Zinc cycling in the Tasman Sea: distribution, speciation and relation to phytoplankton community

Marie Sinoir ^{(1), (2), *}, Michael J. Ellwood ⁽³⁾, Edward C. V. Butler ^{(1), (4)}, Andrew R. Bowie ^{(2), (5)}, Mathieu Mongin ⁽¹⁾, Christel S. Hassler ^{(6), (7)}

⁽¹⁾ CSIRO Marine and Atmospheric Research Laboratories, Castray Esplanade, GPO Box 1538, Hobart TAS 7000, Australia

⁽²⁾ Institute for Marine and Antarctic Studies, University of Tasmania, Hobart TAS 7001, Australia

⁽³⁾ Research School of Earth Sciences, Australian National University, Building 8, 61 Mills Road, Canberra ACT 2000, Australia

⁽⁴⁾ Australian Institute of Marine Science, Arafura Timor Research Facility, PO Box 41775, Casuarina MC, NT 0811, Australia

⁽⁵⁾ Antarctic Climate and Ecosystem Cooperative Research Centre (ACE CRC), University of Tasmania, Hobart TAS 7001, Australia

⁽⁶⁾ Plant Functional Biology and Climate Change Cluster, University of Technology of Sydney, Broadway NSW 2007, Australia

⁽⁷⁾ Present address: Institute F. A. Forel, University of Geneva, 10 rte de Suisse, 1290 Versoix, Switzerland

* Corresponding author: marie.sinoir@gmail.com

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Abstract:

Zinc (Zn) is essential for phytoplankton growth as it plays a central role within enzymatic systems in phytoplankton. Here, we present data for dissolved Zn and Zn chemical speciation for samples collected at four stations in the Tasman Sea east of Australia. Dissolved Zn concentrations determined using isotope dilution inductively coupled plasma – mass spectrometry (ICP-MS) ranged between 0.02 to 0.19 nmol L⁻¹ (15 m depth) and 0.02 to 0.11 nmol L⁻¹ (150 m depth) along a north-south transect. Zn speciation determined using Anodic Stripping Voltammetry (ASV) on four selected profiles from the Tasman Sea indicated that dissolved Zn is strongly complexed to natural organic ligands, with the conditional stability constant for the Zn-ligand complex (log K'_{ZnL}) ranging between 9.3 and 11.4. Dissolved ligand concentrations showed variations with concentrations ranging between 0.8 and 4 nmol L⁻¹. In surface waters, ligand concentrations always exceeded dissolved Zn concentrations, lowering free Zn²⁺ ion concentrations into the picomolar range. Based on comparisons between Zn speciation and the phytoplankton community of the Tasman Sea, we suggest that there is the potential for zinc limitation of certain phytoplankton groups in this region and feedback by phytoplankton on ligands, hence, on Zn biogeochemistry.

Introduction

Zinc is involved in multiple biological and structural functions, especially in relation to carbon and phosphate uptake (Badger 2003; Hoppe 2003; Kupriyanova and Pronina 2011; Sinoir *et al.* 2012). However, phytoplankton species possess different responses to zinc

depletion due to their own specific biological requirements (Sunda and Huntsman 1992). It is well accepted that a good understanding of chemical speciation is required to assess and define a metal's reactivity, but also to understand its bioavailability to marine phytoplankton. Indeed, complexation of metals by organic ligands (L) in seawater can decrease bioavailability, with respect to total dissolved fraction by several orders of magnitude (e.g., Saito *et al.* 2008). Although the concept of bioavailability is often difficult to define (Saito *et al.* 2008; Hassler *et al.* 2012a), the bioavailability of an element is critical to assess micronutrient limitation and its influence on the phytoplankton community. In contrast to iron, there is little information for the marine biogeochemical cycling of Zn, especially concerning chemical speciation and organic complexation. For these many reasons, studies on chemical speciation are one of the priorities of the GEOTRACES research program ('The Marine Biogeochemistry of Trace Elements and Isotopes', SCOR Working Group, 2007). It is essential to explore Zn speciation in seawater in different oceanic regions and throughout the water column in order to understand its marine biogeochemistry and cycling. New data on Zn speciation are recently emerging for different regions of the open ocean (Baars and Croot 2011; Croot *et al.* 2011; Wisniewski-Jakuba 2012; Kim *et al.* 2014), revealing interesting new features in the biogeochemistry of this trace element.

In open ocean waters, total dissolved concentrations of Zn (Zn_{tot}) – operationally defined as Zn species $< 0.2 \mu m$ particle size – are in the nanomolar range (Bruland 1980; Bruland 1989; Bruland and Lohan 2003) and show nutrient-like behaviour, including depletion in surface waters and higher concentrations at depth. In the south Tasman Sea, Ellwood (2008) reported nanomolar dissolved concentrations in winter ($0.38 \pm 0.15 \text{ nmol kg}^{-1}$, 0-100 m), but lower Zn_{tot} concentrations were reported in summer time ($0.16 \pm 0.05 \text{ nmol kg}^{-1}$, 0-125 m, station P3 Ellwood, unpublished data). At these low dissolved Zn

concentrations the bioavailable Zn concentration could be low enough to potentially constrain phytoplankton growth, especially considering a high percentage of dissolved Zn (up to 98%) is complexed by natural strong organic ligands (Bruland 1989; Ellwood and van den Berg 2000; Vraspir and Butler 2009; Hirose *et al.* 2011). The bioavailable forms then fall in the picomolar range in most regions studied (Ellwood and van den Berg 2000; Ellwood 2004; Baars and Croot 2011; Croot *et al.* 2011). Recent studies suggested a relationship between dissolved Zn concentrations and phytoplankton community structure in the subarctic and subantarctic Pacific Ocean, as well as in certain Subantarctic regions showing low levels of silicic acid (Leblanc *et al.* 2005; Hassler *et al.* 2012b; Wisniewski-Jakuba 2012). No study has yet determined Zn speciation and the potential for Zn to constrain phytoplankton in the Tasman Sea.

Here, we present Zn speciation data along a North to South transect in the Tasman Sea, a region especially sensitive to climate change (Hobday *et al.* 2008). Labile Zinc (Zn_{La}) concentration were measured using a metal-addition stepwise voltammetry (ASV) technique for four different stations, while total dissolved concentrations were determined by ICP-MS following preconcentration (Danielsson *et al.* 1978; Bruland *et al.* 1979). Ligand concentrations (L) and conditional stability constant (K'_{ZnL}) were calculated from the titration data using different mathematical techniques. The free Zn^{2+} ion concentrations were, then, derived from L and K'_{ZnL} . The comparison of concentrations of free Zn^{2+} and excess ligands (eL) with chlorophyll *a* and biomarker pigments indicative of dominating phytoplankton groups revealed an important correlation with phytoplankton community structure, suggesting the importance of zinc for the local assemblages during the 2010 austral summer.

