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The extent to which heavy metals and polycyclic aromatic hydrocarbons (PAH) may be transferred up the food chain from sediments to benthic invertebrates and then on to fish species was examined using both laboratory and field techniques. PAHs were shown to bioaccumulate in a chironomid invertebrate (chironomus riparius)to relatively high levels depending on the specific compound. Accumulation in a fish specie (Lepomis macrochirus)that was fed contaminated chironomids was found to be generally low. Mobilization of PAHs from sediments into water was affected by benthic organisms, enhancing the bioavailability of these contaminants to other organisms. In field studies, certain benthic invertebrates and abiotic sediment components were also shown to accumulate heavy metals. This metal accumulation persisted even when metal concentrations in the water were diminishing. It was observed that the metal-tolerant (-accumulating) organisms tended to predominate and served as the major food source for the brown trout (Salmo trutta). This one-year study was well conducted and generated extensive data that should extend the knowledge base related to the transfer and bioaccumulation of toxicants.

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BIOACCUMULATION AND FOOD CHAIN TRANSFER OF POLYCYCLIC AROMATIC HYDROCARBONS AND HEAVY METALS: A LABORATORY AND FIELD INVESTIGATION

FINAL REPORT

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SUMMARY

This research examined bioaccumulation and food chain transfer of contaminants (polycyclic aromatic hydrocarbons and heavy metals) in laboratory microcosms and at impacted sites in the field. In laboratory experiments I measured accumulation of polycyclic aromatic hydrocarbons (PAHs) by Chironomus riparius from sediments and the transfer of these contaminants to the bluegill sunfish, Lepomis macrochirus. Experiments were conducted in laboratory microcosms containing sediments spiked with either benzo[a]pyrene (BAP) or fluoranthene (FLU). PAHs were rapidly accumulated from sediments by chironomids. Concentrations of BAP and FLU in chironomids increased with sediment concentration, however FLU was accumulated to a much greater extent. At sediment concentrations ranging from 47 to 4,040 ug/Kg levels of FLU in chironomids ranged from below detection to 181,000 ug/Kg. In contrast, the maximum concentration of BAP measured in chironomids was 6,030 ug/Kg. Levels of FLU and BAP in Lepomis macrochirus fed contaminated chironomids were generally low, indicating either low uptake or rapid metabolism of these compounds.

Bioturbation of sediments by chironomids decreased water clarity and released sediment-associated BAP to the overlying water. BAP in water and C. riparius increased significantly with chironomid density. In experiments where L. macrochirus was exposed to BAP from water, direct contact with sediments, and chironomids, each source contributed to total body burdens. Results of these laboratory experiments indicate that while sediments are an important sink for PAHs in aquatic systems, these contaminants may be rapidly mobilized and made available to aquatic organisms.

In field studies I examined the transfer of heavy metals (Cd, Cu, Zn) from benthic invertebrates to brown trout (Salmo trutta) at the Arkansas River, Colorado. Levels of metals in water, aufwuchs, benthic invertebrates, and fish were measured at several stations located upstream and downstream from California Gulch, a U.S. EPA Superfund site. Field studies were conducted to estimate the relative contribution of food and water to uptake of heavy metals by brown trout. Feeding habits of brown trout (Salmo trutta) at reference and impacted sites were compared to test the hypothesis that prey availability

influences food chain transfer of contaminants.

Aufwuchs and benthic invertebrates were highly contaminated by heavy metals at stations located downstream from California Gulch. Significant differences ($p<0.05$) in metal concentrations in aufwuchs and benthic macroinvertebrates among upstream (reference) and downstream (impacted) stations were observed. Metal concentrations in aufwuchs and benthic invertebrates remained elevated at downstream some stations, despite decreases in water concentrations. Significant variation among functional groups was also observed, as metal levels in organisms directly associated with aufwuchs (collector-grazers and collector gatherers) generally had the highest metal concentrations. Seasonal variation in metal bioaccumulation was also observed, however this variation was not correlated with metal concentrations in water.

The diet of brown trout at the Arkansas River was dominated by benthic invertebrates. In general, Ephemeroptera, Plecoptera, Trichoptera, and Chironomidae (primarily Orthocladiinae) accounted for between 40-95% of the diet of these organisms. Differences in prey availability between upstream (AR1) and downstream (AR5) stations resulted in differences in the diet of fish. Ephemeroptera comprised a greater portion of the diet of fish collected upstream from CG, whereas metal-tolerant organisms, such as Trichoptera and Orthocladiinae, were more common in the diet of fish from downstream.

Metal concentrations in brown trout were generally similar at stations AR1 and AR5 in liver and kidney tissue. Levels of Zn were significantly higher in gill and gut tissue at the downstream station. Elevated metal levels in water and food at station AR5 resulted in increased metals in gill and gut tissue. These results suggest that despite increased levels of metals at routes of exposure, levels of metals in depositional tissue were similar.

INTRODUCTION

1. Polycyclic Aromatic Hydrocarbons in the Aquatic Environment

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous contaminants of freshwater and marine ecosystems (see reviews by Neff 1979; Johnson et al. 1985; Eisler 1987). Because of their persistence (Neff 1979; Eisler 1987), acute toxicity (Oris and Giesy 1985; Swartz et al. 1990), potential to bioaccumulate (Reichert et al. 1985; Varanasi et al. 1985; Landrum 1989), widespread distribution (Payne et al. 1988; Marcus et al. 1988), and carcinogenicity (Malins et al. 1987; Metcalfe et al. 1988), concern over the fate and effects of these contaminants has increased in recent years. In aquatic environments PAHs are partitioned between water, sediments, interstitial water, and organisms (Eisler 1987). The greatest portion of these contaminants will, however, be associated with sediments (Varanasi et al. 1985; Marcus et al. 1988). Levels of PAHs in sediments and interstitial water are often several orders of magnitude greater than concentrations in overlying water (Neff 1979; Eisler 1987).

Although sediments are a repository for PAHs and other contaminants in aquatic systems, they are not an ultimate sink for these materials (Larsson 1985; Reynoldson 1987). Owing to various physical, chemical, and biological processes, sediment contaminants may be rapidly mobilized and made available to aquatic organisms. Because of their intimate association with sediments and their significance as a food source for bottom-feeding fish, benthic invertebrates play an important role in the transfer of contaminants from sediments. Bioturbation, defined as the movement of sediments by the activity of benthic organisms (Reynoldson 1987), releases sediment contaminants into overlying water.

Although bioaccumulation of PAHs by benthic invertebrates has been demonstrated in several studies (Gerould et al. 1983; Varanasi et al. 1985; Landrum 1989; Landrum et al. 1991), our understanding of how these materials are transferred through aquatic food webs is incomplete (Reynoldson 1987). Uptake of PAHs from water by fish has been demonstrated and is generally the predominant route of exposure for these organisms (Lee et al. 1972; Neff et al. 1976; McCarthy and Jimenez 1985; Kennedy et al.

1989). However, since levels of PAHs in sediments and associated benthic invertebrates are generally much greater than in overlying water, it follows that accumulation from food and sediments may be substantial.

PAHs have been identified in the stomach contents of bottom-feeding fish, indicating the potential for dietary uptake of these compounds (Maccubbin et al. 1985; Malins et al. 1987). Because acute toxicity of those PAHs associated with neoplasia in fish is relatively low (Eisler 1987), many benthic invertebrates are able to survive in areas with high levels of PAHs in sediments. It is likely that these pollution-tolerant prey will accumulate high levels of contaminants; therefore increased utilization by selective predators will increase the potential for food chain transfer of these materials (Dallinger and Kautzky 1985; Dallinger et al. 1987). This phenomenon, which has been called the "food chain effect" (Dallinger et al. 1987), occurs with compounds that do not readily bio-magnify and may account for high levels of these contaminants in aquatic organisms.

2. Bioaccumulation of Heavy Metals in Aquatic Organisms: Importance of Water and Diet

Bioaccumulation of heavy metals in contaminated streams has been demonstrated in algae (Foster 1982; Kelly and Whitton 1989), macroinvertebrates (Smock 1983a; 1983b; Hare et al. 1989; Krantzberg and Stokes 1989) and fish (Ney and Van Hassel 1983; Moriarty et al. 1984; Dallinger and Kautzky 1985). Uptake of metals by fish occurs through three pathways: 1) the gills, 2) the alimentary tract, and 3) through the skin (Dallinger et al. 1987). Dallinger et al. (1987) suggested that the amount of metals absorbed through the skin may be inconsequential. It is generally accepted that gills and the gut provide the primary route for uptake of heavy metals by fish (Dallinger and Kautzky 1985; Dallinger et al. 1987; Giles 1988; Douben 1989). Most evidence derived from laboratory studies indicates that uptake from water is a more important route of exposure than food, particularly for fish (Williams and Giesy 1978; Prosi 1979). However, several recent studies have suggested that dietary accumulation may contribute significantly to total body burdens of heavy metals in these organisms (Dallinger and Kautzky 1985; Hatakeyama and Yasuno 1987; Dallinger et al. 1987; Harrison and Klaverkamp 1989; Douben 1989).

Hatakeyama and Yasuno (1987) reported that 90% of cadmium accumulation in the guppy, Poecilia reticulata, was derived from feeding on contaminated chironomids. Dallinger and Kautzky (1985) demonstrated that rainbow trout accumulated metals primarily through the diet when levels in the water were low. Harrison and Klaverkamp (1989) found that rainbow trout and lake whitefish exposed to cadmium in a continuous water flowing system accumulated significantly greater amounts of cadmium through food rather than water. Patrick and Loutit (1978) and Douben (1989) utilized tubificid worms to provide evidence that a natural food source can influence uptake of heavy metals in fish. This evidence strongly supports the hypothesis that some fraction of heavy metals is elaborated into fish tissue through the food chain.

3. Heavy Metals in Streams of Colorado

Since the discovery of gold and other minerals in the 'Front Range Mineral Belt' of Colorado during the mid 1800's, mining activities have had a major impact on watersheds in the region. These watersheds, which are often located in otherwise undisturbed areas, have the potential to support valuable fish and wildlife resources. The effects of materials from abandoned mines and mine tailings on aquatic organisms are well documented (Sprague et al. 1965; Jefree and Williams 1980; Foster 1982; Chadwick et al. 1986; Lynch et al. 1988; Roline 1988). In one of the earlier comprehensive studies of the impact of mining pollution, Sprague et al. 1968 showed that heavy metals caused large numbers of migrating adult salmon (Salmo salar) to return downstream. Macroinvertebrate communities are also severely affected by heavy metals from mining operations. Downstream communities are usually characterized by reduced species diversity, reduced abundance, and a shift in species composition from sensitive to tolerant taxa (Winner et al. 1980; Chadwick et al. 1986; Lynch et al. 1988; Roline 1988; Clements et al. 1988).

The upper Arkansas River Basin has been recognized as a site of extremely poor water quality for many years. Although several point and non-point sources of impact have been identified, past mining and metallurgical operations in the Leadville area (Leadville, CO) have received the most attention. The Yak tunnel, a U.S. EPA Superfund

site, releases approximately 600 gallons/day of highly contaminated water into California Gulch, which flows directly into the Arkansas River. As a result, levels of zinc (920 ug/Kg), cadmium (180 ug/Kg), copper (16 ug/Kg), and lead (13 ug/Kg) are greatly elevated in the Arkansas River immediately downstream of Leadville, CO. (Colorado Department of Health 1988). Significant acute toxicity (96 h test with Ceriodaphnia dubia) has been observed from stations located 30 Km downstream from California Gulch (Clements, unpublished data). In addition to metals discharged from California Gulch, tributaries draining several mining districts in the area contribute significantly to the total mass load of heavy metals in the upper Arkansas River. Lewis (1987) reported that levels of several metals, including zinc, copper, lead, and cadmium, exceeded aquatic life standards for approximately 300 miles of the upper Arkansas River.

Previous investigations on the upper Arkansas River have demonstrated significant effects of heavy metals on benthic macroinvertebrate and fish populations. Roline (1988) reported reduced diversity and increased abundance of tolerant macroinvertebrates, particularly caddisflies, downstream of California Gulch. Reduced populations and poor survival of brown trout (Salmo trutta) has been attributed to heavy metal contamination of the Arkansas River (Lewis 1987). Several studies have suggested that stress and condition of salmonid species are adversely effected by increases in heavy metal concentrations (Roch et al. 1982, Giles 1988, Spry et al. 1988). Brown trout (Salmo trutta) in the Arkansas River rarely reach the age of 4+ and their condition in all age classes is considered generally poor (Winters 1988). The aquatic invertebrates which represent the major food source for brown trout are also influenced by the presence of metals. Previous investigations suggest that the potential exists in the Arkansas River to support a excellent brown trout fishery. Evaluation of brown trout populations in the Arkansas River indicated that juvenile S. trutta are among the fastest growing trout in streams of Colorado (Nehring and Anderson 1981). This period of rapid growth is, however, followed by very poor survival rates beyond three and four years of age. It has been hypothesized that bioaccumulation of heavy metals, either from water or from the food chain, contributed to the decline of S. trutta populations in the Arkansas River (Lewis 1987).

Heavy metal contamination in the Arkansas River has resulted in increased

abundance of tolerant macroinvertebrates, particularly caddisflies, at stations downstream from California Gulch (Roline 1985). Herrman (1985) noted that the caddisfly Brachycentrus americanus was highly tolerant of heavy metals and abundant at stations immediately downstream from California Gulch. Since these organisms comprise a significant portion of the diet of brown trout (Winters and Anderson 1984), it is likely that dietary uptake of heavy metals may contribute to poor survival of S. trutta in the Arkansas River.

4. The Role of Sediments in the Transfer of Contaminants

Sediments represent an important sink for heavy metals and other contaminants in aquatic systems. Levels of heavy metals in sediments are often several orders of magnitude greater than those in overlying water. Because of their close association with sediments, benthic invertebrates readily accumulate metals and other materials from contaminated sediments (Tatem 1986; Hare et al. 1989) and represent an important link to higher trophic levels. Although most metals show little tendency to biomagnify up food chains, concentrations in fish can reach harmful levels owing to reduced prey diversity and increased consumption of contaminated prey (Dallinger et al. 1987). Many fish are opportunistic feeders and shift their foraging habits based on prey availability. Thus, feeding habits of fish may influence dietary exposure to metals. The concept of this interaction along with its effects was first described by Dallinger et al (1987) as part of the "food chain effect."

Several investigators have shown that feeding habits of fish at impacted sites may be modified to include tolerant prey types (Jefree and Williams 1980; Clements and Livingston 1983; Livingston 1984). In streams polluted by mining effluents, Jefree and Williams (1980) reported that fish switched from pollution-sensitive to pollution-tolerant prey types. I hypothesize that increased utilization of pollution-tolerant prey in the Arkansas River will increase the potential for food chain transfer of heavy metals.

5. Objectives

This research investigated the uptake and transfer of two classes of contaminants (polychclic aromatic hyfrocarbons and heavy metals) in laboratory microcosms and at an field impacted site. The objcetives of the laboratory experiments were 1) to measure uptake of benzo[a]pyrene (BAP) and fluoranthene (FLU) by chironomids and food chain transfer of these contaminants to bluegill; 2) to examine the importance of bioturbation by chironomids in releasing sediment-associated BAP into the water column; and 4) to determine the relative importance of food and water as sources of BAP to bluegill.

In the field study I examined bioaccumulation of metals by aufwuchs and benthic invertebrates and the potential food chain transfer of these contaminants to brown trout, Salmo trutta. The specific objectives of this research were to to test the hypotheses that: 1) concentrations of heavy metals in benthic invertebrates were elevated downstream from California Gulch, a U.S. EPA Superfund site; 2) feeding habits of brown trout will vary among locations due to metal-induced changes in prey availability; 3) metal levels in brown trout tissues are elevated downstream from California Gulch; and 4) benthic invertebrates at the Arkansas River are a potential source of heavy metals to brown trout.

MATERIALS AND METHODS

1. Uptake and Transfer of Polycyclic Aromatic Hydrocarbons

Sediments

Sediments were collected from Acton Lake (Butler Co., OH), washed through a 750 µm sieve to remove indigenous macroinvertebrates, and transferred to the laboratory in a 40-L cooler. Sediments consisted primarily of fine, well-sorted sand. Total volatile solids were determined by measuring weight loss of samples dried at 50 °C for 12 h and then combusted at 500 °C for 1 h. Percent total volatile solids was relatively low, and ranged from 0.4% to 1.2%.

Sediments were spiked by coating the inside of a 4-L jar with either FLU or BAP dissolved in acetone. The jars were filled with 1.5 Kg of sediment and placed on a rolling mill. The sediments were rolled for 4 h, mixed with a metal spatula, and rolled for an additional 2 h.

Experimental Animals

Chironomids (Chironomus riparius) were obtained from laboratory cultures. Organisms were maintained in 19-L aquaria at 20 °C and fed a mixture of trout chow, dog chow, and cerophyl. Juvenile Lepomis macrochirus were obtained from a private fish hatchery. Fish were maintained in a 250-L flow-through tank and fed trout chow.

Experimental Conditions

All experiments were conducted at Miami University in an Environmental Growth Chamber under a 14L:10D light regime. Dechlorinated tap water (pH: 8.06-8.35; hardness:240-280 mg/L as $CaCO_3$; conductivity: 570-590 µmhos/cm; dissolved oxygen: 7.1-7.6 mg/L) was used in all experiments. Water temperature ranged from 19.5-21.0 °C.

Uptake of FLU and BAP by Chironomids and Bluegill

Uptake of FLU and BAP from sediments by Chironomus riparius was measured in 1-L beakers containing 200 ml of sediment and 700 ml of dechlorinated tap water.

Target concentrations of FLU were 100, 500, and 5,000 µg/Kg. Target levels of BAP were 100, 1,000, and 5,000 µg/Kg. Fifty fourth-instar C. riparius were placed into each beaker. Organisms quickly burrowed into the substrate in all beakers.

Sediments and chironomids were sampled after 72 h in the BAP experiments and after 24, 48, and 72 h in the FLU experiments. Sediments were collected from each replicate beaker using a modified 50-cc glass syringe. Three subsamples collected from each beaker were combined and placed in a 30-ml vial (approximately 3.0 g wet weight). Remaining sediments were sieved through a 500-µm mesh net to remove chironomids. Sediments and tissue samples were stored at -60°C.

Dietary transfer of PAHs was measured by feeding bluegill chironomids spiked with FLU or BAP. Chironomids were exposed to BAP and FLU in contaminated sediments for 72 h at the same target levels given above (control, 100, 500, and 5,000 µg/Kg FLU; control, 100, 1,000, and 5,000 µg/Kg BAP). Juvenile bluegill (2.3-3.7 g wet weight) were held in 20-L aquaria and fed 4th-instar chironomids at approximately 5% of their body weight per day (20 per fish per day). After 96 h exposure, fish were depurated in clean water for 24 h. Fish were removed and muscle and gut tissue were dissected and stored at -60 °C.

Differences among treatment levels were analyzed using one-way ANOVA and Duncan's Multiple Range Test on log-transformed data.

Sediment Bioturbation by Chironomids

Bioturbation of sediments by chironomids was measured in 1-L beakers containing BAP-spiked sediments (5,000 µg/Kg). To fill the beakers with minimal disturbance of sediments, a teflon lid was placed directly over the sediments and water was gently added to each beaker. After 24 h fourth-instar C. riparius were placed into the beakers at the following densities: 0, 20, 40, 80, 120, and 200 (two replicates per beaker). Chironomids in the 20, 40, and 80 organisms per beaker treatments quickly buried into the sediment. In the two highest density treatments, chironomids remained clumped on the surface and did not bury into the sediment. These treatments were terminated and excluded from the analysis. After 72 h, a 50-ml water sample was collected from each

beaker and turbidity (using a Helige turbidimeter) and levels of BAP in water were measured. Sediments and chironomids were sampled as described above and stored at -60 °C. Differences between chironomids were analyzed as described above.

