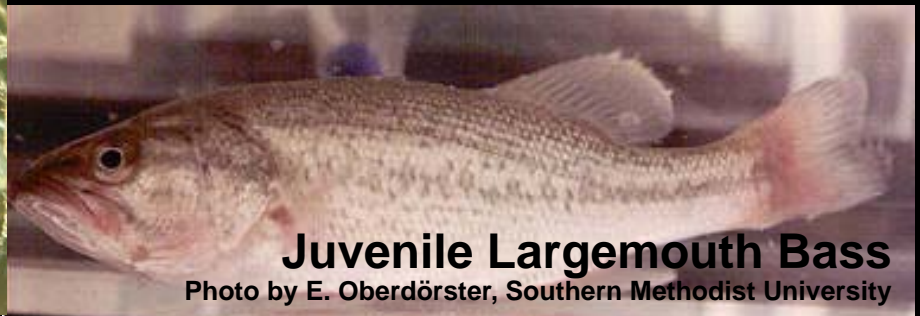
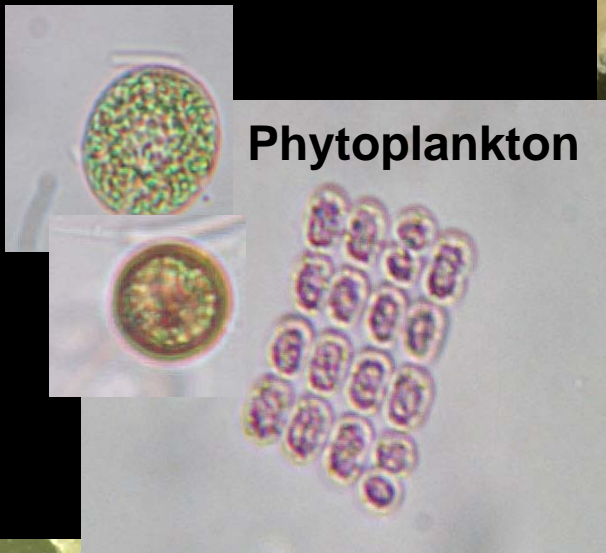


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By James S. Kuwabara, Brent R. Topping, Gerald E. Moon, Peter Husby, Andrew Lincoff, James L. Carter, and Marie-Noële Croteau



U.S. Department of Interior  
U.S. Geological Survey

Scientific Investigations Report  
2005-5037

Internet access at: <http://pubs.water.usgs.gov/sir2005-5037>



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**U.S. GEOLOGICAL SURVEY**

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By James S. Kuwabara<sup>1</sup>, Brent R. Topping<sup>2</sup>, Gerald E. Moon<sup>3</sup>, Peter Husby<sup>4</sup>, Andrew Lincoff<sup>5</sup>, James L. Carter<sup>6</sup>, and Marie-Noële Croteau<sup>7</sup>

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U.S. GEOLOGICAL SURVEY

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## Executive Summary

The water columns of four reservoirs (Almaden, Calero, Guadalupe and Lexington Reservoirs) and an abandoned quarry pit filled by Alamitos Creek drainage for recreational purposes (Lake Almaden; [Table 1](#)) were sampled on September 14 and 15, 2004 to provide the first measurements of mercury accumulation by phytoplankton and zooplankton in lentic systems (bodies of standing water, as in lakes and reservoirs) within the Guadalupe River watershed, California. Because of widespread interest in ecosystem effects associated with historic mercury mining ([Fig. 1](#)) within and down gradient of the Guadalupe River watershed, transfer of mercury to lower trophic-level organisms was examined. The propensity of mercury to bioaccumulate, particularly in phytoplankton and zooplankton at the base of the food web, motivated this attempt to provide information in support of developing trophic-transfer and solute-transport models for the watershed, and hence in support of subsequent evaluation of load-allocation strategies. Both total mercury and methylmercury were examined in these organisms.

During a single sampling event, replicate samples from the reservoir water column were collected and processed for dissolved-total mercury, dissolved-methylmercury, phytoplankton mercury speciation, phytoplankton taxonomy and biomass, zooplankton mercury speciation, and zooplankton taxonomy and biomass. The timing of this sampling event was coordinated with sampling and analysis of fish from these five water bodies, during a period of the year when vertical stratification in the reservoirs generates a primary source of methylmercury to the watershed. Ancillary data, including dissolved organic carbon and trace-metal concentrations as well as vertical profiles of temperature, dissolved oxygen, specific conductance and pH, were gathered to provide a water-quality framework from which to compare the results for mercury. This work, in support of the Guadalupe River Mercury Total Maximum Daily Load (TMDL) Study, provides the first measurements of mercury trophic transfer through planktonic communities in this watershed. It is worth reemphasizing that this data set represents a single “snap shot” of conditions in water bodies within the Guadalupe River watershed to: (1) fill gaps in trophic transfer information, and (2) provide a scientific basis for future process-based studies with enhanced temporal and spatial coverage. This electronic document was unconventionally formatted to enhance the accessibility of information to a wide range of [interest groups](#) ([Appendix 1](#)). The following major observations from interdependent physical, biological, and chemical data were made:

### Physical and Biological Characterizations

- 1. Vertical gradients in the water column:** As a hydrologic context, the five water bodies were sampled within a period of vertical thermal stratification, approximately six months after peak storage ([Fig. 2](#)), but three months before minimal storage for calendar year 2004. The extent of thermal stratification varied with reservoir depth. Sub-oxic bottom-waters (that is, dissolved-oxygen concentrations less than or equal to 2 milligrams per liter) were observed in all reservoirs except Almaden ([Fig. 3](#); [Results and Discussion](#)).
- 2. Phytoplankton communities:** Wide variation between water bodies was observed in both phytoplankton density (8596± 1771 to 112,218 ± 27042 cells per milliliter at Guadalupe Reservoir

and Lake Almaden, respectively) and phytoplankton biovolume ( $430,534 \pm 99,766$  to  $9,268,520 \pm 1,420,683$  cubic micrometers per milliliter at Guadalupe and Almaden Reservoirs, respectively; [Table 1; Results and Discussion](#)). Elevated phytoplankton density at Lake Almaden was due to a sub-surface (6 to 7 meter) bloom of the cyanophyte, *Merismopedia glauca* ([Fig. 4](#)). Despite Almaden Reservoir's second lowest phytoplankton density relative to the other reservoirs, its highest phytoplankton biovolume demonstrates conditions that favor a less diverse community of larger sized species. In contrast, Lexington Reservoir outside the major drainage area of the New Almaden Mercury Mines exhibited a high cell density (only exceeded at Lake Almaden), but a low biovolume (second only to Guadalupe River), reflecting conditions that support smaller phytoplankton species with consequently higher surface to volume ratios. This cell-size effect at Lexington Reservoir relative to the other reservoirs may be due to low mercury-species (toxicant) concentrations or low macronutrient (oligotrophic) conditions. At least an order of magnitude lower analytical detection limits are needed relative to reported nutrient data for the reservoirs to permit a quantitative examination of interdependent constraints on primary productivity due to nutrient availability.

- 3. Zooplankton communities:** As observed with the phytoplankton communities in these five water bodies, the zooplankton communities exhibited wide (that is orders of magnitude) ranges for both density ( $78 \pm 75$  to  $141,047 \pm 70,293$  individuals per cubic meter at Lexington Reservoir and Lake Almaden, respectively) and biomass ( $67 \pm 49$  to  $312,228 \pm 160,225$  micrograms per cubic meter again at Lexington Reservoir and Lake Almaden, respectively; [Table 3; Results and Discussion](#)). Neither zooplankton densities nor biomass were significantly correlated with phytoplankton densities or biovolumes ( $r^2$  consistently less than 0.33, [Tables 2](#) and [3](#)). This suggests that the planktonic communities were at disequilibrium relative to energy transfer or grazing pressures, and hence measured bioaccumulation factors may be skewed relative to a more temporally and spatially intensive sampling design.

Results from this work are the first to report the presence of the invasive species *Daphnia lumholzi* to the South San Francisco Bay region ([Fig. 5](#)).

## Chemical Characterizations

Note: The dissolved-mercury concentrations discussed in this section refer to samples filtered with 0.7-micrometer quartz-fiber filters pre-combusted at 500 degrees Centigrade for 12 hours. In the absence of the modifier "dissolved", the modifier "total" (as in "total mercury" or "total methylmercury") refers to concentrations determined for unfiltered samples (that is, including both particulate and dissolved phases).

- 1. Mercury in the water column:** Concentration trends between the five sampled water bodies were consistent for both total and methylmercury in the water column ([Table 4; Results and Discussion](#)). The lowest concentrations of mercury species were seen in Lexington and Calero Reservoirs, with Lexington typically having the lowest concentrations. Lake Almaden and Guadalupe Reservoirs displayed the highest total mercury concentrations in both the dissolved and particulate phases, but the highest methylmercury concentrations were associated with Lake Almaden and Almaden Reservoir.

Methylmercury in the dissolved and particulate phases was typically less than 10 percent of the total mercury (that is, inorganic and methylmercury). Conspicuous exceptions were noted from Lake Almaden and Almaden Reservoir, previously noted with the highest methylmercury concentrations, where up to 31 percent of the dissolved mercury was methylmercury.

**2. Total mercury and methylmercury in phytoplankton:** Concentrations of total mercury associated with phytoplankton ranged from 22.8 to 172 nanograms per gram dry weight from Lexington and Guadalupe Reservoirs, respectively ([Table 4; Results and Discussion](#)). An overview of regressions that model mercury transfer pathways is provided in [figure 6](#). Bioaccumulation factors, representing diffusive or sorptive assimilation by phytoplankton cells, varied relative to dissolved and total mercury speciation in water, but were of the order of  $10^4$ , approximately four orders of magnitude greater than biomagnification factors (that is, diet driven, trophic-transfer coefficients) between fish trophic levels in this watershed.

Unlike total mercury concentrations, the highest methylmercury concentrations were from Lake Almaden (8.2 nanograms per gram dry weight). Methylmercury consistently represented a small fraction (less than 1 to 11 percent) of total mercury in phytoplankton. This is in stark contrast to methylmercury in fish, which typically represent greater than 90 percent of the total mercury in fish tissue.

**3. Total mercury and methylmercury in zooplankton:** Similar to trends in mercury concentrations measured in phytoplankton, total mercury associated with zooplankton ranged from  $102 \pm 2$  to  $764 \pm 14$  nanograms per gram dry weight from Lexington and Guadalupe Reservoirs, respectively. Methylmercury associated with zooplankton ranged from 84 to 780 nanograms per gram dry weight, as with total mercury, at Lexington and Almaden Reservoirs, respectively. In contrast to trends seen for methylmercury in phytoplankton, and total mercury in zooplankton, Almaden Reservoir and Guadalupe Reservoir (notably, not Lake Almaden) showed the highest methylmercury in zooplankton. Although this shift in uptake pattern cannot be explained with the existing data set, it is important to note that this shift is consistent with the next trophic level (small fish; see section below). That is, the methylmercury assimilated by zooplankton is highly correlated with the mercury (presumably methylmercury) in small fish ( $r^2 = 0.90$ ; [Fig. 6A](#)). In both Almaden and Guadalupe reservoirs, methylmercury in zooplankton already exceeded the U.S. Environmental Protection Agency Water Quality Criteria of 300 nanograms per gram for human consumption of fish, one to three trophic transfers above zooplankton.

Biomagnification factors for zooplankton relative to phytoplankton were inconsistent for total mercury (a range of 2 to 11; [Table 4; Results and Discussion](#)), and even more so for methylmercury (a range of 20 to greater than 1000). These factors, for both mercury species, are one to four orders of magnitude less than those describing the initial accumulation of mercury by phytoplankton from the water column. Only the biomagnification factors for zooplankton relative to total mercury in phytoplankton were similar in magnitude to those reported between fish trophic levels in this watershed.

**4. Associations with mercury in small (prey) fish:** Biomagnification factors for age-1 largemouth bass (prey fish) relative to mercury in zooplankton were consistent within the five lentic systems for methylmercury ( $5.5 \pm 1.0$ ,  $r^2 = 0.90$ ) ([Table 4; Fig. 6A; Results and Discussion](#)) and for total mercury ( $4.8 \pm 0.7$ ,  $r^2 = 0.94$ ; [Fig. 6B](#)). These values were also consistent with those describing mercury transfer between prey and piscivorous fish in this watershed (3.8 to 7.1) and others (estimated at about 0.5 log units), but approximately four orders of magnitude smaller than bioaccumulation factors describing initial accumulation of mercury by phytoplankton from the water column (approximately 26,000 and 19,000 for total and methylmercury, respectively).

## Potential Management Implications

Remediation efforts and Total Maximum Daily Load (TMDL) allocations along the Guadalupe River have linked objectives of decreasing concentrations, loads to down-gradient systems and reducing the bioaccumulation of mercury in biological resources (for example, fish consumed by humans and wildlife). The bioaccumulation factors determined in these studies, consistent with previous mercury trophic-transfer studies in other lakes, indicate that accumulation of mercury is heavily weighted at the base of the food web. This data set provides initial measurements of the phytoplankton and zooplankton bioaccumulation factors for mercury at one time only, during a period of the year marked by dissolved-oxygen stratification, when the reservoirs serve as a significant source of dissolved methylmercury to receiving lotic systems. Therefore, this data may begin to fill an information void in our understanding of mercury trophic transfer, particularly by base trophic levels within the watershed that regulate subsequent mercury accumulation by predatory fish. However, in the absence of any quantification of temporal variability, this limited set of bioaccumulation factors requires prudent interpretation and application ([Fig. 7](#)). Relationships between prey and predator can change over space and time (that is, distributions of prey and predator species vary in quality and quantity). This complexity seriously challenges our ability to accurately model the accumulation of a solute like mercury from one trophic level to the next.

## Background

The Guadalupe River watershed represents a significant spatial component of a Comprehensive Environmental Response Compensation and Liability Act (CERCLA) site affected by drainage from the New Almaden Quicksilver Mines. The economic, historic and geochemical significance of these mines have been thoroughly discussed (Goss, 1958; Johnson, 1963; Lanyon and Bulmore, 1967; Schneider, 1992; Rytuba and Enderlin, 1999; Alpers and Hunerlach, 2000; Topping and others, 2004). Positive regional, national and international contributions of this historic mining operation are adversely offset by the legacy of mercury accumulation in sediment, water and biota. The bioaccumulation of mercury within this watershed has been heretofore poorly quantified.

Many fundamental processes affect the transport, partitioning, speciation and fate of toxic metals like mercury within aquatic systems. These processes, which have been recently examined as part of the Guadalupe River Watershed Mercury TMDL Project (Tetra Tech, 2005), ultimately have a cascading effect on the trophic transfer of mercury (Fig. 7). Biomagnification and subsequent toxicity of methylmercury through the food web is of particular concern (Wren and others, 1995). Typically, trophic transfer of mercury is dominated by the initial accumulation of mercury at the base of the food web (Mason and others, 1995; Mason and others, 1996). Unfortunately, no direct measurements for the bioaccumulation of mercury by phytoplankton or zooplankton have heretofore been available for this study area, or for adjacent, down-gradient ecosystems currently beginning extensive wetland-restoration (California Department of Fish and Game, 2005). This work begins to fill that information gap.

As a result of physical, chemical, and biological processes operating through the water column of lakes and reservoirs, geochemical gradients take on a variety of forms that have been previously reported (Kuwabara and others, 2003a; Fig. 8). Associated gradients in solute concentrations can thereby result in conditions conducive to a net increase in methylmercury concentrations, particularly in hypolimnetic waters of reservoirs within the Guadalupe River watershed (Tetra Tech, 2003). Scientists have recently determined that mercury bioaccumulation predominantly occurs within lower trophic levels (Watras and others, 1998). To complement ongoing geochemical and fish studies, this work provides initial, reservoir-specific bioaccumulation factors for those lower trophic levels.

Water-quality managers are often compelled or required to assess and prioritize remediation strategies for aquatic systems that have been adversely affected by anthropogenic activities. In the case of the Guadalupe River watershed, mercury associated with decades of productive mining at the historic New Almaden Quicksilver Mines has been fluvially transported and deposited in sediment. Frequent demands have been made by regional managers and the public to quantify the connections between fluxes of contaminants and the health, abundance, and distribution of biological resources (Kuwabara and others, 1999). As part of ongoing efforts by the USGS Toxic Substances Hydrology Program to examine processes affecting trace-contaminant transport in San Francisco Bay, this study focuses on a poorly understood, yet potentially important, step in the bioaccumulation of mercury upgradient of the estuary: lower-trophic level mercury uptake by phytoplankton and zooplankton in major reservoirs within the watershed. We hypothesized that the observed exceedances in the mercury concentration of piscivorous fish are driven by the same fundamental processes observed in watersheds unaffected by a legacy of mercury mining, that is, water-column availability of dissolved-mercury species to phytoplankton followed by enhanced methylmercury assimilation by zooplankton.

The results described here followed from the integration of current project studies with information needs identified by the Guadalupe River Watershed Mercury TMDL Working Group, a stakeholder group including environmental-interest, water-quality management, water-supply, and water-treatment organizations. Elevated mercury concentrations in fish, and consequent human-health consumption advisories (California Office of Environmental Health Hazard Assessment, 2005), reflect on the ecological status of aquatic systems within and downstream of the watershed. Quantifying and understanding the magnitude and variability of mercury sources to these fish represent a critical building block to the development of appropriate trophic transfer models and remedial programs for this mining-affected system, and potentially others as well.

To help enable science-based programmatic decisions related to water and ecosystem quality in the Guadalupe River watershed and downstream aquatic systems, the purpose of this study is to provide initial measurements of mercury bioaccumulation by phytoplankton and zooplankton (at the base of the mercury-cycling

food web) and to examine associations between this bioaccumulation to water-quality and mercury concentrations in fish (that is, existing end-member information). In so doing, the intent is to clarify and refine conceptual and numerical models describing mercury dynamics within the watershed.