Methods and material:

Study site

Samples were collected during ‘PINTS’ (Primary productivity induced by Iron and Nitrogen in the Tasman Sea; January and February 2010; voyage ss2010_v01 of R.V. *Southern Surveyor*). Three intensive stations with casts over the full water column and repeated upper water profiles and on-deck incubations experiments were performed during PINTS. These stations were called ‘process stations’ (Hassler *et al.* 2014; P1, P2, and P3, Suppl. Fig. 1; Table 1). For this study, profiles were sampled using trace metal protocols approved for GEOTRACES research voyages from surface to deep waters (GEOTRACES Cookbook at: <http://www.geotraces.org/images/stories/documents/intercalibration/Cookbook.pdf>; accessed 16 Dec 2015). A fourth station (Stn 14; Table 1), a revisit from the ‘SAZ-Sense’ voyage in January-February 2007 (Bowie *et al.* 2011), was also studied.

Sampling

Sample bottles and all labware used during sampling and measuring were soaked in detergent (Decon90, Fisher) overnight and then soaked in acid (10% HCl, Seastar Baseline[®]) for a month. They were then rinsed seven times with Milli-Q water (18 MΩ) in a clean room (ISO Class 5) according to the trace metal protocols recommended for GEOTRACES research voyages and trace metal analysis (Bowie and Lohan 2009; Cutter *et al.* 2012). Samples were collected using Teflon-coated Niskin 1010X bottles (General Oceanics, USA) mounted on an autonomous rosette (Model 1018, General Oceanics, USA), deployed on a Dynex 6mm braid rope (Hampidjan, AU), and sequentially pressure triggered. Water samples for trace metals analyses were filtered (0.2 µm; Pall, AcroPak 200) and then transferred into polypropylene bottles (125 or 150 mL) in a clean container under HEPA-filtered air (ISO Class 5 conditions). Samples were stored in triple plastic bags and frozen without

acidification. Water samples for determination of total dissolved metals were acidified with high-purity 12 M hydrochloric acid (Sigma Aldrich, 2 mL/L sample) and stored at room temperature, also triple bagged. Samples for nutrient analysis and pigments were collected from the standard conductivity-temperature-depth (CTD; SeaBird SBE11) rosette within 1 h of deployment of the autonomous trace metal cast at matching depths. Salinity, temperature, fluorescence and oxygen were obtained from calibrated CTD data using water collected from Niskin bottles (General Oceanics) on the CTD rosette as described in Hassler *et al.* (2014). Macronutrients (reactive phosphorus, PO₄; silicic acid, Si; nitrate-plus-nitrite, NO_x) were determined at sea by CSIRO Hydrochemistry using an automated flow-injection analyser and colorimetric techniques.

Biological data:

The biomass and community structure of phytoplankton assemblages were inferred from chlorophyll *a* (Chl *a*) and biomarker pigments, respectively (Jeffrey and Vesk 1997; Jeffrey and Wright 2006). Water was gently filtered on a GF/F filter (Whatman, < 5mm Hg) and kept in a cryo-vial in liquid nitrogen until further work. Pigments were extracted in the dark and on ice in 100% methanol following two stages of sonication and left overnight at 4°C. Filter debris are removed by centrifugation and the supernatant was analysed for pigments using High Performance Liquid Chromatography (Alliance, Waters) and a photodiode array detector following a modified version of the Van Heukelem and Thomas (2001) method (see Hassler *et al.* 2012*b* for details). Concentrations of Chl *a*, Chl *b* in sample were determined from standards from Sigma, while all other pigment concentrations were determined from standards from DHI (Denmark). Biomarker pigments analysed were Fucoxanthin (Fuco), 19-Butanoyloxyfucoxanthin (But-Fuco), 19 Hexanoyloxyfucoxanthin (Hex-Fuco), Zeaxanthin (Zea), Chl *b*, Peridinin (Perid) and Divinyl Chlorophyll *a* (DVChl *a*).

Biomarker pigments indicate the presence of the following phytoplankton group: Fuco for diatoms; But-Fuco and Hex-Fuco for diatoms and/or haptophytes, Chl *b* for chlorophytes or pelagophytes, Zea for cyanobacteria, Perid for dinoflagellates, DVChl *a* for tropical species such as the cyanobacteria *Prochlorococcus*. The fraction attributable to each class of phytoplankton was established in relation to total chlorophyll *a* (Chl *a*). To obtain an approximation of the local phytoplankton community structure, concentrations of pigments were then integrated from 30–125 m depth for P1, 15 to 70 m depth for P2 and Stn 14 and from 15–80 m depth for P3.

The pigment data were taken from 30 to 125 m for P1, 15 to 70 m for P2, 15 to 79 m for P3 and 15 to 75 m for Stn 14 during the voyage. In that regard, Zn data were considered in accordance with the measured pigments' concentrations for each station, giving a dataset of 16 observations (Suppl. Table 1) for which different correlations were established between Zn speciation and the biology (via pigments). PCA were run to define the correspondence between nutrients and Zn chemistry with biological parameters. All statistical tests (t-tests, PCA and Pearson's correlations) were run using Sigma Plot 11 and R to establish the significant difference between samples or correlations between parameters.

Background on voltammetry technique:

Here we utilised Anodic Stripping Voltammetry (ASV) to determine Zn speciation of Tasman Sea samples (Bruland, 1989; Ellwood, 2004; Wisniewski-Jakuba *et al.*, 2008a). For each sample aliquot the oxidation current (i_p) is measured and is a function of the concentration of labile Zn in solution through the following relationship:

$$i_p = (S\alpha_{Zn}) \times [Zn^{2+}] \quad (1)$$

where *S* is the slope of the linear portion of titration plot (current/concentration added) and α_{Zn} is the side-reaction coefficient for inorganic complexes of Zn in seawater. α_{Zn} was set to

2.1 (Turner *et al.* 1981). Natural organic ligand(s) in solution only react with free Zn^{2+} , not the zinc inorganically complexed, though inorganic zinc complexes are labile and readily dissociate. Hence, natural organic Zn-ligand complexes (ZnL) are formed between the free Zn^{2+} ion and free natural organic ligand (L) present in seawater:

