

Effect of eutrophication on mercury (Hg) dynamics in subtropical reservoirs from a high Hg deposition ecoregion

N. Roxanna Razavi,^{*1} Mingzhi Qu,¹ Dongmei Chen,¹ Yang Zhong,^{2,3} Wenwei Ren,⁴ Yuxiang Wang,¹ Linda M. Campbell⁵

¹Department of Biology, Queen's University, Kingston, Ontario, Canada

²Department of Biology, Tibet University, Lhasa, China

³Institute of Biodiversity Science, School of Life Sciences, Fudan University, Shanghai, China

⁴Key Laboratory of Yangtze River Water Environment, Ministry of Education, College of Environmental Science and Engineering, Tongji University, Shanghai, China

⁵Department of Environmental Science, Saint Mary's University, Halifax, Nova Scotia, Canada

Abstract

Eutrophication can have opposite effects on mercury (Hg) bioavailability in aquatic systems, by increasing methylmercury (MeHg) production but reducing Hg biomagnification. Globally, the effect of eutrophication on Hg dynamics remains largely untested at lower latitudes such as eastern China, a productive subtropical ecoregion with Hg emission and deposition rates that are among the highest worldwide. Here, we quantify Hg (both MeHg and total Hg, THg) concentrations, Hg bioaccumulation, and Hg biomagnification rates in reservoir food webs across a gradient of eutrophication indicated by chlorophyll *a* (Chl *a*), zooplankton density, and total phosphorus (TP). We also assess the effect of hydrogeomorphic (HGM) features on Hg concentrations in water and biota. Water THg concentrations were strongly correlated with TP and were greater in reservoirs at higher elevations with short water retention times (WRT). Zooplankton and top predator THg concentrations were negatively correlated with Chl *a*, suggesting algal biodilution; evidence for zooplankton density dilution was also found when subtropical reservoirs were compared at a global scale with temperate lakes. Mercury bioaccumulation and biomagnification factors, respectively, did not correlate with increasing Chl *a* or zooplankton density suggesting no effect of plankton density on Hg trophic transfer. In subtropical reservoirs, eutrophication is associated with lower Hg concentrations in biota but does not explain Hg biomagnification; HGM features (i.e., elevation, WRT) and land use (i.e., % crop) appear to also influence Hg concentrations and bioaccumulation in reservoir food webs.

Mercury (Hg) is a pollutant that threatens global fisheries due to the toxicity of its organic form, methylmercury (MeHg). Aquatic ecosystems are sites of MeHg production due to geochemical conditions that increase biological methylation by sulphate-reducing bacteria (Lin et al. 2012). From tropical to Arctic latitudes, the process of MeHg transfer across trophic levels depends on food web structure (Lavoie et al. 2013). Through changes in water geochemistry and increases in primary productivity, cultural (i.e., anthropogenic) eutrophication alters food web structure and has the potential to significantly change MeHg bioavailability and biomagnification. Conditions characteristic of eutrophication, such as low hypolimnetic dissolved oxygen concentrations and high dissolved nutrients and sediment organic matter content, promote in situ production of MeHg (Gray

and Hines 2009). However, the visible hallmark of eutrophication, increased algal growth, leads to decreases in MeHg biomagnification through phytoplankton or zooplankton density dilution (Chen and Folt 2005). There remain important questions about what determines the outcome of eutrophication and Hg dynamics. It is also not clear how the changes in MeHg bioavailability and increases in plankton densities may affect the process and rate of Hg transfer between trophic levels and through the food web. Furthermore, most field studies that have measured the effect of eutrophication on Hg dynamics have focused on temperate lakes. This represents a global gap in knowledge regarding the fate of Hg with eutrophication at lower, more productive latitudes such as the subtropics.

Eutrophication is often assessed either by the concentrations of total phosphorus (TP) or chlorophyll *a* (Chl *a*) in the water column. Using these indicators of eutrophication

*Correspondence: roxanne.razavi@queensu.ca

to categorize waterbodies as eutrophic or oligotrophic, authors have documented confounding patterns in MeHg production and Hg bioaccumulation with eutrophication. In temperate and subtropical freshwater systems, rates of Hg methylation were higher in more eutrophic reservoirs, increasing the flux of MeHg to the water column (Gray and Hines 2009; Meng et al. 2010). Despite the possibility for enhanced MeHg availability in water, in natural and experimentally enriched systems Hg concentrations in fish were significantly lower than in oligotrophic lakes (Essington and Houser 2003; Chen and Folt 2005). These lower Hg concentrations in upper trophic level organisms are most often attributed to algal biodilution, the partitioning of Hg into a greater amount of biomass, as suggested by negative correlations between Hg concentrations in biota and Chl *a* (Pickhardt et al. 2002; Chen and Folt 2005). Higher zooplankton densities can also decrease Hg transfer to fish through zooplankton density dilution (Chen and Folt 2005). Other possible mechanisms that can lower fish Hg concentrations in eutrophic systems include shifts in algal cell size (Pickhardt and Fisher 2007) and growth dilution in zooplankton and fish (Simoneau et al. 2005; Karimi et al. 2007). However, lower fish Hg concentrations with eutrophication are not always observed as evidenced by higher Hg concentrations in top predators from temperate eutrophic reservoirs (Stone et al. 2011). Using indicators of eutrophication to predict how nutrient enrichment will affect Hg dynamics is a critical research need to identify fisheries that are at risk of Hg contamination.

It is important to assess not only the effect of eutrophication on Hg concentrations but also the effects on Hg biomagnification between trophic levels (i.e., predator–prey pairs) and across the food web because these processes ultimately determine Hg transfer and can be used for bioaccumulation assessments (Jardine et al. 2013). Whether or not increases or decreases in Hg concentrations, Hg bioaccumulation, and Hg biomagnification rates are expected under scenarios of greater eutrophication remains unclear. This question is especially important in reservoirs that differ markedly from lakes, for example, through water level fluctuations that can increase MeHg bioavailability (Evers et al. 2007) and external nutrient loading. Lower latitudes also deserve attention due to differences in productivity and food web structure compared to temperate regions. For example, in reservoirs of eastern China, intensive stocking of planktivorous Bighead Carp (*Hypophthalmichthys nobilis*) and Silver Carp (*Hypophthalmichthys molitrix*) can have substantial effects on plankton density and species composition, and indirectly affect trophic status of a waterbody (Li and Xu 1995).

In this study, we take a broad-scale, multireservoir approach to examine the link between Hg dynamics and eutrophication in subtropical reservoirs of eastern China. China has among the highest Hg emission and atmospheric

deposition rates in the world. It is estimated that deposition rates in China are 1–2 times and 1–2 magnitudes higher in rural and urban areas, respectively, compared to North America and Europe (Fu et al. 2012). Thus, the present study is akin to a natural “high exposure” treatment relative to studies in other parts of the world where this question has been previously addressed. We assess if measures of eutrophication suggested by Chen and Folt (2005), namely phytoplankton and zooplankton density, could be used to predict Hg concentrations in subtropical reservoirs. We test the predictive ability of indicators of eutrophication (Chl *a*, zooplankton density, and TP) to explain Hg (total Hg [THg] and MeHg) concentrations in the food web, represented by zooplankton, Bighead Carp, and top predatory fishes. Similarly, we ask whether those indicators predict derived variables describing Hg bioaccumulation (i.e., bioaccumulation factor, BAF), Hg biomagnification between a predator and prey pair (i.e., biomagnification factor, BMF), or Hg biomagnification rates (i.e., slope of the log Hg vs. $\delta^{15}\text{N}$ regression) in food webs composed of stocked and wild fishes with various feeding ecologies. Finally, we examine how landscape features, such as hydrogeomorphic (HGM) characteristics at the basin and catchment scale, and land use, predict Hg concentrations in water, zooplankton, and top predators in these subtropical reservoir ecosystems. Our hypotheses regarding the direction of these effects are presented in Table 1. Overall, we find evidence for low MeHg availability in these subtropical reservoirs of eastern China despite their exposure to factors that are known to contribute to elevated MeHg such as elevated inputs of atmospheric Hg deposition, continued water level fluctuations, and susceptibility to eutrophication, all of which can lead to higher MeHg production. This suggests that these factors may be outweighed by biomass or growth dilution associated with eutrophication in subtropical reservoirs.

Methods

Reservoir descriptions

All seven reservoirs in this study (Fig. 1) were located within the same humid to semihumid low-altitude (< 1000 m) ecoregion in eastern China (Zhou and Zheng 2008). This ecoregion has very high population densities and rapid development that have increased nutrient transport to aquatic ecosystems and resulted in the eutrophication of 85% of monitored lakes by 1996 (Zhou and Zheng 2008). The difficulties of managing fisheries in the many hypereutrophic lakes in the region have shifted the focus of fish production to reservoirs, largely because of their oligotrophic to mesotrophic statuses. To ensure that sampling did not occur during the initial period after impoundment when fish Hg concentrations are known to be most elevated, all selected reservoirs for this study were over 30 years old at the time of sampling (construction completed in 1954–1969). Reservoirs

Table 1. Expected outcomes of mercury (Hg) concentrations in water and biota (zooplankton and fish), Hg bioaccumulation factors (BAF_{biota}), and Hg biomagnification (biomagnification factor, BMF or trophic magnification factor, TMF) against effect of eutrophication (i.e., chlorophyll *a* (Chl *a*), total phosphorus (TP), or zooplankton density) and/or hydrogeomorphic (HGM) features at the catchment (HGM:catchment) and basin (HGM:basin) scale.

