Title: Does differential iron supply to algae affect *Daphnia* life history? An ionome-wide study.

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Running Head: Iron effects on *Daphnia* reproduction

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Abstract: The availability of iron (Fe) varies considerably among diet items, as well as ecosystems. Availability of Fe has also changed due to anthropogenic environmental changes in oceanic as well as inland ecosystems. We know little about its role in the nutrition of ecologically important consumers, particularly in inland ecosystems. Physiological studies in several taxa indicate marked effects of dietary Fe on oogenesis. We predicted that differential Fe supply to algae will impact algal Fe concentration with consequences on the life history of the freshwater grazer, *Daphnia magna*. We found that algal Fe concentration increased with Fe supply, but did not affect algal growth, indicating that the majority of experimental Fe additions were likely adsorbed to, or stored in algal cells. Regardless, data indicate that algal Fe impacted the reproductive traits (age and size at maturity) but not juvenile growth rate of *Daphnia*. A subsequent experiment revealed that Fe concentration in eggs was significantly higher than the rest of *Daphnia*. These results indicate that the concentration of Fe in or on algal cells may vary considerably among ecosystems overlying distinct geological formations differing in Fe, possibly with important implications for zooplankton life histories. Understanding the mechanisms underlying this response is unlikely to be accomplished by a strict focus on Fe because we found correlated shifts in the algal ionome, with concomitant ionome-wide adjustments in *Daphnia*. Information on ionome-wide responses may be useful in better understanding the responses of biota to changes in the supply of any one element.

Keywords: ecological stoichiometry, ionomics, nutrient limitation, nutrient balance concept, reproduction, trace metals, zooplankton.

Introduction:

While iron (Fe) is found in vanishingly low concentrations in the open oceans, Fe is relatively abundant on land. Consequently, attention to Fe in inland ecosystems arises more often from concerns about toxicity than limitation (e.g., Saaltink et al. 2017). Although Fe is abundant in the earth's crust, its bioavailability is strongly dependent on physicochemical parameters of the ecosystem (Likens 2009; Schlesinger & Bernhardt 2013). The availability of iron can vary substantially over space and time in ecosystems (e.g., Sterner et al. 2004; Vrede and Tranvik 2006; Lind et al. in review). Nevertheless, we do not know the extent to which Fe impacts taxa inhabiting inland ecosystems, except in cases where Fe supply is very low, limiting primary production (e.g., Sterner et al. 2004), or where Fe supply is very high, approaching or exceeding toxicity thresholds (e.g., van der Welle et al. 2006). Understanding the relevance of Fe supply within these extremes is becoming increasingly important because we are beginning to appreciate the tremendous variation in Fe among ecosystems, even in close proximity, yet overlying distinct geological formations (e.g., Vouri 1995; Lind et al. in review). Moreover, the global Fe cycle is rapidly changing (Hutchins and Boyd 2016), and Fe loading into freshwater lakes has increased over the last few decades (Bjorneras et al. 2017). However, we know little about the extent to which changes in Fe supply impacts the growth and elemental contents of algae (i.e. its nutritional value to primary consumers), and whether such effects are manifested at higher trophic levels.

The vast majority of Fe in lakes (as much as 90%; Morel and Hering 1993) is biologically unavailable because they are bound to chelators preventing its transport through the cell membrane. Iron enters the foodweb when it is taken up by bacteria and phytoplankton via Fe-specific ligands on the cell membrane (Morrissey and Bowler 2012). When Fe bioavailability is low compared to demand, microbes are known to secrete siderophores that increases the bioavailability of Fe (Trick and Wilhelm 1995; Andrews et al. 2003). At high concentrations, iron could also enter the foodweb at higher trophic levels, directly via diffusion or by ingestion of Fe adsorbed to diet items, although we know little about such mechanisms in inland ecosystems (Dave 1984; Bakker et al. 2016). Organisms require Fe because it is a cofactor in several essential biochemical pathways, however, it can also quickly become toxic because it causes oxidative damage to cells if not bound to organic ligands within the cell (Worms et al. 2006). Much of the Fe in marine phytoplankton is thought to be involved in mechanisms that enable organisms to fix gases and in metabolizing substrates to extract energy (Raven 1988), although similar estimates for freshwater phytoplankton that inhabit ecosystems with much higher Fe concentration are unavailable.

In metazoans, there are two major pools of Fe: heme and non-heme. Heme Fe performs critical respiratory functions related to oxygen transport, while non-heme Fe include storage (ferritin) and transport (transferrin) molecules. The levels of these latter two molecules are indicative of Fe demand because they store or mobilize internal Fe depending on Fe supply relative to demand (Ganz and Nemeth 2006), although relatively little is known about Fe dynamics in non-mammalian metazoans. Nevertheless, iron is known to be important for the nutrition of several invertebrates (Yu and Wang 2002; Zhou et al. 2007; Chen et al. 2011; Lind and Jeyasingh 2018). These studies indicate that Fe and Fe-containing proteins may be particularly relevant for reproduction. For example, Chen et al. (2011) in their study of a marine copepod found that dietary Fe impacted fecundity and neonate viability, while work in other invertebrates revealed strong effects of transferrins on oogenesis (Kurama et al. 1995; Dunkov et al. 2002). Perhaps the most direct evidence for the importance of Fe in reproduction was discovered in mosquitoes (Zhou et al. 2007) where 77% of ingested Fe in the form of transferrin was allocated to eggs. Prior work on the freshwater consumer, *Daphnia*, indicated that dietary Fe had a much stronger effect on reproductive traits compared to individual growth rate (Lind and Jeyasingh 2018).

It is also important to note that, similar to other elemental or molecular nutrients, changes in the supply of Fe outside the cell, or the amount of Fe inside the cell do not happen in isolation. Rather, such changes in one element invariably interacts with other elements in the extracellular or intracellular environment, with important correlated impacts on functional traits of organisms (e.g., Baxter 2015; Jeyasingh et al. 2017). Data on such interactions are important to characterize the biogeochemical niches of taxa (Peñuelas et al. 2019), and illuminate a wider set of physiological mechanisms that taxa employ to acclimate or adapt to ecosystems with contrasting biogeochemistry (e.g., Goos et al. 2017; Prater et al. 2019; Rudman et al. 2019), including Fe supply. Indeed, Spijkerman et al. (2007) found that at high concentrations, Fe binds to the cell membrane of *Chlamydomonas* and inhibits transport of bulk elements such as P.