At equilibrium, the relationship between the complexes and the ligand is given by K'_{ZnL} expressed as follows:

$$K'_{\text{ZnL}} = [\text{ZnL}] / ([\text{Zn}^{2+}][\text{L}]) \quad (2)$$

The concentration ZnL is calculated as the difference between Zn_{tot} concentration in the sample and the measured labile Zn concentration. The mass balance equation for total ligand (C_L) is described as follows:

$$C_L = [\text{ZnL}] + [\text{L}] \quad (3)$$

Zn-binding ligands were determined using both the Langmuir relationship (Ružić 1982; van den Berg, 1985) and with the Gerringa method (Gerringa et al., 1995) in order to compare the derived C_L and K'_{ZnL} values (Table 2). The Scatchard method (Scatchard, 1949; Wells *et al.* 2013) was used to verify whether several distinguishable organic ligand classes could be detected with each titration. For the Langmuir relationship method equations (2) and (3) were combined and rearranged to give:

$$([\text{Zn}^{2+}] / [\text{ZnL}]) = (([\text{Zn}^{2+}] / C_L) + 1 / (K'_{\text{ZnL}} C_L)) \quad (4)$$

Values of C_L and K'_{ZnL} are obtained from the slope and the intercept, respectively, of the plot of experimental data $[Zn_{La}]/[ZnL]$ versus $[Zn_{La}]$. Free zinc concentrations ($[Zn^{2+}]$) can be obtained by solving the following quadratic equation:

$$a[Zn^{2+}]^2 + b[Zn^{2+}] - c = 0 \quad (5)$$

$$\text{where } a = \alpha_{Zn}K'_{ZnL}; b = K'_{ZnL}C_L - K'_{ZnL}C_{Zn} + \alpha_{Zn} \text{ and } c = C_{Zn} \quad (6)$$

Instrumentation and mercury film formation:

Zinc speciation was determined using a 663 VA stand (Metrohm, Herisau, Switzerland) with a glassy carbon rotating electrode, a glassy carbon rod counter electrode, and a reference Ag/AgCl electrode equilibrated with a 3M KCl solution. The system was connected to a μ Autolab II and a current amplifier (PGSTAT 12). The software GPES version 4.9 (EcoChimie, The Netherlands) controlled the system and automatically drove the measurement sequence and data collection for samples (Suppl. Table 2). The mercury film required to perform the analysis was plated onto the working electrode. Following this protocol, the working Teflon cup was filled with a solution containing 10 mg L^{-1} of Hg^{2+} (BDH) and 30 mmol L^{-1} for KCl (Suprapur, Merk) and after a 5-min purge with nitrogen gas, the mercury was plated at a potential of -1.0 V for 15 min. The film was replaced before each titration. A cycle of five quick scans without Zn accumulation on the electrode was performed before any titration to check no Zn was detected on the Hg film.

Zinc speciation:

Ten 23-mL Teflon vials (Savillex, USA), used for titration, were conditioned for 24 h with the appropriate spike of Zn in a step-wise sequence from low (background seawater) to a high Zn concentration (10 nmol L^{-1}). This preliminary step is required to saturate the vial

walls with Zn, avoiding the loss of Zn by adsorption during the experiment and preventing experimental artefacts. The Zn standard (34.5 ppm) was prepared by adding 100 μ L of Zn stock solution (ICP-MS std, 1000 mg/L, Sigma Aldrich) and 0.560 μ L of HCl ultrapure (AR grade, Sigma Aldrich) in 100 mL of Milli-Q (Millipore) water. Each sample (125 mL) was thawed overnight at room temperature before measurements and 10-mL aliquots were prepared daily by pipetting 10 mL of sample and an appropriate volume of Zn standard with a minimum equilibration time of 30 min. After a 5-min purge with nitrogen gas (Ultra High Purity, BOC Australia), the Zn in the sample was accumulated onto the mercury film during 300 s at a potential of -1.2 V; this step was followed by a sample equilibration phase for 15 s (Suppl. Table 2). Following the scan in the positive direction, the current peak linked to Zn detection was typically observed at -1.04 V, which was in good agreement with previous studies (Ellwood 2004; Wisniewski-Jakuba 2008a).

The deposition time plays a role in the sensitivity of the method. Therefore, the linearity of the relationship between the deposition time of the sample and the current peak height was checked between 3 and 300 s. The linearity of the peak height was verified and the relationship gave a regression of $R^2 = 0.98$. All data tested were in the 5 % confidence interval of the relationship. In order to achieve greater sensitivity and lower detection limit, a 300 s deposition time was chosen. The peaks also increased with the addition of Zn in the samples (Suppl. Fig. 2). Each titration was preceded by two scans with no Zn additions to ensure that the working electrode was properly conditioned. Following this check, duplicates of scans were performed for each spiked sample. All manipulations in this work were done under a laminar flow hood and in a trace-metal clean area.

Total dissolved zinc determination

Total dissolved Zn concentrations required to calculate Zn speciation and excess ligand were determined using ICP-MS after isotopic dilution following solvent extraction (Danielsson *et al.* 1978; Bruland *et al.* 1979; Ellwood 2008) for the three process stations visited during the PINTS voyage. The detection limit for the method based on a 9 pmol L⁻¹ (3 times the standard deviation of the blank) based on a sample volume of 100 mL. Total dissolved Zn concentrations for the last station measured in this study (Stn 14 or SAZ-Sense), concentrations were determined using an ICP-MS procedure using in-line flow-injection with solid-phase extraction (iminodiacetate sorbent) for preconcentration as per Sullivan *et al.* (2013).

Excess ligand (eL) was simply calculated as the difference between ligand and Zn_{tot} concentrations ($eL = L - Zn_{tot}$)

Results

Study region

The Tasman Front (33–34°S) and Sub-Tropical Front (STF, 43–45°S) were distinguished by a subsurface frontal region in both *T* and *S* between 50–250 m and up to 400 m, respectively (see Hassler *et al.*, 2014 for detail, Fig. 1A). Moving southwards, marked a transition from higher to lower salinity with a distinct STF salinity front from 43–45°S. Even at 1000 m, where salinity gradients were small, there was a frontal region at 44 °S representing the boundary between Antarctic Intermediate Water (AAIW) of southern origin and the higher salinity northern variant which has been modified as it is advected around the South Pacific Gyre (Sokolov and Rintoul, 2000). Therefore, P1, P2 and P3 are all within different water masses; P1 north of the Tasman Front, P2 and P3 south of the Tasman Front, and P3 also south of the STF. Stn 14 and P3 are within the same water mass.

Total dissolved zinc concentrations

During the PINTS voyage, Zn_{tot} was measured from surface to deep waters (0–3500 m) for the three process stations (P1, P2 and P3). Total dissolved Zn concentrations were generally low (~ 0.1 nM) in surface waters and increased with depth in a nutrient-like profile to about 6–7 nM at 3000 m (Fig. 1B). In the first 1000 m, Zn_{tot} concentrations increased slightly poleward along the north-south transect from P1 (0.011 to 1.75 nM), P2 (0.11 to 2.61 nM) to P3 (0.18 to 2.44 nM) and Stn 14 (0.10 to 2.91 nM). The measurements obtained for P3 and Stn 14 are in the same range as the ones obtained by Ellwood (2008) in winter for a north-south transect in the south Tasman Sea transiting the Subtropical Front to the Subantarctic Front.

