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Bioavailability of iron from natural humic complexes in marine phytoplankton

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1. Introduction

In marine coastal areas, atmospheric dust deposition Martin and Fitzwater (1988), continental margin sediments and riverine inputs (Johnson, et al., 1997) are considered as dominant sources of trace elements and nutrients for marine phytoplankton. Among the trace elements, iron is essential for the metabolism where it is required as a component of various enzymes including chlorophyll synthesis (Hudson and Morel, 1990; Martin and Fitzwater, 1988). The hypothesis that shortage of iron limits the primary production in high-nutrient, low-chlorophyll (HNLC) regions of the ocean was first put forward by (Martin and Fitzwater, 1988). In addition to HNLC oceanic regions, some coastal ecosystems (Hutchins and Bruland, 1998) have also been shown to be limited by iron. Nowadays, it is well established that iron along with nitrogen, phosphorus and silicon, plays a key role in phytoplankton dynamics (Boyd, 2007; Moore and Braucher, 2007).

In most marine surface waters, iron occurs at extremely low concentrations (0.02 – 0.50 nmol L^{-1}) (Archer and Johnson, 2000; Krachler, et al., 2010). Inorganic and organic complexation, redox reactions and precipitation and uptake by organisms control the concentration of iron in natural waters. Dissolved iron occurs in seawater in two oxidation states, Fe(II) and Fe(III). These states are involved in the formation of soluble inorganic and organic complexes, colloids and particulate phases. Fe(III) predominates in oxygenated waters and is highly insoluble through the formation of oxyhydroxides (Liu and Millero, 2002). Fe(II) is mainly found in oxygen-free areas; it is thermodynamically unstable in oxygenated seawater and is rapidly oxidized to Fe(III) (Liu and Millero, 2002).

Several studies (Rue and Bruland, 1995; Wu and Luther, 1995) pointed out that only a small fraction of dissolved Fe(III) exists in a free, hydrated or inorganically complexed form and that more than 99 % of the dissolved iron in seawater is strongly complexed by organic ligands. To date, little is known about the specific nature of these ligands, however, phytoplankton growth is controlled by the iron solubility and the iron dissolution rates of colloidal Fe(III) phases (Anderson and Morel, 1982; Wells, et al., 1995).

In consequence of the unknown structure of ligands, Hopkinson and Morel (2009) hypothesized that so-called siderophores (strong iron-binding ligands) may be used by microorganisms to obtain iron from the ligand-bound pool, or that the natural ligands may themselves act as siderophores. Despite their occurrence in marine heterotrophic bacteria, siderophores do not appear to be commonly produced by eukaryotic marine phytoplankton (Amin, et al., 2009; Hopkinson and Morel, 2009). Studies from Hutchins, et al. (1999) and Soria-Dengg, et al. (2001) suggest, that marine phytoplankton appear as iron “pirates”, using siderophores produced by bacteria.

Apparently, marine phytoplankton have multiple iron sources, including siderophores or sedimentary and riverine organic matter in which humic substances (HS) contribute to iron binding (Laglera and van den Berg, 2009). Recent work of Laglera and van den Berg (2009) has shown that HS are ubiquitous hydrophobic components of the natural organic matter present in soil and aquatic environments. The co-variation of iron and HS in coastal areas indicates that iron and HS are transported together from estuarine waters to the open ocean, decreasing both with distance from land and with increasing salinity (Laglera and van den Berg, 2009).

The presence of HS in seawater is in the range of tens to hundreds of µg L^{-1} (Obernosterer and Herndl, 2000). It is thought that the interaction of iron with organic ligands is the cause of a major increase in the solubility of iron in seawater (Johnson, et al., 1997; Kuma, et al., 1999). Most studies on HS have been performed in freshwater systems, and only little is known on the origin and distribution of marine HS (Moran and Hodson, 1994). Elevated iron concentrations in coastal regions may arise from riverine input (Powell and Wilson-Finelli, 2003), but their temporal dynamics are poorly investigated.

In this study, we determined the importance of iron and iron-binding HS for coastal waters. The main question of this study was: Does iron, complexed by natural humic substances, enhance phytoplankton growth in marine coastal systems? The effect of natural HS from a Scottish bog on phytoplankton growth was examined in iron-replete and -starved culture experiments of the chlorophyte *Chlorella salina* Butcher and the coastal marine haptophyte *Isochrysis galbana* Parke. We expected significantly higher cell numbers under iron-replete conditions, as iron is an essential nutrient for enzyme reactions. Additionally, we compared the cell number to the physiological parameter (Fv/Fm) as an indicator for the health conditions of the algae.

2. Material and Methods

2.1 Phytoplankton cultures and maintenance

Experimental studies were conducted with cultures of *C. salina* (clone 211/53) obtained from the Culture Collection of Algae and Protozoa, Dunstaffnage Marine Laboratory, UK (CCAP) and *I. galbana* (clone 927/12) obtained from the Haus des Meeres, Vienna. The two algae species were chosen because of their widespread occurrence and abundance in the Northern Atlantic Ocean (Parke and Dixon, 1976). Growth and iron uptake experiments were performed in modified sterile f/2 medium (Guillard and Ryther, 1962) prepared with 35 ‰ salinity artificial seawater as described by Kester, et al. (1967). All batch cultures were grown in 500 mL glass cylinders supplied with sterile-filtered air at 21°C and pH ~ 8.1 under cool-fluorescent light at a PAR (photosynthetic active radiation) level of 150 µmol quanta m^{-2} s^{-1} and a 16:8 h light : dark cycle. River water containing natural HS was collected directly from the Scottish creek Forsinian Burn (N 58°24.668', W 3°52.405'). The chemical properties of the bog water are described in Table 1.

2.2 Experimental setup and procedures

To obtain different iron concentrations in the f/2 media, a trace element mixture (Guillard and Ryther, 1962) without iron was prepared and depending on the approach, iron and/or chelators were added. The following culture media were prepared: (1) control (f/2 medium only), (2) f/2 medium–($Fe_{(III)}$)+HS, (3) f/2 medium–($Fe_{(III)}$)–HS, (4) f/2 medium+($Fe_{(III)}$)+HS, (5) f/2 medium+($Fe_{(III)}$)–HS (Table 2).

