Title: Chromium uptake and adsorption in marine phytoplankton – implications for the marine chromium cycle

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Abstract

Using the radioisotope ^{51}Cr , we investigated the controls of cellular Cr accumulation in an array of marine phytoplankton grown in environmentally relevant Cr concentrations (1-10 nM). Given the affinity of Cr(III) for amorphous Fe-hydroxide mineral surfaces, and the formation of these mineral phases on the outside of phytoplankton cells, extracellular Cr was monitored in a model diatom species (*Thalassiosira weissflogii*) as extracellular Fe concentrations varied. Extracellular Cr in *T. weissflogii* increased with increasing extracellular Fe, demonstrating that Cr may be removed from seawater via extracellular adsorption to phytoplankton. Short-term Cr(VI) and Cr(III) uptake experiments performed with *T. weissflogii* demonstrated that Cr(III) was the primary oxidation state adsorbing to cells and being internalized by them. Cellular Cr:C ratios ($<0.5 \mu\text{mol Cr mol C}^{-1}$) of the eight phytoplankton species surveyed were significantly lower than previously reported Cr:C ratios in marine particles with a high biogenic component (10 to $300 \mu\text{mol Cr mol C}^{-1}$). This indicates that Cr(III) likely accumulates in marine particles due to uptake and/or adsorption. Mass balance calculations demonstrate that surface water Cr deficits can be explained via loss of Cr(III) to exported particles, thereby providing a mechanism to account for the nutrient depth profile for Cr in modern seawater. Given the large fractionation of stable Cr isotopes during Cr(VI) reduction, Cr(III) associated with exported organic carbon is likely enriched in lighter isotopes. Most sedimentary Cr isotope studies have thus far neglected internal fractionating processes in the marine Cr cycle, but our data indicate that loss of Cr to exported particles may be traced in the sedimentary $\delta^{53}\text{Cr}$ record.

1. Introduction

Chromium (Cr) as oxidized Cr(VI) is a known carcinogen and pervasive industrial contaminant, and its behavior in contaminated aqueous systems has been intensely studied in order to mitigate its impact on human and ecosystem health (reviewed by Cervantes et al., 2001; Zayed and Terry 2003; Costa and Klein 2009; Owlad et al., 2009). However, far less is known about the processes that affect the distribution and speciation of Cr in uncontaminated seawater. Given the recent development and application of the stable Cr isotope system in the marine environment (e.g. Frei et al., 2011; Bonnand et al., 2013; Reinhard et al., 2014; Scheidrich et al., 2015), a full account of the sources, sinks, and internal processes affecting the modern marine Cr cycle is needed.

At equilibrium, Cr(VI), as the soluble oxyanion chromate (CrO_4^{2-}), is predicted to be the dominant oxidation state in oxic, alkaline seawater (Elderfield 1970; Pettine and Millero 1990). However, Cr(III) can sometimes make up more than 50 % of the total dissolved Cr in surface waters (Cranston and Murray 1980; Nakayama et al., 1981ab; Rue et al., 1997; Sander et al., 2003; Connelly et al., 2006), predominantly as Cr(OH)_2^+ and the particle reactive aqua-hydroxy species Cr(OH)_3^0 (Rai et al., 1987). The presence of Cr(III) may be due to reduction of Cr(VI) to Cr(III) mediated by a steady state production of reducing agents (such as organic matter, Fe(II), H_2O_2 , and reactive oxygen species) by biota or light-mediated reactions, followed by slow oxidation of Cr(III) by oxygen (half-life > 100 days; van der Weijden and Reith 1982), and stabilization of Cr(III) by organic ligands (Schroeder and Lee 1975; Nakayama et al., 1981a; van der Weijden and Reith 1982; Hug and Laubscher 1997; Pettine et al., 1998; Sander and Koschinsky 2000; Li et al., 2009).

In oceanic waters, total dissolved Cr concentrations are similar to trace metal nutrient profiles (Cranston 1983; Jeandel and Minster 1987; Mugo and Orians 1993; Rue et al., 1997; Sirinawin et al., 2000; Sander et al., 2003; Connelly et al., 2006). There is a slight Cr drawdown in the surface layer – between 5 and 30 % – and enrichment at depth (Cranston 1983; Jeandel and Minster 1987; Mugo and Orians 1993). This indicates that dissolved Cr in surface waters is drawn down by incorporation into sinking particles, and then remineralized at depth. Indeed, particulate organic carbon (POC) and Cr exported to 3200 m were correlated in the Sargasso Sea (Connelly et al., 2006), and particulate Cr and POC concentrations were correlated in the English Channel (Auger et al., 1999). Sedimentary Cr and organic matter content are often positively correlated (Schroeder and Lee 1975; Brumsack and Geieskes 1983; Francois 1988; Beck et al., 2008), indicating that they may share a common source, such as phytoplankton. Total dissolved Cr concentrations in North Atlantic Deep Water increase progressively as the water mass transits from the north to the south Atlantic (Jeandel and Minster 1987), suggesting particulate associated Cr is released to the dissolved phase during remineralization of organic matter.

While the physiological response of bacteria and algae to Cr toxicity is well documented (reviewed by Wong and Trevors 1988) little is known about Cr incorporation into phytoplankton in unpolluted waters. Few studies have been performed with marine phytoplankton species (Schroll 1978; Frey et al., 1983; Riedel 1984; 1985; Wang and Guo 2000; Wang and Dei 2001). In addition, studies of marine phytoplankton (e.g. Riedel 1984) have used relatively high Cr(VI) concentrations (≥ 100 nM) compared to the total dissolved Cr levels found in surface-ocean waters (1-10 nM). While Cr(VI) may enter marine phytoplankton via inadvertent uptake by a sulfate transporter when Cr(VI) is high (≥ 100 nM) and sulfate concentrations are low (< 2.9 mM), it is likely this pathway is less important in ambient seawater containing high sulfate and

low Cr(VI) concentrations (Riedel 1984; 1985). There is tentative evidence that Cr(III) may be taken up by phytoplankton (Schroll 1978; Wang and Guo 2000), but these authors did not distinguish between Cr(III) internalized and adsorbed to the extracellular matrix. Thus, it is yet unknown whether Cr(VI) or Cr(III) are incorporated into marine phytoplankton inhabiting high salinity and low Cr seawater.

Chromium may also be removed from seawater by directly adsorbing to phytoplankton cell surfaces or onto minerals formed at the cell surface. When inorganic Fe concentrations are high, amorphous Fe-hydroxide minerals like ferrihydrite form on the outside of phytoplankton cells in laboratory studies (Hudson and Morel 1989; Sunda and Huntsman 1995; Ho et al., 2003; Tovar-Sanchez et al., 2003; Sternberg et al., 2005; Tang and Morel 2006) and in natural communities (Tovar-Sanchez et al., 2003; Frew et al., 2006). Chromate can form weak complexes with the surfaces of Fe minerals in groundwater (Zachara et al., 1987). However, competing oxyanions (e.g. sulfate) drastically reduce chromate adsorption to Fe-hydroxide minerals (Cranston and Murray 1978; Nakayama et al., 1981b; Zachara et al., 1987). In contrast, Fe minerals strongly scavenge Cr(III), and are used purposely to scavenge Cr(III) before determinations of Cr(III) concentrations in seawater (Cranston and Murray 1978; Nakayama et al., 1981b). Adsorption of Cr(III) to extracellular Fe-minerals may thus be an important sink for dissolved Cr from surface waters.

