MERCURY UPTAKE PATTERNS OF BIOTA IN A SEASONALLY ANOXIC NORTHERN CALIFORNIA RESERVOIR

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Abstract. Biotic uptake of mercury (Hg) in Davis Creek Reservoir, California increased dramatically in conjunction with the entrainment of anoxic hypolimnetic water into the mixed layer. This indicated a seasonal pulse increase of bioavailable Hg associated with thermal destratification. The effect was more pronounced in juvenile bass (70-200% seasonal increases in muscle Hg concentration), as compared to adults (15-25% increases), and was most distinct in zooplankton, which spiked to concentrations of 3-6 mg/kg, dry weight, immediately following fall destratification (130-270% seasonal increases over pre-fall levels). In addition to the general buildup of methyl Hg in the hypolimnion under anoxic conditions, a dense layer of photosynthetic anaerobic bacteria just beneath the thermocline is implicated as a potentially important seasonal source of methyl Hg to reservoir fish. Hg increases in juvenile and adult fish correspond to late summer and fall entrainment of upper hypolimnetic water, while zooplankton spike increases may be partially related to ingestion or adsorption of Hg-scavenging manganese oxides, which precipitate following full turnover. A simple and effective, syringe-based cold vapor atomic absorption method for total Hg is also described.

1. Introduction

Seasonal variations in mercury (Hg) dynamics have been described in a number of studies of aquatic systems. Because of the complex biogeochemistry of Hg, a variety of factors have been shown to be associated with spatial and temporal variations in both concentrations and production of aqueous methyl Hg in different systems. Variation in this bioaccumulating Hg species has been linked to pH (Winfrey and Rudd, 1990; Bloom *et al.*, 1991), color/humic substances (Meili and Parkman, 1988; Mierle, 1991), temperature (Parks *et al.*, 1986; Matilainen *et al.*, 1991), particulate matter (Hurley *et al.*, 1991), and algal density (Jackson, 1986; Kaiser *et al.*, 1989). Dissolved oxygen levels and accompanying redox potential have been shown to be among the most important factors influencing both aqueous Hg chemistry and the microorganisms which methylate Hg (Korthals and Winfrey, 1987; Iverfeldt, 1988; Regnell, 1990; Watras *et al.*, 1994). Seasonality in the uptake of Hg by higher aquatic organisms has been associated with corresponding aqueous Hg dynamics in individual water bodies (Jackson, 1988b; Wiener *et al.*, 1990; Bodaly, 1993), as well as with physiological and behavioral patterns in the organisms themselves (Kohler *et al.*, 1986; Bodaly, 1993; Parkman and Meili, 1993).

Davis Creek Reservoir, the site of this study, is characterized by strong thermal stratification between spring and mid-fall, with the seasonal development of anoxic conditions throughout the entire hypolimnion by late August. With the breakdown of stratification in the fall, hypolimnetic water is mixed into the surface layer and aerobic mixing occurs, without ice cover, between December and March. In other research at the site, Gill and Bruland (1992) analyzed reservoir water for organo-Hg compounds; i.e. those containing C-Hg bonds and presumed to be dominated by methyl Hg. This work was done over a two year period during which time the anoxic hypolimnion was found to accumulate organo-Hg at concentrations up to an order of magnitude higher than in the surface waters. During the period of complete aerobic mixing, organo-Hg was low throughout the entire

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water column (<2 ng L^{-1}). In this paper we look at the biotic response to the seasonal pattern of organo-Hg in Davis Creek Reservoir, and link the uptake patterns of total Hg by reservoir zooplankton and fish to annual limnological cycles of thermal stratification and destratification in a system with a seasonally anoxic hypolimnion.

2. Materials and Methods

Davis Creek Reservoir (Figure 1) is located in the historic mercury mining region of the California Coast Range, approximately 70 miles north of San Francisco. The reservoir was impounded in 1984-1986, as a water supply for a large gold mining operation. The full reservoir covers 80 hectares, with ~6,000 acre-feet of capacity and a maximum depth of 25 meters. The inflowing creek has historically been impacted by an abandoned Hg mine, resulting in reservoir sediment Hg concentrations of <1-20 mg kg^{-1}, dry weight. The region is hot and dry in the summer, with nearly all precipitation falling between November and April. Epilimnetic water is utilized for mining process work and summer evaporation is a significant loss mechanism. Total surface drop per year is ~3 m.

