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Isotope dilution quantification of $^{200}Hg^{2+}$ and $CH_3{}^{201}Hg^+$ enriched species-specific tracers in aquatic systems by cold vapor ICPMS and algebraic de-convoluting

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Abstract

The aim of this study was to develop an inductively coupled plasma mass spectrometry (ICPMS) method for the determination of enriched species-specific mercury tracers at ng L^{-1} levels (ppt) in zooplankton and aquatic samples from biological tracer experiments. Applying a cold vapor sector field ICPMS method a high sensitivity was obtained, i.e., 10^6 cps for 1 μg L^{-1} of natural mercury measured on $^{202}Hg^+$, which in turn enabled the measurement of mercury isotope ratios with a 0.6–1.4%R.S.D. precision for a 50 ng L^{-1} standard. This method was used to quantify $CH_3{}^{201}Hg^+$ and $^{200}Hg^{2+}$ tracers in zooplankton from a biological tracer experiment with the aim of investigating the effects of algal density and zooplankton density on mercury bioaccumulation in zooplankton in a fresh water system. For quantification purposes a known amount of $^{199}Hg^+$ was added to the zooplankton samples before digestion. The digested samples were analyzed and the resulting ICPMS spectra split into four spectra one for each of the four sources of mercury present in the sample ($CH_3{}^{201}Hg^+$, $^{200}Hg^{2+}$, $^{199}Hg^{2+}$ and natural mercury) using algebraic de-convoluting. The $CH_3{}^{201}Hg^+$ and $^{200}Hg^{2+}$ tracers were quantified using an isotope dilution approach with the added $^{199}Hg^+$. Detection limits were 0.6 and 0.2 ng L^{-1} for $^{200}Hg^+$ and $CH_3{}^{201}Hg^+$, respectively. The coefficient of variation on the tracer determinations was approximately 18% CV estimated from the analysis of real samples with tracer concentrations in the <0.1–100 ng L^{-1} range. The developed method was successfully applied for the determination of species-specific mercury tracers in zooplankton samples from a biological tracer experiment.

Keywords: Mercury; Inductively coupled plasma mass spectrometry; Enriched stable isotopes; Zooplankton; Methylmercury

1. Introduction

Mercury is a well-known environmental pollutant for which the toxicity of organomercury compounds, e.g., methylmercury exceeds that of inorganic and metallic mercury because their lipohilic character enhances transport across the cellular membrane [1]. Mercury in freshwater systems continues to attract attention of researchers because of its bioaccumulation in food webs, which leads to health risks to wildlife and humans. The biomagnification of mercury across trophic levels often results in contamination of predatory fish at levels of concern for human populations. The ultimate accumulation of mercury is influenced by many variables including watershed, soil and vegetation characteristics, water chemistry and food web structure [2–8]. From a biological point of view it then becomes important to investigate and understand in detail the bioaccumulation of mercury and its different species (e.g., CH_3Hg^+ and Hg^{2+}) at every step in a given food web, particularly at lower trophic levels, where mercury enters the food web. One of the most efficient tools to study bioaccumulation, absorption, distribution, and kinetics of mercury in biological and environmental systems is a tracer experiment with enriched isotopes of mercury and mercury species. Various ICPMS techniques have been used for mercury isotope and mercury species analysis as it offers high

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Table 1
Instrumental operating conditions for cold vapor sector field ICPMS

	Cold vapor ICPMS	Standard ICPMS
rf Power	1550 W	–
Plasma gas flow	14.0 L/min	–
Auxiliary gas flow	0.95 L/min	–
Nebuliser gas flow	0.30 L/min	1.05 L/min
Additional gas flow	0.65 L/min	0 L/min
Sample uptake rate	1.0 mL/min	0.4 mL/min
Sample/skimmer cones	Nickel	–
Scan type	Electric	–
Magnet settle time	1 ms	–
Magnet mass	198.968 u	–
Isotopes	^{199}Hg, ^{200}Hg, ^{201}Hg, ^{202}Hg	–
Mass window	80%	–
$^{202}Hg^{+}$ sensitivity (1 μg L^{-1} Hg)	1000000 cps	50000 cps
Dwell time	5 ms	–
Sweeps	300	–
Samples per peak	50	–
Integration window	80%	–
Segment duration	200 ms	–
Measurement time	56 s	–

sensitivity, low detection limits, often in ng L^{-1} range, and a good precision on isotope ratio measurements (<1%R.S.D.) [9-12]. Sector field based ICPMS offers a isotope ratio precision of <0.1%R.S.D. approaching the precision of thermal ionization mass spectrometry (TIMS) the golden standard for isotope analysis, when used in the low mass resolution mode for the analysis of elemental concentrations well above the detection limit [13]. This improvement in isotope ratio precision is beneficial especially for mercury isotope experiments, as the amount of isotope tracer added to a given system can be kept close to natural levels in order not to disturb the natural pathways, as demonstrated by Pickhardt et al. [9] to study bioaccumulation of isotopically labeled methylmercury and inorganic mercury in a freshwater system. The aim of this paper is to present a new technique combining advanced isotope determination (CV-ICPMS) and algebra to create a powerful tool to follow isotopic enriched species-specific mercury tracers ($CH_3{}^{201}Hg^{+}$ and $^{200}Hg^{2+}$) in a freshwater food web.

