THE EFFECTS OF SUPERSATURATION OF DISSOLVED GASES ON AQUATIC INVERTEBRATES OF THE BIGHORN RIVER DOWNSTREAM OF YELLOWTAIL AFTERBAY DAM

by

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APPROVAL

of a thesis submitted by

James Allen Brammer

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VITA

James Allen Brammer, son of Jerry and Fleda Brammer, was born in El Dorado, Kansas, November 8, 1960. He graduated from Broadwater County High School, Townsend, Montana, in May 1979. In September 1979 he entered Montana State University and received a Bachelor of Science degree in Biology in August 1983. He began graduate studies at Montana State University in April 1986.

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ABSTRACT

The objective of this study was to determine if gas supersaturation levels in the Bighorn River adversely affect aquatic invertebrates. Benthic and drift samples were collected in August 1986, and April and September 1987. Sampling sites were 2.4 and 14.5 river kilometers (rkm) below Yellowtail Afterbay Dam, with the upstream site representing an area where higher incidence of gas bubble trauma occurred in fish. Community composition was similar between sites and taxa were representative of those commonly found below deep release impoundments. Community structure was similar between sites during April 1987, but differed strongly during late summer and fall sampling periods. Summer temperature differences between sites coincided with the period of highest gas saturation levels confounding interpretation. Eight invertebrate taxa which differed in abundance between sites and were considered the best candidates for being affected by gas supersaturation were tested in controlled bioassays. Susceptibility differed between taxa, but all those tested were negatively affected by mean delta P levels of 181 mm Hg or greater. *Baetis tricaudatis* was the most susceptible with adverse effects apparent at delta P levels of 113 mm Hg. *Ephemerella inermis* and *Tricorythodes minutus* appeared to have susceptibility thresholds near a delta P of 120 mm Hg. These levels commonly occur in the Bighorn River. Invertebrate sensitivity to high gas saturation levels was expressed through increased buoyancy, which could cause involuntary drift. Total invertebrate densities were greater at rkm 14.5, however densities at rkm 2.4 were not low, ranging from 32,658 to 41,761 organisms/m^2. If gas supersaturation is causing downstream displacement of invertebrates, upstream migration before oviposition appears to be somewhat compensatory.

INTRODUCTION

The solubility of various gases in water is determined by individual properties (mass and partial pressure in the atmosphere) and environmental factors (temperature and pressure). While individual properties of gases are not subject to change, environmental conditions do change, sometimes resulting in dissolved gas supersaturation. Water can become supersaturated with gases in plunge basins below dams, when turbulent water entrains atmospheric gases and carries them to a depth where hydrostatic pressure forces them into solution. Water leaving the plunge basin is then relieved of higher pressures, while maintaining a high concentration of dissolved gases.

Exposure of aquatic organisms to gas supersaturated water often leads to a condition called "Gas Bubble Trauma" (GBT). Bouck (1980) describes GBT as a "physically induced process caused by uncompensated hyperbaric pressure of total dissolved gases". Gorham (1901) was the first to describe external characteristics of GBT in fish, while Marsh and Gorham (1905) offered fundamental insights into the nature of the affliction. Noting gas emboli in the gill filaments of traumatized fish, they attributed death to stasis of the blood. Subsequent research with fish has supported these findings (Bouck 1980; Fidler 1985; Weitkamp and Katz 1980).

Concern for various fisheries, most notably those in the Columbia River system, prompted increased research on effects of gas supersaturation on various species of salmonids (Westgard 1964; Raymond 1968, 1969; Beiningen and Ebel 1970). Literature concerning potential effects on aquatic invertebrates is more limited, although occurrence of GBT in these organisms is well documented (Marsh and Gorham 1905; Evans and Walder 1969; Malouff et al. 1972). Fickeisen and Montgomery (1975) conducted bioassays on effects of gas supersaturated water on six families (representing four orders) of aquatic insects and reported that *Pteronarcys californica* acquired GBT when exposed to 132% and 140% supersaturation for 10 d. Nebeker (1976) noted body distension and gas bubbles throughout the body fluids of stoneflies at 125% saturation but no mortality occurred.

Gas bubble trauma in trout below Yellowtail Afterbay Dam, was initially described in 1973 (Swedberg 1973). It was later determined that the sluiceway gates were largely responsible for the excessive gas entrainment occurring as water passed through Afterbay Dam (Bureau of Reclamation 1973). Subsequent studies validated the need to resolve the problem (Curry and Curry 1981), and in 1982 deflector plates were installed. Although deflector plates reduced dissolved gas supersaturation, they were removed because resulting turbulence pulled rocks into the stilling basin - threatening to undermine the dam. While acceptable alternatives are still being investigated, the problem persists.

In 1981, following a period of closure by the Crow Tribe, the Bighorn River below Afterbay Dam was reopened to public fishing. The value of the fishery grew with the river's growing reputation as one of Montana's premier trout streams. A Bureau of Reclamation funded study was initiated in 1985 by the Montana Cooperative Fishery Research Unit to determine gas supersaturation impacts on the Bighorn River fishery. The research included evaluating influences on the invertebrate community. Specific objectives of the invertebrate research were to:

1) Determine invertebrate abundance and community composition in areas of the Bighorn River where high and low incidence of GBT occur in fish.
2) Define gas supersaturation levels harmful to different invertebrate taxa.
3) Relate findings to gas saturation levels in the river.

DESCRIPTION OF STUDY AREA

Yellowtail Dam is located on the Bighorn River in south-central Montana, approximately 69 air km southeast of Billings. It is a thin-arch concrete structure measuring 160 m high and 442 m long at the crest. Principal uses of the dam include power generation, irrigation, flood control, and fish and wildlife enhancement. The power penstocks are located 63.9 m from the crest of the dam, allowing discharge 57.9 m below the reservoir surface at normal usable capacity (Soltero 1971). This discharge results in essentially no dissolved gas supersaturation.

Yellowtail Afterbay Dam is located 3.5 km downstream of Yellowtail Dam, and was constructed to eliminate river discharge fluctuations resulting from the hydroelectric peaking operations. It is an earthfill embankment, with concrete spillway, sluiceway, and diversion works. The overall crest length is 414.5 m with a 49.4 m wide spillway, having a discharge capacity of 566.4 m^3/s (20,000 cfs) (Bureau of Reclamation 1965). The distance from the river bed to maximum controlled water surface is 16.1 m. Flows are controlled by five radial and three sluiceway gates. The sluiceway gates can release water

6.9 m deeper than the radial gates, so as water levels in the afterbay drop, the sluiceway becomes the primary route for discharge.

The river bottom geology of the Bighorn is mainly alluvial terrace and river floodplain deposits from the quaternary period overlaying various Cretacious shales. Soils have mainly formed in place over sandstone, siltstone, limestone, and shale bedrock and are comprised of sands, clays, and loams in varying proportions (Meshnick et al. 1977).

The study area extended 19.5 km downstream of Yellowtail Afterbay Dam (Figure 1). This stretch of river is braided and has numerous islands. A cottonwood (*Populus spp.*) overstory is common in riparian areas. Important understory shrubs include: wild rose (*Rosa woodsii*), western snowberry (*Symphoricarpos occidentalis*), green ash (*Fraxinus pennsylvanica*), and boxelder (*Acer negundo*). Sandbar willow (*Salix exigua*) is common in moist areas along the stream edge and is the dominant woody species on sand and gravel bars.

In 1986 and 1987 discharge of the Bighorn River commonly ranged between 71 and 127 m^3/s except during run-off, and river temperatures varied between lows of 1-2^{o}C and highs of 19-20^{o}C. White et al. (1988) describe the water as being hard with relatively high alkalinity, conductivity, and total dissolved solid levels.

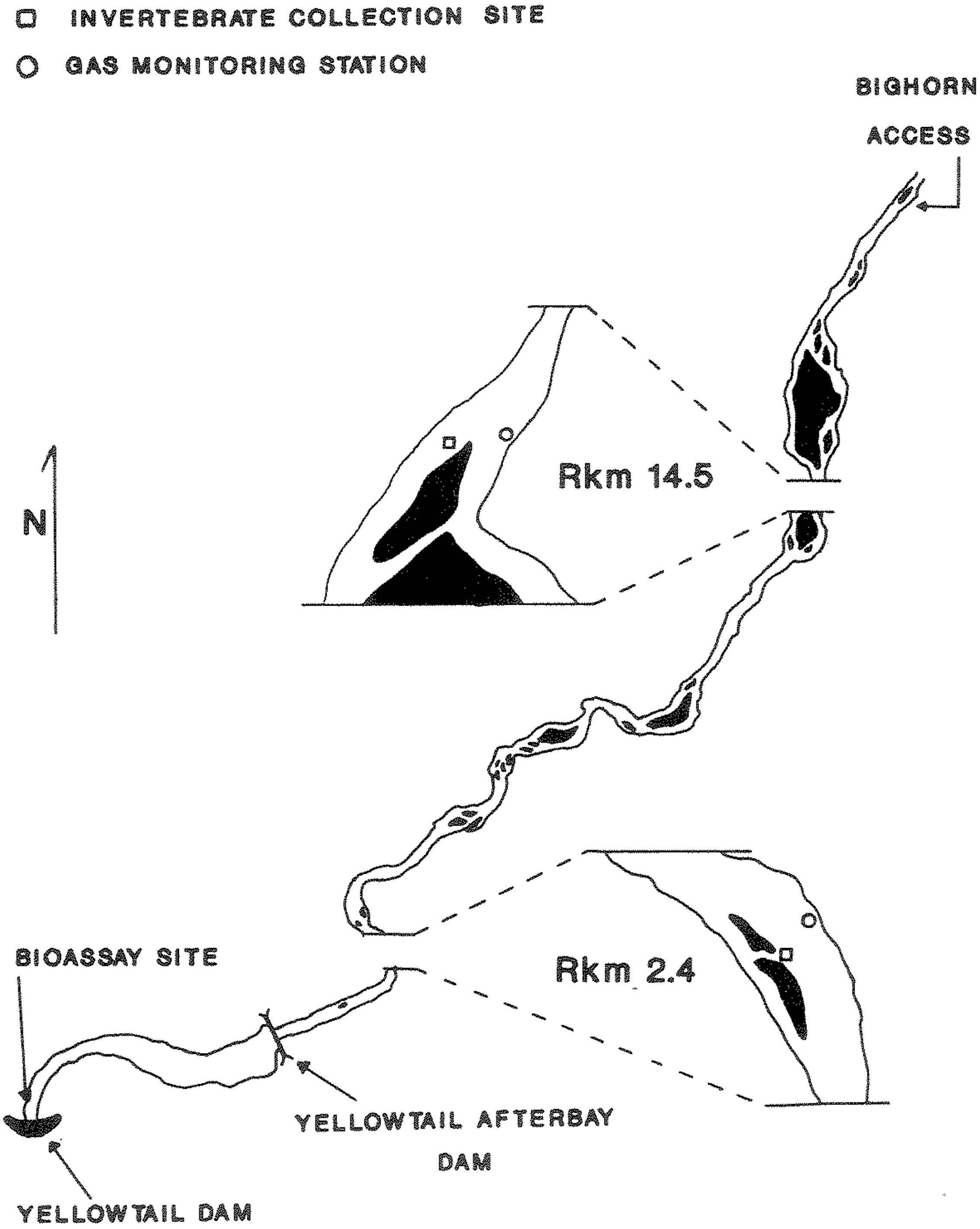

Figure 1. Bighorn River study area, illustrating channel characteristics at invertebrate collection sites and gas monitoring stations.

The Bighorn River is very productive. The bottom is covered with extensive mats of aquatic vegetation. This, coupled with high water clarity, gives the river the appearance of a large spring creek. Instream vegetation is comprised of a mixture of macrophytes, algae and bryophytes. *Potomageton spp*. was the most common macrophyte and *Cladophora* dominated the algae present at invertebrate collection sites.

METHODS

Site Selection

The physical characteristics of four riffle areas were analyzed before selecting two of these as invertebrate sampling sites. Two riffles were near Afterbay Dam and represented areas where high levels of gas bubble trauma (GBT) occurred in fish. The remaining two were further downstream where incidence of GBT was lower. At each riffle, three transects (perpendicular to the current) were established. Water depth and velocity, and substrate size were measured along each transect. Depth and velocity were measured at 1.5 m intervals using a Marsh-McBirney current meter and a top setting rod, while substrate was measured by embedding a 0.085 m^2 modified Hess invertebrate sampler (Waters and Knapp 1961) approximately 3-7 cm into the river bottom at 2 m intervals. All rocks within the sampler with a maximum diameter greater than 6 cm were measured.

Riffles 2.4 and 14.5 river kilometers (rkm) below Afterbay Dam were chosen as invertebrate collection sites due to their physical similarities and their proximity to monitoring stations where gas saturation levels and water temperatures were measured two or three times weekly (Figure 1).

Benthic Invertebrate Sampling

Benthic samples were collected during three periods. Sampling dates were chosen to allow evaluation of invertebrate community structure before run-off and after run-off, the period of highest gas supersaturation and high GBT incidence in trout. In 1986, sampling was done in August (post-runoff). In 1987, samples were taken in April and September. Twenty-five samples per site were collected in August. This was reduced to 15 samples per site in April and September 1987.

Riffles used in benthic sampling (Figure 1) were divided into 1 m^2 grids, and random numbers, generated by computer, were used to determine where samples would be collected within the riffle. Samples were collected by embedding the modified Hess sampler into the substrate to a depth of 5-10 cm. All rocks with periphytic algae were individually scrubbed with a soft bristled brush, examined to ensure no organisms remained, then removed from the sampler. Remaining substrate was agitated with a steel rod to a depth of 7-10 cm, before removal of the sampler. At each sample location and prior to sampling, water velocities (0.6 depth and 5-7 cm above the substrate) and depth were measured using a Marsh-McBirney current meter, and a top-setting rod. Only water velocities at 0.6 depth were collected during the August 1986 sampling period. During all three collection periods, samples were gathered from each riffle over a 2 d interval. Samples were preserved in Kahle's solution (Pennak 1978) containing rose bengal and sorted in the lab at a later date.

Invertebrate sorting was facilitated by straining samples through a series of sieves (8.0, 1.0, and 0.5 mm mesh size) before picking. Due to sample richness, the sieved material smaller than 1.0 mm in the 1987 samples was subsampled before sorting, using a device constructed to divide the material into quarters. Invertebrates were removed from one quarter of the material smaller than 1.0 mm. After sorting, the organisms were identified to family or genus and counted.

Vegetative materials from the samples were dried in an oven at 105°C for 24 h. The dry weight values provided a sample-specific measure as an index to compare vegetative standing crop.

Drift Sampling

Fickeisen and Montgomery (1975) noted that during gas supersaturation bioassays, several invertebrate taxa experienced buoyancy problems due to gas bubbles adhering to their bodies. Thus, drift samples were collected during the April and September sampling periods to evaluate drift rates of various taxa.

Allan and Russek (1985) found that fewer drift samples were required to quantify invertebrate abundance if replicate samples were collected during the period of peak drift, as opposed to other sampling designs. Thus, during the April and September 1987 sampling periods, four synchronized drift samples were taken at the lower end of the two sampling riffles. Nets were placed in the river shortly after dark, when drift is normally greatest (Allen and Russek 1985) and removed 0.5 h later. The nets had 0.15 m^2 openings with a mesh size of 0.5 mm. Each net sampled the entire water column. Water velocities were measured (0.5 depth of the water column) at the mouth of each net at the beginning, middle and end of each sample. Samples were preserved, sieved, subsampled, sorted and counted the same as described for benthic samples.

Periphyton Collection and Analysis

Periphyton samples were taken during both 1987 sampling periods to provide another measure of site similarity. During April, the standing crop of periphyton appeared to vary substantially within collection sites. Thus, upstream and downstream riffles were partitioned into subsections based on visual differences in periphyton abundance, and mapped (Figure 2). Statistical analysis of data by subsection allowed determination of habitat differences within sites and between sites. In September 1987, differences in periphyton abundance were not visually apparent within sites and riffles were not subsectioned.

Periphyton sampling sites were chosen by throwing a steel rod, marked on one end, into the water, and collecting the rock nearest the marked end. Periphyton was scraped off the rock, placed in a labeled plastic bag, put on ice in a cooler, and later frozen for analysis in the lab. The periphytic attachment area was estimated by taking two measurements - essentially length and width - to allow comparison between samples. In April, 42 and 39 samples were collected from the upper and lower sites, respectively, and the number of periphyton samples were proportioned according to subsection area. In September, 40 samples were collected from each site.

Periphyton samples were analyzed for chlorophyll a concentration and ash-free dry weight. To do this each periphytic mass was thawed, blotted with paper towels, and weighed. Three subsamples of approximately 0.2 g were removed for chlorophyll extraction and analysis.

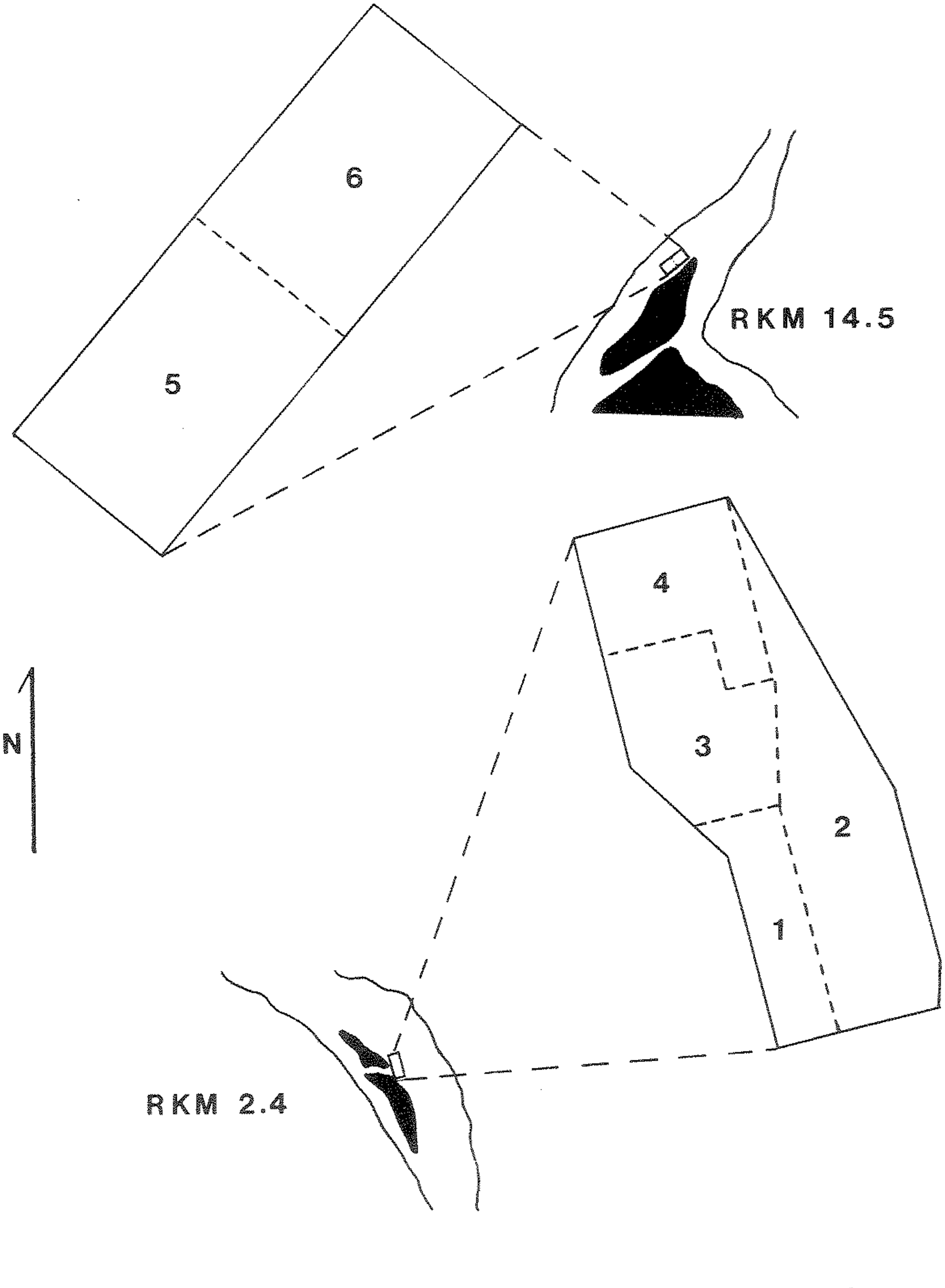

Figure 2. Invertebrate collection sites illustrating how riffles were divided into subsections in April 1987.

Subsamples were placed in a centrifuge tube with 10 ml of extractant (DMSO:90% acetone), agitated, and placed in a dark freezer for 24 h. Upon removal they were centrifuged for 10 min. One milliliter of the chlorophyll solution was then diluted with an additional 5 ml of extractant. Spectrophotometry was done using a spectronic 20 spectrophotometer and methods described by Wilkinson (1983) were used to correct for absorbance by phaeopigments. The following formula (modified from Wilkinson 1983) was used to determine chlorophyll a concentration per unit weight of vegetation:

$$\mathbf{C = (E_a - E_b) \times 27.31 \times v/m \times 1}$$

C = Chlorophyll A concentration (ug/g of vegetation)

E_a = Extract absorbance at 665 nm minus absorbance at 750 nm before acidification

E_b = Extract absorbance at 665 nm minus absorbance at 750 nm after acidification

27.31 = Constant used for extractant

v = Volume, in ml of extractant solution used

m = Blotted wet weight, in grams, of vegetation

l = Path length (usually 1 cm)

The remaining vegetative mass was placed in a drying oven at 105°C for 24 h, weighed and placed in a muffle furnace at 500°C for another 24 h. A final weight was taken to determine ash-free dry weight.

Invertebrate Bioassays

A transportable gas supersaturation unit was constructed so tests could be conducted with water from the Bighorn River. The unit was installed approximately 400 m below the base of Yellowtail Dam (Figure 1) and housed in a 2.44 x 3.05 m metal shed. Supersaturation was achieved by sprinkling water through a vertical, 2.13 m PVC column (20.3 cm in diameter), pressurized by an air compressor. As water passed through the column, high pressure forced the gas into solution (Figure 3). Varying proportions of treated and untreated water were used to achieve mean gas saturation levels which progressively increased from tank 1 through tank 6. Tank 1 received only untreated water and was used as a control. Flows into all tanks (37.9 L aquaria with standpipes maintaining volumes at 30.3 L) were approximately 0.75 L/min exposing organisms to a water turnover time of about 40 min.

Two 7 d (168 h) bioassays were conducted, each testing four invertebrate taxa. The first was run from August 2-9, 1988 and the second from September 8-15, 1988. Test organisms were collected at Bighorn Access

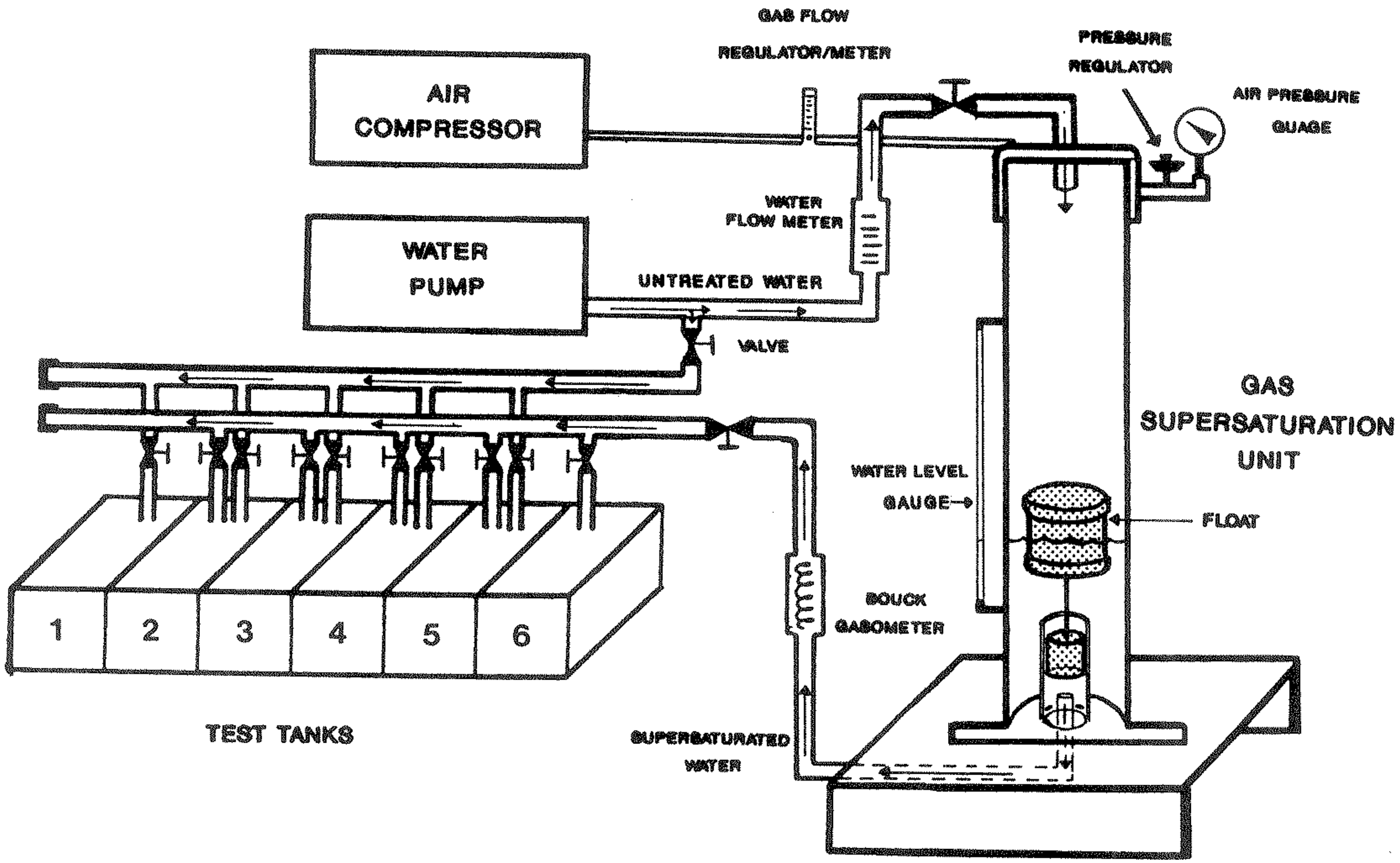

Figure 3. Gas supersaturation system used in aquatic invertebrate bioassay tests.

bottom of the test tank. If unable to successfully swim to the bottom, organisms were classified as stranded. Buoyant animals were examined to determine whether external bubbles coalescing on the body were the cause. If none could be seen, buoyancy was assumed to be physiological and associated with GBT. Organisms were left in the test tanks until they became immobile and unresponsive when disturbed, or were dead. At this time they were recorded as "gas related mortalities", examined under a dissecting scope to determine if visible signs of GBT were present, and preserved. Nonbuoyant dead organisms found on the bottom of a test tank or on the stand-pipe, were classified as "non-gas related mortalities".

Statistical Analysis

Benthic invertebrate data were transformed (ln + 1) for all analyses. All other data were left in raw form and parametric statistics were used, when variances were homogeneous ($p=0.05$). Non-parametric methods were employed when this was not the case.

RESULTS

Benthic Invertebrates

Taxanomic Composition

Twenty-five genera of benthic invertebrates, representing 20 families and 10 orders, were collected from Bighorn River sampling sites. An additional five, three, and two taxa were identified to family, order and class, respectively (Table 1).

Relative abundance in the Bighorn River was dominated by few taxa. Eighty-seven percent of all organisms collected in 107 benthic samples were members of five taxa (Table 2), and only Tricladida, Chironomidae, *Baetis tricaudatis* and *Tricorythodes minutus* were present in all samples. *Isoperla quinquepunctata* was the only stonefly that occurred commonly in Bighorn River benthic samples, and densities were low.

Benthic Sample Variation

Principal components (PC) analysis was performed on benthic data for the 14 taxa/groups exhibiting the greatest variance in abundance between samples (Table 3). Three vectors explained 73.5% of the total variance. When principal components were plotted (Figures 4 and 5), most

Table 1. Aquatic macroinvertebrate taxa collected in benthic and drift samples, 2.4 and 14.5 river kilometers below Yellowtail Afterbay Dam, in the Bighorn River, 1986 and 1987.

Phylum	Class	Order	Family	Genus\species
Annelida	Hirudinea	Rhynchobdellida	Glossiphoniidae	*Glossiphonia complanata* (Linnaeus)
				Helobdella sp.
Oligochaeta				
Arthropoda	Crustacea	Amphipoda	Gammaridae	*Gammarus lacustrus* Sars
			Hyalellidae	*Hyalella azteca* (Saussure)
	Insecta	Diptera	Anthomyiidae	
			Chironomidae	
			Heleidae	
			Simulidae	*Simulium spp.*
			Stratiomyiidae	*Euparyphus spp.*
			Tipulidae	*Tipula spp.*
		Ephemeroptera	Baetidae	*Baetis tricaudatis* Dodds
				Callibaetis sp.
			Ephemerellidae	*Ephemerella inermis* Eaton
				Serratella sp.
			Leptophlebiidae	*Choroterpes sp.*
			Tricorythodidae	*Tricorythodes minutus* Traver
		Plecoptera	Perlodidae	*Isoperla quinquepunctata* (Banks)
			Nemouridae	*Amphinemura banksi* Bauman and Gaufin

(continued)

Table 1 continued.

Phylum	Class	Order	Family	Genus\species
Arthropoda	Insecta	Trichoptera	Brachycentridae	*Amiocentrus aspilus* Ross
			Glossosomatidae	*Glossosoma sp.*
			Hydropshchidae	*Hydropsyche spp.*
			Hydroptilidae	*Hydroptila spp.*
			Limnephilidae	*Dicosmoecus sp.*
			Leptoceridae	*Oecetis sp.*
				Nectopsyche sp.
	Arachnida	Acarina (Trombidiformes)*		
		Collembola		
		Hemiptera		
Mollusca	Gastropoda	Basommatophora	Physidae	*Physella spp.*
			Planorbidae	*Gyraulis sp.*
	Pelecypoda	Heterodonata	Sphaeridae	
Platyhelminthes	Turbellaria	Tricladida		
Nematoda				

* - indicates suborder.

Table 2. Relative abundance of benthic invertebrates, showing the number of samples each taxa was found in, the mean per sample, standard deviation, range, and percent of all organisms.

