UNIVERSITÉ DU QUÉBEC À MONTRÉAL

LES EFFETS DU FER SUR LES COMMUNAUTÉS DE PLANCTON D'EAU DOUCE

MÉMOIRE

PRÉSENTÉ

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PAR

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Fe : Iron

TP : Total phosphorus

GAM : Generalized additive model

LM: Linear model

PCoA: Principal coordinates analysis

USEPA: US environmental protection agency

FEQG: Federal environmental quality guideline

FWQG: Federal water quality guideline

ADN: Deoxyribonucleic acid

DOC: Dissolved organic carbon

EC: effective concentration

LISTE DES SYMBOLES ET DES UNITÉS

ind : individuals

L : liter

ind/L: individuals per liter

μg : micrograms

mg: miligrams

RÉSUMÉ

La biodiversité des lacs et rivières d'eau douce de la planète est menacée par de nombreux types de perturbations anthropogéniques, notamment la pollution des habitats aquatiques associée à l'activité minière. Des recherches approfondies ont été consacrées à la compréhension des sources, du devenir et de la bioaccumulation des métaux lourds issus de la pollution minière dans les écosystèmes aquatiques. La compréhension des effets écologiques du fer (Fe) dans les écosystèmes aquatiques a cependant fait l'objet de beaucoup moins d'attention et ce, malgré l'augmentation alarmante des concentrations de fer dans les eaux de surface d'Europe et d'Amérique du Nord. De plus, nous manquons de connaissances sur la façon dont les ajouts de fer dans l'environnement affectent les organismes pélagiques à la base du réseau alimentaire aquatique, en particulier dans les assemblages de communautés ayant des interactions écologiques. L'objectif de ce projet de mémoire était d'examiner expérimentalement les effets d'un gradient de concentration de particules de Fe sur les assemblages de communautés de phytoplancton et de zooplancton d'un lac naturel. Nous avons prédit que l'ajout de Fe 1) réduirait la biomasse du phytoplancton par une interaction entre le Fe et le phosphore total, 2) favoriserait l'abondance relative des cyanobactéries en raison de leur résistance à l'ombre et de leur capacité à transformer le Fe non biodisponible en une forme biodisponible grâce à l'utilisation de sidérophores, 3) causerait une diminution de la biomasse et la densité des zooplanctons en raison d'une diminution du phytoplancton, et 4) aurait un effet négatif plus important sur les cladocères que sur les copépodes et rotifères en raison de différences dans l'alimentation. Le Fe a eu un impact négatif important sur la densité totale du zooplancton, les copépodes et les rotifères, et il a été particulièrement néfaste pour les cladocères. L'étude fournit des perspectives uniques sur la façon dont la pollution par le Fe peut avoir sur les assemblages de communautés de plancton dans les lacs. Nos résultats mettent en évidence une préoccupation pour les réseaux alimentaires aquatiques confrontés à une exposition élevée au Fe, en raison des effets néfastes précédemment non documentés sur les communautés de zooplancton, et de leur rôle clé dans la liaison entre les producteurs primaires et les consommateurs supérieurs dans les réseaux alimentaires aquatiques.

Mots clés : EC_{50} des communautés, phytoplancton, rotifères, cladocères, copépodes, activité minière.

INTRODUCTION

Les écosystèmes d'eau douce sont parmi les écosystèmes les plus menacés sur Terre (Dudgeon et al., 2006). Les écosystèmes d'eau douce contiennent environ 10% de toutes les espèces animales et environ 30-50% de tous les vertébrés (Geist, 2011). Cependant, malgré leur importance écologique connue, le déclin de la biodiversité dans les écosystèmes d'eau douce dépasse largement la perte de biodiversité dans les écosystèmes terrestres et marins (Pérez-Jvostov et al., 2020; Reid et al., 2019). Une diminution de 83% de l'abondance des populations de vertébrés aquatiques a été observée depuis 1970, et 1/3 de toutes les espèces d'eau douce sont maintenant considérées comme menacées dans la Liste rouge de l'Union Internationale pour la Conservation de la Nature (Pérez-Jvostov et al., 2020). La biodiversité des écosystèmes d'eau douce est particulièrement importante, car elle fournit des services économiques précieux tout en étant un élément de protection contre les événements inattendus qui sont néfastes pour l'environnement (Dudgeon et al., 2006). La biodiversité des écosystèmes d'eau douce est actuellement menacée par cinq grandes catégories de dangers: la modification du débit, l'invasion d'espèces, la surexploitation, la pollution de l'eau et la dégradation de l'habitat (Dudgeon et al., 2006).

La perte de biodiversité en eau douce est exacerbée par une augmentation mondiale des activités d'extraction de métaux à travers le monde. L'extraction du fer peut avoir des répercussions importantes sur les communautés aquatiques et les écosystèmes en raison d'effets toxiques directs et indirects sur l'environnement. L'extraction du fer implique certains des plus grands mouvements de masse et dépôts de déchets qui deviennent oxydés et altérés par l'exposition environnementale, qui s'infiltrent ensuite dans les

ressources aquatiques (Schwarzenbach et al., 2010). Les études concernant les impacts environnementaux aquatiques de l'extraction de minerai de fer se sont le plus souvent concentrées sur le drainage minier acide. Un drainage minier acide est la création de ruissellements très acides produits par l'oxydation de minéraux sulfurés, et leurs métaux lourds associés, lors de l'excavation de gisements minéraux (Hogsden & Harding, 2012). Ultimement, le drainage minier acide peut provoquer l'acidification des milieux d'eau douce, ainsi qu'entraîner une élévation de la concentration de métaux dissous, tels que le fer (Hogsden & Harding, 2012). Le drainage minier acide peut également atteindre des lacs et des cours d'eau de pH neutre par ruissellement, où le Fe(III) peut alors précipiter sous forme d'oxyde de fer ou d'oxyhydroxyde de fer (Kotalik et al., 2019), une forme de fer particulièrement néfaste (Cadmus, Brinkman et al., 2018; Linton et al., 2007). Les oxydes et oxyhydroxydes de fer nocifs peuvent également atteindre les plans d'eau douce de manière atmosphérique via les poussières (Petrovský et al., 2020), et naturellement par des changements hydrologiques environnementaux (Björnerås et al., 2017). Le fer peut également être ajouté intentionnellement dans les écosystèmes d'eau douce comme méthode de restauration des plans d'eau eutrophiques pour contrer la quantité de phosphore (Bakker et al., 2016 ; Immers et al., 2015). Le fer dans l'environnement peut avoir de multiples effets négatifs. Par exemple, les effets toxiques indirects sont décrits comme des processus qui se produisent par la modification physique de l'habitat (Kotalik et al., 2019). Certains des effets toxiques indirects du fer ferrique sont le remplissage de l'espace interstitiel (habitat utilisable pour les organismes benthiques) et la diminution de la production primaire aquatique par étouffement (Kotalik et al., 2019; McKnight & Feder, 1984; Wellnitz & Sheldon, 1995) et réduction de la pénétration de la lumière (Kotalik et al., 2019).

Les gouvernements, à la suite des dangers accrus sur la biodiversité et les activités minières, sont en mesure de créer des limites afin de réduire l'impact écologique. La

recommandation canadienne actuelle est de 0,3 mg/L (CCME, 1987), mais de nouvelles recommandations sont également en cours d'étude. Les Recommandations Fédérales canadiennes pour la Qualité de l'Environnement (RFQE), par exemple, fournissent des points de référence numériques pour la qualité acceptable de l'environnement. En tant qu'outil, les RFQEs sont utilisés pour évaluer, prévenir et gérer les risques. Des RFQEs peuvent sortir des lignes directrices plus précises, telles que les Recommandations Canadiennes pour la Qualité de l'Eau en vue de Protéger la Vie Aquatique (RCQE-PVA). Les RFQEs, dans le cas de la concentration de fer dans les milieux d'eau douce, sont fondées sur des données de toxicité recueillies dans le cadre d'études publiées jusqu'en 2023 portant souvent sur une seule espèce (ECCC, 2024). Cependant, les études utilisées pour les lignes directrices sur la qualité ne semblent pas intégrer suffisamment de données à l'échelle de l'écosystème, où de nombreuses espèces, avec différents stades de vie et habitudes, forment des écosystèmes complexes. Étant donné que le Fe(II) est le plus souvent oxydé en Fe(III) dans les milieux aquatiques naturels dans de nombreuses conditions environnementales, la RFQE a été développée en utilisant le Fe(III). Les RFQE, qui dépendent du pH et du carbone organique dissous (COD), dépendent des paramètres physicochimiques avec lesquels les recommandations peuvent alors être établies (ECCC, 2024). Toutefois, cette recommandation pour la qualité de l'eau est fondée sur les concentrations de toxicité chronique et, surtout, très souvent sur les réponses d'une seule espèce. Pendant ce temps, la concentration de fer recommandée aux États-Unis est de 1 mg/L, encore une fois d'après quelques espèces du réseau trophique (USEPA, 1976). Il y a donc un manque de connaissances sur les réponses écologiques dans les milieux aquatiques à différents niveaux trophiques du réseau trophique.

De nombreuses études ont porté sur les effets toxicologiques du fer au niveau individuel (Bakker et al., 2016; Vuori, 1995), mais peu d'études ont examiné et quantifié les effets indirects du fer sur les communautés aquatiques dans les milieux

d'eau douce (Kotalik et al., 2019). Mon projet de recherche examinera les effets de l'exposition de fer sur les communautés de plancton. Une expérience similaire a été réalisée pour les communautés de macroinvertébrés et de phytoplancton (Kotalik et al., 2019), mais mon expérience sera la première qui étudiera les effets d'une exposition *in situ* au fer avec les changements potentiels de communauté de plancton pélagique. J'ai mis en œuvre une expérience de mésocosmes sur le terrain pour mesurer les effets de l'ajout de fer à plusieurs niveaux trophiques planctoniques, du phytoplancton au zooplancton. Les expériences de mésocosme sont une excellente approche pour répondre aux questions de recherche qui nous permettent de démêler des mécanismes écologiques spécifiques, ainsi que d'intégrer de nombreux niveaux trophiques, comparativement aux tests de toxicité d'une seule espèce, car ils augmentent le réalisme sans sacrifier la causalité (Kotalik, 2020). Les expériences de mésocosme permettent de tester des facteurs de stress précis sur de nombreux taxons avec des histoires de vie complexes (Kotalik et al., 2019), ce qui n'est pas toujours possible avec des expériences en laboratoire. Les mésocosmes sont donc une excellente méthode pour tester les effets de la pollution par le fer sur la production primaire et le potentiel de cascades trophiques, tout en gérant les conditions générales de l'environnement (Kotalik et al., 2019) et permettre la réplication (Spivak et al., 2011). Mon expérience me permettra également d'évaluer la nouvelle RFQEs du Canada et la recommandation des États-Unis concernant le fer total et de voir si elles sont appropriées dans le contexte d'une étude multiespèces.

0.1 Profil de fer et spéciation

Le fer est l'un des éléments les plus abondants de la croûte terrestre (Taylor & Konhauser, 2011), et est un élément essentiel pour presque tous les organismes vivants (Gurzau et al., 2003). Les organismes aquatiques ne font pas exception à cette règle. Le fer est un élément important pour un large éventail de processus cellulaires, tels que

le transport de l'oxygène, la respiration et la synthèse de l'ADN (Le Caire et al., 2006). La plupart de ces processus sont complétés par l'activité de protéines telles que l'hémoglobine, la myoglobine, les cytochromes et les enzymes, des protéines comportant toutes du fer (Le Caire et al., 2006). Le fer est également une partie importante de l'appareil photosynthétique du phytoplancton (Schoffman et al., 2016). Cependant, toutes les sortes de fer ne sont pas égales pour les organismes. Bien que la spéciation du fer soit complexe, le métal se trouve principalement sous deux formes principales. Tout d'abord, le fer ferreux (également appelé Fe^{2+} ou Fe(II)) est la forme où le fer est le plus biodisponible, car c'est aussi la forme la plus soluble (Kotalik et al., 2019). Alternativement, il existe également le fer ferrique (également appelé Fe^{3+} ou Fe(III)), une forme insoluble de fer, qui se produit souvent sous forme de précipités d'oxyde ou d'oxyhydroxyde de fer dans les lacs et les rivières neutres et bien oxygénés (Linton et al., 2007). Cependant, plusieurs autres variables, comme le carbone organique dissous, la lumière UV et le pH, peuvent avoir un impact sur la spéciation du fer (ECCC, 2019).

Bien que le fer soit un élément essentiel, toute concentration de fer qui dépasse la «fenêtre d'essentialité» peut avoir des effets toxiques. À des concentrations élevées, les effets négatifs du fer comprennent des perturbations des membranes cellulaires, des protéines et des pigments, et il peut même causer des dommages à l'ADN et la mort. Cependant, l'oxyde de fer agit différemment sur les organismes par le biais d'effets toxiques directs et indirects (Bakker et al., 2016)

0.2 Effets toxicologiques de l'oxyde de fer dans les milieux aquatiques

Effets toxiques directs

Bien que les oxydes de fer soient moins biodisponibles que les formes de fer dissous, le fer particulaire peut également avoir des effets directs sur la faune aquatique. En effet, on a émis l'hypothèse que les précipités de Fe(III) peuvent causer des effets toxiques directs par l'exposition diététique. Certains animaux, comme les moules, ont même montré la capacité d'absorber les oxydes de fer par endocytose (Vuori, 1995). Cependant, bien que l'effet toxique direct des oxydes de fer soit possible, les effets toxiques indirects sont bien au-delà des plus testés et identifiés (Cadmus, Brinkman and al., 2018; Vuori, 1995). Aucune information concernant les effets toxiques directs des oxydes de fer sur les communautés de plancton n'a été trouvée.

Effets toxiques indirects

Comme il a été présenté précédemment, les oxydes de fer peuvent causer un éventail d'effets indirects négatifs sur les organismes aquatiques. Le premier étant le couvrement de ces précipités sur les surfaces biologiques, conduisant à des effets sur la survie, la reproduction et le comportement des organismes aquatiques (Smith et al., 1973; Smith & Sykora, 1976; Vuori, 1995). Les effets indirects des oxydes de fer sont principalement observés à travers leur accumulation sur les branchies des poissons (Linton et al., 2007; Vuori, 1995). Ces mêmes précipités de fer peuvent également affecter la reproduction, car des études ont montré qu'ils peuvent obstruer la surface des œufs de truite arc-en-ciel et de tête-de-boule (Vuori, 1995; Weatherley et al., 1991), ce qui entraîne une diminution du succès de l'éclosion (Bakker et al., 2016). Cela soulève également la possibilité que ce processus se produise aux zooplanctons, qui peuvent aussi se reproduire par des œufs. Il a également été démontré que les daphnies, connues pour filtrer, peuvent être indirectement affectées par les oxydes de fer en raison d’une diminution de l'efficacité du filtrage (Randall et al., 1999) et l'absorption des nutriments (van Anholt et al., 2002). Un autre effet indirect des oxydes de fer provient des évitements comportementaux effectués par certaines espèces afin de ne pas entrer

en contact avec les particules de fer en suspension (Updegraff & Sykora, 1976; Vuori, 1995). D'autres études importantes sur les précipités d'hydroxyde de fer ont également montré qu'ils pourraient avoir une incidence négative sur la répartition, l'abondance et la diversité des poissons, des invertébrés benthiques et du périphyton (Vuori, 1995). Les précipités de fer dans les milieux d'eau douce modifient donc les caractéristiques physiques et la qualité des habitats benthiques (Vuori, 1995), entraînant des effets toxiques indirects.

Les précipités de fer ont également des effets intéressants sur la disponibilité des nutriments et la pénétration de la lumière. Par exemple, il a été rapporté que les hydroxydes de Fe(III) peuvent adsorber et éventuellement précipiter le phosphate (Vuori, 1995), diminuant sa biodisponibilité. Le phosphore est un élément important pour le phytoplancton, et une diminution de phosphore disponible peut entraîner des diminutions de chlorophylle α et la production primaire (Carpenter et al., 1998). La lumière est un autre effet indirect important des hydroxydes de fer(III). Comme les sédiments, les oxydes de fer causent de la turbidité, ce qui peut limiter la pénétration de la lumière (Kotalik et al., 2019; Niyogi et al., 2002). La lumière est un élément important dans la croissance autotrophe et l'activité photosynthétique (Singh et Singh, 2015), et la limitation soudaine de la lumière dans les lacs pourrait alors causer une inhibition de la croissance des algues et du périphyton (Bakker et al., 2016).

0.3 Effets écologiques des oxydes de fer dans les milieux aquatiques

Réponses écologiques sur les communautés de poissons

Comme décrit précédemment, le fer peut être très endommageable, et parfois même mortel, pour les poissons. Il a été démontré que le fer affecte la répartition des poissons, dans le cas des épinoches à neuf épines et à trois épines, où les épinoches à trois épines

évitaient les environnements riches en fer et les épinoches à neuf épines favorisaient les environnements riches en fer (Verberk et al., 2012). Il a également été démontré que les oxydes de fer réduisent la répartition, l'abondance et la diversité des poissons (Vuori, 1995). Cependant, les effets écologiques précis des oxydes et oxyhydroxydes de fer sur les communautés restent inconnus, car un accent majeur a été mis sur ses effets toxicologiques.

Réponses écologiques sur les communautés de macroinvertébrés

Les macroinvertébrés constituent une partie importante du réseau trophique, car ils sont essentiels au fonctionnement de l'écosystème en raison de leur impact sur la production secondaire et la décomposition (Carlisle & Clements, 2005). Certaines études sur les effets du fer sur les macroinvertébrés ont été réalisées sur des stades larvaires émergents, car ils sont particulièrement sensibles pendant cette période, en raison de leur taux élevé de différenciation cellulaire et de réorganisation tissulaire (Campero et al., 2008). Kotalik et al. (2019) ont constaté que le fer était plus toxique pour les macroinvertébrés larvaires que les adultes émergents, que la toxicité du fer dépendait du stade de vie et que la toxicité du fer entravait davantage les taxons broutteurs-racleurs que les autres taxons de macroinvertébrés. Il a également été démontré que le fer réduit l'abondance de la plupart des macroinvertébrés et modifie la composition de la communauté, la sensibilité étant variable parmi les groupes de macroinvertébrés (Cadmus, Guasch et al., 2018).

Réponses écologiques dans les communautés de phytoplancton et de zooplancton

Peu d'études ont examiné les communautés de phytoplancton et de zooplancton comme réponse écologique à l'ajout d'oxyde de fer. Kotalik et al. (2019) ont comparé la biomasse de phytoplancton à travers un gradient d'oxyde de fer. Ils ont constaté que les diatomées et les algues vertes étaient grandement affectées par l'exposition à l'oxyde

de fer, avec une EC_{20} spécifique au groupe parfois bien en dessous de 1 mg/L Fe, la concentration maximale recommandée de Fe aux États-Unis. Cependant, bien que les diatomées et les algues vertes aient pu être réduites, ils ont également signalé que la biomasse des cyanobactéries augmentait tout au long du gradient d'oxyde de fer. Cette découverte concorde avec d'autres recherches concluant que les cyanobactéries sont les principaux producteurs dans les plans d'eau affectés par le drainage minier acide à un pH neutre ou basique (Bray et al., 2008; Kotalik et al., 2019). Cependant, ces résultats ne sont pas constants d'une étude à l'autre. Certaines études ont révélé que l'ajout de fer avait un impact négatif sur les cyanobactéries et réussissait à réduire les proliférations d'algues nuisibles dans les lacs eutrophes (Molot et al., 2010; Orihel et al., 2016). Les cyanobactéries peuvent être la cause de préoccupations environnementales importantes, car elles ont le potentiel de créer des métabolites secondaires toxiques lorsqu'elles se trouvent à des niveaux élevés de biomasse (Sorichetti et al., 2014). Étant donné que les oxydes de fer semblent affecter différemment les groupes de producteurs primaires, nous nous attendons à d'importants changements dans la composition des communautés de phytoplancton, avec des effets subséquents potentiels sur la biomasse et la composition des espèces plus hautes dans le réseau trophique (Kotalik et al., 2019).

À ma connaissance, aucune étude n'a examiné directement les effets des oxydes de fer sur la composition des communautés de zooplancton. Cependant, certaines études axées sur l'ajout de chlorures de fer et de sulfates de fer comme méthode de restauration pour les lacs eutrophes ont examiné les effets des précipités de fer sur le zooplancton. Une de ces études a montré qu'une exposition aux sulfates de fer a causé une diminution de la biomasse totale de zooplancton (Holz & Hoagland, 1996). Ces résultats étaient semblables à ceux d'une autre étude, qui a montré que la biomasse et l'abondance du zooplancton ont diminué de façon significative à la suite de l'ajout de chlorure de fer dans une expérience de mésocosme, et que ces baisses ont été principalement trouvées dans les rotifères et les cyclopoïdes (Wilson, 2013). Cependant, il ne semble pas y avoir

de consensus clair, car d'autres études n'ont signalé aucun effet néfaste des oxydes de fer sur le zooplancton (Jaeger, 1994). Aucune étude ne semble avoir examiné les effets des oxydes de fer sur des communautés entières de zooplancton dans le contexte d'environnements non eutrophes. Toutes les études trouvées concernant les réponses écologiques ont abordé les effets des oxydes de fer comme méthode pour précipiter le phosphore dans les plans d'eau eutrophes, montrant un manque important de connaissances sur ce sujet.

0.4 Études sur les recommandations de fer d'eau douce

Les études utilisées pour établir la RCQE-PVA concernant la concentration totale de fer ont été principalement effectuées à l'aide de mesures faites sur une seule espèce. Seulement quelques espèces de macroinvertébrés ont été étudiées à l'aide de mésocosmes. Au total, six études portant sur l'effet toxique chronique des oxydes et oxyhydroxydes fer sur 27 espèces aquatiques différentes (cinq poissons, 20 invertébrés, une algue et un amphibien) ont été utilisées pour déduire des valeurs recommandées acceptables. Le Canada recommande des seuils de toxicité en fonction du pH et du DOC des lacs et rivières. Les recommandations selon ces paramètres peuvent être retrouvées sur leur site web (ECCC, 2024). La recommandation de 1 mg/L pour les États-Unis est fondée, quant à elle, sur des essais de toxicité fondés exclusivement sur des espèces de poissons (*Cyprinus carpio*, *Esox lucius* et truites non identifiées) (USEPA, 1976)

0.5 Écosystèmes aquatiques et fer

Comme nous l'avons vu précédemment, les précipitations d'oxyde de fer peuvent avoir une incidence sur la pénétration de la lumière dans les écosystèmes aquatiques. Étant donné que la lumière est un facteur important du métabolisme de l'écosystème par ses

effets sur la production primaire et la respiration (Jankowski et al., 2021), les précipitations d'oxyde de fer ont des effets probables sur le métabolisme des écosystèmes aquatiques. Une étude, réalisée par Kotalik et al. (2019), a démontré que l'ajout de fer entraînait des réductions de la biomasse algale et de la productivité primaire, ce qui entraînait des réductions significatives du métabolisme de l'écosystème. Cela a également été démontré dans une étude de Niyogi et al. (2002), dans laquelle les taux de déposition d'oxydes métalliques (aluminium et fer) ont eu des effets négatifs importants sur la biodiversité, la biomasse d'algues et la production primaire nette. De même, des études axées sur la turbidité ont montré que la turbidité peut avoir un impact sur la production primaire brute par la diminution de la lumière dans la colonne d'eau (Hall et al., 2015; Shen et al., 2019). Cependant, à l'exception de ces études, aucune autre recherche que je connaisse n'a abordé les effets écologiques de l'ajout d'oxyde de fer au milieu aquatique sur les réponses des communautés de plancton, malgré l'importance des communautés de planctons dans le fonctionnement des écosystèmes aquatiques.

0.6 Objectifs et prédictions

L'objectif de ma recherche de thèse de maîtrise est d'évaluer expérimentalement les réponses écologiques des communautés de plancton à l'ajout d'oxyhydroxydes de fer à travers un large gradient de concentration, et de voir si l'addition de fer a des effets sur les nutriments et la physicochimie de l'eau.

Prédiction 1: La biomasse du phytoplancton diminuera avec l'augmentation de l'ajout de fer à travers le gradient de fer, tandis que la composition de la communauté se déplacera vers un écosystème plus dominé par les cyanobactéries. Cet effet pourrait être causé par la réduction de la pénétration de la lumière dans la colonne d'eau, causée par des particules en suspension (Kotalik et al., 2019; Niyogi et al., 2002), qui a à la

fois un effet sur la biomasse du phytoplancton (Kotalik et al., 2019) et la composition de la communauté (Bray et al., 2008; Kotalik et al., 2019).

Prédiction 2: La biomasse du zooplancton diminuera avec l'augmentation de la concentration du fer (Holz & Hoagland, 1996; Wilson, 2013) et la composition de la communauté passera de cladocères de grande taille à des copépodes et des rotifères de petite taille (Wilson, 2013). Cela pourrait être causé par une diminution de la qualité et de la quantité de phytoplanctons en tant que source de nourriture ou par d'autres effets toxiques indirects des oxyhydroxydes de fer, tels que des effets négatifs sur les individus filtreurs (Randall et al., 1999).

Pour atteindre ces objectifs, une expérience de mésocosme a été utilisée. Des mésocosmes ont été exposés à diverses concentrations de fer, allant de 0 à 10 mg/L. Les espèces de zooplanctons et de phytoplanctons du lac Hertel (Mont-Saint-Hilaire) ont été inoculées dans les mésocosmes, et la densité et la biomasse du zooplancton ainsi que la biomasse du phytoplancton ont été analysées. Plusieurs autres variables physicochimiques ont également été examinées, comme le phosphore total, la pénétration de la lumière et la concentration de fer dans la colonne d'eau au fil du temps.

Ce mémoire sera fait d'un article scientifique, présenté au chapitre I. Ce chapitre consiste en une expérience de mésocosme au lac Hertel au cours de l'été 2021. Cette introduction a été écrite afin de donner des informations générales concernant cette étude et montre les nécessités pour mieux comprendre les effets écosystémiques de la pollution par le fer. Le chapitre I sera suivi d'une conclusion générale, où les principaux points de ce projet seront mis en évidence, et quelles autres études pourraient être faites.

CHAPITRE I

EXPERIMENTAL IRON ADDITION TO MESOCOSMS ALTERS FRESHWATER PLANKTON COMMUNITIES: INSIGHTS BASED ON COMMUNITY AND ECOSYSTEM METRICS

L'article sera soumis ultérieurement dans Limnology & Oceanography

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1.1 Abstract

Des augmentations généralisées des concentrations de fer (Fe) se sont produites dans les eaux de surface européennes et nord-américaines au cours des dernières décennies. Pourtant, on sait peu de choses sur les effets écologiques du Fe particulaire sur les communautés de plancton et le métabolisme des écosystèmes aquatiques. Nous avons examiné les effets d'un gradient de concentration expérimental de Fe particulaire sur les assemblages de communautés de plancton et l'équilibre métabolique de la communauté d'un lac naturel. Nous avons prédit que l'ajout de Fe particulaire 1) réduirait la biomasse de phytoplancton grâce à une interaction entre le Fe et le phosphore, 2) favoriserait l'abondance relative des cyanobactéries qui prospèrent sous une disponibilité limitée de la lumière, 3) réduirait la biomasse et la densité du zooplancton crustacé (principalement les cladocères) à mesure que les ressources en phytoplancton seraient épuisées, et 4) induirait des changements dans l'équilibre métabolique de la communauté de plancton vers une hétérotrophie nette à la suite de réductions de la croissance du phytoplancton. Comme prévu, les ajouts de Fe ont eu un impact néfaste sur les densités totales de zooplanctons crustacés ainsi que les densité de copépodes et de rotifères, et ils ont été particulièrement nocifs pour les cladocères. Le gradient des traitements de Fe a eu un effet positif significatif de la biomasse des cyanobactéries et un effet négatif sur la biomasse des chlorophytes, mais aucun effet détectable sur la biomasse totale de phytoplancton. De plus, il n'y avait pas de relation cohérente entre l'exposition au Fe et les changements dans l'équilibre métabolique de la communauté. Bien que les réponses métaboliques restent peu claires, nous montrons que la pollution par le Fe a des effets forts et prévisibles sur le fonctionnement et la composition de la communauté de plancton. Nos résultats mettent en évidence une préoccupation pour les réseaux trophiques aquatiques confrontés à une exposition élevée au Fe en raison des effets néfastes précédemment non documentés du Fe particulaire sur les communautés de zooplancton.

