**Supplementary Materials**

**Contents**

**Trace metal clean techniques** (page 2)

**Figure S1** (page 3)

**Figure S2** (page 4)

**Blank calculations** **and Fe uptake rates equations for SF- and GS-experiments, Figure S3** (page 5)

**Table S1** (page 7)

**Table S2** (page 8)

**Table S3** (page 9)

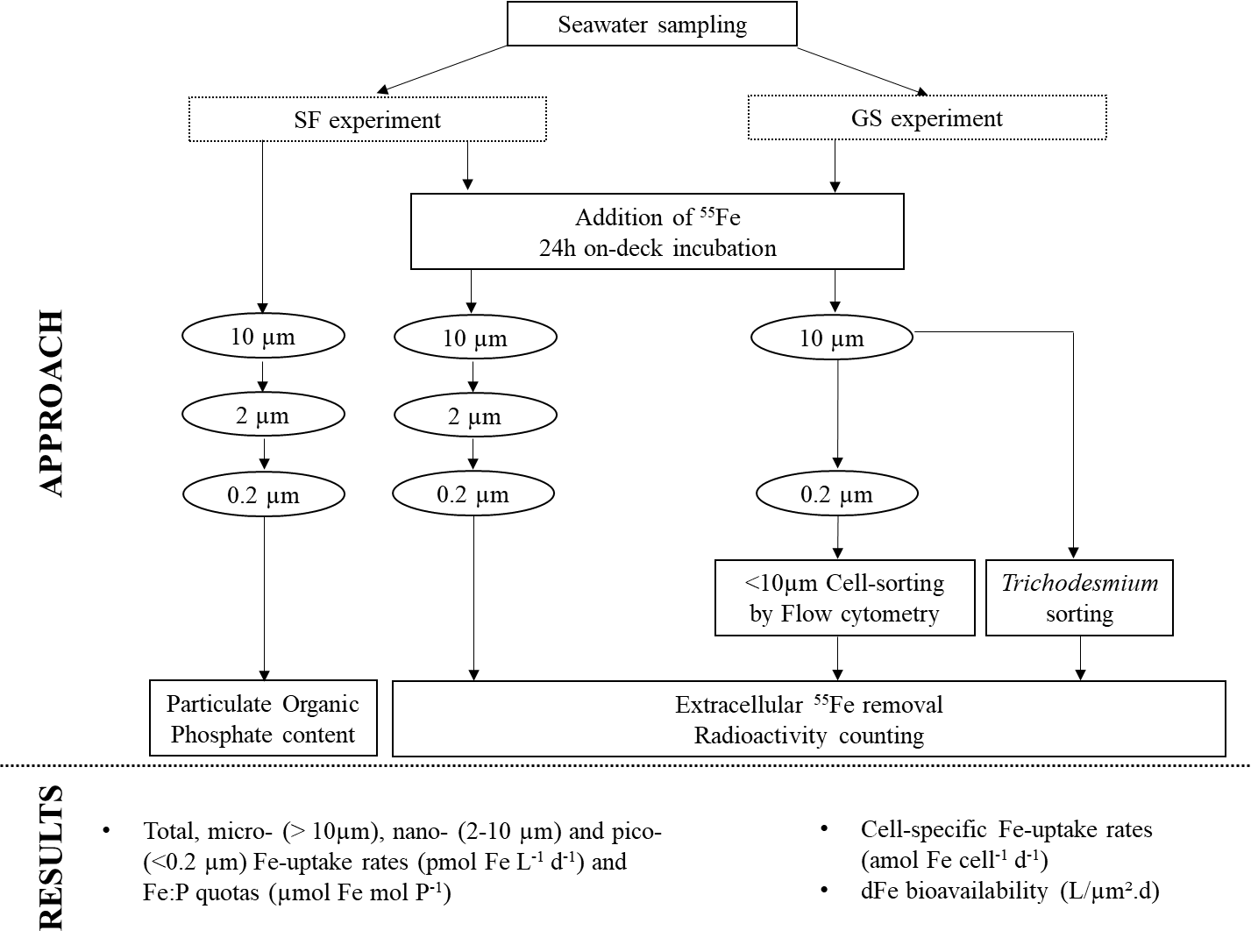
**Table S4** (page 10)