The iron concentration in each bottle of medium was adjusted with a freshly prepared stock solution of $FeCl_3$. Total dissolved iron concentrations in the control media was 0.6 mg Fe L^{-1} (Table 1). All the nutrient stock solutions except the vitamins were sterilized by passing through a 0.2 µm acid-cleaned capsule filter (Sartorius Sartobran 300). After preparation, the media were equilibrated for 24 to 48 h before inoculating the cells.

Prior to starting the experiments, vital pre-cultured clones were transferred from full f/2 medium to the control medium without iron to deplete intracellular iron. Algae were allowed to equilibrate for a period of 7 days and were then inoculated into the experimental media. The acclimated cultures were inoculated in triplicate into 500 mL tubes for three weeks in batch cultures and sampled every other day. Culture vessels were sterilised at 121°C and the glassware was washed in 2M HNO_3 to remove surface iron contamination.

2.3 Determination of the biomass

To determine the growth of phytoplankton the optical density (OD) of the phytoplankton cultures was measured spectrophotometrically at 550 nm wavelength with a Shimadzu RF-1501 spectrophotometer. For chlorophyll-a analyses, triplicate subsamples of the respective culture were filtered through Whatman GF/F filters and then extracted in 3 mL of acetone and stored in darkness at 4°C for approximately 8 h. The absorbance was determined in a 1 cm quartz cell using a Shimadzu RF-1501 spectrophotometer with artificial seawater as a blank at λ= 663 nm (Jeffrey and Humphrey, 1975; Lorenzen, 1975).

The total number of phytoplankton cells present under different nutrient conditions was estimated by measuring the amount of algal cells with a flow cytometer (BD

FACS Aria II) after fixing the cells with formaldehyde (2% final conc.). After measuring the cell volume of *C. salina* and *I. galbana* with an inverted microscope (Utermöhl 1958), the biovolume [$\mu m^3\ L^{-1}$] was calculated.

2.4 Determination of the quantum yield of fluorescence (Fv/Fm)

The quantum efficiency or yield can be used to determine how much solar energy can be converted to fix inorganic carbon. Hence, it describes how well phytoplankton can assimilate light or perform photosynthesis (Kirk, 1994). The maximum (Fv/Fm), can be estimated by measuring the increase in fluorescence yield from dark-adapted minimal fluorescence (Fo) to maximal fluorescence (Fm), which is associated with the closing of photosynthetic reaction centers during saturating light or a photosynthetic inhibitor (Genty, et al., 1989; Kromkamp and Forster, 2003). Therefore, the Fv/Fm ratio is an indicator of nutrient stress.

Every other day, algal samples (3 mL) were dark adapted for 15 min prior to assessing the Fv/Fm with a PAM fluorometer (Walz) zeroed with artificial seawater.

2.5 Determination of the iron concentrations

For total dissolved iron determinations, filtrates of the respective medium (Whatman GF/F) were acidified with HNO_3 to pH ~2 and analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES).

2.6 Statistical analyses

Statistical analyses were performed using SigmaPlot 11.0 and SPSS Statistics 17.0 software packages. Wherever appropriate, a group comparison within Fv/Fm and cell number for each media was conducted by applying one-way analysis of variance

(ANOVA) including a post hoc test after Scheffé to find significant differences between groups. Otherwise, a non-parametric comparison (Kruskal-Wallis-test) at a 95 % significance level was performed.

3. Results

3.1 Cell abundance in cultures with different iron concentrations

All media exhibited concentrations of pH ~8.1 and of conductivity of 52.0 mS cm^{-1}, which are typical values for common seawater. The populations of *C. salina* and *I. galbana* incubated at different iron concentrations exhibited significantly different growth response, however, the cell volume did not decline (*C. salina*: n = 20, 1 - 2: p = 0.378; 1 – 3: p = 0.412; 1 – 4: p = 0.487; 1 – 5: p = 0.562; *I. galbana*: n = 20, 1 - 2: p = 0.289; 1 – 3: p = 0.481; 1 – 4: p = 0.445; 1 – 5: p = 0.573) with decreasing iron concentrations of the media. The cell volume of *I. galbana* (mean 42.75 μm^3) was approximately 3 times smaller than that of *C. salina* (mean 116.29 μm^3). There was a clear effect of iron supply on cell yields for both *C. salina* (n = 3; 1 - 2: p = 0.003; 1 – 3: p = 0.009; 1 – 4: p = 0.031; 1 – 5: p = 0.043; 2 – 3: p = 0.239; 2 – 4: p = 0.16; 2 – 5: p = 0.043; 3 – 4: p = 0.178; 3 – 5: p = 0.047) and *I. galbana* (n = 3; 1 - 2: p = 0.008; 1 – 3: p = 0.021; 1 – 4: p = 0.028; 1 – 5: p = 0.046; 2 – 3: p = 0.198; 2 – 4: p = 0.182; 2 – 5: p = 0.036; 3 – 4: p = 0.149; 3 – 5: p = 0.039). With the exception of the control, iron concentration was below the detection limit (< 10 $\mu g\ L^{-1}$) from the first day on (Fig. 1, 2). During the 21-day culture experiment, cell abundances for both algae species remained fairly constant in the treatments without iron addition. However, in the Fe-amended treatments (control; +Fe+HS; +Fe–HS) cells entered a logarithmic growth phase (day 7 – 13) resulting in highest abundance of *C. salina* and *I. galbana* of 8 x 10^5 mL^{-1} and 7 x 10^5 cells mL^{-1}, respectively, in the control media (Fig. 3, 4). In both phytoplankton species stationary phase was reached at day 13 (Fig. 3, 4). In the control treatment, the optical density and abundance were approximately 2 – 3 times

higher than those in media with HS as chelator. Without any chelator, *C. salina* and *I. galbana* grew at around half their maximum rate (4 x 10^5 cells mL^{-1} and 4 x 10^5 cells mL^{-1}, respectively.

