Animal-mediated elemental cycling across time, space, and animal functional traits

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ABSTRACT

Animal-mediated elemental cycling across time, space, and animal functional traits

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Animals are essential to freshwater biogeochemistry and productivity. Through their excretion, aquatic consumers release bioavailable nutrients and carbon that can vary with animal taxonomic rank, trophic position, and abiotic factors such as light and nutrient supply. In fresh waters, light and nutrient supply is often modulated by dissolved organic matter (DOM), a "murky" component in the water that gives it a brown color and that may indirectly affect animal nutrient and carbon excretion. Additionally, contaminants can impact animal physiology, altering metabolism and inducing stress, further affecting nutrient and contaminant excretion. The size and structure of the ecosystem, including community composition and biomass, can also impact the contribution of aquatic animals to the elemental pool. To understand these dynamics, I examined animal-mediated elemental cycling in freshwater ecosystems across gradients of DOM concentration and composition and under contaminant exposure. I tested fish and invertebrate nitrogen, phosphorus, and DOM excretion across trophic positions during two sampling events in Lake Erie and in naturally DOM-variable streams and lakes. I also investigated the effects of chronic exposure to silver nanoparticles (AgNP) under environmentally relevant conditions on fish nutrient and silver (Ag) release. I found that aquatic animals can be a substantial nutrient contributor to the nutrient pool, particularly when their population biomass is high and ambient nutrient concentrations are low. I also detected nonlinear relationships between animal nutrient excretion and DOM characteristics that varied with taxonomic rank and trophic position and that dampened at larger ecological scales. Importantly, I identified several fish DOM excretion signatures that differed relative to ambient DOM and reported the first fish Ag excretion rates

under AgNPs exposure. My results underscore the context-dependency and variability inherent in animal-mediated elemental cycling, highlighting the critical role of animals as both modifiers and conduits of nutrients, DOM, and contaminants in aquatic ecosystems.

Keywords: animal-mediated elemental cycling, consumer-nutrient driven dynamics, phosphorus, nitrogen, carbon, silver nanoparticles, ecological stoichiometry

RÉSUMÉ

Le cycle des éléments médié par les animaux à travers l'espace, le temps, et les traits fonctionnels des animaux.

Sandra Klemet-N'Guessan

Les animaux sont essentiels à la biogéochimie et à la productivité des eaux douces. Par leur excrétion, les consommateurs aquatiques libèrent des nutriments et du carbone biodisponibles pouvant varier en fonction du rang taxonomique des animaux, de leur position trophique, ainsi que de facteurs abiotiques tels que la lumière et l'apport en nutriments. Dans les eaux douces, la lumière et l'apport en éléments nutritifs sont souvent modulés par la matière organique dissoute (MOD), un composant "trouble" dans l'eau qui lui donne une couleur brune et qui peut indirectement affecter l'excrétion des nutriments et du carbone par les animaux. De plus, les contaminants peuvent avoir un impact sur la physiologie des animaux, altérant le métabolisme et induisant du stress, affectant ainsi davantage l'excrétion des nutriments et contaminants. La taille et la structure de l'écosystème, y compris la composition et la biomasse de la communauté végétale et animale, peuvent également avoir un impact sur la contribution des animaux aquatiques au pool d'éléments. Pour comprendre ces dynamiques, j'ai examiné le cycle des éléments médié par les animaux dans les écosystèmes d'eau douce à travers des gradients de concentration et de composition de MOD et sous l'exposition aux contaminants. J'ai testé l'excrétion d'azote, de phosphore et de MOD par les poissons et les invertébrés à travers les niveaux trophiques durant deux échantillonages dans le lac Érié et dans des ruisseaux et lacs naturellement variables en MOD. J'ai également étudié les effets de l'exposition chronique aux nanoparticules d'argent (AgNP) dans des conditions pertinentes environnementalement sur l'excrétion de nutriments et d'argent (Ag) par les poissons. J'ai constaté que les animaux

aquatiques peuvent être un contributeur important en nutriments, particulièrement lorsque la biomasse de leur population est élevée et que les concentrations ambiantes en nutriments sont faibles. J'ai également détecté des relations non linéaires entre l'excrétion de nutriments par les animaux et les caractéristiques de la MOD, qui variaient en fonction du rang taxonomique et de la position trophique, et qui s'amenuisaient à des échelles écologiques plus importantes. Surtout, j'ai identifié plusieurs signatures d'excrétion de MOD par les poissons qui différaient par rapport à la MOD ambiante et j'ai mesuré les premiers taux d'excrétion d'argent (Ag) par les poissons sous exposition aux AgNP. Mes résultats soulignent l'importance du contexte écologique et la variabilité inhérente au cycle des éléments médié par les animaux, mettant en lumière le rôle critique des animaux en tant que modificateurs et vecteurs de nutriments, de MOD et de contaminants dans les écosystèmes aquatiques.

PREFACE

My dissertation was written in manuscript form and each data chapter is either in review or revision in a peer-reviewed journal, or is being prepared for submission to a peer-reviewed journal. Thus, the style of each manuscript may vary to meet journal requirements. Chapter 2 is nearing submission for Ecological Applications, Chapter 3 is in revision for Functional Ecology, Chapter 4 will be submitted to Functional Ecology, and Chapter 5 is in revision for Royal Society Open Science. My research is a collaborative effort; hence I have listed my co-authors on the title page of each chapter and use the plural 'we' throughout my dissertation.

A fish excreting in a bag at IISD-Experimental Lakes Area © S. Klemet-N'Guessan

Voguons ensemble © S. Klemet-N'Guessan

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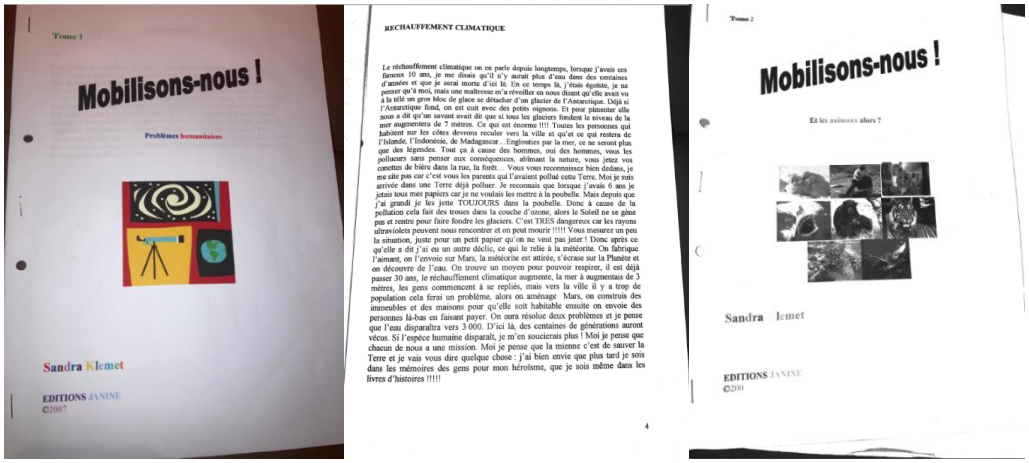
Cette thèse est tout naturellement la suite de ma série « Mobilisons-nous ! » écrite lors de mes 11 ans, il y a exactement 17 ans. Quelques centaines de pages de plus, mais l'essence y est.



Ma première « thèse » écrite à 11 ans © S. Klemet-N'Guessan

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CHAPTER 1

General Introduction

Biogeochemical cycling is the perpetual movement of elements within and between the physical and living environments. It is an essential ecosystem service in which animals play an important role by transforming and transporting elements in freshwater ecosystems. For example, benthic invertebrates and fish release bound nutrients back into their environment through their feeding activities and excretion. Most freshwater animals excrete nitrogen (N) primarily as ammonia (NH_3 or NH_4^+), with fish releasing > 80 % through their gills (i.e., branchial route, Smith 1929, Ip et al. 2001) and bivalves through respiratory epithelia (Thomsen et al. 2016). Similarly, aquatic insects mainly excrete NH_3/NH_4^+ using various organs including Malpighian tubules, anal papilla, the ileum, or the hindgut, depending on the taxon (O'Donnell 2008). While carbon (C) assimilated by aquatic animals is largely released as CO_2 via respiration through the gills, the route for excreting organic C compounds such as dissolved organic matter (DOM) remains unclear (Wright et al. 1989, Darchambeau et al. 2003). Phosphorus (P) excretion pathways are also poorly researched, yet seminal studies indicate that P is primarily released as orthophosphate (PO_4^{3-}) through kidneys (i.e., renal route, Kaune and Hentschel 1987, Bureau and Cho 1999), often measured as soluble reactive phosphorus (SRP).

