

**BIOACCUMULATION OF HEAVY METALS BY BROWN TROUT  
(SALMO TRUTTA) IN THE ARKANSAS RIVER: IMPORTANCE  
OF FOOD CHAIN TRANSFER**

by

**William H. Clements**

A stylized graphic on the left side of the page. It features a black outline of a mountain range with several peaks. Below the mountains, a thick, wavy blue line represents a river. The entire graphic is set against a white background.

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State  
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IMPORTANCE OF FOOD CHAIN TRANSFER**

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**Robert C. Ward, Director**

## ABSTRACT

This study examined uptake and transfer of heavy metals (Cd, Cu, Zn) from benthic invertebrates to brown trout (*Salmo trutta*) at the Arkansas River, Colorado. Metals in water, aufwuchs, benthic invertebrates, and fish were measured at stations located upstream and downstream from California Gulch (CG), a U.S. EPA Superfund site. Field studies were conducted to estimate the relative contribution of food and water to metal uptake by brown trout. 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 some downstream 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.

The diet of brown trout at the Arkansas River was dominated by benthic invertebrates. Ephemeroptera, Plecoptera, Trichoptera, and Chironomidae (primarily Orthoclaadiinae) 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 Orthoclaadiinae, were more common in the diet of fish from downstream.

Elevated metal levels in water and invertebrates at station AR5 resulted in increased metals in gill and gut tissue; however, metal concentrations in brown trout liver and kidney tissue were generally similar at stations AR1 and AR5. These data suggest that fish regulated metal accumulation at the downstream station. The implications of these findings for the recovery of brown trout populations at the Arkansas River are discussed.

## INTRODUCTION

### Bioaccumulation of Metals by Aquatic Organisms

Bioaccumulation of heavy metals in contaminated streams has been demonstrated in algae (Kelly and Whitton 1989), macroinvertebrates (Krantzberg and Stokes 1989; Kiffney and Clements, in press) and fish (Dallinger and Kautzky 1985). Most of the evidence derived from laboratory studies indicates that uptake from water is the predominant route of exposure, particularly for fish (Williams and Giesy 1978). 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) also 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. These studies support the hypothesis that some fraction of heavy metals is elaborated into fish tissue through the food chain.

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 from contaminated sediments (Tatem 1986; Hare et al. 1989) and therefore 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). 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.

## Heavy Metals in Streams of Colorado

The upper Arkansas River Basin in Colorado has been recognized as a site of extremely poor water quality for many years. The Yak Tunnel (Leadville, CO), a U.S. EPA Superfund site, releases large volumes of highly contaminated water into California Gulch, which flows directly into the Arkansas River. Levels of zinc, copper, and cadmium are greatly elevated in the Arkansas River immediately downstream of Leadville, CO. Previous investigations at the upper Arkansas River have demonstrated significant effects of heavy metals on benthic macroinvertebrate and fish populations. In particular, reduced density and poor survival of brown trout (*Salmo trutta*) at the Arkansas River has been attributed to heavy metal contamination. It has been suggested that bioaccumulation of heavy metals, either from water or from the food chain, contributes to the decline of *S. trutta* populations in the Arkansas River.

Heavy metal contamination in the Arkansas River has resulted in increased abundance of tolerant macroinvertebrates, particularly caddisflies, at stations downstream from California Gulch (Clements 1991). In particular, recent experiments conducted in our laboratory demonstrated that the caddisfly *Brachycentrus americanus* is highly tolerant of heavy metals. These organisms are very abundant at stations immediately downstream from California Gulch and comprise a significant portion of the diet of brown trout. Therefore it is likely that dietary uptake of heavy metals may contribute to poor survival of *S. trutta* in the Arkansas River. I hypothesize that increased utilization of pollution-tolerant prey in the Arkansas River will increase the potential for food chain transfer of heavy metals.

This research examined the transfer of heavy metals (Cd, Cu, Zn) from benthic invertebrates to brown trout (*Salmo trutta*) at the Arkansas River, Colorado. **The specific objectives of this research were 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 varied between upstream and downstream stations 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**

### **Study Site**

The study site was located in a valley in central Colorado, between the Sawatch and Mosquito mountain ranges (Fig. 1). Data reported in this study represent part of a long-term monitoring program (Clements, unpublished data) to assess the impacts of heavy metals from the Yak Tunnel, a U.S. EPA Superfund site that discharges into California Gulch (CG) and eventually into the Arkansas River.

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 elevation gradient from Climax to Buena Vista, CO. Water samples and benthic invertebrates were collected at stations upstream and downstream from CG. 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.*).

### **Fish and Invertebrate Sampling**

Brown trout (*Salmo trutta*) were collected from stations AR1 and AR5 using a backpack electroshocker on four sampling occasions: 20-21 April, 8-9 July, 11-12 August, and 5-6 September 1991. On each occasion, sampling was conducted on two consecutive days. After fish had been captured, they were placed in live-baskets. Gut contents were removed with the use of a hand-held stomach pump. Samples were immediately placed on dry ice and frozen for metals analysis.

Stomach samples collected for identification of benthic invertebrates were returned to the laboratory at Colorado State University. Feeding habits have been analyzed for fish collected in April, July, and August. 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.

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. Whole fish were measured, weighed, and immediately frozen on dry ice. In the laboratory, kidney, liver, gut, and gill tissues were taken from each fish and placed in 16.5 ml glass test tubes. All brown trout tissue samples were digested and analyzed as described below.

Benthic invertebrates were collected for metals analysis from each station. Organisms were collected from a riffle area using a D-frame net. All organisms were sorted to genus in the field, except for chironomids which were sorted to tribe. Individual organisms were used for metals analysis when possible, except for chironomids and baetid mayflies, which were pooled because of their small biomass. Each vial was treated as a replicate sample. An effort was made to collect the same species from reference and contaminated sites. All organisms were placed in 25-ml polypropylene scintillation vials and immediately placed on dry ice.

### **Metals Analysis**

Concentrations of Cd, Cu, and Zn were analyzed in water samples collected from all stations. Water samples were collected in a 250 ml acid washed nalgene container and acidified with 1 ml analytical grade HNO<sub>3</sub> in the field. Total metal concentrations were measured using a Instrumentation Laboratory Video 22 graphite furnace atomic absorption spectrophotometer. Accuracy and percent recovery were determined by analyzing National Bureau of Standards bovine tissue, acid blanks, and spikes.

For metals analysis, invertebrates and fish tissue samples were dried in an oven at 50°C 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 and analyzed for Cd, Cu, and Zn as described above.

## **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 in invertebrates. Tukey's HSD test controls maximum experiment error rate and is suitable for unequal samples sizes. Student's t-tests were employed to test for differences in metal levels in fish tissue among locations. All statistical analyses were performed using a PC-version of Statistical Analysis System (SAS). A significant difference was determined to exist at a  $p < 0.05$  level.

## **RESULTS**

### **Concentrations of Heavy Metals in Water**

Concentrations of Cd, Cu, and Zn at the Arkansas River varied among locations and among seasons (Fig. 2). Zinc was the dominant metal measured at all stations on all sampling occasions. Levels of Zn at stations immediately downstream from LMDT (EF5, EF6) and CG (AR3) ranged from 205  $\mu\text{g/L}$  to 8624  $\mu\text{g/L}$ . 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 (defined as periphyton, algae, and associated abiotic material) and macroinvertebrates paralleled those in water (Fig. 3). Zn levels were highest in all organisms, followed by Cu and Cd. Metal levels were highest in aufwuchs and in organisms directly associated with this material (e.g., *Baetis* spp. and *Pteronarcella badia*). Metals in benthic organisms were higher at downstream contaminated stations (AR3 and AR5) compared to upstream reference stations (EF1, AR1, and AR2). Despite greatly reduced levels in water at station AR5 compared to AR3, concentrations of metals in aufwuchs and most invertebrate taxa remained elevated and often increased at station AR5. Metal concentrations in some taxa remained elevated at AR8, the furthest downstream station. For example, concentrations of Zn and Cd in *Baetis* spp. were significantly higher at station AR8 compared to AR1 during May 1991 and September 1990, respectively. In addition, concentrations of copper were higher in *Arctopsyche grandis* at AR8 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 AR1 (spring), Cd and Cu in aufwuchs at EF1 (spring), Cu in *Pteronarcella badia* at AR2 (spring), and Cd in *Rhyacophila* spp. at AR2 (fall).

As with concentrations of metals in water, there was considerable seasonal variability in Cd, Cu, and Zn concentrations in aufwuchs and macroinvertebrates (Fig. 3). Although results of one-way ANOVA indicated that metal levels in benthic communities were generally elevated in the spring, this was dependent on station, 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 levels at EF1 were elevated during spring compared to summer and fall.

## **Feeding Habits of Brown Trout**

Aquatic insects were the dominant prey in the diet of brown trout collected from stations AR1 and AR5 at the Arkansas River on all sampling occasions (Fig. 4).