Using the radioisotope ^{51}Cr , we investigated Cr accumulation by an array of marine phytoplankton species. Due to the possibility of extracellular Cr adsorption, we first tested the efficacies of different extracellular Cr washes. Extracellular Cr in a model diatom species (*Thalassiosira weissflogii*) was also monitored as extracellular Fe concentrations were altered. Short-term Cr(VI) and Cr(III) uptake experiments were performed with *T. weissflogii* to

determine which oxidative state was adsorbed onto and/or internalized into the cells. The results reported herein are the first attempt to quantify cellular Cr concentrations in marine phytoplankton in uncontaminated seawater.

2. Methods

2.1. Phytoplankton Strains and Media Preparation

Phytoplankton isolates were obtained from either the North East Pacific Culture Collection (NEPCC) or the National Center for Marine Algae and Microbiota (NCMA) (formerly the Provasoli-Guillard Culture Center for Marine Phytoplankton; CCMP) (Table 1). The species chosen represent dominant eukaryotic phytoplankton phyla in coastal- and open ocean waters, whose trace metal physiologies are well-studied.

All culture bottles and plasticware (Teflon and polycarbonate) used to prepare the media were soaked in a dilute Extran detergent for 48 h, rinsed with deionized water, soaked in 10% nitric acid for 48 h, and rinsed six times with ultrapure water (18 M Ω cm; Milli-Q, Millipore). Media plasticware (polycarbonate, Nalgene) was first filled with Milli-Q and microwave sterilized before use. All cultures were maintained in the artificial seawater medium Aquil (Price et al., 1989) prepared as described in Maldonado et al., (2006). Synthetic ocean water (SOW) was prepared with the base Aquil salts, and included 10 μ M phosphate, 100 μ M silicic acid, and 300 μ M nitrate. The SOW was passed through a Chelex 100 resin to remove any contaminating trace metals, and microwave sterilized in 2 L Teflon bottles. Once cool, the sterile SOW was amended with filter sterilized vitamins (thiamine, B₁₂, biotin). A suite of trace metals bound to EDTA was also added, and the total dissolved concentrations in the media were: 100 μ M EDTA, 44 nM Co, 10.2 nM Cu, 110 nM Mn, 70 nM Zn, 100 nM MoO₄²⁻, and 10 nM SeO₄²⁻. Iron was

added bound to 1.5-times excess EDTA at a final concentration of 1370, 685, 373, 171, 84, or 42 nM. These Fe additions resulted in EDTA-buffered inorganic Fe concentrations ($[Fe']$) of 1729, 801, 433, 216, 106, and 53 pM, respectively. This includes both thermal and light-mediated dissociation of Fe(III)EDTA at 20 °C (calculated following Sunda et al. 2005). A 50 μ M Cr(VI) stock was made from Na₂CrO₄ (Sigma-Aldrich) in MilliQ, and the final media Cr(VI) concentrations were either 1, 5, or 10 nM. The media was allowed to equilibrate overnight before use.

Background Cr(VI) and Cr(III) concentrations in the media were determined via quadrupole ICP-MS using a modified version of the Mg(OH)₂ co-precipitation method (c.f., Wu and Boyle 1997). Synthetic ocean water was spiked with 5 nM ⁵¹Cr(VI), ⁵¹Cr(III), or an equimolar 10 nM mixture of both. A 2 mL aliquot of ⁵¹Cr-doped SOW was mixed with enough 1 M ammonium hydroxide to produce a small precipitate, and the precipitate was allowed to react for period of time before being centrifuged. Complete extraction of Cr(III) occurred after just 1 minute exposure, while ~5% Cr(VI) was extracted (Table 2). The Cr(VI) extraction was likely due to residual ⁵¹Cr(VI) in the pellet matrix. Cr(III) is favored in acidic conditions, and we determined that complete ⁵¹Cr(VI) reduction occurs in acidified SOW (pH ~2) after 3 weeks. Thus, total background Cr concentrations were determined in acidified media, and Cr(VI) concentrations were determined by the difference between total Cr and Cr(III) concentrations. Background Cr(VI) and Cr(III) concentrations were determined in 50 mL of high Fe media, and were 0.18 ± 0.02 nM ($\pm 1\sigma$; N=3) and 0.06 ± 0.01 nM ($\pm 1\sigma$; N=3), respectively. The specific activity of ⁵¹Cr(VI) and ⁵¹Cr(III) additions to the seawater media were corrected for the background Cr concentrations.

2.2. Culturing Techniques

All culture manipulations and handling occurred inside a Class 100 clean hood using trace metal clean techniques (Maldonado and Price 1996). The phytoplankton isolates were kept in exponential growth phase by serial dilution in 27 mL polycarbonate bottles, grown at 19 ± 1 °C in 24 h continuous light under cool, white fluorescent bulbs ($\sim 130 \mu\text{mol photons m}^{-2} \text{s}^{-1}$). Culture biomass was monitored and growth rates were calculated as previously described (Maldonado and Price 2001). Cell concentrations and diameters were measured using a Coulter Z2 Particle Count and Size Analyzer (Beckman Coulter Inc.). Cell surface area and volume were calculated assuming the cells were spherical.

2.3. Extracellular Cr Washes

Given that Cr(III) adsorbs to Fe precipitates in seawater (Cranston and Murray 1978), we determined the efficacy of previously published cellular washes to remove extracellular Cr. Four washes were tested and compared to a control treatment (10 mL SOW rinse; Table 3). The Ti-citrate-EDTA wash removes extracellular Fe by reduction of the extracellular Fe-precipitate and subsequent complexation of the solubilized Fe(II) by EDTA (Hudson and Morel 1989). Ti(III)-citrate ($E^\circ = -480 \text{ mV}$; Hudson and Morel 1989) should easily reduce CrO_4^{2-} adsorbed to the cell surface ($E^\circ = -130 \text{ mV}$; CRC Handbook 2015). Therefore, if a significant fraction of Cr(VI) is adsorbed to the cell, then the Ti-citrate-EDTA wash should also remove it. The oxalate-EDTA wash dissolves Fe-precipitates by ligand-promoted dissolution (Tang and Morel 2006). Two modifications of the oxalate wash were also tested. First, EDTA was replaced with DTPA, a ligand with a stronger affinity for Fe(III) (and, presumably, Cr(III)). Second, a strong Fe(III) and Cr(III)-binding ligand desferrioxamine B (Duckworth et al., 2014) was added to the oxalate-

EDTA wash. DFB can dissolve amorphous $\text{Cr}(\text{OH})_3$ precipitates and ferrihydrite at similar rates (Duckworth et al., 2014). Oxalate, in the presence of DFB, dissolves the Fe mineral goethite at a rate ten times faster than when DFB is absent (Cheah et al., 2003). Thus, we would expect the oxalate-EDTA-DFB wash to outperform the oxalate-EDTA wash. The washes were prepared and applied as previously described (Table 3; Hudson and Morel 1989; Tang and Morel 2006). The oxalate wash performs optimally at pH 7.0-7.5 (Tang and Morel 2006), and so we chose 7.5 to reduce the risk of damaging the more fragile phytoplankton strains. The Ti-citrate-EDTA wash works optimally at pH 8 (Hudson and Morel 1989). Neither the Ti-EDTA nor the oxalate-EDTA washes cause lysis in *T. weissflogii* (Hudson and Morel 1989; Tang and Morel 2006). Although we did not determine if DTPA or DFB cause cell lysis, this is unlikely during the short exposure time. A similar DTPA wash did not significantly damage *E. huxleyi* (Walsh and Ahner 2014). DFB is commonly used in Fe physiology studies (e.g. Allnutt and Bonner 1987; Maldonado and Price 1999; Shaked et al., 2005; Shi et al. 2013), and has a high specificity for Fe(III).

