Supplementary material: Fe(II) method details

Ferrozine

Ferrozine sample bottles contained 0.1 mL ammonium acetate buffer (made from ammonium hydroxide (Optima grade, Fisher) and acetic acid (Optima grade, Fisher) adjusted to pH 8.0) and 0.1 mL 10 mM ferrozine (3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p'-disulfonic acid, Sigma Aldrich 'for spectrochemical determination of Fe') solution. After addition of seawater to the pre-spiked sample bottles, the combined sample/reagent mixture was loaded into a 2.5 m liquid waveguide capillary cell (LWCC, 3000 Series, World Precision Instruments) using a peristaltic pump (MiniPuls 3, Gilson). Absorbance was measured at 562 nm (Stookey, 1970) and also 700 nm (a non-absorbing wavelength to monitor the stability of the baseline) 3-4 min after the sample collection time using a USB4000 Fiber-optic Spectrometer (Ocean Optics) with a LS-1 tungsten halogen light source (Ocean Optics). A baseline measurement of the sample matrix absorbance (without ferrozine) was also made for every experiment and deducted from measured absorbance. Eight Fe(II) standards were run immediately before, or after, each method comparison experiment encompassing the range of anticipated Fe(II) concentrations. Standards were made using the seawater matrix of each experiment (retained from prior to the Fe(II) spike addition), with ferrozine reagent added to solution prior to the standard Fe(II) spike as per samples. Between samples the LWCC was rinsed sequentially with detergent, 0.1 M HCl and de-ionized water.

Luminol A

FIA using luminol (O’Sullivan et al., 1995; Rose and Waite, 2001; Seitz and Hercules, 1972) without a pre-concentration column (hereafter, ‘luminol A’) was conducted using a system assembled from two 10-port 2-position valves (Valco, VICI), a photomultiplier tube (PMT, H9319-11, Hamamatsu), a glass flow cell with a mirrored base (Waterville Analytical Products) and a peristaltic pump (MiniPuls 3, Gilson). The PMT was secured inside an electrical box to minimize background light and all reagent/sample tubing was opaque (Black PTFE, Global FIA) except polyvinyl chloride peristaltic pump tubing (PVC, Gradko). The analytical setup of the valve, reagent lines and PMT was as per Jones et al., (2013), but with two identical loops loading Fe(II) reagent. Fe(II) reagent solution was made using a premix of 0.26 g luminol (98%, ROTH) and 1.06 g K_2CO_3 (reagent grade, ROTH) in 10 mL de-ionized water, then stored overnight in the dark at 6°C after shaking to ensure complete dissolution. This premix was then added to a 2 L solution of de-ionized water containing 80 mL NH_4OH (trace metal grade, Fisher), to which approximately 22 mL HCl (trace metal grade, Fisher) was added to adjust the final pH to 10.1. The mixed reagent was then allowed to stand for >24 h prior to use to maximize the luminol response (King et al., 1995). During operation, reagent solution and seawater flowed continuously. A loop of luminol reagent (approximately 200 µL) was introduced into the seawater flow before the flow cell every 60 s (flow rates: 5 mL min^{-1} sample seawater and 1 mL min^{-1} reagent). Valve operation and data acquisition were controlled by LabVIEW software. Eight Fe(II) standard additions were made to the seawater matrix of each experiment prior to the Fe(II) spike and used to calibrate chemiluminescence peak height. Standard solutions were each run to produce 5 consecutive peaks.