In some aquatic ecosystems, animal-mediated nutrient cycling can support a substantial proportion of primary production nutrient demand (Vanni et al. 2006), increase phytoplankton (Kelly et al. 2018a), alleviate nutrient limitation (Atkinson et al. 2013), and generate biogeochemical hotspots (McIntyre et al. 2007; Capps and Flecker 2013). Within ecosystems, animals can serve either as an element source, through the ingestion of elements in one habitat

and their excretion in another (e.g. benthic-pelagic coupling; Schindler and Scheuerell 2002) and the decomposition of their carcass (Moore et al. 2007, Nobre et al. 2019); as an element pool, through the storage of elements in animal body tissue and their subsequent acquisition by predators (Vanni et al. 2013); or as an element sink, through an increase of biomass over time (Vanni et al. 2013). The specific role that animals play in the cycling of elements therefore depends on a range of both biotic and abiotic factors.

The relative importance of the nutrients cycled by animals can differ depending on consumer taxonomic classification (Loreau 2000) and functional traits such as consumer body size and trophic position (Vanni et al. 2006). Much of the research in freshwater ecosystems has so far focused on nutrients, specifically nitrogen and phosphorus (Atkinson et al. 2017). Here, I refer to 'functional traits' as a 'well-defined measurable property of an organism' that can influence their performance, including their response to the environment (i.e., response trait) and their effects on ecosystem properties (i.e., effect trait; Mcgill et al. 2006, Violle et al. 2007). Trait types encompass physiological, morphological, life-history, and behavioural (Green et al. 2022). Species vary in several functional traits associated with food acquisition and assimilation, which in turn, can affect somatic growth, reproduction, and body stoichiometry (Atkinson et al. 2017), as well as the release of nutrients. Trophic relationships such as predator-prey interactions can also alter species body stoichiometry, metabolic rates, and behaviour (Atkinson et al. 2017). Species' traits thus shape species-specific roles in nutrient cycling (Atkinson et al. 2017), and these roles may vary among ecosystems (Vanni 2002). Furthermore, the type, size, and physical and chemical characteristics of an ecosystem can play a modulating role in animal-mediated elemental cycling.

The influence of animal-mediated nutrient cycling relative to the overall nutrient supply can differ between streams and lakes. In streams, animal-mediated nutrient cycling may vary with fluctuations in flow velocity (Atkinson et al. 2017) and temperature (Schindler and Eby 1997). In lakes, however, animal-mediated nutrient cycling may change spatially with productivity (Vanni et al. 2006) and seasonality in phytoplankton nutrient quality (Atkinson et al. 2017), in addition to temperature (Schindler and Eby 1997). The size of the ecosystem can also determine food webs structure and biomass (Post et al. 2000), while external nutrients, carbon, and contaminants inputs may affect basal resource quantity and quality which will then affect animal functional traits and ultimately animal-mediated elemental cycling. Considering ongoing global change driven by shifts in climate, land cover and use, and human activities, the provision of nutrients by animals in freshwater ecosystems is likely to change and this change may vary across ecosystem types.

Large lakes present intriguing research opportunities due to their vast size, rich biodiversity, substantial primary production and animal biomass, and significant interactions with human communities (Seehausen et al. 1997, Ludsin et al. 2001, Sterner et al. 2020, Chiblow 2023, Obiero et al. 2023). While nutrient cycling is undoubtedly critical to their function, few studies so far have quantified animal-mediated elemental cycling in large lakes. In the Laurentian Great Lakes, research has primarily focused on quantifying animal nutrient excretion rates of single species, such as dreissenid mussels (*Dreissena spp*.; Arnott and Vanni 1996, Ozersky et al. 2009, Li et al. 2021) or round goby (*Neogobius melanostomus*; Bunnell et al. 2005). The role of animal-mediated nutrient cycling by multi-species assemblages has been seldom researched, with a few studies carried out in the African Great Lakes, including Lake Malawi (André et al. 2003) and Lake Tanganyika (McIntyre et al. 2007). Hence, the collective contribution of both

fish and invertebrate species to nutrient cycling in the Laurentian Great Lakes remains largely unexplored. Analyzing multiple species becomes particularly pertinent when subjected to varying water chemistry conditions, notably influenced by factors like nutrients and DOM.

DOM is a keystone component of the global C cycle. Commonly measured in units of carbon (DOC), DOM regulates several physical, biological, and chemical processes (Solomon et al. 2015). With climate-driven increases in precipitation and temperature, terrestrial DOC is predicted to increase in many aquatic ecosystems in a phenomenon recently coined as "organification" (Rodríguez-Cardona et al. 2023). It is also now widely acknowledged that DOM varies chemically within and among aquatic ecosystems (Wilson and Xenopoulos 2009, Williams et al. 2010) and can change water color in a trend referred to as "browning" (Roulet and Moore 2006). Variable DOM composition (or quality) has been shown to alter microbial activity. Changes in microbial activity can affect C incorporation into microbial biomass and its subsequent availability to animals higher up in the food web (Limberger et al. 2019). DOC may also indirectly affect the food web by controlling light penetration and resource availability to aquatic primary producers, which affects their quantity (i.e., biomass) and quality (i.e., elemental composition; Sterner et al. 1997, Rock et al. 2016, Downs et al. 2016). Variation in primary producer quantity and quality across a DOM gradient can in turn have bottom-up effects on higher trophic levels and alter various parameters such as their population abundance, individual body size, and nutrient limitation (Atkinson et al. 2017). Changes to animal body size and nutrient limitation can then influence the relative quantity and composition of what that animal excretes into the environment, while the population abundance will determine the relative contribution of an animal's population to nutrient cycling in aquatic ecosystems (Stewart et al. 2018).

Documented effects of changes in DOC and DOM have predominantly focused on the traits of individual or a few consumers (e.g., Van Dorst et al. 2020, Bishop et al. 2022), algal and consumer productivity (e.g., Finstad et al. 2014, Rivera Vasconcelos et al. 2018), and energy transfer within food webs following labile C additions (e.g., Scharnweber et al. 2014, Jones et al. 2018, Robbins et al. 2020). However, significant gaps persist in our understanding of nutrient and energy transfers across food webs along DOC and DOM gradients and at various ecological scales (Blanchet et al. 2022). This emphasizes the need of examining the impacts of both DOC and DOM on nutrient and C fluxes across multiple trophic levels, particularly in the context of anticipated shifts in biodiversity due to global change effects (Sala 2000). This will be crucial for determining the future impact of changes in DOC and DOM on the role of consumers in nutrient and C cycles. Furthermore, it is noteworthy that DOM molecules adsorb contaminants, including engineered nanoparticles such as silver nanoparticles (AgNP; Delay et al. 2011), potentially sustaining their presence in aquatic ecosystems (Rearick et al. 2018).

Silver nanoparticles (AgNP) are widely used engineered nanoparticles renowned for their antimicrobial, antifungal, electrical, and optical properties, with applications in diverse fields including medicine, biotechnology, agriculture, electronics, environmental remediation, and the textile industry (Mishra and Singh 2015, Carbone et al. 2016, De Silva et al. 2021, Naganthran et al. 2022). Their introduction into aquatic ecosystems primarily occurs via wastewater discharge and soil runoff, resulting in concentrations typically in the ng/L range (Peters et al. 2018). With global production estimates ranging between 350 and 750 tons annually as of 2024 and a notable upward trajectory since the 2010s (Temizel-Sekeryan and Hicks 2020), AgNP serve as exemplary models for investigating the impact of rising contaminant levels in aquatic environments on organisms and ecosystem processes. The pervasive use of AgNP raises

important concerns regarding their potential ecological implications, particularly in terms of their interactions with biota and subsequent environmental fate (Yu et al. 2013).

Research in the last two decades has advanced our understanding of the accumulation and toxic effects of AgNP and other contaminants on zooplankton and fish at molecular, cellular, and individual levels, predominantly in controlled settings (Tortella et al. 2020). Yet, the influence of contaminants on animal-mediated elemental cycling remains largely unexplored. Notably, only a few studies have investigated the effects of contaminants such as triclosan, mercury, engineered nanomaterials, and trace metals on animal nutrient or contaminants excretion (Yu and Wang 2002, Tsui and Wang 2004, Taylor et al. 2016, Perrotta et al. 2020), with none involving whole ecosystem analyses at ecologically relevant time scales. Exposure to contaminants can increase animal nutrient excretion (Perrotta et al. 2020), possibly through reduced nutrient assimilation efficiency stemming from decreased energy for feeding, reduced animal growth, or disruption in animal metabolic processes (Perrotta et al. 2020, Pearce et al. 2023). Considering the potential negative impact of AgNP exposure on animal physiology and behaviour (Tortella et al. 2020, Hayhurst et al. 2020), there is risk of a negative impact of AgNP on animal nutrient excretion rates. Overall, there is a need for comprehensive analyses of the impact of long-term animal exposure to contaminants, such as AgNP, under natural conditions, and their implications for the role of animals in elemental cycling.