3.2 Photosynthetic efficiency at different iron concentrations

The relationship between Fv/Fm and iron concentration was similar to that observed for cell abundance (Fig. 3-6). The Fv/Fm was significantly higher in the Fe-amended treatments in both *C. salina* (n = 3; 1 - 2: p = 0.029; 1 – 3: p = 0.01; 1 – 4: p = 0.023; 1 – 5: p = 0.373; 2 – 3: p = 0.574; 2 – 4: p = 0.142; 2 – 5: p = 0.034; 3 – 4: p = 0.191; 3 – 5: p = 0.043; Fig. 5) and *I. galbana* (n = 3; 1 - 2: p = 0.038; 1 – 3: p = 0.017; 1 – 4: p = 0.042; 1 – 5: p = 0.402; 2 – 3: p = 0.483; 2 – 4: p = 0.165; 2 – 5: p = 0.049; 3 – 4: p = 0.204; 3 – 5: p = 0.045; Fig. 6). For *C. salina* at day 8, the observed Fv/Fm was 0.73, and without Fe addition, Fv/Fm was 0.25. Maximum Fv/Fm for *I. galbana* was somewhat lower (0.69) but still within the range expected for nutrient-sufficient phytoplankton, and without Fe addition Fv/Fm decreased to 0.42 at day 21. For cultures grown at the lowest concentration of iron of 0.1 mg Fe L^{-1}, Fv/Fm was reduced by approximately 35 – 40% compared to the Fe-amended treatments at day 21 (Fig. 6).

4. Discussion

4.1 Cell abundance in cultures with different iron concentrations

With the exception of the control medium, iron concentration was under the detection limit of the ICP-OES measurement (< 10 µg L-1) from the first day on. This low iron concentration probably resulted from flocculation. The responses to iron limitation by numerous representatives of marine phytoplankton include significant biochemical and physiological changes in their growth characteristics and cell metabolism as demonstrated by Kuma, et al. (1999) and Sunda and Huntsman (1995). The treatments without Fe(III) addition resulted in significantly lower cell abundance than treatments amended with Fe. Although the Fe(III) concentrations of +Fe+HS and +Fe–HS were below the detection limit already at the beginning of our experiments, Fe supply was sufficient to provoke a distinct growth response (Fig. 3, 4). Our findings confirm other studies (Castruita, et al., 2008) showing that low iron concentrations result in a decrease in cell abundance and fluorescence yield in the diatom *Thalassiosira weissflogii* and the coccolithophorid *Emiliana huxleyi*. Sunda and Huntsman (1995) and Shaked, et al. (2005) report reduced growth rates with decreasing iron concentrations in the large oceanic diatoms *Thalassiosira pseudonana, T. oceanica* and *T. weissflogii* and also in other oceanic and coastal phytoplankton taxa. A similar effect on the growth of the diatoms *Chaetoceros* and *Pseudonitzschia* was found (Hutchins and Bruland, 1998). Krachler, et al. (2005) indicated that the “solubility” of organically complexed iron derived from a peat bog in oxic seawater is at least four orders of magnitude higher than the solubility of pure iron-hydroxide.

Recent studies (Shi, et al., 2010) reported that iron uptake by marine phytoplankton depends on the extent of Fe(III) chelation as well as on the nature of the chelating agent. Surprisingly, the addition of HS from bog water instead of the artificial chelator EDTA resulted in a growth of half of their maximum rates in both cultures. The lower growth might be explained by the complication of photosynthetic oxygen release by dissolved HS via electron capture and/or blocking the quinones in photosystem II, resulting in internal oxidative stress as shown by Karasyova, et al. (2007) for the cocoid green algae *Monoraphidium convolutum* and *M. minutum.* Pinto (2003) pointed out that mitochondria and chloroplasts of photosynthetic organisms are highly susceptible to oxidative stress due to intense electron flux in their microenvironment. Castruita et al. (2008) studied the stability of iron-storage proteins and examined whether the EDTA chelation is essential for iron uptake from iron-storage proteins. They discovered that the bioavailability of iron in these storage proteins apparently results from a spontaneously release of Fe(III) into solution of the media.

4.2 Photosynthetic efficiency at different iron concentrations

There is a long history of studies on iron regulation by phytoplankton (Lewin and Chen, 1971; Martin and Fitzwater, 1988). Iron limitation might regulate photosynthetic efficiency (Gerringa, et al., 2000). Iron is essential for many major biogeochemical processes including photosynthesis (Greene, et al., 1991; Kolber, et al., 1994). Raven (1990) estimated that 80 % of the iron required by phytoplankton is located in the photosynthetic electron transfer. Additionally, our results provide strong evidence for a relationship between the physiological state of the phytoplankton and iron availability.

Our laboratory studies showed that Fv/Fm is decreasing when the iron content in the media decreases indicating reduced photosynthetic activity under Fe-depleted conditions.

I. galbana was less responsive to iron stress than *C. salina* (Fig. 5, 6). Greene, et al. (1991) found that iron deficiency induces chlorosis. Also in our study, there was a reduction in photosynthetic pigment content (data not shown). Greene, et al. (1991) explained the decrease in the maximum photosynthetic rate by an increase of minimal turnover time required for an electron to pass from water to CO_2. In conclusion, we have shown that Fe availability to phytoplankton is reduced by humic substances compared to Fe chelated with EDTA.

5. References

Amin, S.A., Green, D.H., Hart, M.C., Kuepper, F.C., G., S.W. and Carranoa, C.J., (2009). 'Photolysis of iron–siderophore chelates promotes bacterial–algal mutualism'. *Environmental Science*, 106:17071-17076.

Anderson, M.A. and Morel, F.M.M., (1982). 'The influence of aqueous iron chemistry on the uptake of iron by the coastal diatom *Thalassiosira weissflogii*'. *Limnol Oceanog*, 27 (5):789-813.

Archer, D.E. and Johnson, K.S., (2000). 'A model of the iron cycle in the ocean'. *Global Biogeochem Cycles*, 14 (1):269-279.

Boyd, P.W., (2007). 'Biogeochemistry: Iron findings'. 446 (7139):989-991.

Breitbarth, E., Achterberg, E.P., Ardelan, M.V., Baker, A.R., Bucciarelli, E., Chever, F., Croot, P.L., S. Duggen, M.G., M. Hassell¨ov, C. Hassler, L. J. Hoffmann, K. A. Hunter, D. A. Hutchins, J. Ingri,, T. Jickells, M.C.L., M. C. Nielsd´ottir, G. Sarthou, V. Schoemann, J. M. Trapp, D. R. Turner, and and Ye17, Y., (2010). 'Iron biogeochemistry across marine systems – progress from the past decade'. *Biogeosciences*, 7:1075-1097.

