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Distribution and Variation of the Trace Metal Iron in the Base of the Pelagic Marine Food Web: A Mesocosm Approach

Trace Metal in marine biology

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ABSTRACT

This study was part of the WAFOW project "*Can waste emission from fish farms change the structure of marine food webs?"* whose objective was to perform mesocosms experiment to simulate the ammonium enriched environment, caused by salmon aquaculture. In order to assess how changes in the stoichiometry of major elements (nitrogen) in the sea affect the distribution of bioactive trace metals, and its effects on the base of the pelagic food web, the distribution and variation in time of the trace metal iron in the water and within the plankton community was studied along a 22 day experiment. It involved 2 types of water (surface and marine systems) each one, with 1 control and 4 NH_4^+ concentrations. Additional samples collected in a river transect and in depth provided the general Fe distribution in the environment. The iron concentration in the water was determine for three fractions as: Chelex labile (Total: TFe_{Ch} and dissolved: DFe_{CH}), DGT labile (Fe_{DGT}) and direct (Total: TFe and dissolved: DFe), whereas the particulate concentration iron in the plankton community was determined both per fraction (PFe_{SF}) and total content ($PFe_{>0.2}$).

Total average per treatments showed higher concentrations for both Tfe_{Ch} and DFe_{Ch} in the marine systems compare to the surface. TFe_{Ch} showed general increasing trend in time and with increase NH_4^+ concentration, with a sharp decrease towards the end of the experiment in both systems. DFe_{Ch} pattern was inverse to TFe_{Ch}, with general decrease over time but lower in magnitude. Fe_{DGT} showed an average lower concentration compare to DFe_{Ch} with no define trend over time. Final Fe_{DGT} concentrations were significantly lower in treatments with artificial NH_4^+ addition. $PFe_{>0.2}$ showed an increasing trend in time and with increased NH_4^+ in both systems. However when normalized to Chlorophyll-a (Chl-a) or particulate organic carbon (POC) the trend inverted, showing that at higher NH_4^+ influx the iron per Chl-a or POC decreases. PFe_{SF} major changes occurred in the marine system where a estimation of the ratio between the 20-140 µm and the 2-20 µm fractions, indicative of the dominant phytoplankton size class, was significantly higher in 2 of the 3 treatments with artificial NH_4^+ addition. This point that the microphytoplankton increased significantly with higher NH_4^+. The variation over time of the concentration of Fe in the water as in the plankton community, indicate that the concentration of NH_4^+ can have positive or negative relation depending on the iron form. Whether via increasing the PFe_{Ch}, or by reducing the uptake by phytoplankton, a modified C:N:P can affect the cycling of iron, which in turn can have negative or positive feedbacks over the major biogeochemical cycles.

LIST OF ABBREVIATIONS

Chl -a Chlorophyll-a

DFe Dissolved iron

DFe_{Ch} Dissolved Chelex labile iron

DGT Diffusive gradients in thin-films

DOM Dissolved organic matter

DON Dissolved organic nitrogen

DOP Dissolved organic phosphorus

Fe_{DGT} DGT labile iron

HR-ICP-MS High Resolution Inductively Coupled Plasma Mass Spectrometry

PE polyethylene

$PFe_{>0.2}$ Particulate iron in plankton community

PFe_{SF} Size fraction iron in plankton community

POC Particulat**e** organic carbon

PON Particulat**e** organic nitrogen

POP Particulat**e** organic phosphorus

Q Iron quota per cell

TFe Total iron

TFe_{Ch} Total Chelex labile iron

UC UltraCLAVE

UP HNO_3 Ultra-pure Nitric Acid

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1. **INTRODUCTION**

According to the FAO, Norway and Chile are the world's two major cultured salmon producers (FAO, 2010). This industry has seen and increased expansion in the last two decades, causing growing concern over the environmental impact this intensive activity can cause into the ecosystems it takes place. Salmon farming releases nutrients as dissolved inorganic species through excretion (Ammonium (NH_4^+) and phosphate (PO_4^{3-})), particulate organic nitrogen (PON) and phosphorus (POP) through defecation, and its dissolved components (DON and DOP) through resuspension from the particulate fractions (Olsen and Olsen, 2008). Oxygen depletion, decreased biodiversity among others, are well documented effects for the marine sediments and benthic fauna. However, current knowledge of how waste release affects the structure and function of the pelagic ecosystems is still scarce (Cloern 2001; Olsen et al. 2006). It has been proposed that this waste release alter nutrient stoichiometry in the seawater determining to some extent how the marine environment, responds to increasing anthropogenic inputs of limiting nutrients (Arrigo 2005).

Major biogeochemical cycles (carbon, nitrogen and phosphorus) in the marine environment, are strongly dependent on marine microbes as this group is directly responsible for approximately half of the earth's primary production (Arrigo 2005). Nutrient uptake by phytoplankton varies among groups and depends on several factors such as kinetics, availability and redox state. In marine ecosystems nitrogen (N), assumed to be the limiting macro-nutrient for biological production (Hecky and Kilham 1988), can be assimilated by phytoplankton as nitrate (NO_3^-), nitrite (NO_2^-), and NH_4^+, occurring at different concentrations in the regions of world oceans. In the open ocean, ecosystems of low productivity, the main source of N is though recycled $NH4^+$, whereas in coastal areas like fjord regions, N is mainly contributed as nitrate NO_3^- with the incoming deep water (nutrient-rich) or in the surface layers through the run-off of in land fertilizers. As this N is in its oxidized form, after phytoplankton uptake a series of metabolic process occur in order to be finally be assimilated as NH_4^+. In this way, NO_3^- undergoes through sequential reduction to nitrite and ammonium, each step involving the assimilatory nitrate reductase and assimilatory nitrite reductase enzymes respectively (Zehr and Ward 2002) Nitrogen incorporated to organic matter is then recycled by biological process (cell lysis, microbial decomposition, excretion,) which liberate N in the organic forms or NH_4^+.

At the same time, these major biogeochemical cycles, involved metabolic process that are dependent on the availability of certain "micro-nutrients". Trace metals such as Mn, Fe, Co, Ni, Cu, Zn and Cd are involved in several biological process influencing carbon cycling in aquatic systems, both directly (e.g. carbon-concentrating mechanism involves the Zn metalloenzyme carbonic anhydrase) and indirectly (e.g. Fe requirements for metalloenzymes in Nitrogen cycle) (Morel and Price 2003). As most of these elements are continuously exported out the photic zone to depth as settling organic biomass, these biological processes (uptake, trophic transfer, regeneration, excretion and decomposition) are critical in controlling the fate of these bioactive metals in the ocean (Wang et al. 2001), thus making a cycle of complex feedback control. Moreover, most of trace metals form complex ligands making them nonreactive and with limited solubility (Morel and Price 2003). Because of the above and due to and effective removal from the water column, concentrations of these elements fall precipitously within short distance of the continental margins making it's recycling even more difficult (Johnson et al. 1997).

Iron specifically, plays a key role in several processes, having effect on major biochemical cycles in the marine environment (Martin 1991; Martin et al. 1991; Morel et al. 1991) . It exists in the ocean mostly in its oxidized form Fe^{3+} (ferric iron) which is virtually insoluble, while the reduced state Fe^{2+}(ferrous ion), the bioavailable form, is less abundant. For instance, Fe is involved in the nutrient uptake by diatoms as it is required as cofactor in the in the reductases in the reduction of NO_3^- to NH_4^+ and thus affecting the nitrogen cycling (Price et al. 1994). The low productivity in Fe-depleted regions of the oceans attributed to low efficiency of the light reaction of photosynthesis, requires a host of Fe-containing electron transfer intermediates. Likewise, electron transfer in respiration also becomes inefficient at low Fe concentrations, affecting carbon conversion into biomass by heterotrophic bacteria (Morel and Price 2003).Fe has been regarded as limiting nutrient for primary production in most regions in open ocean (absence of land based iron supply) and in the so called high-nutrient-low-chlorophyll (HNLC) regions (Boyd et al. 2007). Not being the case in coastal areas, there exist some evidences however that even in coastal systems, phytoplankton production might be iron limited at some extent (Hutchins et al. 1998; Hutchins et al. 2002; Öztürk et al. 2002).

Through this feedback control mechanism between the so called "macro" and "micro" nutrients, it can be expected that enhancement of macronutrient loads (e.g. nitrogen) in a

given environment, may affect in the long term the cycling of trace elements. The biologically "New" versus "Regenerated" production, based on the NO_3^- : NH_4^+ ratio in the water column, is a determinant factor favoring growth rates of certain groups of primary producers (Thompson et al. 1989). Therefore, increased input of dissolved inorganic nutrients (NH_4^+ and PO_4^{3-}) by aquaculture may have a direct effect on the phytoplankton community structure (Olsen and Olsen 2008). The general response from marine pelagic ecosystems to nutrient enrichment, is reflected in increase nutrient uptake by phytoplankton and bacteria as well as growth rate, with the consequent increased autotrophic biomass transfer to higher trophic levels (Olsen and Olsen 2008). However, knowledge on the capacity for phytoplankton to biologically uptake and metabolize these surplus of nutrients, strongly link to the bioavailability of some trace metals, is still scarce. Changes in N supply (e.g. NH_4^+), could potentially affect the cycling or Fe, perhaps by turning coastal waters into Fe limited zones or by changing Fe requirements of phytoplankton community. Thus, understanding how concentration and speciation of certain trace elements in the marine environment are affected by the surplus input of macro nutrients, is fundamental to understand how biological systems (in terms of nutrient uptake, biomass growth, biodiversity, etc…) respond to human induced changes in the environment.

Mesocosm experiments are design to maintain large close environments for periods of weeks, giving the opportunity to simulate natural conditions that otherwise would not be possible, and thus enabling to study from ecological interactions (Granéli and Turner 2002; Dearman et al. 2003; Havskum et al. 2003; Stibor et al. 2004) to pelagic communities responses to environmental perturbations (Olsen et al. 2006) in more realistic perspectives. In this way, the baseline of WAFOW project, consisted in creating the conditions to simulate the nutrient enrichment occurring in fjords ecosystems product of salmon aquaculture in Norway and Chile, to assess the capacity of the pelagic marine planktonic communities to assimilate the incoming nutrient wastes and how it affects the major and minor biogeochemical cycles in fjord ecosystems. Further implications of the outcome research would be to generate the scientific knowledge required to contribute to mitigate possible environmental impacts in these ecosystems.

2. HYPOTHESIS AND OBJECTIVES

2.2 Hypothesis

Environment nutrient enrichment through NH_4^+ and PO_4^{3-} may modify N cycling and the stoichiometry of trace elements linked to it. Therefore, is expected that the NO_3^- to NH_4^+ shift achieved through progressive artificial addition of nutrients imply in the long term, changes in iron requirements by phytoplankton, with a consequent effect on trophic transfer to zooplankton and higher trophic levels.

2.1 Objectives

2.1.1. General objective

Determine the concentration and variation in time of different fractions of the trace element iron in the marine water and the plankton community under experimental conditions of different NH_4^+ concentration, in order to asses positive or negative feedbacks between the nitrogen and iron marine cycles and the possible biological implications in the base of the pelagic marine food web in a fjord ecosystem.

2.1.2. Specifics objectives

Determine the concentration and variation in time of the total and dissolved chelex labile and DGT labile fractions of iron in two types of seawater under experimental conditions with different concentrations of NH_4^+.

Determine the variations in the distribution of trace metal iron within different size fractions of the particulate organic matter, representing the main size classes of the plankton community, under experimental conditions with different concentrations of NH_4^+.

3. THEORY

3.1. Salmon aquaculture and the environment

According to the Food and Agriculture Organization (FAO), the global production of fish, crustaceans and mollusks has reached 144.6 million tons in 2009. While capture production has stayed around 90 million tonnes since 2001, aquaculture production has displayed a substantial growth increasing from 34.6 million tonnes in 2001 to 55.7 million tonnes in 2009, (i.e. average annual growth rate of 6.1 percent) (FAO 2010). The Salmon industry dominates the production of diadromous fish with 1.5 million tonnes (44%), being Norway and Chile the world's leading aquaculture producers of salmonids, with 36 and 28 percent of world production respectively (FAO 2010). Despite the small share (0.8%) of the global aquaculture tonnage production, salmon aquaculture is classified as intensive, which means that fish depend on a diet of artificial feed in pellet form. Because not all the feed is eaten, a significant fraction of these external nutrient inputs can reach the sea bottom where it is eaten by the benthos or decomposed by microorganisms, leading to events of oxygen depletion. This alteration of the natural food web structure can significantly impact the local environment (Soto and Norambuena 2004). Because of the above among other factors, general concern has been expressed about the environmental impacts of salmon farming worldwide. Other impacts include modification of benthic communities, increased nutrient loads in coastal waters and the associated problem of harmful algal blooms, increased harvests of wild fish populations for the production of fish feed, use of different types of chemicals, and escapes of farmed salmon into the wild (Buschmann et al. 2006).

3.1.1. Eutrophication

Salmon farming, among other human activities such as land clearing, production and applications of fertilizers, discharge of human waste, animal production, combustion of fossil fuels, mobilize nutrient elements, mainly nitrogen and phosphorus, to coastal areas (Table 1). As a result fertilization of coastal ecosystems is now a serious environmental problem because it stimulates plant growth and disrupts the balance between the production and metabolism of organic matter in the coastal zone (Cloern 2001). Ecosystem response to the increase nutrient loading (eutrophication) in coastal areas can be seen both in the water (pelagic) quality or in the state of the sea bottom (benthic), though both environments are affected in different ways.

Table 1) Estimated loading rates of organic C and specified nutrient components from a hypothetical salmon CAS producing 1000 tonnes of fish per year (Olsen and Olsen 2008).

Pelagic loading rates	Tonnes farm–1 year–1	g m–3 year–1	mg m–3 day–1 (June–Sept)
OC-loading	20	26	100
NH4-loading	28	36	140
PO4-loading	2.1	2.7	11
Total N-loading	30	39	150
Total P-loading	3	3.9	15
DON + PON loading	17	22	86
DOP + POP loading	6.1	7.9	31

3.1.1.1. Effects on marine sediments

The oxygen concentration at any point in the sediment is dependent on the rate of its uptake, either to fuel aerobic metabolism or to re-oxidise reduced products released from deeper in the sediment. When the oxygen demand caused by input of organic matter exceeds the oxygen diffusion rate from overlying waters, sediments become anoxic and anaerobic processes dominate (Black et al. 2008). Unaltered benthic macrofaunal communities in sediments are highly diverse however, where wastes do accumulate on the sea bottom, oxygen levels are depleted, release noxious gasses in decomposition, ultimately smothering benthic organisms. In some cases, this has led to dramatic changes in the community of animals that live beneath salmon pens, including the reduction of species diversity, leaving only a few species that thrive in polluted conditions (Weber 1997).

As wastes from salmon pens fall directly in the sea bottom, effects on these ecosystem are relatively easy to detect and quantify. Due to this fact, the literature on impacts on sediments and benthic ecosystems is very comprehensive (Black *et al.*, 2008), and there is a general scientific understanding on the requirements to base assessments of state and dynamics, management, and monitoring measures. (Olsen and Olsen 2008).

3.1.1.2. Effects on the water column

Potential impacts of wastes from aquaculture on water column ecosystems is far less studied (Olsen and Olsen 2008). Pelagic ecosystems are primarily affected by inorganic nutrients (NH_4^+ and PO_4^{3-}), one of the main fractions (apart from particulate organic nutrients, and dissolved organic nutrients) released by salmon aquaculture. While the majority of the P is

accumulated in sediments (63%), the majority N wastes is released to open waters (68% of total), prompting plant growth in the pelagic ecosystem (Olsen and Olsen 2008).

One of the most known effect on pelagic ecosystems caused by nutrient enrichment are micro algal blooms (Buschmann et al. 2006). Whether caused by nutrients from salmon farms or from other sources, micro algal blooms can affect both the environment and salmon production mainly by 1.) depleting waters of oxygen, product of remineralization of excess algal biomass post bloom and 2.) accumulation of toxic substances produced by the microalgae (Weber 1997). Pelagic ecosystems have an inherent capacity of persistence, and smaller changes in nutrient input can be mitigated through adaptive responses (Olsen and Olsen 2008). Nevertheless, there is an upper assimilation capacity above which pelagic ecosystems may lose integrity, leading to changes or complete shift of the dominant type of microalgae present in the environment. Knowledge of how these species shift may alter the complete structure of the pelagic food web up to higher trophic levels, or down to the biogeochemical cycling of minor elements, is still scarce.

3.2. The marine nitrogen cycle

The nitrogen biogeochemical cycle in the marine environment is strongly dependent on marine microorganisms as it is composed of multiple transformations of nitrogenous compounds, catalyzed primarily by bacteria and phytoplankton (Zehr and Ward 2002), that ultimately controls the availability of nitrogenous nutrients and thus affecting biological productivity in marine systems. The cycle is "balanced" by biological (metabolic activity) and physical (water masses transport) process that mediate the gains (nitrification, N_2 fixation) and loses (denitrification, Anamox,) occurring at different rates and relevance in the regions of world oceans (Fig. 1).

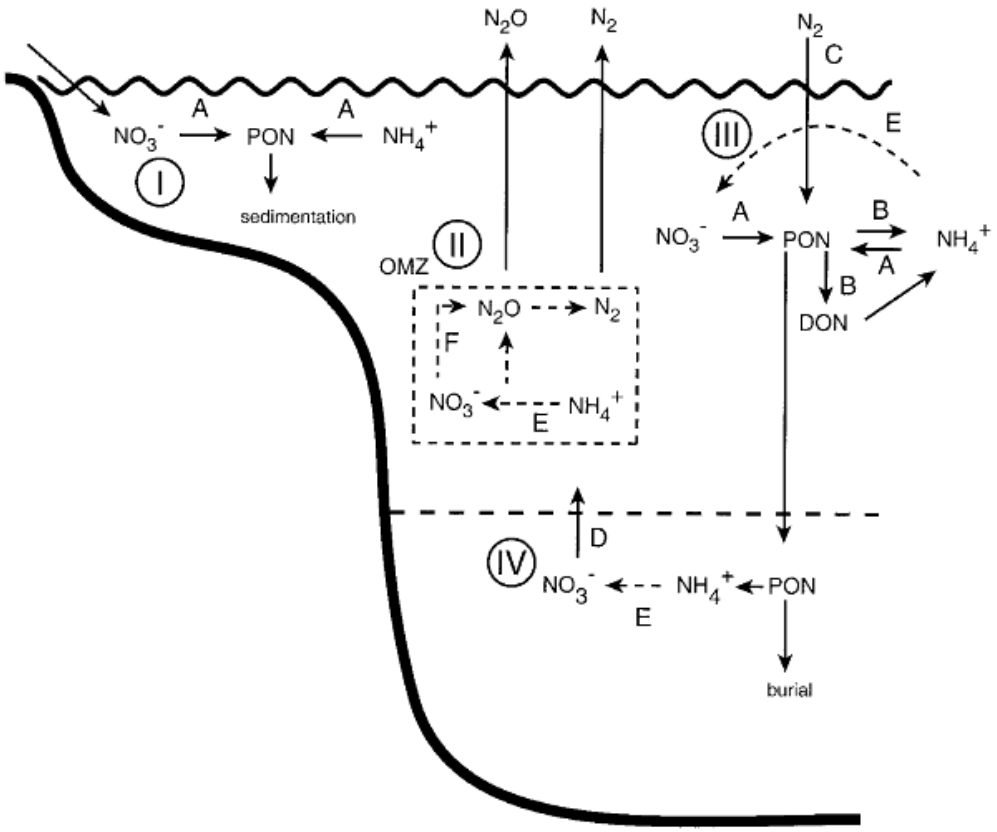


Fig. 1) Diagram of major features of the nitrogen cycle in coastal shelf and upwelling (I), OMZs (II), surface waters of the open ocean (III), and deep water (IV). PON, particulate organic nitrogen. A, DIN assimilation; B, ammonium regeneration; C, nitrogen fixation; D, nitrate diffusion/advection from deep water; E, nitrification; F, nitrification; G, denitrification (Zehr and Ward 2002).

3.2.1. NO_3^- versus NH_4^+ uptake in phytoplankton

NO_3^-, NO_2^- , and NH_4^+, collectively termed dissolved inorganic nitrogen (DIN), can be taken up (via membrane transporters) and assimilated by phytoplankton. However, for NO_3^- to be assimilated, it has to follow sequential reduction to nitrite and ammonium implying additional steps in reduction with an extra energy cost (Zehr and Ward 2002). The preferential uptake by phytoplankton (both eukaryotes and cyanobacteria) of NH_4^+ is normally assumed to represent an adaptation resulting in energetic savings to avoid the necessary to extra steps in reduction of NO_3^- (Thompson et al. 1989; Dortch 1990; Zehr and Ward 2002). However, it has been demonstrated that growth on NO_3^- results in a measurably greater photosynthetic quotient compared to growth on NH_4^+ (Dugdale and Goering 1967), and that the reductant requirement for nitrate does not necessarily result in decreased growth rate (Thompson et al. 1989). So it has become clear that this preference for NH_4^+ is not universal and that not all phytoplankton use nitrate (Zehr and Ward 2002).

Among the numerous sources of nitrogen present in seawater, NH_4^+ and NO_3^- are generally considered to be the most important for regenerated and new production respectively. (Thompson et al. 1989). In most open ocean region, regenerated production is dominated by nanoplankton (including mostly small diatoms and flagellates) (Chavez et al. 1991; Muggli and Harrison 1996), where higher ammonium assimilation rates are generally associated with the smaller (<10 µm) fraction (Le Corre et al. 1996). On the other hand, in coastal areas where upwelling and river runoff generate high input of NO_3^-, new production dominates associated to bloom forming phytoplankton, mainly large diatoms that optimally allocate resources toward production of growth machinery (Arrigo 2005).

3.2.2. NO_3^- and NH_4^+ interactions with Fe

As essential for NO_3^- reduction, the biochemical basis for the relationship for iron and nitrogen it is clear (Morel et al. 1991). It has suggested partially to explain the paradox of HNLC zones as dominant phytoplankton in these regions to adapted very low Fe concentration thus unavailable to use effectively NO_3^- for growth (Martin et al. 1991; Morel et al. 1991). When NO_3^- rather than NH_4^+ is used as the nitrogen source the demands for iron increase, requiring 40% to 60 more iron (Raven 1988; Whitfield 2001). Then is thus expected that oceanic phytoplankton has evolved to achieve a lower iron quota (*Q*) (Brand et al. 1983). This has been confirmed through several cultures experiments (Sunda and Huntsman, 1995). In addition, it has been suggested that the energetic cost for the actual transport of nitrate across the cell membrane is higher than that for ammonium (Turpin and Bruce 1990). Therefore, regardless of the Fe conditions, cells utilizing ammonium should have a theoretical advantage based on energy considerations over cells utilizing nitrate (Muggli and Harrison 1996). Some studies have suggested that under iron replete conditions higher growth rates occurred with NH_4^+ than NO_3^- (Thompson et al. 1989; Levasseur et al. 1993) while other have come out with opposite results (Muggli and Harrison 1996).

3.3. Trace metals

3.3.1. Categorization

Metals exist in a wide variety of forms in natural waters, being present as free / hydrated metal ions, inorganic complexes, organic complexes, attached to colloids or larger particles (Stumm and Morgan 1996). The simplest, most common categorization of metals in water is separation into “dissolved” and “particulate” metal fractions by filtration.

3.3.1.1. “Dissolved” and “particulate” metal species

The fraction that passes through a 0.45 μm filter is typically defined as “dissolved”, while the fraction collected by the filter is termed “particulate”. "Dissolved" is thus operationally defined, and in a strict sense is incorrect, as small particulates (i.e., <0.45μm) will pass through the filter membrane. Rather, the term "filterable" is a more correct term. In practice, the “dissolved” component includes metal species that are truly dissolved, including inorganic species (free metal ions and inorganic metal complexes) and organically complexed metals, but also includes “colloidal” metal species. Metals may be hosted in particulate phases either as part of a mineral lattice, sorbed onto particle surfaces, or as assimilated components in aquatic biota. In general, particulate metal phases tend to be less available, to organisms in comparison to their dissolved counterparts (INAP 2002).

3.3.2. Speciation

According to the International Union of Pure and Applied Chemistry (IUPAC) speciation is defined as “the process yielding evidence of the atomic or molecular form of an analyte” (Hill 1997). It refers to the chemical form or compound for which a element is present, both in living and non-living systems. It can also refer to the quantitative distribution of an element in the environment (Stumm and Morgan 1996). To describe the chemical speciation of an element means to identify its oxidation state, and all the forms of the elements (or cluster of atoms of different elements) has in given solution matrix.

3.3.2.1. Variables affection speciation

The nature of metal speciation is a function of several variables, including the metal of consideration, types and concentrations of metal complexing agents present. Other water quality variables including pH, pE (redox potential), hardness (Ca and/or Mg concentration), alkalinity and salinity are known to influence the speciation of metals to aquatic biota (INAP 2002).

3.3.2.1.1. Complexing agents

Complexation of metals in aquatic systems may occur via reaction with soluble inorganic and organic ligands. The inorganic speciation of the essential trace metals with inorganic ligands (e.g., F^-, Cl^-,HCO_3^-, SO_4^{2-}, HPO_4^{2-}, etc.) is quite well characterized, producing complexes (e.g., carbonates), that dissociate rapidly to the free metal form. In the other hand, organic speciation mediated through organic ligands (e.g., humic substances) present in the DOM, is still poorly understood (Maranger and Pullin 2003). Humic and fulvic acids that have highly reactive aliphatic and aromatic carboxyl and hydroxyl groups that complex with other elements in the water column. They have great relevance as a major component of the DOM pool in coastal and freshwater ecosystems. Organic ligands are also produced by marine microorganisms to complex metal ions to both facilitate uptake of specific ones that occurred in very low concentrations (e.g. Fe) and to mitigate potential toxic effects of others (e.g. Cu) (Vraspir and Butler 2009).

In most cases, complexation with organic ligands reduces metal bioavailability, because most organic-metal complexes are not readily transported across cell membranes. For instance, the bioavailability of several metals (e.g., Cd, Cu, Zn) is reduced in the presence of organic chelators (Zamuda et al. 1985). Yet, further evidence has established two general but opposing views of organic ligands as chelators of metals, one in which binding metals enhances the availability of these elements to planktonic organisms (Sunda and Huntsman 1995) an other stating that chelation actually reduces the availability of these elements. In this way, the presence of organic complexes is a mixed blessing, since can suppress the concentration of potential toxic metals, but they can also reduce the available concentrations of essentials in biological metabolism. (Whitfield 2001).

3.3.2.1.2. pH

Some works have demonstrated an increase in metal toxicity with decreasing pH, due to the increase in free metal-ion activity at lower pH (INAP 2002). Conversely, other studies have shown a decrease in metal toxicity with decreasing pH (Franklin et al. 2000). The latter observations have been attributed to the increased competition of H^+ with trace metals at the cell surface.

3.3.2.1.3. Others

The toxicity of metals to aquatic organisms, for example, generally decreases with increasing water hardness (Di Toro et al. 2001). Two processes have been suggested to account for these observations: 1) Ca and Mg successfully compete with trace metals for membrane transport sites on cellular surfaces; and 2) the complexation of metals with carbonate (CO_3^-) decreases the free metal ion concentration and thus metal bioavailability (INAP 2002).

3.3.3. Bioavailability

Biological availability (bioavailability) is defined as the fraction of total amount of an element that is available for an organism metabolism or that can be absorbed and used or stored in an organism. Bioavailable fraction of metals includes both free metal ions and kinetically–labile metal complexes (i.e., those with rapid dissociation kinetics), the biological response is proportional to the free-metal concentration only (Whitfield 2001). Thus. bioavailability, as well as toxicity, of metals in aquatic systems is strongly dependent on the nature of the metal species present. For this reason, determining the chemical form, or speciation, of metals in the environment is fundamental to predicting impacts to aquatic biota.

