Science of the Total Environment 499 (2014) 36–46

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Effect of eutrophication on mercury, selenium, and essential fatty acids in Bighead Carp (*Hypophthalmichthys nobilis*) from reservoirs of eastern China

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HIGHLIGHTS

- Hg, Se and fatty acids were measured in Bighead Carp from subtropical reservoirs.
- Concentrations and risk–benefit values were compared to degree of eutrophication.
- Se:Hg molar ratios and algal species composition were a function of total phosphorous.
- Eicosapentaenoic acid (EPA) was negatively correlated with chlorophyll-a.
- Changes in Se and EPA with eutrophication may increase risk of exposure to MeHg.

ARTICLE INFO

Article history:
Received 28 April 2014
Received in revised form 1 August 2014
Accepted 8 August 2014
Available online xxxx

Editor: Daniel A. Wunderlin

Keywords:
Methylmercury
Selenium:mercury molar ratios
Asian carp
Fatty acids
Risk–benefit
Hazard quotient

ABSTRACT

Analyses of the risks and benefits of consuming fish assess the content of beneficial fatty acids found in fish relative to harmful pollutants such as methylmercury (MeHg). Quantifying the effect of eutrophication on mercury (Hg), selenium (Se) and essential fatty acids (EFAs) in fish is necessary to determine how measures of risk vary with productivity. Total Hg and MeHg, Se and fatty acids, including the EFA eicosapentaenoic acid (EPA, 20:5n−3) and docosahexaenoic acid (DHA, 22:6n−3), were analyzed in Bighead Carp (*Hypophthalmichthys nobilis*) dorsal muscle tissue from seven subtropical reservoirs of eastern China. Individual elements and fatty acids, as well as derived measures of risk (Se:Hg and hazard quotient, HQ_{EFA}) were regressed against indicators of eutrophication, including total phosphorous (TP), chlorophyll-a (chl-a) and phytoplankton species composition. We found low MeHg concentrations (range = 0.018–0.13 μg/g ww) and Se concentrations (range = 0.12–0.28 μg/g ww), and Se:Hg molar ratios that were well above 1.0, indicating a low risk of Hg toxicity. Bighead Carp had a high content of total polyunsaturated fatty acids ($\sum$ PUFAs = 44.2–53.6%), which included both EPA (6.9–12.5%) and DHA (16.1–23.2%). However, fish had significantly lower Se:Hg molar ratios in reservoirs with high TP, and lower EPA content with increasing plankton density (i.e. higher chl-a). Phytoplankton species composition predicted Se concentrations, but not Hg concentrations or EFA content. Overall, Hg concentrations in Bighead Carp were very low relative to consumption guidelines, and Se concentrations were adequate to confer protective benefits against MeHg toxicity. Our findings suggest that changes to plankton species composition and density with eutrophication may result in fish of lower nutritional value and thus increase risks to fish consumers by changing the availability of Se and EPA relative to MeHg.

1. Introduction

Balancing the benefits and the risks of fish consumption has become difficult (FAO/WHO, 2011). Fish contain omega-3 (n−3) long chain (≥20 carbons) polyunsaturated fatty acids (PUFAs) that humans cannot

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http://dx.doi.org/10.1016/j.scitotenv.2014.08.019
0048-9697/

synthesize in amounts necessary to maintain optimal health and development (Arts et al., 2001). Specifically, fish are important sources of eicosapentaenoic acid (EPA, 20:5n − 3) and docosahexaenoic acid (DHA, 22:5n − 3) referred to as essential fatty acids (EFAs) because of their role in neurological development. Current widespread pollution, especially from mercury (Hg), threatens the benefits imparted by fish to humans and wildlife (NWF, 2006; Mahaffey et al., 2011). Fish species vary in their EFA and Hg contents and consumers are advised to eat fish with higher EFA content relative to Hg to maximize their health benefits while simultaneously minimizing their exposure to the neurotoxicant methylmercury (MeHg; Mahaffey et al., 2011). The toxicity of MeHg may be reduced in the presence of selenium (Se), an essential trace element, with protective effects expected when molar ratios of Se to Hg (Se:Hg) exceed 1 (Ralston, 2008). To provide a balanced risk–benefit analysis of a given fish species, it is useful to quantify EFA and Se content relative to Hg concentrations. For example, in addition to comparing fish Hg concentrations to consumption guidelines, risk can be assessed using the Se:Hg molar ratio, or measures of MeHg relative to beneficial EFA (EPA + DHA) content (such as the hazard quotient, HQ_{EFA}, proposed by Gladyshev et al., 2009). Studies that determine what factors affect how measures of risk vary in aquatic organisms provide critical public health information (Neff et al., 2014).

Cultural eutrophication, the excessive plant growth observed due to nutrient enrichment from human activities, is one of the primary stressors facing aquatic ecosystems (Smith and Schindler, 2009). Eutrophication is likely to impact the outcome of risk–benefit analyses in fish, because primary producers play a key role in determining fatty acid, Hg, and Se availability in aquatic systems. Algae and, to a lesser extent fungi, are the only organisms capable of inserting the n − 3 and omega-6 (n − 6) double bond in fatty acids. Animals cannot synthesize alpha-linolenic acid (ALA; 18:3n − 3) or linoleic acid (LIN; 18:2n − 6) and, although they can convert these fatty acids to their long chain PUFA analogs (EPA, DHA and arachidonic acid, ARA, 20:4n − 6), they do so with variable efficiency.

Not all phytoplankton and zooplankton make the same amounts and kinds of fatty acids. For example, EPA is used as a biomarker of diatoms and cryptophytes due to their high content of this essential fatty acid (Ahlgren et al., 1992). Within the cyanobacteria, some groups have higher amounts of n − 3 fatty acids (e.g. *Oscillatoria* and *Microcystis*) compared to others (e.g. *Anabaena* and *Spirulina*; Ahlgren et al., 1992). Plankton biomass is also important to estimate EPA content (Hartwich et al., 2012), and eutrophic conditions showed reduced EPA and DHA content in seston and zooplankton in both field and experimental settings (Müller-Navarra et al., 2004; Ravet et al., 2012). Thus changes in algal species composition and density with eutrophication affect the availability of fatty acids to higher trophic level consumers. If eutrophication did not affect fish contaminant burden, we expect that the measures of risk posed by contaminants relative to beneficial fatty acids (e.g. HQ_{EFA}, Gladyshev et al., 2009) would increase.

However, eutrophication can also affect the availability of Hg and Se to upper trophic levels because phytoplankton biomass and species composition affect bioaccumulation of these elements from water. Algae can bioconcentrate both Hg (10^4 to 10^5; Pickhardt and Fisher, 2007) and Se (up to 10^6; Stewart et al., 2010). This process involves passive uptake for both inorganic Hg and MeHg (Mason et al., 1996), but is non-passive and also species specific for Se (Stewart et al., 2010). Higher plankton biomass present in eutrophic conditions can reduce Hg biomagnification, a process known as algal biodilution, resulting in lower Hg concentrations in fish (Chen and Folt, 2005). It has yet to be fully determined how Se and eutrophication interact (Young et al., 2010). To date, Se availability in freshwater appears to be a function of habitat type. Lentic habitats enhance the availability and transfer of Se to fish relative to lotic habitats (Orr et al., 2006). This is due to the longer water retention times and more reducing conditions found in lentic environments, which enhance the production of bioavailable (i.e. organic forms) Se (Orr et al., 2006; Stewart et al., 2010). Overall we predict fish Hg concentrations to be lower with increasing eutrophication. If Se concentrations stay constant, this should result in lower risk to fish consumers with increasing eutrophication, as indicated by higher Se:Hg molar ratios. However, given that algal species composition, and thus Se availability, change with eutrophication, we expect these differences to also be reflected in the Se:Hg molar ratios of higher trophic level consumers, such as planktivorous fish.

