Determination of mercury in fish otoliths by cold vapor generation inductively coupled plasma mass spectrometry (CVG-ICP-MS)

Erdal Kenduzler, Mehmet Ates, Zikri Arslan, Melanie McHenry, Paul B. Tchounwou

ABSTRACT

A method based on cold vapor generation inductively coupled plasma mass spectrometry (CVG-ICP-MS) has been developed for determination of inorganic mercury, Hg(II), and total mercury in fish otoliths. Sodium borohydride (NaBH₄) was used as the only reducing agent and its concentration was optimized across an acidity gradient to selectively reduce Hg(II) without affecting methylmercury, CH₃Hg(I). Inorganic Hg was quantitatively reduced to elemental mercury (Hg⁰) with 1 × 10⁻⁶% (m/v) NaBH₄. CH₃Hg(I) required a minimum of 0.5% (m/v) NaBH₄ for complete reduction. Increasing the HCl concentration of solution to 5% (v/v) improved the selectivity toward Hg(II) as it decreased the signals from CH₃Hg(I) to baseline levels. Potassium ferricyanide solution was the most effective in eliminating the memory effects of Hg compared with a number of chelating and oxidizing agents, including EDTA, gold chloride, thiourea, cerium ammonium nitrate and 2-mercaptoethylamine chloride. The relative standard deviation (RSD) was less than 5% for 1.0 µg L⁻¹ Hg(II) solution. The detection limits were 4.2 and 6.4 ng L⁻¹ (ppt) for Hg(II) and total Hg, respectively. Sample dissolution conditions and recoveries were examined with ultra-pure CaCO₃ (99.99%) spiked with Hg(II) and CH₃HgCl. Methylmercury was stable when dissolved was performed with up to 20% (v/v) HCl at 100 ⁰C. Recoveries from spiked solutions were higher than 95% for both Hg(II) and CH₃Hg(I). The method was applied to the determination of Hg(II) and total Hg concentrations in the otoliths of red emperor (CRM 22) and Pacific halibut. Total Hg concentration in the otoliths was 0.038 ± 0.004 µg g⁻¹ for the red emperor and 0.021 ± 0.003 µg g⁻¹ for the Pacific halibut. Inorganic Hg accounted for about 25% of total Hg indicating that Hg in the otoliths was predominantly organic mercury (e.g., methylmercury). However, as opposed to the bioaccumulation in tissues, methylmercury levels in otoliths were very low suggesting a different route of uptake, most likely through the deposition of methylmercury available in the water.

Keywords:
Fish otolith
Inorganic mercury
Total mercury
Cold vapor generation
ICP-MS

1. Introduction

Fish otoliths are calcium carbonate minerals (as aragonite) in the head of fish that aid in balance and hearing to the fish [1,2]. These aragonite minerals grow throughout the life of fish by deposition of calcium carbonate in concentric layers on a proteinaceous matrix. In the meantime, trace elements from the surrounding water successively incorporate into the newly forming aragonite layer. The aragonite polymorph is not susceptible to resorption. Therefore, the temporal concentrations of the trace elements (so-called fingerprints) remain unaltered throughout the fish’s lifetime, and consequently integrate over the fish’s life history when a whole is dissolved [1–7].

Trace elements and heavy metals make up less 1% (by mass) of an otolith. With the exception of Sr, their concentrations range from low ng g⁻¹ to a few µg g⁻¹ [3,8–14]. The utilization of trace elements as robust biological tags in otolith micro-chemical analysis has been largely due to the use of inductively coupled plasma mass spectrometry (ICP-MS) as a highly sensitive tool. Nevertheless, accurate determination of most trace elements and heavy metals, with the exception of relatively abundant Mg, Cu, Mn and Zn, from the otoliths is still a challenging task by direct analysis [3,4,6,8–10]. Various analytical methods, including isotope dilution [4,15], solvent extraction [16], solid phase extraction [6,17–20], co-precipitation [21] and hydride generation [22] have been developed to overcome the difficulties associated with low elemental concentrations in a highly saline calcium matrix.