Mots-clés : Communauté EC_{50}, phytoplancton, rotifère, cladocère, copépode, pollution par le fer, équilibre métabolique de la communauté

1.2 Introduction

Widespread increases in iron (Fe) concentrations have occurred in European and North American surface waters over the last several decades, because of its association with dissolved organic matter (DOM) and hydrological changes **(**Björnerås et al. 2017). This is a concern because Fe can be harmful to aquatic organisms through direct toxicological effects (soluble, ferrous Fe^{2+}), and through indirect effects mediated by ecological interactions in food webs (Baby et al. 2011). Iron oxyhydroxides have a particularly strong impact on benthic organisms through deposition and smothering, alteration of habitat physicochemical properties, and through reduction of basal resources (Cadmus et al., 2018; Kotalik et al., 2019; Linton et al., 2007; Vuori, 1995). Yet, we lack knowledge of the ecological impacts of Fe oxyhydroxides on freshwater biodiversity and ecosystem function, particularly for planktonic organisms (phytoplankton and zooplankton) that are key for aquatic primary production and trophic energy transfer to higher consumers in aquatic food webs (Ogorelec et al., 2021).

Particulate Fe^{3+} addition to aquatic ecosystems has multifaceted effects on phytoplankton that vary depending on environmental conditions. For example, Fe effects on cyanobacterial biomass vary with lake nutrient content (Sorichetti et al., 2016). Cyanobacteria have a competitive advantage over other phytoplankton for nutrients through a unique Fe scavenging system (Murphy et al., 1976; Sorichetti et al., 2014; Wilhelm & Trick, 1994), especially when low concentrations of bioavailable Fe (ferrous Fe^{2+}) occur in eutrophic (Du et al., 2019) lakes and/or low total Fe concentrations exist in oligotrophic lakes (Sorichetti et al., 2014). Fe forms complex with DOM, and cyanobacteria may uniquely access the DOM-bound Fe to overcome Fe limitation (Sorichetti et al., 2014). As such, Fe additions can enhance cyanobacterial

abundance at concentrations near or below the Fe concentration recommendations set by the US government (Kotalik et al., 2019). Yet, diatoms and chlorophytes have also been found to be negatively impacted by Fe addition because of environmental modifications (Kotalik et al., 2019). This can exacerbate water quality problems (Keeler et al. 2012) by enhancing the frequency and intensity of cyanobacterial blooms, which are increasing in extent and severity due to climate change and eutrophication (Taranu et al. 2015; Ho et al. 2019). On the other hand, Fe oxyhydroxide precipitation can suppress cyanobacterial blooms in highly eutrophic lakes (Molot et al., 2010; Orihel et al., 2016). Fe oxyhydroxides adsorb and precipitate phosphorus (P), thus reducing P availability and suppressing phytoplankton biomass (Bakker et al., 2016; Orihel et al., 2016). This effect can be enhanced when benthic processes resuspend lake sediments and Fe precipitates, thereby restricting primary production to extents that lower production at higher trophic levels (Smith et al., 2011). Suspended Fe can also restrict light transmission through the water column, thereby shading phytoplankton, and potentially suppressing primary production by shade-intolerant phytoplankton groups (e.g., chlorophytes) (Kotalik et al., 2019). Further study is required to understand ecological outcomes of particulate Fe addition on phytoplankton communities and for primary production at the base of aquatic food webs.

Knowledge of the effects of FeO(OH) particulates on zooplankton communities is lacking, particularly for copepods and rotifers (Bakker et al., 2016). This knowledge gap is a concern because zooplankton are an important intermediate trophic link between phytoplankton and higher consumers (Ogorelec et al., 2021). An early study found that Fe addition had no direct effect on *Daphnia* sp. mortality (Randall et al., 1999). However, another study in which *Daphnia magna* were fed phytoplankton that had been experimentally exposed to a high concentration of FeO(OH) induced negative effects on reproductive traits, suggesting that Fe exposure can have an indirect toxic effect on *Daphnia* sp. productivity through the food web (Jeyasingh & Pulkkinen,

2019). However, it is possible that Fe particulates may have varying effects on different zooplankton groups, depending on their feeding strategies.

Zooplankton feeding traits can play an important role in the types and quality of particles ingested (Todorova et al., 2015). Filter-feeding cladocerans (Crustacea, Branchiopoda) generally collect microscopic algae or cyanobacteria on their thoracic legs before ingesting them, while other cladocerans, such as chydorids, can feed by scraping surfaces (Dodson et al., 2010). *Daphnia* sp. are highly efficient yet passive filtering grazers with high feeding rates on seston, and therefore consume a high concentration of non-target particles in the process (Bertilsson, 2003). While all copepods are omnivorous (Crustacea, Maxillopoda), most of them are selective feeders (Reid & Williamson, 2010). Calanoid copepods are efficient at selectively feeding on larger, more nutritious prey such as larger phytoplankton cells (Bertilsson, 2003). Cyclopoid copepods, meanwhile, can feed on larger zooplankton and phytoplankton than calanoid copepods, and are mostly known for their raptorial feeding methods (Brandl, 2005; Reid & Williamson, 2010). Meanwhile, rotifers feed through selective foraging and unselective feeding habits, and play an important role in the food webs (Kim, 2000; Obertegger et al., 2011). There is some evidence of differential effects of Fe among zooplankton groups. Immers et al. (2015) found that Fe particulates reduced cladoceran biomass over a yearly exposure, while copepods showed no clear response. Wilson (2013) found that ferric chloride addition altered zooplankton assemblage composition in the short term, before recovering after a month (although no pH adjustment was done, which means that part of the effect may be due to acidification). They also found that rotifer and copepod assemblages were the most impacted zooplankton groups. Therefore, excessive Fe pollution to aquatic environments may pose a conservation concern if it induces shifts in pelagic phytoplankton and zooplankton community structure and functioning.

The objective of our study was to experimentally examine the effects of Fe particulates on plankton community assemblages and their metabolic balance from a natural mesotrophic lake. We predicted that particulate Fe addition would 1) reduce phytoplankton biomass through the P-binding effect of Fe (Bakker et al., 2016; Orihel et al., 2016), 2) favor the relative abundance of cyanobacteria because of their shade resistance (Marzetz et al., 2020) and their ability to metabolize non-bioavailable Fe through the use of siderophores (Årstøl & Hohmann-Marriott, 2019; Du et al., 2019; González et al., 2018; Molot et al., 2014), 3) reduce crustacean zooplankton biomass and density because of their feeding strategies and because phytoplankton resources would also be reduced (Bertilsson, 2003) and 4) reduced gross primary production, thereby shifting community metabolism toward net heterotrophy (Kotalik et al., 2019). We place our findings in the context of Total Fe water quality guidelines for the protection of aquatic life in Canada and the United States, which is of 1 mg/L in the United States (USEPA, 1976) and variable in Canada depending on the pH and dissolved organic carbon (DOC) concentration of a given ecosystem (ECCC, 2024). In doing so, we provide a perspective on the value of measuring community and ecosystem responses in naturalized settings for understanding ecotoxicological thresholds in multi-species assemblages.

1.3 Methods

1.3.1 Study site and mecosm set up

A 28-day field enclosure experiment was conducted using 16x 568 L enclosures with a gradient of iron chloride ($FeCl_3$) treatment exposures (14 levels ranging from 0.1, 0.3, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 mg/L). An additional two enclosures served as treatment controls: 1) no Fe or chloride (Cl^-) addition and 2) only Cl^- addition (Cl^-

concentration equivalent to the highest Fe treatment at 19.04 mg/L Cl^-). The field experiment was conducted at the Gault Nature Reserve (McGill University, Mont-St-Hilaire QC, Canada) from August 23 to September 20, 2021. Lake water and plankton communities were sourced from Lac Hertel (45° 33′ 08″ N, 73° 09′ 16″ W), a mesotrophic lake (annual P and N concentrations of 18 μg/L and 300 μg/L respectively (Hébert et al., 2021)) with a diverse community assemblage of phytoplankton and zooplankton (Table A1). Agricultural grade shade cloth was installed over all enclosures to regulate the water temperatures over the course of each day. The 16 enclosures were filled with 500 L of unfiltered water from Lake Hertel and thereby contained microscopic organisms such as bacteria, protozoans, phytoplankton and a modest amount of zooplankton from the lake. The water was allowed to settle in the enclosures for 24 h before manually adding crustacean zooplankton and rotifers that were gently collected from the pelagic zone of Lac Hertel. Specifically, additional zooplankton were collected using Wisconsin plankton nets (maximum net diameter = 35 cm; mesh size = 54 μm) that were lowered to 5.5 meters depth, and this step was repeated 53 times, following 3 transects near the deepest part of the lake (9 meters). This biomass of the crustacean zooplankton from the lake was first pooled in a common enclosure (320 L) that was gently mixed and then 10 L was subsampled for individual inoculation of each enclosure.

1.3.2 Experimental treatments

The plankton communities were allowed five days to acclimate in the enclosures, after which experimental treatments were added to the enclosures. Experimental Fe treatments were created by adding the total amount of $FeCl_3$ needed in MilliQ water to obtain the final target concentration in each of the 500 L enclosures. The $FeCl_3$-MilliQ water solution was neutralized using 1N or 5N NaOH until the pH was between 7 to 8,

causing Fe precipitation in the form of Fe oxyhydroxides. Chloride treatment to the control enclosure was done by adding HCl in 1L of MilliQ water and neutralized with 5N NaOH until the pH was between 7 to 8. Fe and chloride solutions were then added to appropriate enclosures. Treatment levels were randomly assigned to the enclosures using a random number generator. Submersible water pumps (flow rate of 400 gallons per hour) were put in each enclosure to aid with Fe suspension, and to limit the settling out of precipitates.

Water samples were collected from the enclosures on days 4, 11 and 25 for analyses of total Fe, and on day 25 for analyses of dissolved Fe. Fe concentrations were analyzed with an ICP-MS Agilent 8900 equipped with an SPS4 autosampler. Analysis was done in collision mode with hydrogen gas and Fe56 isotope analysis, at the Université de Montréal.

We measured other potential explanatory variables linked with water chemistry in the enclosures. Using a YSI Pro Plus multiparameter sonde (model 10102030; Yellow Springs Inc.), numerous measures were taken every 2 or 3 days for temperature, dissolved oxygen (DO as % saturation and concentration in mg/L), specific conductivity (SPC, μS/cm) and pH. We ascertained that the pH of water in the enclosures remained above circumneutral, and hence the Fe remained largely insoluble. Total phosphorus (TP) was measured in duplicate before the experiment, and on days 3, 17 and 24 of the experiment, using a standard colorimetric protocol with potassium persulfate digestion (Wetzel and Likens, 2000). An ammonium molybdate solution was then added to the orthophosphates previously produced during the digestion. HOBO data loggers were suspended at mi-depth in the water column of enclosures with 0, 0.1, 0.3, 0.5, 0.75, 1, 4, 7, 10 mg/L of Fe to measure light intensity.

1.3.3 Response variables

Phytoplankton

Phytoplankton community composition and total biomass was analyzed using the FluoroProbe (bbe moldaenke, Germany). This instrument uses different wavelengths to determine relative abundance of phytoplankton groups as well as total abundance (Catherine et al., 2012). Water samples were collected from enclosures on days 0, 2, 5, 7, 9, 12, 14, 16, 18, 21, 23 and 26, and relative abundance and total abundance of phytoplankton were obtained from the fluoroprobe in a dark environment. Phytoplankton data for days 2, 18 and 26 are presented to align with zooplankton results as much as possible.

Crustacean zooplankton

At the beginning of the experiment, the enclosures were inoculated with zooplankton communities of approximately equivalent taxa-specific density and biomass. The initial community was comprised of cyclopoid copepods (omnivorus *Tropocyclops mexicanus* and omnivorous/carnivorous *Acanthocyclops vernalis*, along with juvenile stages), four herbivorous cladoceran species (*Ceriodaphnia lacustris*, *Diaphanosoma brachyurum*, *Bosmina longirostris* and *Daphnia parvula*), and 8 rotifer species (*Monostylla bulla*, *Keratella cochlearis*, *Polyarthra sp*., *Ploesma truncatum*, *Hexarthra mira*, *Conochilus unicornis*, *Kellicottia longispina* and *Gastropus styllifer*) (Table A2 density; Table A3 biomass). Zooplankton samples were collected on days 19 and 28 of the experiment with a bilge pump (12V) and concentrated on a 54 µm filter. For the samples collected on day 19 and 28 at the end of the experiment, a volume of 50 L of enclosure water (10% of the total enclosure volume) and 200 L (40% of the total enclosure volume) was filtered on these respective days. The zooplankton

accumulated on the filter from each enclosure were then preserved at a final concentration of 80% ethanol.

To measure potential change in crustacean zooplankton community composition and diversity, zooplankton were identified to species-level, and species-specific densities were enumerated for all samples collected on days 19 and 28. The crustacean zooplankton were identified to species level with a high-resolution dissecting microscope (6.3-126x; SZ2-IL-ST; Olympus SZ) and species were enumerated. Crustacean zooplankton were counted using a protocol that targets mature individuals that could be identified unambiguously to species, as well as to detect rare species (Mack et al., 2012). Subsamples were taken until either $\geq$ 200 individuals of the most abundant species were counted (excluding copepodids and nauplii), a total of 1000 individuals were counted (excluding copepodids and nauplii), or the total sample was counted. Taxonomic keys included Brooks (1957), Smith and Fernando (1978), De Melo and Hebert (1994), Witty (2004), Thorp and Covich (2010) and Haney et al. (2013). Zooplankton functional traits were assigned following Hébert et al., (2016).

To measure taxon-specific crustacean zooplankton biomass, length-weight regression was applied to estimate the biomass of zooplankton in each enclosure. This method uses published length-weight species-specific relationships (Bottrell et al., 1976; Culver et al., 1985; Dumont et al., 1975; Lawrence et al., 1987; Rosen, 1981) to create a regression where body length measurements determine species-specific biomass. All length data were adapted to zooplankton growth stage and sex. Published length-weight relationships were found for every species of our experiment. The only exceptions were: 1) *Daphnia parvula* for which fitted constants were used for *Daphnia sp*., and 2) *Alona* sp. that we were unable to identify to species level, and so we used the fitted constant for *Alona rectangula*. Weight was determined by using the following formula:

$$W = aL^b$$

Where W is the mass in μg, L is the length in mm, and a and b are fitted constants. The constants a and b vary depending on the species, the development stage and sometimes the sex. Length measurements were obtained at the same time as zooplankton community composition data were gathered. A maximum of 10 individuals were measured per species, and a species-specific average dry weight was obtained for each sample. The species and sample specific biomass data were then multiplied by the density of each species to obtain the biomass per liter of each species.

1.3.4 Community Metabolic Balance (Gross Primary Production: Respiration)

For each enclosure, we calculated the ratio of the rates of gross primary production to ecosystem respiration ratio (GPP:R) using an oxygen stable isotopic mass balance approach paired with ambient measurements of DO concentration (detailed above). GPP:R was measured in each enclosure on weeks 0 and 4 of the experiment. The approach has been described in detail elsewhere (Bocaniov et al., 2012; Bogard et al., 2017; Bogard et al., 2020; Quay et al., 1995) and we provide additional methodological information in the supporting information (Text A1). At GPP:R > 1, the planktonic community is net autotrophic, while GPP:R < 1 indicates the community is net heterotrophic.

1.3.5 Statistical analyses

We estimated Fe concentrations in the enclosures through the conversion of conductivity at the final day of the experiment to chloride concentration, which was related to Fe concentration through its molecular relationship in $FeCl_3$ (Fig. A1 – linear

model and regression; R^2_{adj}.=0.995, r=0.99, P=7.31e^{-12}). We then compared the estimated Fe concentration with the targeted Fe concentration on day 4 of the experiment (or "nominal concentration"; Table A4, Fig. 1.1a), for which there was a close relationship and thus nominal Fe concentration was used as an explanatory variable in all models. The nominal Fe concentrations are hereafter used to reflect the experimental treatments of each enclosure, and the term normal concentration will be referred to as Fe concentration (Table A4, Fig. 1.1a).

We applied multivariate statistical analyses to quantify group-specific effects of Fe on phytoplankton and crustacean zooplankton communities. Group-specific zooplankton biomass and density were obtained by summing the biomass and density of each species within a taxonomic group, and then generalized additive models (GAMs – mgcv R package v1.8-42) were executed on these data. Biomass and density models for phytoplankton or zooplankton were created using the same smooth terms to enable comparison, after first examining Akaike Information Criteria (AIC) and Adjusted R^2 for stepwise construction from simpler to more complex models (Table A5, A6, A7). Simple models were first applied to test for the effect of the experimental Fe treatment on phytoplankton and zooplankton biomass and density. More complex models were then created to see if the effects of Fe were direct, indirect or both. Complex models were made using smooth terms for Fe and TP concentrations, time as a random effect (bs="re") and a tensor product smooth (an interaction term excluding the main effects) between Fe concentration and TP. The Fe concentration and TP were smoothed using a k-value of 5, and all GAMs were modeled using "REML" smoothing parameter estimation. Ultimately, this same model was chosen for every analysis, as it often had the lowest AIC, or the difference between this model and the best one was minimal (maximum 2.6 points) (Burnham & Anderson, 2004). We simplified the aspect of time in the study by categorizing day 2 of the experiment as week 1, day 16 to 18 as week 3, and day 26 to 28 as week 4 of the experiment.

We developed a series of linear models (LM; using function *lme* of nlme R package v3.1-162) and GAMs to consider a range of possible predictors. The model with the lowest AIC between the LM and the GAM was selected (Table A8). All models were created using nominal Fe concentration, which was the target Fe concentration added to the enclosures, and time as a random smooth. For GAMs, diagnostic plots and k-values were verified by using *appraise* and *gam.check* respectively (mgcv R package v1.8-42 and gratia R package v0.8.1). The k-value for the models were changed when the p-value of the k-value of the models were low and the edf close to k'. If edf and k' were not close to each other, the k-value of the smooth term remained unchanged. For linear models, diagnostic plots were verified to ensure model assumptions were met. The graphical representation of GAMs was done using the *getViz* function (mgcViz R package v0.1.9), and each smooth was graphed individually. The models were also graphed using functions *predict.gam* and *ggplot* (mgcv R package v1.8-42 and ggplot2 R package v3.4.1).

Principal Coordinates Analyses (PCoA) were applied to examine the effect of Fe treatment on phytoplankton and crustacean zooplankton community density or biomass data using *capscale* function (vegan R package v2.6-4). Data were modified with Hellinger transformation using *decostand* function (vegan R package v2.6-4). Score data for sites were extracted from the PCoA and modeled using GAMs with Fe concentration as the fixed effect (time as a random effect). Model results were plotted alongside the strongest species or group specific trends apparent in the PCoA graphs, represented by PCoA vectors.

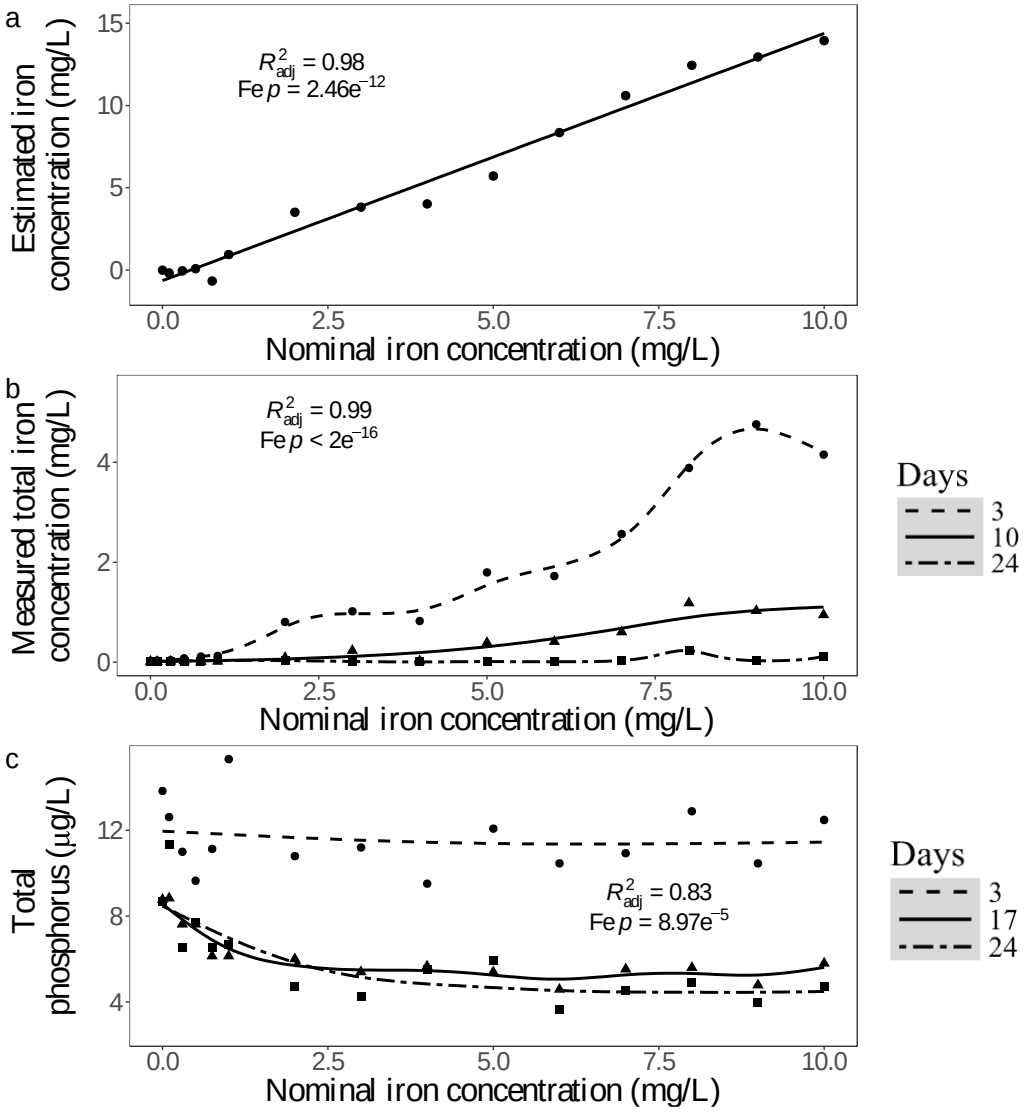

Figure 1.1: a. Relation between nominal and estimated iron concentration (mg/L) at day 4 of the experiment. A linear model was used to follow this trend. b. Change in measured total iron (mg/L) concentrations across time in relation to nominal iron concentration (mg/L) over the 28-day duration of the experiment. A generalized additive model was used to compare the total measured iron concentration across the iron gradient at day 3 (dashed), day 10 (full line) and day 24 (two dash). c. The relationship of total iron concentration (mg/L) with total phosphorus concentration (μg/L) at day 3 (dash), day 17 (full line) and day 24 (two dash) of the 28-day experiment, as revealed by GAMs

GAMs were further applied to determine EC_{50}s, the concentration at which zooplankton density or biomass was 50% lower than in the control. EC_{50}s were only applied if the following conditions were met: (1) Fe concentration impacted density or biomass, (2) density or biomass declined with Fe exposure, and (3) abundance and biomass showed a monotonic decline and remained below 50% across the Fe gradient. If density or biomass models were based on nominal Fe concentration, the EC_{50} was then also determined based on measured Fe concentrations for comparison.

To assess if there was an effect of chloride on plankton communities linked to $FeCl_3$ addition, the biomass and density values of Fe control (no $FeCl_3$ addition) were replaced by the density and biomass values of the chloride control (Cl addition only). Predictions of the new models were then created and graphed, and visual analysis was done to examine possible divergence between the models. While some models slightly differed, no overall effect of chloride was detected (Fig. A2, Fig. A3, Fig. A4).

1.4 Results

Environmental treatments and conditions

Nominal Fe concentrations reflected the levels of experimental Fe treatment that were added to enclosures at the beginning of the experiment (initial exposure). However, Fe oxyhydroxides precipitated out of the water column over the course of the experiment and so this correlation between nominal Fe concentration and actual Fe concentration became increasingly decoupled over time during the experiment. Nonetheless, enclosures with the highest nominal Fe concentrations contained relatively higher measured total Fe concentration in the water column over the course of the experiment (Fig. 1.1b, Fig. A5, Table A4). Overall, measured Fe concentrations declined through

time as precipitated Fe oxyhydroxides settled out of the water column (Fig. 1.1b, Table A4).

There was a strong negative relationship between Fe and TP concentrations that became more enhanced over the duration of the experiment. A negative relationship was apparent by the middle and end of the study – higher concentrations of Fe suppressed TP concentrations in enclosures, with this negative impact being more pronounced at mid Fe concentration (Fig. 1.1c, Fig. A6, Table A4). Finally, light transmission in the enclosures was not consistently affected by the experimental Fe gradient (Fig. A7).

Phytoplankton communities

Experimental Fe addition had variable effects on phytoplankton groups across the Fe concentration gradient (Table 1.1, Fig. 1.2). Positive effects of Fe were observed on cyanobacteria, while a marginal positive effect was found for diatom and a negative effect was found for chlorophyte biomass (Fig. 1.2b, 1.2d, 1.2e, Table 1.1). Complex Fe models showed the mode of action of Fe on the affected phytoplankton group's biomass (Table 1.2, Fig. A8, A9, A10, A11, A12). The negative effect of Fe on chlorophyte biomass was mostly caused through its effect on TP (Table 1.2, Fig. 1.2a, Fig. A9). Chlorophyte biomass responded to TP non-linearly, and these algae were maximal at intermediate to higher TP concentrations across the gradient (Table 1.2, Fig. A9b). An interaction between Fe and TP explained patterns in chlorophyte biomass, but Fe was not a significant independent variable, so Fe only has a significant effect at specific levels of TP (Fig. A9). In contrast, increases in cyanobacteria biomass were mostly driven by particulate Fe (Fig. 1.2b, Table 1.2, Fig. A10a). While the simple Fe model (Table 1.1) showed the marginal positive effect of Fe on diatom biomass, no

elements of the complex model were the apparent cause of the modest increase in diatom biomass. Finally, the Fe treatment had no effect on total phytoplankton (Fig. 1.2a, Table 1.2) and cryptophyte biomass (Fig. 1.2d, Table 1.2).

Table 1.1: Statistical results of simple GAMs conducted on zooplankton density or biomass or phytoplankton biomass where Fe was the sole fixed effect and time was the random effect. Significant effects are bolded.

Response variable	edf	Red.df	F-value	Fe p-value
Total zooplankton density	**2.1289**	**2.631**	**26.04**	**1.27E-6**
Total copepod density	**1**	**1**	**10.138**	**0.00364**
Total cladoceran density	**2.4038**	**2.971**	**20.75**	**1.47E-6**
Total rotifer density	**1.355**	**1.622**	**8.687**	**0.00166**
Total zooplankton biomass	**2.7167**	**3.362**	**20.624**	**1.27E-6**
Total copepod biomass	**1**	**1**	**4.877**	**0.0356**
Total cladoceran biomass	**2.7818**	**3.443**	**23.275**	**1.09E-6**
Total phytoplankton biomass	1	1	0.871	0.35594
Total chlorophyte biomass	**1.001**	**1.002**	**6.255**	**0.0164**
Total cyanobacteria biomass	**1**	**1**	**13.983**	**0.000565**
Total cryptophyte biomass	1.607	1.98	1.519	0.262
Total diatom biomass	**1**	**1**	**4.04**	**0.0511**

For phytoplankton community composition, PCoA axis 1 (Table A9, Fig. 1.3b, Fig. A13 – GAM) and PCoA axis 2 (Table A9, Fig. 1.3b, Fig. A14 - GAM) were significantly explained by Fe addition. Phytoplankton communities shifted from a community dominated by chlorophytes and cryptophytes (Fig. 1.3a, 1.3b) at the beginning of the experiment towards communities more strongly represented by chlorophytes at low Fe concentrations and cyanobacteria at high Fe concentrations by the middle and end of the experiment (Fig. 1.3a, 1.3b).