Response variable	Predictor category	Hypothesized mechanism	Predictor variable(s) and hypothesized direction of effect	References	
Hg _{biota}	Trophic status	Plankton density and somatic growth dilution	Chl <i>a</i> , zooplankton density, TP	–	1,2
		Higher MeHg production with increasing carbon availability and bacterial activity		+	3
BAF _{biota}		Lower MeHg assimilation efficiency with increasing carbon, higher DOC		–	4,5
		Higher MeHg availability due to higher MeHg production		+	6
BMF, TMF		Mechanisms proposed above resulting in higher or lower Hg _{biota}		±	7
Hg _{water}	Land use	Higher % forest cover (lower % crop cover) scavenges atmospheric Hg	% Crop	–	8
	HGM:catchment	Higher atmospheric deposition with larger catchment to surface area	Catchment:surface area (CA : SA)	+	9
	HGM:basin	Higher water temperatures promote methylation	reservoir volume	–	10
Higher nutrient and contaminant input		Water retention time (WRT)	–	11	
Higher atmospheric deposition		Elevation	+	12	
Hg _{biota}	Land use	Higher nutrient input	% Crop		13
				±	
	HGM : catchment	Higher atmospheric deposition	Catchment:surface area (CA : SA)	–	9
		Higher fish metabolic rates in warmer water	Reservoir volume	–	10
	HGM : basin	Higher nutrient and contaminant input	Water retention time (WRT)	–	11
	Higher atmospheric deposition	Elevation	+	12	

¹Chen and Folt 2005; ²Essington and Houser 2003; ³Lin et al. 2012; ⁴Tsui and Wang 2004; ⁵Gorski et al. 2008; ⁶Stewart et al. 2008; ⁷Lavoie et al. 2013; ⁸Evers et al. 2007; ⁹Drevnick et al. 2012; ¹⁰Bodaly et al. 1993; ¹¹Kalff 2002; ¹²Dittman and Driscoll 2009; ¹³Bremigan et al. 2008.

were sampled in the Summer (between late June 2011 and August 2011) and ranged in morphology from deep river—valley (e.g., F, M; Fig.1; Table 2) to shallow lake-plain types (e.g., S, T; Li and Xu 1995) and spanned a wide gradient of surface areas and catchment area to surface area ratios (CA : SA; Table 2). We strove to sample as wide a gradient of eutrophication as possible; however, access to eutrophic reservoirs was limited. Additional information regarding annual water level fluctuations, and water stratification patterns, were also not readily available. We are only aware of a detailed study on stratification patterns for one reservoir sampled here (i.e., Q), which was described as warm monomictic, with a period of thermal stratification found from April until January (Zhang et al. 2014). Two other reservoirs also stratify (i.e., Shu 1964; Zhang et al. 2004) but no data were available for other reservoirs. To the best of our knowledge, there are no direct point sources of Hg into the reservoirs; while there are coal-fired power plants in the direct vicinity (~ 50 km² radius) of most reservoirs (except Q and M), within a larger radius (~ 100 km²) there are between 10 and 15 (i.e., F, M, L, Q) and ~

30 (i.e., H, T, S) coal-fired power plants of varying capacities, representing a substantial nonpoint source of Hg.

The percent of the catchment covered by forests vs. crops was determined using Geographic Information System (ArcGIS Version 10.1). Base maps of watersheds were obtained from BaseCamp 4.1.2 (Garmin International) and Garmin China City Navigator NT 2012 digital elevation model (DEM) and topographic maps at 10 m resolution to locate the approximate study areas. Freely available global digital elevation tiles at 20 m resolution were downloaded (Advanced Spaceborne Thermal Emission and Reflection Global DEM Version 2 <http://asterweb.jpl.nasa.gov/gdem.asp>) and used to delineate the accurate basin areas of reservoirs using hydrological functions in ArcGIS. Land cover data were downloaded from Finer Resolution Observation and Monitoring-Global Land Cover (<http://data.ess.tsinghua.edu.cn/>) using the Landsat Path list option. This dataset was the first 30 m resolution global land cover maps produced using Landsat Thematic Mapper and Enhanced Thematic Mapper Plus data (Gong et al. 2013). The land cover of each

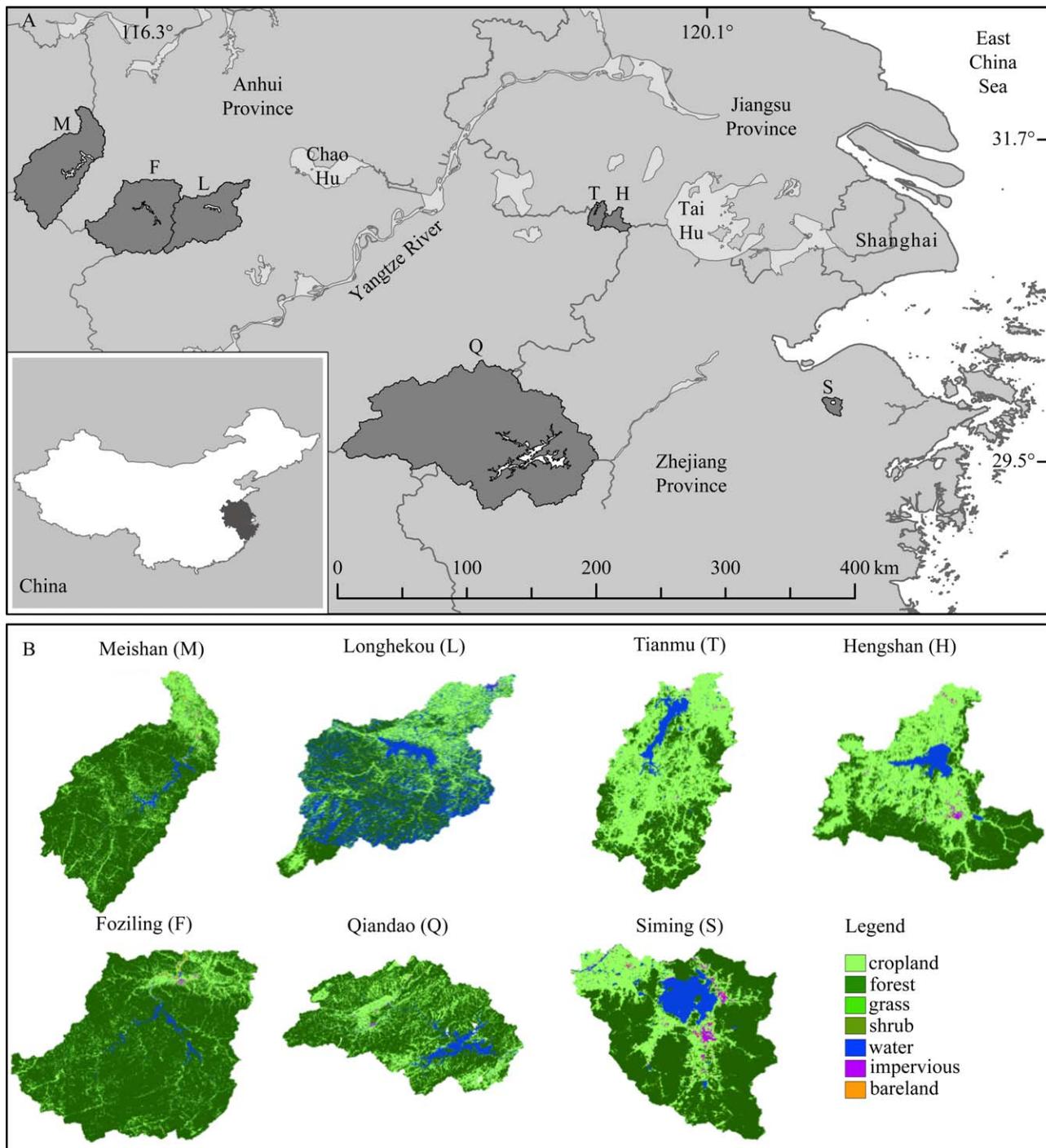


Fig. 1. Location of reservoirs sampled in eastern China. (A) Dark gray area indicates watershed area and (B) colors indicate land use. Reservoir codes are given in parenthesis. Note: reservoirs are not to scale in the lower panel.

reservoir basin was obtained by clipping the corresponding land cover tile with the basin boundary of the reservoir. Percent crop and percent forest cover were summed relative to total land cover. Only in one reservoir (L) was the land cover data assessed as forest when the land cover map indicated

water (land cover data was taken in January), we assumed this to be an error as land cover immediately adjacent was displayed as forested. Land use ranged from highly forested (e.g., F, 85.4% forest cover) to primarily agricultural (e.g., T, 46.8% crop cover; Fig. 1; Table 2).

Table 2. Physical, chemical, and biological characteristics of reservoirs sampled in eastern China (see Fig. 1 for reservoir names and locations). Population density (per km²), surface area (SA, km²), catchment area to surface area ratio (CA: SA), reservoir volume ($\times 10^9$ m³), mean water level (i.e., elevation, m), and water retention time (WRT, days) were taken from published literature. Total phosphorus (TP, mg L⁻¹), total nitrogen (TN, mg L⁻¹), chemical oxygen demand (COD, mg L⁻¹), Secchi depth (SeD, m), chlorophyll *a* (Chl *a*, μ g L⁻¹), zooplankton (zoop) density (ind. L⁻¹) and biomass (μ g L⁻¹), and % forest and crop cover measured in this study.

Reservoir code	Population density*	SA	CA: SA	Volume	Elevation	WRT [†]	TP	TN	COD	SeD	Chl <i>a</i> [‡]	Zoop density	Zoop biomass	% Forest	% Crop
F	15	20	92.0	0.364	124	100	0.069 [§]	1.09 [¶]	2.4	5.0	1.9	6.7	142.5	85.4	10.9
H	158	4.5	34.4	0.112	32	460	0.017	2.45	2.7	4.0	2.9	14.5	62.2	43.9	41.6
L	45	50	22.2	0.516	68	335	0.078	1.13	3.7	2.0	8.2	8.1	58.5	70.7	24.8
M	78	62.9	31.3	1.245	126	323	0.087	1.21	2.8	3.5	1.8	3.8	79.2	77.4	16.2
Q	149	580	18.1	17.84	37	700	0.018	0.62	1.3	5.5	1.5	8.0	71.4	64.2	24.4
S	324	5.5	18.7	0.0795	16	484	0.012	0.68	3.2	1.4	6.5	8.9	66.7	62.6	19.5
T	423	6.7	22.2	0.109	19	580	0.020	1.14	3.6	1.1	23.7	4.0	30.9	35.5	46.8

*Estimate based on total population in the catchment divided by the catchment area.

[†]WRT = reservoir volume: mean outflow rate.

[‡]All samples represent means of replicate samples.

[§]Mean of two samples TP range = 0.067–0.71.

[¶]Mean of two samples TN range = 1.04–1.13.

^{||}Mean of two samples COD range = 2.1–2.6.

Water and zooplankton sampling were done from a small boat in the open water zone of the reservoir in front of the dam. Water was collected for total nitrogen (TN), TP, and chemical oxygen demand (COD) using a one liter Van Dorn water sampler at one meter below the water surface. Samples for TN, TP, and COD were dispensed into acid-cleaned Nalgene bottles and frozen until analysis. Nutrient analysis was carried out by the Shanghai Environmental Monitoring Center (Shanghai, China). TN concentrations were measured according to the alkaline potassium persulfate digestion method (State Environmental Protection of China 1990a) and TP according to the ammonium molybdenum spectrophotometric method with ultraviolet-visible (UV) spectrophotometry following a potassium persulfate digestion (State Environmental Protection of China 1990b). COD was determined using the permanganate index (ISO 8467:1986). Water samples for phytoplankton Chl *a* pigments were dispensed into opaque Nalgene bottles, and 500 mL were filtered in duplicate through 1.2 μ m GF/C filters and stored frozen in tin foil. Chl *a* analyses were completed at McMaster University (Ontario, Canada) following previously published methods (Chow-Fraser 2006). Briefly, frozen filters were placed in 10 mL of 90% reagent-grade acetone and kept in the freezer for two hours prior to centrifugation. Chl *a* content was determined by measuring absorbance by spectrophotometry before and after acidification with hydrochloric acid to account for phaeophytin pigments. The phaeophytin corrected values were used in this study.