3.3.2. Role of trace metals in biology

In living organisms, metal ions regulate an array of physiological mechanisms with considerable specificity and selectivity, as components of enzymes and other molecular complexes. The reactivities of the complexes depend both on the specific properties of the organic molecule to which the metals are joined (e.g., protein), and on the variety and flexibility of the metal's own specific chemical properties (Lippard 1993). The d-block transition metals play a particularly prominent part in enzyme activities of living organisms. Their wide variety of oxidation states, extensive bonding patterns and chemical flexibility allow them to participate extensively in catalysis. The facility with which iron, copper and molybdenum, can undertake one or two electron changes accounts for their importance in the oxidoreductase enzymes (Reilly 2004).

In natural marine environments and freshwaters, trace metals as Fe, Cu, Zn, Ni, Mn, Co, Cd, Mo, Se, Sn, and V are micronutrients that constitute essential dietary components of aquatic organisms (Florence 1982). Such metals are typically present in trace quantities (<10 nM) and are passively and/or actively assimilated by organisms to satisfy physiological requirements.

3.3.3. Biological uptake

As seen, chemical speciation of the elements in sea water have a controlling influence on their biological availability. Generally, for essential trace metals, it is the free metal ion that is most readily assimilated. (Whitfield 2001), whereas particulate and strongly complexed metals are not. Dissolved metals are typically incorporated passively into the cells of organisms via specialized pumps, channels and carriers which operate across the membrane surface (INAP 2002). The regulation of this process in the cell is facilitated by the cell membrane, where globular proteins, dispersed throughout the lipid bilayer, act as conduits for the transport of materials to the cell interior.

Within this process, Whitfield (Whitfield 2001) identify three stages key stages that determine the effectiveness metal uptake, as 1.) Transport of metal species to cell surface (diffusion), 2.) Binding to a biologically-produced ligand (sequestration or capture) and 3. Transfer of complex across cell membrane (internalization) (Whitfield 2001) (Fig. 2) Overall, the uptake process generally follows Michaelis-Menten kinetics, typical for enzyme-mediated reactions of (Hudson and Morel 1990):

$$\rho = \rho max\ [M'] \ /\ K_{\rho} + [M']$$

Where ρ is the uptake rate, K_{ρ} is the half-saturation constant and ρmax corresponds to the maximum uptake rate. [*M'*] is essentially the concentration of free metal ion and kinetically labile complexes adjacent to the cell surface. From K_{ρ} two other parameters that can be derived that are essential in regulating uptake, the ligand exchange rate constant (K_L) and the maximum attainable cell surface ligand concentration $[L1]^{max}$

The development of speciation models (Campbell 1995) has shown an excellent guide to the availability of the trace metals in solution It assumes that the uptake of trace metals by the cell during internalization (3), (Fig. 2) is sufficiently slow that it is rate limiting and the reactions in diffusion (1) and sequestration (2) can reach equilibrium. The free metal ion at the cell surface therefore represents the metal available for uptake, and the concentration of ML1 at the surface is at equilibrium with this concentration (Whitfield 2001).

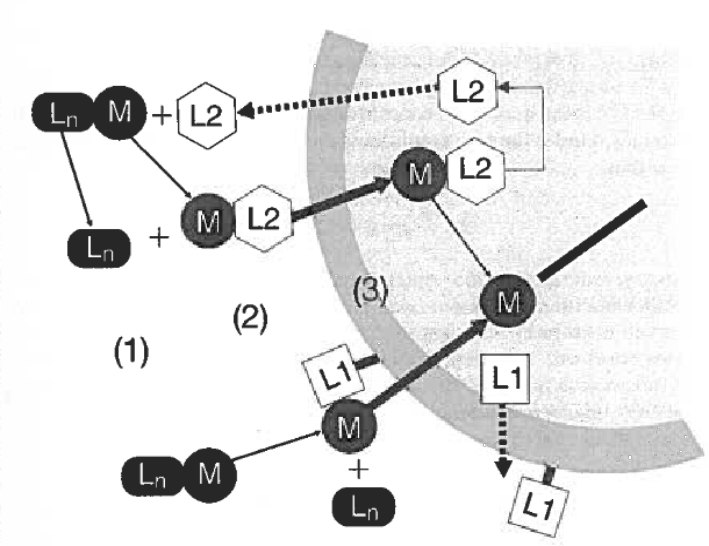


Fig. 2) Uptake of trace metals at the cell surface. (1) Bulk solution where the metal (M) is complexed by inorganic and organic ligands (L_n). (2) Diffusion zone where complexes interact with ligands that are either attached to the cell surface (L1) or released into the bulk solution (L2). (3) Transport zone where the metal—ligand complex is taken into the interior of the cell. Ligands are recycled and returned to the cell surface (dashed lines) (Whitfield 2001).

3.4. Iron

Iron is the second most abundant metal, and the fourth most abundant element in the earth's crust. (Reilly 2004) Belongs to the d-block transition elements and can exist in oxidation states ranging from -2 to +6. However, in biological systems, these are limited to the ferrous (+2), ferric (+3) and ferryl (+4). Three oxides are known, FeO, Fe_2O_3 and Fe_3O_4, representing the Fe(II) and the Fe(III), as well as the mixed Fe(II)–Fe(III) oxide which occurs in nature as the mineral magnetite. With non-oxidising acids, in the absence of air, ferrous salts are formed. Many iron salts, as well as hydroxides, are insoluble in water (Reilly 2004).

Virtually all microorganisms require iron for their respiratory pigments, proteins and many enzymes. Like the other transition metals, with their labile d-electron systems, iron has a rich redox chemistry, which allows it to play a role in a variety of oxido-reductase enzyme activities as well as in electron transfers (Reilly 2004). In the same way, the bioavailability of iron is critically dependent on its redox state. Reduced iron (Fe^{2+}) is highly soluble in water, whereas, its oxidized form (Fe^{3+}) is virtually insoluble (Haese 2005). Iron can also, due to of its unoccupied d-orbitals, bind reversibly to different ligands. This ability to form coordination complexes constitute important chemical property, from biological perspective (Reilly 2004).

3.4.1. Iron marine cycle

Iron is transported to the ocean by four major regimes: fluvial, aeolian, submarine hydrothermal, and glacial, being the fluvial the most important in coastal areas. Iron concentrations in average, show less solubility in marine relative to river water (Haese 2005). Concentrations of both particulate and dissolved Fe species can decrease > 90% at the estuarine mixing zones (Sañudo-Wilhelmy et al. 1996; Öztürk et al. 2002). Dissolved iron is mainly present as Fe (III) oxyhydroxide, which is stabilized in colloidal dispersion by high-molecular-weight humic acids (Hunter 1983). Due to increasing salinity colloids ten to coagulate, resulting in an exponential decreasing gradient between river, estuarine an seawater (Haese 2005).

Most waters are in a fairly oxidized state because of biological activity. Through photosynthesis, autotrophs produce free oxygen and on the other hand organic matter, affecting the redox state of natural waters, therefore iron speciation. Being an essential micronutrient, dissolved iron concentration shows a similar vertical profile in the water column as nitrate being reduced to near zero within the surface layer where PP takes place (Haese 2005). The proximity to iron sources in coastal regions led to the assumption that iron generally is in abundance, occurring 100 to 1000 times higher concentrations in coastal waters (Sunda and Huntsman 1995), decreasing abruptly off continental margins (Johnson et al. 1997) to the extent to become limiting in certain region in the open ocean, reaching concentrations 20 – 30 pM in the High Nutrient Low Chlorophyll (HNLC) zones (Martin 1991; Martin et al. 1991; Morel et al. 1991).

Below the photic zone, where light intensity is not enough to sustain photosynthesis, biologic activity and respiration, thus oxygen consumption sustained by falling organic matter from the photic zone. Product of decomposition and mineralization, dissolve iron concentration increases resembling a nutrient type profile (Johnson et al. 1997). Yet chemistry dictates that iron should adopts a scavenged element profile (lead type), decreasing in concentration with depth due to particle adsorption (Whitfield 2001). If the rate of respiration exceeds downward advection of oxygenated surface water, respiration depletes all available oxygen, suboxic or anoxic conditions are achieved. Under these conditions iron increase due to the mineralization of iron bearing organic matter (Haese 2005).

In sediments, once oxygen is consumed, a variety of microbial activity continue utilizing other oxidants as other than oxygen. We would expect to see oxygen consumed first, followed by reduction of nitrate (denitrification), manganese, iron (ferric iron is reduced to soluble ferrous iron), sulphur, and finally nitrogen (nitrogen fixation). Under these suboxic and anoxic circumstances Fe occurs in its soluble form and concentrations are much higher compared to normal oxic environments. The metal then diffuse upward to the oxic-anoxic boundary in water bodies where they again are oxidized and precipitate (Haese 2005).

3.4.2. Relevance of Iron in biological productivity

Iron is fundamental to the physiology of prokaryotic and eukaryotic cells (Whitfield 2001). Its pivotal role in marine as biological production was tested first in the late 1980s (Martin and Gordon 1988; Martin et al. 1989; Martin 1990; Martin et al. 1990) and have been supported by a series of extensive mass fertilization experiments through the 1990s and during the past decade (Martin et al. 1994; de Baar et al. 2005) and cites there in.

The oxidation-reduction properties of iron make it ideally suited to catalyze electron transfer reactions. In the course of evolution microorganisms have exploited iron for photosynthetic and respiratory functions as well as for the reduction of inorganic nitrogen species, nitrate, nitrite, and nitrogen gas (Morel et al. 1991). It also acts as an acid catalyst in hydrolytic enzymes (Whitfield 2001). Iron is undoubtedly the most versatile and important trace element for biochemical catalysis (Morel et al. 1991) (Table 2).

3.4.3. Iron speciation and uptake by phytoplankton

Iron has a solution chemistry that is dominated (at pH 8) by extensive hydrolysis, which makes it prone to rapid removal by oxyhydroxide colloid formation and effective scavenging onto falling particles (Whitfield 2001). Between 10 and 50% passes through 0.4 μm filter (Martin and Gordon 1988; Martin et al. 1989), and some of this may be colloidal rather than truly dissolved. The particulate and colloidal fraction comprises oxides and aluminosilicates and possibly organic forms. Direct measurements in sea water using electrochemical techniques indicate that a large proportion (usually >97%) of the total dissolved fraction is held in strong organic complexes (Rue and Bruland 1995). The colloidal iron also can be slowly resolubilized by photochemical action in the near-surface layers (Barbeau and Moffett

2000). Concentration of Fe^{3+} is $<10^{-22}$ M or just a handful of molecules per litre (Rue and Bruland 1995). Therefore very little may be in the reduced Fe (II) form rather than the stable Fe(III) in oxygenated water.

Table 2) Metabolic and enzymatic roles of Fe in marine organisms.

Enzyme & proteins	Function
Cythochrome f	Photosynthetic electron transport
Cythochromes b and c	Electron transport in respiration and photosynthesis
Cythochrome oxidase	Mitochondrial electron transport, O2 + 4e- + 4H+ 2H2O
Fe-sulphur proteins	Photosynthetic and respiratory electron transport
Ferrodoxine	Electron transport in photosynthesis and nitrogen fixation
Nitrogenase	Nitrogen fixation
Nitrate and nitrite reductase	Nitrate and nitrite reduction to ammonia
Ribonucleotide reductase	Transforms ribose to deoxyribose (DNA repl. and cell div.)
Fe-superoxide dismutase	Disproportion of O2 - radicals to H2O2 and O2
Catalase	H2O2 breakdown to O2 and H2O
Peroxidase	H2O2 reduction to H2O
Chelatase	Porphyrin and phycobiliprotein synthesis
Succinate dehydrogenase	Fumarate synthesis
Aconitase	Isomerization of citrate
Coprporphyrinogen oxidase	Oxidative decarboxylation of Mg-protoporphrine
Lipooxygenase	Fatty acid oxidation, carotenoid degradation
Glutamate synthetase	Glutamate synthesis
Xantine oxidase	Oxidation xanthine to uric acid
Ferritin	Iron storage
Methane monooxygenase	Methane oxidation
Purple acid phosphatase	Unknown
Alkaline Phosphatase	Formation of phosphate ester

From culture studies it appears that only the dissolved inorganic forms of iron, chiefly the dominant hydrolysis species $Fe(OH)_2^+$, are taken up by marine phytoplankton, therefore the necessity for the iron to be in dissolved inorganic form in order to be available to algae underscores the importance of iron chemistry in surface seawater (Morel et al. 1991). Direct uptake of inorganic iron must involve these soluble hydroxide complexes (Whitfield 2001). Some early studies have claimed that colloidal Fe colloidal might be a usable source of iron for algae (Barbeau and Moffett 2000), while others no (Rich and Morel 1990).

Since iron uptake in marine phytoplankton involves a complexation reaction between iron in the water and an uptake molecule at the cell surface, followed by internalization of the membrane-bound iron (Hudson and Morel 1990), the production of organic ligands to complex iron as bacteria (e.g., siderophore) improve the accessibility to iron. For instance It has been observed that phytoplankton may produce excess ligand in response to an influx of iron (Witter et al. 2000).

In this sense, organic complexes can play key role for phytoplankton by increasing the solubility of iron (Kuma et al. 1996), making it accessible for longer periods, thus greatly reduce the opportunity for removal of the iron by particle scavenging. Moreover, the organic complexes also provide a potential site for the photo reduction of Fe (III) to Fe (II) that is more readily assimilated, provided that it can be accessed in a timescale that is short compared with the oxidation rate (Whitfield 2001).

4. MATERIALS AND METHODS

4.1. Fieldwork

4.1.1. Study Area

The fieldwork took place in Chile as part of the WAFOW project (Can waste emission from fish farms change the structure of marine food webs? A comparative study of coastal ecosystems), between Norway and Chile. Experiments were carried out during the austral summer season between January and February 2011 at the facilities of the Huinay Scientific Field Station (42°22'46"S – 72°25'12"W) in the Comau fjord, Northern Patagonia (Fig. 3).

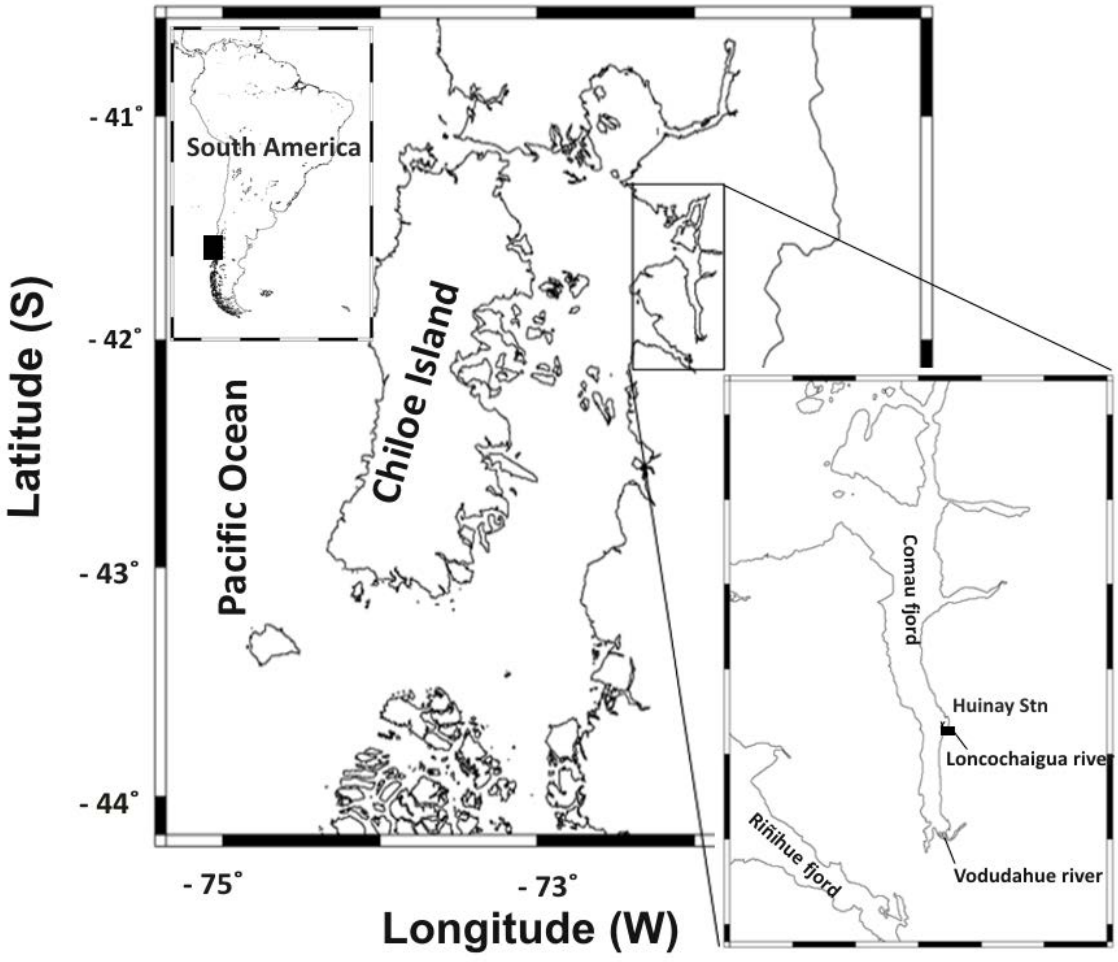


Fig. 3) Study area presenting main freshwater inputs (Loncochaiga and Vodudahue rivers) and the sampling site (Huinay Stn) for the mesocosm experiments in the Comau Fjord, Chile during January-February 2011 (Sanchez et al. 2011).

The study area in Chile corresponds to the fjords region (Patagonia 41 – 55°S), classified as part of the sub Antarctic region and characterized by a marked seasonality with strong climatic changes (Pickard 1971). Scientific knowledge on marine biology and the biogeochemical cycles occurring in the area comes from several oceanographic studies conducted within the last two decades (Pizarro et al. 2000; Palma and Silva 2004; Iriarte et al. 2007; González et al. 2010; González et al. 2011).

4.1.1.1. Hydrography

The Comau fjord hydrography features a two layer system with the presence of a permanent low salinity layer (LSL) between the surface and ~5 m. The mixing of freshwater from precipitation and river runoff (Loncochaigua and Vodudahue) with oceanic water results in a strong halocline, where the salinity regulates the formation of the pycnocline (Fig. 4). This LSL in turn has an effect on the physical characteristics of the water column (e.g., light penetration and nutrient exchange with deeper nutrient-rich water), that exert an important effect on the composition of the planktonic community (Sanchez et al. 2011).

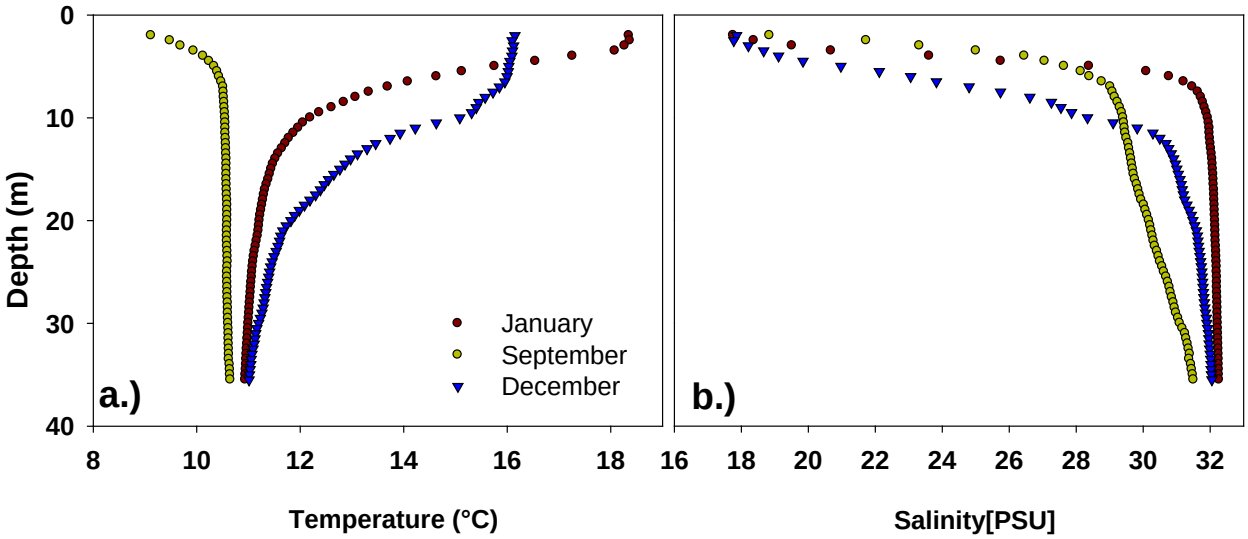


Fig. 4) Typical vertical distribution of a) temperature (°C) and b.) salinity (psu) for three different periods (January, September and December) in the Comau Fjord, Chile (modified from Sanchez et al. 2011).

4.1.2. Mesocosms set up

A mesocosm defines an enclosed space (i.e. body of water) during a period of time for experimental purposes. In the field of aquatic sciences, mesocosm studies have the advantage over standard laboratory tests in that it maintains a natural community under close to natural, self-sustaining conditions. Therefore, the mesocosm approach is often considered the experimental ecosystem closest to the real world, without losing the advantage of reliable reference conditions and replication (Riebesell et al. 2010).

The definitions of size and time duration varied widely among mesocosms experiments and are determined according to the subject of research. Mesocosms range from 1 up to 1000 cubic meters in volume and can be extended over periods of days to a year. For the research considered here and according to Riebesell et al. (2010), our experiment was scaled into a category I-II, that is with multiple units, volume of 1-10 m^3 and days to weeks duration. A total of 10 units (1000 L tanks) were filled half with surface and half with marine water collected at depths of 3 and 10 meters respectively from the fjord constituting five units per water system (without replicates), each one representing one treatment (Fig. 5). Sampling period involved from the 23rd of January until the 14th of February, comprising a total duration of 22 days.

Water pumped into the tanks was collected with a peristaltic pump (Multifix type M80), placed in a peer and using plastic hose (35 mm diameter) projected 30 m offshore. Flowing water were pumped into a 33 L plastic tank (main collector) where afterwards it was equally distributed to each of the five tanks (Fig. 5). Mesocosms where kept as natural as possible without prescreening incoming water, in order to contain different taxonomic groups at the various trophic levels of the natural plankton assemblage.

4.1.3. Nutrient additions

In order to simulate nutrient enrichment occurring in the water column product of salmon aquaculture waste, tanks were supplied with four different concentration (treatments) of macronutrients (nitrogen, phosphorus and silicon) as ammonium chloride (NH_4Cl), sodium dihydrogen phosphate monohydrate ($NaH_2PO_4 \cdot H_2O$) and sodium metasilicate enneahydrate ($Na_2SiO_3 9 \cdot H_2O$) every third day at a fix ratio. Although salmon aquaculture does not add Silicon into the marine environment, it was supplied due to fjords ecosystem in southern Chile has continuous and in excess natural input of it, thus preventing potential nutrient

limiting specifically for diatoms. The five units used per system, were denominated as Control, Natural. Conc1, Conc2 and Conc 3, where “Control” corresponded to the unit with no addition of nutrients, whereas “Natural”, received a nutrient input at the average ratio for N:P:Si, occurring in the natural environment (González et al. 2010). (The three other units received the experimental nutrient concentrations (Table 3).

Fig. 5) Tanks and deployment of the surface and marine systems in the mesocosms experiment in the Comau fjord during January-February 2011.

4.1.4. River and fjord sampling

Additional to the mesocosms, river and seawater samples were collected to have a general background on the concentration and distribution of iron within the study area. The river samples corresponded to the Loncochaigua river, adjacent to the marine station (~ 1 km) (Fig. 3) and involved a three point river to sea transect starting at the location of entirely river water (0 psu), ending at the river sea mix zone. Seawater samples were collected in front of the marine station (~ 2 km offshore), using an acid clean Teflon coated GO-FLO bottle. Samples were collected at 0, 5, 10, 30, 50 and 70 m.

Table 3.) Rate supply (µmol.m^{-3}. d^{-1}) and ratio for the different macronutrients added as NH4Cl for Nitrogen (N), $NaH_2PO4{\cdot}H_2O$ for Phosphorus (P) and $Na_2SiO_3.9H_2O$ for Silicon (Si) in the different treatments for the surface (6-10) and marine systems (1-5), during mesocosms experiment in Comau fjord during January-February 2011. Control units had no nutrient addition representing the base line.

Treatment	Mesocosm	N	P	Si	N:P	N:Si
Control	1 - 6					
Natural	2 - 7	296.0	19.4	146.8	15.3	2
Conc 1	3 - 8	1199.7	49.7	594.7	24.2	2
Conc 2	4 - 9	2991.3	123.8	1483.0	24.2	2
Conc 3	5 - 10	4674.0	193.5	2317.2	24.2	2

4.1.5. Samples collection

Water samples were collected every 3rd day from each tank, according to a specific sampling scheme (Table 4). A total of four different methodological approaches for sea water analysis were employed: chelex-100, DGT, fractionated and direct sampling. Samples for dissolved matter, filtered through 0.2 µm acid washed filters (0.45 + 0.2 µm Sartorious Sartobran 300), were collected by direct sampling and with Chelex-100. All samples processing in the field, were carried out in a closed room under clean air in a Class-100 laminar flow hood (Air Clean Systems 400 Workstation) to avoid contamination. Parallel with the seawater for trace metal analysis, samples to study other chemical (nutrients, (N and P), POC, PON and pH) and biological variables (Chl-a, phytoplankton, zooplankton bacterioplankton) were collected. Further laboratory processing of the samples were done under constant laminar air flow, in the class 100 clean laboratory at the Department of Chemistry at NTNU. All plastic material used both during field and laboratory work, were acid washed according to standard procedures. A total of 6 six extra tubes were run for methodological blanks analysis for Chelex, DGT and direct samples, while 3 filters, per pore size used were set apart for UC-digestion for blank analysis. After laboratory processing, all samples were sent to be analyzed in High Resolution Inductive Coupled Plasma Mass Spectrometry (HR-ICP-MS) Element 2 (Thermo-Finnigan) with PFA-Schott type spray chamber and nebulizer.

Table 4.) Date, sampling day (sday), system sampled and technique used, in the different treatments for the surface and marine systems in the mesocosms experiment in the Comau fjord during January-February 2011.

Date	Sday	System	Technique
23.01.2011	1	Marine	chelex - DGT - fractionation - direct
24.01.2011		Surface	
26.01.2011	2	Marine	chelex - DGT - fractionation - direct
27.01.2011		Surface	
29.01.2011	3	Marine	chelex - DGT - fractionation - direct
30.01.2011		Surface	
01.02.2011	4	Marine	fractionation - direct
02.02.2011		Surface	
04.02.2011	5	Marine	chelex - DGT - fractionation - direct
05.02.2011		Surface	
07.02.2011	6	Marine	fractionation - direct
08.02.2011		Surface	
10.02.2011	7	Marine	fractionation - direct
11.02.2011		Surface	
13.02.2011	8	Marine	chelex - DGT - fractionation - direct
14.02.2011		Surface	

4.2. Techniques

4.2.1. Chelex-100

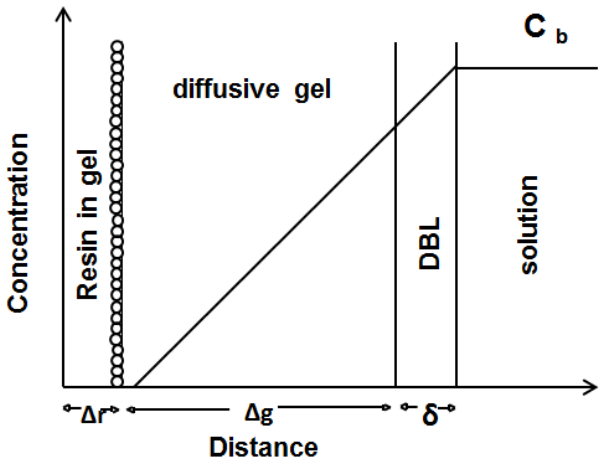
Chellex constitute an ion exchange resin of styrene divinylbenzene copolymers containing paired iminodiacetate ions which act as chelating groups. It has wide applicability among analytical procedures, including those of interest here, the analysis of trace metals in natural waters. The resin exhibits high preference for copper, iron, and other heavy metals over monovalent cations such as sodium and potassium. Also has a very strong attraction for transition metals, even in highly concentrated salt solution. It differs from ordinary exchangers because of its high selectivity for metal ions and its much higher bond strength (Bio-Rad Laboratories).