Taxa/group[a]	# Samples counted	Freq. of occurrence	Mean/ sample	STD	Range min. max.	%
Chironomidae	104	104	923.5	716.8	(75-2975)	21.4
Baetis tricaudatis	107	107	921.9	615.5	(87-2984)	24.0
Tricladida	106	106	522.5	397.5	(63-3025)	13.6
Tricorythodes minutus	107	107	467.5	463.9	(12-1668)	12.2
Physella spp.	107	102	413.1	813.8	(0-4773)	10.8
Simulium spp.	106	103	153.7	335.0	(0-2475)	4.0
Gammarus lacustris	107	98	93.9	185.8	(0-1006)	2.4
Hydropsychidae	107	62	76.3	127.8	(0-604)	2.0
Serratella sp.	106	77	64.4	73.2	(0-311)	1.7
Chironomidae pupa	107	105	51.1	55.8	(0-276)	1.3
Amiocentrus aspilis	107	85	38.4	136.3	(0-1007)	1.0
Optioservus spp.	107	98	25.0	34.2	(0-126)	0.7
Hydroptila	107	80	22.9	41.4	(0-329)	0.6
Hydroptila pupa	107	45	20.1	40.1	(0-162)	0.5
Simulium pupa	107	77	9.0	17.5	(0-112)	0.2
Hyalella azteca	107	92	8.1	15.7	(0-115)	0.2
Glossophonia complanata	107	87	7.9	13.7	(0-84)	0.2
Hydropsyche spp.	107	65	3.6	7.9	(0-66)	0.1
Amiocentrus aspilis pupa	107	54	3.6	6.6	(0-37)	0.1
Ephemerella inermis	107	29	2.0	5.6	(0-36)	0.1
Oecetis sp.	107	31	1.1	3.0	(0-26)	t
Isoperla quinquepunctata	107	20	.9	3.0	(0-22)	t

a - refers to separation of larval and pupal life stages within certain taxa
t - percent abundance at trace levels.

Table 3. Principal components analysis of the 14 taxa/groups[a] which varied most in abundance among 107 benthic samples collected at rkm 2.4 and rkm 14.5, Bighorn River, 1986 and 1987.

Taxa/group[a]	Principal components vector		
	1	2	3
Tricorythodes minutus	0.15	0.11	0.33
Physella spp.	0.41	-0.44	0.34
Simulium spp.	0.34	0.21	-0.17
Simulium spp. pupa	0.08	0.26	-0.17
Gammarus lacustris	0.24	-0.49	-0.32
Hydropsychidae	0.50	0.25	-0.29
Serratella sp.	0.48	-0.04	-0.10
Amiocentrus aspilis	-0.08	0.16	-0.29
Amiocentrus aspilis pupa	0.16	0.15	0.15
Optioservus spp.	-0.07	-0.08	0.40
Hydroptila spp.	-0.14	0.33	0.33
Hydroptila spp. pupa	0.27	0.44	0.23
Hyalella azteca	-0.12	-0.02	0.01
Glossophonia complanata	0.08	-0.14	0.30
Eigen values	14.84	7.90	5.14
Percent of variance	39.17	20.84	13.57
Cumulative percent	39.17	60.01	73.58

a - refers to separation of larva and pupa within certain taxa.

variation in abundance of individual taxa occurred between samples collected during different periods and from different sites.

Principal component 1 explained 39% of the total variance in abundance for those taxa tested and had an eigen value of 14.84, with the heaviest weightings given to *Physella, Simulium*, Hydropsychidae, and *Serratella*. This vector showed a separation between the three

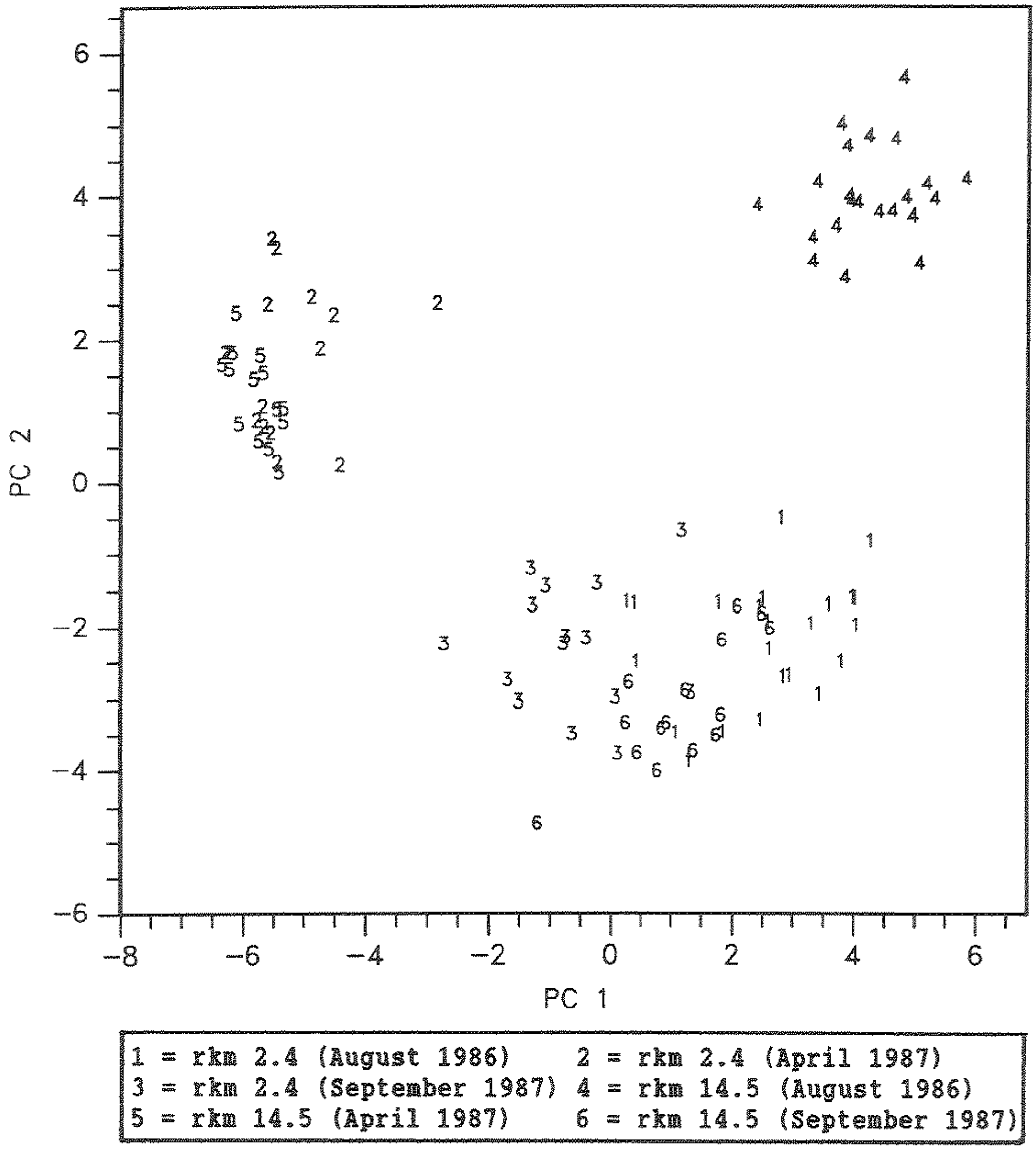

Figure 4. Principal components (PC) of vectors 1 and 2, generated from benthic samples collected at rkm 2.4 and 14.5 during all sampling periods.

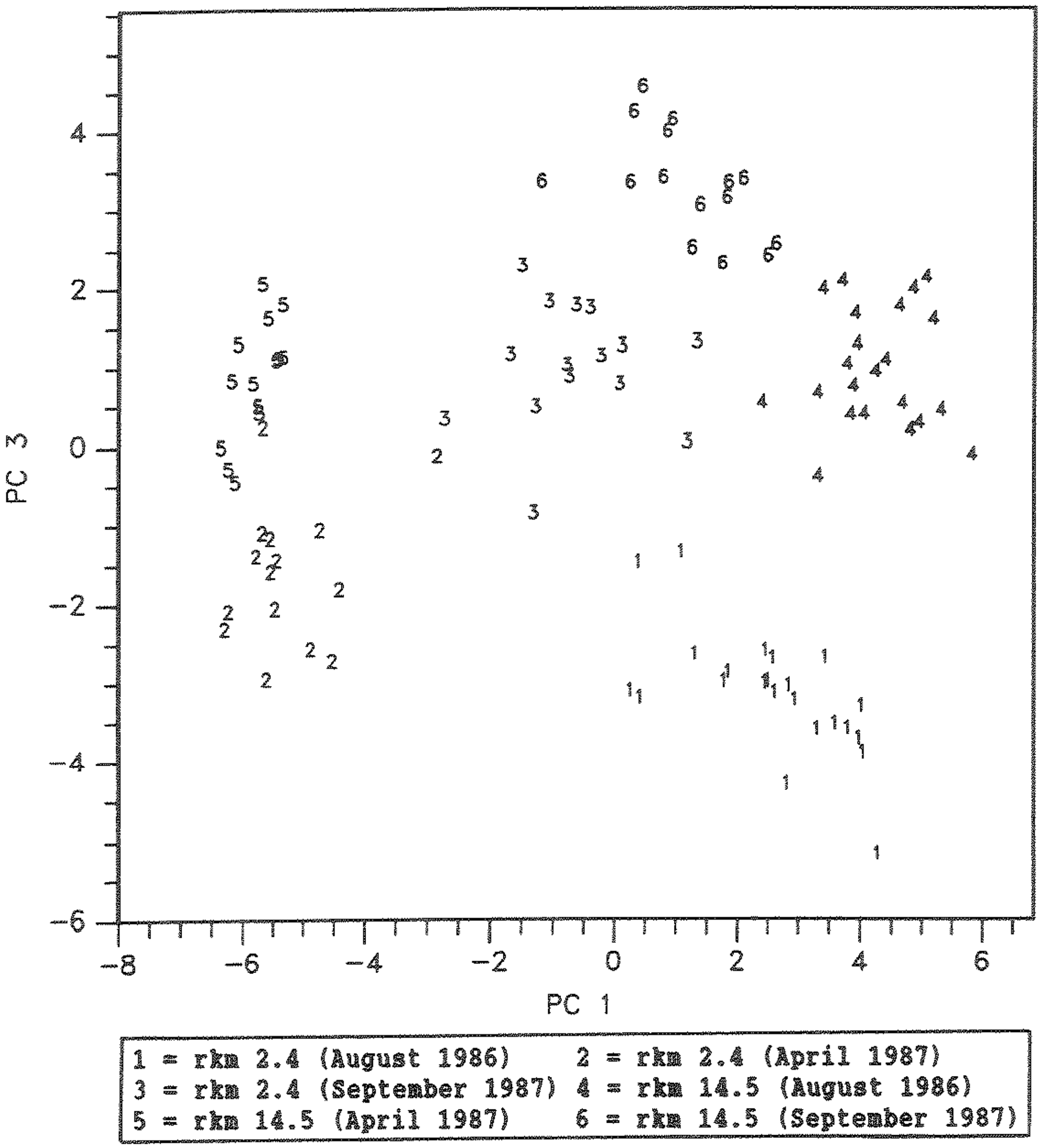

Figure 5. Principal components (PC) of vectors 1 and 3, generated from benthic samples collected at rkm 2.4 and 14.5 during all sampling periods.

Table 4. Estimated density (#/m^2) for taxa/groups[a] commonly occurring in benthic invertebrate samples. Results are provided by site for collection period 1 (August 23-24, 1986), period 2 (April 13-14, 1987) and period 3 (September 22-23, 1987), Bighorn River, MT.

Taxa/group[a]	Period 1 rkm 2.4	Period 1 rkm 14.5	Period 2 rkm 2.4	Period 2 rkm 14.5	Period 3 rkm 2.4	Period 3 rkm 14.5
Amiocentrus aspilis[2]	249	64	2608	60	72	18
Amiocentrus pupa[1 3]	11	145	1	0	50	16
Baetis tricaudatis[1 3]	9603	12356	10354	12519	3061	16290
Chironomidae[1 3]	9783	1664	12109	14511	21966	10117
Chironomidae pupa[1 3]	690	77	1305	981	490	352
Ephemerella inermis[2]	0	0	138	24	0	0
Gammarus lacustrus[1 3]	4157	44	25	25	93	638
Glossophonia complanata[1]	30	56	12	17	194	309
Hyalella azteca[3]	40	26	321	83	145	31
Hydropsyche spp.[1]	3	14	156	52	28	46
Hydropsychidae[1]	1493	2393	7	0	<1	142
Hydroptila spp.[1 2 3]	11	275	352	982	3	224
Hydroptila pupa[1]	1	1053	5	<1	3	18
Isoperla quinquepunctata[2]	0	0	10	70	0	0
Oecetis sp.[3]	0	5	42	10	26	10
Optioservus spp.[1 2 3]	51	80	163	574	181	1016
Physella spp.[1 3]	2162	1387	138	31	3105	24529
Seratella sp.[1]	619	2027	0	0	814	478
Simulium spp.[1]	1686	6236	208	69	431	400
Simulium pupa[1 2]	83	276	156	26	43	10
Tricladida	6001	5036	2756	3575	8924	11483
Tricorythodes minutus[1 2 3]	1506	13358	1792	2847	2132	9578
TOTAL NUMBER[1 3]	38179 N=23	46572 N=25	32658 N=14	36456 N=15	41761 N=15	75670 N=15

a - refers to separation of larva and pupa within certain taxa

1, 2, and 3 - sampling period when abundance was significantly different between rkm 2.4 and 14.5 (p=0.050).

36,456 organisms per square meter at rkm 2.4 and 14.5, respectively.

During August 1986, 16 taxa or groups showed significant differences in abundance between sites

($p \leq 0.05$). Twelve of these were more common at rkm 14.5 (Table 4). Eleven taxa differed significantly in September 1987; six of these were more common downstream. In April 1987, four of seven taxa which differed significantly between sites were more abundant downstream.

Most commonly occurring taxa fell into three general categories with regard to density differences between sites: first, those taxa that were more abundant at rkm 2.4, when significant differences occurred (Group 1); second, those taxa found in significantly greater densities at one site during one period, but at the other site during a subsequent period (Group 2); and third, those taxa that were more abundant downstream, when significant differences occurred (Group 3).

Group 1. Densities of group 1 taxa were hypothesized to be least affected by gas supersaturation, assuming the pattern of influence on invertebrates is similar to that on trout (i.e. increased affliction near Afterbay Dam). However, data for taxa included in this and the other two groups were sometimes confounded by asynchronous life cycles.

Hyalella azteca, *Oecetis*, *Amiocentrus aspilis* larvae, and *Ephemerella inermis* were all more abundant at rkm 2.4 when differences were significant (Table 4). *Amiocentrus aspilis* larvae were significantly more abundant at rkm 2.4

during April 1987 ($p<0.01$). The pupae, however, were more abundant at rkm 14.5 in August 1986 ($p<0.01$) and at rkm 2.4 in September 1987 ($p=0.04$). These differences might result from a lack of synchrony in *A. aspilis*'s life cycle between sites, prompted by differences in water temperatures.

High densities at rkm 2.4 in April 1987 resulted from the presence of many newly hatched larvae. Early instar larvae were not present downstream. Similarly, the difference in August 1986 pupae abundance reflects earlier pupation and/or emergence at rkm 14.5 than at rkm 2.4. Four percent of *A. aspilis* at the upstream site were in the pupal stage versus 69% downstream (Table 4). September 1987 differences were probably also influenced by asynchronous life cycles between sites, since this was the case during the two earlier sampling periods.

Ephemerella inermis was present only in the April 1987 samples (Table 4). Although its life cycle in the Bighorn River was not directly evaluated, late instar nymphs were collected at Bighorn Access (rkm 19.5) the first week in August 1988. Thus, this species was probably present in the Bighorn River through most summer months, but had emerged prior to late August - early September sampling. Given this, a considerable portion of its nymph stage coincided with the periods of highest gas supersaturation levels in the river; any resulting density differences would have been missed.

Group 2. Taxa exhibiting greater abundance at rkm 2.4 and 14.5 during different periods include: *Physella* spp., *Simulium* spp. pupae and *Gammarus lacustris* (Table 4). Multiple species of *Physella* and *Simulium* are present in the Bighorn River. This, along with asynchronous life cycles, made interpretation of density differences difficult.

Physella were more abundant at rkm 2.4 during August 1986 ($p=0.01$) and April 1987, however, differences were not significant during the latter period ($p=0.17$). In September 1987, presence of newly hatched young resulted in snail densities being eight times greater at rkm 14.5. Had the sites been compared at similar life cycle stages, results could have been different.

In April 1987, 43% of the *Simulium* were pupae at rkm 2.4 versus 27% at rkm 14.5 (Table 4). This greater ratio of pupae to larvae contributed to the significant increase in upstream pupae abundance ($p=0.05$), even though total blackfly densities were higher. During August 1986, pupae abundance at both sites corresponded closely with larvae densities.

Gammarus lacustris was 98.9% more abundant at rkm 2.4 than at rkm 14.5 during August 1986 ($p<0.01$) (Table 4).

Differences between sites were again significant during September 1987, although *G. lacustris* was more common downstream.

Group 3. *Baetis tricaudatis, Glossophonia complanata, Hydropsyche* spp., Hydropsychidae, *Hydroptila* spp., *Isoperla quinquepunctata, Optioservus* spp., *Serratella* sp., and *Tricorythodes minutus* all occurred in greater densities at rkm 14.5 when differences were significant (Table 4). Assuming aquatic invertebrates near the dam are more affected by the higher gas supersaturation there, these taxa are the ones most likely being impacted.

Baetis tricaudatis was more abundant downstream during all collections, but only significantly so during August 1986 ($p=0.02$) and September 1987 ($p<0.01$) (Table 4). This taxa had two emergence periods in the Bighorn River. A spring emergence occurred from about mid-April through much of June, and a more limited fall emergence occurred from mid-October through mid-November (Gail Larr personal communication). It is unknown whether this results from two separate cohorts or from a single multivoltine population.

Glossophonia complanata was significantly more abundant at rkm 14.5 in August 1986 ($p<0.01$). It also had greater downstream densities in April and September 1987, but differences were not significant (Table 4).

Hydropsyche spp. and Hydropsychidae were significantly more abundant at rkm 14.5 only in August 1986 ($p=0.03$ and 0.01, respectively). Early instar larvae were identified to family level only. All mid to late instar larvae collected in benthic samples, however, were *Hydropsyche*.

Hydroptila spp. occurred in significantly greater numbers at rkm 14.5 during all sampling periods. Although I was not able to differentiate individual species and their life cycles, pupae to larvae ratios indicate that life cycles between sites were not synchronized. Even so, the magnitude of difference in *Hydroptila* densities during all periods indicates that this taxa is probably more common downstream.

Optioservus spp. also occurred in significantly greater densities at rkm 14.5 during all sampling periods. Most larvae collected are thought to be *O. divergens*.

I. quinquepunctata had emerged prior to the late summer/early fall sampling periods, thus were present only in the spring samples. April 1987 densities were seven times greater at rkm 14.5 ($p<0.01$) (Table 4).

Serratella sp. was present only in late summer and fall collections. Densities were significantly different between sites only during August 1986 ($p<0.01$), with downstream abundance three times greater than upstream (Table 4). Although estimated densities were 1.7 times greater at rkm 2.4 in September 1987, they were not significantly different from densities upstream. Nymphs probably appear in early August and emerge sometime prior to early May. Thus, they are not present in the river during the period of highest gas saturation levels.

Tricorythodes minutus, was one of only three taxa that were significantly more abundant at rkm 14.5 during all three collection periods. Adults emerge for approximately 1 month beginning about the third week in September (Gail Larr personal communication). Thus, nymphs are present during the period of highest gas saturation levels in the Bighorn River. Additionally, August and September sampling was likely done prior to the occurrence of any extensive emergence and probably provide reliable comparisons of *T. minutus* abundance.

Community Structure

Community structure differed markedly between sites during August and September, but was similar during April. In August 1986, percentages of Chironomidae, Tricladida, and *Gammarus lacustris* in the community decreased from 26%, 16%, and 11%, respectively at the upstream site, to 4%, 11%, and 0% at the downstream site (Figure 6). In

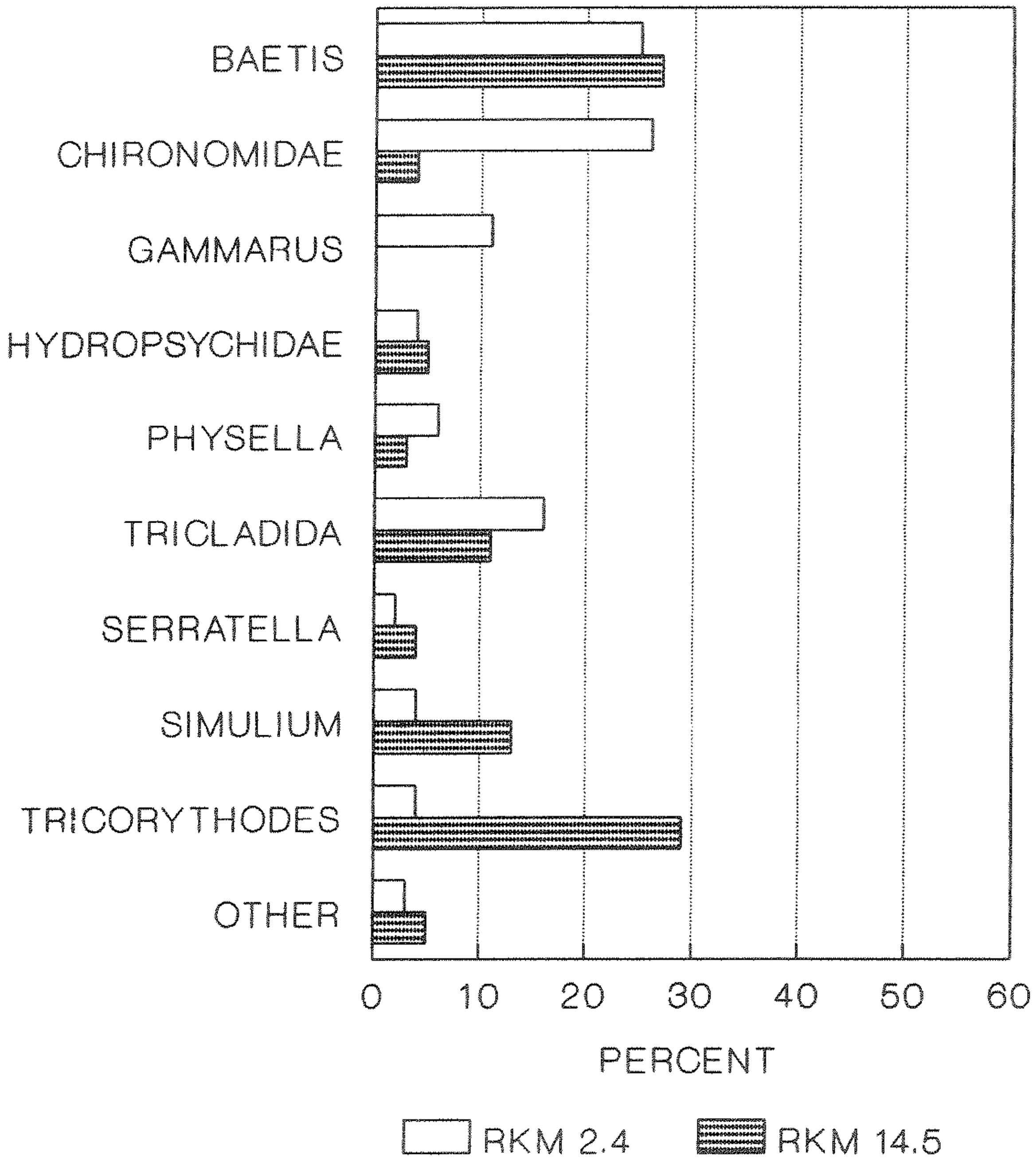

Figure 6. Mean percent of total invertebrate abundance comprised by the nine most common taxa, at rkm 2.4 and 14.5, Bighorn River, August 1986.

contrast, *Tricorythodes* and *Simulium* gained in representation downstream from 4% each, to 29% and 13%, respectively.

In September 1987, abundance of Chironomidae and Tricladida was lower downstream, while abundance of *B. tricaudatis*, *Physella* sp., and *Tricorythodes* was higher (Figure 7). A large number of small individuals was responsible for the increase in *Physella*.

Community structure during April 1987, was remarkably similar between sites. *A. aspilis*, the only taxa exhibiting a major difference in representation, decreased from 8% (2608 organisms per m^2) upstream to less than 1% (60 organisms per m^2) downstream (Figure 8). Slightly greater percent representation of *B. tricaudatis*, Chironomidae, Tricladida, and *T. minutus* at rkm 14.5 resulted from *A. aspilis*' decreased abundance.

Drift

Invertebrate drift was dominated by chironomids (pupae and larvae) and *B. tricaudatis* during both collection periods. In April 1987, drift densities of five invertebrate groups were 0.1 to 12.2 organisms per cubic meter (Table 5). In September 1987, 10 groups dominated the samples. The increase in drift densities coincided with fall increases in total benthic abundance.

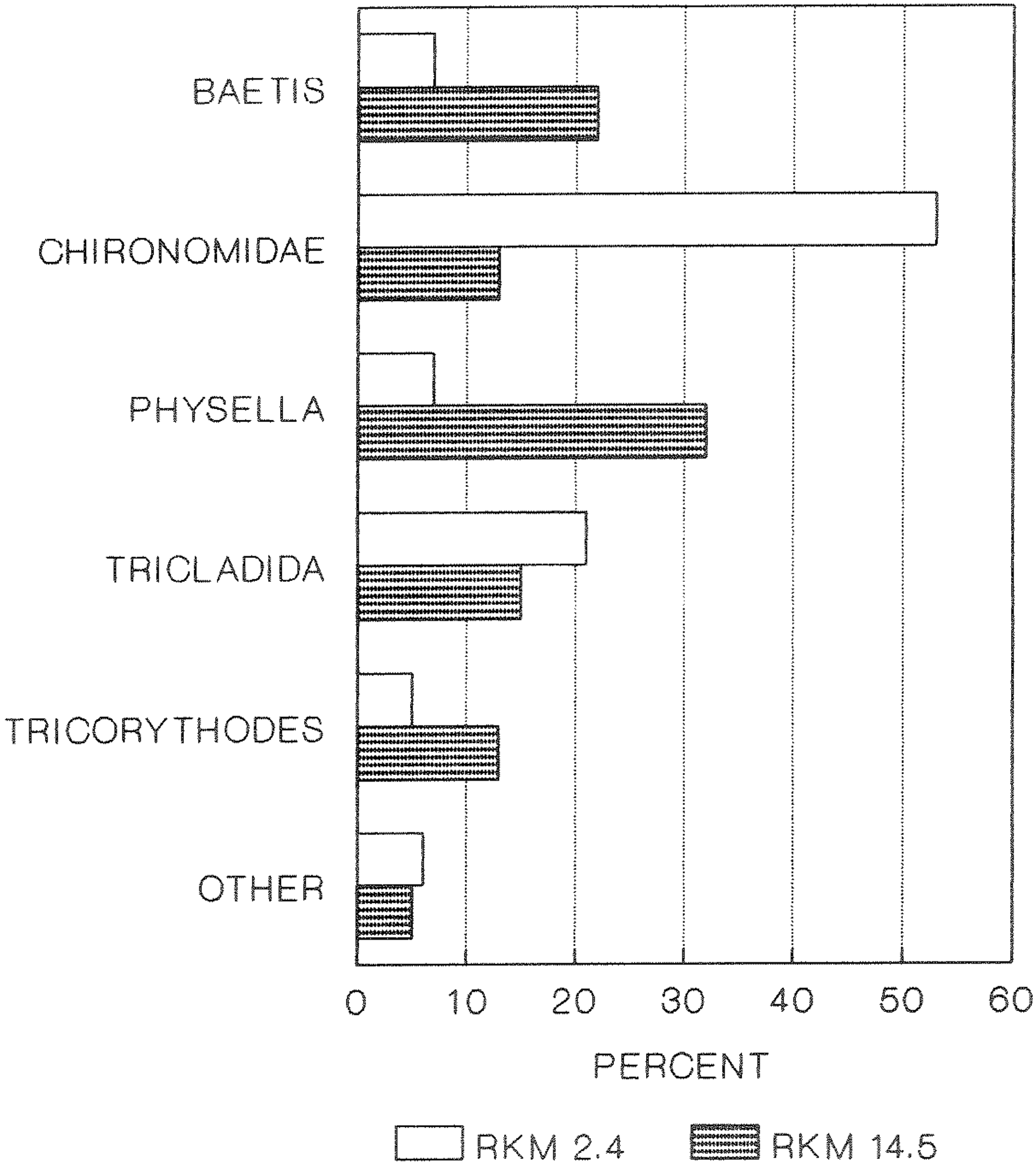

Figure 7. Mean percent of total invertebrate abundance comprised by the five most common taxa, at rkm 2.4 and 14.5, Bighorn River, September 1987.