Uptake of BAP from Food and Water

This experiment measured uptake of BAP by Lepomis macrochirus from sediments, food, and water. The experiment was conducted in 1-L beakers containing 100 ml of BAP-spiked sediment (5,000 ug/Kg). Twelve beakers were randomly assigned to one of three treatments (4 replicates per treatment): 1) chironomids absent (CA); 2) chironomids present (CP); and 3) chironomids present but separated from fish by a 500 um mesh screen placed 2 cm above the sediments (CP/S). One hundred chironomids were placed in each of the CP and CP/S beakers. Higher densities of chironomids were used in these experiments to insure that adequate food would be provided for the fish and that chironomids would be present for analysis at the end of the experiment. Three juvenile bluegill (0.25-0.48 g) were placed in each container. Smaller fish were used in these experiments because of the small containers. Fish in the CA treatment were exposed to BAP from water or from direct contact with sediments. Fish in the CP treatment could actively forage on chironomids and were exposed to BAP from water, direct contact with sediments, and diet. Fish in the CP/S treatment were separated from sediment and chironomids and were exposed to BAP only from water. The experiment was conducted for 96 h, after which fish were removed, placed into clean water, and allowed to depurate for 24 h. Levels of BAP in water sediments, chironomids and whole fish were determined. Individual organs in fish were not analyzed due to the limited amount of tissue in these small fish. Differences between treatments were analyzed as described above.

Analytical Procedures

Water samples for PAH analysis were extracted using bonded reverse-phase cartridges. The cartridges were dried and PAHs eluted using HPLC grade ethyl acetate. Sediment samples were extracted with 10 ml of HPLC grade acetone:cyclohexane (8:2).

Samples were shaken daily for 7 d and then the extracts were filtered through a 0.45 μm glass fiber filter. Bluegill tissue samples and chironomids were ground using a Ten-Broeck tissue grinder and extracted with 5 ml each of acetone, ethyl acetate, and cyclohexane. Concentrations of PAHs in water, sediments, and tissue was measured using HPLC and fluorescence detection. A 10-min gradient from 60% acetonitrile and 40% water to 100% acetonitrile was performed on a reverse phase analytical column. Peaks were identified from known standards and concentrations determined by integration.

2. Uptake and Food Chain Transfer of Heavy Metals at the Arkansas River

Study Site

The study site was located in a valley in central Colorado, between the Sawatch and Mosquito mountain ranges (Fig. 5). The upper Arkansas River is formed by the confluence of two main tributaries, the East Fork of the Arkansas River and Tennessee Creek. Sampling stations were located along a 900 m elevational gradient from Climax to Buena Vista, CO. Stations were located upstream and downstream from California Gulch (CG), a U.S. EPA Superfund site. Three stations (EF1, AR1, and AR2) were located upstream from CG and served as reference sites. Stations AR3, AR5, and AR8 were located 0.3, 6.0, and 45.0 Km downstream from CG, respectively. Substrate consists of mainly gravel-rubble with riffles and runs comprising the majority of stream habitat. Flow is dependent upon snowmelt with high flow occurring during spring runoff. Riparian canopy is scarce, consisting mainly of willow (Salix spp.).

Water Quality

Water samples were collected at all sampling stations. Hardness (48-100 mg/L), alkalinity (35-75 mg/L) and pH (7.6-8.0) of the Arkansas River were typical of soft-water montane streams of the Rocky Mountain region. Hardness and alkalinity increased downstream of EF-1 to a maximum at station AR-5, and then decreased at AR-8. Dissolved oxygen concentrations at all stations were greater than 90% saturation.

A 250-ml sample from each station was acidified with reagent grade nitric acid (HNO_3) for metal analysis. Total metals were determined for each sampling period, except

for May 1991 when both total and dissolved metals were analyzed. Metal concentrations in water (Cd, Cu, and Zn) were measured using graphite furnace techniques on a IL 22 Video Dual Channel Atomic Absorption Spectrophotometer.

Determination of Metals in the Benthic Community

Benthic macroinvertebrates and aufwuchs were collected for metals analysis in fall (October 1990), spring (May 1991), and summer (August 1991). Procedurally-defined aufwuchs, which includes both abiotic and biotic material [9], was scraped from whole rocks collected at each sampling location. Rocks (n=3) were scrubbed with a stiff brush and rinsed with distilled water into a plastic tub. Samples were then transferred into 25 ml polypropylene vials and placed on dry ice.

Immature stages of macroinvertebrate were collected at each station using a kick-net. We collected taxa from several functional feeding groups [17], including grazers (Baetis spp., Ephemeroptera: Baetidae), collector-gatherers (Pteronarcella badia, Plecoptera: Pteronarcyidae), collector-filterers (Arctopsyche grandis, Trichoptera: Hydropsychidae), and predators (Skwala americana, Plecoptera:Perlodidae; Rhyacophila spp., Trichoptera: Rhyacophilidae). Macroinvertebrates were identified to genus or species in the field. All organisms were placed in acid-washed polypropylene scintillation vials (25-ml) and immediately placed on dry ice. Organisms from each sample were placed in a separate vial. We attempted to collect three replicates for each taxa at each station. However, as a result of habitat requirements, life history, and/or metal toxicity some taxa were absent from some stations on some dates. Individual organisms were used for metals analysis when possible, except for Baetis spp., which were pooled because of their small biomass. Twenty mg (dry weight) per replicate was determined to be the minimum amount of tissue necessary for metals analysis.

All samples were thawed to room temperature before metals analysis. Aufwuchs samples were pre-filtered through a 350-μm nitex mesh net to remove macroinvertebrates. Samples were transferred to a 45-μm Gelman metricel filter and rinsed with 15 ml of glass distilled water. Aufwuchs and macroinvertebrates samples were placed into pre-weighed polystyrene tubes, dried to a constant weight at 50 °C, cooled to room temperature, and

weighed to the nearest 0.01 mg. One ml of reagent grade HNO_3 acid was added to each tube, and samples were digested at room temperature for 24 h. Samples were then digested at 50 °C for 4 h, after which 100 µl of H_2O_2 was added to each sample and heated for 1 h to complete the digestion procedure [18]. Tubes were diluted to a final volume of 7 ml with glass-distilled water. All samples were analyzed for Cd, Cu, and Zn on a IL Video 22 Dual Channel Atomic Absorption Spectrophotometer.

Quality control/quality assurance procedures included analysis of National Bureau of Standards (NBS) Bovine Liver tissue (Standard Reference #1577), HNO_3 acid blanks, filter and HNO_3 acid blanks, and distilled water. Samples were analyzed using the protocol described above. Recovery of Cd, Cu, and Zn from NBS Bovine Liver tissue (Standard Reference #1577 n=15) was 92%, 101%, and 111%, respectively. Metals in HNO_3 acid, water, and HNO_3 acid and water blanks were consistently below detection.

Brown Trout Sampling

Brown trout (Salmo trutta) were collected from stations AR1 and AR5 with the use of a backpack electroshocker on four sampling occasions: 20-21 April, 8-9 July, 11-12 August, and 5-6 September 1991. The purpose for collecting these fish was to provide information regarding: 1) foraging preference, 2) concentration of heavy metals in the diet, and 3) concentration of heavy metals in fish tissues (gill, gut, liver, and kidney). The criteria for selecting sampling dates was based on providing the greatest range of temporal information while maximizing sampling efficiency. Winter months were not sampled due to icy conditions. High water prevented efficient sampling during June.

On each occasion, sampling was conducted on two consecutive days. Station AR5 was sampled the first day because of its greater degree of difficulty. Station AR1 was sampled the following day. On all sampling occasions, electroshocking began between 9:00 and 10:00 a.m. M.S.T., and proceeded until 50 brown trout had been captured. Electroshocking fish was conducted at this time to allow the brown trout an opportunity to utilize the major potential food source offered by early morning benthic drift (Elliot 1970). Sampling brown trout at mid-morning should also provide stomach contents containing relatively low numbers of terrestrial insects. The low numbers of terrestrial

insects typically found in the drift during the night should allow a sufficient period of time for the gastric evacuation of most terrestrial invertebrates consumed on the previous day (Elliott 1972).

After fish had been captured, they were placed in live-baskets. To reduce stress and overcrowding, gut contents were taken from groups of fish at random times throughout the collecting procedure. Before extracting stomach contents, brown trout were placed in a five-gallon bucket and anesthetized with MS-222. Gut contents were removed with the use of a hand-held stomach pump. The stomach pump was used to flush stream water into the fish's gut, then the suction of the pump was allowed to extract the mixture of food items and stream water. Gut contents were discharged into a plastic vial containing 100 percent denatured ethanol. This procedure was repeated twice for each fish, and each sample was kept in a separate vial. Distilled water was used in this process to collect stomach contents from fish used to determine heavy metal concentrations in the diet. These samples were immediately placed on dry ice and frozen for metals analysis.

Stomach samples collected for the identification of benthic invertebrates were returned to a laboratory at Colorado State University. Food items were identified to genus, species and enumerated under a dissecting microscope. Dry weights were recorded for each sample to the nearest 0.1 mg using a Sotoris Balance. Gut samples were then allowed to dry for a minimum of 24 hours in an oven at 50°C.

Gut samples for metals analysis were thawed and prey species were identified and enumerated. Only non-metal, acid washed tools were used during this process. Samples were dried in an oven at 50°C as described above and then digested in 1 ml of a 1:1 ratio of concentrated sulfuric and nitric acid. All samples were allowed to predigest for a period of no less than 24 hours. Samples were then heated in a water bath at 50-60°C until digestion was completed. Samples were diluted with 6 ml of distilled water. Metals analysis was carried out at the Colorado Division of Wildlife (CDOW) on a Varian Spectra AA-40.

Metals Concentrations in Fish

On two sampling occasions (July 1991 and September 1991), brown trout collected from each station were sacrificed to determine the concentration of Cu, Cd, Zn in their gills, liver, gut, and kidney. Fish were obtained by electroshocking, anesthetized with MS222, gut contents were collected as described above, and the fish killed with a blow to the head. Whole fish were measured, weighed, and immediately frozen on dry ice. These fish remained frozen until the organs were dissected for metals analysis.

Dissection of brown trout was carried out at Colorado State University immediately after the fish had thawed. All tools used in this procedure were rinsed in a solution of water containing 10% nitric acid then rinsed with distilled water between the dissection of each organ. Entire kidney, liver, gut, and gill filament tissues were taken from each fish and placed in 16.5 ml glass test tubes. Examination of the guts at this time revealed that approximately 90 percent of the food material had been extracted from the foregut of each fish using the stomach pumping technique. Tissue samples were dried and weighed using the procedure previously described. All brown trout tissue samples were digested using 2 ml of a 1:1 ratio of concentrated nitric and sulfuric acid. The procedure used for predigestion and heating of these samples was identical to that previously described for the gut samples. When the digestion process was completed, tissue samples were diluted with 14.5 ml distilled water. Metals analysis was conducted at the Colorado Division of Wildlife.

Benthic Macroinvertebrate Sampling

Macroinvertebrate sampling was done concurrently with electroshocking at each station to provide a quantitative estimate of prey availability to S. trutta. Five replicates were taken at each station using a 0.1 m^2 Hess Sampler. Samples were preserved in 70% ethanol and labeled according to location and date. These samples were taken back to the laboratory and sorted in a white enamel pan. All insects were identified to species (excepted Chironomidae which were identified to tribe) and enumerated under a dissecting microscope.

Statistical Analyses

Because of non-homogeneity of variances all metal concentrations were log-transformed. Analysis of variance (ANOVA) and Tukey's Honest Significant Difference (HSD) multiple range test were performed to determine differences in metal concentrations among taxa and among locations. Tukey's HSD test controls maximum experiment error rate and is suitable for unequal samples sizes. Two-way ANOVA and Tukey's HSD test, with season and taxa as independent variables, was used to evaluate how these factors contribute to differences in metal bioaccumulation. Correlation analyses (Pearson's Correlation Coefficient) were used to analyze the relationship between: water and aufwuchs metal concentrations; water and benthic macroinvertbrate concentrations; and organism size and metal concentrations. All statistical analyses were performed using a PC-version of Statistical Analysis System (SAS) [19]. A significant difference was determined to exist at a $p<0.05$ level.

RESULTS

1. Uptake and Food Chain Transfer of Polycyclic Aromatic Hydrocarbons

Uptake of FLU and BAP by Chironomids and Bluegill

Mean FLU levels in sediment ranged from 47 µg/Kg in controls tc 4040 µg/Kg in the high-FLU treatment FLU sediments levels and were similar among days (Fig. 1). There were no significant differences between controls and the low-FLU treatment on any day. Mean levels of BAP in sediments ranged from 1.1 µg/Kg in controls to 2740 µg/Kg in the high-BAP treatment (Fig. 2). BAP in controls and the low-BAP treatments were not significantly different.

Chironomids rapidly accumulated FLU from treated sediments (Fig. 1). Mean concentration of FLU in chironomids ranged from below detection in controls to 181,000 µg/Kg in the high-FLU treatment (day 2) and increased significantly ($p<0.0001$) with sediment concentration. FLU was detected in chironomids in the low-FLU treatment level only on day 3. Results of two-way ANOVA, with sediment concentration and day as the main factors, indicated that levels in chironomids did not vary significantly among days ($p=0.08$). The sediment x day interaction term from this analysis was marginally significant ($p=0.046$).

BAP was detected in chironomids from all treatments and increased significantly ($P=0.0005$) with sediment levels (Fig. 2). Uptake of BAP by chironomids was considerably less than for FLU, as mean concentrations ranged from 12 µg/Kg in controls to 6030 µg/Kg in the high-BAP treatment.

Levels of PAHs in Lepomis macrochirus fed chironomids exposed to either FLU- or BAP-treated sediments were generally low or below detection (Fig. 1-2). FLU was detected in gut tissue only at the two highest concentrations. FLU in gut tissue did not vary among days, but was significantly higher ($p=0.028$) in fish fed chironomids from the high-FLU treatment compared to the medium-FLU treatment. BAP was not detected in fish from any of the treatments.

Bioturbation

Concentrations of BAP in sediments ranged from 4100 to 5100 ug/Kg and did not vary significantly among the four chironomid densities (Fig. 3). Within 24 h, turbidity in beakers containing chironomids was significantly greater ($p=0.0012$) than in beakers without chironomids (35.5-56.5 NTU versus 5.5 NTU). Increased turbidity was a direct result of chironomid activity, as inspection of these beakers indicated considerable reworking of the upper 10-15 mm of sediment by C. riparius. The concentration of BAP in water increased with chironomid density and was highly correlated with turbidity ($r^2=0.86$; $p<0.01$). Levels of BAP in C. riparius were also affected by chironomid density as BAP in chironomids from the 40- and 80-chironomid treatments was significantly greater than in the 20-chironomid treatment ($p=0.0130$).

Importance of Food and Water as Sources of BAP to Bluegill

Concentration of BAP in sediments from the CA, CP, and CP/S treatments ranged from 3910 to 4290 ug/Kg and did not vary significantly among treatments (Fig. 4). Levels of BAP in water from treatments where fish had direct contact with sediments (CP and CA) were similar and significantly higher than in the CP/S treatment ($p=0.0038$).

Tissue levels of BAP in C. riparius from the CP and CP/S treatments were similar (Fig. 4). Tissue concentrations of BAP in Lepomis macrochirus (whole fish) were significantly greater ($p=0.007$) in containers where fish actively foraged on chironomids (CP). Despite significantly reduced BAP levels in water, BAP in fish from the CP/S treatment was not significantly different from the CA treatment.

2. Uptake and Food Chain Transfer of Heavy Metals

Concentrations of Heavy Metals in Water

Concentrations of Cd, Cu, and Zn at the Arkansas River varied among locations and among seasons (Fig. 6). Zinc was the dominant metal measured at all stations on all sampling occasions. Levels of Zn at stations immediately downstream from Leadville Mine Drainage Tunnel (LMDT) (EF5, EF6) and California Gulch (CG) (AR3) ranged from 205 ug/L to 8624 ug/L. Levels of Cd were also elevated downstream from both sources

of metals; however, Cu concentrations were not influenced by input from LMDT. Concentrations of Cd, Cu, and Zn at station EF1 were generally higher than EF2. The source of metals at station EF1 is not known.

Metal concentrations at stations AR1 and AR2 were elevated above background levels due to input from LMDT. Because of dilution provided by Tennessee Creek, levels of Cd and Zn were generally lower at these two stations compared to EF5 and EF6. Concentrations of most metals were reduced at station AR8, but generally remained above reference station values. An exception to this pattern occurred during spring 1991 when levels of all metals remained elevated at this downstream site.

Seasonal variation in metal concentrations was observed at all stations. In particular, during spring 1991 levels of metals were greatly elevated at all stations downstream from CG. The greatest seasonal variation was observed at station AR3, where levels of Cd, Cu, and Zn were 48X, 107X, and 24X greater in spring 1991 compared to fall 1990.

Metal Concentrations in Aufwuchs and Benthic Invertebrates

The order of metal concentrations in aufwuchs and macroinvertebrates paralleled those in water (Fig. 7; Appendix I). Metals in benthic organisms were generally higher at downstream contaminated stations (AR-3 and AR-5) compared to upstream reference stations (EF-1, AR-1, and AR-2). Despite greatly reduced levels in water at station AR-5 compared to AR-3, concentrations of metals in aufwuchs and most invertebrate taxa remained elevated and often increased at station AR-5. Metal concentrations in some taxa remained elevated at AR-8, the furthest downstream station. For example, concentrations of Zn and Cd in Baetis spp. were significantly higher at station AR-8 compared to AR-1 during May 1991 and September 1990, respectively. In addition, concentrations of copper were higher in Arctopsyche grandis at AR-8 during September 1990.

On a few occasions metal levels were higher at upstream stations compared to downstream stations. This was most frequently observed in the spring, when levels in water were generally greatest. Most notable were the elevated levels of Zn in Baetis spp. and aufwuchs at AR-1 (spring), Cd and Cu in aufwuchs at EF-1 (spring), Cu in

Pteronarcella badia at AR-2 (spring), and Cd in Rhyacophila spp. at AR-2 (fall).

Significant correlations were observed between metal concentrations in water and aufwuchs (Cd: $r=0.89$, $p=0.04$; Cu: $r=0.81$, $p=0.05$; Zn: $r=0.89$, $p=0.02$). Significant correlations between concentrations of metals in water and some invertebrate taxa were also observed, however, this relationship varied considerably among taxa, metals, and sampling periods.

As with concentrations of metals in water, there was considerable seasonal variability in Cd, Cu, and Zn concentrations in aufwuchs and macroinvertebrates (Fig. 8). Although results of one-way ANOVA indicated that metal levels in benthic communities were generally elevated in the spring, this was dependent on stations, taxa, and metals. For example, while Cd levels in aufwuchs were higher in spring, Zn and Cu were generally greatest at downstream stations during fall. As noted above, Cd and Cu at EF-1 levels were elevated during spring compared to summer and fall.

Metal concentrations in organisms collected from station AR-5 (fall, spring, summer) varied significantly among taxa (Fig. 8). The highest concentrations were generally found in the mayfly Baetis spp. and the stonefly Pteronarcella badia, whereas the lowest levels were measured in the two predators, Skwala americana and Rhyacophila spp.. Metal levels in Arctopsyche grandis were generally intermediate between these groups.

Feeding Habits of Brown Trout

Aquatic insects were the dominant prey in the diet of brown trout (Salmo trutta) collected from stations AR1 and AR5 at the Arkansas River (Figs. 9, 6; Appendix II.) Ephemeroptera (Baetis spp., Ephemerella sp.), Plecoptera (Prostoia besametsa, Skwala americana), Trichoptera (Arctopsyche grandis, Rhyacophila spp.), and Chironomidae (Orthocladiinae) dominated the diet of fish collected on all sampling occasions and accounted for between 40-95% of all prey. In particular, Baetis spp. was frequently found in the diet of fish from both stations and on all dates.

Differences in feeding habits of Salmo trutta between upstream and downstream stations were observed (Fig. 10). In particular, mayflies were more common in the diet of

fish collected from AR1 compared to AR5. In contrast, caddisflies and Orthocladiinae were more common in the diet of fish collected downstream from CG.

Differences in feeding habits of S. trutta between stations were a direct result of differences in prey availability. Abundance of Ephemeroptera was greater at the upstream reference station, whereas Trichoptera and Orthocladiinae were more abundant downstream of California Gulch.

Metal Concentrations in Brown Trout

Concentrations of Cd, Cu, and Zn in brown trout tissue varied between stations and dates (Fig. 11). Metal concentrations in liver and kidney tissue were generally similar at stations AR1 and AR5 or greater at the upstream station. In contrast, metal concentrations in gill and gut tissue were often greater at the downstream station. Concentrations and the distribution of metals among different brown trout tissues were similar between summer and fall samples.