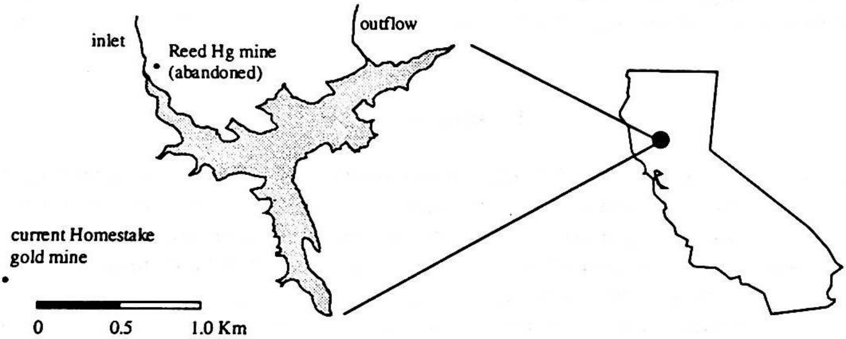

Fig. 1. Davis Creek Reservoir, California.

Limnological monitoring and Hg research have continued, uninterrupted, since reservoir formation (Axler *et al.*, 1988; Slotton, 1991). Broodstock largemouth bass (*Micropterus salmoides*) were placed into the reservoir in 1985. Bass born in the reservoir have been collected since 1985 by gill net, seine, and angling; aging was by scale analysis. Muscle samples were taken from the dorsal-anterior fillet quadrant. Juvenile bass (0.2 - 5.0 g live weight) were collected by seine. Muscle tissue was carefully dissected from these very small fish to maintain comparability with the larger bass; the entire fillet was utilized. Zooplankton were collected in nocturnal tows of a weighted hoop net with a 200 μm mesh basket, and strained through a 500 μm sieve. This separated out the bulk of entrained phytoplankton. Zooplankton biomass was typically dominated by a single cladoceran species, *Daphnia pulex*. Plankton was dried to constant weight at 55 °C and homogenized with teflon and glass instruments prior to analysis.

Bacteriochlorophyll was quantified by scanning spectrophotometer readings of acetone extracts of seston retained on 1.2 μm pore size GF/C filters. Water was collected from various depths by Van Dorn sampler. Dissolved oxygen and temperature profiles were taken with a YSI meter (model 51B). Relative, cumulative volumetric entrainment of hypolimnetic water into the mixed layer was estimated using surface elevation and vertical profiles of temperature and oxygen, together with existing depth/volume curves.

Biota Total Hg Methodology

Total Hg was analyzed in biota samples by cold vapor atomic absorption (CVAA) spectrometry, utilizing a modified micro-delivery system based on a syringe gas-injection procedure first described by Stainton (1971). Samples were digested for 90 minutes with 2:1 concentrated sulfuric (H_2SO_4):nitric (HNO_3) acid in borosilicate test tubes, utilizing heat (95 °C water bath) and pressure (tubes capped securely with teflon-lined screw tops). After cooling tubes in an ice water bath, a consistent volume of 6% potassium permanganate ($KMnO_4$) solution was added, sufficient to leave precipitate in all tubes. Tubes were digested at 80-90 °C for an additional 90 minutes at ambient pressure (without caps) and then topped up to the desired dilution with mercury-free (nitrogen purged) water and capped.

In the detection phase, individual disposable plastic syringes were used to draw 2 ml of well-mixed digest out of each tube, followed by 2 ml of acid reductant mixture. The reductant mixture contained 10 ml H_2SO_4, 5 g hydroxylamine sulfate ($(NH_2OH)_2 \cdot H_2SO_4$), 5 g sodium chloride (NaCl) and 2.5 g stannous chloride ($SnCl_2 \cdot 2H_2O$) per 100 ml, promoting both the rapid dissolution of the permanganate precipitate and reduction of digest mercury to the volatile elemental state. A consistent volume of air was carefully drawn into the syringe, which was quickly capped (needle off) and then mixed by touching the tip to a vortex mixer for 10 seconds. After the permanganate precipitate cleared, the capped syringe was again vortex agitated (15 seconds) and the syringe air was injected into a low-volume, long path-length cuvette, which was mounted in the burner compartment of an atomic absorption spectrophotometer, in the beam path of a mercury lamp set at 253.7 nm. Maximum absorbance (peak height) was reached within 2 seconds; this absorbance was recorded, and the cuvette was readily cleared of mercury for the next sample by flushing with a syringe-full of air. Standards and QA/QC samples were digested and in all other ways treated identically to samples. Exact weights of total digests and injection aliquots were determined by sequential weighings of digest tubes to ± 0.001 g.

