THE ROLE OF NATURAL ORGANIC LIGANDS IN TRANSFORMATIONS OF IRON CHEMISTRY IN SEAWATER AND THEIR EFFECT ON THE BIOAVAILABILITY OF IRON TO MARINE PHYTOPLANKTON.

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Submitted in fulfilment of the requirements for the degree

of Doctor of Philosophy in Science,

Plant Functional Biology and Climate Change Cluster,

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June 2014

CERTIFICATE OF AUTHORSHIP/ORIGINALITY

I certify that the work in this thesis has not been submitted for a degree nor has it been submitted as part of the requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. Furthermore, I certify that all information sources and literature used are indicated in the thesis.

Louisa Norman

ACKNOWLEDGEMENTS

This thesis is dedicated to my parents and my sister who have provided unconditional love and support from the other side of the world for the past 4 years. I don't think any of us ever imagined that the very average high school student would now be sitting here writing the acknowledgements for her Ph.D thesis. Thank you for shaping me into the person I am today as it has allowed me to push myself further than I ever thought possible.

Special thanks are given to my supervisors; Professor Christel Hassler for imparting her wealth of knowledge to me and for her encouragement throughout this process. Her enthusiasm for her research is inspiring. To Assoc. Professor Martina Doblin, for her guidance and motivation which have been invaluable, particularly in the latter stages of this journey. Professor David Waite who provided facilities and intellectual support that were instrumental to the success of part of this thesis, and to Professor Greg Skilbeck for his intellectual contribution to the finished product. My appreciation for what you all have contributed has no measure.

Thank you to Drs Andrew Bowie, Laurie Burn-Nunes, Edward Butler, Nagur Cherukuru, Michael Ellwood, Jason Everett, Carol Mancuso Nichols, Veronique Schoemann, Sutinee Sinutok, Ashley Townsend, and Isabelle Worms, Professors Vera Slaveykova and Grant McTainsh, and Lesley Clementson, Alicia Navidad, Charlotte Robinson, Claire Thompson, Roslyn Watson who have provided support in the form of analysis, data and methodologies that allowed this thesis to come to fruition. Your individual contributions are acknowledged within this thesis.

Thank you to the staff and students at the UNSW Water Research Centre in the School of Engineering for their help and friendship during my few months there. Special thanks are given to Dr An Ninh Pham who, when faced with a biologist in chemists' clothing, provided all the guidance and support I needed to make iron redox chemistry just that bit easier. I sincerely appreciate his time and kindness.

Thanks also are given to the staff and students at the Institut F.-A. Forel, Université de Genève for their encouragement and friendship during my stay in Geneva; particularly Sophie Moisset, Sonia Blanco Ameijeras, Rebecca Flueck and Giulia Cheloni who welcomed me so warmly into their circle.

Warmest thanks are given to all my colleagues within C3 here at UTS. Special thanks are extended to Drs Katherina Petrou and Daniel Nielsen for their friendship, patience, laughs and 'winesday'; to Charlotte Robinson, Kirralee Baker, Dale Radford, Joh Howes and Isobel Cummings, you are all truly amazing people; to Dr Andy Leigh and Peter Jones for their kindness and encouragement, and the provision of a stress reliever in the form of their cat, Chai; and to Professor Peter Ralph and Carolyn Carter who were incredibly kind and supportive when I returned from sea a wounded soldier, and lastly to the wonderful technical staff.

Throughout this research I have been in receipt of financial support from a number of sources and I would like to thank the University of Technology for the provision of an IRS scholarship, and the Australian Research Council which provided the research funds and a stipend that allowed this project to happen (Discovery Project DP1092892 and LIEF grant LE0989539).

And finally, to those endless cups of tea that I have consumed. I have used it to drown sorrows, celebrate achievements; to revive and relax…..it is a most remarkable beverage.

PUBLICATIONS

Publications resulting directly from this thesis:

Chapter 1:
Norman, L., Cabanes, D., Blanco-Ameijerias, S., Moisset, S., Hassler, C.S. 2014. Iron biogeochemistry in aquatic systems: from source to bioavailability. *Chemia.* 68, 764 – 771.

Chapter 4:
Norman, L., Worms, I.A.M., Angles, E., Bowie, A.R., Mancuso Nichols, C., Pham, A.N., Slaveykove, V.I., Townsend, A.T., Waite, T.D., Hassler, C.S. The role of bacterial and algal exopolymeric substances in iron chemistry. *Mar. Chem*. **In press.**

Chapter 4:
Hassler, C.S., **Norman, L**., Mancuso Nichols, C., Clementson, L.A., Robinson, C., Schoemann, V., Watson, R.J., Doblin, M.A. Exopolymeric substances can relieve iron limitation in oceanic phytoplankton. *Mar. Chem*. **In press.**

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bacterioplankton, is represented separately (FeSidTr). Other non-specific uptake pathways (endocytosis, direct permeation and an ion channel) are shown. Once inside the microorganism, Fe (Fe_{int}) reacts with intracellular biological ligands (L_{bio}, e.g. Chlorophyll-*a*), is stored (e.g. vacuole, ferritin) or is involved in cellular homeostasis via gene regulation (grey arrow with ± symbol). Release of Fe biological organic ligands (L_{rel}, such as EPS and siderophores) can exert a feedback in the control of both Fe chemistry and bioavailability. Dotted, dashed and full arrows represent aggregation/disaggregation, transfer, and chemical reaction (complexation, redox), respectively. (From Hassler et al, 2011b).

Fig. 2.1. Sea surface temperature (SST) and Chlorophyll-*a* (μg L^{-1}) plots showing the study area and sampling locations for natural humic substance-like material and nutrient enrichment experiments. Natural samples were collected from at variety of watermass types (river plume, inner shelf, outer shelf, and oceanic (cold-core cyclonic eddy (CCE) and East Australia Current (EAC)), and seawater collected for the nutrient experiments was sampled from the EAC and CCE.

Fig. 2.2 Calibration curve used for the comparison of methods to determine the concentration of electrochemically detected humic substance-like (HS-like) material. Suwannee River Fulvic Acid (SRFA) was used as the HS-like standard in concentrations between 20 and 480 μg L^{-1}. i_p represents the peak height in nA of electrochemically detected Fe′-reactive organic material. Errors = SD of triplicate samples.