Ephemeroptera (*Baetis* spp., *Ephemerella* sp.), Plecoptera (*Prostoia besametsa*, *Skwala americana*), Trichoptera (*Arctopsyche grandis*, *Rhyacophila* spp.), and Chironomidae (Orthoclaadiinae) dominated the diet 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. 5). In particular, mayflies were more common in the diet of fish collected from AR1 compared to AR5. In contrast, caddisflies and Orthoclaadiinae 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 Orthoclaadiinae 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. 6). Cd and Cu levels were generally higher in liver and kidney, whereas concentrations of Zn were higher in gill and gut tissue. Differences in metal concentrations between stations were dependent on tissue type. Metal concentrations in liver and kidney tissue were generally similar or significantly higher at the upstream station. In contrast, metal concentrations in gill and gut tissue were often greater at the downstream station.

The order of metal concentrations in certain brown trout tissue was not the same as that observed for water samples and benthic invertebrates. For example, Cu concentrations were much greater than Zn in liver tissue, despite the fact that ambient Zn levels in water were much higher. Similarly, Cd levels were higher than Cu in kidney tissue.

## DISCUSSION

Results of this study indicate that brown trout collected from the Arkansas River consumed prey that with high levels of heavy metals. In particular, *Baetis* spp. and Orthocladiinae chironomids, which comprised a large portion of the diet at both stations, were highly contaminated. Levels of metals in water, gill tissue, benthic organisms, and gut tissue are shown in Figure 7. Metal levels in each compartment were elevated at the downstream station. In general, metal concentrations in water and brown trout gill tissue were relatively low compared to other compartments. Of the three metals examined, Cd appeared to have the greatest affinity for gill tissue.

Metal concentrations in aufwuchs were greatly elevated, suggesting that this material is a major source of metals at the Arkansas River. This hypothesis is supported by the high levels of metals measured in the mayfly *Baetis* spp., which feeds directly on aufwuchs. Metal concentrations in other dominant brown trout prey were elevated at station AR5 compared to AR1.

Metal levels in brown trout gut tissue were generally lower than in prey items. Although these data indicate that food chain transfer of metals is relatively inefficient, they show that some fraction of these metals are available through the diet. Differences gut tissue concentrations between stations varied among the three metals. Levels of Cd in gut tissue were similar between stations AR1 and AR5, whereas Cu and Zn were elevated at the downstream station.

In general, levels of metals in water and food were greater at station AR5 compared to AR1. As expected, brown trout tissues that were directly exposed to metals in food and water (e.g., gut and gill tissue) 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 at upstream and downstream stations or elevated at the upstream station. Despite similar metal levels measured in brown trout storage tissue at stations AR1 and AR5, I suggest that fish from the downstream station were potentially stressed by metal exposure. Fish at station AR5 are clearly exposed to higher metals in water and

through the diet. The similar levels in fish from these two stations suggest that brown trout regulated metals in these storage tissues. Several researchers have demonstrated that fish regulate metal concentrations in critical organs using metal-binding proteins such as metallothionein (Roch et al. 1982). Since production of these proteins comes at some metabolic cost to the organism, fish from the downstream station must divert energy from other important physiological processes (e.g., growth, reproduction) to metal regulation. Reduced density, growth, and survival of brown trout beyond 3-4 years at locations downstream from California Gulch supports the hypothesis that these fish are stressed by chronic metal exposure.

Based on ambient metal levels, metals in benthic organisms, and feeding habits of brown trout, I propose a conceptual model to explain the distribution and transfer of heavy metals at the upper Arkansas River (Fig. 8). Levels of metals in aufwuchs were much higher than any other compartment. I suggest that dissolved metals and metals associated with particulate materials in the water column 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 and sediments represent major sinks for metals at the Arkansas River and may delay recovery of this system. Current remedial activities at California Gulch are expected to reduce concentrations of heavy metals in water. Despite lower ambient concentrations, metals present in contaminated sediments and periphyton will be bioavailable and will 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 be necessary to evaluate the effectiveness of remediation at the Arkansas River.

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## FIGURE LEGENDS

Figure 1. Map of sampling stations at the upper Arkansas River, CO.

Figure 2. Metal concentrations in water at sampling stations at the Arkansas River. Arrows indicate sources of metals from Leadville Tunnel and California Gulch.

Figure 3. 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 5. Feeding habits of brown trout (Salmo trutta) at stations AR1 and AR5.

Figure 6. Percent composition of dominant macroinvertebrate groups in brown trout diet and in the field at stations AR1 and AR5.

Figure 7. Metal concentrations in liver, kidney, gill and gut tissue of brown trout tissue collected from stations AR1 and AR5 during July, 1991 and September, 1991.

Figure 8. Mean concentrations of metals in water, gills, aufwuchs, prey, and brown trout gut tissue at stations AR1 and AR5.

Figure 9. Conceptual model of heavy metal transfer at the Arkansas River. The size of the arrows indicates the relative importance of different pathways.