Advantages of the approach include: (1) relatively low detection limit: <0.01 mg kg^{-1} Hg in samples; (2) excellent reproducibility and recoveries of spikes and references; (3) ability to re-analyze digests, as only a portion is injected; digests are stable for months; and (4) because stannous chloride never touches digestion tubes, the potential for reductant carry-over is not a concern.

3. Results and Discussion

3.1. ZOOPLANKTON

Zooplankton total Hg was measured 7-13 times per year for four years. We hypothesized that these relatively ephemeral organisms might be useful indicators of potential seasonality in Hg bioavailability. Because they are short-lived, there is no ambiguity as to season of uptake, as is typically the case with adult fish. Furthermore, unlike the reservoir fish, zooplankton were collectable in all seasons.

The single most compelling feature of the zooplankton Hg data record is the presence of extreme seasonal spiking in each of the late fall periods of record (Figure 2). During these times, zooplankton Hg concentrations jumped 2-3 times higher than pre-fall levels, to 3-6 mg kg^{-1} Hg (dry weight). Seasonal spiking coincided with the fall turnover event, as indicated by the cumulative hypolimnetic entrainment curve superimposed in Figure 2. After peaking, zooplankton total Hg subsequently dropped precipitously each winter.

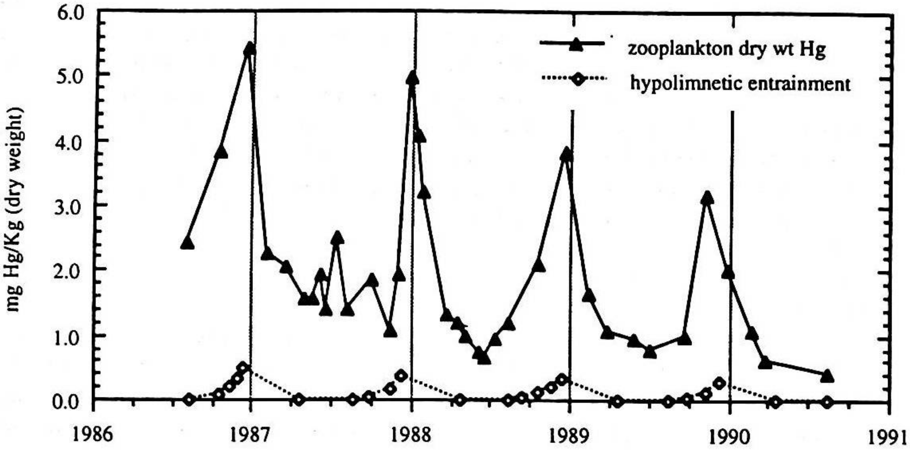

Fig. 2. Zooplankton Hg concentration data record, 1986-1991; with relative, cumulative seasonal entrainment of anoxic hypolimnetic water into the mixed layer superimposed. Peak (100%) entrainment each year has been scaled to 1986 (maximal) hypolimnetic volume, when reservoir was full.

A number of researchers have found anoxic conditions to greatly enhance both Hg methylation (Korthals and Winfrey, 1987; Regnell, 1990; Matilainen *et al.*, 1991) and methyl Hg accumulation in the water (Mason *et al.*, 1993; Watras *et al.*, 1994). Gill and Bruland (1992) demonstrated that the seasonally anoxic hypolimnion of Davis Creek Reservoir was the site of maximal accumulation of dissolved organo-Hg, with concentrations (10-35 ng L^{-1}) an order of magnitude higher than in surface water (1-3 ng L^{-1}). The zooplankton Hg uptake data indicate that this hypolimnetic pool presented a significant pulse of bioavailable Hg to the mixed layer each fall in conjunction with thermal destratification. As zooplankton can accumulate significant proportions of inorganic Hg in addition to organo-Hg (Watras and Bloom, 1992), these total Hg data cannot be attributed solely to uptake of organo-Hg. However, the Gill and Bruland (1992) study found the seasonal accumulations of aqueous Hg in the anoxic hypolimnion to be dominated by dissolved organo-Hg, which accounted for 40-82% of the total (mean = 66%) and 70-92% of the dissolved fraction (mean = 82%).