**Table S5** (page 11)

**References** (page 12)

**Trace metal clean techniques**

Incubation PC bottles were soaked for 1 week in alkaline detergent Decon 90 and rinsed five times with demineralized water followed by ultrapure MilliQ water rinse (3 times) into a trace metal clean laboratory (clean room, ISO5). They were then filled with 10% analytical grade HCl for one week followed by 5 rinses with Ultrapure MilliQ water and filled with 100 mL of 10% Suprapur (Merck) HCl for several weeks until use. Prior to sample collection for incubation experiments, the bottles were vigorously rinsed 3 times with surface seawater from the TMR.



**Figure S1.** Schematic representation of size-fraction (SF) and group-specific (GS) experiments to determine Fe uptake rates by different members of the microbial community during the TONGA cruise. During the GS experiments, specific diazotrophic and non-diazotrophic groups of the microbial community were sorted.

|  |  |
| --- | --- |
| **a)** |  |
| **b)** |  |
| **c)** |  |
| **Figure S2.** Example of cytograms obtained during cell sorting by flow cytometry, showing clusters of *C. watsonii*, *Synechococcus*, *Prochlorococcus*, pico-eukaryotes and heterotrophic bacteria (HB) with high (HNA) and low (LNA) nucleic acid content at **a)** S10-B, **b)** S10-A and **c)** S10-H. | |

**Blank calculations** **and Fe uptake rates equations for SF- and GS-experiments** Size-

Size-Fractionation (SF) Experiment:

At each station, a control bottle was amended with formaldehyde (0.5% final concentration) to stop all biological activity and spiked 15 min later with the same amount of 55Fe and treated the same way as experimental bottles. The radioactivity for each control filter (ASF Blank) corresponds to the non-desorbing adsorption of 55Fe that is considered as background 55Fe uptake. The activity of the blank is subtracted from the activity on the SF-filter (ASF filter) to calculate intracellular 55Fe on the filter (in mol, Eq S1.a), which is injected in Eq S1.b to give the Fe uptake rates for each size fraction (ρpico, ρnano and ρmicro in mol Fe L-1 d-1):

**(Eq S1.a)**

**(Eq S1.b)**

**(Eq S1.c)**

Group-Specific (GS) Experiment:

For each sorted organism, we have plotted the number of disintegrations per minute (dpm) against the number of sorted cells. The y-intercept from the linear curve is considered as the abiotic blank (AGS Blank) (Table in Figure S3). The activity of the blank is subtracted from the activity of the filter containing the cells (Acells) to calculate the intracellular 55Fe uptake (in mol, Eq S2.a) which is injected in Eq S2.b to give group-specific Fe uptake rates (ρTricho, ρ*C.Watsonii*, ρSyn, ρProch, ρpicoeuk, ρHNA and ρLNA, in mol Fe cell-1 d-1):

**(Eq S2.a)**

**(Eq S2.b)**

Fe uptake rates of HB (ρHB, in mol Fe cell-1 d-1) were calculated by using HNA and LNA specific uptake rates (ρHNA and ρLNA) and their abundances (see Table S1), as described in Eq 2.c:

**(Eq S2.c)**

The contribution of each sorted organisms to the bulk Fe uptake is calculated as described in Eq S3.a (e.g. for *Trichodesmium*):

**(Eq S3.a)**

The contribution of ‘others’ is calculated as described in Eq S3.b:

**(Eq S3.b)**

The apparent Fe uptake rate constant kin-app (in L cell-1 d-1) was calculated using the equation from (1) (e.g. for *Trichodesmium*): **(Eq S4)**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | |  |  |  | | --- | --- | --- | |  | Blank  (dpm) | S10-H  (dpm) | | *Trichodesmium* | 1016 | 3460 | | *Crocosphaera Watsonii* | 489 | 3491 | | *Synechococcus* | 333 | 952 | | *Prochlorococcus* | 64 | 144 | | Pico eukaryotes | 50 | 108 | | HB | 99 | 298 | |

**Figure S3.** Example of blank calculation for pico-eukaryotes: the y-intercept corresponds to the remaining 55Fe when no cells of pico eukaryotes uptake Fe. On the righten side, the blank for each sorted organism and the activity of the filter of the sorted organism at S10-H are reported.

