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Fe-binding dissolved organic ligands near the Kerguelen Archipelago in the Southern Ocean (Indian sector)

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Abstract

During the *Ke*rguelen *O*cean and *P*lateau compared *S*tudy (KEOPS; January–February 2005) cruise, the area southeast of the Kerguelen Archipelago in the Indian sector of the Southern Ocean was investigated to identify the mechanisms of natural iron fertilization of the Kerguelen Plateau. In this study, the organic speciation of Fe is described. Samples were determined immediately on board using competing ligand-adsorptive cathodic stripping voltammetry (CL-AdCSV). The dissolved organic ligands were always in excess of the dissolved Fe concentration, increasing the residence time in the water column and the potential availability for phytoplankton. The concentration of the dissolved organic ligands ranged from 0.44 to 1.61 *n*Eq of M Fe (= complexation site for Fe), with an average concentration of 0.91 *n*Eq of M Fe (S.D. = 0.28, $n = 113$) and a mean logarithm of conditional stability constant ($\log K'$) of 21.7 (S.D. = 0.28, $n = 113$). A second weaker dissolved organic ligand group was detected in 32% of the samples, with Fe-binding characteristics at the edge of the detection window of the applied method.

The occurrence of the highest concentrations of dissolved organic ligands in the wind-mixed surface layer and near the sediment at the bottom of the water column indicated that both phytoplankton and the sediment act as sources. Both sources are in concert with the general conclusions from the KEOPS research on the sources of Fe, where Fe was regenerated, organic Fe-binding ligands were formed in the upper layers, and both Fe and ligands were supplied by the sediment.

Keywords: Iron; Organic complexation; Natural iron fertilization; Southern Ocean; Kerguelen Plateau

1. Introduction

The concentration of the essential nutrient Fe in open-ocean seawater is low due to a low solubility of Fe and lack of Fe sources (Kuma et al., 1996; Millero, 1998; Watson, 2001; de Baar and de Jong, 2001). Phytoplankton growth is limited in the high-nutrient low-chlorophyll (HNLC) regions of the oceans, of which the Southern Ocean is the largest. The artificial Fe fertilization experiments EisenEx, EIFEX, SOIREE, and SOFeX proved that Fe is indeed limiting in the Southern Ocean (Boyd et al., 2000; Gervais et al., 2002; Coale et al., 2004; Hoffman et al., 2006).

The concentration of dissolved organic ligands of Fe is for the major part responsible for the potential solubility of Fe in seawater (Kuma et al., 1996; Millero, 1998) above the solubility of 0.2 nM (amorphous) Fe-hydroxides in oxygenated seawater (Liu and Millero, 2002). Over 99% of the total dissolved Fe (Fe_{diss}) is bound to dissolved organic ligands; part of this is probably bio-available. As logical as this may seem, it is still a subject of much debate whether these ligands increase or decrease the availability of Fe for individual phytoplankton species (Hutchins et al., 1999; Sunda, 2001; Rijkenberg, 2005).

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It is not clear whether phytoplankton excrete organic ligands in order to control bio-availability of Fe in seawater. Dissolved organic ligands in the open ocean do originate from phytoplankton blooms and the associated bacteria in the surface waters (van den Berg, 1995; Rue and Bruland, 1997). Marine heterotrophic bacteria and cyanobacteria are known to excrete siderophores, i.e., a direct mechanism 'on purpose' of Fe ligand production (Reid et al. 1993; Wilhelm and Trick, 1994; Benderliev and Ivanowa, 1994; Wilhelm et al., 1996; Martinez et al., 2001). It is less clear whether eukaryotic phytoplankton can excrete siderophores (Fuse et al., 1993; Boye and van den Berg, 2000; McCormack et al., 2003). Only in a few cases has an apparently straightforward relationship between ligand characteristics and phytoplankton biomass been found (Rue and Bruland 1997; Boye et al., 2005; Gerringa et al., 2006). Virus-mediated lysis also might play an important role in the recycling of complexed Fe (Poorvin et al., 2004; Mioni et al., 2005).

Due to the extra energy of the lower wavelength band of solar radiation Fe(III) can be photo-reduced to Fe(II) (Waite et al., 1995; Kuma et al., 1995; Boye et al., 2003; Rijkenberg et al., 2004). In oxygenated waters, short-lived Fe(II) is thought to be one of the fractions of Fe_{diss} that is available for biota (Anderson and Morel, 1980; Takeda and Kamatani, 1989; Maldonado and Price, 2001). The role of the dissolved organic ligands in this process is still unclear; do these substances promote photo-reduction, e.g., by metal–ligand charge transfer, or do they hamper photo-reduction since the Fe(III) is firmly bound by the ligands (Barbeau et al., 2001; Croot et al., 2001; Boye et al., 2003; Rijkenberg et al., 2006a, b; Salmon et al., 2006)?

In contrast to artificial Fe fertilizations, islands in the open ocean are known to continuously supply Fe to the surrounding waters. Blain et al. (2001, 2007, 2008a) showed that Fe and phytoplankton were elevated in the wake of the Kerguelen Archipelago. This so-called "island mass effect" was first observed around the Hawaiian Islands by Doty and Oguri (1965) and since then has also been recognized to have an effect on [Fe_{diss}] near the Galapagos Island by Martin et al. (1994) and in the Southern Ocean by Blain et al. (2001), Bucciarelli et al. (2001), and Croot et al. (2004). In the waters between Crozet and Kerguelen islands, Sedwick et al. (2002) proved that while other limits to phytoplankton growth exist, Fe is always one of them.

The objective of the *Ker*guelen *O*cean and *P*lateau compared *S*tudy (KEOPS; January–February 2005) was to identify the mechanisms of natural iron fertilization of the Kerguelen Plateau. According to Blain et al. (2007, 2008b) and Sarthou et al. (2008), Fe is coming from the plateau, but during phytoplankton blooms is also supplied by the remineralization of sinking particles. Since dissolved organic ligands increase the solubility of Fe, their concentration is critically important. In this specific research of the KEOPS project, the role of dissolved organic ligands was studied in relation to the natural Fe fertilization. Bucciarelli et al. (2001) concluded that near the Kerguelen Islands (less than 200 m water depth) the input of Fe was directly from land by river run-off, whereas further away transport from the sediments was the source of Fe. Either way, dissolved ligands have to be present to keep Fe in the dissolved phase. If the input of Fe(III) exceeds the concentration of empty ligand sites, Fe(III) will precipitate and eventually disappear from the water column. Thus, the question arises whether the ligands are limiting and defining the extent of transport of Fe from the source into the seawater. Another question is whether the ligands have the same source as the Fe and are thus directly part of the natural Fe fertilization or whether ligands are produced by phytoplankton blooms and thus indirectly related to the natural Fe fertilization. Therefore, the subject of this paper is the relationship between the ligand characteristics (concentrations and binding strength) and (i) the presence or absence of phytoplankton, bacteria, and viruses, (ii) the water mass, and (iii) the distance to the bottom sediment.