DISCUSSION

Bioaccumulation and Food Chain Transfer of PAHs

Results of these experiments demonstrate that sediments spiked with PAHs at concentrations commonly observed at impacted field sites (Eisler 1987) are a potential source of contamination to benthic invertebrates and fish. The increased levels of FLU and BAP in Chironomus riparius with higher sediment concentration observed in our study has been reported for amphipods exposed to these contaminants (Landrum et al. 1991). These researchers suggest that increased uptake of PAHs with increased sediment concentrations may have resulted from an increase in the fraction of contaminants that are bioavailable.

C. riparius bioaccumulated FLU to a much greater extent than BAP. The highest concentration of FLU in chironomids was 140,950 ug/Kg, compared with a maximum concentration of 6,030 ug/Kg for BAP. Bioaccumulation factors (concentration in C. riparius/concentration in sediment) for FLU and BAP based on these results were 31.0 and 2.2, respectively. Leversee et al. (1982) reported low bioaccumulation of BAP by C. riparius and noted the ability of these organisms to rapidly metabolize this compound. Lower bioaccumulation of BAP by C. riparius may have resulted from low uptake rate. Landrum et al. (1991) reported that uptake rates of FLU and BAP by amphipods were inversely related to log K_{ow} values (5.20 and 5.98, respectively).

Although it is generally recognized that interstitial water is the major source of sediment-associated PAHs (Swartz et al. 1990), ingestion of contaminated sediments is also a potential route of exposure for benthic organisms, particularly for strongly sorbed materials such as BAP (Landrum 1989). Dietary exposure to sediment-associated contaminants will most likely be greater in organisms feeding on highly enriched sediment particles. C. riparius is a deposit feeder, consuming large amounts of silt and microdetritus (Rasmussen 1984), and therefore it is expected that dietary uptake of BAP in these organisms would be significant.

Results of these experiments indicate that food chain transfer of PAHs from chironomids to Lepomis macrochirus was highly inefficient. Despite high concentrations

of FLU measured in chironomids, FLU was detected only in bluegill gut tissue at the two highest doses. Similarly, BAP was not detected in fish which were individually fed BAP-spiked chironomids. The low dietary assimilation of PAHs by bluegill may have resulted from the short duration (96 h) of these experiments. However, other researchers have also noted that dietary uptake of PAHs is relatively insignificant compared to uptake from water (Jimenez et al. 1987; Niimi and Dookhran 1989).

Bioturbation by C. riparius

Benthic invertebrates interact directly with sediments and have the potential to influence the fate and movement of sediment-associated contaminants (Reynoldson 1987). The role that these organisms play in releasing materials into the water column has received little attention. Burrowing activity of tubificid worms (Limnodrilus hoffmeisteri and Tubifex tubifex) was found to release metals (Boddington et al. 1979) and organics (Karickhoff and Morris 1985) from sediments. Results of our bioturbation experiment indicate that activities of C. riparius, such as feeding, tube-building, and burrowing, disturbed the upper 10-15 mm of sediment. The observed decrease in water clarity and increase in BAP concentration in water with chironomid density support the hypothesis that activity of these organisms was directly responsible for remobilization of these contaminants. The presence of BAP in water from the no-chironomid treatment may have resulted from either leaching from the sediments or disturbance when the beakers were filled with water.

These results have potential implications for field studies at sites impacted by PAHs. Because of the low toxicity of high-molecular weight PAHs such as BAP (Eisler 1987), chironomids, which are tolerant to a wide variety of contaminants (Wiederholm 1984), may thrive in areas with relatively high sediment BAP levels. Densities of C. riparius used in our experiments (2,500-10,000 m^{-1}) were within the range of those reported in the field (Gower and Buckland 1978). We suggest that bioturbation by chironomids and other benthic invertebrates in the field may release large amounts of sediment-associated contaminants into the water column.

The significantly greater concentrations of BAP in C. riparius from the 40- and 80-

chironomid treatments compared to the 20-chironomid treatment may have resulted from increased BAP in water, suggesting that these resuspended contaminants are bioavailable. Alternatively, increased BAP in chironomids at higher densities may have resulted from greater activity of these organisms. Landrum et al. (1991) speculated that increased movement of organisms may increase rate of contaminant accumulation. If C. riparius were more active at higher density, then uptake rates may have increased. Regardless of the specific mechanism, these results have important implications for the design of bioaccumulation studies using benthic invertebrates. If the density of organisms in test chambers affects bioaccumulation, then densities must be standardized among studies.

Uptake of BAP from Food and Water

The relative importance of dietary accumulation of PAHs versus uptake from water is controversial. Some researchers have suggested that uptake from food is important (Dobroski and Epifanio 1980), whereas others have shown that because of low absorption efficiency dietary uptake of PAHs is insignificant relative to uptake from water (McCarthy and Jimenez 1985; Jimenez et al. 1987; Niimi and Dookhran 1989). Because concentrations of PAHs in sediments may be orders of magnitude higher than levels in overlying water (Neff 1979; Eisler 1987), and because benthic invertebrates readily accumulate these materials from sediments (Reichert et al. 1985; Landrum 1989; Landrum et al. 1991; this study), we suggest that dietary uptake by benthic feeding fish may be a significant route of exposure. Incidental ingestion of highly contaminated sediments by benthic feeding fish may also contribute to total body burdens in these organisms (Niimi and Dookhran 1989).

Results of our experiments measuring uptake from food and water simultaneously indicate L. macrochirus accumulated BAP from both sources. The highest levels of BAP in fish were observed in treatments where fish had direct contact with sediments and could actively forage on chironomids (e.g. CP treatment). The large difference in accumulation between the CP and CA/S treatments (121±69 µg/Kg versus 6±5 µg/Kg) suggests that food was the predominant source of BAP. However, our inability to detect

BAP in bluegill during our initial experiments, when fish were exposed only to BAP-spiked chironomids, indicates that direct contact with sediments and/or uptake from water were also important. Direct contact with sediments may have been important in the CA treatment, as fish were observed searching for prey and disturbing sediments.

Levels of BAP in water from the CP and CA treatments were similar. This was unexpected based on previous results showing that chironomids increased levels of BAP in water. The low levels of BAP in water from the CP treatment may have resulted from reduced activity of chironomids in the presence of bluegill. The significantly reduced levels of BAP in the CP/S treatment was most likely a result of reduced water movement across the screen.

Although sediments are an important sink for contaminants in aquatic systems, results of our laboratory experiments indicate that these contaminants may be mobilized and made available to aquatic organisms. Bioturbation and food chain transfer, two important processes mediated by benthic invertebrates, increased availability of sediment-associated PAHs. The pathway of contaminant transfer described in these experiments (sediments to chironomids to bluegill) is realistic, as chironomids comprise a significant portion of the diet of juvenile bluegill in nature (Etnier 1971). Therefore these results may have important implications for field studies. Information concerning the importance of these processes is necessary for evaluating the potential impact of contaminated sediments on aquatic organisms and for implementing remediation procedures.

Metals in Aufwuchs and Benthic Invertebrates

Results of this study demonstrate that metal concentrations in benthic communities at the Arkansas River varied among stations, seasons, and taxa. Depending on taxa and season, Cd, Cu, and Zn were bioavailable for many Km downstream from California Gulch. Moore et al. (1991) reported similar trends at the Blackfoot River (MT) where significant Cd contamination was observed in stoneflies and brown trout more than 75 km downstream from the input of acid mine drainage. Zinc was also observed to be accumulated in some species in the middle reaches of this system.

The concentrations of Cd, Cu, and Zn in aufwuchs from the Arkansas River were

generally higher than macroinvertebrates at all stations. Selby et al. (1985) also observed higher concentrations of Cd in aufwuchs compared to macroinvertebrates and concluded that 75% of the Cd accumulated was associated with abiotic material. Procedurally-defined aufwuchs is a complex mixture of abiotic and biotic components (Newman et al. 1989) and both of these materials concentrate metals. I suggest that metals bound to the abiotic portion of aufwuchs are also bioavailable and may represent a significant source of metals to organisms associated with this material.

Variation in metal concentrations among taxa observed in our study was most likely a result of differences in feeding habits. In general, organisms associated with aufwuchs (collector-gatherers, collector-filterers, grazers) bioaccumulated more metals than predators. These results were similar to other studies that separated organisms according to feeding type (Strong and Luoama 1981; Smock 1983; Burrows and Whitton 1983; Selby et al. 1985; Krantzberg 1989). In particular, the mayfly Baetis spp., which feeds on periphyton and detritus, accumulated significantly more Zn and Cd than other taxa. Burrows and Whitton (1983) found that mayflies as a group (Baetis spp., Rhithrogenia spp., Ecdyonurus venosus, and Ephemerella ignita) accumulated more Zn, Cd, and Pb than other animals in the metal-contaminated River Derewent, England. The higher metal concentrations in Baetis spp. observed in our study was probably a result of exposure to and ingestion of highly-contaminated aufwuchs. In addition, Baetis spp. ingests smaller particles which are often metal-enriched. In contrast to our findings, recent studies have observed predaceous aquatic invertebrates accumulated more metals than other functional groups (Lynch et al. 1988). These results may be attributed to the fact that predators are generally longer lived than other aquatic macroinvertebrates. Regardless of the explanation for variation among functional feeding groups, it is apparent this factor has important implications in monitoring the bioaccumulation of metals in lotic ecosystems.

Organism size was another possible factor influencing metal concentrations. In our study, A. grandis was the only organism showing significant, negative correlations between size and metal concentration. In general, correlation coefficients for organism size and metal concentration were variable, with some positive and some negative. In

contrast to our study, Smock (1983) observed a significant, negative correlation with dry weight of an organism and metal concentration when species were pooled. Similar to our findings other studies have observed considerable variation in body size and metal concentration. Strong and Luoma [26] examined the variation in the correlation of body size with concentration of Cu and Ag in the bivalve Macoma balthica. Strong positive and strongly negative relationships between body size and metal concentration were observed. They concluded correlations appeared to be influenced by the degree of enrichment in tissues, size-dependent differences and seasonal variations in growth rate, and size dependent differences in uptake rates. Krantzberg (1989) observed slopes of age and size regressions with metal content varied among elements and were dependent upon the range in age and size considered. The contrasting results presented in this and other studies demonstrates the need to sample a broad range of age and size classes in order to understand metal contamination and accumulation by aquatic insects.

Uptake of Heavy Metals by Brown Trout

Results of this study indicate that brown trout collected from the Arkansas River consumed prey that were highly contaminated with heavy metals. In particular, metals in Baetis spp. and Orthocladiinae chironomids, which comprised a large portion of the diet at both stations, were greatly elevated. In general, levels of metals in water and food were greater at station AR5 compared to AR1 (Fig. 12). As expected, brown trout tissues that were directly exposed to metals in food (e.g. gut tissue) and water (e.g. gills) generally had higher levels of metals at station AR5 compared to AR1. In particular, Zn was significantly elevated in gill and gut tissue at AR5 on both sampling dates.

In contrast to these findings, levels of Cd, Cu, and Zn in liver and kidney tissue were either similar between upstream and downstream stations or elevated at the upstream station. The similar levels of metals in fish from these two stations, despite greater exposure at AR5, suggest that these fish regulated metals in these storage tissues.

Conceptual Model to of Transfer of Metals at the Arkansas River

Based on results of this investigation, I propose a conceptual model to explain the distribution and transfer of heavy metals at the Arkansas River system (Fig. 13). Levels of metals in aufwuchs (defined as periphyton, algae, and associated abiotic material) were much higher than any other compartment. I suggest that dissolved metals and metals associated with particulate materials were most likely the primary source of contaminants to this material. Levels in organisms directly associated with aufwuchs (e.g. Baetis, and Orthocladiinae chironomids) were also greatly elevated. I suggest that this route (water-----> aufwuchs-----> Baetis and Orthocladiinae) was a primary pathway for the movement of metals in the Arkansas River system. Because of the high levels of metals in Baetis and Orthocladiinae, and because these organisms comprised a significant portion of the diet of brown trout, I suggest that these organisms were an important source of metals to Salmo trutta.

Aufwuchs communities represent a primary sink for metals at the Arkansas River system. Although remedial action at California Gulch is expected to reduce concentrations of heavy metals in water, levels present in contaminated sediments and periphyton may continue to impact this system. Consequently, bioaccumulation of heavy metals by benthic invertebrates and subsequent transfer to brown trout may continue following these cleanup activities. Continued research on the relative importance of diet and water as sources of metals to Salmo trutta will allow evaluation of the effectiveness of remediation at the Arkansas River.

Future Research

Laboratory experiments are currently being conducted to determine the relative importance of aufwuchs and sediments as sources of metal exposure to benthic invertebrates and brown trout. Benthic communities collected from a pristine Colorado stream were exposed to Cd in laboratory microcosms. A two-way experimental design was employed to estimate the relative importance of periphyton and water as sources of metals to benthic invertebrates. The relative importance of food and water as sources of metals in brown trout will be determined by exposing fish to one of four treatments:

controls, food only, water only, food and water. Trout in "water only" streams will be exposed to water collected from California Gulch. Fish in the "food only" streams will be fed benthic invertebrates collected impacted stations at the Arkansas River. Trout in "food and water" streams will be exposed to heavy metals in water and also fed contaminated prey.

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Figure Legends

Figure 1. Levels of fluoranthene in sediment, chironomids, and bluegill from control, low, medium, and high fluoranthene treatments. Treatments with the same letter were significantly different based on results of Duncan's Multiple Range Test.

Figure 2. Levels of benzo(a)pyrene in sediment, chironomids, and fish. Treatments with the same letter were significantly different.

Figure 3. Levels of BAP in sediment, water, and chironomids in microcosms at different chironomid densities. Treatments with the same letter were significantly different based on results of Duncan's Multiple Range Test.

Figure 4. Concentration of BAP in sediment, water, chironomids, and fish from the chironomids-absent (CA), chironomids present (CP), chironomids-present/screen (CP/S) treatments. Treatments with the same letter were significantly different based on results of Duncan's Multiple Range Test.

Figure 5. Map of sampling stations at the upper Arkansas River, CO.

Figure 6. Metal concentrations in water at sampling stations at the Arkansas River. Arrows indicate sources of metals from Leadville Tunnel and California Gulch.

Figure 7. Mean concentrations of Cd, Cu, and Zn in aufwuchs and dominant macroinvertebrate taxa at the Arkansas River. Bars with the same letter were not significantly different ($p<0.05$).

Figure 8. Variation in metal concentrations among dominant taxa and seasons.

Figure 9. Feeding habits of brown trout (Salmo trutta) at stations AR1 and AR5.

Figure 10. Percent composition of dominant macroinvertebrate groups in the diet and at in the field at stations AR1 and AR5.

Figure 11. Metal concentrations in brown trout tissue from stations AR1 and AR5 during July, 1991 and September, 1991.

Figure 12. Mean concentrations of metals in water, gills, aufwuchs, prey, and gut of brown trout at stations AR1 and AR5.

Figure 13. Concpetual model of heavy metal transfer at the Arkansas River. The size of the arrows indicates the relative importance of different pathways.

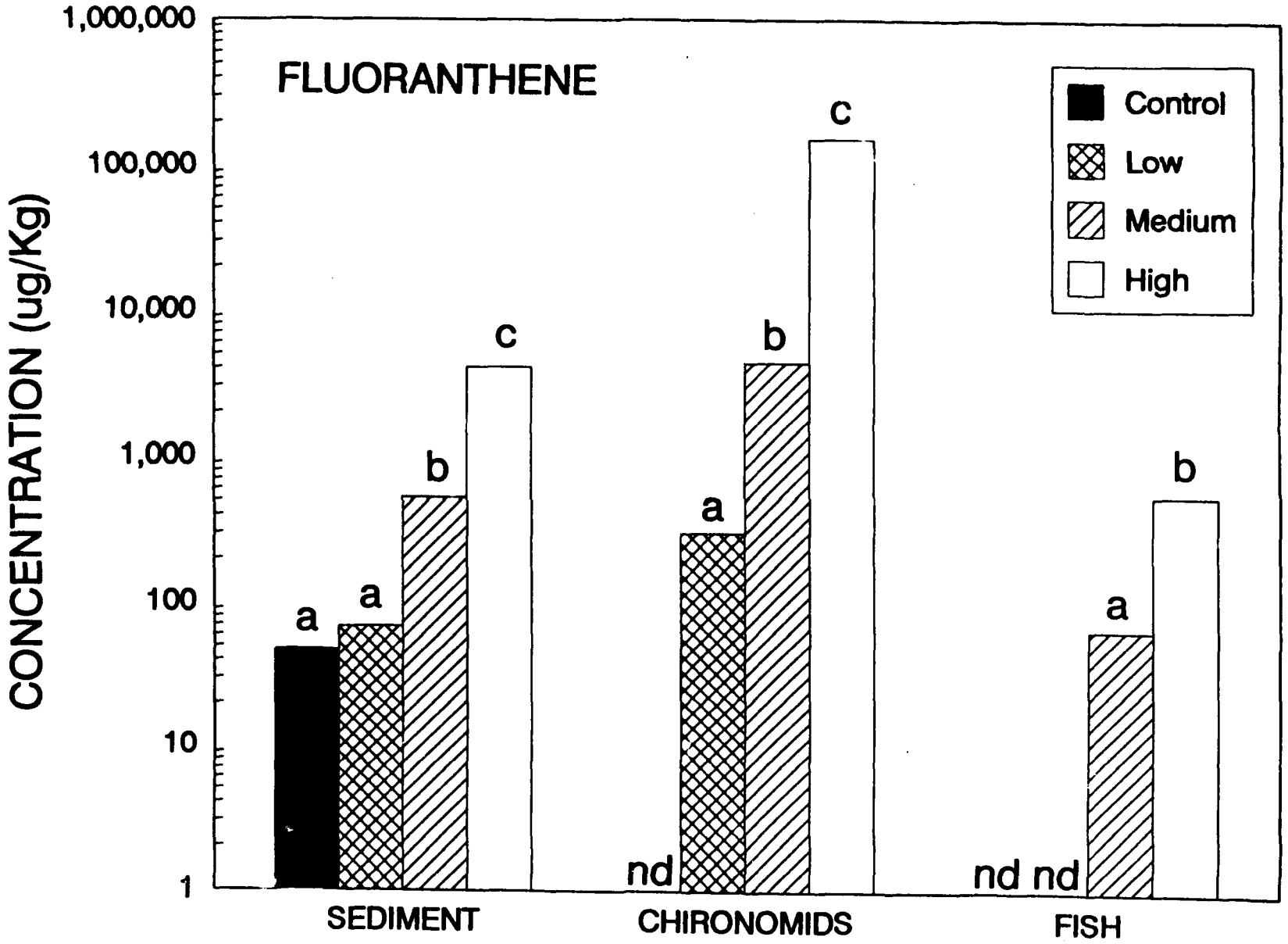

FLUORANTHENE
CONCENTRATION (ug/Kg)
1,000,000
100,000
10,000
1,000
100
10
1
Control
Low
Medium
High
a
a
b
c
nd
a
b
c
nd nd
a
b
SEDIMENT
CHIRONOMIDS
FISH