The main objective of this study was to assess how measures of risk from fish consumption vary with eutrophication. We selected Bighead Carp (*Hypophthalmichthys nobilis*) as a sentinel species because it remains planktivorous at all life stages and therefore directly reflects element and fatty acid accumulation from phytoplankton. Bighead Carp were sampled in subtropical reservoirs of eastern China, a region facing rapid eutrophication, and where Bighead Carp are widely distributed and frequently consumed. The concentrations of total Hg (THg), MeHg, Se and fatty acids were measured, and these concentrations, as well as measures of risk (Se:Hg and HQ_{EFA}) were compared to indicators of eutrophication, specifically total phosphorous (TP), chlorophyll-a (chl-a), and changes in phytoplankton species composition. We assessed overall risk versus benefit by considering, 1) fish Hg concentrations relative to Hg consumption guidelines and a calculation based on tolerable weekly intakes, 2) Se:Hg molar ratios (Se:THg and Se:MeHg), and 3) hazard quotients based on EFA and MeHg (HQ_{EFA}; Gladyshev et al., 2009).

2. Material and methods

2.1. Reservoir descriptions

We sampled Bighead Carp (n = 34) from 7 reservoirs of eastern China in June and August 2011 (Fig. 1, Table 1). Further details regarding these reservoirs are available in Razavi (2014). These reservoirs were oligotrophic to eutrophic (based on chl-a), with algal growth limited by differing factors among reservoirs such that no correlation was observed between chl-a and TP (Razavi, 2014). Hydrogeomorphic features (e.g. water retention time) were an important determinant of nutrient and Hg concentrations in these reservoirs (Razavi, 2014). This caused two clear groupings among reservoirs, those with shorter water retention times that are more lotic-like (high TP reservoirs) and those with longer water retention times that are more lentic-like (low TP reservoirs). Although our indicators of eutrophication, TP and chl-a, were not correlated, we used both of these indicators to represent eutrophication because the lack of correlation between TP and chl-a may be a common occurrence in Chinese subtropical reservoirs (Xu et al., 2010). Furthermore, often studies only use one of these indicators to represent eutrophication so we present both for comparison to the literature. Bioavailability of MeHg and THg from surface water are given in Razavi (2014); determining Hg concentrations in sediments was beyond the scope of this study.

2.2. Field sampling

Bighead Carp were selected for this study because they are planktivorous, a ubiquitous species in Chinese reservoirs, and are among the most frequently consumed fish in the region (Li and Xu, 1995; Zhou et al., 2009; Fang et al., 2012). Bighead Carp are stocked as fingerlings (~13 cm) in reservoirs and then consume natural plankton resources within the reservoir (Li and Xu, 1995). Fish were collected live from fishermen or local markets. We attempted to sample individuals of varying lengths at each reservoir, but in most cases only a narrow range of lengths were available (Table 2). In the field, total length and weight of individual fish were recorded (Table 2), a sample of dorsal muscle tissue taken for Hg and Se analyses, and a separate sample for fatty acid analyses. Tissue samples were immediately frozen in the field, and upon return to the lab those for fatty acid analyses were stored at −80 °C.

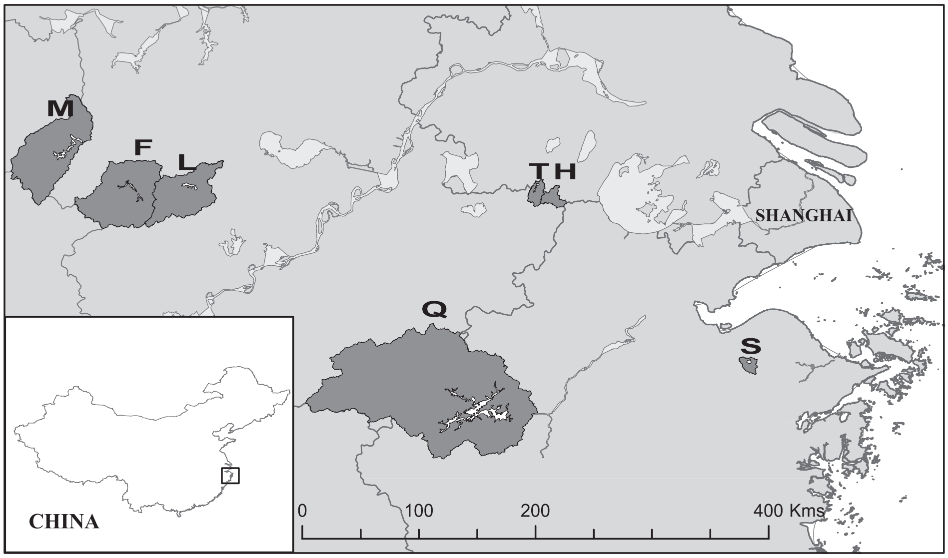

Fig. 1. Location of reservoirs sampled in eastern China. Dark gray indicates reservoir catchment areas. See Table 1 for reservoir codes.

Chlorophyll-a and TP were measured at each reservoir as in Razavi (2014). Briefly, separate surface water samples (1 m depth) taken using a 1-L van Dorn were dispensed into clean brown polyethylene bottles for chl-a analyses and acid-cleaned clear polyethylene bottles for TP analyses. Samples for chl-a were filtered through 1.2 μm GF/C filters in the field and the filters frozen in tin foil. Water samples for TP were frozen in the field until analysis within 1-week of collection. The chl-a analyses were carried out at McMaster University (Ontario, Canada) following Chow-Fraser (2006); sample analyses for TP followed the ammonium molybdenum spectrophotometric method with UV spectrophotometry following a potassium persulfate digestion (State Environmental Protection of China, 1990) at the Shanghai Environmental Monitoring Center (http://www.semc.gov.cn/home/english.aspx Shanghai, China).

2.3. Metal analyses

Total Hg and MeHg concentrations in fish samples were analyzed by the Laboratory for the Analysis of Natural and Synthetic Environmental Toxins (LANSET) at the University of Ottawa (Ontario, Canada). Fish dorsal muscle samples (~1 g) were oven-dried at 60 °C for 48 h and homogenized using a mortar and pestle. Percent water loss was later used to convert individual dry weight (dw) concentrations to wet weight (ww; mean (± standard deviation, SD) of 81.5 ± 4.1% water, n = 34). Total Hg concentrations were measured in samples (~1 mg) using a direct thermal decomposition Hg analyzer, with a method detection limit of 0.012 ng/g. Duplicates had a relative percent difference (the difference between two duplicates divided by the mean) of 4% (n = 24). Quality assurance included National Research Council of Canada certified reference materials (CRM, reported as mean ± SD) DORM-4 (fish protein; 96.5 ± 5.0% recovery, n = 7), DOLT-4 (dogfish liver; 97.3 ± 0.4% recovery, n = 3), and TORT-2 (lobster hepatopancreas; 93.5–99.4% recovery, n = 2). Fish MeHg was extracted into dichloromethane and subsequently quantified by capillary gas chromatography coupled with cold vapor atomic fluorescence spectrometry (GC CVAFS; Cai et al., 1997). Recovery of MeHg in CRM was 95.5 ± 2.7% for DORM-4 (n = 4), 92.6 ± 3.7% for DOLT-4 (n = 3) and 99.8% for TORT-2 (lobster hepatopancreas; n = 1). The method detection limit for MeHg was 0.016 ng/g.

2.4. Selenium analysis

Selenium concentrations were measured following CALA accredited methods at the Analytical Services Unit at Queen's University (Ontario, Canada). Briefly, 0.5 g of tissue was brought to a final volume of 12.5 mL using a hotplate aqua regia digestion method. After digestion and filtration, the samples were analyzed using hydride generation atomic absorption spectroscopy (HG-AAS) on a Varian VGA-77 (Varian

Table 1
Characteristics of reservoirs sampled in eastern China (see Fig. 1 for locations). Surface area (SA), catchment area to surface area ratio (CA:SA), reservoir volume, mean water level (i.e. elevation) and water retention time (WRT) were taken from published Chinese literature. Total phosphorous (TP), secchi depth (SD), and chlorophyll-a (chl-a) were measured in this study.