2. Materials and methods

2.1. Sampling and sample treatment

The KEOPS cruise (Blain et al., 2008a) took place in January and February 2005, in the Southern Ocean near the Kerguelen Archipelago (50°S, 70°E) (Fig. 1) on board the R.V. *Marion Dufresne*. Samples were collected by a metal-clean sampling system, using a winch with an 8-mm Kevlar wire and metal clean modified Teflon-coated PVC GoFlo bottles (General Oceanics). After recovery, the

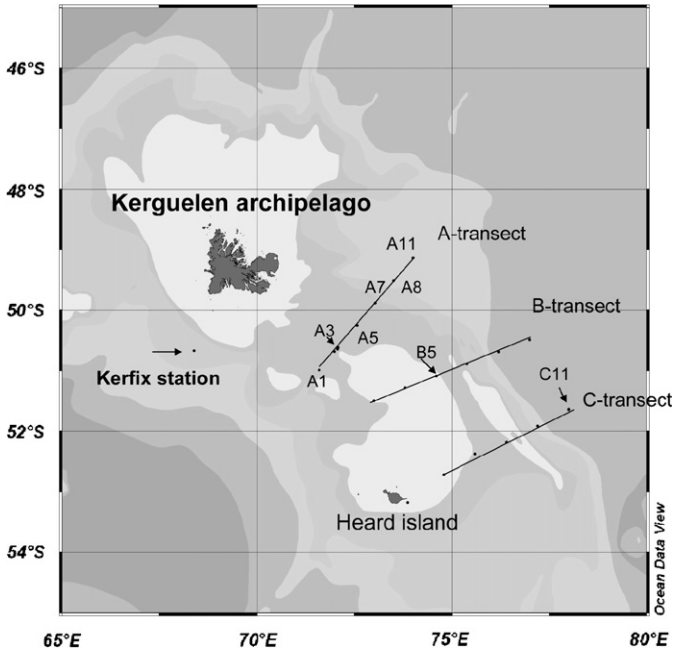

Fig. 1. Map of the area near the Kerguelen Archipelago in the Indian part of the Southern Ocean, with the stations discussed in this research indicated.

GoFlo bottles were brought immediately into an over-pressurized class 100 clean air container, where the samples were filtered in-line through a 0.2-μm filter (Sartorius Sartobran 300 capsule #5231307H5) using N_2 overpressure. Samples for dissolved iron analysis were acidified to pH 2.0 using 1 mL of Ultrapur® hydrochloric acid (HCl, Merck) per 1 L of sample for at least 24 h before analysis.

Dissolved iron concentrations were measured on board according to a chemi-luminescence method adapted from Obata et al. (1993) and Blain et al. (2008b).

The following stations were sampled for the analysis of dissolved organic ligands (Fig. 1): A1, A3 (three visits: I on 19 January 2005, II on 23 January 2005, and III on 4 February 2005), A5, A7, A8, A11, B5 and C11 (two visits: I on 27 January 2005, and II on 6 February 2005). The samples ($n = 86$) were stored at 1 or 2 °C and analyzed as soon as possible on board of the ship.

Three profiles (station A3 (I and III) and station C11 (I)), for a total of 27 samples) were sampled in duplicate and were analyzed both on board and later in the home laboratory after frozen storage (−20 °C) and transport.

During the last visit (12 February 2005) to station A3, pore waters were obtained from cores collected in polycarbonate tubes. The top layers of the sediment cores were sliced in layers of 0.5 cm at 1 °C under a nitrogen atmosphere with a metal-free cutter in a plastic glove compartment. These layers were centrifuged in 50-mL tubes with void space filled with nitrogen. Overlaying water was subsequently filtered with a clean prolypropylene 20-mL syringe over a clean surfactant free cellulose acetate 0.2-μm filter (Viollier et al., in preparation). The pore water samples were stored frozen (−20 °C).

2.2. The voltammetric method

Determination of the organic speciation of iron in seawater was performed using competitive ligand-adsorptive cathodic stripping voltammetry (CL-AdCSV) using 2-(2-thiazolylazo)-*p*-cresol (TAC) (Aldrich, used as received) as the competing ligand (Croot and Johansson 2000) and a mixed NH_3/NH_4OH borate buffer is used to keep the pH at 8.05, near the natural value.

All solutions were prepared using 18.2 MΩ nanopure water. The equipment consisted μAutolab voltammeter (Ecochemie, the Netherlands), a static mercury drop electrode (Metrohm Model VA663), a double-junction Ag/saturated AgCl reference electrode with a salt bridge containing 3 M HCl, and a counter electrode of glassy carbon. All equipment was protected against electrical noise by a current filter (Fortress 750, Best Power). The titration was performed using 0.01 M stock solution of TAC in three time quartz-distilled (3 × QD) methanol, 1 M boric acid (Suprapur, Merck) in 0.3 M ammonia (Suprapur, Merck) (extra cleaning by the addition of TAC after which TAC and $Fe(TAC)_2$ was removed with a C18 SepPak column) to buffer the samples to a pH of 8.05 and a 10^{-6} M Fe(III) stock solution acidified with 0.012 M HCl (3 × QD). Twelve aliquots of 15 mL were spiked with Fe(III) to final concentrations between 0 and 8 nM and allowed to equilibrate overnight (> 15 h) with 5 mM borate buffer and 10 μM TAC. The concentration of $Fe(TAC)_2$ in the samples was measured using the following procedures: (i) removal of oxygen from the samples for 200 s with dry nitrogen gas, a fresh Hg drop was formed at the end of the purging step; (ii) a deposition potential of −0.40 V was applied for 180 s, the solution was stirred to facilitate the adsorption of the $Fe(TAC)_2$ to the Hg drop; and (iii) at the end of the adsorption period, the stirrer was stopped and the potential was scanned using the differential pulse method from −0.40 to −0.90 V at 19.5 mV s^{-1}.

The principle of measuring the binding characteristics of dissolved organic ligands with Fe is extensively described by Gledhill and van den Berg (1994), and by Croot and Johansson (2000). L and K′ of the natural dissolved organic ligands were estimated using the non-linear regression of the Langmuir isotherm (Gerringa et al., 1995),

$$K'_{Fe(TAC)_2} \times \frac{[TAC']^2}{K'_{FeL}} \times [L'] = \frac{[Fe(TAC)_2]}{[FeL]},$$

where K′ is the conditional stability constant of Fe with the ligands, (either TAC or the natural organic ligands (L)) and [L′] and [TAC′] are the concentrations of free (not Fe bound) ligands. $[Fe(TAC)_2]$ and [FeL] are the concentrations of Fe complexes. The inorganic speciation of Fe (Fe′) is described by $[Fe'] = 10^{10} \times [Fe^{3+}]$ (Hudson et al., 1992). The results, including the standard deviations of the regression, are presented in Table 1. Large errors for some of the data (18 samples with standard deviations > 0.3 for log K′) were due to noise in the data. According to technicians on board it was a mechanical interference due to the vibrations of the ship when in full speed. The non-linear regression of the Langmuir isotherm also can be used to detect the presence of two organic ligands, L_1 and L_2, with different conditional stability constants, K'_{FeL_1} and K'_{FeL_2} (Gerringa et al., 1995):

$$K'_{Fe(TAC)_2} \times \frac{[TAC']^2}{(K'_{FeL_1} \times [L'_1] + K'_{FeL_2}) \times [L'_2]} = \frac{[Fe(TAC)_2]}{\Sigma[FeL_i]}.$$

The pore water samples were defrosted before analysis and since the volumes of these samples were only between 4 and 9 mL, they were diluted with seawater from station C11, G25 bottles 3 and 5. To reduce the dilution as much as possible, only 110 mL (instead of the normal 200 mL) for seven subsamples was used as volume for the ligand titration (0, 0.5, 1, 1.5, 2, 4, and 8 nM Fe additions).

