ABSTRACT

A method for the determination of concentrations and speciation of dissolved chromium in seawater was developed in this study and applied to the analysis of chromium in the waters of Saanich Inlet. A magnesium coprecipitation method was used to allow for the preconcentration and isolation of the trivalent fraction, and a reduction step employed to reduce all chromium to its trivalent form to allow for complete scavenging. Graphite furnace atomic absorption (GFAAS) and quadrupole inductively coupled plasma mass spectrometry (ICP-MS) was used for the analysis. The detection limit of the method was a minimum of 0.26 nM on the GFAAS and 1.18 nM on the ICP-MS, with an analytical precision of <1-20% (n=3) for both methods.

The magnesium coprecipitation method was used to examine the seasonal variations in chromium in the Saanich Inlet – a seasonally anoxic fjord on Vancouver Island that is typically subject to renewal events that reoxygenate the bottom of the inlet and alter the dominant redox conditions of the water column. Anoxia was consistent below 100-140 m depths through the months of November 2015, January and February 2016, with the persistence of dissolved hydrogen sulfide in the bottom waters indicative of the lack of strong renewal in 2015. The chromium seasonal cycle appeared to be controlled instead by a combination of biologically-mediated reduction and removal from the surface waters, a diffusive flux of chromium driven by the strong concentration gradient of the two species across the oxycline, and a particulate export of chromium to the sediments. Chromium concentrations were observed to accumulate over the fall and winter months, culminating in a drawdown of the hexavalent fraction and the export of particle-reactive trivalent chromium on organic particulates to the sediment following the onset of a spring bloom. An examination of the speciation of dissolved chromium from the months of February to August may be necessary to provide a more comprehensive understanding of the seasonal cycle of chromium in Saanich Inlet.
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Finally, I would like to thank my family back in Singapore, and my Dance Horizons family here in Vancouver, for dealing with my stressful moments with love and patience. Stephanie – thank you for bearing with me.
1 INTRODUCTION

1.1 Overview

The examination of the biogeochemical distribution and behavior of trace elements in natural waters has continually improved over the last few decades, owing to the consistent improvement of analytical instruments and their associated methods. These trace elements may exist in different forms within the environment, including various oxidation states, forms of complexation (organic and inorganic complexes) and physical states (dissolved, particulate etc) (Bruland and Lohan, 2003). Different forms undergo different biogeochemical processes and have widely varying implications for toxicology (Allen et al., 1980), implying that an understanding of the concentrations of each form is vital, not merely the total concentration of the element of interest.

1.2 Chromium

In the case of chromium, the biological and toxicological effects of the metal are a function of its oxidation state. While chromium can exist in oxidation states ranging from 2- to 6+, only trivalent chromium (Cr(III)) and hexavalent chromium (Cr(VI)) are found in the range of pH and redox potentials typical of seawater (Losi et al., 1994).

1.2.1 Redox Equilibrium and Reaction Kinetics

The distribution of Cr(III) and Cr(VI) is controlled by redox reactions, defined by the equilibrium equation (1), given below (Elderfield, 1970). The ratio of Cr(VI) to Cr(III) can therefore be derived, as shown in equation (2).

\[
\text{Cr(H}_2\text{O)}_4\text{(OH)}_2^{+} \leftrightarrow \text{CrO}_4^{2-} + 6\text{H}^{+} + 2\text{H}_2\text{O} + 3\text{e}^{-} \quad (1)
\]

\[
\log[\text{CrO}_4^{2-} / \text{Cr(H}_2\text{O)}_4\text{(OH)}_2^{+}] = 6\text{pH} + 3\text{pε} - 66.1 \quad (2)
\]

It is typically assumed that oxic seawater has a pH of 8.1 and a pε of 12.5. Under these conditions, Cr(VI) should dominate at a factor of approximately \(10^{20}\). The relationship between pH, pε and the dominant dissolved species of Cr is shown in Figure 1.
The pε of seawater is largely controlled by a dominant redox couple. Under oxidizing conditions, the O$_2$/H$_2$O$_2$ couple at [H$_2$O$_2$] = 0.1 µM results in a pε of 6.5 (Pettine and Millero, 1990), and a subsequent Cr(VI)/Cr(III) ratio of approximately ~100. However, under reducing conditions the SO$_4^{2-}$/H$_2$S couple dominates, resulting in a pε of -4 and an average pH of 7.5, allowing Cr(III) to be greater than Cr(VI) by an approximate factor of $10^{33}$ (Mugo, 1997). This implies that Cr(VI) should be the principal species in oxic conditions, and Cr(III) predominant in reducing, suboxic to anoxic waters.

*Figure 1.* pE-pH relationship for aqueous Cr (Mugo 1997, modified from Cranston 1979)
According to the above redox equilibrium, Cr(VI) should be the dominant form of chromium in oxic waters. However, experimental derivations of chromium speciation have shown an appreciable concentration of Cr(III) relative to Cr(VI) under oxic conditions, in contradiction to the thermodynamic relationship between the two species (Cranston and Murray, 1978; Pettine and Millero, 1990). It has been suggested that this may be due to the slow kinetics of the oxidation of Cr(III) to Cr(VI) using dissolved oxygen, thus allowing for this residual pool of Cr(III) to be measured. Schroeder and Lee (1975) measured a rate of oxidation of 3% in 30 days at room temperature (22°C-26°C). Cr(III) has been shown to oxidize more rapidly in reactions with manganese oxides, with rates dependent on the concentrations of MnO_2 present (Schroeder and Lee, 1975). In contrast, Cr(VI) was found to reduce in seconds when reacting with ferrous ions, and in hours to days when reacting with ferrous iron-bearing minerals, even in the presence of oxygen (Eary and Rai, 1989).

### 1.2.2 Adsorption and Precipitation Processes

The precipitation of the relatively insoluble hydroxo complexes Cr(OH)₃ and (Cr, Fe)(OH)₃ is highly dependent on pH and the concentration of trivalent Cr in the water column, and results in relatively low aqueous concentrations unless solubilized by organic complexation (Rai et al., 1989). Cr(VI) compounds are more soluble in natural waters, and are therefore more likely to remain aqueous (Losi et al., 1994).

Aqueous Cr(III) cations tend to be poorly soluble and adsorb strongly to the negatively-charged surfaces of deprotonated oxides at the ambient pH of natural waters (pH 5-9), while Cr(VI) oxyanions are repelled from these surfaces (Cranston and Murray, 1980). Absorption is thus expected to be a significant process for trivalent Cr species and not for chromate, but is strongly pH-dependent. Cranston and Murray (1980) added a Cr(III) spike to riverine and estuarine water samples from the Columbia River and found that >70% of the spike had been absorbed to container walls and suspended particles in the water within an hour. A spike of Cr(VI) in a parallel experiment resulted in a loss of <10%. Hexavalent Cr is therefore thought to be significantly more mobile in aquatic systems relative to trivalent species.
1.2.3 Toxicology of Chromium

The biological activity, chemical behavior and toxicology of trivalent and hexavalent Cr are markedly different. Cr(III) appears to be a vital component for the control of glucose and lipid metabolism in mammals (Anderson, 1989). Cr(VI) is known to have toxic and carcinogenic effects, causing damage to proteins and DNA owing to its strong oxidizing potential and ability to permeate the biological membrane (Zhitkovitch, 2005). The greatly contrasting effects of these two Cr species indicates that an understanding of its speciation is crucial for the evaluation of toxicity in environmental and biological samples.