Figure 1

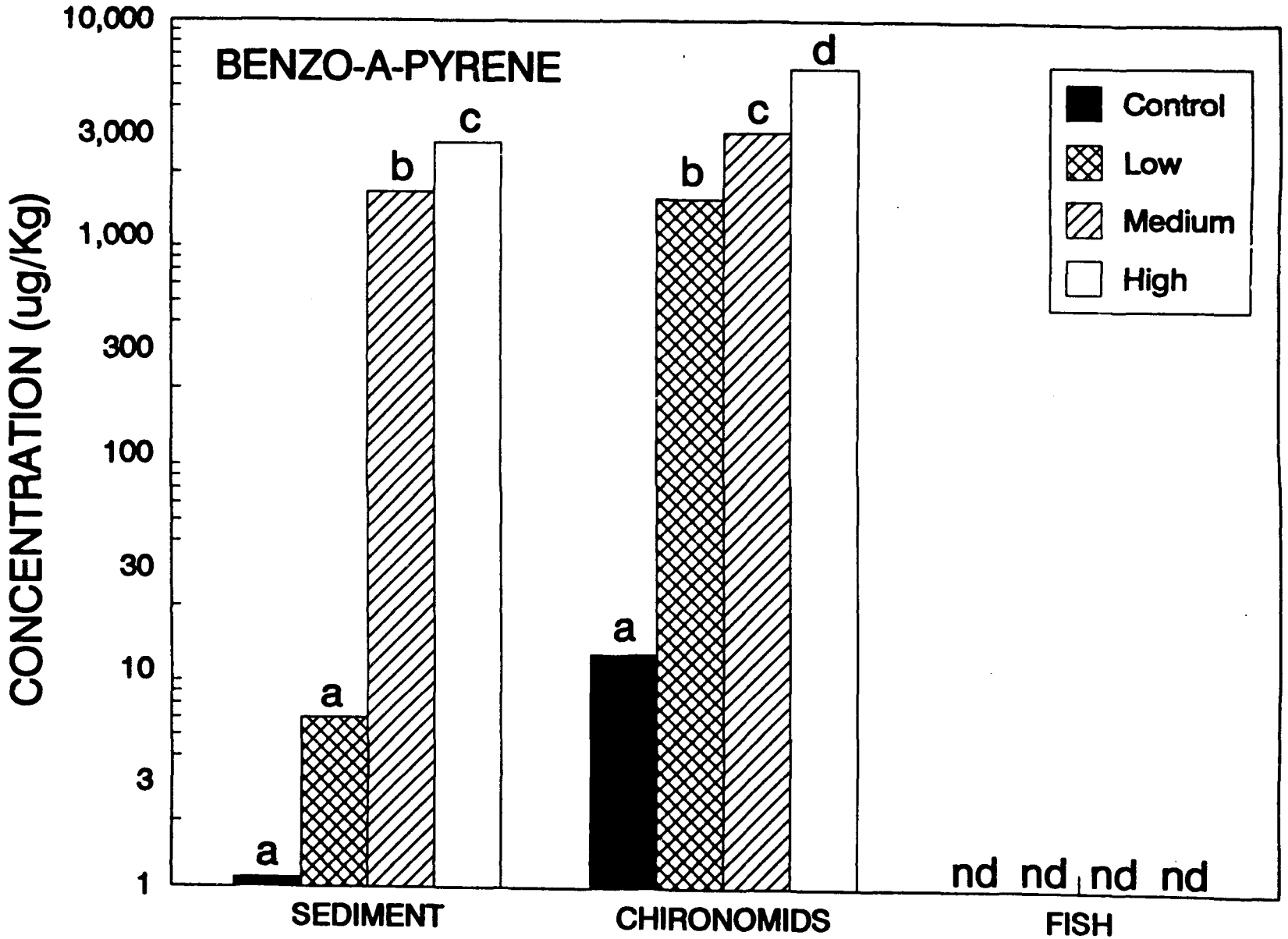

BENZO-A-PYRENE
CONCENTRATION (ug/Kg)
10,000
3,000
1,000
300
100
30
10
3
1
a
a
b
c
a
b
c
d
nd nd nd nd
SEDIMENT
CHIRONOMIDS
FISH
Control
Low
Medium
High

Figure 2

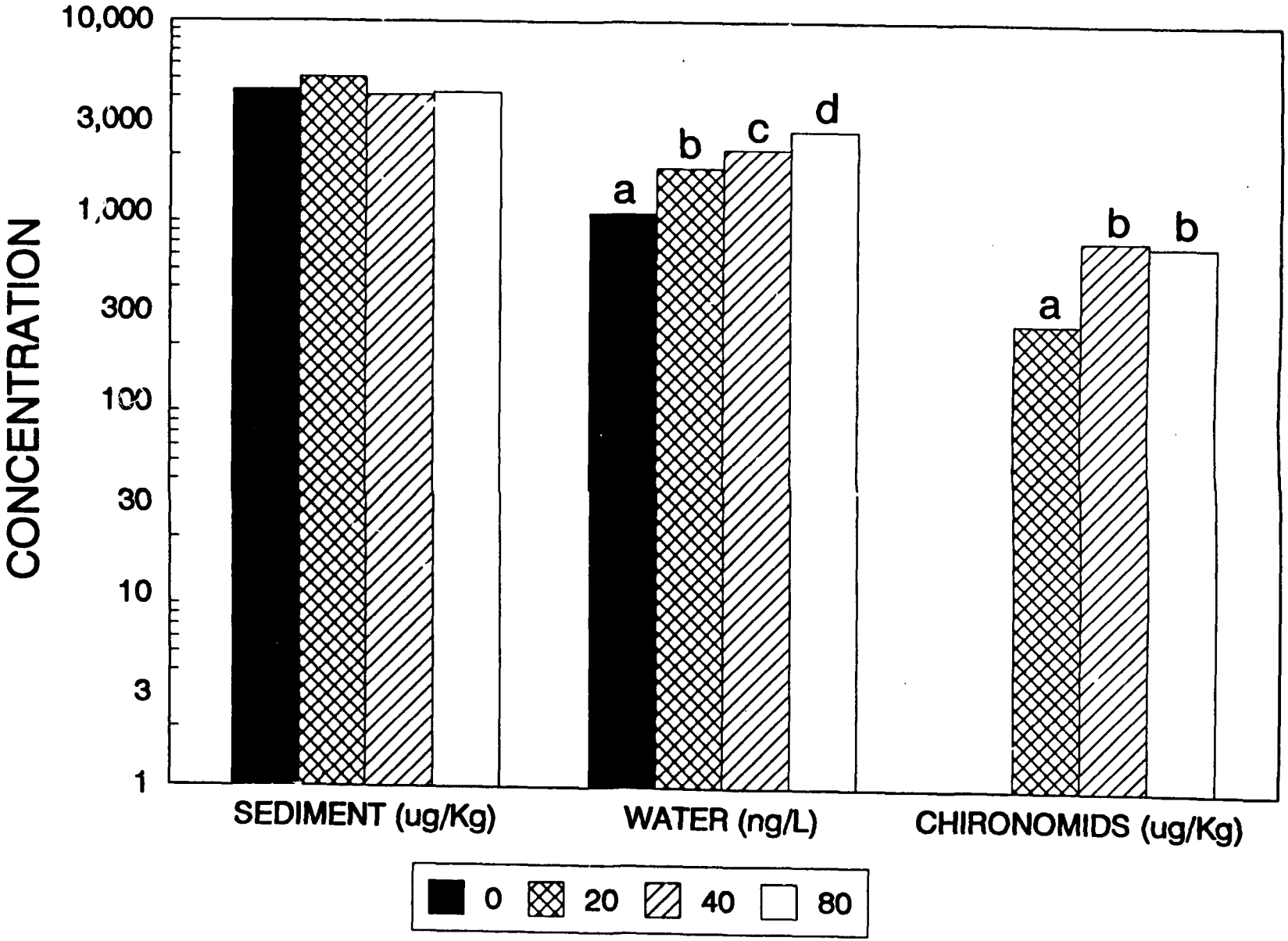

10,000
3,000
1,000
300
100
30
10
3
1
CONCENTRATION
a
b
c
d
a
b
b
SEDIMENT (ug/Kg)
WATER (ng/L)
CHIRONOMIDS (ug/Kg)
0
20
40
80

Figure 2

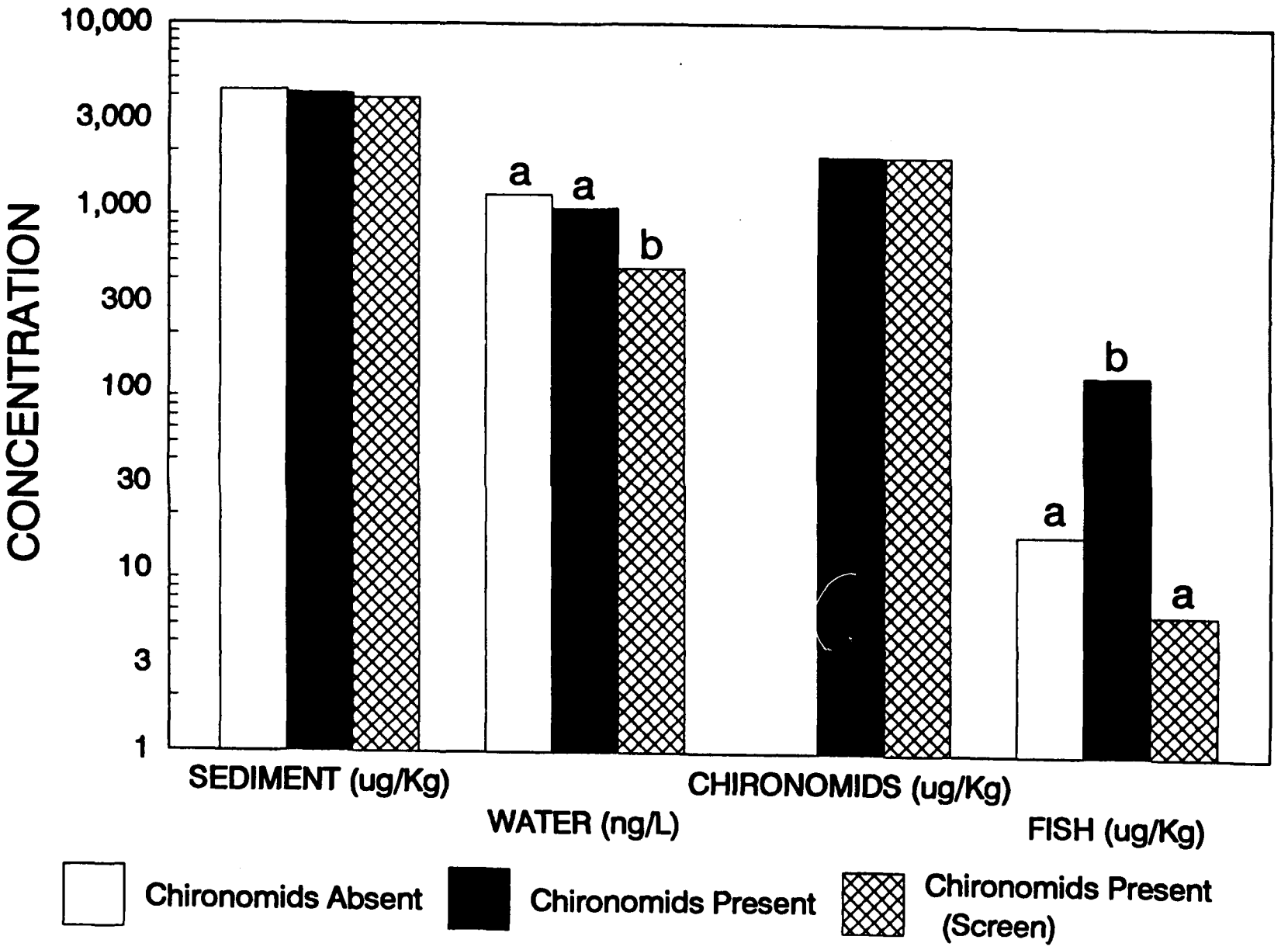
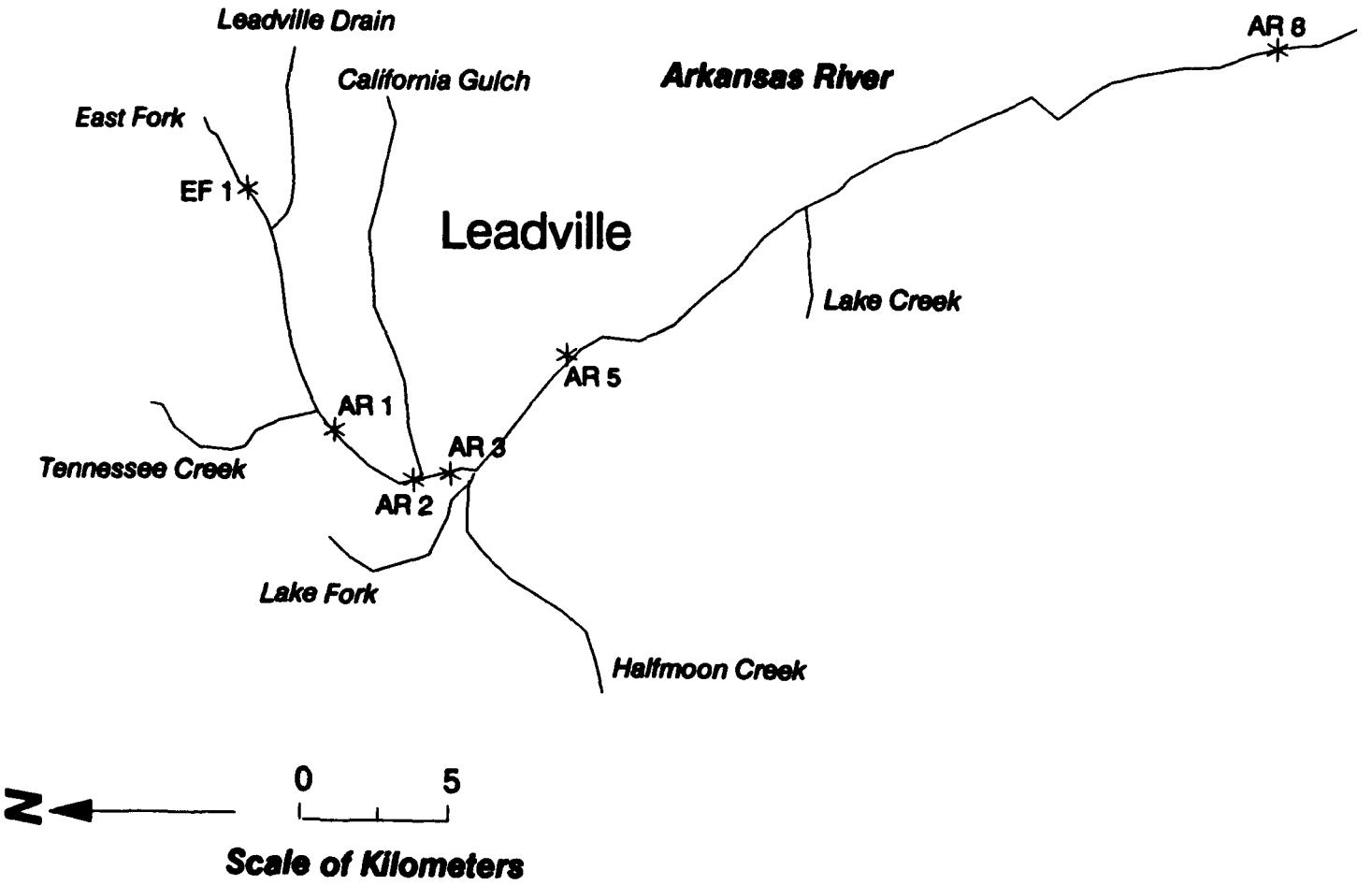

10,000
3,000
1,000
300
100
30
10
3
1
CONCENTRATION
a
a
b
b
a
a
SEDIMENT (ug/Kg)
WATER (ng/L)
CHIRONOMIDS (ug/Kg)
FISH (ug/Kg)
Chironomids Absent
Chironomids Present
Chironomids Present (Screen)

Figure 4.

Upper Arkansas River Basin



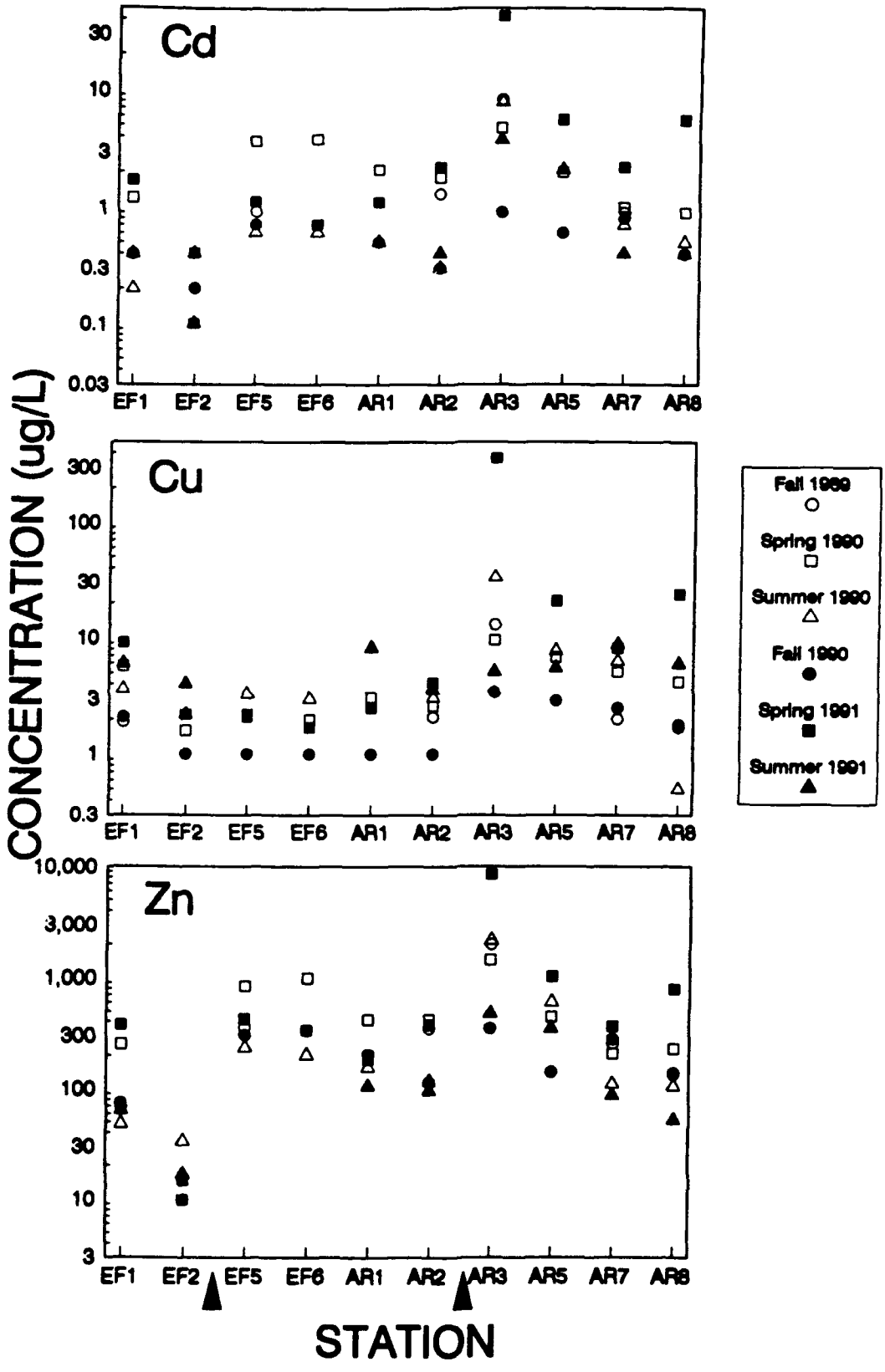

Cd
Cu
Zn
CONCENTRATION (ug/L)
STATION
EF1
EF2
EF5
EF6
AR1
AR2
AR3
AR5
AR7
AR8
Fall 1989
Spring 1990
Summer 1990
Fall 1990
Spring 1991
Summer 1991