2.3. Phytoplankton characteristics, bacteria, and viruses: methods and application into stepwise multiple regression

Automated backward stepwise multiple regression was applied with Systat (version 10, 2000) using default values for fitting (Gerringa et al., 2006). The following parameters

Table 1
Dissolved Fe (nM) and ligand characteristics of the sampled stations, assuming the existence of one dissolved organic ligand

Station and depth	[Fe_{diss}] (nM)	S.D.	[Lt] (*n*Eq M Fe)	S.D.	log K′	S.D.	pFe	log α
A1								
−20	0.103	0.001	1.47	0.20	21.49	0.19	22.6	12.63
−60	0.065	0.003	0.78	0.19	21.28	0.29	22.3	12.13
−100	0.067	0.001	0.83	0.11	21.98	0.26	23.0	12.86
−200	0.074	0.000	0.68	0.15	21.61	0.41	22.5	12.39
−300	0.095	0.006	0.88	0.08	21.73	0.15	22.6	12.62
−400	0.247	0.017	0.5	0.10	21.79	**0.36**	21.8	12.19
−500	0.368	0.010	0.87	0.10	21.6	0.17	21.7	12.30
−550	**0.365**		1.29	0.14	21.45	0.13	21.9	12.42
−600	**0.365**		1.23	0.19	21.58	0.20	22.0	12.52
−620	0.384	0.003	1.38	0.15	21.67	0.16	22.1	12.67
A3, I								
−20	0.147	0.005	1.05	0.11	21.82	0.20	22.6	12.78
−40	0.127	0.015	1.23	0.08	21.62	0.13	22.6	12.66
−60	0.128	0.002	1.03	0.14	21.44	0.14	22.3	12.40
−80	0.117	0.005	1.02	0.09	21.66	0.13	22.5	12.62
−100	0.113	0.003	0.85	0.15	21.18	0.18	22.0	12.05
−150	0.107	0.004	0.88	0.06	22.09	0.15	22.9	12.98
−200	0.119	0.005	1.13	0.12	21.44	0.14	22.4	12.44
−500	0.385	0.028	1.03	0.14	21.42	0.17	21.6	12.23
A3, II								
−20	0.075	0.004	0.7	0.14	21.75	**0.37**	22.7	12.55
−60	0.069	0.002	0.93	0.21	21.39	0.26	22.5	12.33
−80	0.066	0.002	0.74	0.08	22.11	0.23	23.1	12.94
−100	0.059	0.001	0.47	0.10	21.87	**0.35**	22.7	12.48
−150	0.077	0.002	0.79	0.15	21.41	0.26	22.4	12.26
−200	0.142		0.63	0.10	21.85	0.26	22.4	12.54
−300	0.174	0.012	0.45	0.08	21.61	0.29	21.8	12.05
−400	0.236	0.011	0.7	0.15	22.12	0.21	22.4	12.79
−450	0.250	0.003	0.61	0.12	22.11	**0.51**	22.3	12.67
−500	0.316	0.012	1.04	0.13	21.63	0.17	22.0	12.49
A3, III								
−120	**0.112**		1.14	0.09	21.82	0.15	22.8	12.83
−150	0.107	0.001	1.15	0.12	21.67	0.18	22.7	12.69
−200	0.191	0.004	0.995	0.08	21.92	0.15	22.5	12.83
−350	0.242	0.003	0.61	0.08	21.91	**0.31**	22.1	12.48
−400	**0.300**		1.32	0.11	21.51	0.12	22.0	12.52
A5								
−40	0.068	0.000	0.89	0.07	22.25	0.20	23.3	13.16
−80	0.071	0.000	1.13	0.14	21.5	0.17	22.7	12.52
−110	0.074	0.004	0.99	0.14	21.43	0.18	22.5	12.39
−130	0.111	0.000	0.69	0.07	21.67	0.19	22.4	12.43
−150	0.090	0.003	0.69	0.08	21.92	0.27	22.7	12.70
−200	0.123	0.005	0.5	0.07	22.18	**0.42**	22.7	12.76

Table 1 (*continued*)

Station and depth	[Fe_{diss}] (nM)	S.D.	[Lt] (*n*Eq M Fe)	S.D.	log K′	S.D.	pFe	log α
−300	0.149	0.003	0.48	0.10	22.11	**0.47**	22.5	12.63
−350	0.215	0.025	0.65	0.08	22.08	0.26	22.4	12.72
−400	0.194	0.00	0.91	0.10	21.6	0.15	22.2	12.46
−450	0.169	0.02	0.81	0.10	21.65	0.20	22.2	12.46
A7								
−20	0.071	0.004	0.7	0.14	21.67	**0.38**	22.6	12.47
−40	0.094	0.012	1.0	0.12	21.83	0.24	22.8	12.79
−60	0.051	0.003	0.65	0.14	21.57	**0.36**	22.6	12.35
−80	0.054	0.005	0.7	0.07	21.97	0.23	23.1	12.78
−100	0.047	0.004	0.98	0.25	21.3	0.29	22.6	12.27
−150	0.058	0.002	0.95	0.13	21.73	0.20	22.9	12.68
−200	0.087	0.006	0.79	0.10	21.7	0.19	22.6	12.55
−300	0.123	0.011	0.9	0.11	21.73	0.20	22.5	12.62
−400	0.155	0.004	0.5	0.13	21.98	**0.56**	22.3	12.52
−550	0.203	0.015	0.61	0.06	21.92	0.18	22.2	12.53
A8								
−40	0.091	0.002	0.67	0.08	21.85	0.20	22.7	12.61
−60	0.050	0.002	0.83	0.12	21.53	0.18	22.7	12.42
−80	0.055	0.002	0.74	0.09	21.73	0.26	22.8	12.57
−125	0.046	0.000	0.98	0.16	21.34	0.18	22.6	12.31
−200	0.075	0.005	1.01	0.15	21.32	0.18	22.4	12.29
−400	0.120	0.002	0.55	0.33	21.54	**0.56**	22.1	12.17
−600	0.148	0.001	0.61	0.10	21.45	0.22	21.9	12.11
−800	0.185	0.003	0.44	0.07	22.24	**0.47**	22.4	12.65
−1150	0.161	0.005	0.99	0.16	21.29	0.24	22.0	12.21
A11								
−125	0.088	0.004	0.088	0.004	22.03	**0.51**	22.7	12.67
−150	**0.085**				21.48	0.26	22.3	12.23
−200	0.082	0.003	0.082	0.003	21.41	0.21	22.2	12.11
−400	0.088	0.001	0.088	0.001	21.58	0.29	22.2	12.15
−600	0.142	0.010	0.142	0.010	21.11	**1.12**	21.5	11.70
C11, I								
−80	0.066	0.006	0.171	0.002	21.37	0.29	22.6	12.39
−100	0.054	0.002	0.77	0.10	21.84	0.20	23.0	12.70
−150	0.087	0.008	1.05	0.20	21.37	0.24	22.4	12.35
−200	**0.103**		0.88	0.14	21.62	0.26	22.5	12.51
−500	**0.238**		0.74	0.12	21.97	0.29	22.3	12.67
−1500	0.377	0.009	0.9	0.17	21.83	**0.37**	22.0	12.55
−2000	**0.356**		1.17	0.16	21.55	0.17	21.9	12.46
−2500	0.356	0.000	1.37	0.23	21.61	0.26	22.1	12.62
B5								
−40	0.064	0.001	0.97	0.14	21.69	0.25	22.8	12.65
−43	**0.060**		1.26	0.12	21.55	0.15	22.9	12.63
−80	0.051	0.003	0.77	0.26	21.62	0.06	22.8	12.48
−93	**0.052**		0.94	0.12	21.66	0.15	22.9	12.61