Fig. 2.3 Relationship between the concentration of humic substance-like (HS-like) material (log transformed), and Chl-*a* fluorescence (CTD derived) from samples taken during the SS2010-V09 Tasman Sea voyage (*RV Southern Surveyor*, 15th to 31st October 2010, austral spring). Samples were collected at 5 m, 15 m, and the depth of the chlorophyll maximum from water masses comprising river plume, inner shelf and outer shelf waters, and oceanic waters. Panel A indicates the weak positive relationship with Clarence River plume samples included (circled on the plot), Panel B indicates the relationship with these samples excluded.

Fig. 2.4 Relationships between the concentration of humic substance-like (HS-like) material, reduction peak position (E_p, V vs Ag/AgCl electrode) or sensitivity with temperature, salinity, Chl-*a* fluorescence (CTD derived), and turbidity (light transmission) from samples taken during the SS2010-V09 Tasman Sea voyage (*RV Southern Surveyor*, 15th to 31st October 2010, austral spring). Samples were collected at 5 m, 15 m, and the depth of the chlorophyll maximum from water masses comprising river plume, inner shelf and outer

shelf waters, and oceanic waters. Due to extremely high HS-like concentrations from the Clarence River Plume this data was log transformed to allow for clearer graphical representation. Clarence River Plume samples are circled on the plots.

Fig. 2.5 Concentration of macronutrients (ammonia (NH_4), nitrate + nitrite (NO_X), silicic acid ($Si(OH)_4$), and phosphate (PO_4), μmol L^{-1}) in experimental bottles measured at T0 and after 72-h shipboard, nutrient-enrichment experiments undertaken during the SS2010-V09 Tasman Sea voyage (*RV Southern Surveyor*, 15th to 31st October 2010, austral spring). The experiments were conducted in 200–210-μm filtered seawater collected from the depth of the chlorophyll maximum at two sites in the Tasman Sea; East Australia Current (EAC, 29 1 °S 154 3°E), and a cold-core eddy (CCE, 32 2°S 153 8°E). T0 = unamended seawater at the start of the experiment. The treatments were; unamended control (Con), nitrate (N, 10 μM), nitrate + inorganic Fe (NFe, 10 μM + 1 nM), silicate (Si, 10 μM), mixed nutrients (Mix; NO_3 + Fe + Si + P, 10 μM + 1 nM + 10 μM + 0.625 μM), Suwannee River fulvic acid exposed to light (FAL, 200 μg L^{-1}), Suwannee River fulvic acid dark incubation (FAD, 200 μg L^{-1}). Daily additions of nutrients were given to the East Australia Current incubations, and a single initial nutrient addition was given to the cold-core eddy incubations. Errors = SD of triplicate incubations except for EAC FAD where errors represent half interval (range) of duplicates incubations.

Fig. 2.6 Concentration of dissolved Fe (dFe, nM) measured at the conclusion of two 72-h shipboard nutrient-enrichment experiments undertaken during the SS2010-V09 Tasman Sea voyage (*RV Southern Surveyor*, 15th to 31st October 2010, austral spring). The experiments were conducted in 200–210-μm filtered seawater collected from the depth of the chlorophyll maximum at two sites in the Tasman Sea; East Australia Current (EAC, 29 1 °S 154 3°E), and a cold-core eddy (CCE, 32 2°S 153 8°E). Treatments were as per Fig. 2.5. Samples for the analysis of dFe were taken from replicates 1 and 2 of each treatment, therefore duplicate data points are shown for each treatment and experiment.

Fig. 2.7 Cell abundance (cells mL^{-1}) of bacteria (A), and picophytoplankton *Prochlorococcus* (B), *Synechococcus* (C), small eukaryotes (D) and large eukaryotes (E) measured by flow cytometry at T0 and at the conclusion of two 72-h shipboard nutrient-enrichment experiments undertaken during the SS2010-V09 Tasman Sea voyage (*RV Southern Surveyor*, 15th to 31st October 2010, austral spring). The experiments were conducted in 200–210-μm seawater collected from the depth of the chlorophyll maximum at two sites East Australia Current (EAC, 29 1 °S 154 3°E), and a cold-core eddy (CCE, 32 2°S 153 8°E). T0 = unamended seawater at the start of the experiment. Treatments were as per Fig. 2.5. Error = SD of

triplicate incubations except for EAC FAD where errors represent half interval (range) of duplicate incubations. Note differences in y-axis scale.

Fig. 2.8 Concentration of total chlorophyll-*a* (TChl-*a*, mg m^{-3}) measured at T0 and at the end of two 72-h shipboard nutrient-enrichment experiments undertaken during the SS2010-V09 Tasman Sea voyage (*RV Southern Surveyor*, 15th to 31st October 2010, austral spring). The experiments were conducted in 200–210-µm filtered seawater collected from the depth of the chlorophyll maximum at two sites East Australia Current (EAC, 29 1 °S 154 3°E), and a cold-core eddy (CCE, 32 2°S 153 8°E). T0 = unamended seawater at the start of the experiment. Treatments were as per Fig. 2.5. Error = SD of triplicate incubations except for EAC FAD where errors represent half interval (range) of duplicates incubations.

Fig. 2.9 Concentration of biomarker pigments (mg m^{-3}) measured at T0 and at the end of two 72-h shipboard nutrient-enrichment experiments undertaken during the SS2010-V09 Tasman Sea voyage (*RV Southern Surveyor*, 15th to 31st October 2010, austral spring). The experiments were conducted in 200–210-µm filtered seawater collected from the depth of the chlorophyll maximum at two sites East Australia Current (EAC, 29 1 °S 154 3°E), and a cold-core eddy (CCE, 32 2°S 153 8°E). T0 = unamended seawater at the start of the experiment. Treatments were as per Fig. 2.5.