## Results and Discussion

### Physical Data

The five water bodies within the Guadalupe River watershed were sampled in September 2004, months after peak storage (Fig. 2), and during a period of vertical stratification. Maximum depths at the time of sampling ranged from approximately 12 meters (Lake Almaden) to 23 meters (Lexington Reservoir). In terms of thermal stratification, the deepest two reservoirs (Lexington and Guadalupe) exhibited thermoclines at a depth of approximately 10 meters, consistent with observed dissolved oxygen stratification (Fig. 3). Calero Reservoir (16-meter depth) showed depth-uniform temperatures until within a meter of the bottom, well below the oxycline described above. Thermal stratification was minimal (less than 2 degrees Centigrade gradient) at Almaden Reservoir and occurred primarily at a depth of approximately 3 meters, the position of the first dissolved-oxygen-profile step. Lake Almaden was the last water body sampled on September 15, 2004, at dusk. The thermocline at Lake Almaden occurred at approximately 1-meter depth, slightly shallower than the oxycline (Fig. 3B) and at least 5 meters shallower than the subsurface chlorophyll-*a* maximum depth generated by a bloom of the cyanophyte *Merismopedia glauca* (Fig. 4A). At a depth of 2 meters in Lake Almaden, nearer to the observed oxycline and thermocline, the phytoplankton community was dominated by bacillariophytes (diatoms) at cell densities at least an order of magnitude lower than the subsurface *Merismopedia* layer (Fig. 4B). Under approaching darkness, perhaps the cyanophytes, distinctly layered between 6 to 7 meters, were shifting from photosynthetic oxygen production to respiration.

### Biological Data

- 1. Phytoplankton Community:** The depth and vertical extent of the chlorophyll-*a* maximum was highly variable between reservoirs. For example, at Calero and Lexington Reservoirs, a chlorophyll-*a* maximum was not discernable in the water column so sampling was taken at approximately 2 meters (m). In contrast, the chlorophyll-*a* maximum was well defined between depths of 6 to 7 m in Lake Almaden, so water-quality and phytoplankton samples were taken at 6.5 m.

Variability between reservoirs is exhibited by both phytoplankton densities and biovolumes (Fig. 9). Cell densities (that is, abundances) and biovolumes did not correspond between sites ( $r^2 = 0.18$ ). Calero Reservoir, upstream of the mercury-mining area, consistently had cell densities ( $54,000 \pm 15,352$  cells per milliliter) and biovolumes ( $1,887,836 \pm 380,416$  cubic micrometers per milliliter) in the mid-range among reservoirs with significant representation by cyanophytes (blue-green algae), bacillariophytes (diatoms), cryptophytes (for example, *Cryptomonas*), dinophytes (dinoflagellates), and chlorophytes (green algae) (Table 2). Two taxa, *Nannochloris* sp. (Chlorophyta) and *Aphanothece smithii* (Cyanophyta) made up an average of 55.9 percent and 23.6 percent (approximately 80 percent) respectively, of the phytoplankton in Calero Reservoir. A total of 11 different taxa represent at least 1 percent of the total individuals. Although *Nannochloris* sp. and *A. smithii* were numerically dominant, because of their extremely small size they represented just over 2 percent of the algal biovolume in Calero Reservoir. A number of taxa contributed to the total biovolume, including the extremely large dinoflagellate, *Peridiniopsis polonicum* (Dinophyta) and very large centric diatom, *Stephanodiscus tenuis* (Bacillariophyta).

In contrast, Almaden Reservoir had lower cell densities ( $16,552 \pm 3,438$  cells per milliliter) but higher biovolumes ( $9,268,520 \pm 1,420,683$  cubic micrometers per milliliter) due to the dominance of large dinoflagellates and diatoms. *Aphanothece minutissima* (Cyanophyta) made up over 55 percent of the total individuals identified. *Chlorella minutissima* (Chlorophyta) and *Fragilaria crotonensis* (Bacillariophyta), 17.8 percent and 14 percent respectively, made up most of the remaining individuals. Although *A. minutissima* dominated numerically, it is extremely small and therefore made up little of the biovolume. Two taxa, the large dinoflagellate *Ceratium*



*hirundinella* f. *hirundinella* (Dinophyta) and moderate size *Fragilaria crotonensis* (Bacillariophyta), constituted 64.2 and 31.4 percent, respectively, making up approximately 96 percent of the phytoplankton biovolume in Almaden Reservoir. The addition of *Lynghya birgei*, a cyanophyte, *Komma caudate*, a cryptophyte, and *Closterium aciculare*, a chlorophyte made up over 99 percent of the biovolume.

Guadalupe Reservoir, closest to the mercury-mining operation, had both the lowest cell densities ( $8,596 \pm 1,771$  cells per milliliter) and the lowest biovolumes ( $430,534 \pm 99,766$  cubic micrometers per milliliter), and were dominated by chlorophytes. Two taxa, a blue-green alga, *Aphanothece smithii* (41 percent) and a green algal, *Nannochloris* sp. (41 percent) were numerically dominant. However, a diatom, *Fragilaria crotonensis* (Bacillariophyta, 34.4 percent) and golden alga, *Dinobyron divergens* (Chrysophyta, 29.6 percent) made up over 64 percent of the biovolume.

Lexington Reservoir, which is outside the major drainage area of the New Almaden Mercury Mines, had the second highest cell densities among the reservoirs ( $87,028 \pm 19,606$  cells per milliliter), but the second lowest biovolumes ( $487,471 \pm 167,137$  cubic micrometers per milliliter) due to the dominance of small-sized cyanophytes, dinophytes and chlorophytes. The cyanophyte *Aphanothece smithii* (75 percent) dominated numerically with a second cyanophyte, *Dactylococcopsis irregularis* (12 percent), and the chlorophyte, *Nannochloris* sp. (7 percent) making up almost 95 percent of the individuals. Just as in Almaden Reservoir, a large dinoflagellate, *Ceratium hirundinella* f. *carinthiacum* represented 27 percent of the biovolume. Also contributing to the total biovolume were *D. irregularis*, *Peridiniopsis polonicum*, and *Aphanothece smithii*. The total biovolume in Lexington Reservoir was distributed over numerous species.

Lake Almaden had both the highest cell densities ( $16,552 \pm 3,438$  cells per milliliter) and the highest biovolumes ( $9,268,520 \pm 1,420,683$  cubic micrometers per milliliter) due to a visible bloom of the cyanophyte *Merismopedia glauca* and elevated densities of *Cryptomonas erosa*, a genus common to the region (Tetra Tech, 1980). *Merismopedia glauca* dominated both numerically (84 percent) and in biovolume (40 percent). *Aphanothece smithii* (8 percent) was the second most abundant taxon; however, *Cryptomonas erosa* was the next most dominant in biovolume and represented approximate 27 percent of the total algal biovolume of the lake.

Ordination is a mathematical method often used in ecological studies to aid visualizing the similarities and differences among samples that contain too many species to visually compare. Detrended Correspondence Analysis (DCA) was used to ordinate the log-base10 transformed phytoplankton density data. DCA is constrained to represent the greatest amount of variation in the species by site data along the first derived axis, and decreasing amounts of variation on subsequent axes. The axes are highly derived and have the units of mean standard deviations, such that a distance between plotted sites of 4.0 axis units represents almost a complete change in the species present in the compared samples.

Three relationships were clearly identified in the ordination (Fig. 10). First, most of the individual replicates were far more similar within, compared to between reservoirs. This indicated that the replicate samples contained extremely similar algal assemblages compared to the differences in algal assemblages among reservoirs. Second, Calero and Lexington Reservoirs differ greatly from the other three reservoirs. Both of these two reservoirs are dominated numerically by the chlorophyte, *Nannochloris* sp. and the cyanophyte *Aphanothece smithii*. Last, Lake Almaden, dominated by the cyanophyte *Merismopedia glauca*, differs from both Guadalupe and Almaden reservoirs.

In summary, taxonomic analyses of the five water bodies reflect wide variability in phytoplankton community indices (biomass, density, structure and diversity). This work was scheduled to coincide with other bioaccumulation studies during the low-flow period when methylmercury production and transport is of seasonal importance in the watershed (Tetra Tech, 2005). This data set therefore represents the first, but only a single look, at lower trophic-level mercury transfer in these reservoirs. One might therefore expect considerable (that is, order of magnitude) temporal variability in phytoplankton community biomass, composition, spatial distribution and diversity (Tetra Tech, 1980; Kuwabara and others, 2003b).

**2. Zooplankton Community:** The zooplankton community represents the heterotrophic base to food web or trophic-transfer models. Unlike higher-order organisms like fish that predominantly bioaccumulate methylmercury, zooplankton can contain significant concentrations of inorganic mercury forms (Weiner and others, 2005). The zooplankton can therefore represent a significant trophic level of metabolic transitions.

The contrasts between reservoirs is exhibited by both zooplankton abundance and biomass varying over four orders of magnitude (Fig. 11). In contrast to phytoplankton, trends between reservoirs were consistent for zooplankton abundance and biomass ( $r^2 = 0.99$ ) with Lexington Reservoir having the most sparse community, followed by Calero and Guadalupe Reservoirs, and with Almaden Reservoir and Lake Almaden having the highest densities but statistically similar averages (Table 3). Note that the ratio of biomass to abundance (both determined with respect to water-column volume) provides a coarse average of the relative mass (size) per individual in the water body. So in figure 11, zooplankton biomass in excess of the abundance for only Almaden Reservoir, Guadalupe Reservoir and Lake Almaden, in contrast to the other lentic systems, is consistent with observed sparse zooplankton populations in Calero and Lexington reservoirs. The relative differences in the average mass of individuals may also reflect grazing preferences for certain size classes of zooplankton. Within each water body, the importance of at least triplicate sampling is demonstrated here by the fact that even with triplicate sampling the standard deviation for the site was on average between 40 and 50 percent of the site mean (Table 3).

Calero Reservoir was dominated numerically by rotifers (82 percent) that could not be identified to species and the carnivorous rotifer *Asplanchna* (6 percent). Two copepods, *Cyclops* sp. (4.5 percent) and *Acanthodiaptomus siciloides* (3.7 percent) were the next most abundant taxa. Because of the dramatic difference in size between the Rotifera and Copepoda, *Cyclops* sp. and *A. siciloides* represented 32 percent and 31 percent, respectively, of the biomass even though most of the individual copepods were immature. *Asplanchna* and the other rotifers represented a total biovolume of about 13 percent each.

Almaden Reservoir was dominated numerically by *A. siciloides* (43 percent), of which most were mature. Two cladocerans, *Daphnia ambigua* (17 percent) and *Diaphanosoma* sp. (13 percent) were also very abundant. *A. siciloides* also dominated the biomass in Almaden Reservoir and represented almost 71 percent of the biomass. The two most abundant cladocerans (*D. ambigua* and *Diaphanosoma* sp.) contributed 13 percent and 6 percent of the zooplankton biomass.

Three taxa were about equally abundant in Guadalupe Reservoir. Two cladocerans *Simocephalus* (33 percent) and *Bosmina* (26 percent) and the copepod *A. siciloides*. *Cyclops* spp. represented about 7 percent of the individuals. *Simocephalus* sp. represented about 41 percent of the biomass. As in Almaden Reservoir, *A. siciloides* were generally mature and in so represented about 38 percent of the biomass.

Lexington Reservoir was numerically dominated by the rotifer *Asplanchna* (44 percent) and copepod *Cyclops* spp. (23 percent). They both also represented almost half of the biomass (*Cyclops* spp., 27 percent; *Asplanchna*, 22 percent). Most of the remainder of the biomass was represented by the three cladocerans *Simocephalus* (19 percent) and *Bosmina* (10 percent), the non-native cladoceran *Daphnia lumholzii* (6 percent) and the copepod *A. siciloides* (10 percent).

Almaden Lake was dominated numerically and in biomass by the same 4 taxa. *Bosmina* was by far numerically dominant, representing 57 percent of the individuals but only 20 percent of the biomass. *A. siciloides* represented about 15 percent of the individuals but over 41 percent of the biomass. *Simocephalus* and *Cyclops* spp. represented 15 percent and 7 percent of the individuals and 18 percent and 17 percent of the biomass, respectively.

DCA ordination of zooplankton density data was somewhat less clear than the ordination of the phytoplankton data. Within reservoir replicates at Calero and Lexington grouped independently ([Fig. 12](#)), indicating that zooplankton assemblages were somewhat unique to each reservoir. However, differences between Lake Almaden and Almaden and Guadalupe reservoirs were less distinct. These waterbodies had high abundances of the copepod *Acanthodiptomus siciloides* and also were dominated by the cladocerans *Bosmina* and *Simocephalus*.

As a lead into trophic-transfer discussions in later sections, it is important to note that neither zooplankton densities or biomass were significantly correlated with phytoplankton densities or biovolumes. That is, for this single sampling, the transferable energy from the existing standing stock of phytoplankton was at disequilibrium with the zooplankton standing stock. The periodicity of zooplankton biomass is typically out of phase (lags) phytoplankton biomass. How this temporal disequilibrium in energy transfer affects temporal variability in mercury transfer between these trophic levels (that is, biomagnification factors or trophic-transfer coefficients) is beyond the scope of this study, but may be important to quantify when refining water-quality management strategies.

Microscopic examination of zooplankton samples from each site indicated the presence of the invasive species *Daphnia lumholtzi* ([Fig. 5](#)). The rapid spread of this species in the Great Lakes and more recently in the northern San Francisco Bay region has been tracked on the Internet (Stoeckel and Charlebois, 1999; Center for Aquatic Resources Studies, 2004). This initial sighting of the species in the southern San Francisco Bay region was reported on September 22, 2004 and later confirmed by taxonomic analyses (Table 2) for Calero, Almaden and Lexington Reservoirs. The variability in the abundance of *D. lumholtzi* and other less media-prominent species within each water body provides another example in support of at least triplicate zooplankton sampling.

Although an effort was made to collect zooplankton from the Chlorophyll-a maximum depth, the zooplankton sampling differed in many respects from the phytoplankton sampling. First, sampling at a desired depth for zooplankton was not precise because the net drifted vertically over the duration of the tow in response to changes in boat speed and direction. Therefore, the tow vertically and horizontally integrated the zooplankton sample to a much greater extent than the phytoplankton point sample at a fixed depth ([Study Design and Methods](#)). If taxonomic composition of zooplankton assemblages varied with depth as the phytoplankton assemblage did at Lake Almaden ([Fig. 4](#)), the actual pathways for trophic transfer may have been skewed by the sampling methods. In future studies, a more precise stratified sampling with depth would quantify this effect.

## Chemical Data

- 1. Dissolved oxygen and pH profiles:** Vertical profiles in surface waters for dissolved oxygen and pH often correspond during the day because the production of oxygen by photosynthesis is coupled to the consumption of dissolved carbon dioxide (carbonic acid) and hence an increase in pH. The deepest three reservoirs (Lexington, Guadalupe and Calero) exhibited an oxycline at approximately 10 meters ([Fig. 3B](#)) with a consistent decrease in pH below that depth. Almaden Reservoir showed multiple-step oxygen and pH profiles, with both decreasing with depth. The shallowest sampled water body, Lake Almaden, was the only one to exhibit a distinct subsurface dissolved-oxygen maximum at a depth of approximately two meters, coincident with a pH peak. Bottom-waters in all reservoirs except Almaden were oxygen depleted (less than or equal to 2 milligrams per liter).
- 2. Specific Conductance profiles:** In general, specific conductivities for the sampled lentic systems were uniform with depth and similar in magnitude (0.3 to 0.4 millisiemens per centimeter) ([Fig. 3C](#)). The notable exception was Lake Almaden that exhibited elevated specific conductances relative to the four reservoirs (greater than 0.5 millisiemens per centimeter) that increased with depth.
- 3. Mercury in the water column:** As one might expect, the lowest concentrations of mercury species were seen in Lexington and Calero Reservoirs, removed from historic operation of the New Almaden Mercury Mines. However, Calero Reservoir can be hydrologically connected to Almaden Reservoir because as much as 9.3 million cubic meters (7500 acre-feet) per year of water can be transport from Almaden Reservoir to Calero Reservoir (that is, 75 percent of the storage volume for Calero Reservoir) via the Almaden-Calero canal during the months of December through April. Total mercury (dissolved and particulate) ranged from 1.5 to 20.1 nanograms per liter at Lexington Reservoir and Lake Almaden, respectively ([Table 4](#)). Similarly, Lexington Reservoir and Lake Almaden exhibited the concentration extremes for dissolved total mercury that ranged from 0.5 to 2.6 nanograms per liter. Similarly for total methylmercury and dissolved methylmercury, the concentration ranges (0.1 to 1.7 and 0.04 to 0.41 nanograms per liter, respectively) was bracketed by concentrations from Lexington Reservoir at the bottom and Lake Almaden at the top. These trends between lentic systems are consistent with epilimnetic concentrations reported in collaborative studies by Tetra Tech (2004, 2005). Although the highest total-mercury concentrations in both the dissolved and particulate phases were found at Lake Almaden and Guadalupe Reservoir, the highest methylmercury concentrations were associated with Lake Almaden and Almaden Reservoir. The fraction of dissolved total mercury relative to dissolved and particulate total mercury was consistently less than 15 percent ( $14 \pm 7$  percent average), with one exception (Lexington as high as 31 percent). By contrast, the fraction of dissolved methylmercury relative to dissolved and particulate methylmercury was consistently greater than 19 percent ( $26 \pm 4$  percent average).