A 500 mL culture containing 1370 nM total Fe and 5 nM $^{51}\text{Cr}(\text{VI})$ (sodium chromate in saline solution, pH 8; specific activity $\sim 500 \text{ TBq mol}^{-1}$; Perkin Elmer) was inoculated with acclimated *T. weissflogii* growing in mid-exponential phase, and allowed to grow for at least 6 divisions to ensure all the cellular Cr pools were homogenously labeled with ^{51}Cr . In mid-exponential phase ($10,000 \text{ cells mL}^{-1}$), 20 mL aliquots were harvested by gentle vacuum filtration onto 25 mm diameter, 5 μm porosity polycarbonate filters (AMD) placed onto 25 mm GF/F filter backings. Just before the filters went dry, the washes were applied and allowed to completely permeate the filters. For the oxalate wash treatments, two 5 min sequential washes were applied with gentle vacuum filtration between each, followed by a rinse with 10 mL of SOW to remove any loosely associated tracer. The filters were placed in 7 mL borosilicate

scintillation vials and immersed in 5 mL of Scintisafe 50% scintillation cocktail (Fisher) for 24 h in the dark. ^{51}Cr activity was measured in each sample using a Beckman LS65005514 scintillation counter with an internal ^{51}Cr quench curve. Cellular Cr concentrations (mol cell^{-1}) were calculated by dividing the activity on each filter (DPM filter^{-1}) by the specific activity of the ^{51}Cr stock (DPM mol^{-1}), the volume filtered (mL filter^{-1}) and the cell concentration (cells mL^{-1}).

2.4. Steady-State Cellular Cr Concentrations

Cellular Cr concentrations were determined under steady-state conditions primarily using Cr(VI). Although Cr(III) can make up a large portion of the total dissolved Cr pool, particularly in coastal waters and near hydrothermal vent systems, a significant amount of it is likely complexed by organic ligands with unknown thermodynamic properties (Nakayama et al., 1981b; Sander and Koschinsky 2000; Sander et al., 2003). Organic complexes of Cr(III) in seawater are electrochemically inert, and so the concentrations and thermodynamic binding constants of Cr(III) organic ligands are unknown (Sander and Koschinsky 2000). Due to the complexation of Cr(VI) by four oxygen atoms, CrO_4^{2-} is likely inert towards organic complexes, and it is often the dominant oxidation state in open ocean surface waters (Sirinawin et al., 2000; Connelly et al., 2006). Given the relative uncertainty in Cr(III) speciation chemistry in seawater, we determined cellular Cr concentrations in using Cr(VI). Since Cr(III) may be more particle reactive than Cr(VI), this may result in underestimates of cellular Cr concentrations. Thus, for one species, Cr(III) was used in order to compare with the Cr(VI) treatment.

We performed a detailed investigation of the controls of cellular (total and intracellular) Cr concentrations in the model diatom *Thalassiosira weissflogii*. Following the method described

in section 2.3, cellular Cr was determined in *T. weissflogii* grown in media containing constant total dissolved Fe (1370 nM) and varying Cr(VI) concentrations (1, 5, and 10 nM $^{51}\text{CrO}_4^{2-}$). In addition, cellular Cr was measured in media containing constant Cr(VI) concentrations (5 nM $^{51}\text{CrO}_4^{2-}$) and varying $[\text{Fe}^+]$. To characterize interspecies variation, cellular Cr concentrations were determined in eight species (Table 1) grown in media containing 1370 nM Fe and 5 nM $^{51}\text{CrO}_4^{2-}$. Cellular Cr concentrations in the presence of Cr(III) were also determined for *T. weissflogii* grown with 5 nM $^{51}\text{Cr(III)}$ and 1370 nM total dissolved Fe. The Cr(III) would have been complexed by the 100 μM EDTA in the medium, resulting in ~ 0.2 pM inorganic Cr(III) (calculated using MINTEQ version 3.1).

For each experiment, a small inoculum of each acclimated species was added to four replicate 125 mL pre-equilibrated media containing 5 nM ^{51}Cr , and allowed to grow for at least 6 generations (between 3 and 8 days, depending on the growth rate of the species). To ensure there was no loss of ^{51}Cr tracer to the bottle walls during the incubations, we measured the total ^{51}Cr activity (DPM per mL) at the beginning and end of each culture, and found no significant loss for all species and treatments. In mid-exponential growth phase, the cultures were split, and each half was filtered onto a 25 mm polycarbonate filter of appropriate porosity (between 1 and 5 μm). Total cellular Cr was determined on one filter using 10 mL chelexed SOW rinse. Since all washes removed the same amount of Cr (see section 3.1), we opted to use the oxalate-EDTA wash to remove extracellular Cr on the second filter, followed by a 10 mL SOW rinse. Previous work has demonstrated that the oxalate-EDTA wash does not significantly damage cells of the *Thalassiosira* and *Emiliana* strains (Tovar-Sanchez et al. 2003; Tang and Morel 2006; Hassler et al., 2009). Although we did not test the effect of the wash on the remaining strains (*P. pouchetti*, *C. polylepis*, and *P. minimum*), there were no differences between washed and unwashed cellular

Cr concentrations (see Table 4). This indicates that the oxalate-EDTA wash did not cause any significant cell lysis in these strains.

2.5. Short-Term Cr Uptake Rates

Short-term uptake assays (2.5 h time interval) of both $^{51}\text{Cr(VI)}$ and $^{51}\text{Cr(III)}$ were performed with *T. weissflogii* to determine if Cr(VI) or Cr(III) adsorb to or enter the cell. $^{51}\text{Cr(III)}$ was produced by reducing an aliquot of the $^{51}\text{Cr(VI)}$ stock with 200 μM sodium sulfite at pH 2 (using ultraclean HCl, Fisher) for 15 min. The addition of sulfite to the media would have been negligible compared to the total sulfate concentration in the media (28 mM). The reduction of Cr(VI) by sulfite is a second order reaction, and occurs rapidly at low pH (Pettine et al., 2006). The half-life of this reaction is approximately 30 s, and complete reduction of $^{51}\text{Cr(VI)}$ after 10 min was confirmed by co-precipitation of the $^{51}\text{Cr(III)}$ with Mg(OH)_2 after it was added to SOW (Wu and Boyle 1997). The oxidative half-life of Cr(III) is long (> 100 days by O_2 ; van der Weijden and Reith 1982), and so the Cr(III) would not have been oxidized over the course of the assay.