2. Experimental

2.1. Instrumentation

All mercury isotope measurements were performed on an element 1 inductively coupled plasma sector field mass spectrometer (Finnigan MAT GmbH, Bremen, Germany), equipped with a shielded torch and connected to a LI-2 cold vapor sample introduction system (Bjoern Klaue, Verlag fuer Lern- und Informationssysteme, L&I, Breitenfelde, Germany) in operation at the Dartmouth Trace Element Analysis Core Facility. For the determination of exact concentrations of isotopic enriched mercury solutions by isotope dilution a standard sample introduction system comprised of a peristaltic pump (Perimax 12, Spetec, Erding, Germany), a concentric nebulizer (AR35-1-F04, Glass Expansion, Hawthorn, Australia) and a Scott-type spray chamber maintained at 6 °C were used. Even though a sector field ICPMS instrument was used the medium to high mass resolution capabilities were not used. All measurements were carried out in the low mass resolution mode taking advantage of the high sensitivity on the sector field mass spectrometric system compared to most quadrupole ICPMS systems. The instrument settings and the method parameters are summarized in Table 1.

2.2. Standard solutions, chemicals and reagents

All sample digestions, sample acidifications, standard preparations and enriched mercury isotope solution preparations were performed with trace metal grade acids prepared from 69% Optima nitric acid and 33% Optima hydrochloric acid (Fisher Scientific, Pittsburgh, PA, USA) diluted with ultrapure water (>18.2 MΩ cm) produced by a Purelab Plus water purifier (US Filter, MA, USA). Natural mercury standard solutions were prepared from a 1000 mg L^{-1} standard (CPI International, Santa Rosa, CA, USA) by dilution with 0.45 M nitric acid. Isotopically enriched mercury (^{199}Hg, ^{200}Hg and ^{201}Hg) was purchased as Hg(II)O from Oak Ridge National Laboratory, TN, USA. For the synthesis of $Me^{201}Hg$, methylcobalamin, benzene (99.9% HPLC grade) and hexane (99+% Capillary GC grade) all from Sigma–Aldrich, St. Louis, MO, USA were used. In the cold vapor ICPMS system the mercury vapor was generated using a 2% stannous chloride (Certified A.C.S. grade, Fisher Scientific, Pittsburgh, PA, USA) in 1 M hydrochloric acid solution.

2.3. Preparation of enriched mercury isotope solutions

Stock solutions of enriched ^{199}Hg, ^{200}Hg and ^{201}Hg were prepared by dissolution of the entire portion of the enriched

Hg(II)O powder, approximately 15 mg, with 5 mL of aqua regia (HCl:HNO_3 3:1) and diluted to 100 mL with ultrapure water. The resulting mercury solutions (≈200 mg L^{-1}) were stored as a stock solution. More dilute solutions (1–15 μg L^{-1}) for isotopic labeling of the mesocosms (^{200}Hg and Me^{201}Hg) or for isotope dilution calibration purposes (^{199}Hg) were prepared by dilution of the stock solution with 0.16 M HNO_3. The exact concentration of the spike solutions was determined by isotope dilution ICPMS analysis.

2.4. Synthesis of ^{201}Hg enriched methylmercury

Applying a procedure similar to those previously described by Imura et al. [14] and Rouleau and Block [15] ^{201}Hg labeled methylmercury was synthesized by methylation of inorganic ^{201}Hg(II) with methylcobalamin. The synthesis proceeded as follows: (i) A portion of 1 mL of the enriched ^{201}Hg solution (≈200 mg L^{-1}) in 0.2 M HNO_3/0.4 M HCl was placed in a 100 mL separation funnel. Approximately 25 mg methylcobalmin was dissolved in 2 ml 0.01 M HCl and added to the funnel, which was then wrapped in aluminum foil and left to stand for 1 h. (ii) Ten millilitres of benzene/hexane (1:1) was added and the mixture was stirred for 10 min. The extraction was repeated twice and the organic layers combined in a 50 mL conical glass vessel containing 2 mL of 1 M HCl. (iii) The combined organic layers were stirred over the HCl for 10 min, then evaporated slowly by blowing a gentle stream of argon over the surface. The methylmercury was left dissolved in the HCl. The final solution was transferred to a vial, sealed and stored in the freezer (−20 °C). The exact concentration of the synthesized Me^{201}Hg solution was determined by isotope dilution ICPMS analysis.