It has been previously mentioned that these initial data for the lowest trophic levels must be interpreted within the constraints of the sampling design on one date. For example under these low-flow, low suspended sediment conditions, the correlation between dissolved and total methylmercury ( $r^2 = 0.99$ ; [Fig. 6C](#)) and between dissolved and total inorganic mercury ( $r^2 = 0.73$ ) for the five sampled water bodies may suggest a consistent partitioning. Over multiple samplings during the summer of 2004, the correlation between dissolved and total methylmercury ( $r^2 = 0.92$ ) and between dissolved and total inorganic mercury ( $r^2 = 0.47$ ) were lower (Tetra Tech, 2005), but estimates for the logarithm of the partitioning coefficient for methylmercury were generated ( $5.5 \pm 0.5$ ). Suspended sediment concentrations were not determined in this study, but if a typical range is assumed from reservoir sampling during the 2004 summer ( $3.5 \pm 2.5$  milligrams per liter; Tetra Tech, 2005), results from this study indicate a logarithmic partitioning coefficient,  $6.1 \pm 0.4$ , that is consistent with independent investigations in the watershed. On an absolute scale, however, the ratios of total to dissolved inorganic or methylmercury ( $8 \pm 4$  and  $4 \pm 1$ , respectively) were

approximately double that of ratios observed in the coincident study over the entire summer (3.1 and 1.4; Tetra Tech, 2005). This may be due to a more consistent partitioning of mercury species onto and into phytoplankton cells during our sampling relative to the summer in general, suggesting the potential for temporal variability in bioaccumulation factors.

4. **Dissolved Organic Carbon (DOC) in the water column:** Dissolved organic matter, measured as DOC, is a ligand that can compete for mercury complexation in the water, and hence affect the solubility of particulate mercury (Ravichandran, 1998). For example, Kuwabara and others (2002) noted that spatial trends in dissolved-mercury concentrations in Lahontan Reservoir, Nevada, were coincident with DOC. This is not the case for the lentic systems sampled in this study, with poor correlation between dissolved mercury and DOC ( $r^2 = 0.11$ ). Concentrations for DOC varied from  $2.01 \pm 0.02$  to  $4.36 \pm 0.02$  milligrams carbon per liter at Guadalupe and Lexington Reservoirs, respectively (Table 5). It has been suggested that elevated ratios of methyl to total mercury in zooplankton may possibly be related to elevated DOC although a mechanism was not specified (Paterson and others, 1998). In the Guadalupe River watershed, data from this study do not support this hypothesis ( $r^2 = 0.07$ ). In fact, the two highest DOC concentrations were observed in reservoirs removed from the historic mercury-mining activity (that is, Calero and Lexington Reservoirs), and having the lowest zooplankton densities (Fig. 9; Table 2). The net effect of DOC on mercury bioavailability and mercury trophic transfer is confounded by its role as a competing ligand in complexation and partitioning reactions as well as a carbon source to stimulate microbial methylation.
  
5. **Dissolve trace metals in the water column:** In addition to mercury, other trace metals in the dissolved phase can compete for ligands in both dissolved and particulate phases, and hence affect mercury speciation and partitioning. With the exception of cadmium and lead, dissolved-metal concentrations were higher (sometimes by orders of magnitude) than total-dissolved mercury concentrations. Elevated concentrations for dissolved copper, nickel and lead were observed in Guadalupe Reservoir relative to the other reservoirs, but concentration trends between reservoirs were inconsistent for other metals (Table 6). Given that the lowest DOC concentrations were from Guadalupe Reservoir, the speciation of these metals (and mercury, see section above) does not appear to be dominated by organic chelation. Serpentinite formations in the region are a likely contributor to the high dissolved-nickel (11.5 micrograms per liter in Guadalupe Reservoir) and other trace-metal concentrations (Topping and Kuwabara, 2003).
  
6. **Mercury in phytoplankton:** Concentrations of total mercury associated with phytoplankton ranged from 22.8 to 172 nanograms per gram dry weight from Lexington and Guadalupe Reservoirs, respectively. Lake Almaden displayed the second highest total mercury concentration with 74.3 nanograms mercury per gram dry weight. Sample replicates were composited rather than independently analyzed, so a pure error estimate is not available for phytoplankton concentrations, but analytical and replicate precision were both less than 6 percent of the mean for the zooplankton analyses (Appendix 2; Table 4). The bioaccumulation factor between water and phytoplankton relative to total mercury was approximately 26,000 ( $r^2 = 0.84$ ). In view of the magnitude of this calculated bioaccumulation factor for total mercury and the low percentage of methylmercury relative to total mercury in phytoplankton observed within this watershed (a range of less than 1 to 11 percent), it may be prudent to consider the uptake or adsorption of inorganic mercury by phytoplankton as a complementary pathway of site-specific importance for subsequent biomagnification by higher trophic levels. Mason and others (1996) found that methylmercury was assimilated about four times more efficiently than inorganic mercury from diatoms (phytoplankton) to copepods (zooplankton). Given the low ratio of methyl to total mercury in phytoplankton reported here (less than 1 to 11 percent) relative to other studies of lakes not affected by mercury mining (e.g., 13 and 31 percent; Watras and Bloom, 1992), perhaps the supply of inorganic mercury from certain phytoplankton species (adsorbed or cytoplasmic) or



from other suspended particles can significantly contribute to zooplankton assimilation in this mining-affected watershed.

Concentrations of methylmercury associated with phytoplankton ranged from less than 1.50 (the detection limit) to 8.2 nanograms per gram dry weight. Unlike total mercury concentrations, the highest methylmercury concentrations were from Lake Almaden. In fact, of the five sampled water bodies, only two, Lake Almaden and Almaden Reservoir, exhibited phytoplankton-mercury concentrations greater than the detection limit. Given that Lake Almaden and Almaden Reservoir had the highest dissolved and particulate methylmercury concentrations among the lentic systems sampled in this study, this result is not surprising. In contrast, phytoplankton from Guadalupe Reservoir, having the highest total-mercury concentrations, had undetectable methylmercury. The fraction of methylmercury in phytoplankton (less than 1 to 11 percent of total mercury) contrasts the predominance of methylmercury in fish, typically greater than 90 percent of total mercury in fish tissue (Grieb and others, 1990; Bloom, 1992). With a very coarse approximation of 0.75 nanograms per gram for the non-detectable (less than 1.5) methylmercury concentrations in phytoplankton, the accumulation factor between water and phytoplankton relative to methylmercury is estimated at 19,000 ( $r^2 = 0.91$ ). This estimate of the initial diffusive uptake by phytoplankton is consistent with those reported by Watras and others (1998), and approximately four orders of magnitude greater than biomagnification factors between prey and piscivorous fish in this watershed (5.4 with  $r^2 = 0.90$ ; Tetra Tech, 2005). Furthermore, the percentage of methylmercury relative to total mercury in phytoplankton is also well correlated with dissolved methylmercury in the water column ( $r^2 = 0.82$ ; [Table 4](#)). This observation is consistent with those of Lawson and Mason (1998) who noted that cytoplasmic assimilation of methylmercury by phytoplankton was dependent on solution-phase concentration exposure. Watras and Bloom (1992) observed that the percentage of methylmercury in phytoplankton relative to total mercury was inversely correlated with pH (13 and 31 percent methylmercury in lakes at pH 6.1 and 4.7, respectively). They hypothesized that methylmercury through transfer in lower trophic levels is enriched upon acidification. The pH range observed in this study was 6.5 to 9 ([Fig. 3D](#)), routinely higher than those measured by Watras and Bloom (1992). The lower percent methylmercury in phytoplankton reported here (less than 1 to 11 percent, [Table 4](#)) relative to the lakes studied by Watras and Bloom (1992) is consistent with the hypothesis that a higher percentage of inorganic mercury is associated with phytoplankton as pH increases.

Consistent with the observation of others (Watras and others, 1998), we speculate that the lower relative concentration for methylmercury in phytoplankton (less than 1 to 11 percent) compared to fish (greater than 90 percent) is related to the greater exposure of the phytoplankton cell to mercury species in the bulk solution (that is a higher reactive surface to volume ratio relative to higher trophic level organisms for diffusive and sorptive uptake from the water column), in contrast to the dietary assimilation of methylmercury into fish tissue as it grows. There is also a possibility that the filtered phytoplankton samples may have included particulate materials not representative of the natural phytoplankton assemblage (for example, inorganic particulates). Although not observed under microscopic examination, the significant presence of inorganic particles would favor the adsorption of inorganic mercury over methylmercury onto metal-oxide surfaces (Gunneriusson and others, 1995).

In a study of 55 lakes in the United States, Chen and Folt (2005) observed that mercury concentrations in phytoplankton were negatively correlated with phytoplankton densities. They suggested that this phytoplankton “density dilution” underlies the fate of mercury and other contaminants in aquatic systems. Results presented in this report can be used to examine how well the concept of density dilution relates to reservoirs where the primary source of mercury is not atmospheric. There was no significant correlation between methylmercury in phytoplankton and phytoplankton biovolumes or densities ( $r^2 = 0.08$  and  $0.24$ , respectively), indicating an absence of

density dilution during this study, or variability in density dilution between reservoirs that could not be detected by one sampling event.

7. **Mercury in zooplankton:** Consistent with phytoplankton trends between reservoirs, total mercury associated with zooplankton ranged from 102 to 757 nanograms per gram dry weight from Lexington and Guadalupe Reservoirs, respectively. As with the phytoplankton, Lake Almaden displayed the second highest total mercury concentration with 368 nanograms mercury per gram dry weight.

Methylmercury associated with zooplankton ranged from 84 (a single composited sample) to  $764 \pm 14$  nanograms per gram dry weight at Lexington and Guadalupe Reservoirs, respectively. Similarly, Lexington and Guadalupe Reservoirs exhibited the lowest and highest total mercury in zooplankton (102 and 898 nanograms per gram dry weight, respectively). Specific to Almaden Reservoir, mercury concentrations for zooplankton had to be corrected for the presence of algal filaments in the zooplankton-tow (Fig. 13). The mercury concentrations from this assemblage were corrected for algal-concentration dilution based on the relative dry mass of the zooplankton ( $46 \pm 6$  percent dry weight rounded to 50 percent) and phytoplankton. In contrast to trends seen for methylmercury in phytoplankton, Almaden Reservoir and Guadalupe Reservoir (notably, not Lake Almaden) showed the highest methylmercury in zooplankton. Although this shift in uptake pattern cannot currently be explained, it is important to note that this shift is propagated into the next trophic level (that is small fish) as will be discussed in the subsequent section. Perhaps the varied assimilation of methylmercury contributes to this shift because this trophic level appears to represent a transition between uptake pathways. Methylmercury represented 44 to 85 percent of total mercury in zooplankton, a range clearly between those observed for phytoplankton, its prey, and for small fish, its predator (Table 4). The shift in uptake patterns is coincident with 44 percent associated with Lake Almaden and 85 percent associated with Guadalupe Reservoir.

As with phytoplankton, Watras and Bloom (1992) observed that the percentage of methylmercury in zooplankton relative to total mercury was negatively affected by pH (29 and 91 percent methylmercury in lakes at pH 6.1 and 4.7, respectively). Although the pH range observed in this study was 6.5 to 9 (Fig. 3D), the percent methylmercury in zooplankton reported here (44 to 85 percent) is within the range observed by Watras and Bloom (1992). That is, in the Guadalupe River watershed, a lower percentage of methylmercury in reservoir phytoplankton does not carry over to the zooplankton. Feeding rates and assimilation efficiencies for methylmercury into zooplankton may be site specific. Alternatively, the size fractionated samples that were microscopically characterized may have included particulate materials not associated with the natural phytoplankton and zooplankton assemblages. In this critical transition level of trophic transfer, it may be important to confirm and understand whether biomagnification factors are constrained to a sole source of methylmercury in phytoplankton. The importance of characterizing those uptake processes in this watershed may be reflected by the fact that methylmercury in zooplankton, in both Almaden and Guadalupe Reservoirs, already exceeded the U.S. Environmental Protection Agency (USEPA) Water Quality Criteria of 300 nanograms per gram (or 0.3 milligrams per kilogram; USEPA, 2001a) for human consumption of fish, one to three trophic transfers above zooplankton (Fig. 6A).

Methylmercury in zooplankton correlated better with total mercury rather than methylmercury in phytoplankton ( $r^2 = 0.60$  and  $0.01$ , respectively), although both correlations were not significant at the 95-percent confidence level. That is, the biomagnification factors for zooplankton relative to phytoplankton were not consistent for total mercury (a range of 2 to 11; Table 4), as one might expect if only methylmercury was being transferred. But counter intuitively, that phytoplankton-to-zooplankton biomagnification factor was also inconsistent for methylmercury (a range of 20 to greater than 1000). These biomagnification factors, for both mercury species, are orders of

magnitude less than those describing the initial accumulation of mercury by phytoplankton from the water column. However, the biomagnification factors for zooplankton relative to total mercury in phytoplankton are similar in magnitude to biomagnification factors between prey and piscivorous fish in this watershed (Tetra Tech, 2005). A zooplankton tow horizontally and vertically integrates the sample to a much greater degree than a point sample for phytoplankton. This difference in collection method may contribute to the lack of correlation between mercury concentrations in zooplankton and phytoplankton in this study.

In conjunction with similar findings for phytoplankton described above, Chen and Folt (2005) also observed that mercury concentrations in zooplankton were negatively correlated with zooplankton densities. They suggested that this zooplankton “density dilution” is an important biogeochemical effect contributing to the fate of mercury and other contaminants in aquatic systems. As with phytoplankton, results from our five-reservoir study can be used to examine the applicability of the density-dilution concept when atmospheric sources of mercury do not dominate. Consistent with the phytoplankton results, no significant correlation between methylmercury in zooplankton and zooplankton biomass or densities ( $r^2 = 0.08$  and  $0.24$ , respectively) was observed. Perhaps, the density-dilution effect is less discernable when multiple bioavailable mercury sources exist (fluvial and benthic) and atmospheric sources do not dominate. Therefore, the concept of density dilution cannot be extrapolated to the biomagnification of mercury in fish. Based on a negative correlation between mercury concentrations in fish with zooplankton density, Chen and Folt (2005) concluded that the data they examined “provided persuasive evidence that high zooplankton density can reduce the biomagnification of mercury to fish of all trophic levels”. As with phytoplankton and zooplankton results, the hypothesis of density dilution can be tested for fish within the reservoirs of the Guadalupe River watershed. Because a density-dilution effect for methylmercury in zooplankton was not observed in this study, it is not surprising that biomagnification of mercury in small (1-year) fish was not significantly correlated with either the biomass or density of its zooplanktonic prey ( $r^2 = 0.22$  and  $0.17$ , respectively). That is at the time of our sampling, the density dilution effect was not observed at either of the base levels in trophic transfer.

- 8. Associations with mercury in small (prey) fish:** Twenty age-1 largemouth bass (50 to 100 millimeter fork length) were sampled from each of the 5 reservoirs. Total mercury associated with these prey fish, assumed to be predominantly in the form of methylmercury (Grieb and others, 1990; Bloom, 1992), ranged from  $450 \pm 90$  to  $4830 \pm 1220$  nanograms per gram dry weight (that is, 0.5 to 4.8 micrograms mercury per gram dry weight) from Lexington and Almaden Reservoirs, respectively. As with the zooplankton (not phytoplankton), Almaden and Guadalupe Reservoirs displayed the highest total-mercury concentrations in fish, and Lexington and Calero Reservoirs the lowest. In Calero Reservoir, mercury in fish (Table 4) was approximately 3-fold higher than in Lexington Reservoir despite similar mercury concentrations in zooplankton. This suggests either a source of mercury to fish in Calero that was not detected in this study, or that measured mercury concentrations in zooplankton in Lexington Reservoir were higher at the time of collection than during other times of the year. Mercury in small fish was highly correlated with both total and methyl mercury in zooplankton suggesting consistent biomagnification factors within the five sample water bodies ( $4.8 \pm 0.7$ ,  $r^2 = 0.94$ ) and for methylmercury ( $5.5 \pm 1.0$ ,  $r^2 = 0.90$ ) (Fig. 6A). These values were also consistent with those describing dietary mercury transfer between prey and piscivorous fish in this watershed (3.8 to 7.1; Tetra Tech, 2005) and others (estimated at about 0.5 log units or about 3 by Watras and others, 1998), but again about four orders of magnitude smaller than bioaccumulation factors for the initial diffusive or sorptive assimilation of mercury by phytoplankton from the water column (approximately 26,000 and 19,000 for total and methylmercury, respectively). Despite the strong correlation between mercury in small fish and in zooplankton, the temporal and spatial constraints of this data set should be carefully considered when applying these results. McGeer and others (2003) cautioned against the indiscriminate use of biomagnification factors (trophic-transfer coefficients) without an understanding of processes that regulate exposure.



## Study Design and Methods

The protocol described in this section focuses on method applications in this sampling of five lentic systems within the Guadalupe River watershed. Details (for example, quality control specifications) for each analysis are available in Appendix 2 or have been previously documented (Woods and others, 1999; Praskins and others, 2001; Kuwabara and others, 2003a).

Within the Guadalupe River watershed, sampling was performed on 14 and 15 September 2004, at or in close proximity (within 100 meters) of the deepest location in Calero Reservoir, Almaden Reservoir, Guadalupe Reservoir, Lexington Reservoir, and Lake Almaden, respectively ([Fig. 1](#); [Table 1](#)). At each site, the following samples were collected from a U. S. Environmental Protection Agency research vessel:

### Water-column Sampling

After locating and logging the coordinates at each sampling site, a field submersible fluorometer (Turner Designs Self-contained Underwater Fluorescence Apparatus, SCUFA) was lowered into the water column to locate the depth of relative maximum chlorophyll-a concentration. This depth was used to set and deploy devices for subsequent sampling. When a chlorophyll maximum depth was not observed, the water column was sampled at a depth of 2 meters. Vertical profiles were then determined and logged for temperature, specific conductance, dissolved oxygen and pH (YSI Model 600XLM). A Teflon-line Niskin Bottle (General Oceanics) was then used to collect water-column samples for mercury speciation at the depth indicated by prior fluorescence measurements ([Fig. 14A](#)).

### Phytoplankton Sampling

After water samples were collected, phytoplankton samples were collected from the same Niskin bottle sample and preserved with Lugol's Solution for taxonomic and biomass analyses. Phytoplankton cells from the same depth as the Niskin-bottle sample were then peristaltically pumped through an in-line 35-micrometer non-metal prefilter and collected on baked quartz-fiber filters ([Fig. 14B](#)). Flow-through water volumes were recorded for each of the seven replicate filters. After transfer to the laboratory, six samples from each site were lyophilized for subsequent mercury-speciation analysis (three for inorganic-mercury analysis, three for methylmercury analysis). The remaining sample was used for photomicroscopy.