Buesseler, K.O., (2008). 'Ocean Iron Fertilization—Moving Forward in a Sea of Uncertainty'. *Science*, 319:162

Castruita, M., Morel, F.M.M. and Shaked, Y., (2008). 'Availability of iron from iron-storage proteins to marine phytoplankton'. *Limnol Oceanog*, 53 (3):890-899.

Genty, B., Briantais, J.-M. and Baker, N.R., (1989). 'The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence'. *Biochimica et Biophysica Acta (BBA) - General Subjects*, 990 (1):87-92.

Gerringa, L.J.A., de Baar, H.J.W. and Timmermans, K.R., (2000). 'A comparison of iron limitation of phytoplankton in natural oceanic waters and laboratory media conditioned with EDTA'. *Marine Chemistry*, 68 (4):335-346.

Greene, R.M., Geider, R.J. and Falkowski, P.G., (1991). 'Effect of iron limitation on photosynthesis in a marine diatom'. *Limnol Oceanog*, 36:1772-1782.

Guillard, R.R.L. and Ryther, J.H., (1962). 'Studies of marine planktonic diatoms. I. Cyclotella nana Hustedt and Detonula confervacea Cleve'. *Can. J. Microbiol.*, 8:229-239.

Hopkinson, B.M. and Morel, F.M.M., (2009). 'The role of siderophores in iron acquisition by photosynthetic marine microorganisms'. *Biometals*, 22:659-669.

Hudson, R.J.M. and Morel, F.M.M., (1990). 'Iron transport in marine phytoplankton: Kinetics of cellular and medium coordination reactions'. *Limnol. Oceanogr.*, 35 (5):1002-1020.

Hutchins, D.A. and Bruland, K.W., (1998). 'Iron-limited diatom growth and Si:N uptake ratios in a coastal upwelling regime'. *Nature*, 393 (6685):561-564.

Hutchins, D.A., Witter, A.E., Butler, A. and Luther, G.W., (1999). 'Competition among marine phytoplankton for different chelated iron species'. *Nature*, 400 (6747):858-861.

Jeffrey, S.W. and Humphrey, G.F., (1975). 'New spectrophotometric equation for determining chlorophyll a, b, c1 and c2'. *Biochem. Physiol. Pflanz.*, 167:194-204.

Johnson, K.S., Gordon, R.M. and Coale, K.H., (1997). 'What controls dissolved iron concentrations in the world ocean?' *Marine Chemistry*, 57 (3-4):137-161.

Karasyova, T.A., Steinberg, C.E.W., Klose, E.O. and Menzel, R., (2007). 'Natural Organic Matter Differently Modulates Growth of Two Closely Related Coccal Green Algal Species'. *Env Sci Pollut Res*, 14 (2):88–93.

Kester, D.R., Duedall, I.W., Connors, D.N. and Pytkowicz, R.M., (1967). 'Preparation of Artificial Seawater'. *Limnol Oceanog*, 12:176-179.

Kirk, J., (1994). 'Light and photosynthesis in aquatic ecosystems'. *Cambridge University Press*.

Kolber, Z.S., Barber, R.T., Coale, K.H., Fitzwater, S.E., Greene, R.M., Johnson, K.S., Lindley, S. and Falkowski, P.G., (1994). 'Iron limitation of phytoplankton photosynthesis in the equatorial Pacific Ocean'. *Nature*, 371 (6493):145-149.

Krachler, R., Jirsa, F. and Ayromlou, S., (2005). 'Factors influencing the dissolved iron input by river water to the open ocean'. *Biogeosciences*, 2:311-315.

Krachler, R., Krachler R. F., Frank von der Kammer, Altan Süphandag , Franz Jirsa, Shahram Ayromlou, Thilo Hofmann and Keppler, B.K., (2010). 'Relevance of peat-draining rivers for the riverine input of dissolved iron into the ocean'. *Science of the Total Environment*, 408:2402-2408.

Kromkamp, J.C. and Forster, R.M., (2003). 'The use of variable fluorescence measurements in aquatic ecosystems: differences between multiple and single turnover measuring protocols and suggested terminology'. *Eor. J. Phycol*, 38:103-112.

Kuma, K., Tanaka, J. and Matsunaga, K., (1999). 'Effect of natural and synthetic organic-Fe(III) complexes in an estuarine mixing model on iron uptake and growth of a coastal marine diatom, *Chaetoceros sociale*'. *Marine Biology*, 134:761-769.

Kustka, A., Carpenter, E.J. and Sañudo-Wilhelmy, S.A., (2002). 'Iron and marine nitrogen fixation: progress and future directions'. *Research in Microbiology*, 153 (5):255-262.

Laglera, L.M. and van den Berg, C.M.G., (2009). 'Evidence for geochemical control of iron by humic substances in seawater'. *Limnol Oceanog*, 54 (2):610-619.

Lewin, J. and Chen, C.-H., (1971). 'Available Iron: A Limiting Factor for Marine Phytoplankton '. *Limnol Oceanog*, 16 (4):670-675.

Liu, X. and Millero, F.J., (2002). 'The solubility of iron in seawater'. *Marine Chemistry*, 77 (1):43-54.

Lorenzen, C.J., (1975). 'Determination of chlorophyll and phaeopigments: spectrophotometric equations'. *Limnol Oceanog*, 12:343-346.

Martin, J.H. and Fitzwater, S.E., (1988). 'Iron deficiency limits phytoplankton growth in the north-east Pacific subarctic'. *Nature*, 331 (6154):341-343.

Moore, J.K. and Braucher, O., (2007). 'Observations of dissolved iron concentrations in the World Ocean: implications and constraints for ocean biogeochemical models'. *Biogeosciences*, 4:1241-1277.

Moran, M.A. and Hodson, R.E., (1994). 'Support of bacterioplankton production by dissolved humic substances from three marine environments'. *Mar Ecol Prog Ser*, 110:241-247.

Obernosterer, I. and Herndl, G.J., (2000). 'Differences in the optical and biological reactivity of the humic and nonhumic dissolved organic carbon component in two contrasting coastal marine environments'. *Limnol Oceanog*, 45 (5):1120-1129.

Parke, M. and Dixon, P.S., (1976). 'Check-list of British marine algae'. *Journal of the Marine Biological Association of the United Kingdom*, 56:527-594.