2.5. Experimental design

Using a two-way design the effects of algal density and zooplankton density on mercury accumulation in zooplankton (>202 μm), zooplankton (45–202 μm), and particulate samples (0.4–45 μm) were examined with treatments established in sixteen 550 L mesocosm tanks. Only the results from the largest size fraction (>202 μm) are presented in this paper to illustrate the application and validity of the developed method. The tanks were cleaned, filled with water, and inoculated with leaves to buffer the tank and keep the pH in the 7–8 range. Then phytoplankton sampled at Post Pond in Lyme, NH were added and the tanks covered. Low and high algal density treatments were established by addition of dissolved nitrogen and phosphorus at two different levels. The low and high algae treatment were defined by their chlorophyll concentration, the high being seven times that of the low treatment. Nutrients were added 10 days after inoculation of the tanks with algae. Total nutrients in the algal density treatment were 245 μg L^{-1} N plus 20 μg L^{-1} P in the low treatment and 735 μg L^{-1} N and 55 μg L^{-1} P in the high treatment. Enriched stable isotopes of inorganic mercury and methylmercury were added to each tank 22 days after tanks were stocked with algae. A 1 mL solution containing $CH_3{}^{201}$Hg and $^{200}HgCl_2$ was added to each tank to achieve concentrations of 10 ng L^{-1} $CH_3{}^{201}$Hg and 50 ng L^{-1} $^{200}HgCl_2$ in the tanks. These mercury concentrations were in the same order of magnitude as the natural levels found in lakes across the northern New England area. Three days after isotope addition, zooplankton was added to tanks to establish low and high zooplankton density treatments, the high treatment being five times that of the low treatment. The zooplankton was collected in Post Pond, Lyme, NH and contained the general taxa: Bosmina, Daphnia, Cyclops, Mesocyclops, and Leptodiaptomus. A more detailed description of the experimental layout will be published elsewhere.

2.6. Sampling and sample preparation

Aqueous, particulate, and zooplankton samples for mercury isotope analysis were collected on days 2, 6 and 10 after the zooplankton addition. All Teflon vials and sampling equipment were acid cleaned prior to use. Zooplankton samples (>202 μm) were collected by sieving water through a 202 μm nylon mesh. Using clean water the collected zooplankton were washed onto a Teflon filter (30 μm), the filters were placed in Teflon vials and frozen at −25 °C in the dark until digestion and analysis. Typically less than 5 mg of zooplankton were available for analysis. The zooplankton samples were digested by adding 12 mL of a 2:1 HNO_3 and HCl mixture and spiking to 5 pg mL^{-1} with enriched ^{199}Hg before being heated to 70 °C on a hotplate for 12 h. The resulting solutions were analyzed for mercury isotopes (^{199}Hg, ^{200}Hg, ^{201}Hg and ^{202}Hg) using the cold vapor ICPMS system described below without any further dilutions. A more detailed description of the sampling procedures for aqueous and particulate samples (not discussed in the paper) will be published elsewhere.

2.7. Cold vapor ICPMS optimization and measurements

The ICPMS instrument was mass calibrated and optimized daily using a 1 μg L^{-1} natural inorganic mercury solution, and normally a signal with a peak height of minimum 1,000,000 cps on $^{202}Hg^{+}$ was achieved. Before a measurement sequence was started, two blanks and three mercury standards (0.1, 0.5 and 1 μg L^{-1}) were measured in order to determine the background level, the detector dead time and the mass discrimination according to the method outlined by Nelms et al. [16]. Typical values for the detector dead time and mass discrimination were 13 ns and 0.5% u^{-1}. After determination of dead time and mass discrimination the instrument was washed with 0.16 M HNO_3 for 15 min to avoid any carry-over from calibration standards to samples. The mercury concentration in the majority of the samples was in the low ng L^{-1} range and careful background/carry-over control was crucial in order to obtain valid results. Between samples, a 3 min wash with a matrix-matched wash solution was applied. No carry-over of mercury was observed for mercury

Table 2
Sources of mercury and their isotopic composition

Source	^{199}Hg (% abundance)	^{200}Hg (% abundance)	^{201}Hg (% abundance)	^{202}Hg (% abundance)	Atomic weight
Natural Hg (a)[a]	16.87	23.10	13.18	29.86	200.59
Enriched ^{199}Hg (b)	91.95	4.92	0.66	0.73	199.0418
Enriched ^{200}Hg (c)	0.99	96.41	1.46	0.91	199.9927
Enriched $CH_3{}^{201}$Hg (d)	0.10	0.45	98.11	1.18	200.9731