−120	0.055	0.006	0.71	0.10	21.99	**0.35**	23.1	12.81
−150	0.087	0.004	0.85	0.23	21.49	**0.40**	22.4	12.37
−200	0.087	0.003	0.77	0.14	21.6	0.29	22.5	12.43
−300	0.178	0.004	1.34	0.12	21.89	0.15	22.7	12.96
C11, II								
−120	**0.240**		0.999	0.12	21.94	0.21	22.4	12.8
−200	**0.215**		1.39	0.27	21.33	0.22	22.1	12.4
NIOZ								
A3, I								
−20	0.147	0.005	1.58	0.20	21.56	0.15	22.5	12.72
−60	0.127	0.015	1.61	0.07	21.91	0.07	23.0	13.08
−80	0.128	0.002	0.85	0.14	21.41	0.20	22.2	12.27
−100	0.117	0.005	1.19	0.04	22.53	0.09	23.5	13.56
−150	0.113	0.003	0.88	0.06	21.86	0.12	22.7	12.74
−200	0.101	0.004	0.88	0.11	21.5	0.18	22.4	12.39
−400	0.119	0.005	1.02	0.15	21.56	0.20	22.4	12.51
−450	0.266	0.002	0.83	0.10	21.74	0.20	22.1	12.49
−500	0.385	0.028	0.9	0.10	22.22	0.22	22.3	12.93
A3, III								
−40	0.113	0.011	1.49	0.10	21.78	0.12	22.9	12.92
−80	0.117	0.001	1.12	0.20	21.01	0.15	21.9	12.01
−120	**0.112**		1.31	0.16	21.54	0.16	22.6	12.62
−150	0.107	0.001	0.85	0.12	22.04	0.24	22.9	12.91
−200	0.191	0.004	0.99	0.08	21.93	0.14	22.6	12.83
−300	**0.217**		1.02	0.05	21.95	0.10	22.5	12.85
−350	0.242	0.003	0.5	0.05	22.49	0.26	22.5	12.90
−400	**0.300**		1.52	0.17	21.82	0.16	22.4	12.91
−450	**0.300**		0.85	0.13	21.23	0.15	21.5	11.97
C11, I								
−80	0.066	0.006	1.16	0.08	21.71	0.13	22.9	12.75
−100	0.054	0.002	1.15	0.10	22.33	0.18	23.6	13.37
−150	0.087	0.008	1.39	0.12	21.98	0.14	23.2	13.10
−200	**0.103**		0.92	0.08	21.86	0.16	22.8	12.77
−800	**0.272**		0.88	0.07	22.07	0.19	22.4	12.85
−1000	**0.268**		0.9	0.15	21.59	0.21	22.0	12.39
−1500	0.377	0.009	0.58	0.05	22.79	0.36	22.5	13.10
−2000	**0.356**		0.9	0.15	22.01	0.19	22.2	12.75
−2500	0.356	0.000	1.37	0.15	22.04	0.19	22.5	13.05

pFe, negative logarithm of ionic [Fe].
$\log \alpha$, $\log(K' \times [\text{free ligand sites}])$.
S.D., 1 standard deviation. Large S.D. in bold (>0.3) were probably caused by mechanical vibrations of the ship (see Section 2).
Station A3 was sampled three times (I, 18 January; II, 23 January; and III, 4 February).
Station C11 twice (I, 26 January; II, 6 February).
Stations A3(I and III) and C11(I) were analyzed on board and at NIOZ.
See Fig. 1 for the station positions.
Fe concentrations in bold were not validated (not confirmed by a second measurement) or not determined and were obtained by mean of the samples above and below.
Excess ligand and $\log K'$ are independent of $[Fe_{diss}]$, the value for $[L_t]$ is not.

were subjected to multiple regression with the ligand concentration [Lt] or the excess [Lt] over [Fe_{diss}] concentration as dependant variable and [Fe_{diss}], the cell abundance (cells mL^{-1}) of distinguished phytoplankton groups, the chlorophyll-based biomass ($\mu g\,L^{-1}$) of distinguished phytoplankton groups, the nutrients (Si, N, and P, μM) and the abundance of bacteria and of viruses as variables.

To determine the numerical abundance and species composition of the phytoplankton community, freshly taken samples (<1 h after sampling) were analyzed using a flow cytometer (Veldhuis and Kraay, 2000, 2004; Timmermans et al., 2008). Phytoplankton cells were distinguished from other particles based on their chlorophyll fluorescence. For a detailed description of the phytoplankton community structure, see Armand et al. (2008) and Brussaard et al. (2008). In the present study, we used the following populations dominating the phytoplankton community; small eukaryotes (with an estimated spherical diameter (esd) ranging from 2 to 4 μm), predominantly single cells of *Phaeocystis* (esd of 4–9 μm) and a mixture of mostly diatoms (based on size and microscopical determination, esd 8–30 μm). Based on pigment composition and flow cytometric determination of size (Forward Light Scatter), *Synechococcus* cells and pico eukaryotes (esd <2 μm) were identified as separate phytoplankton groups. However, both groups were each less than 3% of the total cell numbers.

Bacteria and viruses were determined using flow cytometry as described by Brussaard et al. (2008). In short, fixed samples (0.5% glutaraldehyde final concentration) were diluted in Tris:EDTA buffer (10:1 mM, pH = 8) upon thawing 5- and 50-fold, respectively. After staining with SYBR Green I (0.5×10^{-4} of commercial stock; Molecular Probes, Invitrogen) for 10 min at 80 °C in the dark and 5 min cooling down to room temperature, the samples were counted using a benchtop Becton Dickinson FacsCalibur Flowcytometer.

A distinction was made in samples above and below the wind-mixed layer and samples above and outside the Kerguelen Plateau (see Section 4). The mixed layer was identified by applying the definition of the depth where the γ = potential density $\sigma - 1000$ differs by 2% from the surface value (Price et al., 1978).

3. Results

3.1. Physical description of the area

Definitions given by Park et al. (1993) on water masses and fronts in the Indian sector of the Southern Ocean are used here. The research area lies south of the Polar Front, as was also observed in other years by scientists (Park et al., 1993, 2008a, b; Park and Gamberoni, 1997; Blain et al., 2001) as well as penguins (Charrassin et al., 2004). The surface water (until around 400 m depth) consisted of Antarctic Surface Water (AASW) in which the top layer was wind mixed with a seasonal variation in temperature between −0.2 °C (WW in C11) and 3.5 °C (A3). Below the wind-mixed layer (WML; 44–128 m thick during KEOPS) the temperature decreased to a minimum (varying between −0.2 °C at C11 and 1.6 °C at A7 and B5) forming the winter water (WW) at about 150–200 m depth (Fig. 2B and D). Below the WW, a permanent thermocline existed to the boundary (B) with the Circumpolar Deep Water (CDW). B is defined as the layer of water where the temperature stops increasing at a steep rate at around 400 m depth (Fig. 2B and D). The CDW is a mixture of water from the three oceans and Antarctic water. In most profiles, Indian deep water (IDW) could be distinguished by its temperature maximum (2–2.3 °C) and oxygen minimum (varying between 4 and 4.7 $mg\,L^{-1}$) at 500–650 m depth (Fig. 2B–D). Only in the deep profiles A11 (not shown) and C11 could the influence of the North Atlantic deep water (NADW) be identified in waters below ~1500 m depth. The NADW is characterized by a temperature decrease with depth and the salinity increase to a maximum of 34.7 for the present KEOPS stations (Fig. 2A and B) (Park et al., 1993). Although in profiles A11 (>2400 m) and C11 (>3000 m) the Antarctic Bottom Water (AABW) was recognized, no samples could be taken at that depth (>2000 m).

The chlorophyll fluorescence data showed that stations A1–A5 and B5 had higher concentrations of phytoplankton compared to station A7–A11 and C11 (Fig. 3) (Armand et al., 2008; Timmermans et al., 2008; Brussaard et al., 2008). The phytoplankton concentrations were quite different during the three visits of station A3 (I on 19 January 2005, II on 23 January 2005, III on 4 February 2005). The bloom existing during the first visit appeared to have decreased somewhat during the second visit and increased again at the third visit (Fig. 4A). The sub-surface maximum in Chlorophyll *a* just below the surface mixed layer is due to sinking of large phytoplankton (diatoms). Unpublished results from Veldhuis showed that the chlorophyll content of the phytoplankton is constant with depth indicating sinking of cells which are not light limited. This was derived from the fact that chloropylly content per cell of the different groups of phytoplankton, as measured with flow cytometry, remained constant over depth. In case of a subsurface chlorophyll maximum caused by light limitation, the chlorophyll per cell would have increased with depth. The vertical sections shown in Park et al. (2008b) point out that subduction is highly improbable, since the isopycnals are horizontal (they do not outcrop to the surface).