Table 1.2: Summary results from the complex models of phytoplankton biomass. Parametric and smooth terms of GAMs models include Fe and TP as smooth effect, time as a random effect smooth and a tensor product smooth between Fe and TP. Significant parametric and smooth terms are indicated in bold and random effect are indicated in italic

Total phytoplankton biomass				
A. parametric coefficients	Estimate	Standard Error	t-value	p-value
(Intercept)	**5.18**	**1.389**	**3.73**	**0.000624**
B. smooth terms	edf	Red.df	F-value	p-value
s(Fe)	1	1	0.328	0.57
s(TP)	**2.258**	**2.779**	**2.813**	**0.0459**
s(Time)	***1.758***	***2***	***11.483***	***0.0000381***
ti(TP, Fe)	**1**	**1**	**7.389**	**0.00983**
R^2_{adj}	0.423			
Deviance explained	50.2%			
Total chlorophyte biomass				
A. parametric coefficients	Estimate	Standard Error	t-value	p-value
(Intercept)	**3.454**	**1.666**	**2.074**	**0.045**
B. smooth terms	edf	Red.df	F-value	p-value
s(Fe)	1	1	0.471	0.497
s(TP)	**2.627**	**3.18**	**4.204**	**0.01**
s(Time)	***1.849***	***2***	***12.584***	***2.56E-5***
s(TP, Fe)	**1**	**1**	**4.345**	**0.0439**
R^2_{adj}	0.469			
Deviance explained	54.7%			
Total cyanobacteria biomass				
A. parametric coefficients	Estimate	Standard Error	t-value	p-value
(Intercept)	**1.037**	**0.3163**	**3.278**	**0.00219**
B. smooth terms	edf	Red.df	F-value	p-value
s(Fe)	**1**	**1**	**9.353**	**0.00401**
s(TP)	1	1	0.608	0.44
s(Time)	***1.633***	***2***	***3.948***	***0.0137***
ti(TP, Fe)	1	1	2.226	0.144
R^2_{adj}	0.358			
Deviance explained	42.6%			
Total cryptophyte biomass				
A. parametric coefficients	Estimate	Standard Error	t-value	p-value
(Intercept)	**0.4032**	**0.179**	**2.253**	**0.03**
B. smooth terms	edf	Red.df	F-value	p-value
s(Fe)	1	1	3.137	0.0843
s(TP)	1.639	2.033	0.805	0.4431
s(Time)	***1.821***	***2***	***14.514***	***7.61E-6***
ti(TP, Fe)	1	1	1.726	0.197
R^2_{adj}	0.613			
Deviance explained	66.1%			
Total diatom biomass				
A. parametric coefficients	Estimate	Standard Error	t-value	p-value
(Intercept)	0.2918	0.2474	1.179	0.245
B. smooth terms	edf	Red.df	F-value	p-value
s(Fe)	1	1	2.484	0.123
s(TP)	1	1	0.166	0.686
s(Time)	***1.844***	***2***	***26.769***	***<2E-16***
ti(TP, Fe)	1	1	1.711	0.198
R^2_{adj}	0.606			
Deviance explained	64.9%			

Zooplankton communities

Total zooplankton (crustacean zooplankton and rotifers), copepod, cladoceran, and rotifer density were all negatively affected by experimental Fe addition (Table 1.1, Fig. 1.4). The reductions of total zooplankton density (Table 1.3, Fig. 1.4a, Fig A15a) and rotifer density (Table 1.3, Fig. 1.4d, Fig A16a) were directly caused by Fe. Meanwhile, patterns in cladoceran density (Table 1.3, Fig. 1.4c, Fig. A17a) were mostly linked to the effect of TP, with modest negative effects found at low TP concentrations (Table 1.3, Fig. 1.4c, Fig. A17b). Finally, it was impossible to determine the biological reason behind the reduction in copepod density, probably caused by a statistical power issue (Table 1.3, Fig. 1.4b, Fig. A18a) .

Fe concentration had an important influence on zooplankton community composition based on relationships with both PCoA axes (Table A10, Fig. 1.5, Fig. A19, Fig. A20). Changes in community structure across the Fe concentration gradient were apparent by week 3 of the experiment for crustacean zooplankton and rotifer densities (Fig. 1.5). Zooplankton communities shifted from dominance by the cladoceran *Bosmina longirostris* and the rotifer *Keratella cochlearis* at low Fe concentrations towards dominance by a single rotifer species, *Euchlanis dilatata*, at the highest Fe concentrations (Fig. 1.5, Table A2). However, by week 4, the enclosures with low Fe concentrations were dominated by *Chydorus sphaericus*, and the high Fe treatment enclosures were dominated by omnivorous/carnivorous copepod *Acanthocyclops vernalis* (Fig. 1.4, Fig. 1.5, Table A2). The species richness of zooplankton communities declined drastically from 15 to 3 species by week 4 of the experiment at the highest Fe concentration (10 mg/L); by contrast, 6 species remained in the control enclosures while 9 species were found in the enclosure with 0.1 mg/L by week 4 (Table A2).

Table 1.3: Summary results from the zooplankton biomass or zooplankton density complex models. Parametric and smooth terms of GAMs models include Fe and TP as smooth effect, time as a random effect smooth and a tensor product smooth between Fe and TP. Significant parametric and smooth terms are indicated in bold and random effect are indicated in italic.

Zooplankton biomass				
Total zooplankton biomass				
A. parametric coefficients	Estimate	Standard Error	t-value	p-value
(Intercept)	**0,6435**	**0,2786**	**2,31**	**0,0311**
B. smooth terms	edf	Red.df	F-value	p-value
s(Fe)	1,0001	1	0,781	0,387
s(TP)	**2,3191**	**2,609**	**3,33**	**0,0232**
s(Time)	***0,9514***	***1***	***19,586***	***0,00015***
ti(TP, Fe)	3,6565	4,798	1,078	0,352
R2-adjusted	0,852			
Deviance explained	89,2%			
Total copepod biomass				
A. parametric coefficients	Estimate	Standard Error	t-value	p-value
(Intercept)	**0,29278**	**0,05463**	**5,36**	**1,48E-05**
B. smooth terms				
s(Fe)	1,2507	1,456	1,254	0,233
s(TP)	**1,0001**	**1**	**12,942**	**0,00138**
s(Time)	***0,7893***	***1***	***3,746***	***0,039***
ti(TP, Fe)	1	1	3,921	0,0588
R2-adjusted	0,397			
Deviance explained	48,1%			
Total cladoceran biomass				
A. parametric coefficients	Estimate	Standard Error	t-value	p-value
(Intercept)	0,4499	0,2754	1,634	0,117
B. smooth terms	edf	Red.df	F-value	p-value
s(Fe)	1,481	1,739	1,364	0,204
s(TP)	2,432	2,761	2,274	0,0655
s(Time)	***0,928***	***1***	***12,887***	***0,00109***
ti(TP, Fe)	2,854	3,698	1,204	0,395
R2-adjusted	0,829			
Deviance explained	87,5%			

Zooplankton density				
Total zooplankton density				
A. parametric coefficients	Estimate	Standard Error	t-value	p-value
(Intercept)	**1,0153**	**0,2063**	**4,922**	**6,42E-05**
B. smooth terms	edf	Red.df	F-value	p-value
s(Fe)	**1**	**1**	**14,618**	**9,27E-04**
s(TP)	2,1752	2,562	2,12	0,0746
s(Time)	***0,8164***	***1***	***4,447***	***0,0271***
ti(TP, Fe)	3,0852	3,822	1,43	0,248
R2-adjusted	0,819			
Deviance explained	86,3%			
Total copepod density				
A. parametric coefficients	Estimate	Standard Error	t-value	p-value
(Intercept)	**0,6024**	**0,1338**	**4,503**	**0,000136**
B. smooth terms	edf	Red.df	F-value	p-value
s(Fe)	1	1	2,68	0,114
s(TP)	1	1	0,271	0,607
s(Time)	*0,5147*	*1*	*1,061*	*0,163*
ti(TP, Fe)	1,5389	1,876	0,971	0,312
R2-adjusted	0,293			
Deviance explained	39,2%			
Total cladoceran density				
A. parametric coefficients	Estimate	Standard Error	t-value	p-value
(Intercept)	0,5711	0,3564	1,602	0,123
B. smooth terms	edf	Red.df	F-value	p-value
s(Fe)	1,457	1,736	1,439	0,194
s(TP)	**2,492**	**2,907**	**2,91**	**0,0376**
s(Time)	***0,96***	***1***	***24,006***	***4,63E-05***
ti(TP, Fe)	1,94	2,423	0,311	0,864
R2-adjusted	0,809			
Deviance explained	85,4%			
Total rotifer density				
A. parametric coefficients	Estimate	Standard Error	t-value	p-value
(Intercept)	**0,38394**	**0,07454**	**5,151**	**2,87E-05**
B. smooth terms	edf	Red.df	F-value	p-value
s(Fe)	**1**	**1**	**5,46**	**0,0281**
s(TP)	1	1	1,253	0,274
s(Time)	*9,06E-06*	*1*	*0*	*0,959*
ti(TP, Fe)	3,09	3,941	1,853	0,158
R2-adjusted	0,457			
Deviance explained	55,3%			

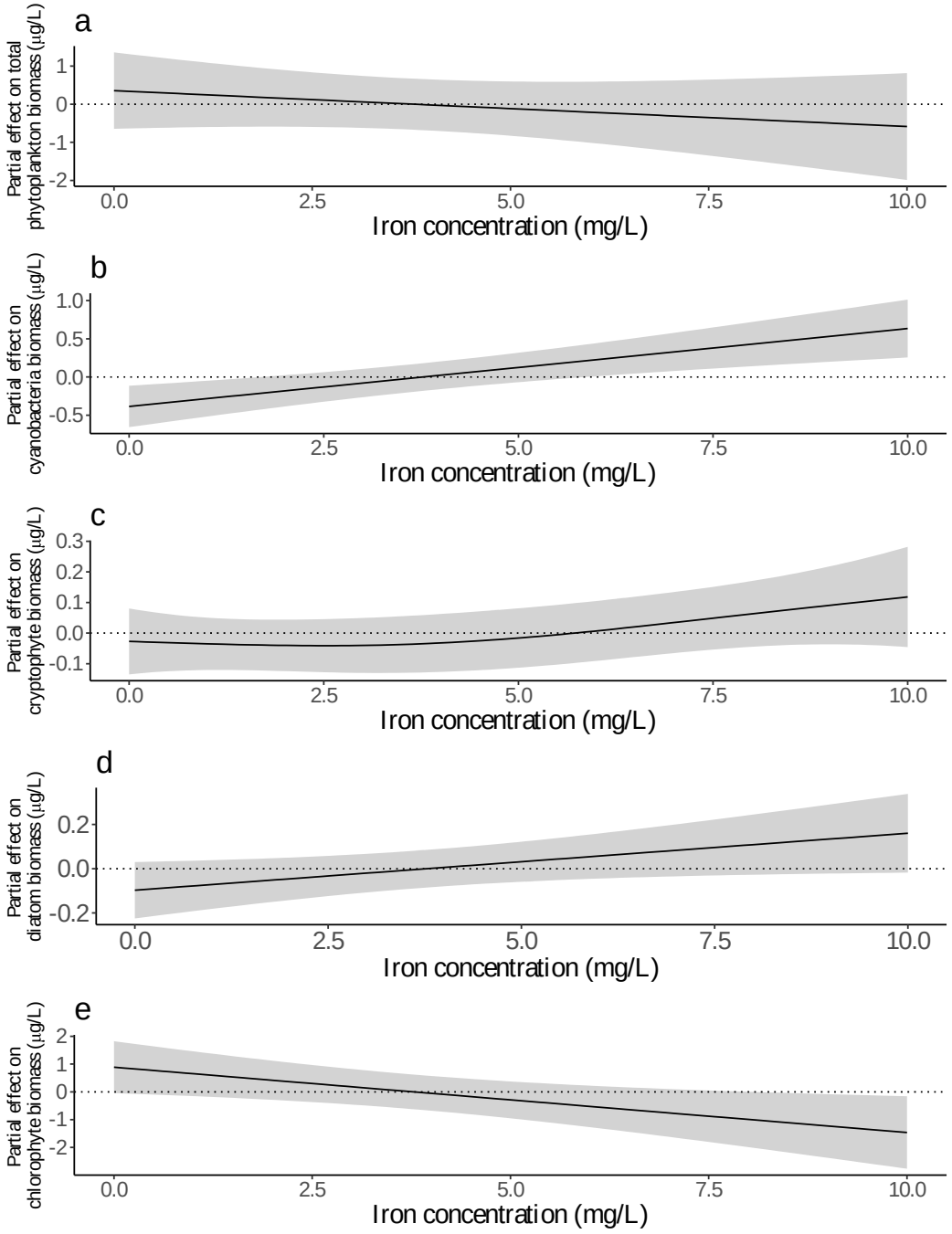

Figure 1.2: Partial effects of experimental iron addition on (a) total phytoplankton biomass, (b) cyanobacteria, (c) cryptophyte, (d) diatom and (e) chlorophyte biomass. Generalized additive models were used.

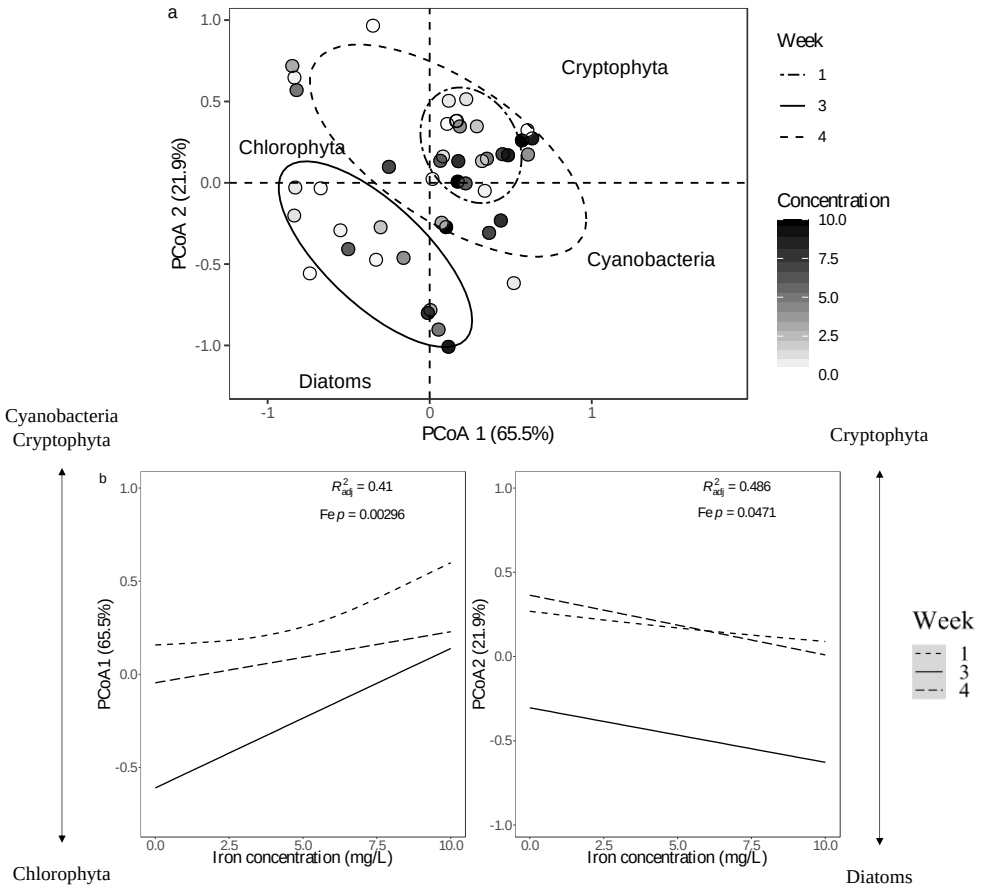

Figure 1.3: (a) Principal coordinate analysis (PCoA) of the phytoplankton communities across the iron gradient and across time. Week 1 is represented by the dashpoint ellipse, while week 3 is represented by the full line ellipse and week 4 by the dashed ellipse. Each enclosure is represented by a circle, with black circles being high iron concentration enclosures and white circles being low iron concentration enclosures (see legend). (b) Changes in phytoplankton community composition based on biomass data across the iron gradient at week 1 (short dash), week 3 (full line) and week 4 (long dash) for PCoA axis 1 and PCoA axis 2. Community data were modified by Hellinger transformation to downweight the influence of dominant species. PCoA axes modeled with iron concentration using GAMs (taxa with highest species scores shows for illustrative purposes).

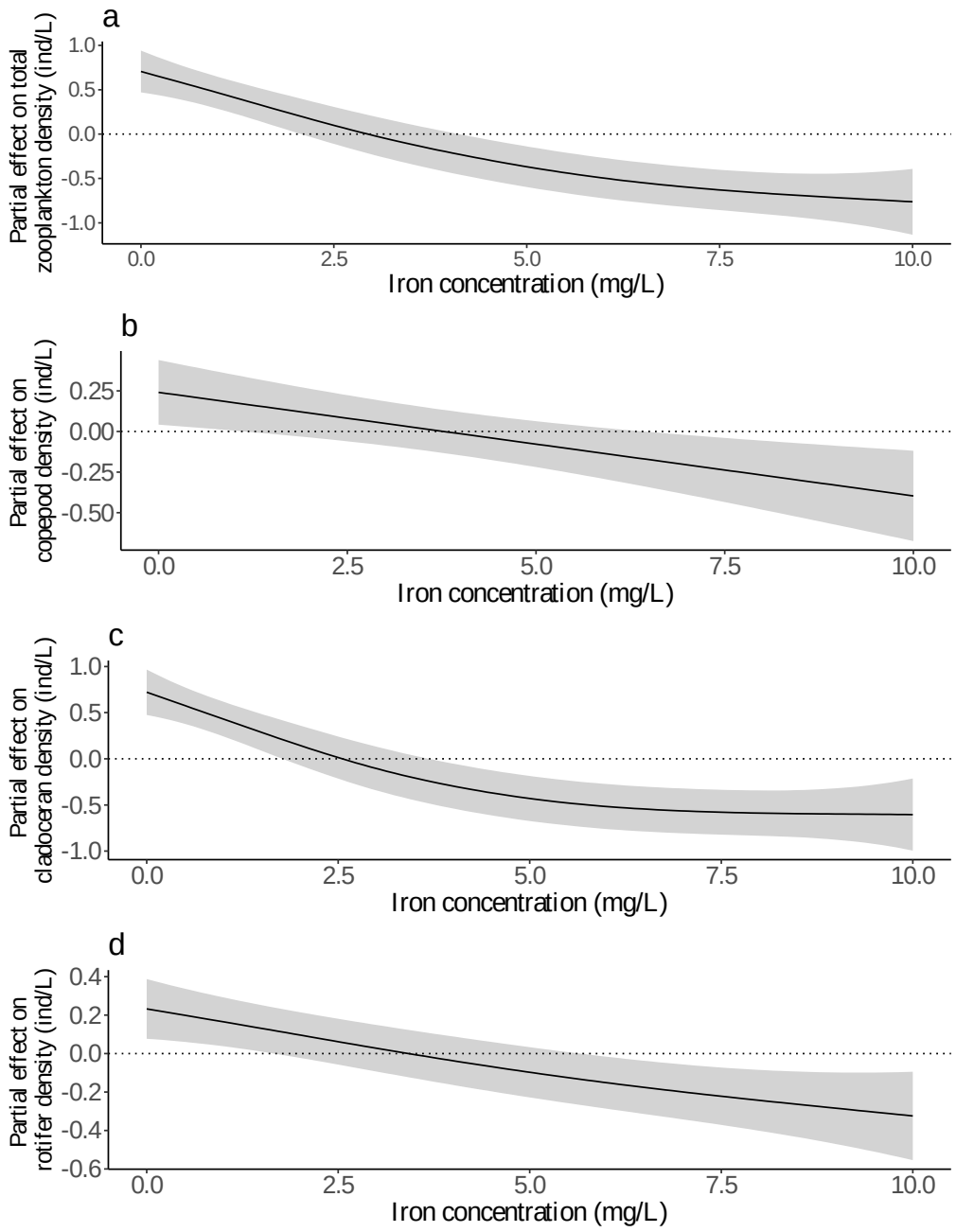

Figure 1.4: Partial effects of experimental iron (Fe) addition on (a) total zooplankton, (b) copepod, (c) cladoceran and (d) rotifer density. Generalized additive models were used.

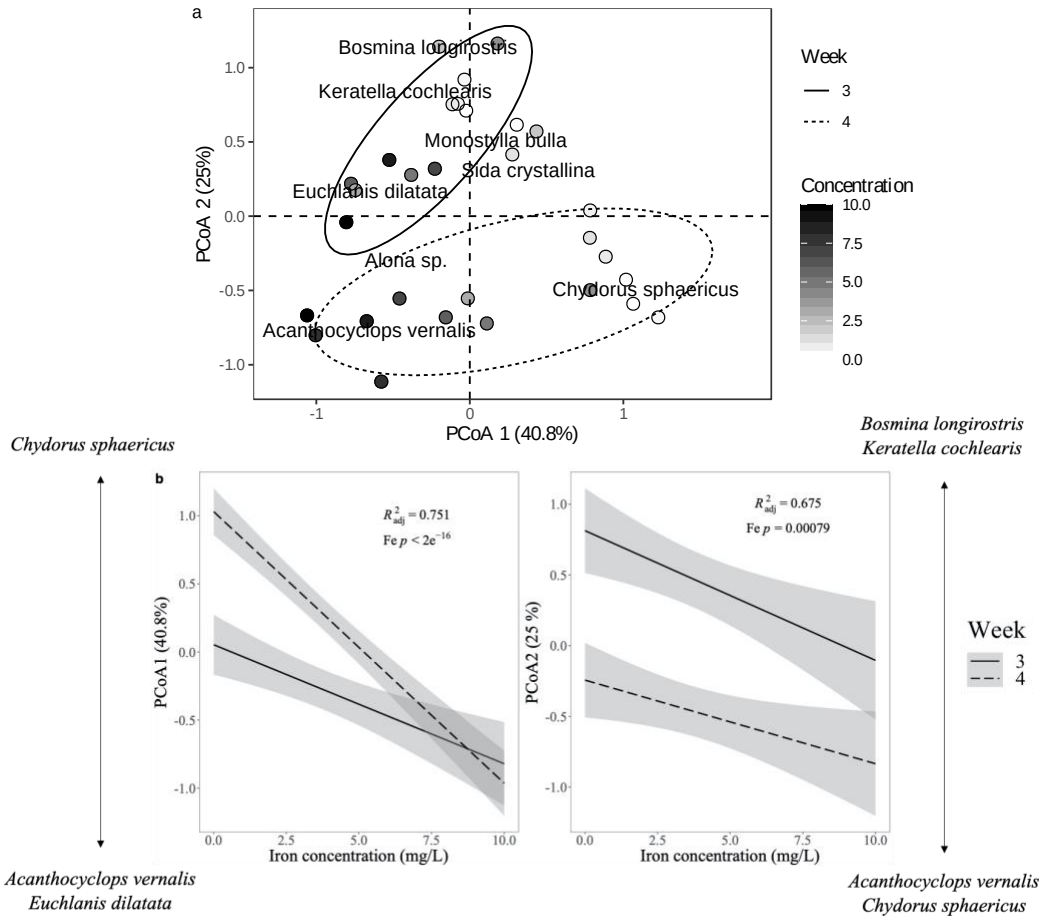

Figure 1.5: a. Principal coordinate analysis (PCoA) of zooplankton community density across the Fe gradient and across time. Week 3 of the experiment is represented by the full line ellipse and week 4 by the dashed ellipse. Each enclosure is represented by a circle, with black circles being high Fe concentration enclosures and white circles being low Fe concentration enclosures (see legend). b. Change in zooplankton community (based on density data) across the iron gradient at the middle (full line) and end (long dash) of the experiment for PCoA axis 1 and PCoA axis 2. Community data was modified using an Hellinger transformation, and PCoA axes modeled with iron concentration using GAMs (taxa with highest species scores shows for illustrative purposes).

Similar to the density results, total zooplankton, copepod and cladocera biomass were all negatively impacted by Fe exposure (Table 1.2, Fig. A21, Fig. A22, Fig. A23, Fig. A24, Fig. A25, Fig. A26, Fig. A27). Total zooplankton and copepod biomass were both affected by TP (Table 1.3, Fig. A25b, Fig. A26b – GAM). We did not detect a significant effect of Fe or TP in the complex models for cladocerans, likely due to weak statistical power. More information can be found in the appendix (Text A2).

Cladocerans were the most impacted group by Fe, followed by rotifers and copepods. EC_{50} values at week 4 for total zooplankton density were 1.2 mg/L (Fig. 1.6a), while EC_{50} values for copepod density were 3.3 mg/L (Fig. 1.6b), cladoceran density were 1 mg/L (Fig. 1.6c) and rotifer density were 2.81 mg/L (Fig. 1.6d). Meanwhile, EC_{50} values for average zooplankton biomass was 0.93 mg/L (Fig. 1.6e), average copepod biomass was 7.76 mg/L (Fig. 1.6f) and average cladoceran biomass was 0.82 mg/L (Fig. 1.6g). Finally, the EC_{50} value for total phytoplankton biomass was > 10 mg/L.

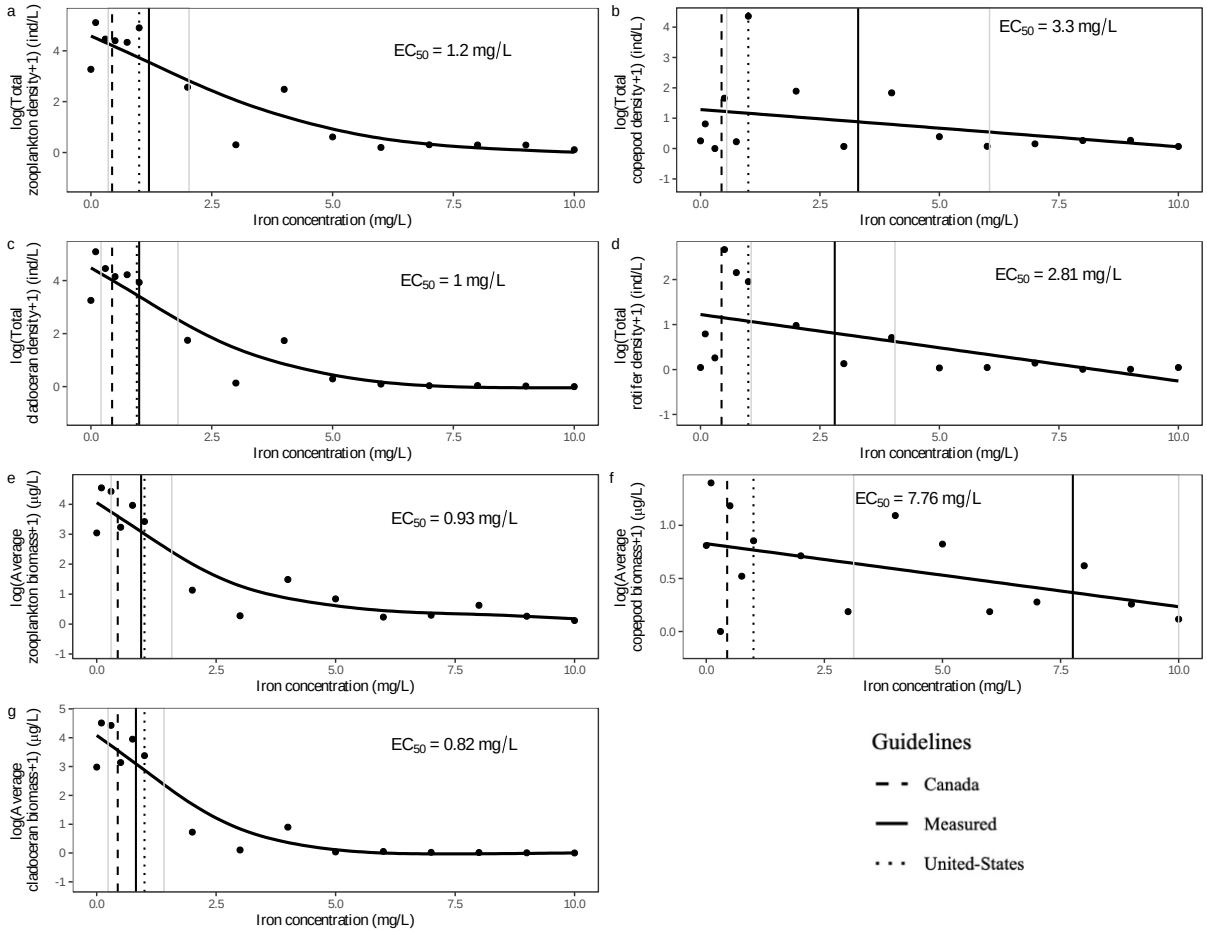

Figure 1.6: EC_{50} values of (a) total zooplankton density, (b) total copepod density, (c) total cladoceran density, (d) total rotifer density, (e) average zooplankton biomass, (f) average copepod biomass and (g) average cladoceran biomass after 4 weeks of Fe exposure. Vertical full lines represent EC_{50} values and the dark gray areas represents the 95% confidence intervals of the EC_{50} values in each panel. The dashed vertical line represents the Canadian recommendation for Lake Hertel (0.44 mg/L) and the dotted vertical line represents the United States recommendation for Fe exposure (1 mg/L). The light gray area around the curve represents the 95% confidence interval. For graph (c), the measured EC_{50} and the United States recommendation are the same (1 mg/L), but the dotted line was slightly shifted for visual clarity.

Community metabolic balance

GPP:R responses were inconsistent across the nominal Fe treatment gradient (Fig. 1.7), and a relationship between GPP:R and nominal Fe concentration was not detected at the beginning or end of experiment. Overall, the enclosures were all heterotrophic (GPP:R < 1) at the beginning of the experiment, and generally increased in GPP:R between the start (week 0) and week 4 of the experiment, including in the iron and chloride controls (Fig. 1.7), indicative of a shift towards autotrophy.