Pinto, E., (2003). 'Heavy metal-induced oxidative stress in algae'. *J. Phycol.*, 39:1008-1018.

Powell, R.T. and Wilson-Finelli, A., (2003). 'Importance of organic Fe complexing ligands in the Mississippi River plume'. *Estuarine, Coastal and Shelf Science*, 58 (4):757-763.

Raven, J.A., (1990). 'Predictions of Mn and Fe use efficiencies of phototrophic growth as a function of light availability for growth and of C assimilation pathway'. *New Phytol.*, 116:1-18.

Rue, E.L. and Bruland, K.W., (1995). 'Complexation of iron(III) by natural organic ligands in the Central North Pacific as determined by a new competitive ligand equilibration/adsorptive cathodic stripping voltammetric method'. *Marine Chemistry*, 50 (1-4):117-138.

Shaked, Y., Kustka, A.B. and Morel, F.M.M., (2005). 'A general kinetic model for iron acquisition by eukaryotic phytoplankton'. *Limnol Oceanog*, 50 (3):872–882.

Shi, D., Xu, Y., Hopkinson, B.M. and Morel, F.M.M., (2010). 'Effect of Ocean Acidification on Iron Availability to Marine Phytoplankton'. *Science*, 327 (5966):676-679.

Soria-Dengg, S., Reissbrodt, R. and Horstmann, U., (2001). 'Siderophores in marine coastal waters and their relevance for iron uptake by phytoplankton: experiments with the diatom *Phaeodactylum tricornutum*'. *Marine Ecology Progress Series*, 220:73-82.

Sunda, W.G. and Huntsman, S.A., (1995). 'Iron uptake and growth limitation in oceanic and coastal phytoplankton'. *Marine Chemistry*, 50 (1-4):189-206.

Timmermans, K.R., Gledhill, M., Nolting, R.F., Veldhuis, M.J.W., de Baar, H.J.W. and van den Berg, C.M.G., (1998). 'Responses of marine phytoplankton in iron enrichment experiments in the northern North Sea and northeast Atlantic Ocean'. *Marine Chemistry*, 61 (3-4):229-242.

Wells, M.L., (2003). 'The level of iron enrichment required to initiate diatom blooms in HNLC waters'. *Mar Chem*, 82:101- 114.

Wells, M.L., Price, N.M. and Bruland, K.W., (1995). 'Iron chemistry in seawater and its relationship to phytoplankton: a workshop report'. *Marine Chemistry*, 48 (2):157-182.

Wu, J. and Luther, G.W., (1995). 'Complexation of Fe(III) by natural organic ligands in the Northwest Atlantic Ocean by a competitive ligand equilibration method and a kinetic approach'. *Marine Chemistry*, 50 (1-4):159-177.

6. Tables and Figures

Table 1: Characteristics of the humic waters collected at Forsinian Burn, Scotland (N 58°24.668', W 3°52.405'), used in the experiments; units in [mg L^{-1}] if not otherwise specified.

pH	Conductivity [µS cm^{-1}]	DOC	Ca^{2+}	Fe^{3+}	K^{+}	Mg^{2+}	Na^{+}	Si^{4+}
6.7	95.0	11.0	2.3	0.3	0.5	2.2	12.0	1.3

Table 2: Experimental media used for the experiments. HS: humic substances; EDTA: ethylene diamine tetra acetic acid

Medium	Control f/2	+Fe+HS	+Fe-HS	-Fe+HS	-Fe-HS
Fe [mg L^{-1}]	0.6	0.6	0.6	0.1	0.1
Chelator	EDTA	HS	-	HS	-

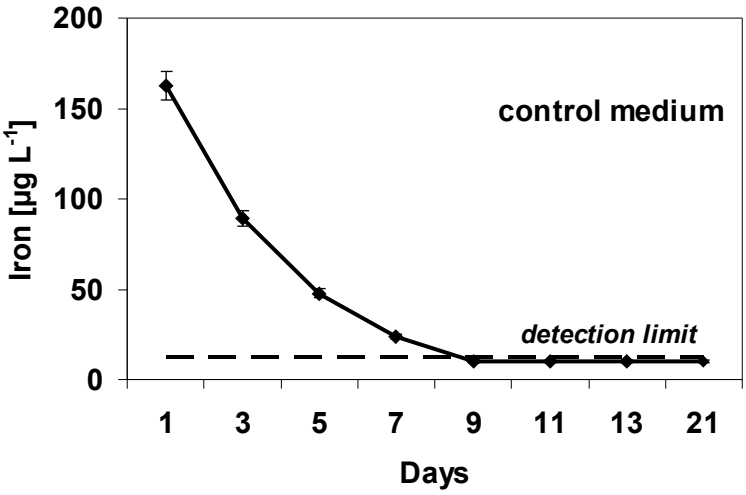

Fig. 1. *C. salina.* Iron concentration [µg L^{-1}] in cultures of *C. salina*. Error bars represent mean ± 1 SD (n=3). ♦: control standard f/2 media. Iron concentrations in all experimental media were under the detection limit (< 10 µg L^{-1}) from the first day on due to flocculation.

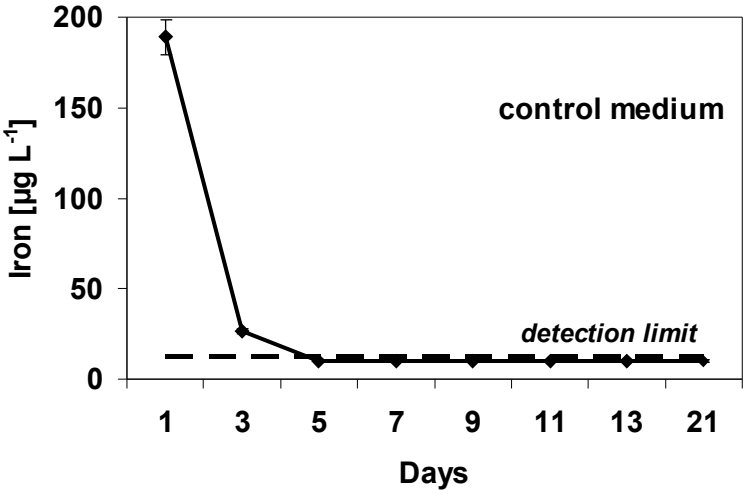

Fig. 2. *I. galbana.* Iron concentration [µg L^{-1}] in cultures of *I. galbana*. Error bars represent mean ± 1 SD (n =3). ♦: control standard f/2 media. Iron concentrations in all experimental media were under the detection limit (< 10 µg L^{-1}) from the first day on due to flocculation.