**Table S1**. Summary of environmental conditions in the study area at 5m depth: N2 fixation and primary production rates, nutrients concentrations and *in situ* abundances and C biomass of the major planktonic groups sorted by flow cytometry in this study. LQ: quantification limit

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **S10 - B** | | | **S10 - A** | **S10 - H** | | | **S11** | | | **S12** | | |
| **N2 fixation rates** (nmol N L-1 d-1) | 64.8 | ± | 15.5 | - | 41.5 | ± | 9.8 | 17.4 | ± | 9.8 | 15.0 | ± | 9.4 |
| **Primary Production** (µmol C L-1 d-1) | 2.49 | ± | 0.73 | - | 0.55 | ± | 0.004 | 1.18 | ± | 0.43 | 0.68 | ± | 0.07 |
| **NO2** (µM) | < LQ=0.01 | | | < LQ=0.01 | < LQ=0.01 | | | < LQ=0.01 | | | < LQ=0.01 | | |
| **NO3** (µM) | < LQ=0.05 | | | < LQ=0.05 | < LQ=0.05 | | | < LQ=0.05 | | | < LQ=0.05 | | |
| **PO4** (µM) | 0.06 | | | 0.05 | 0.06 | | | < LQ=0.02 | | | < LQ=0.02 | | |
| **Si(OH)4** (µM) | 1.15 | | | 1.05 | - | | | - | | | 0.8 | | |
| **DOC** (µM) | 77 | | | 76 | 70 | | | 80 | | | 72 | | |
| ***Trichodesmium***  (filament L-1) | 2637 | | | 2687 | 1851 | | |  | - |  |  | - |  |
| ***Trichodesmium***  (*nifH* gene copies L-1)  (µmol C L-1) | 32 x 106  6.6 | | | - | 8.8 x 106  6.7 | | | 4.0 x 106  4.6 | | | - | | |
| ***C. Watsonii***  (*nifH* gene copies L-1)  (µmol C L-1) | 2.7 x 105  0.2 | | | 7.5 x 105\*  0.55 | 4.0 x 106  2.9 | | | 6.6 x 104  0.05 | | | 4.8 x 104\*  0.04 | | |
| ***Synechococcus***  (cell mL-1)  (µmol C L-1) | 9 946  0.21 | | | 4 417  0.09 | 8 181  0.17 | | | 3 165  0.07 | | | 2 502  0.05 | | |
| ***Prochlorococcus*** (cell mL-1)  (µmol C L-1) | 44 378  0.13 | | | 17 524  0.05 | 52 100  0.16 | | | 26 699  0.08 | | | 6 610  0.02 | | |
| **Pico eukaryotes**  (cell mL-1)  (µmol C L-1) | 346  0.08 | | | 498  0.11 | 249  0.05 | | | 282  0.06 | | | 422  0.09 | | |
| **HNA** (cell mL-1)  (µmol C L-1) | 558 821  0.58 | | | 375 773  0.53 | 332 306  0.34 | | | 417 954  0.43 | | | 522 056  0.54 | | |
| **LNA** (cell mL-1)  (µmol C L-1) | 375 642  0.39 | | | 513 081  0.92 | 281 560  0.31 | | | 290 091  0.30 | | | 466 071  0.48 | | |
| \*Abundance measured by flow cytometry (in cell L-1) | | | | | | | | | | | | | |