As previously observed (Ellwood 2008; Gosnell *et al.* 2012; Wisniewski-Jakuba *et al.* 2012; Hassler *et al.* 2014), there was a tight correlation ($R^2 = 0.98$) between silicic acid concentrations and Zn_{tot} for the four stations (Fig. 1C). The Zn:Si ratio yielded a value of 0.059 ± 0.001 ($n = 57$, $p \leq 0.001$). This correlation agreed with previous results obtained for different regions (e.g., Lohan *et al.*, 2002) and compiled by Croot *et al.* (2011).

Zinc complexation and speciation:

Plots of titration curves and $[Zn^{2+}]/[ZnL]$ versus $[Zn^{2+}]$ are presented (Suppl. Fig. 3) for two samples. Titrations curves (Suppl. Fig. 3 A and B) exhibited a curvature at the lowest Zn concentrations, suggesting the presence of ligand binding the metal. As we increased the Zn concentration with standard additions, the electrode response became linear, indicating that all the Zn-binding ligand(s) was titrated in the sample. $[Zn^{2+}]/[ZnL]$ versus $[Zn^{2+}]$ curves were linear (Suppl. Fig. 3 C and D), meaning the simple one-ligand model (Ružić calculation) could be used for the determination of Zn speciation in our study. The absence of

a second ligand class was verified using the Scatchard method (*data not shown*). Both van den Berg and Gerringa calculations produced very similar results (Table 2).

Dissolved ligand concentrations ranged between 0.23 and 4.19 nM (Table 3) and generally exceeded Zn_{tot} (Fig. 2, panel A to D). However eL revealed exceptions for sample depths of 750 m for P2, 750 m and 1000 m for P3 and those deeper than 500 m for Stn 14 (Fig. 3); at these depths the concentration of Zn_{tot} exceeded eL. P3 and Stn 14 had relatively constant distributions of the ligand throughout the water column, while P2 had a distribution related to biological activity in the upper 200 m. A distinct increase in ligand concentration (4.19 nM) was seen at station P2 at 70 m, where phytoplankton biomass peaked (Fig. 2 B), corresponding to the highest concentration obtained for the excess ligand (Fig. 3). Stability constant values ranged from $\log K'_{ZnL} = 9.3$ to 11.4 (Table 3) and were consistent with those found previously in similar studies (Donat and Bruland 1990; Wisniewski-Jakuba 2008b; Baars and Croot 2011).

Free Zn^{2+} concentrations also increased in a north-to-south gradient with P1 having concentrations ranging from 0.3 to 8.0 pM. Concentrations at P2 ranged from 0.4 to 191 pM, while P3 and Stn 14 had similar ranges with minimum values of 1.7 pM and maximum values between 204 and 239 pM. The strong complexation with organic ligands implied that Zn^{2+} concentrations were close to the concentrations ($[Zn^{2+}]$ less than $\sim 10^{-11}$ mol L⁻¹) that can limit certain phytoplankton growth in surface waters (Sunda and Hunstman 1992; Sinoir *et al.* 2015, *submitted.*).

The concentrations of ligands increased along the PINTS transect, from north to south as for Zn_{tot} , and showed interactions with the biology, especially in the upper 200 m of the water column. In surface waters (0–50 m), excess ligand ($eL = L - Zn_{tot}$) increased from P1 to Stn 14. In waters below 50m (50–125 m), excess ligand increased in the order: P1, Stn 14, P3 and P2, with a higher peak for P2 at 70 m depth corresponding to the identified

maximum of Chl *a* (*data not shown*). The biological interactions are explored further in the next section.

Biological data:

An approximation of the phytoplankton community was established from the pigments relative contribution to total Chl *a* integrated for the sunlit water column (0–125 m for P1; 0–70 for P2; 0 – 80 for P3 and 0 – 75 for Stn 14) revealing differences between stations (Fig. 4). P1 was dominated by haptophytes (Hex-Fuco; 24 %), cyanobacteria (Zea; 22 %) and tropical species (DV Chl *a*; 23%). P2 was also dominated by haptophytes (27%) and cyanobacteria (15 %), while chlorophytes represented 11 %. At P3, haptophytes were still dominant (44 %). Cyanobacteria were not significant with 5 % of the assemblages, while the chlorophytes represented 14 %. Stn 14, a reoccupation of SAZ-Sense P3, was dominated by haptophytes, cyanobacteria and chlorophytes (32, 13 and 15 %). The biomarker pigments of diatoms (Fuco and But-Fuco) were present at low levels for all station (5 to 12 %). Perid, biomarker for dinoflagellates was at very low levels for all stations, as a fraction less than 5%.

Values obtained for Chl *a* at the four studied stations revealed different distributions of biomass in the water column (Table 4). P1 had the lowest range of Tot Chl *a* concentrations (0.12 to 0.36 $\mu\text{g L}^{-1}$), with lowest values in surface waters and with a deep Chl maximum at depth (90–110m). P3 (0.18 to 0.92 $\mu\text{g L}^{-1}$) and Stn 14 (0.12 to 0.57 $\mu\text{g L}^{-1}$) had higher concentrations in the surface coinciding with Chl maximum between 15–25 m depth and low Chl *a* below 35m depth. The Chl *a* distribution at P2 exhibited an intermediate range with values from 0.16 to 0.69 $\mu\text{g L}^{-1}$ and a Chl *a* maximum at the depth of 40–60 m.

Correlations between Zn speciation and phytoplankton

The correlations between the parameters were explored using a PCA (Fig. 5) and Pearson's correlation table (Table 5). Considering the whole dataset (Fig. 5A and B), 61 % was explained along 2 dimensions. The first dimension was defined by Zn organic complexation (eL, K'_{ZnL} , L) as well as Chl *a* and other biomarker pigments (Chl *b*, Fuco, Hex-Fuco, Perid, But-Fuco) whereas the second dimension was described (correlation > 0.5) by dissolved nutrients (PO_4 , NO_x , Zn), Zn^{2+} as well as the biomarker Zeaxanthin (Zea). A third dimension, defined by Zn_{tot} and Si, was required so that all parameters (especially Chl *a*) were statistically represented across these dimensions (i.e. more than 90%). Not surprisingly some parameters were strongly related, such as Zn_{tot} and Si, Zn^{2+} , L, eL and K'_{ZnL} . Interestingly, Zn_{tot} is related to all macronutrients but not to L or eL. This coupling is consistent with the uptake and regeneration of on a similar length scale to that of the macronutrients (Twining et al., 2014). Most eukaryotic biomarker pigments (all except DV Chl *a* and Zea) are grouped around L and eL. Significant correlations were observed for Fuco, Perid and Chl *b*, suggesting that diatoms, pelagophytes and dinoflagellates could be a source of Zn-binding organic ligands in the study region. Fuco was also significantly correlated with Zn_{tot} and K'_{ZnL} , suggesting a capacity to release strong organic ligands, and high Zn requirement for diatoms. High requirements have already been mentioned in previous studies (Sunda and Hunstman 1992, 1995; Twining *et al.*, 2011; Twining and Baines, 2013). However, no correlation was observed between Si and Fuco ($R^2 = 0.027$).