Fig. 2.10 Carbon (C) fixation rates measured from ^{14}C incubations at the end of two 72-h shipboard nutrient-enrichment experiments undertaken during the SS2010-V09 Tasman Sea voyage (*RV Southern Surveyor*, 15th to 31st October 2010, austral spring). The experiments were conducted in 200–210-µm filtered seawater collected from the depth of the chlorophyll maximum at two sites East Australia Current (EAC, 29 1 °S 154 3°E), and a cold-core eddy (CCE, 32 2°S 153 8°E). T0 = unamended seawater at the start of the experiment. Treatments N, NFe, and FAL were as per Fig. 2.5. Error = SD of triplicate incubations.

Fig. 2.11 Concentration of electrochemically detected humic substance-like (HS-like) substances measured at the conclusion of two 72-h shipboard nutrient-enrichment experiments undertaken during the SS2010-V09 Tasman Sea voyage (*RV Southern Surveyor*, 15th to 31st October 2010, austral spring). The experiments were conducted in 200–210-µm filtered seawater collected from the depth of the chlorophyll maximum at two sites, East Australia Current (EAC; 29 1 °S 154 3°E), and a cold-core eddy (CCE; 32 2°S 153 8°E). T0 = unamended seawater at the start of the experiment. Treatments were as per Fig. 2.7. The concentration of HS-like material is expressed as Suwannee River Fulvic Acid equivalents

(SRFA eq) in μg L^{-1}. Error = SD of triplicate incubations except for EAC FAD where errors represent half interval (range) of duplicates incubations.

***** Significantly higher HS-like concentration compared to all other EAC treatments, except FAL and FAD ($p = 0.003$).

† Significantly higher HS-like concentration compared to CCE T0 and control ($p = 0.007$).

‡ Significantly lower HS-like concentration compared to CCE T0 and control ($p = 0.014$).

Fig. 2.12 Relationships between the concentration of humic substance-like (HS-like) material and Silicic acid ($Si(OH)_4$), phosphate (PO_4) and dissolved Fe (dFe) at the conclusion of a 72-h shipboard nutrient-experiment undertaken during the SS2010-V09 Tasman Sea voyage (*RV Southern Surveyor*, 15th to 31st October 2010, austral spring). The experiment was conducted in 200–210-μm filtered seawater collected from the depth of the chlorophyll maximum in the East Australia Current (EAC; 29 1 °S 154 3°E). Treatments were as per Fig. 2.5. Panel A = $Si(OH0)_4$ all data; Panel B = treatments where $Si(OH)_4 < 0.7$ μmol L^{-1}; Panel C = treatments where $Si(OH0)_4 > 20$ μmol L^{-1}; Panel D = PO_4 all data; Panel E = PO_4 enrichment treatment (Mix) excluded; Panel F = dFe all data; Panel G = dFe-enrichment > 10 nM (Mix treatment) excluded. High concentrations, subsequently excluded, are circled to highlight (panels A, D, and F).

Fig. 3.1 Cruise track from the Primary productivity induced by Iron and Nitrogen in the Tasman Sea (PINTS) voyage (RV *Southern Surveyor*, Jan-Feb 2010). Transect stations are shown as circles and process stations as diamonds. Profiles presented in this chapter were from two process stations P1 (30.0 °S, 156.0 °E, also Stn 5) and P3 (46.2 °S, 159.5 °E, also Stn 12) and from Stn 14, 44.6 °S, 149.4 °E. Stn 14 was a reoccupation of process station 3 from the SAZ-Sense expedition (*Aurora Australis*, January–February 2007). Water for the Fe-enrichment experiments was collected stations P1 and P3. Thicker solid lines indicate the East Australian Current (EAC), Tasman Front (TF), and EAC
Extension. The dashed line represents the path of the subtropical front (STF) (From Hassler et al., 2014).

Fig. 3.2 Seawater depth profiles of dissolved nutrients nitrate + nitrite (NO_X; panel A), reactive phosphorus (PO_4; panel B), and silicic acid ($Si(OH)_4$; panel C), measured at stations P1 (30.0 °S, 156.0 °E,), P3 (46.2 °S, 159.5 °E,) and Stn 14 (44.6 °S, 149.4 °E) collected during the PINTS voyage (RV *Southern Surveyor*, Jan-Feb 2010).

Fig. 3.3 Seawater depth profiles of total chlorophyll-*a* (TChl-*a* μg L^{-1}) measured at stations P1 (30.0 °S, 156.0 °E, depths 15 to 125 m), P3 (46.2 °S, 159.5 °E, depths 15 to 80 m) and Stn 14 (44.6 °S, 149.4 °E, depths 15 to 50 m) collected during the PINTS voyage (RV *Southern Surveyor*, Jan-Feb 2010).

Fig. 3.4 Seawater depth profiles (15 to 1000m) from process stations P1 (30.0 °S, 156.0 °E), and P3 (46.2 °S, 159.5 °E) and Stn 14 (44.6 °S, 149.4 °E) collected during the PINTS voyage (RV *Southern Surveyor*, Jan-Feb 2010). Concentration of dissolved Fe (dFe, nM) and the concentration of electrochemically detected Fe′-binding organic ligands (SumL, nM) and their calculated conditional stability constant (Log $K_{Fe'L}$) are presented together with the concentration of humic substance-like (HS-like) material. HS-like material is expressed as Suwannee River Fulvic Acid (SRFA) μg L^{-1}.

Fig. 3.5 Relationship between the concentration of Fe-binding organic ligands (ΣL) and the conditional stability constant (Log $K_{Fe'L}$) for process station P1 (30.0 °S, 156.0 °E), process station P3 (46.2 °S, 159.5 °E) and Stn 14 (44.6 °S, 149.4 °E). Samples were collected during the PINTS voyage (RV *Southern Surveyor*, Jan-Feb 2010; Hassler et al., 2014).

Fig. 3.6 Relationships between the concentration of total chlorophyll-*a* (TChl-*a*) and Fe-binding organic ligands (ΣL), TChl-*a* and ligand conditional stability constant (log $K_{Fe'L}$) at depths between 15 and 125 m at process station P1 (30.0 °S, 156.0 °E), and TChl-*a* and ΣL at depths between 15 and 50 m, humic substance-like (HS-like) material and SumL at depths between 15 and 300 m and at Stn 14 (44.6 °S, 149.4 °E). Samples were collected during the PINTS voyage (RV *Southern Surveyor*, Jan-Feb 2010; Hassler et al., 2014). HS-like material is expressed as Suwannee River Fulvic Acid (SRFA) equivalent in μg L^{-1}.