Unfiltered surface water (one meter) samples for field blanks and THg and MeHg analysis were taken by use of trace metal clean techniques. These concentrations represent

an important, but preliminary assessment: we placed most of our effort into quantifying the fish food web, and due to restrictions on access to reservoirs in the field, we were unable to characterize temporal and spatial variability in water Hg concentrations. Surface water MeHg concentrations measured here may be an underestimate if hypolimnetic conditions are favorable to methylation, as can be observed in reservoirs that stratify. Information on stratification was not available at the time of sampling, and this limits our ability to determine trends in MeHg concentrations in water with indicators of eutrophication. Measurements of BAF (see equation below) that use water MeHg concentrations in the denominator may also be overestimated. The decision to take unfiltered surface waters as done previously by others (Rolfhus et al. 2011) was largely due to lack of access to appropriate ultratrace filtration equipment. Prior to field sampling, Teflon bottles were acid cleaned with concentrated nitric acid, dilute hydrochloric acid (HCl; 10%) and stored in nitric acid (HNO₃; 10%). Bottles were dipped below the surface and the “clean hands and dirty hands” technique (US EPA 1996) was used during sampling to minimize potential for contamination. All water samples for Hg analysis were frozen immediately in the field; those for MeHg analyses were preserved with pure HCl prior to freezing.

Phytoplankton were collected by vertical tows through the water column, using a Wisconsin net (45 μ m nylon mesh; nine tows at one meter); zooplankton were collected with a cone net (202 μ m nylon mesh; three tows at 7–20 m, from one meter above the bottom to the surface, with care taken to avoid collecting sediment). Phytoplankton species composition is given elsewhere and was significantly

correlated to TP concentrations (Razavi et al. 2014a). The size fraction for zooplankton was selected to allow comparison with previously published results (Chen and Folt 2005; published data extracted using Digitizeit 2.0.4, <http://www.digitizeit.de>). Zooplankton samples for enumeration (i.e., zooplankton density) were preserved in 95% alcohol, diluted to a volume of 100 mL, then subsampled and counted (up to 250 individuals per replicate). Zooplankton biomass was calculated on three additional zooplankton tows following Chen and Folt (2005); samples were filtered onto preweighed Whatman filters and then dried at 60°C and weighed. Separate zooplankton samples were collected for THg and MeHg analysis; samples were rinsed with ultraclean water and frozen in Teflon bottles in the field (bottles were prerinsed in acid as described earlier for water samples). Zooplankton samples were freeze-dried (Stewart et al. 2008), homogenized, and split into subsamples for Hg and stable isotope analysis.

We sampled fish from all trophic levels and targeted the same species at all sites. Stocked fish (i.e., raised in ponds and hatcheries to a certain length and released into the reservoir as fingerlings) and wild fish were sampled, but farmed fish were not included as those are exclusively fed with fish food in various types of enclosures, and thus, do not represent the reservoir-wide food web. Details of fish diet items are provided in Razavi et al. (2014b). With the guidance of local fisheries managers who were very familiar with both fish management and fish vendors at each reservoir, we sampled fish from individual fishermen and private fishing enterprises, as well as specific vendors that sold exclusively live fish from the reservoir at local markets. Individual fish weights and total length ranges were measured (Web Appendix, Table A1, www.aslo.org/lo/toc/vol_xx/issue_x/xxxxxa.xxx) and fish dorsal muscle tissues were removed in the field, transported on ice, and frozen at facilities at Fudan University prior to analyses in Canada. Tissues were then dried at a low temperature (between 50°C and 60°C) for 48 h for Hg and stable isotope analysis in a Hg-free oven (no Hg thermometers were ever used and the oven was periodically brought to 180°C to remove residual Hg) at Queen's University (Ontario, Canada). Dried fish tissues were required for the digestion method (see below) and oven drying caused the lowest Hg losses relative to other drying methods (Ortiz et al. 2002).

Stable isotope analyses

A portion of each dried muscle sample was homogenized finely using stainless steel jars and balls on a Mixer Mill MM200 (Retsch GmbH & Co KG), and then weighed into tin capsules (~ 1.2 mg) using a calibrated semi-micro balance (Sartorius AG). Stable isotopes of nitrogen were analyzed at the Stable Isotope Facility at the University of California, Davis by a Sercon-Gas Solid Liquid elemental analyzer interfaced to a Sercon 20-20 isotope ratio mass spectrometer (Sercon). Samples were interspersed with blanks and standards (standard deviation, SD, $\delta^{15}\text{N}$ 0.3‰); the standards were pre-

viously calibrated against National Institute of Standards and Technology (NIST) reference materials. Triplicates of in-house standard materials, defatted Atlantic salmon (mean $\delta^{15}\text{N}$ = 8.8‰, n = 12) and farmed tilapia (mean $\delta^{15}\text{N}$ = 6.1‰, n = 12) were included in each run; SD were less than 0.1‰. A duplicate of a fish sample was run every 10 samples as a check on consistency (coefficient of variation: $\delta^{15}\text{N}$ = 0.8%). Delta values of nitrogen were calculated with the equation $\delta^{15}\text{N} = \left(\left(\frac{^{15}\text{N}}{^{14}\text{N}} \right)_{\text{sample}} : \left(\frac{^{15}\text{N}}{^{14}\text{N}} \right)_{\text{standard}} \right) - 1 \times 1000$. The δ values are expressed in per mil (‰) deviation from international standards (i.e., Air).

Mercury analyses

Unfiltered water samples and zooplankton samples were analyzed for THg and MeHg by the Laboratory for the Analysis of Natural and Synthetic Environmental Toxins research facility at the University of Ottawa (Ontario, Canada). THg concentrations in water samples were analyzed by cold vapor atomic fluorescence spectrometry (CVAFS) using US Environmental Protection Agency (EPA) Method 1631 (US EPA 2002). The reporting limit (lowest point in calibration curve) for THg in water was 0.2 ng L⁻¹. THg concentrations for freeze-dried zooplankton were measured using a direct thermal decomposition Hg analyzer, with a method detection limit of 0.012 ng g⁻¹. Quality assurance for zooplankton included National Research Council of Canada (NRC-CNRC) certified reference materials (CRM) DORM-4 (fish protein; 92.7-101.2% recovery, n = 2), TORT-2 (lobster hepatopancreas; 115.6% recovery, n = 1) and the NIST standard reference material NIST-1566B (oyster tissue, 84.0-85.7% recovery, n = 2). Methylmercury in water was analyzed by capillary gas chromatography (GC) coupled with CVAFS (Cai et al. 1996). Zooplankton MeHg was extracted into dichloromethane and subsequently analyzed by GC CVAFS (Cai et al. 1997). Recovery of CRM for MeHg was 89.5-94.9% for DORM-4 (n = 2) and 97.4-98.1% for TORT-2 (n = 2). The method detection limit for MeHg in water and solids was 0.002 ng L⁻¹ and 0.016 ng g⁻¹, respectively. Recovery of MeHg in spiked water samples was 91.5% ± 2.7% (n = 4). Field blanks [0.2 ± 0.1 ng L⁻¹, n = 4 for THg; 0.009 ng L⁻¹ (n = 1) for MeHg] and procedural blanks revealed no contamination during sampling and analysis.

All fish THg analyses were carried out at Queen's University using CVAFS (Tekran 2600 Total Hg Analyzer, Tekran Instruments, Ontario, Canada) following US EPA Appendix to Method 1631 (US EPA 2001a). All equipment and glassware used were washed in ultrapurified trace-metal quality water (EMD Millipore), placed in an acid bath for a minimum of 12 h, and rinsed again in Millipore water. Oven dried fish tissue (~ 25 mg) was weighed into Teflon vessels, and digested at 200°C for 15 min in trace metal grade acid (5 m L⁻¹ HNO₃) and hydrogen peroxide (5 m L⁻¹ H₂O₂) in a Microwave Accelerated Reaction System (CEM). Samples were diluted using Millipore water, preserved using 0.5% bromine

monochloride, and refrigerated at 4°C for at least 24 h prior to analysis. Quality control was assessed by analysis of CRMs: DORM-3, TORT-2, and DOLT-4 (dogfish liver, NRC-CNRC). Recovery of THg for DORM-3 was $109.4\% \pm 6.9\%$ (mean \pm SD, $n = 22$), for TORT-2 was $113.1\% \pm 5.1\%$ ($n = 13$), and DOLT-4 was $104.5\% \pm 4.6\%$ ($n = 12$). The reporting limit was 0.05 ng L⁻¹ and blank values were all below 0.4 ng. Samples ($n = 33$) spiked with 0.4 ng Hg resulted in a mean spike recovery (88%) and spike precision (13%) that met quality control acceptance criteria (US EPA 2001a).

Statistical analyses

Effect of eutrophication We used Chl *a*, zooplankton density, and TP as indicators of eutrophication, as reported commonly to characterize lake trophic status. These indicators of eutrophication were used as predictor variables of Hg concentrations, Hg bioaccumulation, and Hg biomagnification. As little is known about what limits algal growth within these reservoirs, we used Carlson's (1977) method to determine trophic state index (TSI) that can be calculated based on Chl *a* (TSI_{Chl a}), TP (TSI_{TP}), TN (TSI_{TN}), or Secchi depth (TSI_{SecD}). Trophic state indices were not used as predictor variables but provided insight into the importance of nutrient limitation on algal growth. Note that these estimates are preliminary as they are based on only a single sampling event, and seasonal variations are expected. Primary limitations on algal growth can be inferred based on the deviations of TSIs from TSI_{Chl a} (i.e., subtracting TSI_{TP}, TSI_{TN}, or TSI_{SecD} from TSI_{Chl a}; Matthews et al. 2002). The equations to calculate TSIs are as follows:

$$\text{TSI}_{\text{Chl}a} = 30.6 + 9.81 \ln(\text{Chl}a, \mu\text{g L}^{-1})$$

$$\text{TSI}_{\text{TP}} = 4.15 + 14.42 \ln(\text{TP}, \mu\text{g L}^{-1})$$

$$\text{TSI}_{\text{TN}} = 54.45 + 14.43 \ln(\text{TN}, \text{mg L}^{-1})$$

$$\text{TSI}_{\text{SecD}} = 60 - 14.42 \ln(\text{Secchi depth}, \text{m})$$

Linear regressions were used to test for an effect of eutrophication; Model II slopes were reported (because both variables in the regression equation were random, i.e., the predictor variable was not controlled). All variables were log-transformed where necessary to meet assumptions of normality. Indicators of eutrophication (i.e., Chl *a*, zooplankton density, and TP) were used as independent variables to predict Hg concentrations in zooplankton, Bighead Carp, and top predators and derived variables that describe Hg bioaccumulation and Hg biomagnification. The derived variables included the BAF, BMF, and trophic magnification factor (TMF) and were calculated as follows:

$$\text{BAF (L kg}^{-1}\text{)} = \text{Biota}_{\text{THg or MeHg}} : \text{Water}_{\text{THg or MeHg}}$$