1.3 Speciation Analysis

Methods employed in the speciation of chromium involve the isolation of either Cr(III) and Cr(VI), and the determination of the total Cr. Cr is commonly found in seawater at trace metal concentrations, ranging from approximately 2-10 nM (Mayer, 1988). The salts present in seawater (3.5 wt% total dissolved solids) also result in strong matrix effects on most instruments. Thus, a separation and preconcentration step is required before analytical determination, as well as a suitably sensitive analytical instrument.

1.3.1 Existing Analytical Methods

Various analytical methods for seawater include the use of coprecipitation (Cranston and Murray, 1978; Aydin and Soylak 2009), phase extraction (Béni et al., 2007; Moghadam et al., 2011), stripping voltammetry (Grabarczyk, 2006) and other electrochemical methods (Jin and Yang, 1997), high-performance liquid chromatography (Martinez-Bravo et al., 2001), ion chromatography (Derbyshire et al., 1999), among others. While many of these methods have achieved sufficiently low detection limits and acceptable precision and recovery, a number of them are also tedious, costly, time-consuming, and suffer from a high reagent blank. A simple, low-blank, inexpensive and time-efficient method for the speciation of Cr was therefore needed.

1.3.2 Magnesium Hydroxide Coprecipitation

Magnesium hydroxide coprecipitation has previously been developed as a precise and accurate method for the speciation of various trace metals (Wu and Boyle, 1998; Weiss et al.,
2000; Saito and Schneider, 2006). At the low concentrations of Cr in seawater, precipitation of the analyte itself is difficult. The precipitation of Mg(OH)₂ is induced by the addition of aqueous ammonia to a sample of seawater, raising the pH and hydrolyzing the Mg as an insoluble solid. This coprecipitates the particle-reactive, labile Cr(III) through its inclusion within the lattice structure of the Mg(OH)₂ and adsorption onto the surface of the precipitate, while excluding the less particle reactive Cr(VI) from the scavenging. This coprecipitation allows for the isolation of the Cr(III) fraction of the aqueous Cr in the sample and the removal of the seawater matrix, while providing the preconcentration step needed for the analytical stage. Majority of the existing methods use the inductively coupled plasma mass spectrometer (ICP-MS), but other analytical instruments with sufficient selectivity and sensitivity like the graphite furnace atomic absorption spectrometer (GFAAS) may also be used.

1.4 Thesis Objectives

While the use of the Mg(OH)₂ coprecipitation method has been well-established as an analytical method for trace metals, it has yet to be used for the determination of Cr speciation in seawater. This work will focus on the optimization of existing Mg(OH)₂ coprecipitation methods for the analysis of Cr, as well as the adaptation of the method for use of the GFAAS and the ICP-MS.

2 METHODOLOGY

2.1 Sample Collection and Storage

Seawater samples were collected from the Saanich Inlet, BC, Canada, in the Northeastern Pacific (Figure 2). Ten samples were collected from depths of the water column in the basin, at 10, 40, 75, 90, 100, 110, 120, 135, 150, and 200 m depths. Water samples were collected with 5L Niskin bottles. 4 L of each sample was filtered within six hours of sampling, and transferred to low-density polyethylene (LDPE) Cubitainers™ (Thermo Scientific, USA) for storage.
2.2 Instrumentation and Analytical Procedure

2.2.1 Graphite Furnace Atomic Absorption Spectrometry

A Varian Spectra atomic absorption AA-300 spectrometer (Varian Techtron, Australia), equipped with a Varian Techtron GTA-96 graphite tube atomizer and longitudinal...
Zeeman background correction was used for the determination of dissolved chromium. The spectrometer and autosampler were controlled by an IBM PS-2-30 data station. A Varian Techtron chromium hollow cathode lamp operating at 7 mA (with a slit width of 0.2 nm) was the light source. Partition tubes with pyrolytic graphite coating were used in the furnace. The samples were introduced to the furnace using a Varian Techtron PSC-56 programmable autosampler, with an injection volume of 20 μL. The operating parameters and furnace program used with the GFAAS are outlined in Table 1. Blanks and a 4 ppb calibration solution were run on the instrument every six samples to account for instrumental drift.

Table 1. Operating conditions and the furnace heating program used for the determination of chromium on the Varian Spectra AA-300

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength (nm)</td>
<td>357.9</td>
</tr>
<tr>
<td>Spectral Bandwidth (nm)</td>
<td>0.2</td>
</tr>
<tr>
<td>Lamp Current (mA)</td>
<td>7</td>
</tr>
<tr>
<td>Flow Rate (Ar) (L min⁻¹)</td>
<td>3</td>
</tr>
<tr>
<td>Injection Volume (μL)</td>
<td>20</td>
</tr>
<tr>
<td>Dry Temperature (°C)</td>
<td>85 (Ramp 5.0 s)</td>
</tr>
<tr>
<td></td>
<td>95 (Ramp 40.0 s)</td>
</tr>
<tr>
<td></td>
<td>175 (Ramp 20.0 s)</td>
</tr>
<tr>
<td>Ashing Temperature (°C)</td>
<td>1000 (Ramp 5.0 s, hold 3.0 s)</td>
</tr>
<tr>
<td>Atomisation Temperature (°C)</td>
<td>2600 (Ramp 1.2 s, hold 2.0 s)</td>
</tr>
<tr>
<td>Cleaning Temperature (°C)</td>
<td>2600 (Hold 2.0 s)</td>
</tr>
</tbody>
</table>

2.2.2 Inductively Coupled Plasma Mass Spectrometry

A NexION™ 300D quadrupole inductively coupled plasma mass spectrometer (ICP-MS) (Perkin-Elmer, USA) was also utilized for the analyses. Samples are injected via peristaltic pump to the sample introduction system, consisting a standard concentric glass nebulizer and an O-ring-free cyclonic spray chamber. The operating conditions were determined by tuning with a NexION Setup Solution (Perkin-Elmer, USA). Blanks and certified reference material (CRM) solutions were analysed every 5 samples to account for instrumental drift. A rinse of 1% nitric acid was introduced to the nebulizer for 2 minutes between sample runs to prevent carry over. The monitored ion of interest was $^{52}\text{Cr}^+$. 