Fig. 3.7 Relationships between dissolved Fe (dFe) concentration (nM) and macronutrients nitrate + nitrite (NO_X), phosphate (PO_4) and silicic acid ($Si(OH)_4$) (μmol L^{-1}) at process station P3 (46.2 °S, 159.5 °E) at depths between 15 and 300 m. Samples were collected during the PINTS voyage (RV *Southern Surveyor*, Jan-Feb 2010; Hassler et al., 2014).

Figure 3.8 Concentrations of dissolved Fe (dFe, nM) and relative concentration (%) of labile Fe (Fe_{Labile}) associated with Fe enrichment experiments using phytoplankton communities collected from two sites in the Tasman Sea, P1 (30.0 °S, 156.0 °E, panels A and C) and P3 (46.2 °S, 159.5 °E, panels B and D) during the PINTS voyage (RV *Southern Surveyor*, Jan-Feb 2010). The data presented comes from unamended seawater (T0) and after 4-d incubation in samples with and without the addition of Fe and organic ligands. Treatments measured after 4-d incubation comprised an unamended control (Con), inorganic Fe only (2 nM, Fe),

desferrioxamine B ([15 nM], DFB), glucuronic acid ([15 nM], GLU), natural pelagic bacterial exopolymeric substances ([0.8 nM], EPS), fulvic acid ([100 μg L^{-1}], as Suwannee River Fulvic Acid, FA), and two treatments containing Australian desert dust (D1, 2009 Brisbane dust storm, and D2, red composite, both from the Buronga region, NSW) which were predicted to release ~2 nM Fe. DFB, EPS, GLU and FA treatments were all enriched with 2 nM inorganic Fe. Closed symbols indicate samples with phytoplankton present, open symbols indicate samples where phytoplankton were absent (0.2 μm filtered, single incubations). Error bars represent half-interval of duplicate samples; where no error bars are present the data presented is from a single sample.

Figure 3.9 Concentration of organic ligands and calculated conditional stability constants (log $K_{Fe'L}$) associated with Fe-enrichment experiments using phytoplankton communities collected from two sites in the Tasman Sea, P1 (30.0 °S, 156.0 °E, panels A and C) and P3 (46.2 °S, 159.5 °E, panels B and D) during the PINTS voyage (RV *Southern Surveyor*, Jan-Feb 2010). The data presented comes from unamended seawater (T0) and after 4-d incubation for samples with and without the addition of Fe and organic ligands. Treatments were as per Fig. 3.8. Closed symbols indicate samples with phytoplankton present, open symbols indicate samples where phytoplankton were absent (0.2-μm filtered, single incubations). Where two ligand classes were detected, stronger ligands are indicated by a red symbol and weaker ligands by a blue. Error bars represent half-interval of duplicate samples; where no error bars are present the data presented is from a single sample.

Figure 3.10 Concentration of humic substance-like material (HS-like), expressed as Suwannee River Fulvic Acid equivalents (SRFA eq) in μg L^{-1}, associated with Fe enrichment experiments using phytoplankton communities collected from two sites in the Tasman Sea, P1 (30.0 °S, 156.0 °E, panel A) and P3 (46.2 °S, 159.5 °E, panels B) during the PINTS voyage (RV *Southern Surveyor*, Jan-Feb 2010). The data presented comes from unamended seawater (T0) and after 4-d incubation for samples with and without the addition of Fe and organic ligands. Treatments were as per Fig. 3.8. Closed symbols indicate samples with phytoplankton present, open symbols indicate samples where phytoplankton were absent (0.2-μm filtered, single incubations). Error bars represent half-interval of duplicate samples; where no error bars are present the data presented is from a single sample. Note difference in y-axis scale.

Figure 3.11 Changes in total chlorophyll-*a*, (TChl-*a*) (A) and F_V/F_M (B) from Fe-enrichment experiments after 4-d incubation with and without the addition of organic ligands. Water for the experiments was collected from two sites in the Tasman Sea, P1 (30.0 °S, 156.0 °E)

and P3 (46.2 °S, 159.5 °E) during the PINTS voyage (RV *Southern Surveyor*, Jan.-Feb. 2010). Treatments were as per Fig. 3.8 Error bars represent the half interval of duplicate samples. T0 values not shown; see Table 3.1.

Figure 3.12 Total Chl-*a* concentrations (TChl-*a*) of size fractionated phytoplankton communities from Fe-enrichment experiments after 4-d incubation with and without the addition of organic ligands. Pico-, nano-, microphytoplankton were defined by sequential filtration as > 0.7–2 µm, 2–10 µm, ≥10 µm, respectively. Water for the experiments was collected from two sites in the Tasman Sea, P1 (30.0 °S, 156.0 °E) and P3 (46.2 °S, 159.5 °E) during the PINTS voyage (RV *Southern Surveyor*, Jan-Feb 2010). Treatments were as per Fig. 3.8 Error bars represent the half interval of duplicate samples. Dashed lines represent a comparison of the Fe-ligand complexes with Fe addition only.

Figure 3.13 Size-fractionated biomarker pigment data (measured by HPLC) from Fe enrichment experiments after 4 d incubation with and without the addition of organic ligands. Pico-, nano-, microphytoplankton were defined by sequential filtration as > 0.7–2 µm, 2–10 µm, ≥10 µm, respectively. Water for the experiments was collected from the depth of the fluorescence maximum at two sites in the Tasman Sea, P1 (30.0 °S, 156.0 °E) and P3 (46.2 °S, 159.5 °E) during the PINTS voyage (RV *Southern Surveyor*, Jan.-Feb. 2010). Treatments were as per Fig. 3.8.

Figure 4.1 Molar mass distribution fractograms of exopolymeric substances (EPS) obtained by FFF-RI-UV-ICPMS using a linear decrease in cross-flow rate. Differential refractive index relative intensity, absorbance measured at λ = 254 nm (upper panel of each sub figure), and ^{56}Fe relative intensity (lower panel of each sub figure) from EPS isolated from Antarctic sea ice bacteria, sub-Antarctic zone bloom (SAZ bloom) and axenic algal cultures (*Phaeocystis antarctica* and *Emiliania huxleyi*) . The grey zone following 40-min elution time illustrates the end of applied cross flow and the end of the fractionation corresponding to elution of compounds > 950 kDa as determined using PSS molecular weight calibration.