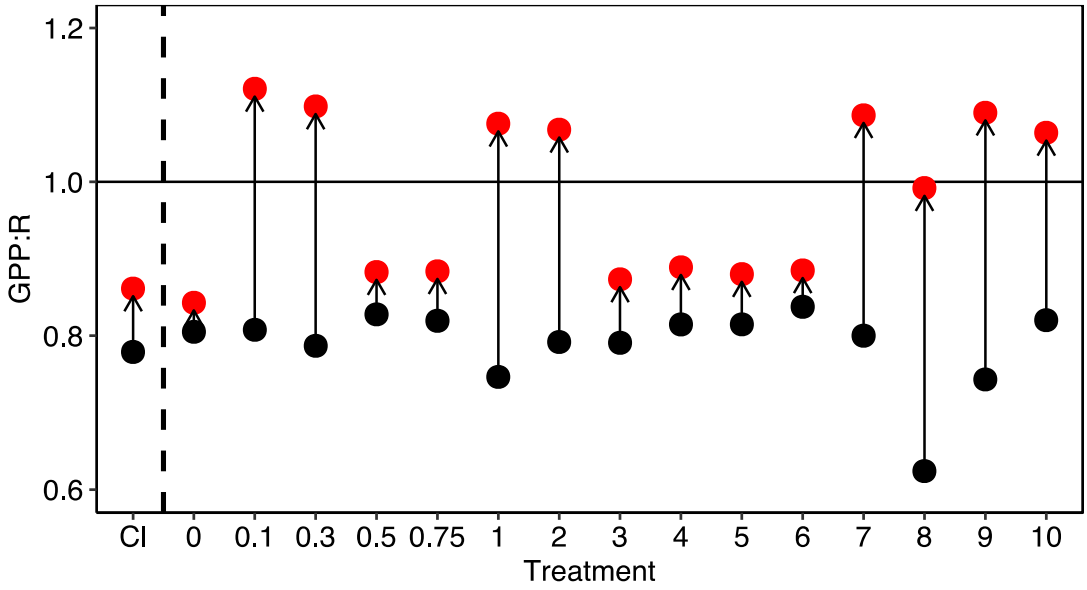

Figure 1.7: Change in GPP:R ratio across the Fe gradient of the experiment and in the chloride control. The black circles represent week 0 and the red circles represent week 4.

1.5 Discussion

We tested the ecological impacts of particulate iron addition on plankton assemblages and metabolic balances using experimental aquatic enclosures in a semi-natural setting. Supporting our predictions, the gradient of experimental Fe addition: 1) favoured the relative abundance of cyanobacteria within the phytoplankton community, 2) strongly reduced density and biomass of crustacean zooplankton, and 3) had a stronger negative effect on cladocerans than copepods; however, rotifer densities were also negatively impacted. Crustacean zooplankton communities shifted towards smaller cladocerans and predatory cyclopoid copepods in enclosures that received higher concentrations of Fe. In contrast with our predictions, there was no detectable effect of Fe addition on the metabolic balance of enclosures, and no enhancement of GPP relative to R. We calculated EC_{50} values for zooplankton, which integrated responses to Fe exposure while accounting for community interactions. EC_{50}s for most zooplankton response variables were similar to guidelines (especially considering error estimates) developed in Canada and the USA based largely on single species tests. Our study highlights the value of measuring community-based responses that integrate ecological interactions for understanding ecotoxicological thresholds in multi-species assemblages to environmental pollution from human activities such as iron mining.

Phytoplankton communities

Our prediction that Fe addition would reduce total phytoplankton biomass through an interaction between Fe and TP (Bakker et al., 2016; Orihel et al., 2016) was not supported. Meanwhile, TP was positively related with total phytoplankton and chlorophyte biomass, which was expected (Bergström & Karlsson, 2019; Frost et al., 2023). Fe oxyhydroxides adsorb and precipitate P from the water column (Bakker et al., 2016; Orihel et al., 2016). This mechanism may have reduced the availability of TP

for primary production and phytoplankton biomass (Bakker et al., 2016; Orihel et al., 2016), but only at the highest concentrations where the relatively lower TP content was observed. Yet our Fe additions did not produce a consistently negative effect on total phytoplankton biomass. Further, no clear shifts were seen in GPP:R, with the highest treatments actually displaying the largest shifts toward autotrophy (Fig. 7). Our results for total phytoplankton biomass therefore contrasted with the findings from other experimental studies (Orihel et al., 2016; Kotalik et al. 2019).

Earlier experimental studies have not considered ecological interactions with zooplankton when evaluating phytoplankton responses to Fe addition. It is possible that Fe had a negative effect on phytoplankton biomass by reducing TP concentrations, but that these losses were offset by the severe reductions in crustacean zooplankton density, especially by large-bodied cladocerans. Therefore, while bottom-up resources in TP for phytoplankton became more limited with the addition of more Fe, the top-down grazing pressure from large-bodied cladocerans was reduced or almost eliminated at high Fe concentrations after two weeks of exposure in our experiment.

Our prediction that Fe addition would alter the composition of phytoplankton communities by favoring the relative abundance of cyanobacteria was supported. While chlorophytes remained the dominant phytoplankton taxon throughout the experiment and at high Fe concentrations, cyanobacteria increased in relative abundance at high Fe concentrations. Cyanobacteria are able to produce siderophores, high affinity Fe chelators, that scavenge Fe from the water column under low Fe conditions (Årstøl & Hohmann-Marriott, 2019; Molot et al., 2014). These findings are consistent with some (Kotalik et al., 2019), but not all (Molot et al., 2010; Orihel et al., 2016) previous studies that tested the effects of Fe addition on phytoplankton biomass and community composition.

Environmental conditions favouring increased cyanobacteria abundance in aquatic ecosystems are a concern, because in the extreme cases, cyanobacterial blooms can disrupt ecosystem functioning, reduce biodiversity and affect human activities such as fisheries (Huisman et al., 2018). In our study, taxa-specific cyanobacteria were not identified. However, previous unpublished (2019) analyses of phytoplankton from the source Lac Hertel found *Aphanizomenon* to be the dominant cyanobacteria over the growing season (Gregory-Eaves, unpublished). Cyanobacteria remained at relatively low biomass within the phytoplankton communities in our experimental enclosures. Yet, cyanobacteria remain an important consideration, as they have lower nutritive value for food webs than other phytoplankton groups (Taipale et al., 2016). Therefore, the slight increases in cyanobacteria that we observed with increasing Fe addition supports Fe as a possible driver of cyanobacterial blooms that appear to be expanding over the northern hemisphere (Ho et al., 2019; Taranu et al., 2015), especially when combined with impacts from eutrophication and climate warming.

Zooplankton communities

We predicted that Fe addition would reduce biomass and density of crustacean zooplankton because of reduced phytoplankton resources. However, we found that phytoplankton biomass was sustained along the Fe treatment gradient, and there was a strong negative relationship between Fe concentration and zooplankton density for both crustacean zooplankton and rotifers. This was a response that we did not expect given that the Fe particulates quickly settled out to the bottom of the enclosures following their addition. Our findings therefore suggest direct toxicological effects of Fe particulates on zooplankton mortality that was independent from phytoplankton resources. Only one other study to our knowledge examined effects of particulate Fe on freshwater zooplankton community response as well as phytoplankton resources (Wilson, 2013). However, unlike our study, Wilson (2013) did not control for

acidification caused by the addition of $FeCl_3$ during their experiment. The loss in zooplankton density associated with particulate Fe addition in our experiment is a concern when considering Fe pollution in the aquatic environment because: 1) crustacean zooplankton are an important trophic link between primary producers and vertebrate consumers (Ogorelec et al., 2021) and 2) rotifers play an important role in linking the transfer of bacterial and phytoplankton carbon to higher trophic levels (Kim, 2000).

We expected differences in the response of zooplankton groups to Fe addition because of differences in feeding strategies. We predicted that cladocerans would be especially negatively affected because of their filter feeding strategy that can indirectly result in the ingestion of non-target particles (Bertilsson, 2003), such as Fe oxyhydroxides. Supporting this prediction, we found that cladocerans were more strongly negatively impacted by Fe addition, and reduced TP, than copepods. *Chydorus sphaericus* was the most abundant cladoceran in our enclosures, and its' density became dramatically reduced with increased Fe concentration (and reduced TP). Copepods were represented by omnivorous/carnivorous *Acanthocyclops vernalis*, which flourished likely by scavenging on dead and dying cladocerans, as well as by their ability to feed selectively (Reid & Williamson, 2010). Other more complex communities with more diverse zooplankton community assemblages, especially with herbivorous calanoid copepods, could potentially produce other results. However, our results clearly show a strong negative effect of Fe addition on cladocerans, which may be even stronger for large daphniids.

Ecosystem metabolic balance

Counter to our prediction, we did not detect a consistent effect of particulate Fe addition on the metabolic balance of enclosures. We randomized the spatial distribution of

treatments among enclosures, and there was no relationship with differences in light transmission among enclosures due to shading from surrounding buildings and trees that could explain higher GPP in certain enclosures and not others (Fig. 7). Contrary to our findings, the single other study that measured ecosystem metabolic balance as a response to particulate Fe addition at neutral pH found that it suppressed GPP (Kotalik et al. 2019). We are unable to estimate the rates of GPP independent from R using the current methodology as we did not quantify air water gas exchange rates that would enable estimates of GPP (supporting text A1). It is possible that rates of GPP therefore declined, while the metabolic balance still shifted toward autotrophy. Further research is required to understand the effects of particulate Fe addition on community metabolic balance in aquatic environments.

Water quality guidelines and community EC_{50}s

Water quality guidelines for long-term exposures to total Fe are 0.3 mg/L in Canada (CCME, 1987), and 1.0 mg/L in the United States (USEPA, 1976). The recent revision of the Canadian guideline now considers site-specific DOC and pH values. Accordingly, the Canadian guideline for our study site, Lake Hertel in mid-summer when the DOC = 3.9 mg/L and pH = 7.7 is slightly higher at 0.44 mg/L total Fe (ECCC, 2024). Water quality guidelines are based on single-species toxicity data and sometimes laboratory experiments, and they do not integrate community and ecosystem–data. To relate water quality guidelines to zooplankton community responses, we calculated EC_{50} values for zooplankton community assemblages. This measure more closely reflects ecological responses in natural communities than single-species laboratory toxicity tests (Dugan & Arnott, 2023).

None of the measured EC_{50} values were below the old (0.3 mg/L) or revised Hertel-specific Canadian freshwater Fe recommendations (Fig. 6) at week 4 of the experiment.

The average zooplankton (0.93 mg/L) and cladocera biomass (0.82 mg/L) EC_{50} values were slightly below the US freshwater Fe recommendation (1 mg/L), but still within the error of our EC_{50} model results. The cladoceran density value was exactly on the US recommendation (1 mg/L) (Fig. 6). Those results showcase that the Canadian Fe recommendations are sound regarding overall plankton health, while the US are less conservative and may lead to cladoceran losses. Cladocera species seem to be most sensitive to the effect of Fe oxyhydroxides, as we hypothesized because of their difference in feeding strategies compared to copepod and rotifer species. This effect was thus visible on the cladoceran density and biomass EC_{50} values that were below the copepod and rotifer EC_{50} values.

1.6 Conclusion

Fe addition to aquatic experimental enclosures had detrimental impacts on zooplankton densities, especially cladocerans. EC_{50} values for cladocera density and crustacean zooplankton biomass occurred at Fe concentrations slightly below water quality guidelines for the protection of aquatic life in the United States. By contrast, Fe addition had negligible effects on total phytoplankton biomass and the metabolic balance of enclosures, but slightly favoured cyanobacterial biomass. Fe impacts on plankton communities and aquatic ecosystem function are understudied. Therefore, our study provides an important first step to uncovering Fe impacts on plankton communities in a semi-natural setting. Further ecosystem-scale verification is needed, especially in landscapes with Fe-rich geology where Fe mining and pollution occurs. This is especially urgent given that there is a broad-scale tendency of increasing Fe concentration in European and North American freshwater ecosystems (Björnerås et al., 2017).

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1.8 References

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CONCLUSION

Cette thèse présente des résultats importants concernant les effets du fer sur les communautés de plancton. La biodiversité d'eau douce est de plus en plus menacée (Albert et al., 2021; Dudgeon et al., 2006; Reid et al., 2019) et la pollution par le fer a considérablement augmenté, à la fois en raison de l'activité humaine et de raisons naturelles (Björnerås et al., 2017; Hogsden & Harding, 2012). Cependant, très peu d'études ont examiné les effets des oxydes de fer sur les écosystèmes d'eau douce, sans parler des effets des oxydes de fer sur les communautés de plancton. De plus, cette étude présente une perspective intéressante sur les effets spécifiques du fer sur le zooplancton, à la fois sur les crustacés et les rotifères. Les gouvernements, à la suite de l'augmentation de la pollution par le fer, peuvent recommander des concentrations maximales de fer dans les écosystèmes d'eau douce. Ces recommandations sont généralement fondées sur des études toxicologiques sur une seule espèce de certains taxons ciblés et tiennent à peine compte de toute la gamme d'organismes présents dans les écosystèmes d'eau douce, comme les copépodes, les rotifères et certains groupes de phytoplanctons (CCME, 1987; ECCC, 2024).

2.1 Sujets traités et objectifs

Les oxydes de fer peuvent avoir des effets toxiques par des méthodes directes et indirectes (Bakker et al., 2016; Vuori, 1995). Alors que les effets toxiques directs agissent sur le corps d'un organisme, les effets toxiques indirects agissent par la modification de l'habitat. Les connaissances sur les effets toxiques directs du fer sur les communautés de plancton sont peu abondantes, mais leurs effets toxiques indirects

potentiels sont bien connus. La plupart des effets toxiques directs ont été testés sur les macroinvertébrés et les poissons (Vuori, 1995), mais restent inconnus pour le plancton. Les effets toxiques indirects, quant à eux, comprennent l'élimination du phosphore de la colonne d'eau et la réduction de la pénétration de la lumière et l'augmentation de la turbidité (Bakker et al., 2016; Vuori, 1995).

Ces effets toxiques directs et indirects peuvent être nocifs pour certains groupes spécifiques des communautés de plancton. Par exemple, les copépodes sont généralement plus mobiles que les cladocères et se nourrissent de manière plus sélective (Bertilsson, 2003; Reid & Williamson, 2010), ce qui leur permet d'éviter potentiellement la précipitation d'oxyde de fer et l'ingestion de particules de fer. Pendant ce temps, les cladocères, tels que *Daphnia sp*., s'alimentent par des méthodes de filtrage non sélectives (Bertilsson, 2003; Dodson et al., 2010). Ce type d'alimentation par filtration pourrait entraîner des problèmes de santé, car les particules d'oxyde de fer pourraient être ingérées ou obstruer leur appareil d'alimentation. Les cyanobactéries sont un autre exemple de groupes de plancton mieux adaptés que d'autres aux effets des oxydes de fer. Les cyanobactéries sont capables d'accéder à des formes de fer non biodisponibles, telles que les oxydes de fer, grâce à l'utilisation de sidérophores: des chélateurs de fer à haute affinité (Årstøl & Hohmann-Marriott, 2019 ; Molot et et., 2014). Les cyanobactéries sont également capables de résister à l'ombrage (Marzetz et al., 2020).

Ces différences interspécifiques n'ont pas été prises en compte lorsque les recommandations gouvernementales en matière de fer ont été établies. En effet, la recommandation du gouvernement des États-Unis de 1 mg/L est fondée exclusivement sur les espèces de poissons (USEPA, 1976), tandis que la recommandation actuelle du gouvernement canadien de 0,3 mg/L ne mentionne aucune des espèces pour en arriver à cette recommandation (CCME, 1987). Entre-temps, les recommandations révisées du

gouvernement canadien sont fondées sur un plus large éventail d'espèces, mais aucune espèce de copépode et de rotifère n'a été prise en compte, tandis qu'une seule espèce d'algue a été prise en compte (ECCC, 2024). Ma thèse permet de tester les effets des oxydes de fer sur un éventail beaucoup plus large d'espèces de plancton, tout en intégrant les interactions communautaires dans un cadre quasi naturel.

Par conséquent, l'objectif de cette thèse était d'évaluer l'impact des oxydes de fer sur les communautés de plancton et de déterminer si les recommandations gouvernementales en matière de fer sont appropriées dans le contexte d'une expérience multiespèces in situ.

2.2 Les résultats importants

J'ai constaté que l'exposition aux oxyhydroxydes de fer n'avait pas d'effet détectable sur la biomasse totale de phytoplancton et de cryptophyte, mais qu'elle avait un effet positif sur la biomasse des cyanobactéries et de diatomées. Le phosphore avait aussi un effet sur la biomasse de chlorophytes. Cet effet peut s'expliquer par l'effet direct du fer sur le phosphore, où le fer peut réduire la quantité de phosphore dans la colonne d'eau (Bakker et al., 2016 ; Orihel et al., 2016). J'ai également constaté que les cyanobactéries étaient plus importantes dans les enclos à des concentrations élevées de fer à la fois au milieu et à la fin de l'expérience, causé par le fer lui-même. Ces résultats contrastent quelque peu avec d'autres études dans le cas de la biomasse totale de phytoplancton (Bakker et al., 2016; Kotalik et al., 2019; Orihel et al., 2016) qui ont constaté que le fer lui-même avait un effet sur la biomasse totale du phytoplancton. L'augmentation de la biomasse des cyanobactéries associée à l'ajout de Fe était en concordance avec certaines études (Kotalik et al., 2019), mais pas à d'autres (Molot et al., 2010; Orihel et al., 2016). Ma thèse montre la complexité des effets de l'oxyde de fer sur les

communautés de plancton, et pourquoi d'autres études sur les effets des oxydes de fer sont nécessaires dans les écosystèmes d'eau douce.

En ce qui concerne le zooplancton, j'ai constaté que la densité totale du zooplancton, des copépodes, des cladocères et des rotifères étaient affectés négativement par l'exposition d'oxyhydroxydes de fer. La densité des cladocères a été affectée négativement à une faible concentration de phosphore total, probablement en raison d'une modification des ressources en phytoplancton. En ce qui concerne la composition, les enclos ont été dominés à la semaine 3 de l'expérience par le cladocère *Bosmina longirostris* et le rotifère *Keratella cochlearis* à de faibles concentrations de fer, tandis qu'à des concentrations élevées, ils étaient dominés par le rotifère *Euchlanis dilatata*. À la semaine 4 de l'expérience, *Chydorus sphaericus* dominait les mésocosmes à faible concentration de fer tandis que *Acanthocyclops vernalis* dominait les mésocosmes à forte concentration de fer. Ces changements communautaires se sont accompagnés de réductions drastiques de la richesse en espèces. D'autres études sur les effets du fer sur les communautés de zooplancton sont inexistantes. Mes résultats indiquent donc que le fer a des effets importants sur les communautés de phytoplancton et de zooplancton en raison de la modification de la composition des communautés et de la perte de l'abondance et de la diversité du zooplancton.

J'ai déterminé les EC_{50} au niveau communautaire pour la densité totale de zooplancton, de copépodes, de cladocères et de rotifères, ainsi que la biomasse totale de zooplancton, de copépodes et de rotifères. J'ai constaté que le EC_{50} de la biomasse totale de zooplancton et de cladocère était inférieur aux recommandations environnementales des États-Unis. Pendant ce temps, le EC_{50} de la densité totale de zooplancton, de copépode, de cladocère, de rotifère ainsi que la biomasse de copépode étaient au-dessus des recommandations gouvernementales. Dans l'ensemble, mes conclusions soulignent que la recommandation des États-Unis sur la qualité de l'eau pour le fer dans les

écosystèmes d'eau douce doit être retravaillée, car elles ne protègent pas adéquatement la biodiversité en eau douce pour le zooplancton. Ces résultats, ainsi que l'étude de Kotalik et al. (2019), qui a trouvé des EC_{20} problématiques pour la chlorophylle a, les diatomées et la biomasse des chlorophytes dans le contexte de la recommandation sur le fer des États-Unis. Ces résultats montrent la nécessité de révisions plus strictes et plus holistiques des recommandations pour la qualité de l'eau, surtout aux États-Unis.

2.3 Possibilités de recherche futures

Ma thèse présente de nouvelles découvertes concernant les effets des oxyhydroxydes de fer sur les communautés de plancton d'eau douce à différents niveaux trophiques, ainsi que sur les EC_{50} communautaires des oxyhydroxydes de fer par rapport aux recommandations de qualité de l'eau pour le fer dans les environnements d'eau douce. Cependant, de nombreuses questions demeurent et mes recherches représentent donc un point de départ pour d'autres recherches futures. Bien que ce projet ait tenu compte des effets des oxyhydroxydes de fer sur l'ensemble de la communauté de plancton, de nombreux groupes d'eau douce n'ont pas été pris en compte dans ce projet, comme les macroinvertébrés et les poissons, qui sont des composantes importantes du réseau trophique. La mise en œuvre d'expériences *in situ* impliquant davantage de niveaux trophiques des écosystèmes d'eau douce, ainsi que des mesures du flux d'énergie et de biomasse entre les niveaux trophiques, à travers un gradient d'exposition au fer produirait des informations importantes. Si le fer séquestre le phosphore au fond du réseau trophique, limitant ainsi potentiellement les producteurs primaires, l'énergie disponible pour le réseau trophique serait limitée, avec des effets en cascade sur le réseau trophique pour les consommateurs plus élevés. D'autre part, si la biomasse de phytoplancton est maintenue par des changements de composition dans la communauté, comme nous l'avons détecté, mais que la mortalité du zooplancton est directement affectée, alors les consommateurs primaires pourraient être limités en ressources (p. ex.

poissons larvaires), ce qui aurait des conséquences pour les populations de consommateurs. La présente étude suggère que la plupart des recommandations gouvernementales en matière de fer pour la protection des habitats d'eau douce, qui sont déduites d'essais toxicologiques d'une seule espèce, sont impropres à la protection de la biodiversité d'eau douce au niveau du zooplancton et, par conséquent, doivent être révisées davantage.

De plus, d'autres études concernant les effets des oxydes de fer sur les communautés et les écosystèmes d'eau douce n'ont pas tenu compte de la saisonnalité. La plupart des autres études, y compris la présente recherche, ont été réalisées au cours de l'été et de l'automne (Cadmus, Guasch et al., 2018; Kotalik et al., 2019; Molot et al., 2010). Seul Orihel et al. (2016), ont examiné les effets du fer sur les communautés de phytoplancton pendant l'hiver et le printemps. L'hiver et le printemps sont des périodes historiquement liées à une toxicité accrue en raison de la fonte des neiges, du ruissellement et des inondations, en particulier dans les zones urbaines (Gillis et al., 2022). La saisonnalité pourrait avoir une incidence sur la quantité, la diversité et le type d'espèces présentes dans les milieux d'eau douce (Shchapov et al., 2021). Étant donné que la saisonnalité pourrait affecter la toxicité différemment, la recherche sur ce sujet doit également être développée.

Enfin, d'autres recherches doivent être effectuées sur le rétablissement potentiel des écosystèmes à la suite d’une exposition aux oxydes de fer. Bien que les oxydes de fer se soient avérés être un outil de restauration utile pour inverser l'eutrophisation dans les lacs, mes recherches ont révélé que les ajouts d'oxyhydroxydes de fer aux écosystèmes d'eau douce peuvent entraîner des changements importants dans la composition des communautés de zooplancton et de phytoplancton, ainsi que dans la densité et la biomasse du zooplancton. Cependant, on en sait peu sur les effets

potentiels à long terme des oxydes de fer sur les écosystèmes d'eau douce ainsi que leur récupération suite à une exposition.

Dans l'ensemble, les oxydes de fer pourraient être considérés comme un outil de restauration utile pour les lacs confrontés à une eutrophisation sévère. Cependant, dans les habitats d'eau douce qui ne sont pas exposés à un excès de nutriments et à une croissance d’algues, le Fe a le potentiel d’avoir des effets négatifs indéniables sur les communautés de plancton, en particulier le zooplancton. Par conséquent, d'autres recherches sont nécessaires pour comprendre l'étendue des impacts écologiques du fer dans les réseaux trophiques aquatiques, la saisonnalité de la toxicité du fer liée au ruissellement, et la capacité de rétablissement de la communauté dans les systèmes naturels. Les recommandations sur la qualité de l'eau pour le fer doivent continuer d'être révisées afin d'établir des seuils qui protègent plus efficacement les organismes d'eau douce.

ANNEXE A

TEXTES, GRAPHIQUES ET RÉSULTATS SUPPLÉMENTAIRES

Supplemental text

Text S1. Oxygen isotope mass balance methods for estimating GPP:R

Here we report the isotopic composition of DO and H_2O in delta (δ) notation:

$$\delta^{18}O = (R_{sample} / R_{VSMOW}) - 1 \qquad (1)$$

where R is the isotope ratio (ratio of number of atoms of ^{18}O, $N(^{18}O)$, to that of ^{16}O, $N(^{16}O)$, in samples (R_{sample}) and in Vienna standard mean ocean water (VSMOW; R_{VSMOW}).

Duplicate samples for $\delta^{18}O$-DO were collected and archived following Bogard et al. (2020) and were analyzed using standard methods at the Veizer Isotope Lab at the University of Ottawa. Samples for $\delta^{18}O$-H_2O were collected and archived until later analysis using cavity ring down spectroscopy (Picarro L2130-i) at the Université du Québec à Montréal GEOTOP facility. As isotopic changes through time were minimal for H_2O, a value of -7.86 ‰ (n = 6, $\pm$1 S.D. = 0.47 ‰) was used for $\delta^{18}O$-H_2O in all calculations. This was the average of three samples collected in week 0 and three in week 4 from the same enclosures. Values of $\delta^{18}O$-DO were used from days 0 and 26 for each enclosure.

Estimates of atom fractions (AF) of ^{18}O, i.e. $N(^{18}O) / [N(^{16}O) + N(^{18}O)]$, were used in mass balance equations (Bogard and others 2020, and references therein):

$$AF = R_{sample} / (1 + R_{sample}) \qquad (2)$$

Assuming an absence of physically driven changes in DO via vertical or lateral exchange of water masses (i.e., each mesocosm is fully mixed), then metabolism and

atmospheric gas exchange are the only processes driving temporal DO and δ^{18}O-DO dynamics, as expressed by both equations:

$$dDO / dt = F_{O2} / Z_{mix} + GPP - R \qquad (3)$$

$$d^{18}O\text{-}DO / dt = k_{O2} \times \alpha_g \times (DO_{sat} \times AF_{air} \times \alpha_s - DO \times AF_{DO}) / Z_{mix} +$$

$$(GPP \times AF_{H2O} \times \alpha_p) - (R \times AF_{DO} \times \alpha_C) \qquad (4)$$

Atom fractions of DO, atmospheric O_2 and H_2O are denoted by AF_{DO}, AF_{air}, and AF_{H2O}, respectively. The δ^{18}O signature of atmospheric air is assumed to be 23.88‰ (Barkan & Luz 2005), updated from previous papers that used a value of 23.5‰ (Bocaniov et al., 2012; Bogard & del Giorgio 2016; Bogard and al., 2017). The gas transfer coefficient is defined as k_{O2}. Isotopic fractionation factors included kinetic gas exchange (α_g = 0.9972) (Knox and others 1992), gas solubility effects (α_s = 1.0007) (Benson & Krause, 1984), and photosynthesis (α_p = 1.000) (Guy and all., 1993). Isotopic fractionation linked to the consumption of DO (α_C) integrates all DO-consuming processes in the mesocosms, which excludes sediment R. Therefore, we used a fractionation factor of $\alpha_C \sim 0.980$, as sediment processes that are absent in this experiment would typically induce a smaller fractionation effect on the DO pool (Bogard et al. 2017 and references therein), so in absence of this effect it is more suitable to use a larger fractionation factor that represents values associated with pelagic R of non-eutrophic systems.