2.2.3 Other Instrumentation

A Sorvall ST16R centrifuge (Thermo Scientific, USA) was used for the centrifugation and separation of the Mg(OH)$_2$ precipitate. Filtration was performed with a Masterflex L/S 7553-70 peristaltic pumping mechanism from Cole-Parmer, USA.

2.3 Materials

Sterile 15 mL polypropylene centrifuge tubes with high-density polyethylene caps were supplied by VWR (USA). Two (2) mL disposable polystyrene autoanalyzer sample cups (VWR, USA) were used with the GFAAS autosampler. Seawater samples were filtered through 0.4 µm Advantec glass fibre filters (Toyo Roshi Kaisha, Japan). Four (4) L LDPE Cubitainers with LDPE foam-lined closures (Thermo Scientific, USA) were used for sample storage following filtration.

The suitability of these materials for trace metal work at the low dissolved concentrations in sea water was tested by the addition of 1 mL of 1% (w/w) HNO$_3$ to a centrifuge tube, shaking, and transferring into a sample cup. Twenty (20) µL of this acid blank was then run on the GFAAS and analyzed for chromium. Chromium present on the materials produced an absorbance signal similar to the direct injection of 20 µL of trace metal-grade 1% (w/w) HNO$_3$, falling below detection limit. Contamination of the samples from the materials was therefore negligible for analysis on the GFAAS, and a cleaning step was not employed.

2.4 Chemicals

Ultrapure water was prepared by a Barnstead MegaPure Glass Still (MP-6A) and Nanopure Diamond system (Thermo Scientific, USA). Trace metal-grade ammonium hydroxide (28% NH$_3$ in H$_2$O) from Sigma-Aldrich was used for the Mg(OH)$_2$ coprecipitation procedure. TraceMetal™ grade 70% HNO$_3$ from Fisher Scientific and analytical-grade Perdrogen™ 30% hydrogen peroxide solution from Sigma-Aldrich was used for the reduction of Cr(VI) to Cr(III). The TraceMetal grade HNO$_3$ was also diluted to 1% of its initial concentration for Mg(OH)$_2$ precipitate dissolution, Cr standard preparation, blank solutions, and rinse solution for the ICP-MS.
Blank solutions for the GFAAS and ICP-MS were prepared by adding the appropriate mass of scandium to 1% HNO₃ to produce a 100 ppb spike. Trivalent and hexavalent chromium standards of 1000 ppm were prepared by dissolving the appropriate masses of chromium (III) chloride hexahydrate and potassium dichromate (Sigma-Aldrich) in 1% (w/w) HNO₃ solution and Milli-Q water respectively. Working standards of 100 ppm to 100 ppb were obtained by serial dilutions of these primary standards. Atomic spectroscopy standard NexION Setup Solution (Perkin-Elmer, USA) was used to tune the ICP-MS before analysis. Multi-element standard Stock-4 solution (Inorganic Ventures, USA) was diluted to produce a standard of 5 ppb Cr.

2.5 Mg(OH)₂ Coprecipitation Procedure

2.5.1 Chromium (III)

Fourteen (14) mL of unacidified seawater was transferred to a 15 mL centrifuge tube and 40 μL of concentrated aqueous ammonia was added, hydrolyzing the Mg in the sample as Mg(OH)₂ and coprecipitating most of the Cr(III) (>95%). After 1.5 minutes of precipitation, the tube was inverted 3 times to mix the precipitate throughout the sample. The sample was centrifuged at a relative centrifugal force (RCF) of 4700 (~5000 rpm) for 2 minutes, and most of the supernatant discarded. Centrifuging and decanting of the precipitate was repeated. To obtain a preconcentration factor of approximately 14, the resulting precipitate was then dissolved in 1 mL 1% (w/w) HNO₃. The final sample solutions were spiked with 100 ppb of Sc as an internal standard for analysis on the ICP-MS. Twenty (20) μL of the solution was then transferred to autosampler cups to be analysed on the GFAAS, or introduced directly to the nebulizer of the ICP-MS via a peristaltic pump. The Cr measured from this represents the labile, inorganic Cr(III) fraction in the sample.

2.5.2 Total Chromium

The scavenging of dissolved chromium during the coprecipitation of Mg(OH)₂ is specific to Cr(III), and an additional step was necessary to reduce the Cr(VI) in the sample for the determination of total dissolved chromium.

Under acidic conditions, H₂O₂ acts as a strong reductant for chromium. Pettine et al. (2002) demonstrated that the rate of the reduction of Cr(VI) with a H₂O₂ addition was a
function of temperature, ionic strength and pH in excess reductant. From the first-order rate constants derived in the study, pH and [H₂O₂] conditions were approximated for the concentrations of Cr(VI) found in the Saanich seawater samples. The pH of the 14 mL sample was first adjusted to approximately 1.8 with the addition of 14 μL concentrated HNO₃. One hundred and forty (140) μL of 9.8 mM H₂O₂ was added and shaken vigorously. The sample was left standing for a minimum of 3 hours to allow for complete reduction of Cr(VI) in the sample to Cr(III). The coprecipitation step was performed with the addition of 140 μL concentrated NH₄OH. A higher volume of NH₄OH is needed for precipitation of the total chromium samples due to the lower pH conditions needed for complete reduction. The sample was then processed with a treatment similar to the previously described Cr(III) treatment (Figure 3). Cr measured from this treatment represents the total labile fraction of inorganic Cr in the sample. The Cr(VI) concentration is calculated from the difference between total labile Cr and labile Cr(III) concentrations.

Figure 3. Schematic flow chart of Mg(OH)₂ analytical coprecipitation method for Cr
2.6 Standard Additions and Calibration

Matrix effects were corrected for by the use of standard additions in the calculation of Cr concentrations in the seawater samples using the method described to isolate Cr(III). Four (4) centrifuge tubes with 14-mL seawater sample volumes were spiked with appropriate volumes of Cr(III) solution to obtain concentrations of 2, 5, 10, 20 ppb following the preconcentration step. Following preconcentration, the Cr(III) concentration was determined by GFAAS and a standard addition curve was constructed.

The linear range for calibration followed the expected range of concentrations of labile Cr in seawater. Five (5) centrifuge tubes were filled with appropriate volumes of 1000 ppm Cr(III) solution and 1% (w/w) HNO₃ to obtain calibration solutions with 1, 2, 4, 8, 15 ppb concentrations. The Cr in each calibration solution was analyzed by GFAAS. The calibration curve constructed from these analyses was used in comparison with the standards additions curve to quantify the effects of the Mg matrix interference (Figure 4). The minimization of the difference between the two curves was used to optimize the method for the GFAAS.