A 500 mL culture containing 1370 nM total dissolved Fe and 5 nM CrO_4^{2-} was inoculated with *T. weissflogii*, and grown under the same conditions as the cellular Cr concentration assays. In late-exponential phase (85 000 cells mL^{-1}), the culture was split into two 250 mL sterile polycarbonate bottles that were amended with either 100 nM $^{51}\text{Cr(VI)}$ or $^{51}\text{Cr(III)}$. Over 2.5 h, duplicate aliquots of each culture (10 mL) were filtered onto 25 mm diameter, 5 μm porosity polycarbonate filters in duplicate for either total cellular ^{51}Cr (SOW rinse) or intracellular ^{51}Cr (oxalate wash + SOW rinse) at each time point. At the end of the assay, filter blanks in each treatment were performed in duplicate by passing 10 mL of the assay solution through a 0.22 μm

filter (Acrodisk), and treating the filtrate as a sample. Filter blanks for the Cr(VI) treatment made up ~18% of the ^{51}Cr signal, while the Cr(III) blanks were negligible (<1%). The ^{51}Cr detected on the filters was used to calculate cellular Cr as described above (subtracting filter blanks). The volumetric ^{51}Cr activity in the assays did not change over the course of the assay, and so loss of the isotope to the container walls was negligible. The uptake assays were performed in ambient laboratory temperature and light.

3. Results

3.1. Efficacy of Cellular Cr Washes

All four washes removed extracellular Cr equally well (Figure 1; ANOVA, $p < 0.05$; Tukey). Using the reductive Ti wash to reduce Cr(VI) to Cr(III) did not significantly increase extracellular Cr removal compared to the non-reductive ligand promoted dissolution washes. Thus, it is likely that negligible Cr(VI) was adsorbing to the cell. The addition of ligands (DTPA and DFB) with high affinity for Fe—and, thus, Cr(III)—had no effect on the removal of extracellular Cr, indicating that the oxalate-EDTA wash was effective at removing nearly all of the extracellular Cr. The majority of Cr(III) that rapidly adsorbed to *T. weissflogii* during the short-term accumulation experiment was also removed by the oxalate-EDTA wash (see section 3.2). Thus, this wash was used for the remaining experiments.

It is possible that a portion of the remaining cellular Cr in the washed treatments is not inside the cell, but rather part of a kinetically inert pool on the outside of the cell. Thus, what we consider to be “intracellular” may be an unknown mixture of intracellular and extracellular Cr. As a result, the reported intracellular Cr concentrations are operationally defined.

3.2. Short-Term Cr(VI) and Cr(III) Accumulation onto and Inside *T. weissflogii*

Short-term accumulation of total Cr(VI) was approximately linear for the first 100 minutes, with a corresponding accumulation rate of $5 \pm 1 \text{ zmol cell}^{-1} \text{ min}^{-1}$ (Figure 2a). No clear trend was observed for intracellular Cr(VI) during the assay, and so an accumulation rate was not calculated. There was no clear difference between intracellular and total cellular Cr(VI) accumulation. However, accumulation of Cr(III) was significantly higher than Cr(VI) (Figure 2a vs. 2b). For example, after 5 min exposure to Cr(III), total cellular and intracellular Cr(III) levels were 19- and 6.5-fold higher, respectively, than in the presence of Cr(VI). By the end of the assay, the difference between Cr(III) and Cr(VI) accumulation increased to 46-fold for total cellular Cr and 32-fold for intracellular Cr levels (Figure 2a and 2b). Total cellular Cr(III) accumulated rapidly within 5 and 30 min (7.1 to $15.5 \text{ amol cell}^{-1}$), and then linearly thereafter (Figure 2b). A smaller but more rapid accumulation of intracellular Cr(III) occurred after 5 min ($2.6 \text{ amol cell}^{-1}$), and intracellular Cr accumulated linearly during the rest of the assay. The rate of total cellular Cr(III) accumulation calculated after 30 min ($122 \pm 19 \text{ zmol Cr cell}^{-1} \text{ min}^{-1}$) was approximately twice as fast as the intracellular accumulation rate ($65 \pm 11 \text{ zmol Cr cell}^{-1} \text{ min}^{-1}$) (Figure 2b), and ~25 times faster than the total cellular Cr(VI) accumulation rate.

3.3. Steady-State Cellular Cr Concentrations and Cr:C Ratios

For the eight phytoplankton species grown in 1370 nM total dissolved Fe and 5 nM $^{51}\text{Cr(VI)}$, total and intracellular Cr concentrations ($\mu\text{mol Cr L}^{-1}$ biovolume) varied 16-fold and 10-fold, respectively (Table 4). The average concentrations were 1.05 ± 1.01 and $0.61 \pm 0.50 \mu\text{mol Cr L}^{-1}$ biovolume for the total and intracellular pools, respectively. Cell-normalized Cr concentrations varied slightly less for the total (12-fold) and intracellular (7-fold) pools, with

average concentrations of 161 ± 118 and 97 ± 75 zmol Cr cell^{-1} , respectively (Table 4). Between 0 and 67% of the cellular Cr was located on the outside of the cell. All three diatoms accumulated extracellular Cr, while only one of the non-diatom species did (*E. huxleyi* 732a).

Cellular Cr:C ratios were calculated using published cellular carbon concentrations (Table 4). Cellular C will vary with nutrient and light availability, and potentially between strains of the same species, and so it is possible that these calculations have a systematic error. We account for this by using cellular C concentrations determined for the same strains grown under nearly identical conditions as used in the present study (i.e. same medium composition, and similar light intensities). Total and intracellular Cr:C ratios ranged between 0.01 and 0.69 $\mu\text{mol Cr mol C}^{-1}$, and were highly variable (34 and 51-fold variation, respectively). In the presence of 1370 nM dissolved Fe, *T. weissflogii* cellular Cr increased ~7.5-fold as $^{51}\text{Cr(VI)}$ concentrations varied from 1 to 10 nM (Table 5), demonstrating that cellular Cr is concentration dependent. Furthermore, cellular Cr in *T. weissflogii* increased ~8-fold when 5 nM $^{51}\text{Cr(VI)}$ was replaced with $^{51}\text{Cr(III)}$ (Table 5).

Cell-normalized intracellular – but not total cellular – Cr concentrations were positively correlated with cell volume ($r^2 = 0.76$, $p < 0.05$) and surface area ($r^2 = 0.72$, $p < 0.05$). If extracellular Fe precipitates were controlling Cr adsorption, then we would expect extracellular Cr concentrations to co-vary with cell surface area. For the four species that accumulated extracellular Cr, there was no significant correlation between surface area and extracellular Cr ($p > 0.05$).

3.4. Extracellular Fe-oxides Accumulate Cr

In the growth media with the three lowest inorganic Fe concentrations (between 53 and 216 pM), Cr did not accumulate on the outside of *T. weissflogii* (Figure 3). Interestingly, the $[Fe']$ in these treatments are near the solubility of Fe' in seawater (~ 0.1 nM; Kuma et al., 1996; Liu et al., 2002). When the inorganic Fe concentration in the media was 433 pM and higher, total cellular Cr was significantly higher than intracellular Cr ($p < 0.05$; one-way t-test, $N=4$). In these treatments, between 22 and 55% of the cellular Cr was extracellular.