### Zooplankton Sampling

At each sampling site, after phytoplankton sampling was completed, the zooplankton community was sampled ([Fig. 14C](#)). Six successive tows were conducted using a conical plankton net having a mesh size of 150-micrometers and a diameter of 50-cm. An effort was made to collect zooplankton from the Chlorophyll-a maximum depth. However, sampling at this depth was not precise because the net drifted vertically over the duration of the tow in response to changes in boat speed and direction. Six zooplankton samples were taken from each reservoir: three replicates for taxonomic and biomass analysis, and three for mercury-speciation analysis and photomicroscopic examination. A calibrated flow meter was used to determine the water volume sampled from each tow. The taxonomic/biomass samples were preserved with Rose Bengal Solution in the field for later examination. The three remaining zooplankton samples were preserved on ice, transported US Geological Survey facilities in Menlo Park, CA and stored at 5°C to wait further processing. Samples were processed within 24 hrs of collection. To remove large extraneous items such as twigs, each mercury/photomicroscopic zooplankton sample was sieved through a 1000-micrometer Nitex screen. To separate zooplankton from smaller organisms such as algal filaments, the sample was then sieved through a 125-micrometer Nitex screen. The zooplankton sample, which was collected on the fine screen, was rinsed, a subsample was removed for photomicroscopic analysis, and the remainder was lyophilized for subsequent mercury-speciation analysis.

## Small-fish Sampling

At each sampling site, large-mouth bass were collected by electroshocking in two size ranges: less than 1 year fish (small fish, 50 to 100 millimeter fork length) and adult fish of legal size, greater than 305 millimeters forklength. For this report, only the data for the smaller-size class (that is, the size class feeding on the zooplankton) will be discussed. Weiner and others (2005) have suggested that one-year-old prey fish can serve as useful bioindicators of methylmercury contamination. After length measurement, the small-sized fish were wrapped in pre-cleaned foil and placed in a plastic bag. The wrapped samples were stored in a cooler with ice until transported back to the laboratory for freezing.

After removal of the gastrointestinal tract, the whole-fish samples were homogenized and analyzed for total mercury using a Milestone DMA-80 Direct Mercury Analyzer, following USEPA Method 7473/ USEPA Region 9 SOP535 (USEPA, 1998).

## Chemical Parameters

1. **Dissolved Mercury and Methylmercury:** Dissolved-mercury and methylmercury samples were processed in a Class-100 laminar-flow hood. Samples from water-column sampling were filtered through a 0.45 micrometer ( $\mu\text{m}$ ) pore-size, 15 millimeter (mm) diameter capsule filter (Gelman Supor 12175). Samples were refrigerated in darkness until analyzed by cold-vapor atomic fluorescence spectrometry (CVAFS, [Fig. 15](#)). Methodological details for total-mercury (Method 1631E, U.S. Environmental Protection Agency (USEPA), 2002) and methylmercury (Method 1630, USEPA, 2001b) were previously reported.
2. **Dissolved organic carbon (DOC):** Dissolved organic carbon was determined by high-temperature, non-catalytic combustion (Qian and Mopper, 1996). Potassium phthalate was used as the standard. Low-DOC water (blanks less than 40 micrograms-organic C per liter) was generated from a double-deionization unit with additional ultraviolet treatment (Milli-Q Gradient, Millipore Corporation) ([Fig. 16](#)).
3. **Dissolved Trace Metals by ICP-MS:** Water-column samples were also collected, filtered (0.2-micrometer polycarbonate membrane) and acidified to provide dissolved trace-metal information for the estuary by flow-injection inductively coupled plasma mass spectrometry (Topping and Kuwabara, 1999; [Fig. 17](#)).

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## Acknowledgments

Mercury-speciation analyses by F. Colich, A. Minecelli, and M. Anderson (Frontier Geosciences, Seattle, WA) are acknowledged. Photomicroscopy by, and discussions regarding zooplankton populations with S.V. Fend are greatly appreciated. This work was undertaken to complement studies associated with the Guadalupe River Watershed Mercury Total Maximum Daily Load (TMDL) Project administered by the Santa Clara Valley Water District and the San Francisco Regional Water Quality Control Board. The U.S. Geological Survey Toxic Substances Hydrology Program, Santa Clara Valley Water District and U.S. Environmental Protection Agency, Region 9 are gratefully acknowledged for support of this work.

Product names are provided for identification purposes only and do not constitute endorsement by the U.S. Geological Survey.

## Appendix 1: Comments on the Report Structure

A major objective of this electronic document is to provide a structure that is easily accessible to a wide range of interest groups. Therefore, pathways within this document have been constructed to be both logical and intuitive. In contrast to typical scientific manuscripts, this report is formatted in a pyramid-like structure to serve the needs of diverse groups who may be interested in reviewing or acquiring information at various levels of technical detail. The report enables quick transitions between the initial [summary information](#) (figuratively at the top of the pyramid) and the later details of [methods](#) or [results](#) (figuratively towards the base of the pyramid) using hyperlinks to supporting figures and tables, and an electronically linked [Table of Contents](#). In addition to hyperlinks within the document to supporting figures and tables, links in Appendices 3 and 4 provide a quick way to directly review and examine all figures and tables.

Although hard copies of this report are available on request, the advantages of the electronic version relative to the hard copy are substantial in many respects, but particularly in the rapid access of information at multiple levels of detail.

Your comments about how this type of Web-based product may be improved to better serve readers are most welcome and may be directed to the major author ([kuwabara@usgs.gov](mailto:kuwabara@usgs.gov)) so that they may be compiled for future revisions and reports.

## Appendix 2: Quality control data for mercury speciation analyses

QA/QC data for water column samples

### Mercury Speciation Results for USGS-Brent Topping

Reported October 13, 2004

Frontier Geosciences Inc., 414 Pontius Ave. N, Seattle WA 98109

#### *Quality Control Data - Matrix Duplicate Report*

| Analyte (ng/L) | Sample QC'd  | Rep. 1 | Rep. 2 | Mean  | RPD |
|----------------|--------------|--------|--------|-------|-----|
| Total Hg       | Res #1 Rep B | 4.04   | 4.08   | 4.06  | 0.9 |
| Methyl Hg      | Res #1 Rep A | 0.239  | 0.237  | 0.238 | 0.8 |

#### *Quality Control Data - Matrix Spike / Matrix Spike Duplicate Report*

| Analyte (ng/L) | Sample QC'd  | Sample Mean | Spike Level | MS    | % Rec. | Spike Dup Level | MSD   | % Rec. | RPD |
|----------------|--------------|-------------|-------------|-------|--------|-----------------|-------|--------|-----|
| Total Hg       | Res #2 Rep A | 6.45        | 24.24       | 30.03 | 97.3   |                 | 29.92 | 96.8   | 0.5 |
| Methyl Hg      | Res #1 Rep A | 0.238       | 6.024       | 6.384 | 102.0  | 6.012           | 6.371 | 102.0  | 0.0 |

MS = Matrix Spike

MSD = Matrix Spike Duplicate

RPD = Relative Percent Difference



### *Quality Control Data - Preparation Blank Report*

| <b>Analyte (ng/L)</b> | <b>PBW1</b> | <b>PBWZ</b> | <b>PBW3</b> | <b>Mean</b> | <b>St. Dev.</b> | <b>R.L.</b> |
|-----------------------|-------------|-------------|-------------|-------------|-----------------|-------------|
| Total Hg              | 0.06        | 0.06        | 0.06        | 0.06        | 0.00            | 0.15        |
| Methyl Hg             | 0.019       | 0.013       | 0.013       | 0.015       | 0.003           | 0.025       |

St. Dev. = Standard Deviation

R.L. = Reporting Limit

### *Quality Control Data - Certified Reference Materials Report*

| <b>Analyte (ng/L)</b> | <b>CRM Identity</b> | <b>Cert. Value</b> | <b>Obs. Value</b> | <b>% Rec.</b> |
|-----------------------|---------------------|--------------------|-------------------|---------------|
| Total Hg              | NIST 1641d          | 1601000            | 1574000           | 98.3          |
| Methyl Hg             | DORM-2              | 4470               | 4597              | 102.8         |

CRM Identity = Certified reference material identity

Cert. Value = Certified value

Obs. Value = Experimental result

% Rec. = Percent recovery

QA/QC data for phytoplankton and zooplankton analyses

## Mercury Speciation Results for USGS-James S. Kuwabara

Reported November 10, 2004

Frontier Geosciences Inc., 414 Pontius Ave. N, Seattle WA 98109

### *Quality Control Data - Matrix Duplicate Report*

| <b>Analyte (ng/g)</b> | <b>Sample QC'd</b> | <b>Rep. 1</b> | <b>Rep. 2</b> | <b>Mean</b> | <b>RPD</b> |
|-----------------------|--------------------|---------------|---------------|-------------|------------|
| Total Hg              | Almaden Reservoir  | 279.4         | 296.0         | 287.7       | 5.8        |
| Methyl Hg             | Almaden Reservoir  | 278.4         | 266.3         | 272.4       | 4.4        |

### *Quality Control Data - Matrix Spike / Matrix Spike Duplicate Report*

| <b>Analyte (ng/g)</b> | <b>Sample QC'd</b>  | <b>Sample Mean</b> | <b>Spike Level</b> | <b>MS</b> | <b>% Rec.</b> | <b>Spike Dup Level</b> | <b>MSD</b> | <b>% Rec.</b> | <b>RPD</b> |
|-----------------------|---------------------|--------------------|--------------------|-----------|---------------|------------------------|------------|---------------|------------|
| Total Hg              | Guadalupe Reservoir | 757.0              | 2151               | 2831      | 96.3          | 1923                   | 2522       | 91.8          | 4.9        |
| Methyl Hg             | Lake Almaden        | 160.4              | 571.5              | 721.2     | 98.1          | 569.2                  | 696.6      | 94.2          | 4.1        |

MS = Matrix Spike

MSD = Matrix Spike Duplicate

RPD = Relative Percent Difference

### *Quality Control Data - Preparation Blank Report*

| <b>Analyte (ng/g)</b> | <b>PBW1</b> | <b>PBW2</b> | <b>PBW3</b> | <b>Mean</b> | <b>St. Dev.</b> | <b>R.L.</b> |
|-----------------------|-------------|-------------|-------------|-------------|-----------------|-------------|
| Total Hg              | 0.49        | 0.39        | 0.45        | 0.50        | 0.05            | 0.45        |
| Methyl Hg             | 2.06        | -0.18       | 0.09        | 0.80        | 1.22            | 1.50        |

St. Dev. = Standard Deviation

R.L. = Reporting Limit

### *Quality Control Data - Certified Reference Materials Report*

| <b>Analyte (ng/g)</b> | <b>CRM Identity</b> | <b>Cert. Value</b> | <b>Obs. Value</b> | <b>% Rec.</b> |
|-----------------------|---------------------|--------------------|-------------------|---------------|
| Total Hg              | DOLT-S              | 3370               | 3529              | 104.7         |
| Methyl Hg             | DORM-2              | 4470               | 4429              | 99.1          |

CRM Identity = Certified reference material identity

Cert. Value = Certified value

Obs. Value = Experimental result

% Rec. = Percent recovery

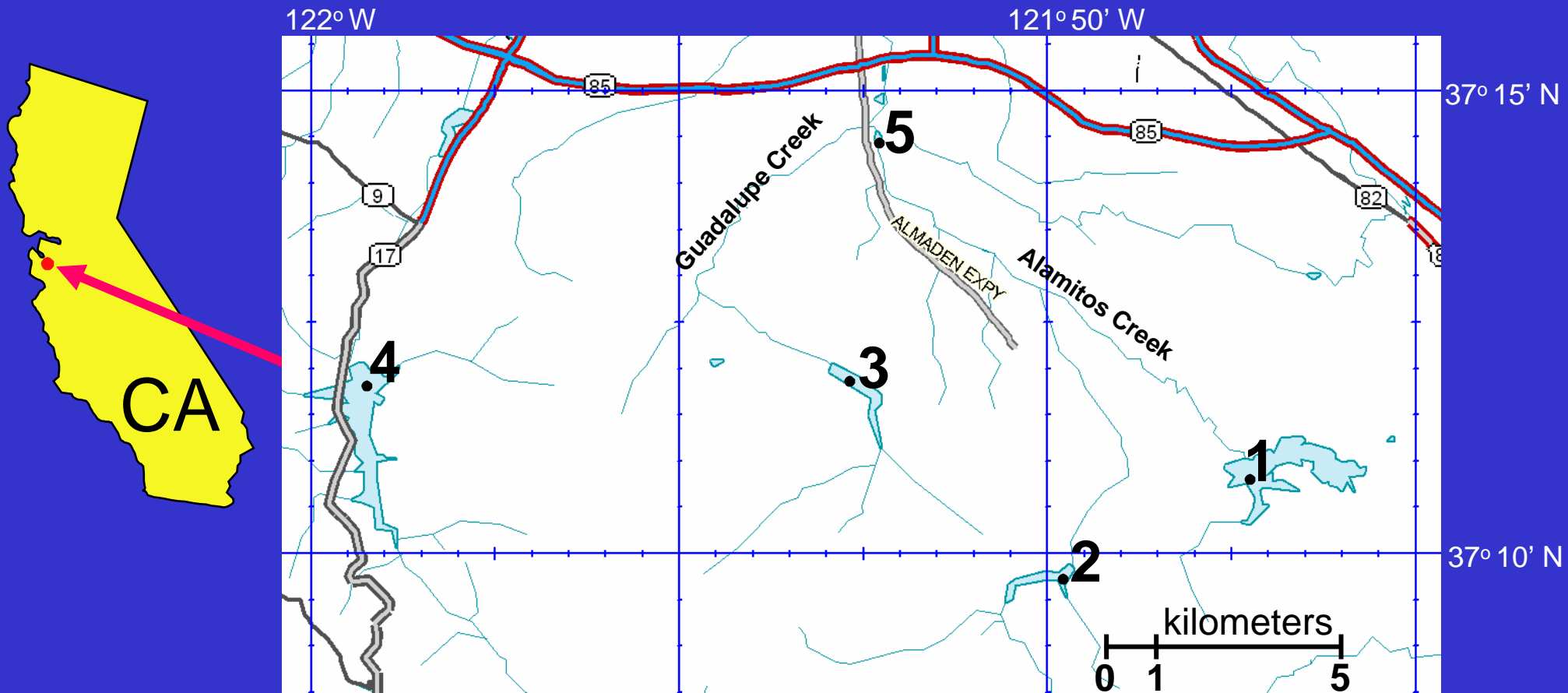
## Appendix 3: List of Figures

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## Appendix 4: List of Tables

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Figure 1. Sampling sites (Numbered as sampled)



1 - Calero Reservoir

2 - Almaden Reservoir

3 - Guadalupe Reservoir

4 - Lexington Reservoir

5 - Lake Almaden

Figure 2. Variation in storage for sampled reservoirs in water year 2004.

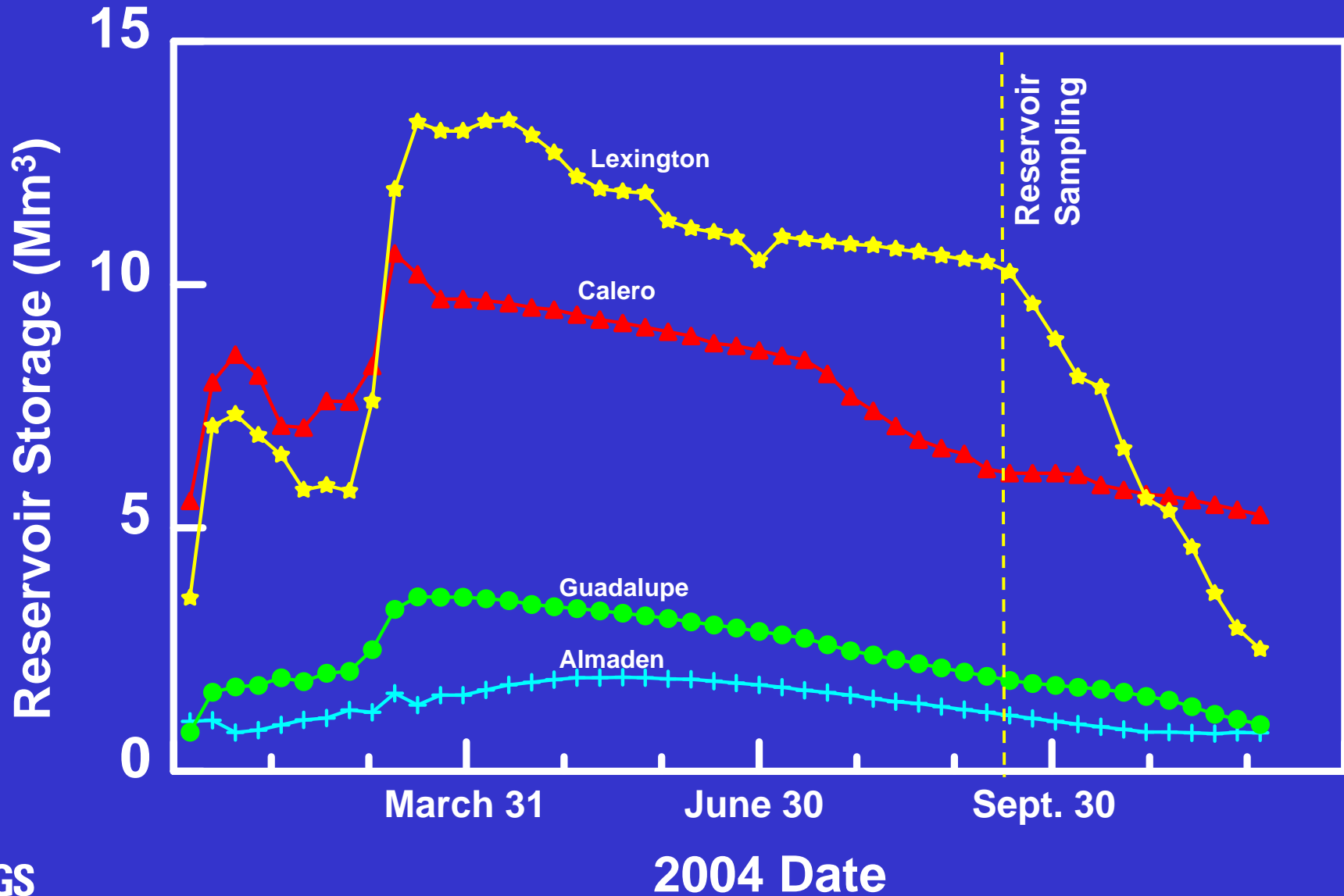


Figure 3. Depth profiles in sampled water bodies for: A) Temperature, B) Dissolved oxygen, C) Specific Conductance, and D) pH.

