ELEMENTAL STOICHIOMETRY OF MARINE HETEROTROPHIC BACTERIA AND ITS RESPONSE TO CHANGING COPPER AVAILABILITY

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INTRODUCTION

- **Marine heterotrophic bacteria** are significant players in the energy and nutrient cycles of marine ecosystems.
- These microbes rely on a suite of **bioactive metals such as iron (Fe), copper (Cu), manganese (Mn), zinc (Zn) and cobalt (Co)** for biochemical functions. Yet, little is known of their physiological responses to trace metal shortage.
- In isolates belonging to major bacterial clades associated with phytoplankton blooms, we examined growth, carbon metabolism and elemental stoichiometry across a gradient of **Cu availability,** from deplete to replete, representative of unpolluted coastal and open ocean surface waters..
- We present a summary of the 1) **physiological response** to varying Cu, 2) bacterial **elemental stoichiometry**, as well as 3) a preliminary comparison of the **elemental stoichiometry** of our **bacteria** and **eukaryotic algae** from literature.

METHODS

Three strains of marine heterotrophic bacteria were isolated from surface waters (25m) different locations along the coastal-open ocean transect, Line P, in the Northeast Pacific in 2012. They include:

- coastal strain of ***Pseudoalteromonas* sp. PAlt-P2** (station P2)
- oceanic strain of ***Dokdonia* sp. Dokd-P16** (midway station P16)
- oceanic strain of ***Pseudoalteromonas* sp. PAlt-P26** (iron-limited station P26).
- while ***Ruegeria pomeroyi,*** **DSS-3** strain was obtained from ATCC culture collection (DMS 15171, isolated from coastal Georgia).

Strains from Line P were isolated on agar plates as previously described (Grager & Price, 1999). Physiological measurements were obtained from acclimated batch-cultures in mid- to late-exponential phase grown in Aquil media modified for heterotrophic bacteria (Granger & Price, 1999). Copper levels were varied from 0.1-50 nmol L^{-1} ($[Cu'] = 4.6$-388.9 fmol L^{-1}). Bacterial metals and phosphorus were obtained with ICP-MS analysis of digested bacterial pellets (7 h in 3 mL of ultrapure HNO_3 at 120°C, evaporated to dryness and heated in 1 mL of ultrapure H_2O_2 at 120°C for 4 h). Carbon and nitrogen were obtained from 10-20 mL of culture filtered onto pre-combusted GFF filters and analysed with CNH analyzer. Sulfur data was obtained with ICP-OES analysis of the same samples as those for ICP-MS analysis. Bacterial metals were normalized to cell numbers (obtained with flow cytometry, data not shown), mol P (obtained simultaneously during ICP-MS analysis) or to mol C (cellular metals [metal/cell] were converted to metal: mol C using Cu quota [mol/cell] determined with CNH analysis and flow cytometry).

RESULTS

PHYSIOLOGICAL RESPONSE

Dokd-P16, reduces growth, C metabolism* and Cu quotas, but increases Mn quota when grown in low-Cu media . This indicates a **high dependence on Cu** availability in this *Flavobacteriia* strain

Pseudoalteromonas **spp (PAlt-P2 and PAlt-P26)** do not alter growth or C metabolism (PAlt-P26)*, with no significant effects on metal quotas, except for Zn quotas of PAlt-P26.

R. pomeroyi maintains constant growth but alters its C metabolism*, reduces its Cu and Co quota , while increasing its Mn and Fe quota in response to low Cu.

No significant effects were found for **cellular macronutrients** (umol C, N, S, P per cell) or macronutrient ratios (C:N, S:P) in response to variable Cu concentrations for any of the strains (data not shown).