4. Discussion

4.1. Extracellular Cr Accumulation

The formation of amorphous Fe-hydroxide minerals on the cell surface can make up 10 to 65% of the total cellular Fe pool in *T. weissflogii* (Hudson and Morel 1989; Ho et al., 2003; Tang and Morel 2006) when grown in inorganic Fe concentrations similar to those used here (30 to 450 pM). Given the tendency of Cr to adsorb to Fe precipitates, in our study, extracellular Cr in *T. weissflogii* increased from 22 to 55% as $[Fe']$ increased significantly above the solubility of Fe in seawater (~ 100 pM; Kuma et al., 1996; Liu et al., 2002). Furthermore, when grown at a constant, and high inorganic Fe concentration (1729 pM $[Fe']$), extracellular Cr levels in *T. weissflogii* increased ~ 8 -fold as $^{51}Cr(VI)$ increased from 1 to 10 nM (Table 5). This indicates that accumulation of Cr onto cells is also dependent on Cr concentration. For the four species that accumulated extracellular Cr, between 26 and 67% was extracellular, suggesting that extracellular Cr can make up a significant fraction of total cellular Cr. Thus, when $[Fe']$ is high, extracellular Fe-oxides can scavenge Cr out of solution; and this scavenging process is both dependent on Fe' and Cr concentrations.

Although such inorganic dissolved Fe concentrations are uncommon in surface seawater environments due to strong complexation by organic ligands (e.g., 0.1 to 1 pM; Sander et al., 2015), between 20 and 80% of particulate Fe in surface waters are associated with Fe-hydroxide mineral phases (Tovar-Sanchez et al., 2003; Frew et al., 2006; Rauschenberg and Twining 2015). Since Cr(III) rapidly adsorbs to Fe-hydroxide minerals in seawater, while the chromate oxyanion does not (Cranston and Murray 1978; Nakayama et al., 1981b), it was our aim to determine whether Cr(III) or Cr(VI) was adsorbing to extracellular Fe-hydroxide minerals on *T. weissflogii*. The short-term Cr uptake assay confirmed that Cr(III) rapidly adsorbed to *T. weissflogii*, while Cr(VI) adsorbed to the cell 25-times more slowly. This is likely due to competition between Cr(VI) with the high sulfate concentration in seawater (Cranston and Murray 1978; Nakayama et al., 1981b). Similarly, the Cr concentration of *T. weissflogii* grown with 5 nM $^{51}\text{Cr(III)}$ instead of $^{51}\text{Cr(VI)}$ was ~8-fold higher, despite the low Cr(III) (~0.2 pM) buffered by the EDTA in the medium. Thus, the majority of extracellular Cr likely accumulates as Cr(III).

Cr(VI) was added to the majority of the experiments, so if Cr(III) is the dominant oxidation state associated with the extracellular matrix of *T. weissflogii*, then Cr(VI) reduction must occur outside the cell prior to adsorption. Li et al., (2009) observed that phytoplankton-mediated Cr(VI) reduction is dependent on light, and occurred outside of the cells. This reduction was likely catalyzed by photo-induced production of reducing agents such as oxygen and organic carbon free radicals, H_2O_2 , or Fe(II) (Schroeder and Lee 1975; van der Weijden & Reith 1982; Douglas et al., 1986; Kaczynski and Kieber 1994; Hug and Laubscher 1997; Pettine et al., 1998; Sander and Koschinsky 2000). Phytoplankton can either directly or indirectly produce all these reducing agents (e.g. Palenik et al., 1987; Maldonado and Pride 2001; Kustka et al., 2005; Marshall et al., 2005). The light-dependency of Cr(VI) reduction by phytoplankton

can account for the indistinguishable extracellular Cr in *T. weissflogii* during the short-term $^{51}\text{Cr(VI)}$ accumulation assay. These experiments were performed under ambient laboratory light ($\sim 10 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$), which is significantly lower than the incubator light intensity used ($\sim 130 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$).

If Cr accumulation onto the extracellular, amorphous Fe hydroxide minerals was due to passive adsorption of $^{51}\text{Cr(VI)}$ from the media onto the mineral surface, then we would expect extracellular Cr to increase as $[\text{Fe}^{\text{II}}]$ increased. This has been observed for other elements that adsorb to extracellular amorphous Fe-hydroxide minerals (e.g. Ba, P, V) (Sternberg et al., 2005; Tang and Morel 2006). Although extracellular Cr increased between 216 and 801 pM inorganic Fe, it did not continue to increase at the highest $[\text{Fe}^{\text{II}}]$ treatment. We calculate that less than 0.2% of the total dissolved Cr was incorporated into the cells in the highest inorganic Fe treatment, and so this plateau was not limited by total dissolved Cr availability. Since the extracellular Cr is likely to be Cr(III), adsorption sites for Cr(III) could have been scarce due to competition with other ions present at much higher concentrations (e.g. 10 μM phosphate). As extracellular Fe increased, more adsorption sites would become available to scavenge Cr(III). Thus, extracellular Cr(III) would increase as extracellular Fe increases, but only until all of the Cr(III) produced in the medium is scavenged.

Extracellular reduction and the subsequent adsorption of Cr(III) to phytoplankton may be an important removal pathway for dissolved Cr in seawater, and published field data support these laboratory results. The dissolved Cr(III):Cr(VI) ratios in surface waters of the Sargasso sea were correlated with phytoplankton productivity during some months (Connelly et al., 2006), suggesting that phytoplankton are imparting a kinetic control on the oxidation state of Cr in seawater. Interestingly, only the diatoms and *E. huxleyi* 732a accumulated extracellular Cr,

suggesting that the other four species were unable to reduce Cr(VI). If Cr(III) is the primary oxidation state adsorbing to marine phytoplankton, the diatoms were more effective at reducing Cr(VI), and thus may impart a greater influence on the oxidation state of Cr in natural waters. In support of this, the only diatom (*Phaeodactylum tricornutum*) examined by Li et al., (2009) was able to reduce ~4-times more Cr(VI) than the green and red algae species surveyed. Li et al., (2009) did not examine prymnesiophytes or dinoflagellates, but our data suggest they enzymatically reduce Cr(VI) to a lesser extent than diatoms.. This may explain why Connelly et al., (2006) only observed correlations between phytoplankton productivity and the dissolved Cr(III):Cr(VI) ratio in some months (September 1994, March and June 1995). During these months, diatom abundance was elevated compared to other months (Steinberg et al., 2001). Thus, phytoplankton species composition will likely influence the reduction of Cr(VI) to Cr(III) in surface waters.

4.2. Intracellular Cr Accumulation

The average intracellular Cr concentration for the eight species surveyed (0.61 ± 0.50 $\mu\text{mol Cr L}^{-1}$ biovolume) is much lower than most bioactive metals (3 to 700 $\mu\text{mol L}^{-1}$ biovolume; Ho et al., 2003). While some animals require trace amounts of Cr for sugar metabolism (Anderson 1989; 1997), there is no known metabolic Cr requirement in plants. Thus, the low intracellular Cr concentration in phytoplankton is likely due to incidental Cr transport into the cell (e.g. via sulfate transporters; Riedel et al., 1984; 1985) or adsorption to unknown cell wall components. This is supported by the positive correlations between cell-normalized intracellular Cr concentrations and cell surface area and volume, whereby intracellular Cr concentrations scale with cell size despite surveying diverse phytoplankton species.