We predicted that Fe supply will alter Fe concentration in algae, consequently impacting the life history of *Daphnia magna* consuming the algae. Further, we predicted that Fe concentration of eggs will be higher compared to the rest of the *Daphnia* soma. Finally, we expected correlated changes in the concentrations of multiple elements in algae due to experimental manipulation of Fe concentration in the algal culture medium, which will be reflected in the ionomes (i.e. the entire collection of elements in an individual; Salt et al. 2008) of daphniids.

Methods:

<u>*Algal culturing:*</u> *Acutodesmus* sp. was cultured in WC medium (Guillard and Lorenzen 1972) without vitamins at three iron levels: 3.7 µmol L^{-1} (i.e. the standard medium), 0.37 µmol L^{-1}, or 0.15 µmol L^{-1} $FeCl_3$ (henceforth referred to as HiFe, MedFe, and LoFe, respectively). The chemical equilibrium modeling software, Visual MINTEQ (v 3.1, Gustafsson 2018), was used to assess the impact of differential Fe supply on the forms and bioavailability of Fe as well as multiple other elements. Semi-batch cultures (4L) were inoculated with fresh algae and kept at ~20ºC with a 16:8 light dark cycle at ~120 µmol m^{-2} s^{-1}. After cultures reached stable state (~ 6d), 2L of culture was decanted and used for experiments and replaced with 2L of fresh medium. This process was repeated every 2d throughout the experiment. Density of harvested algae was

estimated using microscopy, and stored at 4ºC in the dark for feeding daphniids. Harvested algae were also frozen in triplicate 15mL trace-metal clean centrifuge tubes for quantifying the algal Fe and other elements.

Elemental contents of algae: Tubes were thawed and 10mL was filtered onto pre-combusted and pre-weighed glass fiber filters, dried at 60ºC for at least 48h, and loaded onto a CN analyzer (varioMicro Cube; Elementar Americas, Mt. Laurel, NJ, USA). Duplicate filter and media blanks were run per standard procedure. To determine the concentrations of the other elements, we used an automated ICP-OES analyzer (iCAP 7400; Thermo Scientific, Waltham, MA, USA). Ten mL of thawed algae were filtered onto cellulose acetate filters and digested using 800 µL of HNO_3 and 400 µL of H_2O_2 for 24h at room temperature, diluted to a volume of 10 mL with ultrapure (Type 1) water, and injected into the ICP. Validation and calibration of the ICP-OES was achieved by using multi-element external reference standards (CCV Standard 1, CPI International, Santa Rosa, CA, USA). Additionally, an in-line Yttrium internal standard (Peak Performance Inorganic Y Standard, CPI International, Santa Rosa, CA, USA) was used to correct for any instrument drift or matrix effects. For each treatment duplicate filter and digestion blanks, consisting of diluted HNO_3 and H_2O_2 with no sample, were also run to correct for background concentrations.

Daphnia *life history:* The *Daphnia magna* clone studied was isolated from a pond in Belgium (Decaestecker et al. 2005) and maintained in the laboratory at ~20ºC and 18:6 light:dark cycle. Life history experiments were started from great granddaughters of females taken from stock cultures. Twenty-seven third-clutch neonates born within 24 h were individually transferred into beakers with 100 mL of modified ADaM medium (Klüttgen et al. 1994; containing 0.33 g/L of Meersalz© which has ~6ng of Fe per kg) and fed respective algae (HiFe= 9; MedFe= 9; LoFe=

9) at a concentration of 10 x 10^6 cells $mL^{-1}day^{-1}$ (approximating to 1 mg C $mL^{-1}day^{-1}$ which is well above incipient limiting concentration; Lampert 2011). Life history of individuals were characterized following Jeyasingh & Weider (2005). Briefly, animals were measured on day 0 and day 4 to obtain juvenile growth rate (as mm day^{-1}) and upon first reproduction. For length measurement, animals were transferred in a drop of ADaM onto a glass slide under a stereomicroscope. Upon reproduction, offspring were counted and discarded, and the mother transferred into a fresh jar. Animals were transferred to fresh jars daily for the first 4 days, and subsequently after each clutch was birthed. The experiment was ended when a female had produced the 3rd clutch.

Iron content of eggs and Daphnia *soma:* We tested whether Fe content of *Daphnia* eggs is higher than the rest of the body. Because we were unable to generate enough egg mass to reliably measure Fe concentration using the ICP-OES from the individuals used for the life history experiment described above, this experiment was done approximately two years later at Oklahoma State University using a different clone of *D. magna* (Lind and Jeyasingh 2018) fed *Scenedesmus obliquus* cultured in a continuous flow chemostat in the same HiFe medium used for the aforementioned experiments. We isolated a gravid female and propagated its third clutch progeny for three generations in 500 mL jars fed *S. obliquus* at a concentration of 1 mg C L^{-1}. 250 eggs from approximately 20 third generation progeny with their third clutch of eggs were dissected following Trubetskova and Lampert (1995). These eggs were dried in batches of 50 eggs in 1.5mL microfuge tubes at 60ºC for 48h, weighed and processed for ICP analyses as described above for *Daphnia* samples. The remaining tissue (soma) was transferred onto five 1.5mL microfuge tubes and processed similarly for quantifying Fe content.

Elemental contents of Daphnia *in high and low iron treatments:* We investigated the effect of dietary iron on *Daphnia* Fe content at two iron levels (high and low), omitting the medium Fe treatment due to logistical constraints of generating enough individuals. Juveniles born within 24 h were collected into six groups of 15. Three groups were allocated to high Fe treatment, and 3 groups to the low Fe treatment. To ensure enough biomass for ionomic analyses, the animals were fed 2 mg C $L^{-1}day^{-1}$ of respective algae (i.e. HiFe or LoFe). Offspring were removed and discarded daily. On day 18, all remaining animals were rinsed in fresh ADaM and placed in microcentrifuge tubes and stored frozen for elemental analyses.