Changes in climate, land use and cover, and human activity are altering the productivity of large lakes, DOC and DOM composition, and the presence of contaminants in aquatic ecosystems. To address the effects and responses in animal-mediated elemental cycling to these three environmental changes, I examine in my dissertation animal-mediated nutrient, carbon, and contaminant excretion and cycling in streams and lakes across various levels of biological

organization and spatial scales (Tables 1 and 2), gradients of DOC concentration and DOM composition, and under AgNP exposure. Through four chapters, I test the hypotheses that animal-mediated elemental excretion rates and stoichiometric ratios vary relative to (1) an animal taxonomic classification and trophic position due to differences in elemental demand, ingestion, and assimilation across species, (2) the ecosystem type (i.e., streams vs. lakes), influenced by the presence of flow in streams and diverse foraging habitats in lakes, (3) DOM-induced changes in light and nutrient availability which can affect the quantity and quality of basal resources and prey for animals, and (4) AgNP exposure which can impact animal physiology and behaviour, potentially altering animal elemental demand, ingestion, and assimilation. Chapters 2 to 5 encompass three main objectives aimed at:

1) Quantifying animal-mediated N, P, and C cycling across trophic positions in a large lake, in streams, and in small lakes, and comparing it to ambient concentrations and external loading.
2) Assessing the indirect effects of high light and low nutrients in low DOC environments and of low light and high nutrients in high DOC environments on animal-mediated N, P, and C excretion at the individual, population, and community levels in both streams and lakes.
3) Testing the effects of AgNP on animal-mediated N, P, and Ag excretion rates and ratios at the individual level in lakes.

In addition to these objectives, I address previously identified knowledge gaps in animal-mediated nutrient cycling (Atkinson et al. 2017) including the effect of diversity in community assemblage on individual-level nutrient cycling (Chapters 1 and 4); the ecosystem impact of differences in N vs. P cycling by animals (Chapters 2 to 5); and the direct and indirect influence

of abiotic factors (here, temperature, DOM, and AgNP) on the excretion of various elements by animals, including contaminants understudied in animal-mediated elemental cycling research such as Ag (Chapters 3 to 5).

In Chapter 2, I quantified animal-mediated nutrient cycling in a large productive lake, Lake Erie, at three levels of biological organization (individual, population, community, Table 1), two spatial scales (basin and lake wide, Table 1) using an assemblage of nine fish species and two benthic invertebrates referred to jointly as dreissenids. I demonstrated that both differences in species identity and biomass were important in shaping interspecific differences in individual- and population-level nutrient excretion rates. As expected, dreissenids were the highest nutrient contributors, recycling nutrients in less than three days and surpassing external and internal nutrient loads by several orders of magnitude. Fish comparatively lower mass-normalized nutrient excretion rates resulted, nonetheless, in nutrient loads exceeding external P inputs in the Western basin of Lake Erie. Based on my findings, I strongly urge researchers and ecosystem managers to incorporate animal-derived N and P loads as an internal source of nutrients in future nutrient budget assessments of large lakes.

In Chapter 3, I examined the relationships between fish and mayfly nutrient excretion and both DOC (concentration) and DOM (composition) in 11 streams, at three levels of biological organization (individual, population, community, Table 1), two spatial scales (reach and watershed, Table 1), and using animal groups defined by taxonomic classification or trophic position. I found that animal nutrient excretion had linear and nonlinear relationships with spatial variations in DOC and DOM across levels of biological organization, space, and animal groups. Animal nutrient excretion was also most variable at smaller relative to larger ecological scales.

My results demonstrated that integrating various ecological scales and animal groupings was critical for a more comprehensive assessment of DOM effects on ecological responses.

In Chapter 4, I replicated my experiments in Chapter 3 in lakes with a few modifications including a focus on fish only and the measurement of both DOC and DOM composition excretion rates. Specifically, I explored the relationships between fish nutrient, DOC, and DOM excretion and DOC and DOM in 11 lakes at the individual level. I found that variations in fish N and P excretion rates along the lake DOC gradient were opposite to what was found in streams, and that fish excreted P and N:P at particularly high and low rates, respectively, given the low ambient conditions. I also demonstrated that fish excreted DOC and terrestrially- and microbially-derived DOM in varying amounts relative to ambient conditions. I concluded that fish were active contributors of diverse nutrients and DOM molecules and could adjust their contributions based on environmental constraints mediated by ambient nutrients, DOC, and DOM composition.

In Chapter 5, I used a whole-lake AgNP addition experiment to test the effects of long-term AgNP exposure on fish-mediated elemental cycling. I examined N, P, and Ag excretion rates and ratios by fish in both the experimental and a reference lake and used two mathematical models to predict N and P excretion rates under uncontaminated conditions to compare them to my empirical results. I found that fish P and P:Ag excretion rates increased in Year 2 of AgNPs addition, although model-based predictions diverged from empirical P excretion rates in both the experimental and reference lakes. Most importantly, fish high Ag excretion rates relative to their tissue Ag content suggested that fish acted as a source and a conduit for Ag resuspension in the water column, thereby prolonging Ag exposure and uptake by aquatic organisms. These findings

highlighted the benefits of whole-lake contaminants exposure studies for assessing long-term effects of contaminants on organisms in their natural environment.

Finally, Chapter 6 summarizes and compares findings from Chapters 2-5, highlights studies limitations, and proposes promising avenues for future research.

Tables

Table 1. Terminology used in the dissertation for elemental excretion rates calculated at three levels of biological organization and two spatial scales.

Term	Definition	Units	Proximal drivers
(i) Levels of biological organization			
Individual-level elemental excretion	Elemental excretion rates and stoichiometric ratios by an individual animal expressed as either per capita, mass-specific, or mass-normalized (see Table 2)	Unit mass element individual^{-1} h^{-1} *or* Unit mass of element g^{-1} h^{-1} *or* molar	Animal functional traits
Population-level elemental excretion	Elemental excretion rates and stoichiometric ratios by the total population of individuals of a given species at a given spatial scale	Unit mass of element m^{-2} h^{-1}	Population biomass and size structure
Community-level elemental excretion	Elemental excretion rates by the aggregation of several populations to generate an animal assemblage at a given spatial scale expressed as either turnover time, elemental load, or volumetric elemental excretion	Days *or* Unit mass of element yr^{-1} *or* Unit mass of element L^{-1}	Community species composition and biomass
(ii) Spatial scale			
Reach- or basin-scale elemental excretion	Individual-, population-, or community-level elemental excretion rates scaled to the surface area of a stream reach or lake basin	Unit mass of element m^{-2} h^{-1}	Population biomass and size structure
Lake wide or watershed-scale elemental excretion	Community-level elemental excretion rates scaled to the surface area of a lake or a stream network expressed as elemental load or recycling ratio of ambient water elemental concentrations to community-level elemental excretion	Unit mass of element yr^{-1} *or* Unitless	Community species composition and biomass

Table 2. Size-scaling methods used for three measurements of individual-level elemental excretion rates and stoichiometric ratios.