Luminol B

A second FIA method (hereafter ‘luminol B’, as opposed to ‘luminol A’ described above), using a 8-hydroxyquinoline (8-HQ) pre-concentration column (Landing et al., 1986), was also used. For this method, a 50 mM luminol stock was prepared by dissolving 0.177 g luminol (98%, ROTH) and 0.250 g Na_2CO_3 (Sigma-Aldrich) in 20 mL of de-ionized water which was then stored overnight at 6°C prior to use. A 2 M NaOH (trace metal grade, Sigma- Aldrich) stock solution was prepared in 200 mL de-ionized water. A 0.1 M stock solution of dimethylglyoxime (DMG) (Fluka, >99%), used to mask the interference caused by Co(II) (Klopf and Nieman, 1983; Ussher et al., 2009), was prepared in methanol (Acros Organics, 99.9%). A 40 mM sulphite standard was prepared from sodium sulphite (Acros Organics, 98.5%) in de-ionized water. A 10 µM luminol working solution was prepared as required by dissolving 15 g of Na_2CO_3 (Acros Organics, 99.5%) in 500 mL de-ionized water, to which 200 µL of luminol stock, 5 mL NaOH stock and 200 µL DMG stock were added and then the solution made up to 1 L with de-ionized water. The luminol reagent solution was then passed through a Chelex 100 column, which was pre-cleaned with 0.5 M HCl (Fisher, trace metal grade) followed by de-ionized water, flowing at approximately 2 mL min^{-1} and allowed to stabilize for >24 h before use (Bowie et al., 1998). A 50 mM HCl elution acid was made by diluting HCL (UPA grade, Romil) with de-ionized water. 2 M ammonium acetate buffer stock was prepared from NH_4OH (Optima grade, Fisher) and CH_3COOH (Optima grade, Fisher) in de-ionized water and adjusted to pH 5-5.5. To make a working buffer solution, 200 mL of buffer stock was diluted with de-ionized water to a final volume of 1 L. The luminol B apparatus setup included 3 peristaltic pumps (Gilson, Minipuls3) with 2 stop PVC accu-rated pump tubing (Elkay). All other manifold tubing was fluorinated ethylene propylene (Cole-Palmer). The manifold used a solenoid valve to control sample/buffer and wash flows, a 6-port 2-position injection valve (Valco, VICI) to control loading and eluting cycles, and a PMT (Thorn EMI B2F/RFI+C634). Valve/pump timings and data acquisition were controlled by LabVIEW software with a data acquisition module (Ruthern Instruments, Bodmin, UK). A 120 s pre-loading time ensured any previous sample left in the line was flushed to waste. Sample was mixed with the buffer and then loaded over the 8-HQ column (for 60 s) which was then rinsed with de-ionized water and eluted (for 80 s) with 50 mM HCl (Table 2). The 80 s elution period, longer than needed to generate a peak, was maintained to prevent carry over between replicate loading/unloading cycles. 5 Fe(II) standard additions were used to calibrate the system by standard addition to a seawater matrix that was buffered to pH 5.5. Standard solutions were each run for 5 consecutive sample cycles. Sampling was continuous during the oxidation experiments producing a sample peak every 3.5 min (Supplementary Table 1).

	Pre-load	Sample loading	Column washing	Column eluting
Sample pump	120 s	60 s	Off	Off
Wash pump	Off	Off	30 s	Off
Reagent pump	120 s	60 s	30 s	80 s

Supplementary Table 1. Flow injection analysis cycle for the Fe(II) flow injection analysis pre-concentration system (luminol B).

Voltammetry

The voltammetry estimation of Fe(II) was carried out by determination of the difference between reactive Fe(III) concentrations in the presence and absence of the Fe(II) binding ligand 2,2'-dipyridyl (Dp). Reactive Fe(III) is defined as the concentration of Fe(III) available to complex with the added electroactive ligand (which binds both Fe(III) and Fe(II)) (Waska et al., 2016) 1-nitroso-2-napthol. Prior addition of Dp to a sample has been shown to mask Fe(II) from the determination, allowing the contribution of Fe(II) to the electrochemical Fe(III) signal to be estimated (Gledhill and Van Den Berg, 1995). In this study, the method of Gledhill and van den Berg (1995) was modified by omitting the catalytic oxidant H_2O_2 and the surfactant sodium dodecyl sulfate. Sample bottles for the determination of reactive Fe(III) (Fe_{RIII}) containing 50 µL of 2 mM Dp were pre-prepared so that the Fe(II) was fixed by Dp as the sample was collected. Sample bottles for the determination of total reactive Fe (Fe_R, the sum of reactive Fe(III) and reactive Fe(II)) contained no added reagents prior to sample aliquot collection. In order to control time differences between sample collection and analysis a calibration curve was established using the experimental water pre Fe(II) addition. For the calibration curve nine 10 mL aliquots of experimental water were pipetted into separate 15 mL fluorinated ethylene propylene centrifuge tubes. 1-Nitroso-2-napthol (NN) was added to a final concentration of 20 µM and the sample buffered at pH 7.0 through the addition of Hepes (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, Sigma) to a final concentration of 10 mM. Standard additions of 10 nM Fe(III) were added to 3 aliquots, and 20 nM Fe(III) to 3 separate aliquots. The samples were left to equilibrate for >30 min prior to analysis. All experimental samples were analyzed in triplicate. Fe_{RIII} was determined between 30 min and 1 h after sampling and Fe_R determined subsequent to Fe_{RIII}, 30 min to 1 h after the addition of NN. Close control of the analysis times for all samples was considered necessary as determination of Fe_R is operational and can also be influenced by the kinetics of NN and Fe complexation in seawater (Laglera and Filella, 2015). For all samples the voltammetry conditions were as follows: N_2 Purge time 180 s, deposition potential -0.15 V, deposition time 30 s, quiescence time 8 s, potential scan from -0.25 V to -0.6 V using sampled DC with an interval time of 0.1 s and a step potential of 0.00255 to give a scan rate of 25 mV s^{-1}. Prior to experiments the response to Fe(II) of Dp was checked and found to be equivalent to Fe(III) (0.6 nA $nmol^{-1}$).

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