Reservoir	Reservoir code	Closest town/city	Population density in catchment (per km^2)[a]	SA (km^2)	CA:SA	Volume ($\times 10^9$m^3)	Elevation (m)	WRT (days)[b]	TP (mg/L)	SD (m)	Chl-a (μg/L)[d]	Trophic state[e]	Evidence of P limitation of algal growth[f]
Foziling	F	Foziling	15	20	92.0	0.364	124	100	0.069[c]	5.0	1.9	Oligotrophic	No
Hengshan	H	Yixing	158	4.5	34.4	0.112	32	460	0.017	4.0	2.9	Oligotrophic	No
Longhekou	L	Wanfohu	45	50	22.2	0.516	68	335	0.078	2.0	8.2	Mesotrophic	Yes
Meishan	M	Meishan	78	62.9	31.3	1.245	126	323	0.087	3.5	1.8	Oligotrophic	No
Qiandao	Q	Chun'an	149	580	18.1	17.84	37	700	0.018	5.5	1.5	Oligotrophic	No
Siming	S	Lianglong	324	5.5	18.7	0.0795	16	484	0.012	1.4	6.5	Mesotrophic	Yes
Tianmu	T	Tianmuhu	423	6.7	22.2	0.109	19	580	0.020	1.1	23.7	Eutrophic	Yes

[a] Total population in the watershed/catchment area.
[b] WRT = reservoir volume/mean outflow rate.
[c] Mean of 2 samples TP range = 0.067–0.71.
[d] All samples represent means of replicate samples.
[e] Determined using trophic status index based on chl-a (Razavi, 2014).
[f] Determined using deviations of trophic state indices (Razavi, 2014).

Table 2
Mean (±SE) and range of total length (cm), weight (kg), total mercury (THg), methylmercury (MeHg) and selenium (Se) concentrations (μg/g ww), and molar ratios of Se:THg and Se:MeHg in Bighead carp by reservoir. The Se:THg and Se:MeHg molar ratios presented here were calculated on each individual sample and then averaged by location. Letters indicate significant differences among reservoirs in Bighead carp total length using analysis of variance. See Table 1 for reservoir codes.

Reservoir code	n	Total length	Weight	THg	MeHg	Se	Se:THg	Se:MeHg
F	5	45.5 ± 1.2[a] 41.4–48.7	1.0 ± 0.1 0.8–1.3	0.14 ± 0.03 0.086–0.25	0.13 ± 0.03 0.086–0.23	0.14 ± 0.01 0.08–0.16	3.07 ± 0.61	3.41 ± 0.66
H	5	57.6 ± 2.3[a,b] 53.5–64.0	2.1 ± 0.2 1.4–2.8	0.028 ± 0.002 0.023–0.032	0.024 ± 0.001 0.020–0.027	0.26 ± 0.01 0.23–0.28	23.92 ± 2.19	30.28 ± 3.39
L	5	69.9 ± 5.9[b] 59.6–86.2	4.4 ± 1.2 2.4–8.4	0.059 ± 0.01 0.036–0.072	0.053 ± 0.01 0.031–0.069	0.12 ± 0.01 0.08–0.16	5.26 ± 0.40	6.45 ± 0.72
M	5	73.2 ± 2.7[b,c] 64.0–80.0	4.8 ± 0.5 3.0–5.9	0.073 ± 0.01 0.049–0.12	0.070 ± 0.01 0.045–0.12	0.14 ± 0.01 0.09–0.18	5.61 ± 1.16	6.43 ± 1.42
Q	5	88.2 ± 5.1[c] 73.5–102.0	9.2 ± 1.1 6.5–12.2	0.052 ± 0.01 0.035–0.063	0.043 ± 0.01 0.029–0.061	0.28 ± 0.01 0.26–0.30	14.27 ± 1.43	19.38 ± 2.53
S	4	44.8 ± 3.7[a] 39.0–55.0	1.1 ± 0.3 0.7–1.8	0.024 ± 0.004 0.018–0.035	0.018 ± 0.004 0.013–0.030	0.15 ± 0.01 0.13–0.18	17.76 ± 2.89	25.15 ± 4.31
T	5	50.2 ± 0.9[a] 48.0–53.2	1.4 ± 0.1 1.2–1.7	0.055 ± 0.01 0.032–0.079	0.050 ± 0.01 0.030–0.075	0.22 ± 0.02 0.16–0.27	11.40 ± 2.61	13.54 ± 3.00

Australia Pty. Ltd, Mulgrave, Victoria, Australia). Recovery of CRM DOLT-4 (88 ± 6% recovery, n = 4) and TORT-2 (82–98% recovery, n = 2), and the relative percent difference of duplicates (average = 24%, n = 6) met quality control criteria. The detection limit was <0.2 μg/g dw. We report Se concentrations as ww, using moisture content for individual fish as described above.

2.5. Lipid and fatty acid analyses

Fish samples stored at −80 °C were freeze-dried and weighed using a microbalance precise to 1 μg (dry weight of sample range = 23.615–47.896 mg; mean ± SD = 37.317 ± 8.068 mg). Lipids were extracted from tissues by first homogenizing the samples in 2 mL of 2:1 choloroform:methanol (Folch et al., 1957). The lipid extract was brought to a final volume of 8 mL with 2:1 chloroform:methanol. Next, 1.6 mL of NaCl (0.9%) was added, the phases were mixed, centrifuged (2300 rpm at 4 °C), and the upper layer removed. The solvent layer was evaporated under nitrogen gas, redissolved in 2 mL of 2:1 chloroform:methanol, and the % total lipid (on a dw tissue basis) was determined gravimetrically. Fatty acid methyl esters were quantified by adding sulfuric acid in methanol (1:100 mixture), flushing the headspace with nitrogen gas, and incubating 16 h in a water bath at 50 °C. Once the samples had cooled, potassium hydrogen carbonate, hexane: diethyl ether (1:1), and butylated hydroxyl toluene (0.01%) were added, and the vials then vortexed and centrifuged. The upper organic layer was transferred to another centrifuge tube; hexane:diethyl ether (1:1) was added to the original tube, which was shaken, vortexed and centrifuged. Fatty acid methyl esters were evaporated under nitrogen gas, dissolved in hexane, and transferred to amber glass GC vials. Analysis was carried out by gas chromatography equipped with a flame-ionization detector (GC-FID; Agilent HP6890, Agilent Technologies, Inc., Wilmington, Delaware, USA) as described previously (Kim et al., 2014; Neff et al., 2014). We reported any individual fatty acid that exceeded 3% (>1 μg/mg) of total lipids. We were especially interested in the EFA eicosapentaenoic acid (EPA; 20:5n−3), docosahexaenoic acid (DHA;22:6n−3), and arachidonic acid (ARA; 20:4n−6) and their precursors linoleic acid (LIN;18:2n−6c), γ-linolenic acid (GLA; 18:3n−6), and α-linoleic acid (ALA; 18:3n−3). The total saturated fatty acids (∑SAFAs), total monounsaturated fatty acids (∑MUFAs) and total polyunsaturated fatty acids (∑PUFAs), as well as total omega-3 (∑n−3) and omega-6 (∑n−6) and the ratio of total omega-3 to omega-6 (n−3:n−6) were also reported.

2.6. Statistical analysis

All statistical analyses were performed using JMP (Version 11.0; SAS Institute Inc., Cary, North Carolina, USA). Where necessary, variables were log-transformed to meet assumptions of normality for parametric tests. An analysis of variance (ANOVA) revealed significant differences in fish total length among sites (ANOVA, $F = 20.6$, $p < 0.0001$, $n = 34$). Due to non-overlap in fish total length among reservoirs, no direct among-site comparisons (i.e. ANOVA) of Hg or fatty acids were made. One individual fish had a fatty acid profile determined to be an outlier (all fatty acid concentrations an order of magnitude higher than all other individuals) and was removed from all analyses and summary statistics of fatty acids. However, this individual fish was not an outlier for Hg or Se and was included in data for those elements. The fatty acid concentrations of Bighead Carp dorsal muscle tissue are presented as μg fatty acid/mg dry tissue (abbreviated as μg/mg dw) and as relative proportions compared to the total fatty acid (expressed as %).

Selenium:mercury molar ratios (Se:THg and Se:MeHg) of individual fish were calculated (by dividing Se, THg and MeHg concentrations (μg/g) by 78.96, 200.59 and 215.63, respectively) and then averaged to get a mean Se:Hg value for each reservoir. Sample sizes were too small for within reservoir comparisons of Se:Hg molar ratios and fish length, and when all fish from all reservoirs were combined no significant effect of fish total length was found for either Se:THg or Se:MeHg ($p > 0.05$). This lack of correlation could be possibly due to differences in growth rates among reservoirs, but we do not have growth rates to test this assumption.