where biota = zooplankton or fish (Bighead Carp or top predator)

$$\text{BMF} = \text{Bighead Carp}_{\text{THg or MeHg}} : \text{Zooplankton}_{\text{THg or MeHg}}$$

$$\text{TMF} = 10^m$$

where *m* equals the rate of food web Hg biomagnification by trophic level (see below for equation). BAF values were calculated on zooplankton Hg concentrations in dry wt and on fish Hg concentrations in wet wt; BAF values were log-transformed. To derive the BMF, we assumed that Bighead Carp feed 100% on zooplankton. In reality the diet of Bighead Carp can be comprised of both phytoplankton and zooplankton, although most studies assume the largest fraction of Bighead Carp diet is from zooplankton (Zhou et al. 2009). Zooplankton Hg concentrations were measured in dry wt so BMF values were also expressed on a dry wt basis. Note that the Hg concentrations in fishes are more integrative than the zooplankton Hg concentrations and as a result may under or overestimate BMF. To be able to compare fish Hg concentrations across reservoirs, we first tested for total length as a covariate and made length adjustments when necessary in the species of interest. We chose to focus on species at the bottom and top of the food web that were found in all reservoirs, that is, the low trophic level Bighead Carp, and the top predators (selection criteria below). Bighead Carp were of significantly different total lengths among reservoirs, but small sample sizes within each reservoir resulted in insignificant regressions of log THg vs. log total length. Bighead Carp THg concentrations were significantly and positively correlated with log total length across reservoirs if an outlier (F) was excluded, but using the size-adjusted Bighead Carp in the remaining reservoirs ($n = 6$) did not change the outcome of the Bighead Carp vs. eutrophication comparisons. Therefore, Bighead Carp from all reservoirs were included without any size adjustments. Top predators were selected as the species with the highest $\delta^{15}\text{N}$ within each reservoir (note that in two reservoirs [i.e., S and H] the number of predatory fishes available for collection was low relative to other reservoirs); all top predators that met this criteria belonged to the same genus (i.e., *Chanodichthys*, see Web Appendix, Table A1 for the species selected by reservoir). This was expected based on previous research of their feeding habits and trophic position in Qiandao Hu (Razavi et al. 2014b), a subtropical reservoir (Q) also included in this study. However, a significant difference in total length in *Chanodichthys* among reservoirs was found (ANOVA, $R^2 = 0.53$, $F_{6,24} = 4.60$, $p = 0.003$, $n = 31$). As sample sizes were too small to test for a linear relationship between log THg and log length for individual *Chanodichthys* species within reservoirs, all the top predators were combined to test for total length as a covariate. Log Hg concentrations increased with log fish total length (linear regression analysis, $R^2 = 0.40$, $F_{1,29} = 17.71$, $p = 0.0002$, $n = 31$). Therefore, we size-adjusted Hg concentrations in top predators using analysis of covariance (ANCOVA). To run the ANCOVA, sample

Table 3. Concentrations of total Hg (THg) and methylmercury (MeHg) in water (ng L^{-1}), zooplankton, Bighead Carp, and top predators (ng g^{-1}) from subtropical reservoirs sampled in eastern China. See Fig. 1 for reservoir codes.

Reservoir code	Water*			Zooplankton [†]			Bighead Carp [‡]		Top predator [§]
	THg	MeHg	%MeHg	THg	MeHg	%MeHg	THg \pm SE	MeHg \pm SE	Log THg \pm SE
F	40	0.02	0.04	73.0	43.8	60.0	804 \pm 272	758 \pm 244	776 \pm 1
H	0.8	0.01	0.9	27.6	8.50	30.8	140 \pm 7.77	119 \pm 6.17	398 \pm 1
L	40	0.01	0.04	34.2	20.4	59.6	306 \pm 20.0	276 \pm 28.8	363 \pm 1
M	20	0.01	0.04	48.3	17.4	36.0	348 \pm 39.2	335 \pm 44.0	692 \pm 1
Q	0.6	0.01	1	71.3	25.5	35.8	276 \pm 31.9	229 \pm 39.8	646 \pm 1
S	1	0.01	0.7	56.9	27.8	48.9	119 \pm 20.1	93.4 \pm 19.4	275 \pm 1
T	1	0.02	2	14.9	5.80	38.9	310 \pm 43.5	281.2 \pm 41.9	363 \pm 1

* $n = 1$ at each reservoir.

[†] $n = 1$ (combined from three tows) at each reservoir.

[‡]Bighead Carp are presented as raw data (not size-adjusted), see text for details.

[§]Size-adjusted values (least squares means) \pm standard error (SE) based on dry wt, see text for details.

sizes of top predators had to be increased for some reservoirs. The additional top predators were selected based on the species with the next highest mean $\delta^{15}\text{N}$ value (Web Appendix, Table A1). In only one reservoir (S), we added a similar predatory species *Siniperca chuatsi* ($n = 1$; Web Appendix, Table A1) to make size-adjustment possible. The combination of fish species was necessary to determine the Hg concentrations at the highest possible level of the fish food web (see similar method used in Chen and Folt 2005). The ANCOVA was first run with an interaction term (log total length \times reservoir). No significant effect of the interaction term was found ($p > 0.05$), so the ANCOVA was rerun assuming homogeneity of slopes. The ANCOVA was significant (ANCOVA, $R^2 = 0.62$, $F_{7,41} = 9.59$, $p = 0.0001$); LS Means were used to represent size-adjusted (adj) Hg concentrations for top predators (mean total length = 30.9 cm across all reservoirs, antilog THg concentrations presented in Table 3).

Mercury biomagnification. The Hg biomagnification rate was assessed by the slope from the regression of log THg vs. $\delta^{15}\text{N}$ using all fish sampled (i.e., individual [ind.] concentrations) at each reservoir. The Model I slope (assumes predictor variable contains no error) was reported for consistency with the literature. The slope was multiplied by 2.8, the average increase in $\delta^{15}\text{N}$ per trophic level for a tropical fish assemblage (Jepsen and Winemiller 2002) to obtain the final TMF. An ANCOVA was used to test for differences in Hg biomagnification rates among all reservoirs using log THg as the dependent variable, $\delta^{15}\text{N}$ as the covariate and reservoir as an independent categorical variable. A significant interaction term indicated a significant difference among slopes. Separate ANCOVA were run between reservoirs to determine which slopes were significantly different.

Effect of HGM features and land use. We tested for an effect of HGM features (at the catchment and basin scale) and land use on Hg concentrations in water, zooplankton, and top

predators (Table 1). We conducted multiple linear regressions for each response variable (Hg_{water} and $\text{Hg}_{\text{zooplankton}}$ or $\text{Hg}_{\text{top predator}}$) using only predictors from a single category at a time (land use, HGM:catchment, or HGM:basin). We then conducted multiple linear regression models that combined the predictor variables that were significant ($p < 0.05$) from all the categories, including significant predictors from the trophic status category (i.e., indicators of eutrophication). Only the most significant models are reported. All statistical analyses were done using JMP 10.0 (SAS Institute).

Results

Trophic status

Chl *a* concentrations were not significantly correlated with TP or TN concentrations in water ($p > 0.05$). Trophic State Indices (Table 4) suggest that several of these reservoirs were likely not nutrient limited at the time of sampling. Phosphorus limitation, indicated by $\text{TSI}_{\text{Chl } a} - \text{TSI}_{\text{TP}} > 0$ and $\text{TSI}_{\text{TN}} > \text{TSI}_{\text{TP}}$ was found in 3/7 reservoirs (L, S, and T; Table 4). Partial evidence of nitrogen limitation, indicated by $\text{TSI}_{\text{Chl } a} - \text{TSI}_{\text{TN}} > 0$ and $\text{TSI}_{\text{TN}} < \text{TSI}_{\text{TP}}$, was found in 1/7 reservoirs (T, but $\text{TSI}_{\text{TN}} > \text{TSI}_{\text{TP}}$; Table 4). Limitation by nonalgal turbidity (i.e., light), indicated by $\text{TSI}_{\text{Chl } a} - \text{TSI}_{\text{SeD}} < 0$ was found in 5/7 reservoirs and the remaining two reservoirs had values close to 0 (Table 4; Matthews et al. 2002). Using $\text{TSI}_{\text{Chl } a}$ most reservoirs (4/7) classify as oligotrophic ($\text{TSI} < 45$, Table 4; Matthews et al. 2002). Alternatively, using TP concentrations alone (Environment Canada 2004) reservoirs classify as mesotrophic (4/7, criteria = 10-20 $\mu\text{g L}^{-1}$ TP) or eutrophic (3/7; criteria = 35-100 $\mu\text{g L}^{-1}$ TP; Table 2).

Effect of eutrophication

Mercury concentrations at different levels of the food web were significantly negatively correlated with Chl *a* (Fig. 2), but no significant correlations were found for the other two indicators of eutrophication (i.e., zooplankton density and

Table 4. Trophic state indices (TSI) for subtropical reservoirs sampled in eastern China. Phosphorus (P) limitation is indicated by $TSI_{Chl\ a} - TSI_{TP} > 0$ and $TSI_{TN} > TSI_{TP}$; nitrogen (N) limitation is indicated by $TSI_{Chl\ a} - TSI_{TN} > 0$ and $TSI_{TN} < TSI_{TP}$. Limitation by non-algal turbidity (i.e., light) is expected when $TSI_{Chl\ a} - TSI_{SeD} < 0$. The trophic state is assigned based on $TSI_{Chl\ a}$. See Fig. 1 for reservoir codes.

Reservoir code	$TSI_{Chl\ a}$	TSI_{TP}	TSI_{TN}	TSI_{SeD}	$TSI_{Chl\ a} - TSI_{TP}$	$TSI_{Chl\ a} - TSI_{TN}$	$TSI_{Chl\ a} - TSI_{SD}$	Trophic state	Possible limiting factor
F	36.5	65.2	55.7	36.8	-28.7	-19.2	-0.3	Oligotrophic	Nonalgal turbidity
H	40.4	45.0	67.4	40.0	-4.6	-27.0	0.4	Oligotrophic	—
L	49.9	34.1	56.2	50.0	15.8	-6.3	-0.1	Mesotrophic	P, nonalgal turbidity
M	36.0	68.5	57.2	41.9	-32.6	-21.2	-5.9	Oligotrophic	nonalgal turbidity
Q	34.3	45.8	47.6	35.4	-11.5	-13.2	-1.1	Oligotrophic	nonalgal turbidity
S	47.8	40.0	48.9	55.1	7.8	-1.1	-7.4	Mesotrophic	P, nonalgal turbidity
T	59.7	47.3	56.3	58.6	12.3	3.3	1.0	Eutrophic	P, N

TP). We found that increasing densities of phytoplankton, as indicated by higher Chl *a* values, predicted lower THg concentrations in zooplankton (Fig. 2, $R^2_{adj} = 0.54$, $p = 0.037$, $n = 7$) and top predators (Fig. 2, $R^2_{adj} = 0.50$, $p = 0.046$, $n = 7$). No significant relationship was found between THg concentrations in Bighead Carp and Chl *a*, or with MeHg and %MeHg concentrations in zooplankton or Bighead Carp and Chl *a* ($p > 0.05$). Overall, MeHg concentrations were very low in water (Table 3), but %MeHg in zooplankton and Bighead Carp were within the range expected (31-60% and 77-96%, respectively).