The selectivity of Chelex-100 for metal cations corresponds to that of iminodiacetic acid, and it is dependent on the pH, ionic strength, and the presence of other complex-forming species. Particularly, the pH affects the quantity of cations exchanged, being very low below pH 2, while it reaches its maximum above pH 4. For the purposes here, pH was not variable affecting the results as the experiments were carried out in natural waters (Bio-Rad Laboratories).

4.2.2. Diffusive gradients in thin-films (DGT)

Diffusive gradients in thin-films constitute a simple, but precision plastic device capable of accumulate dissolved substances in a controlled way. For trace metals analysis, the technique of provides an in situ means of quantitatively measuring labile species in aqueous systems (Zhang and Davison 1995). Since both the mechanism of metal assimilation in aquatic organisms and the mode of metal uptake by DGT are governed by labile metal concentrations in solution, a correlation between DGT metal concentrations and the bioavailable fraction would be expected.

The DGT technique principle is based on Fick's first law of diffusion. Each DGT unit consists of 1.) a layer of polyacrylamide hydrogel of known thickness Δg (cm), is backed by 2.) a layer of ion-exchange resin (Chelex-100) of thickness Δr (cm). Between the diffusive gel and the bulk solution there is 3.) a diffusive boundary layer (DBL), of thickness δ, where transport of ions is solely by molecular diffusion (Zhang and Davison 1995) (Fig. 6).



(Fig. 6) Schematic representation of the free concentration of ionic species in a hydrogel assembly in contact with aqueous solution, where the concentration is ***Ct,*** (DBL is diffusive boundary layer). The rate of diffusion is assumed to be the same in the gel and solution (Zhang and Davison 1995)

The δ here was assumed negligibly compared to Δg due to effective stirring of the solution in the shaker. Then, the flux F (mol. cm^{-2} s^{-1}) of metal ions diffusing through the gel layer to the resin can be expressed by X-1

$$F = D\,(C_b - C')\,/\,\Delta g \qquad \text{X-1}$$

Where D is the diffusion coefficient (E-6 $cm^{-2}.s^{-1}$) of the element in the gel, C_b the free concentration of a metal ion in bulk solution, and C' the free concentration of the metal ion in the resin gel layer. If the free metal ions are in rapid equilibrium with the resin, with a large binding constant, C' is effectively zero providing the resin is not saturated. Therefore X-1 can be simplified to X-2

$$F = D\, C_b \,/ \Delta g \qquad \text{X-2}$$

According to the definition of flux (F = M/At), the mass diffused through an area A (cm^2), after given time t (sec) should be

$$M = D\, Cbt\, A \,/ \Delta g \qquad \text{X-2}$$

The mass of the diffused ion M (ng)**,** can be obtained by X-3

$$M = (\, C_e\, (V_g + V_e) / f_e \qquad \text{X-3}$$

Where Ce is the concentration (µgr L^{-1}) of ions in the acid eluent obtained from the results on HR-ICP-MS analysis, V_g the is the volume (L) of gel in the resin gel layer, V_e the is the elution volume (L) of acid and f_e the ratio of the eluted to bound metal, known as the elution factor and here assumed 0.9 as extraction proceeded with 2M UP HNO_3 (Ardelan *pers. comm.*). Obtaining M, the concentration of the ion in the bulk solution can be quantified by rewriting

$$Cb = M\Delta g \,/\, Dt\, A\, g \qquad \text{X-2}$$

4.2.3. Size fraction filtration

Constitute a separation method based on predefined (pore size) criteria. In the field of aquatic sciences, it has applicability on the study mainly on biological and certain chemical variables. Separation by size range are defined "artificially", usually based on the biological feature of the size distribution of the living organisms comprised in the plankton size spectrum. Determining the distribution of trace metals within different size fractions in water and plankton in aquatic ecosystems, can be obtained from the dissolved matter (<0.2 µm), separation living from nonliving matter, passing through Picoplankton (e.g., Bacteria) up to the macroplankton (e.g., fish larvae). The size fractions are associated to specific groups of organisms (e.g., taxonomically related) or different trophic levels within the food web.

Size fraction filtration can be performed either by independent simple filtration or sequential fractionation. In the first case, different samples of water of the same volume are filtered through a determined pore size filter. Assuming that each filter size retain all particles bigger than the pore size, and let through the smaller ones, values obtained (e.g., trace metal concentration) in each filter size are subtracted from the previous (bigger pore) filter size (e.g., value in 20 µm filter – value in 60 µm filter) to obtain the specific amount present in each fraction (e.g., < 60 µm > 20 µm). For the second case, the sample water sample is filtered sequentially through an in-line system of filters, starting from the bigger pore size to the smaller one. Though water is the same, the volume filtered may not be strictly the same as bigger pore size can filter higher volume at high speed, while smaller fraction tend to get clogged. In that way, volume of filtered water may follow a decreasing fashion towards the smaller pore size filters. Having sequential filters in an in-line holder rather than performing independent filtrations through each filter vastly simplifies field operations in which many such samples must be collected while minimizing handling and potential contamination of individual filters (Cullen and Sherrell 1999).

4.3. Laboratory work

4.3.1. Chelex samples

Sample for dissolved Chelex labile (DFe_{Ch}) and Total Chelex labile (TFe_{Ch}) iron were collected in acid washed plastic bottles. A volume (90 – 150 ml) of water sample is transferred, were 0.8 mL (shaken beforehand) of the Chelex-100 solution (Ammonium Acetate buffer ($C_2H_4O_2.NH_3$)) is transferred to the sample using an automatic pipette. For the DFe_{Ch}, 0.2 µm acid washed filters (0.45 + 0.2 µm Sartorious Sartobran 300) and syringe were used to filter the water sample. Afterwards, each sampling bottle was placed in a plastic bag to ensure a clean environment and then in a shaker (65 - 80rpm) for 48 – 72 hours. After this period, each sample was transferred to an acid-washed plastic PE column (Bio-Rad Laboratories), where the water was washed out through the column, and the Chelex-100 containing the material was restrained by a resin present at the end of the column. Remains of samples, were first washed with Milli-Q water and secondly with~ 10mL of 0.1M $C_2H_4O_2.NH_3$ to remove the residue of seawater matrix. After washing out the water, columns were locked and stored at low temperature (4˚C) until transport.

In the laboratory, columns containing the sample were placed in grid, and extraction of trace metals were done in a two-step acidifying process: 1 mL of 2M UP HNO_3 was added, wait for 5 minutes and then gently shaken to re-suspend the Chelex-100. After 15 more minutes, content of the column was poured into acid-washed PE tubes and 4 mL of 0.25M UP HNO_3 was added again to the columns. After 10 minutes, final content was poured into the PE tubes, obtaining a total 5 mL sample (Öztürk et al. 2002; Ardelan et al. 2010).

4.3.2. DGT samples

DGT unit consists of a 0.4 μm pore-size cellulose acetate filter, a polyacrylamide hydrogel diffusion layer, and a Chelex-100 impregnated binding phase. Samples for the DGT labile iron (Fe_{DGT}), were collected placing three DGT samplers where placed in acid washed plastic containers with a volume (1500 – 2000 mL) of water. Afterwards, each plastic container was placed in a plastic bag to ensure a clean environment and then in a shaker (60 - 80rpm) for 48 – 72 hours. After completing the time period, DGT samplers were taken out of the sample water and stored in a freezer until transport (Ardelan et al. 2009).

In the laboratory, all DGT samplers processing were done over a Teflon sheet, where for each sampler was opened and first two layers (filter and gel) were removed. The third layer, corresponding to the gel holding the resin was transferred to an acid washed PE tube and 4 mL 3M UP HNO_3 was added. PE tubes containing the resin were put on a shaker at (60 - 80rpm) for a 12 hour period. Afterwards, HNO_3 in the PE tubes were transferred to new acid-washed PE tubes, keeping the resin in the old one. To assured total transfer of all material, 4 mL (1 – 3 ml) of Milli-Q water were added to old tubes and then poured into the new ones, thus obtaining 5 mL samples final volume.

4.3.3. Size fraction filtration

To determine concentration and distribution of the particulate total ($PFe_{>0.2}$) and in different size fractions (PFe_{SF}) iron content within the plankton community present in the mesocosms, filtration with sequential fractionation was performed encompassing a range of six size classes: 0.2 – 2 μm (picoplankton), 2 - 10 μm (nanoplankton), 10 – 20 μm (larger nanoplankton), 20 – 140 μm (microplankton), >140 μm (mesozooplankton) and > 220 μm (larger mesozooplankton) (Fig. 7). Filtration up to the 10 μm was performed with a simple filtration system fitted to a peristaltic pump and using acid washed polycarbonate filters (25

and 54 mm diameter), whereas filtration from 20, 140 and 220 μm fractions were performed with acid washed plastic sieves with different pore size Nitex meshes with the retained material then afterwards washed into 0.2 μm filters. Filtration volumes ranged from ≥ 2000 mL for bigger fractions to a 100 mL for the smaller ones. Samples were kept frozen and sent back to Trondheim.

In NTNU laboratory, samples were defrosted to perform UC-digestion. The filters with the organic material were placed into Teflon tubes adding 5 mL of UP (50%) HNO_3. The rack with tubes was placed into the UC to follow digestion process (APPENDIX 1.) After two hours process, samples were set to final dilution by pouring the remnant on the Teflon tubes into a dilution bottle and filled it up to 61 ± 0.3 mg. Subsample volume was recalculated (density of ultra-pure water 0.998 $gr.cm^{-3}$ at room temperature), then divided by total original volume filtered to obtain final concentration.

4.3.4. Direct samples

Direct samples for dissolved (DFe) and total iron (TFe) were collected, transferring ~10 mL of water to an acid-washed PE tubes and one drop of 1M UP HNO_3 (Optima Grade, Sigma) was added to the sample to make pH lower than 1.5 and then stored. For dissolved matter samples, 0.2 μm acid washed filters (0.45 + 0.2 μm Sartorious Sartobran 300) and syringe were used to filter the water sample. In the laboratory, samples were diluted ten times by adding 1 ml of each sample in new PE tube, then adding 9 ml of ultra-pure water (Milli-Q) and concentrated UP HNO_3 was added to bring final concentration to 0.1 M HNO3. A total of six tubes were run for blank analysis.

4.3.5. Blanks and detection limits

The limit of detection is a part of the quality control of an analytical method, and is defined as the lowest concentration that is statistically different from the instrumental blank value (Grasshoff et al. 1999), The detection limit used here is three times the standard deviation calculated from the measured method blank values, which correspond to the random errors associated to methodological procedures. All values reported here, lie above the blank value determined, first subtracted from the blanks obtained from HR-ICP-MS values and then calculated to the appropriate concentration. Blanks and detection limits of the analysis performed in HR-ICP-MS for each of the technique are presented in Table 6. For accuracy and precision of techniques, refer to Ardelan et al. (2010). Extended tables (APPENDIX 2).

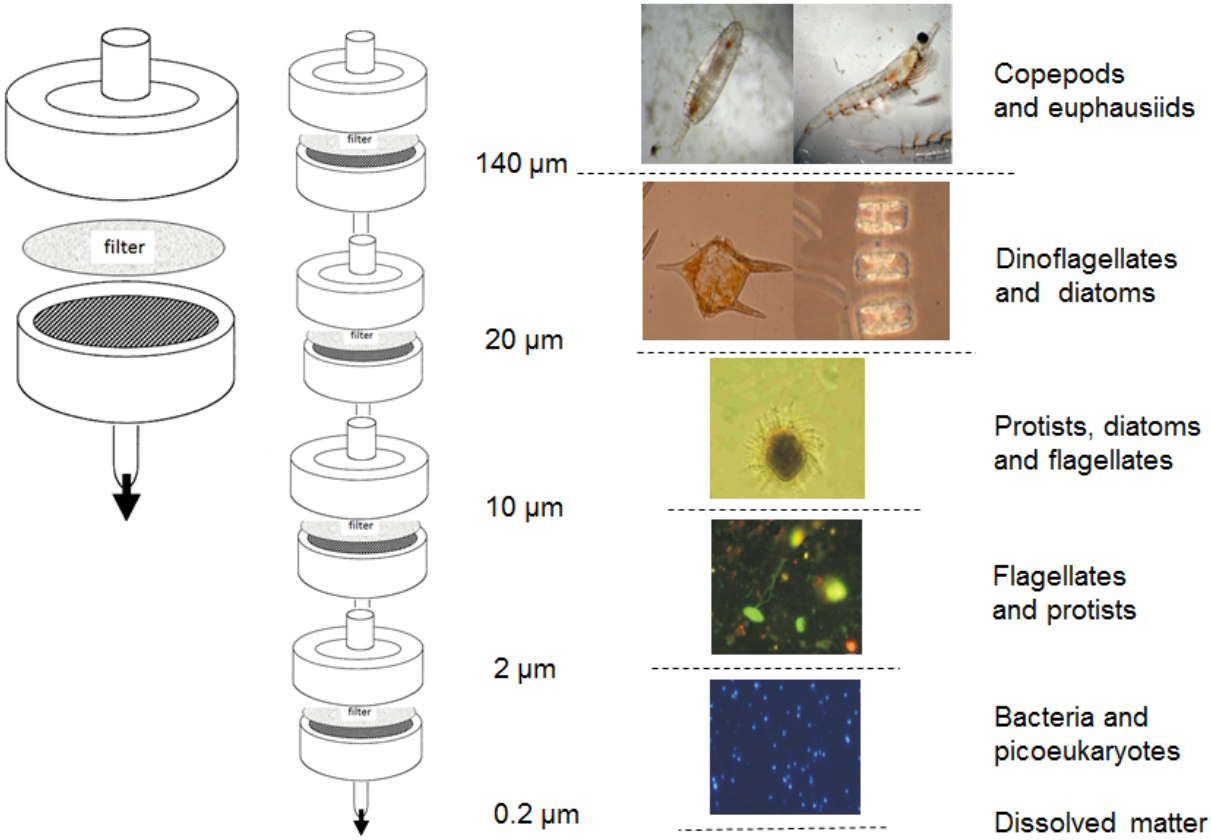


Fig. 7) Schematic representation of the sequential filtration system with filters sizes used and the type of microorganisms that are retained in the Fe_{SF} samples.

4.3.6. Statistical analysis

Statistical analysis were performed using the software package Microsoft Excel 2010 and SYSTAT Sigma Plot V. 11. When Normality and Equal Variance tests requirements were met, Parametric tests (1- way ANOVA with Holm-Sidak test) and correlation analysis between the final concentrations of the different treatments or between the mean concentration and the NH_4^++ loading, were performed. Otherwise non-parametric approach were used (Rank ANOVA (Kruskall- Wallis) (APPENDIX 3).

Table 6). Concentration (nmol.L^{-1}) and relative standard deviation (RSD %), for the blanks analyzed in HR-ICP-MS for the direct, chelex, DGT and fractionation samples in the mesocosms experiment in the Comau fjord, Chile during January-February 2011. Filter: Filter pore size. Std: Standard deviation. C. Int 95%: Confidence interval 95 %. C. Int. 95% (%): Confidence interval 95 % percentage.

Technique	**Direct**		**Chelex**		**DGT**		**Fractionation**		
Replicate	**nmol.L^{-1}**	**RSD**	**nmol.L^{-1}**	**RSD**	**nmol.L^{-1}**	**RSD**	**nmol.L^{-1}**	**RSD**	**Filter**
1	0.08	0.07	0.02	0.01	0.04	0.05	0.02	0.15	0.8
2	0.10	0.04	0.01	0.10	0.02	0.08	0.01	0.06	0.8
3	0.06	0.04	0.02	0.01	0.02	0.03	0.02	0.14	0.8
4	0.14	0.14	0.03	0.09	0.03	0.05	0.01	0.02	2
5	0.07	0.13	0.04	0.05	0.03	0.08	0.02	0.12	2
6	0.06	0.08			0.04	0.08	0.01	0.01	2
7					0.03	0.05	0.01	0.03	10
8					0.03	0.09	0.02	0.01	10
9							0.01	0.08	10
Average	0.08	0.08	0.02	0.05	0.03	0.06	0.02	0.07	
Std	0.03	0.04	0.01	0.04	0.01	0.02	0.00	0.06	
Rsd (%)	35.91	52.31	39.07	80.89	23.79	34.52	30.86	80.44	
C. Int. 95%	0.03	0.04	0.01	0.04	0.01	0.02	0.00	0.04	
C. Int.95% (%)	32.12		39.07		17.98		21.82		
Number	6	6	5	5	8	8	9	9	

5. RESULTS

5.1. Iron distribution in the natural environment

Concentrations of TFe_{Ch}, DFe_{Ch} and Fe_{DGT} in a river to sea transect and in a depth profile are presented to describe the general distribution pattern of these three fractions of iron in natural waters in the Comau fjord during the mesocosms experiments (Fig. 8).

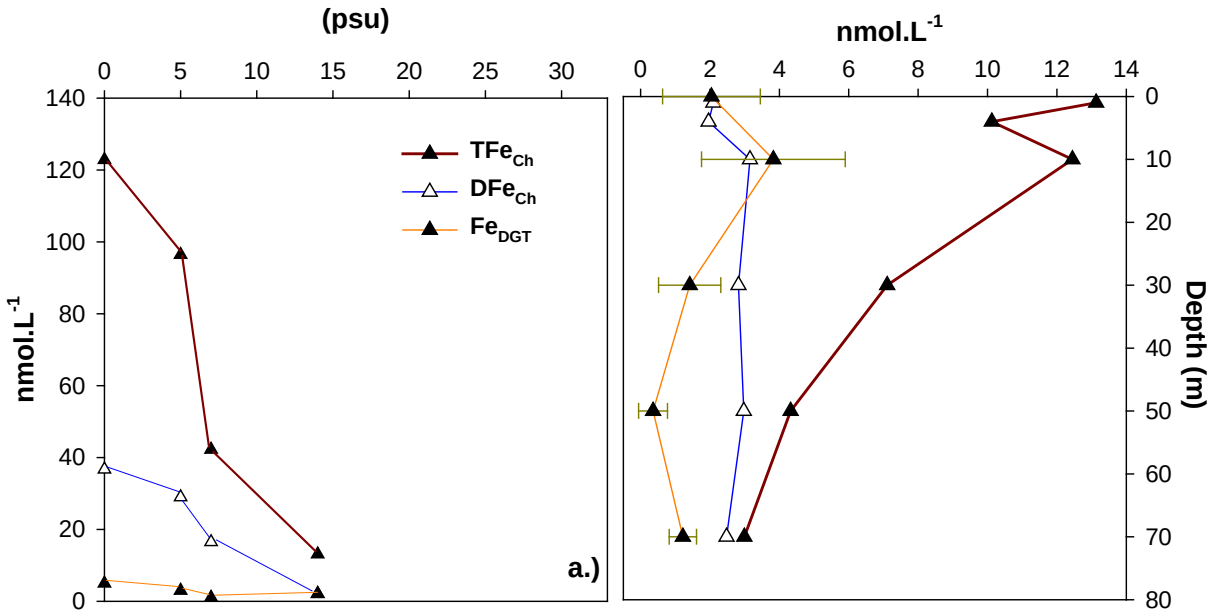


Fig. 8) Distribution of the TFe_{Ch}, DFe_{Ch} and Fe_{DGT} ($nmol.L^{-1}$) for a.) river to sea transect (psu) and b.) vertical profile (0 - 70m) in the Comau fjord, Chile during January-February 2011. Error bars: standard deviation (n=3).

Concentration for these three fractions of iron measured show that riverine constituted a main source, presenting the highest values at the most inland point sampled with salinity 0 psu (river water), while decreasing exponentially towards higher salinity and the sampling point. Concentrations ranged 122.8 to 42.2 $nmol.L^{-1}$, 36.7 to 16.5 $nmol.L^{-1}$ and 5.0 to 1.0 $nmol.L^{-1}$ for TFe_{Ch}, DFe_{Ch} and Fe_{DGT} fractions respectively. Fe_{DGT} presented the highest variation with a sharp decrease (79. 3 % reduction) at the mix zone (7 psu) in the fjord. Fe_{DGT} values represented 13.5, 10.3 and 6% of the DFe_{Ch} fraction for the upper, mid and down river respectively. TFe_{Ch} and DFe_{Ch} showed less steep gradient with concentrations 65.6 and 55.1 % lower relative to upper river values (Fig. 8a).

The vertical profile down to 70 meters, showed similar pattern to the river for all three variables (PFe_{Ch} > DFe_{Ch} > Fe_{DGT}), with the highest values at surface and lowest in depth (Fig. 8b). TFe_{Ch} presented the highest decrease in concentration (77.2 %) from 13,3 nmol.L^{-1} at the surface to 2.99 nmol.L^{-1} at 70 m. DFe_{Ch} presented a different pattern with an slightly increase from 0 to 10 m (2.01 nmol.L^{-1} to 3.15 nmol.L^{-1}), then after remaining without major changes until 70 m. The Fe_{DGT} fraction at the surface presented higher values (2.04 ± 1.4 nmol.L^{-1}) than the mean concentration at the river-sea mix point (1.0 ± 0.1 nmol.L^{-1}) and the DFe_{Ch} concentration at the surface (2.01 nmol.L^{-1}). At 10m, concentration increased (3.82 ± 2.1 nmol.L^{-1}), followed by a decrease until 50m (0.36 ± 0.4 nmol.L^{-1}). At 70m presented a final increase (1.2 ± 0.4 nmol.L^{-1}).

5.2. Iron variability in the water in the mesocosms

Measurements for TFe_{Ch}, DFe_{Ch} and Fe_{DGT} collected at 3 and 10 m that represented the initial conditions (sday 1) of the mesocosm experiments (Fig. 9). TFe_{Ch} fraction presented higher values in the marine layer (13.06 nmol.L^{-1}) compared to surface (8.34 nmol.L^{-1}), whereas DFe_{Ch} presented higher values in the surface layer (8.24 nmol.L^{-1} versus 6.11 nmol.L^{-1}). Fe_{DGT} fraction showed similar concentrations with values of 4.7 ± 1.9 nmol.L^{-1} and 4.1 ± 1.4 nmol.L^{-1} for the marine and the surface layer respectively.

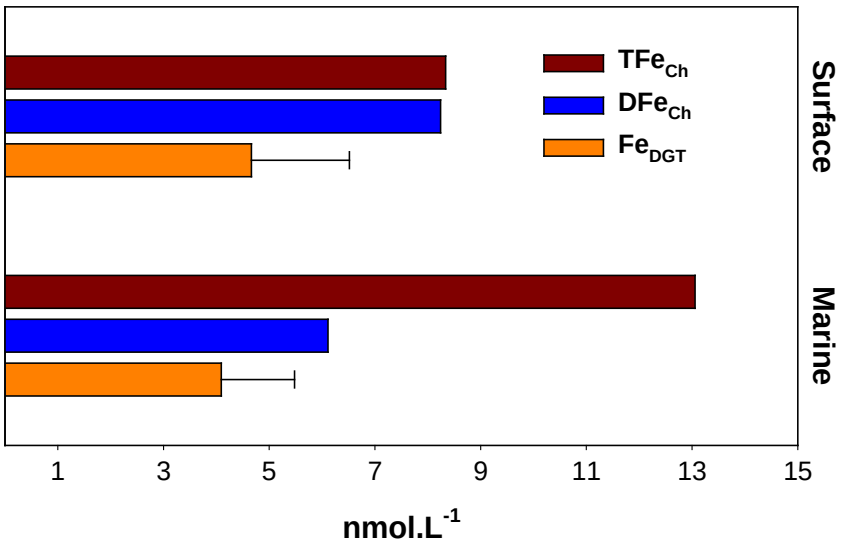


Fig. 9) Distribution of the TFe_{Ch}, DFe_{Ch} and Fe_{DGT} (n.mol.L^{-1}) at two depths representing the surface and marine layer initial conditions for all treatments in the mesocosms experiment in the Comau fjord, Chile during January-February 2011. Error bars: standard deviation (n=3).

5.2.1. Chelex Labile Iron (Fe_{Ch})

Along the 22 days of the experiment, TFe_{Ch} and DFe_{Ch} fractions presented overall higher concentrations on the marine system compared to the surface. The great means for all five treatments were 13.10 ± 6.3 and 16.80 ± 6.5 nmol.L^{-1} for TFe_{Ch} and 5.13 ± 2.3 and 5.67 ± 1.4 nmol.L^{-1} for DFe_{Ch} in the marine and surface systems respectively (Fig. 10 and Fig. 11).

5.2.1.1. Surface system

TFeCh

TFe_{Ch} trends for all treatments (except Conc 2) resembled a "bell shape" distribution with an initial increase, followed by a maximum and a posterior decrease (Fig. 10). Total mean TFeCh concentration for the Control and Natural treatments presented the lowest values with 7.69 ± 2.9 nmol.L^{-1} and 11.88 ± 2.8 nmol.L^{-1} respectively. All treatments with artificially NH_4^+ addition (Conc 1, Conc 2 and Conc 3) reached maximum concentrations between sampling 3 and 5, to then after decrease. The highest NH_4^+ supply treatment (Conc 3) reached its maximum concentration earlier (sday 3) than others, yet the highest concentration in time (25.6 nmol.L^{-1}) and the highest mean of all treatments (16.03 ± 8.8 nmol.L^{-1}) occurred in Conc 2.

DFeCh

DFe_{Ch} distribution in the surface system, exhibited lower range in concentrations between treatments compared to the marine (range: 2.03 nmol.L^{-1} to 8.24 nmol.L^{-1}) (Fig. 10). Final concentrations for all treatments presented lower values compared to the initial conditions (8.24 nmol.L^{-1}), reflected in a decreasing trend showed by all (except Natural) treatments. Natural presented a temporal upward trend (sday 2 to 5), then decreasing until the end.

5.2.1.2. Marine system

TFeCh

Very high concentration in Conc 3 treatment in sday 2 (isolated point), was attributed to some contamination, given that such high variation in short time is unlikely to occur (Fig. 11). All treatments (except Control) resembled the bell shape distribution showed by TFe_{Ch} in the surface layer. The control, followed an initial increase to then decrease steadily. Highest concentrations (>30 nmol.L^{-1}) were reached by treatments Conc 1 (31.6 nmol.L^{-1}) and Conc 2 (30.3 nmol.L^{-1}) in sday 5. Final concentrations for Control, Natural and Conc 3 treatments were lower than initial conditions (13.07 nmol.L^{-1}).