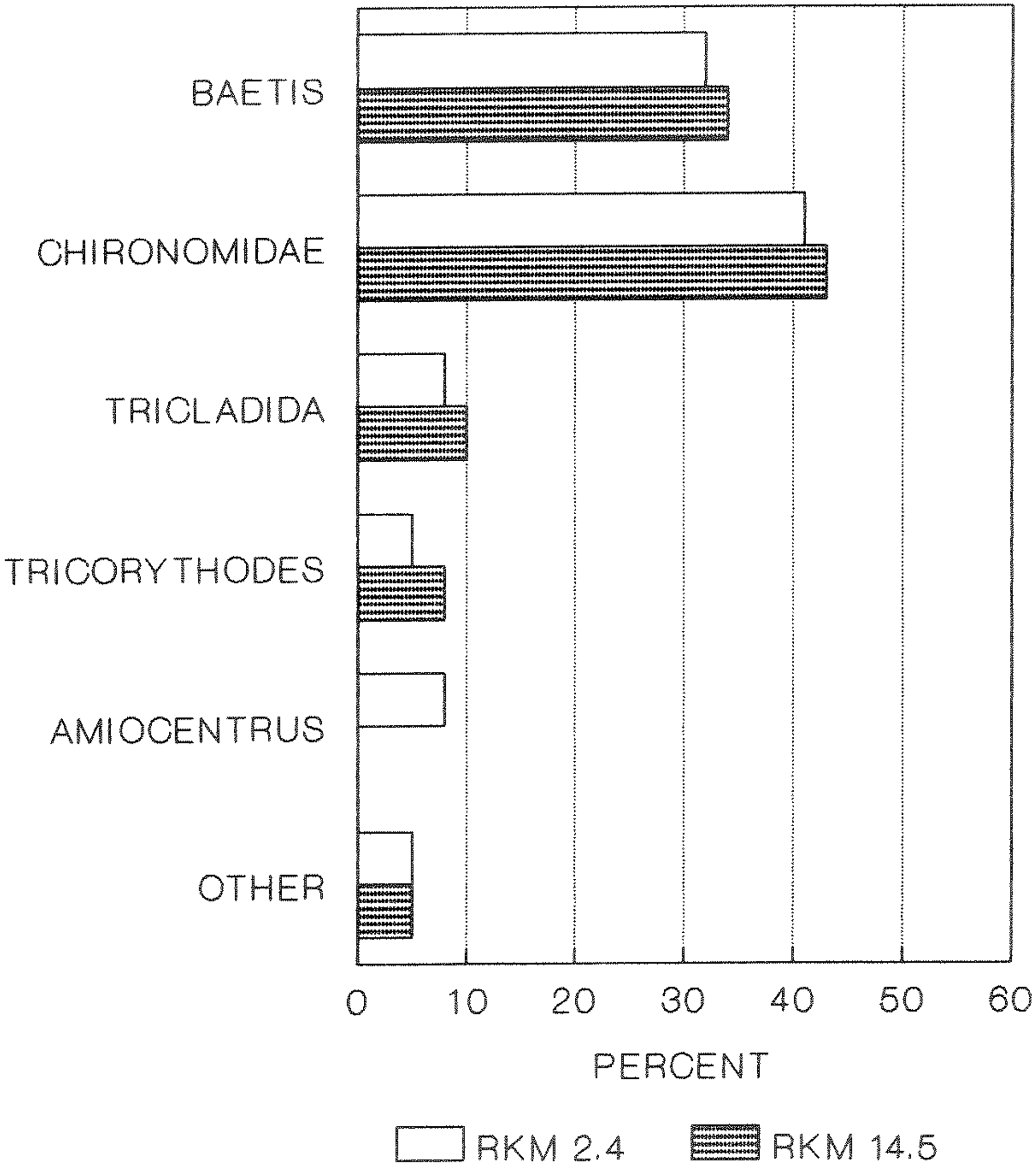

Figure 8. Mean percent of total invertebrate abundance comprised by the five most common taxa, at rkm 2.4 and 14.5, Bighorn River, April 1987.

Table 5. Mean drift densities (#/m^3) and standard deviations, at rkm 2.4 and 14.5, for those taxa most commonly present in the drift during collection periods 2 and 3, Bighorn River.

Taxanomic group	APRIL 15, 1987				SEPTEMBER 24, 1987			
	RKM 2.4		RKM 14.5		RKM 2.4		RKM 14.5	
	$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.
Baetis	4.1	2.4	11.7	2.8	9.8	2.8	112.2	36.7
Tricorythodes	0.1	0.0	p/	---	2.3	1.4	2.2	1.1
Serratella	a/	---	a/	---	1.6	0.4	2.3	1.0
Physella	a/	---	a/	---	2.7	1.1	12.6	10.2
Chironomidae (L)	12.2	7.5	7.5	3.0	8.2	2.1	3.9	3.2
Chironomidae (P)	4.6	1.9	5.8	1.4	7.8	1.8	5.4	1.6
Simulium (L)	0.1	0.1	0.1	0.1	0.5	0.2	0.1	0.1
Gammarus	p/	---	p/	---	0.4	0.3	0.5	0.2
Hyalella	p/	---	p/	---	0.6	0.2	0.1	0.1
Tricladida	p/	---	a/	---	3.1	2.0	3.5	3.2

a/ absent from drift samples
p/ present in sample(s), but mean drift densities were much less than 0.1 organism per cubic meter
(L) larvae
(P) pupae

These increases were commonly due to recruitment of early instar organisms.

To determine whether differences in drift densities between rkm 2.4 and 14.5 resulted from different benthic densities, drift rate at each site was compared to benthic abundance. In April 1987, *Baetis*, chironomid pupae, and *Simulium* exhibited proportionately greater drift rates at rkm 14.5, relative to their benthic densities (Figure 9). Although gas saturation levels were not measured when drift samples were collected (April 13 and 14), delta P trends indicate that they were greater at this site (Appendix D: Figure 39).

In September, drift densities of *B. tricaudatis*, chironomid larvae and pupa, *Physella, Hyalella* and triclads were proportionately close to benthic densities at rkm 14.5 (Figure 10). This suggests that drift rate differences between sites, for these taxa, are associated with differences in benthic abundance.

Tricorythodes, *Simulium*, and *Gammarus* had lower drift rates downstream relative to their benthic abundance, while *Serratella* drift rates were higher. The delta P trend during the sampling period (September 22 and 23) indicates that gas saturation levels were higher at the upstream site (Appendix D: Figure 40).

Site Characteristics

Mean daily discharges were assumed to be the same at both sites during each sampling period. Discharges near Afterbay Dam remained at 85 m^3/s during sampling in August 1986 and at 99 m^3/s in September 1987. In April 1987, discharges decreased from 56 m^3/s on the first sampling day to 50 m^3/s the following day.

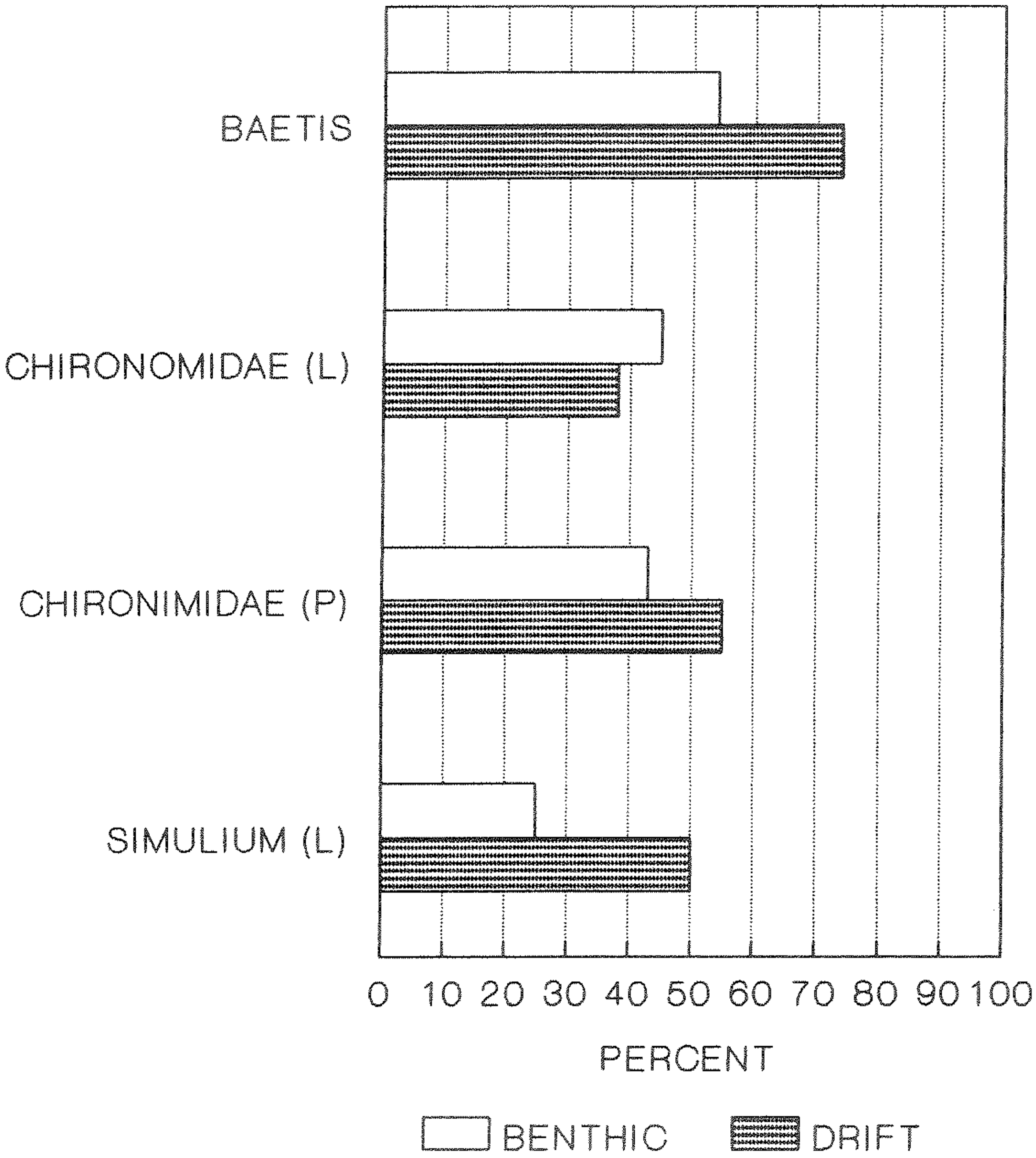

Figure 9. Mean percent of *Baetis tricaudatis*, Chironomidae larvae and pupae, and *Simulium* larvae collected at rkm 14.5, in benthic and drift samples, Bighorn River, April 1987.

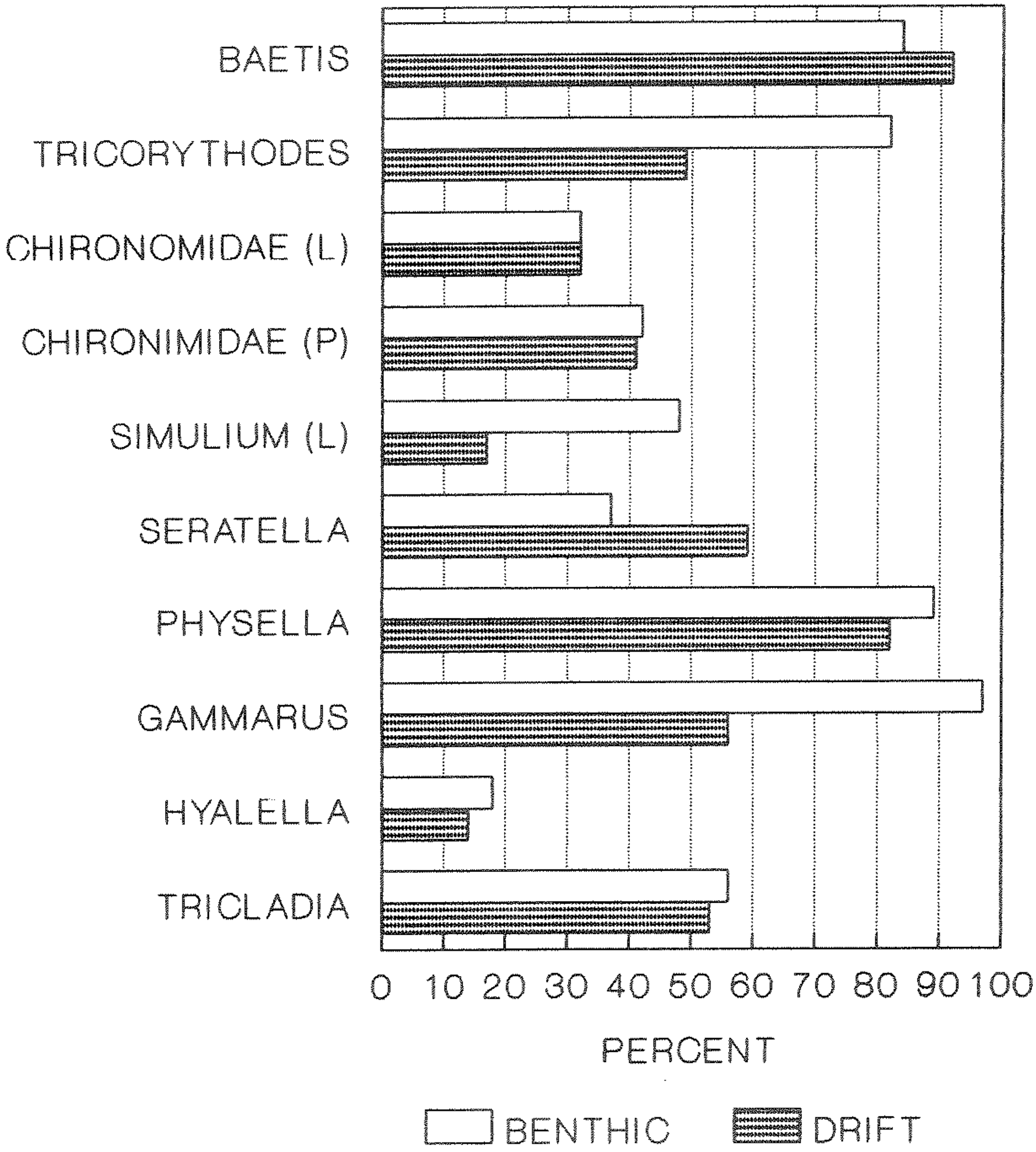

Figure 10. Mean percent of nine taxa collected at rkm 14.5 in benthic and drift samples, Bighorn River, September 1987.

Other site specific characteristics are addressed in four parts: 1) "Associated Habitat Characteristics" - includes water velocities, water depths, and dry weights of vegetation (DWV) collected in each benthic sample. 2) Periphyton - includes periphytic ash-free dry weights (AFDW), and chlorophyll a concentrations. Ash free dry weights were used to quantify standing crops of aquatic plants at sampling riffles, and to determine if DWV accurately depicted vegetation abundance; 3) Temperature; and 4) Gas saturation.

Associated Habitat Characteristics

The greatest variation in water depth, water velocity, and DWV (Table 6) occurred between sampling periods, although some differences between sites during a single sampling period were significant. Of the habitat characteristics measured during August 1986, only water depth varied significantly between sites ($p=0.05$), averaging 0.1 m deeper at rkm 2.4. Water velocity differences at 0.6 depth approached significance ($p=0.08$) with the faster water upstream, while differences in DWV were insignificant ($p=0.72$).

In April 1987, differences in water depth neared significance ($p=0.07$), with greater depths occurring at rkm 2.4 (Table 6). Mean water velocities at 0.6 depth were greater, although not significantly different at the

Table 6. Mean water depths, water velocities (M = 0.6 depth; B = 3 to 5 cm above the substrate), and dry weight of vegetation (DWV), measured for each benthic sample, according to site and collection period. Ranges are in parentheses.

Period/site	N	Water depth (m)	Water velocity M (m/sec)	Water velocity B (m/sec)	DWV (g/Hess sample)
August 1986					
rkm 2.4	23	0.3	0.8	---	5.56
		(0.1-0.3)	(0.2-1.1)	---	(2.43-13.09)
rkm 14.5	25	0.2	0.7	---	5.95
		(0.1-0.2)	(0.5-0.9)	---	(1.56-21.17)
April 1987					
rkm 2.4	15	0.3	0.4	0.1	12.29
		(0.1-0.4)	(0.1-0.9)	(0.0-0.3)	(2.21-26.56)
rkm 14.5	14	0.2	0.5	0.2	10.91
		(0.2-0.3)	(0.2-0.7)	(0.0-0.5)	(2.44-23.52)
September 1987					
rkm 2.4	15	0.2	0.3	0.1	6.62
		(0.1-0.2)	(0.2-0.5)	(0.0-0.2)	(2.34-15.94)
rkm 14.5	15	0.2	0.6	0.2	12.98
		(0.1-0.2)	(0.2-0.9)	(0.0-0.3)	(7.56-24.35)

downstream site. Velocities near the substrate, however, were significantly different (p=0.02), averaging 0.1 m/s faster at rkm 14.5. Means for DWV were similar, indicating comparable vegetative standing crops between riffles. Correlations between water velocity and individual taxa in the Bighorn River, however, were poor. Oddly, invertebrate covariance correlations were commonly higher with velocities at 0.6 depth, than with velocities at the substrate. This may result from the lack of an effective means of measuring substrate velocities.

The largest differences in physical characteristics (Table 6) between sites occurred during September 1987. Average water velocity (0.6 depth) and vegetation abundance (DWV) were approximately 100% greater at the downstream site ($p<0.01$). Correlations between invertebrate numbers and DWV were not strong, possibly indicating that the specific type of vegetation, not overall abundance, was important for individual taxa.

Sampling sites were subdivided in April 1987 (Figure 2) to determine if certain subsections from each site were more comparable than others. Some differences between subsections (Table 7) were present for each characteristic measured. However, comparable subsections changed with the physical parameter used to evaluate them. Thus, no unique combination of subsections could be used to compare upstream and downstream invertebrate communities. These results did show that some parameters differed significantly within sites but not between sites when the entire riffles were compared.

Periphyton.

Ash-free dry weight (AFDW) (Table 8) showed trends similar to those from dry weights of vegetation (DWV) (Table 7). Some differences occurred among subsections

Table 7. Mean water depths, water velocities (M = 0.6 depth; B = 3 to 5 cm above the substrate), and dry weight of vegetation (DWV) associated with benthic samples collected in April 1987 in each riffle subsection. Ranges are in parentheses.

Site/ subsection	N	Water depth (m)	Water velocity M (m/sec)	Water velocity B (m/sec)	DWV (g/Hess Sample)
rkm 2.4					
1	3	0.2 (0.1-0.3)	0.2 (0.1-0.3)	0.1 (0.0-0.1)	5.30 (4.98-5.87)
2	5	0.4 (0.3-0.4)	0.5 (0.2-0.9)	0.1 (0.0-0.2)	15.64 (11.79-17.97)
3	4	0.2 (0.2-0.3)	0.6 (0.6-0.7)	0.1 (0.0-0.3)	13.43 (2.21-26.56)
4	3	0.2 (0.1-0.2)	0.3 (0.1-0.4)	0.1 (0.0-0.2)	12.18 (3.80-18.82)
rkm 14.5					
5	7	0.2 (0.2-0.3)	0.5 (0.2-0.6)	0.2 (0.0-0.5)	5.52 (2.44-10.22)
6	7	0.2 (0.2-0.2)	0.5 (0.4-0.7)	0.2 (0.1-0.5)	16.30 (9.91-23.52)

in April 1987 (Tables 7 and 8), probably because of smaller sample size. Both AFDW and DWV indicated that sampling sites had similar vegetation abundance in April 1987, but variance was large. In September 1987 a significantly greater standing crop of vegetation was present at rkm 14.5 (p=0.01).

In April 1987, chlorophyll a concentrations were similar between sites, but differences were present between subsections within the upstream site. Mean concentrations in subsections 4 and 5 were 60% and 71% of

Table 8. Mean and standard deviation of periphytic AFDW ($g/cm^2 \cdot 10^{-3}$) and Chlorophyll *a* concentration (µg/g of blotted wet weight) at rkm 2.4 and 14.5 for April and September 1987 sampling periods. Data are also presented by subsection for April 1987.

Period/site/ subsection	Ash-free dry weight mean	Ash-free dry weight S.D.	Ash-free dry weight N	Chlorophyll *a* mean	Chlorophyll *a* S.D.	Chlorophyll *a* N
April 1987						
rkm 2.4						
1	6.34	3.40	6	683.0	324.8	6
2	38.52	50.62	15	659.9	172.2	18
3	13.47	8.27	5	697.4	104.3	5
4	17.75	27.61	7	428.3	94.7	6
SITE	**24.47**	**38.17**	**33**	**628.1**	**199.3**	**35**
rkm 14.5						
5	11.64	6.79	20	509.0	155.1	20
6	65.61	60.30	13	718.8	196.1	11
SITE	**32.90**	**45.91**	**33**	**583.5**	**196.1**	**31**
Sept. 1987						
rkm 2.4	11.34	10.90	34	---.-	---.-	--
rkm 14.5	17.92	10.11	37	---.-	---.-	--

those in subsection 6 (Table 8) ($p<0.01$). Chlorophyll *a* data were not collected in September 1987.

Water Temperature

Water temperatures at rkm 14.5 began to exceed those at rkm 2.4 in early spring during both years of the study. These differences persisted until late fall (Figures 11 and 12). Temperature disparity between sites was probably

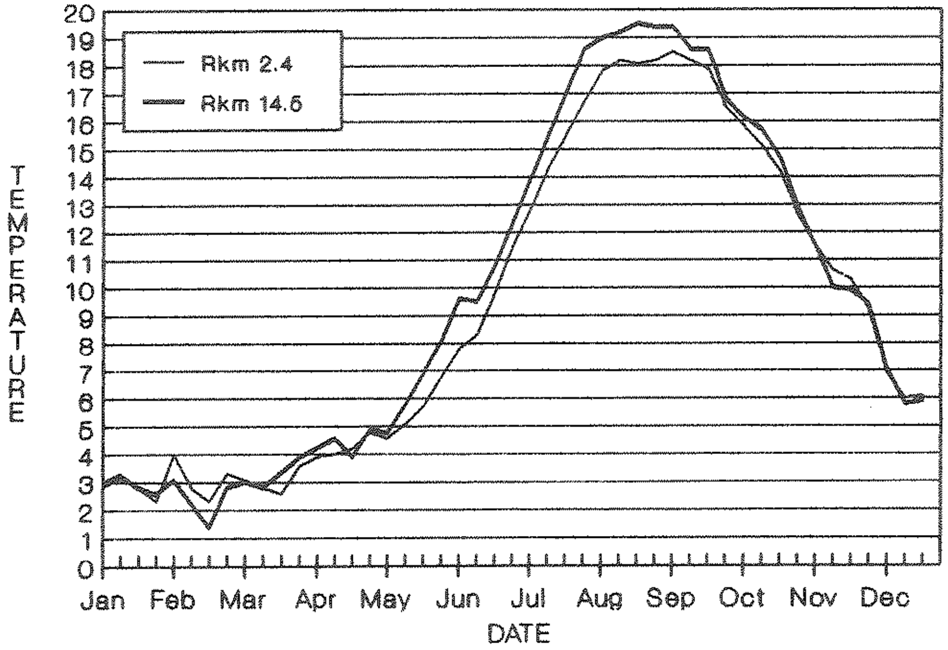

Figure 11. Mean weekly, Bighorn River water temperatures (°C) at rkm 2.4 and 14.5, 1986.

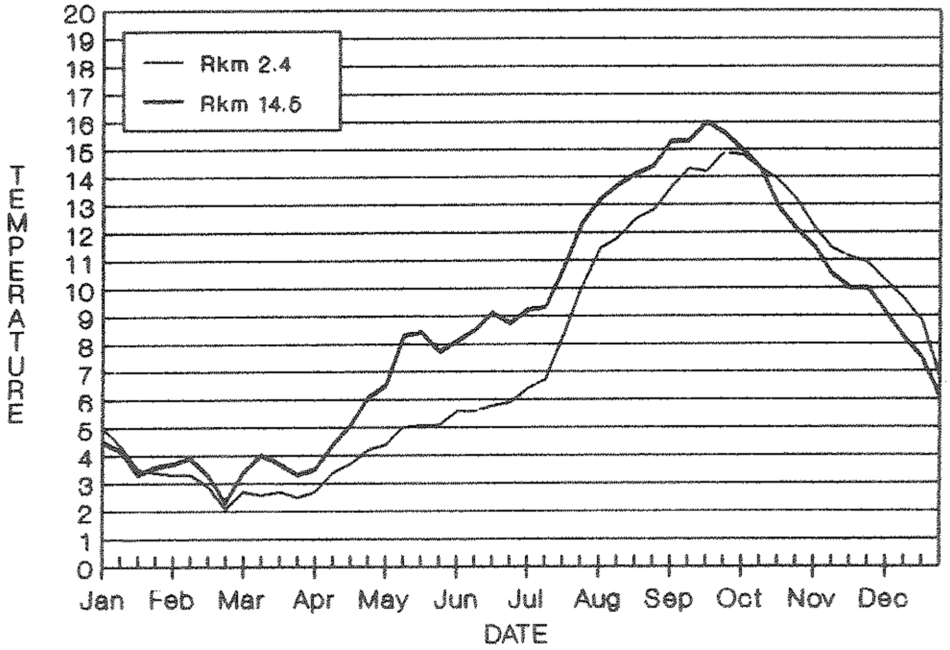

Figure 12. Mean weekly, Bighorn River water temperatures (°C) at rkm 2.4 and 14.5, 1987.

the primary cause of asynchronous life cycles in many of the taxa collected.

In 1986 the highest and lowest mean weekly water temperatures occurred at rkm 14.5 (Figure 11). Temperatures were similar at both sites in January, but were cooler downstream during February. Water temperatures were warmer at Rkm 14.5 from the second week in March through the third week of October.

Temperatures were warmer downstream by the third week in January 1987 (Figure 12). This difference persisted until the third week in October. The minimum average weekly temperature was similar between sites, while the maximum was approximately 1°C warmer at rkm 14.5 (Figure 12). Temperature differences between sites were substantially larger in 1987, likely associated with lower flows during the second year of the study.

Gas Saturation

Gas saturation levels were generally higher at rkm 2.4 than at rkm 14.5 during 1986, except for a few periods between late June and mid-August (Figure 13). The highest gas saturation levels occurred at rkm 14.5 on June 30 and July 2, when delta P's reached 156 and 159 mm Hg, respectively. Mean monthly gas levels were greater upstream throughout the year, except in July when rkm 14.5 delta P's averaged 2.1 mm Hg higher (Figure 14).

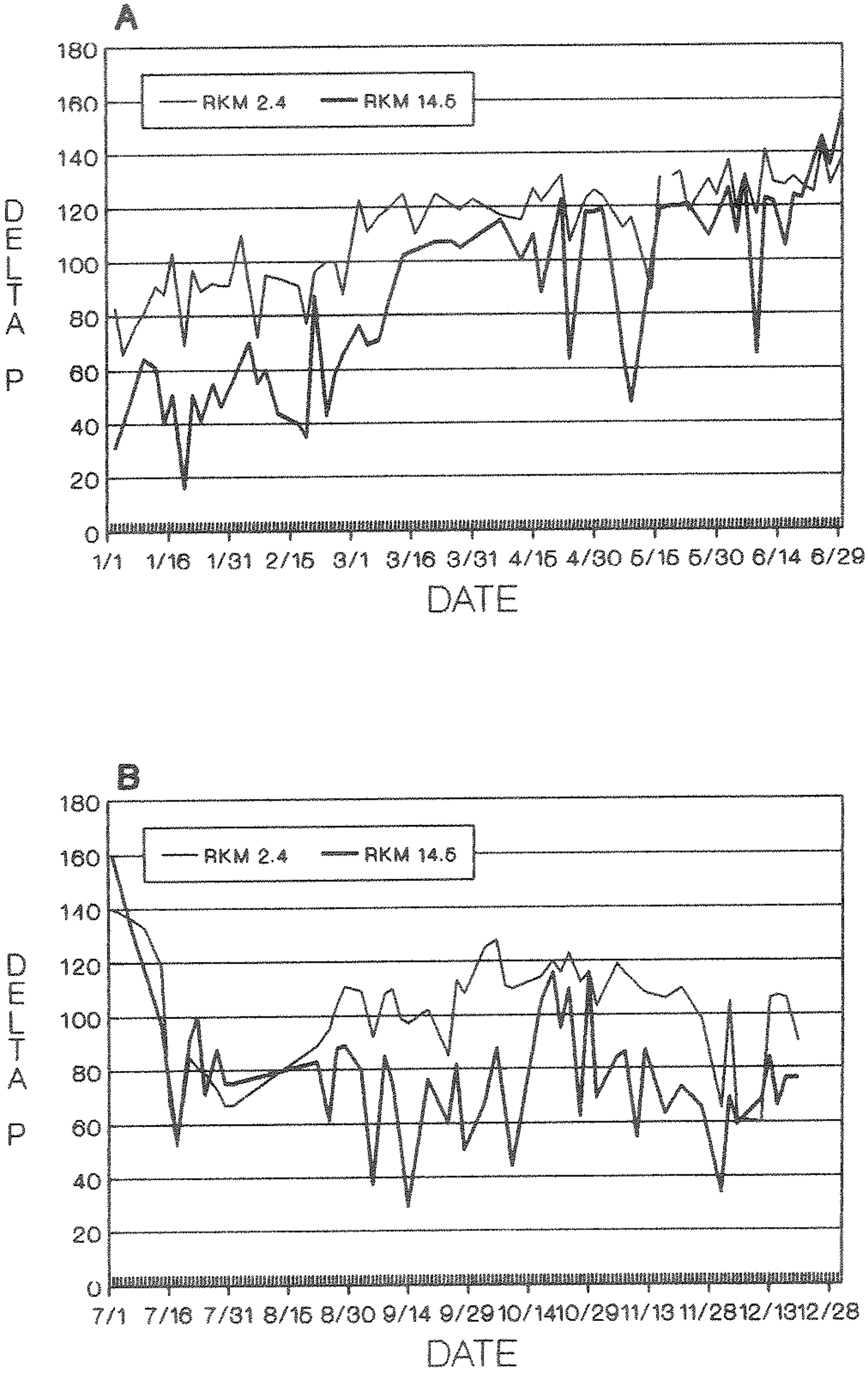

Figure 13. Delta P levels (mm Hg) measured two or three times weekly on the Bighorn River, at rkm 2.4 and 14.5, from January 1 through June 30 (A) and from July 1 through December 31 (B) 1986.