Risk from fish consumption was calculated taking into account the provisional tolerable weekly intake, an endpoint used for food contaminants with cumulative effects (WHO, 2004). Using this endpoint, the amount of fish that can be safely consumed was calculated as in Ouédraogo and Amyot (2013) as follows:

$$A = W \times I/C$$

where A = the amount (g) of a fish that can be safely eaten on a weekly basis, W = average body weight (65 or 70 kg for an adult woman or man, respectively), I = tolerable weekly intake of MeHg (1.6 μg/kg body weight, WHO, 2004) and C = MeHg concentration in fish (μg/g ww).

A measure of risk vs. benefit was calculated following Gladyshev et al. (2009). The hazard quotient (HQ_{EFA}) for fish consumption takes into account the recommended dose of EFA as follows:

$$HQ_{EFA} = \frac{R_{EFA} \times C_{MeHg}}{C_{EFA} \times RfD \times AW}$$

where R_{EFA} = a recommended dose of 500 mg/day of EFA (EPA + DHA; Kris-Etherton et al., 2009), C_{MeHg} = metal content in a given fish (μg/g ww), C_{EFA} = EPA + DHA content in a given fish (mg/g ww), RfD = reference dose for MeHg of 0.1 μg/kg/day (U.S. EPA, 2001a) and AW = average weight of an adult individual (kg), set to 70 kg. An $HQ_{EFA} < 1$ indicates no risk (Gladyshev et al., 2009). For this calculation only, we used EPA and DHA concentrations on a ww basis for consistency with the literature, converted using individual fish moisture content as described above for Hg and Se.

To test for differences with eutrophication, THg and MeHg, Se, fatty acid and Se:Hg molar ratios and HQ_{EFA} were plotted against log chl-a and log TP using linear regression analyses. Phytoplankton community composition was quantified from species presence/absence data (Table S1 and Fig. S1) using principal component analysis (PCA) on covariances. We then took the PC1 axis score and used it as a predictor variable to test for an effect of changes in species composition on Hg, Se, fatty acid content and Se:Hg molar ratios and HQ_{EFA}. The significance level for all tests was set at $p < 0.05$.

3. Results

3.1. Mercury, selenium and fatty acid content

Mean THg concentrations in Bighead Carp dorsal muscle tissue varied from 0.024 to 0.14 μg/g, and mean MeHg concentrations varied from 0.018 to 0.13 μg/g. This represents more than a 7-fold variation in MeHg concentrations among reservoirs (Table 2). Within reservoir variation (i.e. the range in Hg concentrations among individuals from a given reservoir) varied from ~1.5 to 3-fold for both THg and MeHg. Mean % MeHg ranged between 77 and 96% among reservoirs. All individuals sampled were well below the FAO/WHO (2007) limit of 0.5 μg/g THg and the U.S. EPA (2001b) consumption guideline of 0.3 μg/g THg.

Bighead Carp mean Se concentrations ranged from 0.12 to 0.28 μg/g, with among and within site variation ranging from ~3-fold to up to a 2-fold difference, respectively (Table 2). The mean molar ratios of Se:Hg among sites ranged from 3.07 to 23.92 for Se:THg and 3.41 to 30.28 for Se:MeHg (Table 2); only one individual fish had Se:Hg < 1.

Total fatty acid content ranged from 16.5 to 27.8 μg/mg dw at all reservoirs (Table 3); mean % lipid (includes all lipids, in addition to fatty acids) determined by gravimetric analysis ranged between 4 and 5% of dry weight. The mean percent of EPA and DHA ranged from 6.9 to 12.5% and 16.1 to 23.2%, respectively (see Table 3b for content in μg/mg dw). The mean percent of ARA ranged from 8.0 to 12.1% (Table 3), while the mean percent of ALA, GLA and LIN were each below 3% at all reservoirs (not reported). At all reservoirs, the mean ∑SAFA (~27–29%) exceeded the mean proportion of ∑MUFA (~13–22%). The mean ∑PUFA proportion ranged from ~44 to 54% (Table 3). The total n−3 and n−6 among all reservoirs ranged between 5.3 to 9.3 and 1.9 and 3.3 μg/mg dw, respectively. The n−3:n−6 varied from 2.1 to 3.1 (Table 3), suggesting FA profiles are ideal for human consumption (i.e. n−3:n−6 > 1, Neff et al., 2014).

3.2. Effect of eutrophication

Chl-a and TP showed no significant correlations with Hg (THg and MeHg) and Se concentrations. However, both molar ratios of Se:THg ($r^2 = 0.79$, $F = 19.18$, $p = 0.007$, $n = 7$, not shown) and Se:MeHg showed significant negative correlations with log TP (Fig. 2A; $r^2 = 0.79$, $F = 18.73$, $p = 0.008$, $n = 7$). The reservoirs clearly grouped into low and high TP reservoirs (Fig. 2A.); these same 2 groups were evident in the correlation between PC1 scores based on phytoplankton composition and log TP (Fig. 2B; $r^2 = 0.86$, $F = 30.61$, $p = 0.003$, $n = 7$). A positive trend, but non-significant, was observed between log HQ_{EFA} and log TP ($p = 0.097$). Log chl-a was a significant negative predictor of EPA (Fig. 3; $r^2 = 0.65$, $F = 9.31$, $p = 0.028$, $n = 7$). Log chl-a was not a significant predictor of Se:THg or Se:MeHg or HQ_{EFA} ($p > 0.05$). No other fatty acids presented in Table 3 were significantly correlated with either log chl-a or log TP. Phytoplankton species composition (as determined by PC1 scores) showed a positive trend with log

Table 3
Fatty acid composition (±SE) of Bighead Carp by reservoir, presented as a. concentration (μg/mg dw) and b. percent (%). See Table 1 for reservoir codes.