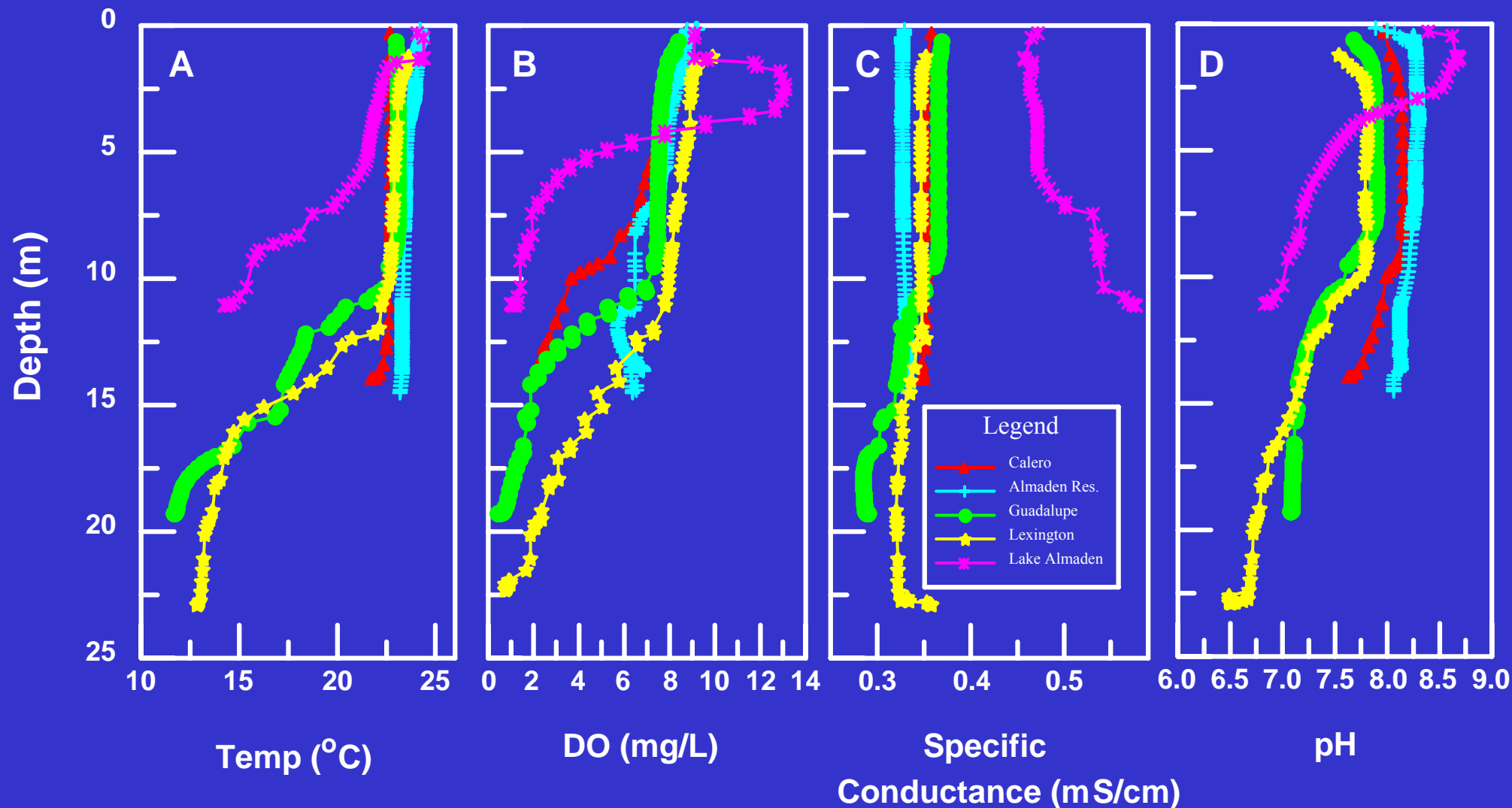
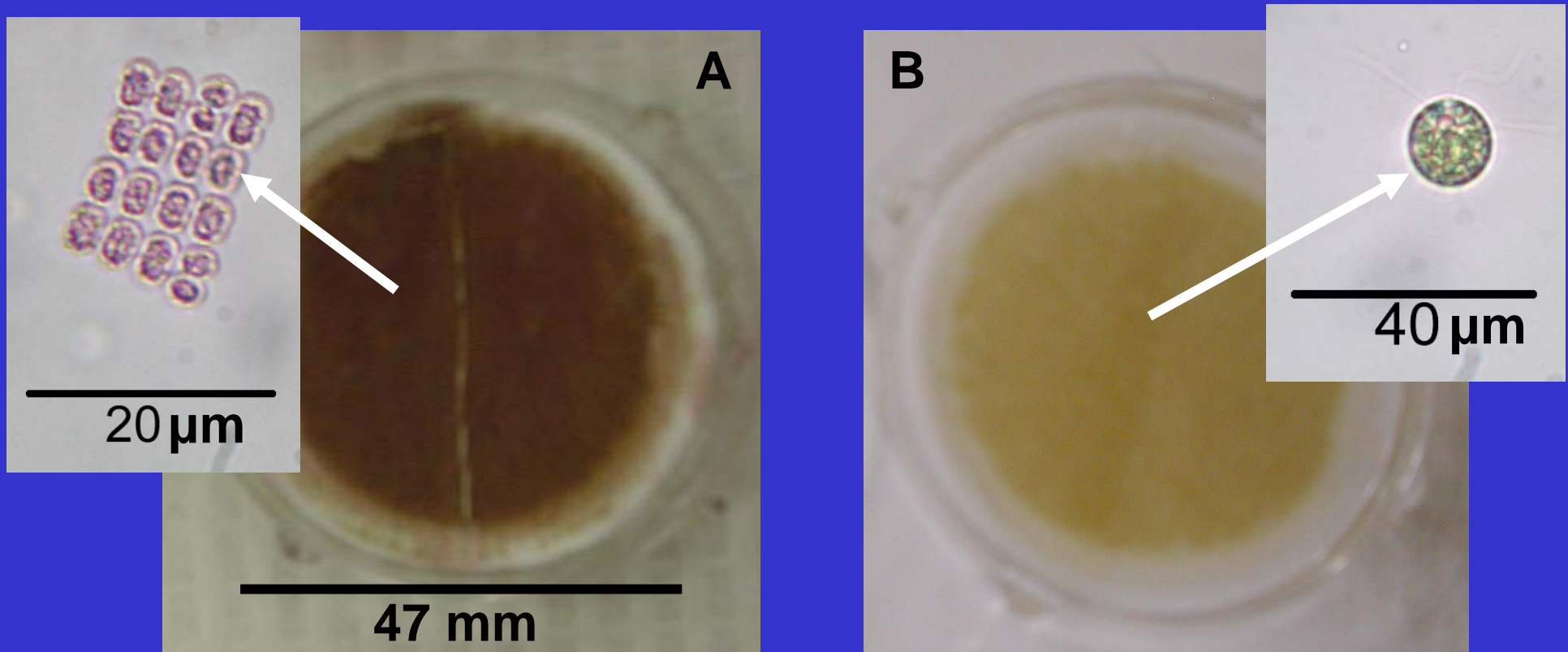


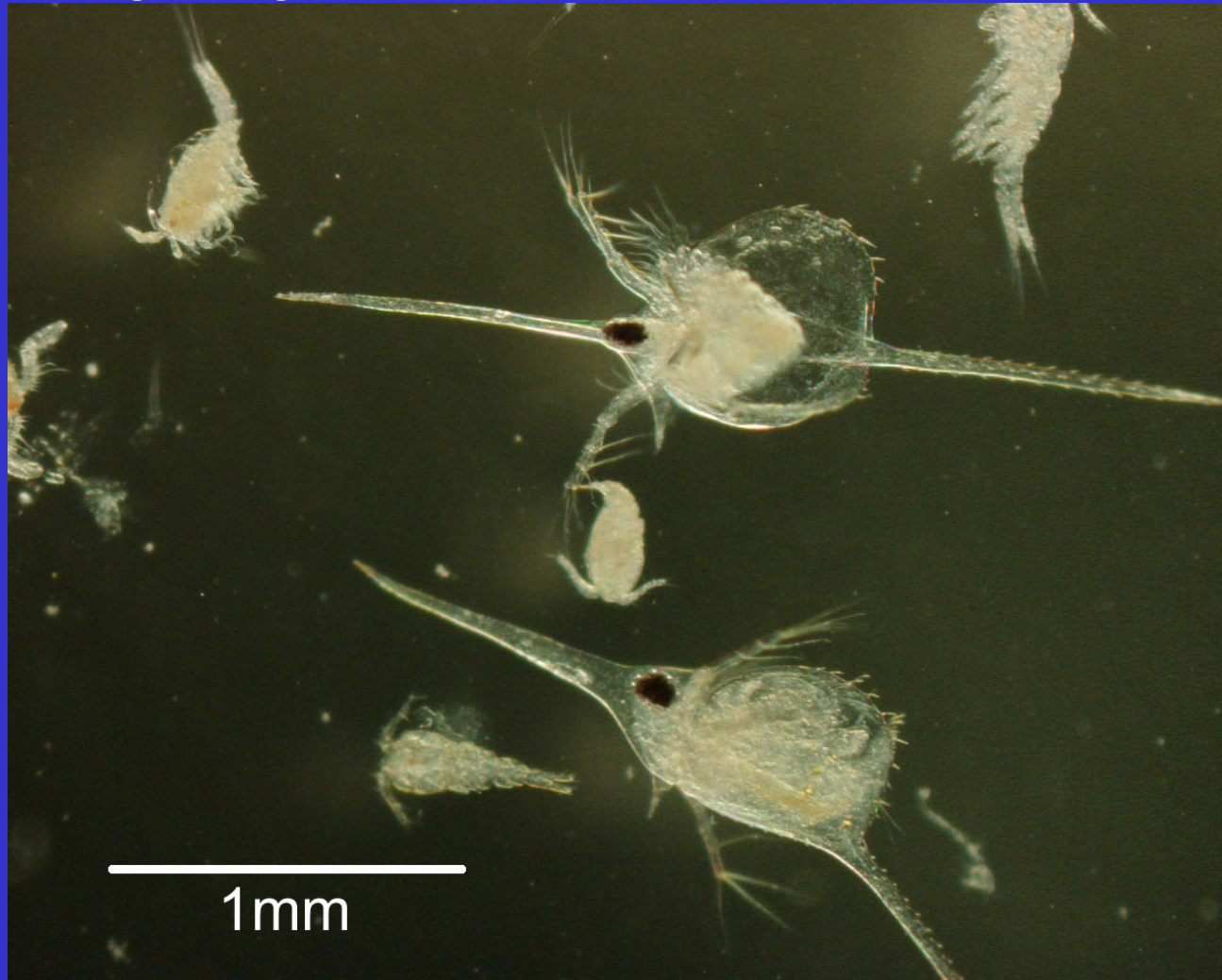


Figure 4. Photographs and photomicrographs of a stratified bloom of *Merismopedia glauca* in Lake Almaden: A) Cyanophyte-dominated community at 6 meters depth and B) Diatom-dominated community at 2 meters depth.



Photographs by F. Colich and S. Fend

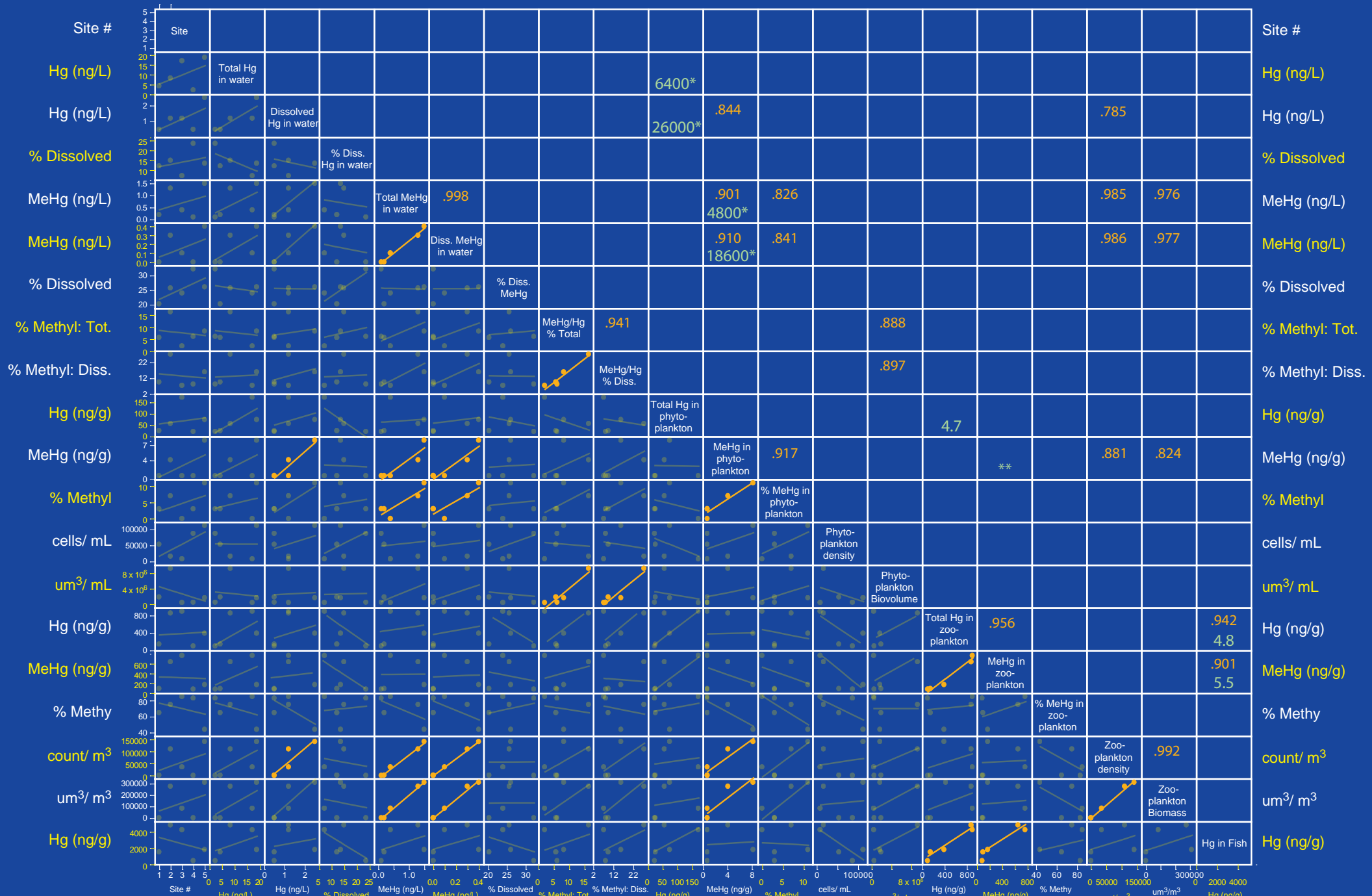
Figure 5. Photomicrograph of the initial South Bay sighting of *Daphnia lumholtzi*.



Photomicrograph by S.V. Fend

**Figure 6. Mercury trophic-transport matrix**

For P less than 0.05, **orange** numerals in cells represent coefficients of determination (r-squared). **Green** numerals denote calculated bioaccumulation or biomagnification factors. Highlighted regression lines are shown for those parameters correlated with P less than 0.05.