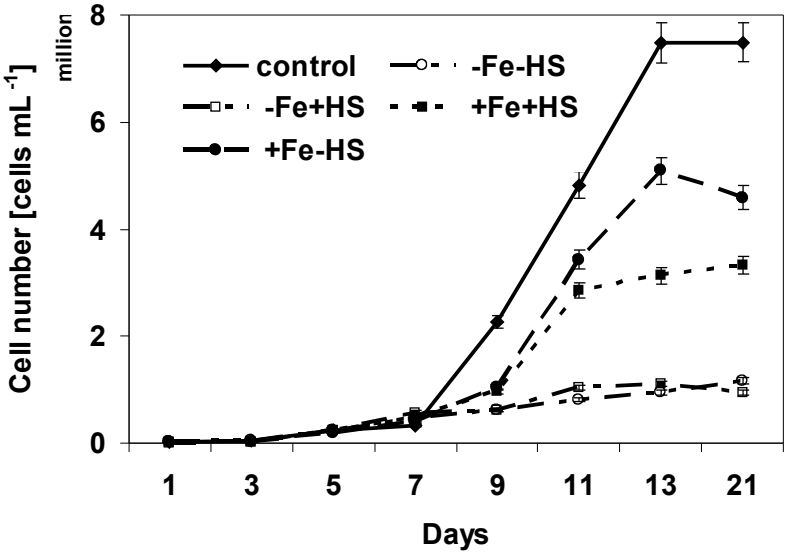

Fig. 3. *C. salina*. Cell numbers [cells mL^{-1}] in batch cultures of *C. salina* grown in the different treatments. Error bars on cell concentrations represent mean ± 1 SD (n =3). ♦: control standard f/2 media; ■: Fe, with HS from a Scottish bog; □: no Fe addition, with HS; ●: Fe, no HS; ○: most starved media: neither Fe or HS.

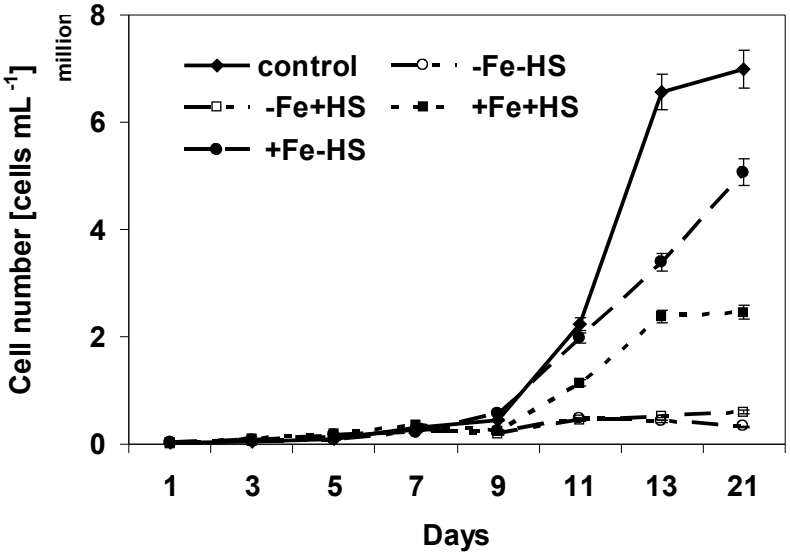

Fig. 4. *I. galbana*. Cell numbers [cells mL^{-1}] in batch cultures of *C. salina* grown in the different treatments. Error bars on cell concentrations represent mean ± 1 SD (n =3). ♦: control standard f/2 media; ■: Fe, with HS from a Scottish bog; □: no Fe addition, with HS; ●: Fe, no HS; ○: most starved media: neither Fe or HS.

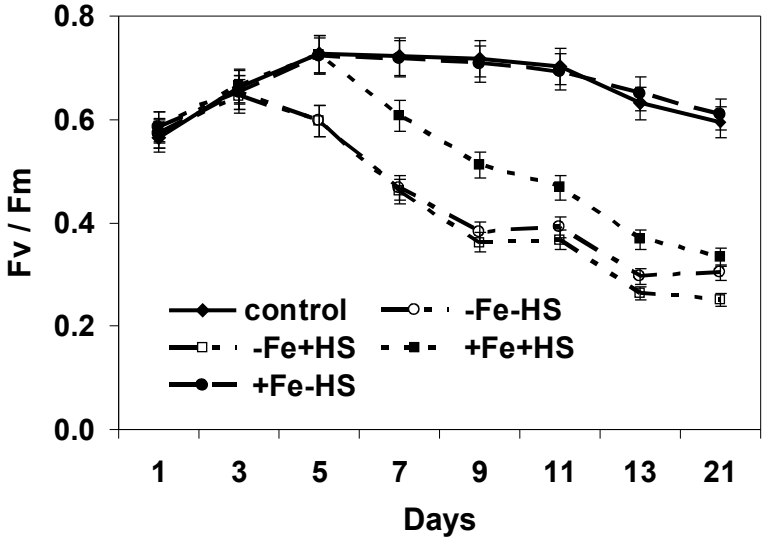

Fig. 5. *C. salina*. Fv/ Fm in batch cultures of *C. salina* grown in the different treatments. Error bars on cell concentrations represent mean ± 1 SD (n =3). ♦: control standard f/2 media; ■: Fe, with HS from a Scottish bog; □: no Fe addition, with HS; ●: Fe, no HS; ○: most starved media: neither Fe or HS.