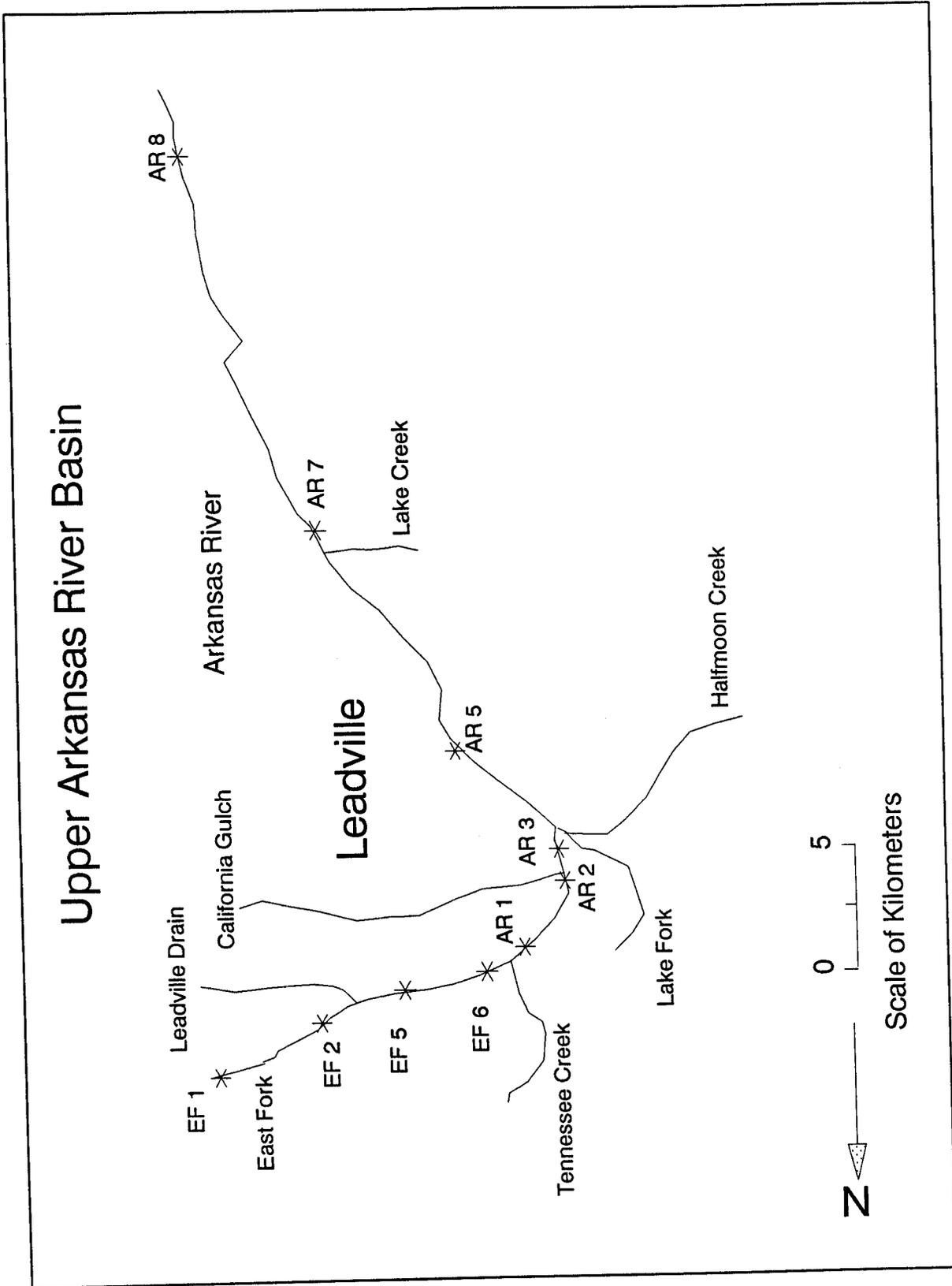


Figure 1

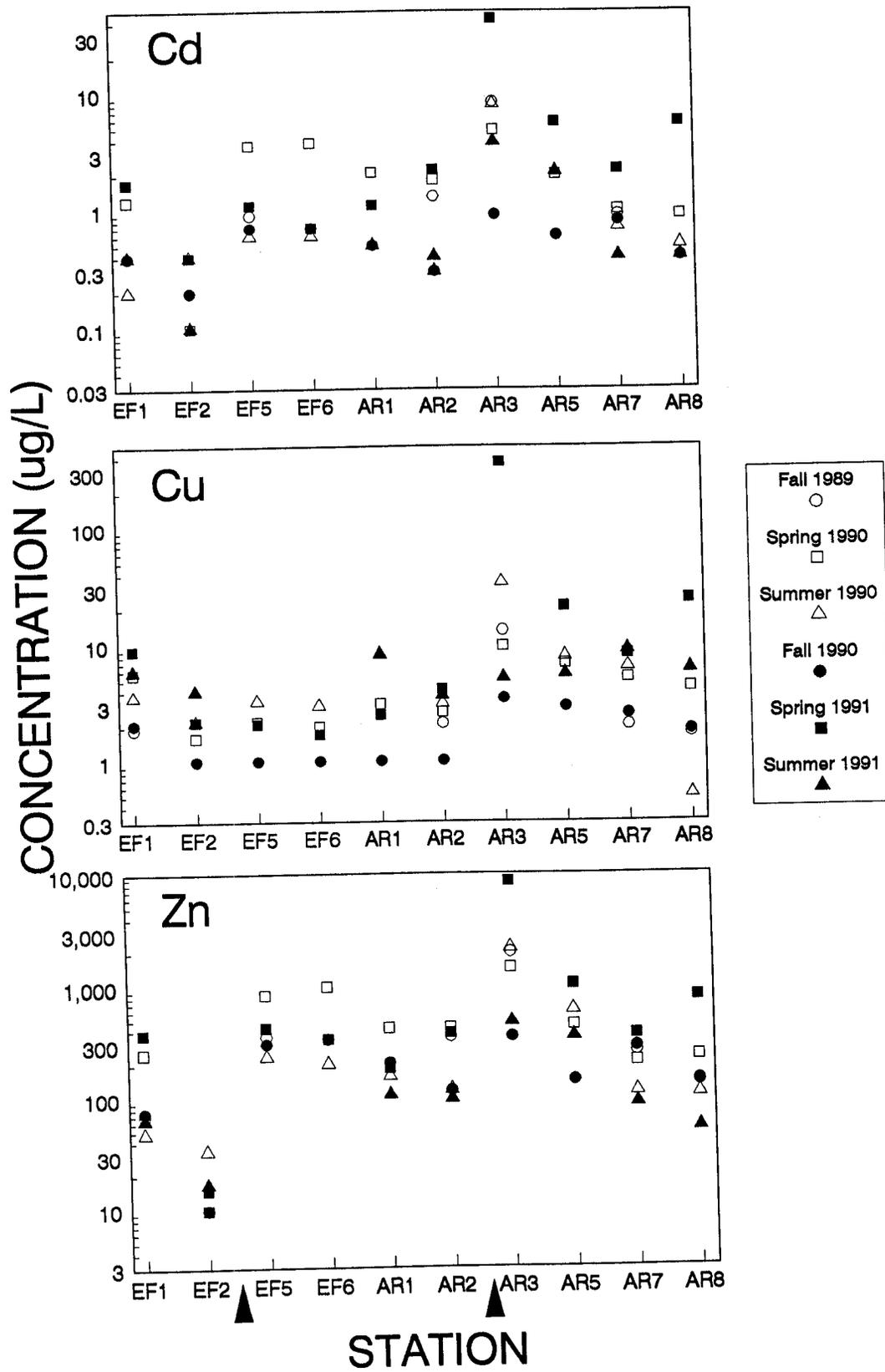


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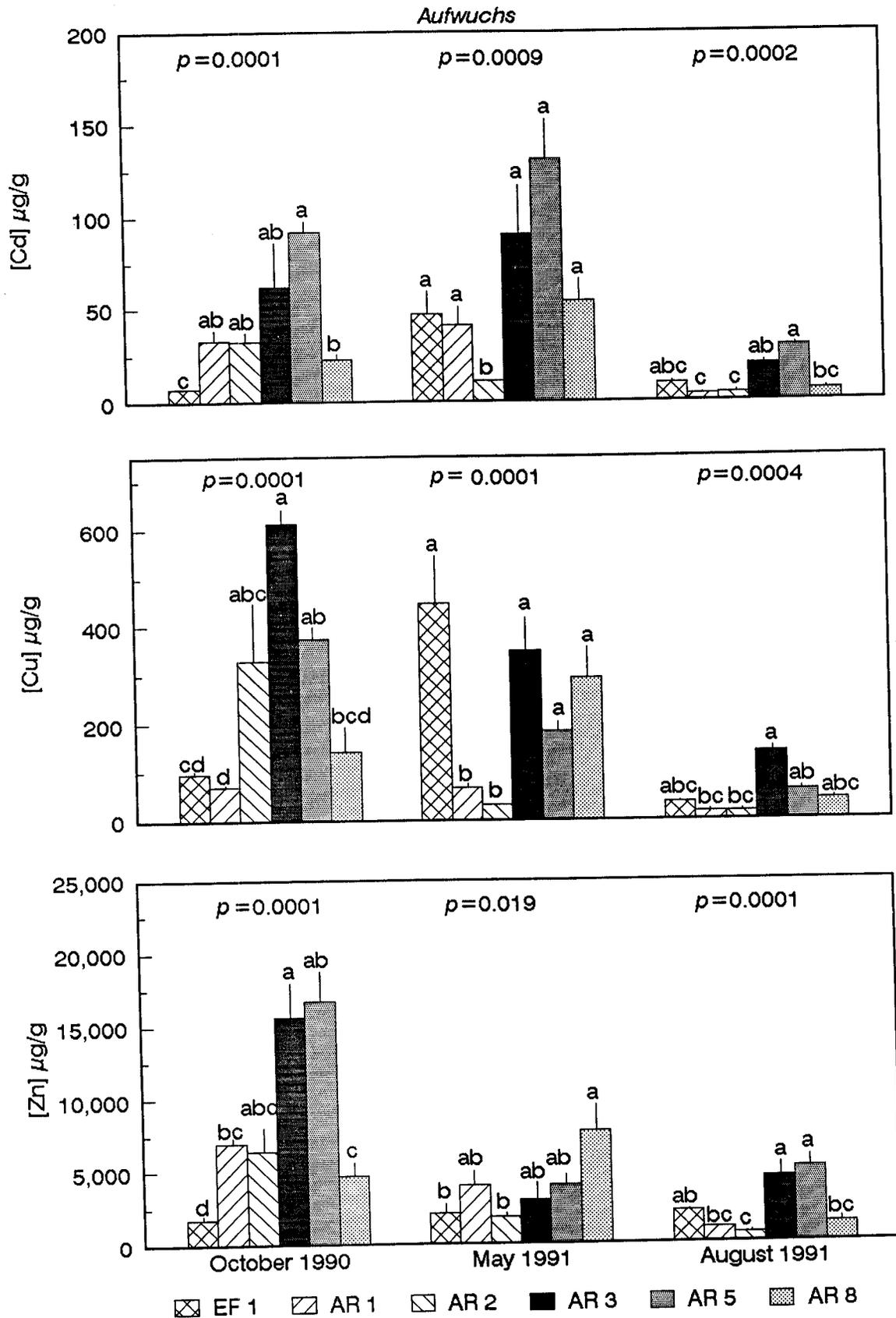