* not shown

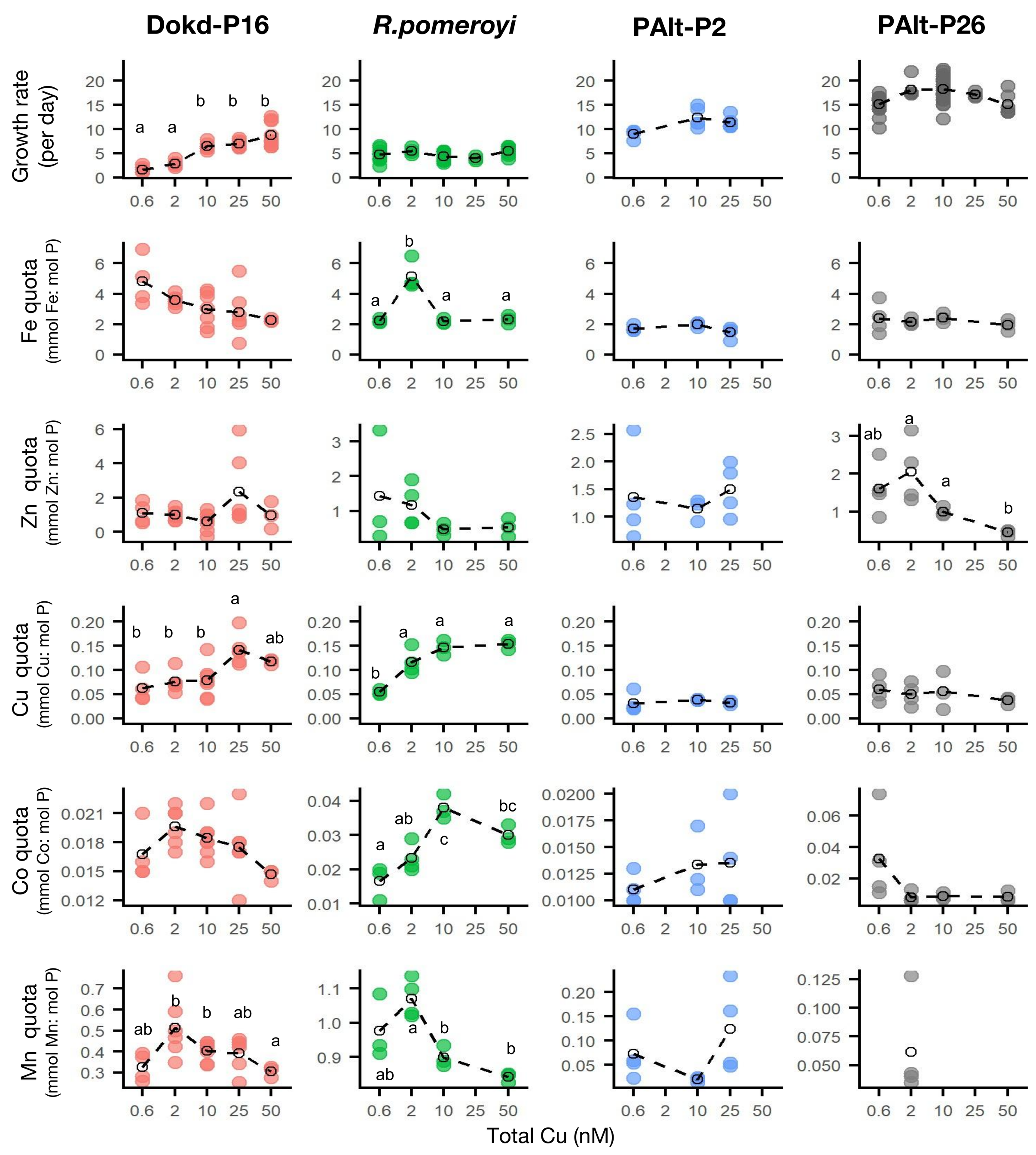


Figure 1. Metal quotas of marine heterotrophic bacteria at various Cu levels. Open circles represent mean metal to phosphorus ratio and the colored points are individual measurements.. Different letters indicate that treatments were significantly different using post-hoc analysis (p <0.05), while for plots with no letters data was not statistically significant (one-way ANOVA). Note x axis is not linear.