![Sensitivity plot](image)

**Figure 4.** Example plot of calibration and standard addition curves generated during the same run. The sensitivity is defined as the slope of the two curves.
3 DISCUSSION OF ANALYTICAL DEVELOPMENT

3.1 Applications of the Mg(OH)$_2$ Coprecipitation Method to Spectrometry

Both the GFAAS and ICP-MS are robust, precise analytical tools for trace metal detection. The magnesium hydroxide coprecipitation method was first optimized to the GFAAS before being directly applied to the ICP-MS. A direct comparison between the two in terms of ease of application, average run time and reliability of results could be drawn.

3.1.1 GFAAS for Chromium in Seawater

The GFAAS was selected for the optimization of the method due to its relatively lower operating cost and ease of maintenance. Initial runs demonstrated that the relatively high concentrations of Mg$^{2+}$ present in the matrix of the final solutions from the dissolution of the Mg(OH)$_2$ precipitate resulted in appreciable overlap of the background and analyte signals following atomization. Despite the automatic background correction function of the instrument, there was a notable reduction in sensitivity of the standard addition curves relative to the calibration curves measured on the GFAAS (Figure 4), which could be explained by the instrument overcompensating for the background signal overlap in the final absorbance output. The automatic background correction program could not be altered via the data station controlling the GFAAS, and the effect of this program on the analyses was therefore not investigated.

Spiking a calibration solution with Mg$^{2+}$ in concentrations approximately equal to that of the standard addition solutions produced a similar decrease in sensitivity. In the interest of avoiding these matrix effects, the mass of the isolated Mg(OH)$_2$ precipitate was reduced by minimizing the volume of NH$_4$OH added to each sample and the time allowed for coprecipitation to occur. A final precipitate mass of 0.070±0.002g was obtained, equating to approximately 3.5±0.1% Mg(OH)$_2$ by weight in the final solutions. With the smaller precipitate, the difference in sensitivity could be minimized to approximately 15% relative to the calibration curve (Figure 4), but this value was not consistent. Due to the variations in discrepancy between the sensitivities of the standard addition and calibration curves, the sensitivity of the calibration curve was not used to derive the concentration of Cr in the samples. Three standard addition curves were generated from three different depths of the
main station to obtain an average sensitivity (Figure 5), which could then be applied to all samples. The preparation of these additional standard addition curves resulted in a significantly longer run time per depth profile generated.

\[
\text{Average Sensitivity} = 0.0135
\]

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{average_sensitivity.png}
\caption{Average standard addition curve for November 2015, derived from the average of three standard addition curves generated from 10, 110, 200 m from Saanich Inlet.}
\end{figure}

Despite the optimization of the coprecipitation procedure, considerable issues with the ashing of the final solutions remained. The high concentrations of dissolved $\text{Mg(OH)}_2$ resulted in the ejection of smoke and residual salt particles from the furnace chamber following atomization, and the buildup of salts on the graphite tube. This buildup appeared to be causing dramatic non-linear drift of the GFAAS, with a 5-20% decrease in sensitivity for every 15-20 runs (Figure 6). This drift appears to increase in severity with continued use of the same graphite tube, and quantifying the variations in drift with time has proven to be difficult. Replacing the graphite tube with greater frequency has shown to limit the effects of this, but this translates into a higher running cost of the procedure.
The similar robustness and ease of use of the ICP-MS allowed for the direct application of the Mg(OH)$_2$ coprecipitation method following its optimization on the GFAAS. The multi-element and isotope capabilities of the ICP-MS allows for the inclusion of a Sc internal standard and for the isolation of $^{52}$Cr$^+$ from the high-Mg matrix, both of which would serve to minimize the same matrix effects that were seen on the GFAAS. The internal Sc spike also serves to account for instrumental drift. It should be noted that $^{52}$Cr$^+$ is subject to several polyatomic interferences that have not been accounted for as a result of the use of argon as a plasma gas and the HNO$_3$ solvent ($^{36}$ArO, $^{38}$Ar$^{14}$N, $^{40}$Ar$^{12}$C) (Ardini et al., 2011), which may need to be examined in future work.

The use of an internal standard allowed for the correction of variability in sensitivity between samples. The ICP-MS is able to complete a single analysis of a sample in several seconds, compared to the 78.2 seconds per run on the GFAAS. Both of these factors result in

\[ \text{Absorbance} = \frac{\text{Concentration (ppb)}}{\text{Sensitivity}} \]

**Figure 6.** Calibration curves generated during analysis for November 2015. Calibration curve #1 represents the initial sensitivity of the GFAAS. Calibration curves #2 and #3 were generated after 15 and 30 accumulative sample runs respectively.

### 3.1.2 ICP-MS for Chromium in Seawater

The similar robustness and ease of use of the ICP-MS allowed for the direct application of the Mg(OH)$_2$ coprecipitation method following its optimization on the GFAAS. The multi-element and isotope capabilities of the ICP-MS allows for the inclusion of a Sc internal standard and for the isolation of $^{52}$Cr$^+$ from the high-Mg matrix, both of which would serve to minimize the same matrix effects that were seen on the GFAAS. The internal Sc spike also serves to account for instrumental drift. It should be noted that $^{52}$Cr$^+$ is subject to several polyatomic interferences that have not been accounted for as a result of the use of argon as a plasma gas and the HNO$_3$ solvent ($^{36}$ArO, $^{38}$Ar$^{14}$N, $^{40}$Ar$^{12}$C) (Ardini et al., 2011), which may need to be examined in future work.

The use of an internal standard allowed for the correction of variability in sensitivity between samples. The ICP-MS is able to complete a single analysis of a sample in several seconds, compared to the 78.2 seconds per run on the GFAAS. Both of these factors result in
a drastically faster run time for the method on the ICP-MS. Overall, the application of the method to the ICP-MS presents a simpler and faster sample processing procedure, which may make the Mg(OH)$_2$ coprecipitation method more viable on the ICP-MS.

3.2 Procedural Blanks, Detection Limit, and Precision

3.2.1 GFAAS

Due to the minimal volumes of reagents added to the sample, the reagent blank netted from the GFAAS were below or at detection limit, with an average peak area value of approximately 0.000. The reagent blank was obtained by the direct analysis of diluted HNO$_3$, H$_2$O$_2$ and NH$_4$OH into centrifuge tubes on the GFAAS. The analytical blank was obtained by the injection of 20-µL of 1% (w/w) HNO$_3$. This was used to calculate the detection limit of the method, computed as 3 times the standard deviation of 5 analytical blanks, which was an average absorbance of 0.000837 for a new tube. This resulted in an approximate detection limit of 0.26 nM for Cr. It should be noted that due to instrumental drift with continued use of a single graphite tube, the decrease in sensitivity results in a corresponding increase in detection limit. After approximately 100 runs, the detection limit increased by approximately 77%, to 0.45 nM.

The reproducibility of the procedure was assessed by the use of five replicates of seawater samples from different depths in the Saanich Inlet. The relative standard deviation of all five values was satisfactory, at 4.4%. The precision of the samples was derived from the average of the RSD% of triplicates of each sample, which ranged from <1-20%.