Mercury (Hg) in the aquatic ecosystems mainly originates from the deposition of atmospheric Hg released from the anthropogenic
activities [23–25]. Inorganic Hg in water is converted by bacteria to highly toxic methylmercury that accumulates in the sediments [23,25–27]. While microorganisms, such as phytoplankton and zooplankton ingest methylmercury (CH₃HgCl) from water, dietary uptake is the major route of exposure of fish to methylmercury [25,28–30]. It is now well-documented that most Hg in fish tissue is in the form of methylmercury, although total body burden could vary with geological, biological and physiological differences among species [31–33].

The concentrations for a number of minor and major elements (Al, Na, Cl, Sr, Ca, Si) in bluefin tuna otoliths were reported to vary with total Hg body burden [34]. Further, laboratory exposures conducted with different fish indicate that the uptake of Hg into otoliths is related with its concentration in the water [35]. To date, however, Hg has not been considered as a biological tracer in otolith microchemistry, which is due in part to measurement difficulties and relatively low sensitivity of solution-based ICP-MS to this element. Thus, there is no information about the chemical forms of Hg in fish otoliths and whether otolith Hg could aid in population studies or not.

In this study, we have developed a cold vapor generation method for determination of Hg(II) and total Hg in otoliths by ICP-MS in an attempt to elucidate the chemical forms of Hg in fish otoliths, and to provide an insight about its source and utility in otolith microchemistry. Sodium borohydride (NaBH₄) was used as reducing agent to discriminate between the Hg(II) and total Hg levels. Studies were performed with a number of chelating and oxidizing reagents to eliminate the memory effects. Effects of acid dissolution on the stability and recoveries of Hg species were examined by spiking Hg(II) and CH₃HgCl to ultra-pure calcium carbonate. The method was applied to the determination of Hg(II) and total Hg in otolith samples from two different oceanic fish species, red emperor and Pacific halibut.

2. Experimental

2.1. Reagents and solutions

Deionized water produced by Barnstead™ E-Pure system with minimum resistivity of 17.8 MΩ cm was used throughout. A 1.0 μg mL⁻¹ Hg(II) solution was prepared from a 1000 μg mL⁻¹ standard solution (Sigma Aldrich) and stored in 5% (v/v) HNO₃ (Trace metal grade, Fisher Scientific). Methylmercury chloride (CH₃HgCl) solution (1000 μg mL⁻¹ in water) was purchased from Alda Aesar (99.99%). A 1.0 μg mL⁻¹ CH₃HgCl stock solution was prepared and stored in water. All experimental solutions and calibration standards were prepared from these standard solutions. Trace metal grade hydrochloric acid (HCl, BDH Chemicals) was used for dissolution of samples and preparation of experimental solutions. Sodium borohydride (NaBH₄, 99.9%, Sigma Aldrich) was used as reducing agent. Aqueous solutions of NaBH₄ were stabilized in 0.1% (m/v) NaOH (99.9%, Sigma Aldrich). All other reagents, including ethylenediaminetetraacetic acid (EDTA), potassium ferricyanide [K₃Fe(CN)₆], thiourea, l-cysteine, 2-mercaptopethamline chloride, cerium(IV) ammonium nitrate [(NH₄)₂Ce(NO₃)₆], and gold chloride (HAuCl₄·3H₂O) were reagent grade.

2.2. Instrumentation

A Varian 820MS ICP-MS instrument (Varian, Australia) was used. The instrument was equipped with a peltier-cooled double-pass glass spray chamber, micromist nebulizer, standard one-piece, low flow, ball-and-socket connection quartz torch, standard Ni sampler and skimmer cones, patented Collision Reaction Interface (CRI), a unique 90° ion mirror delivering exceptional sensitivity, all-digital detector; Discrete Dynode Electron Multiplier (DDDM, Model AF250, ETP Australia) providing nine decades of linear dynamic range. Samples were introduced manually. The instrument was optimized daily for sensitivity, doubly charged ions (<2%) and oxides (<3%) with 5 μg L⁻¹ 118Ba, 25Mg, 115In, 140Ce, 208Pb solution. Data collection was achieved by ICP-MS Expert software package (version 2.2.1b126). The operating parameters of the instrument are summarized in Table 1.