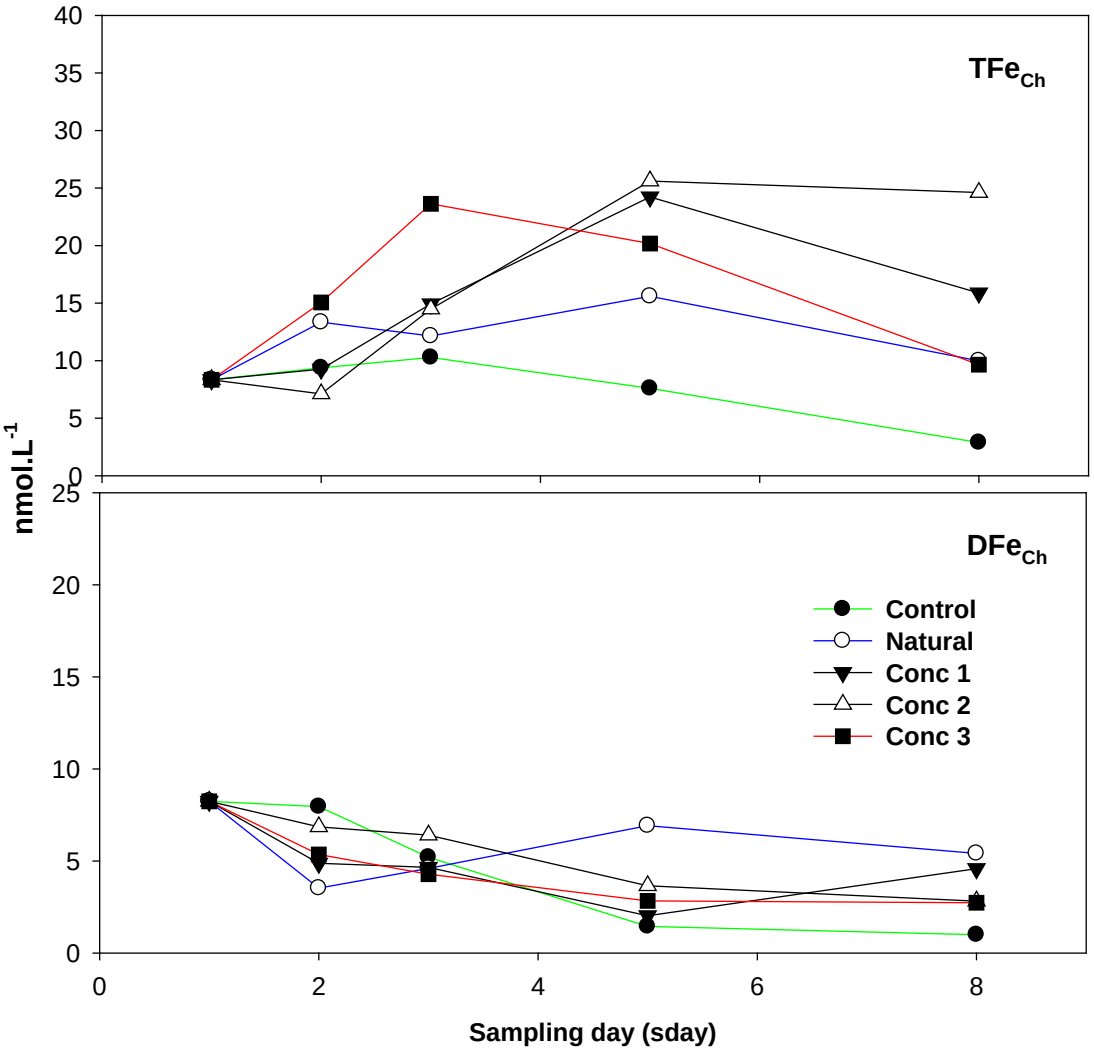


Fig. 10) Distribution of TFe_{Ch} and DFe_{Ch} ($nmol.L^{-1}$), per sampling day (sday), for all treatments in the surface system in the mesocosms experiment in the Comau fjord, Chile during January-February 2011. Note different scale.

DFeCh

As occurred in sday 2 for TFeCh, very high concentrations in treatments Conc 1, Conc 2 and Conc 3, appeared to be product of contamination, likely to happened during sample manipulation (Fig. 11). Disregarding these outliers on sday 2, DFeCh distribution for all treatments followed an overall decreasing trend along time as occurred with the same fraction in the surface system, yet with narrower range (~ 2.5 to 8 $nmol.L^{-1}$) in concentration. At the end, all treatments (except Control) showed significant decrease compare to initial concentrations.

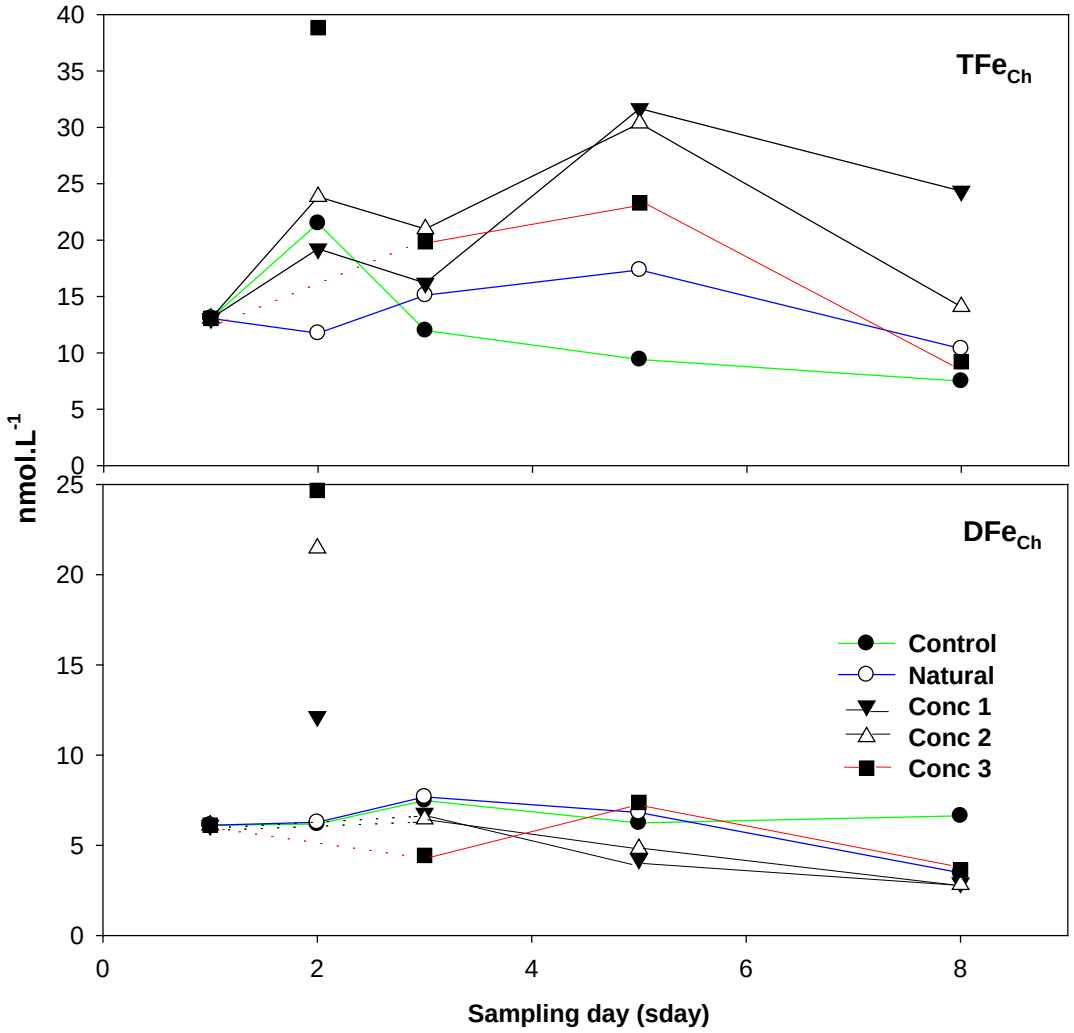


Fig. 11) Distribution of TFe_{Ch} and DFe_{Ch} ($nmol.L^{-1}$), per sampling day (sday), for all treatments in the marine system in the mesocosms experiment in the Comau fjord, Chile during January-February 2011. Dash line: estimated value.

5.2.1.3. TFe_{Ch} versus DFe_{Ch} and NH_4^+ loading

Relation between TFe_{Ch} and DFe_{Ch} per treatment, appeared to be not significant neither for the surface nor in the marine system (Fig. 12). Yet, when coupling together TFe_{Ch} and DFe_{Ch} only for treatments with artificial nutrient addition (Conc 1, Conc 2 and Conc 3), a negative correlation (R^2 = 0.535) appears for the surface system, suggesting an inverse relation for these variables. TFe_{Ch} and DFe_{Ch} for the marine treatments, pictured as well a negative correlation, however this one was weak (R^2 =0.266) (Fig. 13).

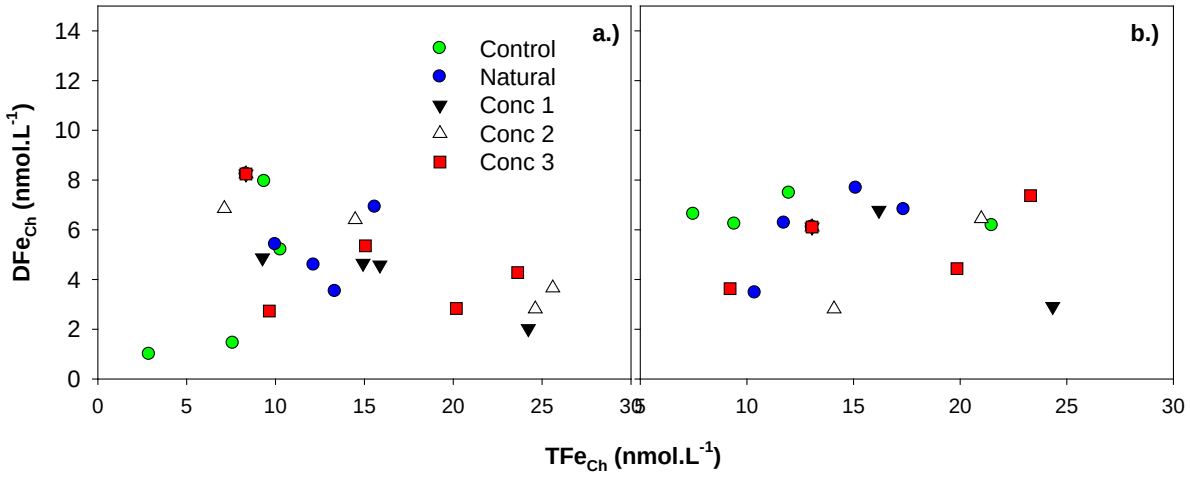


Fig. 12) TFe_{Ch} versus DFe_{Ch} for a.) surface and b.) marine systems, for all treatments in the mesocosms experiment in the Comau fjord, Chile during January-February 2011.

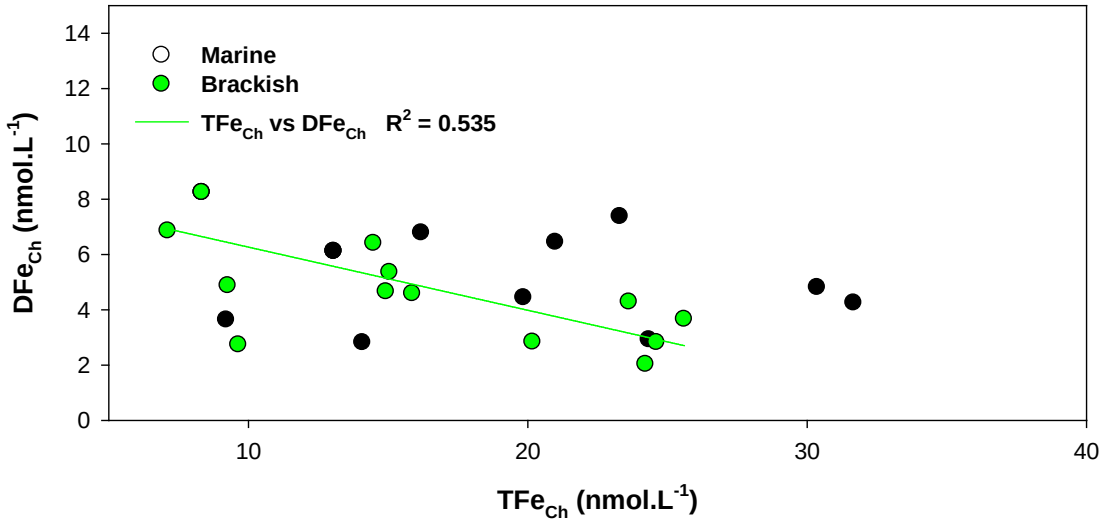


Fig. 13) TFe_{Ch} versus DFe_{Ch} for treatments with artificial nutrient addition (Conc 1, Conc 2 and Conc 3), in the mesocosms experiment in the Comau fjord, Chile during January-February 2011.

Concentrations of TFe_{Ch} relative the NH_4^+ loading presented positive linear correlation for both the surface ($R^2 = 0.606$) and marine ($R^2 = 0.839$) systems, reflecting the increasing trend time observed with increasing NH_4^+ concentration. Contrary to TFe_{Ch}, the DFe_{Ch} was poorly correlated in both systems (Fig. 14).

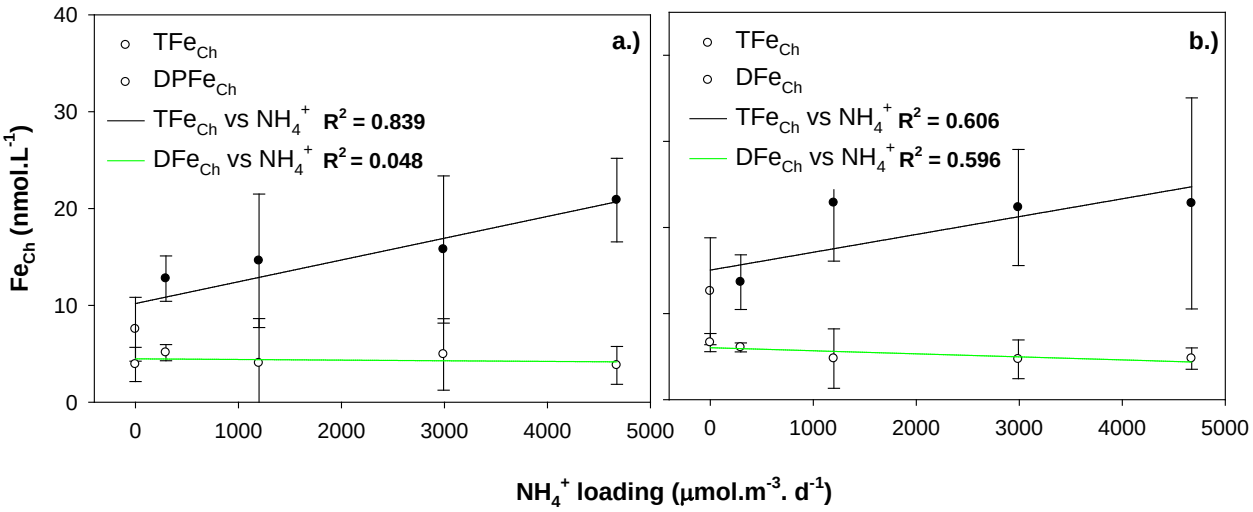


Fig. 14) TFe_{Ch} and DFe_{Ch} versus NH_4^+ loading for a.) surface and b.) marine systems, for all treatments in the mesocosms experiment in the Comau fjord, Chile during January-February 2011. Error bars: standard deviation (n=5).

5.2.2. DGT labile (Fe_{DGT})

Different from TFe_{Ch}, the DFe_{Ch}, Fe_{DGT} average concentration for all treatments showed no appreciable difference between the surface (4.02 ± 2.4 nmol.L^{-1}) and marine (3.94 ± 2.3 nmol.L^{-1}) system (Fig. 15).

5.2.2.1. Surface system

Values in the surface system ranged from 0.27 ± 0.1 nmol.L^{-1} to 6.6 ± 2.2 nmol.L^{-1}. The overall temporal pattern for all treatments in the surface system, shows a decrease in concentration from initial conditions (until sday 2), followed by an increase (until sday 5), and a final decrease. The lowest final concentration occurred in treatment Conc 3 (0.93 ± 0.8 nmol.L^{-1}), while the highest occurred in Natural treatment (4.34 ± 1.9 nmol.L^{-1}). A comparison between treatment's final concentrations showed significant differences between the Natural and the three artificial nutrient addition treatments (Conc 1, Conc 2 and Conc 3) (1-way ANOVA; DF: 14, Holm-Sidak test). Moreover, lower concentrations in a gradient (Conc 1: 2.40 ± 0.4 nmol.L^{-1} > Conc 2: 2.37 ± 0.78 nmol.L^{-1} > Conc 3: 0.93 ± 0.8 nmol.L^{-1}) relative to NH_4^+ concentration, depict a negative relation between the NH_4^+ and Fe_{DGT} concentrations.

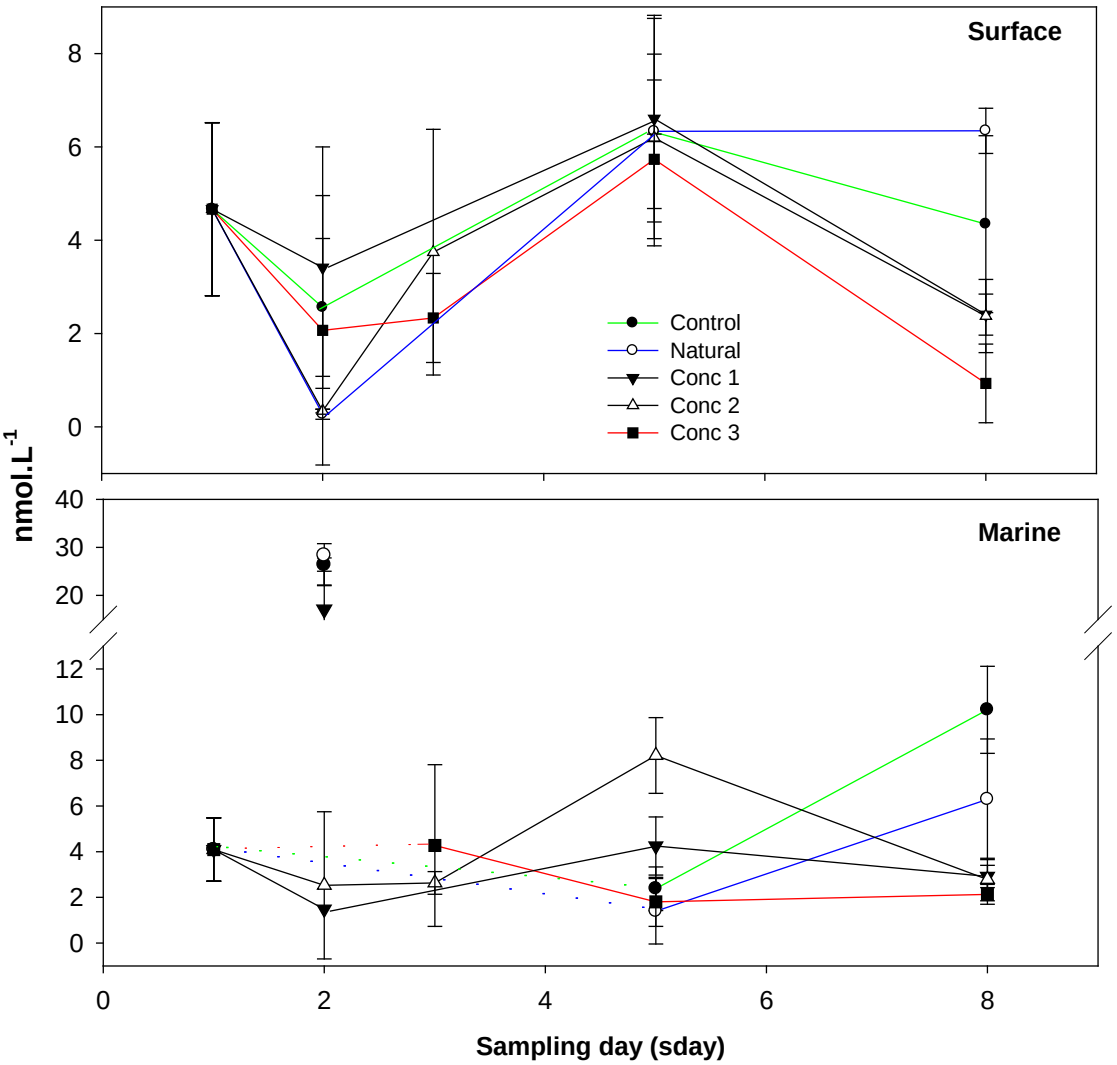


Fig. 15) Distribution of the Fe_{DGT} in surface (top) and marine (bottom) system, for all treatments in the mesocosms experiment in the Comau fjord, Chile during January-February 2011. Error bars: standard deviation (n=3). Conc 2 and Conc 3 have extra samples in sday 3 Note different scale. Dash line: estimated value.

5.2.2.2. Marine system

In the marine layer Fe_{DGT} presented very the high concentrations in the Control, Natural and Conc 3 treatments for sday 2 (isolated points) (Fig. 15). This values were attributed to some sort of contamination during sample manipulation, given that such values correspond to discrete points in time and way off the range expected to be found determined in the Fe_{DGT} fraction. Therefore concentration for this period is assumed here is the average concentrations between sday 1 and 5 for Control and Natural and between sday 1 and sday 3 for Conc 3 (dash line).

Despite the above, Fe_{DGT} presented values in a broader range compare to the surface layer (up to 9.52 ± 2 nmol.L^{-1}). From sday 5, concentrations for Control and Natural treatments showed an increase until the end of the experiment. As occurred for the surface system, a comparison between treatment's final concentrations showed significant differences between the Natural and the three artificial nutrient addition treatments (Conc 1, Conc 2 and Conc 3) (1-way ANOVA; DF:14, Holm-Sidak method), suggesting same negative relation showed in the surface system, between the concentration of nutrient and of this fraction of iron.

5.2.1.3. Direct

Concentrations for DFe and TFe measured along the mesocosm experiments for both the surface and marine systems presented data without trend or reliable values, compared to data obtained by the other techniques (APPENDIX 4). Data from direct samples was not used for further analysis.

5.3. Iron variability in the plankton community in the mesocosms

5.3.1. Particulate iron per size fraction (PFe_{SF})

5.3.1.1. Surface system

PFe_{SF} (all fractions included) in the Control treatment showed decreasing with an initial total concentration of 95.9 nmol.L^{-1} and a final of 32.9 nmol.L^{-1} (65.7% reduction). Natural treatment present no trend. All treatments with artificial nutrient addition, showed higher total mean concentration than Control and Natural treatments. Mean total PFe_{SF} concentrations of 168.8 ± 54.4, 176.0 ± 40.9 and 183.0 ± 116.4 nmol.L^{-1} for Conc 1, Conc 2 and Conc 3 respectively (mean ± SD), exhibited an increasing trend with increased NH_4^+ loading (Fig.16).

PFe_{SF} percentage in the 0.2 - 2µm fraction remained in the same range for most treatments. Only Conc 2 showed lower average (12%) compare to Control and Natural (27.5 %), but with no significant differences (1-way ANOVA, P: 0.497). PFe_{SF} in the fraction >140 µm showed increase proportion in all treatments with artificial nutrient addition (Conc 1: 24.9% Conc 2: 46.7 % and Conc 3: 16.7 %) compare to Control (11.6 %) and Natural (14.9 %), yet not significant.

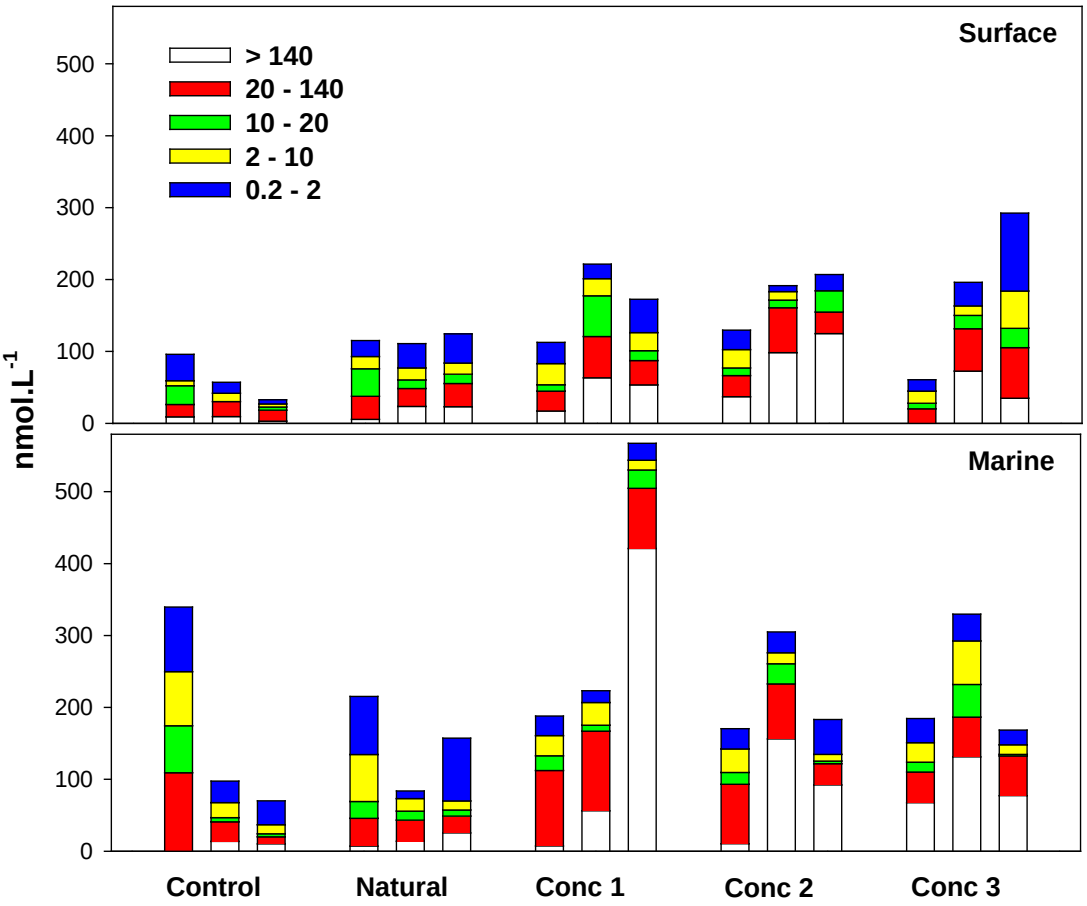


Fig. 16) Distribution of the Fe_{SF}, representing the size structure (µm) of the plankton community (see Fig.7), in the surface (top) and marine (bottom) systems for all treatments in the mesocosms experiment in the Comau fjord, Chile during January-February 2011.

5.3.1.2. Marine system

PFe_{SF} (all fractions included) in Control and Natural treatments, resembled the pattern exhibited in the surface system i. e. decrease trend in time for Control (79.4% reduction) and no trend for Natural treatment (Fig. 16). Different to the surface system, PFe_{SF} contained in the 0.2 - 2µm fraction in all treatments with artificial nutrient addition showed a lower proportion (Conc 1: 24.9 % Conc 2: 46.7 % and Conc 3: 16.7 %) compare to Control (34.8 %) and Natural (35.3 %) treatments. As in the surface system, PFe_{SF} in the > 140 µm fraction, showed an increased proportion for Con1, Con 2 and Con 3, but proven not statistically meaningful.

5.3.1.3. PFe_{SF} in the 20-140 µm and 2-20 µm fraction

To compare PFe_{SF} contained in plankton community in the size range 2 - 140 µm, the fractions 2-10 µm and 10–20 µm (containing the nanoplankton) were added and then

compare to 20 – 140 µm fraction (containing the microplankton) (Fig. 17). In the surface system, the microplankton – nanoplankton ratio (µ/n ratio) presented no significant differences between treatments (1-way ANOVA, DF: 14 P: 0.589).