3.2.2 ICP-MS

The analytical blank was obtained by the injection of 1% (w/w) HNO$_3$. This was used to calculate the detection limit of the method, computed as 3 times the standard deviation of 5 analytical blanks, which resulted in an approximate detection limit of 1.18 nM for Cr. The precision of the samples derived from the average RSD% of triplicate samples ranged from 1.5 to 10%.
3.3 Recovery Efficiency

The recovery of the Mg(OH)$_2$ coprecipitation method has been shown to be highly efficient. Wu and Boyle (1998) demonstrated that recovery did not diminish with smaller precipitates, and that the slightest precipitation of Mg(OH)$_2$ resulted in a recovery efficiency of approximately 100%. This was confirmed by the addition of radioactive Cr ($^{54}$Cr) to a seawater sample and subsequent gamma counting (D. Semeniuk, personal communication, December, 2015). Recovery was therefore not evaluated in this study.

3.4 Comparisons to Other Analytical Methods for Chromium

To assess the applicability of the method to the derivation of chromium concentrations in seawater, the detection limit of the Mg(OH)$_2$ coprecipitation method was compared to the those of existing methods in the literature that utilize either the GFAAS or the quadrupole ICP-MS (Table 2). The detection limit of the developed method was lower or similar to most of the methods listed, indicating its suitability for the determination of dissolved chromium in seawater. The coprecipitation method is straightforward in its sample preparation procedure, requires no enriched isotope spike or reagent purification, and has a relatively short run-time and low running cost. The minimal number of reagents added to the method minimizes the potential for contamination. Overall, the Mg(OH)$_2$ coprecipitation is a simple, cost-effective and robust method that eliminates the need for expensive instrumentation and complex protocol.

Table 2. Comparision of detection limits for the speciation of dissolved chromium in seawater using various GFAAS and ICP-MS analytical techniques

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Protocol</th>
<th>Detection Limit (nM)</th>
<th>Cr Species</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFAAS</td>
<td>Mg(OH)$_2$ coprecipitation</td>
<td>0.26 - 0.45</td>
<td>Cr(III), Cr(tot)</td>
<td>This work</td>
</tr>
<tr>
<td>GFAAS</td>
<td>Fe(OH)$_3$ coprecipitation</td>
<td>0.20</td>
<td>Cr(III), Cr(tot)</td>
<td>Cranston and Murray, 1978</td>
</tr>
<tr>
<td>GFAAS</td>
<td>Liquid-liquid extraction</td>
<td>0.96</td>
<td>Cr(VI), Cr(tot)</td>
<td>Béni et al., 2007</td>
</tr>
<tr>
<td>GFAAS</td>
<td>Chitosan extraction</td>
<td>0.90</td>
<td>Cr(VI), Cr(tot)</td>
<td>Xue et al., 2000</td>
</tr>
<tr>
<td>ICP-MS</td>
<td>Mg(OH)$_2$ coprecipitation</td>
<td>1.18</td>
<td>Cr(III), Cr(tot)</td>
<td>This work</td>
</tr>
<tr>
<td>ICP-MS</td>
<td>High-Performance Liquid Chromatography</td>
<td>2.50</td>
<td>Cr(VI)</td>
<td>Martinez-Bravo et al., 2001</td>
</tr>
</tbody>
</table>
4. Applications of the Method: Saanich Inlet, BC

4.1 Seasonal Stratification and Anoxia of Saanich Inlet

Saanich Inlet is a seasonally anoxic fjord located on Vancouver Island, British Columbia. The inlet has a shallow sill at its mouth that restricts flow to the deeper inner basin. Freshwater runoff from the surrounding watershed results in stratification of the upper 120 m of the water column (Figure 7), isolating deep waters from atmospheric gas exchange and oxygen renewal (Herlinveaux, 1962). High surface primary productivity also contributes to anoxic conditions at depth through the oxidation of sinking organic matter, which results in extensive sulfate reduction and the accumulation of hydrogen sulfide (Anderson and Devol, 1973). In the late summer or early fall, the upwelling of cold, saline waters along the coast of British Columbia mixes with the warm, less saline waters of the Strait of Georgia, forming a dense intermediate water mass in Haro Strait. During a typical year, the higher density of this water mass allows it to spill over the sill of Saanich Inlet, displacing some or all of the anoxic deep waters and reoxygenating the bottom of the inlet. This seasonal process is referred to as deep water renewal (Anderson and Devol, 1973). The upward displacement of anoxic bottom waters in the basin during a renewal results in a shallower suboxic layer in the late summer, and a reduction of H$_2$S levels (Zaikova et al., 2009).

Sampling was conducted from the months of April 2015 to February 2016. H$_2$S was detected qualitatively through its characteristic smell for depths below 150 m for all months. This was confirmed through the detection of H$_2$S for the months of November, January and February (M.T. Beltran, unpublished data, March 2016), indicating that anoxic bottom conditions were prevalent and that deep water renewal had been minimal.

4.2 Hydrographic Variations in Saanich Inlet

Seawater was sampled from the main station on four separate occasions: August 12 2015, November 22 2015, January 13 2016, and February 4 2016. Temperature, salinity, dissolved oxygen and dissolved hydrogen sulfide were measured in addition to dissolved Cr concentration and speciation.
Temperature and salinity measurements provided insight into the degree of deep water renewal in Saanich Inlet. The slight increase in density in August indicates that a dense water mass did enter the inlet, but the restriction of this elevated density to the upper 60 m of the water column (Figure 7) suggests that this intrusion was not dense enough to cause extensive displacement of deeper waters. The persistence of anoxic conditions and H₂S at depth confirms the minimal nature of the renewal event in August 2015 (Figures 10-13).

However, a closer examination of the temperature, salinity and resulting density measurements in the anoxic zone of the water column (120-200 m) indicate that some limited renewal did take place (Figures 7-9). Deep water temperatures increased by approximately...
2°C with salinity increasing by slightly less than 0.1 PSU from August to November 2015 (Figures 8-9). This corresponded to an increase in density (σ) of approximately 0.02 (Figure 7), which is likely due to the addition of denser water over the sill of Saanich Inlet. It should be noted that there appeared to be weak but continued renewal from November 2015 to January 2016 (Figure 7).

![Saanich Inlet salinity profiles](image)

*Figure 8.* Saanich Inlet salinity profiles for all four months sampled. Inset profiles zoom in on the salinity variations below 100 m.
Figure 9. Saanich Inlet temperature profiles for all four months sampled. Inset profiles zooms in on the temperature variations below 100 m.