The annual cycle of zooplankton Hg spike increases and subsequent declines to baseline very closely matches the cycle of Mn precipitation and sedimentation noted at this reservoir. Gill and Bruland (1992) found that annual water column turnover introduced large pulses of both aqueous organo-Hg and dissolved Mn to the mixed layer from the anoxic hypolimnion at turnover. The subsequent cycle of precipitation and sedimentation of Mn was linked to removal of aqueous organo-Hg from the water column, presumably through adsorption onto particulate Mn. The timing of the zooplankton seasonal Hg pattern suggests that the large increases may be partially due to ingestion or adsorption of Mn precipitates by these cladoceran filter feeders.

3.2 YOUNG-OF-YEAR JUVENILE BASS

Seasonality in Hg uptake was also investigated in young-of-year juvenile largemouth bass (Table I, Figure 3). Muscle Hg concentration was utilized in this seasonal work rather than whole body Hg burden due to difficulties in consistently compositing such small individual fish (0.2 - 5.0 g), as well as for comparability with the adult fish and other regional fish data. Unlike zooplankton, which can accumulate significant proportions of inorganic as well as methyl Hg (Watras and Bloom, 1992), fish muscle Hg has repeatedly been demonstrated to consist almost entirely of methyl Hg (Bloom, 1992). In other work, we have found muscle tissue to be the repository of >90% of the total mercury and >95% of the methyl mercury body burden in largemouth bass (Suchanek *et al.*, 1992). Thus, changes in muscle total Hg concentration in rapidly growing juvenile bass provide a good measure of changes in overall methyl Hg uptake and bioavailability.

TABLE I

Juvenile bass total Hg data (wet weight muscle mg Hg kg^{-1} from individual fish)

	Date	n	Mean Hg	Std. Dev.	95% Confidence Interval of Mean
1988	11-Jul	12	0.66	±0.17	0.55 - 0.77
	8-Aug	10	0.74	±0.18	0.61 - 0.87
	10-Sep	23	0.61	±0.15	0.55 - 0.68
	21-Oct	15	1.31	±0.40	1.09 - 1.53
1989	1-Jul	10	0.44	±0.08	0.39 - 0.50
	13-Aug	19	0.65	±0.12	0.59 - 0.71
	26-Sep	19	0.93	±0.22	0.82 - 1.04
1990	29-Jun	23	0.35	±0.12	0.30 - 0.40
	4-Aug	23	0.35	±0.08	0.32 - 0.39
	15-Sep	12	0.82	±0.24	0.66 - 0.97
	24-Oct	10	1.10	±0.16	0.98 - 1.21

Newly hatched juvenile bass taken in early July of 1988 weighed less than 0.5 g and, yet, had accumulated muscle Hg concentrations with a mean of 0.66 mg kg^{-1}, highlighting the overall high level of Hg bioavailability in this young reservoir. Individuals collected in August were approximately double the size of the July set, but muscle Hg concentration remained at a similar level, indicating similar levels of Hg bioavailability throughout the period. Juveniles collected 10-September consisted of larger/older individuals (1.3-4.5 g) than the July-August samples, but had muscle Hg concentrations which were statistically identical to those seen earlier in the summer. However, the October sample set demonstrated a significant departure from the previous uniform trend of muscle Hg concentrations, with concentrations approximately double those found earlier. It is notable that the elevated Hg concentrations in the October 1988 samples varied inversely with size and age, with highest levels in the youngest individuals (Figure 3). These smallest individuals, of a size similar to early season young and almost certainly of a late spawn, were those whose growth and Hg accumulations were