Assuming steady state conditions over the day to multi-day timescales, we can solve equation 4 for GPP and R sequentially (as done by Bogard et al. 2020). Here, because we are interested in the ratio of GPP:R only, we combine and simplify this calculation, obtaining the following equation as in Quay et al. (1995) and Bogard &

del Giorgio (2016), but with a modification where atom fractions are used in place of $^{18:16}O$ ratios:

$$GPP : R = AF_{H2O}\,\alpha_p - G \;/\; AF_{DO}\,\alpha_r - G \qquad (5)$$

where G represents the net flux of atmospheric O_2 across the air-water interface:

$$G = \alpha g\,(AF_{air}\alpha s - (AF_{DO}\_DO / DO_{sat}) \;/\; (1 - DO / DO_{sat}) \qquad (6)$$

Text S2. Effects of Fe exposure on zooplankton community biomass

Fe addition impacted zooplankton biomass community composition on PCoA axis 1 (Table A11, Fig. A22, Fig. A23 – GAM), but not on PCoA axis 2 (Table A11, Fig. A22, Fig. A24 – GAM). Species-specific biomass at the beginning of the experiment was dominated by *Ceriodaphnia lacustris* and *Diaphanosoma brachyurum*, followed by *Acanthocyclops vernalis* and *Bosmina longirostris* across all enclosures (Table A3). By week 3 and week 4 of the experiment, divergent patterns were observed across the Fe gradient: low Fe enclosures were dominated by cladocerans *Bosmina longirostris* and *Chydorus sphaericus*, respectively, and high Fe enclosures were dominated by *Acanthocyclops vernalis* at both time points (Fig. A22, Table A3). Overall, increasing Fe concentration did not alter zooplankton biomass, although the biomass of certain taxa were favoured, such as omnivorous/carnivorous cyclopoid copepods (Fig.A22, Table A3).

Table A1**:** Zooplankton species and phytoplankton groups detected in Lake Hertel (August 2021).

Zooplankton	**Group**
Tropocyclops mexicanus	Copepod
Acanthocyclops vernalis	Copepod
Ceriodaphnia lacustris	Cladocera
Diaphanosoma brachyurum	Cladocera
Bosmina longirostris	Cladocera
Daphnia parvula	Cladocera
Chydorus sphaericus	Cladocera
Alona sp.	Cladocera
Acroperus harpae	Cladocera
Sida crystallina	Cladocera
Monostylla bulla	Rotifer
Euchlanis dilatata	Rotifer
Keratella cochlearis	Rotifer
Polyarthra sp.	Rotifer
Trichocerca sp.	Rotifer
Ploesoma truncatum	Rotifer
Hexarthra mira	Rotifer
Conochilus unicornis	Rotifer
Kellicottia longispina	Rotifer
Gastropus stylifer	Rotifer
Ascomorpha saltans	Rotifer
Trichotria tetractis	Rotifer
Platyias patulus	Rotifer
Phytoplankton	
Green algae	
Blue green algae	
Diatoms	
Cryptophyta	

Table A2: Species-specific zooplankton densities (individuals/L) at the beginning, middle and end of the experiment.

			Density (ind/L)															
			Start	Middle (Day 19)														
Iron Concentration	Family	Feeding habits	N/A	0	0,1	0,3	0,5	0,75	1	2	3	4	5	6	7	8	9	10	Chloride
Nauplii		N/A	18,99	2,78	4,04	14,66	5,16	9,28	9,22	2,94	1,74	4	3,28	1,12	1,78	0,1	0,3	0,6	3,44
Immature cyclopoid	Cyclopidae	N/A	10,47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tropocyclops mexicanus	Cyclopidae	Omnivore	1,56	0	0	0	0,02	0	0	0	0	0	0	0	0	0	0	0	0,02
Acanthocyclops vernalis	Cyclopidae	Omnivore-Carnivore	2,18	0,12	0,16	0,1	0,12	0,24	0,16	0,3	0,04	0	0,1	0,1	0,1	0,26	0,18	0,22	0,06
Ceriodaphnia lacustris	Daphniidae	Herbivore	51,95	0	0	0	0	0,02	0	0,04	0	0	0,02	0	0	0	0	0	0
Diaphanosoma brachyurum	Sididae	Herbivore	12,46	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bosmina longirostris	Bosminidae	Herbivore	4,53	2,6	2,96	1,06	4,88	0,86	0,72	5,54	0	0,14	0,06	0,06	0,02	0	0,04	0	1,84
Daphnia parvula	Daphniidae	Herbivore	0,21	0	0	0	0	0	0	0	0	0	0	0	0	0,02	0	0	0
Chydorus sphaericus	Chydoridae	Herbivore	0	0,46	1,02	0,58	0,94	0,3	1,28	0,02	0	0,04	0,04	0	0,06	0	0,02	0	0,26
Alona sp.	Chydoridae	Herbivore	0	0	0	0	0,04	0	0	0	0	0	0	0	0	0	0	0	0
Acroperus harpae	Chydoridae	Herbivore	0	0,12	0,02	0	0	0	0	0,02	0	0	0	0	0,02	0	0	0	0,02
Sida crystallina	Sididae	Herbivore	0	1,24	0	0	0	0,42	0,02	0,02	0	0	0	0	0	0	0	0	0,02
Monostylla bulla	Lecanidae	N/A	0,02	0,56	1,54	0,64	2,58	0,36	0,1	0,24	0,06	0	0,02	0,02	0,18	0	0,12	0,12	1,06
Euchlanis dilatata	Brachionidae	N/A	0	1,34	0,04	1,28	3,82	0,34	0,28	0,48	0,02	0,02	0,52	0,06	0,26	0,04	0,68	0,04	1,22
Keratella cochlearis	Brachionidae	N/A	0,51	0	0	2,68	0,28	0,26	0,1	3,72	0,02	0,4	0,02	0	0,04	0	0,12	0,04	0,3
Polyarthra sp	Synchaetidae	N/A	10,41	0	0	0,02	0	0	0	0,02	0	0	0	0	0	0	0	0	0
Trichocerca sp.	Trichocercidae	N/A	1,16	0	0	0,02	0	0	0	0	0	0,02	0	0	0	0	0	0	0
Ploesoma truncatum	Synchaetidae	N/A	4,18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hexarthra mira	Hexarthridae	N/A	1,46	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Conochilus unicornis	Conochilidae	N/A	26,75	0	0	0	0	0	0,16	0	0	0	0	0	0	0	0	0	0
Kellicottia longispina	Brachionidae	N/A	0,32	0	0	0	0	0	0	0,02	0	0	0	0	0	0	0	0	0
Gastropus stylifer	Gastropidae	N/A	0,16	0	0	0	0	0	0	0	0	0,2	0	0	0	0	0	0	0
Ascomorpha saltans	Gastropidae	N/A	0	0	0	0	0	0	0	0,6	0	0	0	0	0	0	0,08	0	0
Trichotria tetractis	Brachionidae	N/A	0	0,02	0,04	0,26	0,04	0,04	0,12	0	0	0,06	0	0	0,02	0	0	0	0
Platyias patulus	Brachionidae	N/A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Iron Concentration	Family	Feeding habits	Start	End (Day 28)															
Nauplii		N/A	18,99	0	0	0	3,25	0	77	5,40	0,005	5,02	0,11	0,01	0,055	0,015	0,205	0,01	2,75
Immature cyclopoid	Cyclopidae	N/A	10,47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tropocyclops mexicanus	Cyclopidae	Omnivore	1,56	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acanthocyclops vernalis	Cyclopidae	Omnivore-Carnivore	2,18	0,29	1,25	0	1	0,25	0,25	0,22	0,065	0,24	0,365	0,065	0,11	0,285	0,105	0,06	0,25
Ceriodaphnia lacustris	Daphniidae	Herbivore	51,95	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diaphanosoma brachyurum	Sididae	Herbivore	12,46	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bosmina longirostris	Bosminidae	Herbivore	4,53	0,071	2,75	0	3	0	0,5	2,14	0	0	0,005	0	0	0	0,005	0	0,5
Daphnia parvula	Daphniidae	Herbivore	0,21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chydorus sphaericus	Chydoridae	Herbivore	0	24,79	157,75	85	59,5	67	48,75	2,54	0,145	4,53	0,33	0,065	0,03	0,005	0	0	107,75
Alona sp.	Chydoridae	Herbivore	0	0	0,25	0,25	0	0	0,25	0,021	0	0,082	0	0,035	0,005	0,035	0,02	0	0,25
Acroperus harpae	Chydoridae	Herbivore	0	0,071	1	0	0	0,25	0,5	0,014	0	0,061	0,005	0	0	0	0	0	0,75
Sida crystallina	Sididae	Herbivore	0	0	0	0	0	0	0,25	0,041	0	0	0	0	0	0	0	0	1,25
Monostylla bulla	Lecanidae	N/A	0,02	0,04	1,1	0,025	12,11	7	2,06	0,15	0,005	0,01	0,005	0,01	0,005	0,005	0,005	0	3,14
Euchlanis dilatata	Brachionidae	N/A	0	0	0,015	0	0	0,38	2,11	0,068	0,135	0,07	0,025	0,035	0,15	0	0	0,045	0,33
Keratella cochlearis	Brachionidae	N/A	0,51	0,005	0,015	0,27	0,22	0,10	0,04	1,42	0	0,005	0	0	0	0	0	0	0,016
Polyarthra sp	Synchaetidae	N/A	10,41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trichocerca sp.	Trichocercidae	N/A	1,16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ploesoma truncatum	Synchaetidae	N/A	4,18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hexarthra mira	Hexarthridae	N/A	1,46	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Conochilus unicornis	Conochilidae	N/A	26,75	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Kellicottia longispina	Brachionidae	N/A	0,32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gastropus stylifer	Gastropidae	N/A	0,16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ascomorpha saltans	Gastropidae	N/A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trichotria tetractis	Brachionidae	N/A	0	0	0,075	0	1,06	0,13	1,86	0,014	0	0,95	0,005	0	0	0	0	0	0
Platyias patulus	Brachionidae	N/A	0	0	0	0	0	0	0	0,0068	0	0	0	0	0	0	0	0	0

Table A3: Species-specific zooplankton biomass (µg/L) at the beginning, middle and end of the experiment.

			Biomass (ug/L)																
			Start	Middle (Day 19)															
Iron Concentration	Family	Feeding habits	N/A	0	0,1	0,3	0,5	0,75	1	2	3	4	5	6	7	8	9	10	Chloride
Nauplii	N/A	N/A	2,39	0,20	0,29	1,21	0,39	0,67	0,68	0,21	0,12	0,26	0,23	0,08	0,57	0,12	0,023	0,041	0,27
Acanthocyclops vernalis	Cyclopidae	Omnivore-Carnivore	6,82	0,19	0,36	0,23	0,25	0,37	0,41	0,54	0,24	0,00	0,18	0,38	0,17	0,86	0,26	0,69	0,18
Tropocyclops mexicanus	Cyclopidae	Omnivore	0,53	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ceriodaphnia lacustris	Daphniidae	Herbivore	36,82	0	0	0	0	0	0	0	0	0	0,021	0	0	0	0	0	0
Diaphanosoma brachyurum	Sididae	Herbivore	32,44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bosmina longirostris	Bosminidae	Herbivore	4,15	3,19	3,91	1,95	5,65	1,07	0,60	6,04	0	0,15	0,060	0,062	0,020	0	0,040	0	2,20
Acroperus harpae	Chydoridae	Herbivore	0	0,23	0	0	0	0	0	0	0	0	0	0	0,020	0	0	0	0
Chydorus sphaericus	Chydoridae	Herbivore	0	0,29	0,55	0,48	1,03	0,14	0,76	0	0	0,029	0,020	0	0,035	0	0	0	0
Daphnia parvula	Daphniidae	Herbivore	0,56	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sida crystallina	Sididae	Herbivore	0	5,89	0	0	0	1,93	0,045	0	0	0	0	0	0	0	0	0	0
Alona sp.	Chydoridae	Herbivore	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Feeding habits	**Start**	**End (Day 28)**															
Nauplii	N/A	N/A	2,39	0	0	0	0,26	0	0	0,44	0,0003	1,36	0,0075	0,00061	0,0053	0,0010	0,0246	0,00088	0,1886
Acanthocyclops vernalis	Cyclopidae	Omnivore-Carnivore	6,82	1,25	3,05	0	2,01	0,68	1,35	0,60	0,21	0,62	1,27	0,20	0,32	0,86	0,27	0,12	1,05
Tropocyclops mexicanus	Cyclopidae	Omnivore	0,53	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ceriodaphnia lacustris	Daphniidae	Herbivore	36,82	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diaphanosoma brachyurum	Sididae	Herbivore	32,44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bosmina longirostris	Bosminidae	Herbivore	4,15	0,11	3,46	0	3,63	0	0,62	0	0	0	0,010	0	0	0	0	0	0,73
Acroperus harpae	Chydoridae	Herbivore	0	0,14	1,96	0	0	0,52	0,94	0,029	0	0,11	0,0086	0	0	0	0	0	1,43
Chydorus sphaericus	Chydoridae	Herbivore	0	18,48	84,74	82,75	18,44	50,46	26,67	0,84	0,11	1,32	0,021	0,042	0,023	0,0035	0	0	31,95
Daphnia parvula	Daphniidae	Herbivore	0,56	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sida crystallina	Sididae	Herbivore	0	0	0	0	0	0	0	0,19	0	0	0	0	0	0	0	0	6,02
Alona sp.	Chydoridae	Herbivore	0	0	0,17	0,047	0	0	0,12	0	0	0,024	0	0,015	0,0011	0,012	0,0052	0	0,12

Table A4**:** Water chemistry - Fe and TP statistical models for parametric and smooth terms of GAMs and LMs. GAMs included a smooth effect for Fe and a random effect smooth for time. Significant parametric and smooth terms are indicated in bold and random effects are indicated in italic. Estimated iron concentration represents the Fe concentration estimated using conductivity.

Estimated iron concentration				
A. parametric coefficients	Estimate	Standard Error	t-value	p-value
(Intercept)	-0,63666	0,30702	-20,74	0,0585
Nominal Fe	**1,50214**	**0,06053**	**24,817**	**2,46E-12**
R2-adjusted	0,98			
Measured total iron concentration				
A. parametric coefficients	Estimate	Standard Error	t-value	p-value
(Intercept)	**-2,087**	**0,9509**	**-2,195**	**0,0345**
B. smooth terms	edf	Red.df	F-value	p-value
s(Nominal Fe)	**4,849**	**5,908**	**55,7**	**<2E-16**
s(Time)	***1,984***	***2***	***145***	***<2E-16***
R2-adjusted	0,986			
Deviance explained	94,7%			
Measured dissolved iron concentration				
A. parametric coefficients	Estimate	Standard Error	t-value	p-value
(Intercept)	**0,0059743**	**0,0007676**	**7,783**	**3,02E-06**
Nominal Fe	**-0,0005348**	**0,0001513**	**-3,534**	**0,00367**
R2-adjusted	0,45			
Total phosphorus				
A. parametric coefficients	Estimate	Standard Error	t-value	p-value
(Intercept)	**7,955**	**1,835**	**4,334**	**9,9E-05**
B. smooth terms	edf	Red.df	F-value	p-value
s(Nominal Fe)	**2,889**	**3,577**	**8,498**	**8,97E-05**
s(Time)	***1,978***	***2***	***90,983***	***<2E-16***
R2-adjusted	0,828			
Deviance explained	84,7%			

Table A5**:** AIC and R^2_{adj} of possible zooplankton density models. For each response variable, a term was removed until arriving with most simple model possible. The selected model was bolded.

Total zooplankton density				
Model No.	Variables	AIC	R^2_{adj}	Term removed
0	**FE + TIME + TP + FExTIME**	**21,45**	**0,819**	**-**
1	FE + TIME + TP	20,03	0,827	FExTIME
2	FE + TIME	31,91	0,703	TP
3	FE + TIME (linear)	45,18	0,663	GAM
Total cladoceran density				
Model No.	Variables	AIC	R^2_{adj}	Term removed
0	**FE + TIME + TP + FExTIME**	**25,33**	**0,809**	**-**
1	FE + TIME + TP	24,97	0,806	FExTIME
2	FE + TIME	32,87	0,722	TP
3	FE + TIME (linear)	50,8	0,686	GAM
Total copepod density				
Model No.	Variables	AIC	R^2_{adj}	Term removed
0	**FE + TIME + TP + FExTIME**	**33,08**	**0,293**	**-**
1	FE + TIME + TP	32,53	0,292	FExTIME
2	FE + TIME	30,64	0,286	TP
3	FE + TIME (linear)	42,43	0,339	GAM
Total rotifer density				
Model No.	Variables	AIC	R^2_{adj}	Term removed
0	**FE + TIME + TP + FExTIME**	**10,18**	**0,293**	**-**
1	FE + TIME + TP	7,08	0,292	FExTIME
2	FE + TIME	11,68	0,286	TP
3	FE + TIME (linear)	24,15	0,346	GAM

Table A6**:** AIC and R^2_{adj} of possible zooplankton biomass models. For each response variable, a term was removed until arriving with most simple model possible. The selected model was bolded.

Total zooplankton biomass				
Model No.	Variables	AIC	R^2_{adj}	Term removed
0	**FE + TIME + TP + FExTIME**	**8,07**	**0,852**	**-**
1	FE + TIME + TP	7,3	0,854	FExTIME
2	FE + TIME	22,24	0,724	TP
3	FE + TIME (linear)	42,98	0,623	GAM
Total cladoceran biomass				
Model No.	Variables	AIC	R^2_{adj}	Term removed
0	**FE + TIME + TP + FExTIME**	**17,91**	**0,829**	**-**
1	FE + TIME + TP	19,17	0,815	FExTIME
2	FE + TIME	24,89	0,751	TP
3	FE + TIME (linear)	47,28	0,641	GAM
Total copepod biomass				
Model No.	Variables	AIC	R^2_{adj}	Term removed
0	**FE + TIME + TP + FExTIME**	**-39,11**	**0,397**	**-**
1	FE + TIME + TP	-36,49	0,344	FExTIME
2	FE + TIME	-31,1	0,118	TP
3	FE + TIME (linear)	-15,45	0,143	GAM

Table A7**:** AIC and R^2_{adj} of possible phytoplankton biomass models. For each response variable, a term was removed until arriving with most simple model possible. The selected model was bolded.

Total phytoplankton biomass				
Model No.	Variables	AIC	R^2_{adj}	Term removed
0	**FE + TIME + TP + FExTIME**	**199,77**	**0,423**	**-**
1	FE + TIME + TP	205,71	0,298	FExTIME
2	FE + TIME	208,32	0,241	TP
3	FE + TIME (linear)	215,53	0,329	GAM
Total Chlorophyta biomass				
Model No.	Variables	AIC	R^2_{adj}	Term removed
0	**FE + TIME + TP + FExTIME**	**191,2**	**0,469**	**-**
1	FE + TIME + TP	193,48	0,443	FExTIME
2	FE + TIME	201,99	0,261	TP
3	FE + TIME (linear)	208,9	0,326	GAM
Total Cyanobacteria biomass				
Model No.	Variables	AIC	R^2_{adj}	Term removed
0	**FE + TIME + TP + FExTIME**	**90,14**	**0,358**	**-**
1	FE + TIME + TP	90,85	0,335	FExTIME
2	FE + TIME	90,58	0,326	TP
3	FE + TIME (linear)	102,08	0,373	GAM
Total diatom biomass				
Model No.	Variables	AIC	R^2_{adj}	Term removed
0	**FE + TIME + TP + FExTIME**	**24,35**	**0,606**	**-**
1	FE + TIME + TP	24,28	0,598	FExTIME
2	FE + TIME	22,4	0,607	TP
3	FE + TIME (linear)	40,87	0,692	GAM
Total Cryptophyta biomass				
Model No.	Variables	AIC	R^2_{adj}	Term removed
0	**FE + TIME + TP + FExTIME**	**1,8**	**0,613**	**-**
1	FE + TIME + TP	1,23	0,606	FExTIME
2	FE + TIME	-0,8	0,619	TP
3	FE + TIME (linear)	18,64	0,697	GAM

Table A8: Model selection: for each response variable, a linear model (LM) and a generalized additive model (GAM) were compared, and the best model is bolded. Random variables are in italic.

Model No.	Variables	Versus model No.	AIC	Term removed
	Estimated iron concentration			
0	Fe (GAM)	-	39,49	-
1	**Fe (LM)**	**0**	**39,49**	**GAM**
	Measured iron concentration			
0	**Fe + *Time* (GAM)**	**-**	**-84,76**	**-**
1	Fe + *Time (LM)*	0	118,37	GAM
	Dissolved iron concentration			
0	Fe (GAM)	-	-140,25	-
1	**Fe (LM)**	**0**	**-140,25**	**GAM**
	Total phosphorus			
0	**Fe + *Time* (GAM)**	**-**	**159,05**	**-**
1	Fe + *Time* (LM)	0	181,14	GAM

Table A9: Phytoplankton community composition (PCoA axes) in relation with iron concentration and time as a random effect (italic) in the GAMs. Statistically significant variables are indicated in bold and random effects are indicated in italic.

Phytoplankton PCoA1				
A. parametric coefficients	Estimate	Standard Error	t-value	p-value
(Intercept)	3,37E-16	0,1747	0	1
B. smooth terms	edf	Red.df	F-value	p-value
s(Fe)	**1**	**1**	**9,988**	**0,00296**
s(Time)	***1,83***	***2***	***10,789***	***9,05E-05***
R2-adjusted	0,41			
Deviance explained	44,8%			
Phytoplankton PCoA2				
A. parametric coefficients	Estimate	Standard Error	t-value	p-value
(Intercept)	**4,83E-15**	**0,2135**	**0**	**1**
B. smooth terms				
s(Fe)	**1**	**1**	**4,189**	**0,0471**
s(Time)	***1,901***	***2***	***19,22***	***9,22E-07***
R2-adjusted	0,486			
Deviance explained	52%			

Table A10: Zooplankton (crustacean zooplankton and rotifers) community composition of crustaceans and rotifers (PCoA axes) in relation with Fe concentration and time as a random effect (italic) in the GAMs. Statistically significant variables are bolded.

Zooplankton density PCoA1				
A. parametric coefficients	Estimate	Standard Error	t-value	p-value
(Intercept)	1,61E-16	0,2768	0	1
B. smooth terms	edf	Red.df	F-value	p-value
s(Fe)	**1**	**1**	**67,38**	**<2E-16**
s(Time)	***0,9544***	***1***	***20,92***	***7,23E-05***
R2-adjusted	0,751			
Deviance explained	76,7%			
Zooplankton density PCoA2				
A. parametric coefficients	Estimate	Standard Error	t-value	p-value
(Intercept)	**1,86E-15**	**0,4669**	**0**	**1**
B. smooth terms				
s(Fe)	**1**	**1**	**14,29**	**0,00079**
s(Time)	***0,9791***	***1***	***46,74***	***<2E-16***
R2-adjusted	0,675			
Deviance explained	69,7%			

Table A11: Statistical summary of parametric and smooth terms of the generalized additive models with PCoA axis for zooplankton biomass PCoA in relation with Fe concentration and time as a random effect (italic). Statistically significant variables are bolded.

Zooplankton biomass PCoA1				
A. parametric coefficients	Estimate	Standard Error	t-value	p-value
(Intercept)	4,69E-17	0,0594	0	1
B. smooth terms	edf	Red.df	F-value	p-value
s(Fe)	**1**	**1**	**84,16**	**<2E-16**
s(Time)	*5,28E-06*	*1*	*0*	*0,822*
R2-adjusted	0,741			
Deviance explained	75%			
Zooplankton biomass PCoA2				
A. parametric coefficients	Estimate	Standard Error	t-value	p-value
(Intercept)	**-1,33E-15**	**0,5021**	**0**	**1**
B. smooth terms				
s(Fe)	1	1	0,003	0,96
s(Time)	***0,9789***	***1***	***46,29***	***<2E-16***
R2-adjusted	0,61			
Deviance explained	63,6%			

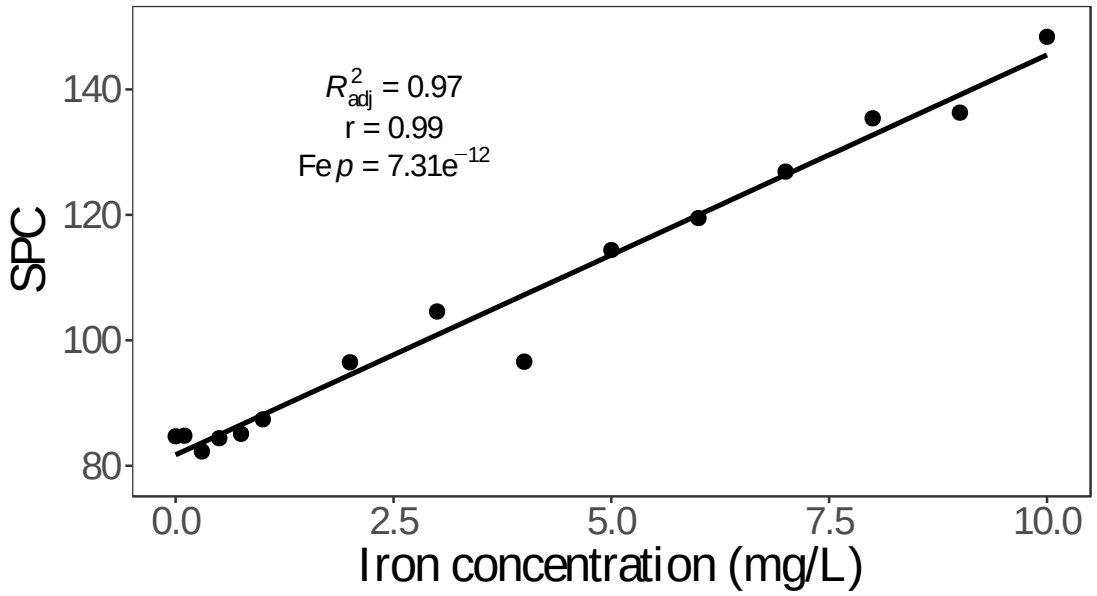

Figure A1**:** Graphical representation of Pearson's correlation between iron concentration and specific conductivity (SPC) on day 28 of the experiment. A linear model was used for adjusted R^2 and iron p-value.

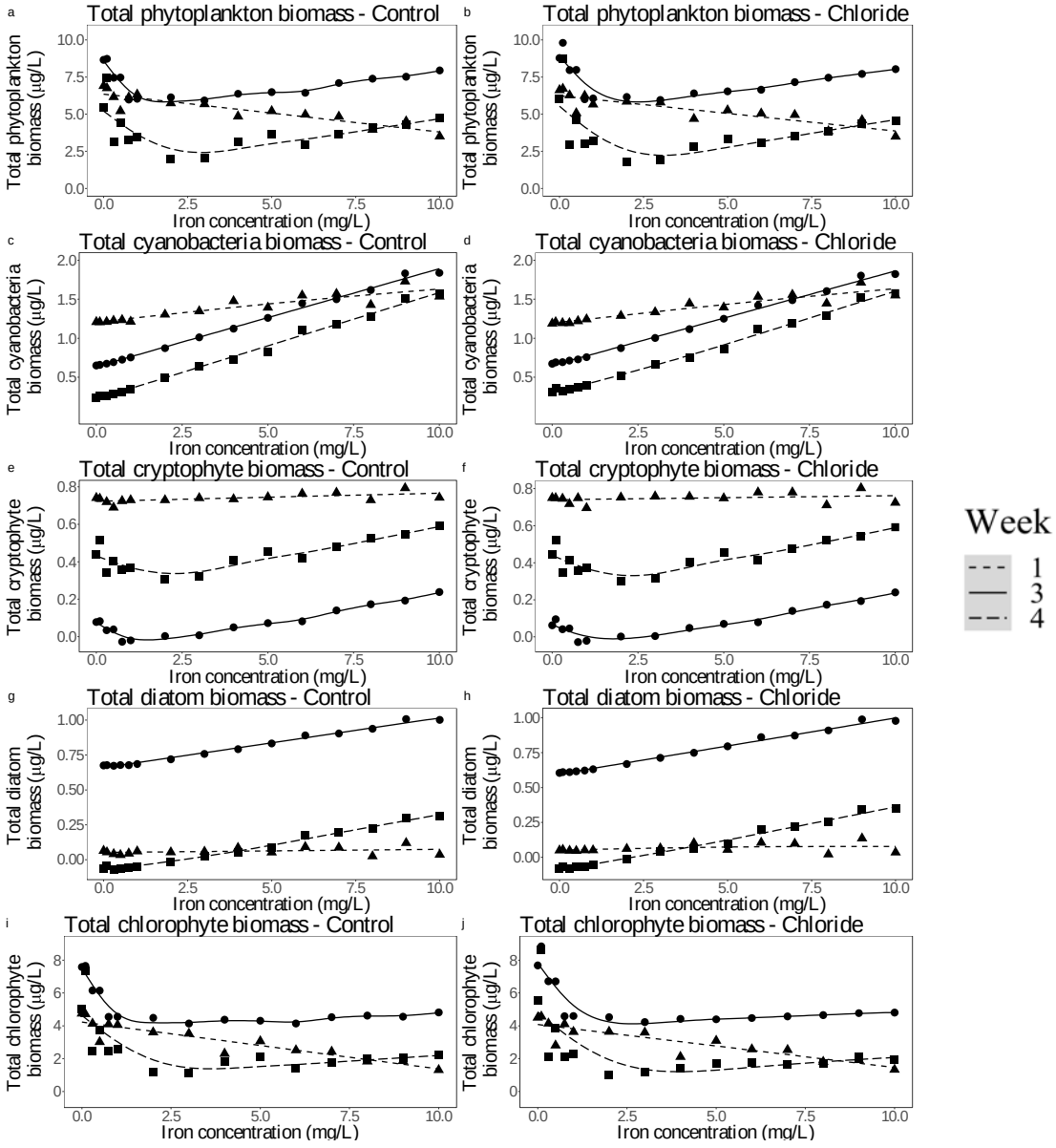

Figure A2: Comparison of total phytoplankton biomass (a & b), cyanobacteria biomass (c & d), cryptophyte biomass (e & f), diatom biomass (g & h) and chlorophyte biomass (i & j) model predictions depending on the control data selected. Week 1 is represented by the short dashed line, week 3 is represented by the full line and week 4 by the long dashed line. Two different models, one with the Fe control and the other with the chloride control, were used to compare similarity. Subsequent prediction was done on those models, and visual analysis was done to see if models greatly changed. The shaded area represents the 95% confidence interval.