3.2. Dissolved organic ligand concentrations

The dissolved Fe-binding organic ligands had a mean concentration of 0.91 *n*Eq of M Fe ($n = 113$, S.D. = 0.28) and a mean excess over [Fe_{diss}] of 0.75 *n*Eq of M Fe ($n = 113$, S.D. = 0.28) (Fig. 5; Table 1). The mean logarithm of the conditional stability constant ($\log K'$) was 21.73 ($n = 113$, S.D. = 0.32).

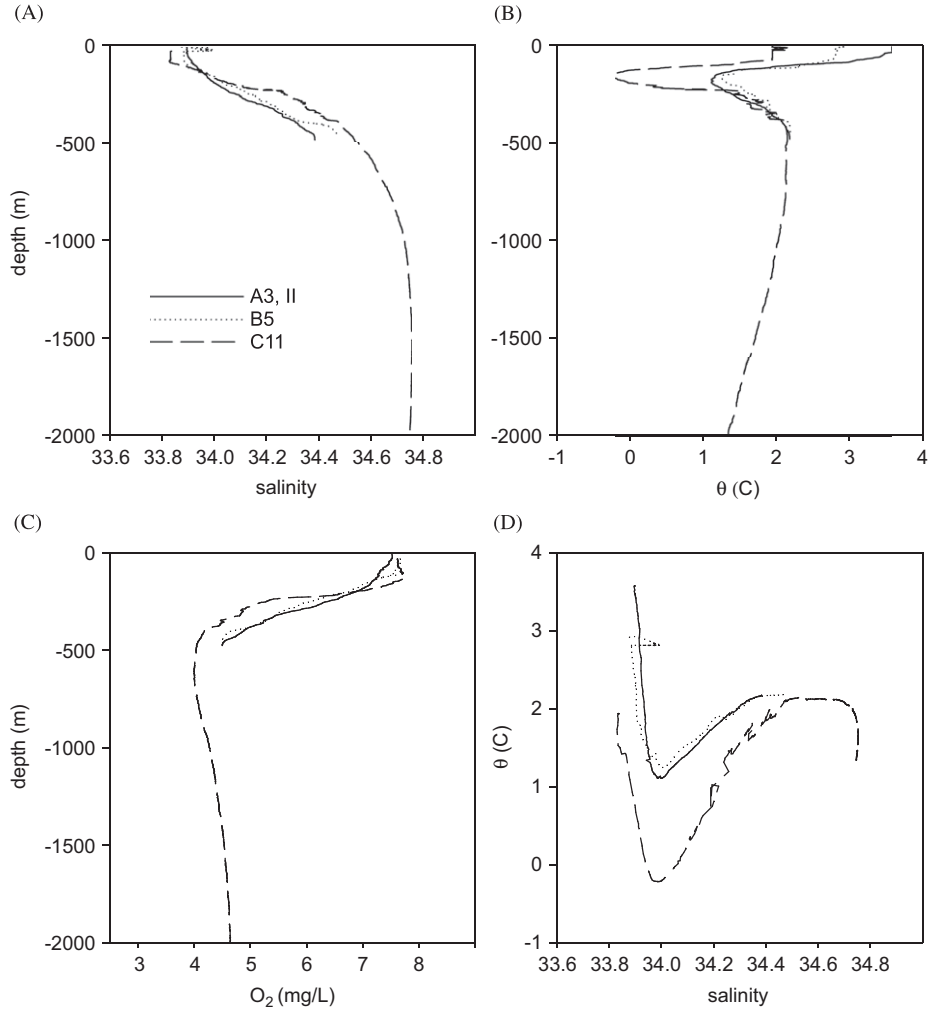

Fig. 2. Hydrographic data (ctd) of the stations A3 (II = second visit on 23 January 2007), B5 and C11 (only data used until 2000 m depth): (A) salinity; (B) potential temperature (θ °C); (C) oxygen (mg L^{-1}); and (D) the θ–S property–property plot.

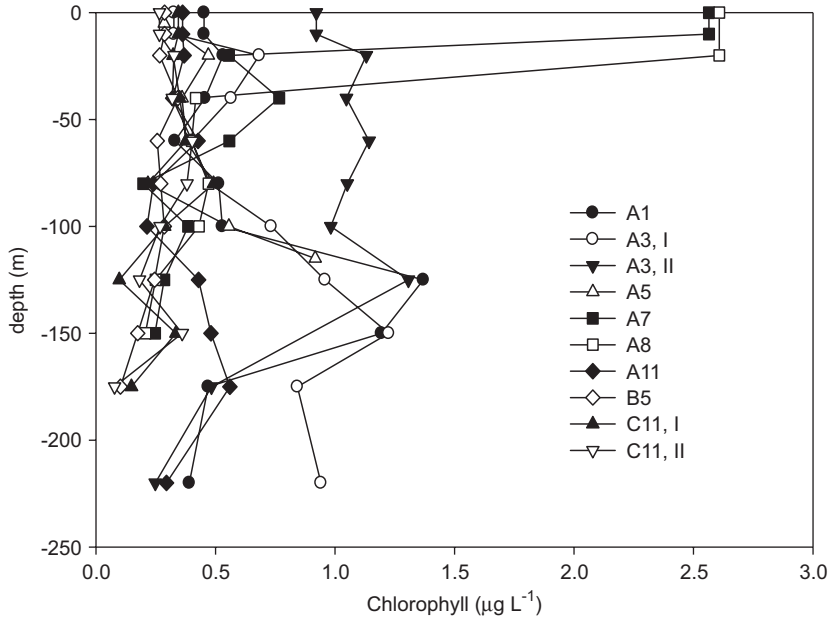

Fig. 3. Chlorophyll-based biomass (µg L^{-1}) of the phytoplankton as determined by flow cytometry for all stations.

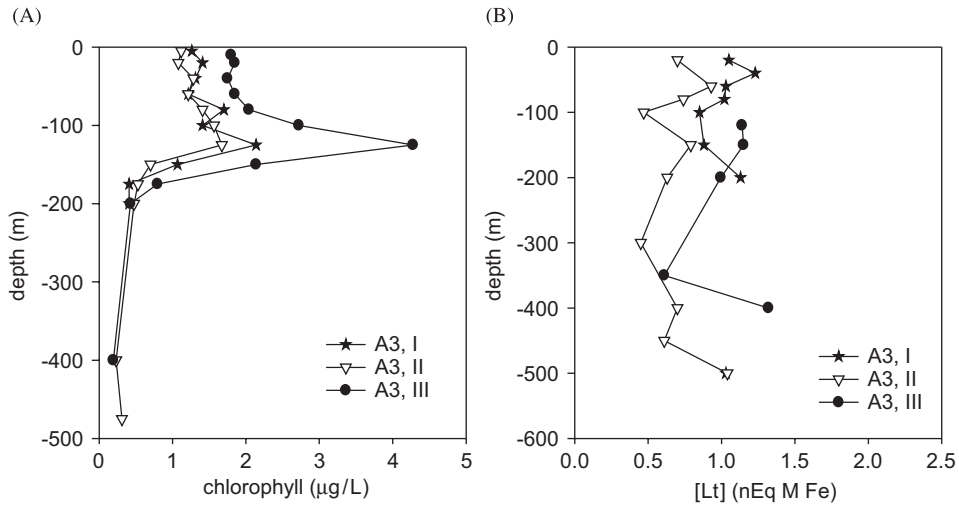

Fig. 4. Chlorophyll fluorescence and the dissolved organic ligand concentrations of station A3 during three different visits: (A) chlorophyll fluorescence ($\mu g\,L^{-1}$); (B) the ligand concentration [Lt] (*n*Eq of M Fe) (values measured on board).