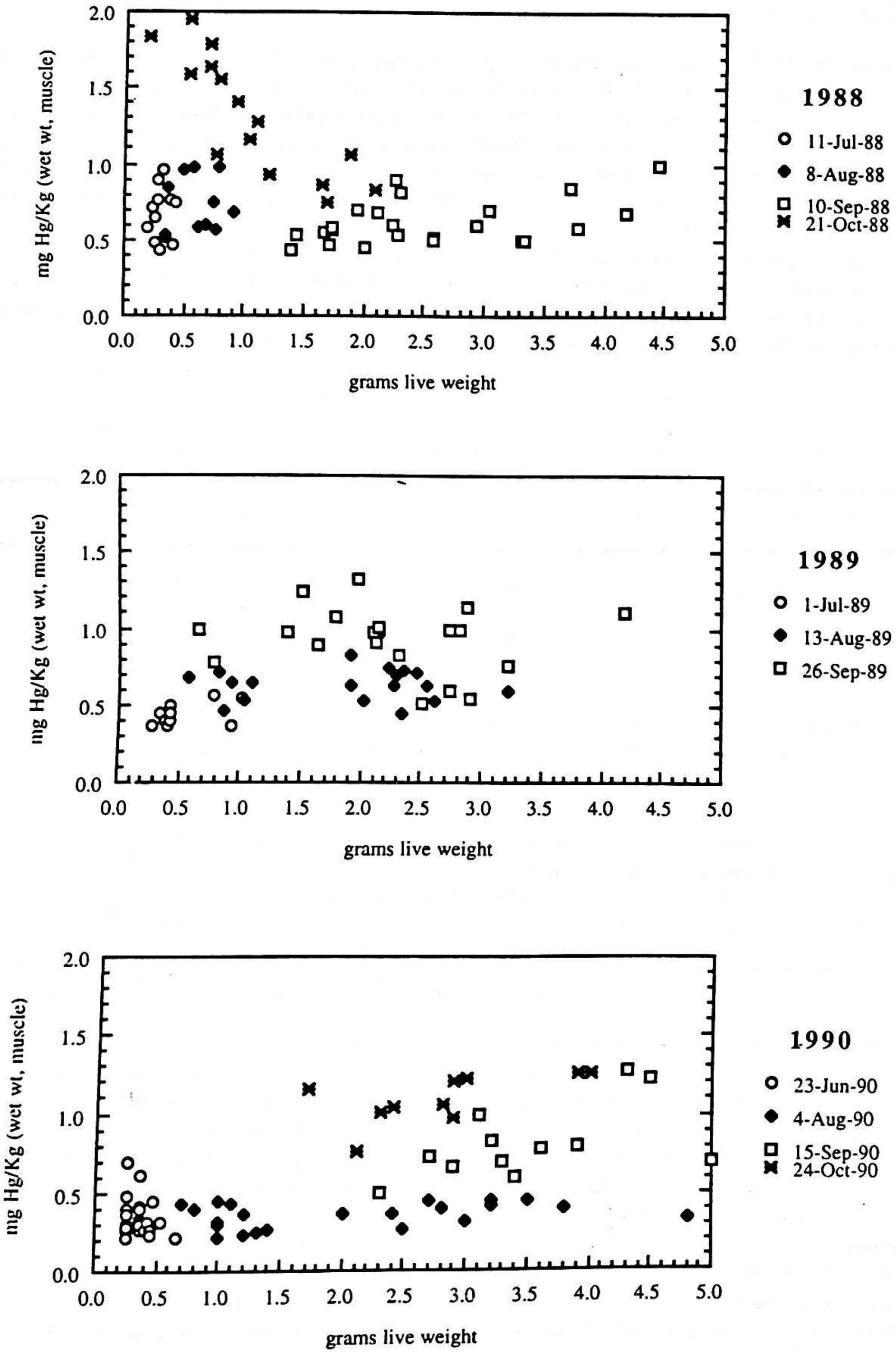

Fig. 3. Young-of-year bass muscle mercury, summer through fall, 1988, 1989, 1990.

proportionately most dominated by the period when anoxic hypolimnetic water was being entrained into surface waters.

A similar pattern was observed in 1989 and 1990. Juvenile bass taken in late September or October of each of the three years of record, and in mid-September of 1990, had significantly elevated Hg concentrations relative to samples taken June through August (Table I). Bass were dormant and unobtainable between mid-fall and spring.

The annual seasonal increases in juvenile bass Hg were noted in September and October, *before* the most significant lowering of the thermocline typically occurred. However, loss of epilimnetic volume due to water usage and evaporation dropped the water surface level by ~0.5 m per month at these times, moving the thermocline a corresponding amount into the upper hypolimnion even when the mixed layer remained a constant depth. In addition, the absolute depth of the mixed layer typically increased somewhat throughout the late summer and early fall due to wind action and lowering temperatures. Together, these two processes resulted in the progressive entrainment of anoxic water from just beneath the thermocline into the mixed layer, from late summer until turnover was complete by late November or early December (Figure 4, also Figure 2). Because the bass were collected prior to turnover (when they became dormant each year), the seasonal increases in muscle Hg corresponded mainly with entrainment of anoxic water from the *upper* hypolimnion.