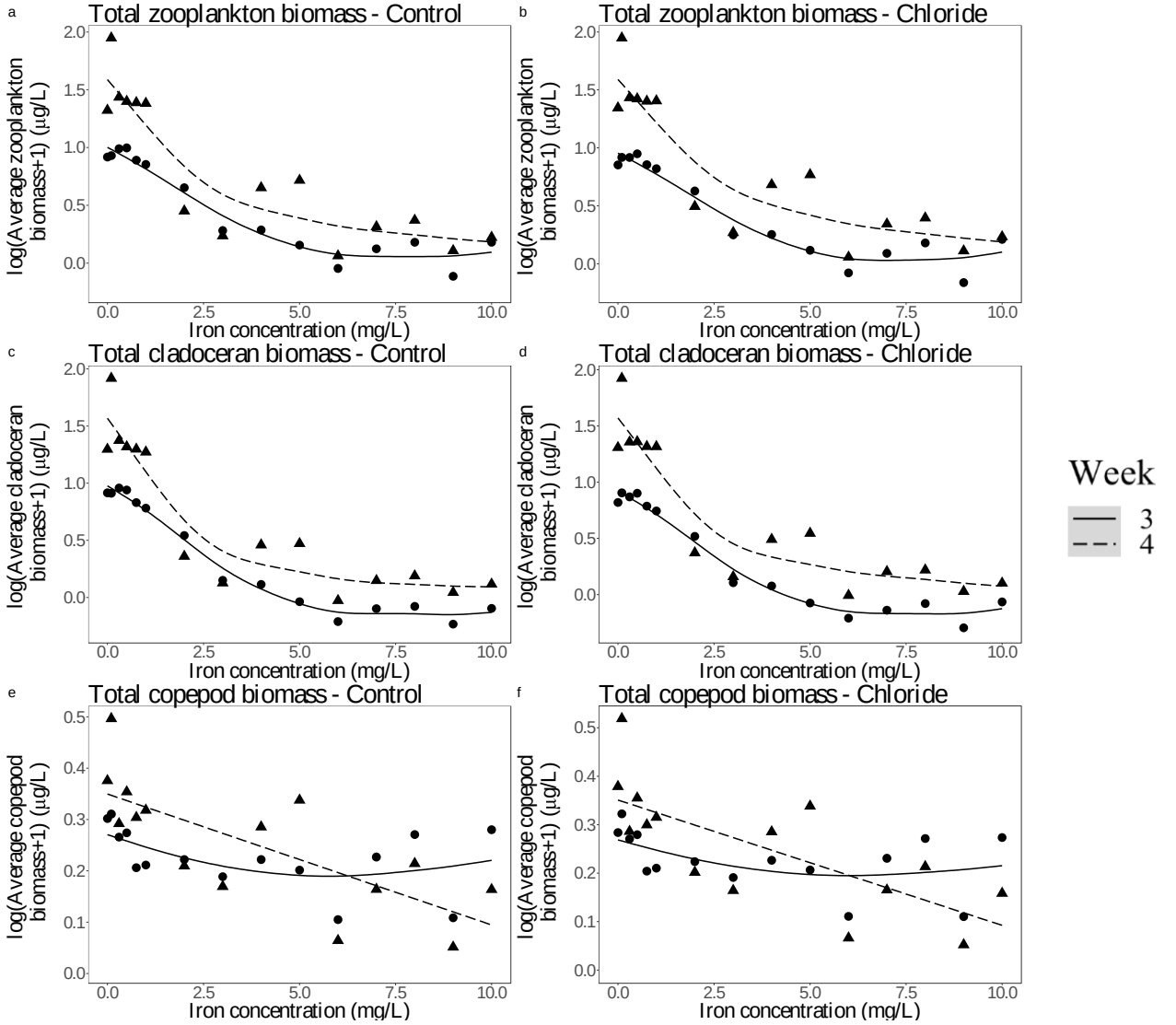

Figure A3: Comparison of total zooplankton biomass (a & b), total cladoceran biomass (c & d) and copepod biomass (e & f) models depending on the control data selected. Week 3 is represented by a full line and week 4 is represented by a dashed line. Two different models, one with the Fe control and the other with the chloride control, were used to compare similarity. Subsequent prediction was done on those models, and visual analysis was done to see if models greatly changed. The shaded area represents the 95% confidence interval of the general additive models.

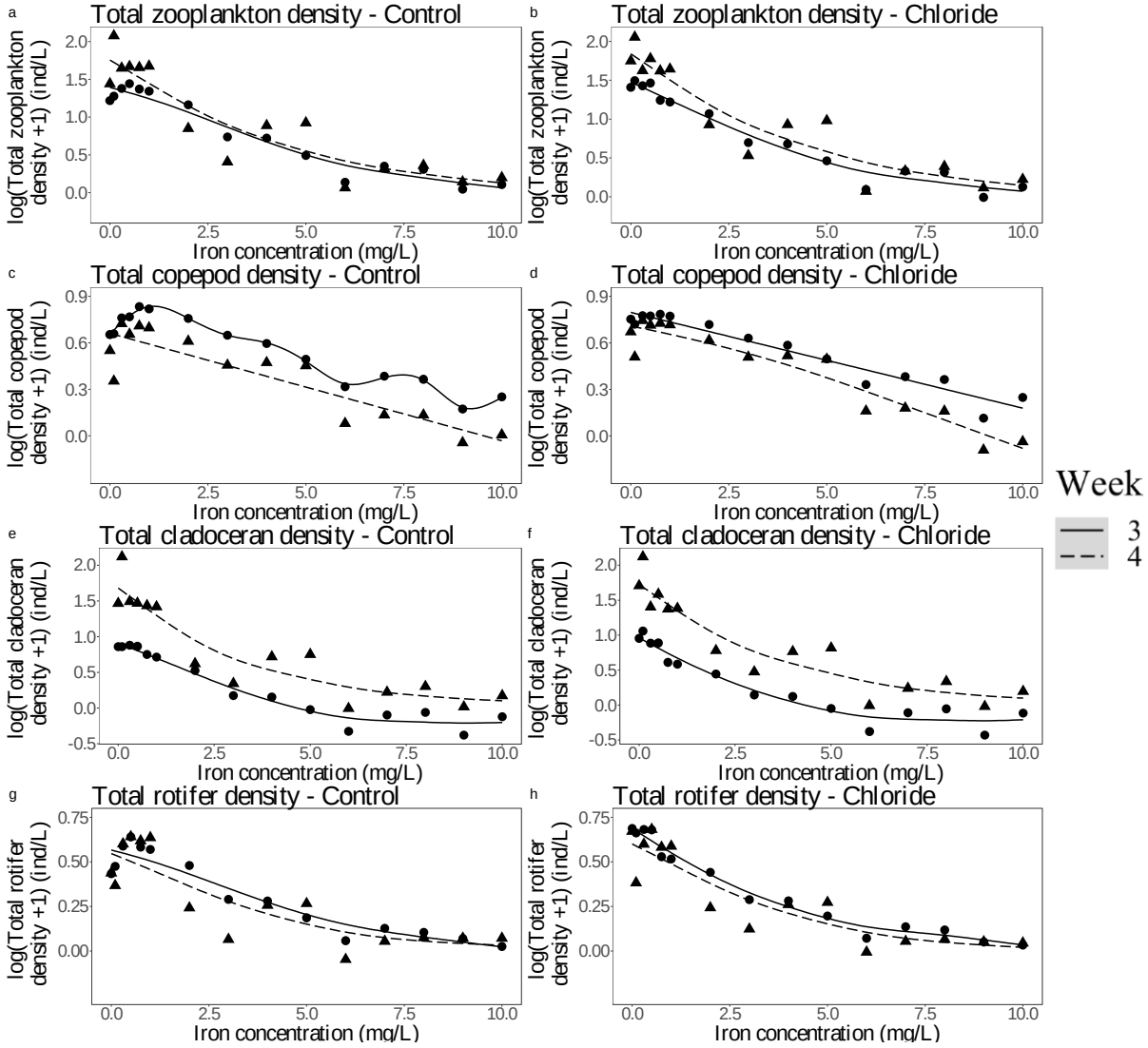

Figure A4: Comparison of total zooplankton density (a & b), total cladoceran density (c & d), total copepod biomass (e & f) and total rotifer density (g & h) models depending on the control data selected. Week 3 is represented by a full line and week 4 is represented by a dashed line. Two different models, one with the Fe control and the other with the chloride control, were used to compare similarity. Subsequent prediction was done on those models, and visual analysis was done to see if models greatly changed. The shaded area represents the 95% confidence interval of the general additive models.

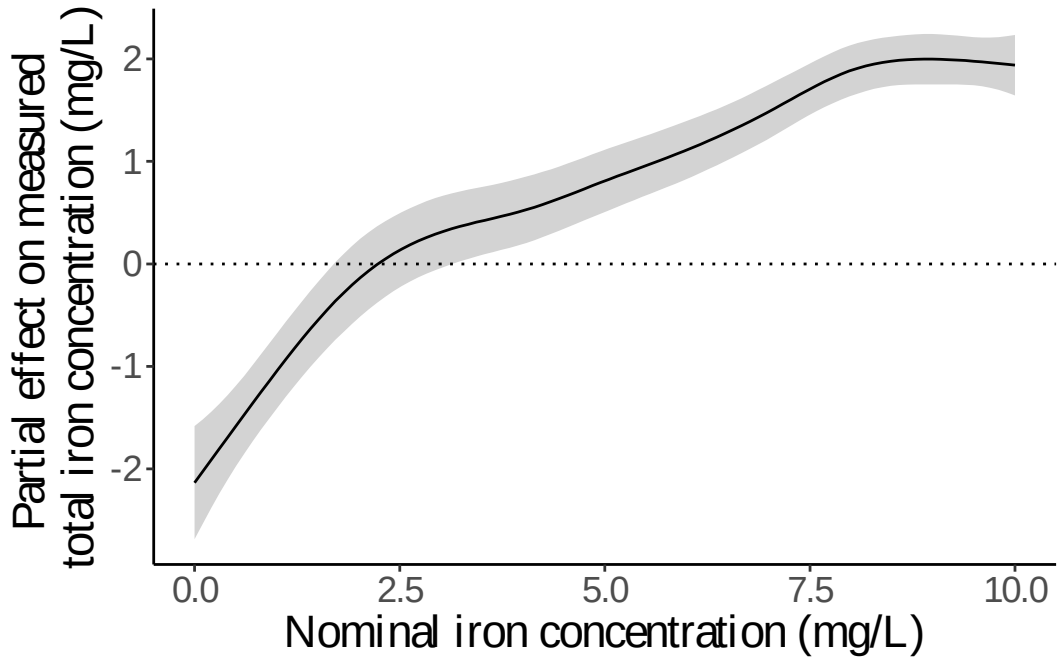

Figure A5. Generalized additive model partial smooth of nominal iron concentration (mg/L) on measured total iron (mg/L). The grey area represents the 95% confidence interval, the dashed line represents the null effect and the rug plot the distribution of the data.

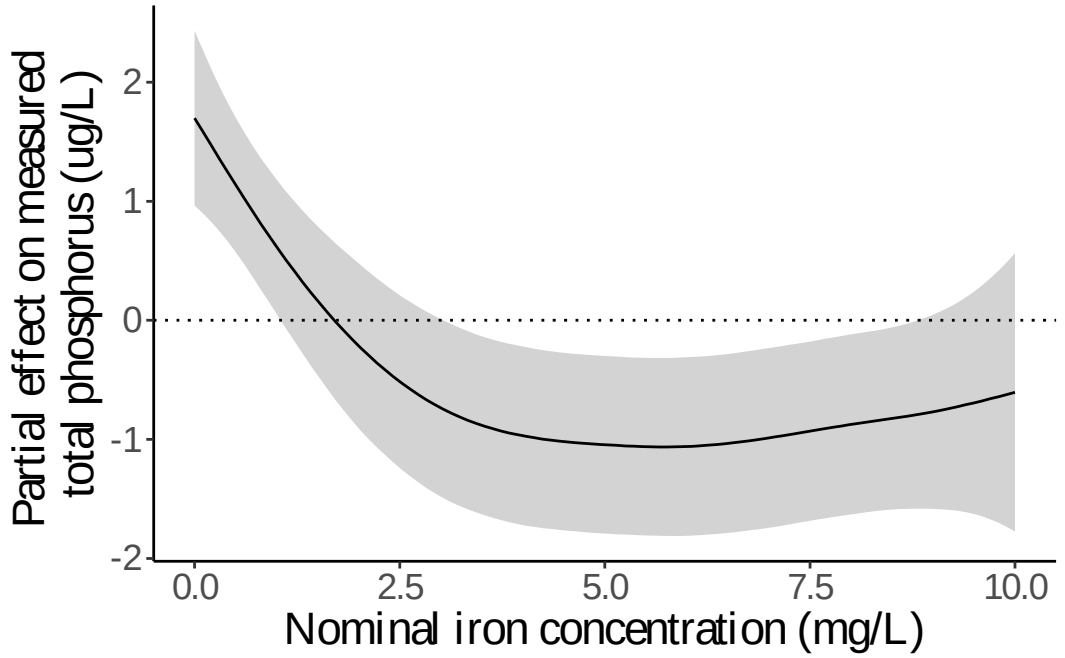

Figure A6: Generalized additive model partial smooth of nominal iron concentration (mg/L) on total phosphorus (ug/L). The grey area represents the 95% confidence interval, the dashed line represents the null effect and the rug plot the distribution of the data.

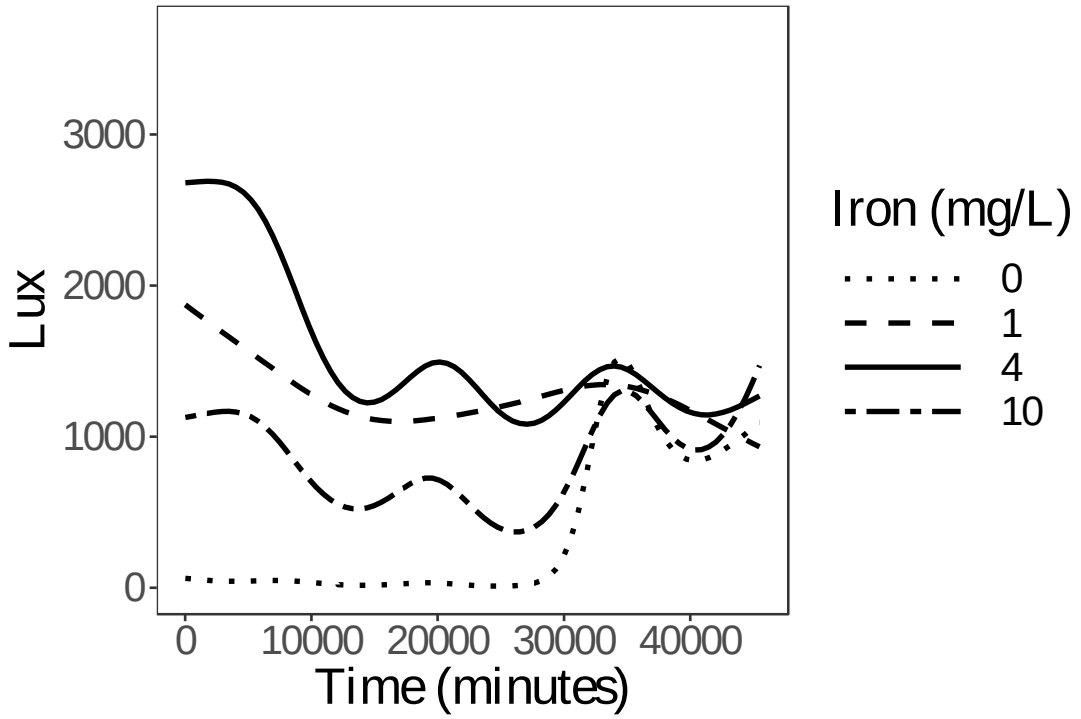

Figure A7: Light intensity (Lux) in the enclosures across the iron concentration (mg/L) gradient and time (minutes). Generalized additive models (GAMs) were used to graph the data. HOBO data loggers were put in the middle of the water column of select enclosures across the iron gradient and were used to measure light intensity.

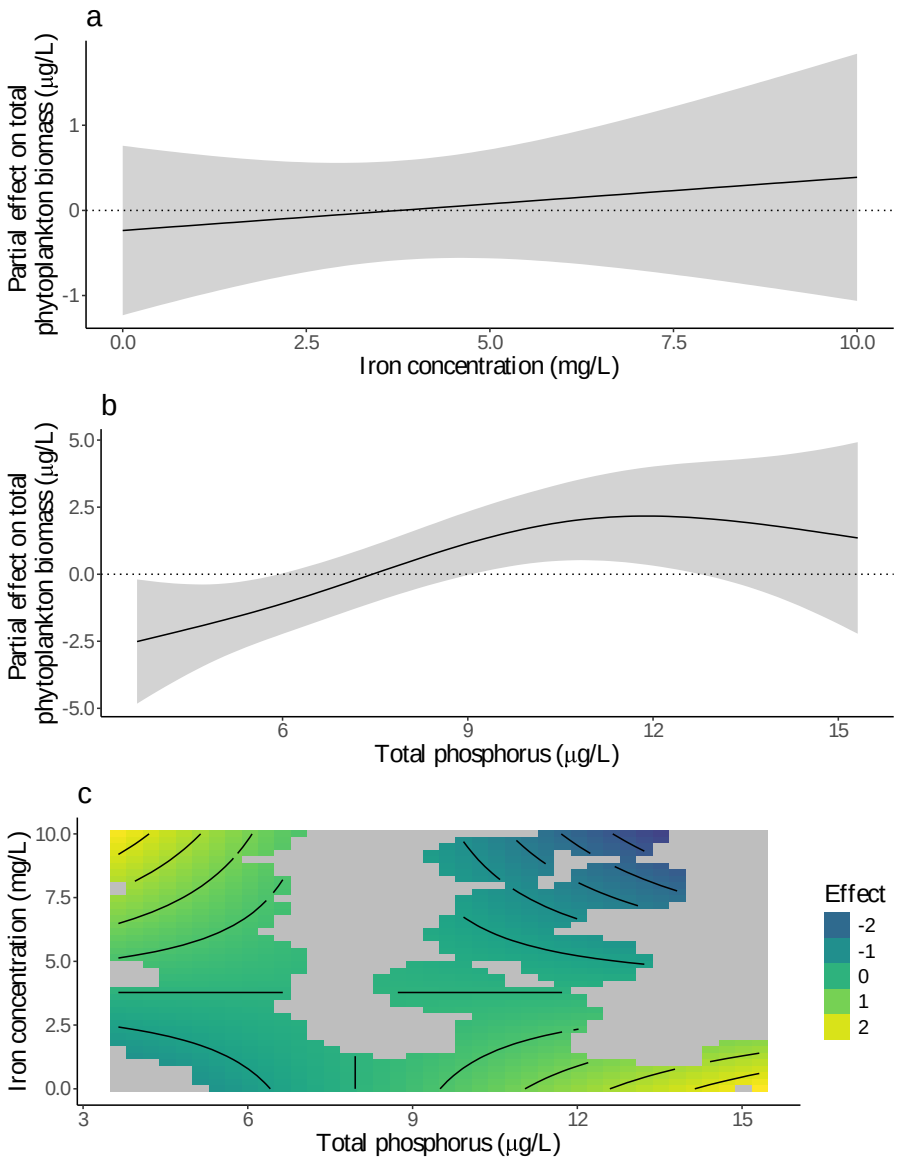

Figure A8: a. Generalized additive model partial smooth of iron concentration (mg/L) on total phytoplankton biomass (µg/L). The grey area represents the 95% confidence interval, the dashed line represents the null effect and the rug plot the distribution of the data. b. Generalized additive model partial smooth of total phosphorus (µg/L) on total phytoplankton biomass (µg/L). The grey area represents the 95%, the dashed line represents the null effect and the rug plot the distribution of the data. c. Tensor product smooth of the relation between total phosphorus (µg/L) and iron concentration (mg/L) on total phytoplankton biomass (µg/L). Dark blue represents negative effects on total phytoplankton biomass, while yellow represents positive impacts on total phytoplankton biomass.

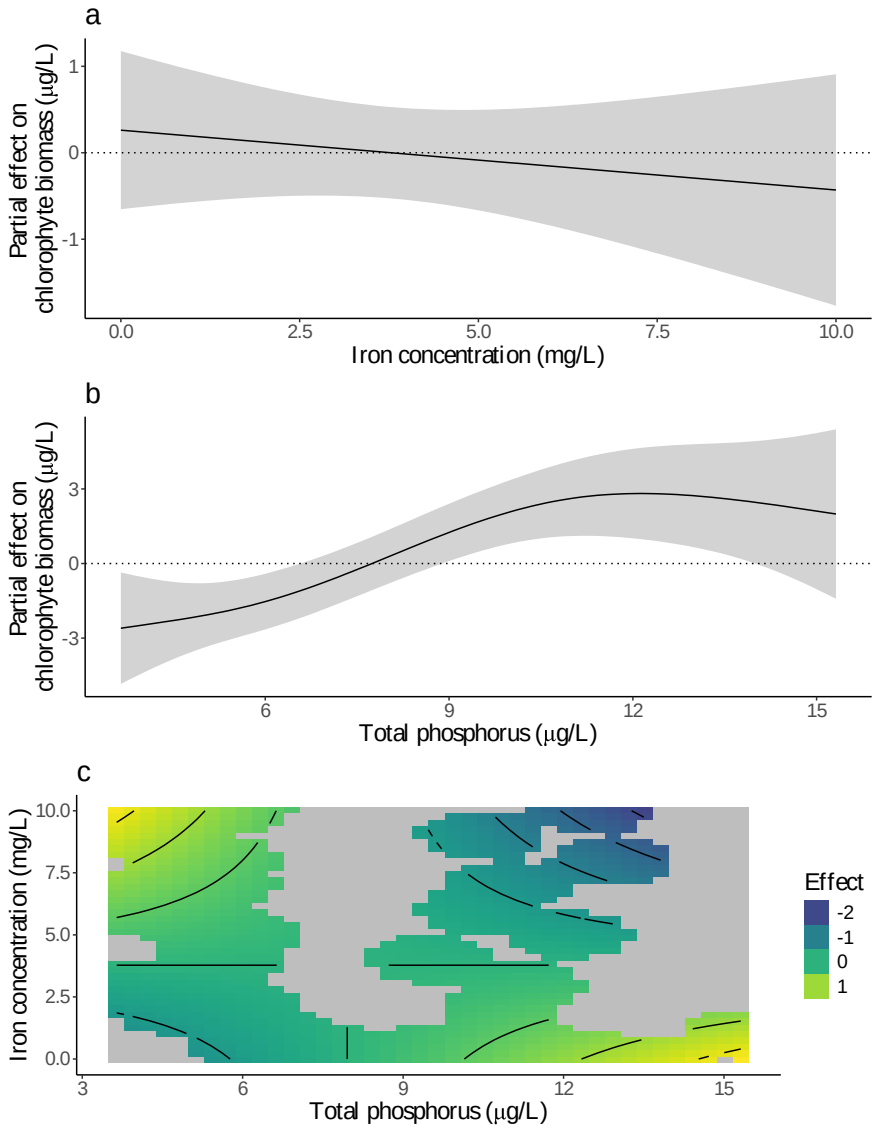

Figure A9: a. Generalized additive model partial smooth of iron concentration (mg/L) on chlorophyte biomass (µg/L). The grey area represents the 95% confidence interval, the dashed line represents the null effect and the rug plot the distribution of the data. b. Generalized additive model partial smooth of total phosphorus (µg/L) on chlorophyte biomass (µg/L). The grey area represents the 95%, the dashed line represents the null effect and the rug plot the distribution of the data. c. Tensor product smooth of the relation between total phosphorus (gµ/L) and iron concentration (mg/L) on chlorophyte biomass (µg/L). Dark blue represents negative effects on chlorophyte biomass, while yellow represents positive impacts on chlorophyte biomass.

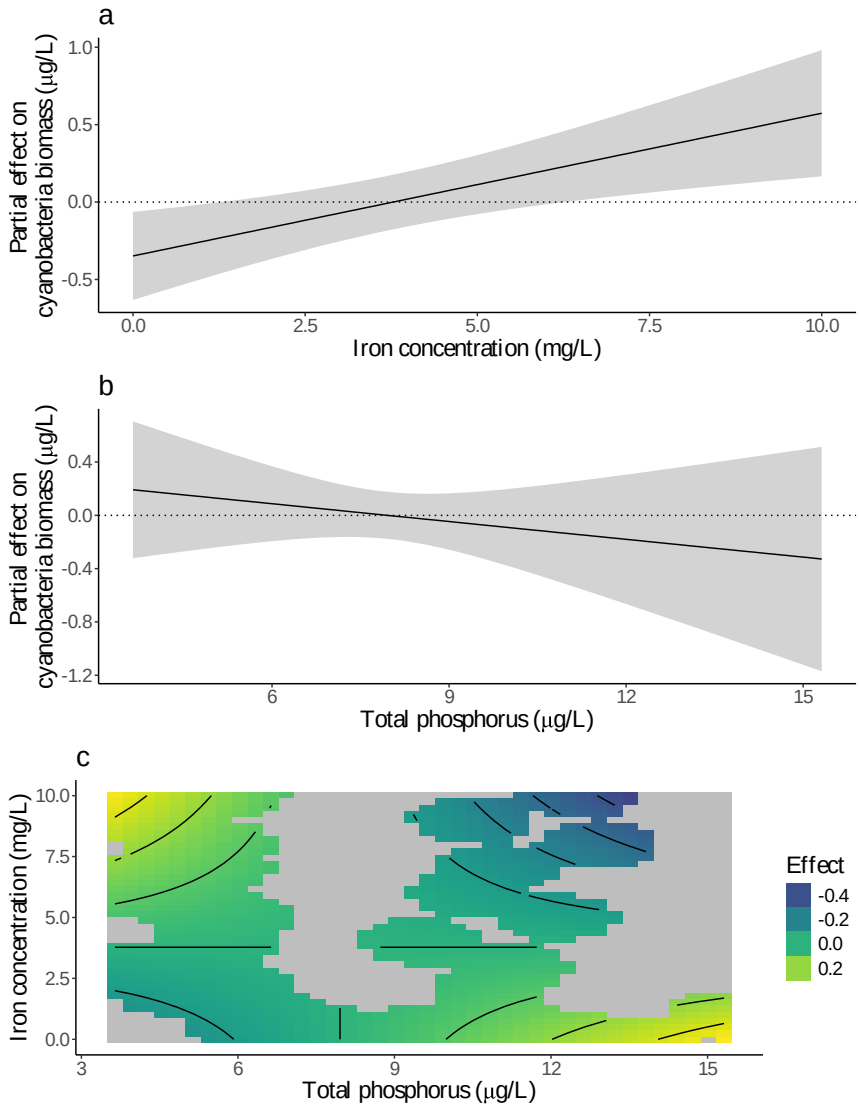

Figure A10: a. Generalized additive model partial smooth of (Fe) concentration (mg/L) on cyanobacteria biomass (µg/L). The grey area represents the 95% confidence interval, the dashed line represents the null effect and the rug plot the distribution of the data. b. Generalized additive model partial smooth of total phosphorus (µg/L) on cyanobacteria biomass (µg/L). The grey area represents the 95%, the dashed line represents the null effect and the rug plot the distribution of the data. c. Tensor product smooth of the relation between total phosphorus (µg/L) and (Fe) concentration (mg/L) on cyanobacteria biomass (µg/L). Dark blue represents negative effects on cyanobacteria biomass, while yellow represents positive impacts on cyanobacteria biomass.