In 32% of the samples, two groups of dissolved organic ligands could be distinguished, with a mean conditional stability constant of the relatively strong ligand group ($\log K'_1$) of 22.06 ($n = 36$, S.D. = 0.38) and a mean ligand concentration $[L_1] = 0.57$ *n*Eq of M Fe ($n = 36$, S.D. = 0.14), whereas the relatively weak ligand group had $\log K'_2 = 19.71$ ($n = 36$, S.D. = 0.65), a mean $[L_2] = 2.29$ *n*Eq of M Fe ($n = 36$, S.D. = 1.55). The weak ligand group has an alpha value ($\log \alpha = \log(L \times K') = 11.07$) at the edge of the detection window (DW) of the applied method, assuming that DW was one order of magnitude around the alpha value of the competing ligand TAC ($\log DW = 12.4$) (van den Berg and Donat, 1992). We therefore only considered the results obtained calculating one dissolved organic ligand group.

The duplicate series obtained by a second analysis in the home laboratory (A3 I, A3 III, and C11; Table 1) showed the same trends with the exception of the top layer of A3 I for which the analyses at the home laboratory gave higher ligand concentrations. Either the two upper samples were slightly contaminated with Fe on board or during thawing, and/or Fe was retained on the bottle walls.

The ligand concentrations were highest in the surface layer where phytoplankton lived and in samples near the sediment with a minimum around 300–400 m depth as is most distinct in profiles A1–A5 (Fig. 5). In station A7, the variation with depth of the ligand concentration was less than in the other stations. In order to get a clearer picture of the influence of different parameters in the water column on the ligand concentrations, we distinguished six different water types and calculated mean values for their Fe and ligand characteristics (Fig. 6). Within the AASW, a distinction was made between water containing more than $0.1\,\mu g\,L^{-1}$ chlorophyll *a* (type 1) from the euphotic zone characterized by the presence of phytoplankton, and the WW (type 2) characterized by the temperature minimum and the absence of living phytoplankton ($<0.1\,\mu g\,L^{-1}$ chlorophyll *a*). The boundary (B) with the CDW at around 400 m depth forms type 3, this water type was not sampled in every station. The IDW, the oldest water with the oxygen minimum at 500–650 m depth is type 4. Type 5 is characterized by the proximity of the sediment on the plateau (A1–A5, B5, samples taken within 50 m of the sediment surface). Type 6, where the influence of NADW is recognized, is found at depths below around 1500 m depth. For all stations on the plateau, the distributions of the types 1–5 waters increased directly with depth. Only at the deepest off-plateau stations (A11 and C11) was type 6 present and there the sequence with depth was types 1–4 and then type 6.

With the application of the multiple regression no relevant relationships between the ligand concentration and other parameters such as $[Fe_{diss}]$, the cell abundance ($cells\,mL^{-1}$) of distinguished phytoplankton groups, the chlorophyll-based biomass ($\mu g\,L^{-1}$) of distinguished phytoplankton groups, nutrient concentrations (Si, N, and P) and the abundance of bacteria and of viruses were found, not even after dividing the samples into different groups such as within versus below the mixed layer, and over versus off the plateau.

4. Discussion

4.1. The dissolved organic ligand concentration

The total ligand concentration was always in excess of $[Fe_{diss}]$ above the plateau (A1–5 and B5) but also in waters where the influence of the plateau was assumed to be absent (A11 and C11) (Table 1; Fig. 5). In the wake of the islands, Bucciarelli et al. (2001) found high $[Fe_{diss}]$ above the solubility of inorganic Fe and assumed (following the approach of Nolting et al. (1998) that the Fe was organically complexed with the ligand concentra-

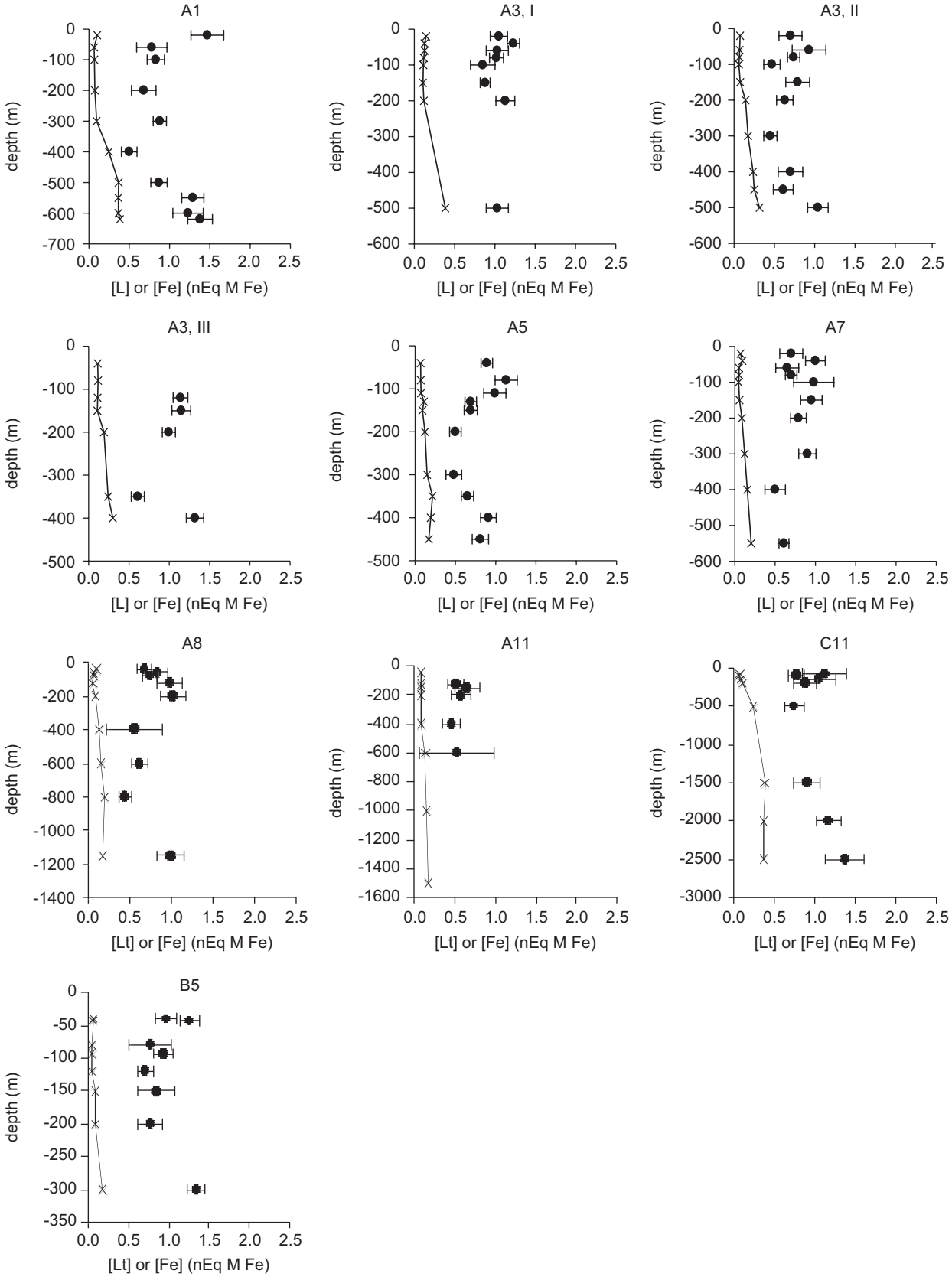

Fig. 5. The dissolved Fe (nM) and organic ligand concentrations [Lt] (*n*Eq of M Fe). The standard deviations from the calculation of the ligand concentration are given.