Fig. 6

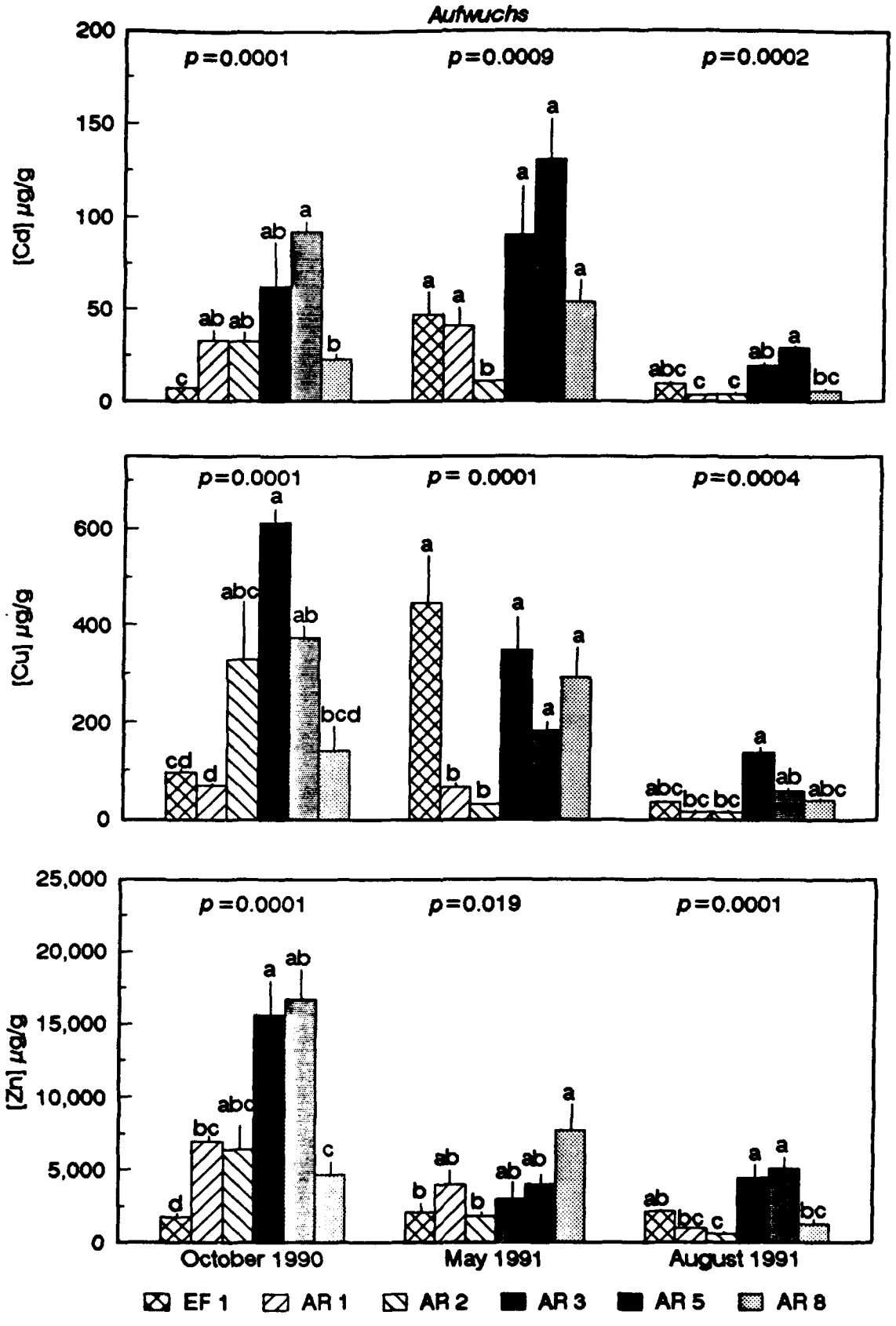

Aufwuchs
p=0.0001
p=0.0009
p=0.0002
[Cd] µg/g
200
150
100
50
0
p=0.0001
p= 0.0001
p=0.0004
[Cu] µg/g
600
400
200
0
p=0.0001
p=0.019
p=0.0001
[Zn] µg/g
25,000
20,000
15,000
10,000
5,000
0
October 1990
May 1991
August 1991
EF 1
AR 1
AR 2
AR 3
AR 5
AR 8

Fig. 7

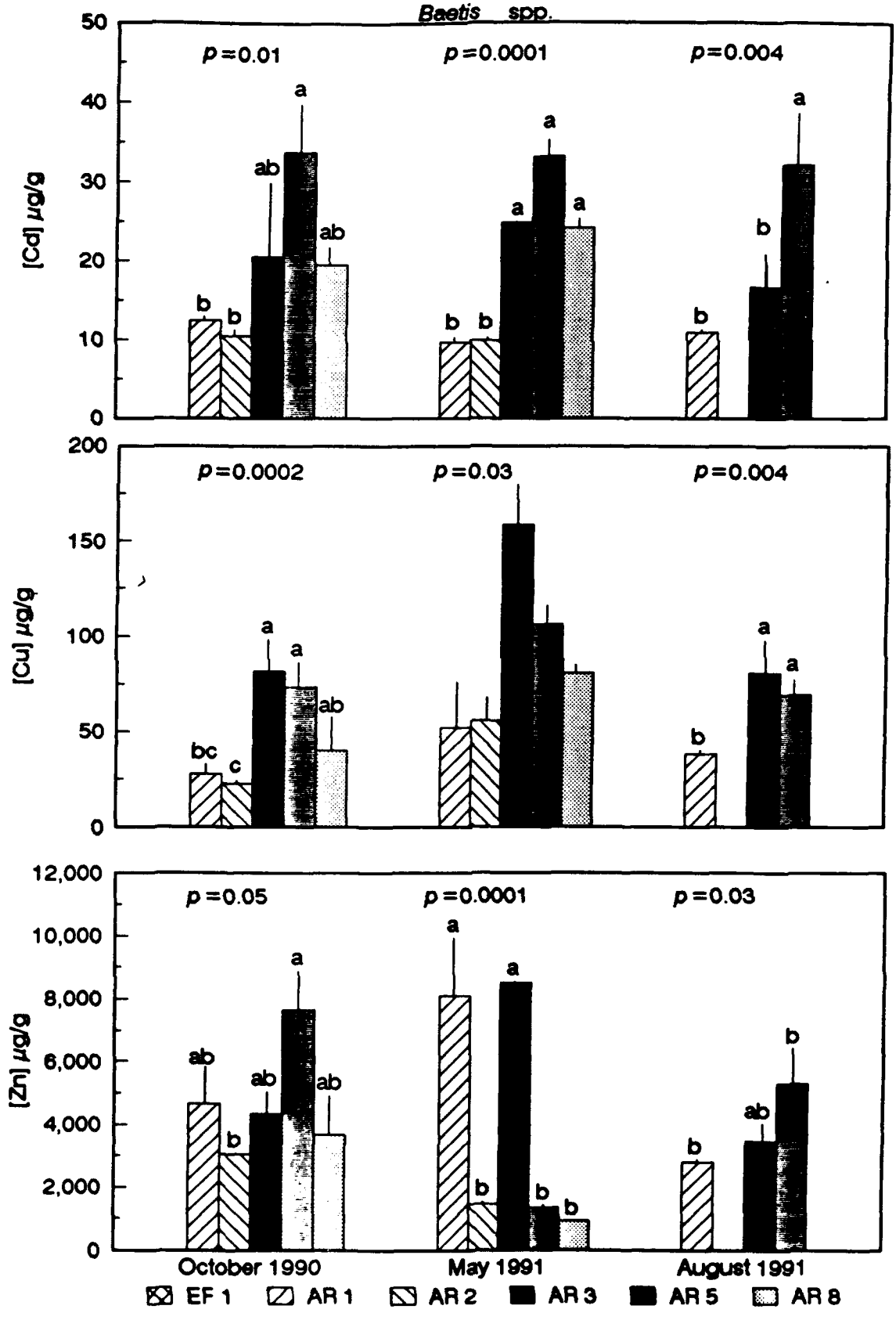

Baetis spp.
p=0.01
p=0.0001
p=0.004
[Cd] µg/g
50
40
30
20
10
0
b
b
ab
a
ab
b
b
a
a
a
b
b
a
p=0.0002
p=0.03
p=0.004
[Cu] µg/g
200
150
100
50
0
bc
c
a
a
ab
b
a
a
p=0.05
p=0.0001
p=0.03
[Zn] µg/g
12,000
10,000
8,000
6,000
4,000
2,000
0
ab
b
ab
a
ab
b
a
b
b
b
b
ab
b
October 1990
May 1991
August 1991
EF 1
AR 1
AR 2
AR 3
AR 5
AR 8

Fig. 7

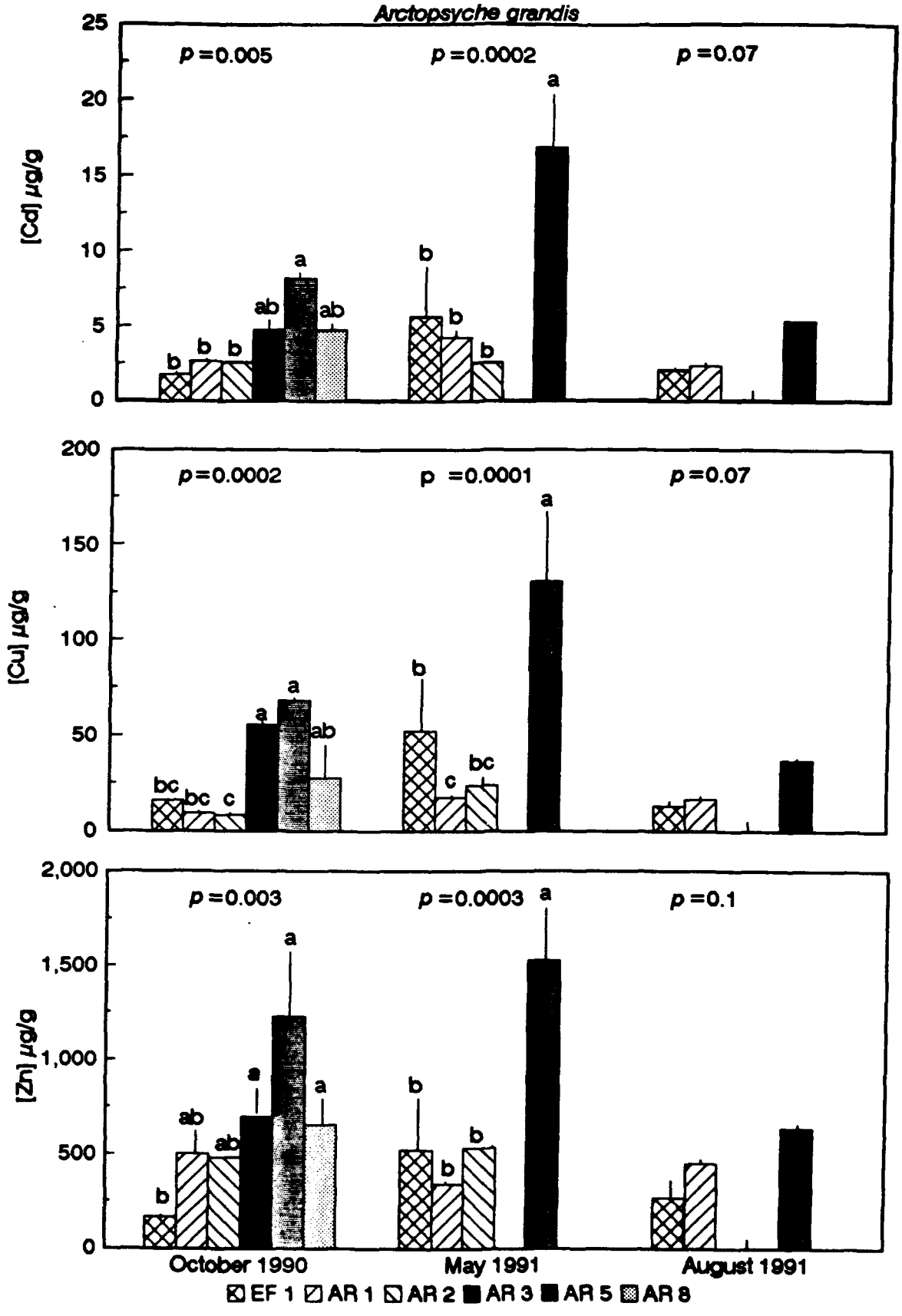

Arctopsyche grandis
p =0.005
p =0.0002
p =0.07
[Cd] µg/g
25
20
15
10
5
0
b b b ab a ab
b b b
a
p =0.0002
p =0.0001
p =0.07
[Cu] µg/g
200
150
100
50
0
bc bc c a a ab
b c bc
a
p =0.003
p =0.0003
p =0.1
[Zn] µg/g
2,000
1,500
1,000
500
0
b ab ab a a
b b b
a
October 1990
May 1991
August 1991
EF 1 AR 1 AR 2 AR 3 AR 5 AR 8

Fig. 7

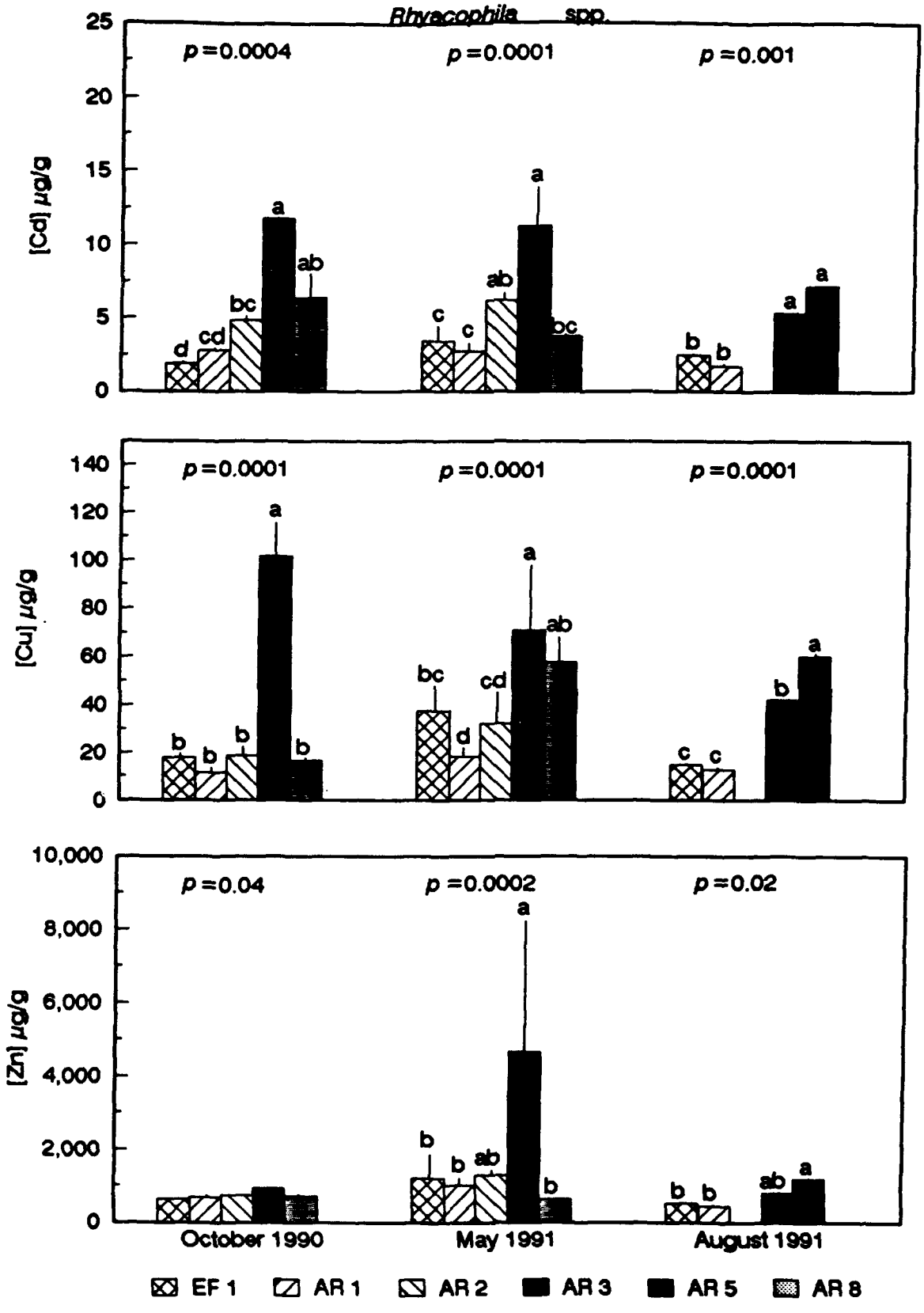

Rhyacophila spp.
p =0.0004
p =0.0001
p =0.001
[Cd] µg/g
25
20
15
10
5
0
d cd bc a ab
c c ab a bc
b b a a
p =0.0001
p =0.0001
p =0.0001
[Cu] µg/g
140
120
100
80
60
40
20
0
b b b a b
bc d cd a ab
c c b a
p =0.04
p =0.0002
p =0.02
[Zn] µg/g
10,000
8,000
6,000
4,000
2,000
0
b b ab a b
b b ab a
October 1990
May 1991
August 1991
EF 1
AR 1
AR 2
AR 3
AR 5
AR 8

Fig. 7

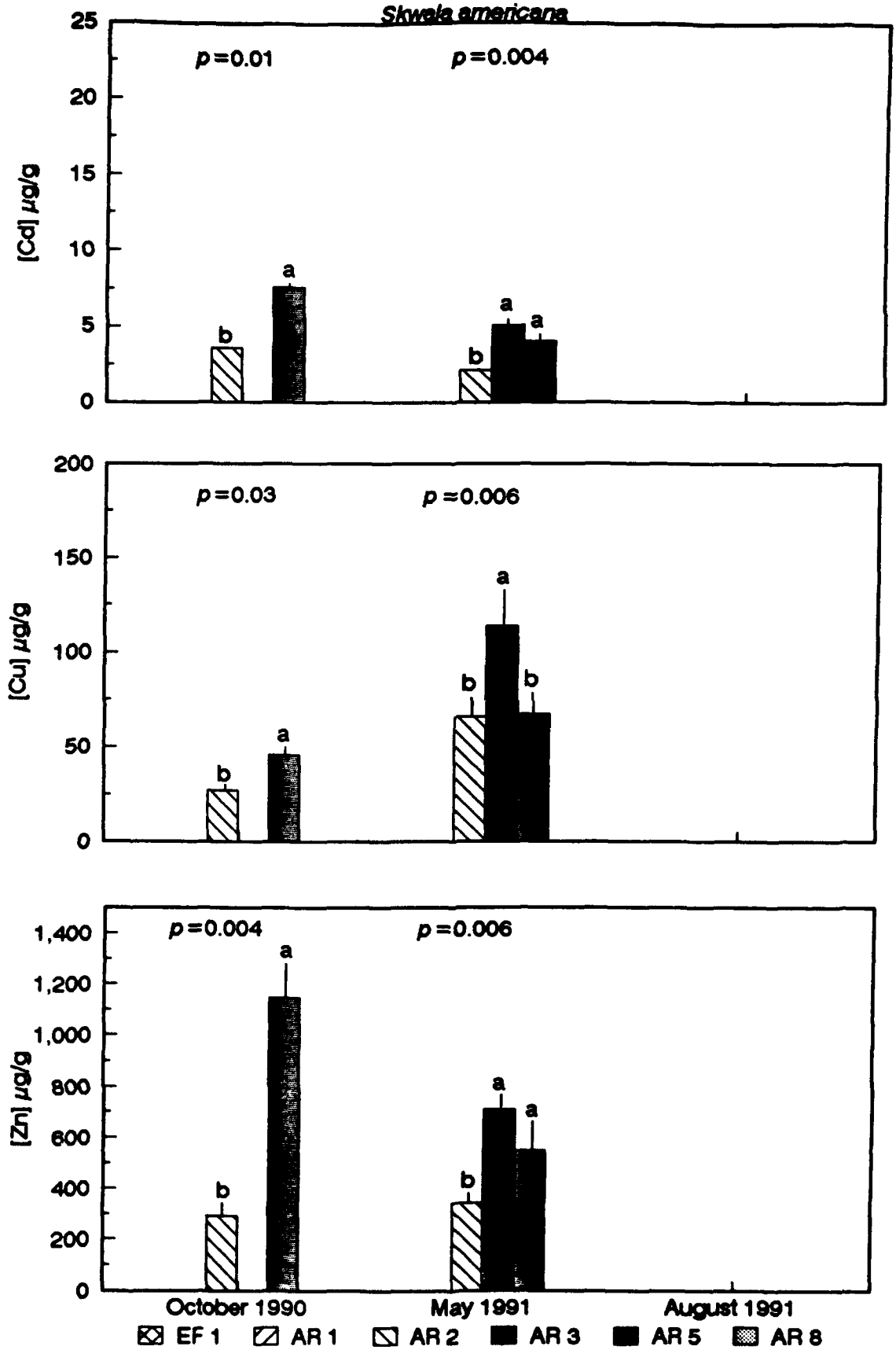

Skwala americana
p=0.01
p=0.004
[Cd] µg/g
25
20
15
10
5
0
a
b
a
b
a
p=0.03
p=0.006
[Cu] µg/g
200
150
100
50
0
a
b
a
b
b
p=0.004
p=0.006
[Zn] µg/g
1,400
1,200
1,000
800
600
400
200
0
a
b
a
b
a
October 1990
May 1991
August 1991
EF 1
AR 1
AR 2
AR 3
AR 5
AR 8