Mercury bioaccumulation values, as indicated by BAF values, were also only significantly correlated with one indicator of eutrophication (i.e., TP). Increasing concentrations of TP predicted significantly lower THg log BAF in zooplankton (Fig. 3A, $R^2_{adj} = 0.86$, $p = 0.002$, $n = 7$), Bighead Carp ($R^2_{adj} = 0.80$, $p = 0.004$, $n = 7$, not shown), and top predators ($R^2_{adj} = 0.78$, $p = 0.005$, $n = 7$, not shown). There was a clear separation of the reservoirs based on TP concentrations (Fig. 3A) that drove the strong linear relationship whether in zooplankton, Bighead Carp, or top predators. As TP separated the reservoirs into groups, we performed an ANOVA using TP as a categorical variable. We found the same results: TP as a categorical independent variable did not predict Hg concentrations but did predict BAF values in zooplankton, Bighead Carp, and top predators (ANOVA, $p < 0.05$). As the results were not different, we present TP as a continuous variable as originally intended. No significant regressions were found using BAF calculated with MeHg concentrations in zooplankton or fish ($p > 0.05$). High TP reservoirs also had high THg concentrations in water (Fig. 3B; $R^2_{adj} = 0.91$, $p = 0.0006$, $n = 7$) providing evidence as to what may underlie the BAF-TP relationship (see discussion later).

Mercury biomagnification, as indicated by the THg or MeHg BMF between Bighead Carp and zooplankton, was not significantly predicted by any of the indicators of eutrophication (e.g., Fig. 4, BMF vs. Chl *a*, $p > 0.05$). Furthermore, the rates of Hg biomagnification across the food web, measured

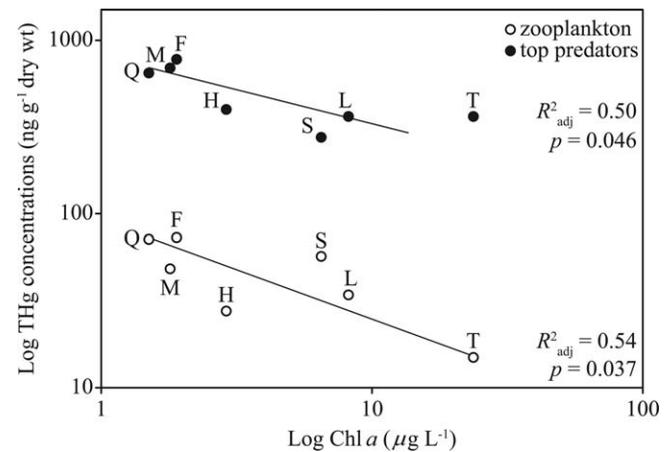


Fig. 2. Log total mercury (THg) concentrations in zooplankton and size-adjusted top predators vs. log chlorophyll *a* (Chl *a*). See Fig. 1 for reservoir codes.

using TMF, were also not significantly predicted by any of the indicators of eutrophication ($p > 0.05$). There were significant differences in Hg biomagnification rates across all reservoirs (ANCOVA, $F_{7,464} = 55.9$, $p = 0.0001$, with significant interaction term), but this appeared to be driven by one reservoir (S) whose Hg biomagnification rate was nonsignificant (Table 5).

Predictors of Hg concentrations including HGM features and land use

Significant effects of HGM features and land use on Hg concentrations were found (Table 6). For THg concentration in water, the best models included HGM:basin features, with water retention time (WRT) and elevation each explaining the same degree of variance (Table 6). No predictors were significant for MeHg concentrations in water. Zooplankton THg and MeHg concentrations were significantly negatively correlated with land use (i.e., % crop). The addition of Chl *a* increased the amount of variance explained compared to % crop or Chl *a* alone (Table 6). As with water THg concentrations, top predator THg concentrations were significantly

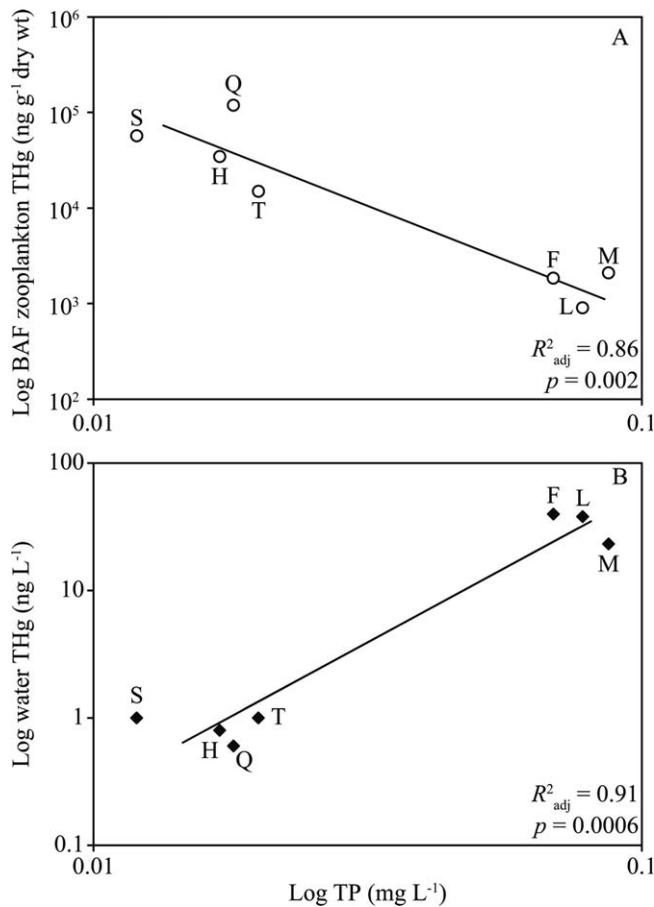


Fig. 3. (A) Log bioaccumulation factor (BAF) for total mercury (THg) in zooplankton vs. log total phosphorus (TP) and (B) Log THg concentrations in water vs. log TP. See Fig. 1 for reservoir codes.

positively correlated with elevation. Elevation explained the same degree of variance as Chl *a* alone; the combination of the two predictors yielded a slightly improved variance explained but the model was no longer significant (Table 6).

Discussion

The prevailing view about the effect of eutrophication on Hg dynamics is that it will lead to lower Hg concentrations in aquatic organisms (Driscoll et al. 2012). As most field studies that assess eutrophication and Hg bioaccumulation and biomagnification have been conducted in temperate systems, multireservoir studies in subtropical, high Hg deposition ecoregions such as eastern China are akin to a global “high exposure” treatment. We hypothesized that subtropical reservoirs may have greater MeHg availability if they are under high Hg deposition rates and/or undergo frequent water level fluctuations, leading to elevated fish Hg concentrations. Instead, relatively low Hg concentrations were observed in subtropical reservoir fishes of eastern China with the exception of a few higher trophic level fish species. Mer-

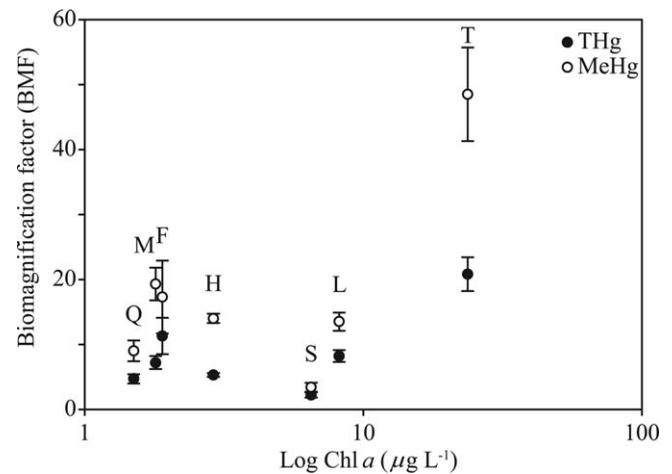


Fig. 4. Mean (\pm SE) biomagnification factor for total mercury (THg) and methylmercury (MeHg) vs. log chlorophyll-*a* (Chl *a*). See Fig. 1 for reservoir codes.

cury concentrations in biota decreased with increasing Chl *a* across all reservoirs, suggesting that eutrophication can lower rates of Hg bioaccumulation. However, Hg biomagnification between a predator and prey pair or across the food web was not predicted by eutrophication. Differences in HGM features at the basin scale, such as elevation and WRT, as well as land use explained abiotic and biotic THg concentrations in addition to trophic status. Our study indicates that both eutrophication and HGM features are important controls on Hg dynamics in subtropical reservoir food webs.

Effect of eutrophication on Hg dynamics

The negative relationship observed between THg concentrations in biota and Chl *a* (Fig. 2) shows that increasing phytoplankton densities can reduce Hg accumulation into zooplankton and top predators from subtropical reservoirs. Chl *a* concentrations measured here spanned from low (1.5–1.9 μg L⁻¹), medium (6.5–8.2 μg L⁻¹) to high (23.7 μg L⁻¹). THg concentrations in zooplankton varied nearly fivefold over that range (14.9–73.0 ng g⁻¹ dry wt), while size adjusted THg concentrations in top predators varied about threefold (275–776 ng g⁻¹ dry wt). Several studies have observed significantly lower Hg concentrations in lake and reservoir fishes with increasing Chl *a*. These decreases in THg concentrations with increasing Chl *a* are attributed to algal biodilution (Chen and Folt 2005) and/or growth dilution (Simoneau et al. 2005), mechanisms that in field experiments are difficult to tease apart because of their potential to interact (Foe and Louie unpubl.). Chl *a* measurements can serve as a practical tool to determine the potential of Hg to biomagnify in temperate lakes (Chen and Folt 2005), and based on our findings, appears valid for subtropical reservoir environments.

BAFs describing Hg transfer from water to biota were not, however, significantly predicted by Chl *a*. We expected a

Table 5. Linear regression of log total mercury (THg) (ng g^{-1} dry wt) vs. $\delta^{15}\text{N}$ (‰). Letters indicate regression slopes that are significantly different based on analysis of covariance (ANCOVA). Abbreviations: trophic magnification factor (TMF); confidence interval (CI). See text for details and Fig. 1 for reservoir codes.