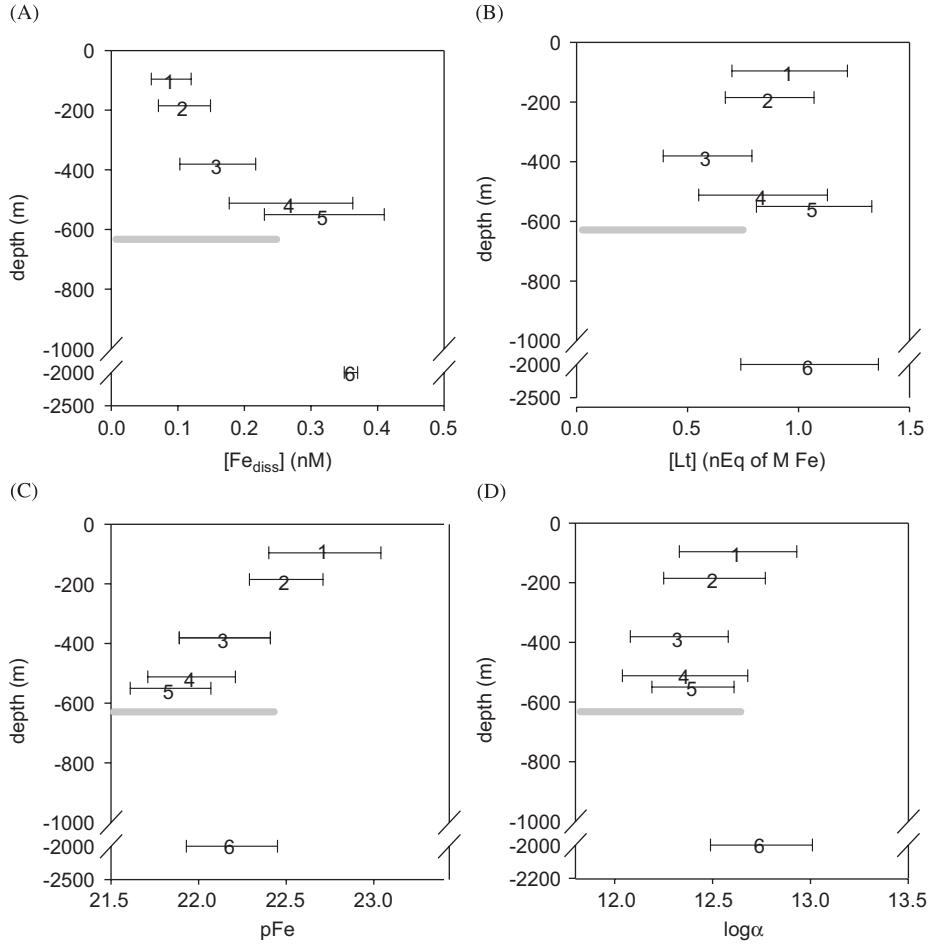

Fig. 6. Mean values of dissolved Fe [Fe_{diss}], dissolved ligands [Lt], pFe and the alpha factor of the ligand(s) of the six distinguished water types. Standard deviations are indicated. Water type characterization: 1, AASW containing >0.1 μg chlorophyll ($n = 50$); 2, WW characterized by the temperature minimum in AASW ($n = 15$), 3, the boundary (B) with the Circumpolar Deep Water (CDW) ($n = 8$); 4, IDW ($n = 8$), characterized by a temperature maximum (2–2.3 °C) and an oxygen minimum; 5, bottom water ($n = 4$) on the plateau (A1–A5 and B5), samples taken within 50 m of the sediment; 6, NADW ($n = 6$) characterized by a salinity maximum ($S = 34.6$–34.7). The position of the plateau is indicated by the gray bar at a mean depth of 620 m.

tions between 3 and 10 times higher than [Fe_{diss}]. Our results confirm their assumption since we found that the ligand concentration was on average 5.9 times [Fe_{diss}] ($n = 113$, maximum [Lt]/mean[Fe]$_{diss} = 10.4$, minimum [Lt]/mean[Fe]$_{diss} = 2.8$).

The ligand concentrations and conditional stability constants compare well with values found by others (Gledhill and van den Berg, 1994; Rue and Bruland, 1995, 1997; Croot and Johansson, 2000; Boye et al., 2001, 2003, 2005). The excess with mean values between 0.2 and 1.4 *n*Eq of M Fe were in concert with data from the Southern Ocean in between 0°E and 21°E, (0.5 *n*Eq of M Fe, Boye et al., 2001; 0.5–0.7 *n*Eq of M Fe, Boye et al., 2005; 1.1–2.6 *n*Eq of M Fe, Croot et al., 2004).

There was no distinct gradient in the ligand concentration from more coastal stations to open-ocean stations (Table 1; Fig. 5). Since the main current over the plateau is from the southwest (Park et al., 2008a), a marine origin of the ligands is likely. A marine origin also was concluded by Powell and Donat (2001), who did research in the South and Equatorial Atlantic to detect the source of ligands (terrestrial versus marine source), and possible relations between ligand characteristics and water type. Their profiles showed a similar variability in ligand concentration to those found in the present study, with high but variable concentrations, in the top few hundred meters; lower values below these depths, and a small increase in ligand concentrations to the bottom. In contrast, Powell and Donat (2001) did not find more than one ligand group in the South and Equatorial Atlantic. We did find a second relatively weak ligand group in 32% of the samples. However, the ligand characteristics of this relatively weak group were at the edge of the applied detection window and could not be determined accurately. The presence of these weak ligands could not be related to features such as, water type, depth or as observed by Rue and Bruland (1995, 1997) the presence of phytoplankton.

4.2. Possible sources of dissolved organic ligands

Although the variation in ligand concentration in the profiles (Table 1; Fig. 5) were large it is clear that the concentrations were higher in the upper 200 m, showed a minimum near 300–400 m depth and were higher again near the sediment. This indicates two possible sources of organic ligands: one from the top layer, probably the phytoplankton and bacteria and one from below from the sediment. To confirm this observation, mean [Fe_{diss}] and mean ligand characteristics were calculated for the six distinguished water types (Fig. 6). Type 6, (NADW) only sampled in station C11, contains slightly stronger ligands with a mean $\log K' = 21.9$ versus $\log K' = 21.6$–21.7 in the other water types (Table 1). The differences in the mean [Fe_{diss}] and pFe (negative logarithm of [Fe^{3+}], i.e., the Fe not bound to the organic ligands and hydroxides) between water type 1 and types 4 and 5 were statistically significant according to the *t*-test ($P<0.001$), whereas differences in mean ligand concentration were gradual and not statistically significant between adjacent water types (Fig. 6). Excluding water type 6, the mean [Fe_{diss}] and the mean ionic Fe concentration (shown as pFe), increased sharply with depth near the sediment. This indicates depletion of Fe by the phytoplankton in the upper layer and a supply from the sediment.

This perspective is in keeping with other studies of Fe during KEOPS and elsewhere. According to Sarthou et al. (2008), the rate of Fe regeneration accounts for half of the Fe demand by the phytoplankton. That regeneration of Fe plays an important role (more than 50%) in the upper ocean (in contrast to Zn) was also concluded from experiments in Bering Sea water by Hurst and Bruland (2007). Blain et al. (2007, 2008b) concluded that apart from the regeneration from sinking biogenic material, the sediment forms also an important source for Fe_{diss}. Atmospheric input of Fe can be neglected according to Blain et al. (2008b). According to Timmermans et al. (2008), translocation experiments, using natural phytoplankton as indicator of bio-available Fe, provide proof that Fe is coming from the Kerguelen Plateau.

As for Fe_{diss}, two sources are suggested located in the top layer and near the bottom.