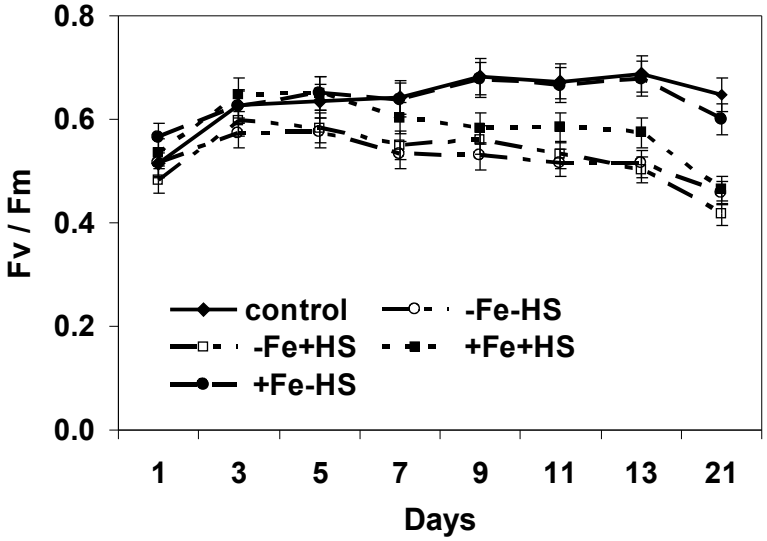

Fig. 6. *I. galbana*. Fv/ Fm in batch cultures of *C. salina* grown in the different treatments. Error bars on cell concentrations represent mean ± 1 SD (n =3). ♦: control standard f/2 media; ■: Fe, with HS from a Scottish bog; □: no Fe addition, with HS; ●: Fe, no HS; ○: most starved media: neither Fe or HS.

7. Abstract

The trace element iron is highly insoluble in sea water. Its availability often controls the productivity of photoautotrophs in the ocean. Interestingly, to date the mechanisms of iron cycling and transport in the sea remain poorly understood. We studied the effects of dissolved iron and humic substance-iron complexes on phytoplankton growth in culture experiments of the chlorophyte *Chlorella salina* and the coastal marine haptophyte *Isochrysis galbana*. There was a positive correlation between iron availability and cell numbers for both *C. salina* and *I. galbana*. During the 21-day culture experiments, maximum cell numbers of 8 x 10^5 cells mL^{-1} at the stationary growth phase (day 13 – 21) for *C. salina* and 7 x 10^5 cells mL^{-1} for *I. galbana* were obtained in the control media f/2, containing iron chelated with EDTA. Low cell abundance for both algal species was observed in media with no addition of iron. Media containing iron promoted rapid growth during the logarithmic growth phase (day 7 – 13), however, to a different degree depending on the chelator type. Iron chelated with EDTA supported higher growth than iron chelated with humic substances derived from a Scottish bog). Furthermore, our results provide strong evidence for a relationship between the physiological status of the marine phytoplankton and iron availability in the media. Our laboratory studies have shown that the maximum fluorescence yield (Fv/Fm) decreased (*C. salina*: 0.73 to 0.25; I*. galbana*: 0.69 to 0.42) significantly with decreasing iron concentrations in the media. Our findings indicate that organically complexed iron supports phytoplankton growth in seawater.

8. Conclusion

In this study we could show that the riverine input of organically bound iron, such as humic substances, stimulates phytoplankton growth. The generally low availability of iron for phytoplankton communities has important ecological consequences, including the limitation of primary productivity (Breitbarth, et al., 2010; Kolber, et al., 1994) and influencing the nitrate reduction (Timmermans, et al., 1998) and nitrogen fixation in the marine environment (Kustka, et al., 2002). The investigation of the bioavailability of organically bound-iron in other species of phytoplankton allows a better understanding of the physiological limitation of essential metabolic processes, and also the elucidation of an important feedback mechanism between the chemistry of iron in the environment and its biological utilization.

9. Zusammenfassung

Eisen ist ein höchst unlösliches Spurenelement im Meerwasser und seine Verfügbarkeit kontrolliert die Produktivität von photoautotrophen Organismen in den Meeren. Obwohl Eisen das vierthäufigste Element in der Erdkruste ist, ist es in weiten Teilen des Meeres nur in geringen Konzentrationen vorhanden (0,02 – 1 nM) (Hudson and Morel, 1990; Martin and Fitzwater, 1988). Daher stellt Eisen, gemeinsam mit Stickstoff, Phosphor und Silizium eine Schlüsselrolle für das Vorkommen und Wachstum von Phytoplankton in den Ozeanen dar (Boyd, 2007; Moore and Braucher, 2007). An Küstengebieten der Meere gelten Staubablagerungen aus der Luft, Sedimente der Kontinentalränder, sowie Flußablagerungen als vorherrschende Quellen für Spurenelemente und Nährstoffe für marines Phytoplankton (Johnson, et al., 1997; Martin and Fitzwater, 1988). Eisen ist für Mikroalgen essentiell, da diese es für den Stoffwechsel, wo es als Bestandteil unterschiedlicher Enzyme, unter anderem für die Photosynthese erforderlich ist, benötigt wird (Hudson and Morel, 1990).

Studien von Martin und Fitzwater (1988) haben jene Gebiete im Ozean untersucht, die sich durch hohe Nährstoffkonzentrationen auszeichnen, deren Phytoplankton-Biomasse aber dennoch gering ist - die so genannten HNLC-Gebiete (high nutrient - low chlorophyll). Aufgrund von Labor- und Feldexperimenten weiß man, dass vor allem Eisen das Phytoplanktonwachstum in diesen Regionen kontrolliert (Wells, 2003). Ausgedehnte HNLC-Regionen liegen im äquatorialen Pazifik, im Nordpazifik sowie im Südpolarmeer.

Bereits seit den 90ger Jahren wurden in verschiedenen Ozeanregionen *in situ* Eisendüngungsexperimente durchgeführt, um zu testen, ob Eisendüngung geeignet wäre dem Anstieg der atmosphärischen Kohlendioxidkonzentration entgegen zu wirken (Buesseler, 2008). Obwohl diese Experimente gezeigt haben, dass Eisenzugabe das Phytoplanktonwachstum kurzzeitig stimuliert (Buesseler, 2008), wurde keine nachhaltige Sedimentation von organischem Kohlenstoff in die Tiefsee erzielt.

In vorangegangene Studien unter anderem von Krachler et al. (2005) wurde festgestellt, dass Eisen auch auf natürliche Art, zum Beispiel durch Regen oder durch Flusswasser, ins Meer gelangt. In Hochmooren in Schottland wurden bioanorganische, an Verwitterungsprozessen beteiligte Stoffe (Huminstoffe) entdeckt, die eine derart hochkomplexe Struktur aufweisen, dass sie Eisen stark an sich binden können und somit das an ihnen gebundene Eisen ins Meer gelangt.