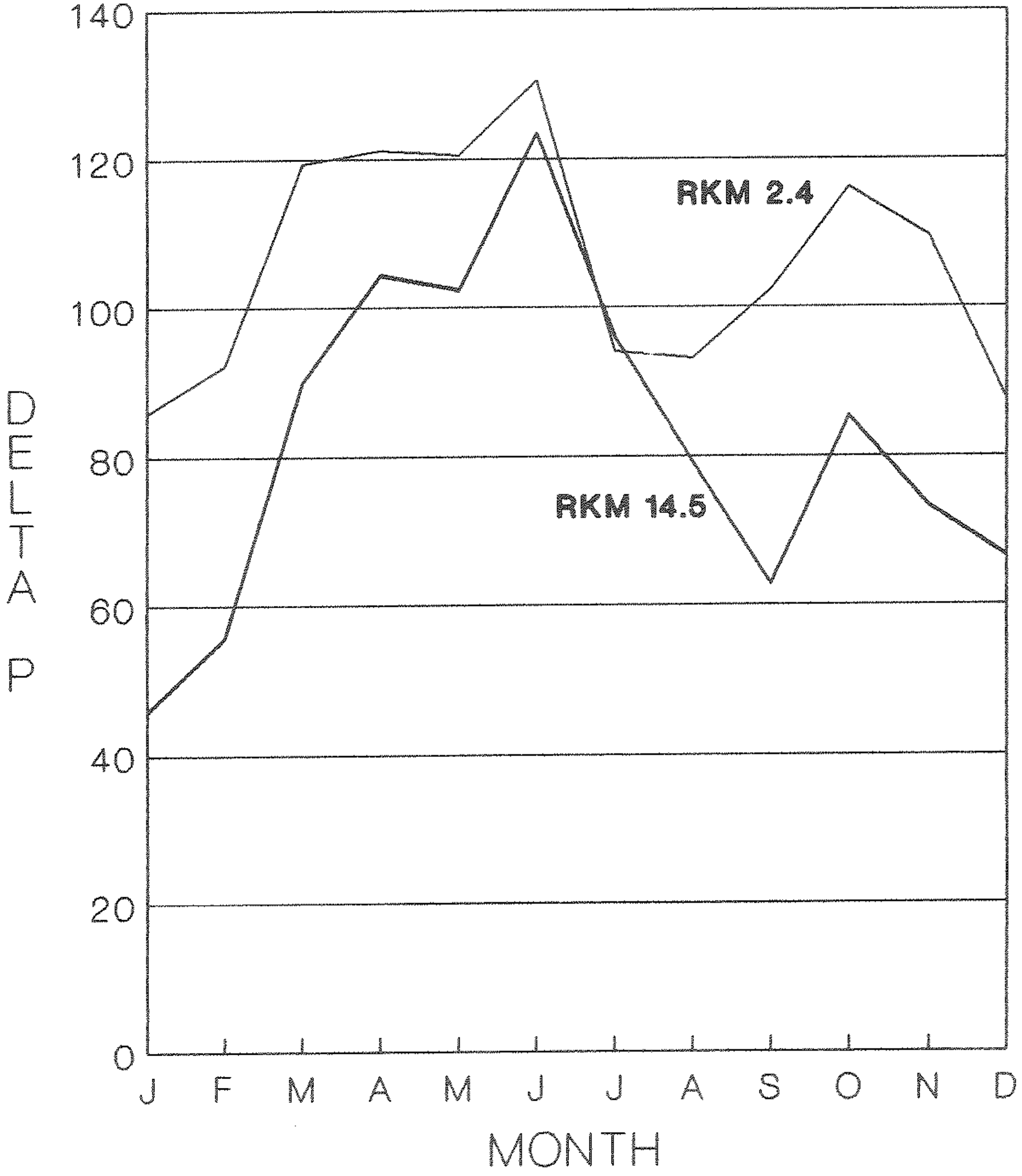

Figure 14. Mean monthly delta P levels (mm Hg) at rkm 2.4 and 14.5, Bighorn River, 1986.

Gas saturation levels during 1987 at rkm 14.5 exceeded those at rkm 2.4 much more frequently than the previous year (Figure 15). Higher downstream delta P's were observed on at least one occasion in each of the first 10 months of the year. As in 1986, the highest delta P levels were recorded at rkm 14.5, with gas pressures reaching 191 mm Hg. The highest delta P recorded at the upstream site was 154 mm Hg.

Mean monthly gas saturation levels were generally higher in 1987 than for the same months in 1986, with less disparity between sites. Downstream average delta P's exceeded those at rkm 2.4 during April, July, and August (Figure 16).

The composition of supersaturated gases varied between sites, with oxygen (O_2) partial pressures always influencing delta P's more at rkm 14.5 than at rkm 2.4. In 1986, the percentage of total delta P due to O_2 at rkm 14.5 ranged from 36% in January to 76% in August. Values for the same months at rkm 2.4 were 11% and 17%, respectively (Figure 17). This trend was repeated in 1987. In 5 of 12 months, the contribution of O_2 to total delta P's averaged 70% or more downstream, but averaged no higher than 28% in any month at rkm 2.4 (Figure 18).

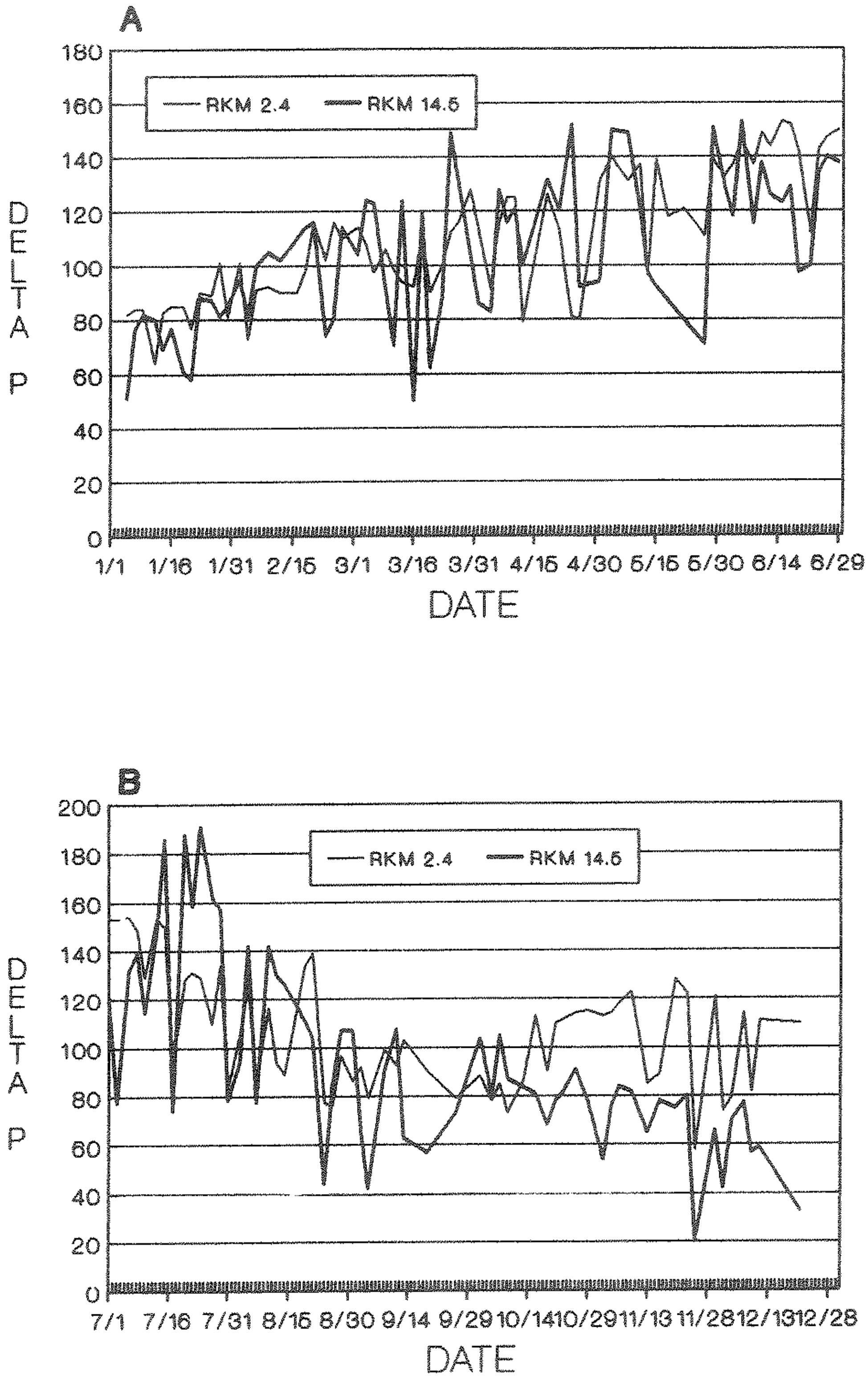

Figure 15. Delta P levels (mm Hg) measured two or three times weekly on the Bighorn river, at rkm 2.4 and 14.5, from January 1 through June 30 (A) and from July 1 through December 31 (B), 1987.

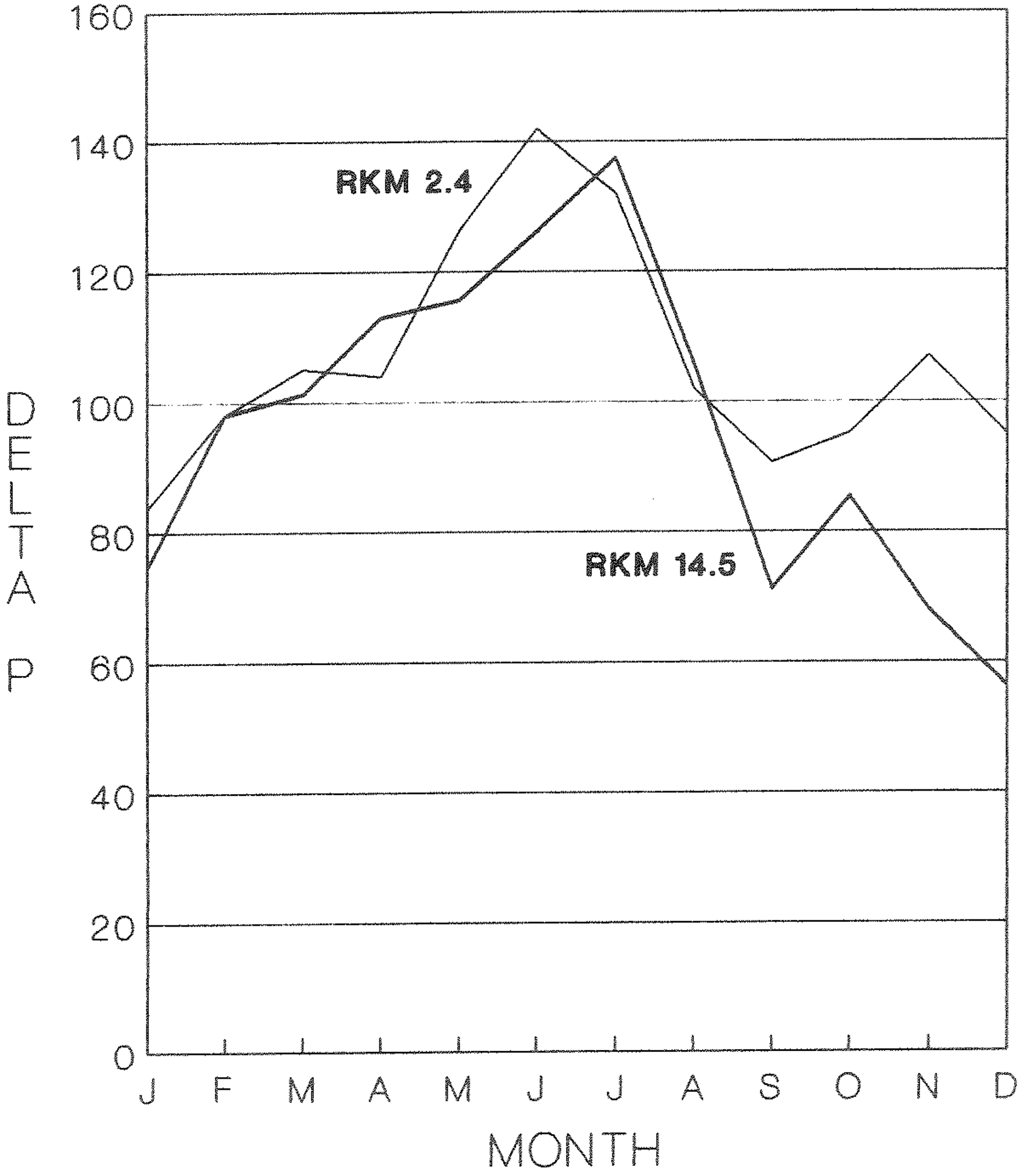

Figure 16. Mean monthly delta P levels (mm Hg) at rkm 2.4 and 14.5, Bighorn River, 1987.

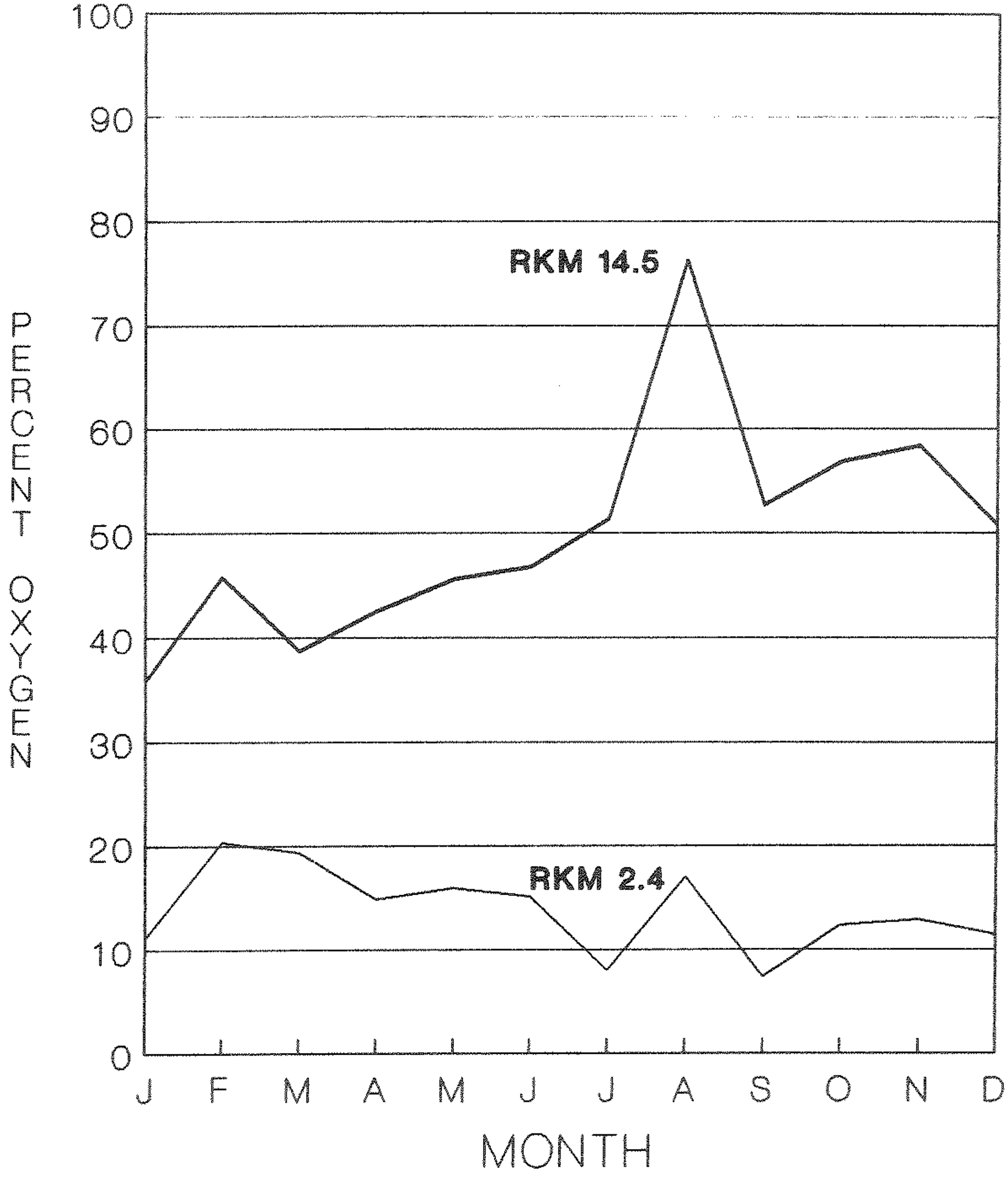

Figure 17. Percent of mean monthly delta P levels comprised of oxygen at rkm 2.4 and 14.5, Bighorn River, 1986.

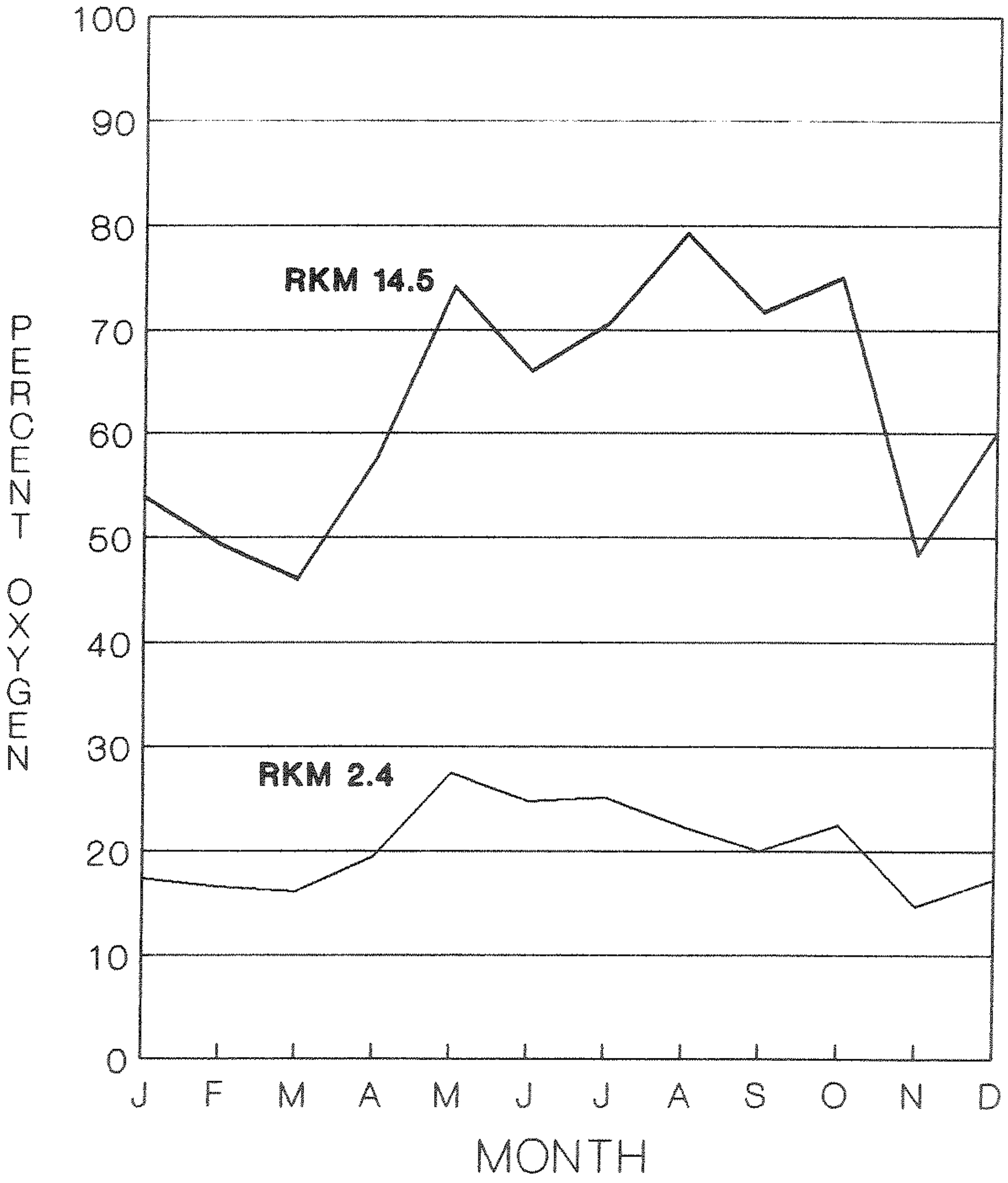

Figure 18. Percent of mean monthly delta P levels comprised of oxygen at rkm 2.4 and 14.5, Bighorn River, 1987.

Invertebrate Bioassays

Eight macroinvertebrate taxa were tested under controlled bioassay conditions. Taxa were selected to include representatives from groups 1, 2 and 3. *Amiocentrus aspilis* and *Ephemerella inermis* were significantly more abundant at rkm 2.4 when differences occurred (group 1). *Physella* and *Gammarus lacustris* had significantly greater densities at one site during one period, but at the other site during a subsequent period (group 2). *Baetis tricaudatis, Isoperla quinquipunctata, Hydropsyche spp.* and *Tricorythodes minutus* were all more abundant downstream when differences were significant (group 3).

The initial effect on all taxa exposed to high levels of gas supersaturated water was buoyancy which resulted in organisms becoming stranded at the water surface. Buoyancy was caused by gas bubbles forming and clinging to the body externally or forming internally within the organism. Stranding from external bubbles was temporary, lasting until the organism floated to the surface where the bubbles would eventually burst. This was most prevalent during the first 24 h.

Buoyancy from internal gas accumulation (gas bubble trauma or GBT) was more permanent than externally caused buoyancy. Recovery from GBT was not observed to occur within the 168 h test periods. Gas bubbles were observed at various locations within appendages and body cavities of afflicted organisms (Appendix A). Severity increased with increasing delta P levels and with time of exposure. Advanced GBT symptoms included protraction of the head from the thorax, separation of abdominal segments (i.e. distension), and loss of torsal mobility. Death was common in organisms exhibiting these symptoms.

Mean delta P levels were lower in all tanks during bioassay #2 (Figure 19). This made comparing susceptibility of taxa tested in different bioassays difficult.

Bioassay #1

Although delta P levels fluctuated during tests, the overlap between tanks was small except between the two highest gas levels tested (Table 9). This overlap resulted from one measurement of 270 mm Hg in tank 5. Excluding this, mean delta P, standard deviation, and range would be 221.6, 9.9, and 204-237, respectively, in tank 5. With this exception, delta P varied more at low supersaturation levels, probably reflecting varying gas pressures in the untreated water. Delta P fluctuations at high saturation levels were attributed to gases coming out of solution and impeding water flow through the valves (i.e. vapor lock), rather than to variations in gas levels

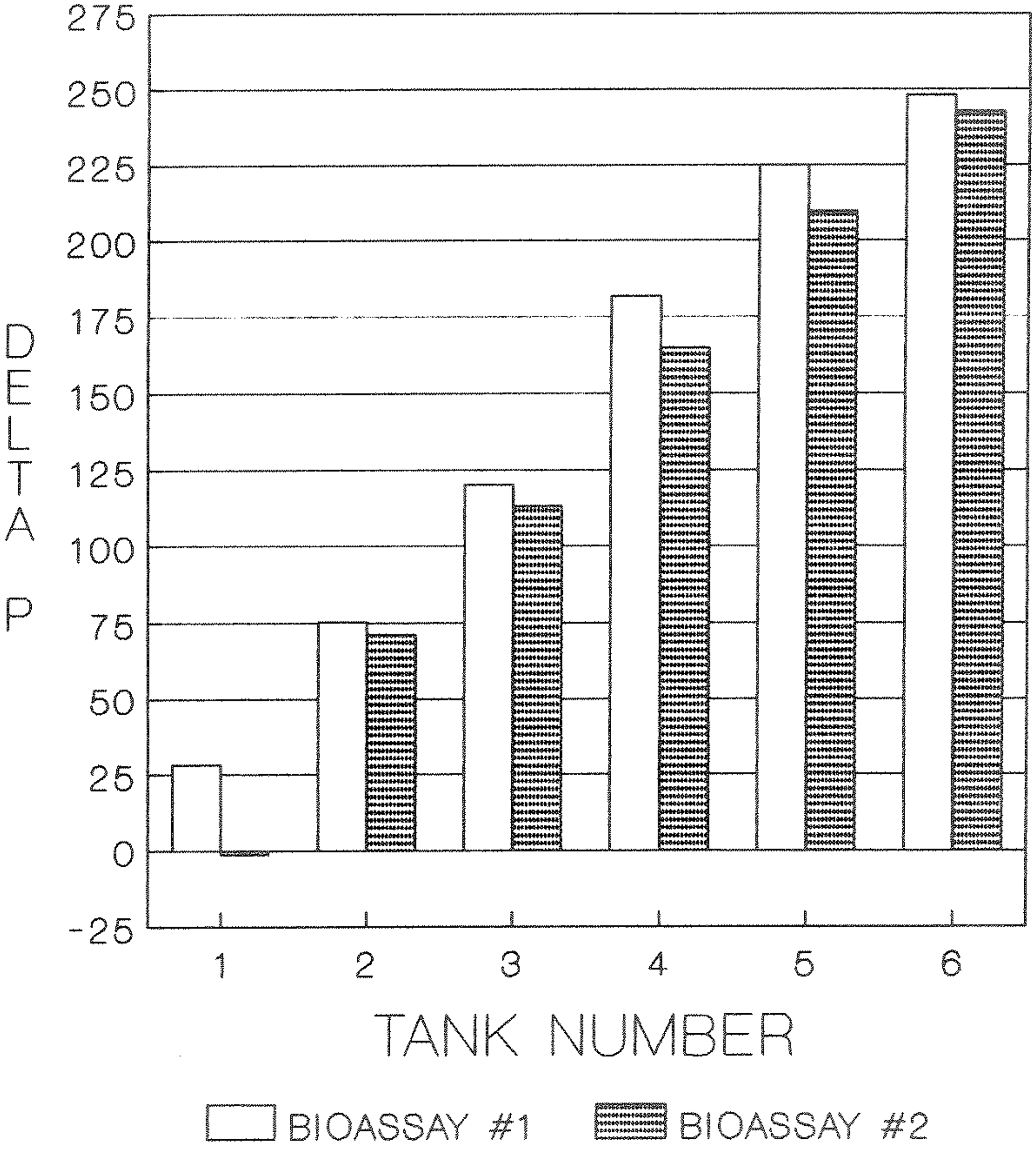

Figure 19. Comparison of mean delta P levels (mm Hg) in all test tanks during bioassays 1 and 2.

Table 9. Means, standard deviations (S.D.), and ranges of Delta P (mm Hg), dissolved oxygen (mg/L), and temperature (°C) during bioassay #1, August 2 to 9, 1988.

Tank	Delta P			Dissolved oxygen			Temperature		
	mean	S.D.	range	mean	S.D.	range	mean	S.D.	range
1	28.4	13.6	14-57	8.0	0.29	8.5-9.4	8.5	0.6	7.8-8.9
2	75.5	11.8	54-92	9.8	0.23	9.2-10.0	8.7	0.6	7.8-10.0
3	120.3	8.1	104-134	10.8	0.34	10.2-11.5	8.7	0.6	7.8-10.0
4	181.6	6.9	170-193	11.9	0.24	11.3-12.2	8.9	0.6	7.8-10.6
5	225.0	16.0	204-270	13.3	0.48	12.2-13.8	9.0	0.7	7.8-10.6
6	248.2	4.3	244-258	14.7	0.29	14.1-15.0	9.2	0.8	8.3-11.1

produced by the column. This problem was never rectified, but was partially overcome by periodically "bleeding" the water line.

Water temperatures also varied between tanks (Table 9). Average temperatures increased with the proportion of treated water used, but differences were small and the ranges similar. Water chemistry (Table 10) was similar to that reported for the Bighorn River (White et al. 1986).

I. quinquepunctata, A. aspilis, E. inermis, and *Hydropsyche spp.* were tested during bioassay #1. Individuals of three of the four taxa suffered GBT induced stranding (Figure 20) and death (Figure 21) at

Table 10. Water chemistry measurements taken the day following gas supersaturation bioassays, Bighorn River, Montana 1988.

Parameter	Bioassay #1	Bioassay #2
Total hardness ($CaCo_3$)	332 mg/L	309 mg/L
Ca hardness	205 mg/L	200 mg/L
pH	8.3	8.3
Total alkalinity	178 mg/L	216 mg/L

delta P's of 181 mm Hg and greater, but none were negatively influenced by delta P's of 75 or less.

Isoperla quinquepunctata. The lowest susceptibility to GBT induced stranding during bioassay #1 occurred in *I. quinquepunctata*. Of the 20 nymphs tested, three (15%), four (20%) and six (30%) became permanently stranded at delta P's of 181, 225, and 248 mm Hg, respectively (Figure 20). A similar trend in mortality occurred (Figure 21). The threshold for GBT induced stranding and death in this taxa appears to be between a delta P of 181 and 120 mm Hg.

Eight *I. quinquepunctata* emerged during the test. Emergence was observed from tanks 1, 2, 3 and 6. Only one stonefly (tank 3) died during emergence. During molts prior to the final molt for emergence, one *Isoperla* died, and another became buoyant due to external bubbles.

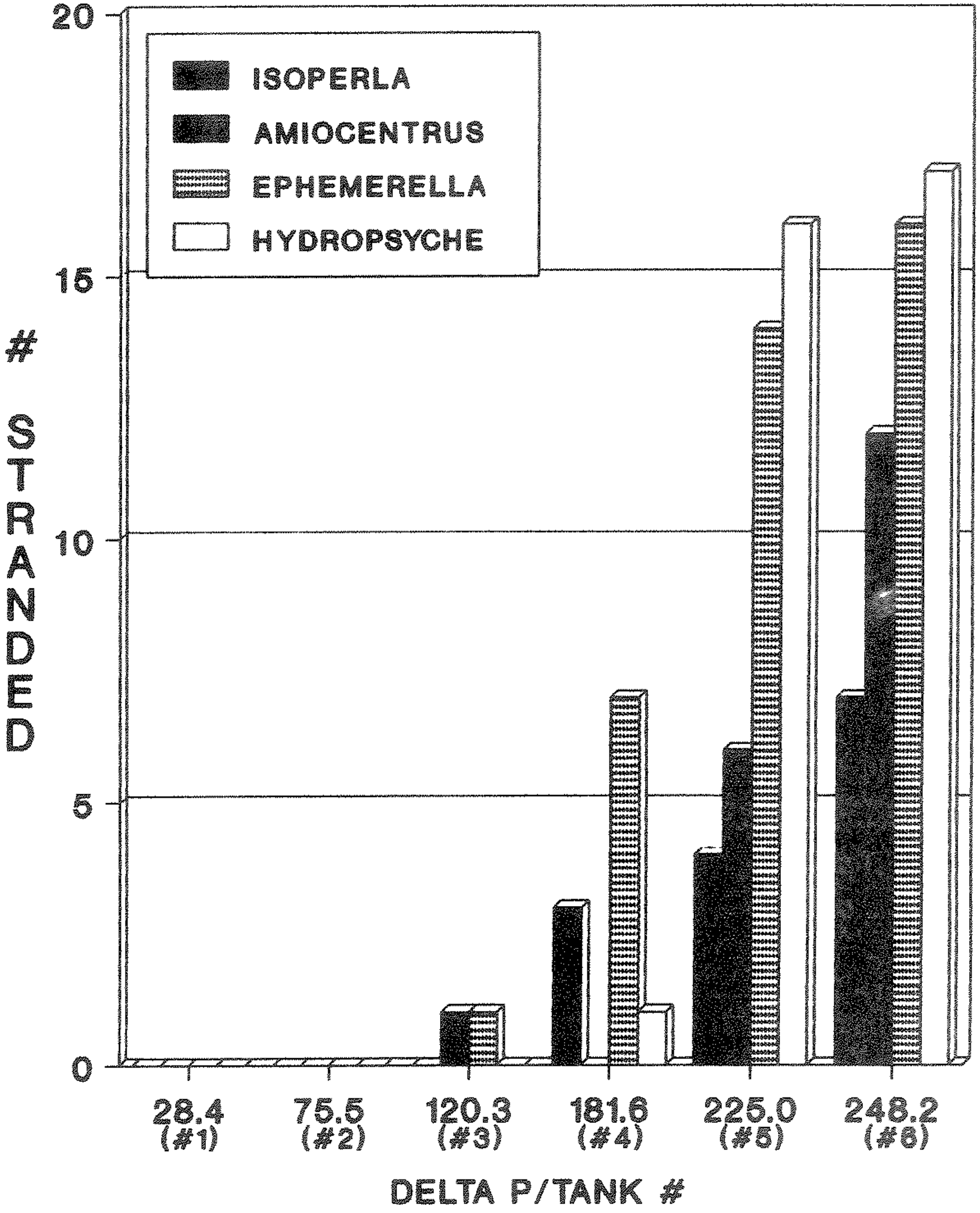

Figure 20. Susceptibility of four taxa to stranding at six delta P levels used in bioassay #1. N = 20.