Short-term Cr(VI) internalization by *T. weissflogii* was low relative to Cr(III) and not linear, while there was a small accumulation of total cellular Cr(VI) during the first 100 min of the assay. However, there was no clear difference between intracellular and total cellular Cr. This suggests that the trace amount of Cr(VI) associated with *T. weissflogii* might be due to a combination of uptake via sulfate transporters and non-specific adsorption to cell wall material, but not adsorption to extracellular Fe-oxides. We are unable to rule out either possibility.

Similarly, the low cellular Cr concentrations in the steady-state experiments could be partially due to a combination of non-specific extracellular adsorption and intracellular uptake of Cr(VI). Short-term and steady-state cellular accumulation rates – calculated by multiplying the cellular quota (zmol Cr cell^{-1}) by growth rate (d^{-1}) – should be the same when the cells in each assay are experiencing the same conditions (Morel 1987). The calculated steady-state accumulation rate for *T. weissflogii* grown with $10 \text{ nM } ^{51}\text{Cr(VI)}$ was $231 \pm 46 \text{ zmol Cr cell}^{-1} \text{ d}^{-1}$, or equivalent to $0.01 \text{ amol Cr cell}^{-1} \text{ h}^{-1}$. Assuming this uptake rate scales linearly with Cr(VI) concentrations up to 100 nM , then the steady-state accumulation rate of 100 nM Cr(VI) would be $\sim 0.1 \text{ amol Cr cell}^{-1} \text{ h}^{-1}$. Thus, over the course of the 2.5 h short-term assay, we would expect the intracellular Cr concentration to be $\sim 0.25 \text{ amol Cr cell}^{-1}$. This is similar to the range of $^{51}\text{Cr(VI)}$ that accumulated in *T. weissflogii* during the short-term assay (0.4 to $0.6 \text{ amol Cr cell}^{-1}$). Cr(VI) may be an important species entering phytoplankton – via incidental uptake by sulfate transporters – inhabiting polluted estuarine environments (with high Cr and low sulfate) (Riedel 1984; 1985). However, little Cr(VI) likely enters phytoplankton in unpolluted seawater environments (with low Cr and high sulfate), due to competition with sulfate (Riedel 1984; 1985).

In contrast to Cr(VI), Cr(III) accumulated inside *T. weissflogii* rapidly and linearly. The quick association of $^{51}\text{Cr(III)}$ within 5 min in the washed cells indicates that a small pool of Cr(III) was not removed by the oxalate wash (e.g. Cr(III) associated with cell-surface transporters). Given the rapid rate of Cr(III) internalization in *T. weissflogii*, Cr(III) is likely the dominant oxidation state entering phytoplankton in most marine environments. Although little is known about Cr transport in eukaryotic phytoplankton, trivalent metals are known to be taken up. For example, Al(III) can enter freshwater phytoplankton species (Crémazy et al., 2013), and Fe(III) is internalized by Fe(III) permeases in marine and freshwater phytoplankton (Hudson and Morel 1990; Maldonado et al., 2006; Glaesener et al., 2013).

4.3. Comparisons with Field Estimates of Biogenic Cr

There are few published studies of particulate Cr concentrations in surface seawater (Cranston 1983; Dauby et al., 1994; Auger et al., 1999; Sander et al., 2000). Only one (Dauby et al., 1994) reports particulate Cr concentrations along with particulate organic carbon (POC) concentrations. Particulate matter sampled at stations in the Celtic Sea (stations 29-34 in Dauby et al., 1994) was dominated by phytoplankton derived organic carbon (determined by $\delta^{13}\text{C}$ consistent with marine phytoplankton; -22 to 24 ‰) and had the smallest contributions of lithogenic metals (characterized by lower Al (<2.2 mg Al g sediment⁻¹) and Ti (<47 µg Ti g sediment⁻¹) concentrations). Median particulate Cr/Al and Cr/Ti ratios at these stations were ~20-times higher than the remaining stations that were dominated by lithogenic and terrigenous particle sources. These stations were also the farthest offshore, and are unlikely contaminated by industrial sources. Thus, the particulate Cr and organic carbon at these stations were dominated

by marine sources, and the Cr:C ratios are likely representative of Cr in autochthonous marine biogenic particles.

The total particulate Cr:C ratios at the Celtic Sea stations (mean: 65; median: 21; range: 10 to 297 $\mu\text{mol Cr mol C}^{-1}$) are between 15 and 15,000 times higher than the total cellular Cr:C ratios measured in our laboratory cultures. Since so few measurements of particulate Cr:C_{org} have been reported, it is difficult to compare our lab data with the Celtic Sea samples.

Interestingly, the marine fraction of particulate Cr and C_{org} was carefully determined for recently exported particulate matter in the Cariaco basin, and the Cr:C_{org} (10 to 40 $\mu\text{mol Cr mol C}^{-1}$) were similar to the Celtic Sea (Calvert et al. 2015). Sediment trap material was collected at 225 m. This depth is below the permanent pycnocline in the basin (~150 m), but above the anoxic zone (~275 m) (Scranton et al. 2001). Thus, the Cr:C_{org} values reported for the Cariaco Basin – and, thus, the Celtic Sea – are likely representative of exported biogenic Cr:C_{org}.

The field Cr:C estimates indicate that our laboratory work largely underestimates the cellular Cr concentrations of natural plankton communities. Since we added $^{51}\text{Cr(VI)}$ to most of our experiments, and Cr(VI) associates with phytoplankton cells at insignificant rates, this suggests that Cr accumulation by natural plankton communities is due to either Cr(III) internalization or adsorption to cells. Indeed, cellular Cr in *T. weissflogii* increased nearly 10-fold when $^{51}\text{Cr(VI)}$ was replaced with $^{51}\text{Cr(III)}$, despite the majority of the $^{51}\text{Cr(III)}$ being chelated by the high EDTA concentration in the medium. Inorganic Cr(III) can make up to 50% of the total dissolved Cr pool in surface seawater (e.g. Nakayama et al., 1981b). Thus, Cr(III) adsorption and/or internalization by phytoplankton are likely important loss terms for dissolved Cr in marine waters

4.4. Implications for Marine Cr Biogeochemistry

We have shown that adsorption and internalization of Cr(III) by marine phytoplankton can occur, and Cr in marine biogenic particles is likely Cr(III). Using the northeast subarctic Pacific as a case study, we can demonstrate that the loss of Cr to biogenic particles can account for the majority of the dissolved Cr lost from surface waters during an annual cycle. Mugo and Orians (1993) report total dissolved Cr concentrations in the sunlit mixed layer at Ocean Station Papa (OSP; 50°N 145°W) of ~2.7 nM, and this increases to 4 nM at 500 m depth. Thus, approximately 1.3 nM was exported from surface waters with sinking particulate matter and remineralized at depth. The average yearly carbon export rate at 200 m (just below the permanent pycnocline in this region at ~150 m which sets the depth of the winter mixed layer; Gargett 1991) is $\sim 4 \text{ mol C m}^{-2} \text{ y}^{-1}$ (Timothy et al., 2013). Mixed layer depths at OSP shoal to 40 m throughout the spring and summer due to warming (Whitney and Freeland 1999), with an average depth of ~80 m. Using the median estimate ($21 \text{ } \mu\text{mol Cr mol C}^{-1}$) of Cr:C ratio for marine biogenic particles reported for the Celtic Sea (Dauby et al., 1994), we estimate that $\sim 1 \text{ nmol Cr L}^{-1} \text{ y}^{-1}$ would be lost from an 80 m mixed layer depth. This value corresponds well with the observed 1.3 nM draw down of dissolved Cr. Thus, loss of Cr(III) to phytoplankton in surface waters can account for a significant portion of dissolved Cr exported below the pycnocline in this region over an annual period.