**Table S2.** Detailed data of the SF-Experiment: DPM (in triplicates) measured for each size fraction and blanks, 55Fe added, *in situ* dFe concentrations and Fe uptake rates calculated using Equations S1.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Stations** | **Filter porosity**  *µm* | **DPM** | **specific activity** *dpm/mol* | **55Fe added** *nM* | **dFe in situ** *nM* | **Fe uptake rate** *nmol Fe/L.d* |
| S10-B | 10 | 10506 | 1.38E+16 | 0.20 | 0.49 | 4.62E-03 |
| S10-B | 2 | 1997 | 1.38E+16 | 0.20 | 0.49 | 7.28E-04 |
| S10-B | 0.2 | 3410 | 1.38E+16 | 0.20 | 0.49 | 1.44E-03 |
| S10-B | 10 | 3022 | 1.38E+16 | 0.20 | 0.49 | 1.03E-03 |
| S10-B | 2 | 5278 | 1.38E+16 | 0.20 | 0.49 | 2.30E-03 |
| S10-B | 0.2 | 21547 | 1.38E+16 | 0.20 | 0.49 | 1.01E-02 |
| S10-B | 10 | 13163 | 1.38E+16 | 0.20 | 0.49 | 5.89E-03 |
| S10-B | 2 | 9509 | 1.38E+16 | 0.20 | 0.49 | 4.32E-03 |
| S10-B | 0.2 | 42735 | 1.38E+16 | 0.20 | 0.49 | 2.03E-02 |
| Blank S10-B | 10 | 859 |  |  |  |  |
| Blank S10-B | 2 | 476 |  |  |  |  |
| Blank S10-B | 0.2 | 398 |  |  |  |  |
| S10-A | 10 | 4421 | 1.38E+16 | 0.20 | 1.56 | 5.13E-03 |
| S10-A | 2 | 5511 | 1.38E+16 | 0.20 | 1.56 | 6.69E-03 |
| S10-A | 0.2 | 14804 | 1.38E+16 | 0.20 | 1.56 | 1.84E-02 |
| S10-A | 10 | 3759 | 1.38E+16 | 0.20 | 1.56 | 4.29E-03 |
| S10-A | 2 | 5355 | 1.38E+16 | 0.20 | 1.56 | 6.49E-03 |
| S10-A | 0.2 | 5682 | 1.38E+16 | 0.20 | 1.56 | 6.86E-03 |
| S10-A | 10 | 1551 | 1.38E+16 | 0.20 | 1.56 | 1.49E-03 |
| S10-A | 2 | 1887 | 1.38E+16 | 0.20 | 1.56 | 2.09E-03 |
| S10-A | 0.2 | 4897 | 1.38E+16 | 0.20 | 1.56 | 5.87E-03 |
| Blank S10-A | 10 | 377 |  |  |  |  |
| Blank S10-A | 2 | 236 |  |  |  |  |
| Blank S10-A | 0.2 | 271 |  |  |  |  |
| S10-H | 10 | 9825 | 1.38E+16 | 0.18 | 0.35 | 3.91E-03 |
| S10-H | 2 | 6121 | 1.38E+16 | 0.18 | 0.35 | 2.42E-03 |
| S10-H | 0.2 | 15504 | 1.38E+16 | 0.18 | 0.35 | 6.23E-03 |
| S10-H | 10 | - | 1.38E+16 | 0.18 | 0.35 | - |
| S10-H | 2 | - | 1.38E+16 | 0.18 | 0.35 | - |
| S10-H | 0.2 | - | 1.38E+16 | 0.18 | 0.35 | - |
| S10-H | 10 | 8768 | 1.38E+16 | 0.18 | 0.35 | 3.49E-03 |
| S10-H | 2 | 10668 | 1.38E+16 | 0.18 | 0.35 | 4.26E-03 |
| S10-H | 0.2 | 23751 | 1.38E+16 | 0.18 | 0.35 | 9.56E-03 |
| Blank S10-H | 10 | 147 |  |  |  |  |
| Blank S10-H | 2 | 129 |  |  |  |  |
| Blank S10-H | 0.2 | 109 |  |  |  |  |
| S11 | 10 | 5959 | 1.38E+16 | 0.16 | 0.41 | 2.25E-03 |
| S11 | 2 | 974 | 1.38E+16 | 0.16 | 0.41 | 1.82E-04 |
| S11 | 0.2 | 2533 | 1.38E+16 | 0.16 | 0.41 | 1.06E-03 |
| S11 | 10 | 4287 | 1.38E+16 | 0.16 | 0.41 | 1.39E-03 |
| S11 | 2 | 3017 | 1.38E+16 | 0.16 | 0.41 | 1.30E-03 |
| S11 | 0.2 | 11481 | 1.38E+16 | 0.16 | 0.41 | 5.64E-03 |
| S11 | 10 | 8084 | 1.38E+16 | 0.16 | 0.41 | 3.33E-03 |
| S11 | 2 | 2867 | 1.38E+16 | 0.16 | 0.41 | 1.23E-03 |
| S11 | 0.2 | 6337 | 1.38E+16 | 0.16 | 0.41 | 3.01E-03 |
| Blank S11 | 10 | 1576 |  |  |  |  |
| Blank S11 | 2 | 474 |  |  |  |  |
| Blank S11 | 0.2 | 470 |  |  |  |  |
| S12 | 10 | 10347 | 1.38E+16 | 0.16 | 0.24 | 3.39E-03 |
| S12 | 2 | 5799 | 1.38E+16 | 0.16 | 0.24 | 1.91E-03 |
| S12 | 0.2 | 10992 | 1.38E+16 | 0.16 | 0.24 | 3.75E-03 |
| S12 | 10 | 1907 | 1.38E+16 | 0.16 | 0.24 | 4.32E-04 |
| S12 | 2 | 2289 | 1.38E+16 | 0.16 | 0.24 | 6.83E-04 |
| S12 | 0.2 | 12152 | 1.38E+16 | 0.16 | 0.24 | 4.16E-03 |
| S12 | 10 | 11992 | 1.38E+16 | 0.16 | 0.24 | 3.96E-03 |
| S12 | 2 | 2157 | 1.38E+16 | 0.16 | 0.24 | 6.37E-04 |
| S12 | 0.2 | 1793 | 1.38E+16 | 0.16 | 0.24 | 5.33E-04 |
| Blank S12 | 10 | 672 |  |  |  |  |
| Blank S12 | 2 | 337 |  |  |  |  |
| Blank S12 | 0.2 | 271 |  |  |  |  |