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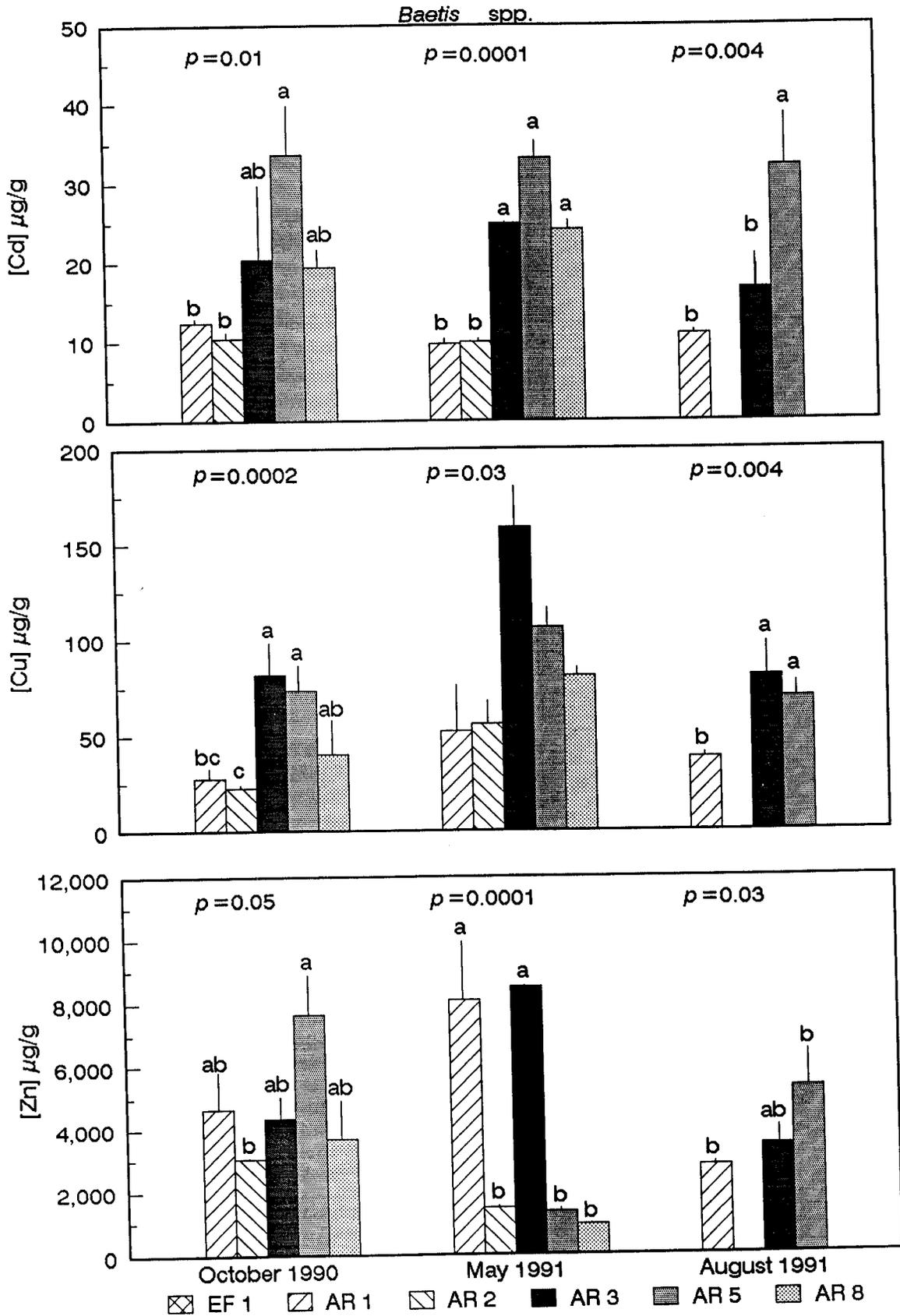


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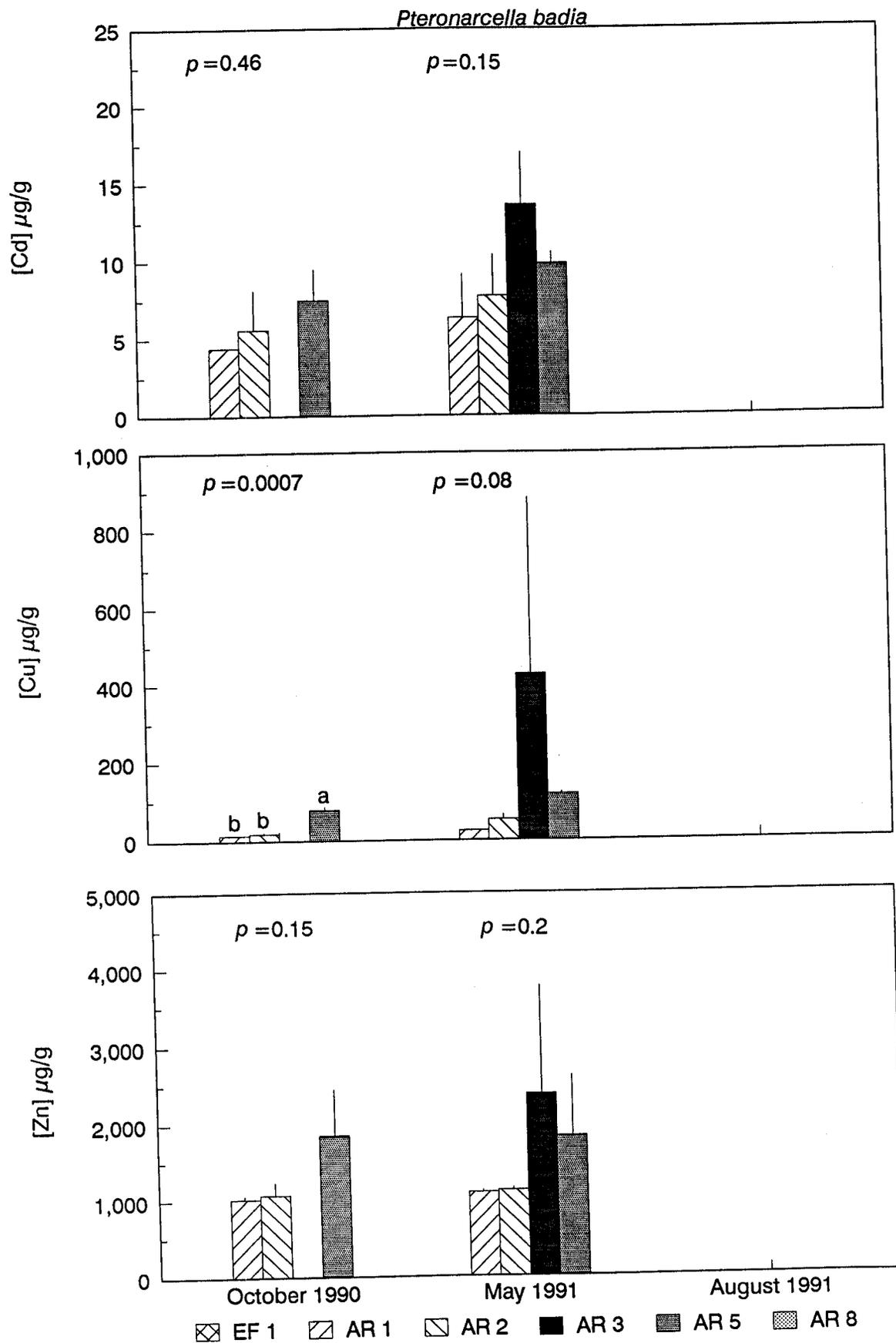


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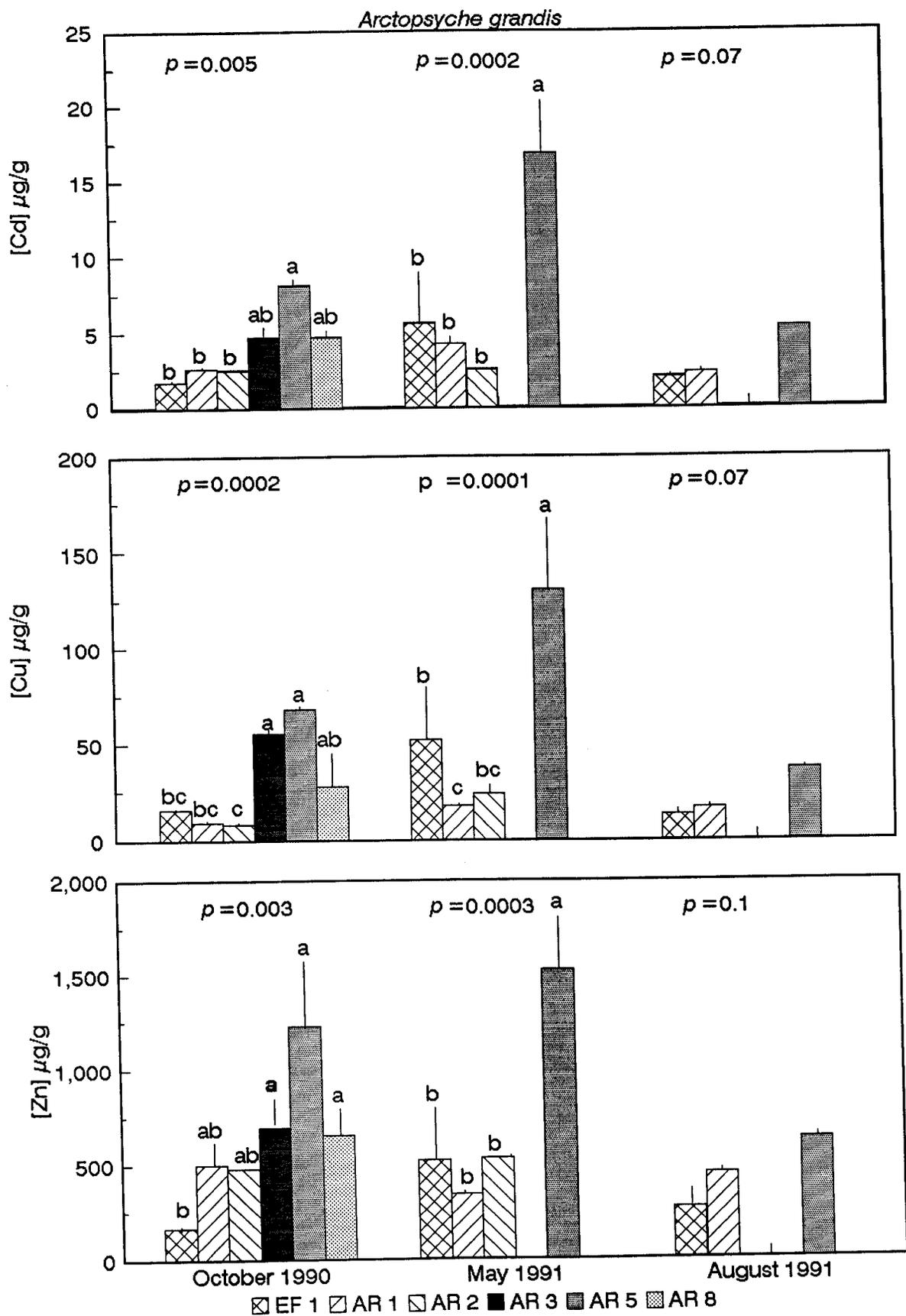