Measurement	**Size-scaling method**	**Calculation**
Per capita elemental excretion rate and ratio (Ex_{pc})	None	Ex_{pc} = (final elemental concentration in the water sample – initial elemental concentration in the water sample) x experimental bag water volume / incubation time in minutes x 60 minutes / number of individuals
Mass-specific elemental excretion rate and ratio (Ex_{ms})	None	$Ex_{ms} = Ex_{pc}$ / dry $mass_{sp}$
Mass-normalized elemental excretion rate and ratio (Ex_{mn})	(1) Size-scaling coefficients (m) generated from the slope of the log_{10}-log_{10} relationship between per capita excretion rates (values > 0 only) for a *given* species and element, and population mean dry mass for *a given* species (dry $mass_{sp}$) (2) Size-scaling coefficients (m) generated from the slope of the log_{10}-log_{10} relationship between per capita excretion rates (values > 0 only) across *all* species and for a given element, and population mean dry mass across *all* species (dry $mass_{sp}$)	$Ex_{mn} = Ex_{pc}$ / (dry $mass_{sp}$)^{m}

CHAPTER 2

The missing piece to the large lake nutrient dynamics equation: animal-mediated nutrient cycling

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Abstract

Nutrient cycling in freshwater ecosystems is an essential ecosystem service, partly facilitated by animals through the transport and release of bioavailable nutrients critical for sustaining primary production. In lakes, animal-mediated nutrient cycling may vary spatially with productivity and seasonally due to changes in temperature and resource availability. To date, the importance of animal-mediated nutrient cycling remains poorly understood in large lakes. Here, we quantified nitrogen (N) and phosphorus (P) excretion rates and ratios of nine ecologically, socially, and economically important fish species and two dreissenid mussels (*Dreissena* spp.) in the western basin of Lake Erie and compared these rates to ambient and external nutrients. We found interspecific variability in mass-specific N and P excretion that was particularly high in dreissenids. Individual-level nutrient excretion rates were also compounded by differences in biomass, leading to the dominance of population nutrient excretion rates by white perch (*Morone americana)* and dreissenids. At the community-level, we demonstrated that despite low individual nutrient excretion rates, fish and dreissenids contribute to the rapid turnover of ambient dissolved nutrient concentrations to exceed external P loads from the catchment. These findings underscore the critical role of animals in driving internal nutrient fluxes within large productive lakes, with implications for ecosystem management. We recommend the inclusion of animal-mediated nutrient cycling as an internal nutrient source in future nutrient budgets and ecosystem models.

Keywords: animal-mediated nutrient cycling, internal load, phosphorus budget, nitrogen, Lake Erie

Introduction

Animals are key modifiers and conduits of nutrients in lakes. Through their excretion, animals recycle nutrients within their habitat and can translocate them from one habitat to another (Vanni 2002). Animals can be a stable source of nutrients relative to other external sources (Williamson et al. 2018). Nonetheless, on a shorter temporal scale such as seasons, animal nutrient excretion rates can be more variable due changes in temperature, resource availability, and population recruitment (Peng et al. 2020, Sharitt et al. 2021, Villnäs et al. 2022). Given that seasonal changes in ambient temperature are often concomitant to changes in resource availability, animal feeding ecology is also likely an important driver of seasonal effects on nutrient excretion rates.

Animal feeding ecology, including their trophic position and energy source often inferred from stable isotopes $\delta^{15}N$ and $\delta^{13}C$, respectively, could shape the uptake and release of nutrients (Atkinson et al. 2010). The isotopic signature of $\delta^{15}N$ and δ^{13} of consumer body tissue and their food sources can indicate the degree of trophic fidelity (Atkinson et al. 2010) and potential preferences for a given food source (Perrotta et al. 2020). However, the influence of animal body stoichiometry on nutrient excretion rates is not universally accepted (Allgeier et al. 2015, Durston and El-Sabaawi 2019, Villnäs et al. 2022), with some studies suggesting that disparities in animal nutrient excretion rates are instead related to ontogenic changes in diet and body nutrient requirements (Pilati and Vanni 2007) or body size (Oliveira-Cunha et al. 2022). Furthermore, animals typically exhibit N:P excretion ratios close to the Redfield ratio of 16:1, a threshold that theoretically marks the shift between N and P limitation in phytoplankton (André et al., 2003; Vanni et al., 2006; Ptacnik et al., 2010). However, it's worth noting that the N:P excretion of animals might deviate from the Redfield ratio when accounting for different ontogenic stages (Pilati and Vanni, 2007). Understanding these disparities in individual-level

responses to environmental conditions and animal functional traits is critical, especially when considering larger organizational scales such as populations and communities (Atkinson et al. 2017).

Animal-mediated nutrient cycling at the population and community levels are largely governed by community composition and biomass (Hopper et al. 2023). Taxonomic and species-based differences in functional traits, such as food acquisition and assimilation, can shape their role in nutrient cycling, potentially leading to population and community-level variations in nutrient excretion rates depending on the level of functional dissimilarity within the community assemblage. A comprehensive data synthesis by Vanni and McIntyre (2016) revealed that vertebrates excrete nutrients at higher per capita rates and ratios than invertebrates, despite their larger body size, indicating a potentially greater role in nutrient recycling than previously recognized. This hypothesis finds support in other studies as well (Parr et al. 2019, Atkinson et al. 2019). However, due to their comparatively large biomass, invertebrates may contribute more nutrients to the ambient pool at the population level (Hopper et al. 2023). There is also a growing body of evidence that supports biomass as a driver of population- and community-level nutrient excretion rates and fluxes (Atkinson et al. 2017, 2019, Frauendorf et al. 2020, Hopper et al. 2020). This may be especially important in some of the world's largest lakes such as the Laurentian Great Lakes which have undergone significant shifts in their food webs (DuFour et al. 2023, O'Donnell et al. 2023) and nutrient trends (Singh et al. 2023) in recent decades.

Lake Erie is the most biodiverse and productive of the Laurentian Great Lakes, yet faces numerous environmental threats, notably high external nutrient loading and recurring harmful algal blooms (HAB). Despite efforts to reduce external P loading, high nutrient inputs from tributaries persist, leading to HAB resurgence and hypoxic zones threatening fish (Vanderploeg

et al. 2009, Kraus et al. 2015). The persistence of HAB is largely attributed to increasing decadal trends in soluble reactive phosphorus (SRP) in tributaries, despite decreasing trends in total phosphorus (TP; Baker et al. 2014, Jarvie et al. 2017, Singh et al. 2023). While there has been a strong focus thus far on reducing external P inputs, research in the last decade suggests that internal nutrient loading such as nutrient release from the sediments may be an important source of bioavailable N and P (Wang et al. 2021, Bocaniov et al. 2023).

The role of animal-derived nutrients in Lake Erie internal nutrient cycling has, however, received comparatively less attention despite its demonstrated relevance for some taxa. For example, dreissenid mussels (*Dreissena* spp.) significantly contribute to nutrient flux in the Western basin (Conroy et al. 2005, Ozersky et al. 2009, Li et al. 2021), whereas round gobies (*Neogobius melanostomus*) minimally impact phosphorus cycling in the Central basin (Bunnell et al. 2005). Animal relative contribution to nutrient cycling may be especially large when external nutrient supply is high due to their high biomass (Vanni et al. 2006, Wilson and Xenopoulos 2011). Additionally, climate-induced increases in ambient temperatures in Lake Erie could affect the recruitment and growth rates of both fish and invertebrates (Karatayev et al. 2018, Dippold et al. 2020, Marcek et al. 2021), potentially impacting animal biomass and population nutrient excretion rates. Understanding animal-mediated nutrient recycling across levels of biological organization is thus crucial for untangling large lakes complex nutrient dynamics, especially in the context of climate change.

Using nine fish species and dreissenids, our objectives were to 1) quantify animal N, P, and N:P excretion rates at the individual, population, and community levels at two different times of the year, summer, and fall, and 2) compare animal-mediated nutrient supply to ambient conditions and external loads. We hypothesized that individual-level nutrient excretion rates and

ratios would vary across species due to differences in species functional traits associated with nutrient uptake, assimilation, and storage, although not directly tested in this study. Moreover, we predicted that individual-level nutrient excretion rates and ratios would increase with temperature and therefore decrease from summer to fall. Considering that resource nutrient content generally increases with trophic levels and thus increasing $\delta^{15}N$ (Sterner et al. 2002, Fry 2006) and that ambient nutrient conditions increase from west to east in Lake Erie, with increasing $\delta^{13}C$ (Heuvel et al. 2024), we predicted that individual-level nutrient excretion rates and ratios would increase with tissue $\delta^{15}N$ and N, and decrease with tissue $\delta^{13}C$ and C:N due to higher resource nutrient content. We also predicted that population nutrient contribution would increase with biomass and would be the highest for dreissenids given their documented high biomass and influence on nutrient cycles in the Laurentian Great Lakes (Li et al. 2021). Lastly, we predicted that community-level nutrient contribution would either match or surpass the magnitude of ambient conditions and external loads.