Figure 4.2 ^{56}Fe eluograms (lower panel of each sub figure) showing hydrodynamic radius (nm) of components of exopolymeric substances (EPS). For comparison refractive index relative intensity (lower panels), absorbance measured at λ = 254 nm (UV, upper panels), and fluorescence (fluo, upper panels) are shown. EPS were isolated from Antarctic sea ice bacteria, sub-Antarctic zone bloom (SAZ bloom) and axenic algal cultures (*Phaeocystis antarctica* and *Emiliania huxleyi*).

Figure 4.3 ^{56}Fe eluogram after *in silico* deconvolution of Fe distribution associated with Antarctic sea ice bacterial EPS. Maximum Fe signal intensity (red line) associated with components with hydrodynamic radii (R_h) of ~29 nm. Three further prominent components measured with R_h of ~ 26 nm, 40 nm and 60 nm (green lines).

Fig. 4.4 The solubility of Fe in the presence or absence of bacterial or algal exopolymeric substances (EPS) in both the colloidal (0.02 um to 0.2 um) and soluble (<0.02 um) size fractions. EPS isolates were from an Antarctic sea ice bacteria, a natural phytoplankton bloom from the sub-Antarctic zone (SAZ bloom), and from axenic algal cultures of *Phaeocystis antarctica* and *Emiliania huxleyi*. An experimental control solution of inorganic Fe only is also presented. Experimental medium was synthetic seawater (pH 8.0). Error bars indicate half interval, n=2.

Fig. 4.5 The effect of Fe associated with EPS (Fe-EPS) on the growth of the Southern Ocean diatom, *C. simplex,* over 187-h incubation at 4 °C and at 50 µmol photons m^{-2} s^{-1}. Growth curve in terms of cells numbers (A) and maximum quantum yield (F_V/F_M, B) are presented. Fe concentration in the Tasman Sea surface seawater (seawater) medium was 0.56 nM. The growth of *C simplex* in the presence of Fe bound to EPS was compared to both inorganic Fe and seawater control. Additions of EPS and inorganic Fe provided an additional 1 nM Fe to the seawater medium. EPS isolates were from an Antarctic sea ice bacteria, a natural phytoplankton bloom from the sub-Antarctic zone (SAZ bloom), and from axenic algal cultures of *Phaeocystis antarctica and Emiliania huxleyi*. Error bars indicate standard deviation, *n*=3.

Fig. 5.1 Relative concentration of 10 µM TAC-Labile Fe (Fe_{Labile}) as a percentage of the total dissolved Fe measured in the 0.2-µm and 0.02-µm filtered fractions of experimental samples from two replicate experiments simulating the wet deposition of Australian mineral dust into the Southern Ocean. The dust used was collected during a large dust storm over Brisbane, QLD., and resuspended in rainwater collected in the Tasman Sea (31° 35.849'S 178° 00.00'E, GP13 GEOTRACES voyage, 27/05/2011) before being exposed to UV + visible light (UV, 2000 µE), visible light only (VIS, 2000 µE), or kept in darkness (Dark). Resuspended, treated dust was added to synthetic seawater to give a dust enrichment of 0.5 mg L^{-1}. Where no bars are present the concentration of Fe_{Labile} was below detection limit (0.05 nM) after synthetic seawater Fe correction.

Fig. 5.2 Concentration of Fe'-binding organic ligands (nM; A) and conditional stability constants (Log $K_{Fe'L}$; B) in the 0.2-µm and 0.02-µm-filtered fractions of experimental

samples from two replicate experiments simulating the wet deposition of Australian mineral dust into the Southern Ocean. Treatments were as per Fig. 5.1. Ligand concentration and Log $K_{Fe'L}$ were calculated using total dissolved Fe concentrations.

Fig. 5.3 Concentration of electrochemically detected Fe'-binding humic substance-like material (HS-like; μg L^{-1} SRFA equivalent) in unfiltered, 0.2-μm and 0.02-μm filtered fractions of experimental samples from two replicate experiments simulating the wet deposition of Australian mineral dust into the Southern Ocean. Treatments were as per Fig. 5.1. Errors = standard deviation of triplicate samples. Where no bars are present the concentration of HS-like was below detection limit (1.49 μg L^{-1} SRFA Eq.).

Fig. 5.4 Concentration of total hydrolysable saccharides (reported as μM C) measured in 0.2-μm and 0.02-μm filtered experimental samples from two experiments simulating the wet deposition of Australian mineral dust into the Southern Ocean. Treatments were as per Fig. 5.1. Error = standard deviation of triplicate samples.

Figure 5.5 The effect of Fe associated with Australian mineral dust on the growth of the Southern Ocean diatom *C. simplex* over 326 h incubation period at 4 °C and 50 μmol photons m^{-2} s^{-1}. Growth curves (A), Cell volume, μm^3 (B) and F_V/F_M (C) were compared to an inorganic Fe (1 nM) incubation. The dust used was collected during a large dust storm over Brisbane, QLD., and resuspended in rainwater collected in the Tasman Sea (31°35.849'S 178°00.00'E, GP13 GEOTRACES voyage, 27/05/2011) before being exposed to UV + visible light (UV, 2000 μE), visible light only (VIS, 2000 μE), or kept in darkness (Dark). Resuspended, treated dust was added to synthetic seawater to give a dust enrichment of 0.5 mg L^{-1}. Error = standard deviation of triplicate samples.

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µm filtered seawater collected from the depth of the chlorophyll maximum at two sites A) East Australia Current (EAC; 29 1 °S 154 3°E), and B) cold-core eddy (CCE; 32 2°S 153 8°E). The treatments included in these analyses were; unamended control, nitrate (NO_3, 10 µM), nitrate + inorganic Fe (NO_3 + Fe, 10 µM + 1 nM), silicate (Si, 10 µM), mixed nutrients (Mix; NO_3 + Fe + PO_4, 10 µM + 1 nM + 0.625 µM).