Table 1. Operating parameters for Varian 820-MS ICP-MS instrument for cold vapor generation.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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<tbody>
<tr>
<td>RF power (kW)</td>
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<tr>
<td>Plasma Ar flow (L.min⁻¹)</td>
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<td>Auxiliary Ar flow (L.min⁻¹)</td>
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<td>10</td>
</tr>
<tr>
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<td>0</td>
</tr>
<tr>
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<tr>
<td>Hg²⁰⁹ and Hg²⁰²</td>
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</tbody>
</table>

Vapor generation

Sample acidity (% (v/v) HCl) | 5.0  |
Sample flow rate (ml min⁻¹)  | 2.0  |
NaBH₄ flow rate (ml min⁻¹)    | 1.0  |

2.3. Otolith samples

Fish otolith certified reference material (CRM 22) was purchased from the National Institute of Environmental Studies (NIES), Japan. The material was prepared from sagittal otoliths.
of emperor snapper (Lutjanus sebae) collected from the northwest coast of Western Australia. It is certified for Ba, Ca, K, Mg, Na and Sr along with indicative values for Cd, Cu, Pb and Zn. Otolith samples of adult Pacific halibut (Hippoglossus stenolepis) collected from the eastern Pacific Ocean were kindly provided by NOAA James Howard Marine Laboratory, Sandy Hook, NJ.

2.4. Otolith dissolution procedure

The otolith sample of emperor snapper was received as fine powder. Those of Pacific halibut were ground using agate mortar and mill, passed through 120 mesh (125 μm) stainless steel sieves, and stored in an acid-cleaned plastic container. Approximately 50 mg powdered otolith sample was placed in teflon tubes (60 mL inner volume). Each sample was first wetted with water and slowly dissolved with 0.5 mL of 5% (v/v) HCl. Then, 3 mL of 20% (v/v) HCl was added to the pre-dissolved samples. The tubes were screw-capped tightly and heated 100 °C for 1 h, cooled to room temperature and then collapsed to 12 mL with water. Three replicate measurements were made for both Hg species by CVG-ICP-MS.

3. Results and discussion

3.1. Optimum conditions for vapor generation

Cold vapor generation (CVG) has been the preferred method for determination of Hg by atomic absorption (AAS) and atomic fluorescence spectrometry (AFS) as it offers improved sensitivity and lower detection limits [36–41]. Unlike many other heavy metals, direct determination of Hg by ICP-MS suffers from memory effects due to the adsorption of Hg throughout the sampling system. In addition, ICP-MS exhibits relatively low sensitivity to Hg due to the low abundances of natural Hg isotopes and its high first ionization potential (10.4 eV). Thus, CVG has also been interfaced to ICP-MS as an attractive sample introduction method to enhance the sensitivity in determination of this ubiquitous and toxic element [42–45]. Acidic stannous chloride (SnCl₂) solution is frequently used as reductant provided that all Hg species in the sample are converted to Hg(II) by means of vigorous acid digestion or reacting with strong oxidizing agents (e.g., KMnO₄ [38,41,43]). In this study, we opted for NaBH₄ as it can reduce both inorganic and organic forms of Hg [36,39,44]. Further, the reaction of NaBH₄ with acids produces water vapor and hydrogen gas as by-products, which is also advantageous to avoid possible contamination to the sampling interface of ICP-MS instrument from using SnCl₂ at percent levels.

Initial experiments showed that the generation of Hg vapor from Hg(II) and CH₃HgI took place robustly in a wide acidity interval (Fig. 2A). Signals increased up to 1% (v/v) HCl and remained almost flat with further increase to 5% (v/v) HCl. The signal intensity from 10 μg L⁻¹ CH₃HgCl was always about 80% of that from 10 μg L⁻¹ Hg(II), which was proportional to the stoichiometric composition of Hg in CH₃HgCl. However, Fig. 2A clearly indicated that CH₃Hg(I) was quantitatively converted Hg vapor as was Hg(II) when solution acidity was above 1% (v/v) HCl.