Methods

Study system and species

Lake Erie (*Waabishkiigoo-gichigami* in Anishnaabemowin, the Ojibwe language) is a large but shallow lake that hosts the richest and most abundant fish fauna of the Laurentian Great Lakes (*Nayaano-nibiimaang Gichigamin*; Bunnell et al. 2014). We sampled benthic macroinvertebrates and nine fish species spanning a range of trophic positions in summer and fall 2021. Sampled macroinvertebrates included zebra and quagga mussels (*Dreisseina polymorpha* and *D. rostriformis bugensis*) referred to jointly as dreissenids. Fish species included the omnivores gizzard shad (*Dorosoma cepedianum*), white perch (*Morone americana*), brown bullhead (*Ameiurus nebulosus*), yellow bullhead (*Ameiurus natalis*), and goldfish (*Carassius auratus*); the

invertivores logperch (*Percina caprodes*) and round goby (*Neogobius melanostomus*), and the invertivores/piscivores yellow perch (*Perca flavescens*) and largemouth bass (*Micropterus salmoides*). Sampling was done during two periods and at two locations following the Canadian Council for Animal Care's guidelines for best practices (Trent U. AUP #26601). The first sampling was done at the end of the summer in the nearshore on August 12-15th 2021 close to Point Pelee (42.0267802, -82.6712699) in the Western basin of Lake Erie. The second sampling was done during the fall on October 18-20th 2021 in the Detroit River (42.34357, -82.92858). The Detroit River is the river mouth and largest source of water entering Lake Erie via the Western basin, channeling both nutrients and fish to Lake Erie (Raby et al. 2018, Colborne et al. 2019), and can therefore be used as a proxy for the Western basin of Lake Erie. The Detroit River was selected for our second sampling instead of Lake Erie due to logistical and weather constraints.

Sampling and nutrient excretion experiment

Animal nutrient excretion rates were tested using three to twenty individuals of each fish species (< 40 cm total length) and seven sets of five to ten dreissenids of different sizes to account for body size and species-specific variability. Based on previous experiments in lakes, we aimed to test 20 individuals of varying sizes from each fish species but were limited by fish availability during either of the two sampling periods (André et al. 2003, Higgins et al. 2006). The sample size and number of individuals per sample for dreissenids was determined from both prior work using dreissenids and pilot work testing the optimal number of individual dreissenids of varying sizes and incubation time to maximize detection and minimize stress and fasting effects on nutrient excretion estimates (André et al. 2003, Conroy et al. 2005). Fish were collected mainly using an electrofishing boat, with occasional seine netting deployed in shallow waters. Dreissenids were hand-picked from buoys in the nearshore and briefly scrubbed with distilled

water. Following collection, each test unit (one fish or one set of five to ten dreissenids) was placed in a whirl-pak bag filled with 0.2 to 6L of base water collected from the Detroit River (both samplings, N = 18.6 ± 6.5 µg N/L, P = 9.4 ± 3.6 µg P/L) that was prefiltered through 1 µm borosilicate glass microfiber filter. Bags containing sampled individuals and two additional bags without individuals (controls) were incubated in a holding tank with ambient Lake Erie water that was renewed frequently to maintain ambient temperature (± 0.5 °C). Tested individuals were incubated for 29 to 91 min for fish and for 1h 8 to 1h 58 min for dreissenids depending on the ambient temperature and the size of the organisms collected in the bag (i.e. warmer temperatures and larger body sizes would have lower incubation times).

Following incubation, individuals were removed from the bags and fish were weighed (wet mass). We converted fish wet mass to dry mass using a conversion factor of 0.25 (Vanni et al. 2017). All dreissenids were placed on ice to be dried without their shells and weighed in the laboratory. Shortly after collection, water samples from both the nutrient excretion experiments and ambient lake or river water were filtered through pre-ashed Whatman GF/F glass microfiber filters (nominal pore size 0.7 µm), then through 0.22 µm polycarbonate membrane filters and stored at 4°C prior to analysis. During our second sampling, one to 18 individual fish were kept on ice for stable isotopes analyses. For our first sampling analysis, we used stable isotope and tissue nutrient content estimates generated by the Ontario Ministry of Natural Resources and Forestry (OMNRF) bottom trawl surveys in August 12th-28th 2019. Estimates for white perch, gizzard shad, and yellow perch were averaged across individuals of a given species and a single species-specific mean value for stable isotope and tissue nutrient content was used for each fish tested in the first sampling.

Fish biomass analysis

To estimate population-level nutrient excretion rates, we used lake wide fish population abundance data collected from interagency gill net and bottom trawl surveys done by the Ohio Department of Natural Resources (ODNR), the Ohio United States Geological Survey (USGS), and the OMNRF in the summer and early fall months of 2016 to 2021. Similarly, we fish biomass estimates (kg/ha) in the Western Basin of Lake Erie for gizzard shad, logperch, round goby, white perch, and yellow perch from 2021, as provided by the Lake Erie Committee Forage Task Group. These were calculated from abundance and biomass data collected during annual interagency fish community bottom trawl surveys conducted by the ODNR, and OMNRF that occurred in August 2021. Fish biomass was also derived for the Western basin of Lake Erie from a database maintained by the USGS Lake Erie Biological Station integrating data from bottom trawl surveys from 2011 to 2020 (Keretz et al. 2022, DuFour et al. 2023). Dreissenid biomass was retrieved from 1992 to 2019 from Karatayev et al. (2021b) as total wet biomass (total wet weight, tissue with shell, g/m^2). Wet biomass was converted to dry biomass by using a conversion factor of 0.25 for fish (Vanni et al. 2017) and 0.025 for dreissenids (tissue without shell; Karatayev et al. 2022)

Nutrient and stable isotope analyses

Nutrient excretion samples were quantified as both total dissolved nitrogen (TDN, µg N/L) and total dissolved phosphorus (TDP, µg P/L) and ammonia (NH_4^+, µg N L^{-1}) and soluble reactive phosphorus (SRP, µg P L^{-1}). TDN was estimated using spectrometry via the second derivative method following persulfate digestion (Crumpton et al. 1992), and TDP was quantified using the molybdate blue method following persulfate digestion (Murphy and Riley 1962). NH_4^+ and SRP were analyzed using the phenate (Solórzano 1969) and molybdate blue methods (Murphy and

Riley 1962), respectively, on a spectrophotometer. Ambient water samples were analyzed as TDN and TDP.

To quantify $\delta^{13}C$ and $\delta^{15}N$ in fish muscle was freeze-dried while dreissenids whole-body tissue without the shell was dried in the oven at 60 °C. All animal tissue was then homogenized into a fine powder and assessed for $\delta^{15}N$ and $\delta^{13}C$ using a subsample (0.4-1.1 mg for fish, 0.6-0.7 mg for dreissenids) on a Delta V Advantage Thermoscientific Continuous Flow Mass Spectrometer (Thermo Scientific, Bremen, Germany) coupled to a 4010 Elemental Combustion System (Costech Instruments, Valencia, CA, USA) and a ConFlo IV gas interface. Instrument accuracy during the period of sample analysis was based on NIST standards (NIST 8573, NIST 8547, and NIST 8574 for $\delta^{15}N$ and NIST 8543, NIST 8573, NIST 8574 for $\delta^{13}C$; n=6 for all). The mean difference from the certified values were 0.02, 0.2, and -0.19‰ for $\delta^{15}N$ and 0.14, -0.1, and -0.2‰ for $\delta^{13}C$, respectively. Precision of $\delta^{13}C$ and $\delta^{15}N$ were determined by running a set of four lab standards (bovine liver (NIST 1577c), internal lab tilapia muscle, USGS 40, and Urea IVA 33802174) every 16 samples, and measured ≤0.20‰ for $\delta^{13}C$ and ≤0.18‰ for $\delta^{15}N$ for all standards. Animal %C and %N were generated from stable isotope analyses. Dreissenid tissue %P was determined using a second subsample (0.6-1.1 mg) of tissue powder which was acid hydrolyzed, mixed with distilled water, titrated to pH 7 with NaOH, and analyzed for P using persulfate digestion followed by the molybdate blue method (Murphy and Riley 1962, Sterner and George 2000). Tissue %P was not determined for fish.

Estimating nutrient excretion rates and P loads

Per capita nutrient excretion rates (µg N or P/individual/h) were calculated for both the dissolved (TDN and TDP) and bioavailable (NH_4^+ and SRP) fractions of nutrients by subtracting the N or P

concentration in the prefiltered base water in the control bags (i.e., initial concentration) from the N or P concentration in the water samples in the experimental bags post-incubation (i.e., final concentration) and correcting for sample water volume, number of individuals, and time. Per capita nutrient excretion rates were then divided by mass to generate mass-specific nutrient excretion rates (µg N or P/g/h). To scale up individual-level mass-specific nutrient excretion rates to population-level nutrient excretion rates, mass-specific nutrient excretion rates for five fish species and dreissenids were multiplied by yearly mean fish biomass data (kg/ha) from 2016 to 2021 to calculate population nutrient excretion rates (µg N or P/m^2/h). All subsequent analyses except for the community-level nutrient turnover time were done using NH_4^+ and SRP nutrient excretion rates.