Table 2.5 Instrument sensitivity and position of the reduction peak position ($E_{p,}$ V vs Ag/AgCl electrode) derived from the determination of humic substance-like (HS-like) material. Samples analysed were from nutrient-enrichment experiments at T0 (unamended seawater) and after 4 d incubation in samples with and without the addition of nutrients. The experiments were conducted in 200–210-µm filtered seawater collected from the depth of the chlorophyll maximum at two sites A) East Australia Current (EAC, 29 1 °S 154 3°E), and B) a cold-core eddy (CCE, 32 2°S 153 8°E). Treatments were as per table 2.4.

Table 2.6 Concentration range of humic-substance like (HS-like) material measured using cathodic stripping voltammetry by Laglera et al. (2007) and Laglera and van den Berg (2009).

Table 3.1 Depth of chlorophyll maximum (Cmax), and in situ concentration of total chlorophyll *a* (TChl-*a*), nutrients (silicate (Si), nitrate + nitrite (NO_x), phosphate (PO_4)), and F_V/F_M from process stations P1 (30.0 °S, 156.0 °E), and P3 (46.2 °S, 159.5 °E). Water was collected at the depth of the fluorescence/chlorophyll-*a* max (Cmax) from these two stations to conduct Fe-enrichment experiments during the PINTS voyage (RV *Southern Surveyor*, Jan-Feb 2010).

Table 3.2 Daily uptake of dissolved Fe (dFe, nM) from Fe enrichment experiments at the conclusion of a 4-day incubation in samples with and without the addition of organic ligands. Water for the experiments was collected from two sites in the Tasman Sea, P1 (30.0 °S, 156.0 °E) and P3 (46.2 °S, 159.5 °E) during the PINTS voyage (RV *Southern Surveyor*, Jan-Feb 2010). Treatments measured after 4-d incubations comprised an unamended control (Con), two treatments containing Australian desert dust (D1, 2009 Brisbane dust storm, and D2, red composite from the Buronga region) which were predicted to release ~2 nM Fe, inorganic Fe only (2 nM), and organic ligands desferrioxamine B (DFB [15 nM]) natural pelagic bacterial exopolymeric substances (EPS, [0.8 nM]), glucuronic acid (GLU [15 nM]), and fulvic acid (FA, [100 µg L^{-1}], as Suwannee River Fulvic Acid). DFB, EPS, GLU and FA treatments also contained 2 nM inorganic Fe. Unfiltered = incubations where phytoplankton were present, filtered = incubations where phytoplankton was absent (0.2-µm filtered).

Errors are the half interval of duplicate samples. Where no errors are stated the values are from a single sample.

Table 3.3 Instrument sensitivity (expressed as Suwannee River Fulvic Acid equivalents (SRFA eq) in μg L^{-1}) and the reduction peak potential ($E_{p,}$ V vs Ag/AgCl electrode) from the determination of humic substance-like (HS-like) material from Fe enrichment experiments at T0 (unamended seawater) and after 4-d incubation in samples with and without the addition of organic ligands. Water for the experiments was collected from two sites in the Tasman Sea, P1 (30.0 °S, 156.0 °E) and P3 (46.2 °S, 159.5 °E) during the PINTS voyage (RV *Southern Surveyor*, Jan-Feb 2010). Treatments were as per Table 3.2. Unfiltered = incubations where phytoplankton were present, filtered = incubations where phytoplankton absent (0.2-μm filtered). Errors are the half interval of duplicate samples. Where no errors are stated the values are from a single sample.

Table 3.4 Variability in instrument sensitivity between experimental treatments after 4-d incubations in samples with and without the addition of organic ligands. Water for the experiments was collected at process station P3 (46.2 °S, 159.5 °E) in the Tasman Sea during the PINTS voyage (RV *Southern Surveyor*, Jan-Feb 2010). Treatments were as per Table 3.2. Statistically significant differences ($p \leq 0.05$) are highlighted in bold type.

Table 4.1 Constituents of synthetic seawater used for humic substance-like analysis. Based on AQUIL media as per Price et al. (1989) using major salt only. Final pH = 8.00

Table 4.2 Composition of exopolymeric substances (EPS) isolated from an Antarctic sea ice bacteria, a natural sub-Antarctic zone bloom (SAZ bloom) and axenic algal cultures (*Phaeocystis antarctica* and *Emiliania huxleyi)*. Relative concentration (%) of protein, uronic acid and neutral sugars present are shown together with total hydrolysable saccharides (reported as mmol C g^{-1} EPS).

Table 4.3 Mass distribution parameters for differential refractive index (DRI), UVD, and ^{56}Fe in the low molar mass (LMM) region of the respective signal fractograms exopolymeric substances (EPS) isolated from Antarctic sea ice bacteria, sub-Antarctic zone bloom (SAZ bloom) and axenic algal cultures (*Phaeocystis antarctica* and *Emiliania huxleyi*). *Mw* = weight average molar mass, *Mn* = number average molar mass, *Mp* = maximum peak intensity. Calculation for molar mass dispersity ($Đ_M$), $Đ = Mw/Mn$.

Table 4.4 Concentration of macronutrients (NO_X, NO_2, NH_3, PO_4) present in exopolymeric substances (EPS) isolated from Antarctic sea ice bacteria, a sub-Antarctic zone bloom (SAZ

bloom) and axenic algal cultures (*Phaeocystis antarctica* and *Emiliania huxleyi*). Data reported as nmol g^{-1} EPS.

Table 4.5 Concentration of trace metals present in exopolymeric substances (EPS) isolated from sea ice bacteria, a natural sub-Antarctic zone bloom (SAZ bloom) and axenic algal cultures (*Phaeocystis antarctica* and *Emiliania huxleyi*). Data reported as nmol g^{-1} EPS. <DL = below detection limit.