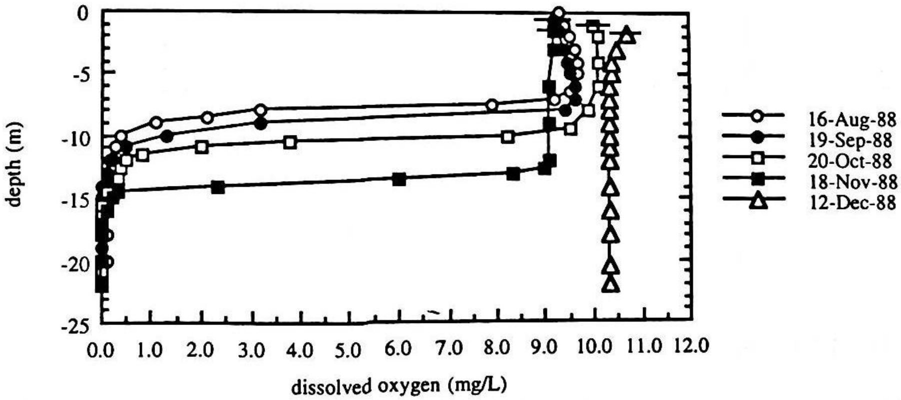

Fig. 4. 1988 profiles of dissolved oxygen throughout period of hypolimnetic entrainment.

The upper hypolimnetic layer, just beneath the thermocline, was of particular interest as this was the location of a dense seasonal lens of photosynthetic anaerobic bacteria (Figure 5). This narrow and well defined layer of purple or green sulfur bacteria was found where light levels were highest in conjunction with anoxia, following the thermocline down from late August through November during each year of our study. While Gill and Bruland (1992) indicated the bottom of Davis Creek Reservoir to be the major source and/or accumulation location of organo-Hg, conclusions from that study are constrained by a coarse sampling interval (2-5 m) which may have missed narrow strata with elevated concentrations (G. Gill, personal communication). An interesting possibility is that the narrow but extremely dense seasonal layer of photosynthetic anaerobes immediately below the thermocline may have been a significant site of Hg methylation and/or accumulation. Indeed, Mason *et al.* (1993) found this to be the case in another system, utilizing fine-resolution (0.1 m) depth profiles of aqueous Hg species and bacteriochlorophyll in an anoxic estuarine kettle basin. In that study, bacteriochlorophyll and methyl Hg both increased more than 50-fold immediately below the pycnocline in a ~2 m thick lens. Watras *et al.* (1994) and Verta and Matilainen (1994) also describe elevated methyl Hg concentrations associated with layers of similar bacteria. All of these researchers indicate that the photosynthetic anaerobes themselves are almost certainly not the direct sources of elevated methyl Hg, but that co-existing sulfate reducers may be involved. Matilainen (1994) presents experimental evidence for this. At Davis Creek Reservoir, the dense layer of photosynthetic anaerobes developed immediately below the thermocline during the time of year when this stratum was being progressively entrained into the mixed layer where fish were actively growing. The juvenile bass Hg data indicates that this layer may be a seasonally important source of bioavailable Hg to surface waters.

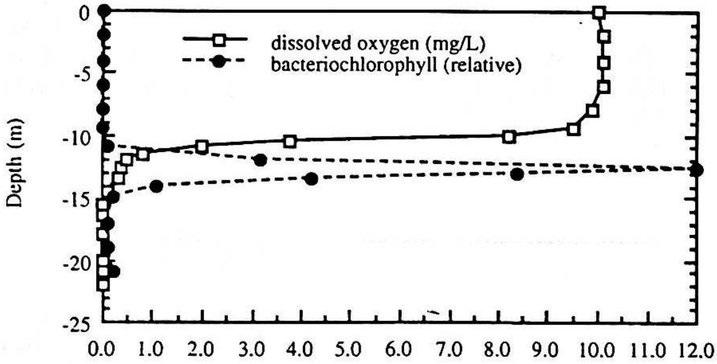

Fig. 5. Distribution of photosynthetic anaerobes in anoxic upper hypolimnion, 20-Oct-88.