4.3 Chromium in Saanich Inlet

4.3.1 Depth Profiles for Chromium and Seasonal Variations

Speciation analysis was performed on samples for the months of August 2015, November 2015, January 2016 and February 2016, using the Mg(OH)$_2$ coprecipitation method with both the GFAAS and the ICP-MS. The GFAAS was used to analyse samples from August and November, and the ICP-MS for the analysis of January and February. Four depth profiles were generated from these analyses, presented in Figures 10 to 13.
Figure 10. Saanich Inlet depth profiles for August 2015: (A) Dissolved chromium speciation; (B) Dissolved oxygen concentrations (L. Pakhomova, unpublished data).

Figure 11. Saanich Inlet depth profiles for November 2015: (A) Dissolved chromium speciation; (B) Dissolved oxygen and hydrogen sulfide concentrations (O$_2$: L. Pakhomova, unpublished data; H$_2$S: M.T. Beltran, unpublished data).
Figure 12. Saanich Inlet depth profiles for January 2016: (A) Dissolved chromium speciation; (B) Dissolved oxygen and hydrogen sulfide concentrations (O$_2$: L. Pakhomova, unpublished data; H$_2$S: M.T. Beltran, unpublished data).

Figure 13. Saanich Inlet depth profiles for February 2016: (A) Dissolved chromium speciation; (B) Dissolved oxygen and hydrogen sulfide concentrations (O$_2$: L. Pakhomova, unpublished data; H$_2$S: M.T. Beltran, unpublished data).
The variations in seasonality in speciation between months appeared to be depth-specific, with relation to the position of the oxycline. Total dissolved Cr concentrations varied seasonally, with maximum surface concentrations of 3.50 nM, and minimum concentrations at depth of 1.40 nM. Cr(III) was the dominant species below the oxycline for all four months (80-100%), but the proportion of Cr(VI) in the oxic zone of the water column was more seasonally variable. Cr(VI) accounted for 70-95% of the total dissolved chromium.

Figure 14. Comparison plots for depth profiles for Saanich Inlet for the months of August and November 2015, January and February 2016: (A) Dissolved total chromium; (B) Dissolved trivalent chromium; (C) Dissolved hexavalent chromium;
above the oxycline for the months of August and September 2015, but only 33-72% in January and February 2016.

### 4.3.1.1 Upper water column (0-100 m)

Seasonal variations between the four depth profiles appear to show an accumulation of Cr through fall and winter (August to January), followed by a drawdown in the early spring (February). This pattern is most obvious in the Cr(tot) profiles, with surface concentrations increasing from 2.36 nM in August to 3.50 nM in November, followed by a sharp decrease from 3.18 nM in January to 2.22 nM in February (Figure 14). The largest increase in Cr(tot) between August and November occurred in the upper 60 m of the water column, which coincides with the intrusion of higher density water above the sill of Saanich Inlet (Figure 7). This feature likely documents the input of high-Cr water, presumably originating from upwelling along the Pacific coast. The similarity of Cr(tot) determined in August 2015 to the data from February 2016 suggests that these low Cr concentrations are maintained over the summer (Figure 14), but the precise monthly variations in speciation should be analyzed before a conclusion can be made.

Surface Cr(III) concentrations detected in January and February (1.65 nM and 0.84 nM; Figures 12 and 13) were elevated relative to those of August and November (0.22 nM and 0.16 nM; Figures 10 and 11). Cr(III) concentrations appeared to be relatively uniform throughout the upper 100 m for each month (Figure 14), indicating that much of the variability in trends for total dissolved Cr was a result of the variation in Cr(VI) trends between the sampled months. This is particularly clear in Figure 11, as the lack of an increase in trivalent Cr concentrations that may correspond to the total Cr increase in November implies that this was driven by an input of Cr(VI).

Likewise, in January 2016 (Figure 12), total Cr and Cr(VI) concentrations were elevated between 60-100 m, implying either a lateral advection of dissolved Cr at those depths.

### 4.3.1.2 Oxycline (100-140 m)

The onset of anoxic and euxinic conditions below depth interval results in the reduction of Cr(VI) to dissolved Cr(III), increasing in concentration at depth. Cr(VI)
decreased significantly just above the oxycline (80-100 m) for all four months, while Cr(III) concentrations remained largely uniform in this same interval. This suggests that the removal of Cr(VI) did not correspond to a reduction to dissolved Cr(III), which may imply that the loss of total Cr at the oxycline is driven by the partial precipitation of relatively insoluble Cr(III) hydroxides that fall out of the water column. The decrease of Cr(VI) and increase of Cr(III) across the oxycline would likely develop a downward and upward concentration gradient respectively, which may explain the persistence of Cr(VI) and Cr(III) in redox conditions where the species are out of thermodynamic equilibrium.

The diffusional flux across the oxycline is visible in Figure 10, where Cr(VI) concentrations are substantially higher at depth despite the dominance of Cr(III) (~42% Cr(VI)). Concentrations of hexavalent Cr vary from 2.3 nM in August to 0.13 nM in February. The fluctuations in concentrations at this interval between months could be explained by a combination of reductive processes, and Cr flux between the oxycline and the deeper waters below.

**4.3.1.3 Anoxic zone (>140 m)**

Total dissolved Cr concentrations were lowest in August 2015 (1.94 nM), but appeared to increase up to 3.19 nM in January, before undergoing a drawdown to 2.66 nM in February 2016 (Figure 14). There was little variation in the dominance of Cr(III) in the anoxic zone for all four months, with consistent concentrations of 2.67-2.90 nM from November to February.

**4.3.1.4 Comparisons to existing dataset**

The most extensive time series of dissolved Cr speciation was completed by Mugo (1997). Compared to the drastic variations in Cr(III) concentrations between the oxic and anoxic zones of Saanich Inlet measured in this study, labile Cr(III) concentrations recorded in the 1994-1995 dataset were significantly more consistent for all depths from August 1994 to February 1995. Cr(VI) was determined to be the dominant species of dissolved Cr for this same time period throughout the water column, accounting for 60% to 82% of the total labile Cr at 200 m depth. In contrast, Cr(III) accounted for more than 90% of the total dissolved Cr at 200 m depth for all profiles generated with the Mg(OH)$_2$ coprecipitation method, with
trivalent Cr dominating the anoxic zones in all months. The variation in total Cr concentrations recorded by Mugo (1997) was also significantly greater, with a range of 0.74 nM to 7.20 nM.

There was evidence of strong seasonal deep water renewal occurring in the depth profiles generated by Mugo, with the deepening of the oxic-anoxic boundary in late summer and the oxygenation of the anoxic zone in the subsequent months. It is possible that the environmental conditions of the bottom waters of Saanich Inlet may change in the absence of a renewal event, increasing the saturation level for Cr(III) hydroxides below the oxycline to allow for the accumulation of labile Cr(III) at depth. This may explain the difference in dominant Cr species between the two datasets, as an undersaturated water column would keep Cr(III) in solution. Alternatively, the presence of Cr(VI) in deep water reported by Mugo may be a consequence of this strong deep water renewal.