[a] Ref. [20].

concentrations below 1 μg L^{-1}. As an extra precaution, samples and matrix-matched blanks were alternated throughout the sequence to insure that no carry-over of mercury took place. All sample solutions were analyzed using the method outlined in Table 1. The integrated data were exported into a spreadsheet program, where all tracer calculations, including background correction, detector dead time correction, mass discrimination correction, algebraic de-convoluting, and isotope dilution calculations were performed. It is important to note that Hg(0) vapor is not formed with the same efficiency from methylmercury and inorganic mercury in the cold vapor system. Accurate and reliable determination of isotope labeled methylmercury can therefore only be achieved after digestion of samples with nitric acid in order to convert all methylmercury into inorganic mercury.

2.8. Isotope dilution ICPMS optimization and measurements

The instrument was mass calibrated and optimized as explained above, normally achieving a minimum sensitivity of 50,000 cps for $^{202}Hg^+$ with standard pneumatic nebulization and a Scott-type spray chamber. Typical values for the detector dead time and mass discrimination were 13 ns and 0.3% u^{-1}. The enriched isotope mercury solutions (^{199}Hg, ^{200}Hg or ^{201}Hg) were diluted to a concentration in the 2–10 μg L^{-1} range and spiked with a small volume of a 100 μg L^{-1} natural mercury standard solution. The volume added was adjusted to obtain an isotope ratio of interest (^{199}Hg/^{202}Hg, ^{200}Hg/^{202}Hg or ^{201}Hg/^{202}Hg) close to 1, in order to minimize the uncertainty contribution from the detector dead time correction. When performing analysis at μg L^{-1} level with a Scott-type spray chamber, the carry-over of mercury was substantial and a 10 min wash time with 0.16 M HNO_3 was applied to avoid carry-over between samples. All sample solutions were analyzed using the method outlined in Table 1. The integrated data were exported into a spreadsheet program, where all calculations, including background correction, detector dead time correction, mass discrimination correction and isotope dilution calculations were performed.

2.9. Calculation of enriched tracer concentrations in zooplankton using algebraic de-convoluting

The digested zooplankton samples from the mesocosms contain mercury from four different sources: natural mercury (a) regarded a contaminant in our system, enriched ^{199}Hg (b) for calibration purposes, and enriched inorganic ^{200}Hg (c) and $CH_3{}^{201}$Hg (d) as tracers. All of the four sources of mercury have a known unique isotopic composition, see Table 2. This system with four sources of mercury can be fully described from the determination of the intensity of four mercury isotopes, i.e., $^{199}Hg^+$ (1), $^{200}Hg^+$ (2), $^{201}Hg^+$(3) and $^{202}Hg^+$ (4). The contribution from each of the mercury sources to the four measured isotopes and thereby the concentration of each mercury source in the sample can be calculated using matrix algebra in a way similar to the approach described by Hintelmann and Evans [10]. The total intensity measured for a single isotope is the sum of the individual contributions from the four sources (a–d):

$$\sum {}^{199}\mathrm{Hg} = {}^{199}\mathrm{Hg(a)} + {}^{199}\mathrm{Hg(b)} + {}^{199}\mathrm{Hg(c)} + {}^{199}\mathrm{Hg(d)}$$

In a systematized form the system with four isotopes (1–4) and four sources (a–d) can be described using the following equations:

$$\sum {}^{199}\mathrm{Hg} = 1\mathrm{a} + 1\mathrm{b} + 1\mathrm{c} + 1\mathrm{d},$$

$$\sum {}^{200}\mathrm{Hg} = 2\mathrm{a} + 2\mathrm{b} + 2\mathrm{c} + 2\mathrm{d},$$

$$\sum {}^{201}\mathrm{Hg} = 3\mathrm{a} + 3\mathrm{b} + 3\mathrm{c} + 3\mathrm{d},$$

$$\sum {}^{202}\mathrm{Hg} = 4\mathrm{a} + 4\mathrm{b} + 4\mathrm{c} + 4\mathrm{d}$$