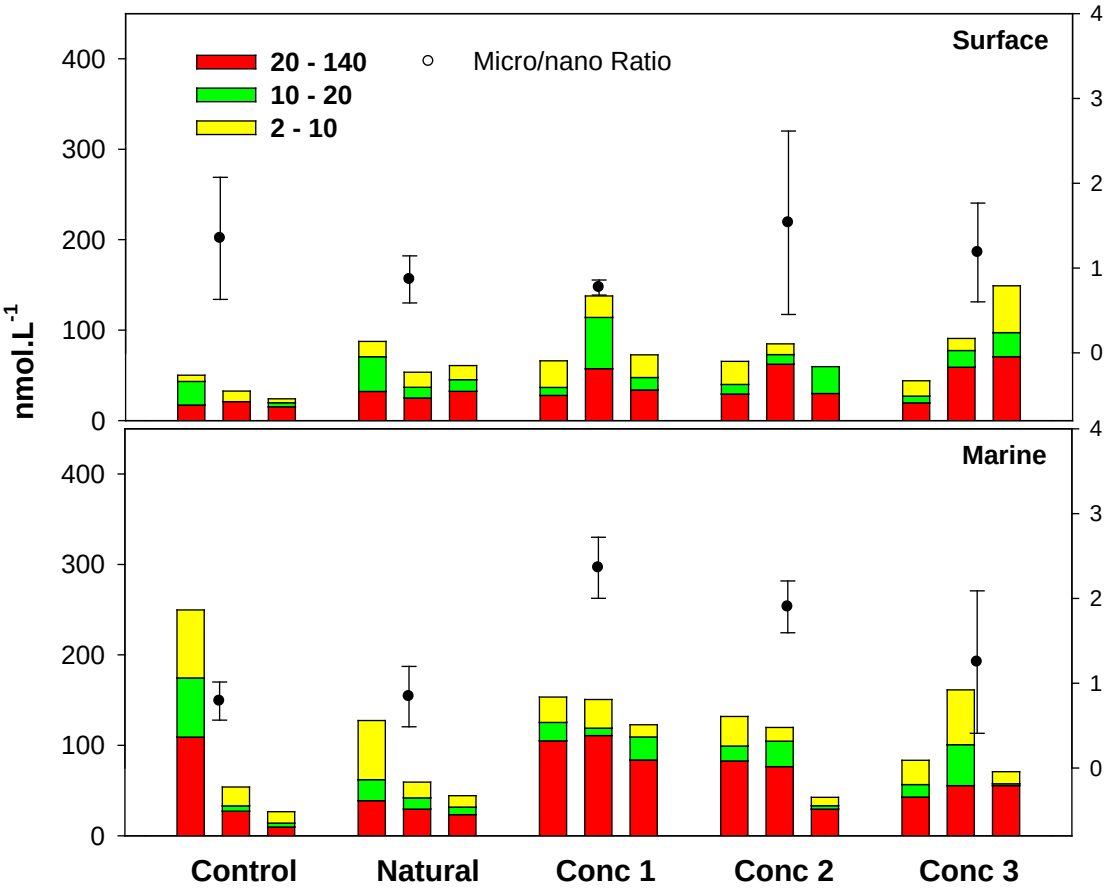


Fig. 17) Distribution of PFe_{SF} in three fractions (2 – 10 µm, 10 – 2 µm and 20 - 140 µm), representing the size structure of the plankton community (Left axis). Ratio between PFe_{SF} in the 20 – 140 µm and 2 – 10 + 10 – 20 µm fractions, representing the ratio between microplankton and nanoplankton (Right axis), in the surface and marine systems, for all treatments in the mesocosms experiment in the Comau fjord, Chile during January-February 2011. Error bars: standard deviation (n=3).

In the marine system, the Control and Natural treatments, presented mean ratios of (0.78 ±0.2 and 0.84 ± 0.4 respectively), while Conc 1, Conc and Conc 3 mean ratios values were 2.36 ± 0.4, 1.90 ± 0.3 and 0.93 ± 0.4 respectively. Mean ratio for Conc 1 and Conc 2 presented significant differences respect to the Control and Natural (1-way ANOVA, DF: 14 P: < 0.002; Holm-Sidak method). Significant higher ratios imply that the PFe_{SF} in the 20 – 140 µm fraction increased its proportion relative to the 2-20 µm thus reflecting possible increase of microplankton over the nanoplankton at certain NH_4^+ loading.

5.3.2. Particulate total iron ($PFe_{>0.2}$)

$PFe_{>0.2}$ in all treatments presented a higher mean value (116.25 ± 75.1 nmol.L^{-1}) in the marine compare to the surface system (80.63 ± 44.6 nmol.L^{-1}) Likewise the total values of PFe_{SF}, $PFe_{>0.2}$ exhibited and confirmed, through increased number of sampling points, the same trend of increased absolute content of iron in the plankton biomass along time and with increased NH_4^+ loading for both surface and marine systems (Fig. 18a and Fig. 19a).

However, when $PFe_{>0.2}$ standardized by the total Chlorophyll (Chl-a) content, reflected an inverse trend with a decrease in the $PFe_{>0.2}$ per Chl-a within time and increase NH_4^+ loading in both systems (Fig. 18b and Fig. 19b). Same change in trend occurred when $PFe_{>0.2}$ is standardized by the particulate organic carbon (POC) (Fig. 18c and Fig. 19c). This trend is further supported by the relation of the $PFe_{>0.2}$ standardized and plotted either against the Chl-a or POC (Fig. 20). The three artificial addition treatments showed well fitted (R^2: 0.507 to 0.960) negative exponential correlation with the two variables. The latter, would imply that iron content per organism (phytoplankton cell) or at least within certain groups in the plankton community in both systems would tend to reduce the iron uptake with increased NH_4^+ concentration.

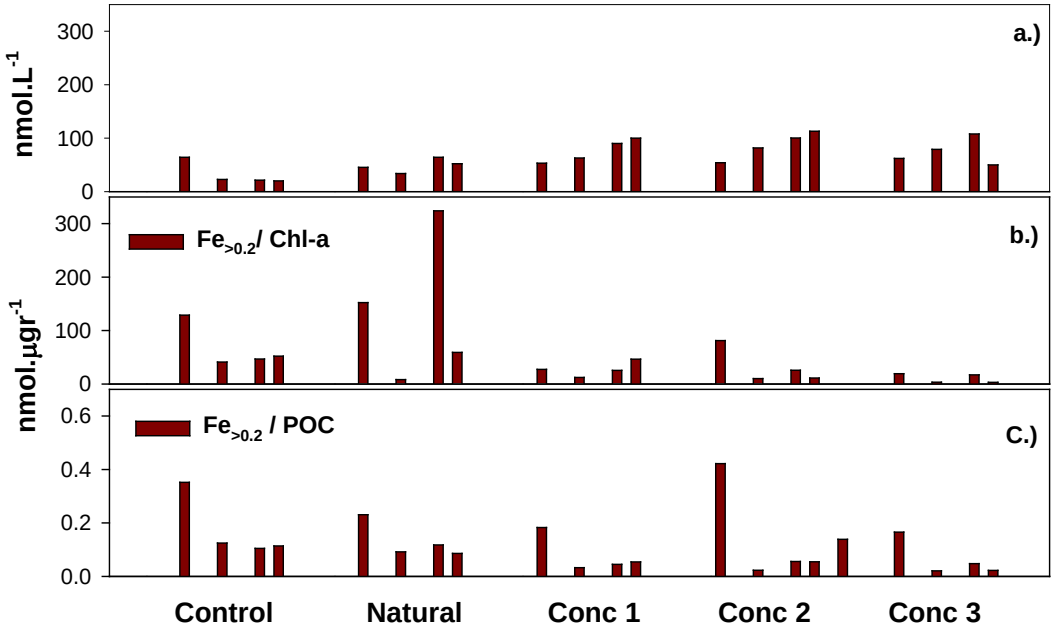


Fig. 18) Distribution of the $PFe_{>0.2}$ for a.) absolute values (nmol.L-^{1}), b.) standardized by Chlorophyll a (Chl-a) (nmol.ugr^{-1} Chl-a) and c.) standardized by the Particulate Organic Carbon (POC) (nmol.ugr^{-1} POC), for all treatments in the surface system in the mesocosms experiment in the Comau fjord, Chile during January-February 2011.

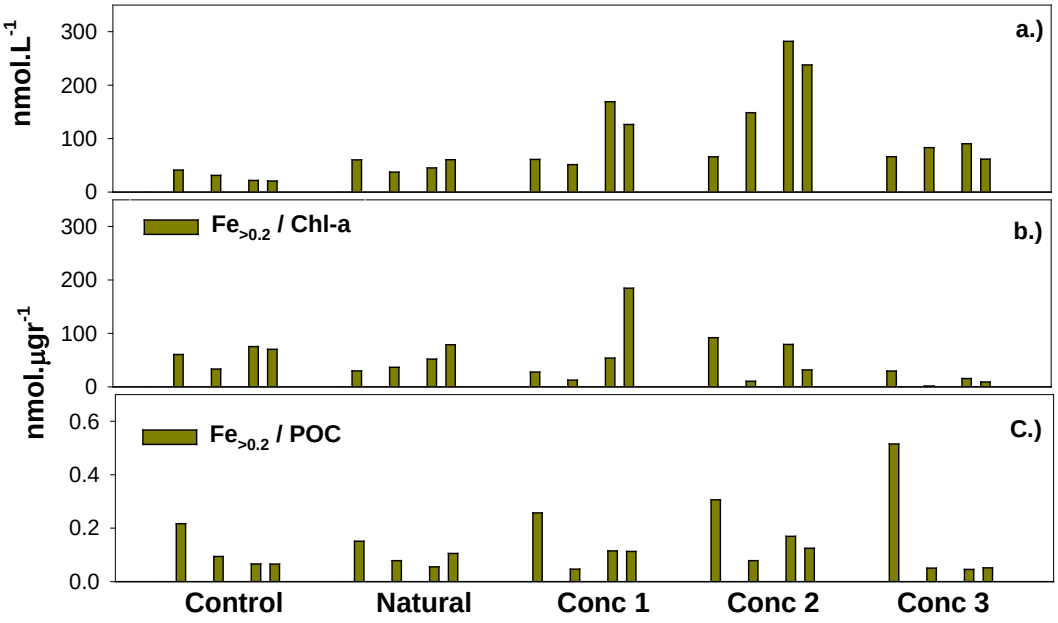


Fig. 19) Distribution of $PFe_{>0.2}$ for a.) absolute values (nmol.L-^{1}), b.) standardized by Chlorophyll a (Chl-a) (nmol.ugr^{-1} Chl-a) and c.) standardized by particulate organic carbon (POC) (nmol.ugr^{-1} POC), for all treatments in the marine system in the mesocosms experiment in the Comau fjord, Chile during January-February 2011.

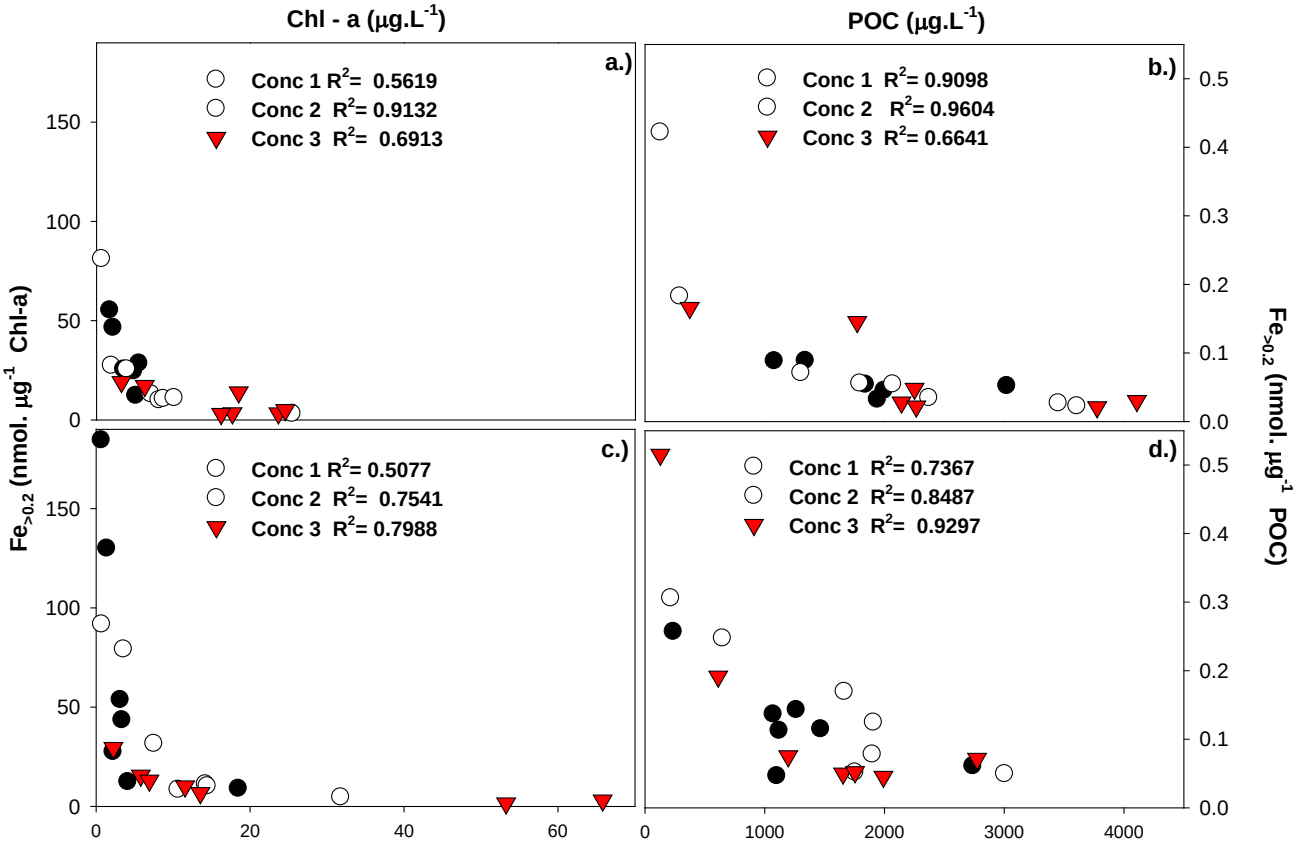


Fig. 20) Chlorophyll concentration (Chl-a) (ugr.L^{-1}) and Particulate Organic Carbon (POC) (ugr.L^{-1}) versus the $PFe_{>0.2}$ in a.- b.) the surface and c. – d.) the marine systems, for treatments with artificial nutrient addition (Conc 1, Conc 2 and Conc 3), in the mesocosms experiment in the Comau fjord, Chile during January-February 2011.

6. DISCUSSION

6.1. Iron in the natural environment

As for all coastal areas, riverine input can be regarded as a main source of iron in the Comau fjord. Whereas aeolian input of iron in coastal areas is regularly considered negligible compared to fluvial (Haese 2005). Yet, some measurements in the Norwegian coastal area, aeolian Fe flux estimation about 33.6±16 μmol m^{-2} day^{-1}, constituted a rather high level of atmospheric iron flux (Öztürk et al. 2003). The river concentrations, although higher relative to the marine water, presented low values compare to other data from river discharges or low salinity portions of estuarine zones. Values for dissolved (filterable) iron from 254 – 344 nmol.L^{-1} nearby San Francisco bay, U.S.A. (Sañudo-Wilhelmy et al. 1996), 260 - 470 nmol.L^{-1} in the Congo river, Congo (Haese 2005), 400 - 1100 nmol.L^{-1} in Galveston bay, U.S.A. and 900 – 1500 nmol.L^{-1} in the Nidelva river, Norway (Öztürk et al. 2002), are far above the highest value measured for the Loncochaigua river in the Comau fjord (36.7 nmol.L^{-1}).

The exponential decrease in concentration described for iron and most bioactive metals within estuarine conditions with increasing salinity (Sañudo-Wilhelmy et al. 1996; Wen et al. 1999; Wells et al. 2000), was also a feature of iron in the study area (Fig. 3). Although the concentration gradient could not be followed up to marine waters (32-33 psu) in the surface, only reaching a ~15 psu at the sampling point in front of the marine station, it was clear the sharp decline in concentration up to one order of magnitude for some of the fractions of iron measured. The mechanism modulating this pattern acts within the colloidal fraction and its interaction with the dissolved organic matter (DOM), both variables which were not part of main objectives to achieve in this study. Yet, the colloidal constitute an essential fraction that can account for > 90 % of the dissolved fraction (Wen et al. 1999). Iron is mainly present as Fe(III) oxyhydroxide, which is stabilized in this colloidal phase by high-molecular-weight humic acids. Also evidence indicates that some fraction of metal complexing organic ligands reside in the marine colloidal phase (Muller 1996). On the other hand, due to increasing salinity and thus increasing ionic strength the colloidal dispersion destabilized, which results in the coagulation of the fluvial colloids (Haese, 2005). In this way, the relevance of the colloidal iron is highlighted, as a dual role it can accomplish by enhancing the metal availability over extended time periods, but also facilitating rapid metal removal from surface waters by aggregating (Wells et al. 2000).

The Fe_{DGT} fraction considered to represent the readily bioavailable one, averaged 9.9% of the $DFe_{Ch.}$ Assuming that DFe_{Ch} represent the total amount of dissolved fraction, Fe_{DGT} values fit within in the range reported in literature where, more than 90 % of the dissolved fraction might be strongly complexed in colloids (Kuma et al 1998, Wells et al., 2000), therefore probably not bioavailable. Besides, the percentages of DFe_{Ch} showed a decreasing trend from river to seawater suggesting decreasing trend of the bioavailable fraction with increased salinity. In contrast to the river sea mix zone, in the sampling point Fe_{DGT} showed twofold increase, accounting for the entire dissolved fraction at 10 m. A change in the dissolved fraction could be attributed to biological action through the interaction with organic ligands released eventually increasing solubility of the dissolved fraction, thus having a positive feedback on phytoplankton (Whitfield 2001).

6.2. Iron variability in the plankton community in the mesocosms

6.2.1. Chelex and DGT labile fractions

The FeCh pattern exhibited in time both in the surface layer and the marine layer are directly related to biological control, through the trends follow by POC and Chl-a (Fig. 21). The biological production reflected in these two variables, increased proportionally to the NH_4^+ input (i.e. Conc 3, > Conc 2, > Conc 1) in which basically the control and natural treatments didn't showed drastic changes on time, whereas the treatments with artificial nutrient addition did. This general increase was followed by drastic reduction in the second half of the experiment probably due to nutrient exhaustion, in which case preliminary analysis point to PO_4^{3-}.

TFeCh and DFeCh followed this pattern with opposite trends. The TFe_{Ch} showed remarkable trend that followed the POC particularly with the correspondence increase and decay, while the DFeCh showed a decreasing trend. Compared to the natural levels of Chl-a and POC in the region (Gonzalez et al. 2010; Gonzalez et al. 2011), the concentrations observed in the treatments, determined a high rate formation of particulate material, (that could be appreciated at simple observation). These type of aggregation would have enhanced the adsorption of colloidal iron into particles, therefore removing portion of the soluble fraction and transforming into particulate.

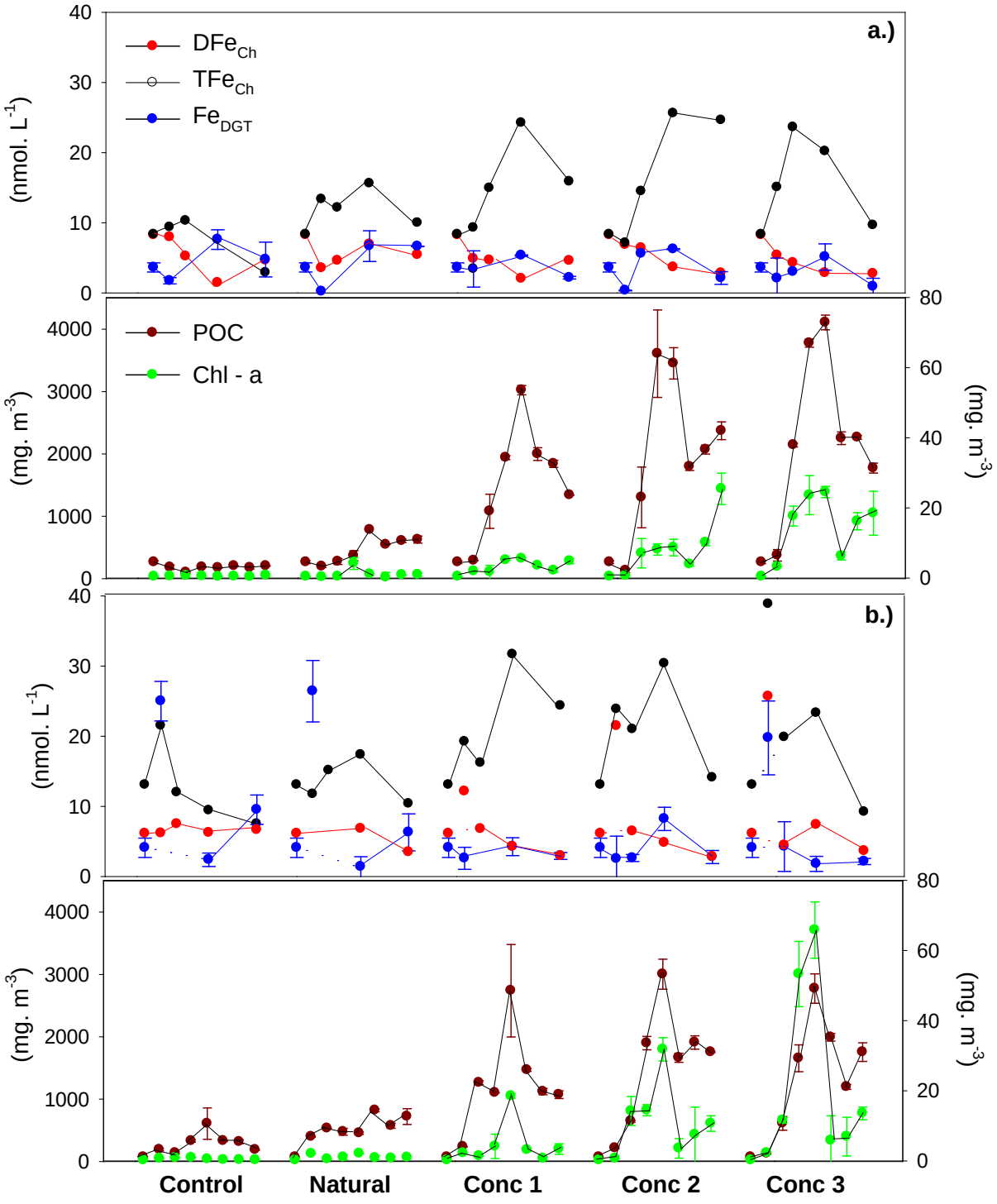


Fig. 21) Total Chelex labile iron (TFe_{Ch}), dissolved Chelex labile iron (DFe_{Ch}), DGT labile iron (Fe_{DGT}) (nmol.L^{-1}), Chlorophyll (Chl-a) and Particulate Organic Carbon (POC) (mg.m^{-3}) concentrations in a.) surface and b.) marine systems, for all treatments in the mesocosms experiment in Comau fjord, Chile during January-February 2011. Isolated points: contamination outliers. Dash line: Estimated values. Error bars: standard deviation (n=3).

Wong et al. (2006) in a mesoscale experiment reported a quick transformation of the dissolved iron to particulate forms, with as much as 70% of the added iron transformed in the non-dissolved form after less than 24 h. The trend described lower colloidal iron percentages as the experiment progressed while the particulate fractions increased. The mechanism alleged to be involved, could be a combination of biological uptake (Nodwell and Price 2001; Chen et al. 2003) or simply adsorption of colloidal iron to the plankton cell surfaces as well as aggregation of oxyhydroxides (Wong et al. 2006). In our case, the second mechanism seems to be the likely driver for changes in the distribution of dissolved and total forms of iron. The colloidal iron although not measured here could account for the reduction within the DFe_{Ch}.

Probably related to this, was the observed decoupled increase of PFe_{Ch} relative $DFe_{Ch,}$ when it could be expected that the increase of the former would have correspond to an equal decrease in the concentration of the latter, involving a change in the physical phase. Several factors could account for the poor correlation between these two variables, certainly mechanical artifact could be referred as an important one. It is known that the colloids, encompasses a size range from 1 to 1000 nm in diameter (Wells 1998), lying within the boundaries of the dissolved and particulate matter, thus subjected possible bias via artificial manipulation. At the same time, the colloid production rates can be enhance by biological action, presumably through a combination of cell exudation and lysis, microbial degradation of particulate organic matter, and 'sloppy' feeding and excretion by zooplankton (Wells and Goldberg 1994).

Taking into account the above, the fact of the TFe_{Ch} increase could be due to colloidal Fe large enough to be retained in the filter invoking the technical artifact. The formation rate of this colloid could be increased by enhanced aggregation of particulate material in the system, thus progressively increasing the amount transformed into the particulate labile pool, matching the trend observed in the POC in time and with NH_4^+ concentration. In the other hand, biological activity within time might have induced changes in the speciation in a fraction of iron previously not accounted for chelex (not soluble or complexed), then afterwards progressively transformed in the PFe_{Ch}.

This factors coupled or independently could reflect the uncoupled increase of TFe_{Ch} relative to the small decrease in the dissolved fraction, while at the same time, accounting for the

positive good linear correlation with NH_4^+ input and TFeCh, but the poor one for $DFe_{Ch.}$ Finally, the decrease observed in TFe_{Ch} might represent the settlement of the dying phytoplankton and without observing a any increases in the dissolved fraction, could suggest that the iron transformed to "particulate" fraction was eventually exported to the bottom of the mesocosms, thus prevented from being recycled back into the system.

The average concentration for Fe_{DGT} were lower both in the surface and in the marine systems for all treatments compare to the $DFe_{Ch,}$. In broad terms would indicate that all the dissolve fraction in this case, the chelex labile, was probably not readily bioavailable. Nevertheless, when looking at the trends in time between Fe_{DGT} and DFe_{Ch}, it can be noticed high variation, exhibiting at some points opposite trends, even with Fe_{DGT} values higher than the DFe_{Ch} (Fig. 21). If a ratio between DFe_{Ch} and Fe_{DGT} is estimated to look for possible a gross pattern, it results in higher ratio for the marine (1.44) compared to the surface (1.27), indicating there was a lower proportion of the bioavailable fraction in the marine system. But again, given the high variability the latter cannot be determine significant. Observing the trends in time, it can be appreciated that the DFe_{Ch} and in particular Fe_{DGT} high variability, might the response to a dynamic system in which biological (release of organic ligands) and chemical (kinetics and equilibrium) forcing determine changes in the iron speciation in short periods of time. Nonetheless, and regardless all the variation in time, at the end of the experiment, the significant lower Fe_{DGT} concentrations for all the treatments with NH_4^+ compared to Natural one reflected the decrease of the bioavailable fraction of iron, therefore reflected in increase uptake by the growing phytoplankton biomass.

Different to river, the DFe_{Ch}:Fe_{DGT} ratio in both systems was not constant. Disregarding possible biological effects, may be still difficult to make compare between the concentrations obtained in river and seawater, as DGT exhibit different performance in each type of water (INAP 2002). Ionic strength, and pH are an important variables that can affect the rates of diffusion of elements and the structure of the polyacrylamide hydrogel structure among others. In pristine rivers waters with low ionic strength, compare to seawater, the effect of variables mentioned would be different over DGT performance (INAP 2002).

Several other factors could have led to source of error or possible wrong interpretations. Although cautiously planned and carefully performed, the fieldwork carried out was not under ultra clean conditions, leaving more room form possible contamination. In fact, what

high peaks obtained for both TDe_{Ch} and DFe_{Ch} samples of appeared to be clear sample artifact. On the other hand, provided no methodological error are involved, a source of high variation can constitute the enclosed system by itself. Mesocosms experiment, although a good approach to study trends in time at ecosystem level, they do not resemble the natural pattern, thus interpretations or drawn from results, must be taken with caution for further extrapolation. An example of this, constitute the NH_4^+ rate supply (4.6 µmol.L^{-1}.d^{-1}) applied in treatment Conc 3. A significant high concentration (Olsen et al. 2006) was applied to expect for possible toxic effects, in that way exposing the plankton community to drastic changes in short periods of time that would rather not occurred in natural environment.

6.2.2. Iron content in planktonic organisms

Determination of the iron content within the planktonic organisms, through size fractionation PFe_{SF} and total content $PFe_{>0.2}$ revealed changes of the distribution of the particulate content of iron within the plankton community in time. $PFe_{SF,}$ although not standardized neither by POC nor Chl- a due to lack of fractionated data for these variables, showed for the marine system a significant change in time of the ratio of absolute iron content between two fractions of the plankton community, representing the microplankton and the nanoplankton. Complementary to PFe_{SF} , was the data provided by the $PFe_{>0.2}$. The total content of iron within the particulate fraction in the plankton community standardized both by POC and Chl-a provided and insight over the content of iron relative to the carbon pool in the system. In the absence of information on composition and abundance of the planktonic community (temporary unavailable), both variables can relate to a rough estimate of the iron content per organism or cell (iron quota Q). The two fractions of iron analyzed indicate that the plankton community, and it could be infer in particular phytoplankton, faced changes both in terms of composition and iron uptake (thus Q) with an increased NH_4^+ over time.