Die vorliegende Studie untersuchte die Bioverfügbarkeit von diesem Eisen aus natürlichen Huminstoff-Komplexen auf die Phytoplankter: *Chlorella salina* Butcher und *Isochrysis galbana* Parke. Wie wirkt sich Eisenmangel auf das Wachstum und die Physiologie von Phytoplankton aus? Ist das an Huminstoffe gebundenes Eisen überhaupt bioverfügbar für die Algen bzw. kann das Wachstum von Phytoplankton in Küstengebieten dadurch angekurbelt werden? Da Meeresplankton häufig Wachstumslimitation durch fehlendes Eisen aufweist, könnte die Zufuhr von komplex gebundenem Eisen zu einer Ankurbelung der photoautotrophen Primärproduktion führen. In den Laborexperimenten konnten nach 21 Tagen in beiden Kulturen signifikante Unterschiede zwischen den Eisen-mangel und -gesättigten Medien festgestellt werden. Minimale Zellzahlen wurden für beide Algenspezies in jenen Medien ohne Eisenzugabe (2×10^5 Zellen mL^{-1}) beobachtet, während Medien mit

Eisenzugaben ein rapides Wachstum (*C. salina*: 8 x 10^5 Zellen mL^{-1}; *I. galbana*: of 7 x 10^5 Zellen mL^{-1}) zeigten, unterschiedlich vom Liganden (EDTA oder Huminstoffe). Weiters zeigten die Ergebnisse einen starken Zusammenhang zwischen dem physiologischen Zustand der Zellen und der Eisenverfügbarkeit in den Medien. Der Fluoreszenz-Ertrag, der als Indikator für Stress durch Nährstoffmangel herangezogen wurde, fiel bei fehlendem Eisen deutlich von 0,73 auf 0,25 bei *C. salina* und von 0,69 auf 0,42 bei *I. galbana*. In der Studie wurde herausgefunden, dass Meerwasser, welches durch Moorwasser mit verschiedenen Fe-Konzentrationen angereichert wurde, ein Algenwachstum bedingt. Diese Arbeit, deutet darauf hin, dass an Huminstoffe gebundenes Eisen aus Moorwasser wie eine „Auffrischungsimpfung“ auf das Phytoplanktonwachstum im Meerwasser wirken kann.

Curriculum Vitae

JOHANNA ZEILINGER

Date of birth: July 23rd 1981
Place of birth: Krems a. d. Donau, Austria
Nationality: Austrian
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EDUCATION

Since 2008
Master thesis at the Dept. of Marine Biology:
Bioavailability of iron from natural humic complexes in marine phytoplankton
Supervisor: Ao. Prof. Dr. M. Schagerl, Advisor: Ao. Prof. Dr. R. Krachler, Dr. F. Jirsa, Institute of Inorganic Chemistry

since 2002
Studies in biology (ecology and zoology) with special emphasis on Marine Ecology at the University of Vienna, Department of Marine Biology/ Department of Limnology

1996 - 2002
Federal commercial high school in Innsbruck, Tirol
Project work: Actual state analysis of solar thermal systems in Tyrolean townships

RESEARCH VISITS AND MARINE BIOLOGY COURSES

October 2008
Competition experiment between Gobiodon histrio and Gobiodon rivulatus
Investigation of Acropora densities and occupation by Gobiodon spp.
Excursion "Coral Reef Course Red Sea"
Dahab Association for Environmental Development, Dahab, Egypt
Advisors: Dr. J. Herler and Ao. Prof. Dr. W. Waitzbauer, University of Vienna

September 2007
Fluorescence characteristics and absorption patterns of Padina pavonica at two different growth depths
Excursion "Marine Ecological field course, Mediterranean"
STARESO Research Institute, Calvi, Corsica, France
Advisors: Prof. Dr. J. Ott and Ao. Prof. Dr. M. Schagerl, University of Vienna

February 2007
Adhesion of limpets in tidal flat and rocky intertidal
Excursion "Coastal habitats of California"
Bodega Marine Laboratory, Bodega Bay, California
Advisors: Prof. Dr. J. Ott, University of Vienna

June 2006
Diversity and classification in marine Mesopsammon
Wadden Sea Research Station, Sylt; Alfred Wegener Institute for Polar and Marine Research, Germany
Advisor: Ao. Prof. Dr. G. Steiner, University of Vienna

July 2005
Introduction to the marine biology of the Mediterranean
Centre for Marine Research, Rovinj, Croatia
Advisor: Ao. Prof. Dr. G. Steiner, University of Vienna

JOHANNA ZEILINGER

WORK EXPERIENCE

July 2010
Tutor at a course in freshwater ecology, Riegersburg, Austria
Course part "Primary Productivity"
Instructor: Ao. Prof. Dr. Hubert Keckeis, University of Vienna

April – September 2010
Participant in the project: Development of appropriate indicators of the relationship between organic/low-input farming and biodiversity (BioBio project) Dept. of Agricultural Economics and Engineering, Università di Bologna, Italy
Instructor: Prof. Dr. Thomas Frank, University of Natural Resources and Life Sciences (BOKU), Vienna

2007 - 2010
Scientific support of the culture collection of Algae, University of Vienna
Instructor: Ao. Prof. Dr. M. Schagerl, Dept. of Marine Biology, University of Vienna

2007 – 2010
Laboratory assistant within the project:
Zwirn M, Chen C, Schagerl M
Induction of sexual reproduction in the genus *Spirogyra* (Zygnemataceae, Streptophyta)
Instructor: Ao. Prof. Dr. M. Schagerl, Dept. of Marine Biology, University of Vienna

SKILLS AND QUALIFICATIONS

Languages
German – first language
English - fluent in speech and writing
French – basics

Computer literacy
MS Office, Powerpoint, Statistica, SPSS, Sigma Plot

Additional qualifications
Electron microscopy (TEM and SEM)
Stable Isotope Probing (SIP)
Fluorescence measurements with Pulse Amplitude Modulation (PAM)
S.C.U.B.A. (Padi AOWD, Rescue diver), underwater fieldwork and photography