Fatty acid common name and molecular formula	Reservoir code						
	F (n = 5)	H (n = 4)	L (n = 5)	M (n = 5)	Q (n = 5)	S (n = 4)	T (n = 5)
a.							
Hexadecanoic acid (16:0)[a]	2.92 ± 0.24	2.99 ± 0.34	2.84 ± 0.22	3.64 ± 0.36	4.53 ± 1.11	2.50 ± 0.24	2.41 ± 0.24
Octadecanoic acid (18:0)	1.37 ± 0.06	1.60 ± 0.17	1.30 ± 0.06	1.60 ± 0.12	1.69 ± 0.25	1.59 ± 0.12	1.75 ± 0.14
∑SAFAs	**4.9 ± 0.3**	**5.4 ± 0.6**	**4.9 ± 0.4**	**6.1 ± 0.6**	**7.7 ± 1.8**	**4.7 ± 0.4**	**4.9 ± 0.4**
Palmitoleic acid (16:1)	0.44 ± 0.05	0.50 ± 0.12	0.88 ± 0.27	1.40 ± 0.32	1.79 ± 0.80	0.32 ± 0.08	0.20 ± 0.01
Elaidic and Oleic acid (18:1)[b]	1.68 ± 0.07	2.32 ± 0.28	2.33 ± 0.37	3.26 ± 0.29	4.20 ± 0.97	2.22 ± 0.40	1.75 ± 0.13
∑MUFAs	**2.4 ± 0.2**	**3.1 ± 0.4**	**3.5 ± 0.7**	**5.0 ± 0.6**	**6.4 ± 1.8**	**2.8 ± 0.5**	**2.2 ± 0.2**
Arachidonic acid (ARA; 20:4n−6)	2.13 ± 0.12	2.17 ± 0.24	1.62 ± 0.06	2.06 ± 0.16	2.08 ± 0.13	1.28 ± 0.08	1.97 ± 0.18
Eicosapentaenoic acid (EPA; 20:5n−3)	2.30 ± 0.09	1.55 ± 0.20	1.99 ± 0.17	2.48 ± 0.33	2.74 ± 0.57	1.32 ± 0.05	1.20 ± 0.08
Docosahexaenoic acid (DHA; 22:6n−3)	4.01 ± 0.32	3.49 ± 0.36	3.89 ± 0.26	4.92 ± 0.57	4.14 ± 0.45	3.23 ± 0.08	4.04 ± 0.35
∑PUFAs	**9.9 ± 0.4**	**8.9 ± 1.1**	**9.3 ± 0.8**	**12.4 ± 1.6**	**12.5 ± 2.2**	**7.2 ± 0.2**	**8.4 ± 0.7**
∑omega-3 (n−3)[c]	7.2 ± 0.3	6.0 ± 0.7	7.0 ± 0.7	9.3 ± 1.3	9.2 ± 1.8	5.3 ± 0.2	5.9 ± 0.5
∑omega-6 (n−6)[d]	2.7 ± 0.1	2.9 ± 0.3	2.2 ± 0.1	3.1 ± 0.3	3.3 ± 0.4	1.9 ± 0.0	2.5 ± 0.2
n−3:n−6	2.7 ± 0.2	2.1 ± 0.0	3.1 ± 0.2	3.0 ± 0.2	2.7 ± 0.2	2.8 ± 0.2	2.4 ± 0.1
Total lipid	**18.4 ± 0.6**	**18.8 ± 2.2**	**18.9 ± 1.8**	**24.8 ± 2.8**	**27.8 ± 5.9**	**16.5 ± 1.1**	**17.3 ± 1.2**
b.							
Hexadecanoic acid (16:0)[a]	15.8 ± 0.9	16.1 ± 0.2	15.1 ± 0.3	14.8 ± 0.3	15.9 ± 0.5	15.1 ± 0.5	13.8 ± 0.5
Octadecanoic acid (18:0)	7.5 ± 0.2	8.9 ± 0.4	7.0 ± 0.4	6.6 ± 0.4	6.4 ± 0.5	9.7 ± 0.4	10.1 ± 0.1
∑SAFAs	**26.6 ± 0.7**	**29.0 ± 0.5**	**26.1 ± 0.3**	**24.9 ± 0.4**	**27.1 ± 0.7**	**28.2 ± 0.7**	**27.9 ± 0.7**
Palmitoleic acid (16:1)	2.4 ± 0.3	2.5 ± 0.3	4.3 ± 0.9	5.3 ± 0.8	5.4 ± 1.3	1.8 ± 0.4	1.1 ± 0.0
Elaidic and Oleic acid (18:1)[b]	9.1 ± 0.4	11.8 ± 0.6	12.1 ± 0.7	13.3 ± 0.7	14.8 ± 1.1	13.2 ± 1.5	10.1 ± 0.3
∑MUFAs	**13.2 ± 0.9**	**15.4 ± 1.0**	**17.9 ± 1.6**	**20.1 ± 0.9**	**21.6 ± 2.1**	**16.5 ± 2.0**	**12.8 ± 0.3**
Arachidonic acid (ARA; 20:4n−6)	11.6 ± 0.6	12.1 ± 0.5	8.8 ± 0.8	8.5 ± 0.5	8.4 ± 1.2	8.0 ± 1.0	11.3 ± 0.4
Eicosapentaenoic acid (EPA; 20:5n−3)	12.5 ± 0.5	8.5 ± 0.3	10.6 ± 0.3	9.9 ± 0.4	9.9 ± 0.1	8.1 ± 0.3	6.9 ± 0.2
Docosahexaenoic acid (DHA; 22:6n−3)	21.7 ± 1.3	19.9 ± 1.2	20.8 ± 0.6	20.0 ± 1.2	16.1 ± 1.8	19.8 ± 1.0	23.2 ± 0.6
∑PUFAs	**53.6 ± 0.6**	**49.1 ± 1.9**	**49.3 ± 0.7**	**49.8 ± 0.8**	**46.4 ± 1.8**	**44.2 ± 1.6**	**48.6 ± 0.7**
∑omega-3 (n−3)	38.9 ± 0.7	33.1 ± 1.3	37.3 ± 0.4	37.2 ± 0.9	33.6 ± 1.0	32.6 ± 0.9	34.1 ± 0.6
∑omega-6 (n−6)	14.7 ± 0.6	16.0 ± 0.5	12.0 ± 0.7	12.6 ± 0.5	12.8 ± 1.0	11.6 ± 0.9	14.5 ± 0.4

[a] Sum of 16:0 and 16:0i.
[b] Sum of 18:1n−9t, 18:1n−9c and 18:1n−7.
[c] Sum of 18:3n−3, 20:3n−3, 20:5n−3, 22:5n−3c, 22:6n−3, 18:4n−3.
[d] Sum of 18:2n−6t, 18:2n−6c, 18:3n−6, 20:3n−6, 20:4n−6, 22:4n−6, 22:5n−6, 20:2n−6c, 22:2n−6c.

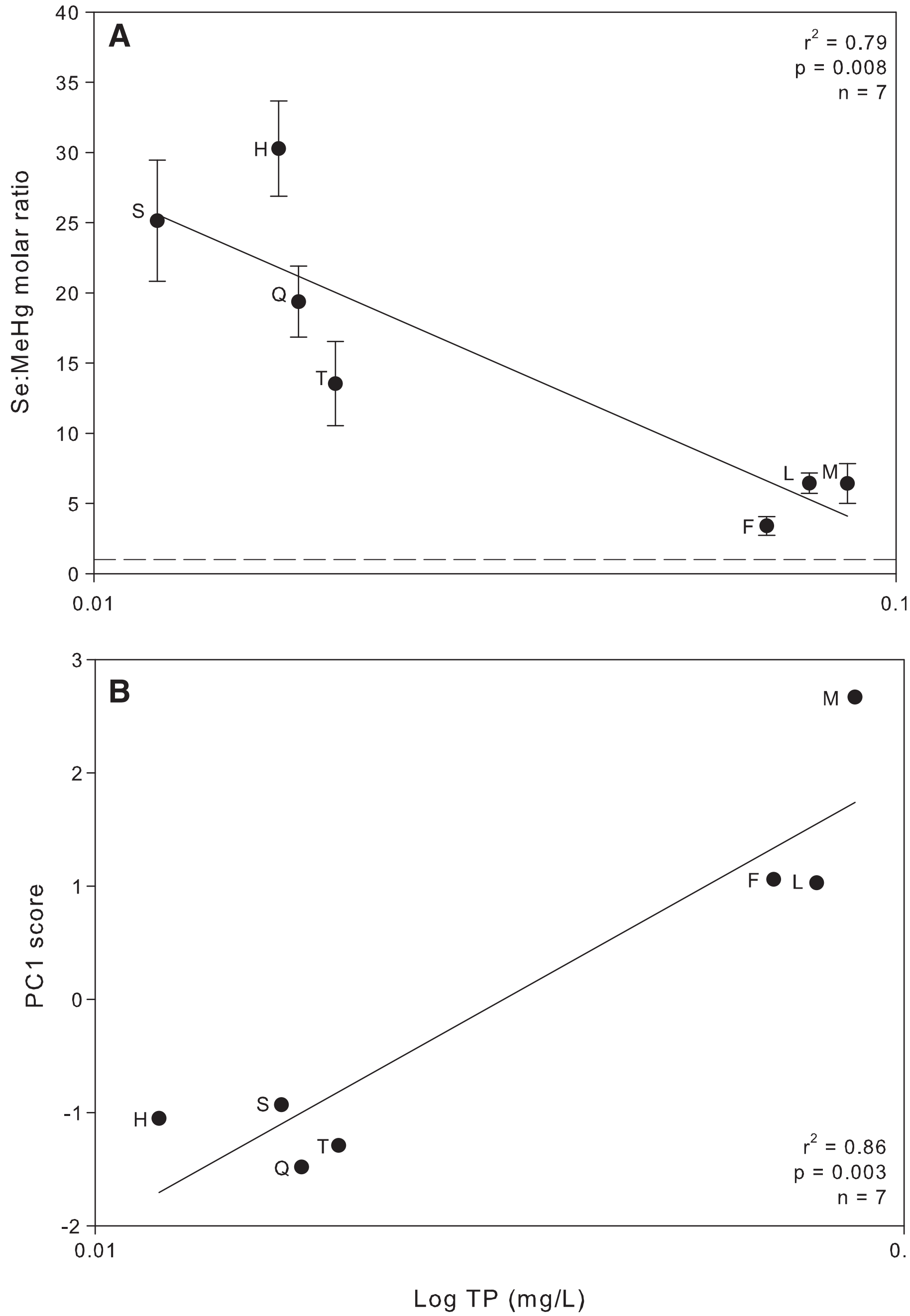

Fig. 2. A. Mean (±SE) of selenium to methylmercury (Se:MeHg) molar ratio in Bighead Carp dorsal muscle tissue vs. log total phosphorous (TP). Molar ratios above 1 are expected to be protective against MeHg toxicity B. Principal component analysis axis 1 scores (PC1) based on phytoplankton species composition (see text and Supplementary information for details) vs. log TP. See Table 1 for reservoir codes.