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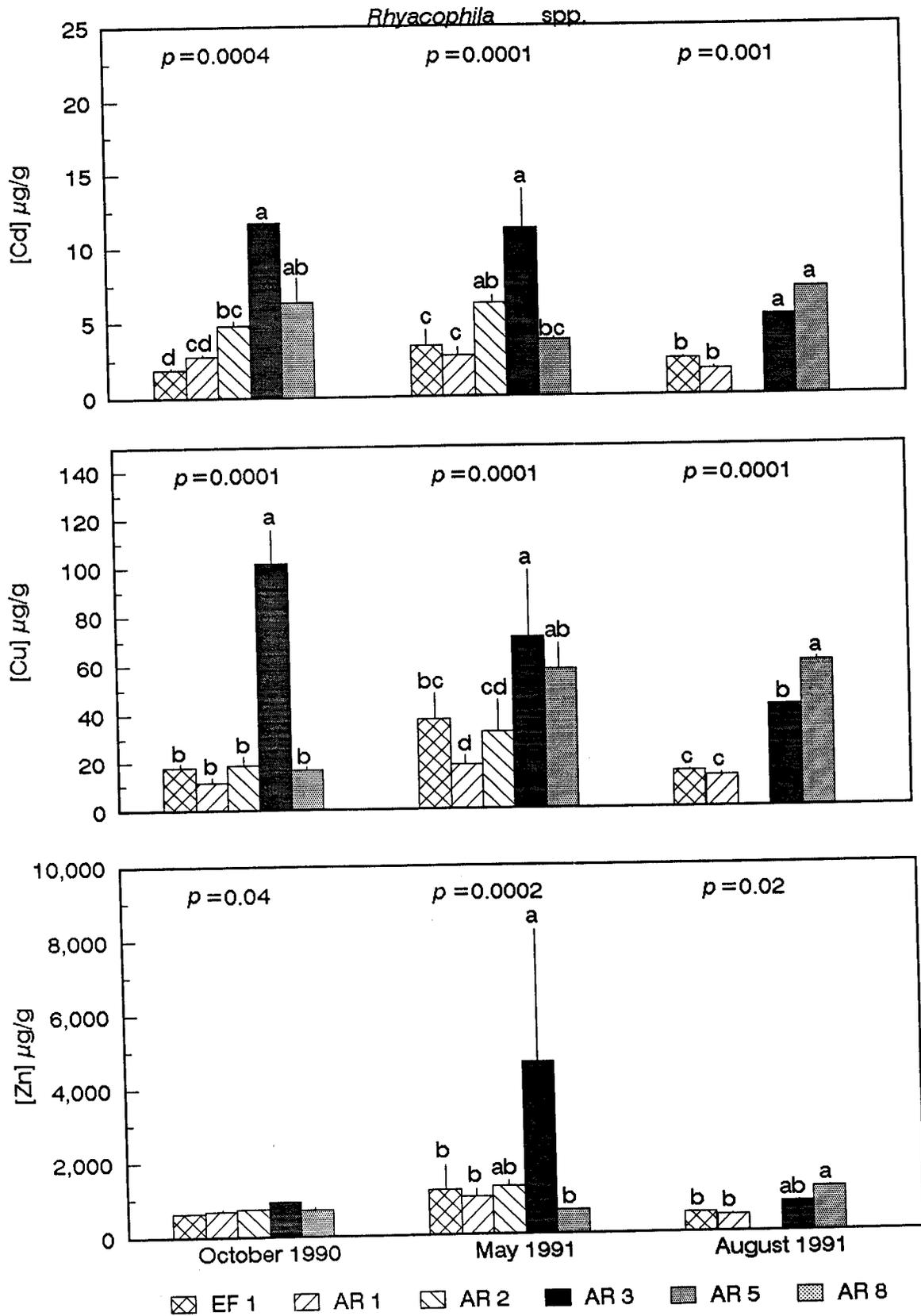
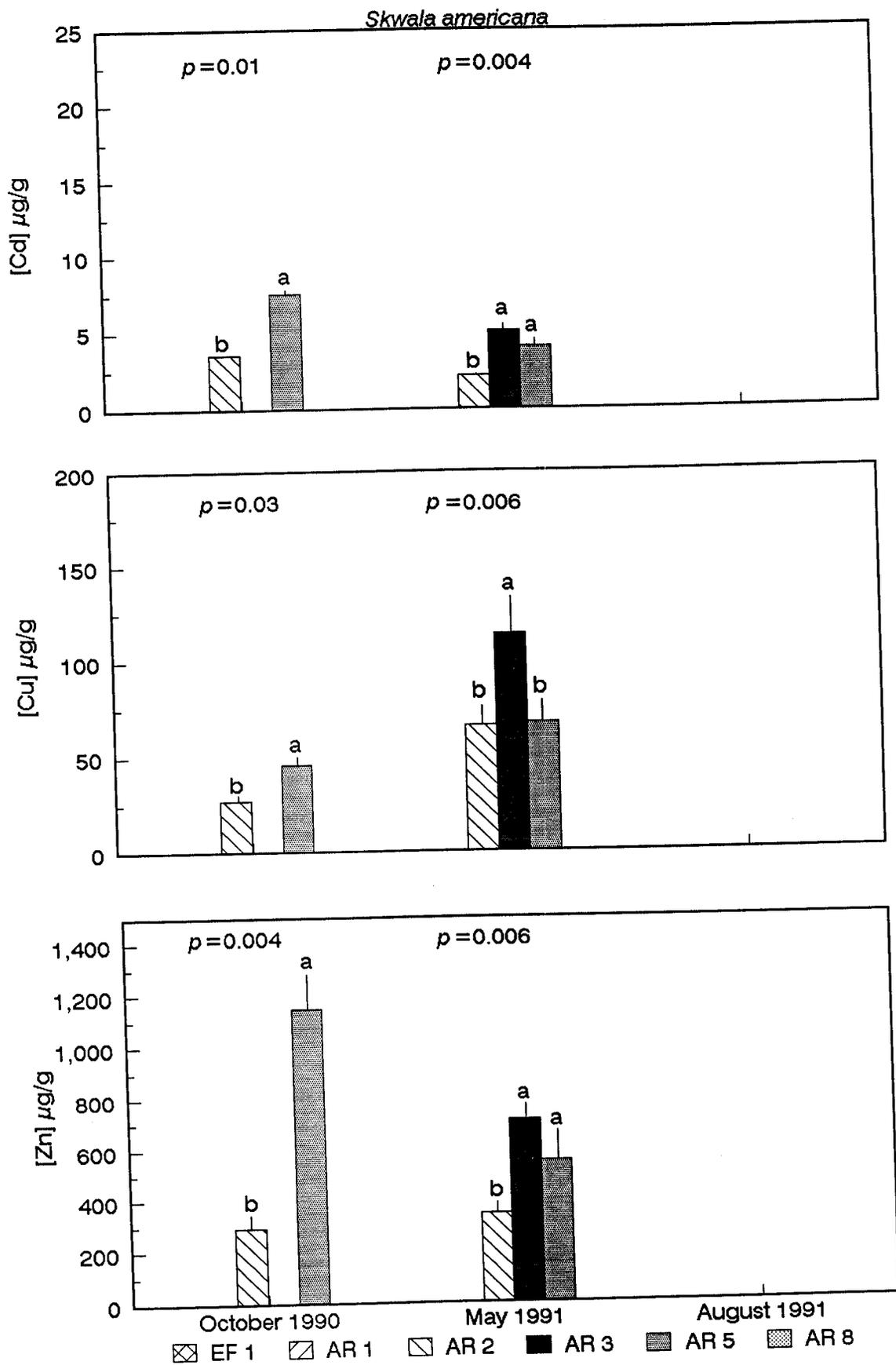
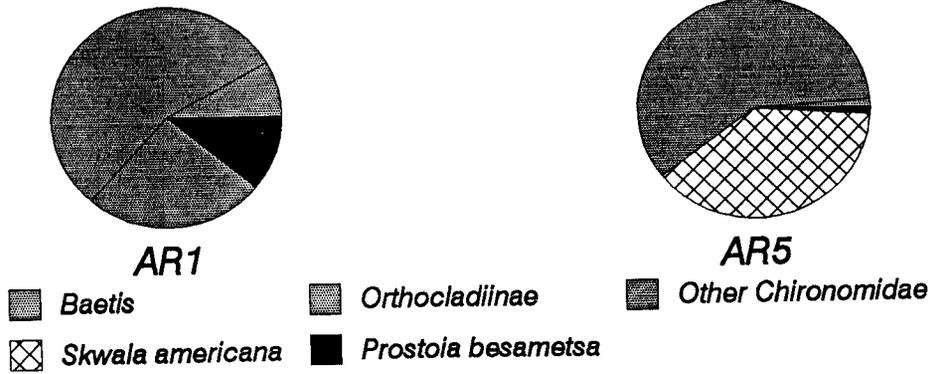