METAL SIGNATURE

Metal quotas (Me:P) follow the order of:

Fe > Zn, Mn> Cu>Co	*R. pomeroyi*
Fe> Zn > Mn >Cu >Co	Dokd-P16
Fe > Zn > Cu >Mn> Co	PAlt-P26 & PAlt-P2

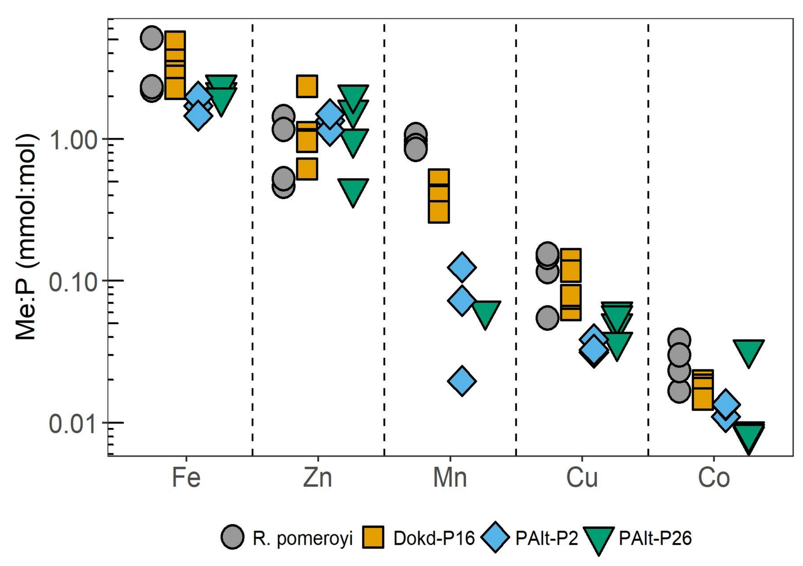


Figure 2. Summary of the metal signature of marine heterotrophic bacteria. Data points represent mean metal to phosphorus ratio measured at each Cu level for the four strains (From Fig.1) Errors were omitted from the plot for clarity.