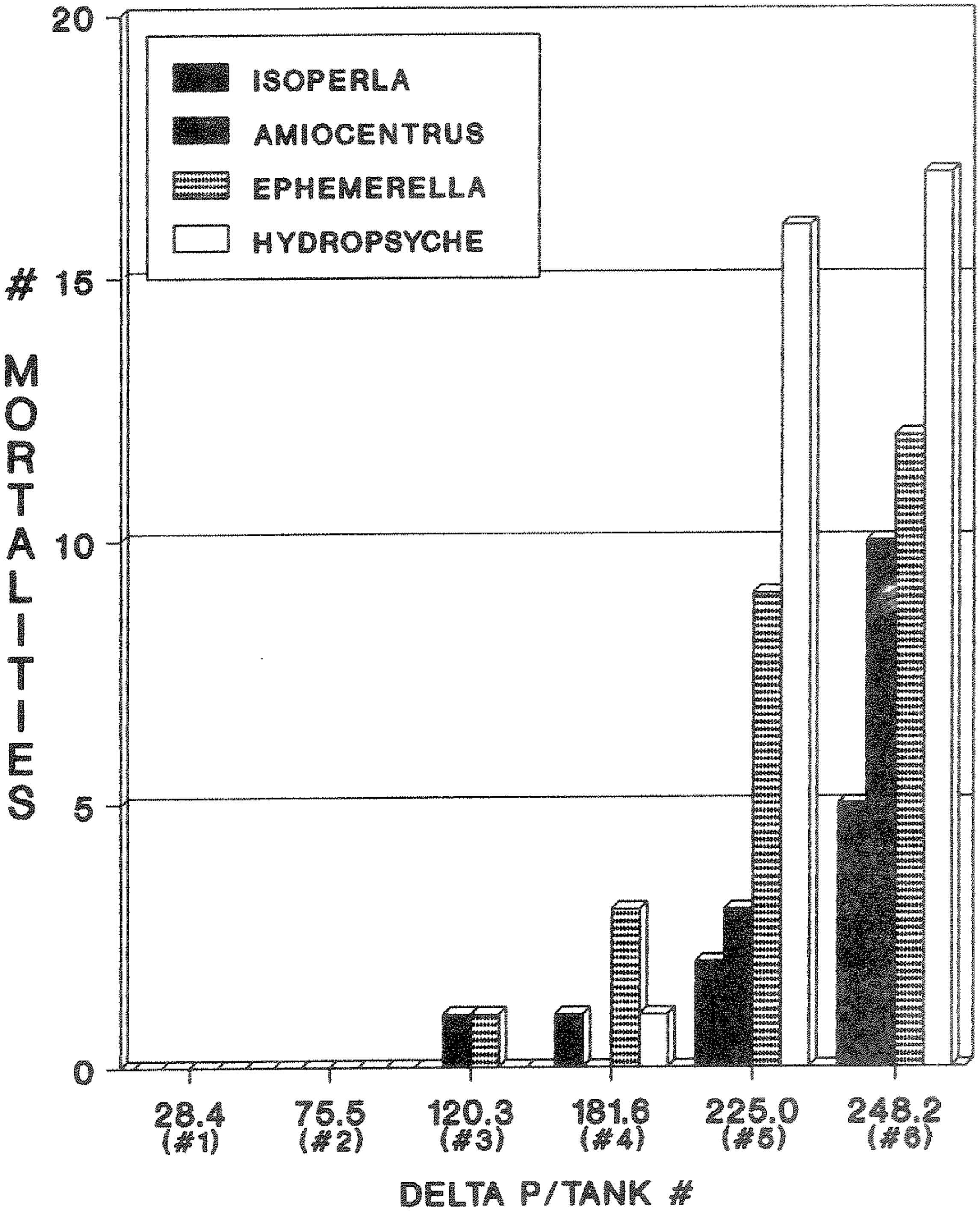

Figure 21. Gas related mortalities for four taxa tested at six delta P levels during bioassay #1. N = 20.

Amiocentrus aspilis. The case of *A. aspilis* inhibited detection of the onset of GBT and may have resulted in some GBT caused mortalities not being observed. When gas accumulation became severe, however, symptoms were readily visible (Appendix A).

Nineteen *A. aspilis* were stranded during the test; 18 were in tanks 5 and 6 (Figure 20). The single stranded larvae in tank 3 that subsequently died, suffered from external gas bubbles and not GBT. Death resulted from stranding above the water surface due to water level fluctuations. Total GBT related mortality was 13 for the test, with 3 (15%) and 10 (50%) occurring in tanks 5 and 6, respectively (Figure 21).

The threshold for GBT induced stranding and death appeared to be above a mean delta P of 181 mm Hg. Pupation decreased at higher gas saturation levels. Nine, 8, and 2 *Amiocentrus* larvae pupated in tanks 1, 2 and 5, respectively.

Ephemerella inermis. *E. inermis* nymphs were more susceptible to stranding during bioassay #1 than any other taxa. One (5%), 7 (35%), 14 (70%) and 16 (80%) nymphs became stranded in tanks 3, 4, 5, and 6, respectively (Figure 20). Total *E. inermis* mortality caused by GBT was 25 (Figure 21). Non-gas related mortality ranged from three (15%) in tanks 3 and 5 to four (20%) in tanks 1 and 2, and was not correlated with mean gas supersaturation levels.

The threshold for GBT-induced stranding appeared to be near a delta P of 120 mm Hg. Longer exposure at lower gas levels, however, could affect this species.

Extremely high delta P levels could also affect this species' ability to mature and/or emerge. Successful emergence was seen in tanks 1 and 2, while observed attempts to emerge from tanks 5 and 6 were unsuccessful. Also, two nymphs died while trying to molt in tank 6.

Hydropsyche _spp._ *Hydropsyche* was the taxon most susceptible to GBT at the highest gas saturation levels. Stranding occurred almost entirely in tanks 5 and 6 (delta P = 225 and 248.6) (Figure 20). By the end of the test, only five larvae remained in these two tanks. A single larvae developed GBT in tank 4, indicating that *Hydropsyche*'s threshold is probably around 181 mm Hg, and that it is less susceptible to GBT than *Ephemerella inermis* at lower delta P's.

Incidence of mortality (Figure 21) and of stranding (Figure 20) were identical. This occurred because larvae remained in their nets near the tank bottom until they became quite distended. By the time larvae were noted at the water surface, GBT had been severe for some time and they were near death. Other taxa became stranded when

GBT was less severe and remained on the water surface longer before death.

No larvae attempted to pupate during the test. Thus, potential gas supersaturation effects on pupation are unknown.

Bioassay #2

Physella spp., *Gammarus lacustris, Tricorythodes minutus*, and *Baetis tricaudatis* were tested during bioassay #2. All taxa had individuals that became permanently stranded during the test. *Physella* was least susceptible to GBT-induced stranding while *B. tricaudatis* was most susceptible.

Water chemistry was similar to that observed after bioassay #1 (Table 9). Total hardness was 23 mg/L less, while total alkalinity had increased from 178 mg/L to 216 mg/L $CaCO_3$.

Large variations in delta P (Table 11) occurred during bioassay #2. Temperature ranges were large, but mean temperatures were more similar between tanks than during bioassay #1. Tanks 1 through 5 averaged 11.2°C, while tank 6 averaged 11.3°C.

Physella sp.. *Physella* was the least affected of the organisms tested in bioassays #1 and #2. Four were stranded at the end of the test (Figure 22). These data,

Table 11. Means, standard deviations (S.D.), and ranges for Delta P (mm Hg), dissolved oxygen (mg/L), and temperature (°C) during bioassay #2, September 8 to 15, 1988.

Tank	Delta P			Dissolved oxygen			Temperature		
	mean	S.D.	range	mean	S.D.	range	mean	S.D.	range
1	-1.5	4.7	-8-12	7.2	0.24	6.7-7.4	11.2	0.4	10.6-11.7
2	71.3	9.9	59-88	8.6	0.12	8.5-8.7	11.2	0.4	10.6-11.7
3	113.1	9.2	94-135	9.4	0.15	9.2-9.6	11.2	0.4	10.6-11.7
4	164.7	12.7	141-189	10.6	0.25	10.2-10.9	11.2	0.5	10.0-11.7
5	209.6	9.0	199-222	11.7	0.32	11.1-12.1	11.2	0.5	10.0-12.2
6	242.6	7.3	222-254	13.9	0.24	13.6-14.2	11.3	0.5	10.0-12.2

however, are somewhat misleading. Although snails were commonly seen stranded during the test, none remained that way.

The randomness exhibited by the number of buoyant gastropods from observation to observation (Appendix B: Figure 38) suggested that *Physella* may have been able to alter its buoyancy. This in turn suggests that criteria used for determining gas supersaturation effects might have been unreliable for this taxon.

Physella commonly clung to the tank walls and were less frequently associated with the bottom than other taxa tested. Those in the upper one-half of the tanks were dislodged during each observation (4 h intervals) to

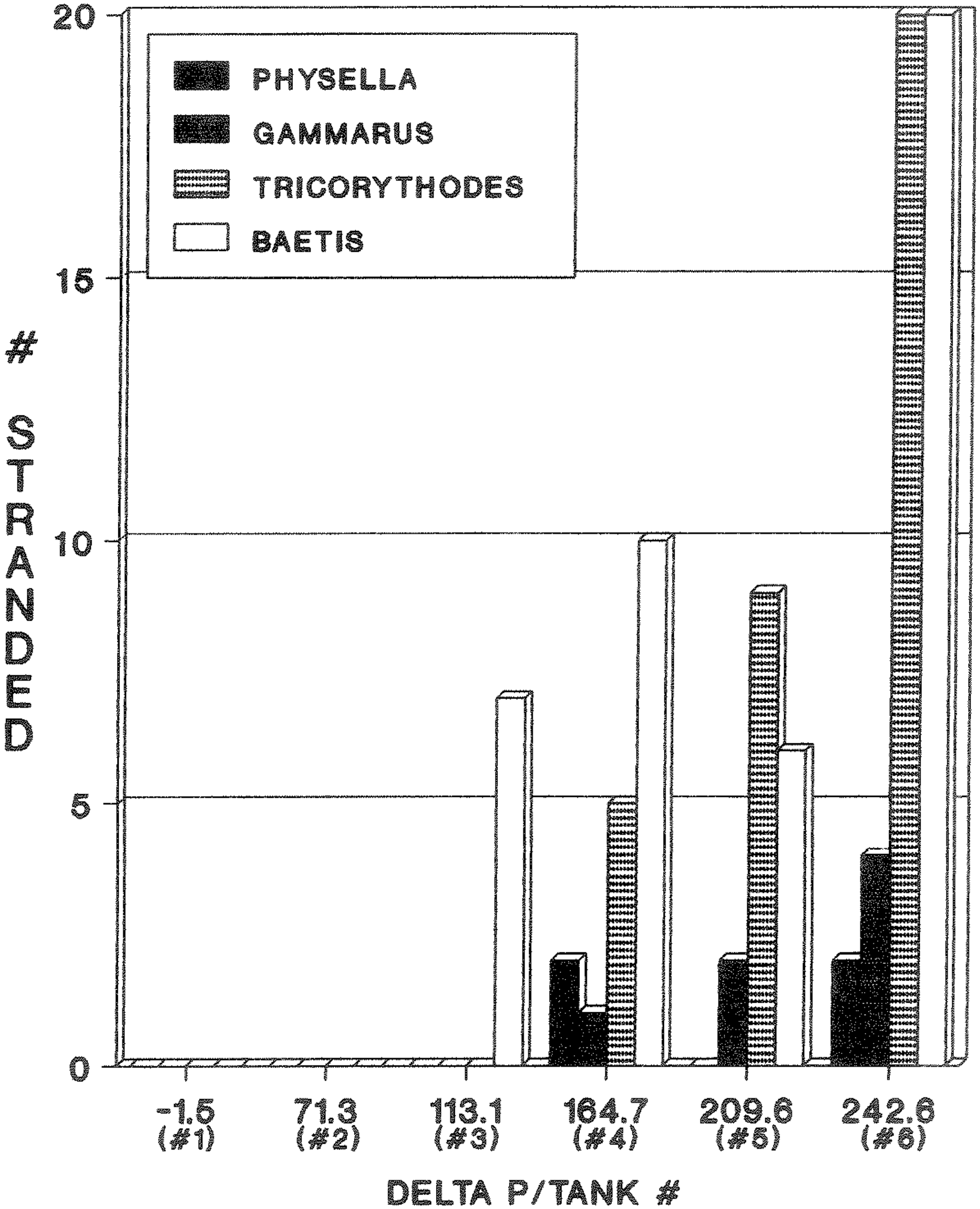

Figure 22. Susceptibility of four taxa to stranding at six delta P levels used in bioassay #2. N = 20.

determine if they would float or sink. Thus, the status of most gastropods was regularly checked. Although some individuals became stranded in tanks 1, 2, and 3, most stranding occurred in tanks 4, 5 and 6 (Figure 22). Thus, while stranding was temporary, incidence increased at delta P's of 164 mm Hg and greater.

One gas-caused mortality occurred in tank 6 (Figure 23). It appeared that gas accumulated between the shell and the mantle, forcing the organism out of the shell.

Gammarus *lacustris*. Relatively few *Gammarus* became permanently stranded during the test. Only one (5%), two (10%), and four (20%) of the amphipods were restricted to the water surface at delta P's of 164, 209, and 242 mm Hg, respectively (Figure 22). Internal bubbles were not observed in individuals exposed to gas saturation levels of 113 mm Hg or less.

Substantial buoyancy was necessary to strand *Gammarus* at the surface. Because of their strong swimming ability, some individuals with internal bubbles were able to return to the tank bottom. Thus, GBT was often severe before the organism became helpless, leaving little time between stranding and death. For this reason, GBT induced mortality (Figure 23) was identical to stranding results (Figure 22).

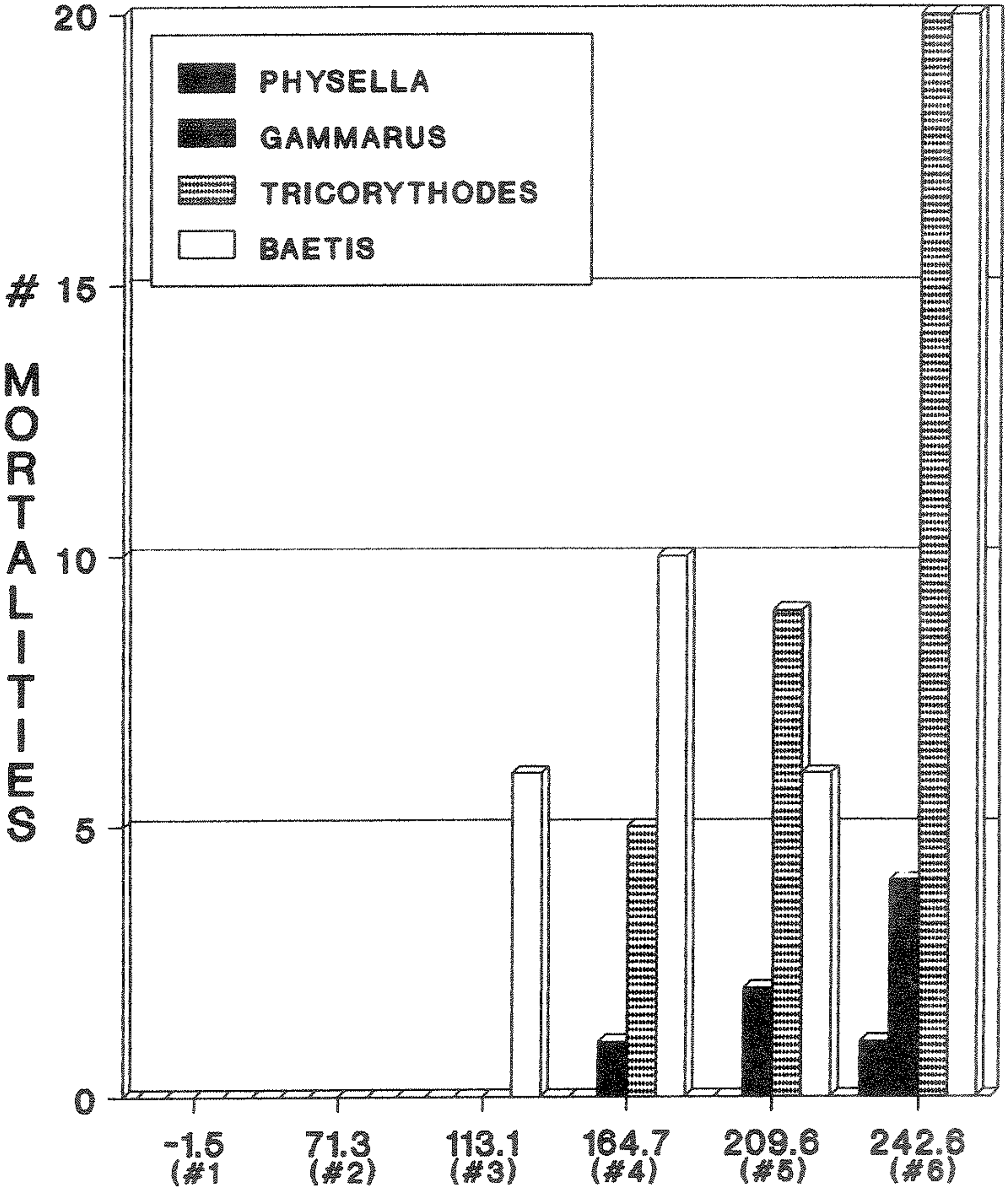

Figure 23. Gas related mortalities for four taxa tested at six delta P levels during bioassay #2. N = 20.

As with *Physella*, applicability of criteria used to determine gas supersaturation effects on *Gammarus* was also suspect. While stranding was not common, *Gammarus* appeared to more commonly occupy the upper portion of tanks 4, 5 and 6, compared to tanks with lower delta P's.

To determine if differences in their positions were related to increasing delta P's, the number of individuals in the upper one-fourth of each tank was noted in all observations after hour 84 (N = 20). The mean number (per observation) of amphipods occupying the top one-fourth of the tanks was 0.63, 0.21, 0.16, 2.95, 4.79, and 5.95 for tanks 1 through 6, respectively.

These results indicate that gas supersaturation levels in tank 4 affected *Gammarus* more than indicated by the numbers stranded (Figure 22). These data also suggest that the threshold for behavioral response of *Gammarus* to gas supersaturation levels occurs somewhere between a mean delta P of 113 and 164 mm Hg.

Tricorythodes _minutus_. Susceptibility of *Tricorythodes* to GBT was high at mean delta P levels of 164.7 mm Hg or greater. Stranded individuals comprised 20, 45, and 100% of all *T. minutus* in tanks 4, 5, and 6, respectively (Figure 22). Mortality and stranding results were identical (Figure 23). No stranding or mortality from GBT occurred at delta P levels of 113 mm Hg or less.

Thus, the threshold appears to lie between delta P's of 113 and 165. No nymphs attempted to emerge during the test.

T. minutus nymphs were much less mobile than *E. inermis*, *I. quinquepunctata* and *B. tricaudatis*. Thus, their ability to overcome initial buoyancy was inferior to other, more mobile, invertebrate taxa tested.

Gammarus was observed feeding on *T. minutus* nymphs once in tank 5, and three times in tank 6. Only one of the nymphs was alive at the time of observation; all nymphs being fed upon were buoyant. Most GBT afflicted *T. minutus* were removed and classified as mortalities when body movement and response to disturbance was nearly absent, but before death. Thus, predation rather than scavenging probably occurred. Predation likely occurred in other tanks as well. Only 15, 14, and 17 *Tricorythodes* were accounted for in tanks 1, 2, and 4, respectively.

Baetis _tricaudatis_. Of the taxa tested, *B. tricaudatis* were the most sensitive to gas supersaturation. One (5%), six (30%), seven (35%), and 14 (70%) of the individuals in tanks 3 through 6 were classified as "stranded" by hour 8 of the test (Appendix B: Figure 36). At the end of the test seven (35%), 10 (50%), 6 (30%), and 20 (100%) were stranded in these same tanks (Figure 22). Mortalities from gas supersaturation ranged from six in tank 3 to 20 in tank 6 (Figure 23).

Disappearance of nymphs from tanks 1 through 5 confounded the results. At the end of the test, the number of *Baetis* accounted for was 5, 8, 14, 12, 8, and 20, in tanks 1 through 6, respectively. Most of the depletion occurred early in the test. By hour 80 few *Baetis* nymphs remained in the tanks.

Emergence and predation were the probable cause of *B. tricaudatis*'s depletion. Eight nymphs are known to have successfully emerged; four from tank 2, and two from each of tanks 3 and 4. Since most nymphs were not late instar, predation appears more important than emergence in explaining disappearance from the tanks.

Baetis nymphs were strongly affected by GBT early in the bioassay, and exhibited little time between initial buoyancy and loss of mobility. Stranded individuals became easy prey for an agile predator. *Gammarus* were observed eating nymphs and were likely the main predator. Seven of the nine partially eaten nymphs recovered, were buoyant.

Early depletion in tank 5 appeared to cause the decrease in stranded nymphs from 35% at h 8 to 30% at h 168, the end of the test (Appendix B: Figure 36). All organisms in tank 6 were accounted for. This likely resulted from the short time required for stranding and death (Appendix B: Figures 36 and 37). Also, delta P's averaging 242 mm Hg may have decreased *Gammarus*'s predatory behavior.

The pattern of gas related mortality for *B. tricaudatis* (Appendix B: Figure 37) was similar to the number stranded, in that mortality in tank 4 surpassed that in tank 5 by h 32. This was probably due to predation. Total GBT associated mortality was 42 and would have probably been higher had depletion of *Baetis* not occurred.

Baetis's threshold for GBT induced stranding appears closer to a delta P of 71 than 113 mm Hg. One nymph in tank 2 was strongly buoyant at h 80 (Appendix B: Figure 36), but not in subsequent observations, possibly because it was preyed upon. The short time required for depletion of *Baetis* may have prevented other individuals from becoming stranded in tank 2.

DISCUSSION

Invertebrate Community Characteristics

Community Composition

Community composition in the Bighorn River below Yellowtail Afterbay Dam was typical of other tailwater communities. In a survey of regulated streams in six major drainage basins in the Rocky Mountains of Colorado, Zimmerman and Ward (1984) found that *Tricorythodes minutus, Baetis spp., Ephemerella infrequens* and chironomids were often present in high numbers below deep-release impoundments. *Isoperla quinquepunctata* was one of two plecopterans that were commonly found. Other common groups included amphipods, snails, oligochaetes, turbellarians, sphaeriid clams, and water mites.

The Bighorn River invertebrate community was dominated by five taxa: Chironomidae, *Baetis tricaudatis,* Tricladida, *Tricorythodes minutus, and Physella spp.*. Community dominance by few taxa has been documented below other deep release impoundments (Spence and Hynes 1971; Ward and Short 1978; Ward and Stanford 1979). Ward (1976) found that mayflies, *Baetis sp.* and *Ephemerella inermis,* along with dipterans, *Cardiocladius sp. and Simulium arcticum,* accounted for 87% of the organisms in a study section on the South Platte River below Cheesman Lake in Colorado. He suggested that diurnal and seasonal temperature constancy, summer cold and winter warm conditions, and delayed seasonal temperature maximums are primarily responsible for the limited success of some species and the elimination of others below deep release dams.

In addition to temperature patterns, the Bighorn River's abundant aquatic vegetation is important in determining invertebrate community composition and abundance. Total invertebrate densities increase (Hynes 1970) while species diversity tends to decrease (Yount 1956) with increased vegetative productivity.

The Bighorn River invertebrate fauna is comprised of taxa typically associated with aquatic vegetation. Most taxa are considered "collectors", which depend primarily on fine particle detritus for food. Abundant vegetation benefits members of this trophic group by filtering particulate organic matter from the water (Armitage 1984) and providing a source of recruitable food.

Other commonly occurring Bighorn River taxa have differing trophic habits, but still tend to rely on aquatic vegetation. *Physella spp., Hyalella azteca*, and *Gammarus lacustris*, are considered omnivores, but may depend heavily on detritus or live plants (Pennak 1978).

Hydroptila is considered a herbivore along with certain species of *Oecetis*, while *Isoperla quinquepunctata* and *Glossophonia* are predators and probably associate with vegetation indirectly through their prey.

Invertebrate Densities and Community Structure

Certain invertebrate community similarities between upstream (rkm 2.4) and downstream (rkm 14.5) sites in spring, were absent during late summer-early fall in the Bighorn River. Total benthic densities were not statistically different between sites during April 1987, but were significantly greater downstream during August 1986 and September 1987. Likewise, invertebrate community structure differed considerably between sites during late summer/fall sampling periods, but not in the spring (Figures 6, 7, and 8).

Principal components analysis showed that a major portion of the variation in individual taxa abundance resulted from samples being collected at different times and from different sites. Physical and chemical habitat factors control invertebrate distribution and abundance (Cummins 1975). Thus, the loss of invertebrate community similarities between sites in the fall undoubtedly resulted from habitat changes during summer. Attempts to link site differences (other than gas supersaturation) to changes in invertebrate communities, however, were not completely successful. Correlations between individual taxa and water depth, water velocity or vegetation abundance were invariably poor and inconsistent. Because a complex set of conditions influences invertebrate densities, it is understandable why individual habitat parameters inadequately explained all differences that occurred. In spite of this, habitat data provided some description of the physical variability between sites and, when related to the ecology of individual taxa, allowed some inference into potential effects on the invertebrate community.

Habitat Comparisons Between Sites

During each sampling period, at least one of the habitat parameters measured differed significantly between sites. In general, however, both sites were similar with regard to variability and range of conditions provided. Comparisons between sites in August 1986, indicated that only water depth was significantly different. Hynes (1970) indicates current speed, temperature, and substratum as the most important factors regulating distribution of aquatic invertebrates. Microhabitat distribution is primarily determined by food availability, nature of the sediments, and current velocity (Cummins 1975). It seems unlikely, then, that a slight difference in depths would cause substantial variation in invertebrate abundance. The influence of depth is probably more indirect than direct, first affecting aquatic vegetation through light availability, which in turn directly influences invertebrates. With the high water clarity of the Bighorn River, small changes in depth would have little influence on light availability.

Mean water velocity near the substrate (Table 6) was significantly different between sites in April 1987 (0.1 m/sec faster at rkm 14.5); at 0.6 depth it was significantly different between sites in September 1987 (0.3 m/sec faster at rkm 14.5). Changes in water velocity can alter invertebrate communities by influencing the occurrence and densities of species (Hynes 1970). Specific responses of invertebrate communities to differing water velocities at Bighorn River sampling sites, however, were difficult to determine. Correlations with densities of individual taxa were always poor, although they were commonly better with velocities at 0.6 depth than those near the substrate. Poor correlations suggest that factors other than water velocity were more important in determining distribution. Since water velocities approach zero near the substrate, velocities near mid-depth may better reflect velocities associated with food resources and oxygen.

In summary, it is unknown whether velocity differences present between sampling sites were sufficient to significantly alter the invertebrate communities. The data only indicate that differences existed, and thus the potential to influence the invertebrate community was present.

Greater abundance of downstream vegetation in September 1987, was likely responsible for some of the increase in invertebrate density. Increases in algae and macrophytes alter the character of the substrate, providing increased surface area for invertebrate colonization and retention of particulate organic materials, through the filtering action of the plants (Armitage 1984). The trophic habits of most taxa common to the Bighorn River indicate that increases in benthic vegetation are beneficial. Correlations between the taxa that increased at rkm 14.5 and dry weights of vegetation, however, were not strong, suggesting that specific types of vegetation may be more important than overall abundance.

In April 1987, total invertebrate abundance and invertebrate community structure were similar between sites. Vegetative standing crop was also comparable between sites. A variety of periphytic plants existed on sampling riffles, but different types were not quantified.

Analysis showed that chlorophyll a concentrations significantly differed within both sites, but not between them. Concentrations of chlorophyll a differ among species of periphytic plants (Wilkinson 1983). Thus, a patchy distribution of different algae and macrophytes might explain differences in chlorophyll a concentrations within riffles. Alternately, the lack of difference between sites could suggest a similarity in vegetative types and distributions at rkm 2.4 and rkm 14.5.

Temperature is often used by aquatic invertebrates as an environmental cue to synchronize life cycles. Thus, a single species occurring at sites having different temperature regimes can exhibit considerable life history differences (Merritt and Cummins 1984). Different aspects of aquatic invertebrate life histories that can be affected include the rhythm of life cycles, emergence times (Hynes 1970), and the length of the emergence period (Ide 1935).

Temperature differences between invertebrate sampling sites were largest from early spring through late fall, with temperatures increasing downstream. These differences were undoubtedly responsible for asynchronous life cycles exhibited by *Amiocentrus aspilis, Physella* spp., *Simulium* spp., and *Hydroptila sp.* during late summer and early fall sampling periods. This made interpretation of benthic data difficult. Abundance differences could exist between sites at any one time, while densities at similar stages of the life cycle could be similar. Had densities been compared at a similar time in the life cycles of these taxa, results may have differed. Other species likely had asynchronous life cycles that were not detected at the level of sampling in this study.

Temperature can also affect distribution of species (Merritt and Cummins 1984; Hynes 1970). Plecopterans are often negatively affected by altered temperature regimes downstream of deep release dams (Zimmerman and Ward 1984; Spence and Hynes, 1971). *Isoperla quinquepunctata* was the only stonefly that occurred commonly in Bighorn River benthic samples. Densities were low at both sites, but greater downstream. It seems likely that the more normal downstream diurnal and seasonal temperature patterns are favorable for this species.

Optioservus divergens was significantly more abundant at rkm 14.5 during all sampling periods. This species is a habitat generalist, becoming very abundant under the right conditions (Dan Gustafson personal communication). In Limekiln Creek, a tributary of Afterbay Reservoir, densities were considerably greater than those in the Bighorn River (Dan Gustafson personal communication). Limekiln Creek exhibits temperature patterns typical of prairie streams. From this, it seems likely that downstream temperatures are more suitable for this species than those near Afterbay Dam.