Table 4.6 Fe biogeochemistry associated with exopolymeric substances (EPS) isolated from an Antarctic sea ice bacteria, a natural sub-Antarctic zone bloom (SAZ bloom) and axenic algal cultures (*Phaeocystis antarctica* and *Emiliania huxleyi*). The overall % of labile iron (Fe_{Labile}) and the concentration of ligands associated with strong binding affinities ([L_1]), weaker binding affinities ([L_2]) and the sum of all ligands ([sumL]), together with the calculated conditional stability constant relative to inorganic iron (log $K_{Fe'L1}$, log $K_{Fe'L2}$ or log $K_{Fe'sumL}$) is presented. Electrochemically detected humic substance-like (HA-like) material is also shown and expressed as Suwanee River Fulvic Acid (SRFA) equivalents. Results are from a sample set measured after 24 h equilibration, and a further set measured after 9 weeks. Both sets were equilibrated at 4 °C in the dark.

Table 4.7 Pseudo-first-order rate constant (k' s^{-1}) and half-life ($t_{½}$) for Fe(II) (30 nM) oxidation at ambient laboratory temperature (22 °C) and 4 °C in 0.2-µm filtered seawater only (pH 8.09 ± 0.02) and in the presence of model saccharides and isolated natural bacterial and algal exopolymeric substances (EPS). Model ligands = Dextran (DEX, polysaccharide) in concentrations 50 – 500 nM (Ligand-to Fe-ratio, L: Fe 1.66 to 16.6), and Glucuronic acid (GLU, monosaccharide) in concentrations 50–5000 nM (L: Fe 1.66 to 166). EPS isolates = Antarctic sea ice bacteria, natural phytoplankton bloom from the sub-Antarctic zone (SAZ bloom), axenic algal culture of *Emiliania huxleyi*. EPS were added at a concentration to give L:Fe of 1.66. Seawater only n = 12 (22 °C) and 6 (4 °C), all ligands n = 3 for both temperatures.

Table 4.8 The effect of Fe associated with EPS (Fe-EPS) on the growth of the Southern Ocean diatom, *C. simplex,* over 187-h incubation at 4 °C and 50 µmol photons m^{-2} s^{-1}. Fe concentration in the Tasman Sea surface seawater medium was 0.56 nM. The growth of *C simplex* in the presence of Fe bound to EPS was compared to both inorganic Fe and seawater control. Growth rate (µ d^{-1}, calculated between 48-h and 118-h when all incubations were in exponential growth phase), final biomass at 187-h (cells ml^{-1}), and the bioavailability (in %) of Fe-EPS relative to inorganic Fe (assumed 100% bioavailable) is presented. Additions of Fe-EPS and inorganic Fe provided an additional 1 nM Fe to the seawater medium. EPS

isolates were from an Antarctic sea ice bacteria, a natural phytoplankton bloom from the sub-Antarctic zone (SAZ bloom), and from axenic algal cultures of *Phaeocystis antarctica and Emiliania huxleyi.* Errors represent the standard deviation of triplicate samples.

Table 5.1 Constituents of synthetic seawater (SS) based on AQUIL media as per Price et al. (1989) using major salts only. Final pH = 8.00. Background dissolved Fe = 0.73 ± 0.02 nM, n = 4

Table 5.2 Concentration of macronutrients (phosphate (PO_4), silicic acid ($Si(OH)_4$; µM) and trace metals (Iron (Fe), zinc (Zn) and copper (Cu); nM) present in filtrates of experimental samples simulating the wet deposition of Australian mineral dust into the Southern Ocean. The dust used was collected during a large dust storm over Brisbane, QLD., and resuspended in rainwater collected in the Tasman Sea (31°35.849'S 178°00.00'E, GP13 GEOTRACES voyage, 27/05/2011) before being exposed to UV + visible light (UV, 2000 µE), visible light only (VIS, 2000 µE), or kept in darkness (Dark). Resuspended, treated dust was added to synthetic seawater to give a dust enrichment of 0.5 mg L^{-1}. Data for single 0.2 µm and 0.02 µm filtered samples are presented. **Bold type** = Exp 2, non-bold type = Exp 3. Errors for PO_4 and $Si(OH)_4$ are the standard deviation of triplicate samples. Fe, Zn and Cu data is from a single sample. Concentrations measured in the dust treatments are the combined contribution of rainwater and dust. < DL = below detection limit.

Table 5.3 Fe size fractionation (soluble < 0.02-µm, colloidal 0.02- to 0.2-µm and particulate > 0.2-µm) of Fe associated with rainwater and Australian continental dust in experimental samples from two replicate experiments simulating the wet deposition of Australian mineral dust into the Southern Ocean. Solubilities of each size fraction are calculated using the total acid leachable concentration (372.1 nM) of Fe present in 0.5 mg L^{-1} dust. Treatments were as per Table 5.2. Relative concentrations (%) are presented. **Bold type** = Exp I, non-bold type = Exp II.

Table 5.4 Relative size distribution (%) of humic substance-like (HS-like) material in experimental samples from two replicate experiments simulating the wet deposition of Australian mineral dust into the Southern Ocean. Particulate = > 0.2-µm, Colloidal = 0.02- to 0.2-µm, soluble = < 0.02-µm. Treatments were as per Table 5.2. **Bold type** = Exp 2, non-bold type = Exp 3.

Table 5.5 Relative retention of Fe on C_{18} resin of Fe associated with Australian mineral dust. For comparison model ligands (humic acid (HA); desferrioxamine B (DFB), 15 nM; DTPA, 100 nM; glucuronic acid (GLU), 100 nM) are presented. Experimental medium was

synthetic seawater (pH 8.0). UV- and Dark-treated dust enrichments are presented. Unfiltered, 0.2-µm filtered and 0.02-µm filtered were measured to assess the nature of the organic ligands in each size fraction.

Table 5.6 The effect of Fe associated with Australian desert dust on the growth of the Southern Ocean diatom *C. simplex.* Bioavailability (%) of Fe associated with Australian mineral dust relative to inorganic Fe (assumed 100% bioavailable) measured after 24-h. Growth rate (µ d^{-1}, calculated between 136 h and 232 h when all incubations were in exponential phase) and final biomass after 326 h incubation period at 4 °C and 50 µmol photons m^{-2} s^{-1} are also presented. Treatments were as per Table 5.2. Error = standard deviation of triplicate samples.