**Table S3.** Detailed data of the GS-Experiment: DPM for each sorted organisms and blanks, 55Fe added, *in situ* dFe concentrations and cellulare Fe uptake rates calculated using Equations S2.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Stations** | **Sorted organisms** | **DPM** | **specific activity** *dpm/mol* | **55Fe added** *nM* | **dFe in situ** *nM* | **Fe uptake rate** *amol Fe/cell.d* |
| S10-B | *Trichodesmium* | 7571 | 1.38E+16 | 0.20 | 0.49 | 32.510 |
|  | *C. Watsonii* | 1876 | 1.38E+16 | 0.20 | 0.49 | 0.464 |
|  | *Synechoccocus* | 488 | 1.38E+16 | 0.20 | 0.49 | 0.015 |
|  | *Prochloroccocus* | - | 1.38E+16 | 0.20 | 0.49 | - |
|  | Pico eukaryote | 334 | 1.38E+16 | 0.20 | 0.49 | 0.271 |
|  | HNA | 190 | 1.38E+16 | 0.20 | 0.49 | 0.005 |
|  | LNA | 184 | 1.38E+16 | 0.20 | 0.49 | 0.002 |
| S10-A | *Trichodesmium* | 1319 | 1.38E+16 | 0.20 | 1.56 | 5.974 |
|  | *C. Watsonii* | 715 | 1.38E+16 | 0.20 | 1.56 | 0.361 |
|  | *Synechoccocus* | 911 | 1.38E+16 | 0.20 | 1.56 | 0.005 |
|  | *Prochloroccocus* | 192 | 1.38E+16 | 0.20 | 1.56 | 0.376 |
|  | Pico eukaryote | 129 | 1.38E+16 | 0.20 | 1.56 | 0.557 |
|  | HNA | 133 | 1.38E+16 | 0.20 | 1.56 | 0.001 |
|  | LNA | 116 | 1.38E+16 | 0.20 | 1.56 | 0.001 |
| S10-H | *Trichodesmium* | 3460 | 1.38E+16 | 0.18 | 0.35 | 13.487 |
|  | *C. Watsonii* | 3491 | 1.38E+16 | 0.18 | 0.35 | 1.213 |
|  | *Synechoccocus* | 952 | 1.38E+16 | 0.18 | 0.35 | 0.027 |
|  | *Prochloroccocus* | 144 | 1.38E+16 | 0.18 | 0.35 | 0.036 |
|  | Pico eukaryote | 108 | 1.38E+16 | 0.18 | 0.35 | 0.271 |
|  | HNA | 467 | 1.38E+16 | 0.18 | 0.35 | 0.005 |
|  | LNA | 129 | 1.38E+16 | 0.18 | 0.35 | 0.001 |
| S11 | *Trichodesmium* | 4636 | 1.38E+16 | 0.16 | 0.41 | 4.020 |
| S12 | *Trichodesmium* | 24657 | 1.38E+16 | 0.16 | 0.24 | 15.025 |