Se concentrations (Fig. 4; $r^2 = 0.57$, $F = 6.28$, $p = 0.054$, $n = 7$). This relationship was significant when PC1 scores were derived using functional groups (Table S1) as opposed to species ($r^2 = 0.61$, $F = 7.78$, $p = 0.038$, $n = 7$, not shown) which gives weight to the relationship observed in Fig. 4. PC1 scores (using phytoplankton species) were not significant predictors of Hg concentrations, Se:Hg molar ratios, HQ_{EFA} or any fatty acid presented in Table 3.

3.3. Risk–benefit analyses

Two of the three risk–benefit analyses of Bighead Carp fish consumption suggested that the overall risk was low. None of the Bighead Carp Hg concentrations exceeded consumption guidelines set by the U.S. EPA (2001b) or the FAO/WHO (2007). Considering the risk relative to the provisional tolerable weekly intake for MeHg, the mean allowable fish consumption amount for Bighead Carp was >2500 g/week for both an average adult woman and man (Table 4). When considering the effect of Se as well as Hg, all the reservoirs had Se:Hg ratios that exceeded the protective ratio of 1, while Se concentrations remained below toxicity thresholds. Only the third measure of risk, HQ_{EFA}, which takes into account the fish consumption required to achieve a daily-required EFA (EPA + DHA) dose of 500 mg/day, exceeded the risk threshold in all 7 reservoirs (Table 4).

4. Discussion

While many studies report Hg, Se and fatty acid content of fish, fewer studies report these concentrations simultaneously, and fewer

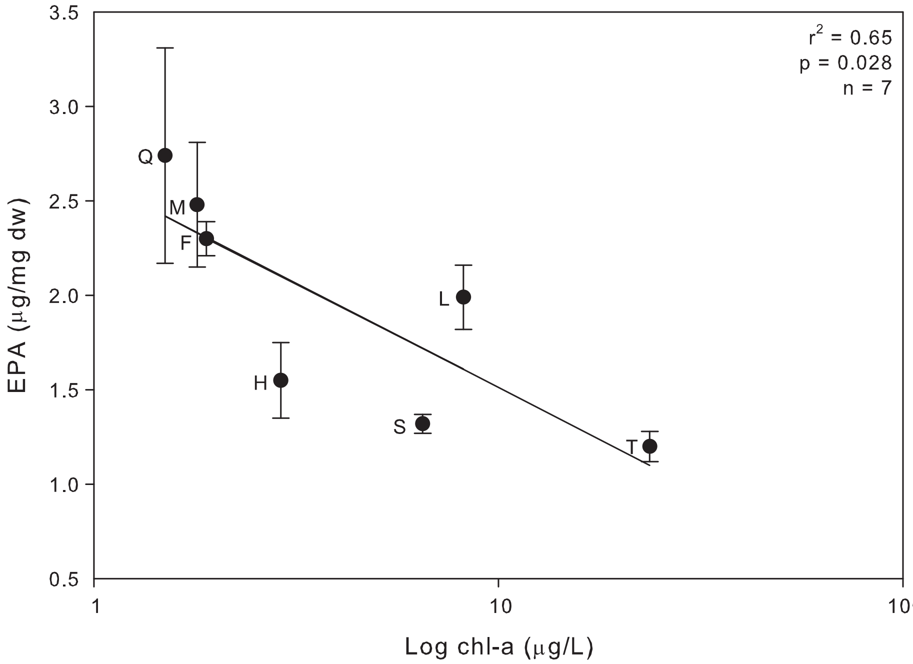

Fig. 3. Mean (±SE) concentration of eicosapentaenoic acid (EPA) in Bighead Carp dorsal muscle tissue vs. log chlorophyll-a (chl-a). See Table 1 for reservoir codes.

still report how eutrophication affects these concentrations and derived measures of risk. Our first two objectives were to characterize the Hg, Se and fatty acid content of a ubiquitous planktivore from subtropical reservoirs, and then to determine the effect of eutrophication (through changes in chl-a, TP and phytoplankton species composition) on those concentrations. Eastern China has among the highest Hg emission and deposition rates in the world (Fu et al., 2012), yet the muscle of Bighead Carp in our study contained low Hg concentrations. Selenium concentrations were adequate against MeHg toxicity (i.e. high Se:Hg molar ratios), but were not themselves at toxic concentrations. Bighead Carp contained moderately high EFA content (average EPA + DHA of all Bighead Carp in this study = 119 mg/100 g) compared to terrestrial meats (EPA + DHA range = 5–30 mg/100 g, Hibbeln et al., 2006), and similar EFA content to other Cypriniformes (EPA + DHA range = 70–214 mg/100 g, Gladyshev et al., 2012). Overall, these findings demonstrate that Bighead Carp from eastern Chinese reservoirs are a source

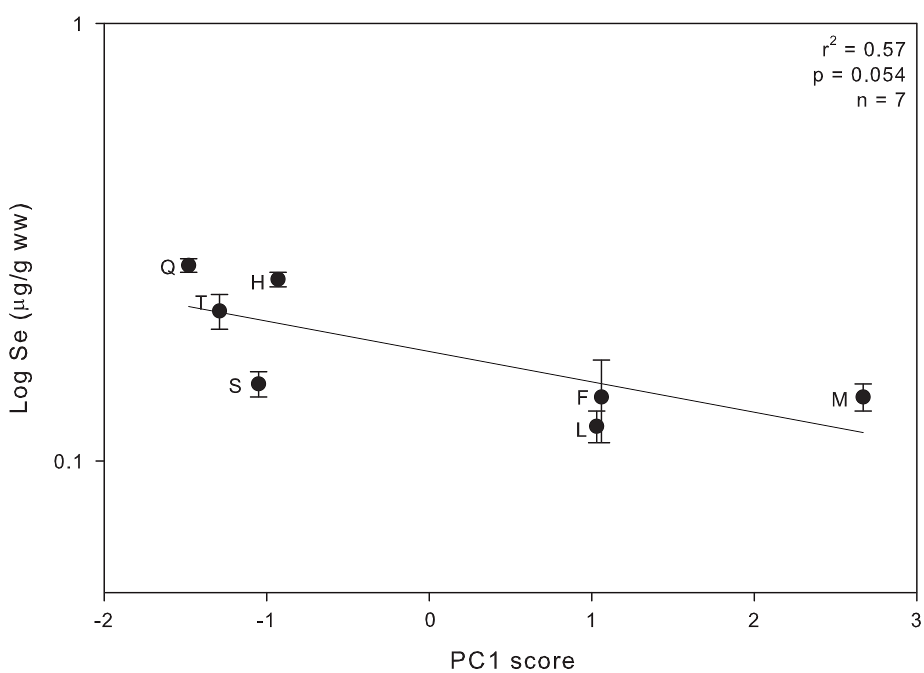

Fig. 4. Mean (±SE) concentration of selenium (Se) in Bighead Carp dorsal muscle tissue vs. the principal component analysis axis 1 scores (PC1) based on phytoplankton species composition (see text and Supplementary information for details). See Table 1 for reservoir codes.

Table 4
Estimates for 1) allowable Bighead Carp consumption based on methylmercury (MeHg) concentrations in dorsal muscle tissue. Calculations use average weights for an adult woman (65 kg) and man (70 kg), see text for details on calculations; 2) a risk–benefit ratio expressed as hazard quotient (HQ_{EFA}), calculated using essential fatty acids (EFAs), i.e. the sum of eicosapentaenoic acid (EPA; 20:5n − 3) and docosahexaenoic acid (DHA; 22:6n − 3) relative to MeHg concentrations. Hazard quotients were first calculated for individual fish. See Table 1 for reservoir codes.