Daphnia were isolated from experimental jars in groups of 15, washed in triplicate with ultrapure (Type 1) water before being dried at 60°C for at least 72h. *Daphnia* for CN analyses were dried in pre-weighed tin boats, weighed to 0.0001 mg accuracy using a microbalance (XP2U, Mettler Toledo, Columbus, OH, USA), and introduced into an elemental analyzer as above for algae. *Daphnia* for ICP were dried in pre-weighed cellulose-based paper (Spectrum Inc., Gardena, CA, USA) and transferred to 15 ml trace metal-free polypropylene conical centrifuge tubes (VWR International, Radnor, PA, USA). Samples were digested in 200 μL of trace metal grade 67-70% HNO_3 (BDH Aristar ® Plus, VWR International, Radnor, PA, USA) and 100 μL of trace metal grade 30-32% H_2O_2 (BDH Aristar ® Ultra, VWR International, Radnor, PA, USA). Each sample was then allowed to digest overnight at room temperature (~22°C) until the solution was clear. All digested *Daphnia* samples were then diluted to a final volume of 5 mL with ultrapure (Type 1) water, and injected into the ICP-OES as described above for algae.

Statistical analyses: We used ANOVAs to test the effects of iron supply treatment on algal cell counts, and algal Fe content. A MANOVA was used to test the effects of iron supply treatment on *Daphnia* life history traits (JGR=Juvenile Growth Rate, AFR=Age at First Reproduction, SFR=Size at First Reproduction, fecundity), if the MANOVA was significant, we did subsequent ANOVAs to test the effect of Fe supply treatment on each life history trait. A t-test was used to test the effect of iron treatment on the iron content of *Daphnia* soma and eggs.

To explore correlated changes in the ionomes of algae and *Daphnia* due to iron treatment (and associated shifts in algal and daphniid iron contents), we utilized a nutrient balance approach (Parent et al. 2013). As elaborated in Prater et al. (2019), this approach enables unbiased estimates of multivariate relationships among elements, which avoid violating common statistical assumptions and can be used to describe and interpret elemental interactions in organismal tissues. Nutrient balances represent orthogonal log contrasts of elements derived from binary partitions of multivariate elemental data projected into Euclidean space. To construct these balances, we first performed at PCA on algal or *Daphnia* ionomes. We set the cutoff at 0.5 for factor loading scores, and identified orthogonal elements. We constructed balances along the first three PC axes that cumulatively explained over 90% of variation in both algae and *Daphnia*. Nutrient balances were calculated following Parent et al. (2013): Balance = $\sqrt{(rs \div r + s)\ ln\ [g(c^{+}) \div g(c^{-})]}$, where *r* and *s* represent the number of elements on the left and right side of the balance, and $g(c^{+})$ and $g(c^{-})$ represent the geometric mean of elemental percentages on the left- and right-hand side of the balance, respectively. The effects of iron treatment on the balances thus constructed were analyzed using ANOVAs. All analyses were done in JMP® (Version Pro 13, SAS Institute Inc., Cary, NC, 1989-2019).

Results:

Although several chemical species differed slightly among the three media, the most striking differences were related to Fe (Table S1). The majority of Fe (~70%) was predicted to be present as iron hydroxide in all three media (Table S1, row 73), and the % of FeOH chelated to EDTA increased with media Fe concentration (~2% in LoFe and MeFe and 28% in HiFe; Table S1, row 127).

Concentration of Fe in the culture medium did not impact algal density (Fig. 1a; $F_{2, 51}$= 146.91, P= 0.62), although concentration of Fe in algae differed significantly among all three Fe supply treatments, increasing with Fe concentration of media (Fig. 1b; $F_{2, 6}$= 285.20, P< 0.001). All filter/digestion blanks were below LOD for both the CN analyzer and ICP-OES and thus not used to correct data.

MANOVA revealed significant effects of iron on *Daphnia* life history (Fig. 2; Table 1). Subsequent ANOVAs revealed that juvenile growth rate (JGR) and fecundity were not affected, while size at first reproduction (SFR) and age at first reproduction (AFR) were significantly affected by iron treatment. Post-hoc tests revealed that SFR decreased with increasing Fe, while AFR was approximately 10 days earlier in the HiFe treatment compared to the MedFe and LoFe treatments.

Iron concentration in *Daphnia* was significantly higher in HiFe treatment compared to the LoFe treatment (Fig. 3a; $F_{1, 4}$= 58.50, P= 0.001). Moreover, eggs of *Daphnia* (which wre measured only under HiFe conditions) contained significantly more Fe than the rest of the soma (Fig. 3b; t= 2.96; df= 4; P= 0.041).

In addition to iron, we measured 14 other elements in algae (Fig. S1) and *Daphnia* (Fig. S2). Iron treatment affected the concentration of multiple elements in both algae (Fig. 4a; Table 2) and *Daphnia* (Fig. 5a; Table 3) as captured by the principal components analyses. We constructed two nutrient balances for algae (Fig. 4b), and three for *Daphnia* (Fig. 5b) based on the criteria set (see Methods). Subsequent ANOVAs on these balances revealed significant effects of iron treatment on one balance each for algae and *Daphnia.* Algae in LowFe treatment exhibited substantially lower balance score for the Al,Ca,Cu,K,Mg,Na,S,Si,Sr,Zn,P,N | Fe balance ($F_{2, 6}$= 410.38, P< 0.0001) compared to the MedFe and HiFe treatments (which did not differ from each other). This means that as algal Fe increased with increasing Fe supply (Fig. 1b) the concentration of the 12 elements in the numerator decreased. *Daphnia* growing on LowFe algae exhibited significantly higher values for the Ca,K,Mg,Mn,Na,S,Sr,Zn | Cu,Fe,Si balance compared to counterparts in HiFe algae ($F_{1, 4}$= 16.85, P= 0.01). This means that as Fe concentration in *Daphnia* increased when fed HiFe algae (Fig. 3a) the concentration of Cu and Si also increased, while that of the other 8 elements decreased.