Community-level nutrient excretion rates were estimated using two approaches. The first approach relied on determining nutrient turnover times (min) in the Western basin by dividing ambient areal TDN and TDP (µg N or P/m^2) by aggregate population TDN or TDP excretion rates (Conroy et al. 2005). Ambient areal TDN and TDP were estimated by assuming constant concentrations across the water column and multiplying epilimnetic TDN and TDP by the Watern basin mean depth (8.5 m; Bocaniov et al. 2023). The second approach consisted in calculating fish and dreissenid nutrient excretion loads (tonnes/yr) both in the Western basin and lake wide by converting aggregate population NH_4^+ and SRP excretion rates to loads using mean mass-specific nutrient excretion rates (Western basin) or species-specific mass-specific nutrient excretion rates (lake wide) and Western basin and lake wide surface areas (25657 and 3284 ha, respectively; Bocaniov et al. 2023). We then compared Western basin fish and dreissenid N excretion loads to external total Kjeldahl N (TKN; comprising organic N and NH_4^+) loads, as well as both Western basin and lake wide fish and dreissenids P excretion loads to external P

loads. External TKN loads were from the main United States (US) tributaries in 2019 (NCWQR 2022). External P loads comprised TP loads in 2019 and mean TP loads from 2011 to 2020, combining both Canadian and US sources (Environment and Climate Change Canada 2021), as well as TP loads from the main Canadian and US tributaries (Environment and Climate Change Canada 2021, NCWQR 2022). Given that SRP loads were only provided for Canadian sources, we approximated total SRP loads for both Canadian and US sources by assuming that SRP comprise on average 33% of TP loads (Maccoux et al. 2016).

Statistical analyses

We compared individual-level mass-specific N, P, and N:P excretion rates across nine fish species and dreissenids by running a two-way analysis of variance (ANOVA), followed by posthoc tests (emmeans package; Lenth 2021). Given that our tested species assemblage differed between the two sampling periods and locations, we assessed the confounded effects of sampling period (summer vs. fall) and location (Lake Erie vs. Detroit River), referred as "first" and "second" on mass-specific nutrient excretion rates and ratios. We thus ran an ANOVA using both sampling and species as fixed factors with an interaction effect. Then, we ran similar ANOVA with a subset of species including species tested in both samplings with at least three observations in each sampling. To test the effects of temperature or stable isotope composition ($\delta^{15}N$ and $\delta^{13}C$) on mass-specific nutrient excretion rates and ratios, linear mixed effects models were used (lmer; lme4 package, Bates et al. 2015). Model selection was done using several indices of model performance (compare_performance, performance package; Lüdecke et al. 2021) and model diagnostics (check_model, lindia package; Lee and Ventura 2017). Fixed factors included sampling with an interaction effect with temperature or stable isotope, while species was added as a random effect. The final model was selected based on the highest

performance score and AIC values relative to the null model (model structure: *mass-specific nutrient excretion rates ~ temperature or stable isotope + (1|Species)*).

Population-level nutrient excretion rates were visualized using a time-series from 2016 to 2021 for fish and 1992 to 2019 for dreissenids, assuming constant mass-specific nutrient excretion rates. Community-level turnover times and P loads in the Western basin from 2011 to 2020 and lake wide in 2019 were visualized along with total and tributary P loads using bargraphs. Nutrient excretion rates and ratios across the three levels of biological organizations were log-transformed to meet assumptions of normality of residuals. Final plots were generated (tidyverse package; Wickham et al. 2019) and arranged to visualize results coherently (ggarrange, ggpubr package Kassambara 2023). All statistical analyses were done using R Statistical Software (v4.1.3; R Core Team 2023) and RStudio (v2022.2.1.461 RStudio Team 2022) on a Windows PC (v 22H2).

Results

The Western basin of Lake Erie in the summer had an average (mean ± SD) ambient temperature of 25.7 ± 1.3 ºC and nutrient concentrations varying from 349.5 to 483.7 µg TDN/L (414.1 ± 40.8) and from 11.1 to 14.5 µg TDP/L (13.6 ± 1.5). In the second sampling, the Detroit River ambient temperature averaged 16.4 ºC ± 0.4 but no nutrients were measured. At the individual level, fish mean mass-specific N and P excretion rates were particularly low but had relatively low variance, while dreissenid mean mass-specific N and P excretion rates were six times higher than those of fish (Table S1, Fig. 1a, b). This resulted in low N:P excretion rates averaging in the first sampling 5.06 ± 8.37 for fish and 3.33 ± 1.78 for dreissenids, and in the second sampling

5.10 ± 4.77 for fish, much lower than the Redfield ratio of 16 (Table S1, Fig. 1c). Fish tested in the first sampling were on average larger and with marginally higher tissue %N than those tested in the second sampling (Table S1). Fish also had a lower tissue C:N ratio relative to dreissenids likely due to lower lipid content (Table S1). At the population level, given that dreissenid biomass was on average 109 times higher than that of the five tested fish populations, dreissenid mean population nutrient excretion rates were more than 600 times higher for dreissenids relative to those of fish (Table S2).

Animal nutrient excretion rates at the individual level

Significant differences in mass-specific N, P, and N:P excretion rates were found across dreissenids and fish species at the individual level (Table S3). Dreissenid mass-specific N excretion rates in the summer were the highest, followed by yellow perch in the summer, although there was only one observation (Fig. 1a). Brown bullhead and largemouth bass had the lowest mass-specific N excretion rates which were significantly different from white perch (Table S4, Fig. 1a). Species mass-specific P excretion rates were more variable and showcased more interspecific differences. Dreissenids mass-specific P excretion rates in the first sampling, followed by gizzard shad in both samplings were significantly higher than most other species and the mean mass-specific P excretion rates (Table S4, Fig. 1b). White perch mass-specific P excretion rates were also significantly higher than a few species, and 1.2 times higher than the species average (Table S1). The lowest mass-specific P excretion rates were observed in brown and yellow bullhead, largemouth bass, and yellow perch (Table S4, Fig. 1b). Interestingly, largemouth bass and yellow perch were the only species with mass-specific N:P excretion rates higher than the species average (Fig. 1c).

Effects of sampling and animal feeding ecology on animal nutrient excretion rates

Fish and mussels excreted N at higher rates in the first sampling period, which had higher temperatures relative to the second sampling, but was also carried out in Lake Erie relative to the Detroit River (Tables S1 and S5, Fig. 2a and S1b). However, this difference was likely driven by the particularly high N excretion rates observed in dreissenids, white perch, and yellow perch (Fig. 1a, 2a). We did not find any significant effect of sampling nor temperature independently from species on mass-specific P and N:P excretion rates (Table S5, S7, Fig. 2b, c, S1a). Due to logistical constraints, our tested species assemblage differed between samplings, with only four out of ten species (i.e., goldfish, gizzard shad, largemouth bass, and yellow perch) captured during the two sampling periods, and only two (i.e., largemouth bass and gizzard shad) with more than one observation in the first sampling (Fig. 1). To account for the collinearity between sampling and species, we tested the interactive effects of sampling and species on largemouth bass and gizzard shad nutrient excretion rates and did not find any significant effect of sampling (Table S6, Fig. S2). While there were fewer observations in the first sampling relative to the second, mass-specific N excretion rates appeared to be higher in the first sampling relative to the second while mass-specific P excretion rates appeared higher in the second sampling relative to the first (Fig. S2).

We did not find any significant relationship between mass-specific N, P, and N:P excretion rates and tissue $\delta^{15}N$, $\delta^{13}C$, %N, and C:N (Table S8, Fig. 3). Accordingly, none of the lmer models assessing the relationship between animal mass-specific nutrient excretion rates and tissue stable isotopes or nutrient content fit better than null models (Table S9). Nonetheless, there appeared to be a clear separation in the $\delta^{13}C$ signature of animals collected in the first and second sampling, except for gizzard shad (Fig. 3), with first sampling individuals showing lower $\delta^{13}C$. We also noted two main isotopic clusters, with brown bullhead, goldfish, logperch, round goby,

and yellow perch in the second sampling having the lowest $\delta^{15}N$ and the highest $\delta^{13}C$, and white perch in the first sampling having the highest $\delta^{15}N$ and lowest $\delta^{13}C$ (Table S3, Fig. 3). Two species diverged from these trends, with dreissenids $\delta^{15}N$ comprising the first cluster but $\delta^{13}C$ comprising the second cluster and gizzard shad showing wide variations in both $\delta^{15}N$ and $\delta^{13}C$ (Table S3, Fig. 3).