While Chl-a only account for the autotrophic component of the plankton community (i.e. diatoms, dinoflagellates and flagellates), the POC render the complete amount of carbon in the whole community (i.e. including bacteria and protists). Accordingly, to make inferences about the nutrient uptake by phytoplankton, chlorophyll rather than carbon content should be the parameter to compare with for possible effects over iron uptake in presence of excess NH_4^+. However estimation for an iron to carbon ratio cannot be made based on Chl-a. Given that both parameters related to the iron content showing same trend (exponential decrease), Fe:C ratio estimation was obtain based on POC.

Further assumptions must be made when attempting to estimate the Fe:C ratio. The values used are from the POC and particulate Fe concentrations obtained in field experiments. Is clear that determination of POC include al organic carbon present even if it might imply allochthonous particulate material. This is of particular consideration in the surface system, as the surface layer in fjords generally receive constant input of river origin (Vargas et al. 2011). Moreover, contrast to relatively constant "Redfield" ratios of C: N: P, the cellular Fe: C ratios vary markedly (by a factor of 30) as a function of the iron available (Bruland et al. 2001). Hence, most of the data available on estimation of trace element quotas come from laboratory cultures (e.g., Sunda and Huntsman 1995), with still few data from natural environments.

Both differences and similarities observed in the surface and marine systems are related to a great extent to biological attributes characteristic of the surface and marine layer present in the Comau fjord. In turn, the biology is profoundly influenced by constant physical forcing i.e. presence of the permanent LSL, in addition to others hydrographic parameters proper of fjord ecosystems (Pickard 1971). This LSL can exert considerable effect on both the physical and biological features of the water column, reducing light penetration, nutrient exchange and limiting wind-induced mixing, during period of strong water column stratification (Gibbs 2001). The results of these environmental partitioning, is often less productive (primary productivity) surface layer based on nutrient recycling, dominated regularly by the nanoplankton size class, whereas below the halocline, a marine layer with peak productivity in (10-15m) based more on constant nutrient input (oceanic nutrient-rich waters) and with microphytoplankton as the dominant component (Sánchez et al. 2011).

Base on the above, a plankton community dominated by big diatoms and dinoflagellates, was expected to be resembled in the marine system in the mesocosms. The increase in the μ/n ratio provided evidence that microphytoplankton, diatoms in particular, might be taking advantage of the NH_4^+ input at less energy expense, outweighing the growth of the nanoplankton fraction. Studies with the diatom *Thalassiosira pseudonana* showed 8% increase in growth based on NH_4^+ compared to NO_3^-, under saturating light and Fe-replete conditions. (Thompson et al. 1989.) Similarly Levasseur et al. (1993) found higher growth rates for ammonium-grown cells than for nitrate-grown cells of several species under the same conditions. In the other hand, an oceanic diatom isolated from the subarctic Pacific was found to have no difference in the growth rates of nitrate- and ammonium- grown cells under

Fe-replete conditions (Muggli et al. 1996). It is worth noting that NO_3^-, even though not added (not objective of the study), concentrations in the marine system showed a decreasing trend in the Control and Natural, while treatments with artificial addition showed higher NO_3^- content at high NH_4^+ input (data not shown), thus supporting the idea of preferential ammonium uptake. An alternate scenario in which the diatoms would not be growing, but rather increasing the iron Q seems unlikely as literature reports that in presence of exceed NH_4^+ diatoms would require less iron. Nevertheless, Price et al. (2005), found contrasting results growing when diatoms with NO_3^- and NH_4^+ under high and low Fe-mediums. While the iron quota was higher for NO3- under low Fe, at high Fe, was higher for NH_4^+ grow cells (Price 2005). The author referenced to a number of experiments performed in laboratory, pointing mainly that growth conditions and species differences may be responsible for the contrasting results.

The decrease total iron content observed in $Fe_{>0.2}$ was for both the surface and marine systems. Furthermore this reduction uptake appeared to be exponential with the lowest ratio at maximum NH_4^+ in both systems. Nevertheless, if composition and abundance of planktonic community was different, or at least the proportion of dominant groups between the surface and marine systems, it could be expected differences in the rate of decrease in iron uptake. In this way looking into the average Fe:C ratio per treatment in both systems, we obtain a relation that in fact differs for each system (Fig. 22). It can be observed that the reduction of Fe:C was larger in the surface system. Given that the average was obtained from all measurements in time for each treatment, the spread of the data increase considerably due to the fact that initial conditions were equal in all treatments. So the standard deviation here is likely to resemble the differentiation achieved in time within each treatment according to the NH_4^+ addition. Despite this, a consistent trend can be observed pointing that the dominant group of phytoplankton within the surface system exhibited a higher rate of decrease of iron uptake. If nanophytoplankton were to be dominant in this system, it would seem plausible to draw a connection between this two. Nano- or pico-plankton, with higher surface to volume ratios, are more efficient exploiting at low nutrient concentrations (Chisholm 1992; Price et al. 1994), therefore thriving in environments were the main source of nitrogen is recycled, like the NH_4^+ uptake conditions reflected here. In a scenario where NH_4^+ is supplied in excess, the phytoplankton could have less requirement for iron, therefore reducing its uptake (Sunda and Huntsman 1995). Though, reports on

Emiliana Huxleyi, a flagellate type of oceanic phytoplankton more adapted to thrive in nutrient recycle environment, therefore resembling more the type expected to dominate in the SLS of the Comau fjord, showed that Fe quotas normalized to Carbon, where no significant different between nitrate- and ammonium-grown cells neither at high Fe nor a Fe-stressed conditions (Muggli and Harrison 1996).

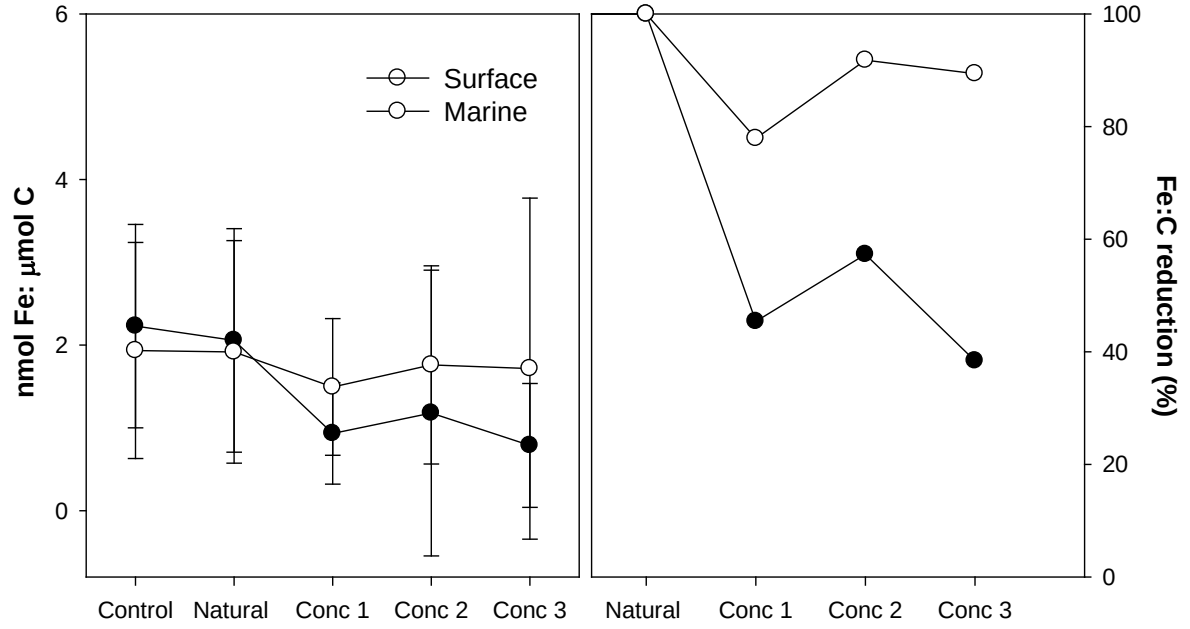


Fig. 22) Iron to Carbon ratio (nmol Fe; µmol C) and Fe:C reduction (%), assuming the ratio in the Natural treatment as 100%, measured in the $PFe_{>0.2}$ in the surface and marine systems, for all treatments in the mesocosms experiment in the Comau fjord, Chile during January-February 2011. Error bars: standard deviation (n=8).

On the other hand, the less pronounce decrease in Fe:C ratio in the marine system could be linked to dominance of microphytoplankton. The success of diatoms growing on NH_4^+ basis reports on literature, and here assumed in the increased µ/n ratio observed, could be translated into less preference over nitrate uptake, and therefore reduced iron requirements for this group. Yet, diatoms in general have a higher requirement on iron to satisfy certain metabolic demands (Bruland et al. 2001). Mostly, coastal diatoms have been shown to have an order-of-magnitude higher iron requirement (on an Fe: C basis) than oceanic diatoms (Sunda and Huntsman l995). This could thus accounting for the rather smoothly decrease in Fe:C ratio observed.

In other words, in a scenario observed of sustained supply of NH_4^+ at high concentration the Q reduction the dominant group in the surface layer reached up to 60 % while on the

marine only a maximum reduction of 20 % was observed. In despite the higher percentage reduction on Fe:C ratio in the surface system, both ratios estimated for the plankton community in Comau fjord during the experiment accounted very low values (0.5 to 6.2 µmol:mol^{-1}) compared to what is reported on the literature for coastal phytoplankton resembling more the ratio of oceanic species (Sunda and Huntsman 1995; Ho et al. 2003; Sarthou et al. 2005). Range of Fe:C ratio from oceanic to coastal phytoplankton span 2.3 to 370 µmol:mol^{-1}, positioning our values in the lowest range possible. To give further support or contrast our findings, values from the literature of Chl-a to C ratio were used to estimate a range of values of Fe:C (Table. 7). Values for natural and Conc 3 treatments (more divergent estimations) showed higher values than the Fe: C based on POC, but still in the low range for coastal phytoplankton. Despite this, the trend is consistent, thus seeing a markedly decrease in the iron Q with increased NH_4^+, giving further support to the initial findings.

Table 7) Chl-a to C ratio (nmol:mol^{-1}) for a range of values for *T. weissfloggi* (Sunda and Huntsmann, 1995), Fe to Chl-a ratio (nmol:µg^{-1}) and estimated Fe to C ratio (µmol:mol^{-1}) for the control and the highest NH_4^+ system in both the surface and marine system, in the mesocosms experiment in the Comau fjord, Chile during Jauary-February 2011.

System	Chl:C^{-1}	Control		Conc 3	
		Fe:Chl-a^{-1}	Fe:C	Fe:Chl-a^{-1}	Fe:C
	mmol:mol^{-1}	nmol:µg^{-1}	µmol:mol^{-1}	nmol:µg^{-1}	µmol:mol^{-1}
Surface	0.127 - 0.431	135.9	15.4	10.7	1.2
			52.3		4.1
Marine	0.127 - 0.431	112.8	12.8	11.4	1.3
			43.4		4.4

Determination of Q is important to infer over the physiological state, adaptation to environment or possible growth limitation in phytoplankton (Whitfield 2005 and cites therein). In the same way, any iron induced changes in the Redfield proportions of phytoplankton should affect in a similar way the biogeochemical cycling of C, N, and P (Price 2005). In this context the a relation between carbon and iron can be establish through the Fe use efficiency (1/Q), which can relate how efficient can be the export of carbon from surface to deep water. On example of this, are the differences observed over artificial enrichment of iron versus the natural enrichment in polar regions, where it has been claimed that the latter present higher sequestration efficiency (Blain et al. 2007; Pollard et al. 2009).

6.3. Implications for higher trophic levels

The most visible effect iron variability in the mesocosms regarding higher trophic levels, was the accumulation of PFe iron in the fraction > 140 um. Until this size fraction is fair to consider the top limit size for phytoplankton, as major diatoms and dinoflagellates such be contained in the previous fraction (20-140µm). Nonetheless, the possibility for a high the occurrence of large chain forming diatoms that could have been retained in the > 140 µm mesh size could no excluded. Despite not been significant, due to high variability, PFe_{SF} > 140 um was more than 50 % of total PFe at the end of the experiment (see Fig. 16 Conc 1 and Conc 2 marine). The accumulation followed the large increase in phytoplankton and thus food availability, as it was expected.

Among factors regulating the metal uptake in zooplankton, food concentration is one regarded as determinant. Previous studies has demonstrated that food quantity did not affect the assimilation of several trace elements by copepods (Wang et al. 1996). But more recent ones on copepods (Xu and Wang 2001), clearly showed that assimilation of metals increased with a decrease in food abundance, while in cladocerans (Yu and Wang 2002) has been proposed as factor. This factor in turn may affect the rate at which a metal passed through the digestive tract (Wang 2002). Accordingly, we could expect to see effects on the ingestion and assimilation efficiency by the zooplankton community present in both the surface and marine systems do to an increase food availability.

Zooplankton grazing can strongly influence the fate of trace metals associated with phytoplankton biomass (Fowler and Knauer 1986). However the effect exert over the plankton community, trace metals and major biogeochemical cycles, is strongly dependent on the type dominant group. In the case of the zooplankton community structure in the Comau fjord, dominance occur both the more brackish cladocerans and the more copepods, both groups with different ecological role and thus effects over biogeochemical cycles (Sánchez et al. 2011). Whether the cycling of iron, already modified by NH_4^+ induced changes in phytoplankton, would be further affected by the zooplankton, depends on factors as assimilation efficiency (Wang and Fisher 1998), export through pellet formation (Hutchins et al. 1995; Sarthou et al. 2005), regeneration (Hutchins et al. 1993), complexation capacity (Hutchins et al. 1999; Sato et al. 2007) all of which needs to assessed in the study area.

7. CONCLUSIONS

The addition of NH_4^+ in our experimental set up showed there was an effect in the distribution of the different forms of iron measured, in the water as well as in the particulate matter representing the content of iron in the plankton community, for both the surface and marine systems. And that this effect depending on the iron form, was correlated either positively or negatively in time and with increased NH_4^+ concentration.

The addition of NH_4^+ effect over the iron content in the plankton proved to be significant and complementary by both fractions measured. The evidence provided suggest that in a system normally NO_3^- based, if NH_4^+ is provided in excess is readily taken as the main source for phytoplankton. Moreover, that a lower energy cost nitrogen source as ammonium can account for high growth rate, here reflected in the Chl-a values reached. In the same way, high NH_4^+ concentration could be regarded as the main factor for the low estimations of Fe: C ratio, in a phytoplankton community that appeared to have already natural low Fe:C for coastal phytoplankton.

Despite being mesocosms, results here obtained after all are product of enclosed systems manipulated and thus interpretations must follow careful screening. Natural systems are complex, and this could be seeing from the differences observed in the surface and marine systems. Effects of NH_4^+ were for both systems significant but different in magnitude. In presence of excess nutrients similar responses could be expected, but in fact Fe:C ratio and others parameters like Chl-a and POC were considerably different. This suggests that biological component is key factor within each system and therefore in the natural environment. A phytoplankton community with a low Fe:C but rather high Chl-a yield, as seeing here, would result in higher efficiency in carbon export, something that might be considered as beneficial from several perspectives. However, to fully understand the consequences and spinoffs that the increase input of NH_4^+ by salmon aquaculture, can cause to the pelagic ecosystem in the fjords of Chile, more emphasis need to be allocated to study the links between macro and micro-nutrients cycles and the role of biota within it.

Here by, the hypothesis proposed in this study is supported empirically up to phytoplankton level, but further assumptions can be drawn for possible implications for zooplankton and higher trophic levels.

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APPENDIX 1. UltraCLAVE digestion

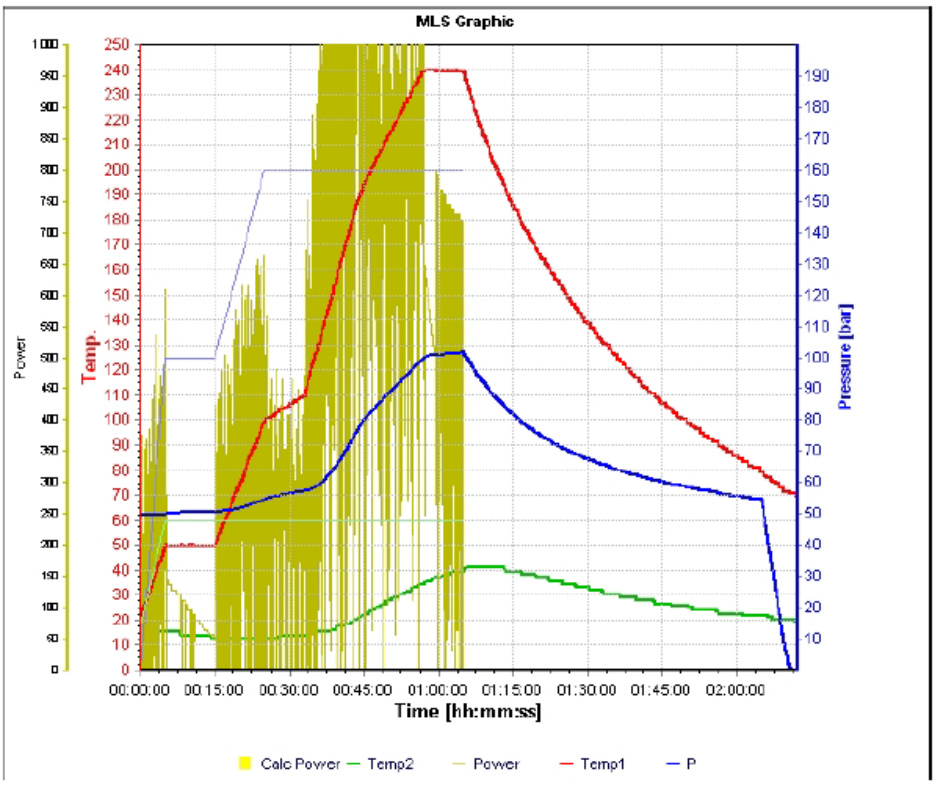
MLS Microwave Report

Application: ultraCLAVE

Report 01.03.2012 15:20:43 **Operator:** Administrator

Filename: M:\A\2012\PROJECTS NOT FINNISHED\KJEMI\MASTER\Anni Vera Wafo Chile\562-121-160-080911-Nico- Mads-Sediments.dpr

MLS Miles
www.milestonesrl



Parameter

```
Signature name  :
Signature date  :
Signature func. :
Operator        :Administrator
Date            :
Method filename
:Sediment-100mg-til-108ml.mpr
Create run      :Administrator
Microwave Power :pulse
Load pressure   :50.0 bar
Release temp.   :80.0 °C
Release pressure:10.0 bar/min
Cooling         :ON
Auto open       :OFF
Cooling on Temp.:40.0 °C
Ground load     :300ml MQ + 2ml
H2SO4 + 30ml H2O2
Ventilation time:01:07:21
```

Remark:

Sediments and soil max 130mg smp, digested with 9ml 50% v/v HNO3 ultra pure, digested according to temp. profile in this method, the diluted to 108ml (109.8g), matrix 0.6M HNO3 analyzed on Element 2

APPENDIX 2. Detection limits and results for iron samples

Chelex blank values and concentrations corrected by blank

									Date of analyses: 19 og 20.10.11 sekvens 48 + sekv				
									Counting digits = 3				
								Check	Isotope	Fe56(MR)			
									Parameteres	Conc.			
Sampling d	Type of san	Metho d	Acid matrix	High	Dilut	Project-In	number		Sample ID	µg/L	RSD, %		
Start formulas									Start statistical calculations				
	Chelex blan	Chelex	0.6M HNO3			287	#VALUE!		0287-start-dgt-blank	0.98	0.5		
	Chelex blan	Chelex	0.6M HNO3			288	0		288	0.76	5.7		
	Chelex blan	Chelex	0.6M HNO3			289	0		289	1.38	0.8		
Discarded	Chelex blan	Chelex	0.6M HNO3			290	1		289	1.41	4.8		
	Chelex blan	Chelex	0.6M HNO3			309	#VALUE!		0309-start-dgt-blanks	2.12	2.6		
	Chelex blan	Chelex	0.6M HNO3			310	0		310				
	Chelex blan	Chelex	0.6M HNO3			311	0		311				
	Chelex blan	Chelex	0.6M HNO3			312	0		312				
	Chelex blan	Chelex	0.6M HNO3			313	#VALUE!		0313-end-dgt-blanks				
									Stop statistical calculations				
Stop formulas						9			Average	1.33	2.9		
					Blank values subtracted samples,				empty cells means < instr	1.33	2.9		
Av	#DIV/0!	#DIV/0!	#DIV/0!			301			Min	0.76	0.5		
									Max	2.12	5.7		
									Std	0.52	2.3		
									Rsd % <5, 5-10, >10	39.1			
									Confidence interval 95%	0.52	2.3		
									Confidence interval 95% (%)	39.1			
									Number	5	5		

Results corrected for blanks

									Date of analyses: 19 og 20.10.11 sekvens 48 + sekv				
									Counting digits = 3				
								Check	Isotope	Fe56(MR)			
									Parameteres	Conc.			
Sampling d	Type of san	Metho d	Acid matrix	High	Dilut	Project-In	number		Sample ID	µg/L	RSD, %		
Start formulas									Start statistical calculations				
23/01/2011	Chelex sam	Chelex	0.6M HNO3			1	#VALUE!		start-pnr-1-313-Nico-Anivera	23.59	7.5		
23/01/2011	Chelex sam	Chelex	0.6M HNO3			2	#VALUE!		2-celex-1-86	11.71	2.7		
23/01/2011	Chelex sam	Chelex	0.6M HNO3			3	0		3	12.97	3.9		
23/01/2011	Chelex sam	Chelex	0.6M HNO3			4	0		4	10.77	0.9		
24/01/2011	Chelex sam	Chelex	0.6M HNO3			5	0		5	14.46	5.0		
24/01/2011	Chelex sam	Chelex	0.6M HNO3			6	0		6	15.11	2.2		
26.01.2011	Chelex sam	Chelex	0.6M HNO3			7	#VALUE!		7-esi-exe-kom	40.10	4.5		
26.01.2011	Chelex sam	Chelex	0.6M HNO3			8	0		8	11.70	28.2		
26.01.2011	Chelex sam	Chelex	0.6M HNO3			9	0		9	22.38	3.5		
26.01.2011	Chelex sam	Chelex	0.6M HNO3			10	0		10	28.47	11.3		
26.01.2011	Chelex sam	Chelex	0.6M HNO3			11	0		11	32.46	2.4		
26.01.2011	Chelex sam	Chelex	0.6M HNO3			12	0		12	24.32	4.2		
26.01.2011	Chelex sam	Chelex	0.6M HNO3			13	0		13	38.60	4.0		
26.01.2011	Chelex sam	Chelex	0.6M HNO3			14	0		14	41.81	4.8		
26.01.2011	Chelex sam	Chelex	0.6M HNO3			15	0		15	62.37	5.0		
26.01.2011	Chelex sam	Chelex	0.6M HNO3			16	0		16	46.85	4.9		
27.01.2011	Chelex sam	Chelex	0.6M HNO3			17	0		17	17.25	3.5		
27.01.2011	Chelex sam	Chelex	0.6M HNO3			18	0		18	15.94	7.5		

27.01.2011	Chelex sam	Chelex	0.6M HNO3			19	0		19	26.57	7.6
27.01.2011	Chelex sam	Chelex	0.6M HNO3			20	0		20	6.04	2.6
27.01.2011	Chelex sam	Chelex	0.6M HNO3			21	0		21	16.89	5.8
27.01.2011	Chelex sam	Chelex	0.6M HNO3			22	0		22	8.96	3.7
27.01.2011	Chelex sam	Chelex	0.6M HNO3			23	0		23	14.95	8.1
27.01.2011	Chelex sam	Chelex	0.6M HNO3			24	0		24	12.51	7.0
27.01.2011	Chelex sam	Chelex	0.6M HNO3			25	0		25	29.96	7.4
27.01.2011	Chelex sam	Chelex	0.6M HNO3			26	0		26	10.23	10.2
29.01.2011	Chelex sam	Chelex	0.6M HNO3			27	0		27	24.98	4.4
29.01.2011	Chelex sam	Chelex	0.6M HNO3			28	0		28	14.86	5.2
29.01.2011	Chelex sam	Chelex	0.6M HNO3			29	0		29	30.14	7.9
29.01.2011	Chelex sam	Chelex	0.6M HNO3			30	0		30	41.20	3.6
29.01.2011	Chelex sam	Chelex	0.6M HNO3			31	0		31	33.02	13.2
29.01.2011	Chelex sam	Chelex	0.6M HNO3			32	0		32	8.86	2.9
29.01.2011	Chelex sam	Chelex	0.6M HNO3			33	0		33	37.61	1.2
29.01.2011	Chelex sam	Chelex	0.6M HNO3			34	0		34	11.97	6.2
29.01.2011	Chelex sam	Chelex	0.6M HNO3			35	0		35	35.83	9.5
29.01.2011	Chelex sam	Chelex	0.6M HNO3			36	0		36	8.40	2.2
30.01.2011	Chelex sam	Chelex	0.6M HNO3			37	0		37	13.85	5.9
30.01.2011	Chelex sam	Chelex	0.6M HNO3			38	0		38	7.57	11.5
30.01.2011	Chelex sam	Chelex	0.6M HNO3			39	0		39	17.36	1.0
30.01.2011	Chelex sam	Chelex	0.6M HNO3			40	0		40	7.15	2.5
30.01.2011	Chelex sam	Chelex	0.6M HNO3			41	0		41	18.86	8.7
30.01.2011	Chelex sam	Chelex	0.6M HNO3			42	0		42	6.87	4.7
30.01.2011	Chelex sam	Chelex	0.6M HNO3			43	0		43	20.67	5.3
30.01.2011	Chelex sam	Chelex	0.6M HNO3			44	0		44	9.53	3.7
30.01.2011	Chelex sam	Chelex	0.6M HNO3			45	0		45	32.59	6.5
30.01.2011	Chelex sam	Chelex	0.6M HNO3			46	0		46	6.44	3.1
04.02.2011	Chelex sam	Chelex	0.6M HNO3			47	0		47	17.38	4.9
04.02.2011	Chelex sam	Chelex	0.6M HNO3			48	0		48	8.79	6.9
04.02.2011	Chelex sam	Chelex	0.6M HNO3			49	0		49	22.20	6.5
04.02.2011	Chelex sam	Chelex	0.6M HNO3			50	0		50	9.11	3.0
04.02.2011	Chelex sam	Chelex	0.6M HNO3			51	0		51	41.54	3.1
04.02.2011	Chelex sam	Chelex	0.6M HNO3			52	0		52	5.91	1.4
04.02.2011	Chelex sam	Chelex	0.6M HNO3			53	0		53	52.16	8.5
04.02.2011	Chelex sam	Chelex	0.6M HNO3			53	#VALUE!	53-reanal		51.69	1.2
04.02.2011	Chelex sam	Chelex	0.6M HNO3			54	0		54	8.46	0.4
04.02.2011	Chelex sam	Chelex	0.6M HNO3			55	0		55	44.47	0.2
04.02.2011	Chelex sam	Chelex	0.6M HNO3			56	0		56	11.93	1.7
04.02.2011	Chelex sam	Chelex	0.6M HNO3			57	0		57	15.87	1.4
05.02.2011	Chelex sam	Chelex	0.6M HNO3			58	0		58	3.21	2.2
05.02.2011	Chelex sam	Chelex	0.6M HNO3			59	0		59	27.48	1.0
05.02.2011	Chelex sam	Chelex	0.6M HNO3			60	0		60	12.78	2.1
05.02.2011	Chelex sam	Chelex	0.6M HNO3			61	0		61	41.43	2.3
05.02.2011	Chelex sam	Chelex	0.6M HNO3			62	0		62	3.90	0.9
05.02.2011	Chelex sam	Chelex	0.6M HNO3			63	0		63	49.91	1.7
05.02.2011	Chelex sam	Chelex	0.6M HNO3			64	0		64	6.96	1.9
05.02.2011	Chelex sam	Chelex	0.6M HNO3			65	0		65	32.80	1.1
05.02.2011	Chelex sam	Chelex	0.6M HNO3			66	0		66	5.65	1.4
05.02.2011	Chelex sam	Chelex	0.6M HNO3			67	0		67	10.40	1.8
13.02.2011	Chelex sam	Chelex	0.6M HNO3			68	0		68	8.71	1.4
13.02.2011	Chelex sam	Chelex	0.6M HNO3			69	0		69	15.06	1.3