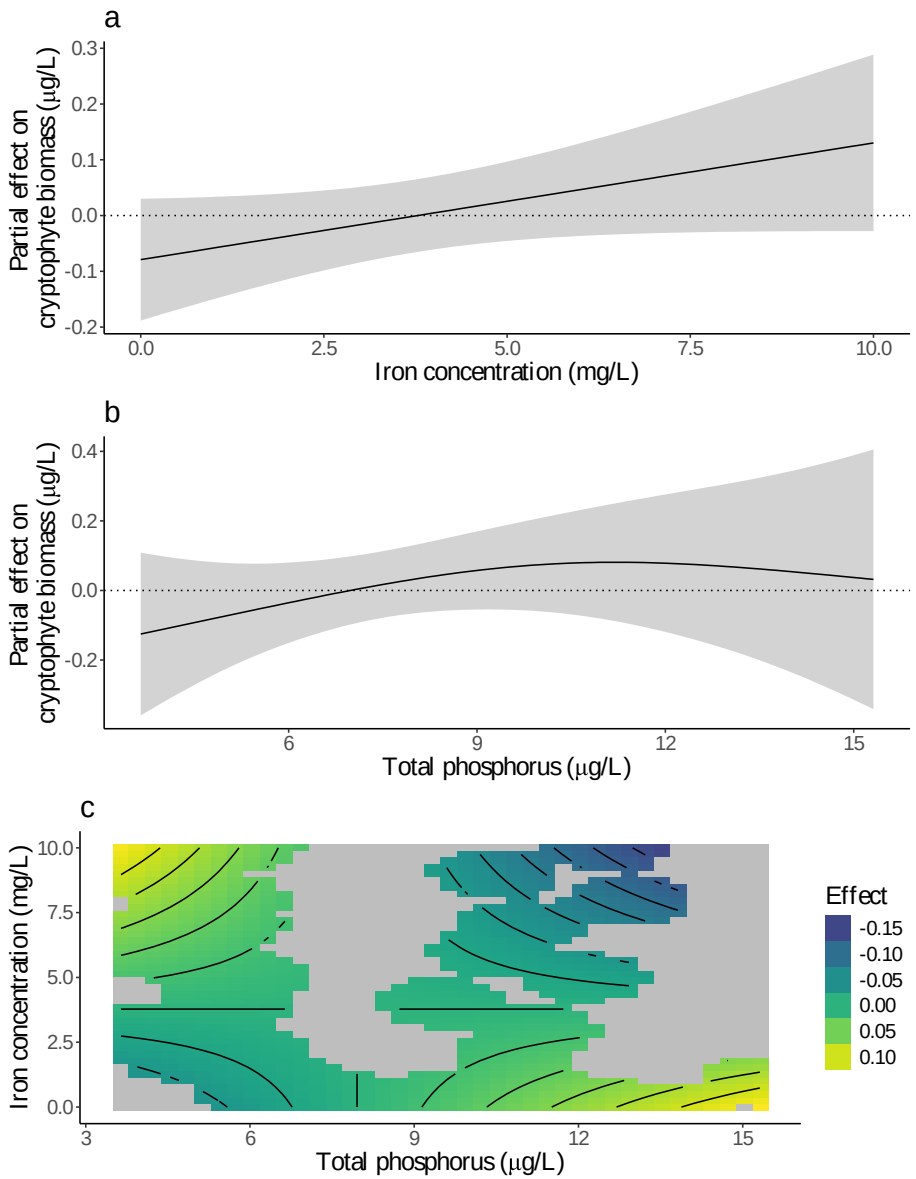

Figure A11: a. Generalized additive model partial smooth of iron concentration (mg/L) on cryptophyte biomass (µg/L). The grey area represents the 95% confidence interval, the dashed line represents the null effect and the rug plot the distribution of the data. b. Generalized additive model partial smooth of total phosphorus (µg/L) on cryptophyte biomass (µg/L). The grey area represents the 95%, the dashed line represents the null effect and the rug plot the distribution of the data. c. Tensor product smooth of the relation between total phosphorus (µg/L) and iron concentration (mg/L) on cryptophyte biomass (µg/L). Dark blue represents negative effects on cryptophyta biomass, while yellow represents positive impacts on cryptophyte biomass.

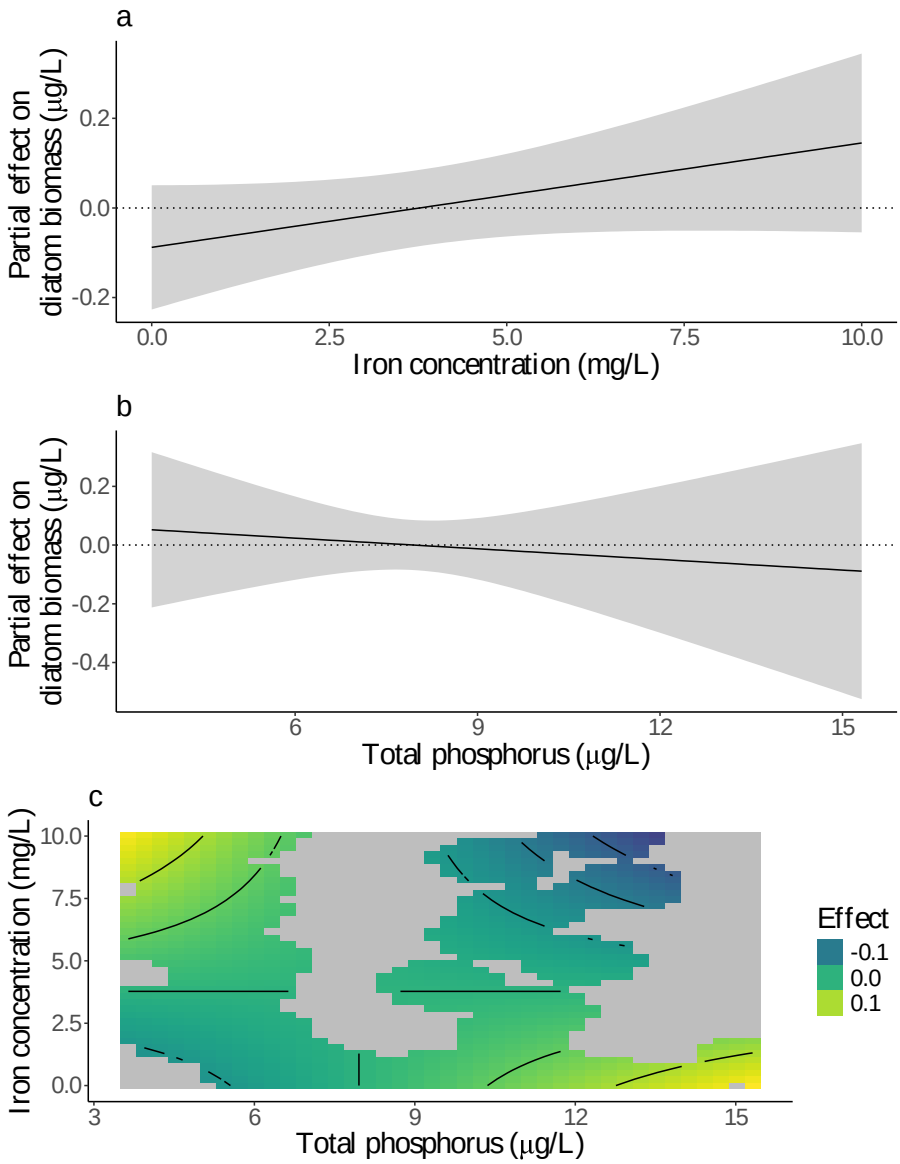

Figure A12: a. Generalized additive model partial smooth of (Fe) concentration (mg/L) on diatom biomass (µg/L). The grey area represents the 95% confidence interval, the dashed line represents the null effect and the rug plot the distribution of the data. b. Generalized additive model partial smooth of total phosphorus (µg/L) on diatom biomass (µg/L). The grey area represents the 95%, the dashed line represents the null effect and the rug plot the distribution of the data. c. Tensor product smooth of the relation between total phosphorus (µg/L) and (Fe) concentration (mg/L) on diatom biomass (µg/L). Dark blue represents negative effects on diatom biomass, while yellow represents positive impacts on diatom biomass.

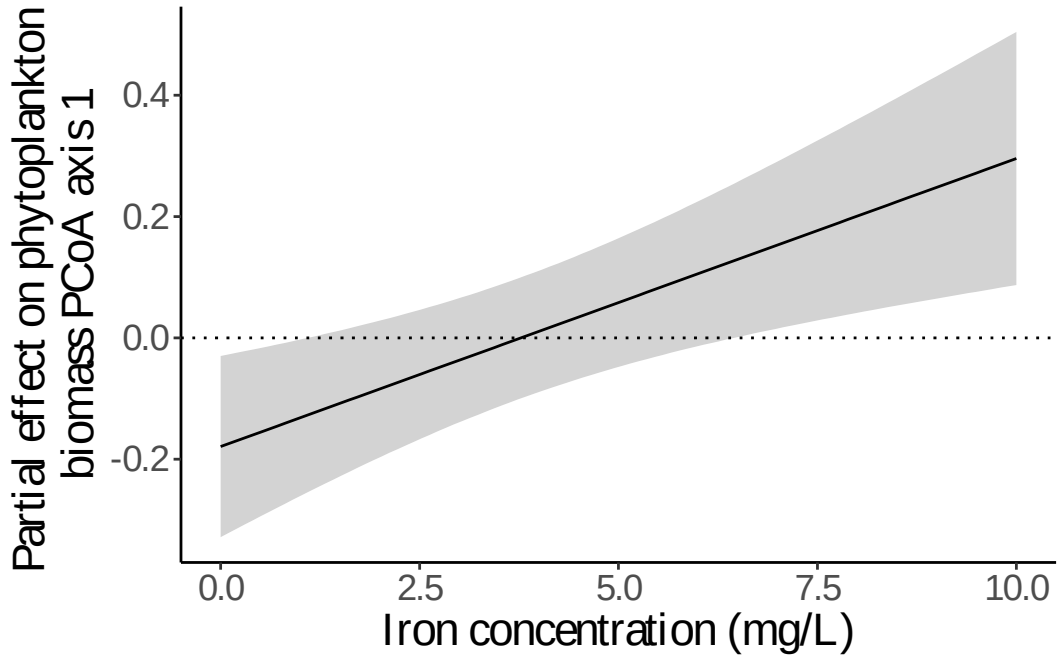

Figure A13: Generalized additive model partial smooth of Fe concentration (mg/L) on the PCoA axis 1 scores of the phytoplankton biomass PCoA. The grey area represents the 95% confidence interval, the dashed line represents the null effect and the rug plot the distribution of the data.

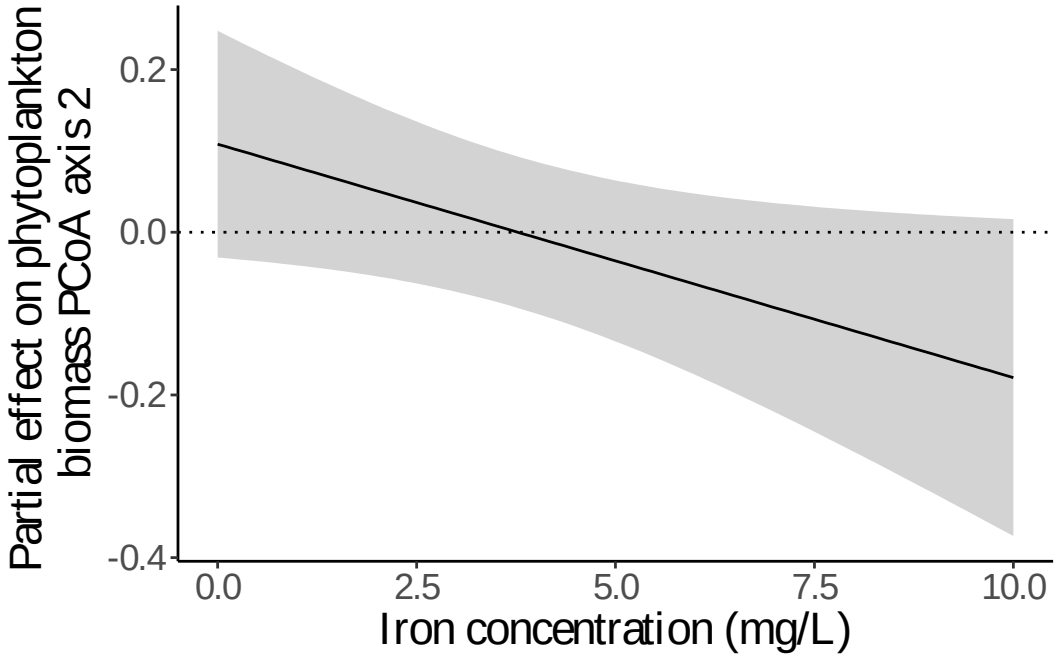

Figure A14: Generalized additive model partial smooth of (Fe) concentration (mg/L) on the PCoA axis 2 scores of the phytoplankton biomass PCoA. The grey area represents the 95% confidence interval, the dashed line represents the null effect, and the rug plot represents the distribution of the data.

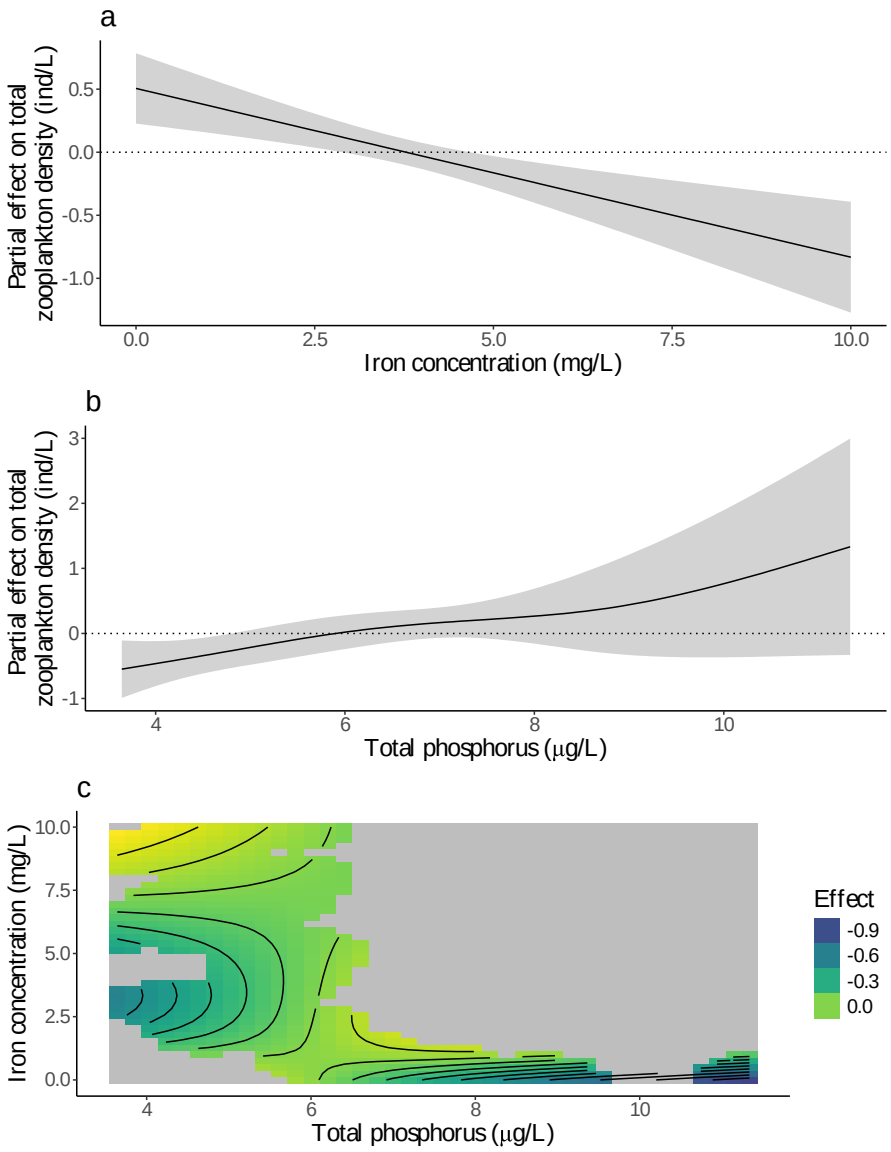

Figure A15: a. Generalized additive model partial smooth of iron concentration (mg/L) on total zooplankton density (ind/L). The grey area represents the 95% confidence interval, the dashed line represents the null effect and the rug plot the distribution of the data. b. Generalized additive model partial smooth of total phosphorus (µg/L) on total zooplankton density (ind/L). The grey area represents the 95%, the dashed line represents the null effect and the rug plot the distribution of the data. c. Tensor product smooth of the relation between total phosphorus (µg/L) and iron concentration (mg/L) on total zooplankton density (ind/L). Dark blue represents negative effects on total zooplankton density, while yellow represents positive impacts on total zooplankton density.

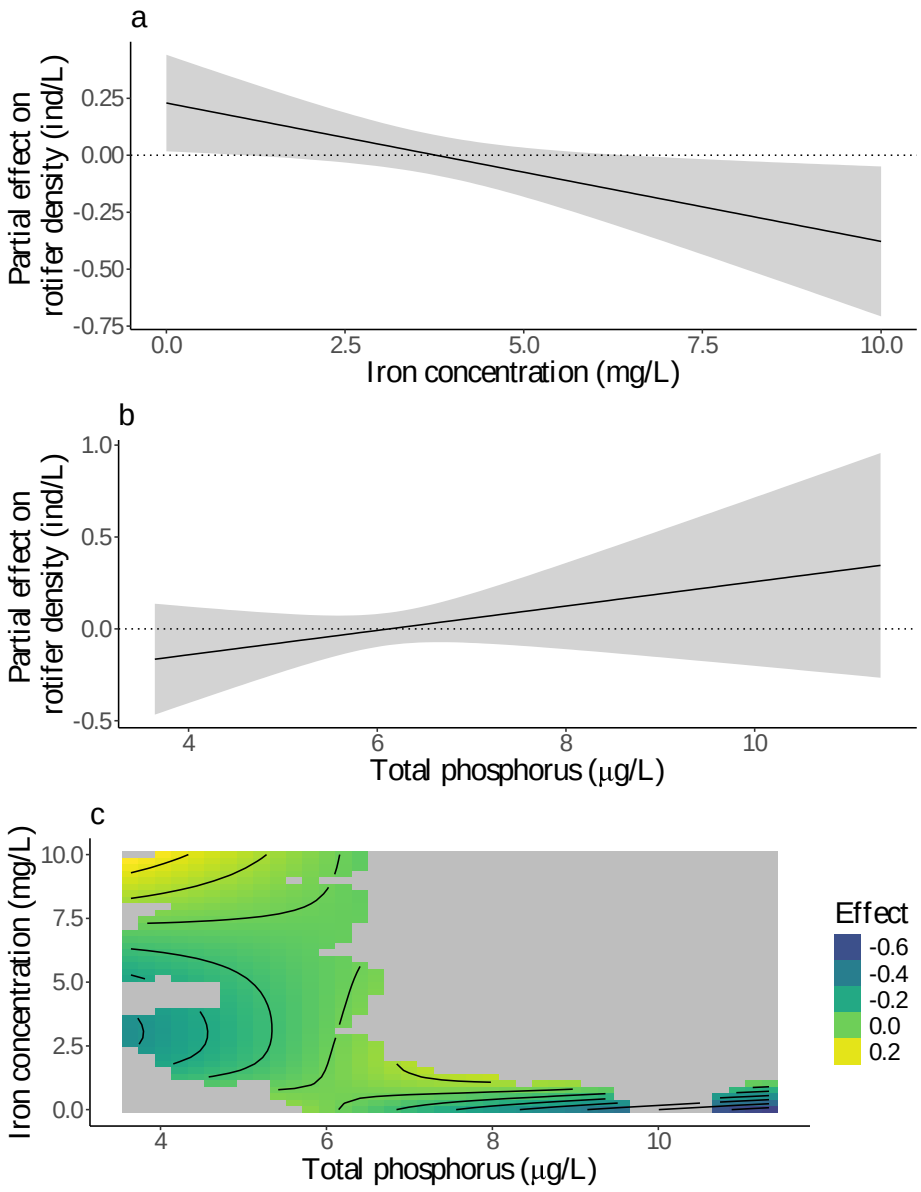

Figure A16: a. Generalized additive model partial smooth of Fe concentration (mg/L) on rotifer density (ind/L). The grey area represents the 95% confidence interval, the dashed line represents the null effect and the rug plot the distribution of the data. b. Generalized additive model partial smooth of total phosphorus (µg/L) on rotifer density (ind/L). The grey area represents the 95%, the dashed line represents the null effect and the rug plot the distribution of the data. c. Tensor product smooth of the relation between total phosphorus (µg/L) and Fe concentration (mg/L) on rotifer density (ind/L). Dark blue represents negative effects on rotifer density, while yellow represents positive impacts on rotifer density.

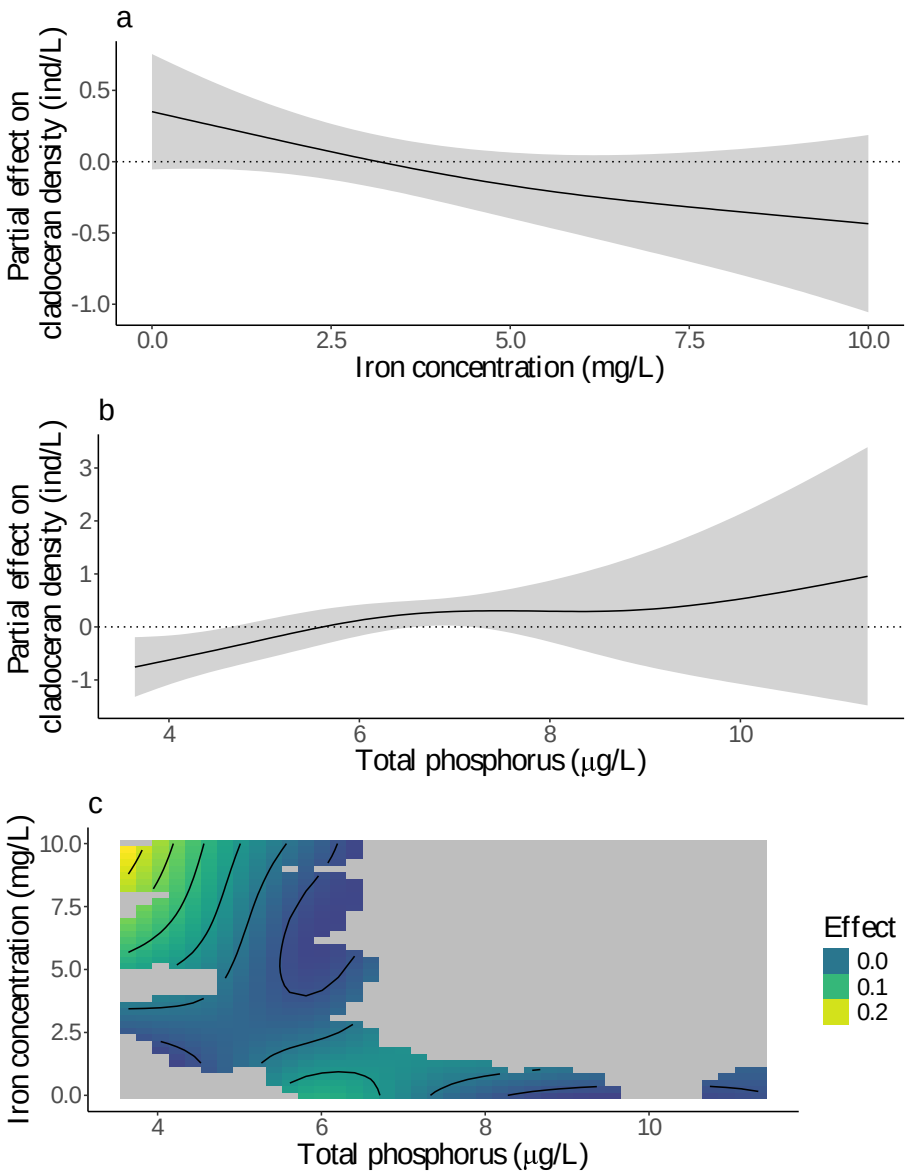

Figure A17: a. Generalized additive model partial smooth of Fe concentration (mg/L) on cladoceran density (ind/L). The grey area represents the 95% confidence interval, the dashed line represents the null effect and the rug plot the distribution of the data. b. Generalized additive model partial smooth of total phosphorus (µg/L) on cladoceran density (ind/L). The grey area represents the 95%, the dashed line represents the null effect and the rug plot the distribution of the data. c. Tensor product smooth of the relation between total phosphorus (µg/L) and Fe concentration (mg/L) on cladoceran density (ind/L). Dark blue represents negative effects on cladoceran density, while yellow represents positive impacts on cladocera density.

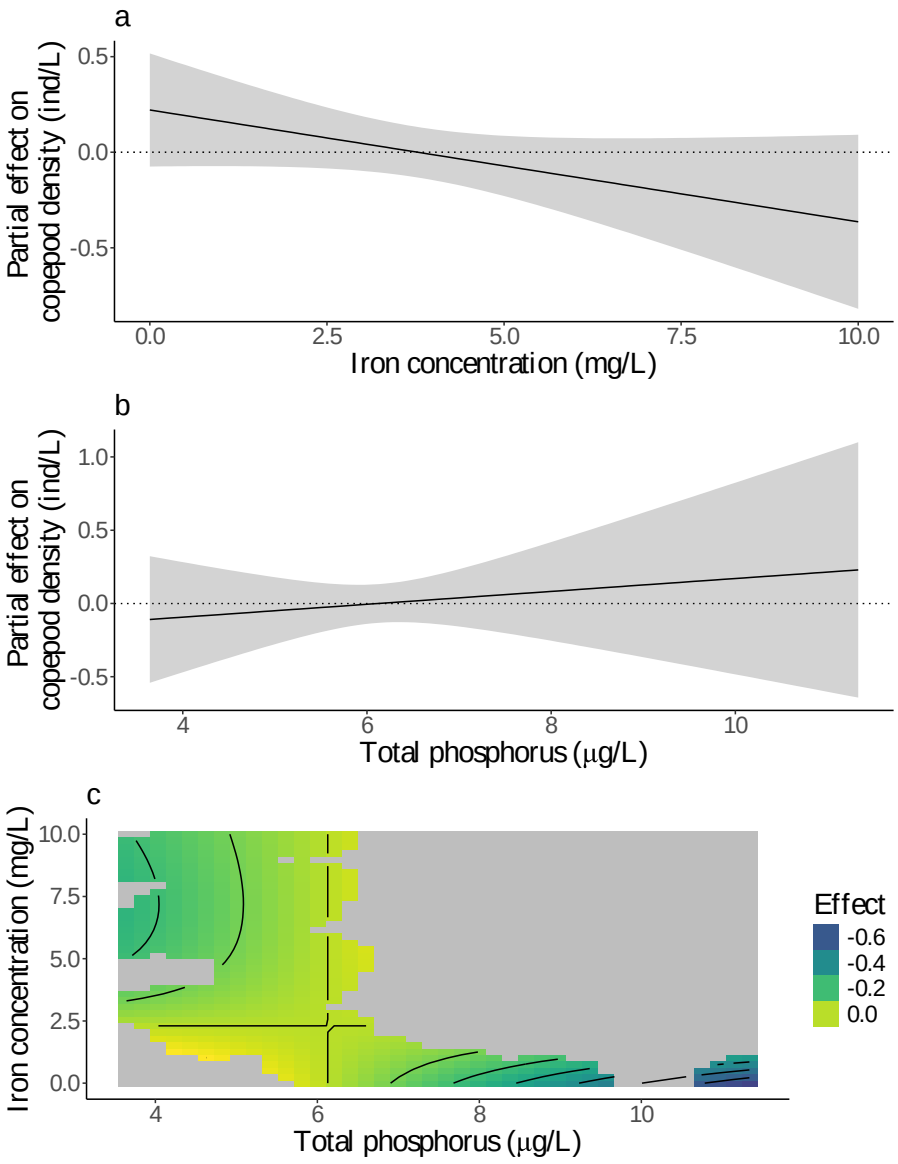

Figure A18: a. Generalized additive model partial smooth of Fe concentration (mg/L) on copepod density (ind/L). The grey area represents the 95% confidence interval, the dashed line represents the null effect and the rug plot the distribution of the data. b. Generalized additive model partial smooth of total phosphorus (µg/L) on copepod density (ind/L). The grey area represents the 95%, the dashed line represents the null effect and the rug plot the distribution of the data. c. Tensor product smooth of the relation between total phosphorus (µg/L) and Fe concentration (mg/L) on copepod density (ind/L). Dark blue represents negative effects on rotifer density, while yellow represents positive impacts on copepod density.