Reservoir code	n	MeHg concentrations (μg/g ww) ± SE	Allowable consumption				Hazard quotient	
			Amount per week (g)		Ratio of consumption[a]		EPA + DHA (mg/g ww) ± SE	HQ_{EFA} Mean ± SE
			Woman	Man	Village	City		
F	5	0.13 ± 0.03	800	862	4.7–5.1	1.8–2.0	1.19 ± 0.13	9.2 ± 3.5
H	5[b]	0.024 ± 0.001	4374	4710	25.7–27.7	9.9–10.7	1.03 ± 0.13	1.7 ± 0.2
L	5	0.053 ± 0.01	1972	2124	11.6–12.5	4.5–4.8	1.12 ± 0.09	3.4 ± 0.4
M	5	0.070 ± 0.01	1484	1598	8.7–9.4	3.4–3.6	1.57 ± 0.30	3.3 ± 0.4
Q	5	0.043 ± 0.01	2422	2608	14.2–15.3	5.5–5.9	1.31 ± 0.20	2.5 ± 0.5
S	4	0.018 ± 0.004	5649	6084	33.2–35.8	12.8–13.8	0.90 ± 0.03	1.5 ± 0.3
T	5	0.050 ± 0.01	2091	2252	12.3–13.2	4.8–5.1	0.93 ± 0.08	4.0 ± 0.7

[a] Amount allowable/amount consumed. Fish consumption estimates (170 g/week for a village; 440 g/week for a city) are based on the most recent national survey data in Zhai and Yang (2006).
[b] Sample size is n = 4 for the EPA + DHA concentrations and the hazard quotient calculations as described in the text.

of EFAs that presents a low risk of MeHg exposure. These results will be discussed with respect to Bighead Carp diet and physiology, and fishing pressure in Chinese reservoirs.

4.1. Mercury concentrations

Bighead Carp are known to feed on phytoplankton and zooplankton and thus occupy a low trophic level (Zhou et al., 2009). The low Hg concentrations observed here are consistent with low Hg accumulation in low trophic level fish compared to top predators (Cabana et al., 1994). Furthermore, Bighead Carp grow quickly and as such may experience growth dilution that can further reduce Hg burden in fish (Simoneau et al., 2005). Fast growth rates are thought to account for low Hg concentrations in tropical lake fishes, despite elevated Hg in water from industrial pollution (Poste et al., 2012). Total Hg concentrations reported here were within the range found for Bighead Carp in many studies of subtropical reservoirs from southwestern China (e.g. THg range = 0.007–0.315 μg/g, mean = 0.069 μg/g; Yan et al., 2010) but were higher than a hypereutrophic subtropical lake in eastern China (THg range = 0.003–0.023 μg/g, mean = 0.011 μg/g; Wang et al., 2012). The capture of Bighead Carp at a young age by intense fisheries (Li and Xu, 1995) may explain these low Hg concentrations, as age is also a well-known predictor of Hg accumulation in fishes (Lange et al., 1994).

4.2. Effect of eutrophication on Hg concentrations

We expected to find a negative relationship between Hg concentrations in Bighead Carp and chl-a, as found for other fishes (Simonin et al., 2008). Lower fish Hg concentrations with chl-a are often attributed to algal biodilution, whereby a constant amount of MeHg is distributed in a larger biomass of algae, resulting in reduced Hg biomagnification (Chen and Folt, 2005). Although the effects of eutrophication on MeHg bioavailability are complex, and can also potentially increase bioavailability (Razavi, 2014), evidence for algal biodilution was previously documented within the planktonic food web of a subtropical Chinese reservoir (Wang et al., 2011), and THg concentrations in both zooplankton and top predators in the same reservoirs as the present study were negatively predicted by chl-a (Razavi, 2014). The lack of relationship between Bighead Carp THg (or MeHg concentrations) and chl-a (or zooplankton density, Razavi, 2014) may be due to differences in growth rates among reservoirs, as accumulated Hg is 'diluted' in fish that grow faster (Simoneau et al., 2005). Further research is needed to understand how factors such as growth rate and age determine Hg accumulation in Bighead Carp from reservoirs in Eastern China.

4.3. Selenium concentrations

As with Hg, diet is also the primary exposure route for Se in fish. Se concentrations reflect algal species composition because of interspecific variation in Se content in primary producers (Stewart et al., 2010). We found that phytoplankton species composition differed between high and low TP reservoirs, and that Bighead Carp Se concentrations reflected this change in species composition. Organisms such as Bighead Carp that remain planktivorous at all life stages are ideal sentinels of Se availability (Ponton and Hare, 2013). The Se concentrations found here suggest no general risk to wildlife or human consumers due to Se toxicity in these reservoirs. We are unaware of any previous work on Se concentrations in reservoir fish from China. Our results are congruent with Se concentrations (0.30 μg/g) reported for the planktivorous Silver Carp (*Hypophthalmichthys molitrix*) collected in markets from major Chinese cities (Du et al., 2012). A study of a eutrophic lake in the Yangtze River watershed impacted by a chlor-alkali plant found high Se concentrations (Se range = 0.4–1.5; Jin et al., 2006) in Silver Carp. Higher Hg concentrations were also found in fish from that impacted lake (Jin et al., 2006). This suggests the reservoirs studied here are not impacted directly by industrial pollution.

4.4. Effect of eutrophication on Se concentrations

In addition to diet, Se availability is affected by hydrology (Stewart et al., 2010). Our previous work on the reservoirs studied here showed that TP concentrations were higher in reservoirs with short water retention times (i.e. more lotic; Razavi, 2014). We show here that phytoplankton species composition reflects differences in hydrology among reservoirs (as inferred from Fig. 2B), with high TP reservoirs (i.e. more lotic) exhibiting a different phytoplankton species composition compared to low TP reservoirs (i.e. more lentic). We also find that phytoplankton species composition is a predictor of Se concentrations in Bighead Carp. These lines of evidence suggest that Se availability, as inferred from fish Se tissue concentrations (Orr et al., 2006), is a function of hydrology and phytoplankton species composition in subtropical reservoirs. These results are consistent with findings from a temperate watershed, where fish from lentic habitats had higher Se concentrations than fish from lotic habitats (Orr et al., 2006). The temperate study found that lentic habitats showed a greater formation of organoselenium and Se bioaccumulation in fish relative to lotic habitats (Orr et al., 2006). Under controlled conditions, phosphorous was found to limit Se accumulation by a freshwater alga and subsequent Se transfer to zooplankton (Yu and Wang, 2004). Thus, interactions among nutrients, algal species composition and Se availability with habitat may explain our observation of significant decreases in Se:Hg molar ratios in high TP (i.e. short water retention time) reservoirs.

4.5. Fatty acid concentrations

Fish diet also determines, in part, EFA content in fish because algae are the primary organisms capable of inserting the n − 3 and n − 6 double bond in fatty acids. The overall lipid content of Bighead Carp suggests these are low fat fish (lipids comprised 4–5% dry weight). Lipid content of Bighead Carp did not vary widely, in contrast to the tropical planktivore Tilapia (*Oreochromis niloticus*) whose % lipid (dry weight) varied by up to 12-fold (Zenebe et al., 1998). This large variation, which was greater than omnivorous species in that study, was hypothesized to be due to differences in phytoplankton species consumed (Zenebe et al., 1998). Thus, our results suggest that there is little variation in total lipid of plankton among reservoirs.