Potentially Affected Taxa

Assuming that invertebrates are most susceptible to gas bubble trauma (GBT) near Afterbay Dam where nitrogen saturation is highest, taxa that were more abundant downstream are candidates for being negatively impacted by gas supersaturation. Significantly greater densities at rkm 14.5 occurred with *Tricorythodes minutus, Hydroptila* spp., *Optioservus divergens, Hydropsyche* spp., *Isoperla quinquepunctata, Baetis tricaudatis,* and *Glossophonia complanata.*

Increased abundance of *O. divergens* and *I. quinquepunctata* at rkm 14.5 is likely related to temperature rather than gas supersaturation, for reasons discussed earlier. Multiple species of *Hydroptila* and *Hydropsyche* occur in the Bighorn River, confounding interpretation. *Hydroptila*, however, was one of only three taxa (along with *Tricorythodes minutus* and *Optioservus divergens*) that was significantly more abundant downstream during all sampling periods, indicating that all Bighorn River species within this genera could be affected by high gas saturation levels.

Ephemerella inermis, was only present in the April 1987 benthic samples, occurring in significantly greater densities at rkm 2.4. It had emerged prior to the late summer/early fall sampling periods, but was present in the river during the period of highest gas saturation levels. Because abundance differences caused by gas supersaturation would have been missed, this taxon also might be affected.

Invertebrate Bioassays

Eight potentially affected taxa were tested during bioassays. These included: *Amiocentrus aspilis*, *Ephemerella inermis*, *Physella*, *Gammarus lacustris*, *Isoperla quinquepunctata*, *Hydropsyche*, *Tricorythodes minutus*, and *Baetis tricaudatis*.

Externally Induced Buoyancy

Invertebrates tested varied in tolerance to elevated delta P's, but at some level tested, all experienced externally and/or internally induced buoyancy. Fickeisen and Montgomery (1975) found that supersaturated water from 107 to 123%, or water with a high concentration of entrained gas bubbles induced invertebrate buoyancy through bubbles adhering to the body surface. In my study externally induced buoyancy seemed to result from gas bubbles coming out of solution and forming on test organisms, rather than the adherence of entrained bubbles. If entrained gas bubbles cause invertebrates to become buoyant in the Bighorn River, those in the stilling basin and immediately downstream would be most affected. Gas in solution, however, potentially threatens invertebrates for miles downstream of Afterbay Dam. Setae and/or short spines common on many aquatic insects, as well as silt or organic matter adhering to the body, may provide nucleation sites for bubble formation directly on organisms.

All taxa tested experienced some buoyancy due to external gas bubbles, which generally occurred at delta P's ≥ 120 mm Hg. *A. aspilis, I. quinquepunctata*, and *B. tricaudatis* were most affected. Bubbles occurred on the terminal end of *A. aspilis's* case and often persisted until larvae floated to the surface since there was no way for the larvae to dislodge them. Usually stranding occurred only during the first 12 h of the bioassay (Appendix B: Figure 30), although bubbles were observed throughout the test. After a short time, most larvae had secured themselves to the tank walls or bottom.

Species behavior and location of bubble formation may determine the extent to which externally induced buoyancy influences invertebrates of the Bighorn River. Bubbles primarily occurred on the wings and abdomen of *Isoperla* and *Baetis* nymphs. After test organisms became familiar with their new surroundings, they became more stationary and the incidence of stranding decreased. It is possible that abdominal movements used for ventilation (i.e. gill beating in Ephemeroptera) or during swimming could encourage formation of external bubbles. *Ephemerella* was also affected by external buoyancy, but did not seem to be as susceptible as *Baetis*, even though it uses similar ventilation and swimming movements.

In contrast, mobility associated with an organism's strength as a swimmer may help moderate the incidence of permanent stranding. *Isoperla* was sometimes able to dislodge external bubbles while swimming. Fickeisen and Montgomery (1976) observed this in *Cleon*. *I. quinquepunctata* and *G. lacustris* were the strongest swimmers tested and were able to overcome slight positive buoyancy and swim to the tank bottom. Swimming strength alone could not compensate for extreme buoyancy caused by supersaturated water, but it may allow certain taxa to do better than others.

It is conceivable that buoyant organisms modify their behavior to reduce the chance of involuntary drift. If so, this is probably the most important factor in determining what effects externally induced buoyancy will have on invertebrates. In bioassays, all taxa seemed to better cope with externally induced buoyancy after 24 h, by becoming more stationary. Movements after this time did not appear sluggish and organisms were assumed to be healthy. *E. inermis* and *I. quinquepunctata* nymphs were also observed attempting to dislodge bubbles while clinging to the bottom of the tank. Under natural conditions, these types of behavior would minimize the occurrence of externally induced stranding, when gas saturation levels in the river exceed the thresholds required for bubble formation.

Tolerance to external bubbles likely depends on the location of bubble formation. Fickeisen and Montgomery (1975) report that bubbles were located around the mouth of *Cleon*, which may have inhibited their feeding. This would likely cause increased activity due to discomfort. None of the taxa that I tested, however, exhibited this symptom.

Isoperla, *Baetis* and probably other invertebrates could be affected by externally induced stranding in the Bighorn River. The potential for impacts are likely greater at the upstream site since delta P's exceeded 120 mm Hg more frequently than downstream (Appendix C: Tables 12 and 13). However, the most extreme gas supersaturation levels typically occurred at rkm 14.5 and the composition of gases differs there. It is difficult to know how these factors would affect the incidence of externally induced stranding downstream. Bioassay results indicated, however, that invertebrates minimize stranding by becoming more stationary. Thus, while some involuntary drift due to external buoyancy probably occurs, I do not believe this significantly reduces invertebrate densities in the Bighorn River.

Internally Induced Buoyancy

In bioassays, buoyancy of organisms without external gas bubbles resulted from gas accumulation in the body cavity. Gas bubble trauma was more permanent and seemed less tolerable than external buoyancy. Problems were first expressed by the organism becoming stranded on the water surface. Advanced GBT was evident by anatomical distensions and loss of abdominal mobility.

The mode in which internal gas accumulation occurs is not totally understood. Most hemi- and holometabolous immature aquatic insects have tracheal gills for oxygen uptake and a network of trachea for transporting gases to various parts of the body. In addition, respiration may occur through the body surface. Cuticles of immature aquatic insects are thinner than in most adults, and are underlain by a rich network of tracheae (Pennak 1978).

In terrestrial insects, tracheae originate at spiracles. Spiracle valves open and close the passageway for oxygen uptake. A pressure gradient, created when oxygen diffuses through tracheal walls and is taken up by cells, results in air uptake and expulsion of carbon dioxide during a series of valve openings and closures (Hainsworth 1981). In immature aquatic insects, reduced pressure in the tracheal system likely occurs as oxygen is utilized. No valve is present, however, to isolate the tracheal system from the aquatic environment. As partial pressures of gases in solution increase, pressures in the tracheal system must climb until equilibrium is reached. If pressures become great enough to cause gases to diffuse through tracheal walls faster than cells can utilize them, or the tracheal walls rupture, gas accumulation in the body cavity of the organism would result. Pressures in the body cavity then increase until equilibrium, or near equilibrium with the partial pressures of the gases in solution is achieved.

While the manner in which organisms are affected is different between external and internal gas bubbles, both result in buoyancy and potential downstream displacement. In April, *Baetis* drift rates were greater at rkm 14.5 in proportion to benthic densities than at rkm 2.4. Downstream delta P's ranged from 100 to 127 mm Hg during a 10 d period that included the days that drift was collected (Appendix D: Figure 39). These gas levels are in a range that may exceed *Baetis*'s GBT threshold (between 71 and 113 mm Hg), leaving gas supersaturation as a possible cause. Because of limited sampling, other potential causes for increased drift can not be addressed.

In September 1987, *Tricorythodes* and *Gammarus* exhibited disproportionately higher drift rates at rkm 2.4. Gas saturation levels ranged between a delta P of 79 and 90 mm Hg (Appendix D: Figure 40), well below the GBT thresholds determined for these two taxa during bioassays. Thus, other factors were likely responsible for their drift patterns.

Although drift sampling results did not conclusively show that drift rates increase because of high delta P's, combined results from bioassays, benthic sampling and river gas saturation data suggest that *B. tricaudatis*, *E. inermis* and *T. minutus* were the most likely affected. The GBT threshold for *Baetis*, determined from bioassay #2, was near a delta P of 71 mm Hg. From February through October 1987, gas saturation levels at rkm 2.4 always exceeded this level. This was also true at rkm 14.5 from April through July (Appendix C: Tables 14 and 15). *E. inermis*'s GBT threshold was near a delta P of 120 mm Hg (Figure 22). *T. minutus*'s GBT threshold was between 113 and 164 mm, however, the trend established by the numbers stranded indicated that it was probably closer to a delta P of 113 mm Hg (Figure 22). Gas saturation levels equal to or greater than this were also common at both sites in 1986 and 1987.

T. minutus was significantly more abundant at rkm 14.5 during all three sampling periods. Densities were 58% greater downstream in April 1987 (prior to extended periods of high gas saturation levels) increasing to 350% greater in September. Similarly, *Baetis* densities at rkm 14.5 were 21% greater than those upstream in April 1987, and 430% greater in September. It seems likely that a similar trend would have been documented with *E. inermis* if late summer sampling had occurred prior to their emergence.

Although observed thresholds were exceeded at both rkm 2.4 and 14.5, they were exceeded over longer periods of time at the upstream site. During bioassays some organisms became affected within 1 or 2 h. Others, however, did not become noticeably affected for days, especially at gas saturation levels near their thresholds. Thus, increased densities at the downstream site would be consistent with these observations.

Benthic abundance patterns in *Gammarus, Physella* and *Amiocentrus* suggested that the distributions of these taxa are little affected by high gas levels in the Bighorn River. Bioassay results supported this, as GBT thresholds for these taxa were rarely or never exceeded in the River (Appendix B: Tables 12, 13, 14, and 15). Also, *Isoperla*'s relatively low susceptibility to GBT, seems to support the assumption that temperature, rather than gas supersaturation is determining its distribution.

Delta P fluctuation patterns and the partial pressures of individual supersaturated gases must be considered when assessing "in stream" impacts on the invertebrate community. I attempted to maintain constant delta P levels during bioassays. Gas saturation levels in the Bighorn River, however, exhibited large fluctuations over relatively short periods (Figures 8, 9, 11, and 12). Nebeker et al. (1976) noted that stoneflies exposed to gas supersaturation levels of 135% for 7 d, acquired internal gas bubbles. After 4 h at a lower gas level, however, symptoms disappeared. Thus, invertebrates in the river might tolerate exposure to delta P's above their susceptibility threshold when periods of lower delta P's commonly occur.

The composition of supersaturated gases in water may also determine the extent of influence on aquatic invertebrates. During bioassays, the ratio of dissolved oxygen to nitrogen was similar to that in supersaturated water immediately below Afterbay Dam. Downstream, however, the oxygen component of total dissolved gases increased. In fish, nitrogen is more important in causing GBT than oxygen (Marsh and Gorham 1905). Thus, while total gas saturation levels were sometimes higher downstream, the incidence of GBT in trout was lower (White et al. 1987). This may or may not be the case with invertebrates. How supersaturated levels of oxygen and nitrogen separately affect aquatic invertebrates must be answered before specific "in river" effects can be understood.

Conclusion

Total invertebrate densities and invertebrate community structure were similar between sites in the spring but were quite different by the end of summer. Temperature and certain physical habitat differences were responsible for the density patterns of most taxa. However, gas saturation levels in the Bighorn River frequently exceed thresholds for formation of external gas bubbles and for acquiring gas bubble trauma in *Baetis tricaudatis, Ephemerella inermis,* and *Tricorythodes minutus*. Significantly greater downstream abundances of *Baetis* and *Tricorythodes* suggest that some displacement from upstream is occurring and it is likely due to high gas saturation levels. Late summer distribution patterns in *Ephemerella* could not be determined because nymphs had emerged prior to sampling, but they were likely similar to *Baetis* and *Tricorythodes*.

After being exposed to gas supersaturated water for less than 1 d, most test organisms exhibited behavior that minimized the occurrence of involuntary drift (stranding in the bioassay tanks) due to external gas bubbles. Invertebrates in the river might also adjust their behavior when afflicted with externally induced buoyancy, thus limiting displacement.

Most taxa tested did not acquire gas bubble trauma until gas saturation levels became extremely high. Once afflicted, however, internally induced buoyancy was physically more harmful to test organisms than external gas bubbles, causing death in some.

From a management perspective the primary concern is the well being of the trout populations. Benthic invertebrate densities were very high at both sampling sites and invertebrate community composition was comparable to other rivers downstream of deep release impoundments. If downstream displacement occurs as a result of high gas saturation levels, it appears that upstream migration by adults before ovipositing compensates for losses that occur. Increased invertebrate drift caused by gas supersaturation might be beneficial in terms of food availability for trout so long as invertebrate populations are not depleted.

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APPENDICES

APPENDIX A

Invertebrate gas bubble trauma symptoms.

Bioassay #1

Ephemerella inermis

E. inermis afflicted with GBT usually floated on the water surface, ventral side up, suggesting initial gas accumulation occurred ventrally within the nymph. Under magnification, bubbles could occasionally be seen in the ventral thorax, abdomen and appendages. During early stages of GBT, organisms readily responded to disturbance. Gills appeared to function properly and nymphs swam, albeit upside down, with the undulatory abdominal movement common to swimming mayflies. As GBT became more severe, response to disturbance was greatly reduced, gills were flared, the head protruded from the thorax, and the abdomen often became distended (segments separated making the membranes joining them clearly visible). In the latter stages, wing pads filled with air and some gills ceased to move. Movement at this stage was sluggish, principally restricted to the appendages, and the organism was near death.

Decreases in the number of individuals classified as stranded (i.e. in tank 5 at h 60, Figure 24) does not suggest that buoyant organisms recovered. Once on the surface, *Ephemerella* were carried to the standpipe by current. Most remained, but some crawled down the standpipe to the floor of the tank. These individuals were indistinguishable from those unaffected. At the conclusion of the test, all nymphs remaining on tank floors were examined. Two were buoyant in tank 6 and one in tank 4.

Six partially eaten nymphs (three buoyant) were recovered during the test: one in each of tanks 1, 4, 5 and 6, and two in tank 3. Four of these were tied to *Amiocentrus* cases, but it is unknown whether they were preyed upon or scavenged.

Isoperla quinquepunctata

Gas bubble trauma symptoms exhibited by *I. quinquepunctata* were similar to those of *E. inermis*. Stranded nymphs floated ventral side up and response to disturbance declined as symptoms became more acute. As GBT progressed, movement of the abdomen was restricted, limiting nymphs' ability to swim. Wing pad inflation, however, did not occur in this species.

I. quinquepunctata's posture also changed with increasing severity of GBT. Unaffected stonefies maintained a low vertical profile, with their entire thorax and abdomen resting against the tank bottom. The profile of nymphs with advanced GBT varied from the abdomen being arched away from the attachment surface to having the entire thorax and abdomen lifted off the surface. This could be detrimental in running water by subjecting organisms to greater water velocities.

During the first 24 h of the test, some *I. quinquepunctata* nymphs at the three highest gas levels (delta P > 181.6 mm Hg) became temporarily stranded at the water surface, due to external bubble formation. External bubbles were observed on nymphs later in the tests, but fewer individuals became stranded. *I. quinquepunctata*, as well as individuals of other taxa tested, were most active early in the test, likely due to unfamiliarity with their surroundings. Externally induced buoyancy may not have forced these stonefly nymphs to become detached from the tank bottom or sides, but likely became a factor when they released their foothold to move.

Reductions in the number of stranded *Isoperla* nymphs occurred several times during the test. This was the most mobile taxa tested and some individuals suffering from Gas bubble trauma apparently crawled or swam to the tank bottom. At the conclusion of the test, six stonefly nymphs on the bottom of tank 5 were strongly buoyant. Thus, stoneflies classified as stranded during the bioassay likely remained buoyant throughout the test. When buoyant, *Isoperla*'s agility may be an advantage compared to less mobile taxa.

Hydropsyche spp.

Hydropsyche with GBT experienced gas accumulation severe enough to cause distension; sometimes to the extent that the abdomen and thorax became translucent. A hole was noted behind the head of one dead larvae. It is unknown if this resulted from the organism bursting or if it had been preyed upon. Gas bubbles were also noted in the anal claws, meso- and metathoracic legs, and the metathorax, of various individuals.

Predation on *Hydropsyche* was not documented, however one *Amiocentrus* and one *Isoperla* were seen eating dead larvae. Because larvae often remained inside their nets until they were near death, it seems more likely that they were scavenged.

Amiocentrus aspilis

Amiocentrus was quite susceptible to buoyancy from external gas bubbles, particularly during the first day of the bioassay. Seventeen larvae with numerous external gas bubbles were stranded in tank 6 during the first 8 h of the test. Their buoyancy differed from that of other organisms tested in that gas bubbles were attached to the caddis case and frequently became lodged in the posterior opening of the case. The case surface likely provided numerous nucleation sites for gas bubble formation and growth. Once present, there was little the larvae could do to dislodge them. By h 20, all larvae had attached themselves to the tank, preventing surface stranding.

External gas bubbles primarily occurred when the average delta P was 120.3 mm Hg and greater (tanks 3 - 6). Larvae began to develop GBT sometime prior to h 104. When GBT became severe, bloating prevented them from retreating into their cases when disturbed. Like in *Hydropsyche*, inflation became severe enough to make their thorax nearly transparent.

Bioassay #2

Gammarus lacustrus

Gas bubble trauma in *Gammarus* was first observed in tank 4 at h 52; a bubble had formed in the marsupium of a female. As the test progressed, many amphipods occupying positions in the upper portion of tanks 4, 5 and 6 suffered this affliction. Distally hooked stiff hairs border amphipod oostegites, helping to retain eggs in the marsupium (Pennak 1978). These hairs may act as nucleation sites for bubble formation and likely inhibit *Gammarus*'s ability to dislodge the bubble once present.

Gas bubbles in the marsupium affected buoyancy, but did not appear to affect survival. More severe GBT was indicated by gas accumulation in the gut. All *Gammarus* that died had a bubble or bubbles running the length of the gut. In one individual, bubbles were also present in the head. By the end of the test, most *Gammarus* in tanks 5 and 6 had bubbles in the marsupium or gut.

Short term stranding due to external bubbles occurred in several individuals during the test. When bubbles broke or were dislodged, they returned to the tank bottom. Externally induced buoyancy occurred with increasing frequency in tanks 4, 5, and 6, but *Gammarus* exposed to lower gas levels (≤ 113.1 mm Hg) were unaffected.

Tricorythodes minutus

Tricorythodes, like *Ephemerella* and *Isoperla*, floated ventral side up. Response to disturbance was good initially, but degraded as GBT symptoms became more severe. Gas accumulation in the body cavity was the primary avenue of GBT affliction. Separation of abdominal segments and protraction of the head away from the body were observed in affected nymphs. In addition, later stages of GBT were characterized by inflation of the wing pads, and by flared gills. Gas bubbles were seen dorsally in the thorax, in the wings, and posteriorly in the abdomen. In one individual, a bubble ran the entire length of the body cavity.

A decreasing trend in the number of stranded *Tricorythodes* occurred in tank 5 during the fifth day of the bioassay. This was probably due to predation and to accidental transfer of nymphs. At the conclusion of the test only 14 of 20 nymphs in tank 5 were accounted for, however, three extra nymphs were found in tank 6. Gas supersaturation levels were measured in sequence from tank 1 through tank 6. Although the Bouck gasometer was examined after each measurement, it appears that some organisms were not detected and some transfer occurred.

Baetis tricaudatis

Gas bubble trauma symptoms in *Baetis* were the same as those exhibited by *Ephemerella* and *Tricorythodes*. Mortality, unrelated to GBT, was high. Nineteen non-gas mortalities occurred during the test. Seven, five, and four of these occurred in tanks 3, 1, and 2, respectively. Seven mortalities occurred in the first 8 h and may have been due to handling. *Baetis*'s size and mobility made handling more difficult than other taxa.

Physella sp.

Single non-gas mortalities occurred in tanks 1, 2, 3, 4, and 6. All organisms except the one in tank 3 had crushed shells when they were discovered. Injuries probably occurred when the Bouck gasometer was placed in the tanks to measure gas saturation levels.

APPENDIX B

Figures related to bioassay results

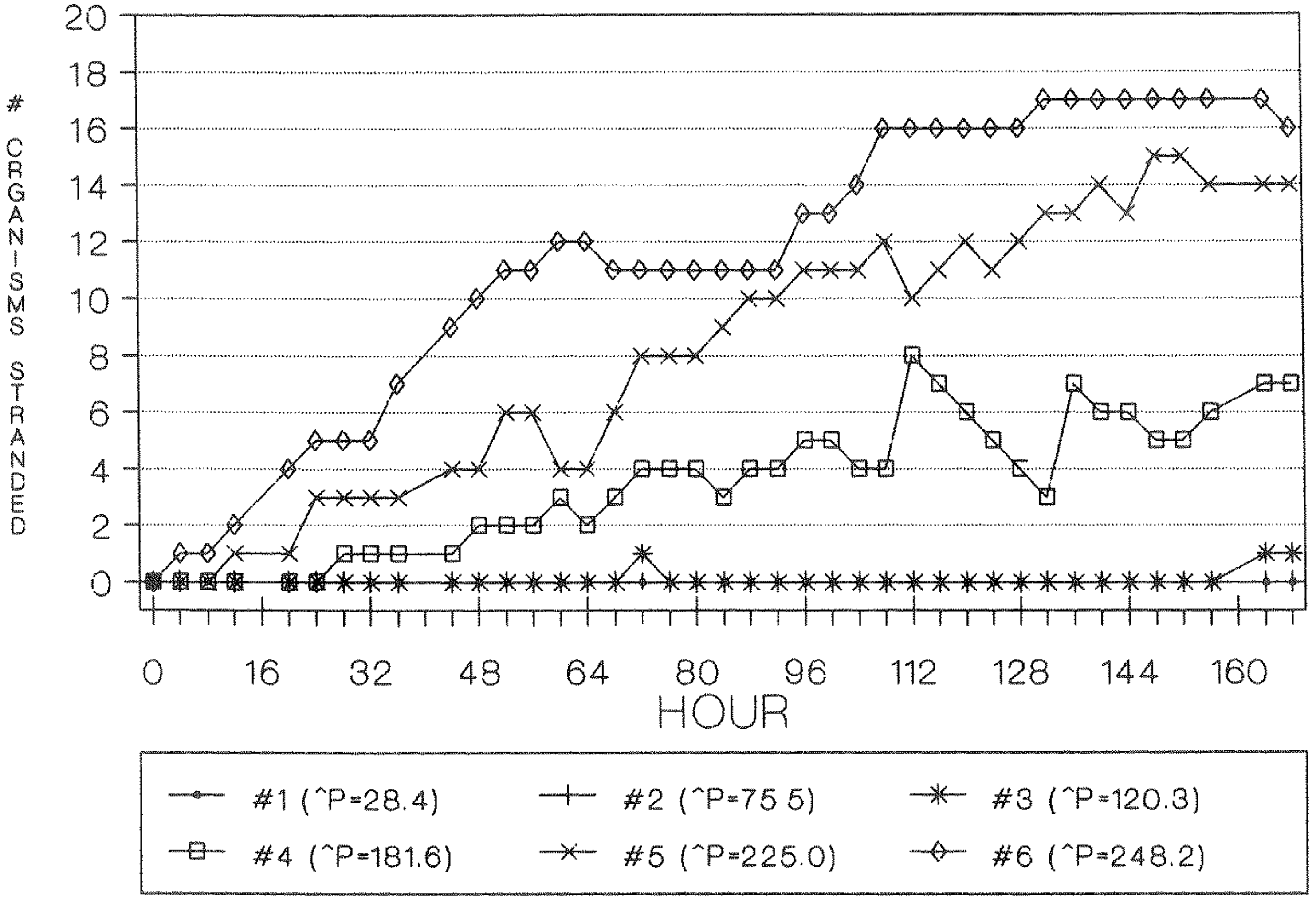

Figure 24. The number of stranded <u>Ephemerella inermis</u>, by hour, in test tanks 1-6, where mean delta P's ranged from 28.4 to 248.2 mm Hg. Individuals that died while stranded, continued to be counted as stranded for the remainder of the test.

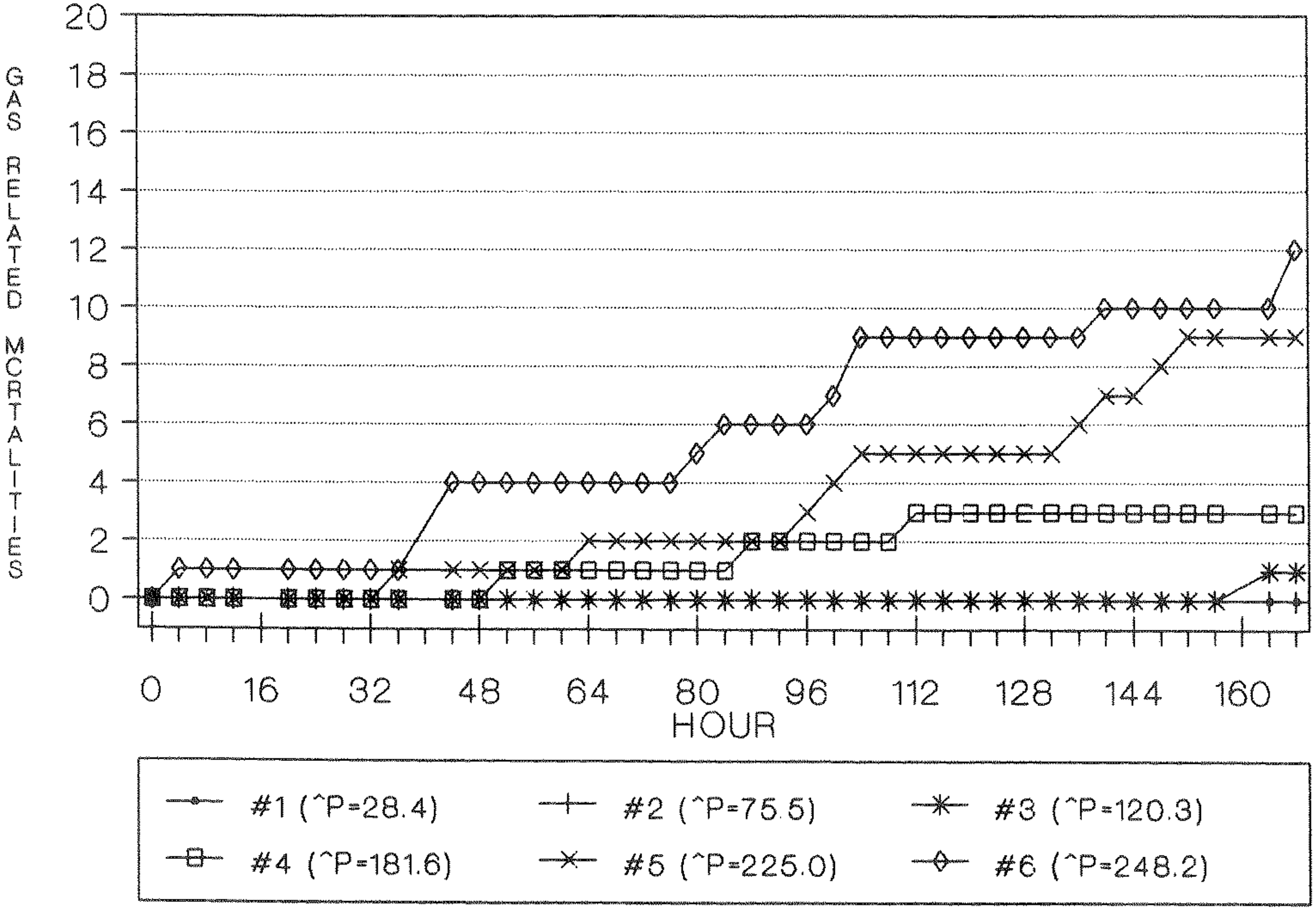

Figure 25. Cumulative mortality of <u>Ephemerella inermis</u> exposed to six delta P levels ranging from 28.4 to 248.2 mm Hg for 168 hours. Non-gas related mortalities are not included.

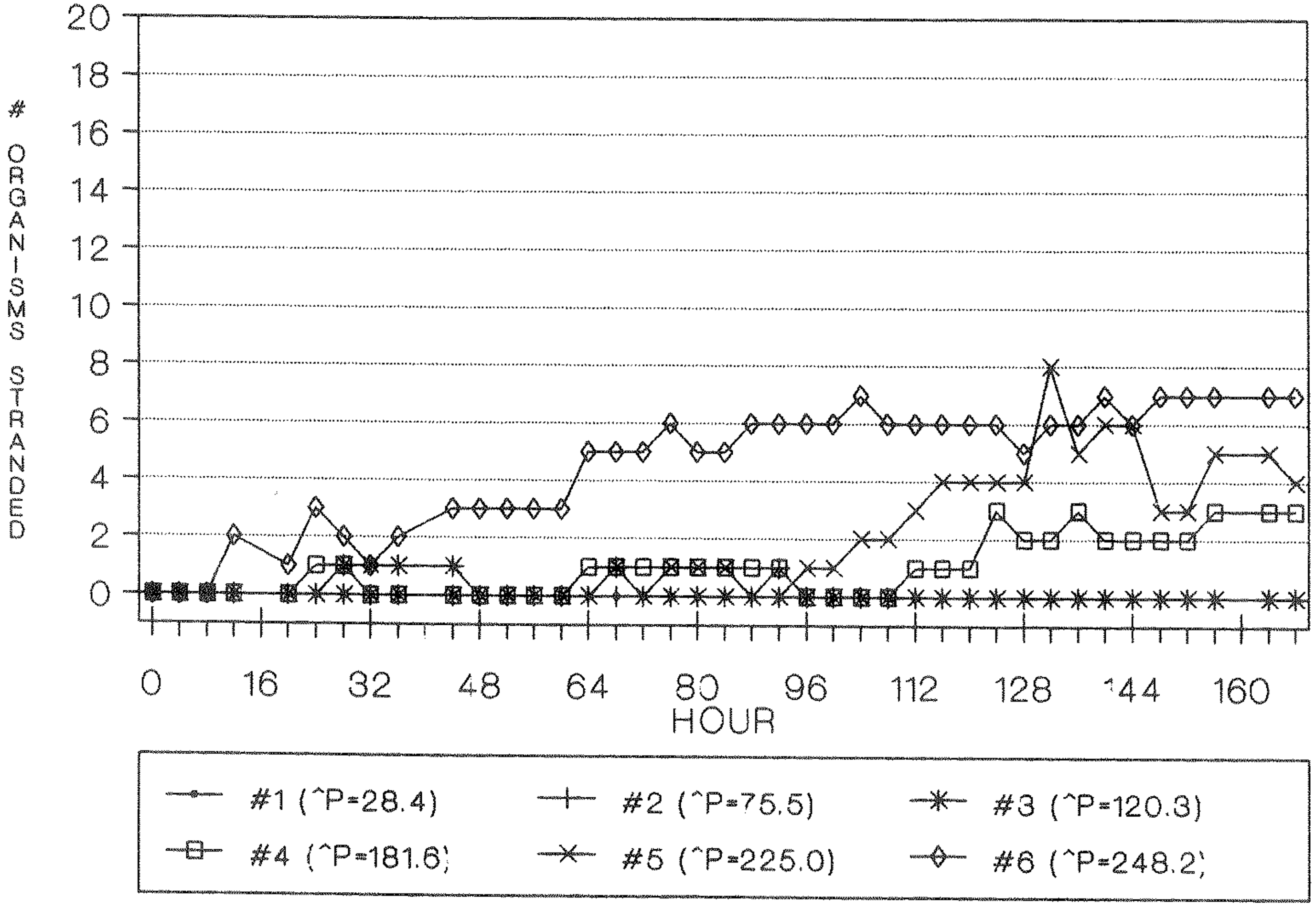

Figure 26. The number of stranded Isoperla quinquepunctata, by hour, in test tanks 1-6, where mean delta P's ranged from 28.4 to 248.2 mm Hg. Individuals that died while stranded, continued to be counted as stranded for the remainder of the test.