Reservoir code	TMF	THg vs. $\delta^{15}\text{N}$	R^2	R^2_{adj}	p	n
	Mean (95% CI)	Mean Slope (95% CI)				
F	1.7 (1.3-2.2)	0.08 (0.04-0.12) ^a	0.19	0.18	0.0001	73
H	1.5 (1.0-2.2)	0.06 (0.00-0.12) ^{ab}	0.30	0.23	0.0651	12
L	2.0 (1.6-2.6)	0.11 (0.07-0.15) ^a	0.28	0.27	<0.0001	88
M	1.7 (1.2-2.2)	0.08 (0.03-0.12) ^{ab}	0.15	0.13	0.0022	62
Q	2.3 (1.8-2.8)	0.13 (0.09-0.16) ^a	0.32	0.31	<0.0001	93
S	1.1 (1.0-1.4)	0.02 (0.00-0.05) ^b	0.03	0.01	0.1467	83
T	1.6 (1.2-2.0)	0.07 (0.03-0.11) ^a	0.17	0.16	0.0008	61

Table 6. Significant multiple linear regression models explaining variation in total mercury (THg) and methylmercury (MeHg) concentrations in water, zooplankton, and top predators from reservoirs of eastern China using predictor variables from three categories: trophic status, hydrogeomorphic (HGM) features, and land use (see Table 1). Number of observations for all models is 7; slope coefficient estimates and standard error (SE) are provided. σ^2 is the residual sum of squares divided by the number of observations. Abbreviations: water retention time (WRT), chlorophyll a (Chl a), Akaike's Information Criterion (AIC_c).

Response variable	Predictor category	Predictor variable	Parameter estimates			σ^2	Model			
			Estimate	SE	p		AIC_c	R^2	R^2_{adj}	p
Log water THg	HGM:basin	WRT	-0.0038	0.00		0.16	20.99	0.75	0.70	0.012
		log elevation	2.0	0.53		0.16	20.97	0.75	0.70	0.012
Log zooplankton THg	Land use	% Crop	-0.017	0.00		0.01	2.66	0.78	0.74	0.0080
	Trophic status	log Chl a	-0.45	0.16		0.02	6.68	0.62	0.54	0.037
	Land use and Trophic status	% Crop and log Chl a	-0.012	0.00	0.040	0.01	12.42	0.88	0.82	0.014
Log zooplankton MeHg	Land use	% Crop	-0.022	0.0038		0.01	1.98	0.87	0.84	0.0022
Log top predator THg	HGM:basin	log elevation	0.37	0.13		0.01	1.58	0.61	0.53	0.038
	Trophic status	log Chl a	-0.30	0.11		0.01	2.11	0.58	0.50	0.046
	HGM:basin and Trophic status	log elevation and log Chl a	0.24	0.15	0.18	0.01	12.58	0.75	0.62	0.064

decrease in BAF with increasing eutrophication by several possible mechanisms. First, a direct effect of increasing Chl a can be to decrease MeHg assimilation efficiency of herbivores, as observed under lab conditions for *Daphnia magna* (Tsui and Wang 2004). Reduced MeHg assimilation efficiency was observed at a Chl a concentration equivalent to about $7 \mu\text{g L}^{-1}$ (Tsui and Wang 2004; Foe and Louie unpubl.). Second, increasing Chl a can also signal a change in phytoplankton cell size that occurs with eutrophication. Larger phytoplankton cells characteristic of eutrophic conditions have a lower uptake of Hg from water than smaller cells characteristic of oligotrophic conditions and fish Hg concentrations were highly predicted by these changes in algal cell size (Mason et al. 1996). Lastly, eutrophic conditions often result in a higher abundance of inedible algal species that could reduce Hg accumulation by zooplankton. A larger gra-

dient is needed to test for the possible effect of increasing algal density on BAF. Expanding the gradient of eutrophication beyond the range explored here to include additional eutrophic reservoirs is an important future need.

The BAF for zooplankton and fish were, however, negatively correlated with TP, and we propose this is driven by the strong correlation between water THg concentrations and TP (Fig. 3). This clear distinction in Hg bioaccumulation between low and high TP reservoirs may be a spurious correlation, given the nearly 1:1 relationship between water THg concentrations and TP (i.e., ratios regressed against the denominator of that ratio will result in a negative correlation). However, others (Jardine et al. 2013) have interpreted Hg bioaccumulation or biomagnification expressed as ratios vs. a mathematical argument in that ratio, and tests for spurious correlations have revealed that causal explanations are

valid (Lindström 2001). Alternatively, this finding suggests that there is a common factor for all reservoirs with elevated TP that leads to lower Hg bioaccumulation. We hypothesize that inorganic Hg and TP in water are correlated because both are sorbed onto suspended particulates, such as inorganic particles and organic matter. Inorganic Hg and MeHg have a high affinity for particles, and suspended particulate matter and TP can also be highly correlated (Lindström 2001). Thus, reduced Hg bioavailability could arise in high TP reservoirs due to Hg associated with particulates lost through sedimentation. The results from the analysis of HGM features on water Hg concentrations (*see below*) lend support to this hypothesis.

Indicators of eutrophication did not explain differences in predator-prey (i.e., BMF; Fig. 4) and food web Hg biomagnification rates (i.e., TMF). Abiotic variables are expected to influence BMF and TMF, yet few studies compare physical or chemical characteristics to these measures of biomagnification (Jardine et al. 2013). With respect to eutrophication, lower trophic transfer efficiency of Hg, coupled with the expectation of enhanced growth rates of organisms at higher trophic levels, is hypothesized to lower the overall Hg biomagnification rate (Lavoie et al. 2013). The most comprehensive global meta-analysis to date finds that lower Hg biomagnification rates are associated with higher productivity (as indicated by TP) but only when coupled with the effect of other variables (dissolved organic carbon, DOC, pH; Lavoie et al. 2013). The global comparison of food web Hg biomagnification rate with Chl *a* as an indicator of eutrophication was inconclusive (Lavoie et al. 2013). The similarity in the biomagnification rates (Table 5) measured in all but one of our subtropical reservoirs demonstrates the need for a larger gradient and number of observations. Alternatively, food web Hg biomagnification rates may be independent of water quality and more dependent on trophic structure.

How HGM and land use affect Hg availability

HGM features are important predictors of trophic status and food web interactions (Bremigan et al. 2008). We find HGM features mediate both the input of nutrients and Hg to subtropical reservoirs as evidenced by the strong covarying relationship between water THg concentrations and TP. The HGM feature, WRT, was a significant predictor of water THg concentrations (that also holds for TP given the nearly 1 : 1 relationship; Fig. 3B). WRT is a strong surrogate of nutrient and contaminant inputs to aquatic systems (Kalff 2002). Mercury accumulation in sediments was a positive function of the ratio between catchment area to surface area (CA : SA; Drevnick et al. 2012). As CA : SA and WRT are inversely correlated (Kalff 2002), this implies that at lower WRT, a higher sedimentation rate, presumably from the presence of higher amounts of particulates, could be expected. This lends support to our hypothesis that the reservoirs with high TP and high water THg concentrations may have higher particulate loads.

At the basin scale, elevation was an HGM feature found to be an important determinant of Hg concentrations in both water and top predators. This is likely due to the higher forest cover found in reservoirs at higher elevations. Forest canopies enhance dry deposition and as a result forest cover is a key factor predicting landscapes that are Hg sensitive (Evers et al. 2007). This study finds preliminary evidence to suggest a trend of increasing exposure to atmospheric Hg deposition with forests at higher elevation in eastern China.

Land use affects nutrient and Hg transport to aquatic systems (Bremigan et al. 2008; Betemariam et al. 2013) and was an important HGM predictor for THg and MeHg concentrations in zooplankton. The negative relationship of zooplankton Hg concentrations with increasing % crop is consistent with the effect of % crop on fish Hg concentrations from lakes in agriculture and open areas of Washington State (Mattieu et al. 2013). In subtropical reservoirs, increasing crop cover could lead to higher nutrient inputs and enhanced algal growth that may result in algal biodilution and/or somatic growth dilution. Although % crop cover was not correlated with TN, TP, or Chl *a* in these subtropical reservoirs, indirect evidence suggests a higher input of nitrogen as zooplankton $\delta^{15}\text{N}$ values showed a positive trend with % crop ($R^2_{\text{adj}} = 0.31$, $p = 0.11$, data not shown). Higher $\delta^{15}\text{N}$ values are observed in lower trophic levels and can reflect anthropogenic nitrogen inputs from agricultural sources. In the reservoirs with watersheds dominated by crop cover (i.e., H, T), we observed tea plantations and rice paddies, both crops known to contribute high nutrient loads (e.g., due to low nitrogen use efficiency) to reservoirs, especially relative to pine and bamboo plantations in forested watersheds. Another possibility is that Hg lost from watersheds with higher % crop cover is less bioavailable, as would be the case if associated with high concentrations of DOC. Further work is needed to determine if higher nutrients and/or DOC may explain the lower Hg concentrations in zooplankton from subtropical reservoirs with higher % crop in the watershed.

The importance of all these HGM features on Hg inputs is illustrated by one reservoir (i.e., F; Table 2). This reservoir was at the highest elevation, and had the highest CA : SA (92.0) and shortest WRT (100 d). Water THg concentrations were also highest (39.6 ng L^{-1}) and Chl *a* concentrations were low ($1.9 \text{ } \mu\text{g L}^{-1}$). The low phytoplankton density and/or low % crop cover possibly inhibited the process of algal biodilution, resulting in the highest Hg concentrations in zooplankton (73.0 ng g^{-1} dry wt and 43.8 ng g^{-1} dry wt THg and MeHg, respectively), and highest mean Hg concentrations in Bighead Carp (824 ng g^{-1} dry wt and 758 ng g^{-1} dry wt THg and MeHg, respectively), and size-adjusted top predators (776 ng g^{-1} dry wt THg; Table 3). As the food web did not support a significantly different food web Hg biomagnification rate compared to other reservoirs, this suggests that eutrophication and reservoir HGM features may be

more important in determining Hg concentrations in biota than biomagnification rates in these subtropical food webs.

Characterizing eutrophication

The finding that Chl *a* and TP were not significantly correlated highlights the importance of studying the effect of eutrophication on Hg dynamics at different latitudes. Although positive TP and Chl *a* relationships are expected in both temperate and subtropical systems, factors other than nutrient limitation can affect algal abundance in reservoirs, such as mean depth, light limitation, and WRT. In the Three Gorges Dam in China, a multiyear study found that nonalgal turbidity affected light availability and limited algal abundance, as opposed to TN or TP, especially at short WRT (Xu et al. 2010). Here, less than half of the sampled reservoirs had evidence of phosphorus limitation, while almost all had evidence to suggest limitation by nonalgal turbidity (i.e., light; Table 4). This may explain the lack of relationship between Chl *a* and TP in our reservoirs, although larger samples sizes are needed to confirm this trend and to comprehensively assess the role of nutrients and additional factors such as zooplankton grazing. Biotic factors can also regulate algal growth through top-down control, and especially for reservoirs in China with active fisheries, stocking density of planktivorous carp could have a large effect. In some reservoirs with lower stocking densities of Bighead and Silver Carp, zooplankton grazing may also be a factor regulating algal abundance. Unfortunately, information on stocking densities for each reservoir was not available. Overall, the lack of relationship between TP and Chl *a* seen in subtropical reservoirs appears to be driven by nonalgal turbidity and hydrology. This differs from the primarily nutrient-limited algal growth expected in most temperate systems. As these factors can affect Hg cycling directly or indirectly by affecting algal growth, this highlights the need for further study into the interaction between eutrophication and Hg dynamics in subtropical systems.

