolomieui)*	C	O
Largemouth Bass *(Micropterus salmoides)*	O	R
Walleye *(Stizostedion vitreum)*	R	R
Northern Pike *(Esox lucius)*	R	R

[a]Relative Abundance: A-Abundant, C-Common, O-Occasional, R-Rare.

centrations recovered to levels below the state standard within 770 m of the effluent discharge point.

Un-ionized ammonia nitrogen concentrations exceeded the .02 mg/1 EPA recommended criteria at most stations throughout all periods of the study except period 5. Temperatures and pH values were lowest during period 5, causing much of the total ammonia nitrogen to exist in the ionized or non-toxic form.

Wild Fish Data

Table 4 lists the 38 fish species collected from the Iowa River during 1976-77, with an indication of relative abundance based on actual field data. The spotfin shiner (*Notropis spilopterus*) and common shiner (*Notropis cornutus*) were the most common species caught. The channel catfish (*Ictalurus punctatus*) and small mouth bass (*Micropterus dolomieui*) were the most common gamefish species, carp (*Carpiodes carpio*) and river carpsucker (*Cyprinus carpio*) the most common nongame fish.

Both above and below the effluent outfall, the fish community of the Iowa River was generally well balanced, with a number of carnivores, omnivores, predators, forage species, and bottom feeders. No significant elimination or replacement of species below the outfall was noted, nor was there any significant change in the species composition in comparing results during the 1976-77 study with those of Olson (1975).

In general, diversity index values for the fish collections indicated an overall healthy fish community and were not consistently depressed by the effluent input. There was some reduction noted in gamefish species below the effluent. This may have been due to the effluent input as well as to changes and restrictions in available fishery habitats during summer and winter.

Winter fish collections emphasized that, even with the extreme stress conditions of low stream flow, low dissolved oxygen and continuing high ammonia levels present during the winter sampling, the composition of the fishery below the effluent outfall was not significantly changed compared with the summer fishery. Overall catch was somewhat reduced during the winter, but this probably was due in part to the sharply reduced water volume present and the number of areas containing very low levels of dissolved oxygen.

Comparing average results above and below the outfall, the number of species per station above was 27; below, 21. Average diversity above was 3.45; below, 3.12. Both average values generally are considered indicative of a healthy fishery.

Caged-Fish Toxicity Tests

During summer and fall 1976, 9 4-day toxicity tests were conducted. Daily un-ionized ammonia values ranged from .01 mg/1 to 1.09 mg/1 while total ammonia nitrogen values ranged from 0.08 mg/1 to 21.78 mg/1. Despite high un-ionized ammonia levels, only 4 mortalities among 630 test fish were recorded, and these occurred over a wide range of un-ionized ammonia levels (0.09 mg/1, 0.15 mg/1, 0.17 mg/1, 0.93 mg/1) and during 3 different tests. The remaining 4 toxicity tests were conducted during winter, 1976-1977, when un-ionized ammonia concentrations were the lowest. Un-ionized ammonia levels ranged from 0.001 mg/1 to 0.09 mg/1 while total ammonia nitrogen levels ranged from 0.1 mg/1 to 23.8 mg/1. A total of 19 fish died during the winter toxicity tests, but none of the deaths was due to high un-ionized ammonia levels. Nine fish died at station 4 during 15-19 November due to lack of water in the cage. Low dissolved oxygen values ranging from 1.5 mg/1 to 3.0 mg/1 were responsible for 10 mortalities at station 1 during 24-28 January 1977. A heavy ice cover and low river flow caused this situation at the upstream control station. Surprisingly, the effluent's warmer temperatures prevented a thick ice cover developing downstream, facilitating aeration of the sewage effluent and river water.

Macroinvertebrates

Chironomid (Diptera) larvae and hydropsychid (Trichoptera) larvae (*Cheumatopsyche* sp., *Hydropsyche frisoni*, and *Hydropsche orris*) were the dominant macroinvertebrates collected from the artificial substrate samplers (Table 5). Two species of mayflies, *Baetis* sp. and *Isonychia* sp., exhibited a significant ($P < 0.05$) reduction in abundance at station 4 immediately below the sewage outfall (Moore, 1977). In contrast, the hydropsychids showed no significant

Table 5. Mean number of macroinvertebrate species found on artificial substrate samplers during both 3 week sampling periods, 1976. Mean diversity, mean density, and standard errors at each station for each sampling period.