For optimization of NaBH₄ concentration, Hg(II) and CH₃HgCl solutions were acidified to 2% (v/v) HCl. The signal profiles from Hg(II) and CH₃Hg(I) are illustrated in Fig. 3. The reduction of Hg(II) occurred even in the presence of trace levels of NaBH₄, while signals for CH₃Hg(I) increased steeply with increasing NaBH₄ up to 0.1% (m/v) and then leveled off above 0.2% (m/v) NaBH₄. For quantitative reduction of CH₃Hg(I), 0.5% (m/v) NaBH₄ was sufficient. The inset figure is the blow-up illustration of signal profiles up to 0.002% (m/v) NaBH₄. This pattern indicated that NaBH₄ concentration of about 1 × 10⁻⁸% (m/v) was adequate to achieve quantitative reduction of Hg(II) to Hg vapor. These results are consistent with those reported by Segade and Tyson [39] who first utilized this approach for determination of Hg(II) and total Hg content in various samples. Under same conditions, the reduction of CH₃Hg(I) was negligible (ca. 3–4 × 10⁶ cps) which was approximately 2% of the signal from 10 μg L⁻¹ Hg(II). Considering ng g⁻¹ levels of total Hg in otoliths, the contribution from CH₃Hg(I) could be significant to confound accurate determination of inorganic Hg levels as well as deteriorate the detection limits. Thus, the solution acidity was reexamined for the Hg(II) and CH₃HgCl solutions using 1 × 10⁻⁸% (m/v) NaBH₄ as reductant. The results are illustrated in Fig. 2B. As occurred with 1% (m/v) NaBH₄ (see Fig. 2A), signals for Hg(II) increased with increasing acidity up to 2% (v/v) HCl and then remained relatively constant. In contrast, signals from CH₃Hg(I) were at the maximum when CH₃HgCl was in water (e.g., 0%, v/v) and rapidly decreased with
increasing HCl concentration. For 5% and 6% (v/v) HCl solutions, signals were around $5 \times 10^3$ to $1 \times 10^4$ cps that were within the vicinity of blanks signals from 5% (v/v) HCl solutions. The concentration of HCl was increased up to 12% (v/v), but did not show any improvement in the background Hg signals, and thus the solutions were maintained at 5% (v/v) throughout.

The flow rates of the carrier argon and sample solution were optimized for Hg(II) solutions in 5% (v/v) HCl. Optimum flow rate for sample solution was around 2 mL min$^{-1}$ when reacted on-line with 1% (m/v) NaBH$_4$ solution running at 1 mL min$^{-1}$. Signals did increase to some extent with higher flow rates but at the cost of more sample solution, which was not desirable as it would require dilution of otoliths containing very levels of Hg. The flow rate of carrier argon was more influential on signal intensity and stability. Optimum value was 1.2 mL min$^{-1}$ for both 1.0 $\times$ 10$^{-2}$% and 1% (m/v) NaBH$_4$. Plasma stability was not affected from the vigorous reaction of 1% (m/v) NaBH$_4$ with 5% (v/v) HCl since water vapor was removed efficiently by the peltier-cooled spray chamber (3°C).

### 3.2. Memory effects

Mercury determination by ICP-MS is known to suffer from memory effects that are attributed to mainly adsorption of Hg(II) through the sampling interface and sample introduction system [43, 46, 47]. In conventional solution-based measurements, the memory effects were eliminated by adding gold chloride (HAuCl$_4$) to sample and washout solutions [48]. l-Cysteine was also reported to be effective among a number reagents used to facilitate the washout of Hg from the sampling interface of ICP-MS instrument [47]. The memory effects in CVG-ICP-MS were said to be lower because of the gaseous nature of Hg introduced to the spectrometer and the reduced surface area of sample introduction components interacting with ionic Hg [42, 43]. Our results, however, showed that memory effects in CVG were severe just as in solution mode to deteriorate the precision and detection limits. The residual signals from the introduction 10 μg L$^{-1}$ Hg(II) solution varied between 300,000 and 400,000 cps. This background gradually decreased to about 50,000 cps within 10 wash cycles with 5% (v/v) HCl or 5% (v/v) HNO$_3$, but was still far above the blank signals observed from 5% (v/v) HCl (~5000 cps). It was interesting to note that such background was not observed from CH$_3$HgCl that was washed out from the sampling and CVG components readily.