DV Chl *a* and Zea are not related to other biomarker pigments. DV Chl *a* is significantly negatively correlated to Zn_{tot} and K'_{ZnL} , whereas Zea was anti-correlated to Zn^{2+} , suggesting that prokaryotes were favoured by low Zn availability. Zea was also anti-correlated with the other nutrients suggesting that multi-nutrient control might be at play. Amongst the discrete data points, four were not well represented (points 1, 5, 7 and 8; Table 4) by this PCA. P1 data were grouped, P2 data were close to P1, suggesting geographical

variations. All Chl *a* max data points are grouped, except for data point 5 (P1) which was, not surprisingly, more aligned with DV Chl *a* as *Prochlorococcus* were dominating at P1 (Hassler *et al.*, 2014).

To explore further how differences in Chl *a* affected the relations between parameters, data were separated into two groups (Table 4): the high Chl *a* regime (Chl *a* > 0.19 $\mu\text{g L}^{-1}$, P1 and P2 deep water; P3 and Stn 14 surface waters, Fig. 5C and D, Suppl. Table 3a) and the low Chl *a* regime (P1 and P2 surface water > 15m; P3 and Stn 14 deep water < 50 m; Fig. 5E and F, Suppl. Table 3b). The PCA using high and low Chl *a* data points showed interesting differences in the relationship between parameters, but for both Chl *a* regimes data points north and south of the Tasman Front were clearly differentiated (Fig. 5 D and F). When only high Chl *a* data points were considered, Chl *a* was not significantly related to any Zn parameters, but a significant correlation was measured for PO_4 and NO_x . All Zn chemical speciation parameters (Zn_{tot} , Zn^{2+} , K'_{ZnL}) are grouped and related to Fuco and Hex-Fuco, but a significant correlation was only measured between Fuco and Zn_{tot} (Suppl. Table 3). Overall, Hex-Fuco, But-Fuco and Fuco were also significantly related to PO_4 and NO_x . L and eL are closely related to Chl *b* with significant correlations. When considering low Chl *a* data points (Table 4), Chl *a* was not well represented in the PCA (< 60% along the two dimensions). For the low Chl *a* regime, Fuco and Perid were more closely related to Zn^{2+} , as well as Zn_{tot} and L. In that Chl *a* regime, Fuco, Hex-Fuco and Chl *b* were statistically related to Zn_{tot} (Suppl. Table 3). Chl *b* was also related to K'_{ZnL} and macronutrients (NO_x and PO_4).

As observed for the complete dataset, Zea and DV Chl *a* were clearly separated from eukaryotic pigments and anti-correlated to macronutrients for both Chl *a* regimes. However, significant correlations were only observed in the high Chl *a* regime, where DV Chl *a* was anti-correlated with Zn_{tot} and K'_{ZnL} and Zea was anti-correlated with NO_x , suggesting that

low NO_x availability could limit the growth of cyanobacteria of the type *Synechococcus*. Linear relationships between pigments, Zn_{tot} and Zn²⁺ are represented in Fig. 6 whereas relationships between pigments L and eL are represented in Fig. 7. Both figures illustrate the previous discussion on the different relationships explored in this paper.

Discussion:

Studies investigating the link between Zn and plankton community structure have produced interesting results with some studies suggesting there is a potential for Zn-limitation of phytoplankton growth although results differ regionally (e.g. Ellwood 2004; Lohan *et al.* 2005; Shaked *et al.* 2006; Wisniewski-Jakuba *et al.* 2008*a* and 2008*b*; Hassler *et al.* 2012*b*; Wisniewski-Jakuba *et al.* 2012). From all studies, Zn seems to be influencing the local community by either favouring or inhibiting certain phytoplankton and possibly other microbial taxa, and by acting subtly on the productivity or the composition of the assemblages (Crawford *et al.* 2003, Cullen and Sherrell 2005). This study in the Tasman Sea extends this assessment of the influence of Zn over oceanic, surface-water ecosystems.

One of the primary findings of this work is the observation of a gradient of concentration for all Zn species and associated ligands along the north-south transect and clear differences between stations south and north of the Tasman Sea according to all the parameters measured in this study. In surface waters (0–100 m), Zn was more related to the biology than the physical parameters in this region as previously indicated by Hassler *et al.* (2012*b*) for the Sub-Antarctic Zone (SAZ) region. Increasing Zn_{tot} concentrations matched the increasing ligand concentrations; therefore, the fraction of Zn associated with the strong Zn-ligand complex also increased, leaving concentrations of Zn²⁺ close to limiting levels for phytoplankton growth. This was especially true for P2, P3 and Stn 14 (59 to 98% of Zn

associated with a strong Zn-ligand). It has been shown in laboratory studies that the threshold for limiting the growth of the coastal diatom *Thalassiosira weissflogii* and the open-ocean diatom *Thalassiosira oceanica* was a concentration of Zn^{2+} lower than 10 pM and 2 pM, respectively (Sunda and Huntsman 1992, 1995; Morel *et al.* 1994). Therefore, surface concentrations of Zn^{2+} at P2 (0.5 to 3 pM) were close to the limiting threshold previously reported for oceanic diatoms, a marginal group of phytoplankton at northern PINTS stations (12 and 5 % from the pigments' data). However, Zn^{2+} concentrations at P3 (6 to 17 pM) and Stn 14 (4 to 18 pM) were potentially able to sustain diatom growth. The high degree of complexation, suggesting low bioavailability, is in good agreement with previous studies for different global regions (Bruland 1989, Donat and Bruland 1990, Ellwood 2004, Baars and Croot 2011).

The north-to-south gradient observed for the free and labile Zn species, and ligand concentrations was also observed for biological activity with Tot Chl *a* increasing from P1 to Stn 14. However, Chl *a*, a surrogate for phytoplankton biomass, was not significantly correlated to any Zn parameters, as observed by Wisniewski-Jakuba *et al.* (2012). Here diatoms (inferred from Fuco) were the only phytoplankton groups that were significantly related to dissolved Zn, suggesting a limiting role for Zn. Although Zn^{2+} is considered as the chemical species available for phytoplankton (Sunda and Huntsman 1992, 1995), some species have the ability to access organically complexed Zn to fulfil their requirements (Saito *et al.* 2002; Aristilde *et al.* 2012; Xu *et al.* 2012), including diatoms, *Nitzschia Closterium* CS-1, originating from the Tasman Sea (Sinoir *et al.*, 2015 *submitted*).