Species	STATIONS 1	4	7	8	9
Ephemeroptera					
Baetis brunneicolor	2.0	.3	3.5	2.3	7.3
Baetis frondalis	0	.2	.2	.6	.2
Baetis intercalaris	.8	0	2.6	3.8	5.8
Baetis sp.	7.1	5.7	10.7	16.6	23.5
Caenis sp.	.4	.6	1.1	.2	.8
Isonychia sp.	4.0	.8	1.5	2.3	2.8
Pseudocloeon dubium	0	0	.2	0	0
Pseudocloeon parvulum	0	0	0	.4	1.8
Pseudocloeon punctiventris	0	.2	0	.6	.2
Pseudocloeon sp.	0	0	0	.2	.5
Tricorythodes sp.	1.1	.4	1.1	.5	1.8
Heptagenia sp.	.3	0	0	0	4.1
Stenonema sp.	4.8	3.1	4.3	5.0	.2
Trichoptera					
Cheumatopsyche sp.	43.0	53.0	53	41.7	85.5
Hydropsyche betteni	1.8	2.8	4.6	3.5	9.0
Hydropsyche bifida	1.2	.6	.6	.2	.4
Hydropsyche frisoni	18.3	24.5	39.6	69.8	64.7
Hydropsyche orris	35.2	58.0	128.5	157.7	130.1
Hydropsyche simulans	.7	1.0	1.0	3.3	3.0
Mayatrichia sp.	3.6	.2	7.3	7.6	13.3
Coleoptera					
Stenelmis sp.	.4	.2	.2	.2	.5
Diptera					
Chironomidae	248	727.0	585.8	230.8	223.2
Hemerodromia sp.	9.3	2.8	2.8	1.3	2.5
Simulium sp.	24.3	4.7	9.8	7.6	7.6
Diversity					
Midsummer, 1976	2.70 (±.13)	0.95*(±.10)	2.36 (±.14)	2.37 (±.14)	2.37 (±.24)
Late summer, 1976	1.29* (±.05)	1.05* (±.13)	1.07*(±.04)	1.07 (±.04)	1.92 (±.09)
Density					
Midsummer, 1976	279* (±17)	872* (±38)	605 (±45)	543 (±17)	600 (±87)
Late summer, 1976	607 (±97)	867* (±47)	1114* (±54)	653 (±18)	566 (±55)

*Values are significantly different from other station means during same sampling time. Least Significant Difference Test ($P<0.05$).

abundance differences immediately below the sewage outfall. This is consistent with Tarzwell's (1965) findings showing *Hydropsyche* sp. occurring where total ammonia nitrogen levels exceeded 10 ppm.

A significant reduction (Table 5) in macroinvertebrate diversity occurred at station 4 during midsummer, 1976. Stations 7, 8, and 9 were not significantly different from station 1. These data suggest that the macroinvertebrate community diversity during midsummer, 1976, recovered to its natural conditions by station 7 located 770 m below the sewage outfall. During the late summer sampling period, station 1 exhibited an unusually low mean diversity index. Because a shifting sandbar threatened to bury samplers at station 1, this station was moved 2 days before sampler retrieval, probably dislodging a number of species and thus lowering the mean diversity index. The mean diversity values at stations 8 and 9 better approximate the real diversity value of station 1. Provided stations 8 and 9 exhibited community structures similar to the real conditions at station 1, a diversity recovery occurred by station 8 located 1550 m below the sewage outfall.

DISCUSSION

Fish and invertebrate fauna in the Iowa River below Marshalltown were not adversely affected by the Marshalltown municipal sewage effluent despite high total ammonia and un-ionized ammonia concentrations from July 1976 through August 1977. Evidence supporting this conclusion includes: no consistent depression in wildfish diversity below the Marshalltown sewage effluent, no change in fish species composition since the work of Olson in 1973, and only 2% channel catfish mortality during field toxicity tests. Macroinvertebrate diversity data indicated a 770-1550 m recovery zone during July-September 1976. This trend was supported by a cluster analysis, which grouped river stations according to biological and chemical similarity (Moore, 1977). The 770-1550 m recovery zone for macroinvertebrate diversity is quite short when compared with those of similar studies in which a return to normal diversities occurred 2 km or farther downstream from the sewage discharge (Egloff and Brakel, 1973; Zimmer, 1972; Avery, 1970).

These findings are particularly noteworthy because the Iowa River at Marshalltown during this study had flows 3 times lower than the 7-day 10-year low flow prediction. Therefore, the ratio of sewage effluent to river water was maximized. Fish and invertebrate survival during low flow conditions and high un-ionized ammonia concentrations was severely tested.

Why was a noticeable effect on aquatic life not seen? The sewage effluent enters the Iowa River on the south bank and closely hugs this bank, with little mixing. At station 6, 300 m downstream, a river bend thrusts the effluent plume into the mainstream, and complete mixing begins. Perhaps the confined effluent plume and gradual mixing zone minimize effluent impacts.