Discussion:

Increasing the concentration of Fe in algal culture medium produced algae with higher Fe contents (Fig. 1b). Experimental Fe amendment impacted *Daphnia* life history, particularly its reproductive traits as opposed to juvenile growth (Fig. 2; Table 1). Concentration of Fe in *Daphnia* was higher in the HiFe treatment (Fig. 3a), and Fe content of eggs was higher compared to the soma (Fig. 3b). Together, these results clearly show that changes in Fe supply to algae impacts Fe concentration in algae, and *Daphnia* ingesting such algae exhibit differences in life history, potentially due to higher Fe demand of eggs. Furthermore, changes in the supply of one element invoked shifts in the availability (Table S1) and concentration (Figs. 4 and 5; Tables 2 and 3) of multiple other elements.

The significant increase in Fe concentration of algae (Fig. 1b), with no corresponding changes in the density of algal cells among Fe treatments (Fig. 1a) indicates that much of the Fe was likely adsorbed to the surface of algal cells or stored inside cells, and not utilized by algae for anabolic purposes, although little is known about either process in freshwater algae in general, and *Acutodesmus* in particular. In natural waters, bioavailability of Fe is strongly dependent on chelators (e.g., DOC; note that EDTA performs this role in artificial media; Kilham et al. 1998), and such chelators play an important role in protecting phytoplankton from trace metal toxicity (Worms et al. 2006). Even in the pelagic zones of oceans where Fe concentration is vanishingly low, significant proportions of Fe can be adsorbed to the surface of algal cells (Hassler and Schoemann 2009). As such, it is likely that zooplankton in high Fe lakes ingest more Fe with important implications for life history. Note that total Fe in oligotrophic lakes ranges from 0.03 - 33µg/L (Sterner et al. 2004; Vrede and Tranvik 2006; North et al. 2007), and Lind et al. (in review) found total Fe concentrations in eutrophic Oklahoma reservoirs ranging between 30 - 1100µg/L.

Juvenile growth rate was not altered in response to the concentration at which Fe was supplied to their algal diet (Fig. 2), supporting prior observations (Lind and Jeyasingh 2018). Moreover, reproductive traits (Fig. 2) responded similarly to prior work reporting impacts of Fe on the reproductive traits of daphniids (Lind and Jeyasingh 2018), other crustaceans (Chen et al. 2011), and insects (Zhou et al. 2007), indicating iron-intensive oogenesis in arthropods. Data on Fe concentration in the soma of *Daphnia* (Fig. 3a) and eggs (Fig. 3b) further indicate an important role for Fe in *Daphnia* oogenesis. In addition, the roughly ten day difference in maturity between the HiFe treatment compared to the LowFe and MedFe treatments (Fig. 2b) suggests large impacts on demographics and population growth parameters of daphniids inhabiting lakes with differing Fe supplies, potentially with important ecological ramifications (Boersma 1997).

Reflecting biochemical and physiological complexities, changes in the supply of one invokes correlated shifts in the supplies and processing of multiple other elements (Baxter 2015). Decreasing concentrations of some elements in HiFe algae (Fig. 4) could be a function of differential availability. While increased Fe decreases P availability in oxygenated waters (Likens 2010), similar interactions of Fe with other elements have been observed to occur at various levels (e.g., Mn, Chua and Morgan 1996; Cu, Gulec and Collins 2014; Si, Mloszewska et al. 2018), although such pairwise analyses and interpretation can be misleading. The observation that trace metals such as Cu, Mn, Zn were higher in LoFe, in addition to the lack of changes in cell density (Fig. 1a), indicate that Fe supply was not limiting algal growth. The concentrations of these trace metals should increase with rates of protein synthesis and growth because they act as cofactors when bound to enzymes, while causing oxidative stress when they are not bound to ligands (Worms et al. 2006). Such post-assimilatory interactions among nutrient can be more important for nutrition than intake (O'Dell 1989).

Results from the nutrient balance analyses (Parent et al. 2013) revealed that while algae in the LowFe treatment were lower in Fe concentration compared to MedFe and HiFe treatments, they had higher concentrations of Al, Ca, Cu, Fe, K, Mg, Mn, N, Na, P, S, Si, Sr, and Zn (i.e. all elements measured, except C). This observation indicates that increased Fe supply in the MedFe and HiFe treatments may inhibit uptake and/or retention of multiple other elements, although such shifts were not substantial enough to limit algal growth (Fig. 1a). Higher Fe availability can hijack or impede other metal-specific as well as bulk element transporters (e.g., Sousa et al. 2018), potentially explaining lower concentrations of these other elements. However, other mechanisms are likely contributing to this pattern, including the possibility of excess Fe inhibiting protein synthesis by diverting ATP to pumping out Fe from protein anabolism (Worms et al. 2006), a pattern consistent with our observation of lower bulk element (e.g., N, P, S) concentration.

Daphniid ionomes varied significantly with iron treatment (Fig. 5). The nutrient balance revealed that LowFe *Daphnia* contained lower concentrations of Fe, Cu and Si while concentrations of Ca, K, Mg, Mn, Na, S, Sr, and Zn increased compared to daphniids in the HiFe treatment. In the context of algal ionomic shifts (Fig. 4), patterns in the *Daphnia* ionome indicate that Fe, Ca, K, Mg, Mn, Na, S, Sr, Zn contents in *Daphnia* tracked algal ionomic patterns. While this may be surprising because *Daphnia* do not depend on dietary supply for some of these major ions (e.g., Ca; Tan & Wang 2009), interactions between major ions and trace elements are known to be of importance for *Daphnia* (e.g., Karimi & Folt 2006; Lind & Jeyasingh 2018). On the other hand, Cu and Si occupied opposite sides of the balance in algae and *Daphnia*, potentially indicating strong regulation of these elements in daphniids because they are known to be toxic (e.g., de Schamphelaere & Janssen 2002; Karimi et al. 2019). Such ionomic data should be useful in understanding the mechanisms by which Fe impacts expression of life history traits in ecologically important taxa, and in deciphering vast transcriptomic differences among life stages (Campos et al. 2018). Finally, note that the contents of C, N, P, and Al in *Daphnia* were not part of any nutrient balances constructed, indicating that they are relatively homeostatic. A common observation particularly for C, N, and P (Sterner & Elser 2002).