Our data showed that the correlation between Zn_{tot} and Fuco, a biomarker for diatoms was also more significant than the one for Zn^{2+} . In this regard, we can suggest that diatoms could rely more on weakly organically complexed Zn rather than solely on Zn^{2+} .

The relation between biology and Zn speciation was confirmed by the study of excess ligands. In their review, Gledhill and Buck (2012) hypothesized that excess ligands are indicative of the metal interaction with the biology. That said, with an excess of ligands reported in the upper water column (P1, P2, P3 and Stn 14), our results (Suppl. Table 1) are in good agreement with this perspective. Moreover, the highest excess of ligands (P2, 70 m) corresponds well with a Chl *a* maximum for this station. The persistent excess of ligands for P1, associated with low levels of Chl *a*, could suggest that the phytoplankton seasonal peak at P1 had already passed at the time of sampling and the bloom was in decline (Fig. 1).

The community of the four stations was dominated by haptophytes (e.g. *E. huxleyi* as picked up from the MODIS calcite composite map from the study region during the voyage, Mark Baird, pers. comm.). Aside from this group, cyanobacteria were the other main phytoplankton community members in the northern station with a switch towards chlorophytes moving southward. Although abundance of diatoms increased from P1 to P3, they never exceeded the abundance of haptophytes at time of sampling. Haptophytes might rely on Zn^{2+} to fulfill their requirements, even under low Zn concentrations. It is also known that species such as *E. huxleyi* can alleviate Zn depletion by replacing Zn by cobalt (Co) or cadmium (Cd) (Timmermans *et al.* 2001; Xu *et al.* 2007), which could explain their ability to grow under all conditions encountered in this study. Dissolved labile Co and Cd concentrations measured as per Sullivan *et al.* (2013; Butler *et al.* unpublished results) from surface water to the deep chlorophyll maximum for the study region, suggested that 3-15 pM Co and up to 10 pM Cd could be sufficient to complement Zn biological requirement.

The consistent domination of haptophytes might also be related to the high ligand concentrations. Indeed, the concentrations of ligands at P2 and P3 found in this study were some of the highest encountered in studies done with ASV (Table 3). Although higher ligand

concentrations have been previously reported in incubations after Zn additions (Lohan *et al.* 2005), we suggest that other phytoplankton species from the natural community could have been producing organic ligand exudates. Our data showed a significant relationship between the L and eL with diatoms, pelagophytes and dinoflagellates, suggesting that these phytoplankton are able to either produce or benefit from the presence of Zn organic ligands. Vasconcelos *et al.* (2002) and Vasconcelos and Leal (2008) have suggested that *E. huxleyi* growth can be promoted due to the nature of such exudates, especially chlorophyte exudates. If *E. huxleyi* growth is indeed promoted by exudates and facilitated by its lower requirements for Zn, this could also explain the dominance of small phytoplankton species in the Tasman Sea assemblage.

Here, our data hints at contrasting relationships between eukaryotic and prokaryotic phytoplankton with Zn chemistry. Cyanobacteria, which can grow without Zn, but do have a biological requirement for Co (Saito *et al.* 2002; Saito and Goepfert 2008) were negatively correlated to Zn chemical species, suggesting that Zn was not limiting but could be deleterious. The different relationships obtained between Zn chemistry and pigments revealed the further possibility of a link between the biology and speciation. Where they are present, cyanobacteria can overcome species like diatoms in assemblages due to their low requirements for Zn and the impossibility for large diatoms to efficiently substitute Zn by Co or Cd (Timmermans *et al.* 2001). When Zn allows the development of more nutritionally demanding species or species excreting/benefiting from organic biologically produced ligands, cyanobacteria could be out-competed. Moreover, the size of phytoplankton can be affected by Zn availability. For example, it has been shown that smaller diatoms can grow even under lower Zn²⁺ concentrations where larger ones cannot (Sunda and Huntsman 1992, 1995). The possibility of a phytoplankton community with small diatoms could explain their

presence at low Zn_{tot} concentrations, while the large diatoms could be Zn limited (Ellwood 2004, Lohan *et al.* 2005).

Furthermore, strong relationships with macronutrients observed here, suggested a multiple and complex nutrient limitation, in addition to co-limitation of other trace elements as previously suggested for the study region (Hassler *et al.*, 2012b; Hassler *et al.* 2014). In the Tasman Sea, the low abundance of diatoms arose from a strong Fe-Si limitation; in which case, the two together—Zn and Fe-Si co-limitation—could explain the marginal abundance of diatoms found throughout during the PINTS study (Hassler *et al.*, 2014; this study). No obvious relation between Si and the diatoms (Fuco) was observed. This lack of a relation is surprising as their association has previously been demonstrated in other regions (Wiesniewski-Jakuba *et al.* 2012).

Iron limitation has not been taken into account here to discuss the influence of Zn on phytoplankton assemblages. Several studies have previously shown the link between Zn and Fe limitation in deck incubations and cultures. De la Rocha *et al.* (2000) showed the influence of Zn-Fe limitation on Si uptake by phytoplankton while Franck *et al.* (2003) showed it on nitrate-nitrogen (NO_3) uptake. The metal-metal interactions, Zn-Fe as well as Zn-Co-Cd, and their influence on macronutrient uptake have not been explored here and further work is needed to relate speciation of micronutrients, availability and composition of phytoplankton in this region.

This study showed that different patterns and relationships could be observed when considering a high and a low Chl *a* regime, suggesting different dynamics and inter-dependency under oligotrophic vs. bloom situations. However, more studies are required to substantiate these findings.

This study has also shown the importance of differentiating chemical species and members of the phytoplankton community to understand the role of complexation in

establishing the bioavailability of Zn to the different phytoplankton taxa present. The corollary is that such observational and experimental refinement can demonstrate that the bioavailability of Zn influences the composition of the phytoplankton community (or more broadly, the assemblage of micro-organisms in oceanic, surface-water ecosystems) and vice versa, as different microorganisms are believed to affect and define the bioavailability of Zn and other metals by producing organic complexing ligands. Here we found that diatoms, pelagophytes, as well as dinoflagellates, could potentially excrete Zn-binding organic ligands. Some phytoplankton, such as *E. Huxleyi*, could benefit from the presence of these organic ligands that could specifically enhance Zn bioavailability. Clearly, more study is required to identify which phytoplankton excrete organic ligands and which phytoplankton benefit from this.

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Table captions:

Table 1: Location of the four stations studied. The depths indicated are the ones analysed in this study and in parentheses are the depths sampled for trace elements at each station.