**Table S4**. Bulk Fe uptake rates, POC-normalized Fe uptake rates and contribution of the picoplanktonic fraction (0.2–2 µm) to the bulk Fe uptake in this study compared to published studies of natural surface waters in other oceanic regions.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Reference** | **Oceanic region** | **dFe** | **Bulk Fe uptake** | | **POC-normalized Fe uptake** | **0.2 - 2 µm fraction** | |
| (nM) | (pmol Fe  L-1 d-1) | | (µmol Fe  mol C-1 d-1) | (pmol Fe  L-1 d-1) | (% of total) |
| **This study** | WTSP | 0.24-1.56 | 6.5–19 | | 1.2–3.5 | 2.8–10.6 | 43–63 |
| *Ellwood et al. 2020* (2) | SO (Subantarctic zone) | 0.02–0.06 | 11–27 | |  | 6.7–17.3 | 20–45 |
| *Fourquez et al. 2020* (3) | SO (Subantarctic zone) | 0.08 | 90–193 | | 48-102 | 20–46\*\* | 60–73\*\* |
| *Fourquez et al. 2015* (4) | SO (Kerguelen Plateau) | 0.06–0.38 | 19–40 | | 2.5–5.3 |  | 2 |
| *Strzepek et al. 2005* (5) | SO (SE of New Zealand) | 0.05 | 26–101 | |  |  | 1.4–4.3\*\* |
| *Mioni et al. 2007* (6) | ESP | 0.08-0.3\* | 10–567 | |  | 8–403\* |  |
| *King et al. 2012* (7) | South West Pacific (NE of New Zealand) | 0.03-0.60 |  | |  | 5.1–14.2 | 17–60 |
| \*rates for 0.2-1 µm fraction, \*\*rates for 0.2-0.8 µm fraction | | | |  |  |  |  |

**Table S5.** Estimation of Fe:C quotas for diazotrophs (*Trichodesmium* and *C. Watsonii*), non-diazotrophic cyanobacteria (*Synechococcus* and *Prochlorococcus*) and HB, calculated from the equation (3) in (8). Calculations are based on the cellular Fe uptake rates measured in this study and growth rates (µ in d-1) from the literature. For diazotrophs, we estimated the growth rates based on cellular N2 fixation rates and cellular N content measured for *C. Watsonii* and *Trichodesmium* in the WTSP (9). With respect to the Fe-limitation conditions (when dFe <0.6 nM, phytoplankton is considered Fe-limited) suggested by (10), we considered stations S10-H, S10-B, S11 and S12 for the calculations of Fe:C quotas but we excluded S10-A (dFe concentration >0.6 nM)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  | **µ** | **Quotas Fe:C** (µmol Fe mol C-1) | | | |
| **References** | (d-1) | S10-B | S10-H | S11 | S12 |
| ***Trichodesmium*** | *Bonnet et al., 2018* (9) | 0.01 | 159 | 66 | 20 | 73 |
| ***C. Watsonii*** | *Bonnet et al., 2018*(9) | 0.05 | 13 | 34 |  |  |
| ***Synechococcus*** | *Worden and Binder* (11) | 0.37 | 1.9 | 3.5 |  |  |
| ***Prochlorococcus*** | *Worden and Binder* (11) | 0.36 | n/a | 37.8 |  |  |
| **HB** | *Van Wambeke et al., 2018* (12) | 1.92 | 1.8 | 3.0 |  |  |

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