\* Slopes account for 1000-fold difference between the units ug/L and ug/g. These are listed as such for consistency with standard respective units (for example, ug/L for aqueous samples).  
 \*\* Slope not given because negative correlation misrepresents BMF

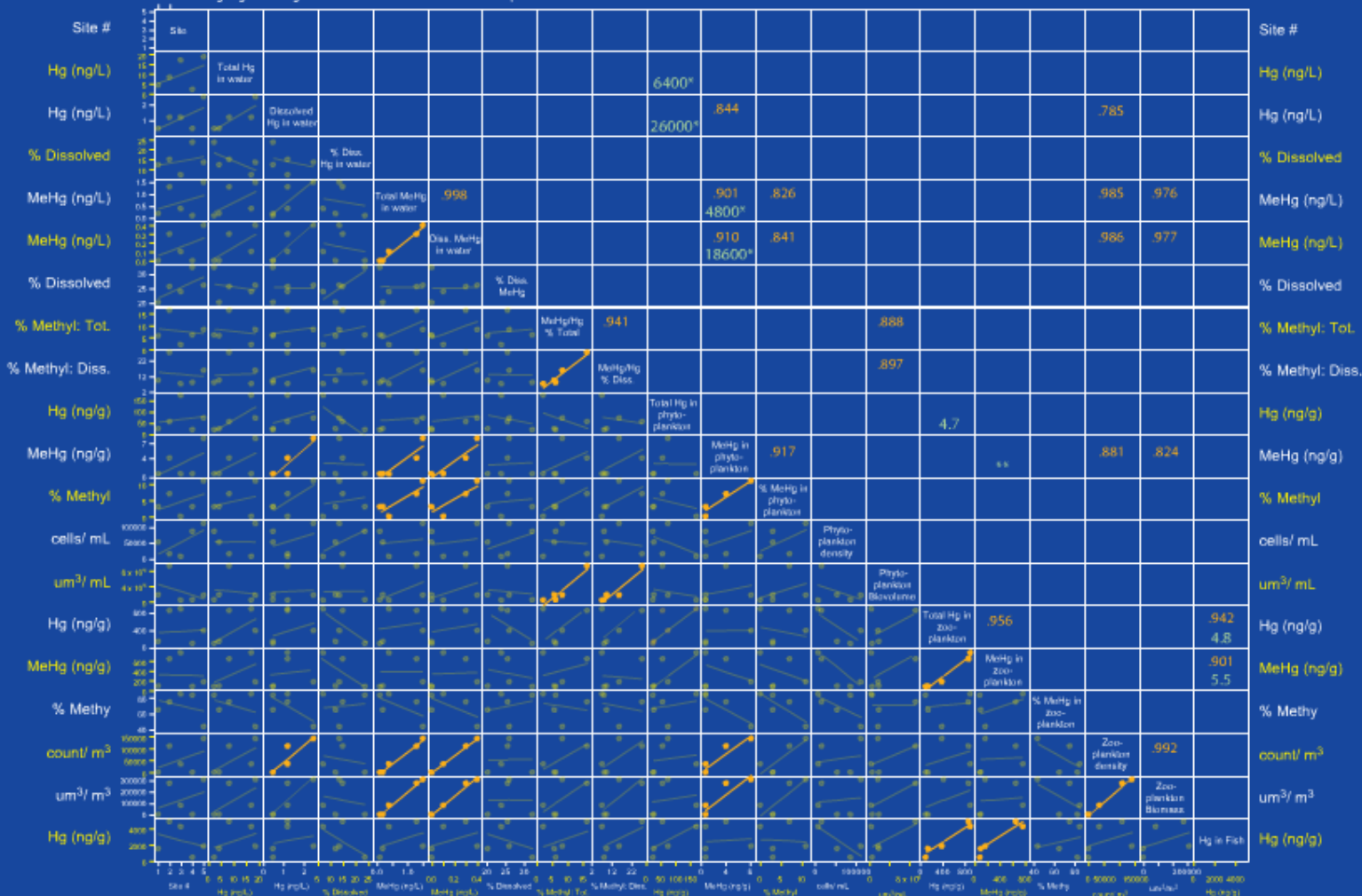
Figure 6A. Empirical relationship between methylmercury concentrations in zooplankton and in small fish.

The animation begins with the overview matrix (Fig. 6), then zooms in on the highlighted cell describing the biomagnification of methylmercury between zooplankton and age-1 largemouth bass in the five sampled water bodies. In the final graphic of the animation, a light blue rectangle is shown at the bottom left corner of the plot within which the EPA Water Quality Criterion for methylmercury in fish and organisms is met.

To replay, pause, or resume the animation, use the buttons below. To print, right-click on the desired frame, select "Print".

Figure 6. Mercury trophic-transport matrix

For P less than 0.05, orange numerals in cells represent coefficients of determination (r-squared). Green numerals denote calculated bioaccumulation or biomagnification factors. Highlighted regression lines are shown for those parameters correlated with P less than 0.05.



\* Shows correlation for 1000 fold difference between the water and fish. These are based on mean for consistency with our published articles. For example, a 1000 fold increase in water would result in a 1000 fold increase in fish. \*\* Slope not given because regression correlation not significant. RMR



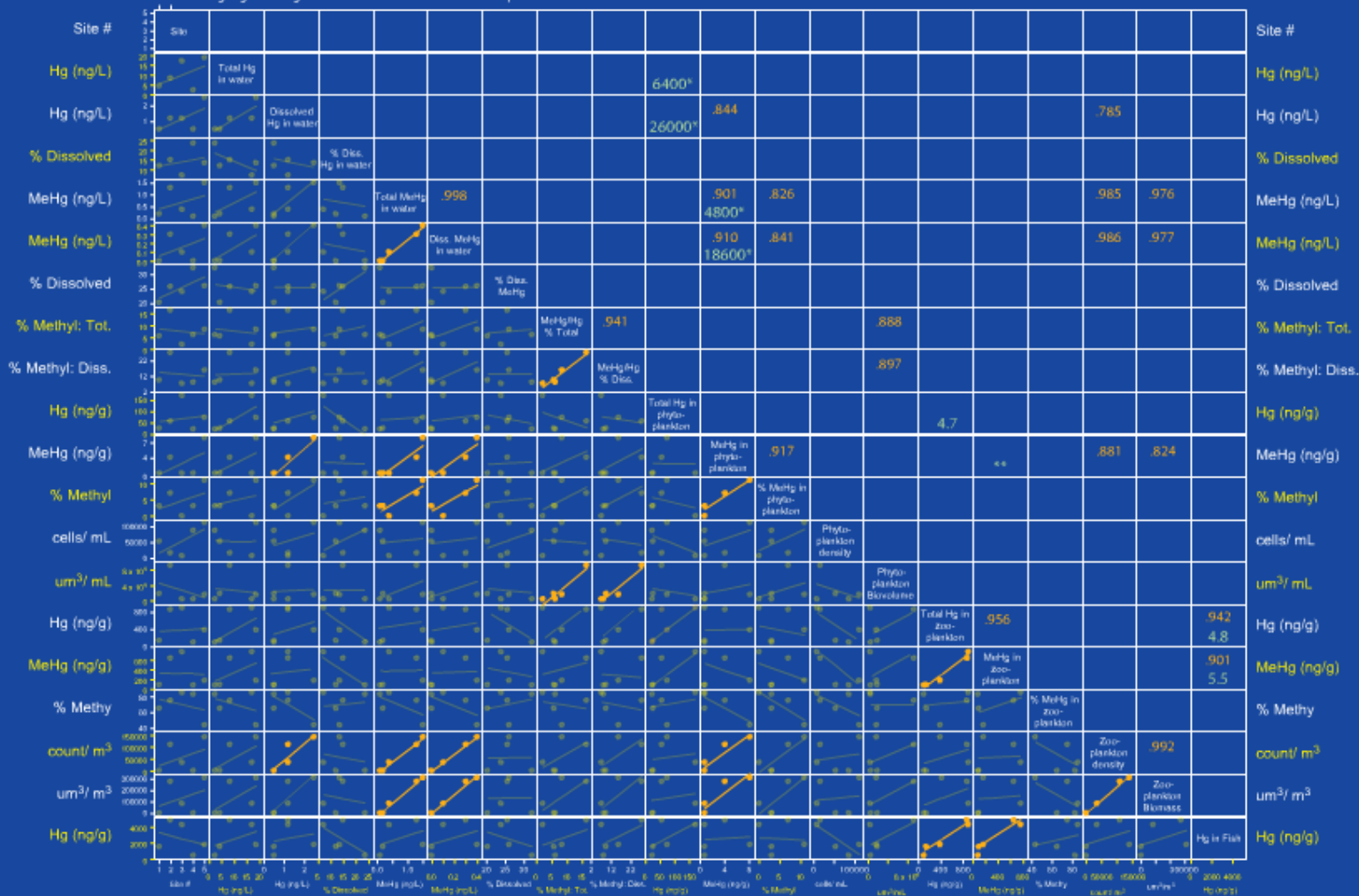
Figure 6B. Empirical relationship between total mercury concentrations in zooplankton and in small fish.

The animation begins with the overview matrix (Fig. 6), then zooms in on the highlighted cell describing the biomagnification of total mercury between zooplankton and age-1 largemouth bass in the five sampled water bodies.

To replay, pause, or resume the animation, use the buttons below. To print, right-click on the desired frame, select "Print".

Figure 6. Mercury trophic-transport matrix

For P less than 0.05, orange numerals in cells represent coefficients of determination. Green numerals denote calculated bioaccumulation or biomagnification factors. Highlighted regression lines are shown for those parameters correlated with P less than 0.05.



\*\* Spear's rho test for 1200 field observed parameter (7 months) and log<sub>10</sub> data. These are based on only the consistency with standard and respective units for water vs. up's for aquatic samples.

\*\* Spear's rho test for 1200 field observed parameter (7 months) and log<sub>10</sub> data. These are based on only the consistency with standard and respective units for water vs. up's for aquatic samples.

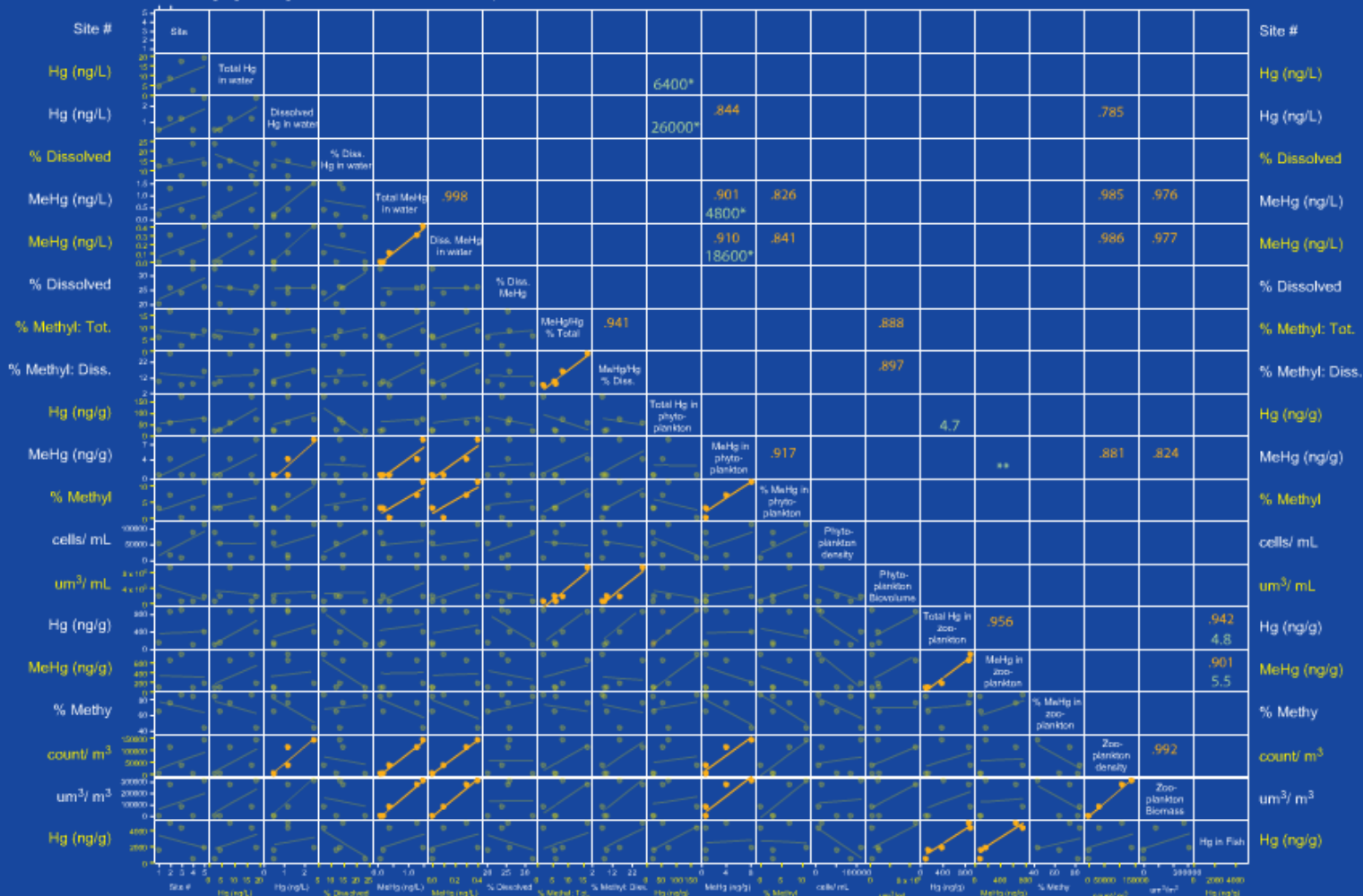
### Figure 6C. Methylmercury in dissolved and particulate phases.

The animation begins with the overview matrix (Fig. 6), then zooms in to focus on the consistent relationship between dissolved and total (that is dissolved and particulate) methylmercury in the water column of the five sampled water bodies.

To replay, pause, or resume the animation, use the buttons below. To print, right-click on the desired frame, select "Print".

**Figure 6. Mercury trophic-transport matrix**

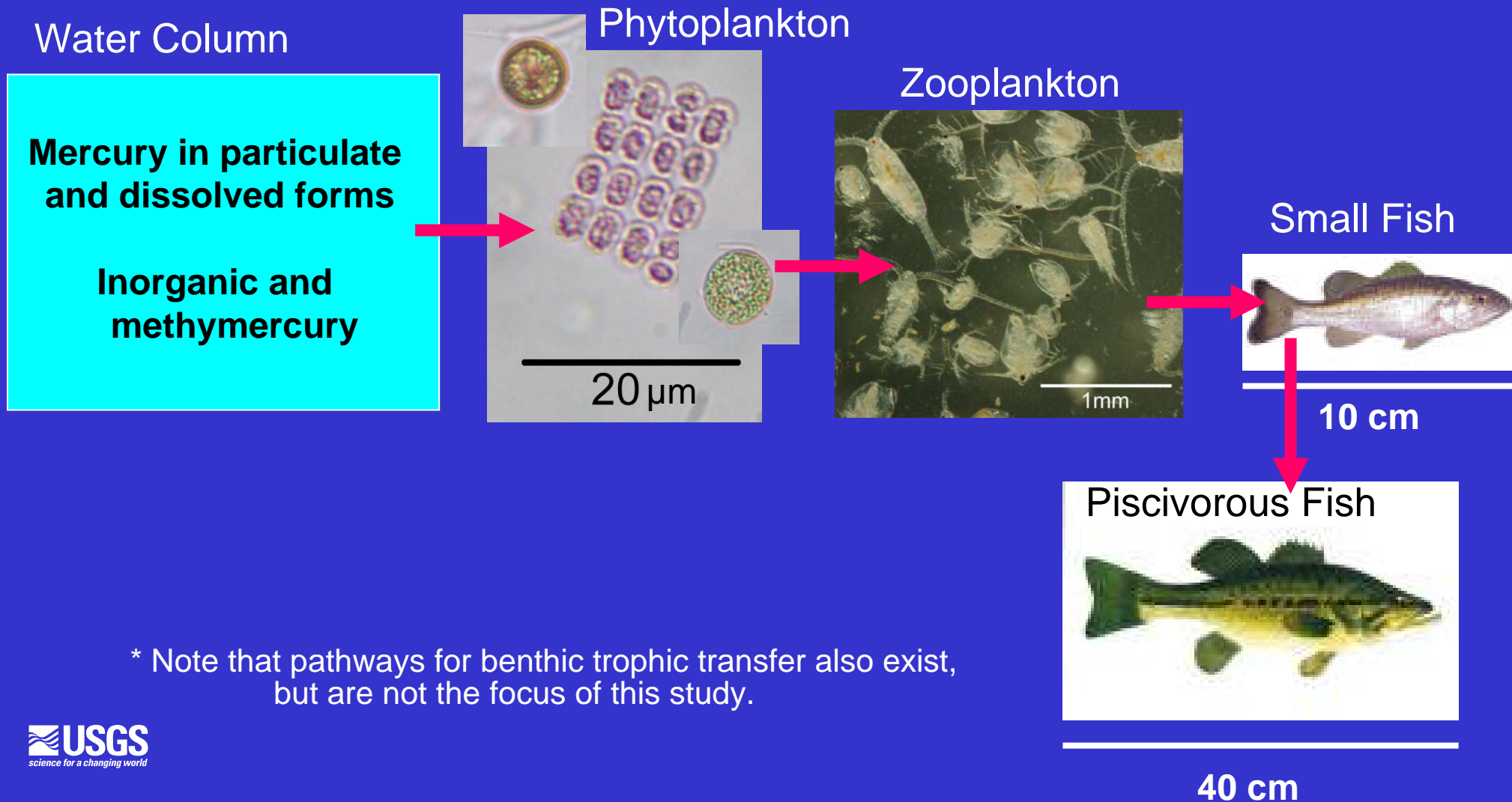
For P less than 0.05, orange numerals in cells represent coefficients of determination. Green numerals denote calculated bioaccumulation or biomagnification factors. Highlighted regression lines are shown for those parameters correlated with P less than 0.05.



\* Spearman's rho for 1000 bootstrapped permutations between the units (ng/L) and (ng/g). These are listed on each cell corresponding with the units in question with the strength (ng/L) for comparison purposes.

\*\* Slope does not cover the basic range of the correlation, interpret cautiously. (50)

# Figure 7. The pelagic pathway examined for mercury trophic transfer\*



\* Note that pathways for benthic trophic transfer also exist, but are not the focus of this study.