Table 2: Comparison between van den Berg and Gerringa calculations. Ligand concentration "[L]", Zn_{tot} concentrations and stability constant "logK" are presented for all depths analysed in this study at P3 as well as differences between both methods.

Table 3: Comparison of Zn complexation results obtained in this study and in previous oceanic studies. Adapted from Baars and Croot (2011).

Table 4: Distinction between the two groups as a function of Chl *a*. This table presents all Chl *a* data per station and depth and, as a result, the separation between the high Chl (Chl *a* > 0.19 $\mu g L^{-1}$, P1 and P2 deep water; P3 and Stn 14 surface waters) and low Chl (P1 and P2 surface water > 15m; P3 and Stn 14 deep water < 50 m) groups. This table also serves for the PCA results.

Table 5: Results of Pearson's correlation between Zn species – Zn_{tot} and Zn^{2+} –, ligands (L), excess ligand (eL) and the different biomarkers used in this study. The upper number corresponds to the Pearson's coefficient and the italic number below is the *p* value. Significant correlations are in bold and highlighted.

Figure captions:

Fig. 1: A: Temperature versus salinity plot for the four PINTs stations studied here. B: Profiles of dissolved Zn concentrations (Zn_{tot}) for each station sampled and analysed in this study. Depth has been put in log scale for improved clarity. C: Relationship between Zn and

Si for each station sampled. The 1:1 line indicates $Zn_{tot} = Si$, meaning above it $Zn_{tot} > Si$ and below $Zn_{tot} < Si$.

Fig. 2: Profile of Zn_{tot} , Zn^{2+} and L concentrations as a function of depth for the four stations analysed here. The error bars present for the L and Zn^{2+} concentrations are the standard error between duplicates of scans. Chl *a* concentrations have been added as a green line with a different scaling to identified the biomass distribution throughout the water surface waters (0–125m).

Fig. 3: Excess ligand ($eL = L - Zn_{tot}$) concentration as a function of Zn_{tot} concentration.

Values for the four stations analysed here have been represented on the same graph. The 1:1 line indicates $Zn_{tot} = L$, above $Zn_{tot} > L$ and below $Zn_{tot} < L$.

Fig. 4: Composition of phytoplankton community based on pigment composition for each station in the top 100 m of the water column. Fractions were calculated from Tot Chl *a* and pigments data.

Fig. 5: PCA correlation circles between Zn speciation, biological data and dissolved nutrients applied to all data (A and B). Results from the distinction between the high Chl and the low Chl regimes are presented in panel C, D and E, F, respectively. A, C and E correspond to correlations obtained for continuous data (arrows) while B, D and F were obtained for discrete data (dots numbered). The dataset used to perform the PCA is found in Table 4 and Suppl. Table 1.

Fig. 6: Relationship between the biological indicators and Zn_{tot} and Zn^{2+} . A, E show the Tot Chl *a* as a function of $[Zn_{tot}]$ and $[Zn^{2+}]$, respectively. B, C represent two biomarkers for

haptophytes (Hex-Fuco and But-Fuco) as a function of Zn_{tot} concentration. F, G represent the two biomarkers as a function of Zn^{2+} concentration. D and H consider the biomarker for diatom, Fuco, as a function of Zn_{tot} and Zn^{2+} concentration, respectively. The black dots (●) correspond to depth where the Tot Chl *a* was the lowest for each station and the white dots (○) corresponded to the depth where the Tot Chl *a* was the highest in the water column for each station. Regression coefficients have been added to the corresponding curve and the significant regressions have been designated with an asterisk.

Fig. 7: A: Chl *a* as a function of eL. Only surface water depth (30-100 m) have been represented to be consistent with Chl *a* data. B: Correlations between L concentrations (nmol L⁻¹) and pigments (µg L⁻¹) from the different relevant phytoplankton groups. Only positive and/or significant correlation are represented for clarity.

Supplementary material captions:

Supplementary Table 1: Instrumental settings for the voltammetry measurements.

Supplementary Table 2: Zn species' and complexing ligand concentrations for samples analysed in this study. Ecological data (pigments and chlorophyll-a concentrations) are found in this table as well for the surface waters (30-100m) of the visited stations. The stations numbered are the 16 observations used for studying the interactions between biology and Zn speciation.

Supplementary Table 3: Results of Pearson' correlation between Zn species – free and labile-, ligands, excess ligand and the different biomarkers used in this study. The upper number

corresponds to the Pearson's coefficient and the italic number below is the p value.

Significant correlations are in bold. Suppl. Table *3a* corresponds to the high Chl level (HChl) and Suppl. Table *3b* corresponds to the low Chl level (LChl).

Suppl. Fig. 1: Google Earth NASA Modis image, colour composite of chlorophyll concentration and location of the stations analysed in this work for the period 28/01/2010 to 14/02/2010.

Suppl. Fig. 2: A typical stripping voltammogram representing the entire range of the scan obtained for a sample with increasing Zinc concentrations.

Suppl. Fig. 3: Titration curves obtained for two different samples at P2 (E52) and Stn 14 (9). A, B show the peak current (nA) as a function of [Zn] (nM: $Zn_{tot} + Zn_{added}$). C, D show the van den Berg linearisation of the data using Ruzic calculation ($\alpha = 2.1$)

Station name	Longitude	Latitude	Depth
			(m)
P1	165.0 E	30 S	15–300 m
			(15–3000 m)
P2	162.1 E	39.6 S	15–750 m
			(15–4500 m)
P3	159.5 E	46.2 S	15–1000 m
			(15–3500 m)
SAZ-Sense Station 14	153.2 E	45.6 S	15–1000 m
			(15–3750 m)