Fig. 7

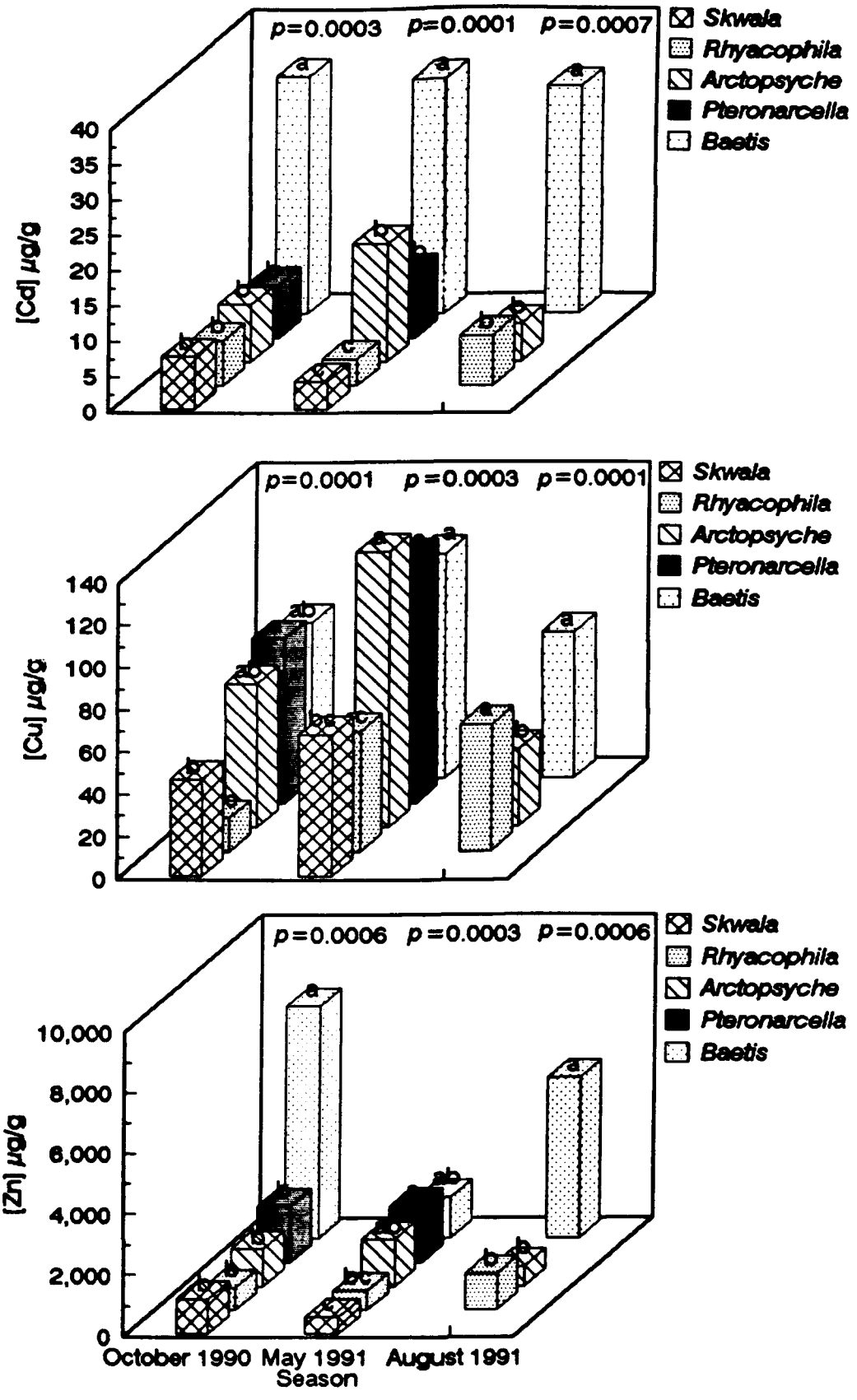
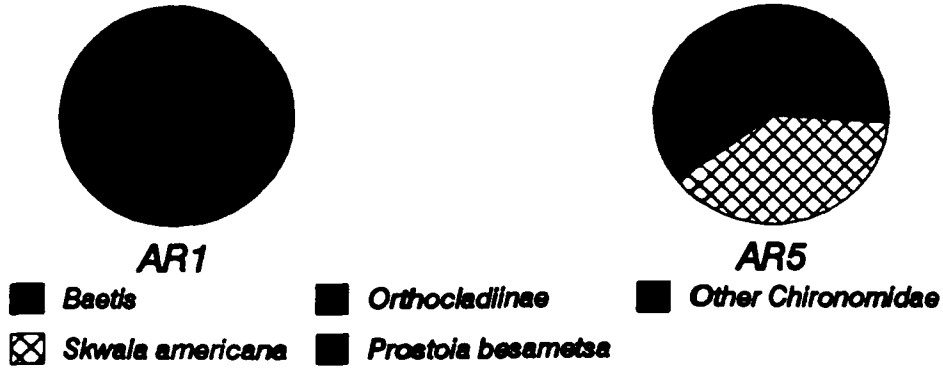

p=0.0003 p=0.0001 p=0.0007
[Cd] µg/g
40
35
30
25
20
15
10
5
0
Skwala
Rhyacophila
Arctopsyche
Pteronarcella
Baetis
p=0.0001 p=0.0003 p=0.0001
[Cu] µg/g
140
120
100
80
60
40
20
0
p=0.0006 p=0.0003 p=0.0006
[Zn] µg/g
10,000
8,000
6,000
4,000
2,000
0
October 1990 May 1991 August 1991
Season

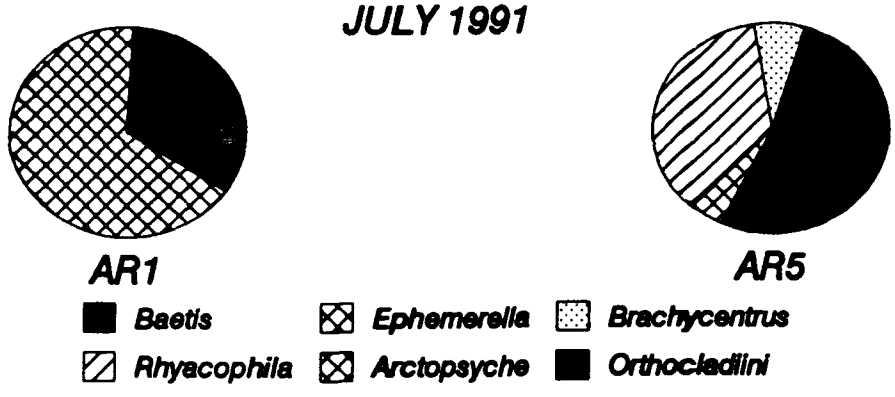
Fig. 8

FEEDING HABITS OF BROWN TROUT

APRIL 1991

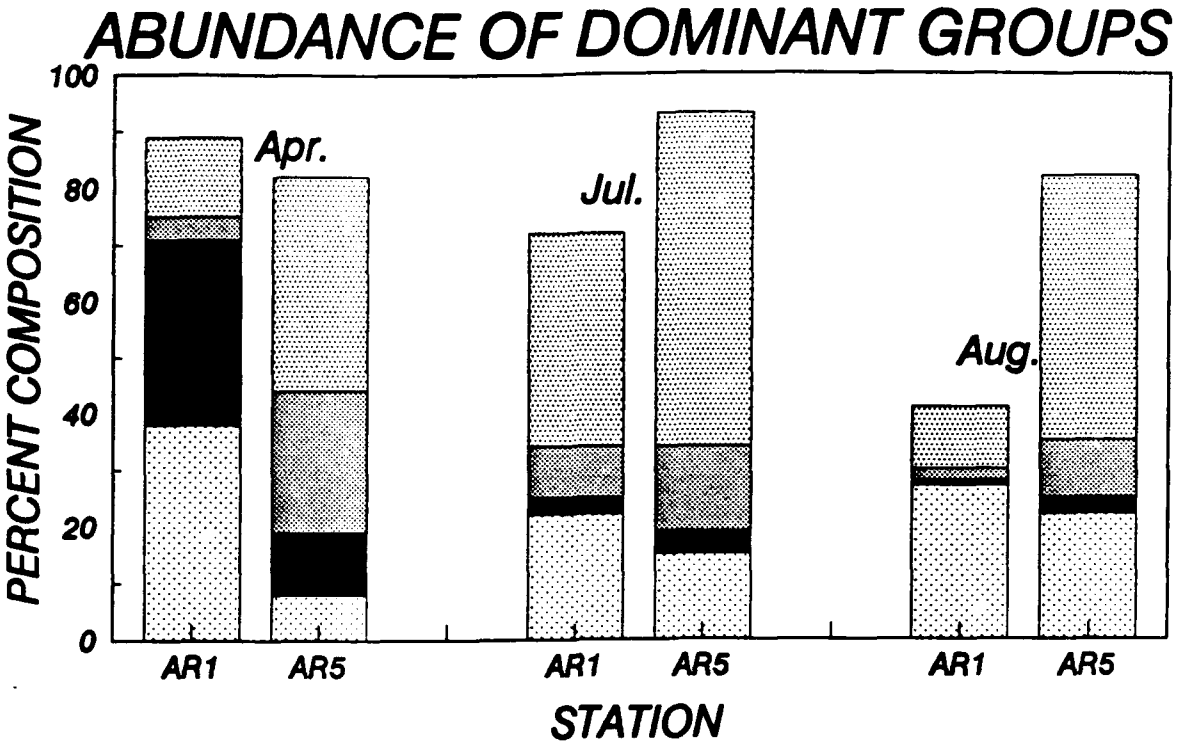


JULY 1991



August 1991

Fig 9


ABUNDANCE OF DOMINANT GROUPS
PERCENT COMPOSITION
100
80
60
40
20
0
Apr.
Jul.
Aug.
AR1
AR5
AR1
AR5
AR1
AR5
STATION

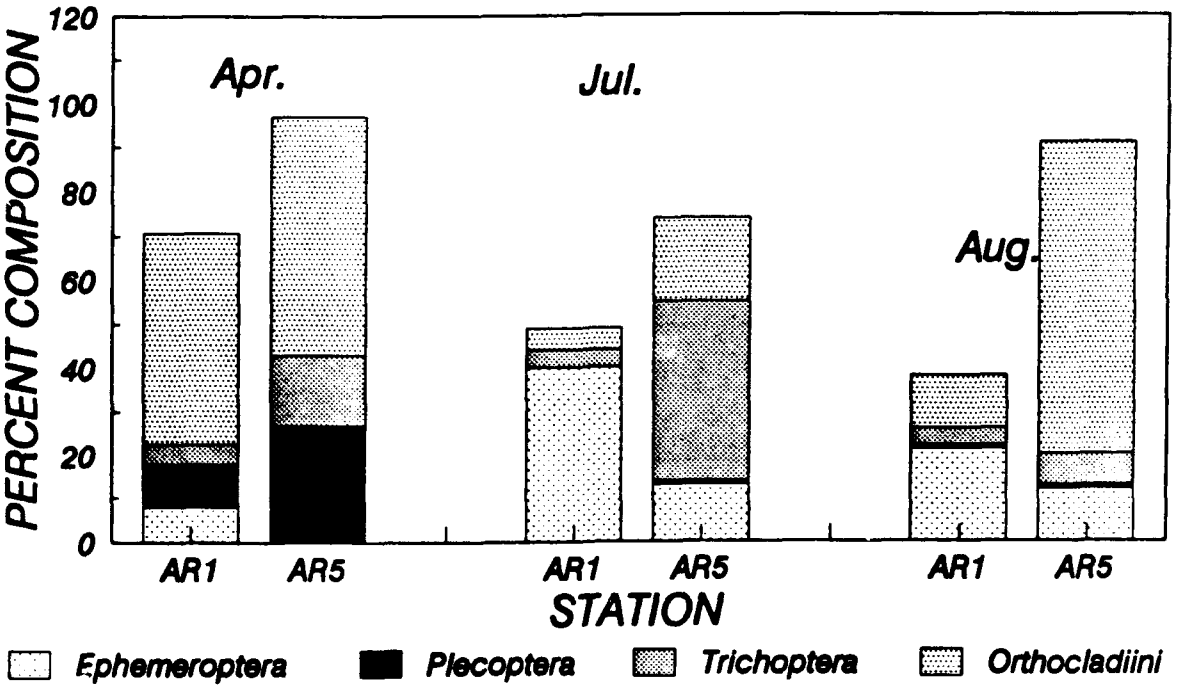

FEEDING HABITS OF BROWN TROUT
PERCENT COMPOSITION
120
100
80
60
40
20
0
Apr.
Jul.
Aug.
AR1
AR5
AR1
AR5
AR1
AR5
STATION
Ephemeroptera
Plecoptera
Trichoptera
Orthocladiini

Fig. 10

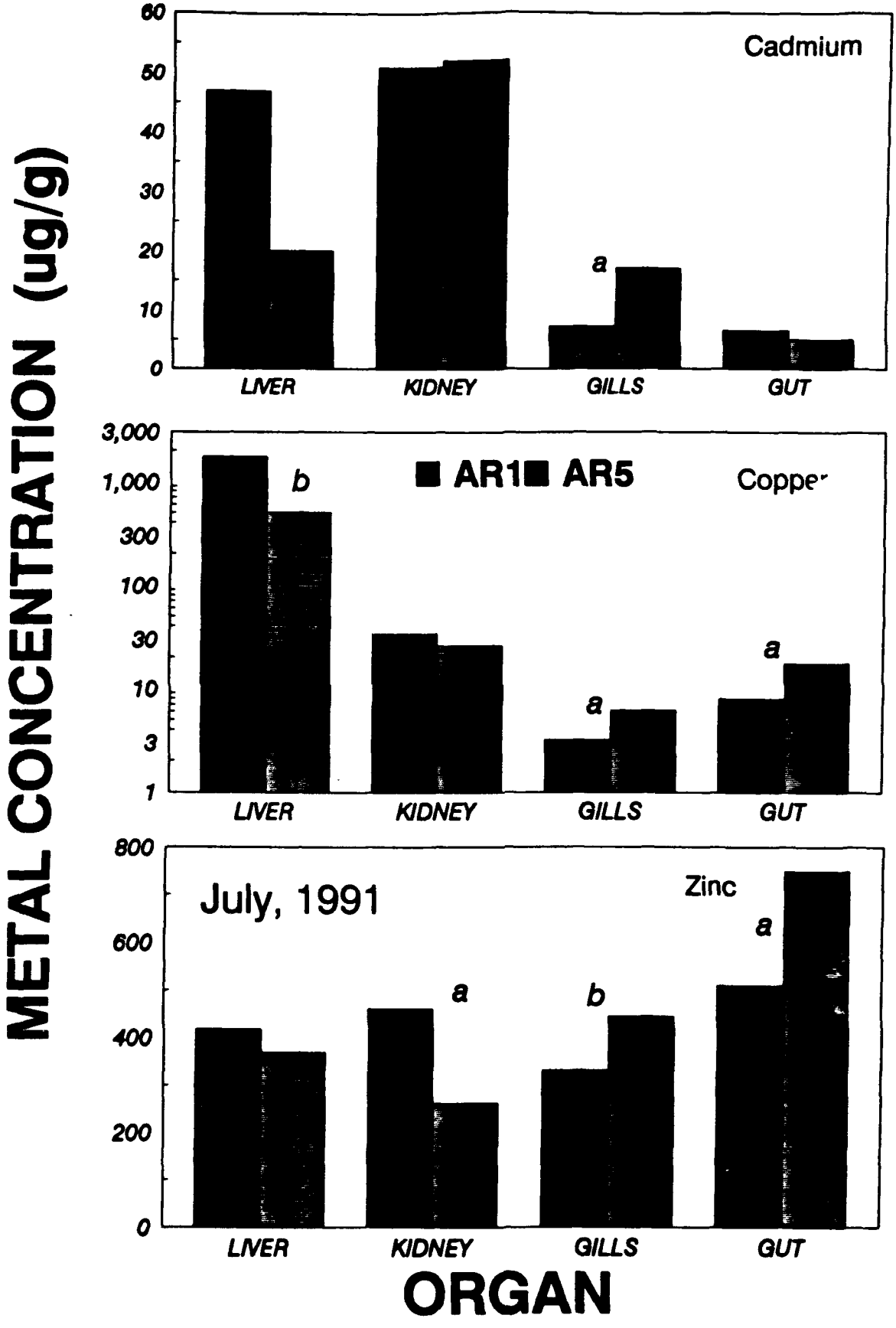

METAL CONCENTRATION (ug/g)
Cadmium
60
50
40
30
20
10
0
a
LIVER
KIDNEY
GILLS
GUT
3,000
1,000
300
100
30
10
3
1
b
AR1 AR5
Copper
a
a
LIVER
KIDNEY
GILLS
GUT
800
600
400
200
0
July, 1991
Zinc
a
a
b
LIVER
KIDNEY
GILLS
GUT
ORGAN

Fig. 11

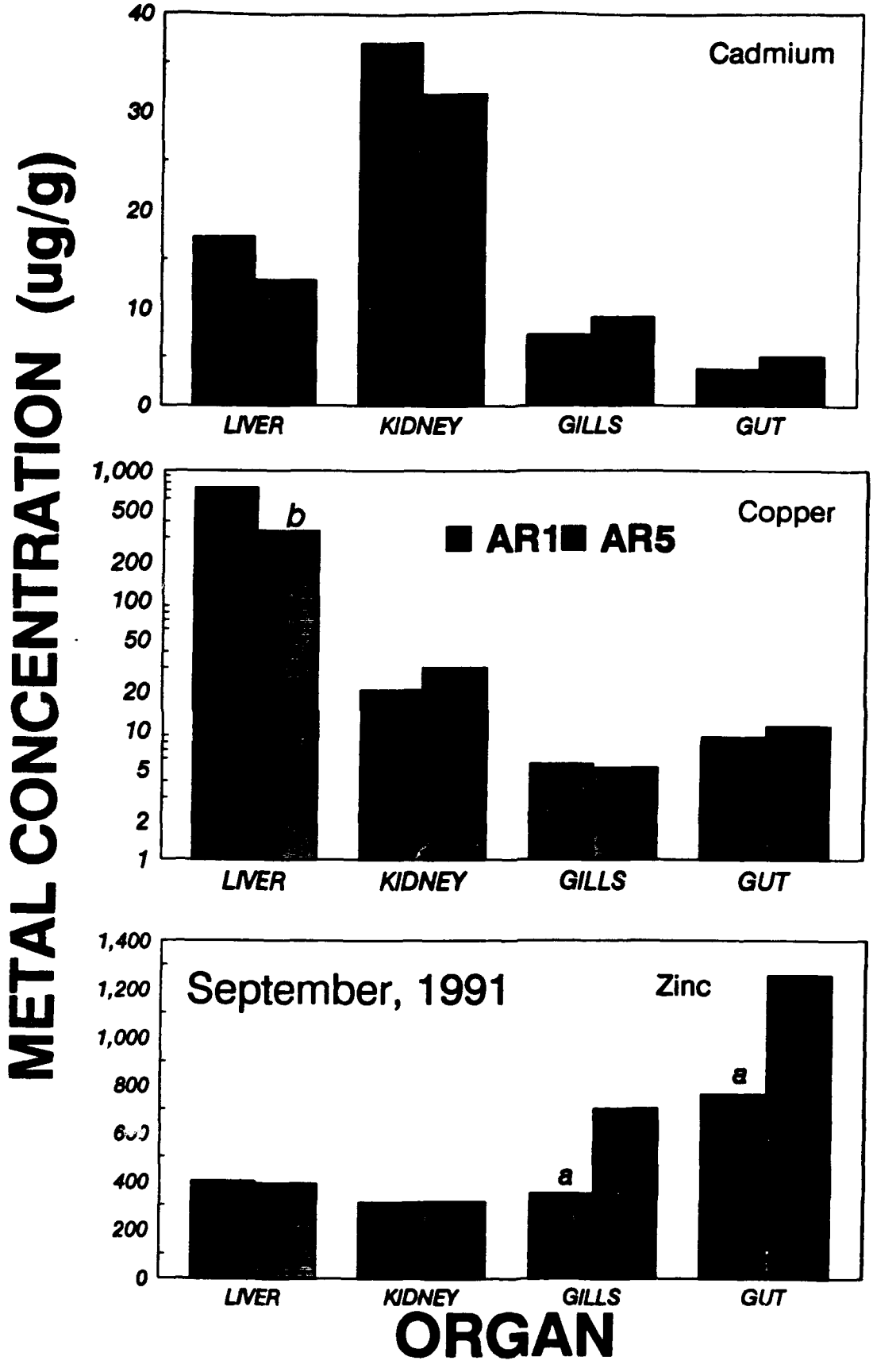

METAL CONCENTRATION (ug/g)
Cadmium
40
30
20
10
0
LIVER
KIDNEY
GILLS
GUT
Copper
AR1 AR5
1,000
500
200
100
50
20
10
5
2
1
b
LIVER
KIDNEY
GILLS
GUT
September, 1991
Zinc
1,400
1,200
1,000
800
400
200
0
a
a
LIVER
KIDNEY
GILLS
GUT
ORGAN