SUMMARY

It is widely accepted that the complexation of iron (Fe) with organic compounds is the primary factor that regulates Fe reactivity and its bioavailability to phytoplankton in the open ocean. Despite considerable efforts to unravel the provenance of the many organic ligands present in the 'ligand soup' much of this pool remains largely unresolved and the ligands remain grouped into either strong (L_1) or weak (L_2) types. The Tasman Sea and Southern Ocean are areas of particular interest as both regions are subject to Fe limitation or co-limitation and are likely to be severely affected under climate change scenarios. The predictions of dryer conditions in central Australia suggest that the Tasman Sea may be subject to changes in the intensity and frequency of atmospheric dust deposition and, in consequence, enhanced Fe deposition into the surface waters. This thesis aims to improve our knowledge of a) how natural organic ligands affect Fe solubility, chemistry, and bioavailability, and b) which forms of Fe are available to phytoplankton.

Natural seawater samples (surface and profiles to 1000m) revealed that electrochemically detected HS-like material, which are thought to make up a proportion of the weaker L_2 class of ligands, account for a very small fraction of the Fe-binding organic ligand pool. The distribution of HS-like material in coastal, shelf and offshore regions associated with the EAC does not exhibit a nearshore to offshore (high to low) concentration gradient, likely because of low riverine HS-like input. Higher concentrations of HS-like material were generally found at, or adjacent to, the chlorophyll maximum (Cmax). However, little correlation with chlorophyll-*a* (Chl-*a*) was observed and so these higher concentrations are more likely linked to degraded algal material and microbial activity rather than direct primary productivity. Perturbation experiments using water collected offshore in the EAC and a cold core cyclonic eddy (CCE) indicated that the *in situ* utilisation and production of HS-like material, and its character, differ depending on the phytoplankton and microbial communities present, and reflect the biological activities of these different communities, as well as photochemical transformations. The addition of a model HS (Suwannee River fulvic acid) enhanced Chl-*a* concentration in both communities, particularly in the EAC, likely due to the remineralisation of Fe and other nutrients via photochemical and bacterial transformation of this material.

Seawater depth profiles from the northern and southern Tasman Sea indicate Fe limitation (or co-limitation) at the stations sampled. Dissolved Fe (dFe), organic ligand concentrations and conditional stability constants were consistent with previous studies (showing the

presence of mostly L_2 ligands) with higher ligand concentrations and conditional stability constants close to the Cmax. Ligand concentration, as previously reported, is in excess of dFe throughout the water column, although no correlation between dFe and ligand concentration was observed.

Fe-enrichment experiments using two contrasting phytoplankton communities investigated how the communities respond, in terms of biomass and community structure, to inorganic Fe delivered alone or bound to an organic ligand (siderophore, saccharides, bacterial exopolymeric substances (EPS)) or dust-borne Fe from two dust samples (D1 and D2) originating from the Australian continent. Overall, Fe bound to a strong Fe-binding siderophore was much less available to both phytoplankton communities; whereas, Fe bound to bacterial EPS (lowest conditional stability constant) induced the greatest increase in overall phytoplankton biomass. Dust D1 did not have the highest rate of dFe uptake, or result in the greatest increase Chl-*a*, but did induce the greatest shift in community structure. Whilst one ligand (L_2) was measured in most incubations, both L_1 and L_2 ligands were detected in the D1 and inorganic Fe incubations, indicating *in situ* biological production of Fe-binding ligands (i.e. siderophores or EPS) in response to Fe addition and an added ligand component from the dust. The greater response of the phytoplankton to the EPS and D1 led to further laboratory experiments.

Analysis of 4 EPS isolates (1 bacterial, 1 mixed natural community, and 2 microalgal laboratory cultures) showed that both bacterial and algal EPS contain functional components known to bind Fe (uronic acid, saccharides). The bacterial EPS was made up of mainly high molecular mass components, whereas the algal EPS were of low molecular mass. Most EPS contained components that were measured as both L_1 and L_2 ligands, with the L_1 ligands having an affinity for Fe close to that of bacterial siderophores. EPS greatly enhanced Fe solubility in seawater, however, it may also accelerate Fe(II) oxidation, and thus, Fe(II) removal from the system. Other trace elements and macronutrients were associated with the EPS that may be accessible to phytoplankton and could help to relieve nutrient limitation. Bioaccumulation experiments indicated that Fe bound to all EPS used was highly bioavailable to the Southern Ocean diatom *C. simplex* (50 to > 100%) relative to the bioavailability of inorganic Fe (assumed 100% bioavailable). This enhanced bioavailability was likely due to increased Fe solubility, and possible formation of more bioavailable forms of Fe.

Further experiments using dust D1, and rainwater collected in the Tasman Sea, revealed that despite low fractional solubilities (< 1%), the dust represents, potentially, an important

source of Fe and other vital macronutrients and trace elements. Both the rainwater and dust were associated with ligands in the L_2 class that helped to maintain the solubility of Fe. Light exposure, particularly UV, can a) have a substantial effect on the Fe chemistry of the Fe-laden dust, lowering the conditional stability constant and altering the size distribution of both Fe and ligands (including saccharides and HS-like material), and b) improve the bioavailability of dust-borne Fe to *C. simplex*.

The perturbation experiments in the EAC, CCE and north and south Tasman Sea demonstrated that organic ligands play an important role in regulating the nutrient dynamics of marine systems. They show that the bioavailability of Fe to phytoplankton is dependent on the various Fe species and Fe sources (i.e. inorganic Fe, organically bound, dust-borne), and that this differs between phytoplankton size fractions and from one bacterio- or phytoplankton species to another. The Tasman Sea and Southern Ocean receive, possibly increasing, periodic inputs of atmospheric dust from the source region of D1, which initiated a substantial community shift in perturbation experiments. However, the impact that dust-borne Fe will have on a natural phytoplankton community will be dependent on the duration and intensity of the dust deposition event, and the nutritive state and community structure of the resident phytoplankton. Bacterial siderophores have previously been suggested as key players in Fe biogeochemistry, however, in remote regions bacterial and algal EPS could play a significant role in the biogeochemical cycling of Fe and other nutrients, and their contribution should also be considered to further our understanding of the dynamics of Fe-limited oceans.