Sample ID	[Zn] _{tot} (nmol L ⁻¹)	Differences						Averages							
		VandenBerg		Gerringa method		VB vs Gerringa		VandenBerg				Gerringa			
		[L]	logK'ZnL,Zn'	[L]	logK'ZnL,Zn'	[L]	logK'ZnL,Zn'	[L]	logK'ZnL,Zn'	[L]	logK'ZnL,Zn'	[L]	logK'ZnL,Zn'	[L]	logK'ZnL,Zn'
								average	stddev	average	stddev	average	stddev	average	stddev
E71-15m	0.18	1.66	10.17	1.68	10.18	0.02	0.00	1.58	0.12	10.07	0.15	1.61	0.09	10.08	0.14
E71-15m		1.49	9.96	1.55	9.98	0.06	0.02								
E233-28m	0.17	2.02	10.34	2.02	10.15	0.00	0.19	2.13	0.15	10.15	0.28	2.10	0.11	10.12	0.05
E233-28m		2.23	9.95	2.17	10.08	0.06	0.14								
E126-50m	0.24	2.09	9.75	2.16	9.96	0.07	0.21	2.03	0.08	9.84	0.12	2.09	0.09	10.06	0.15
E126-50m		1.98	9.92	2.03	10.16	0.06	0.24								
E26-79m	0.11	1.71	10.07	1.69	10.25	0.02	0.17	1.76	0.07	9.97	0.14	1.75	0.08	10.23	0.02
E26-79m		1.81	9.88	1.80	10.22	0.00	0.34								
E66-121m	0.12	1.74	10.07	1.66	10.31	0.08	0.24	1.64	0.15	10.45	0.54	1.62	0.06	10.39	0.11
E66-121m		1.54	10.83	1.58	10.46	0.04	0.37								
E44-146m	0.11	0.99	10.58	1.14	10.15	0.14	0.43	1.18	0.26	10.05	0.74	1.27	0.19	9.94	0.29
E44-146m		1.36	9.53	1.41	9.73	0.05	0.20								
9-300m	0.28	1.45	10.09	1.65	9.37	0.20	0.73	1.51	0.08	9.94	0.21	1.66	0.02	9.45	0.12
9-300m		1.57	9.79	1.67	9.54	0.10	0.26								
7-500m	0.55	1.79	9.30	1.69	9.33	0.10	0.03	1.81	0.02	9.24	0.08	1.72	0.04	9.25	0.10
7-500m		1.82	9.18	1.74	9.18	0.08	0.00								
5-750m	1.23	1.83	9.16	1.51	9.53	0.32	0.37	1.74	0.13	9.10	0.09	1.43	0.12	9.48	0.08
5-750m		1.64	9.03	1.34	9.42	0.30	0.39								
3-1000m	2.44	2.91	9.48	2.70	9.63	0.20	0.15	3.71	1.13	9.24	0.35	3.43	1.03	9.34	0.42
3-1000m		4.51	8.99	4.16	9.04	0.35	0.05								

0.10 0.24 pool stddev 0.22 0.27 0.18 0.15

[L] in nmol/L

logK'ZnL,Zn' inorganic conditional stability constant

Region	Sample	[L] (nmol L ⁻¹)	log K' _{ZnL}	References	Method
North Pacific	Profile	1.0 – 1.5	10.7– 11.3	Bruland (1989)	ASV
North Pacific	60 and 150m	1.7–2.3	11.0 – 11.5	Donat and Bruland (1990)	ASV
	60 and 150m	1.6 – 2.3	10.1 – 10.5	Donat and Brulan (1990)	CSV
North Pacific	15 m	0.7	10.5	Lohan <i>et al.</i> (2005)	CSV
North Atlantic	Surface	0.4 – 2.5	10.0 – 10.5	Ellwood and van den Berg (2000)	CSV
North Atlantic	Profile	0.9 – 1.5	9.8 – 10.5	Jakuba <i>et al.</i> (2008)	FF - ASV
	Profile	0.6 – 1.3	9.8 – 11.3	Jakuba et al. (2008)	FF- ASV
South Pacific	20 m	1.3	10.6	Ellwood (2004)	ASV
	20 m	1.2	10.2	Ellwood (2004)	CSV
Southern Ocean	10–200 m	1.4 – 5.3	9.1 – 10.4	Baars and Croot (2011)	ASV
Drake Passage	25–100 m	1.4 – 2.5	9.7 – 9.8	Baars and Croot (2011)	ASV
Zero Meridian	10–200 m	2.5 – 5.3	9.1 – 10.4	Baars and Croot (2011)	ASV
Tasman Sea					
P1	15–300m	0.23 – 1.48	9.3 – 11.0	This study	ASV
P2	Profile	0.55 – 4.19	9.3 – 11.1	This study	ASV
P3	Profile	1.18 – 3.71	9.4 – 10.8	This study	ASV
Stn 14	Profile	1.23 – 2.65	9.6 – 11.4	This study	ASV

Stn	Depth (m)	Chl <i>a</i> (µg/L)	Number all data	Number high Chl <i>a</i>	Number low Chl <i>a</i>
P1	30	0.112	1		1
	50	0.195	2	1	
	72	0.291	3	2	
	100	0.13	4		2
	125	0.374	5	3	
P2A	15	0.168	6		3
	30	0.196	7	4	
	50	0.29	8	5	
	68	0.509	9	6	
P3	15	0.575	10	7	
	30	0.429	11	8	
	50	0.137	12		4
	80	0.115	13		5
Stn14	15	0.421	14	9	
	30	0.319	15	10	
	50	0.118	16		6

	But- Fuco	Fuco	Hex- Fuco	Zea	DVChla	Perid	Chlb	Chla
Zn _{tot}	-0.149 <i>0.581</i>	0.563 0.023	0.243 <i>0.365</i>	-0.284 <i>0.286</i>	-0.594 0.015	-0.101 <i>0.711</i>	0.178 <i>0.509</i>	0.065 <i>0.812</i>
L	0.194 <i>0.471</i>	0.565 0.023	0.415 <i>0.110</i>	0.031 <i>0.908</i>	-0.098 <i>0.718</i>	0.539 0.031	0.763 0.001	0.441 <i>0.088</i>
eL	0.214 <i>0.425</i>	0.525 0.037	0.402 <i>0.122</i>	0.060 <i>0.825</i>	-0.042 <i>0.878</i>	0.565 0.023	0.768 0.001	0.447 <i>0.083</i>
Zn ²⁺	-0.141 <i>0.603</i>	0.007 <i>0.981</i>	-0.102 <i>0.706</i>	-0.577 0.019	-0.156 <i>0.563</i>	-0.353 <i>0.180</i>	-0.224 <i>0.403</i>	-0.308 <i>0.246</i>
K' _{ZnL}	-0.243 <i>0.403</i>	0.572 0.033	0.408 <i>0.148</i>	0.191 <i>0.513</i>	-0.640 0.014	0.422 <i>0.133</i>	0.393 <i>0.165</i>	0.270 <i>0.350</i>
Si	0.134 <i>0.622</i>	-0.019 <i>0.945</i>	-0.041 <i>0.880</i>	-0.543 0.030	-0.035 <i>0.898</i>	0.036 <i>0.895</i>	0.354 <i>0.178</i>	-0.076 <i>0.781</i>
Nox	0.138 <i>0.610</i>	0.134 <i>0.621</i>	0.066 <i>0.809</i>	-0.726 0.001	-0.330 <i>0.212</i>	-0.159 <i>0.556</i>	0.215 <i>0.423</i>	-0.100 <i>0.713</i>
PO4	0.184 <i>0.495</i>	0.303 <i>0.253</i>	0.184 <i>0.496</i>	-0.702 0.002	-0.468 <i>0.068</i>	-0.078 <i>0.773</i>	0.305 <i>0.250</i>	0.010 <i>0.970</i>

p value in italic, significant correlation in bold
and highlighted

P1

P2

P3

Stn 14