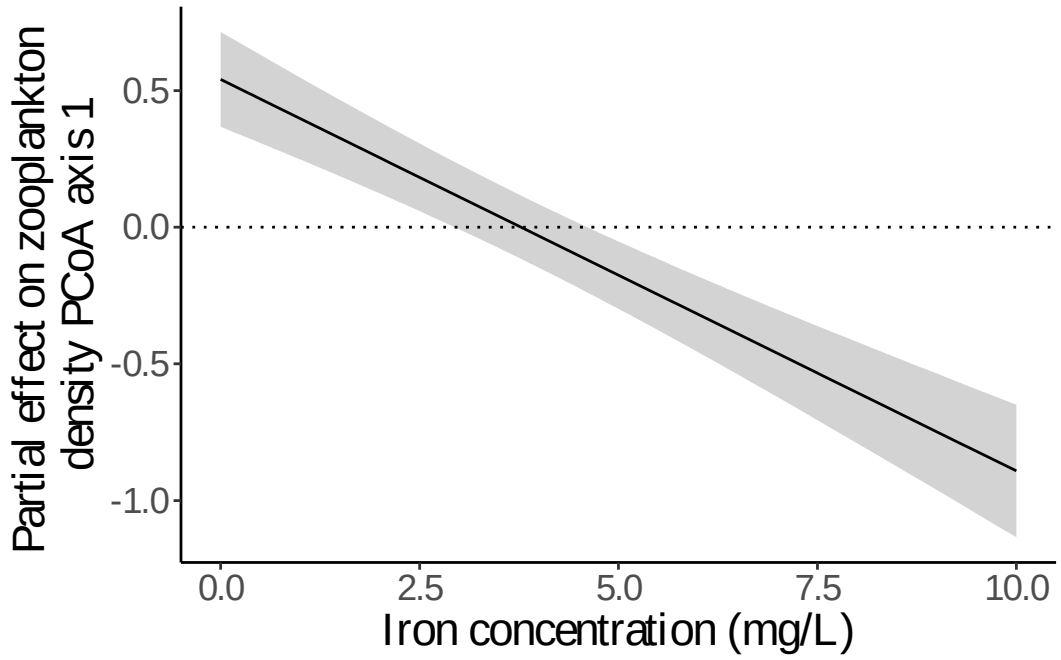

Figure A19: Generalized additive model partial smooth of Fe concentration (mg/L) on the PCoA axis 1 scores of the zooplankton density PCoA. The grey area represents the 95% confidence interval, the dashed line represents the null effect and the rug plot the distribution of the data.

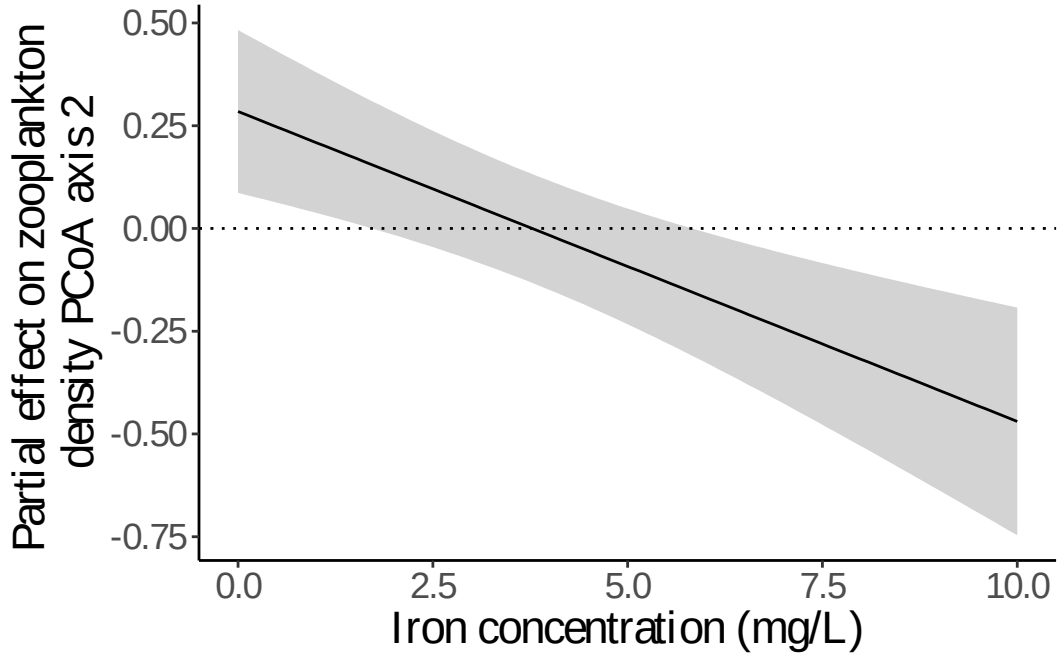

Figure A20: Generalized additive model partial smooth of Fe concentration (mg/L) on the PCoA axis 2 scores of the zooplankton density PCoA. The grey area represents the 95% confidence interval, the dashed line represents the null effect and the rug plot the distribution of the data.

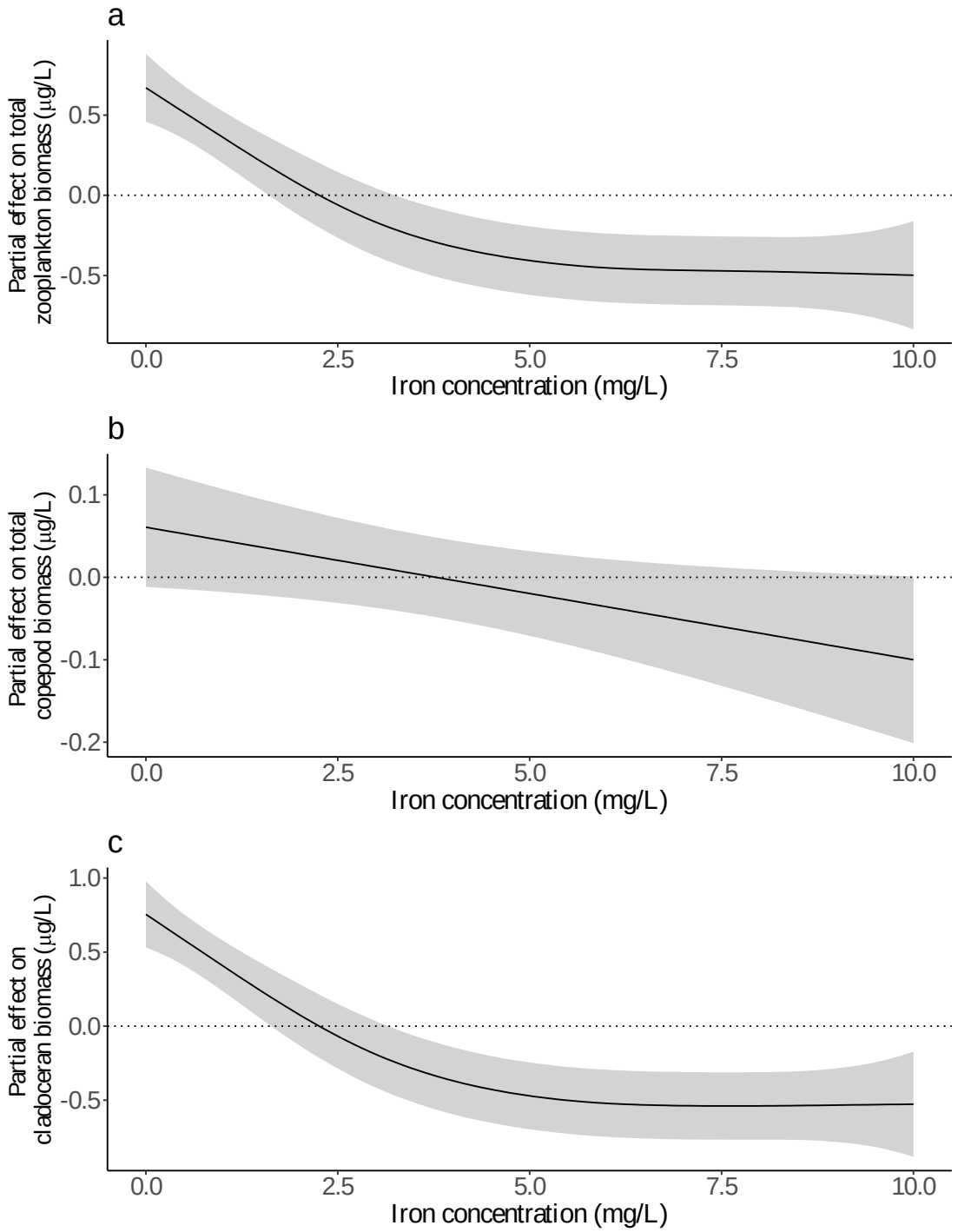

Figure A21: Partial effects of experimental Fe addition to (a) total zooplankton biomass, (b) total copepod biomass and (c) cladoceran biomass. Generalized additive models were used.

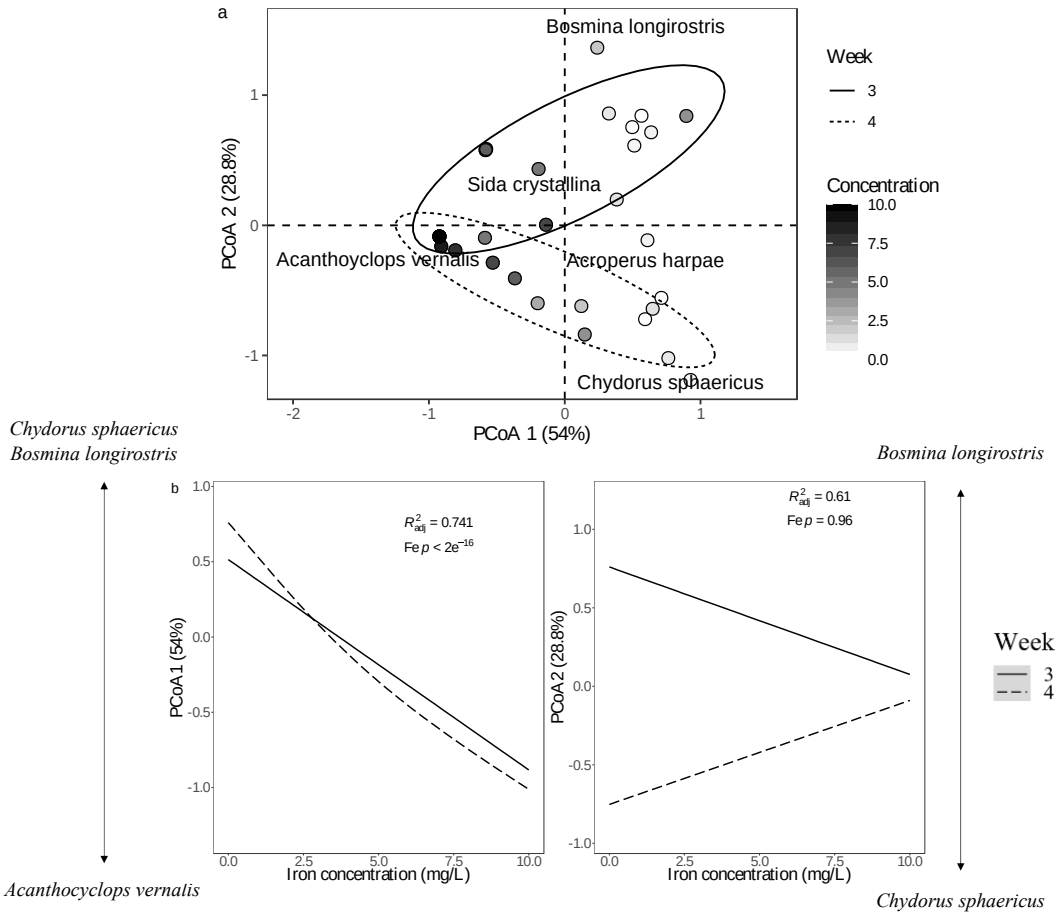

Figure A22: a. Principal coordinate analysis (PCoA) of zooplankton community biomass across the iron gradient and across time. Week 3 of the experiment is represented by the full line ellipse and week 4 by the dashed ellipse. Each enclosure is represented by a circle, with black circles being high iron concentration enclosures and white circles being low iron concentration enclosures. b. Change in species-specific zooplankton biomass community across the iron gradient at week 3 (full line) and week 4 (long dash) of the experiment for PCoA axis 1 and PCoA axis 2. Community data was modified using Hellinger transformation, and the highest species scores were retained for each PCoA axis and modeled with iron concentration using GAMs.

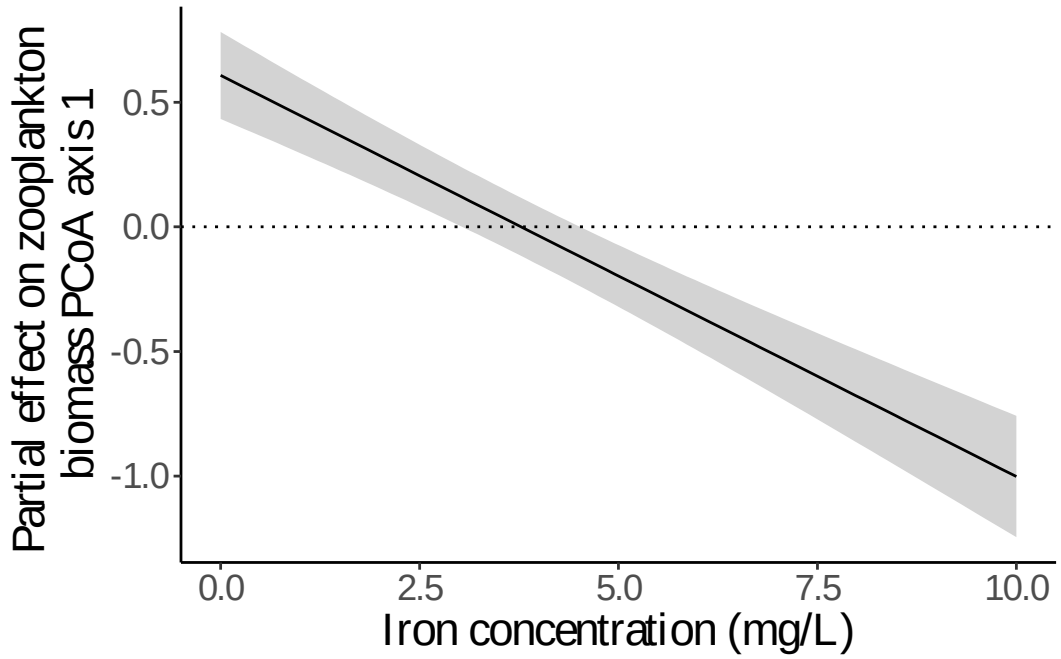

Figure A23: Generalized additive model partial smooth of Fe concentration (mg/L) on the PCoA axis 1 scores of the zooplankton biomass PCoA. The grey area represents the 95% confidence interval, the dashed line represents the null effect, and the rug plot represents the distribution of the data.

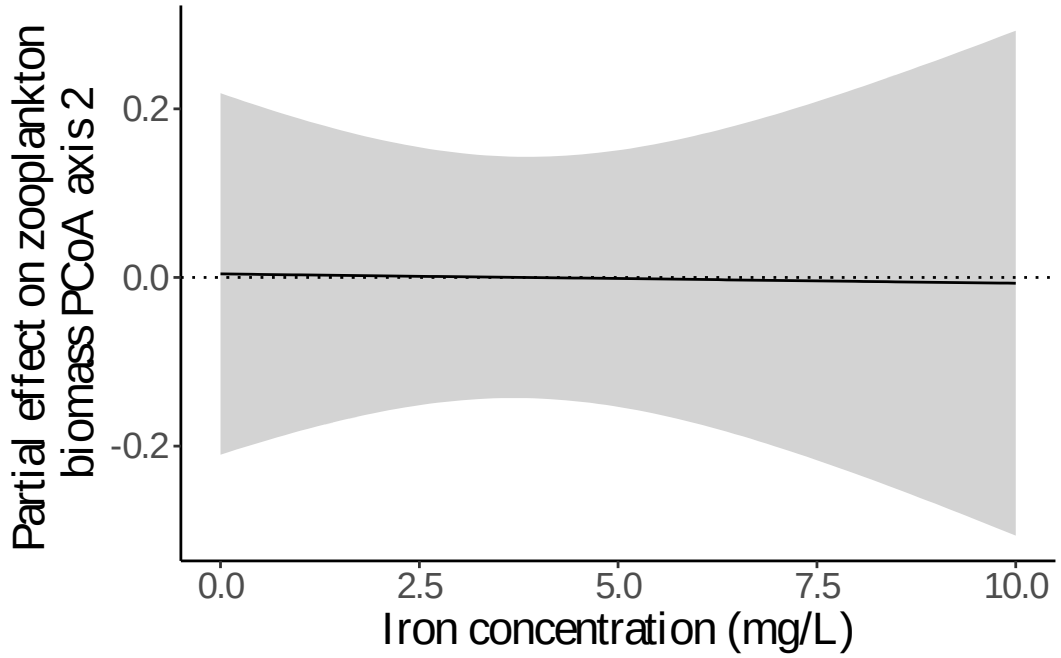

Figure A24: Generalized additive model partial smooth of Fe concentration (mg/L) on the PCoA axis 2 scores of the zooplankton biomass PCoA. The grey area represents the 95% confidence interval, the dashed line represents the null effect, and the rug plot represents the distribution of the data.

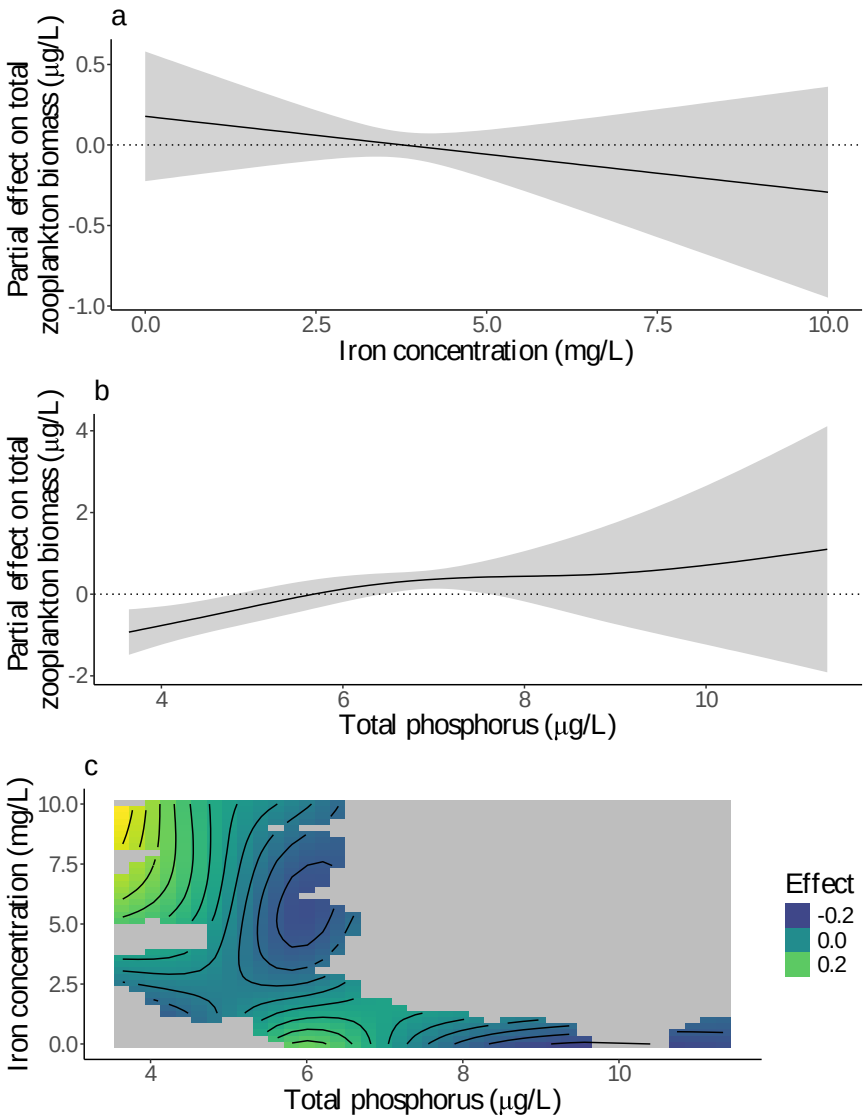

Figure A25: a. Generalized additive model partial smooth of Fe concentration (mg/L) on total zooplankton biomass (µg/L). The grey area represents the 95% confidence interval, the dashed line represents the null effect and the rug plot the distribution of the data. b. Generalized additive model partial smooth of total phosphorus (µg/L) on total zooplankton biomass (µg/L). The grey area represents the 95%, the dashed line represents the null effect and the rug plot the distribution of the data. c. Tensor product smooth of the relation between total phosphorus (µg/L) and Fe concentration (mg/L) on total zooplankton biomass (µg/L). Dark blue represents negative effects on total zooplankton biomass, while yellow represents positive impacts on total zooplankton biomass.

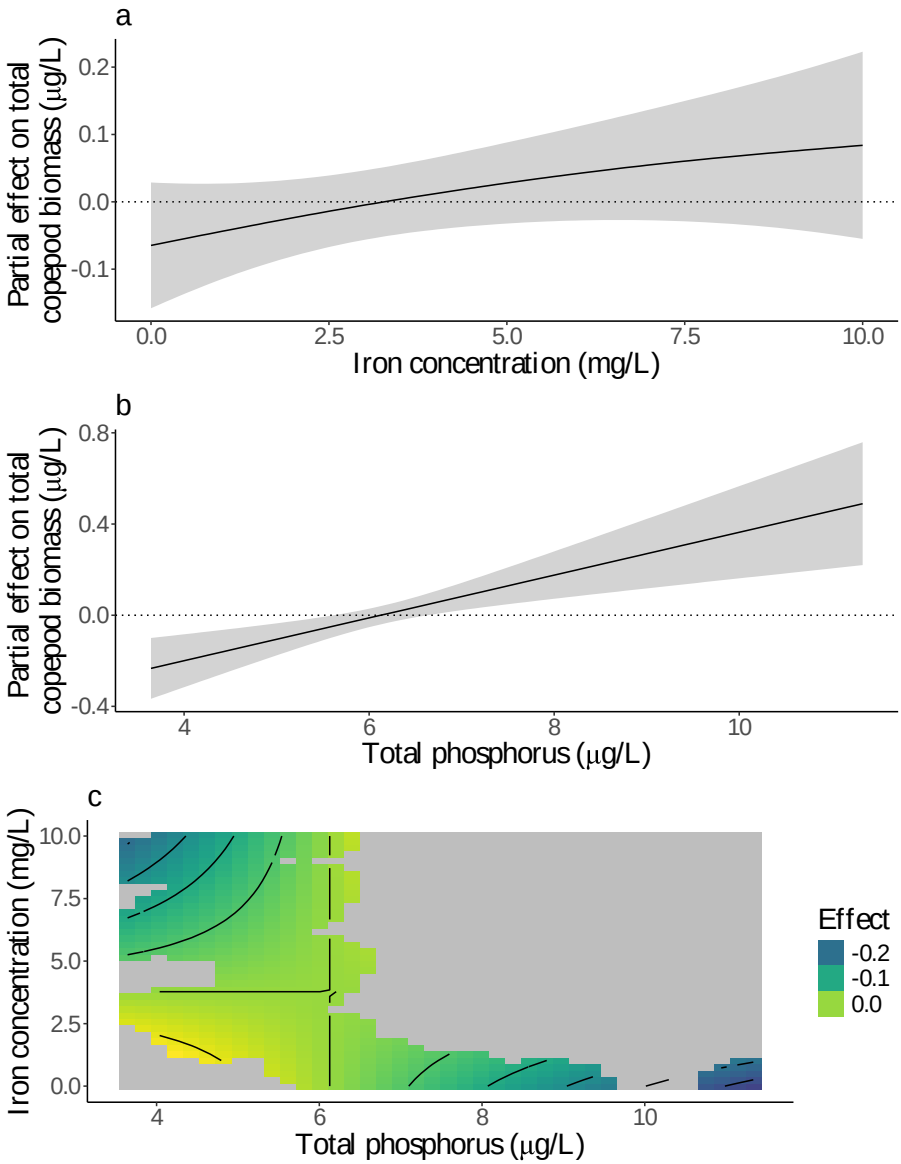

Figure A26: a. Generalized additive model partial smooth of Fe concentration (mg/L) on copepod biomass (µg/L). The grey area represents the 95% confidence interval, the dashed line represents the null effect and the rug plot the distribution of the data. b. Generalized additive model partial smooth of total phosphorus (µg/L) on copepod biomass (µg/L). The grey area represents the 95%, the dashed line represents the null effect and the rug plot the distribution of the data. c. Tensor product smooth of the relation between total phosphorus (µg/L) and Fe concentration (mg/L) on copepod biomass (µg/L). Dark blue represents negative effects on copepod biomass, while yellow represents positive impacts on copepod biomass.

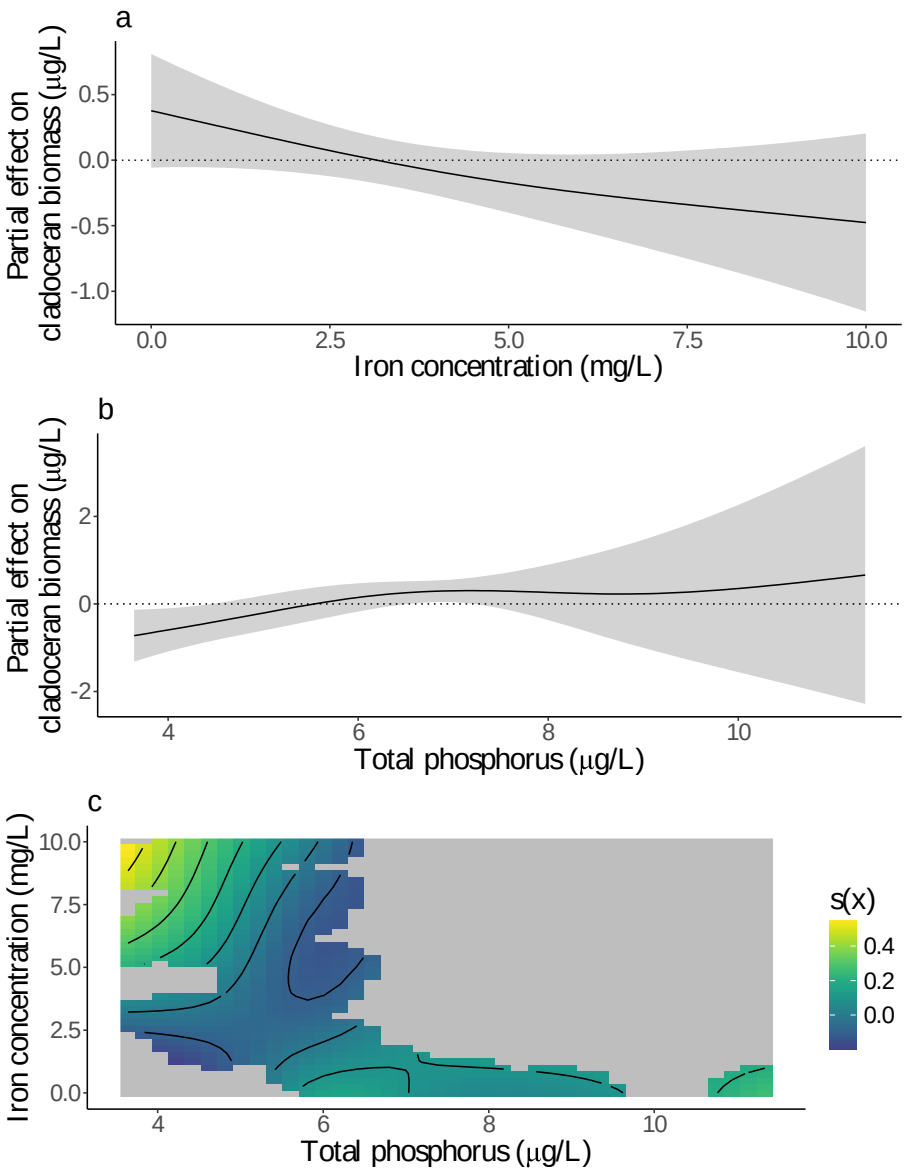

Figure A27: a. Generalized additive model partial smooth of Fe concentration (mg/L) on cladoceran biomass (µg/L). The grey area represents the 95% confidence interval, the dashed line represents the null effect and the rug plot the distribution of the data. b. Generalized additive model partial smooth of total phosphorus (µg/L) on cladoceran biomass (µg/L). The grey area represents the 95%, the dashed line represents the null effect and the rug plot the distribution of the data. c. Tensor product smooth of the relation between total phosphorus (µg/L) and Fe concentration (mg/L) on cladoceran biomass (µg/L). Dark blue represents negative effects on cladocera biomass, while yellow represents positive impacts on cladocera biomass.

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