Comparisons with other systems

China has the highest Hg emissions in the world, and documented observations of lower fish Hg concentrations than reported for temperate fishes appear paradoxical. Low fish Hg concentrations in China are especially surprising when they occur in reservoirs, environments favorable to Hg methylation (Yan et al. 2010). Our study confirms that Chinese subtropical reservoirs show low Hg bioaccumulation in many fish species especially in stocked species under intense fishing pressure that have rapid growth rates and are caught at a young age (Yan et al. 2010; Razavi et al. 2014b). Here, we found only 3.6% (17/473) of fish had THg concentrations above the $0.3 \mu\text{g g}^{-1}$ wet wt limit to protect all exposed populations (US EPA 2001b) and 1.3% (6/473) of fish had THg concentrations above the $0.5 \mu\text{g g}^{-1}$ wet wt limit for nonpredatory fish (FAO/WHO 2007). Those fish had elevated THg concentrations from

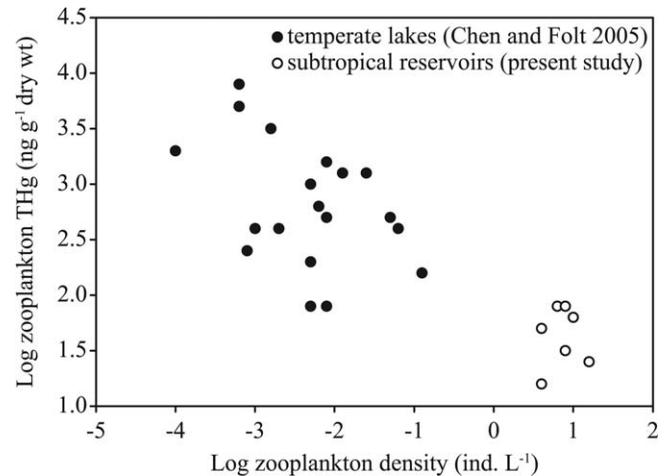


Fig. 5. Log zooplankton THg vs. zooplankton density (individuals L^{-1}). The same zooplankton size fraction ($> 202 \mu\text{m}$) was sampled in temperate lakes from Chen and Folt 2005 and subtropical reservoirs from this study.

their piscivorous diets and higher trophic position in the food web.

As with algal bloom dilution, zooplankton density dilution is a process that can also decrease Hg transfer through the food web. Decreasing THg concentrations in both zooplankton and fish were correlated with increasing zooplankton density in a temperate multilake study (Chen and Folt 2005). We did not find any correlations with THg or MeHg concentrations in biota and zooplankton density. This is likely due to the similarity in zooplankton density among reservoirs. However, put in a global context relative to temperate lakes in Chen and Folt (2005), our data supports that lower zooplankton Hg concentrations are associated with higher zooplankton densities (Fig. 5). This comparison would benefit from additional data, but while many studies report THg or MeHg concentrations in zooplankton, most do not report the density (ind. L^{-1}) measurements or same mesh sizes ($> 202 \mu\text{m}$) to have allowed for a broader comparison. Furthermore, this result could be due to inherent differences between the levels of turbidity (i.e., more Hg associated with particulate matter not bioavailable to zooplankton) in the natural lakes studied in Chen and Folt (2005) and the subtropical reservoirs studied here. Future studies should also assess zooplankton species composition to determine if the higher Hg concentrations in zooplankton at lower densities may not be due to a greater abundance of hyperaccumulator species such as *Daphnia*. On a global scale, greater zooplankton densities found in subtropical reservoirs may have a higher capacity for zooplankton density dilution.

MeHg availability to zooplankton and their diet items is critical to understanding food web Hg bioaccumulation in reservoirs, and appears to be directly linked to MeHg

availability in water (Stewart et al. 2008). Preliminary evidence suggests MeHg concentrations (and %MeHg) in surface waters of eastern subtropical reservoirs are very low (Table 3). These results are comparable to observations of highly elevated water THg concentrations (6.8 ng L⁻¹ to 83 ng L⁻¹) but low water MeHg concentrations (and %MeHg) in unfiltered water in nearby Lake Taihu (water % MeHg ranging from 0.12% to 3.5%, Wang et al. 2012). Low MeHg concentrations in Chinese surface waters may be due to algal biodilution or high MeHg demethylation rates (Wang et al. 2012). These are plausible mechanisms to explain low MeHg concentrations observed in the shallower reservoirs sampled here that do not stratify. However, low surface water MeHg concentrations found here may be an underestimate of MeHg exposure to biota in deeper reservoirs because hypolimnetic waters can have higher MeHg concentrations in reservoirs that stratify. Thus, detailed characterization of stratification and water MeHg concentrations at different spatial and temporal scales remains a research priority for reservoirs in eastern China.

Low water MeHg concentrations found for here were reflected in BAF values. Mean log BAF values for MeHg of 6.21 (range 5.53-6.59) for subtropical reservoirs were comparable (~ 0.5 log units higher) to mean log BAF values for MeHg in zooplankton from a temperate reservoir (Stewart et al. 2008) and experimentally flooded systems (Rolfhus et al. 2011). This was in contrast to the high trophic transfer efficiency of MeHg between a predator and prey pair (Bighead Carp–zooplankton). Calculating BMF between predator and prey pairs can be difficult in subtropical reservoirs due to omnivory in many fishes (Razavi et al. 2014b); Bighead Carp were the best candidate for determining a BMF given their consistent planktivorous diet (Zhou et al. 2009). The mean BMF based on MeHg concentrations was ~ 12.7 (range 3.4-19.3, excluding an outlier of 48.5; Fig. 4) and were higher than TMF in all reservoirs (Table 5). These BMF values are higher than temperate stream and lake fishes with BMF means of ~ 4.5 (range 2-9.5; Jardine et al. 2013) and ~ 6.5 (range 4-8.5; Chen et al. 2000), respectively. We expect BMF values would be even higher had we accounted for phytoplankton in Bighead Carp diet (smaller denominator for $Hg_{\text{predator}}: Hg_{\text{prey}}$) because of the lower trophic level of phytoplankton compared to zooplankton. These high BMF values may reflect the elevated feeding rates of Bighead Carp, but we presume that concomitant rapid growth rates keep Bighead Carp from accumulating high Hg concentrations through growth dilution.

To our knowledge, this study is the largest dataset of subtropical reservoirs to find evidence that eutrophication, as assessed by Chl *a* and TP, can lower Hg concentrations and Hg bioaccumulation. We find that HGM features are important determinants of nutrient and Hg inputs, and reservoirs most susceptible to Hg contamination are in high elevation, forested watersheds, with short WRT. We hypothesize these

reservoirs receive higher atmospheric Hg deposition and input of nutrients that are not bioavailable, preventing algal blooms that could reduce Hg transfer in the food web. Further research is needed in subtropical reservoirs to characterize factors affecting algal growth and MeHg distribution in water, to aid in the identification of reservoirs prone to lower Hg bioavailability for developing fisheries.

References

- Betemariam, H. H., C. M. McCutcheon, A. D. Davis, L. D. Stetler, T. M. DeSutter, M. R. Penn, and J. J. Stone. 2013. Geochemical behavior and watershed influences associated with sediment-bound mercury for South Dakota lakes and impoundments. *Water Air Soil Pollut.* **224**: 1-14. doi:10.1007/s11270-013-1497-1
- Bodaly, R. A., J. W. M. Rudd, R. J. P. Fudge, and C. A. Kelly. 1993. Mercury concentrations in fish related to size of remote Canadian Shield lakes. *Can. J. Fish. Aquat. Sci.* **50**: 980-987. doi:10.1139/f93-113
- Bremigan, M. T., and others. 2008. Hydrogeomorphic features mediate the effects of land use/cover on reservoir productivity and food webs. *Limnol. Oceanogr.* **53**: 1420-1433. doi:10.4319/lo.2008.53.4.1420
- Cai, Y., R. Jaffé, A. Alli, and R. D. Jones. 1996. Determination of organomercury compounds in aqueous samples by capillary gas chromatography-atomic fluorescence spectrometry following solid-phase extraction. *Anal. Chim. Acta* **334**: 251-259. doi:10.1016/S0003-2670(96)00309-1
- Cai, Y., G. Tang, R. Jaffé, and R. Jones. 1997. Evaluation of some isolation methods for organomercury determination in soil and fish samples by capillary gas chromatography—atomic fluorescence spectrometry. *Int. J. Environ. Anal. Chem.* **68**: 331-345. doi:10.1080/03067319708030499
- Carlson, R. E. 1977. A trophic state index for lakes. *Limnol. Oceanogr.* **22**: 361-369. doi:10.4319/lo.1977.22.2.0361
- Chen, C. Y., and C. L. Folt. 2005. High plankton densities reduce mercury biomagnification. *Environ. Sci. Technol.* **39**: 115-121. doi:10.1021/es0403007
- Chen, C. Y., R. S. Stemberger, B. Klaue, J. D. Blum, P. C. Pickhardt, and C. L. Folt. 2000. Accumulation of heavy metals in food web components across a gradient of lakes. *Limnol. Oceanogr.* **45**: 1525-1536. doi:10.4319/lo.2000.45.7.1525
- Chow-Fraser, P. 2006. Development of the Water Quality Index (WQI) to assess effects of basin-wide land-use alteration on coastal marshes of the Laurentian Great Lakes, p. 137-166. *In* T. P. Simon and P. M. Stewart [eds.], *Coastal wetlands of the Laurentian Great Lakes: Health, habitat, and indicators*. Indiana Biological Survey.
- Dittman, J. A., and C. T. Driscoll. 2009. Factors influencing changes in mercury concentrations in lake water and yellow perch (*Perca flavescens*) in Adirondack lakes. *Biogeochemistry* **93**: 179-196. doi:10.1007/s10533-009-9289-9