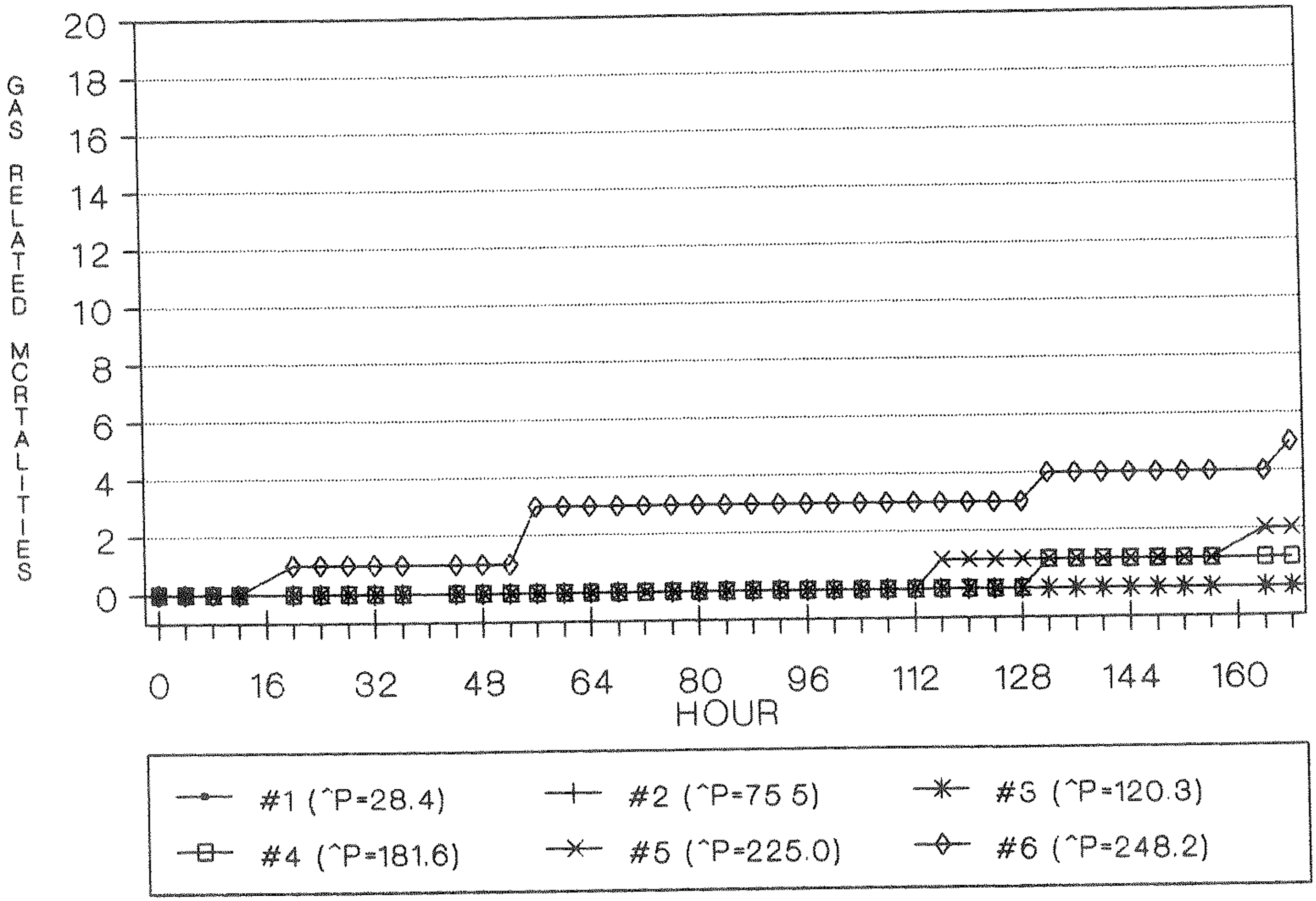

Figure 27. Cumulative mortality of <u>Isoperla quinquepunctata</u> exposed to six delta P levels ranging from 28.4 to 248.2 mm Hg for 168 hours. Non-gas related mortalities are not included.

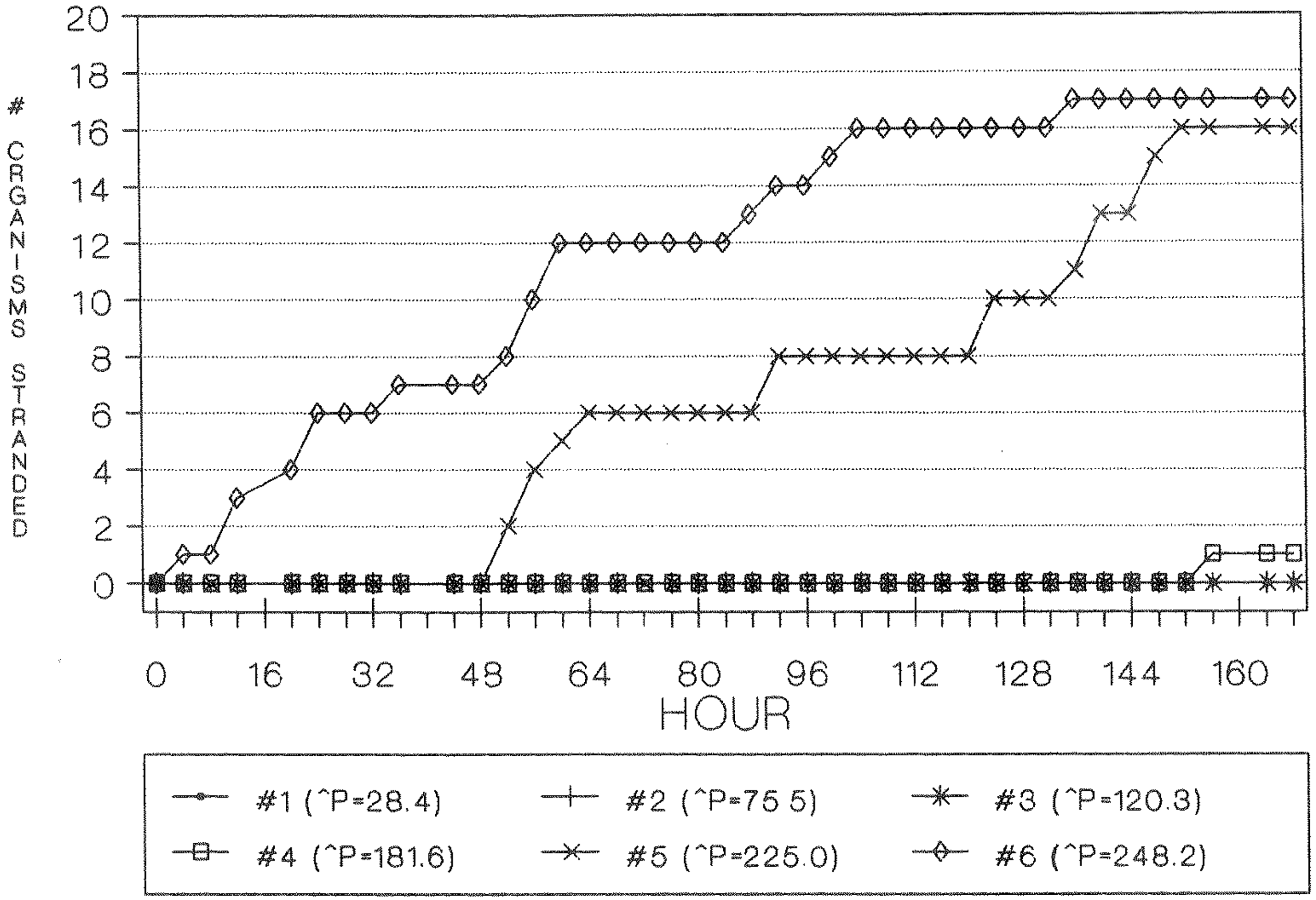

Figure 28. The number of stranded Hydropsyche by hour, in test tanks 1-6, where mean delta P's ranged from 28.4 to 248.2 mm Hg. Individuals that died while stranded, continued to be counted as stranded for the remainder of the test.

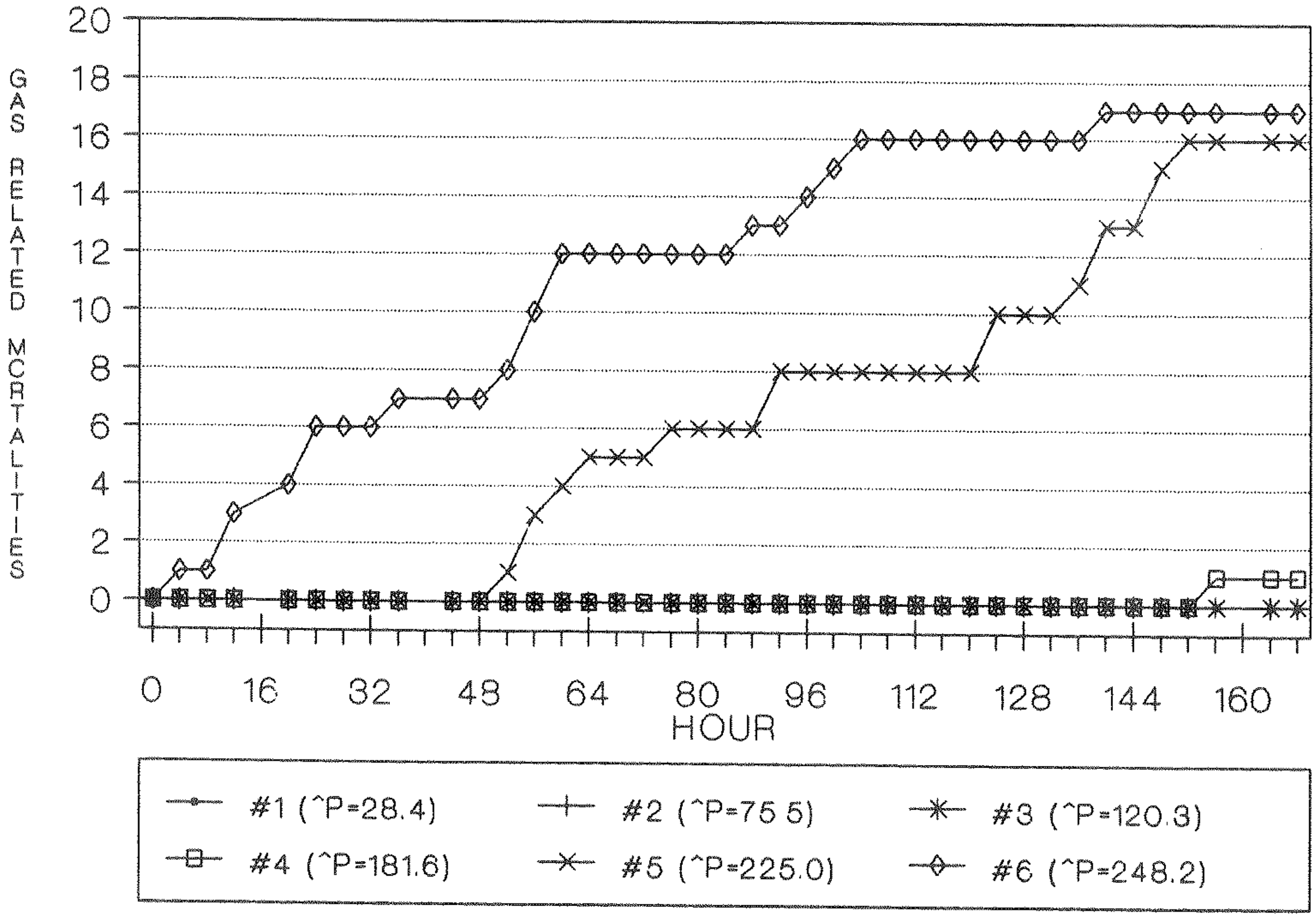

Figure 29. Cumulative mortality of <u>Hydropsyche</u> exposed to six delta P levels ranging from 28.4 to 248.2 mm Hg for 168 hours. Non-gas related mortalities are not included.

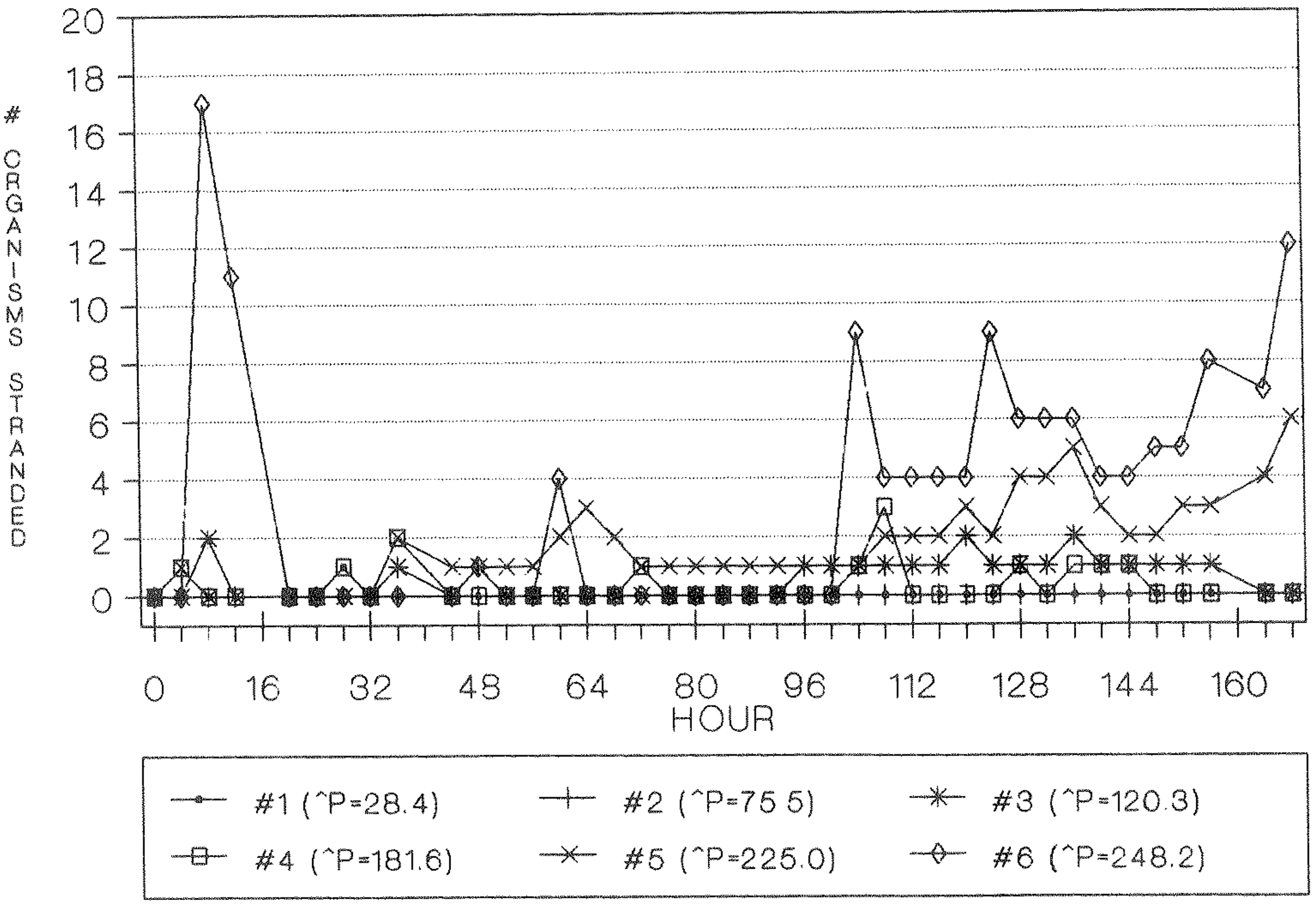

Figure 30. The number of stranded Amiocentrus aspilis by hour, in test tanks 1-6, where mean delta P's ranged from 28.4 to 248.6 mm Hg. Individuals that died while stranded, continued to be counted as stranded for the remainder of the test.

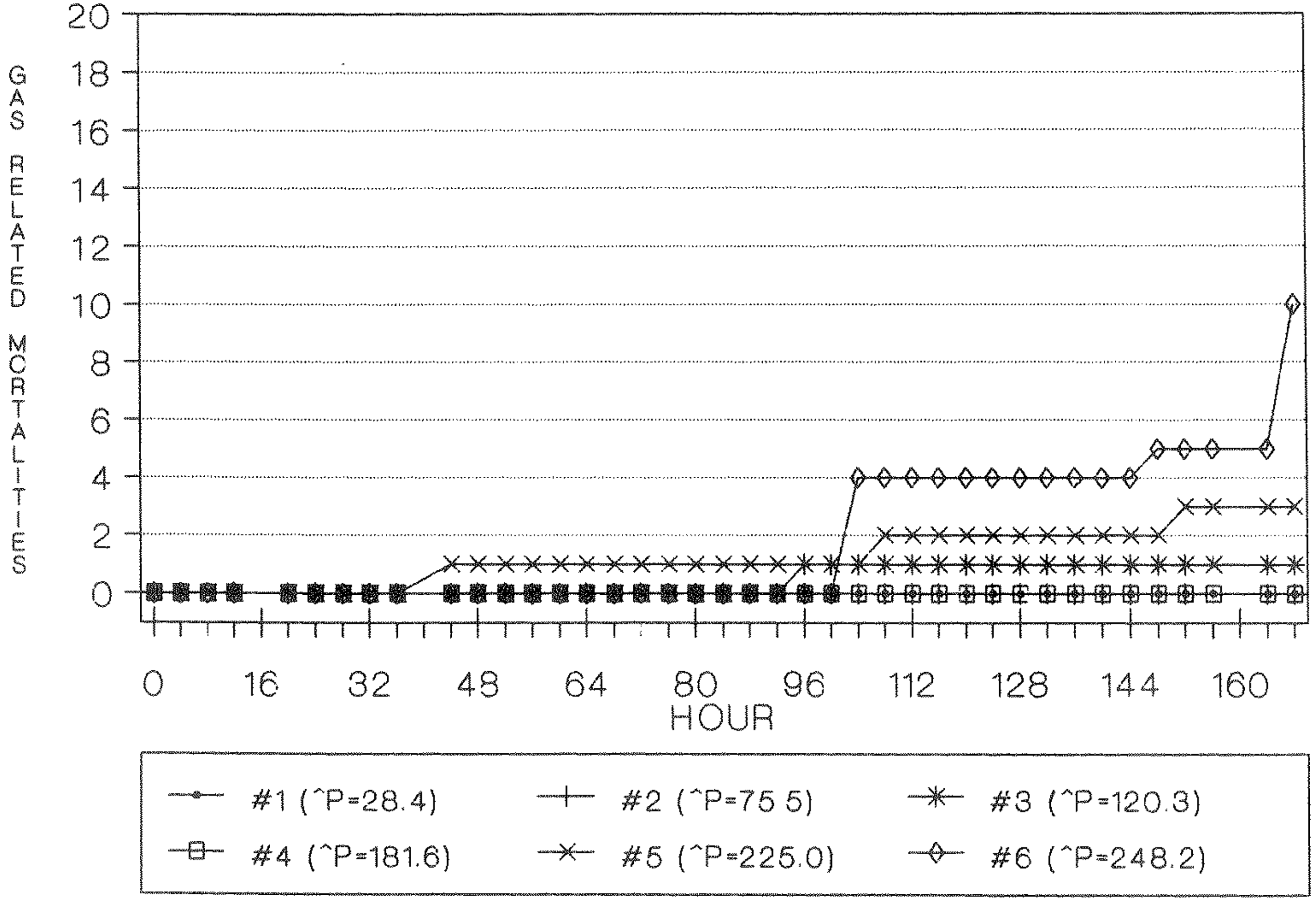

Figure 31. Cumulative mortality of <u>Amiocentrus aspilis</u> exposed to six delta P levels ranging from 28.4 to 248.2 mm Hg for 168 hours. Non-gas related mortalities are not included.

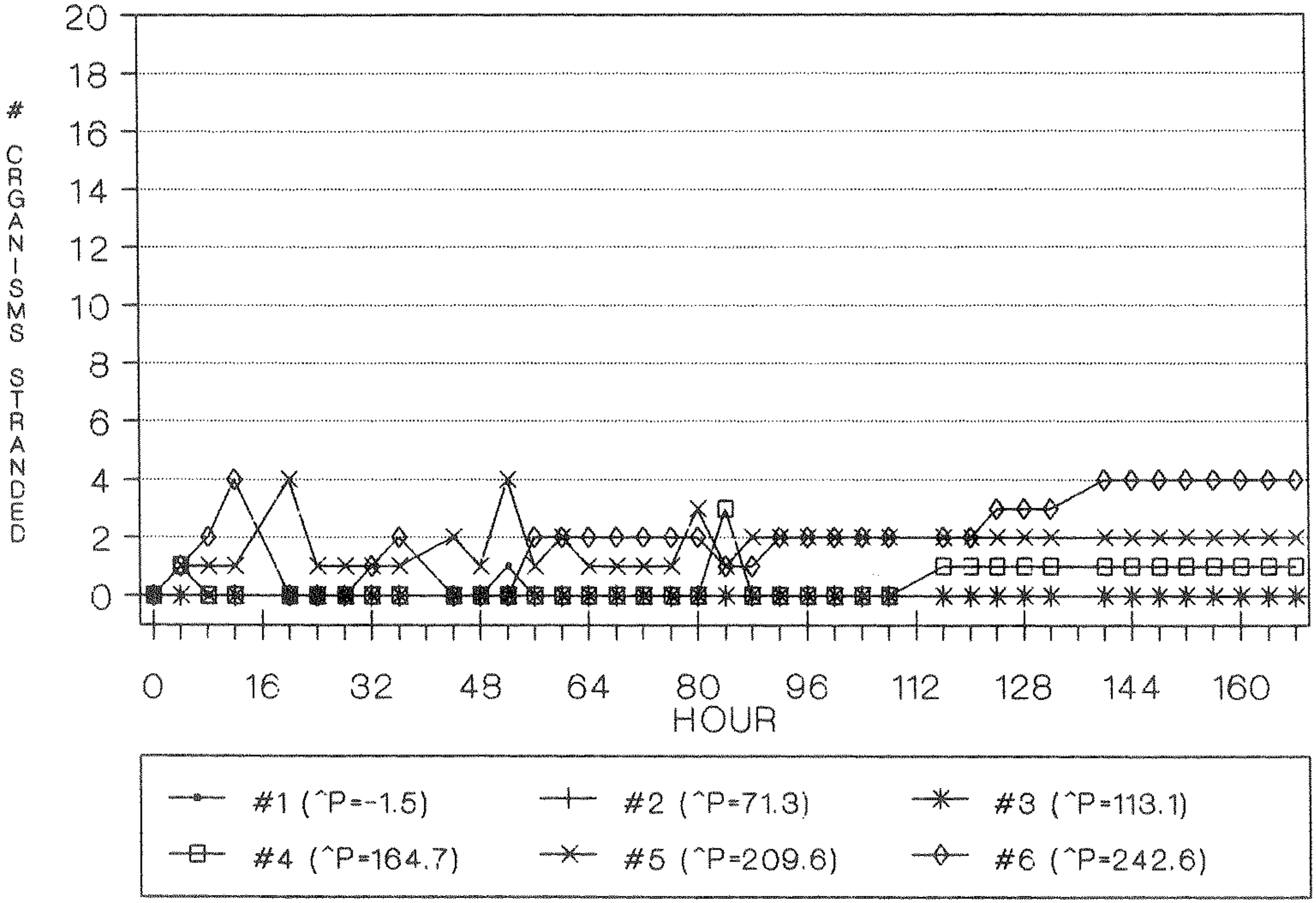

Figure 32. The number of stranded <u>Gammarus lacustrus</u> by hour, in test tanks 1-6, where mean delta P levels ranged from 1.5 to 242.6 mm Hg. Individuals that died while stranded, continued to be counted as stranded for the remainder of the test.

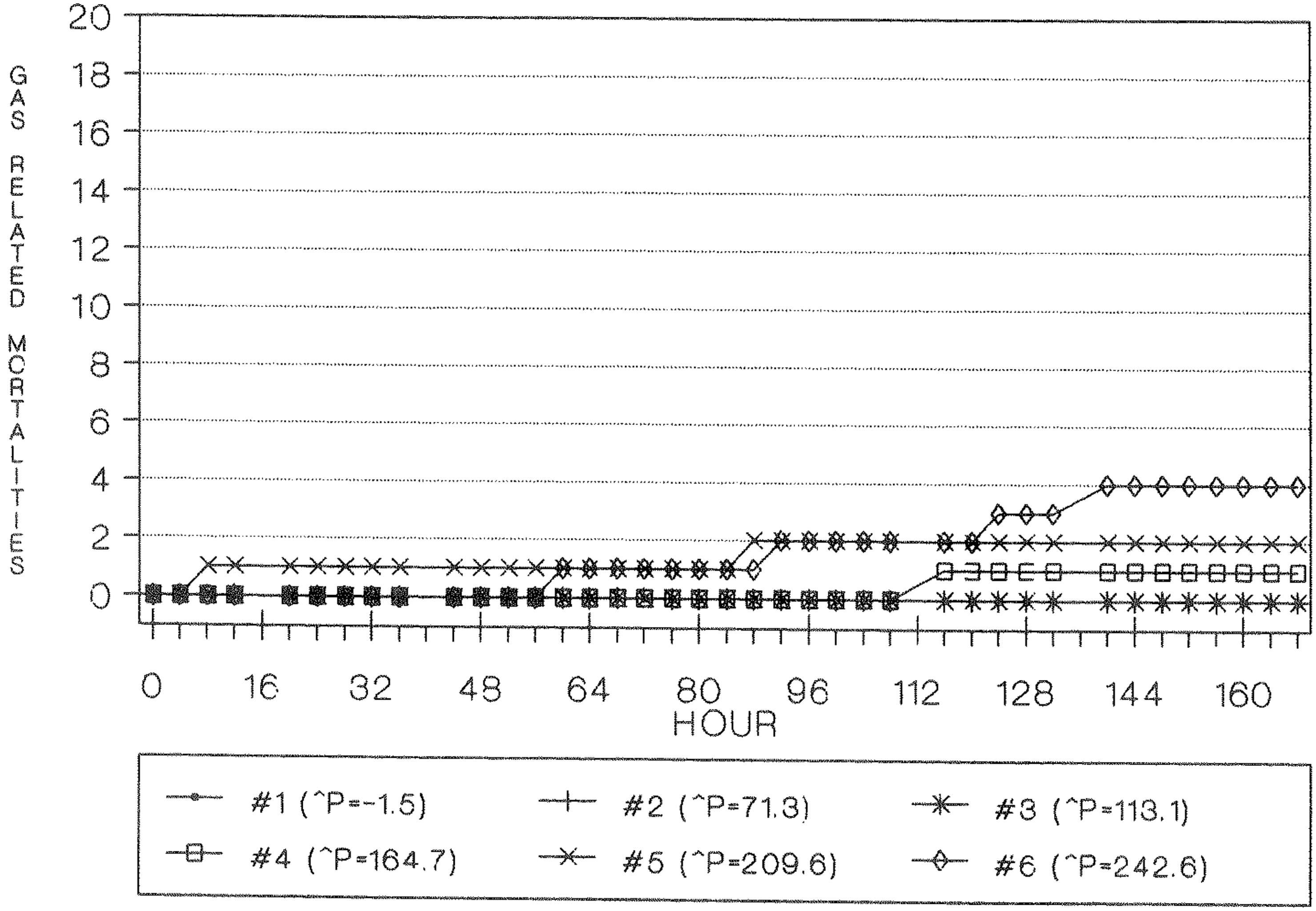

Figure 33. Cumulative mortality of <u>Gammarus lacustrus</u> exposed to six delta P levels ranging from 1.5 to 242.6 mm Hg for 168 hours. Non-gas related mortalities are not included.