The signal profiles from three replicate runs for 10 μg L$^{-1}$ Hg(II) and the performances of washout solutions are illustrated in Fig. 4. Despite the fact that gold chloride (HAuCl$_4$) is the most referred reagent for Hg washout, signal stability was highly variable when gold chloride solution (10 μg mL$^{-1}$ Au) was run through the system (Fig. 4A). Signals repeatedly decreased in each washout, which could be due to the fact that Hg(II) amalgamated with residual gold throughout the sampling tubing and fittings. Signals tended to increase in subsequent runs, but the variation in signal intensity was substantial. Moreover, the depression in Hg signals was so prominent after repeated washout such that it was necessary to replace the pump tubing and teflon lines to recover the sensitivity. Washout with 0.5% (m/v) l-cysteine did not cause such depression, but the cleaning of residual Hg was inefficient as can be seen from the high background signals (~500,000 cps) following the washout with 0.5% (m/v) l-cysteine solution (Fig. 4B). Cerium(IV) ammonium nitrate (0.5% m/v), as a strong oxidizing agent, performed better than l-cysteine. However, extended wash was necessary to decrease the signals to baseline levels (Fig. 4C).

The other washout solutions, including 0.2% (m/v) EDTA, 0.5% (m/v) 2-mercaptoethylamine chloride, and 0.5% (m/v) thiourea performed similarly to l-cysteine, and thus were not effective in removing the memory effects. Potassium ferricyanide solution, 0.5% (m/v) K$_3$Fe(CN)$_6$ in 5% (v/v) HCl, was the most effective among

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**Fig. 2.** The effect of HCl concentration on Hg signals from 10 μg L$^{-1}$ Hg(II) and CH$_3$HgCl solutions. (A) Initial optimization of HCl concentration using 1% (m/v) NaBH$_4$. (B) Re-examination of HCl concentration on signal profiles with 1 $\times$ 10$^{-2}$% (m/v) NaBH$_4$.

**Fig. 3.** The effect of NaBH$_4$ concentration on Hg signal from 10 μg L$^{-1}$ Hg(II) and CH$_3$HgCl solutions in 2% (v/v) HCl. The inset figure is the blow-up from 0% to 0.002% (m/v) NaBH$_4$. 
the solutions examined (Fig. 4D). The signal intensity abruptly increased when the ferricyanide solution was run and reacted with 1% (m/v) NaBH₄ solution, suggesting that the Hg adsorbed along the sampling system was brought to a form, most likely Hg(II), to react with the reductant. The signals decayed during the washout for about 90 s then immediately dropped to the background levels during 5% (v/v) HCl run (see Fig. 4D), indicating that K₃Fe(CN)₆ solution effectively removed the residual Hg from the sampling components. Washout with potassium ferricyanide solution was also advantageous as it afforded better sensitivity and stability in signals. Signals for Hg were stable during the replicate runs (RSD < 5%), unlike the steady increase occurred after the washout with gold chloride solution. Additional experiments to optimize the concentration of K₃Fe(CN)₆ solution showed that 0.2% (m/v) solution was sufficient for successful washout of the residual Hg within 45–60 s.

3.3. Effects of acid dissolution on methylmercury stability

Otoliths are primarily calcium carbonate with about 3–4% organic matrix that could contain elemental species, including inorganic Hg and methylmercury. Digestion in acid is thus essential for complete extraction of elemental species to the solution. In case of Hg, it is also critical to utilize acid digestion/dissolution conditions without altering the organic mercury (e.g., CH₃Hg) levels in the otoliths. Table 2 summarizes the composition of Hg(II) and CH₃Hg(I) in solutions when CH₃HgCl (10 μg L⁻¹) is digested in various HCl solutions at 100 °C. Inorganic Hg was measured by using 1% (m/v) SnCl₂ solution in 5% (v/v) HCl as reductant, whereas 0.5% (m/v) NaBH₄ was adopted for CH₃Hg(I). The concentration of Hg(II) was not significant when CH₃HgCl was digested in up to 20% (v/v) HCl. Average Hg(II) concentration was about 0.14 μg L⁻¹, indicating that dissolution of an otolith sample in 20% (v/v) HCl would not induce any significant changes to methylmercury levels. Treatment with 50% (v/v) HCl was not suitable as it yielded higher levels of Hg(II) up to 0.72 μg L⁻¹ due to the mineralization of CH₃HgCl.