13.02.2011	Chelex sam	Chelex	0.6M HNO3		70	0		70	4.94	3.9
13.02.2011	Chelex sam	Chelex	0.6M HNO3		71	0		71	32.34	1.5
13.02.2011	Chelex sam	Chelex	0.6M HNO3		72	0		72	4.36	2.0
13.02.2011	Chelex sam	Chelex	0.6M HNO3		73	0		73	19.44	2.1
13.02.2011	Chelex sam	Chelex	0.6M HNO3		74	0		74	4.20	1.2
13.02.2011	Chelex sam	Chelex	0.6M HNO3		75	0		75	10.82	1.9
13.02.2011	Chelex sam	Chelex	0.6M HNO3		76	0		76	4.80	1.2
13.02.2011	Chelex sam	Chelex	0.6M HNO3		77	0		77	4.25	3.4
14.02.2011	Chelex sam	Chelex	0.6M HNO3		78	0		78	5.92	1.1
14.02.2011	Chelex sam	Chelex	0.6M HNO3		79	0		79	13.44	2.1
14.02.2011	Chelex sam	Chelex	0.6M HNO3		80	#VALUE!	80-end-rack-1		6.13	3.7
14.02.2011	Chelex sam	Chelex	0.6M HNO3		81	#VALUE!	81-start-rack-3		20.24	2.7
14.02.2011	Chelex sam	Chelex	0.6M HNO3		82	0		82	6.16	3.4
14.02.2011	Chelex sam	Chelex	0.6M HNO3		83	0		83	28.37	3.4
14.02.2011	Chelex sam	Chelex	0.6M HNO3		84	0		84	4.17	2.3
14.02.2011	Chelex sam	Chelex	0.6M HNO3		85	0		85	13.02	1.1
14.02.2011	Chelex sam	Chelex	0.6M HNO3		86	#VALUE!	86-end-chelex		3.94	3.2
14.02.2011	Chelex sam	Chelex	0.6M HNO3		87	#VALUE!	87-start-river-chelex		3.68	2.3
	River Chele	Chelex	0.6M HNO3		88	0		88	4.63	3.9
	River Chele	Chelex	0.6M HNO3		89	0		89	18.31	2.5
	River Chele	Chelex	0.6M HNO3		90	0		90	3.09	0.7
	River Chele	Chelex	0.6M HNO3		91	0		91	13.98	3.8
	River Chele	Chelex	0.6M HNO3		92	0		92	3.16	3.7
	River Chele	Chelex	0.6M HNO3		93	0		93	5.38	1.6
	River Chele	Chelex	0.6M HNO3		94	0		94	4.56	0.9
	River Chele	Chelex	0.6M HNO3		95	0		95	10.33	0.8
	River Chele	Chelex	0.6M HNO3		96	0		96	4.19	4.3
	River Chele	Chelex	0.6M HNO3		97	0		97	3.75	1.6
	River Chele	Chelex	0.6M HNO3		98	0		98	3.72	1.0
	River Chele	Chelex	0.6M HNO3		99	0		99	13.62	4.7
	River Chele	Chelex	0.6M HNO3		100	0		100	54.30	3.3
	River Chele	Chelex	0.6M HNO3		101	0		101	66.31	3.5
	River Chele	Chelex	0.6M HNO3		102	0		102	8.69	4.0
	River Chele	Chelex	0.6M HNO3		103	0		103	14.98	2.7
	River Chele	Chelex	0.6M HNO3		104	#VALUE!	104-end-river-chelex		18.86	4.5
	River Chele	Chelex	0.6M HNO3		105	#VALUE!	105-start-wafow-chelex		63.31	3.7
Stop formulas					106		Average		18.67	3.1
Av	40566.333	#DIV/0!	#DIV/0!		53		Min		3.09	0.8
							Max		66.31	4.7
							Std		15.30	1.4
							Rsd % <5, 5-10, >10		81.9	
							Confidence interval 95%		2.99	0.9
							Confidence interval 95% (%)		16.0	
							Number		106	11

DGT blanks values and concentrations corrected by blank

								Counting digits = 3			
								Isotope		Fe56(MR)	
						Check		Parameteres		Conc.	
Sampling da	Type of san	Metho d	Acid matrix	High	Diluti	Project-Ini	number	Sample ID		µg/L	RSD, %
Start formulas								Start statistical calculations			
	DGT blanks	DGT	0.6M HNO3			287	#VALUE!	0287-start-dgt-blank		2.30	2.6
	DGT blanks	DGT	0.6M HNO3			288	0		288	1.32	4.2
	DGT blanks	DGT	0.6M HNO3			289	0		289	1.35	1.5
Discarded	DGT blanks	DGT	0.6M HNO3			290	#VALUE!	0290-end-dgt-blanks			
	DGT blanks	DGT	0.6M HNO3			309	#VALUE!	0309-start-dgt-blanks		1.59	2.8
	DGT blanks	DGT	0.6M HNO3			310	0		310	1.73	4.7
	DGT blanks	DGT	0.6M HNO3			311	0		311	2.42	4.2
	DGT blanks	DGT	0.6M HNO3			312	0		312	1.58	3.0
	DGT blanks	DGT	0.6M HNO3			313	#VALUE!	0313-end-dgt-blanks		1.55	5.0
								Stop statistical calculations			
Stop formulas						9		Average		1.52	3.5
				Blank values subtracted samples, empty cells means < instr						1.52	3.5
Ave	#DIV/0!	#DIV/0!	#DIV/0!			301		Min		1.32	1.5
								Max		2.42	5.0
								Std		0.41	1.2
								Rsd % <5, 5-10, >10		27.1	
								Confidence interval 95%		0.31	0.9
								Confidence interval 95% (%)		20.5	
								Number		8	8
Results corrected for blanks											
23/01/2011	DGT's	DGT	0.6M HNO3			133	0		133	3.42	59.7
23/01/2011	DGT's	DGT	0.6M HNO3			134	0		134	5.56	4.2
23/01/2011	DGT's	DGT	0.6M HNO3			135	0		135	2.21	0.9
23/01/2011	DGT's	DGT	0.6M HNO3			136	0		136	1.68	4.9
23/01/2011	DGT's	DGT	0.6M HNO3			137	0		137	3.66	3.5
23/01/2011	DGT's	DGT	0.6M HNO3			138	0		138	1.25	1.1
23/01/2011	DGT's	DGT	0.6M HNO3			139	0		139	1.52	3.3
23/01/2011	DGT's	DGT	0.6M HNO3			140	0		140	2.62	2.4
23/01/2011	DGT's	DGT	0.6M HNO3			141	0		141	3.11	1.0
23/01/2011	DGT's	DGT	0.6M HNO3			142	0		142	1.97	10.3
23/01/2011	DGT's	DGT	0.6M HNO3			143	0		143	1.93	3.1
23/01/2011	DGT's	DGT	0.6M HNO3			144	0		144	0.24	12.0
23/01/2011	DGT's	DGT	0.6M HNO3			145	0		145	1.04	3.6
23/01/2011	DGT's	DGT	0.6M HNO3			146	0		146	0.95	1.0
23/01/2011	DGT's	DGT	0.6M HNO3			147	0		147	0.61	0.8
23/01/2011	DGT's	DGT	0.6M HNO3			148	0		148	1.94	3.7
23/01/2011	DGT's	DGT	0.6M HNO3			149	0		149	0.72	4.1
23/01/2011	DGT's	DGT	0.6M HNO3			150	0		150	0.06	5.8
23/01/2011	DGT's	DGT	0.6M HNO3			151	0		151	5.31	1.2
23/01/2011	DGT's	DGT	0.6M HNO3			152	0		152	1.65	0.6
23/01/2011	DGT's	DGT	0.6M HNO3			153	0		153	0.26	3.2
23/01/2011	DGT's	DGT	0.6M HNO3			154	0		154	0.69	1.5
23/01/2011	DGT's	DGT	0.6M HNO3			155	0		155	2.14	3.5
23/01/2011	DGT's	DGT	0.6M HNO3			156	0		156	-0.19	4.7
24/01/2011	DGT's	DGT	0.6M HNO3			157	0		157	1.53	4.5
24/01/2011	DGT's	DGT	0.6M HNO3			158	0		158	1.08	3.3
24/01/2011	DGT's	DGT	0.6M HNO3			159	0		159	0.18	4.2

24/01/2011	DGT's	DGT	0.6M HNO3		160	#VALUE!	160-end-rack-3		1.72	1.2
24/01/2011	DGT's	DGT	0.6M HNO3		161	#VALUE!	0161-start-rack-1-nr-2		7.80	2.6
24/01/2011	DGT's	DGT	0.6M HNO3		162	0		162	1.81	3.1
24/01/2011	DGT's	DGT	0.6M HNO3		163	0		163	0.86	1.5
24/01/2011	DGT's	DGT	0.6M HNO3		164	0		164	0.80	3.4
24/01/2011	DGT's	DGT	0.6M HNO3		165	0		165	-0.10	0.0
24/01/2011	DGT's	DGT	0.6M HNO3		166	0		166	0.37	2.5
24/01/2011	DGT's	DGT	0.6M HNO3		167	0		167	0.23	1.4
24/01/2011	DGT's	DGT	0.6M HNO3		168	0		168	8.74	3.2
26.01.2011	DGT's	DGT	0.6M HNO3		169	0		169	7.21	1.0
26.01.2011	DGT's	DGT	0.6M HNO3		170	0		170	8.90	2.1
26.01.2011	DGT's	DGT	0.6M HNO3		171	0		171	9.78	2.5
26.01.2011	DGT's	DGT	0.6M HNO3		172	0		172	9.39	3.1
26.01.2011	DGT's	DGT	0.6M HNO3		173	0		173	7.10	1.5
26.01.2011	DGT's	DGT	0.6M HNO3		174	0		174	1.23	0.9
26.01.2011	DGT's	DGT	0.6M HNO3		175	0		175	-0.20	5.5
26.01.2011	DGT's	DGT	0.6M HNO3		176	0		176	0.49	2.3
26.01.2011	DGT's	DGT	0.6M HNO3		177	0		177	1.59	1.5
26.01.2011	DGT's	DGT	0.6M HNO3		178	0		178	-0.20	3.4
26.01.2011	DGT's	DGT	0.6M HNO3		179	0		179	0.08	3.8
26.01.2011	DGT's	DGT	0.6M HNO3		180	#VALUE!	180-re		8.56	1.2
26.01.2011	DGT's	DGT	0.6M HNO3		181	#VALUE!	181-re		5.67	0.4
26.01.2011	DGT's	DGT	0.6M HNO3		182	0		182	5.42	1.6
26.01.2011	DGT's	DGT	0.6M HNO3		183	0		183	1.41	2.3
27.01.2011	DGT's	DGT	0.6M HNO3		184	0		184	0.47	1.1
27.01.2011	DGT's	DGT	0.6M HNO3		185	0		185	0.68	2.1
27.01.2011	DGT's	DGT	0.6M HNO3		186	0		186	0.12	1.1
27.01.2011	DGT's	DGT	0.6M HNO3		187	0		187	0.06	2.6
27.01.2011	DGT's	DGT	0.6M HNO3		188	0		188	-0.21	3.1
27.01.2011	DGT's	DGT	0.6M HNO3		189	0		189	-0.48	1.8
27.01.2011	DGT's	DGT	0.6M HNO3		190	0		190	1.75	0.8
27.01.2011	DGT's	DGT	0.6M HNO3		191	0		191	0.53	4.7
27.01.2011	DGT's	DGT	0.6M HNO3		192	0		192	-0.11	2.7
27.01.2011	DGT's	DGT	0.6M HNO3		193	0		193	-0.10	4.0
27.01.2011	DGT's	DGT	0.6M HNO3		194	0		194	-0.13	1.9
27.01.2011	DGT's	DGT	0.6M HNO3		195	0		195	-0.23	1.2
27.01.2011	DGT's	DGT	0.6M HNO3		196	0		196	1.55	0.7
27.01.2011	DGT's	DGT	0.6M HNO3		197	0		197	0.01	0.8
27.01.2011	DGT's	DGT	0.6M HNO3		198	0		198	1.60	3.5
29.01.2011	DGT's	DGT	0.6M HNO3		199	0		199	1.22	2.8
29.01.2011	DGT's	DGT	0.6M HNO3		200	0		200	3.64	1.5
29.01.2011	DGT's	DGT	0.6M HNO3		201	0		201	0.95	0.7
29.01.2011	DGT's	DGT	0.6M HNO3		202	0		202	0.78	1.3
30.01.2011	DGT's	DGT	0.6M HNO3		203	0		203	2.33	1.6
30.01.2011	DGT's	DGT	0.6M HNO3		204	0		204	0.69	1.9
30.01.2011	DGT's	DGT	0.6M HNO3		205	0		205	5.39	0.9
30.01.2011	DGT's	DGT	0.6M HNO3		206	0		206	1.06	2.5
04.02.2011	DGT's	DGT	0.6M HNO3		207	0		207	0.89	2.5
04.02.2011	DGT's	DGT	0.6M HNO3		208	0		208	0.44	1.9
04.02.2011	DGT's	DGT	0.6M HNO3		209	0		209	0.41	2.5
04.02.2011	DGT's	DGT	0.6M HNO3		210	0		210	0.97	1.7
04.02.2011	DGT's	DGT	0.6M HNO3		211	0		211	-0.02	2.3

04.02.2011	DGT's	DGT	0.6M HNO3		212	0		212	1.91	1.4
04.02.2011	DGT's	DGT	0.6M HNO3		213	0		213	1.23	2.7
04.02.2011	DGT's	DGT	0.6M HNO3		214	0		214	1.12	0.5
04.02.2011	DGT's	DGT	0.6M HNO3		215	0		215	2.40	2.0
04.02.2011	DGT's	DGT	0.6M HNO3		216	0		216	2.45	3.4
04.02.2011	DGT's	DGT	0.6M HNO3		217	0		217	3.38	1.4
04.02.2011	DGT's	DGT	0.6M HNO3		218	0		218	0.19	1.4
04.02.2011	DGT's	DGT	0.6M HNO3		219	0		219	0.80	1.1
04.02.2011	DGT's	DGT	0.6M HNO3		220	0		220	0.82	1.0
04.02.2011	DGT's	DGT	0.6M HNO3		221	0		221	2.11	1.8
05.02.2011	DGT's	DGT	0.6M HNO3		222	0		222	3.74	1.6
05.02.2011	DGT's	DGT	0.6M HNO3		223	0		223	4.85	1.1
05.02.2011	DGT's	DGT	0.6M HNO3		224	0		224	3.20	1.9
05.02.2011	DGT's	DGT	0.6M HNO3		225	0		225	4.64	2.0
05.02.2011	DGT's	DGT	0.6M HNO3		226	0		226	2.89	2.5
05.02.2011	DGT's	DGT	0.6M HNO3		227	0		227	5.17	2.2
05.02.2011	DGT's	DGT	0.6M HNO3		228	0		228	3.05	3.3
05.02.2011	DGT's	DGT	0.6M HNO3		229	0		229	2.97	2.9
05.02.2011	DGT's	DGT	0.6M HNO3		230	0		230	3.46	3.3
05.02.2011	DGT's	DGT	0.6M HNO3		231	0		231	3.49	3.4
05.02.2011	DGT's	DGT	0.6M HNO3		232	0		232	3.55	5.2
05.02.2011	DGT's	DGT	0.6M HNO3		233	0		233	3.93	3.6
05.02.2011	DGT's	DGT	0.6M HNO3		234	0		234	3.64	1.2
05.02.2011	DGT's	DGT	0.6M HNO3		235	0		235	2.14	1.1
05.02.2011	DGT's	DGT	0.6M HNO3		236	0		236	2.77	1.1
13.02.2011	DGT's	DGT	0.6M HNO3		237	0		237	3.79	0.8
13.02.2011	DGT's	DGT	0.6M HNO3		238	0		238	7.44	1.3
13.02.2011	DGT's	DGT	0.6M HNO3		239	0		239	2.76	1.3
13.02.2011	DGT's	DGT	0.6M HNO3		240	#VALUE!	0240-end-rack-3-nr-2		2.62	0.8
13.02.2011	DGT's	DGT	0.6M HNO3		241	#VALUE!	0241-start-rack-3		1.12	2.6
13.02.2011	DGT's	DGT	0.6M HNO3		242	0		242	0.90	4.2
13.02.2011	DGT's	DGT	0.6M HNO3		243	0		243	1.20	1.0
13.02.2011	DGT's	DGT	0.6M HNO3		244	0		244	0.92	2.8
13.02.2011	DGT's	DGT	0.6M HNO3		245	#VALUE!	0245-Na-1-smp-herfra		1.31	1.3
13.02.2011	DGT's	DGT	0.6M HNO3		246	0		246	0.88	6.5
13.02.2011	DGT's	DGT	0.6M HNO3		247	0		247	0.68	3.6
13.02.2011	DGT's	DGT	0.6M HNO3		248	0		248	0.87	1.9
13.02.2011	DGT's	DGT	0.6M HNO3		249	0		249	0.75	2.5
13.02.2011	DGT's	DGT	0.6M HNO3		250	0		250	0.58	2.5
13.02.2011	DGT's	DGT	0.6M HNO3		251	0		251	1.19	3.5
14.02.2011	DGT's	DGT	0.6M HNO3		252	0		252	2.21	0.6
14.02.2011	DGT's	DGT	0.6M HNO3		253	0		253	1.02	2.3
14.02.2011	DGT's	DGT	0.6M HNO3		254	0		254	1.97	2.4
14.02.2011	DGT's	DGT	0.6M HNO3		255	0		255	9.05	0.7
14.02.2011	DGT's	DGT	0.6M HNO3		256	0		256	2.25	0.9
14.02.2011	DGT's	DGT	0.6M HNO3		257	0		257	3.70	1.9
14.02.2011	DGT's	DGT	0.6M HNO3		258	0		258	0.69	1.8
14.02.2011	DGT's	DGT	0.6M HNO3		259	0		259	0.78	4.0
14.02.2011	DGT's	DGT	0.6M HNO3		260	0		260	0.98	0.2
14.02.2011	DGT's	DGT	0.6M HNO3		261	0		261	0.93	4.4
14.02.2011	DGT's	DGT	0.6M HNO3		262	0		262	0.50	1.1

14.02.2011	DGT's	DGT	0.6M HNO3		263	0	263	0.34	2.8
14.02.2011	DGT's	DGT	0.6M HNO3		264	0	264	0.59	6.0
14.02.2011	DGT's	DGT	0.6M HNO3		265	#VALUE!	0265-end-dgt	0.02	1.1
14.02.2011	DGT's	DGT	0.6M HNO3		266	#VALUE!	0266-start-depth-profile-dgt	0.56	2.7
09.02.2011	Depth Profil	DGT	0.6M HNO3		267	0	267	4.96	2.9
09.02.2011	Depth Profil	DGT	0.6M HNO3		268	0	268	1.63	0.1
09.02.2011	Depth Profil	DGT	0.6M HNO3		269	0	269	2.38	4.2
09.02.2011	Depth Profil	DGT	0.6M HNO3		270	0	270	2.06	2.3
09.02.2011	Depth Profil	DGT	0.6M HNO3		271	0	271	0.65	3.9
09.02.2011	Depth Profil	DGT	0.6M HNO3		272	0	272	0.87	3.7
09.02.2011	Depth Profil	DGT	0.6M HNO3		273	0	273	0.88	2.6
09.02.2011	Depth Profil	DGT	0.6M HNO3		274	0	274	0.17	2.8
09.02.2011	Depth Profil	DGT	0.6M HNO3		275	0	275	0.12	2.7
09.02.2011	Depth Profil	DGT	0.6M HNO3		276	0	276	4.88	1.8
09.02.2011	Depth Profil	DGT	0.6M HNO3		277	0	277	0.00	6.1
09.02.2011	Depth Profil	DGT	0.6M HNO3		278	0	278	0.75	4.2
09.02.2011	Depth Profil	DGT	0.6M HNO3		279	0	279	0.49	3.0
09.02.2011	Depth Profil	DGT	0.6M HNO3		280	0	280	0.41	3.2
09.02.2011	Depth Profil	DGT	0.6M HNO3		281	0	281	0.51	0.6
09.02.2011	River sampl	DGT	0.6M HNO3		282	0	282	0.38	4.7
09.02.2011	River sampl	DGT	0.6M HNO3		283	0	283	0.46	4.5
09.02.2011	River sampl	DGT	0.6M HNO3		284	0	284	2.46	2.7
09.02.2011	River sampl	DGT	0.6M HNO3		285	0	285	2.09	3.3
09.02.2011	River sampl	DGT	0.6M HNO3		286	#VALUE!	0286-end-depth-profile-dgt	1.90	4.4
09.02.2011	River sampl	DGT	0.6M HNO3		291	#VALUE!	0291-start-wafow-dgt	0.46	1.5
							Stop statistical calculations		
Stop formulas					155		Average	2.01	2.9
Ave	40566.3333	#DIV/0!	#DIV/0!		210.0258		Min	-0.48	0.0
							Max	9.78	59.7
							Std	2.21	4.9
							Rsd % <5, 5-10, >10	110.2	
							Confidence interval 95%	0.36	0.8
							Confidence interval 95% (%)	17.8	
							Number	155	155

Filtration blank values and concentrations corrected by blank

Blanks used for corrections

Date of analyses: 13.01.12 sekvens nr 1

Counting digits = 3

UltraClave											Isotope	Fe56(MR)	
Date	Ves	Vial vol.(r	Sa	Type of sam	Id filter	Fiter type	R	Serial nr.	Project-Inr		Parameteres	Conc.	
	3	(61±0.3g)							ID	nec-der id	Sample ID	µg/L	SD, %
Start formulas											Start statistical calculations		
23/05/2011	41	60.00		1				522	UC-blanks	##	Blank-522-1	2.7	2.4
23/05/2011	42	60.00		2				522	UC-blanks	##	Blank-522-2	0.7	1.6
23/05/2011	43	60.00		3				522	UC-blanks	##	Blank-522-3	0.4	4.6
23/05/2011	44	60.00		3				522	UC-blanks	##	Blank-522-4	0.7	1.4
23/05/2011	45	60.00		3				522	UC-blanks	##	Blank-522-5	0.6	3.9
25/05/2011	49	60.00		1				524	UC-blanks	##	Blank-524-1	0.4	5.2
25/05/2011	50	60.00		2				524	UC-blanks	##	Blank-524-2	1.1	2.2
25/05/2011	51	60.00		3				524	UC-blanks	##	Blank-524-3	0.5	5.5
26/05/2011	41	60.00		1			N	525	UC-blanks	##	Blank-525-1	0.3	5.4
26/05/2011	42	60.00		2				525	UC-blanks	##	Blank-525-2	0.7	2.6
26/05/2011	43	60.00		3				525	UC-blanks	##	Blank-525-3	0.2	5.9
27/05/2011	44	60.00		1				526	UC-blanks	##	Blank-526-1	0.2	2.0
27/05/2011	45	60.00		2				526	UC-blanks	##	Blank-526-2	0.2	4.6
27/05/2011	46	60.00		3				526	UC-blanks	##	Blank-526-3	0.2	1.7
30/05/2011	47	60.00		1				527	UC-blanks	##	Blank-527-1	0.1	0.6
30/05/2011	48	60.00		2				527	UC-blanks	##	Blank-527-2	0.2	3.3
30/05/2011	49	60.00		3				527	UC-blanks	##	Blank-527-3	0.2	5.3
23/06/2011	50	60.00		1			N	535	UC-blanks	##	Blank-535-1	0.6	2.8
23/06/2011	51	61.57		2				535	UC-blanks	##	Blank-535-2	0.0	10.6
23/06/2011	52	60.00		3				535	UC-blanks	##	Blank-535-3	0.2	5.6
24/06/2011	41	60.00		1				536	UC-blanks	##	Blank-536-1	0.2	5.6
24/06/2011	42	60.00		2				536	UC-blanks	##	Blank-536-2	0.5	4.1
24/06/2011	43	60.00		3				536	UC-blanks	##	Blank-536-3	0.3	0.9
											Stop statistical calculations		
											Average	0.5	3.8
											Blank values subtracted samples, empty cells means < instrumental d	0.5	3.8
											Min	0.0	0.6
											Max	2.7	10.6
											Std	0.5	2.3
											Rsd % <5, 5-10, >10	109.9	
											Confidence interval 95%	0.2	1.0
											Confidence interval 95% (%)	46.9	
											Number	23	23

Blanks used for corrections

Date of analyses: 13.01.12 sekvens nr 1

Counting digits = 3

UltraClave												Isotope	Fe56(MR)	
Date	Ves	Whal vol.(r	Sa	Type of sam	Id filter	Fiter type	R	Serial nr.	Project-Inr			Parameteres	Conc.	
	3	(61±0.3g)							ID		der id	Sample ID	µg/L	SD, %
Start formulas												Start statistical calculations		
24/06/2011	48	60.00		Filterblanks	0.8 - 1	0.8		536	520	0		520	1.0	8.2
24/06/2011	49	60.00		Filterblanks	0.8 - 2	0.8		536	521	0		521	0.8	3.4
24/06/2011	50	60.00		Filterblanks	0.8 - 3	0.8		536	522	0		522	1.3	7.9
24/06/2011	51	60.00		Filterblanks	2.0 - 1	2		536	523	0		523	0.7	1.3
24/06/2011	52	60.00		Filterblanks	2.0 - 2	2		536	524	0		524	0.9	6.9
24/06/2011	53	60.00		Filterblanks	2.0 - 3	2		536	525	0		525	0.8	0.5
24/06/2011	54	60.00		Filterblanks	10 - 1	10		536	526	0		526	0.6	1.6
24/06/2011	55	60.00		Filterblanks	10 - 2	10		536	527	0		527	1.3	0.7
24/06/2011	56	60.00		Filterblanks	10 - 3	10		536	528	##		0528-siste-prove	0.5	4.4
												Stop statistical calculations		
												Average	0.9	3.9
												Blank values subtracted samples, empty cells means < instrumental d	0.9	3.9
												Min	0.5	0.5
												Max	1.3	8.2
												Std	0.3	3.1
												Rsd % <5, 5-10, >10	30.9	
												Confidence interval 95%	0.2	2.2
												Confidence interval 95% (%)	21.8	
												Number	9	9

Results corrected for filter-blanks, related to filtertype, this because there is more tehn less .

a) Filter-blanks includes UltraClave blanks

b) all filter-blanks are used without dividing into different blanks gruops

no signifcant difference between the blanks

Results calculated back to absolute quantity in approx. 60ml, the orignal concentration in the sample