- Drevnick, P. E., H. Yang, C. H. Lamborg, and N. L. Rose. 2012. Net atmospheric mercury deposition to Svalbard: Estimates from lacustrine sediments. *Atmos. Environ.* **59**: 509-513. doi:10.1016/j.atmosenv.2012.05.048
- Driscoll, C. T., and others. 2012. Nutrient supply and mercury dynamics in marine ecosystems: A conceptual model. *Environ. Res.* **119**: 118-131. doi:10.1016/j.envres.2012.05.002
- Environment Canada. 2004. Canadian guidance framework for the management of phosphorus in freshwater systems. National Guidelines and Standards Office, Water Policy and Coordination Directorate, Environment Canada. Report No.1-8.
- Essington, T. E., and J. N. Houser. 2003. The effect of whole-lake nutrient enrichment on mercury concentration in age-1 yellow perch. *Trans. Am. Fish. Soc.* **132**: 57-68. doi:10.1577/1548-8659(2003)132<0057:TEOWLN>2.0.CO;2
- Evers, D. C., and others. 2007. Biological mercury hotspots in the northeastern United States and southeastern Canada. *Bioscience* **57**: 29-43. doi:10.1641/B570107
- Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO). 2007. Evaluation of certain food additives and contaminants. Sixty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series 940.
- Fu, X., X. Feng, J. Sommar, and S. Wang. 2012. A review of studies on atmospheric mercury in China. *Sci. Total Environ.* **421-422**: 73-81. doi:10.1016/j.scitotenv.2011.09.089
- Gong, P., and others. 2013. Finer resolution observation and monitoring of global land cover: First mapping results with Landsat TM and ETM+ data. *Int. J. Remote Sens.* **34**: 2607-2654. doi:10.1080/01431161.2012.748992
- Gorski, P. R., D. E. Armstrong, J. P. Hurley, and D. P. Krabbenhoft. 2008. Influence of natural dissolved organic carbon on the bioavailability of mercury to a freshwater alga. *Environ. Pollut.* **154**: 116-123. doi:10.1016/j.envpol.2007.12.004
- Gray, J. E., and M. E. Hines. 2009. Biogeochemical mercury methylation influenced by reservoir eutrophication, Salmon Falls Creek Reservoir, Idaho, USA. *Chem. Geol.* **258**: 157-167. doi:10.1016/j.chemgeo.2008.09.023
- Jardine, T. D., K. A. Kidd, and N. O'Driscoll. 2013. Food web analysis reveals effects of pH on mercury bioaccumulation at multiple trophic levels in streams. *Aquat. Toxicol.* **132-133**: 46-52. doi:10.1016/j.aquatox.2013.01.013
- Jepsen, D. B., and K. O. Winemiller. 2002. Structure of tropical river food webs revealed by stable isotope ratios. *Oikos* **96**: 46-55. doi:10.1034/j.1600-0706.2002.960105.x
- Kalff, J. 2002. *Limnology: Inland water ecosystems*. Prentice-Hall.
- Karimi, R., C. Y. Chen, P. C. Pickhardt, N. S. Fisher, and C. L. Folt. 2007. Stoichiometric controls of mercury dilution by growth. *Proc. Natl. Acad. Sci. USA* **104**: 7477-7482. doi:10.1073/pnas.0611261104
- Lavoie, R. A., T. D. Jardine, M. M. Chumchal, K. A. Kidd, and L. M. Campbell. 2013. Biomagnification of mercury in aquatic food webs: A worldwide meta-analysis. *Environ. Sci. Technol.* **47**: 13385-13394. doi:10.1021/es403103t
- Li, S., and S. Xu. 1995. Culture and capture of fish in Chinese reservoirs. International Development Research Centre and Southbound.
- Lin, C. C., N. Yee, and T. Barkay. 2012. Microbial transformations in the mercury cycle, p. 155-191. In G. Liu, Y. Cai, and N. O'Driscoll [eds.], *Environmental chemistry and toxicology of mercury*. Wiley.
- Lindström, M. 2001. Distribution of particulate and reactive mercury in surface waters of Swedish forest lakes—an empirically based predictive model. *Ecol. Model.* **136**: 81-93. doi:10.1016/S0304-3800(00)00382-3
- Mason, R. P., J. R. Reinfelder, and F. M. M. Morel. 1996. Uptake, toxicity, and trophic transfer of mercury in a coastal diatom. *Environ. Sci. Technol.* **30**: 1835-1845. doi:10.1021/es950373d
- Matthews, R., M. Hilles, and G. Pelletier. 2002. Determining trophic state in Lake Whatcom, Washington (USA), a soft water lake exhibiting seasonal nitrogen limitation. *Hydrobiologia* **468**: 107-121. doi:10.1023/A:1015288519122
- Mattieu, C. A., C. V. Furl, T. M. Roberts, and M. Friese. 2013. Spatial trends and factors affecting mercury bioaccumulation in freshwater fishes of Washington State, USA. *Arch. Environ. Contam. Toxicol.* **65**: 122-131. doi:10.1007/s00244-013-9882-8
- Meng, B., and others. 2010. Influence of eutrophication on the distribution of total mercury and methylmercury in hydroelectric reservoirs. *J. Environ. Qual.* **39**: 1624-1635. doi:10.2134/jeq2009.0440
- Ortiz, A. I. C., Y. M. Albarrán, and C. C. Rica. 2002. Evaluation of different sample pre-treatment and extraction procedures for mercury speciation in fish samples. *J. Anal. At. Spectrom.* **17**: 1595-1601. doi:10.1039/b207334j
- Pickhardt, P. C., and N. S. Fisher. 2007. Accumulation of inorganic and methylmercury by freshwater phytoplankton in two contrasting water bodies. *Environ. Sci. Technol.* **41**: 125-131. doi:10.1021/es060966w
- Pickhardt, P. C., C. L. Folt, C. Y. Chen, B. Klaue, and J. D. Blum. 2002. Algal blooms reduce the uptake of toxic methylmercury in freshwater food webs. *Proc. Natl. Acad. Sci. USA* **99**: 4419-4423. doi:10.1073/pnas.072531099
- Razavi, N. R., M. T. Arts, M. Qu, B. Jin, W. Ren, Y. Wang, and L. M. Campbell. 2014a. Effect of eutrophication on mercury, selenium, and essential fatty acids in Bighead Carp (*Hypophthalmichthys nobilis*) from reservoirs of eastern China. *Sci. Total Environ.* **499**: 36-46. doi:10.1016/j.scitotenv.2014.08.019
- Razavi, N. R., M. Qu, B. Jin, W. Ren, Y. Wang, and L. M. Campbell. 2014b. Mercury biomagnification in subtropical reservoir fishes of eastern China. *Ecotoxicology* **23**: 133-146. doi:10.1007/s10646-013-1158-6

- Rolfhus, K. R., B. D. Hall, B. A. Monson, M. J. Paterson, and J. D. Jeremiason. 2011. Assessment of mercury bioaccumulation within the pelagic food web of lakes in the western Great Lakes region. *Ecotoxicology* **20**: 1520-1529. doi:10.1007/s10646-011-0733-y
- Shu, H.-S. 1964. On the variation of water temperature in Meishan reservoir. *Oceanol. Limnol. Sin.* **6**: 135-151.
- Simoneau, M., M. Lucotte, S. Garceau, and D. Laliberté. 2005. Fish growth rates modulate mercury concentrations in walleye (*Sander vitreus*) from eastern Canadian lakes. *Environ. Res.* **98**: 73-82. doi:10.1016/j.envres.2004.08.002
- State Environmental Protection of China. 1990a. Water quality-determination of total nitrogen-alkaline potassium persulfate digestion: UV spectrophotometric method. GB 11894-89. Beijing, China.
- State Environmental Protection of China. 1990b. Water quality-determination of total phosphorus: Ammonium molybdate spectrophotometric method. GB 11893-89. Beijing, China.
- Stewart, A. R., M. K. Saiki, J. S. Kuwabara, C. N. Alpers, M. Marvin-DiPasquale, and D. P. Krabbenhoft. 2008. Influence of plankton mercury dynamics and trophic pathways on mercury concentrations of top predator fish of a mining-impacted reservoir. *Can. J. Fish. Aquat. Sci.* **65**: 2351-2366. doi:10.1139/F08-140
- Stone, J. J., C. M. McCutcheon, L. D. Stetler, and S. R. Chipps. 2011. Interrelationships between fish tissue mercury concentrations and water quality for South Dakota Natural lakes and impoundments. *Water Air Soil Pollut.* **222**: 337-349. doi:10.1007/s11270-011-0828-3
- Tsui, M. T. K., and W.-X. Wang. 2004. Uptake and elimination routes of inorganic mercury and methylmercury in *Daphnia magna*. *Environ. Sci. Technol.* **38**: 808-816. doi:10.1021/es034638x
- United States Environmental Protection Agency (US EPA). 1996. Method 1669 Sampling ambient water for trace metals at EPA Water Quality Criteria levels. U.S. Environmental Protection Agency Office of Water. Washington, D.C.
- United States Environmental Protection Agency. 2001a. Appendix to Method 1631: Total mercury in tissue, sludge, sediment, and soil by acid digestion and BrCl oxidation. U.S. Environmental Protection Agency Office of Water. EPA-821-R-01-013. Washington, D.C.
- United States Environmental Protection Agency. 2001b. Freshwater criterion for fish [accessed 2014 March 25]. Available from <http://www.epa.gov/fedrgstr/EPA-WATER/2001/January/Day-08/w217.htm>
- United States Environmental Protection Agency. 2002. Method 1631 Revision E: Mercury in water by oxidation, purge and trap, and cold vapor atomic fluorescence spectrometry. U.S. Environmental Protection Agency Office of Water. EPA-821-R-02-019. Washington, D.C.
- Wang, S., D. Xing, Y. Jia, B. Li, and K. Wang. 2012. The distribution of total mercury and methyl mercury in a shallow hypereutrophic lake (Lake Taihu) in two seasons. *Appl. Geochem.* **27**: 343-351. doi:10.1016/j.apgeochem.2011.09.029
- Xu, Y., Q. Cai, X. Han, M. Shao, and R. Liu. 2010. Factors regulating trophic status in a large subtropical reservoir, China. *Environ. Monit. Assess.* **169**: 237-248. doi:10.1007/s10661-009-1165-5
- Yan, H., and others. 2010. Total mercury in wild fish in Guizhou reservoirs, China. *J. Environ. Sci.* **22**: 1129-1136. doi:10.1016/S1001-0742(09)60228-X
- Zhang, Y., Z. Wu, M. Liu, J. He, K. Shi, M. Wang, and Z. Yu. 2014. Thermal structure and response to long-term climatic changes in Lake Qiandaohu, a deep subtropical reservoir in China. *Limnol. Oceanogr.* **59**: 1193-1202. doi:10.4319/lo.2014.59.4.1193
- Zhang, Y.-L., W. M. Chen, D.-T. Yang, W.-Y. Huang, and J. Jiang. 2004. Monitoring and analysis of thermodynamics in Tianmuhu Lake. *Adv. Water Sci.* **15**: 61-67.
- Zhou, B., and B. Zheng. 2008. Research on aquatic ecoregions for lakes and reservoirs in China. *Environ. Monit. Assess.* **147**: 339-350. doi:10.1007/s10661-007-0125-1
- Zhou, Q., P. Xie, J. Xu, Z.-X. Ke, L.-G. Guo, and T. Cao. 2009. Seasonal variations in stable isotope ratios of two biomanipulation fishes and seston in a large pen culture in hypereutrophic Meiliang Bay, Lake Taihu. *Ecol. Eng.* **35**: 1603-1609. doi:10.1016/j.ecoleng.2007.11.009

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