The 2nd and 3rd explanations assume that un-ionized ammonia is the toxic compound in the Marshalltown sewage effluent. Perhaps the aquatic organisms in the Iowa River are more tolerant of un-ionized ammonia concentrations at high pH values ($pH \geq 8$). Laboratory toxicity work by Tomasso et al. (1980) showed channel catfish tolerating 25% greater un-ionized ammonia values at pH 8 than at pH 7. A careful analysis of 6 toxicity studies indicates this same trend (Szumski et al., in press); at higher pH values, coldwater fish, warmwater fish, and invertebrates are more tolerant to un-ionized ammonia concentrations. This presumably occurs because a pH shift in the gill chamber causes a reduction in un-ionized ammonia at the gill surface. Szumski et al. (in press) also mentions that alkalinity can lessen the toxic effects of un-ionized ammonia by buffering the water to pH change. In the Iowa River study, the highest un-ionized ammonia values occurred as expected during the summer (period 1) when pH values were highest, ranging from 7.2 to 9.3. Because more un-ionized ammonia exists at higher pH values, it would be selectively advantageous for aquatic organisms to develop an increased tolerance at higher pH values.

The 3rd explanation is that warmwater species are more tolerant of un-ionized ammonia. The difference between 4-day LC_{50} values for warmwater fish and salmonids suggests that is true, but further research analyzing the differential tolerance of the 2 fish groups is needed. Again, a genetically bred tolerance to un-ionized ammonia concentrations among warmwater fish, which are the most likely to experience higher concentrations, seems selectively advantageous.

Finally, adverse changes in the Iowa River fauna may have passed undetected by the field methods chosen. Admittedly, this study was not a long-term study, nor could it detect subtle physiological or behavioral alterations. Possibly, effluent ammonia concentrations have rendered a short section of the Iowa River unsuitable for fish reproduction or some other period of the fish life cycle. However, the simultaneous presence of an active sport fishery and the sewage effluent has existed for many years. Furthermore, a comparison of Olson's 1973 study with Govro's (1977) data collected over the same stations on the Iowa River did not indicate reductions in catch or species diversity over a 3-year period, despite continuous effluent discharges containing high concentrations of total ammonia nitrogen. This comparison suggests that adverse long term changes in the Iowa River fauna have not occurred.

The EPA 0.02 mg/l un-ionized ammonia criterion is not realistic for the Iowa River below Marshalltown, Iowa. Not only was this criterion exceeded during 2 or more periods by all downstream station means, but even the upstream control station exhibited mean values exceeding the criterion. During periods with high un-ionized ammonia concentrations, the dissolved oxygen levels never dropped below 5 mg/l, indicating that the nitrification of ammonia did not exert a harmful oxygen demand. Despite un-ionized ammonia concentrations up to 50 times higher than the recommended criterion, the fish fauna in the Iowa River seemed unaffected by the sewage effluent, and the macroinvertebrate diversity quickly recovered. We agree with the American Fisheries Society's evaluation of this criterion (Thurston et al., 1979). We believe that the appropriateness of the un-ionized ammonia criterion for nonsalmonid fishes and other aquatic organisms has not been demonstrated or verified in field situations. Because of dissimilar response to ammonia among warmwater and cold-water aquatic species, a multiple criterion for different temperature habitats should be considered.

The Iowa seasonal standard for total ammonia nitrogen is less conservative than the EPA criterion and recognizes seasonal changes in ammonia toxicity due to temperature and pH. None of the total nitrogen mean period values at the upstream control station exceeded the Iowa stream standard. This suggests that the stream standard is realistic for Iowa waters. However, a total ammonia nitrogen standard does not address the specific fraction—un-ionized ammonia—that is toxic to aquatic life. For example, during the summer, high un-ionized ammonia levels (greater than 0.10 mg/l) often occurred when total ammonia concentrations met the Iowa water quality standards. Likewise, during the winter, when total ammonia levels exceeded the 5 mg/l state standard, un-ionized ammonia levels were very low (less than 0.08 mg/l) and often below the EPA criterion of 0.02 mg/l. The data from this study indicate that the Iowa stream standard is lax during the summer months and too strict during the winter months. By adopting a seasonal un-ionized ammonia standard, this conflict could be resolved. Not only would this standard address the toxic fraction of ammonia, but it would offer reasonable protection for biological life at all seasons and prevent an overly stringent standard from requiring additional ammonia removal by municipal wastewater control plants.

We feel an Iowa seasonal un-ionized ammonia standard must recognize the state's warmwater streams. Further research using Iowa fish and water quality conditions is needed to establish this seasonal standard.

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