Overall, these results, along with prior work on a variety of taxa indicate that differential Fe supply to primary producers impact reproductive traits in metazoan consumers (Kurama et al. 1995; Dunkov et al. 2002; Zhou et al. 2007; Chen et al. 2011; Lind and Jeyasingh 2018). Such large effects of dietary Fe on reproductive traits should alter demographic parameters, with potentially important ecological consequences particularly as Fe supply to lakes increases (Bjorneras et al. 2017). Further, this work highlights the importance of considering interactions among elements in impacting the life history of ecologically important taxa as global biogeochemistry continues to change rapidly. Importantly, such correlated shifts in the ionomes of taxa in response to changes in Fe supply as documented here may also be relevant for changes in other elements such as P (Jeyasingh et al. 2017; in review). Such ionome-wide data may be essential for a robust understanding of biotic responses to changes in biogeochemical supplies (Jeyasingh et al. 2014; Peñuelas et al. 2019).

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Table 1 *Daphnia* life-history responses to iron treatment. The MANOVA testing for significant effect of Fe treatment was significant (Pillai's Trace for effect of iron treatment= 1.75; F= 38.35; P< 0.0001). Results of subsequent ANOVAs on Type III sums of squares of each trait are furnished in the table. JGR = juvenile growth rate (mm day^{-1}), SFR = size at first reproduction (mm), AFR = age at first reproduction (days), Fecundity = total number of neonates in the first three clutches. Significant Fe treatment effects are represented in bold.

Variable	*F*	*df*	*P*
JGR	1.137	2,23	0.338
SFR	88.624	2,23	**0.000**
AFR	116.060	2,23	**0.000**
Fecundity	2.333	2,23	0.120

Table 2 Loading scores along the first three principal component axes accounting for 93.66% variation in the algal ionome.

Element	PC1 (70.13%)	PC2 (13.71%)	PC3 (9.81%)
Al	0.71212	0.04897	0.59083
Ca	0.80251	0.33836	-0.2515
Cu	0.94853	0.03932	0.07147
Fe	-0.91337	0.30304	0.16367
K	0.94777	-0.10586	-0.10237
Mg	0.95077	-0.16828	0.10434
Mn	0.97434	-0.20075	-0.06352
Na	0.70092	0.17841	-0.65389
S	0.96634	-0.08973	0.08049
Si	0.94298	-0.30896	-0.10695
Sr	0.84148	-0.18789	0.49642
Zn	0.68864	0.50848	-0.38403
P	0.98427	-0.01329	-0.00178
N	0.64483	0.64301	0.38066
C	0.05035	0.96135	0.13764

Table 3 Loading scores along the first three principal component axes accounting for 95.76% variation in the *Daphnia* ionome.

Element	PC1 (51.39%)	PC2 (29.56%)	PC3 (14.80%)
Al	0.0396	-0.90137	0.30674
Ca	0.99205	-0.05794	0.11149
Cu	-0.67232	0.31122	-0.61471
Fe	-0.56989	0.79042	0.20931
K	0.99198	0.04471	0.11597
Mg	0.91751	0.30168	0.21738
Mn	0.70032	-0.41867	-0.54991
Na	0.97788	-0.20547	0.03728
S	0.9523	0.22885	-0.13497
Si	-0.56069	-0.33807	0.64883
Sr	0.98581	-0.06559	0.15007
Zn	0.5195	0.77803	0.15748
P	0.31433	0.81034	0.41326
N	-0.25465	-0.50686	0.78671
C	-0.21642	0.94706	0.22904

List of Figures

Fig. 1 (**a)** Density of *Acutodesmus* cells per µL in the three Fe supply treatments. Boxes indicate 1st and 3rd quartiles, with the line representing the median, while the whiskers indicate the maximum and minimum observed values (n= 12-21). No differences among treatments were found. (**b)** Concentration (mean ± 1SD; n= 3) of Fe in algae cultured in the three Fe supply media. Concentration differed significantly among all three treatments.

Fig. 2 *Daphnia* life-history traits in response to iron supply (n= 8-9). (**a)** JGR = juvenile growth rate (mm day^{-1}), (**b)** SFR= size at first reproduction (mm), **(c)** AFR= age at first reproduction (days), and (**d)** Fecundity (total neonates in first three clutches). Boxes indicate 1st and 3rd quartiles, with the line representing the median, while the whiskers indicate the maximum and minimum observed values (see Table 1 for MANOVA results).

Fig. 3 (**a)** Iron content of *D. magna* in low and high Fe treatments (n= 3). (**b)** Iron content of eggs and soma of *D. magna* in HiFe conditions (n= 5). Mean (µg Fe per mg of dry *Daphnia* mass) ± 1 standard deviation are presented.

Fig. 4 (a) Plot of the first two principal components axes accounting for variation in 15-element algal ionome. Circles indicate locations of each sample. Open circles are from LowFe algae, while closed circles are from MedFe (grey) and HiFe (black) treatments, respectively. **(b)** Mean ± 1SD of the nutrient balances constructed from the PCA. The Al|Na balance did not significantly differ among treatments, while the other balance differed significantly between LoFe compared to the MedFe and HiFe treatments.

Fig. 5 (a) Plot of the first two principal components axes accounting for variation in 15-element *Daphnia* ionome. Open circles indicate locations of each sample from the LowFe treatment, while closed circles are from the HiFe treatment. **(b)** Mean ± 1SD of the nutrient balances constructed from the PCA. The Ca,K,Mg,Mn,Na,S,Sr,Zn | Cu,Fe,Si balance value was significantly different between the two Fe treatments, while the other two balances were not.