3.4. Interferences and analytical performance

The effect of calcium matrix on the recoveries of Hg(II) and CH₃Hg(I) is shown in Table 3. These results were obtained from the dissolution of ultra-pure CaCO₃ (99.99%) samples in 20% (v/v) HCl that were spiked with Hg(II) and CH₃HgCl solutions. No significant interference was observed from up to 20,000 μg mL⁻¹ Ca(II) (e.g., 2%, m/v). The recoveries for Hg(II) and CH₃Hg(I) were better than 95% by using the aqueous external calibration.

The memory effects were minimal at Hg concentrations less than 1.0 μg L⁻¹ when 0.2% (m/v) K₃Fe(CN)₆ solution was run

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**Fig. 4.** Removal of residual Hg with various washout solutions. Signals during continuous CVG runs: a: 5% (v/v) HCl; b: 10 μg L⁻¹ Hg(II); c: washout solution. Washout solutions are: (A) gold chloride (10 μg mL⁻¹ Au) in 5% (v/v) HCl; (B) 0.5% (m/v) L-cysteine in 2% (v/v) HCl; (C) 0.5% (m/v) cerium(IV) ammonium nitrate in 2% (v/v) HCl; (D) 0.5% (m/v) potassium ferricyanide in 5% (v/v) HCl.

**Table 2**

<table>
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<th>HCl concentration (% v/v)</th>
<th>Hg(II) (μg L⁻¹)</th>
<th>CH₃HgCl (μg L⁻¹)</th>
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<tbody>
<tr>
<td>5</td>
<td>0.04 ± 0.01</td>
<td>9.6 ± 0.4</td>
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<tr>
<td>10</td>
<td>0.12 ± 0.05</td>
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<td>20</td>
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<tr>
<td>50</td>
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**Table 3**

<table>
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<th>Calcium concentration (μg mL⁻¹)</th>
<th>Recovery Hg(II) %</th>
<th>Recovery CH₃Hg(I) %</th>
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<tr>
<td>0</td>
<td>100 ± 5</td>
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<tr>
<td>200</td>
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<tr>
<td>2000</td>
<td>90 ± 1</td>
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Table 3

<table>
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<th>Ca(II) (μg ml⁻¹)</th>
<th>Hg(II) (%)</th>
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<td>20,000</td>
<td>98.1 ± 2.6</td>
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Laboratory experiments indicate that uptake of heavy metals, including Cu, Pb and Hg into fish otoliths is proportional to their concentrations in the water [35, 49, 50]. On the other hand, mercury in fish tissue is mainly from the dietary uptake at the base of the food chain and is known to bioaccumulate (as methylmercury) two to three orders of magnitude higher levels than its concentration in the water. For instance, total Hg as methylmercury in tissues of Pacific halibut ranges from 0.15 to 0.45 μg g⁻¹ averaging to about 0.241 μg g⁻¹ [51, 52]. These concentrations are about an order of magnitude higher than total Hg (0.021 ± 0.003 μg g⁻¹) found in the otoliths of adult halibut, although most Hg in the otoliths is also methylmercury. Such a large difference could point to the fact that Hg in otoliths may not be influenced from the dietary uptake, nor could it be related to Hg levels in tissues. As such, it is presumably related with the Hg available in the water.

Evidently, the information gathered from the otoliths of red emperor and Pacific halibut demonstrated that Hg in the otoliths was predominantly organic mercury (e.g., methylmercury). Inorganic Hg was present only at sub-ng g⁻¹ levels. Further, total Hg levels were distinctly different between the two otolith samples, which could be attributed to the geo-chemical differences between the habitats of the two fish species (e.g., eastern Pacific Ocean for halibut and northwest coast of Western Australia for the red emperor) [2, 3, 6, 8, 50]. This result is consistent with the laboratory exposure studies [35] and also supports the assumption above that Hg content in otoliths is most likely mediated by Hg available in the water.