All the individual isotope concentrations can now be expressed as the ratio of the individual isotopes over one common isotope for each source, e.g., R_{21} is ^{200}Hg/^{199}Hg isotope ratio in source a:

$$\begin{array}{llll} 1\mathrm{a}/1\mathrm{a} = 1, & 1\mathrm{b}/2\mathrm{b} = R_{12}, & 1\mathrm{c}/3\mathrm{c} = R_{13}, & 1\mathrm{d}/4\mathrm{d} = R_{14}, \\ 2\mathrm{a}/1\mathrm{a} = R_{21}, & 2\mathrm{b}/2\mathrm{b} = 1, & 2\mathrm{c}/3\mathrm{c} = R_{23}, & 2\mathrm{d}/4\mathrm{d} = R_{24}, \\ 3\mathrm{a}/1\mathrm{a} = R_{31}, & 3\mathrm{b}/2\mathrm{b} = R_{32}, & 3\mathrm{c}/3\mathrm{c} = 1, & 3\mathrm{d}/4\mathrm{d} = R_{34}, \\ 4\mathrm{a}/1\mathrm{a} = R_{41}, & 4\mathrm{b}/2\mathrm{b} = R_{42}, & 4\mathrm{c}/3\mathrm{c} = R_{43}, & 4\mathrm{d}/4\mathrm{d} = 1 \end{array}$$

Substituting this set of ratios into the above set of equations yields the equations below, a system of four equations with four unknowns. The sums of the individual isotopes are measured and the isotope ratios are constants known from the isotopic composition of the four mercury sources. The coefficients 1a, 2b, 3c, and 4d are the unknowns to be calculated:

$$\sum {}^{199}\mathrm{Hg} = 1\mathrm{a} + R_{12}2\mathrm{b} + R_{13}3\mathrm{c} + R_{14}4\mathrm{d},$$

$$\sum {}^{200}\mathrm{Hg} = R_{21}1\mathrm{a} + 2\mathrm{b} + R_{23}3\mathrm{c} + R_{24}4\mathrm{d},$$

$$\sum {}^{201}\mathrm{Hg} = R_{31}1\mathrm{a} + R_{32}2\mathrm{b} + 3\mathrm{c} + R_{34}4\mathrm{d},$$

$$\sum {}^{202}\mathrm{Hg} = R_{41}1\mathrm{a} + R_{42}2\mathrm{b} + R_{43}3\mathrm{c} + 4\mathrm{d}$$

These coefficients can be calculated using a matrix inversion approach. The equations can be rewritten on a matrix form: $\mathbf{AX} = \mathbf{B}$:

$$\mathbf{A} = \begin{bmatrix} 1 & R_{12} & R_{13} & R_{14} \\ R_{21} & 1 & R_{23} & R_{24} \\ R_{31} & R_{32} & 1 & R_{34} \\ R_{41} & R_{42} & R_{43} & 1 \end{bmatrix}, \qquad \mathbf{X} = \begin{bmatrix} 1a \\ 2b \\ 3c \\ 4d \end{bmatrix},$$

$$\mathbf{B} = \begin{bmatrix} \sum {}^{199}\mathrm{Hg} \\ \sum {}^{200}\mathrm{Hg} \\ \sum {}^{201}\mathrm{Hg} \\ \sum {}^{202}\mathrm{Hg} \end{bmatrix}$$

The solution vector (**X**) can now be found using the inverse of the **A** matrix as $\mathbf{X} = \mathbf{A}^{-1}\mathbf{B}$. The inverse of **A** ($\mathbf{A}^{-1}$) can be calculated using the excel function MINVERSE. The matrices **A** and $\mathbf{A}^{-1}$ are the same for all the samples measured as the mercury sources and their isotopic compositions are constant with only their contribution to individual samples changing from sample to sample. The two matrices **A** and $\mathbf{A}^{-1}$ can be calculated from the data in Table 2 and need only to be calculated once for the complete study:

$$\mathbf{A} = \begin{bmatrix} 1 & 18.69 & 0.68 & 0.08 \\ 1.37 & 1 & 66.03 & 0.38 \\ 0.78 & 0.13 & 1 & 83.14 \\ 1.77 & 0.15 & 0.62 & 1 \end{bmatrix},$$

$$\mathbf{A}^{-1} = \begin{bmatrix} -0.00423 & -0.00527 & -0.00688 & 0.57448 \\ 0.05376 & -0.00027 & 0.00031 & -0.03030 \\ -0.00073 & 0.01526 & 0.00007 & -0.01142 \\ -0.00004 & -0.00013 & 0.01209 & -0.00521 \end{bmatrix}$$

When the sum of the signal intensities for the four isotopes has been determined in the samples (**B**) using the ICPMS system, the information needed to calculate **X** is available. The concentration of the different mercury sources is calculated using isotope dilution with enriched ^{199}Hg and therefore based on isotope ratios of the measured isotopes and not on absolute count rates. Therefore, the small 5–10% reduction in instrument sensitivity over the course of a day's analysis is less critical to the final results than variations in the background level, as the level of mercury is very low, often less than 20 ng L^{-1}. As a consequence, every single sample is bracketed by two matrix-matched blank solutions. The true number of counts for each isotope is then calculated as the detector dead time and mass bias corrected count rate subtracted the average of the two blanks bracketing the sample.