Date of analyses: 13.01.12 sekvens nr 1

Counting digits = 3

UltraClave												Isotope	Fe56(MR)	
Date	Ves	Final vol.(r	Sa	Type of sam	Id filter	Fiter type	R	Serial nr.	Project-Inr			Parameteres	Conc.	
	3	(61±0.3g)							ID	ec-	der id	Sample ID	g/60ml	SD, %
Start formulas												Start statistical calculations		
23/05/2011	49	60.00	26	Zoo pl.	1	0.2		522	314	##		Nico-Ani-Vera-pnr314-528-fil	8.0	4.9
23/05/2011	49	60.00	26	Zoo pl.	1	0.2		522	314	##		314-repating-test	8.0	5.5
23/05/2011	50	60.00	26	Tank 600 m	2	0.2		522	315	0		315	4.7	5.5
23/05/2011	51	60.00	26	Brackish 0,	3	0.2		522	316	0		316	4.2	5.0
23/05/2011	52	60.00	26	Filtration	4	0.2		522	317	0		317	1.8	3.7
23/05/2011	53	60.00	26	Filtration	5	0.2		522	318	0		318	2.7	2.0
23/05/2011	54	60.00	26	Filtration	6	0.2		522	319	0		319	2.0	2.7
23/05/2011	55	60.00	26	Filtration	7	0.2		522	320	##		0320-Se-mo-fra-14-24	2.2	1.6
23/05/2011	56	60.00	26	Filtration	8	0.2		522	321	0		321	2.2	5.3
23/05/2011	57	60.00	27	Filtration	9	0.2		522	322	0		322	2.2	3.1
23/05/2011	58	60.00	27	Filtration	10	0.2		522	323	0		323	1.5	3.1
23/05/2011	59	60.00	27	Filtration	11	0.2		522	324	0		324	1.8	3.1
23/05/2011	60	61.19	27	Filtration	12	0.2		522	325	0		325	1.8	1.6
23/05/2011	61	60.82	27	Filtration	13	0.2		522	326	0		326	2.1	1.3
23/05/2011	62	60.00	29	Filtration	14	0.2		522	327	0		327	11.9	2.2
23/05/2011	63	60.00	29	Filtration	15	10		522	328	0		328	2.2	3.4
23/05/2011	64	60.00	29	Filtration	16	2		522	329	0		329	2.5	4.6
23/05/2011	65	60.00	29	Filtration	17	0,2		522	330	0		330	12.8	6.0
23/05/2011	66	60.00	29	Filtration	18	0.2		522	331	0		331	0.8	3.5
23/05/2011	67	60.00	29	Filtration	19	0.2		522	332	0		332	4.5	4.4
23/05/2011	68	60.00	29	Filtration	20	10		522	333	0		333	0.8	3.7
23/05/2011	69	60.00	29	Filtration	21	2		522	334	0		334	2.0	1.8
23/05/2011	70	60.00	29	Filtration	22	0,2		522	335	0		335	2.3	3.3
23/05/2011	71	60.00	29	Filtration	23	0.2		522	336	0		336	0.8	5.1
23/05/2011	72	60.00	29	UC BLANK	24	0.2	F	522	337	0		337	0.3	0.5
23/05/2011	73	60.00	29	Filtration	25	10		522	338	0		338	0.7	1.7
23/05/2011	74	60.00	29	UC BLANK	26	2	C	522	339	0		339	0.0	7.9
23/05/2011	75	60.00	29	Filtration	27	0,2		522	340	0		340	0.7	2.1
23/05/2011	76	60.00	29	Filtration	28	0.2		522	341	0		341	1.2	2.0
23/05/2011	77	60.00	29	Filtration	29	0.2		522	342	0		342	9.2	4.6
23/05/2011	78	60.00	29	Filtration	30	10		522	343	0		343	0.6	3.5
23/05/2011	79	60.00	29	Filtration	31	2		522	344	0		344	1.1	2.5
23/05/2011	79	60.00	29	Filtration	31	2		522	344	##		344-repeat-e-pls	1.1	4.1
23/05/2011	80	60.00	29	Filtration	32	0,2		522	345	##		345-re-pls-forrige	0.7	7.1
25/05/2011	41	60.00	29	Filtration	33	0.2		524	346	0		346	7.5	6.2
25/05/2011	42	60.00	29	Filtration	34	0.2		524	347	0		347	4.8	6.8
25/05/2011	43	60.00	29	Filtration	35	10		524	348	0		348	0.5	5.5
25/05/2011	44	60.00	29	Filtration	36	2		524	349	0		349	0.9	13.6
25/05/2011	45	60.00	29	Filtration	37	0,2		524	350	0		350	0.8	5.8
25/05/2011	45	60.00	29	Filtration	37	0,2		524	350	##		Repeat-smp-350	0.9	3.5

25/05/2011	46	60.00	30	Filtration	38	0.2		524	351	0			351	1.0	4.7
25/05/2011	47	60.00	30	Filtration	39	0.2		524	352	0			352	1.9	6.9
25/05/2011	48	60.00	30	Filtration	40	10		524	353	0			353	0.8	6.2
25/05/2011	52	60.00	30	Filtration	41	2		524	354	0			354	0.2	4.3
25/05/2011	53	60.00	30	Filtration	42	0,2		524	355	0			355	0.9	2.2
25/05/2011	54	60.00	30	Filtration	43	0.2		524	356	0			356	0.6	15.9
25/05/2011	55	60.00	30	Filtration	44	0.2		524	357	0			357	3.6	2.7
25/05/2011	56	60.00	30	Filtration	45	10		524	358	0			358	0.7	4.6
25/05/2011	57	60.00	30	Filtration	46	2		524	359	0			359	0.3	7.9
25/05/2011	58	60.00	30	Filtration	47	0,2		524	360	0			360	0.4	2.2
25/05/2011	58	60.00	30	Filtration	47	0,2		524	360	##			Glemt-360	0.4	2.8
25/05/2011	59	60.00	30	Filtration	48	0.2		524	361	##			Glemt-361	1.9	3.6
25/05/2011	60	60.00	30	Filtration	49	0.2		524	362	##			Glemt-362	3.1	2.9
25/05/2011	61	60.62	30	Filtration	50	10		524	363	##			Glemt-363	0.3	1.8
25/05/2011	62	60.00	30	Filtration	51	2		524	364	##			Glemt-364	0.7	0.4
25/05/2011	63	60.00	30	UC BLANK	52	0,2	C	524	365	##			Glemt-365	0.0	7.2
25/05/2011	64	60.00	30	Filtration	53	0.2		524	366	##			Glemt-366	4.1	1.6
25/05/2011	65	60.00	30	Filtration	54	0.2		524	367	##			Glemt-367	3.3	1.6
25/05/2011	66	60.00	30	Filtration	55	10		524	368	##			Glemt-368	0.4	4.5
25/05/2011	67	60.00	30	Filtration	56	2		524	369	##			Glemt-369	0.6	5.7
25/05/2011	68	60.00	30	Filtration	57	0,2		524	370	##			Glemt-370	0.6	1.1
25/05/2011	69	60.00	30	Filtration	58	0.2		524	371	##			Glemt-371	6.5	1.1
25/05/2011	69	60.00	30	Filtration	58	0.2		524	371	0		#	**371**	7.3	10.1
25/05/2011	70	60.00	30	Filtration	59	0.2		524	372	##			Glemt-372	2.2	1.8
25/05/2011	70	60.00	30	Filtration	59	0.2		524	372	0		#	**372**	2.5	1.5
25/05/2011	71	60.91	30	Filtration	60	10		524	373	0	sert	#	**373**	0.3	3.7
25/05/2011	72	60.00	30	Filtration	61	2		524	374	0	sert	#	**374**	0.4	10.8
25/05/2011	73	60.00	30	Filtration	62	0,2		524	375	0	sert	#	**375**	0.3	2.2
25/05/2011	74	60.00	01	Filtration	63	0,2	L	524	376	0	sert	#	**376**	0.0	####
25/05/2011	75	61.28	01	Filtration	64	0,2		524	377	0		#	**377**	1.3	1.8
25/05/2011	75	61.28	01	Filtration	64	0,2		524	377	0			377	1.3	0.8
25/05/2011	76	60.00	01	Filtration	65	0,2		524	378	0		#	**378**	1.9	5.6
25/05/2011	76	60.00	01	Filtration	65	0,2		524	378	0			378	1.7	4.1
25/05/2011	77	60.00	01	Filtration	66	0,2		524	379	0		#	**379**	5.2	3.9
25/05/2011	77	60.00	01	Filtration	66	0,2		524	379	0			379	5.0	2.8
25/05/2011	78	60.00	01	UC BLANK	67	0,2	C	524	380	0		#	**380**	0.0	14.3
25/05/2011	79	60.00	02	Filtration	68	0,2		524	381	0		#	**381**	0.8	7.4
25/05/2011	79	60.00	02	Filtration	68	0,2		524	381	0	kvens		381	0.8	3.1
25/05/2011	80	60.00	02	Filtration	69	0,2		524	382	0		#	**382**	1.2	6.7
25/05/2011	80	60.00	02	Filtration	69	0,2		524	382	0	kvens		382	1.1	2.7
26/05/2011	44	60.00	02	Filtration	70	0,2		525	383	0		#	**383**	2.1	6.6
26/05/2011	44	60.00	02	Filtration	70	0,2		525	383	0	kvens		383	2.1	2.7
26/05/2011	45	60.00	02	Filtration	71	0,2		525	384	0		#	**384**	1.8	8.8
26/05/2011	45	60.00	02	Filtration	71	0,2		525	384	0	kvens		384	1.8	1.7
26/05/2011	46	60.00	02	Filtration	72	0,2		525	385	0		#	**385**	1.9	8.3
26/05/2011	46	60.00	02	Filtration	72	0,2		525	385	0	kvens		385	1.8	0.6
26/05/2011	47	60.00	04	Filtration	73	0.2		525	386	0	kvens		386	1.5	4.5
26/05/2011	48	60.00	04	Filtration	74	0.2		525	387	0		#	**387**	3.2	3.6
26/05/2011	48	60.00	04	Filtration	74	0.2		525	387	0	kvens		387	3.0	0.5
26/05/2011	49	60.00	04	Filtration	75	10		525	388	0		#	**388**	0.2	2.5
26/05/2011	49	60.00	04	Filtration	75	10		525	388	0	kvens		388	0.2	2.2
26/05/2011	50	60.00	04	Filtration	76	2		525	389	0		#	**389**	1.0	5.8
26/05/2011	50	60.00	04	Filtration	76	2		525	389	0	kvens		389	0.7	3.3
26/05/2011	51	60.00	04	Filtration	77	0,2		525	390	0			390	1.0	2.6
26/05/2011	52	60.00	04	Filtration	78	0.2	T	525	391	0			391	1.5	1.5
26/05/2011	53	60.00	04	Filtration	79	0.2		525	392	0			392	3.3	1.8
26/05/2011	54	60.00	04	Filtration	80	10		525	393	0			393	0.4	4.5
26/05/2011	55	60.00	04	Filtration	81	2		525	394	0			394	0.5	2.8

26/05/2011	55	60.00	04	Filtration	81	2	525	394	0	394	0.5	2.8
26/05/2011	56	60.00	04	Filtration	82	0,2	525	395	0	395	0.3	4.3
26/05/2011	57	60.00	04	Filtration	83	0.2	525	396	0	396	6.3	4.4
26/05/2011	58	60.00	04	Filtration	84	0.2	525	397	0	397	12.4	2.2
26/05/2011	59	60.00	04	Filtration	85	10	525	398	0	398	0.9	1.6
26/05/2011	60	60.00	04	Filtration	86	2	525	399	0	399	0.5	3.8
26/05/2011	61	60.00	04	Filtration	87	0,2	525	400	0	400	0.3	0.6
26/05/2011	62	60.00	04	Filtration	88	0.2	525	401	0	401	17.4	3.8
26/05/2011	63	60.00	04	Filtration	89	0.2	525	402	0	402	4.3	4.1
26/05/2011	64	60.00	04	Filtration	90	10	525	403	0	403	0.5	1.9
26/05/2011	65	60.00	04	Filtration	91	2	525	404	0	404	0.3	1.0
26/05/2011	66	60.00	04	Filtration	92	0,2	525	405	0	405	0.5	2.4
26/05/2011	67	60.00	04	Filtration	93	0.2	525	406	0	406	7.3	3.2
26/05/2011	68	60.00	04	Filtration	94	0.2	525	407	0	407	3.1	2.7
26/05/2011	69	60.00	04	Filtration	95	10	525	408	0	408	0.6	1.6
26/05/2011	70	60.00	04	Filtration	96	2	525	409	0	409	0.7	2.1
26/05/2011	71	60.00	04	Filtration	97	0,2	525	410	0	410	0.4	8.6
26/05/2011	72	60.00	05	Filtration	98	0.2	525	411	0	411	1.0	3.7
26/05/2011	73	60.00	05	Filtration	99	0.2	525	412	0	412	2.3	2.5
26/05/2011	74	60.00	05	Filtration	100	10	525	413	0	413	0.0	74.4
26/05/2011	75	60.00	05	Filtration	101	2	525	414	0	414	0.3	3.5
26/05/2011	76	60.00	05	Filtration	102	0,2	525	415	0	415	0.2	1.2
26/05/2011	77	60.00	05	Filtration	103	0.2	525	416	0	416	2.6	3.2
26/05/2011	78	60.00	05	Filtration	104	0.2	525	417	0	417	1.4	3.9
26/05/2011	79	60.92	05	Filtration	105	10	525	418	0	418	0.3	1.5
26/05/2011	80	60.00	05	Filtration	106	2	525	419	0	419	0.3	1.8
27/05/2011	41	60.00	05	Filtration	107	0,2	526	420	0	420	0.4	2.0
27/05/2011	42	60.00	05	Filtration	108	0.2	526	421	0	421	7.1	2.3
27/05/2011	43	60.00	05	Filtration	109	0.2	526	422	0	422	3.2	3.1
27/05/2011	47	60.00	05	Filtration	110	10	526	423	0	423	1.1	0.8
27/05/2011	48	60.00	05	Filtration	111	2	526	424	0	424	0.3	3.3
27/05/2011	49	60.00	05	Filtration	112	0,2	526	425	0	425	0.2	4.6
27/05/2011	50	60.00	05	Filtration	113	0.2	526	426	0	426	11.0	4.5
27/05/2011	51	60.00	05	Filtration	114	0.2	526	427	0	427	3.5	4.1
27/05/2011	52	60.00	05	Filtration	115	10	526	428	0	428	0.2	0.9
27/05/2011	53	60.00	05	Filtration	116	2	526	429	0	429	0.1	2.7
27/05/2011	54	60.00	05	Filtration	117	0,2	526	430	0	430	0.1	0.5
27/05/2011	55	61.44	05	Filtration	118	0.2	526	431	0	431	4.1	3.1
27/05/2011	56	60.00	05	Filtration	119	0.2	526	432	0	432	1.6	4.4
27/05/2011	57	60.00	05	Filtration	120	10	526	433	0	433	0.4	6.8
27/05/2011	58	60.00	05	Filtration	121	2	526	434	0	434	0.1	0.9
27/05/2011	59	60.00	05	Filtration	122	0,2	526	435	0	435	0.2	1.9
27/05/2011	60	60.00	07	Filtration	123	0,2	526	436	0	436	0.6	4.9
27/05/2011	61	60.00	07	Filtration	124	0,2	526	437	0	437	1.3	1.6
27/05/2011	62	60.00	07	Filtration	125	0,2	526	438	0	438	1.9	4.2
27/05/2011	63	60.00	07	Filtration	126	0,2	526	439	0	439	3.2	1.7
27/05/2011	64	60.00	07	Filtration	127	0,2	526	440	0	440	1.0	3.5
27/05/2011	65	60.00	08	Filtration	128	0,2	526	441	0	441	0.6	3.8
27/05/2011	66	60.00	08	Filtration	129	0,2	526	442	0	442	1.8	3.3
27/05/2011	67	60.00	08	Filtration	130	0,2	526	443	0	443	1.5	2.2
27/05/2011	68	60.00	08	Filtration	131	0,2	526	444	0	444	1.1	6.2
27/05/2011	69	60.49	08	Filtration	132	0,2	526	445	0	445	1.2	2.8
27/05/2011	70	60.91	10	Filtration	133	0,2	526	446	0	446	0.6	1.7
27/05/2011	71	60.00	10	Filtration	134	0,2	526	447	0	447	1.7	4.1
27/05/2011	72	60.00	10	Filtration	135	0,2	526	448	0	448	1.4	7.4
27/05/2011	73	60.00	10	Filtration	136	0,2	526	449	0	449	2.7	1.5
27/05/2011	74	60.00	10	Filtration	137	0,2	526	450	0	450	0.7	5.3
27/05/2011	75	60.00	11	Filtration	138	0,2	526	451	0	451	0.6	2.1

30/05/2011	61	60.00	13	Filtration	158	0.2		527	471	0		471	10.3	3.0
30/05/2011	62	60.00	13	Filtration	159	0.2		527	472	0		472	1.6	3.2
30/05/2011	63	60.00	13	Filtration	160	10		527	473	0		473	0.1	2.1
30/05/2011	64	60.36	13	Filtration	161	2		527	474	0		474	0.1	1.3
30/05/2011	65	60.00	13	Filtration	162	0,2	(1	527	475	0		475	0.6	3.6
30/05/2011	66	60.00	13	Filtration	163	0.2		527	476	0		476	8.6	1.1
30/05/2011	67	60.90	13	Filtration	164	0.2		527	477	0		477	1.5	5.1
30/05/2011	68	60.00	13	Filtration	165	10		527	478	0		478	0.0	2.5
30/05/2011	69	60.00	13	Filtration	166	2		527	479	0		479	0.2	2.9
30/05/2011	70	60.00	13	Filtration	167	0,2		527	480	0		480	0.2	3.1
30/05/2011	71	60.00	14	Filtration	168	0.2		527	481	0		481	0.3	0.4
30/05/2011	72	60.00	14	Filtration	169	0.2		527	482	0		482	0.9	0.8
30/05/2011	73	60.00	14	Filtration	170	10		527	483	0		483	0.1	2.7
30/05/2011	74	60.00	14	Filtration	171	2		527	484	0		484	0.1	1.1
30/05/2011	75	60.00	14	Filtration	172	0,2		527	485	0		485	0.1	3.1
30/05/2011	76	60.00	14	Filtration	173	0.2		527	486	0		486	2.6	1.9
30/05/2011	77	60.00	14	Filtration	174	0.2		527	487	0		487	1.8	2.6
30/05/2011	78	60.00	14	Filtration	175	10		527	488	0		488	0.3	2.7
30/05/2011	79	60.00	14	Filtration	176	2		527	489	0		489	0.2	5.1
30/05/2011	80	60.00	14	Filtration	177	0,2		527	490	0		490	0.5	2.7
23/06/2011	44	60.00	14	Filtration	178	0.2		535	491	0		491	6.0	4.7
23/06/2011	45	60.00	14	Filtration	179	0.2		535	492	0		492	1.9	2.8
23/06/2011	46	60.00	14	Filtration	180	10		535	493	0		493	0.2	2.6
23/06/2011	47	60.00	14	Filtration	181	2	M	535	494	0		494	0.1	1.2
23/06/2011	48	60.00	14	Filtration	182	0,2		535	495	0		495	0.5	3.3
23/06/2011	49	60.00	14	Filtration	183	0.2		535	496	0		496	13.9	2.4
23/06/2011	53	60.00	14	Filtration	184	0.8		535	497	0		497	1.7	2.1
23/06/2011	54	60.00	14	Filtration	185	10		535	498	0		498	0.2	1.9
23/06/2011	55	60.00	14	Filtration	186	2	L(	535	499	0		499	0.0	1.4
23/06/2011	56	60.00	14	Filtration	187	0.8		535	500	0		500	0.2	1.7
23/06/2011	57	60.00	14	Filtration	188	0.8		535	501	0		501	2.9	4.0
23/06/2011	58	60.00	14	Filtration	189	0.8		535	502	0		502	2.0	1.6
23/06/2011	59	60.00	14	Filtration	190	10		535	503	0		503	0.2	3.1
23/06/2011	60	61.47	14	Filtration	191	2		535	504	0		504	0.2	3.9
23/06/2011	61	60.49	14	Filtration	192	0.8		535	505	0		505	0.3	1.9
23/06/2011	62	60.00	14	Filtration	193	0.8		535	506	0		506	649.1	0.3
23/06/2011	63	60.00	14	Filtration	194	0.8		535	507	0		507	1,496.2	2.4
23/06/2011	64	60.00	14	Filtration	195	0.8		535	508	0		508	211.8	3.0
23/06/2011	65	60.00	14	Filtration	196	0.8		535	509	0		509	370.1	4.0
23/06/2011	66	60.00	14	Filtration	197	0.8		535	510	0		510	223.2	1.5
23/06/2011	67	60.00	14	Filtration	198	0.8		535	511	0		511	176.4	3.0
23/06/2011	68	60.00	14	Filtration	199	0.8		535	512	0		512	368.9	1.3
23/06/2011	69	60.49	14	Filtration	200	0.8		535	513	0		513	147.3	6.5
23/06/2011	70	60.00	14	Filtration	201	0.8		535	514	0		514	206.1	0.8
23/06/2011	71	60.00	14	Filtration	202	0.8		535	515	0		515	97.7	4.2
24/06/2011	44	60.00		Filtration	52	0.2	M	536	516	0		516	0.7	0.2
24/06/2011	45	60.00		Filtration	67	0.2	M	536	517	0		517	1.9	3.9
24/06/2011	46	60.00		Filtration	162b	0.2	M	536	518	0		518	0.2	4.1
24/06/2011	47	60.00		Filtration	181b	2	M	536	519	0		519	0.1	4.1

Stop statistical calculations		
Average	19.8	4.5
Min	0.0	0.2
Max	1,496.2	6.5
Std	117.2	12.2
Rsd % <5, 5-10, >10	593.1	
Confidence interval 95%	48.9	5.1
Confidence interval 95% (%)	247.4	
Number	24	24

APPENDIX 3. Statistical Analysis

Nonlinear Regression

Fig. 14.) Surface system TFe_{Ch} regression

Data Source: Data in Results (4nd)
Equation: Polynomial, Linear
f = y0+a*x

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9164	0.8399	0.7865	2.2345

	Coefficient	Std. Error	t	P
y0	10.1870	1.4413	7.0677	0.0058
a	0.0022	0.0006	3.9665	0.0286

Analysis of Variance:

Analysis of Variance:

	DF	SS	MS
Regression	2	1101.9857	550.9928
Residual	3	14.9792	4.9931
Total	5	1116.9649	223.3930

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	1	78.5546	78.5546	15.7328	0.0286
Residual	3	14.9792	4.9931		
Total	4	93.5338	23.3835		

Statistical Tests:

Normality Test (Shapiro-Wilk) Passed (P = 0.5705)

W Statistic= 0.9262 Significance Level = 0.0500

Constant Variance Test Failed (P = 0.0160)

Nonlinear Regression

Fig. 14.) Surface system DFe_{Ch} regression

Data Source: Data in Results (4nd)
Equation: Polynomial, Linear
f = y0+a*x

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.2124	0.0451	0.0000	0.6969

	Coefficient	Std. Error	t	P
y0	4.4775	0.4495	9.9604	0.0022
a	-6.6573E-005	0.0002	-0.3765	0.7316

Analysis of Variance:

Analysis of Variance:

	DF	SS	MS
Regression	2	94.9230	47.4615
Residual	3	1.4571	0.4857
Total	5	96.3801	19.2760

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	1	0.0689	0.0689	0.1418	0.7316
Residual	3	1.4571	0.4857		
Total	4	1.5259	0.3815		

Statistical Tests:

Normality Test (Shapiro-Wilk) Passed (P = 0.0544)

W Statistic= 0.7794 Significance Level = 0.0500

Constant Variance Test Passed (P = 0.0500)

Nonlinear Regression

Fig. 14.) Marine system TFe_{Ch} regression

Data Source: Data in Results (4nd)
Equation: Polynomial, Linear
f = y0+a*x

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.7785	0.6061	0.4748	3.7992

	Coefficient	Std. Error	t	P
y0	15.0494	2.4506	6.1410	0.0087
a	0.0021	0.0010	2.1484	0.1209

Analysis of Variance:

Analysis of Variance:

	DF	SS	MS
Regression	2	1842.0217	921.0109
Residual	3	43.3019	14.4340
Total	5	1885.3236	377.0647

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	1	66.6234	66.6234	4.6157	0.1209
Residual	3	43.3019	14.4340		
Total	4	109.9253	27.4813		

Statistical Tests:

Normality Test (Shapiro-Wilk) Passed (P = 0.0946)

W Statistic= 0.8083 Significance Level = 0.0500

Constant Variance Test Passed (P = 0.0500)

Nonlinear Regression

Fig. 14.) Marine system DFe_{Ch} regression

Data Source: Data in Results (4nd)
Equation: Polynomial, Linear
f = y0+a*x

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.7724	0.5966	0.4621	0.6626

	Coefficient	Std. Error	t	P
y0	6.0320	0.4274	14.1133	0.0008
a	-0.0004	0.0002	-2.1062	0.1258

Analysis of Variance:

Analysis of Variance:

	DF	SS	MS
Regression	2	146.8470	73.4235
Residual	3	1.3171	0.4390
Total	5	148.1641	29.6328

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	1	1.9476	1.9476	4.4361	0.1258
Residual	3	1.3171	0.4390		
Total	4	3.2647	0.8162		

Statistical Tests:

Normality Test (Shapiro-Wilk) Passed (P = 0.7524)

W Statistic= 0.9521 Significance Level = 0.0500

Constant Variance Test Passed (P = 0.0500)

One Way Analysis of Variance

Fe_{DGT} Surface system 1 way - ANOVA

Data source: Data 2 in Results (4nd)

Normality Test: Passed (P = 0.582)

Equal Variance Test: Passed (P = 0.665)

Group Name	N	Missing	Mean	Std Dev	SEM
Control	3	0	4.677	1.608	0.929
Natural	3	0	6.343	0.484	0.280
Conc. 1	3	0	2.404	0.439	0.254
Conc. 2	3	0	2.374	0.784	0.453
Conc. 3	3	0	0.930	0.844	0.487

Source of Variation	DF	SS	MS	F	P
Between Groups	4	55.262	13.815	15.913	<0.001
Residual	10	8.682	0.868		
Total	14	63.944			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

Multiple Comparisons versus Control Group (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
Natural vs. Conc. 3	5.412	7.114	<0.001	0.013	Yes
Natural vs. Conc. 2	3.969	5.217	<0.001	0.017	Yes
Natural vs. Conc. 1	3.939	5.177	<0.001	0.025	Yes
Natural vs. Control	1.665	2.189	0.053	0.050	No

One Way Analysis of Variance

Fe_{DGT} Marine system 1 way - ANOVA

Data source: Data 2 in Results (4nd)

Normality Test: Passed (P = 0.213)

Equal Variance Test: Passed (P = 0.633)

Group Name	N	Missing	Mean	Std Dev	SEM
Control	3	0	10.213	1.905	1.100
Natural	3	0	6.294	2.639	1.524
Conc. 1	3	0	2.918	0.487	0.281
Conc. 2	3	0	2.782	0.933	0.539
Conc. 3	3	0	2.133	0.437	0.252

Source of Variation	DF	SS	MS	F	P
Between Groups	4	138.694	34.673	14.577	<0.001
Residual	10	23.786	2.379		
Total	14	162.480			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 0.999

Multiple Comparisons versus Control Group (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
Natural vs. Conc. 3	4.160	3.304	0.008	0.013	Yes
Natural vs. Control	3.919	3.112	0.011	0.017	Yes
Natural vs. Conc. 2	3.512	2.789	0.019	0.025	Yes
Natural vs. Conc. 1	3.376	2.681	0.023	0.050	Yes

One Way Analysis of Variance

Fig 17.) u/ n plankton ratio 1-way ANOVA

Data source: Data 3 in Results (4nd)

Dependent Variable: Mar Mi/Na

Normality Test: Passed (P = 0.216)

Equal Variance Test: Passed (P = 0.992)

Group Name	N	Missing	Mean	Std Dev	SEM
Control	3	0	0.788	0.225	0.130
Natural	3	0	0.842	0.356	0.206
Conc 1	3	0	2.366	0.355	0.205
Conc 2	3	0	1.900	0.305	0.176
Conc 3	3	0	0.931	0.363	0.209

Source of Variation	DF	SS	MS	F	P
Between Groups	4	6.245	1.561	14.776	<0.001
Residual	10	1.057	0.106		
Total	14	7.302			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 0.999

Multiple Comparisons versus Control Group (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor: **Treatment**

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
Control vs. Conc 1	1.577	5.943	<0.001	0.013	Yes
Control vs. Conc 2	1.112	4.189	0.002	0.017	Yes
Control vs. Conc 3	0.143	0.538	0.602	0.025	No
Control vs. Natural	0.0538	0.203	0.844	0.050	No

APPENDIX 4. Direct samples