4. Conclusion

A cold vapor generation procedure has been developed and applied to the determination of Hg species and their composition in fish otoliths by ICP-MS. It is demonstrated that careful optimization of NaBH₄ concentration as a strong reducing agent affords selective determination of Hg(II) and total Hg levels. Potassium ferricyanide provides rapid and effective removal of residual Hg from the sample introduction system. It proved to be better washout solution for CVG determinations of Hg compared with gold chloride and thiol-containing chelating agents. The procedure is highly robust, free of memory effects and offers sufficiently low detection limits for accurate determination of small concentrations of Hg in otoliths by ICP-MS.

The results suggest that fish otoliths contain Hg at ng g⁻¹ levels, which is largely methylmercury. In addition, despite its very low concentrations, Hg content in the otoliths could be significantly different provided that the habitats of the fish are different as in the case of red emperor (L. sebace) and Pacific halibut (H. stenolepis). It is therefore assumed that Hg concentration in fish otoliths could be a useful chemical tag in otolith microchemistry. It is important to note that this assumption is based on the fact that otolith Hg originates through deposition from the water, and is not biomagnified (e.g., methylated) in the otolith. Although the results ascertain the presence of methylmercury in the otoliths, more studies should be conducted through controlled laboratory exposures to elucidate if methylmercury concentration in otoliths is related to its concentration in the water or is derived from the methylation of inorganic Hg.

Acknowledgements

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Fig. 5. Inorganic Hg and total Hg content in the otoliths of red emperor (CRM 22) and pacific halibut. Values are mean ± standard deviation for five replicate measurements.

through the CVG manifold for 45–60 s. Under these conditions, the detection limits based on the 3 times the standard deviation of blank (n = 12) were 4.2 ng L⁻¹ and 6.4 ng L⁻¹ for Hg(II) and total Hg. The relative standard deviation (n = 5) was lower than 5% at 1.0 μg L⁻¹ level for Hg(II) and CH₃Hg(I). The detection limits were mainly limited by the background signals of the blank solution (5% (v/v) HCl). Yet, they were comparable to those obtained by isotope dilution (ID) CVG-ICP-MS (−1 ng L⁻¹) using SnCl₂ as reductant [42, 43], which offers better precision and reproducibility due to the nature of ID analysis.

3.5. Mercury composition of fish otoliths

The otolith certified reference material of red emperor (CRM 22) and the otolith samples extracted from Pacific halibut were digested according to the optimized dissolution conditions and were analyzed for Hg(II) and total Hg by using 1 x 10⁻⁴% (v/v) NaBH₄ and 0.5% (v/v) NaBH₄, respectively. Calibration was performed with standards that contained Hg(II) and CH₃HgCl at equal concentrations from 0 to 1.0 μg L⁻¹. The results for Hg(II) and total Hg are illustrated in Fig. 5. The concentrations were at sub-μg g⁻¹ levels for the otoliths of both species. For red emperor, Hg(II) and total Hg concentrations were 0.010 ± 0.001 μg g⁻¹ and 0.038 ± 0.004 μg g⁻¹, respectively. Mercury levels in the Pacific halibut otoliths were even lower; 0.006 ± 0.001 μg g⁻¹ for Hg(II) and 0.021 ± 0.003 μg g⁻¹ for total Hg. Because of these significantly low concentrations, Hg(II) levels were verified with an alternate method using 1% (v/v) SnCl₂ in 5% (v/v) HCl as a highly selective reductant that reduces only Hg(II) to elemental Hg. The results were 0.008 ± 0.001 μg g⁻¹ for the red emperor and 0.004 ± 0.001 μg g⁻¹ for the Pacific halibut. These values were not significantly different from those obtained with 1 x 10⁻⁴% (v/v) NaBH₄ and thus confirmed that the contribution to Hg(II) from organic Hg was not significant.
References