Figure 1

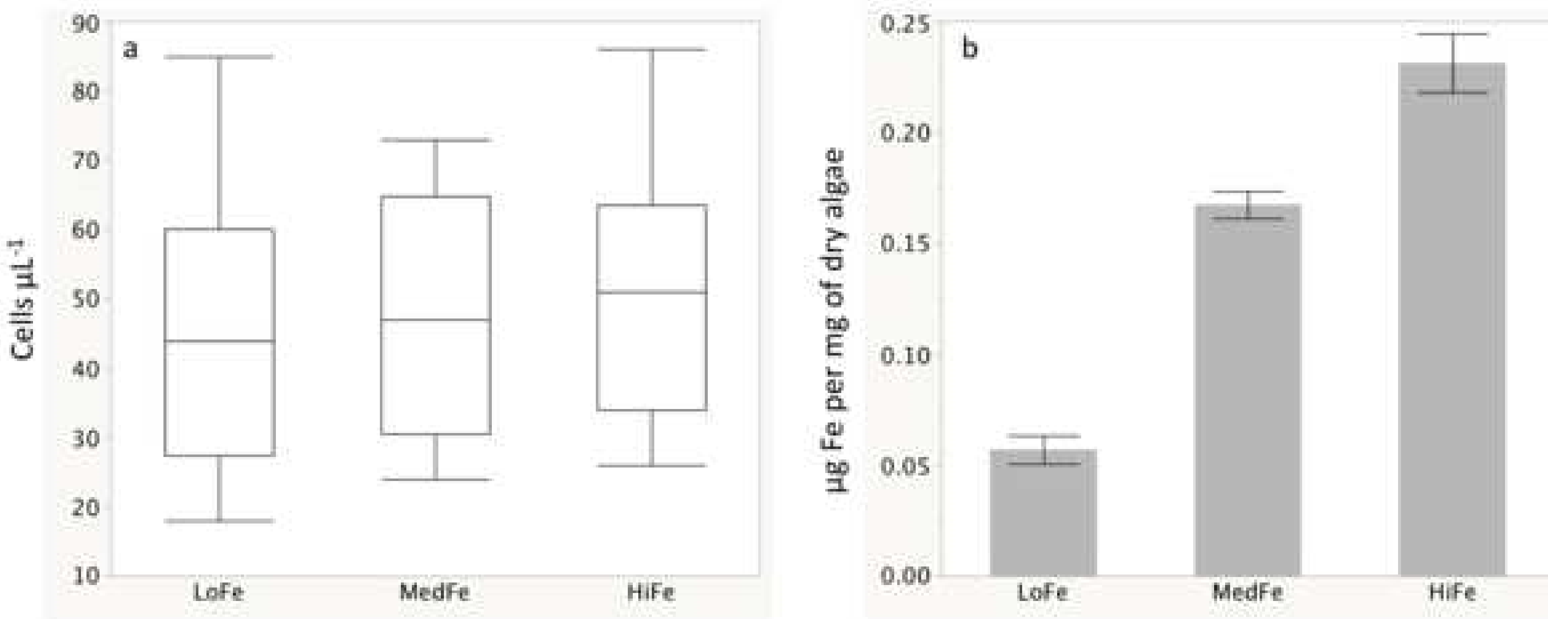

a
Cells µL⁻¹
90
80
70
60
50
40
30
20
10
LoFe
MedFe
HiFe
b
µg Fe per mg of dry algae
0.25
0.20
0.15
0.10
0.05
0.00
LoFe
MedFe
HiFe