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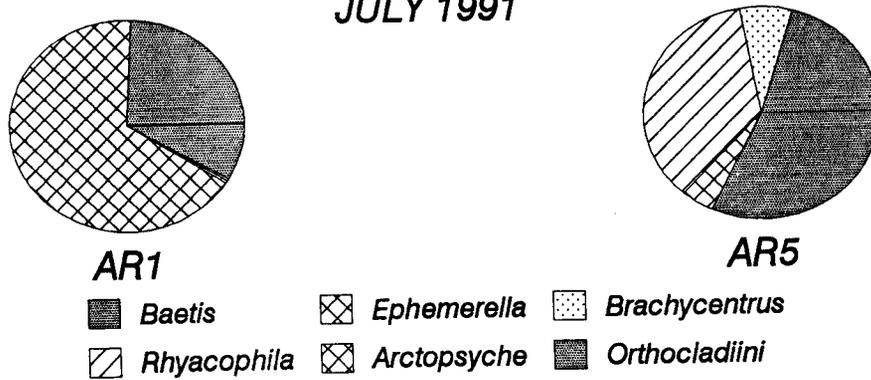


# FEEDING HABITS OF BROWN TROUT

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## JULY 1991



## August 1991

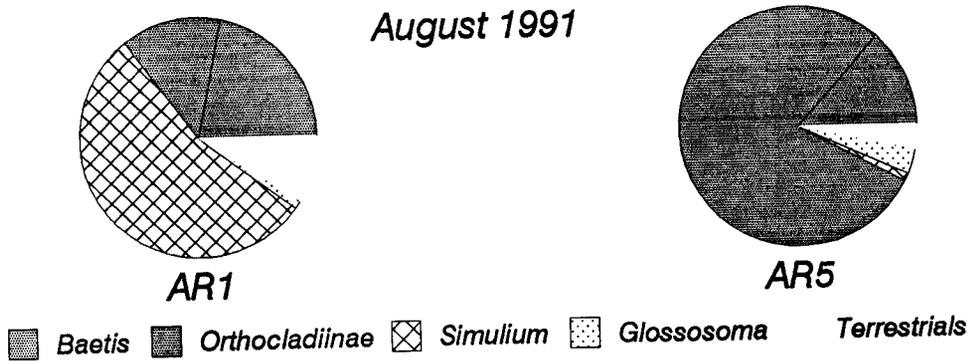
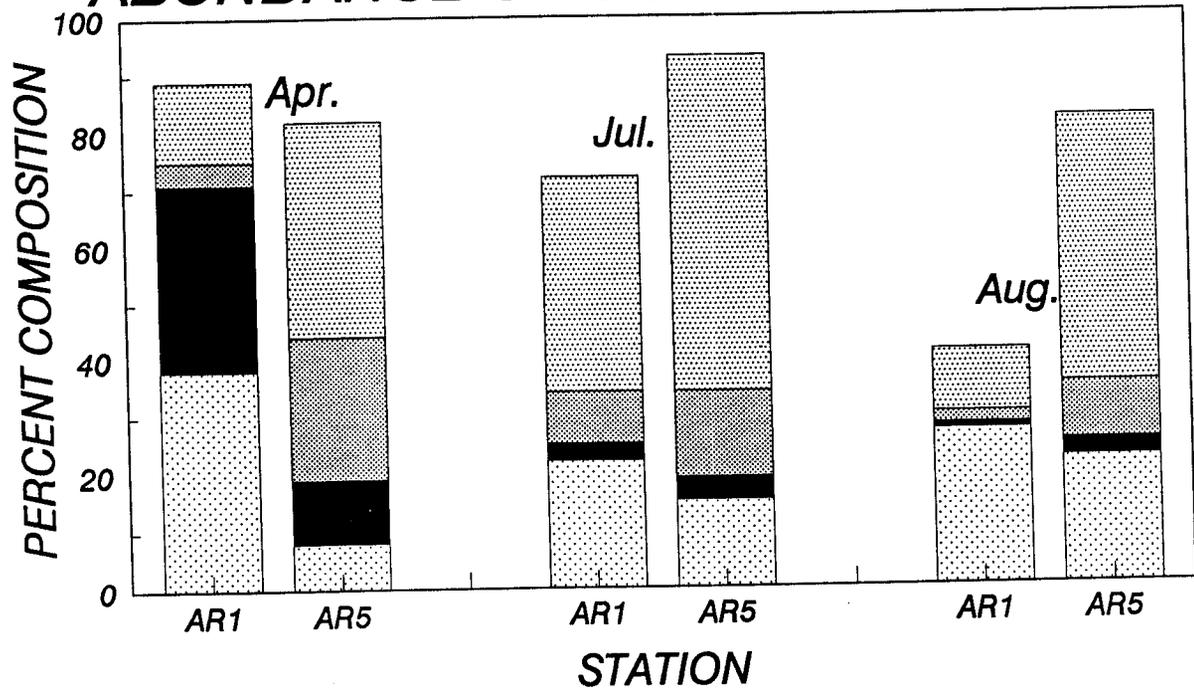


Figure 4.

## ABUNDANCE OF DOMINANT GROUPS



## FEEDING HABITS OF BROWN TROUT

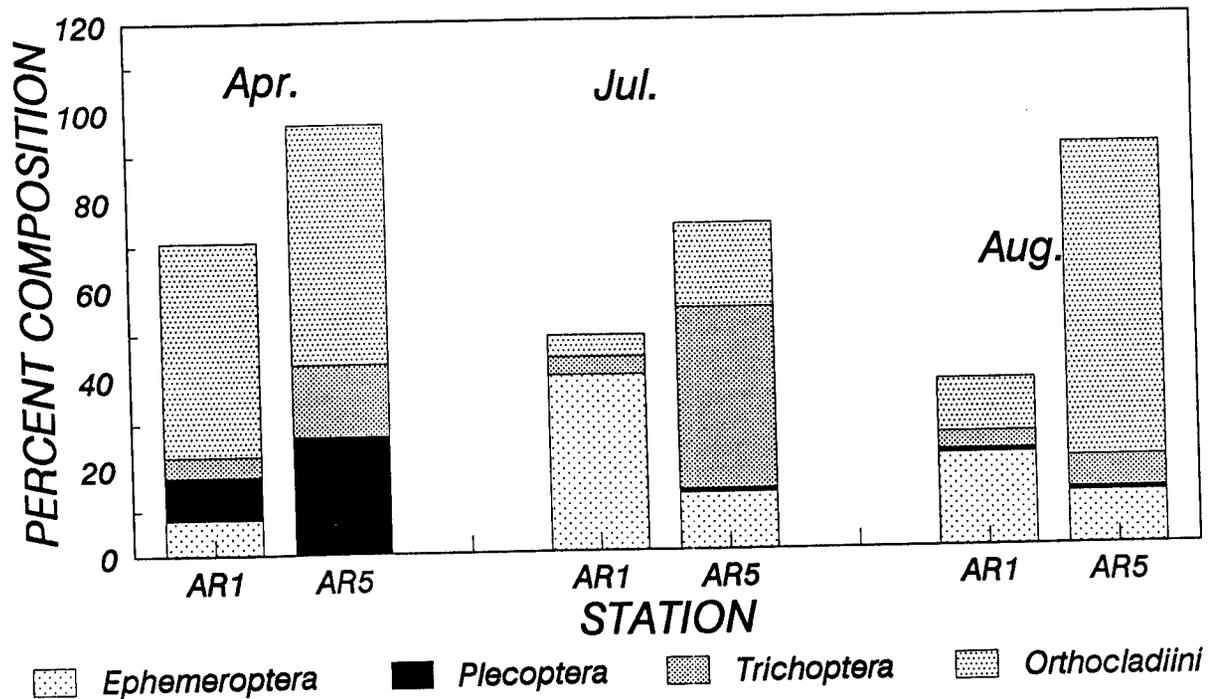


Figure 5.