ELEMENTAL STOICHIOMETRY

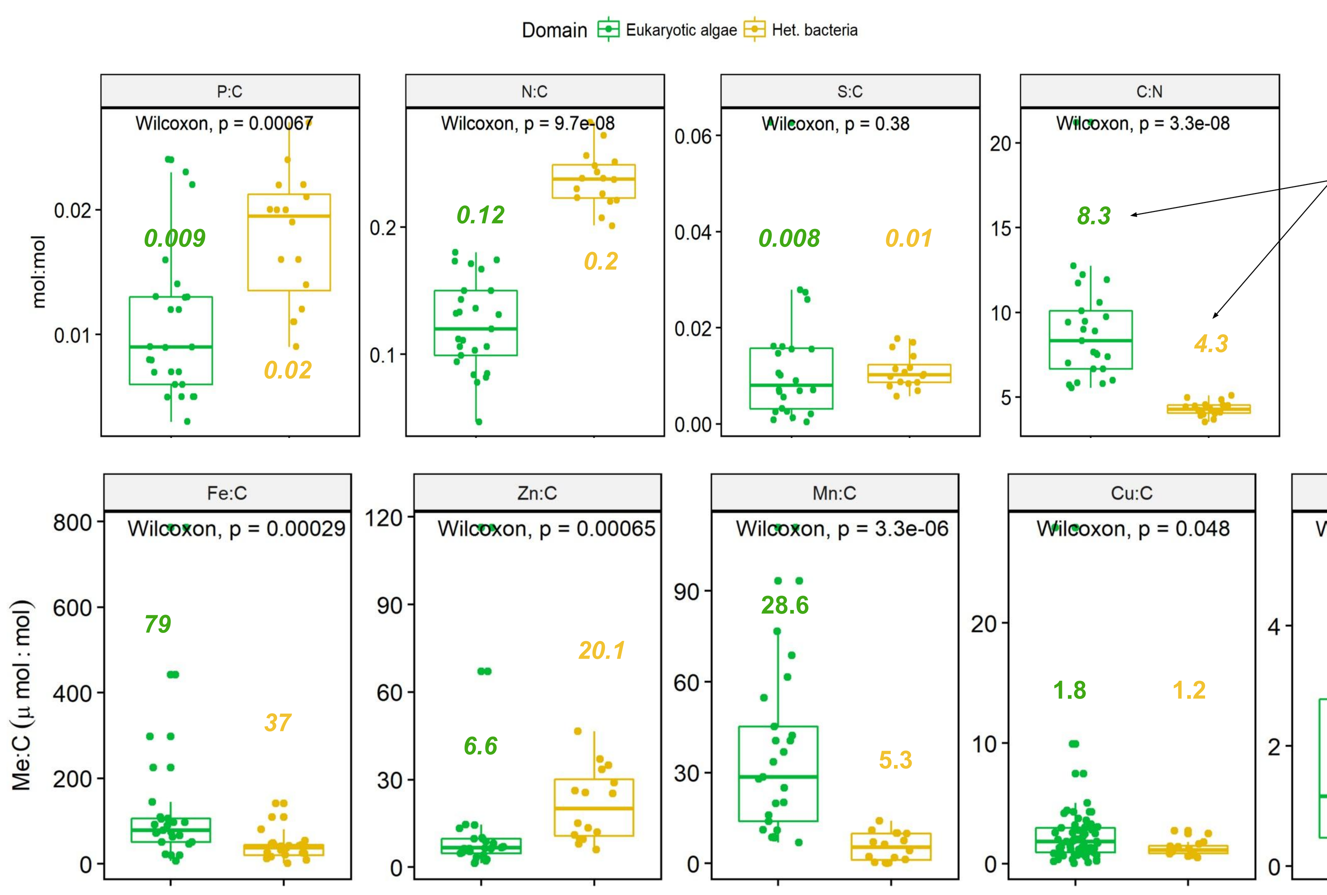
Summary of molar ratios using the medians shown in box plots

EUKARYOTIC ALGAE

$(P_{0.009}\,N_{0.12}C_{1}S_{0.008})_{1000}\;Fe_{0.08}\,Zn_{0.007}Mn_{0.03}Cu_{0.002}Co_{0.0012}$

HETEROTROPHIC BACTERIA

$(P_{0.02}\,N_{0.2}\,C_{1}\,S_{0.01})_{1000}\;Fe_{0.04}\,Zn_{0.02}\,Mn_{0.005}Cu_{0.001}Co_{0.00003}$



Numbers next to the boxplots represent **medians**

Figure 3. Boxplots of elemental stoichiometries of heterotrophic marine bacteria and eukaryotic algae. Data sources:

Phytoplankton

- **Fe, Zn, Mn, Cu, Co quotas** for a variety of species cultured in **metal replete** Aquil from Ho et al. 2003 & Quigg et al. 2010.
- **Cu quotas** measured under **replete and limiting Cu conditions** from Annett et al. 2008, Guo et al. 2012, Sunda & Huntsman, 1995

Heterotrophic bacteria:

- **Fe data** from Fourquez et al. 2016 for Fe-replete coastal and oceanic *A.macleodii*
- **Fe, Zn, Mn, Cu and Co** of all four strains in this study measured at the Cu treatments in Fig. 1

PRELIMINARY comparison suggests that compared to eukaryotic algae heterotrophic bacteria have :

- **higher P** and **N** per C biomass
- **higher Zn** per biomass
- **lower Fe, Mn** and **Co** per biomass
- **What about Cu?**

References

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Any questions?

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