When the true number of counts is found, **X** can be calculated using the above equation. Using the calculated numbers for **X** all the coefficients and terms in the four equations with the four unknowns can be calculated and the measured ICPMS spectrum split into four spectra each representing one source, as illustrated in Fig. 1a and b.

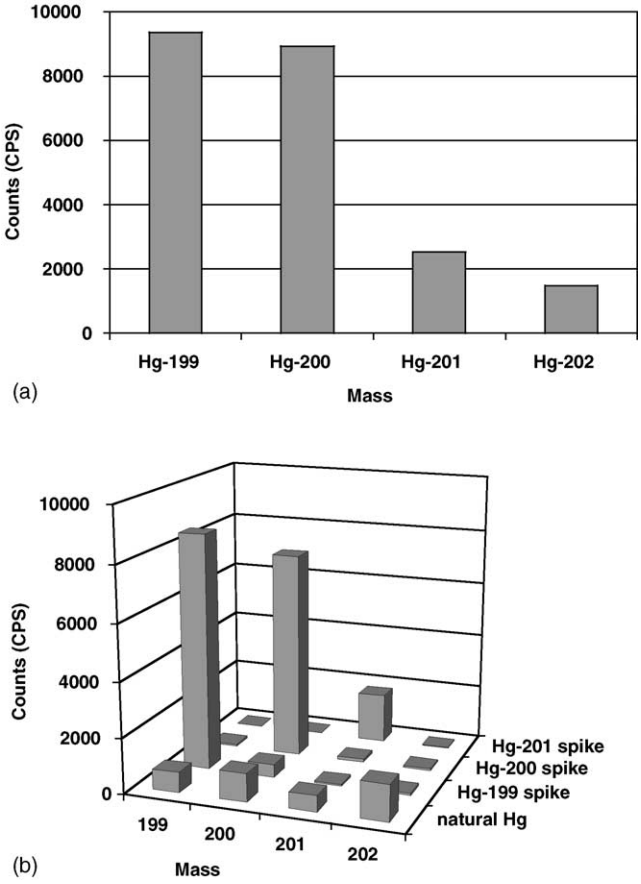

Fig. 1. Algebraic de-convoluting of mercury spectra: (a) original ICPMS spectrum; (b) de-convoluted spectrum showing the isotopic composition of the four mercury sources.

The exact concentration and mass of the ^{199}Hg spike added before sample digestion is known, therefore pairing the ^{199}Hg spectra with each of the three other spectra, the concentration of natural Hg, ^{200}Hg spike and $CH_3{}^{201}$Hg spike can be calculated applying a standard isotope dilution approach, e.g., when the concentration of the ^{200}Hg source is calculated the ^{199}Hg/^{200}Hg ratio (X/Y) is calculated from the combined enriched ^{199}Hg and ^{200}Hg source spectra and applied in the following general equation for isotope dilution calibration. In this calculation the measured ratio of the calibration isotope (^{199}Hg) over the spike isotope ($R_{x/y}$), the mass of the original sample (m_{smp}) plus the mass (m_{sp}) and concentration (c_{sp}) of the ^{199}Hg spike added are used:

$$c_{\mathrm{smp}} = \frac{m_{\mathrm{sp}} c_{\mathrm{sp}} A_{\mathrm{smp}} (R_{x/y} Y_{\mathrm{sp}} - X_{\mathrm{sp}})}{m_{\mathrm{smp}} A_{\mathrm{sp}} (X_{\mathrm{smp}} - R_{x/y} Y_{\mathrm{smp}})}$$

where A is the atomic weight, X and Y the isotopic abundances. All of the above equations and calculations are conveniently built into a spreadsheet, which calculates the concentrations of the enriched inorganic ^{200}Hg and enriched $CH_3{}^{201}$Hg, the two mercury tracers that originally were added to the mesocosms.