Fig. 11

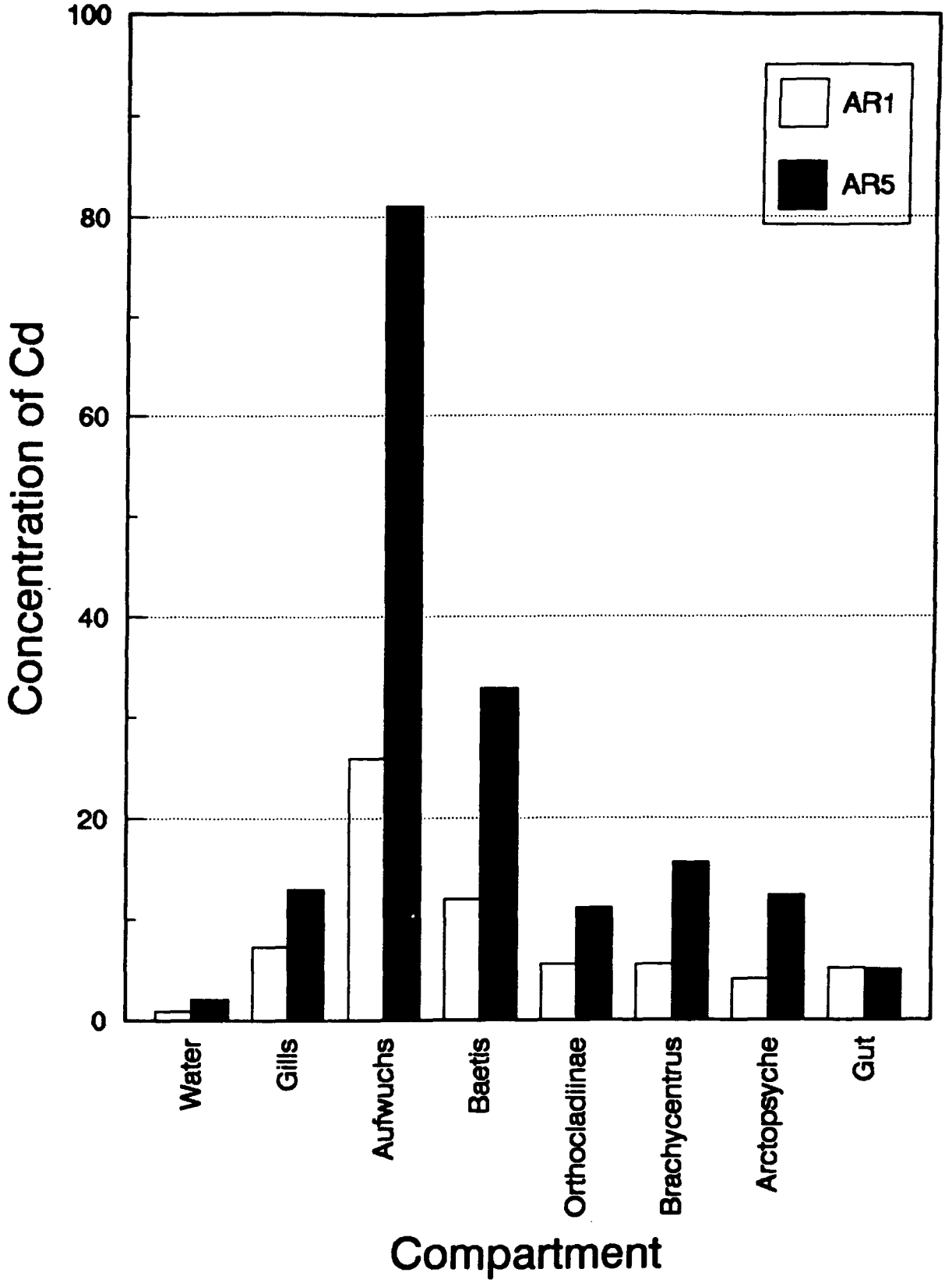

100
80
60
40
20
0
Concentration of Cd
AR1
AR5
Water
Gills
Aufwuchs
Baetis
Orthocladiinae
Brachycentrus
Arctopsyche
Gut
Compartment

Fig. 12

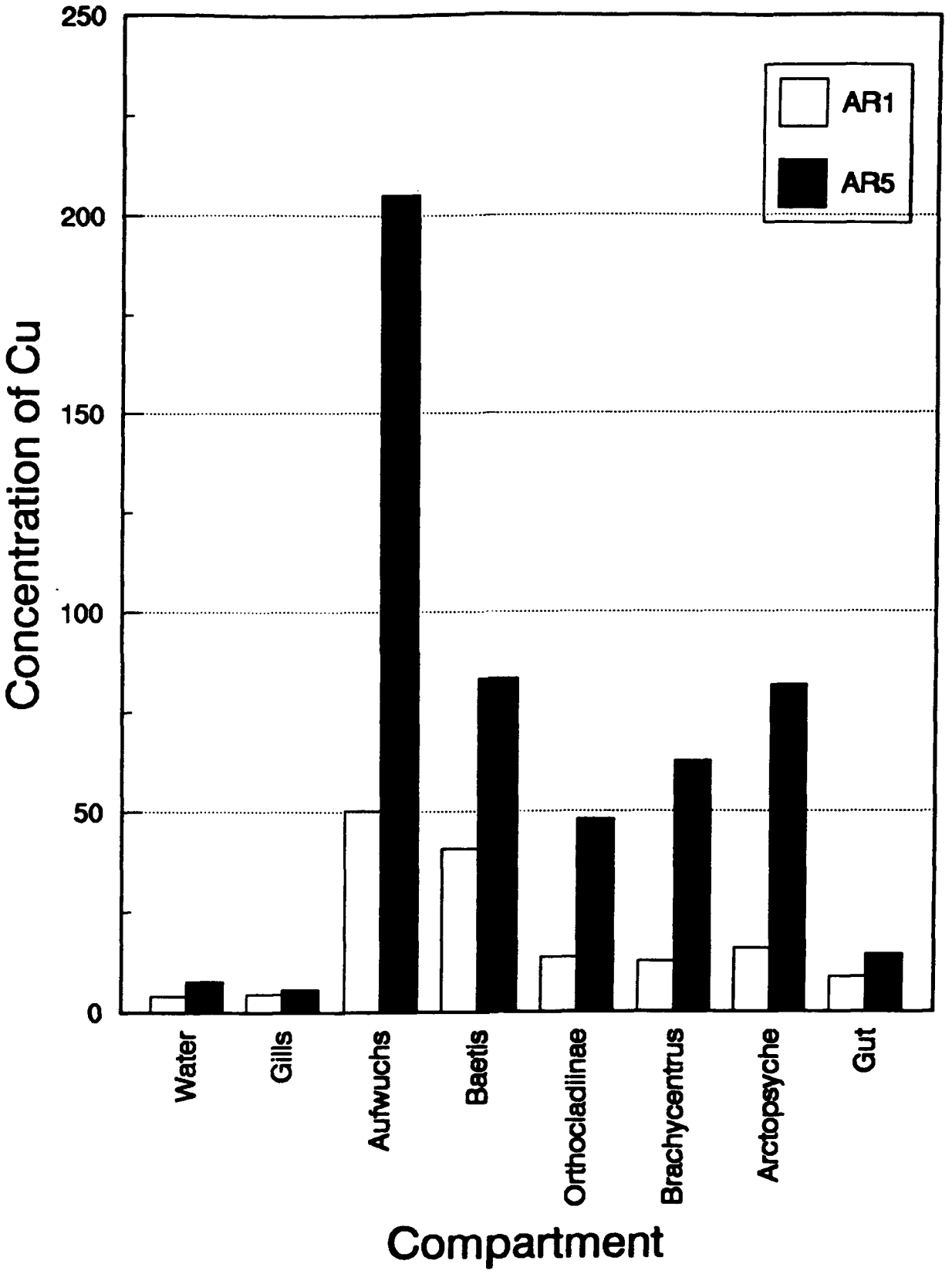

250
200
150
100
50
0
Concentration of Cu
AR1
AR5
Water
Gills
Aufwuchs
Baetis
Orthocladiinae
Brachycentrus
Arctopsyche
Gut
Compartment

Fig. 12

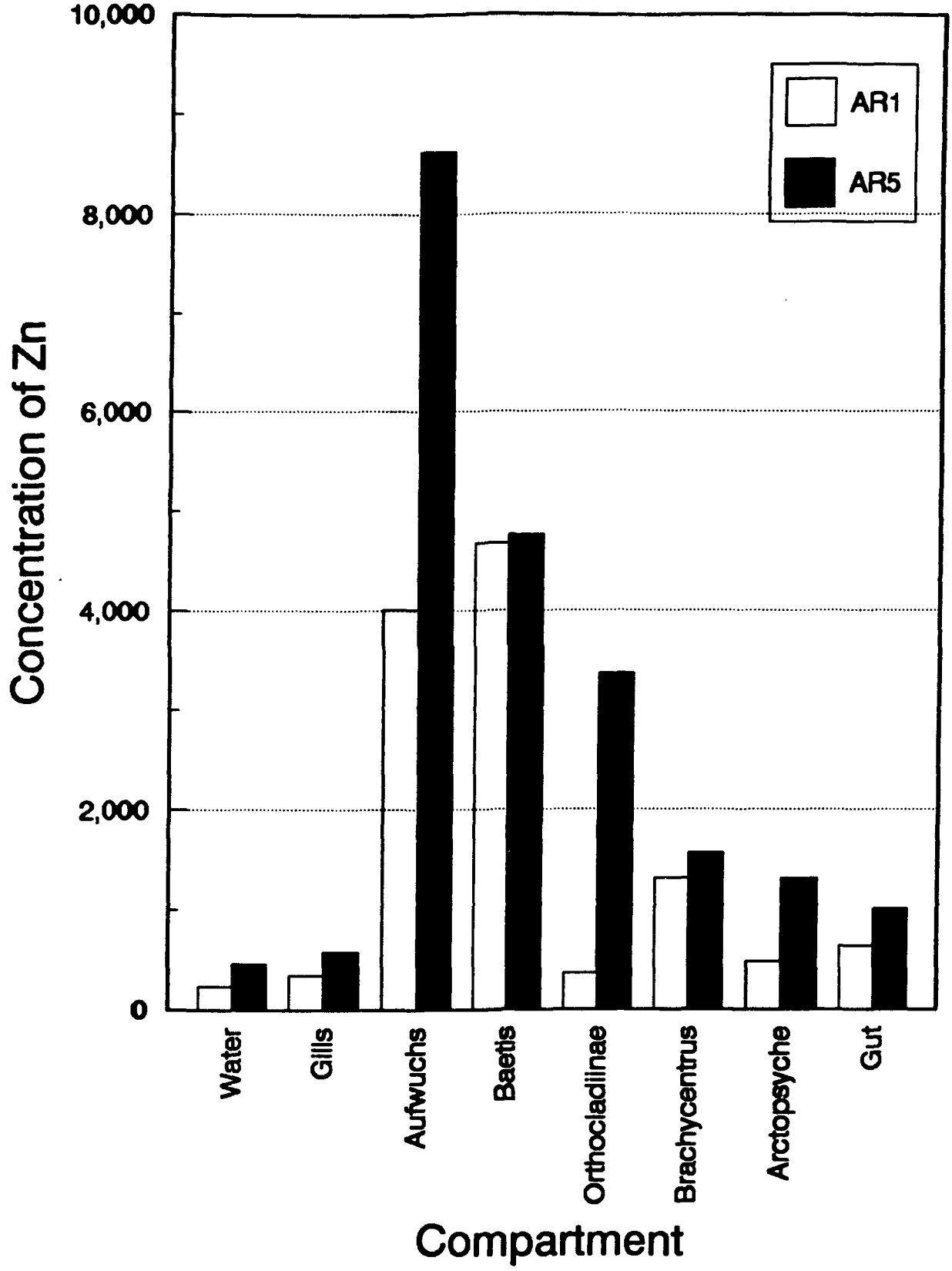

10,000
8,000
6,000
4,000
2,000
0
Concentration of Zn
AR1
AR5
Water
Gills
Aufwuchs
Baetis
Orthocladiinae
Brachycentrus
Arctopsyche
Gut
Compartment

Fig. 12

HEAVY METALS IN THE ARKANSAS RIVER

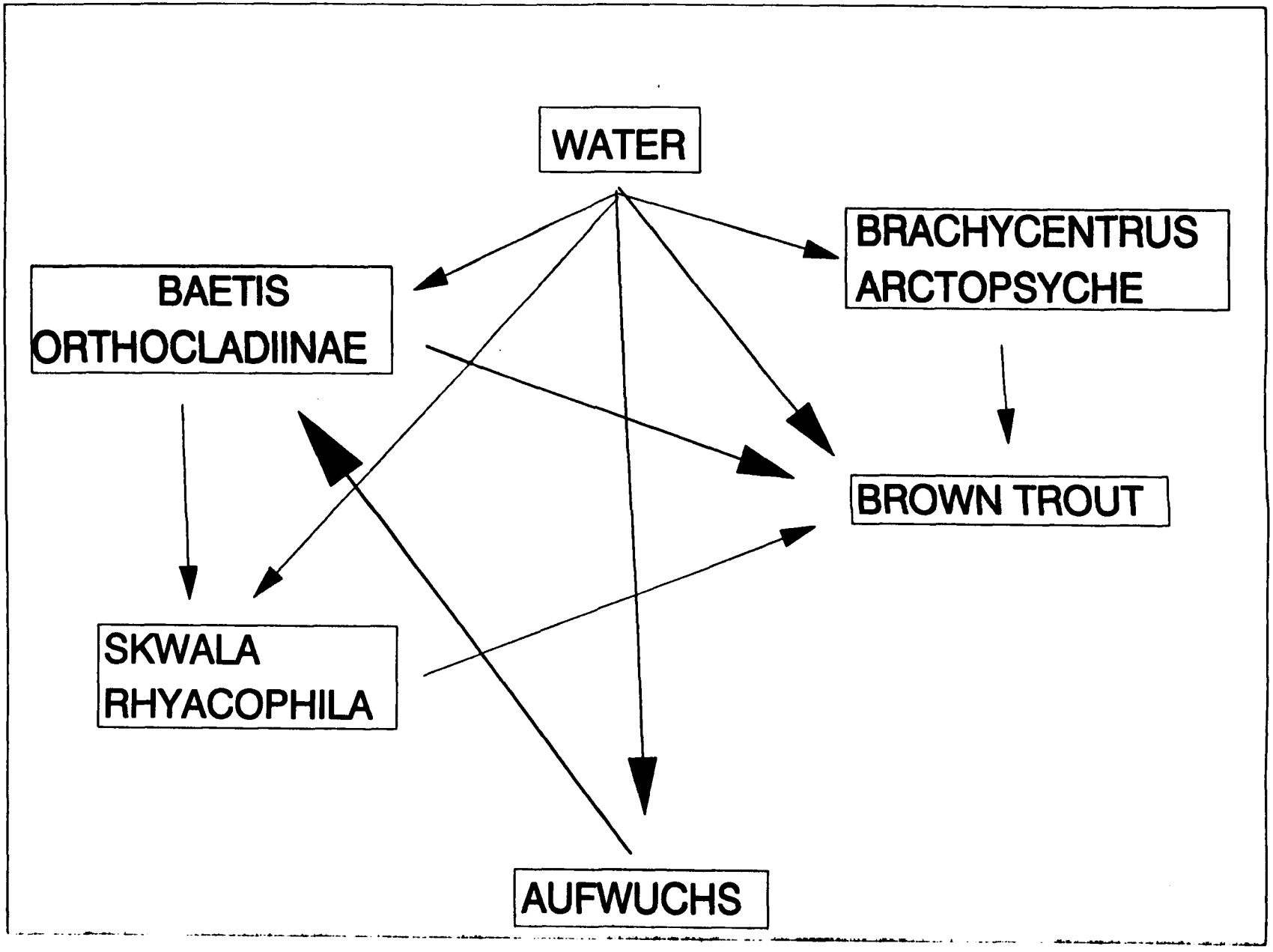


Fig. 13

APPENDIX I.

: Concentration of cadmium, copper, and zinc (ug/g), respectively, in Arctopsyche spp. collected from the Arkansas River, CO. Values equal mean concentrations ± 1 standard error.

Cadmium

MONTH	STATIONS EF 1	AR 1	AR 2	AR 3	AR 5A	AR 8
August 1990	1.95± 0.93	6.13± 0.78			16.00± 2.50	5.37± 0.02
November 1990	1.65± 0.40	2.55± 0.55	2.46± 0.34	4.65± 1.45	8.06± 0.54	4.73± 1.27
May 1991	5.55± 2.67	4.23± 1.26	2.51± 0.17		20.30± 3.90	
August 1991	1.95± 0.50	3.32± 0.60			5.26± 0.27	

Copper

MONTH	EF 1	AR 1	AR 2	AR 3	AR 5A	AR 8
August 1990	20.45± 3.4	19.67± 2.45			91.01± 6.36	28.38± 8.18
November 1990	15.85± 1.95	9.30± 1.10	8.30± 1.90	55.60± 1.70	68.23± 4.16	27.66± 9.24
May 1991	51.89± 21.11	17.60± 1.65	23.79± 4.80		130.39± 13.80	
August 1991	13.10± 4.57	16.25± 1.68			36.96± 1.33	

Zinc

MONTH	EF 1	AR 1	AR 2	AR 3	AR 5A	AR 8
August 1990	348.0± 13.00	625.67± 18.67			1871± 296.6	609.5± 60.90
November 1990	162.4± 15.00	500.5± 100.1	478.5± 2.55	695.6± 140.3	1226.2± 257.2	652.9± 163.2
May 1991	517.7± 208.5	340.6± 33.00	528.2± 25.23		1526± 216.9	
August 1991	265.5± 100.5	447.5± 20.50			634.5± 22.50	

Concentration of cadmium, copper, and zinc (ug/g), respectively, in Rhyacophila spp. collected from the Arkansas River, CO. Values equal mean concentrations ± 1 standard error.

Cadmium

	STATIONS					
MONTH	EF 1	AR 1	AR 2	AR 3	AR 5A	AR 8
August 1990	3.39± 1.08	5.14± 0.51	2.82± 0.21	14.20± 1.59		
November 1990	1.90± 0.27	2.76± 0.43	4.83± 0.89	11.70± 0.45	6.30± 1.70	
May 1991	3.34± 0.58	2.71± 0.39	6.24± 0.45	11.20± 0.94	3.74± 0.41	
August 1991	2.44± 0.15	1.72± 0.30		5.34± 0.09		

Copper

MONTH	EF 1	AR 1	AR 2	AR 3	AR 5A	AR 8
August 1990	21.01± 1.37	28.40± 8.17		252.84± 20.82		
November 1990	17.95± 1.45	11.50± 2.68	18.60± 4.85	101.73± 8.75	16.40± 1.15	
May 1991	37.37± 5.39	18.23± 2.11	31.93± 5.41	70.93± 8.67	57.30± 5.03	
August 1991	14.65± 0.25	12.89± 0.99		42.06± 0.08		

Zinc

MONTH	EF 1	AR 1	AR 2	AR 3	AR 5A	AR 8
August 1990	645.0± 61.34	1015± 95.5	804.0± 64.35	2381.2± 233.14		
November 1990	644.8± 8.40	668.1± 64.90	733.1± 42.72	937.7± 43.70	728.5± 79.10	
May 1991	1201± 316.80	1009± 118.70	1293± 103.20	4650± 229.0	1526± 216.85	
August 1991	553.50± 100.50	448.0± 95.00		796.5± 96.5		

concentrations ± 1 standard error.