4.3. Phytoplankton as source of dissolved organic ligands

Parallel increases between dissolved organic ligands and chlorophyll in the water column were observed in the vertical (Boye et al., 2001) and in time after an Fe enrichment (Rue and Bruland, 1997; Croot et al., 2001, 2004; Boye et al., 2005). Although it is known that ligands are formed as an active response upon Fe release (Fuse et al., 1993), it is not easy to find a relationship between ligand characteristics and phytoplankton biomass. Only in a few studies has a straightforward relationship been found in natural waters (Rue and Bruland, 1997; Boye et al., 2005; Gerringa et al., 2006). Multiple linear regression on profiles from the Canary Basin (Gerringa et al., 2006) revealed that ligand concentrations were related to phytoplankton biomass, mainly diatoms. bacterial and viral abundance or other parameters.

In our KEOPS study, we were not able to identify a clear link between ligand concentrations and these parameters. Possibly the standing stock of the phytoplankton and bacteria was not related to production of ligands because of grazing. Moreover, the variations in ligand concentrations in the upper 200 m are a result of a balance between production and breakdown processes, including photo-chemical reactions which can obscure a possible relationship between [L] and the biomass parameters (Croot et al., 2001; Boye et al., 2003; Rijkenberg et al., 2004, 2006b).

From Fig. 6, it can be concluded that Fe is depleted in the AASW, but the ligand concentration is relatively high compared to the deeper WW and B thus resulting in high pFe values. A relatively high ligand concentration indicates a production of ligands in the surface layer, suggesting that ligand production is a mechanism to keep Fe available for phytoplankton. Increasing ligand concentrations increase pFe (decrease [Fe^{3+}]), which is a measure for the available Fe. This contradiction reflects the issue whether ligands increase or decrease the availability of Fe for phytoplankton (Hutchins et al., 1999; Sunda, 2001; Rijkenberg, 2005). More research is needed in order to elucidate the exact source of dissolved organic ligands and its effect on Fe availability in the AASW, but also elsewhere.

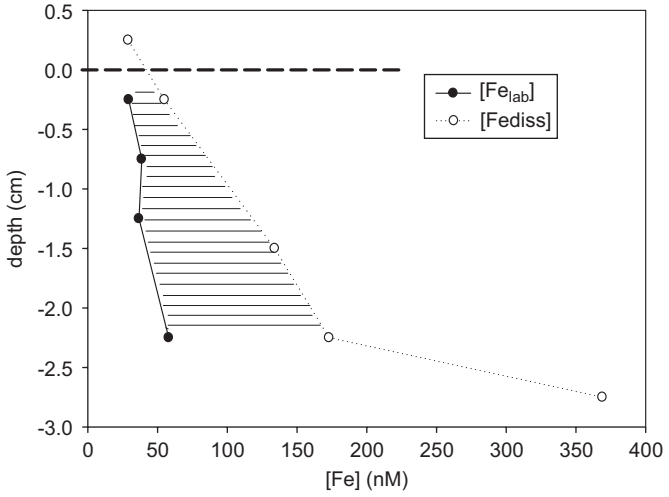

Fig. 7. Total dissolved Fe ([Fe_{diss}]) concentrations of sediment pore waters and above standing water at station A3 (sampled 23 January 2005) and labile Fe ([Fe_{lab}]) concentrations in the pore waters of station A3 (sampled 12 February 2005). Labile refers to the method applied, with respect to 10 μM TAC after equilibration during the night. The difference between [Fe_{diss}] and [Fe_{lab}] (shaded area) is assumed to be [FeL] equal to [Lt], since the ligands are saturated. The dashed line marks the sediment surface. Oxygen penetration was 7 mm.

4.4. The sediment as source of dissolved organic ligands

A flux of organic ligands from estuarine sediments has been observed before by Skrabal et al. (1997) for Cu binding organic ligands and by Croot and Johansson (2000) for Fe-binding organic ligands. Boye et al. (2003) found a parallel increase in Fe and ligands between the Atlantic and the shallower waters of the North Sea, and suggested that both had a common source in admixed bottom waters. On the Kerguelen Plateau the relatively higher [Lt] near the sediment (water type 5) indicate diffusive transfer of organic ligands from the plateau sediments (Fig. 7). Pore waters from station A3 in this research analyzed for dissolved organic ligands showed that [Fe_{diss}] was in excess of the dissolved organic ligands; from 29.4 to 58.1 nM of Fe_{lab} (Fe_{lab} = labile Fe with respect to equilibration overnight with 10 μM TAC) from 0.25 to 2.25 cm (Fig. 7). Such highly labile concentrations of Fe(III) can only exist as Fe(II), or as Fe(III) complexed with relative weak ligands at the edge of the detection window, or as colloids. A distinction between colloidal and truly dissolved Fe and Fe-binding ligands was not made in this study. With the method applied in the present study, TAC competes with truly dissolved organic ligands but also with organic and inorganic colloids. Fe bound to the surface of colloids or freshly formed inorganic Fe colloids might be bound (or complexed) more weakly than with TAC (10 μM). Therefore, Fe′ in Fig. 7 represents Fe that is either weakly adsorbed on colloidal material, fresh colloidal inorganic or mixed organic/inorganic Fe or Fe dissociated from relatively weak dissolved organic ligands.

Dissolved organic ligands of Fe(III) as well as Fe(II) in pore waters of a salt marsh were measured by Luther et al. (1996) in different size fractions. The organic complexes of Fe(III) were present in the higher size fractions (>5000 MW). From the EisenEx Fe experiment, it is known that the colloidal fraction was subject to rapid changes with time. After the enrichment the colloidal-Fe fraction was highest but declined rapidly (Boye et al., 2005; Nishioka et al., 2005; Croot et al., 2005).

Labile Fe was not observed in the seawater above the Kerguelen Plateau sediment. Apparently, Fe(III) can diffuse out of the sediment passing the oxidized layer (the oxygen penetration was 7 mm; Viollier et al., in preparation) without being trapped by precipitation as oxides. To stay in the dissolved phase, this Fe must be firmly complexed by organic ligands (either colloidal or truly dissolved) or be incorporated in inorganic/organic colloids. The Fe is thought to come from degradation of sinking or resuspended particles and directly out of the sediment. Diffusive fluxes of Fe estimated from pore water gradients by Viollier et al. (in preparation) compare within the same order of magnitude with diffusive fluxes measured along the Californian coast (50–150 nmol m^{-2} yr^{-1}, <600 m depth; Elrod et al., 2004). Benthic chamber fluxes of Fe measured also by Elrod et al. (2004) are, however, 1 order of magnitude above diffusive fluxes. Such enhancement might be due to bio-irrigation operating in sediment at the chamber scale. Such discrepancy already has been shown for benthic oxygen consumption fluxes.

The sediment thus functions as source for both dissolved Fe and ligands directly and due to processes affecting resuspended material.

5. Conclusions

The ligands in the water column are always in excess of [Fe_{diss}], enlarging the solubility and potentially increasing bio-availability of Fe. Since no distinct gradient in the ligand concentration from more coastal stations to open-ocean stations was found and the main current over the plateau is from the southwest, a marine origin of the ligands is likely. The exact nature of the Fe-binding ligands is not known, the ligands are probably partly colloidal and possibly mixtures of inorganic and organic colloids.

Where Fe is regenerated in the surface layer (Sarthou et al., 2008) Fe-binding dissolved organic ligands were present. These ligands were probably produced by the blooming diatoms. During our research on the natural Fe fertilization around the Kerguelen Islands similar coinciding increases of diatoms and organic ligands were found to those as reported after Fe enrichments in surface. However, no statistically significant direct relationship was found, presumably as a result of other processes influencing the ligand and the diatom concentrations, such as grazing, export, photo-chemical reactions, nutrient limitations, progression of the bloom, etc.

The sediments acted as a source of both Fe and dissolved organic ligands, enabling transport of complexed Fe through the oxic sediment layer and into bottom waters. In the pore waters, ligands were saturated; so that excess [Fe_{diss}] was either Fe(II) or existed as inorganic colloids or weak organic ligands. In the water above, the sediments the Fe-binding ligands were in excess over [Fe_{diss}].

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