# Figure 8. Conceptual Model of Solute Transport

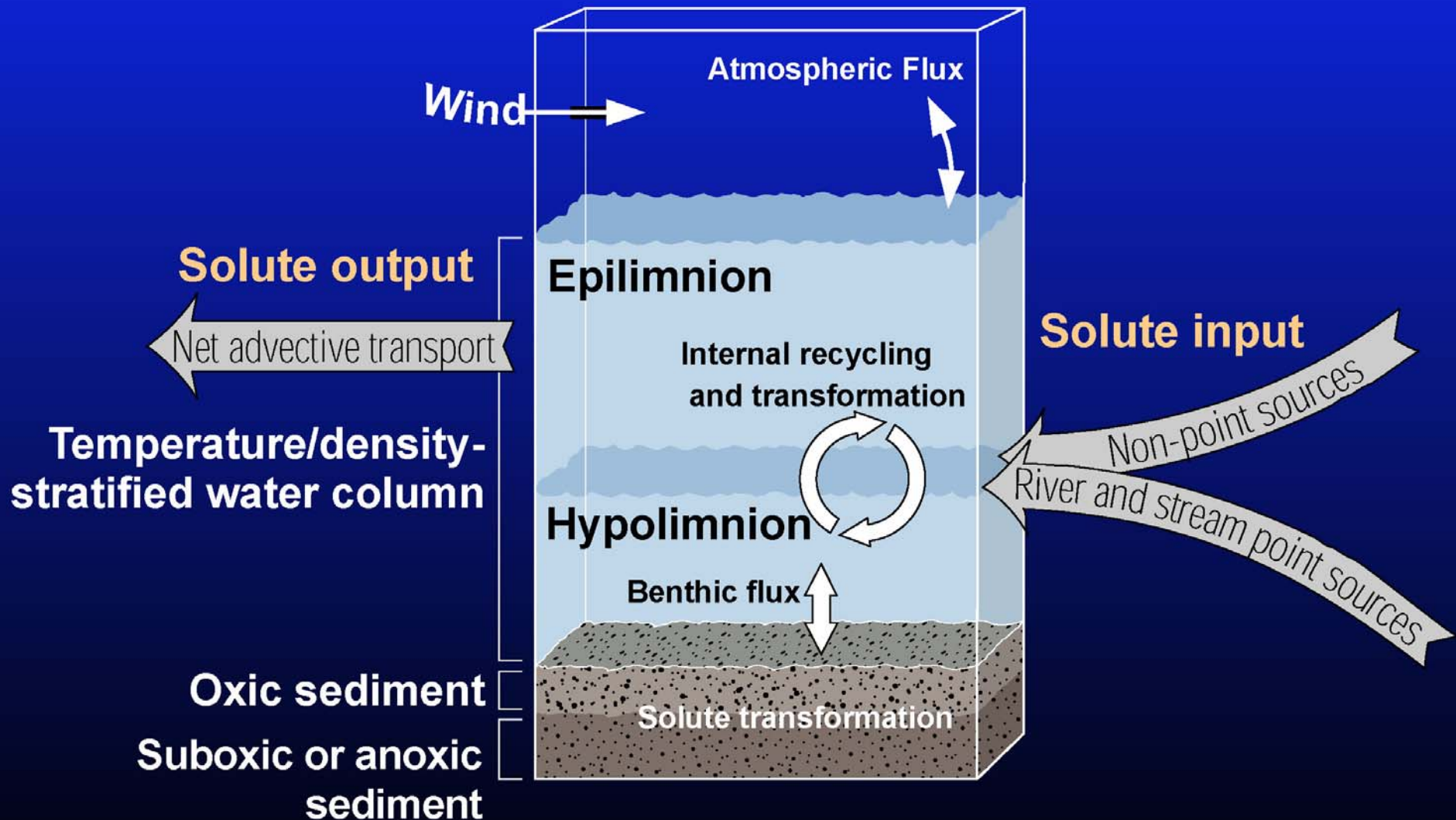




Figure 9. Trends in the phytoplankton communities between lentic systems.  
Error bars depict standard deviations about the mean, centered at the marker.

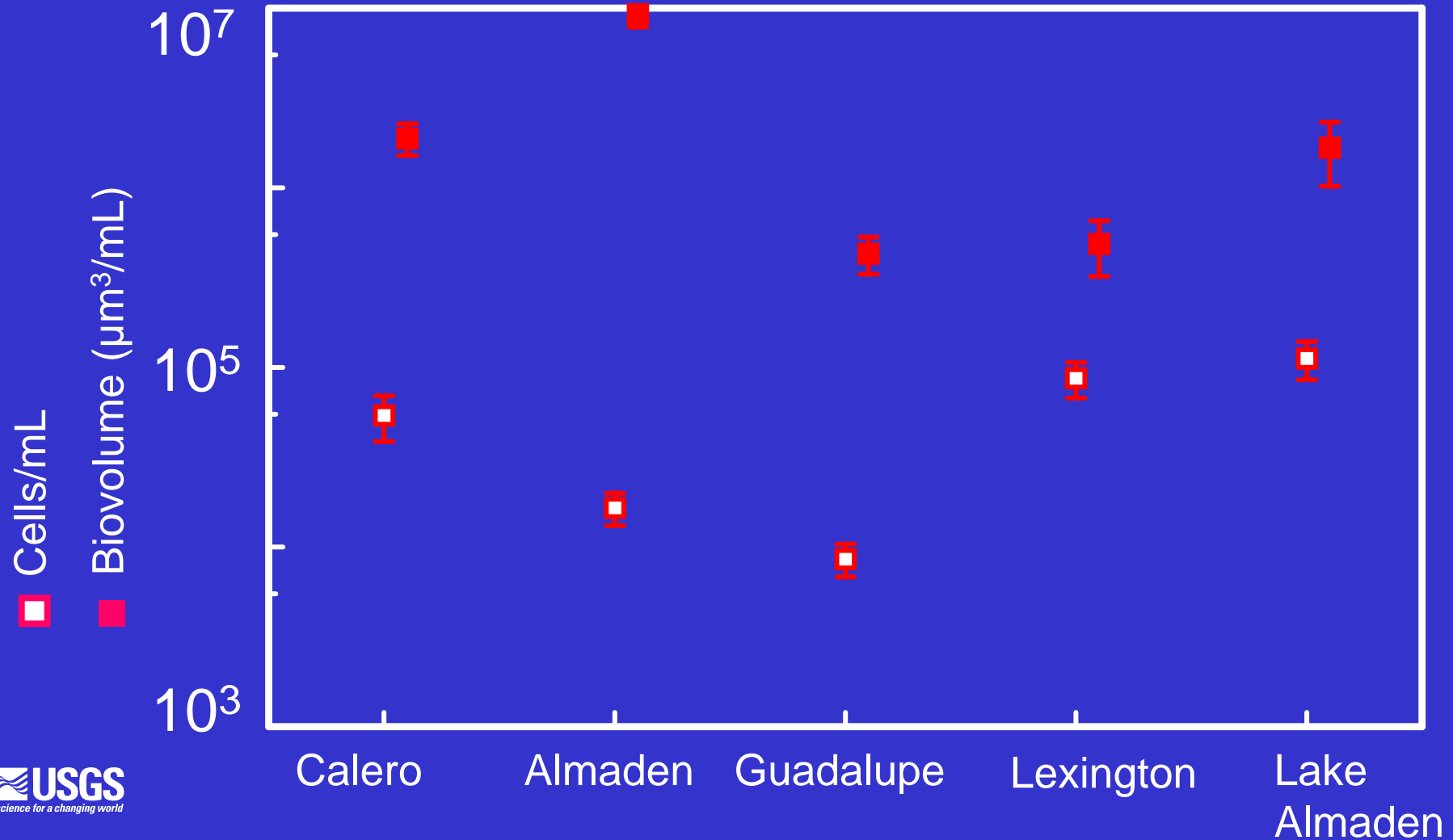


Figure 10. Graph showing detrended correspondence analysis (DCA) of phytoplankton taxonomic data.

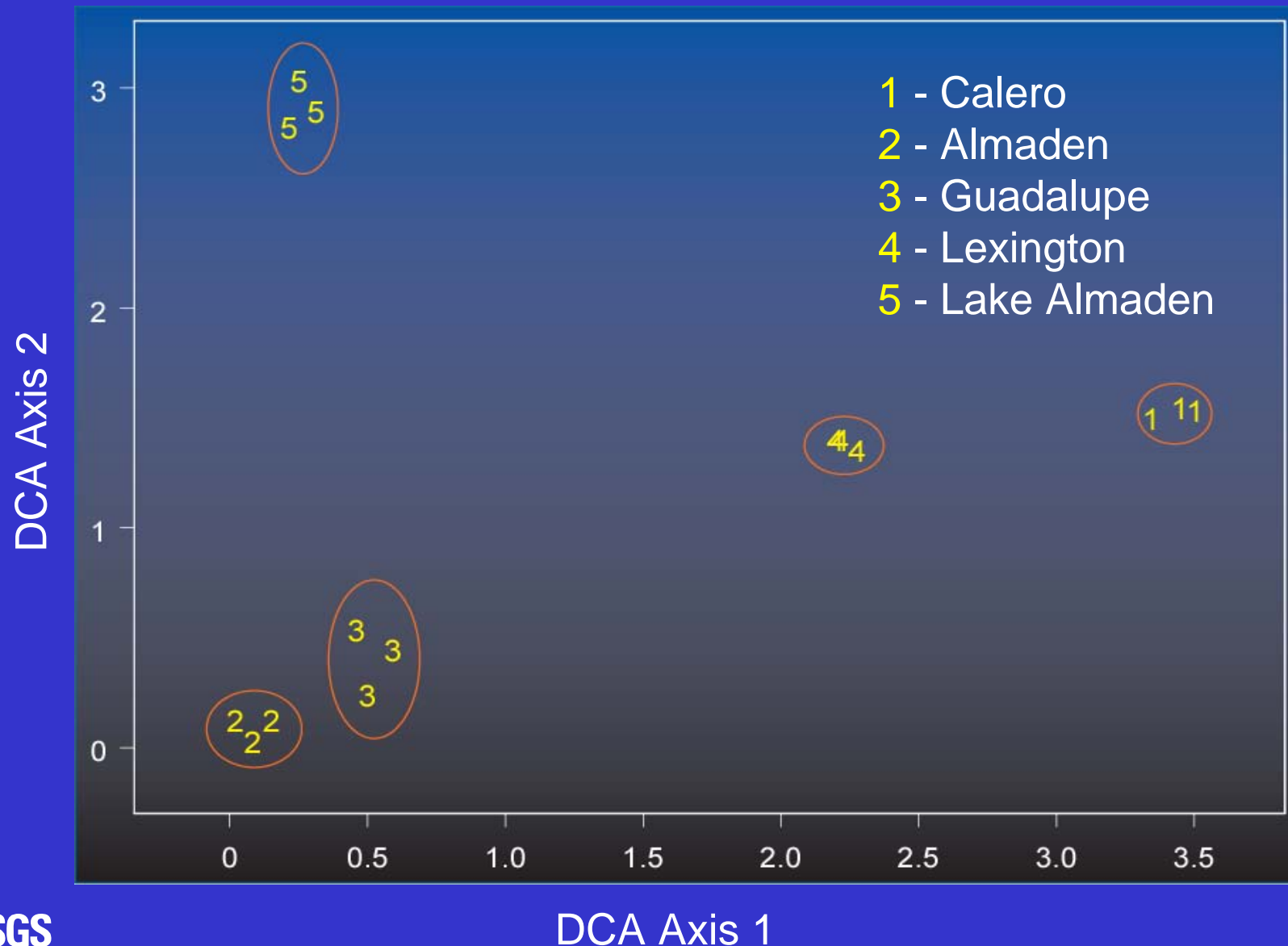


Figure 11. Trends in the zooplankton communities between lentic systems.

Error bars depict standard deviations about the mean, centered at the marker.

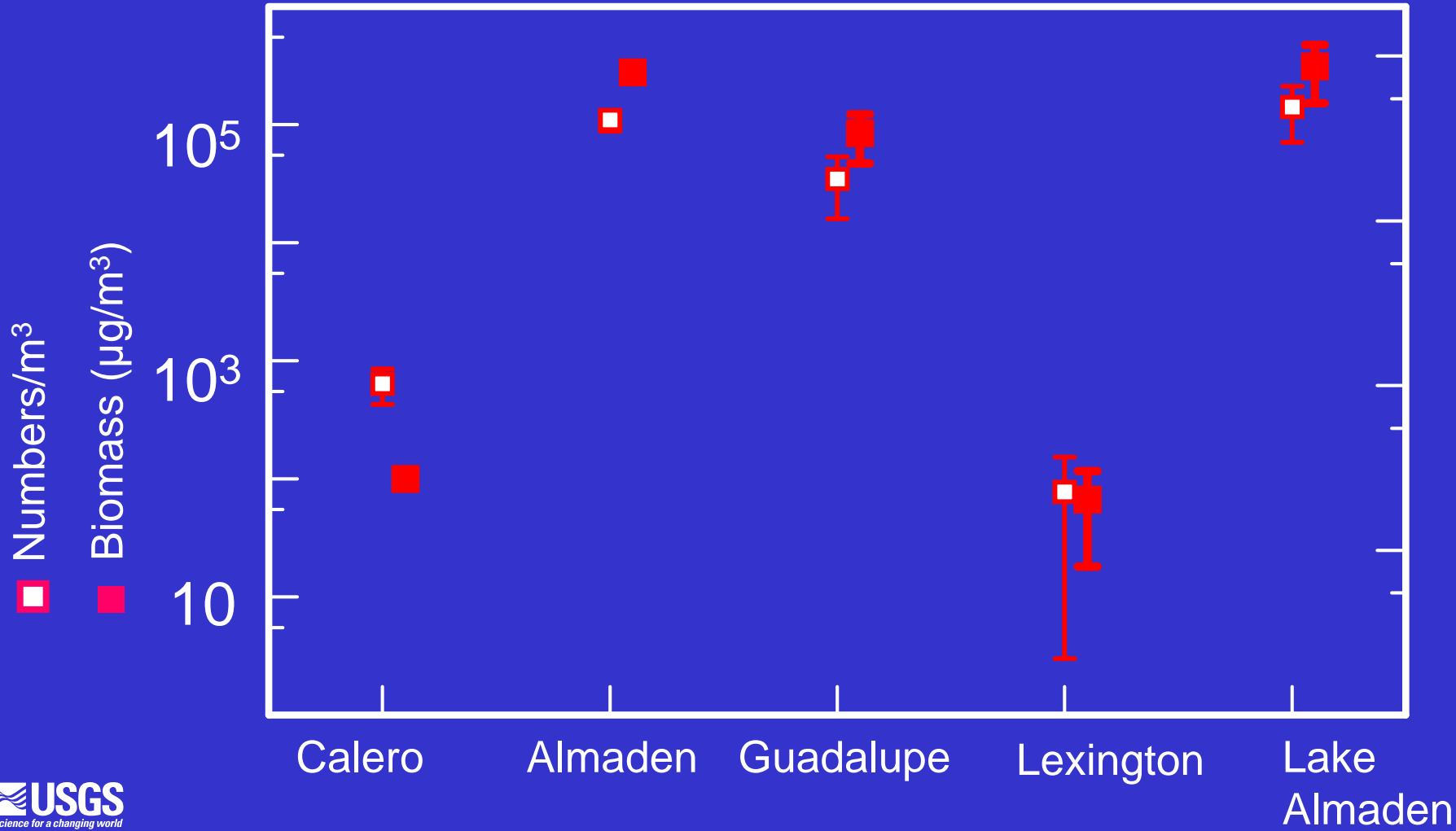


Figure 12. Graph showing detrended correspondence analysis (DCA) of zooplankton taxonomic data.

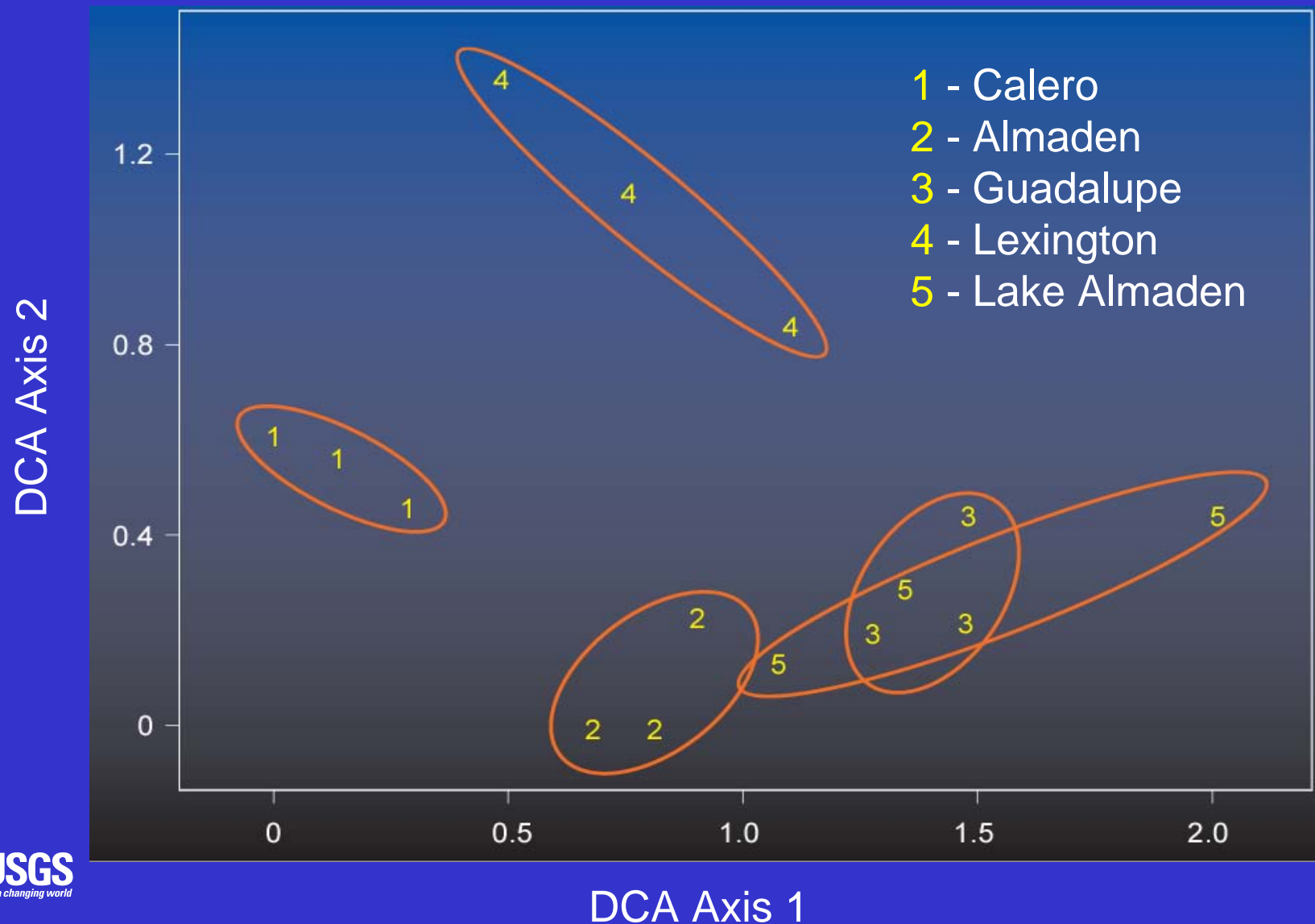
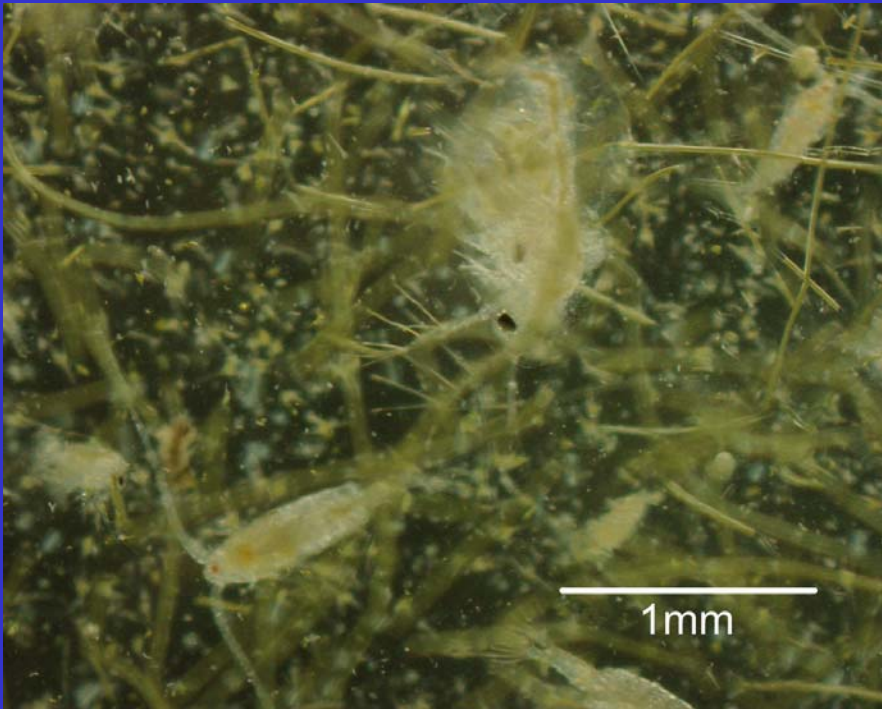
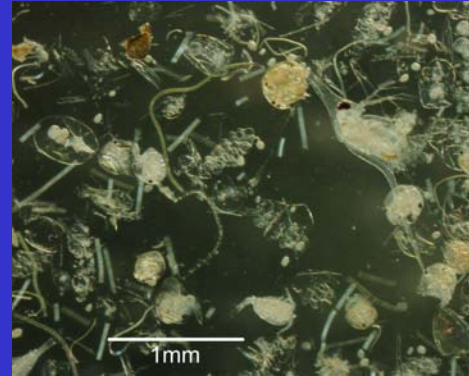