**METAL CONCENTRATION (ug/g)**

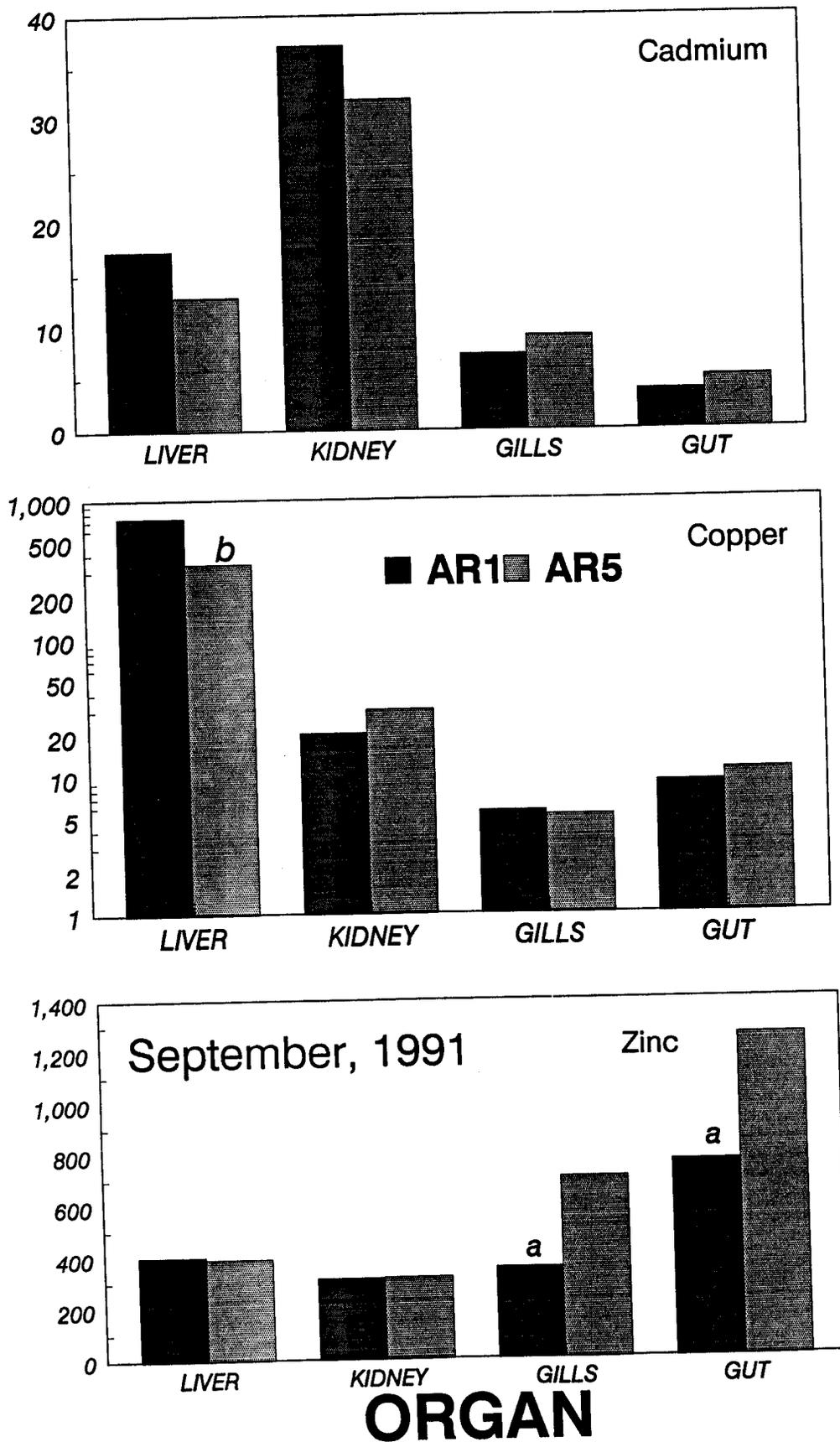
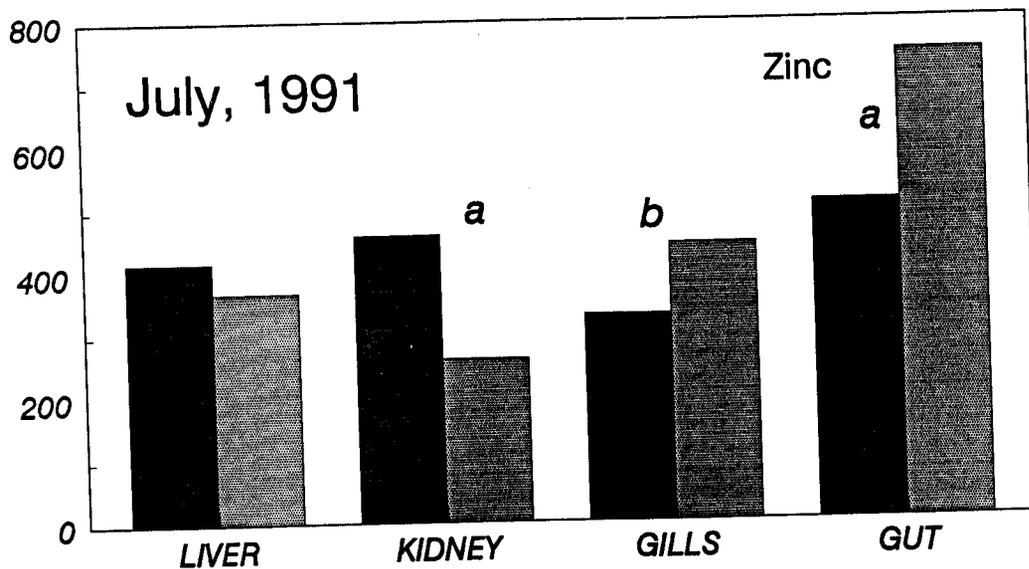
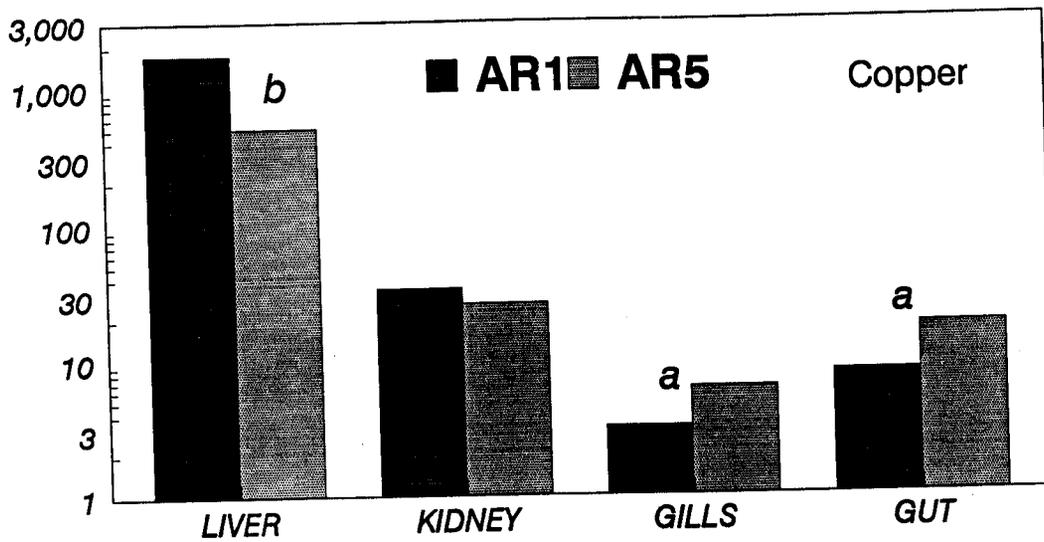
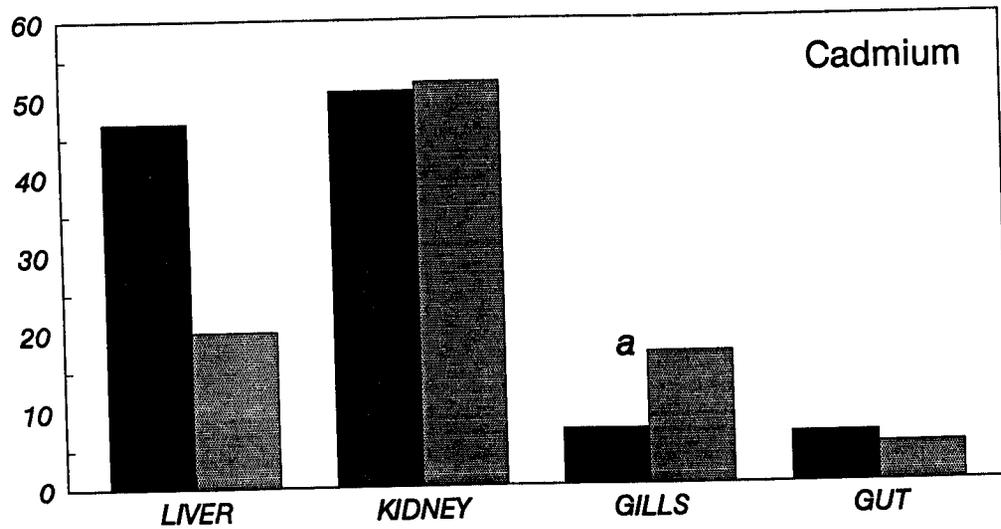


Figure 6.