4.4. Reframing Marine Stable Cr Isotope Systematics

The use of stable Cr isotope systematics in the marine environment relies on understanding the possible sources, sinks, and redox transformations of Cr in seawater, as well as the fractionation factors associated with these processes. Cr(VI) is fractionated more than most

transition metals during biotic and abiotic reduction, and fractionation factors can be up to -4‰ (Cr(III) favoring the lighter isotopes) (Ellis et al., 2002; Zink et al. 2010; Han et al., 2012; Jamieson-Hanes et al., 2012; Kitchen et al., 2012; Basu et al., 2014; Wang et al. 2015). Thus, stable Cr isotopes may provide information about redox chemistry in past environments (e.g. Schoenberg et al., 2008; Frei et al., 2009; Crowe et al., 2013; Planavsky et al., 2014).

Chromium in igneous rocks is primarily found as Cr(III), and is oxidized during continental weathering to produce isotopically heavy Cr(VI) in surface and subsurface aqueous environments (Crowe et al., 2013). This isotopically heavy Cr(VI) is eventually transported to seawater, via submarine groundwater discharge and continental run-off, where it is well-mixed in the oceans (residence time of >10,000 years; Campbell and Yeats 1984; Reinhard et al., 2014). Thus, stable Cr isotopes in marine sediments have been used to track the oxygenation of the atmosphere and ocean in earth's history (Frei et al., 2009; 2011; Crowe et al., 2013; Frei and Polat 2013; Reinhard et al., 2014). However, these studies did not consider how internal processing of Cr in the ocean might alter its isotopic composition. Deep water dissolved $\delta^{53}\text{Cr(VI)}$ in seawater is enriched in lighter Cr compared to surface waters, and has a range of 1‰ across the major global basins (Scheiderich et al., 2015). Dissolved Cr(VI) isotopes undergo Rayleigh fractionation at a global scale, and Scheiderich et al. (2015) suggest that the fractionation effect ($\sim 0.8\text{‰}$) could be due to the removal of isotopically light Cr(III) from surface waters via adsorption to particles (Figure 4). A portion of these particles is exported to depth by the biological pump. Isotopic exchange between dissolved Cr(VI) and particulate Cr(III) has little impact on dissolved $\delta^{53}\text{Cr(VI)}$ in systems like seawater with relatively high dissolved [Cr(VI)] and low particulate Cr(III) (Wang et al. 2015). Thus, a portion of the Cr(III) is either oxidized back to the less particle reactive Cr(VI) by Mn-oxides (Eary and Rai 1987; Tang

et al., 2014), or undergoes reversible exchange (as for other particle reactive metals, like thorium; Bacon and Anderson 1982) to increase $\delta^{53}\text{Cr}$ in the dissolved pool. This would tend to remove light Cr isotopes from surface waters, and export them to deeper waters. In support of this, we have demonstrated that loss of dissolved Cr via association with phytoplankton material can account for the observed dissolved Cr deficits in the subarctic Pacific surface ocean. Cr(III) is rapidly sequestered onto and within phytoplankton cells, and Cr(III) is likely the dominant oxidation state associated with marine biogenic particles. Additional work exploring the $\delta^{53}\text{Cr}$ of suspended particulate matter would confirm if isotopically light Cr is associated with exported material.

5. Conclusion

A number of workers have suggested that the biological pump is responsible for the nutrient-like depth profiles of dissolved Cr in seawater (Jeandel and Minster 1987; Sirinawin et al., 2000; Scheiderich et al. 2015), yet a mechanism accounting for the loss of Cr has not been described. Here, we have demonstrated that phytoplankton can sequester dissolved Cr(III) via extracellular adsorption and direct internalization into cells. Using previously published data, we calculated that Cr loss to particles can account for observed deficits of Cr in the upper water column, suggesting that phytoplankton may be an important removal vector for Cr in surface waters. The use of stable Cr isotopes in the marine sedimentary record depends on there being little internal isotope fractionation of dissolved Cr among ocean basins (Frei et al., 2009; Crowe et al., 2013; Reinhard et al., 2014). However, the pool of dissolved Cr(VI) is only partially reduced in surface waters, and the resulting Cr(III) is subsequently removed by incorporation into particles. Given the isotope fractionation associated with Cr(VI) reduction, the Cr(III)

exported with organic matter would be enriched in light isotopes compared with the source of dissolved Cr(VI). This would lead to the isotope fractionation of dissolved Cr with depth and between ocean basins, as has been recently reported (Scheiderich et al., 2015). Thus, internal oceanic processes, like the biological pump, and interbasinal fractionation may play an important role in determining $\delta^{53}\text{Cr}$ variations reported in past sediments.

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Table 1. Isolated laboratory phytoplankton species, strains, classes, provenance, and cell diameters determined with a coulter counter. Surface area and volumes were calculated assuming the cells were approximately spherical.

Species	Strain	Class	Provenance	Diameter (μm)	Surface Area (μm^2)	Volume (fL)
<i>Thalassiosira weissflogii</i>	Actin CCMP 1336	Bacillariophyceae	Coastal	9-11.5	305-409	501-777
<i>Thalassiosira pseudonana</i>	3H NEPCC 58	Bacillariophyceae	Coastal	5.8	104	100
<i>Thalassiosira oceanica</i>	CCMP 1003	Bacillariophyceae	Oceanic	5.7	102	96
<i>Prorocentrum minimum</i>	NEPCC 541	Dinophyceae	Coastal	12.2	503	1060
<i>Emiliana huxleyi</i>	NEPCC 646	Prymnesiophyceae	Coastal	9.7	378	692
<i>Emiliana huxleyi</i>	NEPCC 732a	Prymnesiophyceae	Oceanic	4.0	50	33
<i>Phaeocystis pouchetti</i>	NEPCC 225	Prymnesiophyceae	Oceanic	4.8	73	58
<i>Chrysochromulina polylepis</i>	NEPCC 242	Prymnesiophyceae	Oceanic	4.9	76	63

Table 2. Extraction efficiencies for 5 nM ^{51}Cr in synthetic ocean water using the $\text{Mg}(\text{OH})_2$ co-precipitation method (Wu and Boyle 1997). See section 2.1 for a description.