Population-level nutrient excretion rates varied temporally from 2016 to 2021 with changes in biomass for both fish and dreissenids (Fig. 4). Given that white perch had the highest biomass, white perch population N and P excretion rates were consistently the highest among all five fish species (Fig. 4a, c). Gizzard shad population N excretion rates fluctuated with yellow perch population N excretion rates due to more or less similar biomass, exceeding it in 2017 and 2020, and remaining under it all other years (Fig. 4a). Conversely, gizzard shad population P excretion rates were continuously higher than yellow perch population P excretion rates (Fig. 4c). Logperch and round goby population N and P excretion rates followed similar trends, although fluctuations in round goby nutrient excretion rates were more subtle and remained higher than those by logperch (Fig 4). Dreissenid population N and P excretion rates were visualized from 1992 to 2019, spanning a much larger period than fish population nutrient excretion rates, and were three orders of magnitude higher than rates observed in fish (Fig. 4). Dreissenid population nutrient excretion rates had two major fluctuation periods. The first period consisted of an increase in population nutrient excretion rates from 1992 to 2002, followed by a decrease to 2011 to similar levels observed in 1992 (Fig. 4b, d). The second period was much shorter and comprised a rise in population nutrient excretion rates from 2011 to 2014 to levels lower than the maximum observed in 2002, followed by a small decline from 2014 to 2019 (Fig. 4b, d).

We estimated the turnover of ambient dissolved nutrient concentrations to be less than a year, at the community level, and to exceed external TP and SRP loads when the total biomass was considered (Fig. 6). In the western basin, turnover of TDN and TDP was 235 and 34 days for fish and 5 and 3 days for dreissenids, respectively (Fig. 6a, b). When comparing N and P loads excreted by fish and dreissenids relative to total and tributary sources in both the western basin and lake wide we found that fish excreted more than half of tributary TKN loads as NH_4^+ while dreissenids surpassed tributary TKN loads by one order of magnitude (Table S10, Fig. 6c). Given their lower biomass relative to the Western basin, SRP loads by the five fish species tested lake wide were at least one order of magnitude lower than external P loads (Table S10, Fig. 6d). In the western basin, fish SRP loads were 3.4 to 4 times higher than total and tributary SRP loads, respectively, and close to total and tributary TP loads (Table S10, Fig. 6e). Dreissenids SRP loads consistently exceeded total and tributary TP and SRP in both the western basin and lake wide by one to 1.5 orders of magnitude (Table S10, Fig. 6d, e). Dreissenids remained the highest N and P animal contributor with N and P loads about one to two orders of magnitude higher relative to fish in the Western basin and lake wide, respectively (Table S10, Fig. 6c, d, e).

Discussion

Little attention has been paid to the role of animals in internal nutrient cycling in large lakes. Here, we present the first comprehensive analysis of animal-mediated nutrient cycling in one of the world's largest lakes, Lake Erie. We found that dreissenids had by far the highest N excretion rates, while both dreissenids and gizzard shad excreted the most P. We noted higher mass-specific N excretion during our first sampling period that was warmer and in Lake Erie relative to the second sampling period that was cooler and in the Detroit River. This difference was unlikely to be explained by species differences in tissue stable isotopes and elemental

stoichiometry. Turnover of animal excreted nutrients in the Western basin was rapid and ranged between 3 and 235 days. N and P loads excreted from fish in the Western basin exceeded half of the total TKN loads from US main tributaries and were 3.4 times higher than total SRP loads, while dreissenids alone surpassed all external N and P loads by one to two orders of magnitude. Despite individual-level nutrient excretion rates of fish in Lake Erie appearing modest compared to other freshwater ecosystems of comparable trophic states, our study underscores their pivotal role in internal nutrient loads within large lakes, and one that should be considered for future nutrient management.

Mass-specific nutrient excretion rates varied across species. Notably, we found that gizzard shad had the highest mass-specific P excretion rates among fish and the lowest N:P excretion rates, consistent with prior comparative analyses involving various fish species, including gizzard shad and logperch (Torres and Vanni 2007). However, in our study, we observed higher mass-specific P excretion rates for gizzard shad compared to logperch, contrary to previous findings (Torres and Vanni 2007). We estimated dreissenid nutrient excretion rates within the range of previously measured rates for dreissenids in Lake Erie during similar sampling periods (Arnott and Vanni 1996, Naddafi et al. 2008), yet up to 3.5 times higher than rates reported in other studies carried out in Lake Erie, Lake Ontario, or Lake Simcoe (Conroy et al. 2005, Ozersky et al. 2009, 2015). This discrepancy could be related to methodological differences, such as longer incubation times (6h vs. < 2h in this study and Ozersky et al. 2009) which would skew results due to nutrient uptake by microbes (Ozersky et al. 2009). The elevated mass-specific nutrient excretion rates found in dreissenids partly accounted for the substantial variance in mass-specific nutrient rates detected between dreissenids and fish.

Mass-specific nutrient excretion rates were relatively low for fish compared to dreissenids and other mesotrophic to eutrophic ecosystems (e.g., Torres and Vanni 2007). Our overall low mass-specific fish P excretion rates may be attributed to differences in dietary P requirements and assimilation efficiency, influenced by food quality and ingestion rates (Glaholt Jr and Vanni 2005, Czamanski et al. 2011). In P-rich systems like Western Lake Erie, where P is not limiting for fish, their dietary P requirements and assimilation efficiency may be low, leading to preferential P release as egestion rather than excretion (Halvorson and Atkinson 2019). Moreover, much of the bioavailable P in Lake Erie may be sequestered in biomass, resulting in less P available for mass-specific excretion. Lastly, we noted that our mass-specific N:P excretion rates averaged 8.37. Interestingly, if we were to combine both N:P excretion and egestion, which were not measured in this study, this value could potentially align more closely with the classic Redfield ratio of 16:1 as has been reported previously (André et al. 2003). Even so, our observed low mass-specific N:P excretion rates highlight that animals in Lake Erie excrete disproportionally more P than N possibly due to the ambient conditions or animal functional traits associated with nutrient ingestion, assimilation, allocation, and release.

We measured higher mass-specific N excretion rates during our first sampling period which was warmer, although this could have been confounded with the difference in sampling location between the two sampling periods. Consistent with our findings, previous studies have also demonstrated that animal N excretion rates are more responsive to temperature changes compared to P excretion rates (Devine and Vanni 2002, Peng et al. 2020). The difference in incubation temperatures between the two periods was 8.8°C, which is near the 10°C Q_{10} coefficient used to estimate how biological rates change with temperature. We thus estimated Q_{10} for mass-specific N and P excretion rates to be 3.1 and 1.9, respectively, which falls within the

Q_{10} range of ~ 2 to 3 for most biological systems (Reyes et al. 2008). However, given that our study focused on fish under 40 cm in total length, we captured nutrient excretion rates across various life stages, likely with a relatively smaller representation of adults for larger fish species. Our mass-specific P excretion rates may have been particularly sensitive to differences in P demand across ontogeny (Pilati and Vanni 2007), thereby hindering potential changes between our two sampling periods. For example, our first sampling gizzard shad cohort mostly comprised adults, whereas our second sampling cohort consisted of juveniles smaller in size. The observed higher mass-specific excretion rates in the second sampling may be attributed to ontogenic stage differences, as nutrient excretion rates scale up allometrically with size, and we only corrected for mass. Overall, due to metabolic constraints associated with size and the lower nutritional demand in adult vertebrates relative to juveniles (May and El-Sabaawi 2022), our recorded nutrient excretion rates could potentially overestimate the true population excretion rates.

Mass-specific nutrient excretion rates were not related to trophic position, based on $\delta^{15}N$, nor pelagic or benthic feeding, based on $\delta^{13}C$, of the fish species, suggesting that feeding ecology is not an important driver of N and P excretion. However, inter- and intraspecific variability in tissue stable isotopes and spatial heterogeneity in the physical-chemical conditions between the two sampling periods (Heuvel et al. 2019, 2023) and/or slow tissue turnover rates, particularly in fish (Dodds et al. 2014) could mask a relationship between stable isotopes and mass-specific nutrient excretion rates. A significant relationship between tissue C:N and mass-specific N excretion rates was found, although not for %C or %N nor for P. Our findings align with the growing recognition that animal elemental composition, particularly among vertebrates, poorly correlates with elemental excretion rates (McManamay et al. 2011, Durston and El-Sabaawi

2019), as the latter should reflect instead the stoichiometric mismatch between elemental demand and dietary elemental content (May and El-Sabaawi 2024).