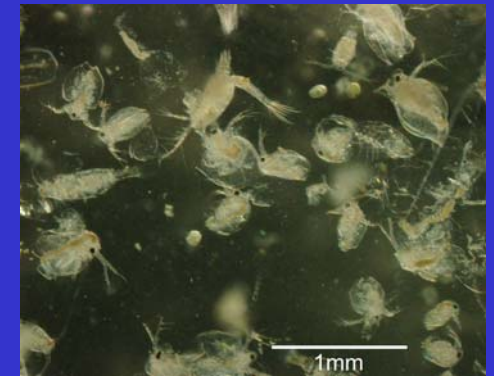
Figure 13. Photomicrographs showing a contrast in zooplankton-sample composition at Almaden Reservoir relative to other sampling sites.



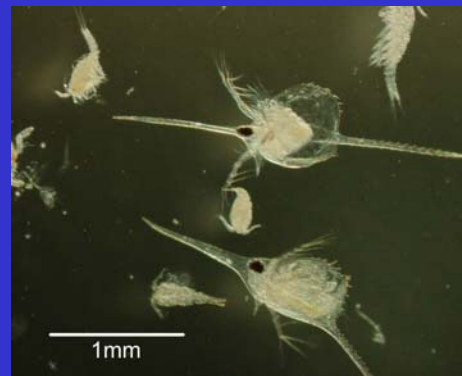
Almaden Reservoir  
(Abundance of algal filaments)



Calero Reservoir



Guadalupe Reservoir



Lexington Reservoir



Lake Almaden



Figure 14. Field methods used in this study for sampling: A) the water column, B) phytoplankton, and C) zooplankton.



Figure 15. Photograph showing mercury analysis by cold-vapor atomic fluorescence spectroscopy (CVAFS).



Figures of merit for mercury analysis  
Typical method detection limit  
0.2 pM

Figure 16. Photograph showing equipment used for dissolved organic carbon (DOC) analysis by high-temperature, non-catalytic oxidation.



Figures of merit for DOC analysis

Detection limit

0.1 mg/L



Figure 17. Photograph showing equipment used for dissolved-metal analysis by inductively coupled plasma mass spectrometry (ICP-MS)



Figures of merit  
for metal analyses

Detection limit

Cu 0.01 ug/L

Ni 0.05 ug/L

**Table 1. Specifications for size and location of five lentic systems sampled in the Guadalupe River watershed for this study, September, 2004.**

| Reservoir                 | Date Sampled | Starting Time (hr) Sampled | Location     |               | Surface Area (acre) | Storage Volume (acre-feet) | Surface Area (km <sup>2</sup> ) | Storage Volume (Mm <sup>3</sup> ) |
|---------------------------|--------------|----------------------------|--------------|---------------|---------------------|----------------------------|---------------------------------|-----------------------------------|
|                           |              |                            | Latitude (N) | Longitude (W) |                     |                            |                                 |                                   |
| Calero                    | 9/14/2004    | 0900                       | 37° 10.874'  | 121° 47.250'  | 349                 | 9934                       | 1.4                             | 12.3                              |
| Almaden                   | 9/14/2004    | 1400                       | 37° 09.816'  | 121° 49.756'  | 57                  | 1586                       | 0.2                             | 2.0                               |
| Guadalupe                 | 9/15/2004    | 0800                       | 37° 11.884'  | 121° 52.657'  | 74                  | 3415                       | 0.3                             | 4.2                               |
| Lexington                 | 9/15/2004    | 1400                       | 37° 11.952'  | 121° 59.198'  | 412                 | 19044                      | 1.7                             | 23.5                              |
| Lake Almaden <sup>1</sup> | 9/15/2004    | 1630                       | 37° 14.493'  | 121° 52.286'  | NA                  | NA                         | NA                              | NA                                |

<sup>1</sup> Dimensions were not available, but Lake Almaden, an abandoned quarry pit along Alamitos Creek that currently serves as a recreational area, was visibly the smallest, shallowest (Fig. 3) water body sampled.





**Table 4. Mercury speciation in the water column and biota.**

Results from Frontier for Guadalupe Watershed sampling on 9/14/04 and 9/15/04

**Water-column Samples**

| Reservoir            | Site # | Rep. | Inorganic and Methyl Hg |           |                   | Methyl Mercury    |                  |           | Percent           |                   |                |           |
|----------------------|--------|------|-------------------------|-----------|-------------------|-------------------|------------------|-----------|-------------------|-------------------|----------------|-----------|
|                      |        |      | Total Hg (ng/L)         |           | Percent Dissolved | Ratio Total:Diss. | Methyl Hg (ng/L) |           | Percent Dissolved | Ratio Total:Diss. | Methyl / Total |           |
|                      |        |      | Total                   | Dissolved |                   |                   | Total            | Dissolved |                   |                   | Total          | Dissolved |
| <b>Calero</b>        | 1      | A    | 4.57                    | 0.52      | 11                | 9                 | 0.239            | 0.051     | 21                | 5                 | 5              | 10        |
|                      |        | B    | 4.04                    | 0.52      | 13                | 8                 | 0.248            | 0.048     | 19                | 5                 | 6              | 9         |
| <b>Almaden</b>       | 2      | A    | 6.45                    | 1.17      | 18                | 6                 | 1.430            | 0.367     | 26                | 4                 | 22             | 31        |
|                      |        | B    | 10.10                   | 1.18      | 12                | 9                 | 1.070            | 0.275     | 26                | 4                 | 11             | 23        |
| <b>Guadalupe</b>     | 3      | A    | 17.50                   | 1.11      | 6                 | 16                | 0.360            | 0.087     | 24                | 4                 | 2              | 8         |
|                      |        | B    | 16.40                   | 1.28      | 8                 | 13                | 0.365            | 0.087     | 24                | 4                 | 2              | 7         |
| <b>Lexington</b>     | 4      | A    | 1.54                    | 0.47      | 31                | 3                 | 0.123            | 0.040     | 33                | 3                 | 8              | 9         |
|                      |        | B    | 2.96                    | 0.49      | 17                | 6                 | 0.122            | 0.039     | 32                | 3                 | 4              | 8         |
| <b>Lake Almaden</b>  | 5      | A    | 20.10                   | 2.62      | 13                | 8                 | 1.670            | 0.410     | 25                | 4                 | 8              | 16        |
|                      |        | B    | 17.20                   | 2.40      | 14                | 7                 | 1.410            | 0.390     | 28                | 4                 | 8              | 16        |
| Average =            |        |      | 10.09                   | 1.18      | 14                | 8                 | 0.70             | 0.18      | 26                | 4                 | 8              | 14        |
| Standard Deviation = |        |      | 7.07                    | 0.78      | 7                 | 4                 | 0.62             | 0.16      | 4                 | 1                 | 6              | 8         |

**Mean Concentrations for Summer, 2003 (Tetra Tech, 2003)**

| Reservoir | Total Hg (ng/L) |           | Methyl Hg (ng/L) |           | epilimnion  | Reservoir    |
|-----------|-----------------|-----------|------------------|-----------|-------------|--------------|
|           | Total           | Dissolved | Total            | Dissolved |             |              |
| Calero    | 2.0             | 0.25      | 0.8              | 0.14      | epilimnion  | Calero       |
|           | 3.4             | 2.02      | 3.1              | 1.25      | hypolimnion |              |
|           | 5.6             | 1.36      | 2.3              | 0.61      | epilimnion  | Almaden      |
| Guadalupe | 5.9             | 1.05      | 2.3              | 0.56      | hypolimnion |              |
|           | 10.6            | 1.05      | 3.3              | 0.49      | epilimnion  | Guadalupe    |
|           | 7.6             | 1.63      | 2.9              | 0.74      | hypolimnion |              |
| Lexington | 1.4             | 0.20      | 0.6              | 0.07      | epilimnion  | Lexington    |
|           | 2.2             | 0.69      | 1.3              | 0.74      | hypolimnion |              |
|           | 25.4            | 4.4       | 17.9             | 1.72      | epilimnion  | Lake Almaden |
|           | n/a             | n/a       | n/a              | n/a       | hypolimnion |              |

**Biological Samples** (All biological concentrations relative to dry weight.)

| Reservoir                  | Site # | Rep. | Phytoplankton   |                  |          | Zooplankton     |                  |          | Small Fish      |            |                |
|----------------------------|--------|------|-----------------|------------------|----------|-----------------|------------------|----------|-----------------|------------|----------------|
|                            |        |      | Total-Hg (ng/g) | Methyl-Hg (ng/g) | % Methyl | Total-Hg (ng/g) | Methyl-Hg (ng/g) | % Methyl | Total-Hg (ng/g) | STD (ng/g) | Replicates (n) |
| <b>Calero</b>              | 1      | A    | 25.2            | <1.50            | <6       | 146.0           | 95.1             | 65       | 1520            | 1790       | 20             |
|                            |        | B    | NA              | NA               | NA       | NA              | NA               | NA       |                 |            |                |
|                            |        | C    | NA              | NA               | NA       | NA              | NA               | NA       |                 |            |                |
| <b>Almaden<sup>1</sup></b> | 2      | A    | 56.6            | 4.11             | 7        | 861.9           | 647.9            | 75       | 4830            | 1220       | 20             |
|                            |        | B    | NA              | NA               | NA       | 859.9           | 639.9            | NA       |                 |            |                |
|                            |        | C    | NA              | NA               | NA       | NA              | NA               | NA       |                 |            |                |
| <b>Guadalupe</b>           | 3      | A    | 172.0           | <1.50            | <1       | 885.0           | 756.0            | 85       | 4240            | 560        | 20             |
|                            |        | B    | NA              | NA               | NA       | 890.0           | 755.0            | NA       |                 |            |                |
|                            |        | C    | NA              | NA               | NA       | 918.0           | 780.0            | NA       |                 |            |                |
| <b>Lexington</b>           | 4      | A    | 22.8            | <1.50            | <7       | 102.0           | 84.2             | 83       | 450             | 90         | 20             |
|                            |        | B    | NA              | NA               | NA       | NA              | NA               | NA       |                 |            |                |
|                            |        | C    | NA              | NA               | NA       | NA              | NA               | NA       |                 |            |                |
| <b>Lake Almaden</b>        | 5      | A    | 74.3            | 8.20             | 11       | 383.0           | 179.0            | 44       | 1820            | 370        | 20             |
|                            |        | B    | NA              | NA               | NA       | 391.0           | 178.0            | NA       |                 |            |                |
|                            |        | C    | NA              | NA               | NA       | 405.0           | 162.0            | NA       |                 |            |                |
| Average =                  |        |      | 70              |                  |          | 631             | 492              | 70       | 2572            | 806        |                |
| Standard Deviation =       |        |      | 61              |                  |          | 358             | 314              | 17       | 1875            | 690        |                |

<sup>1</sup> The netted zooplankton sample from Almaden Reservoir was an assemblage of both zooplankton and significant amounts of algal filaments (Fig. 13). The mercury concentrations from this assemblage were corrected for algal-concentration dilution based on the relative dry mass of the zooplankton (46 ± 6 % dry weight rounded to 50%) and phytoplankton.

**Table 5. Dissolved organic carbon (DOC) concentrations at the chlorophyll-a maximum depth<sup>1</sup>.**

| <b>Water Body</b>   | <b>Sampling<br/>Depth<br/>(meters)</b> | <b>DOC (uM)</b> |        | <b>DOC (mg-C/L)</b> |        |
|---------------------|--|-----------------|--------|---------------------|--------|
|                     |  | mean            | 95% ci | mean                | 95% ci |
| Calero Reservoir    | 2                                      | 363             | 2      | 4.36                | 0.02   |
| Almaden Reservoir   | 2                                      | 182             | 4      | 2.18                | 0.04   |
| Guadalupe Reservoir | 2                                      | 168             | 2      | 2.01                | 0.02   |
| Lexington Reservoir | 2                                      | 256             | 1      | 3.07                | 0.01   |
| Lake Almaden        | 6                                      | 224             | 3      | 2.69                | 0.03   |

<sup>1</sup> When a discernable peak in chlorophyll-a concentration was not observed in the depth profile, the water column was sampled at a depth of 2 meters.

**Table 6. Dissolved trace-metal concentrations (micrograms per liter) at the chlorophyll-a maximum depth<sup>1</sup>.**

| <b>Water Body</b>   | <b>Copper</b> |       | <b>Cadmium</b> |       | <b>Zinc</b> |       | <b>Nickel</b> |       | <b>Iron</b> |       | <b>Lead</b>  |       | <b>Manganese</b> |       | <b>Cobalt</b> |       | <b>Vanadium</b> |       |
|---------------------|---------------|-------|----------------|-------|-------------|-------|---------------|-------|-------------|-------|--------------|-------|------------------|-------|---------------|-------|-----------------|-------|
|                     | mean          | 95%ci | mean           | 95%ci | mean        | 95%ci | mean          | 95%ci | mean        | 95%ci | mean         | 95%ci | mean             | 95%ci | mean          | 95%ci | mean            | 95%ci |
| Calero Reservoir    | <b>1.210</b>  | 0.027 | <b>0.002</b>   | 0.000 | <b>0.85</b> | 0.06  | <b>2.00</b>   | 0.01  | <b>41.5</b> | 1.4   | <b>0.006</b> | 0.000 | <b>0.341</b>     | 0.006 | <b>0.069</b>  | 0.000 | <b>6.674</b>    | 0.000 |
| Almaden Reservoir   | <b>0.392</b>  | 0.004 | <b>0.000</b>   | 0.001 | <b>0.60</b> | 0.00  | <b>1.15</b>   | 0.01  | <b>48.6</b> | 0.7   | <b>0.008</b> | 0.000 | <b>0.775</b>     | 0.005 | <b>0.079</b>  | 0.000 | <b>2.376</b>    | 0.000 |
| Guadalupe Reservoir | <b>0.740</b>  | 0.001 | <b>0.002</b>   | 0.000 | <b>0.73</b> | 0.01  | <b>11.46</b>  | 0.04  | <b>65.5</b> | 1.5   | <b>0.015</b> | 0.000 | <b>0.348</b>     | 0.003 | <b>0.049</b>  | 0.001 | <b>3.168</b>    | 0.001 |
| Lexington Reservoir | <b>0.725</b>  | 0.002 | <b>0.002</b>   | 0.000 | <b>1.32</b> | 0.01  | <b>1.12</b>   | 0.02  | <b>58.7</b> | 0.9   | <b>0.004</b> | 0.000 | <b>0.381</b>     | 0.002 | <b>0.047</b>  | 0.001 | <b>1.456</b>    | 0.001 |
| Lake Almaden        | <b>0.680</b>  | 0.002 | <b>0.000</b>   | 0.000 | <b>1.08</b> | 0.02  | <b>1.94</b>   | 0.03  | <b>48.2</b> | 0.4   | <b>0.005</b> | 0.001 | <b>1.822</b>     | 0.009 | <b>0.052</b>  | 0.000 | <b>1.988</b>    | 0.000 |

<sup>1</sup> When a discernable peak in chlorophyll-a concentration was not observed in the depth profile, the water column was sampled at a depth of 2 meters.