Species	Reaction Time (min)	% Extraction ($\pm 1\sigma$, N=6)
Cr(VI)	1	5.0 ± 1.3
	3	4.9 ± 1.0
	5	4.9 ± 1.1
Cr(III)	1	99.8 ± 1.8
	3	101.6 ± 1.8
	5	103.1 ± 2.4

Table 3. Extracellular Cr washes examined with *Thalassiosira weissflogii*

Wash	Reagents	Application	Reference
Control	SOW Rinse pH 8.2	10 mL rinse	
Ti-citrate-EDTA	TiCl ₃ 50 mM Na ₂ EDTA 50 mM Na ₃ Citrate 0.6 M NaCl 0.01 M KCl pH 8.0	2 min soak in 2 mL wash 10 mL SOW ^a rinse	Hudson and Morel (1989)
Oxalate-EDTA	100 mM Na ₂ Oxalate 50 mM Na ₂ EDTA 0.3 M NaCl 0.01 M KCl pH 7.5	5 min soak in 2 mL wash, twice 10 mL SOW rinse	Tang and Morel (2006)
Oxalate-DTPA	100 mM Na ₂ Oxalate 50 mM DTPA 0.3 M NaCl 0.01 M KCl pH 7.5	5 min soak in 2 mL wash, twice 10 mL SOW rinse	Modified Tang and Morel (2006)
Oxalate-EDTA-DFB	100 mM Na ₂ Oxalate 50 mM Na ₂ EDTA 0.1 mM DFB 0.3 M NaCl 0.01 M KCl pH 7.5	5 min soak in 2 mL wash, twice 10 mL SOW rinse	Modified Tang and Morel (2006)

^aSOW is synthetic ocean water, the base seawater of the Aquil medium (Price et al. 1989)

Table 4. Mean ($\pm 1\sigma$; N=4) cellular Cr concentrations (zmol Cr cell⁻¹ and $\mu\text{mol Cr L}^{-1}$ of biovolume) and Cr:C ratios ($\mu\text{mol Cr mol C}^{-1}$; calculated using previously reported cellular carbon concentrations) for the phytoplankton species grown with 1370 nM total dissolved Fe and 5 nM Cr(VI). Total cellular Cr concentrations that are significantly greater than the intracellular Cr concentrations are in bold ($p < 0.05$; one-way student's t-test), and the percent extracellular Cr contents are listed. The mean ($\pm 1\sigma$; N=8) cellular Cr concentration for the species grown with 5 nM Cr(VI) is provided.

Species	Cellular Cr ($\mu\text{mol Cr L}^{-1}$ biovolume)		Cellular Cr (zmol Cr cell ⁻¹)		Cr:C Ratio ($\mu\text{mol Cr mol C}^{-1}$)		%	Cellular carbon (mol C L ⁻¹ biovolume)
	Total	Intracellular	Total	Intracellular	Total	Intracellular		
<i>T. weissflogii</i>	0.40 $\pm$ 0.05	0.24 $\pm$ 0.03	301 $\pm$ 39	167 $\pm$ 24	0.04 $\pm$ 0.01	0.02 $\pm$ 0.003	45	10 ^a
<i>T. pseudonana</i>	1.50 $\pm$ 0.48	0.50 $\pm$ 0.04	145 $\pm$ 44	48 $\pm$ 4	0.08 $\pm$ 0.03	0.03 $\pm$ 0.002	67	18 ^b
<i>T. oceanica</i>	3.07 $\pm$ 0.63	1.22 $\pm$ 0.14	286 $\pm$ 58	114 $\pm$ 12	0.23 $\pm$ 0.05	0.09 $\pm$ 0.01	60	13 ^b
<i>P. minimum</i>	0.29 $\pm$ 0.04	0.24 $\pm$ 0.05	300 $\pm$ 33	243 $\pm$ 51	0.02 $\pm$ 0.03	0.02 $\pm$ 0.004		12 ^c
<i>E. huxleyi</i> 646	0.19 $\pm$ 0.06	0.12 $\pm$ 0.03	126 $\pm$ 37	84 $\pm$ 20	0.02 $\pm$ 0.01	0.01 $\pm$ 0.003		10 ^c
<i>E. huxleyi</i> 732a	2.15 $\pm$ 0.42	1.60 $\pm$ 0.38	70 $\pm$ 13	52 $\pm$ 12	0.69 $\pm$ 0.14	0.51 $\pm$ 0.12	26	3 ^c
<i>P. pouchetii</i>	0.45 $\pm$ 0.18	0.59 $\pm$ 0.19	26 $\pm$ 10	33 $\pm$ 10	0.03 $\pm$ 0.01	0.03 $\pm$ 0.01		18 ^c
<i>C. polylepis</i>	0.64 $\pm$ 0.22	0.55 $\pm$ 0.25	38 $\pm$ 12	33 $\pm$ 14	0.05 $\pm$ 0.02	0.04 $\pm$ 0.02		13 ^c
Mean:	1.05 $\pm$ 1.01	0.61 $\pm$ 0.50	161 $\pm$ 118	97 $\pm$ 75	0.15 $\pm$ 0.23	0.09 $\pm$ 0.17		

^aPrice 2005; ^bAnnett et al., 2008; ^cGuo et al., 2012

Table 5. Mean ($\pm 1\sigma$; N=4) cellular Cr concentrations (zmol Cr cell⁻¹ and $\mu\text{mol Cr L}^{-1}$ of biovolume) and Cr:C ratios ($\mu\text{mol Cr mol C}^{-1}$; calculated using previously reported cellular carbon concentrations) in *T. weissflogii* grown in 1370 nM total dissolved Fe and various Cr(VI) and Cr(III) treatments. Total cellular Cr concentrations that are significantly greater than the intracellular Cr concentrations are in bold ($p < 0.05$; one-way student's t-test), and the percent extracellular Cr contents are listed.

Cr Treatment	Cellular Cr ($\mu\text{mol Cr L}^{-1}$ biovolume)		Cellular Cr (zmol Cr cell ⁻¹)		Cr:C Ratio ($\mu\text{mol Cr mol C}^{-1}$)		% Extracellular
	Total	Intracellular	Total	Intracellular	Total	Intracellular	
1 nM Cr(VI)	0.08 $\pm$ 0.02	0.05 $\pm$ 0.01	57 $\pm$ 17	36 $\pm$ 8	0.008 $\pm$ 0.002	0.005 $\pm$ 0.001	30
5 nM Cr(VI)	0.40 $\pm$ 0.05	0.24 $\pm$ 0.03	301 $\pm$ 39	167 $\pm$ 24	0.04 $\pm$ 0.01	0.02 $\pm$ 0.003	45
10 nM Cr(VI)	0.52 $\pm$ 0.04	0.40 $\pm$ 0.07	411 $\pm$ 54	287 $\pm$ 52	0.05 $\pm$ 0.01	0.03 $\pm$ 0.01	37
5 nM Cr(III)	3.58 $\pm$ 0.75	1.75 $\pm$ 0.36	2783 $\pm$ 574	1288 $\pm$ 287	0.36 $\pm$ 0.17	0.17 $\pm$ 0.04	54

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Figure 1

Figure 2

Figure 3

Figure 4