3. Results and discussion

3.1. Precision of the isotope measurements

Since the quantification of the mercury tracers are performed using an isotopic dilution approach, it is important the ICPMS instrument is setup to measure isotopic ratios with the best possible accuracy and precision. Therefore, a relative high number of sweeps ($n = 300$) across the four mercury isotopes are used to cancel out random noise, primarily from the sample introduction system. The precision of the isotopic ratio determinations applying the standard ICPMS setup with a pneumatic nebulizer was evaluated by repeated analysis ($n = 10$) of a 500 ng L^{-1} natural mercury standard. The $^{199}Hg/^{200}Hg$, $^{199}Hg/^{201}Hg$ and $^{199}Hg/^{202}Hg$ ratios can be measured with a precision in the 0.3–0.5%R.S.D. range, approximately twice the theoretical precision as calculated from Poisson statistics. For the cold vapor ICPMS system the precision was 0.6–1.4%R.S.D. ($n = 10$) for repeated analysis of a 50 ng L^{-1} standard solution, again approximately twice the theoretical precision. The sensitivity of the cold vapor ICPMS system is more than an order of magnitude higher than that of the pneumatic nebulization system and therefore is it advantageous for the analysis of the zooplankton samples, as the mercury concentration in the digests of these samples are in the low ng L^{-1} range. On the other hand, the pneumatic nebulization ICPMS system is preferred for the measurement of the exact concentration of the spike isotopes by isotope dilution as the isotope ratio determinations are more precise than for the cold vapor generation ICPMS system. Furthermore, the mercury concentration of the sample solutions can be adjusted to minimize the impact of background subtraction and detector dead time correction on the precision and accuracy of the measurement, typically in the 2–10 μg L^{-1} range. Finally the operation of the pneumatic system is simpler and faster.

A good accuracy on the isotope ratio determinations is ensured by mass bias correction of the observed isotope ratios. The mass bias correction factor ($f = R_{true}/R_{measured}$) is estimated daily from the analysis of mercury standard solutions. A consistent and systematic higher mass bias of 0.5% u^{-1} versus 0.3% $u^{-1}$was found for the cold vapor generation system compared to the pneumatic nebulization system. The most likely explanation for this difference is that the observed mass bias is dependent on the total ion current produced from a sample in the plasma [17], which is clearly different for the two sample introduction systems as one is introducing a gas into the plasma, the other an aerosol.

3.2. Figures of merit for tracer determinations

The detection limit for the determination of the $^{200}Hg^{2+}$ and the $CH_3{}^{201}Hg^+$ tracers in the aquatic samples was calculated as three times the standard deviation of concentrations in 12 water samples taken from the mesocosms before the mercury tracers were added. The detection limits were 0.6 and 0.2 ng L^{-1} for ^{200}Hg and $CH_3{}^{201}Hg$, respectively.

The coefficient of variation (%CV) for the method was estimated from the determination of the $^{200}Hg^{2+}$ and $CH_3{}^{201}Hg^+$ tracers in eight real samples on two different days. The standard deviation on the results was estimated as $S^2 = \sum d^2/2n$ [18], where d is the difference between two measurements of the same sample on different days. The %CV was 19% for $^{200}Hg^{2+}$ and 17% for $CH_3{}^{201}Hg^+$, which is relatively high, but acceptable considering that the tracer concentrations were in the 0.4–100 ng L^{-1} range for $^{200}Hg^{2+}$ and in the <0.2–18 ng L^{-1} range for $CH_3{}^{201}Hg^+$.

3.3. Determination of ^{200}Hg and $CH_3{}^{201}Hg$ tracers in aquatic samples

The developed ICPMS method for the determination of species-specific mercury isotope tracers was applied in a biological experiment designed to study the effects of algal density and zooplankton density on mercury accumulation in zooplankton. The data presented here are a subset of the complete dataset, chosen to show that the developed method successfully can be applied for the determination of enriched mercury isotopes at ng L^{-1} levels in biological tracer experiments. The full results for the biological study will be published elsewhere. One of the hypotheses tested was that the algal biomass would have an influence on the uptake of methylmercury and inorganic mercury by zooplankton and that methylmercury would be bioaccumulated to higher con-

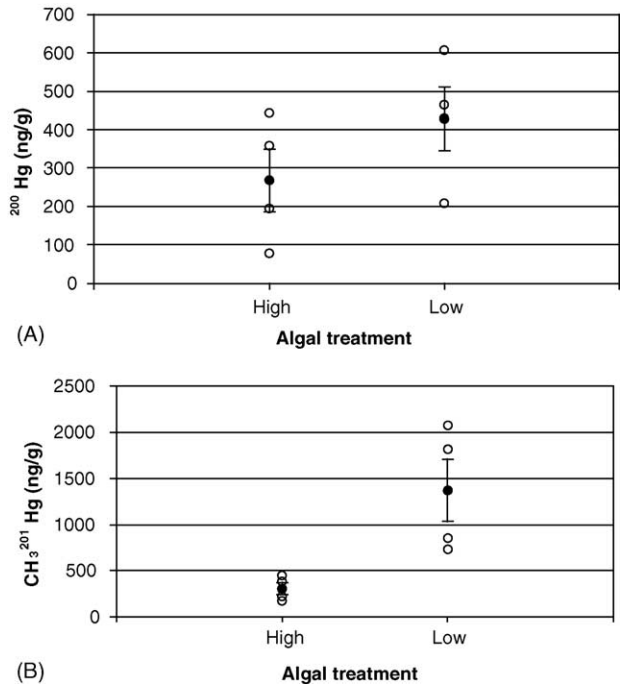

Fig. 2. Concentration of enriched labeled $^{200}Hg^{2+}$ (A) and $CH_3{}^{201}Hg^+$ (B) in zooplankton as a function of low and high algal treatment. Individual measurements (○) and the average (●) with one standard error (S.E.M. = $s/\sqrt{n}$) error bars.