Figure 2

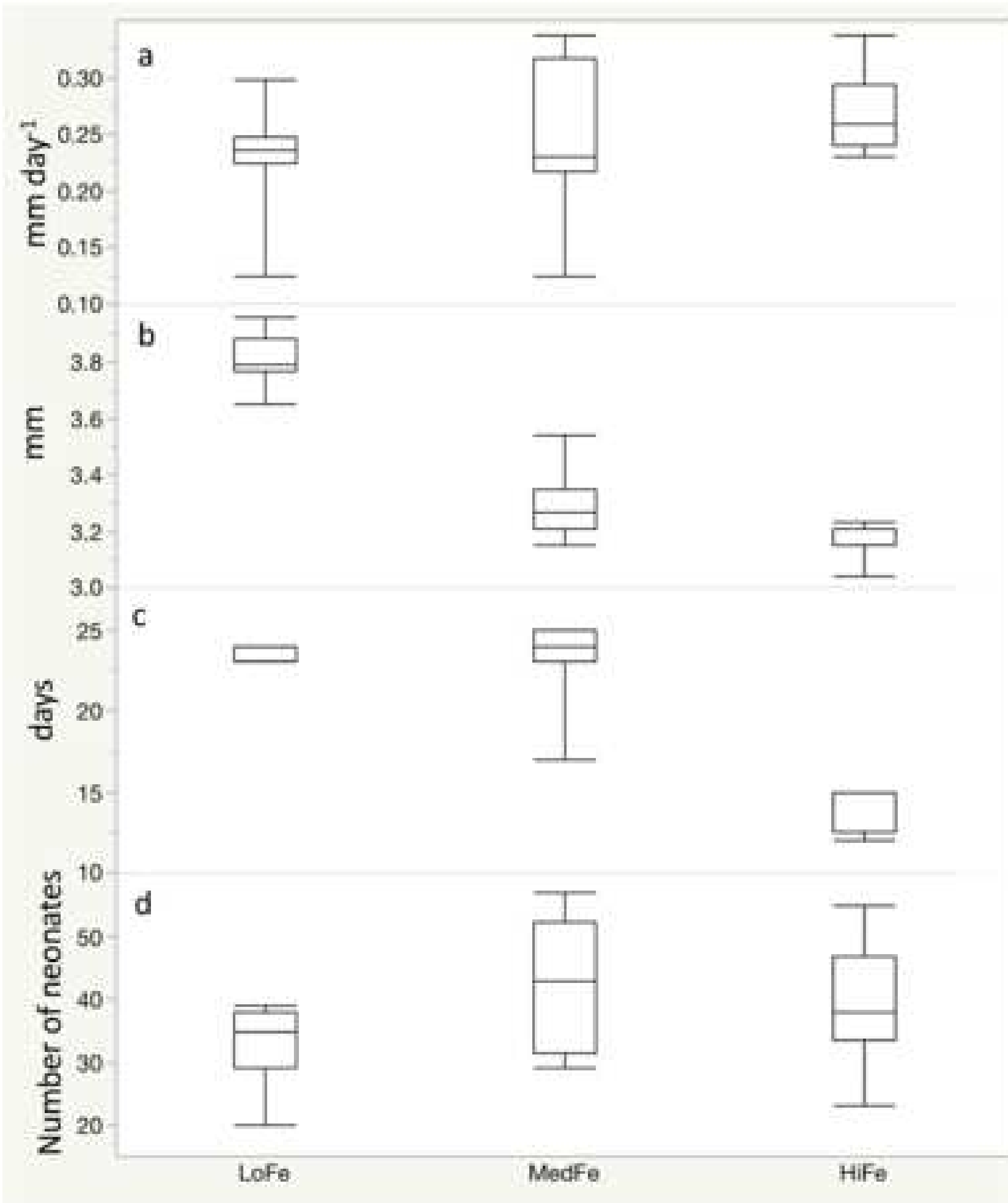
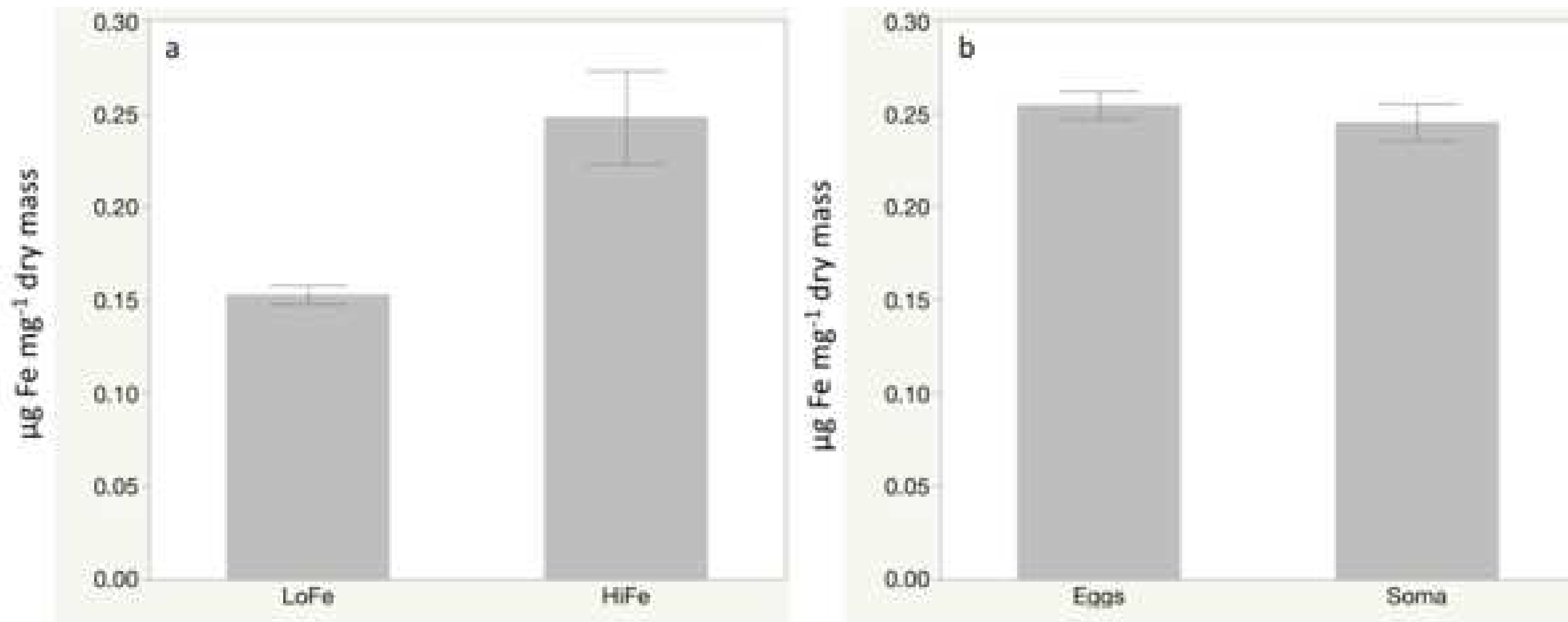

a
b
c
d
mm day-1
mm
days
Number of neonates
0.30
0.25
0.20
0.15
0.10
3.8
3.6
3.4
3.2
3.0
25
20
15
10
50
40
30
20
LoFe
MedFe
HiFe