3.3 ADULT BASS

Four to 25 bass from the initial 1985 cohort of reservoir-born fish were analyzed for Hg at each of 24 sampling events throughout the 5 years of this study, with average size increasing from 20 g in year 1 to >750 g in year 5. In addition to the overall very high Hg levels (2-4 mg kg^{-1}, wet weight muscle), these data give the indication of a seasonal pattern, though clearly not statistically significant, superimposed on the general decline in muscle Hg concentrations in this cohort of bass as they grew in the newly impounded reservoir (Figure 6). The overall year-to-year declines in mean Hg levels in all the data sets presented throughout this paper can be attributed to declining mercury bioavailability following initial peak levels associated with impoundment and newly flooded soils (Abernathy and Cumbie, 1977; Bodaly *et al.*, 1984; Jackson, 1988a). In each year, bass muscle Hg concentrations declined during thermal stratification between spring and mid summer. The general decline was interrupted in each of the years beginning in the late summer to mid fall, when muscle Hg concentrations typically increased by ~0.5 mg kg^{-1}. This coincided with the juvenile bass seasonal increases. Following turnover, when the bass became dormant, their muscle Hg concentrations remained steady or increased further during the winter, as evidenced by highest annual levels typically occurring in the first spring collection of each year. This was despite the fact that, soon after fall turnover, aqueous concentrations of organo-Hg have been shown to plummet in this reservoir in conjunction with the precipitation and sedimentation of manganese oxides (Gill and Bruland, 1992). As Hg depuration is relatively minimal in these fish (Slotton, 1991), significant short term decreases in muscle Hg levels could only occur through growth dilution under lower Hg bioavailability. We believe that this process explains the declines noted in bass muscle Hg concentrations during each spring through mid-summer season after initial impoundment. However, with metabolic-based weight loss (as opposed to growth) during November-March dormancy, tissue Hg could become slightly more concentrated, despite lowest aqueous Hg concentrations occurring in that season.

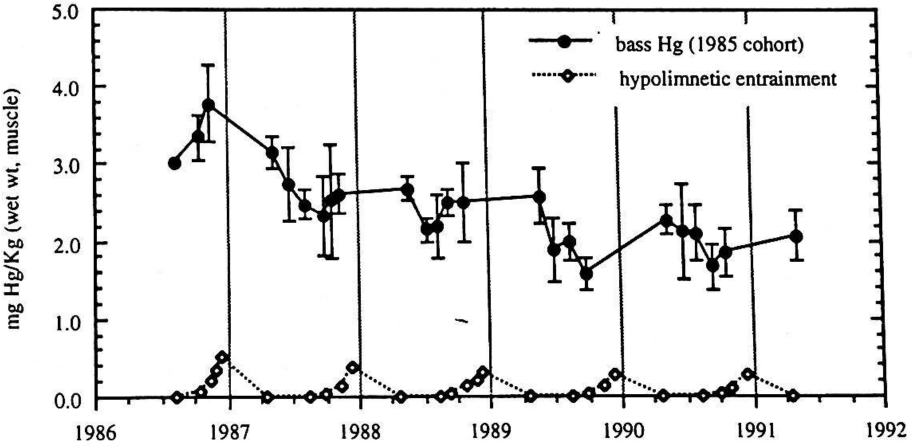

Fig. 6. Bass muscle Hg with 95% confidence intervals, 1986-1991; all plotted points from 1985 cohort. Relative, cumulative seasonal entrainment of anoxic hypolimnetic water into the mixed layer superimposed. Peak (100%) entrainment each year has been scaled to 1986, when full.

4. Conclusions

These biotic data records demonstrate that Hg bioavailability in the surface waters of Davis Creek Reservoir increases in conjunction with the seasonal entrainment of anoxic hypolimnetic water. Zooplankton spike increases coincide with full destratification and may be linked, in part, to ingestion or adsorption of Hg-scavenging manganese oxides, which precipitate following turnover. As the bass become dormant at turnover, destratification-related seasonal increases in bass muscle Hg correspond with the entrainment of anoxic water from the upper hypolimnion, prior to full turnover. Consequently, the dense layer of photosynthetic anaerobic bacteria located just beneath the thermocline is implicated as a potential seasonal source region for methyl Hg, in addition to general hypolimnetic accumulations which peak toward the bottom.

Seasonal response in adult fish Hg concentration is necessarily damped because an uptake time scale of weeks to months represents a relatively small portion of the entire lifetime Hg accumulation, as compared to zooplankton and young-of-year fish. Zooplankton and young-of-year fish are clearly better indicators of seasonal variation in Hg bioavailability than are adult fish.

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