centrations at low algal densities than at high densities, as previously suggested by Pickhardt et al. [9]. Similarly in a field study, Chen and Folt [19] found that algae density was negatively correlated with total mercury in zooplankton. The preliminary data from this study shown in Fig. 2 support this hypothesis as no significant difference in the zooplankton (>202 μm) uptake of inorganic mercury ($^{200}Hg^{2+}$) were found between high and low algal concentrations collected on the 2nd day after the zooplankton was added to the mesocosms, while methylmercury uptake ($CH_3{}^{201}Hg^{+}$) was greater at a low algal biomass than high ($P = 0.02$). The explanation for this is two-fold: (1) algae and/or zooplankton selectively assimilate methylmercury over inorganic mercury and (2) higher algal biomass results in a lower mass-specific concentration of methylmercury in algae [9], and because zooplankton preferentially assimilate CH_3Hg^{+} over inorganic Hg, higher algal biomass results in lower methylmercury in zooplankton on a biomass basis.

4. Conclusions

ICPMS offers good capabilities for isotope analysis combined with high sensitivity to allow determination of mercury isotope ratios at low concentration levels. This makes the technique particularly useful for conducting mercury tracer experiments at concentrations representative of natural freshwater systems. Using a cold vapor sector field ICPMS method followed by algebraic de-convoluting of the obtained mass spectra, species-specific enriched isotope mercury tracers at ng L^{-1} levels were successfully quantified in zooplankton.

References

[1] R.G. Fernandez, M.M. Bayon, J.I.G. Alonso, A. Sanz-Medel, J. Mass Spectrom. 35 (2000) 639.
[2] R.C. Back, P.R. Gorski, L.B. Cleckner, J.P. Hurley, Sci. Total Environ. 304 (2003) 349.
[3] P.R. Gorski, L.B. Cleckner, J.P. Hurley, M.E. Sierszen, D.E. Armstrong, Sci. Total Environ. 304 (2003) 327.
[4] H. Parkman, M. Meili, Can. J. Fish. Aquat. Sci. 50 (1993) 521.
[5] A. Tremblay, M. Lucotte, Can. J. Fish. Aquat. Sci. 54 (1997) 832.
[6] N.C. Kamman, P.M. Lorey, C.T. Driscoll, R. Estabrook, A. Major, B. Pientka, E. Glassford, Environ. Toxicol. Chem. 23 (2004) 194.
[7] R. Stemberger, C. Chen, Can. J. Fish. Aquat. Sci. 55 (1998) 339.
[8] C. Chen, R. Stemberger, N.C. Kamman, B. Mayes, C. Folt, Ecotoxicology, submitted for publication.
[9] P.C. Pickhardt, C. Folt, C. Chen, B. Klaue, J. Blum, Proc. Natl. Acad. Sci. U.S.A. 99 (2002) 4419.
[10] H. Hintelmann, R.D. Evans, Fresenius J. Anal. Chem. 358 (1997) 378.
[11] L. Lambertsson, E. Lundberg, M. Nilsson, W. Frech, J. Anal. At. Spectrom. 16 (2001) 1296.
[12] R.C.R. Martin-Doimeadios, E. Tessier, D. Amouroux, R. Guyoneaud, R. Duran, P. Caumette, O.F.X. Donard, Mar. Chem. (2004) (published online June).
[13] F. VanHaecke, L. Moens, R. Dams, P.D.P. Taylor, Anal. Chem. 68 (1996) 567.
[14] N. Imura, E. Sukegawa, S.-K. Pan, K. Nagao, J.-Y. Kim, T. Kwan, T. Ukita, Science 172 (1971) 1248.
[15] C. Rouleau, M. Block, Appl. Organomet. Chem. 11 (1997) 751.
[16] S.M. Nelms, C.R. Quetel, T. Prohaska, J. Vogl, P.D.P. Taylor, J. Anal. At. Spectrom. 16 (2001) 333.
[17] K.G. Heumann, S.M. Gallus, G. Radlinger, J. Vogl, J. Anal. At. Spectrom. 13 (1998) 1001.
[18] R.L. Anderson, Practical Statistics for Analytical Chemistry, Van Nostrand Reinhold Company Inc., New York, 1987.
[19] C. Chen, C. Folt, Environ. Sci. Technol., in press.
[20] K.J.R. Rosman, P.D.P. Taylor, Pure Appl. Chem. 70 (1998) 217.