4.3.2 Controls on Cr Speciation and Abundance

The speciation of Cr in seawater is largely controlled by the presence of oxidizing or reducing agents. The increase in proportion of Cr(III) relative to the total labile Cr with increasing depth can be explained as a rapid and thorough reduction of Cr(VI) to Cr(III), possibly due to the occurrence of H₂S in the anoxic bottom waters of Saanich Inlet (Pettine et al., 1994). The seasonal accumulation and flushing of H₂S with periodic stratification and deep water renewal would result in a greater change in the ratio of Cr(III)/Cr(VI) between months, but the lack of a recent strong renewal event could explain the persistence of H₂S and Cr(III) below the oxycline (Figures 11-13). The change in dominant redox couple in the anoxic zone is likely to be the most important determinant of the relative abundance of the two species, but the speciation of Cr could also be a function of the input and removal processes involved in Saanich Inlet.

The accumulation of Cr(tot) in the upper 100 m of the water column from August to November 2015 could be explained by the circulation of water masses in Saanich Inlet and the Strait of Georgia. Circulation in Saanich Inlet itself is slow due to weak winds and tidal currents, and flow is restricted due to the 75 m deep sill at the mouth of the fjord (Gargett and Whitney, 2003). Surface water enters Saanich Inlet by flowing over the sill from the Strait of Georgia. In the fall, the onset of storms and winds could induce mixing and the upwelling of
intermediate or deep waters from the Pacific that may have a higher concentration of dissolved Cr due to accumulation and remineralization over the summer. This high-Cr water can then flow into Saanich Inlet, acting as an input of regenerated labile Cr(VI) to the surface layer and resulting in the gradual increase in Cr(tot) concentrations over the winter. This inflow of water from the Strait of Georgia is typically associated with the sinking of the denser intermediate water mass in Haro Strait (Anderson and Devol, 1973), but the persistence of H$_2$S at depth in the winter of 2015 indicates that the base of the water column has not been reoxygenated through a deep water renewal event. This suggests that the influence of the intrusion of high-Cr intermediate water from the Strait of Georgia was minimal and largely limited to the upper layer of the inlet.

It has been demonstrated that the occurrence of elevated primary production in the form of a phytoplankton bloom may be accompanied by the biologically-mediated photoreduction of iron (III) to iron (II), producing reducing agents that could reduce Cr(VI) to Cr(III) (Li et al., 2009). An early increase in primary productivity in January 2016 in the form of a bloom may explain the increase in Cr(III) and drawdown of Cr(VI) in the upper 40 m of the water column. The subsequent drawdown of Cr(III) without a corresponding oxidation to Cr(VI) in February 2016 may be a result of the increased scavenging and removal of the Cr species with an increase in downward particle flux arising from a phytoplankton bloom, as Cr(III) is significantly more particle-reactive than Cr(VI) (Cranston & Murray, 1980). Further data on the level of primary productivity in the form of fluorescence or chlorophyll concentrations will be needed to substantiate this claim.

It should be noted that the developed method involves the filtering of seawater samples before coprecipitation, thus preventing the determination of particulate Cr concentrations. The more particle-reactive Cr(III) has a greater tendency towards a particulate form, which implies that the reduction of Cr(VI) removes the Cr(III) in the water column and precipitates it as a solid. This process appeared to be most significant at the oxycline, where the sharp reduction in total Cr concentrations for all four months (Figure 10-13) suggests that the reduction of Cr(VI) to particulate Cr(III) resulted in its exclusion from Cr speciation measurements performed. The development of a concentration gradient for both Cr species across the oxycline may induce the diffusional flux of Cr(VI) into euxinic
waters and its subsequent rapid reduction, which may provide a significant mechanism for the removal of Cr from oxic waters.

Following from this, there appears to be two main mechanisms for Cr removal from the mixed layer of the water column to the deeper anoxic zone: the biologically-mediated reduction of Cr(VI) during a phytoplankton bloom and the subsequent scavenging of the reduced Cr(III), and the diffusive flux of Cr(VI) into euxinic waters in combination with the consequent reduction and precipitation of insoluble Cr(III) hydroxides. The relative importance of these two processes appear to be seasonally variable and strongly dependent on the annual cycle of productivity of the inlet.

Changes to the speciation of Cr at depth is likely to be a function of the transferromg of Cr from the surface to the deeper anoxic zone via the two mechanisms mentioned above, and the scavenging of Cr(III) to the sediment. In the absence of strong deep water renewal adding high-Cr water to the anoxic zone of Saanich Inlet, the accumulation of total Cr at 200 m from August to January (Figure 14) indicates that the addition of Cr from surface removal processes is greater than the scavenging of Cr at depth during this time period. Conversely, the reduction of total dissolved Cr concentrations in February at this same depth (Figure 14) suggests that removal of Cr(III) to the sediments is higher following a period of high primary production. This can be attributed to the higher settling flux of particulate organic matter and other biogenic particles following a phytoplankton bloom (Timothy et al., 2003), which may increase the concentration of particles at depth and allow for greater scavenging of Cr(III).

4.4 Comparisons with Other Anoxic Environments

Few studies of anoxic to euxinic marine basins have determined the variations in dissolved chromium speciation with depth. Seasonally anoxic lakes have been much more thoroughly examined, and may serve as a proxy for a comparison to the highly stratified water column of Saanich Inlet. Achterberg (1997) analysed the changes in trace metal speciation of a seasonally anoxic lake (Esthwaite Water, UK) upon the onset of anoxic conditions in summer. In the months of August and September 1997, there was a visible depletion of Cr(VI) below the hypolimnion, with %Cr(III) values approaching 100% close to the bottom of the lake. The greatest increase in the ratio of Cr(III) to Cr(VI) occurred at the approximate depth of the hypolimnion, which was similar to the contrast in speciation
between oxic and anoxic zones seen in Saanich Inlet. The drastic change in dominant species of labile Cr could be explained by the accumulation of Fe(II) below the hypolimnion in Esthwaite Waters (Achterberg 1997), in which the reducing agent acts to extensively reduce labile Cr(VI) present in the water column to its trivalent form (Rai et al., 1989). The variations in ratio between the two Cr species is driven largely by a change in the dominant redox couple of the various layers of the water column in this locality, which is proposed to also be the most important determining factor for the speciation of Cr in Saanich Inlet.

4.5 Summary and Conclusions

Dissolved Cr speciation in Saanich Inlet is largely a factor of the prevailing redox couple present, and the different redox conditions between the oxic surface and deep anoxic layers of the water column result in a sharp contrast in Cr(III) and Cr(VI) concentrations across the oxycline. The persistence of euxinic conditions at depth in the months of November 2015, January and February 2016 indicate the lack of recent strong renewal from the intrusion of denser, oxygenated, high-Cr water from the Strait of Georgia. In the absence of significant deep water renewal, the seasonal variations in Cr speciation and concentration is likely to be driven instead by a combination of biologically-mediated removal and diffusive flux.