**METAL CONCENTRATION (ug/g)**



**ORGAN**

Figure 6 (cont.)

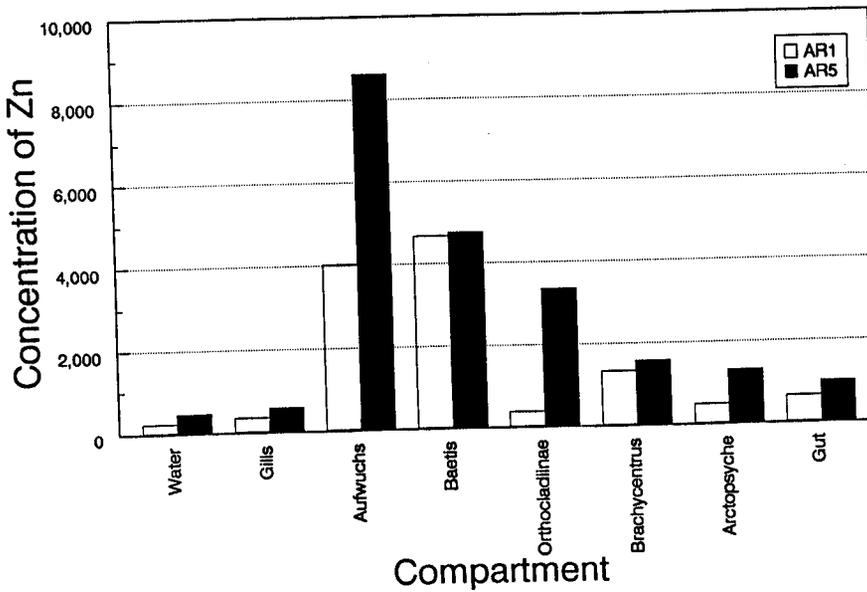
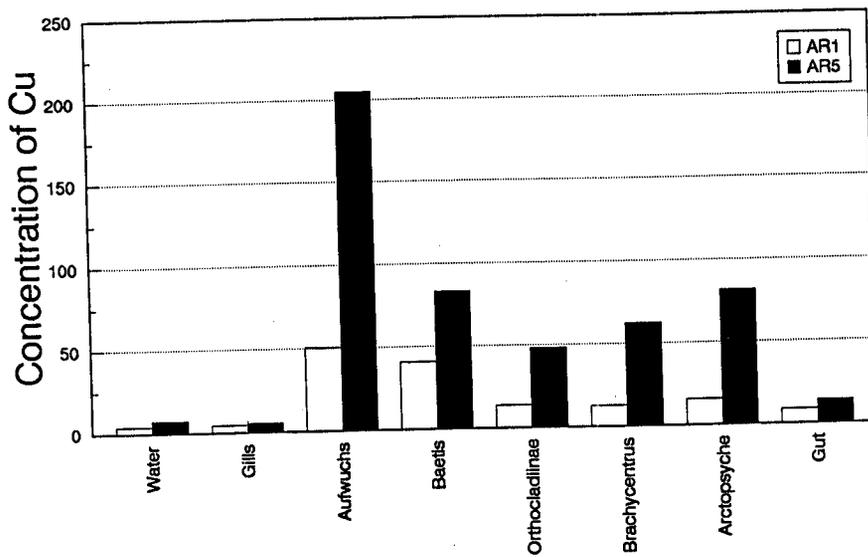
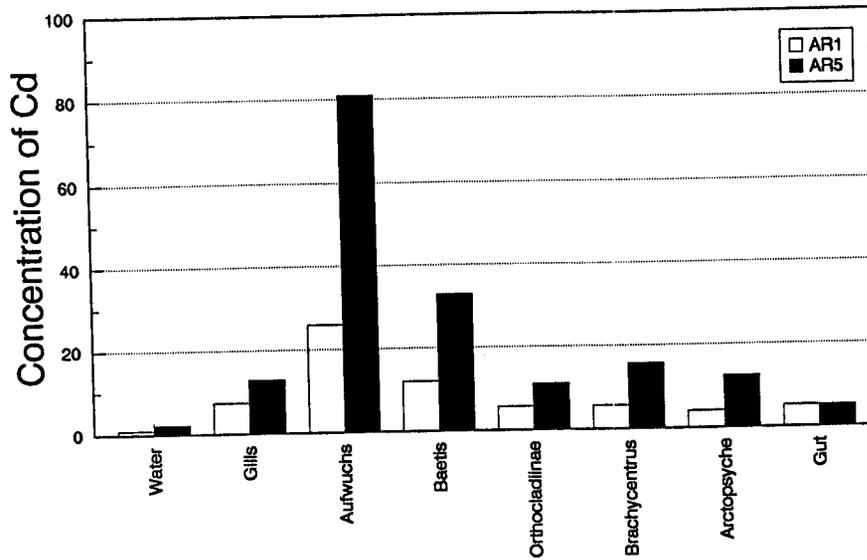


Figure 7.

# HEAVY METALS IN THE ARKANSAS RIVER A CONCEPTUAL MODEL

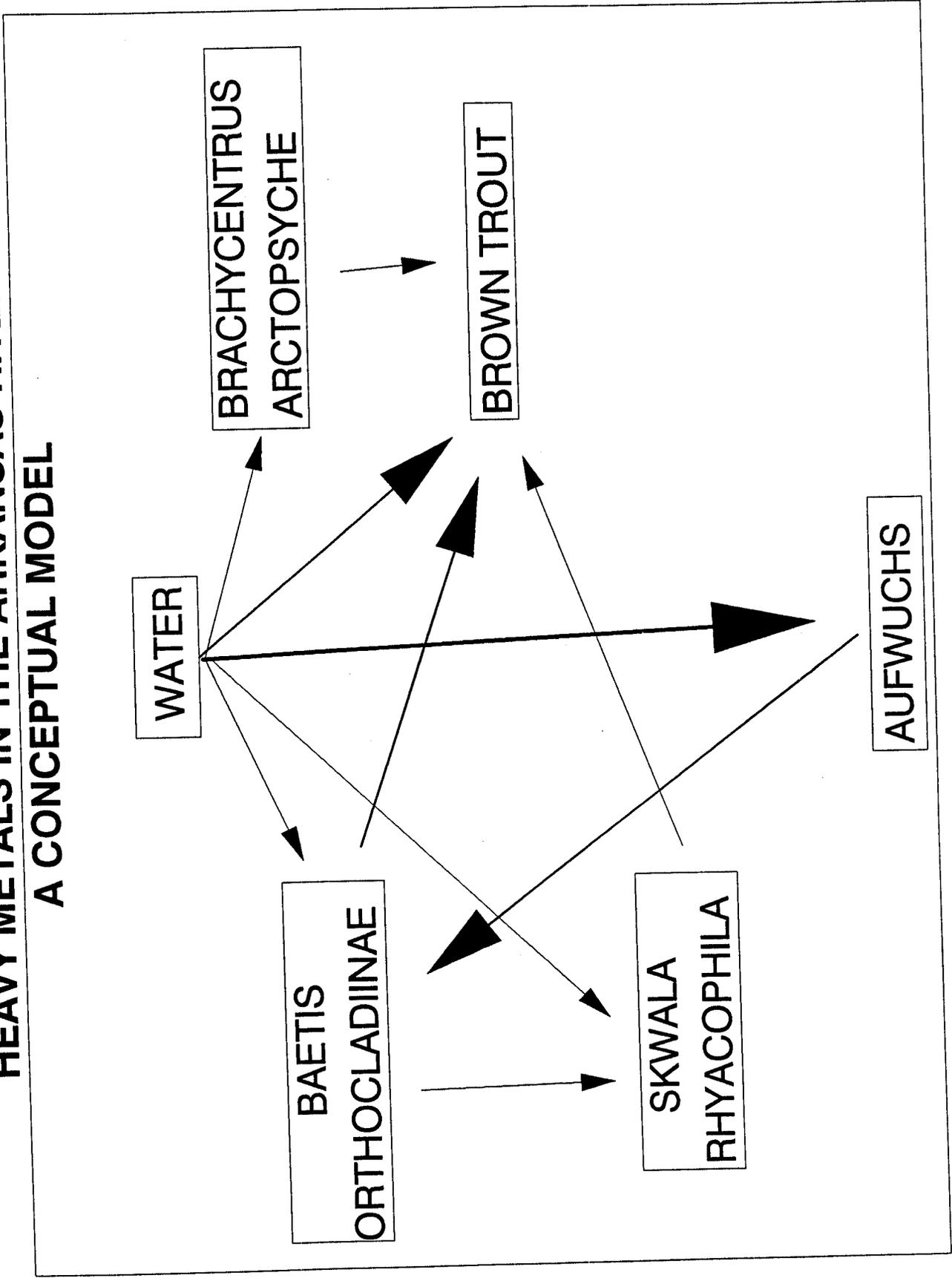


Figure 8.