In contrast, the fatty acid composition of plankton may differ among reservoirs. We found that n − 3:n − 6 ranged between 2.1 and 3.1 (mean (±SE) = 2.7 ± 0.1, n = 33), in good agreement with the mean n − 3:n − 6 of 2.8 ± 1.1 for temperate lake fishes (Ahlgren et al., 2009). Significant differences were observed in n − 3:n − 6 among feeding guilds in temperate lake fishes, with herbivorous–omnivorous fish containing the lowest n − 3:n − 6 (2.0) compared to carnivorous–piscivorous (2.5) and carnivorous–benthivorous fish (3.7); Ahlgren et al. (2009). The n − 3:n − 6 we found fell within the range of all the feeding groups described by Ahlgren et al. (2009), despite Bighead Carp's consistent diet of zooplankton and phytoplankton. High n − 3 fatty acids (calculated in the same way as our study) in Bighead Carp dorsal muscle tissue were also found in the hypereutrophic Lake Taihu (Zhang et al., 2012a), which the authors proposed could be due to varying proportions of *Microcystis* availability in Bighead Carp diet. This algal group showed a highly variable n − 3:n − 6 ranging between 2 and 5 (Hayakawa et al., 2002). Thus our fatty acid results for Bighead Carp suggest these fish are eating different species or different proportions of phytoplankton species among reservoirs.

4.6. Effect of eutrophication on fatty acids

Changes in phytoplankton species composition with eutrophication can affect fatty acid availability due to interspecific differences in phytoplankton fatty acid content. For example, diatoms, cryptophytes and ciliates contain high amounts of EPA relative to other algal groups (Hartwich et al., 2012). We observed that EPA content of Bighead Carp decreased with increasing chl-a (i.e. phytoplankton density). Although this requires further investigation with larger sample sizes, this result is consistent with a decrease of EPA in seston with increasing lake productivity (as indicated by TP in Müller-Navarra et al., 2004). We were unable to show that phytoplankton species composition (based on presence/absence data) was correlated with EPA or DHA content in Bighead Carp. Plankton biomass and species composition were successfully used to reconstruct past seston EPA content in Lake Constance (Hartwich et al., 2012). Future studies that account for abundances or biomass of certain plankton groups, especially of ciliates and diatoms, may provide further insight into the differences responsible for the varying EPA content of Bighead Carp found here with phytoplankton density.

4.7. Implications for human health

Our third objective was to measure the overall risk of consumption of Bighead Carp. Considering Hg concentrations alone relative to consumption guidelines (U.S. EPA, 2001b; FAO/WHO, 2007), we found no evidence for risk to human health. The MeHg concentrations in Bighead Carp were low, such that large amounts of Bighead Carp dorsal muscle (g/week; Table 4) could be safely consumed by adults without exceeding the MeHg tolerable weekly intake of 1.6 μg/kg body mass (WHO, 2004). This is in agreement with a risk–benefit analysis of freshwater and marine fish species from coastal city markets in China that found low risk due to Hg contamination, and large allowable daily consumption amounts for Silver Carp, a similar species to the Bighead Carp (Du et al., 2012). The average daily consumption from the most recent national survey (Zhai and Yang, 2006) is estimated to be 23.7 g and 62.3 g in villages and large cities, respectively. This is equivalent to about 170–440 g of aquatic products per week. A study conducted in the main village of one of our reservoirs (Qiandao), found most respondents (~46%) estimated they ate between 50 and 100 g of fish per week, while ~17% of respondents estimated they ate >250 g per week (Fang et al., 2012). These consumption values are 2-fold to more than 10-fold lower than the amount of Bighead Carp we calculated could be safely consumed (Table 4). The observation that hair THg concentrations in women (17–46 years) were below thresholds in the Qiandao study (Fang et al., 2012), suggests that Hg may not be a contaminant of concern for women with similar fish consumption patterns in eastern China. Further studies of human populations living near reservoirs are needed to confirm this finding.

Considering Hg concentrations without accounting for Se concentrations can produce overly cautious risk assessments (Ouédraogo and Amyot, 2013). The low Bighead Carp Hg concentrations found in the present study were further mitigated by the presence of Se. The Se:Hg molar ratios indicated no concern for any individual fish consumed (except one fish from Foziling). All mean ratios, irrespective of reservoir, remained above 1, accepted as the protective threshold (Ralston, 2008). We did observe a high variation in Se:Hg, typical for freshwater fishes (Burger et al., 2012). Larger intraspecific than interspecific differences make it difficult to use Se:Hg values for risk assessment purposes, such that the overall usefulness of Se:Hg ratios is still debated and requires further research (Burger et al., 2012). Attempts, for example, to find a pattern between fish size and Se:Hg molar ratios may work for marine fish (larger fish generally have lower Se:Hg molar ratios), but this has not been clearly shown for freshwater species (Burger et al., 2012). At lower latitudes, low trophic level fish like Bighead Carp can often be very large, such that Se:Hg remains high despite their large size. Overall, it appears that there is sufficient, but not excessive, Se relative to Hg to protect against the toxicity of MeHg in fish in the reservoirs studied here. Evidence of sufficient Se to Hg concentrations was also found in a human hair study of women from one of our study reservoirs (Fang et al., 2012).

Unlike the other two risk analyses, the third calculation that considered MeHg relative to EFAs (EPA + DPA) concluded that among all reservoirs, the amount of Bighead consumption needed to achieve the adequate EFA dose (500 mg/day) would exceed the risk threshold (i.e. $HQ_{EFA} > 1$). The reason for these high hazard quotients is because Bighead Carp have only moderate EFA concentrations. Freshwater fish in general are found to have lower EFA content relative to marine species (Gladyshev et al., 2012). This result illustrates that even a low level of Hg contamination, as found here for Bighead Carp, can compromise this resource if it is the sole source of beneficial EFAs. Our findings suggest that eutrophication may further decrease the EFA content (by reducing EPA concentrations), thereby requiring higher consumption to achieve a given EFA requirement. This would mean a greater exposure to MeHg in a eutrophic reservoir to achieve the same dietary EFA requirement as an oligotrophic reservoir. However, it is important to note that consumers most likely derive EFA from other dietary sources in addition to Bighead Carp. Thus a full assessment of the MeHg exposure from all dietary EFA sources is needed to achieve a complete understanding of the risk of MeHg exposure to eastern Chinese populations.

The risk assessments presented here were completed on dorsal muscle samples alone. Future studies should consider among-tissue variation to better inform Chinese Bighead Carp consumers. In China, this species is most often prepared as a soup, where the dorsal muscle tissue as well as brain and skin are eaten. Both Hg and Se can vary by tissue, and brain tissues were found to contain the lowest Hg and Se values relative to other tissues for a marine top predator (Burger et al., 2013).

Bighead Carp were found to have higher EPA and DHA content in dorsal muscle compared to ventral and tail tissue (Zhang et al., 2012b) but no data are available on EFAs, Hg or Se concentrations in brain tissues or skin of Bighead Carp. Understanding the among-tissue variation in Hg, Se, Se:Hg molar ratios and EFAs will help further improve fish consumption advisories.

5. Conclusions

This study addresses an important and still largely untested question regarding changes in risk based on measures such as Se:Hg and HQ_{EFA} with eutrophication. In reservoirs of eastern China, Bighead Carp appear to be a source of EPA and DHA that also have a low overall Hg content and sufficient Se to confer protective benefits against MeHg toxicity (i.e. high Se:Hg molar ratios). However, eutrophication may reduce Se and EPA content through changes to phytoplankton species composition and plankton biomass, respectively, decreasing the overall nutritional value of these fish relative to Hg content. Future research needs include expanding sample sizes and the eutrophication gradient, as well as assessing the Hg, Se and fatty acid content of other subtropical reservoir species, especially higher trophic level fish that can accumulate greater Hg burdens.

Acknowledgments

We thank the School of Life Sciences at Fudan University (Shanghai, China) for field support and lab space; Drs. E Yumvihoze and A Poulain (University of Ottawa) for MeHg analyses, and J Chao and A Kling (Environment Canada) for assistance with fatty acid analyses. We also thank Drs. R Lavoie and P Hodson (Queen's University), L Campagna (Cornell University), and two anonymous reviewers for providing comments on earlier versions of this manuscript. Funding for this work was provided by an International Doctoral Research Award from the International Development Research Centre (105407-99906075-087), Ontario Graduate Scholarships and the Department of Biology at Queen's University to NR Razavi, and by a Natural Sciences and Engineering Research Council of Canada Discovery Grant and a Queen's University (RGPIN/311786 (2005–2010, 2010, 2012–2017)) Chancellor Award to LM Campbell.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2014.08.019.

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