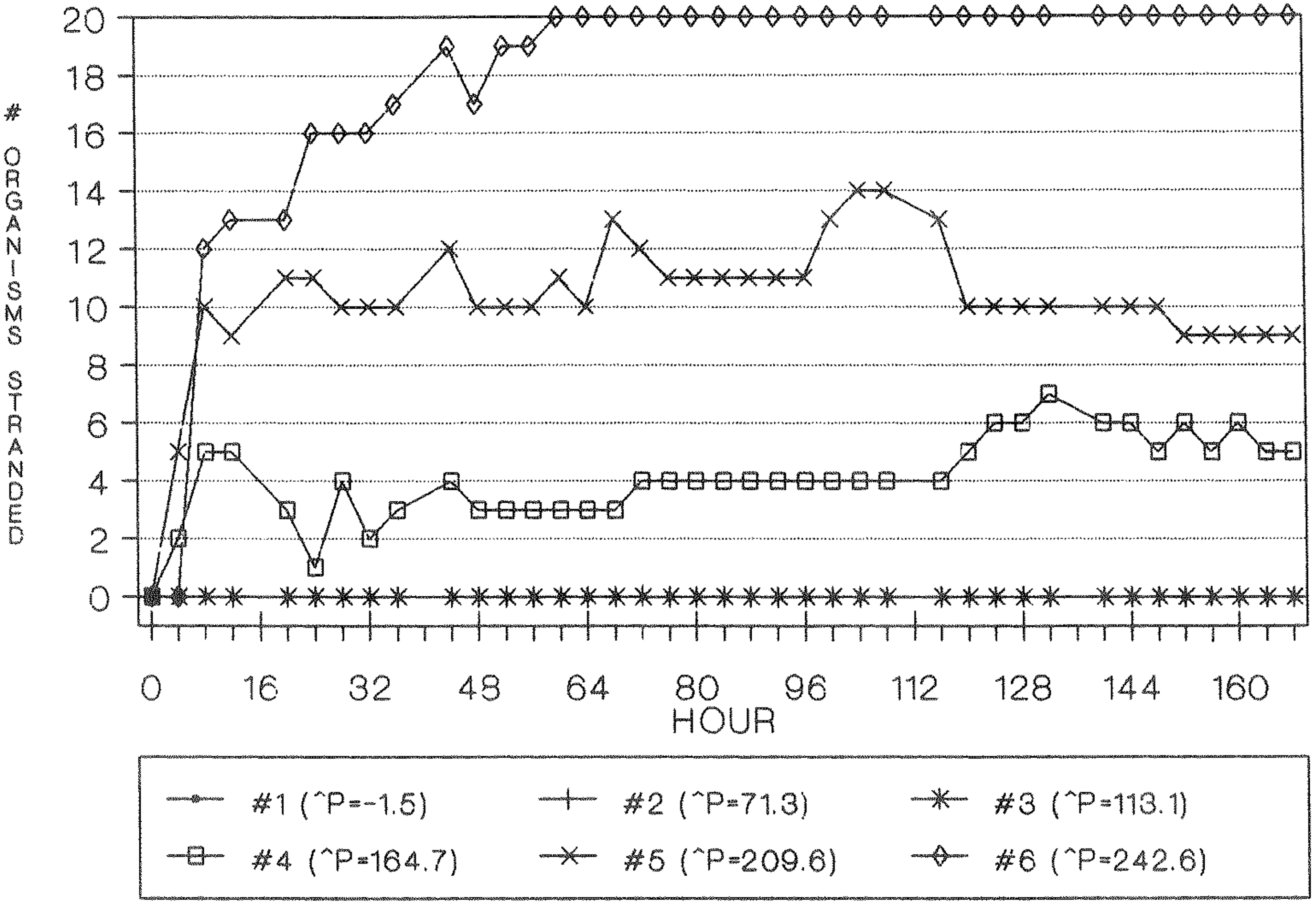

Figure 34. The number of stranded Tricorythodes minutus by hour, in test tanks 1-6, where mean delta P levels ranged from 1.5 to 242.6 mm Hg. Individuals that died while stranded, continued to be counted as stranded for the remainder of the test.

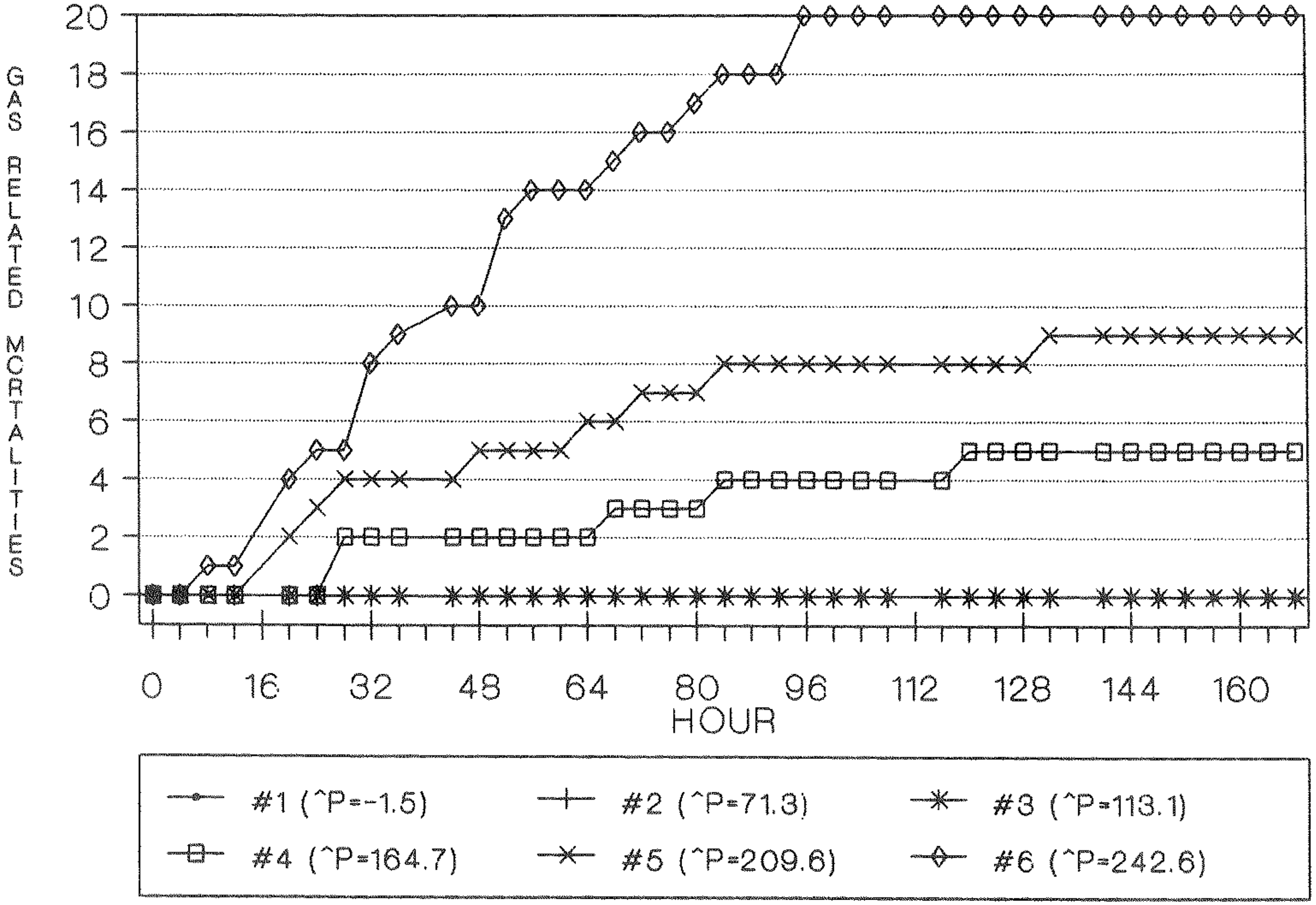

Figure 35. Cumulative mortality of <u>Tricorythodes</u> <u>minutus</u> exposed to six delta P levels ranging from 1.5 to 242.6 mm Hg for 168 hours. Non-gas related mortalities are not included.

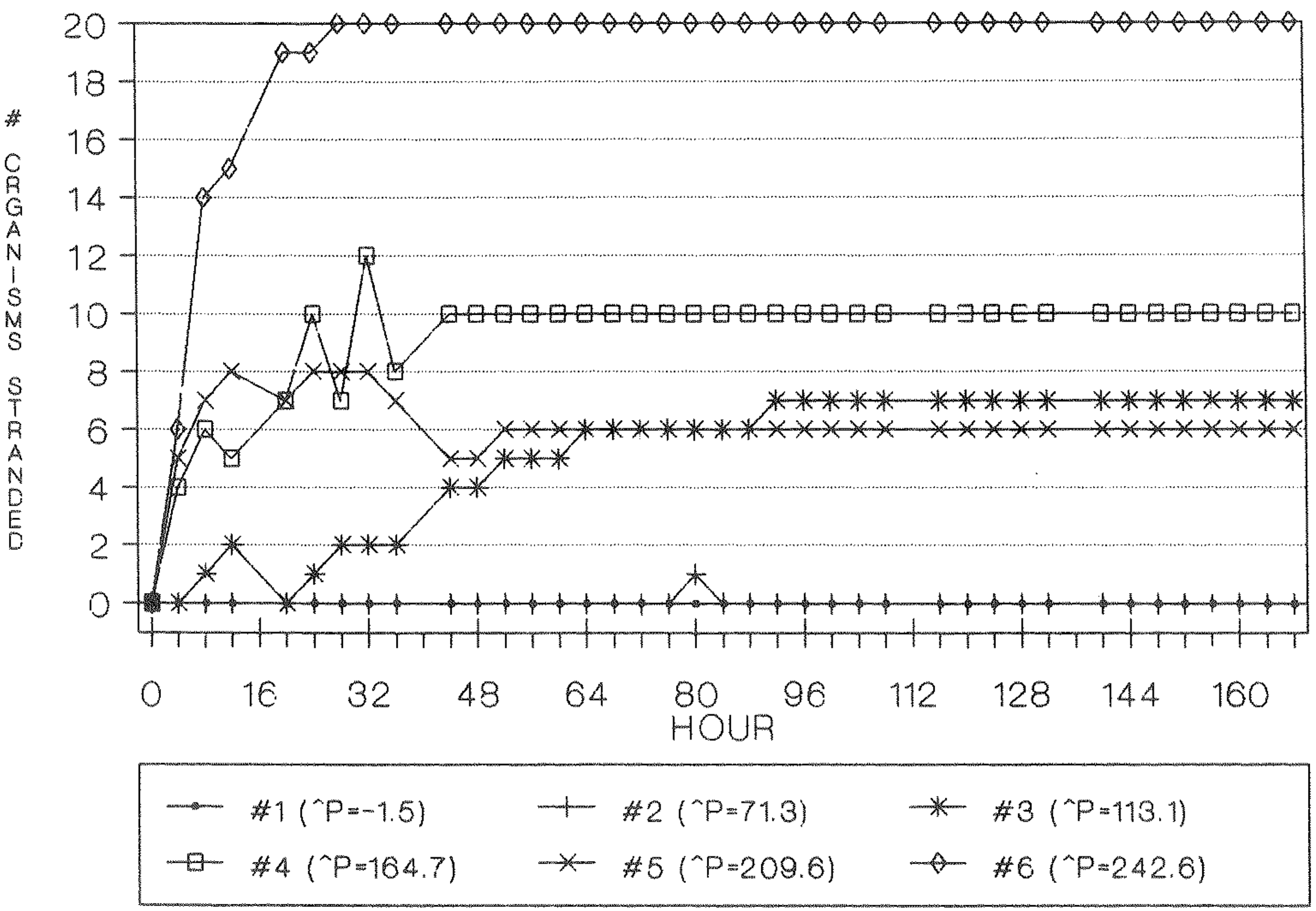

Figure 36. The number of stranded <u>Baetis tricaudatis</u>, by hour, in test tanks 1-6, where mean delta P levels ranged from 1.5 to 242.6 mm Hg. Individuals that died while stranded, continued to be counted as stranded for the remainder of the test.

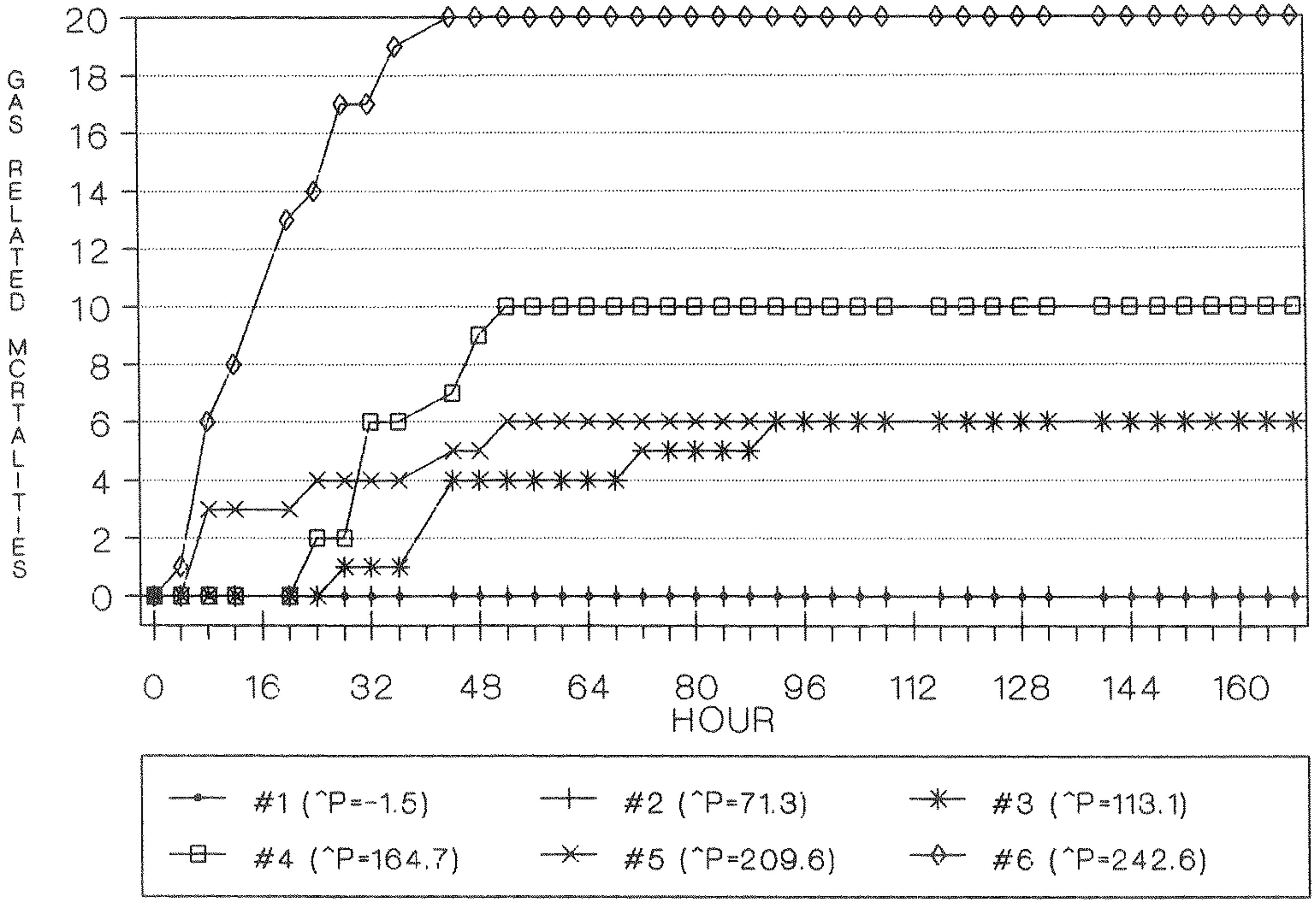

Figure 37. Cumulative mortality of Baetis tricaudatis exposed to six delta P levels ranging from 1.5 to 242.6 mm Hg for 168 hours. Non-gas related mortalities are not included.

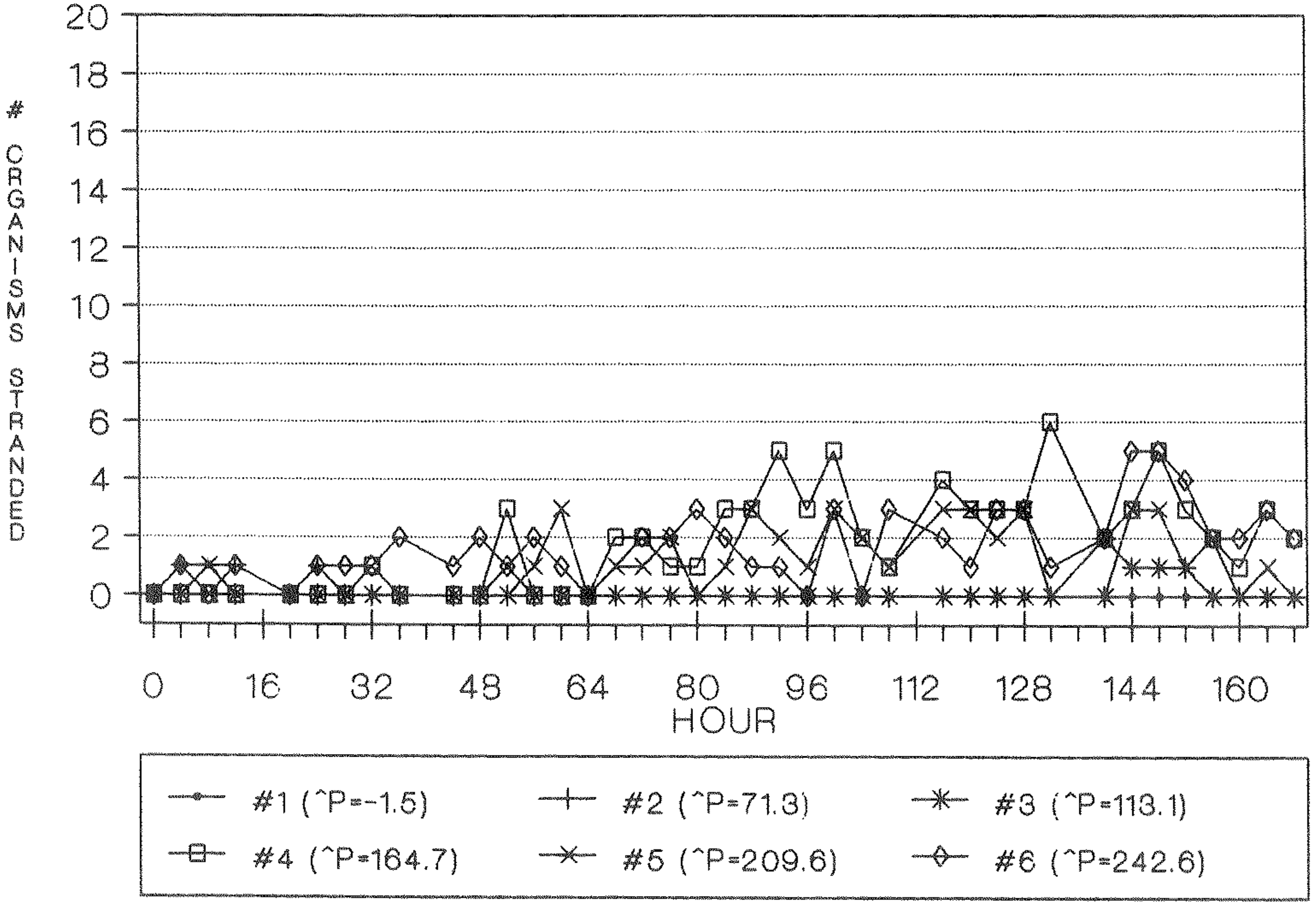

Figure 38. The number of stranded <u>Physella</u>, by hour, in test tanks 1-6, where mean delta P levels ranged from 1.5 to 242.6 mm Hg. Individuals that died while stranded, were counted as stranded for the remainder of the test.

APPENDIX C

Tables showing Bighorn River Delta P frequencies.

Table 12. The frequencies (percent of measurements taken) that Bighorn River delta P's at rkm 2.4 equaled or exceeded mean delta P levels (mm Hg) in test tanks 2-6 during bioassay #1. Results are provided for 1986 and 1987 by month.

	Test tank delta P levels				
Year/month	75	120	181	225	248
1986					
January	79	0	0	0	0
February	90	0	0	0	0
March	100	50	0	0	0
April	100	63	0	0	0
May	100	55	0	0	0
June	100	85	0	0	0
July	73	27	0	0	0
August	80	0	0	0	0
September	100	0	0	0	0
October	100	36	0	0	0
November	100	0	0	0	0
December	63	0	0	0	0
1987					
January	92	0	0	0	0
February	91	0	0	0	0
March	100	8	0	0	0
April	100	36	0	0	0
May	100	70	0	0	0
June	100	92	0	0	0
July	100	85	0	0	0
August	100	25	0	0	0
September	100	0	0	0	0
October	100	0	0	0	0
November	86	40	0	0	0
December	83	0	0	0	0

Table 13. The frequencies (percent of measurements taken) that Bighorn River delta P's at rkm 14.5 equaled or exceeded mean delta P levels (mm Hg) in test tanks 2-6 during bioassay #1. Results are provided for 1986 and 1987 by month.

	Test tank delta P levels				
Year/month	75	120	181	225	248
1986					
January	0	0	0	0	0
February	10	0	0	0	0
March	75	0	0	0	0
April	88	13	0	0	0
May	82	27	0	0	0
June	92	77	0	0	0
July	60	18	0	0	0
August	50	0	0	0	0
September	9	0	0	0	0
October	43	0	0	0	0
November	38	0	0	0	0
December	63	0	0	0	0
1987					
January	75	0	0	0	0
February	90	0	0	0	0
March	70	50	0	0	0
April	100	40	0	0	0
May	88	50	0	0	0
June	100	69	0	0	0
July	93	71	21	0	0
August	92	25	0	0	0
September	33	0	0	0	0
October	90	0	0	0	0
November	60	0	0	0	0
December	17	0	0	0	0

Table 14. The frequencies (percent of measurements taken) that Bighorn River delta P's at rkm 2.4 equaled or exceeded mean delta P levels (mm Hg) in test tanks 2-6 during bioassay #2. Results are provided for 1986 and 1987 by month.

	Test tank delta P levels				
Year/month	71	113	164	209	242
1986					
January	85	0	0	0	0
February	100	0	0	0	0
March	100	80	0	0	0
April	100	88	0	0	0
May	100	82	0	0	0
June	100	100	0	0	0
July	73	36	0	0	0
August	80	0	0	0	0
September	100	10	0	0	0
October	100	36	0	0	0
November	100	29	0	0	0
December	63	0	0	0	0
1987					
January	92	0	0	0	0
February	100	18	0	0	0
March	100	23	0	0	0
April	100	56	0	0	0
May	100	80	0	0	0
June	100	92	0	0	0
July	100	85	0	0	0
August	100	33	0	0	0
September	100	0	0	0	0
October	100	30	0	0	0
November	90	70	0	0	0
December	100	17	0	0	0

Table 15. The frequencies (percent of measurements taken) that Bighorn River delta P's at rkm 14.5 equaled or exceeded mean delta P levels (mm Hg) in test tanks 2-6 during bioassay #2. Results are provided for 1986 and 1987 by month.

Year/month	Test tank delta P levels 71	113	164	209	242
1986					
January	0	0	0	0	0
February	10	0	0	0	0
March	88	0	0	0	0
April	88	50	0	0	0
May	82	56	0	0	0
June	100	77	0	0	0
July	91	27	0	0	0
August	80	0	0	0	0
September	50	0	0	0	0
October	55	18	0	0	0
November	57	0	0	0	0
December	38	0	0	0	0
1987					
January	75	0	0	0	0
February	100	27	0	0	0
March	70	50	0	0	0
April	100	50	0	0	0
May	100	50	0	0	0
June	100	85	0	0	0
July	100	79	21	0	0
August	92	25	0	0	0
September	43	0	0	0	0
October	90	0	0	0	0
November	60	0	0	0	0
December	20	0	0	0	0

APPENDIX D

Gas saturation trends during sampling periods

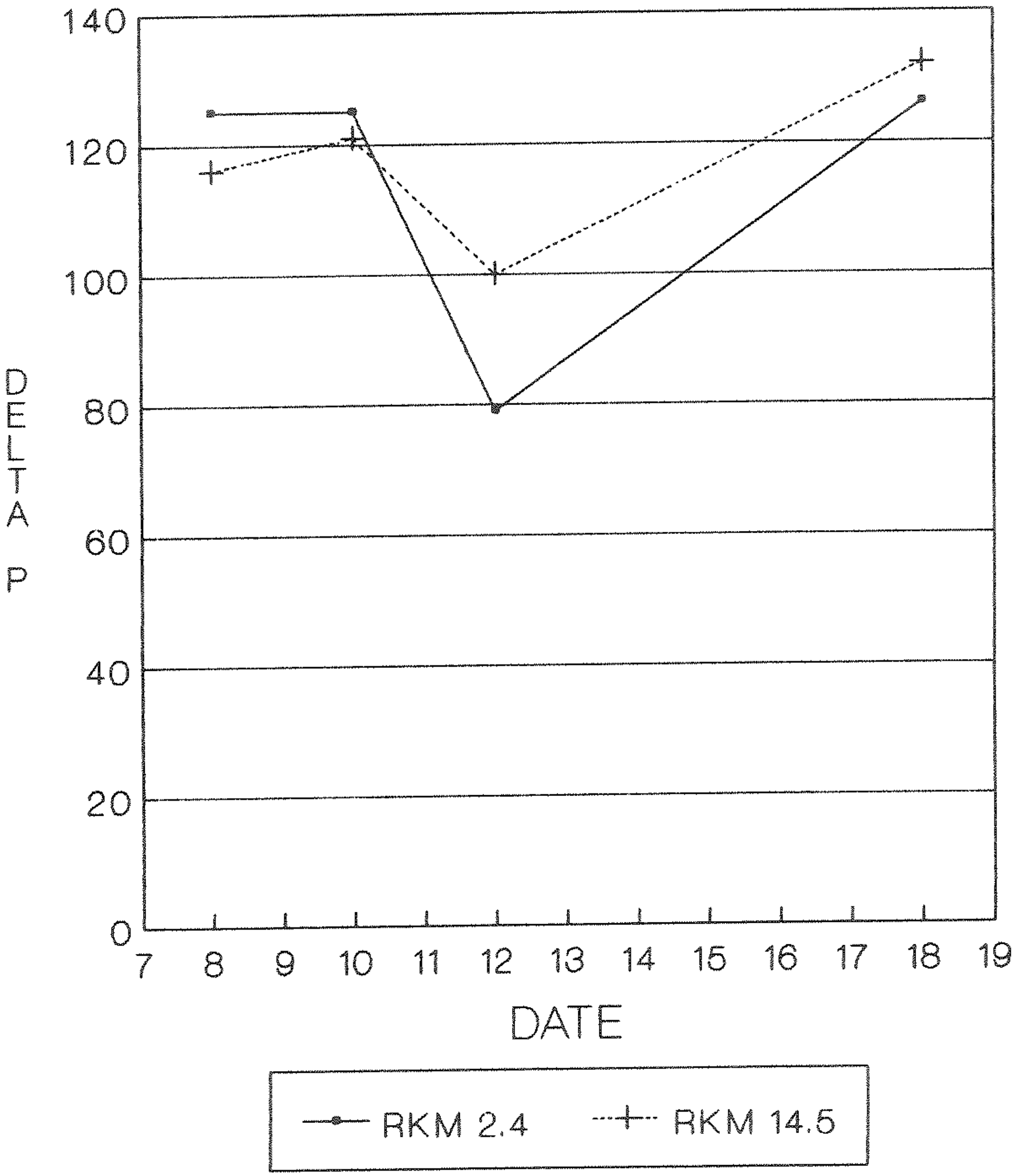

Figure 39. Comparison of Bighorn River delta P's measured at rkm 2.4 and 14.5 from April 8 to April 19, 1987.

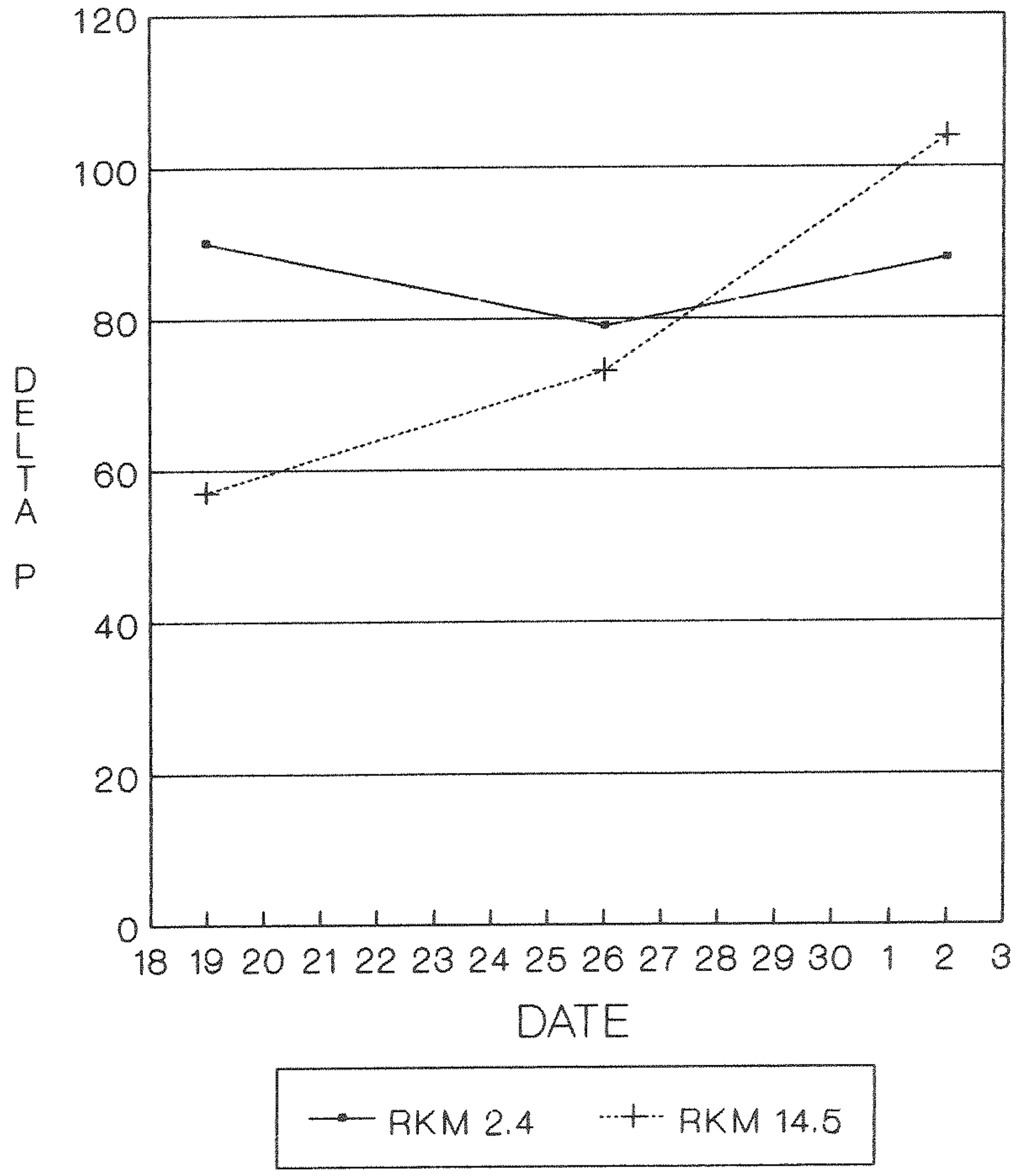

Figure 40. Comparison of Bighorn River delta P's measured at rkm 2.4 and 14.5 from September 19 to November 2, 1987.