Concentration of cadmium, copper, and zinc (ug/g), respectively, in Baetis spp. collected from the Arkansas River, CO. Values equal mean concentrations ± 1 standard error.

Cadmium

		STATIONS				
MONTH	EF 1	AR 1	AR 2	AR 3	AR 5A	AR 8
August 1990		15.10± 3.45				36.34± 1.06
November 1990		12.30± 0.93	10.30± 1.60	20.43± 4.60	33.57± 4.34	19.40± 2.55
May 1991		9.64± 0.85	9.91± 0.75	24.76± 0.34	33.08± 2.75	24.09± 1.76
August 1991		10.92± 0.66		16.61± 2.89	32.04± 4.61	

Copper

MONTH	EF 1	AR 1	AR 2	AR 3	AR 5A	AR 8
August 1990		44.29± 8.70				92.56± 8.25
November 1990		28.06± 3.30	22.65± 1.25	81.80± 10.55	73.65± 9.05	48.60± 5.40
May 1991		52.49± 12.01	56.03± 8.06	158.84± 22.10	106.9± 6.24	80.95± 3.82
August 1991		38.15± 0.99		80.77± 12.77	69.53± 5.08	

Zinc

MONTH	EF 1	AR 1	AR 2	AR 3	AR 5A	AR 8
August 1990		3133± 31.50				6374± 89.11
November 1990		4657± 771.6	3048± 53.50	4352± 431.4	7657± 873.5	3676± 800.9
May 1991		8111± 826.7	1485± 15.70	8518± 5.07	1342± 51.70	926.6± 33.81
August 1991		2800± 100.0		3466± 448.5	5300± 781.0	

Concentration of cadmium, copper, and zinc (ug/g), respectively, in Brachycentrus spp. collected from the Arkansas River, CO. Values equal mean concentrations ± 1 standard error.

Cadmium

	STATIONS					
MONTH	EF 1	AR 1	AR 2	AR 3	AR 5A	AR 8
August 1990		8.38± 0.38			14.99± 2.98	
November 1990		2.60± 0.20			14.96± 3.60	31.83± 0.75
May 1991					14.90± 1.57	
August 1991					17.88± 0.89	

Copper

MONTH	EF 1	AR 1	AR 2	AR 3	AR 5A	AR 8
August 1990		22.46± 5.15			54.91± 6.89	
November 1990		2.65± 0.15			53.70± 11.50	62.00± 1.45
May 1991					88.62± 7.29	
August 1991					53.51± 1.02	

Zinc

MONTH	EF 1	AR 1	AR 2	AR 3	AR 5A	AR 8
August 1990		2525± 236.6			1476± 229.1	
November 1990		111.5± 12.50			1357± 366.8	
May 1991					2153± 381.8	
August 1991					1306± 17.5	

Concentration of cadmium, copper, and zinc (ug/g), respectively, in Orthocladiinae spp. collected from the Arkansas River, CO. Values equal mean

Cadmium

MONTH	EF 1	AR 1	AR 2	AR 3	AR 5A	AR 8
			STATIONS			
August 1990					20.23± 3.10	
November 1990				5.30± 0.20	7.85± 1.15	
May 1991		5.47± 1.35		7.35± 2.51	5.30± 0.76	6.22± 0.62
August 1991				8.26± 1.44		10.52± 1.15

Copper

MONTH	EF 1	AR 1	AR 2	AR 3	AR 5A	AR 8
August 1990					60.59± 11.08	
November 1990		11.80± 1.80	58.30± 27.60		31.75± 5.85	62.00± 1.45
May 1991		15.44± 1.03		79.22± 31.18	52.03± 2.75	67.91± 4.89
August 1991				100.31± 17.39		53.37± 8.85

Zinc

MONTH	EF 1	AR 1	AR 2	AR 3	AR 5A	AR 8
August 1990					8448± 552.0	
November 1990				777.5± 202.5	1076± 196.5	
May 1991		360.8± 62.28		857± 171.0	566.1± 37.10	709.7± 142.2
August 1991				1712± 275.5		1436± 297.0

Concentration of cadmium, copper, and zinc (ug/g), respectively, in Skwala spp. collected from the Arkansas River, CO. Values equal mean concentrations ± 1 standard error.

Cadmium

	STATIONS					
MONTH	EF 1	AR 1	AR 2	AR 3	AR 5A	AR 8
August 1990			4.20± 0.82		6.94± 0.54	2.05± 0.19
November 1990			3.50± 0		7.46± 0.84	
May 1991			2.13± 0.23	4.99± 0.66	4.03± 0.54	
August 1991						

Copper

MONTH	EF 1	AR 1	AR 2	AR 3	AR 5A	AR 8
August 1990					59.36± 4.18	62.41± 4.70
November 1990			27.30± 4.00			46.10± 3.07
May 1991			66.00± 7.80	113.73± 10.22	67.26± 6.08	
August 1991						

Zinc

MONTH	EF 1	AR 1	AR 2	AR 3	AR 5A	AR 8
August 1990			603.7± 21.30		1089± 43.67	
November 1990			293.5± 52.50		1152± 88.33	
May 1991			345.0± 24.20	713.2± 33.34	553.7±7. 90	
August 1991						

Concentration of cadmium, copper, and zinc (ug/g), respectively, in periphyton collected from the Arkansas River, CO. Values equal mean concentrations ± 1 standard error.

Cadmium

MONTH	STATIONS EF 1	AR 1	AR 2	AR 3	AR 5A	AR 8
November 1990	7.13± 0.61	32.90± 7.30	32.70± 6.81	62.10± 17.5	81.93± 11.93	23.00± 4.72
May 1991	47.00± 11.80	41.00± 10.80	10.80± 3.30	90.00± 30.40	130.60± 21.30	54.00± 11.50
August 1991	9.3± 1.3	3.6± 1.0	4.3± 0.9	19.7± 3.5	28.7± 4.1	5.9± 1.8

Copper

MONTH	EF 1	AR 1	AR 2	AR 3	AR 5A	AR 8
November 1990	96.90± 23.14	69.50± 15.30	330.5± 125.0	613.6± 56.40	375.3± 32.50	143.0± 56.50
May 1991	447.2± 114.0	66.10± 25.80	32.00± 4.00	348.0± 87.60	183.0± 15.80	293.0± 59.00
August 1991	35.5± 5.3	16.4± 2.2	15.9± 5.0	138.0± 20.1	58.1± 8.9	39.9± 12.1

Zinc

MONTH	EF 1	AR 1	AR 2	AR 3	AR 5A	AR 8
November 1990	1744± 237.00	6992± 1332	6397± 2254	15647± 2617	16714± 2617	4696± 1212
May 1991	2136± 551.0	4023± 1088	1823± 446.0	2999± 974.0	4022± 790	7722± 1751
August 1991	2150± 80.8	1028± 18.6	629± 139.1	4525± 726.4	5136± 531.9	1269± 363.2

APPENDIX II.

12 AUGUST 1991
STATION AR1

	Pooled from 50 Brown Trout		Hess Samples	
	Number of Individuals	Proportion of Diet	Mean #/.1m^2	Proportion of Benthos
Baetis bicaudatus*	0	0	48.2	0.112
Baetis tricaudatus	77	0.157	58.4	0.136
Baetis hageni	0	0	0.2	0
Drunella grandis	2	0.004	0	0
Drunella doddsi	1	0.002	2	0.005
Ameletus sp.	0	0	0.2	0
Rhithrogena sp.	0	0	4.2	0.01
Cinygmula sp.	1	0.002	0	0
Epeorus longimanus	2	0.004	3.4	0.008
Ephemerella infrequens	0	0	0.8	0.002
Accentrella carolina	1	0.002	0.4	0.001
Pteronarcella badia	2	0.004	0	0
Zapada cinctipes	1	0.002	0	0
Utacapnia logana	0	0	0.2	0
Kogotus modestus	0	0	0.2	0
Sweltsa coloradensis	0	0	5.4	0.013
Suwallia sp.	0	0	0	0
Triznaka signata	0	0	0.2	0
Skwala americana	0	0	0	0
Isoperla fulva	0	0	0	0
Isoperla mormona	0	0	0.2	0
Arctopsyche grandis	3	0.006	5.2	0.012
Hydropsyche oslari	0	0	0	0
Hydropsyche cockerelli	0	0	0	0
Brachycentrus americanus	8	0.016	0.2	0
Brachycentrus occidentalis	0	0	0	0
Rhyacophila brunnea	4	0.008	3.8	0.009
Rhyacophila angelita	0	0	1.2	0.003
Oligophlebodes minutus	0	0	0	0
Hydroptila sp.	2	0.004	0	0
Lepidostoma ormea	1	0.002	0	0
Hesperophylax occidentalis	0	0	0	0
Agapetus boulderensis	0	0	0	0
Glossosoma sp.	3	0.006	0.4	0.001
Orthocladiinae	59	0.12	47.2	0.11
Tanytarsini	0	0	0.4	0.001
Tanypodinae	1	0.002	0.4	0.001

	Pooled from 50 Brown Trout		Hess Samples	
	Number of Individuals	Proportion of Diet	Mean #/.1m^2	Proportion of Benthos
Simulium sp.	233	0.475	231	0.538
Bezzia sp.	2	0.004	2.2	0.005
Tipula sp.	0	0	0	0
Hexatoma sp.	2	0.004	0.8	0.002
Antocha sp.	0	0	0.6	0.001
Dicranota sp.	0	0	0	0
Chelifera sp.	0	0	2.2	0.005
Atherix pachypus	0	0	0	0
Bibiocephala grandis	0	0	0.8	0.002
Dytiscidae sp. 1	1	0.002	0	0
Optioservus sp.	0	0	0	0
Heterlimnius corpulentus	4	0.008	8.6	0.02
Hydracarina	3	0.006	0	0
Oligochaeta	4	0.008	0	0
Nematoda	5	0.01	0	0
Baetis sp. (Adult)	14	0.029	0	0
Chironomidae (Adult)	13	0.026	0	0
Terrestrials/Miscellaneous	42	0.086	0	0
Total Number of Individuals	491		2145	
Mean # of Individuals/Sample	14.44118		429	
Mean # of Taxa/Sample	3.617647		16.2	
	1.985458		2.925748	
	3.970915		5.85149[illegible]	

*Found only as early instars.

STATION AR5
12 August 1991

	Pooled from 50 Brown Trout		Hess Samples	
	Number of Individuals	Proportion of Diet	Mean #/.1m^2	Proportion of Benthos
Baetis bicaudatus*	0	0	75.4	0.081
Baetis tricaudatus	25	0.018	129	0.139
Baetis hageni	0	0	0	0
Drunella grandis	0	0	0	0
Drunella doddsi	0	0	0.6	0.001
Ameletus sp.	0	0	0	0
Rhithrogena sp.	0	0	0	0
Cinygmula sp.	0	0	0	0
Epeorus longimanus	0	0	0	0
Ephemerella infrequens	0	0	0	0
Accentrella caroline	0	0	0	0
Pteronarcella badia	2	0.001	1.4	0.002
Zapada cinctipes	0	0	0	0
Utacapnia logana	0	0	0	0
Kogotus modestus	0	0	0	0
Sweltsa coloradensis	0	0	4.6	0.005
Suwallia sp.	6	0.004	0.4	0
Triznaka signata	0	0	1.4	0.002
Skwala americana	1	0.001	19	0.02
Isoperla fulva	0	0	4.2	0.005
Isoperla mormona	0	0	0	0
Arctopsyche grandis	4	0.003	36.6	0.039
Hydropsyche oslari	0	0	1.2	0.001
Hydropsyche cockerelli	0	0	0.2	0
Brachycentrus americanus	8	0.006	21.4	0.023
Brachycentrus occidentalis	18	0.013	15	0.016
Rhyacophila brunnea	7	0.005	2.8	0.003
Rhyacophila angelita	2	0.001	2.8	0.003
Oligophlebodes minutus	0	0	4.4	0.005
Hydroptila sp.	3	0.002	3.4	0.004
Lepidostoma ormea	0	0	0	0
Hesperophylax occidentalis	3	0.002	0	0
Agapetus boulderensis	0	0	1.2	0.001
Glossosoma sp.	41	0.03	0.4	0
Orthocladiinae	976	0.708	433.6	0.466
Tanytarsini	0	0	0.2	0
Tanypodinae	11	0.008	4.6	0.005
Simulium sp.	11	0.008	137.2	0.148
Bezzia sp.	0	0	0.2	0
Tipula sp.	1	0.001	1.2	0.001

	Pooled from 50 Brown Trout		Hess Samples	
	Number of Individuals	Proportion of Diet	Mean #/.1m^2	Proportion of Benthos
Hexatoma sp.	3	0.002	0.2	0
Antocha sp.	0	0	0	0
Dicranota sp.	0	0	1.2	0.001
Chelifera sp.	1	0.001	3	0.003
Atherix pachypus	2	0.001	0	0
Bibiocephala grandis	0	0	0.4	0
Dytiscidae sp. 1	0	0	0	0
Optioservus sp.	0	0	0.6	0.001
Heterlimnius corpulentus	0	0	11.2	0.012
Hydracarina	0	0	0	0
Oligochaeta	0	0	10.6	0.011
Nematoda	1	0.001	0	0
Baetis sp. (Adult)	147	0.107	0	0
Chironomidae (Adult)	42	0.03	0	0
Terrestrials/Miscellaneous	63	0.046	0	0
Total Number of Individuals	1379		4648	
Mean # of Individuals/Sample	55.16		929.6	
Mean # of Taxa/Sample	5.48		24.2	
	2.531719		4.578209	
	5.063438		9.156419	

*Found only as early instars.

6 SEPTEMBER
STATION AR1

	Pooled from 50 Brown Trout		Hess Samples	
	Number of Individuals	Proportion of Diet	Mean #/.1m^2	Proportion of Benthos
Baetis bicaudatus*	3	0.003	28.4	0.131
Baetis tricaudatus	204	0.192	20.4	0.094
Drunella grandis	3	0.003	0	0
Drunella doddsi	0	0	0.8	0.004
Ameletus sp.	3	0.003	0	0
Rhithrogena sp.	0	0	11.8	0.054
Cinygmula sp.	2	0.002	0	0
Epeorus longimanus	0	0	0.6	0.003
Ephemerella infrequens	0	0	3.4	0.016
Accentrella caroline	4	0.004	0.2	0.001
Paraleptophlebia sp.	1	0.001	0	0
Pteronarcella badia	9	0.008	0	0
Zapada cinctipes	1	0.001	0	0
Sweltsa coloradensis	0	0	1.4	0.006
Suwallia sp.	1	0.001	0	0
Trizneka signata	0	0	0.8	0.004
Skwala americana	1	0.001	0.4	0.002
Isoperla fulva	0	0	0	0
Megarcys signata	0	0	0.2	0.001
Arctopsyche grandis	12	0.011	4	0.018
Hydropsyche oslari	0	0	0	0
Hydropsyche cockerelli	0	0	0	0
Brachycentrus americanus	3	0.003	0.4	0.002
Brachycentrus occidentalis	0	0	0	0
Rhyacophila brunnea	12	0.011	0.6	0.003
Rhyacophila angelita	1	0.001	0.2	0.001
Rhyacophila valuma	0	0	0	0
Rhyacophila verrula	0	0	0.4	0.002
Oligophlebodes minutus	0	0	0	0
Agrevlea multipunctata	1	0.001	0	0
Hydroptila sp.	2	0.002	0.6	0.003
Lepidostoma ormea	1	0.001	0	0
Glossosoma sp.	7	0.007	0	0
Orthocladiinae	117	0.11	17.4	0.08
Tanytarsini	0	0	0	0
Tanypodinae	1	0.001	0.6	0.003
Chironomini	1	0.001	0	0
Simulium sp.	315	0.297	119.4	0.551
Bezzia sp.	16	0.015	0	0
Tipula sp.	0	0	0	0
Hexatoma sp.	2	0.002	0	0
Antocha sp.	0	0	0	0
Oreogeton sp.	0	0	0	0
Chelifera sp.	1	0.001	0	0
Pericoma sp.	0	0	1.4	0.006

	Pooled from 50 Brown Trout		Hess Samples	
	Number of Individuals	Proportion of Diet	Mean #/.1m^2	Proportion of Benthos
Bibiocephala grandis	3	0.003	0.8	0.004
Dytiscidae sp. 1	0	0	0	0
Optioservus sp.	0	0	0	0
Heterlimnius corpulentus	3	0.003	2.4	0.011
Hydracarina	28	0.026	0.2	0.001
Oligochaeta	0	0	0	0
Nematoda	32	0.03	0	0
Baetis sp. (Adult)	33	0.031	0	0
Chironomidae (Adult)	126	0.119	0	0
Terrestrials/Miscellaneous	112	0.106	0	0
Total Number of Individuals	1061		1084	
Mean # of Individuals/Sample	21.22		216.8	
Mean # of Taxa/Sample			14.4	
			1.356466	
			2.712932	

*Found only as early instars.

6 SEPTEMBER 1991
STATION AR5

	Pooled from 50 Brown Trout		Hess Samples	
	Number of Individuals	Proportion of Diet	Mean #/.1m^2	Proportion of Benthos
Baetis bicaudatus*	0	0	14.2	0.026
Baetis tricaudatus	957	0.343	73.8	0.133
Drunella grandis	0	0	0	0
Drunella doddsi	0	0	0	0
Ameletus sp.	0	0	0	0
Rhithrogena sp.	0	0	0	0
Cinygmula sp.	0	0	0	0
Epeorus longimanus	0	0	0	0
Ephemerella infrequens	0	0	0.2	0
Accentrella caroline	0	0	0	0
Paraleptophlebia sp.	0	0	0	0
Pteronarcella badia	11	0.004	2.2	0.004
Zapada cinctipes	0	0	0	0
Sweltsa coloradensis	1	0	0.6	0.001
Suwallia sp.	0	0	0	0
Triznaka signata	1	0	4.2	0.008
Skwala americana	77	0.028	8.2	0.015
Isoperla fulva	2	0.001	7.8	0.014
Megarcys signata	0	0	0	0
Arctopsyche grandis	56	0.02	19.4	0.035
Hydropsyche oslari	1	0	2	0.004
Hydropsyche cockerelli	2	0.001	1.6	0.003
Brachycentrus americanus	8	0.003	44.8	0.081
Brachycentrus occidentalis	29	0.01	42.6	0.077
Rhyacophila brunnea	2	0.001	2.2	0.004
Rhyacophila angelita	1	0	0.4	0.001
Rhyacophila valuma	1	0	0	0
Rhyacophila verrula	0	0	0	0
Oligophlebodes minutus	0	0	0.6	0.001
Agrevlea multipunctata	0	0	0	0
Hydroptila sp.	0	0	5.4	0.01
Lepidostoma ormea	0	0	0	0
Glossosoma sp.	6	0.002	5.6	0.01
Orthocladiinae	1114	0.399	259.4	0.467
Tanytarsini	1	0	0.2	0
Tanypodinae	4	0.001	0.8	0.001
Chironomini	1	0	0	0
Simulium sp.	41	0.015	0.8	0.001
Bezzia sp.	0	0	0	0
Tipula sp.	8	0.003	1.2	0.002
Hexatoma sp.	3	0.001	0	0
Antocha sp.	0	0	1.6	0.003
Oreogeton sp.	1	0	1.4	0.003
Chelifera sp.	4	0.001	6.2	0.011
Pericoma sp.	0	0	0.2	0
Bibiocephala grandis	0	0	0	0

	Pooled from 50 Brown Trout		Hess Samples	
	Number of Individuals	Proportion of Diet	Mean #/.1m^2	Proportion of Benthos
Dytiscidae sp. 1	8	0.003	0	0
Optioservus sp.	0	0	0.8	0.001
Heterlimnius corpulentus	1	0	0	0
Hydracarina	1	0	4.2	0.008
Oligochaeta	0	0	37.6	0.068
Nematoda	35	0.013	5.4	0.01
Baetis sp. (Adult)	214	0.077	0	0
Chironomidae (Adult)	87	0.031	0	0
Terrestrials/Miscellaneous	112	0.04	0	0
Total Number of Individuals	2790		2778	
Mean # of Individuals/Sample	55.8		555.6	
Mean # of Taxa/Sample			22.2	
			2.56125	
			5.122499	

*Found only as early instars.