The seasonal cycle of Cr in Saanich Inlet when not controlled by renewal events can be described as a series of input and removal processes between the upper oxic mixed layer and the deeper anoxic zone. The accumulation of Cr as Cr(VI) over the fall and winter results in increasing concentrations in the mixed layer, which results in a strong diffusive flux driven by the downward concentration gradient of Cr(VI) across the oxycline. Cr concentrations correspondingly accumulate at depth with the additive effect of this strong diffusion of Cr. The drawdown of Cr(VI) and reduction to Cr(III) in the mixed layer may be due to the onset of a spring bloom, which increases the particle flux to the sediment and the scavenging of Cr(III) at the surface and at depth, resulting in lower concentrations overall. The variations in speciation and concentration of Cr from the months of February to August have not been examined in this study, and a year-long time series may provide a more complete understanding of the seasonal Cr cycle in Saanich Inlet.
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Inlets, British Columbia, Canada: Sources and seasonal patterns. \textit{Progress in


Appendix A

A.  Protocol for dissolved Cr(III) and Cr(tot) determination

A.1  Materials
0.4 µm glass fibre filters; 15mL polypropylene centrifuge tubes; autoanalyzer sample cups;

A.2  Chemicals
Trace metal-grade 70% nitric acid (HNO₃); trace metal-grade aqueous ammonia (28% NH₃ in H₂O); analytical-grade 30% hydrogen peroxide (H₂O₂); chromium (III) chloride hexahydrate; potassium dichromate; NexION Setup Solution; Stock-4 solution, Scandium (Sc) internal standard solution;

A.3 Mg(OH)₂ Coprecipitation Procedure
1. Dilute concentrated HNO₃ in Milli-Q to obtain two 1% (w/w) HNO₃ solutions. Add a 100 ppb Sc internal standard spike to one dilute HNO₃ solution. Dilute 30% H₂O₂ in Milli-Q to obtain a 9.8mM solution.
2. Working standards of 100 ppm obtained from the serial dilution of chromium (III) chloride hexahydrate in 1% HNO₃ and potassium dichromate in Milli-Q water.
3. Dilute the appropriate masses of 100 ppm Cr(III) standard solution to create a calibration solution series with concentrations of 1, 2, 4, 8, and 15 ppb.
4. Transfer two aliquots of 14 mL of unacidified seawater to a 15 mL centrifuge tube.
5. Transfer eight other 14 mL aliquots of the same seawater and add the appropriate masses of 100 ppm Cr(III) solution to obtain standard addition solutions with approximate concentrations of 0.0714, 0.179, 0.357, 0.714 ppb (to obtain final concentrations of 2, 5, 10 and 20 ppb following the preconcentration step).
6. Weigh each tube with a mass balance to determine its initial mass.
7. (For Cr(tot) determination) Adjust the pH of one 14 mL aliquot and one standard additions solution series to 1.8 with the addition of 14 µL of concentrated HNO₃.
8. (For Cr(tot) determination) Add 140 µL of 9.8 mM H₂O₂ to each of the five solutions and shake vigorously. Leave standing for at least 3 hours to allow for full reduction.
9. *(For Cr(tot) determination)* Add 140 μL of concentrated NH₄OH to the treated solutions after full reduction of Cr(III). Allow for 1.5 minutes of precipitation before inverting the tubes 3 times to mix the precipitate evenly throughout the sample.

10. *(For Cr(III) determination)* Add 40 μL of concentrated NH₄OH to one untreated 14 mL aliquot, and to each solution for one standard additions series. Allow for 1.5 minutes of precipitation before inverting the tubes 3 times to mix the precipitate evenly throughout the sample.

11. Centrifuge the samples and standard addition solutions at 4700 RCF (~5000 rpm) for 2 minutes. Decant the supernatant. Repeat this centrifugation and decantation step to remove as much of the supernatant as possible.

12. Dissolve the precipitates with the addition of 1 mL of 1% HNO₃ to each centrifuge tube. If samples are to be analyzed on the ICP-MS, use the 1% HNO₃ solution with the 100 ppb Sc internal standard.

13. Weigh each of the final solutions. Use the difference between the initial and final masses of each sample to obtain the concentration factors for each sample.

14. *(For GFAAS analysis)* Transfer 20 μL of each sample, standard addition, calibration, and blank (1% HNO₃) solution to autosampler cups.

15. *(For GFAAS analysis)* Begin each analysis by generating a calibration curve from the calibration solutions. Run the sample and standard addition solutions consecutively, with a blank run every 3 samples and another calibration curve every 15 runs.

16. *(For ICP-MS analysis)* Spike the multi-element Stock-4 standard and blank (1% HNO₃) solutions with a 100 ppb Sc internal standard. Dilute the Stock-4 solution to 5 ppb Cr with Milli-Q.

17. *(For ICP-MS analysis)* Introduce the sample solutions to the ICP-MS directly. Blank solutions should be run on the instrument every 6 samples, and Stock-4 solution at the beginning and end of each analysis. A rinse solution of 1% HNO₃ should be run on the instrument for 2-3 minutes between each sample to prevent carry over.
Appendix B

Table B1. Dissolved chromium measurements from Saanich Inlet, August 2015.

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>Cr(III) (nM)</th>
<th>Cr(III) Error (nM)</th>
<th>Cr(tot) (nM)</th>
<th>Cr(tot) Error (nM)</th>
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Table B2. Dissolved chromium measurements from Saanich Inlet, November 2015.

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>Cr(III) (nM)</th>
<th>Cr(III) Error (nM)</th>
<th>Cr(tot) (nM)</th>
<th>Cr(tot) Error (nM)</th>
<th>Cr(VI) (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.15725</td>
<td>0.03891</td>
<td>3.50442</td>
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<td>3.34716</td>
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<td>3.03944</td>
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<td>0.03952</td>
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</tr>
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</tr>
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</tr>
<tr>
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Table B3. Dissolved chromium measurements from Saanich Inlet, January 2016.

<table>
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<th>Depth (m)</th>
<th>Cr(III) (nM)</th>
<th>Cr(III) Error (nM)</th>
<th>Cr(tot) (nM)</th>
<th>Cr(tot) Error (nM)</th>
<th>Cr(VI) (nM)</th>
</tr>
</thead>
<tbody>
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Table B4. Dissolved chromium measurements from Saanich Inlet, February 2016.

<table>
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<th>Cr(III) (nM)</th>
<th>Cr(III) Error (nM)</th>
<th>Cr(tot) (nM)</th>
<th>Cr(tot) Error (nM)</th>
<th>Cr(VI) (nM)</th>
</tr>
</thead>
<tbody>
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<td>0.07789</td>
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<td>0.22055</td>
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<td>0.12158</td>
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<tr>
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<td>0.22055</td>
<td>0.03690</td>
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