Figure 3



a
b
0.30
0.25
0.20
0.15
0.10
0.05
0.00
μg Fe mg-1 dry mass
LoFe
HiFe
Eggs
Soma

Figure 4

Click here to access/download;Figure;4 Algal ionome.tiff

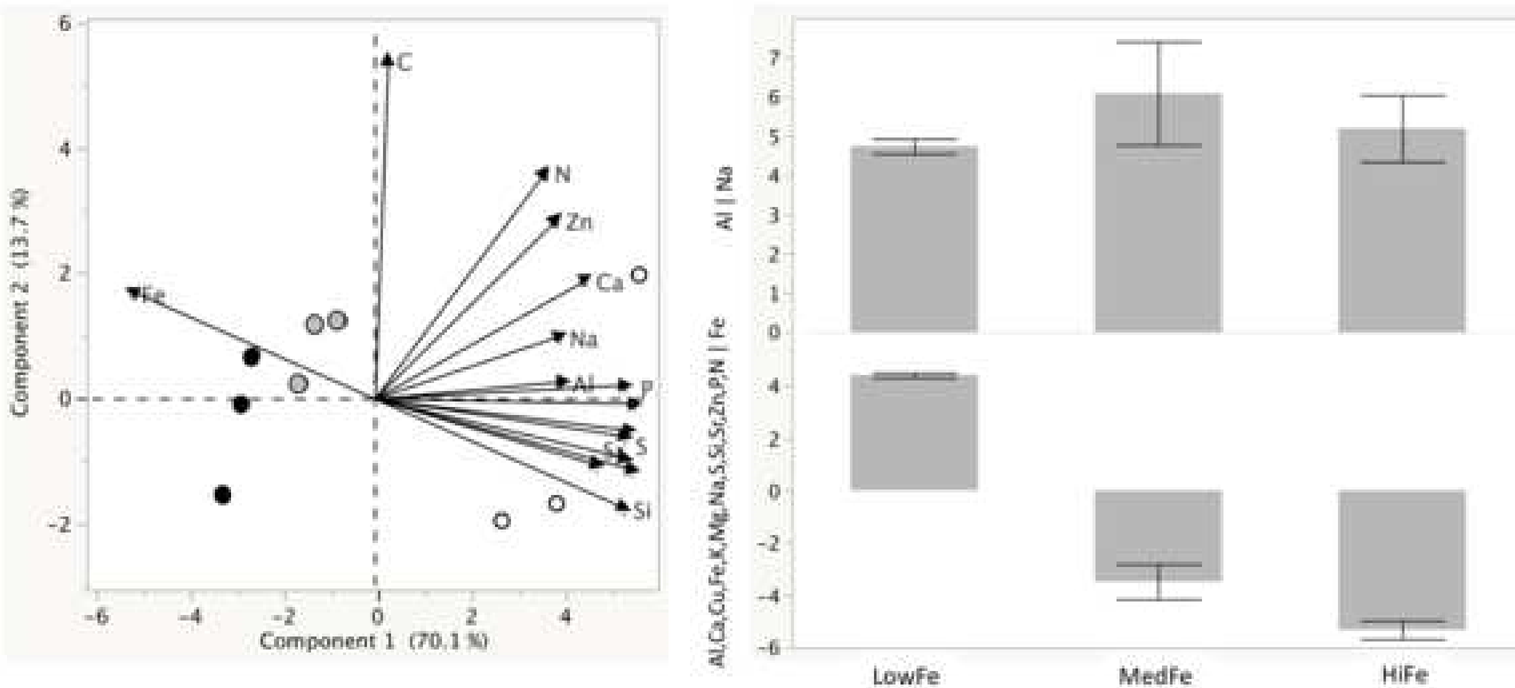
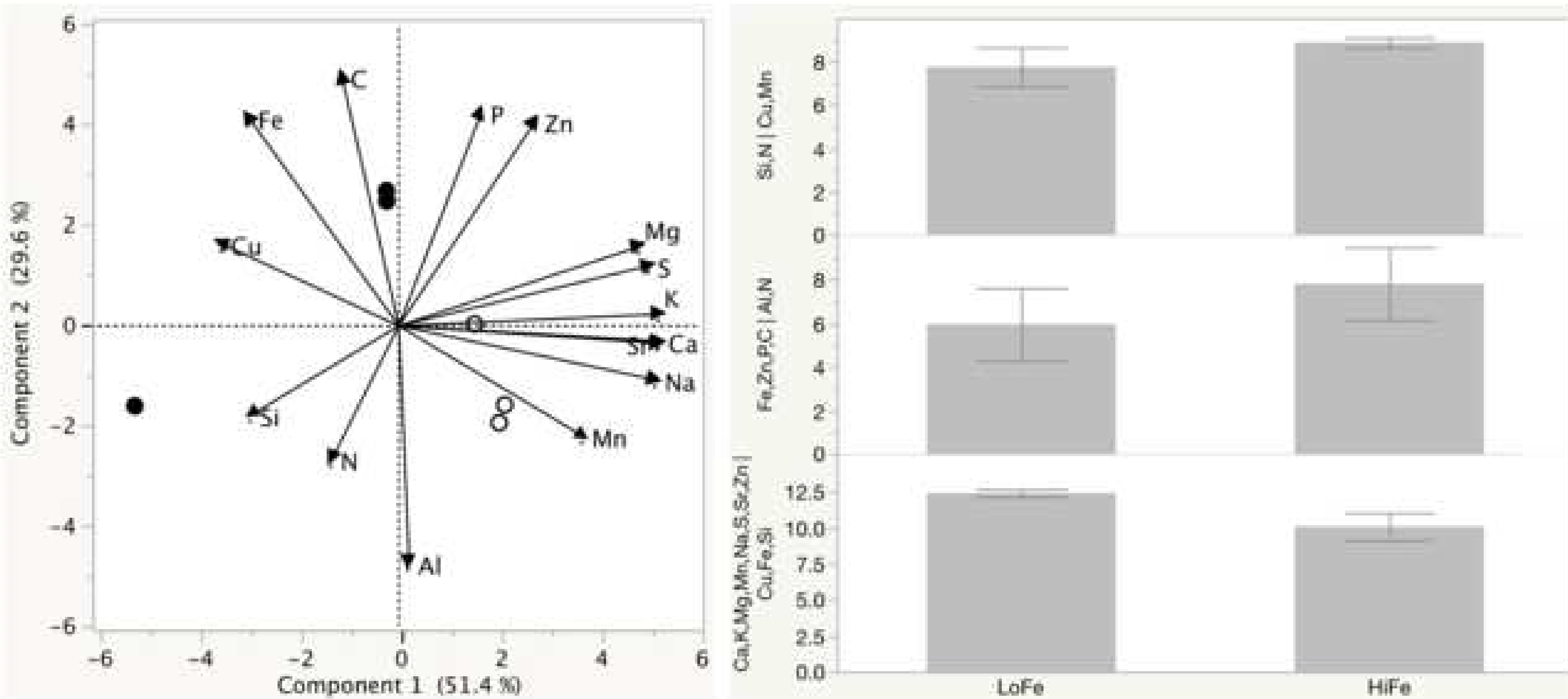


Figure 5

Click here to access/download;Figure;5 Daphnia ionome.tiff



ESM1

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