Although no significant relationship was found between tissue stable isotopes and mass-specific nutrient excretion rates, we observed distinct isotopic signals. For example, the isotopic niche of gizzard shad overlapped with white perch and exhibited significant variations, especially in the second sampling, indicating their broad resource use, likely encompassing both zooplankton and detritus and spanning pelagic and littoral habitats, with a pronounced reliance on pelagic environments. The substantial variation in $\delta^{13}C$ further suggests that through their feeding and excretion activities, gizzard shad facilitate nutrient translocation from the sediments to the water column (Vanni et al. 2006), or between pelagic and benthic habitats depending on individual trophic fidelity. Incorporating fish movement ecology into feeding ecology and animal-mediated nutrient cycling analyses could provide deeper insights into spatial nutrient and energy dynamics (Cooke et al. 2022), particularly relevant for migratory species such as walleye (*Sander vitreus*; Wang et al. 2007).

Dreissenids and white perch dominated population nutrient excretion rate estimates due to their high biomass. Furthermore, we noted similar temporal patterns in population nutrient excretion rates between competing endemic and non-endemic species. Both white perch and round gobies had parallel trends in population nutrient excretion rates compared to yellow perch and logperch, respectively, reflective of their biomass, while consistently having higher rates than their endemic counterparts. Similarities in population nutrient excretion rates likely stem from similarities in species ecology, supported by comparable trends in mass-specific nutrient excretion rates and tissue stable isotopes. This suggests functional redundancy in nutrient excretion traits between white perch and yellow perch, as well as round goby and logperch

(Villéger et al. 2017). Additionally, projections of fish population recruitment under anticipated climate change and agricultural conservation practices indicate that white perch population nutrient excretion is likely to remain higher than that of yellow perch, owing to the anticipated stabilization or increase in white perch recruitment and decrease in yellow perch recruitment (Dippold et al. 2020). Future research could integrate variations in mass-specific nutrient excretion rates across years to better integrate temporal changes in population and ultimately community nutrient excretion rates.

Fish and dreissenids together supplied considerable amounts of nutrients through excretion, with the potential for rapid turnover of dissolved nutrients in the Western basin. Fish and dreissenid N and P loads constituted at least half of external N loads and were 2.5 to 39 times higher than external P loads. When compared to estimates of internal P loads from sediments, fish and dreissenid-derived P loads surpassed sediment-derived P loads by anywhere from 3 to 88-fold in the Western basin and lake wide (Matisoff et al. 2016, Wang et al. 2021, Bocaniov et al. 2023). The considerable P loads from dreissenids are also in agreement with previous findings in Lake Erie and Lake Ontario (Arnott and Vanni 1996, Ozersky et al. 2009, Li et al. 2021). While P loads from the five fish species we assessed across the lake were found to be lower than those from external sources, we argue that community estimates derived from the total fish biomass have the potential to surpass external SRP loads lake wide, especially when accounting for the abundant freshwater drum (*Aplodinotus grunniens*) and the large piscivore walleye (Forage Task Group 2023). Additionally, the spatial variability in animal biomass and movement across basins is likely to result in differential impacts on animal-mediated nutrient cycling within each basin. However, considering the reported decline in fish and dreissenid biomass over the last decade and potential shifts in external P loads due to climate-induced

changes in precipitation patterns and temperature, and increased agricultural conservation practices (Dippold et al. 2020, Karatayev et al. 2021b, Forage Task Group 2023, Fraker et al. 2023), the future impacts of animals on internal P loads remains uncertain.

Our study highlights the substantial N and P contributions from animals in Lake Erie could, providing valuable insights into the potential sources fueling the persistent high trophic states and recurrent harmful algal blooms (HABs) occuring in the Western and Central basins. We contend that animal-derived nutrients may be especially important in years and basins with lower external nutrient inputs and enhanced in-lake nutrient enrichment via sediment sources (Boedecker et al. 2020, Wu et al. 2022). Our findings support previous claims that eutrophication management strategies focusing solely on reducing P inputs may exacerbate existing N:P imbalances in lakes, necessitating a comprehensive consideration of both N and P dynamics (Boedecker et al. 2020, Wu et al. 2022). Evidently, much of the available nutrients supplied from both external and internal sources are eventually consumed and sequestered within primary production and consumer biomass in particulate form. Nonetheless, we argue that animal-mediated nutrient cycling could have profound implications for internal nutrient processes in large productive lakes and should be considered in future nutrient budget assessments.

Acknowledgements

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Figures

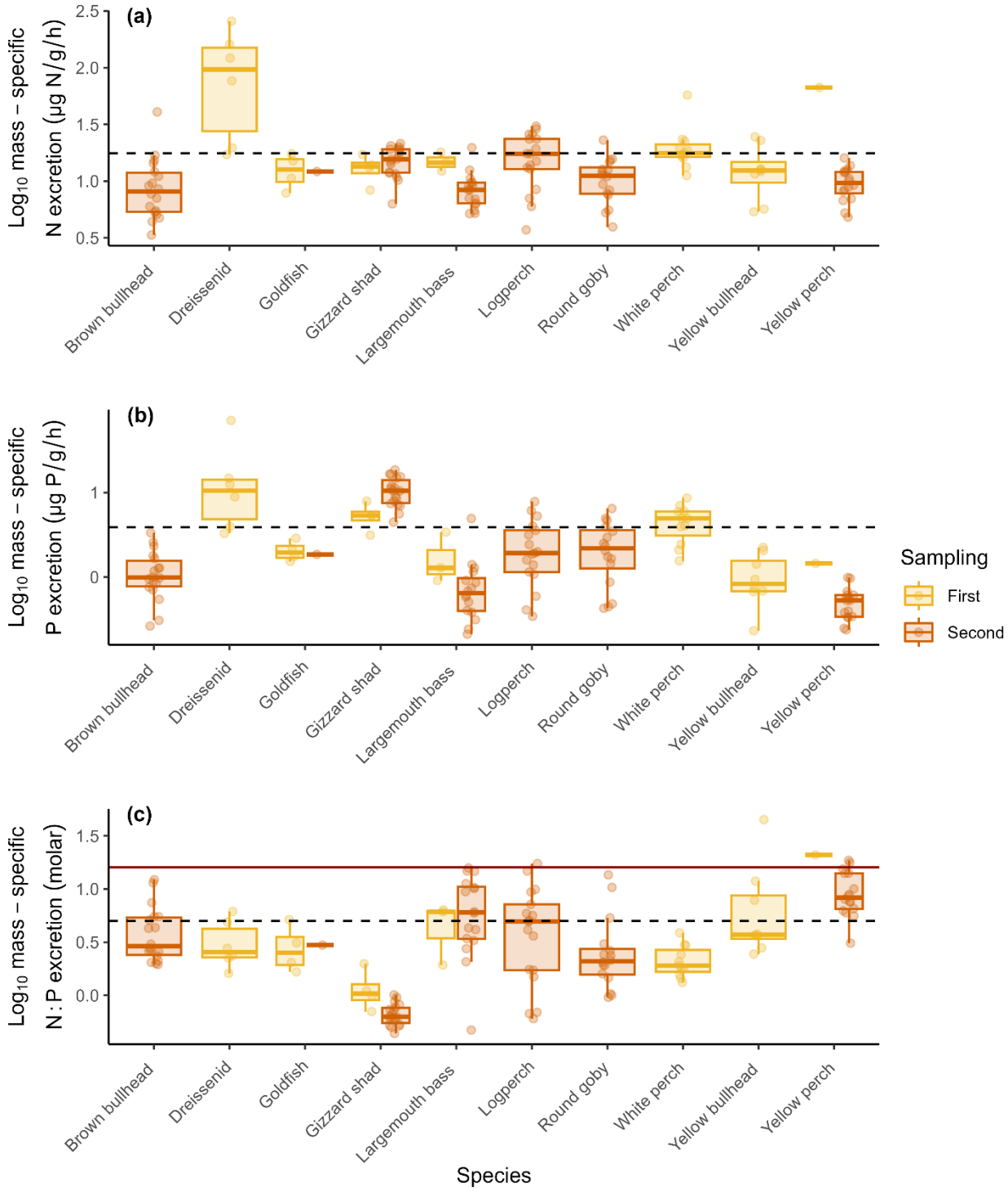
(a)
Log10 mass – specific N excretion (µg N/g/h)
2.5
2.0
1.5
1.0
0.5
(b)
Log10 mass – specific P excretion (µg P/g/h)
1
0
(c)
Log10 mass – specific N:P excretion (molar)
1.5
1.0
0.5
0.0
Brown bullhead
Dreissenid
Goldfish
Gizzard shad
Largemouth bass
Logperch
Round goby
White perch
Yellow bullhead
Yellow perch
Species
Sampling
First
Second

Figure 1. Dreissenids excrete N at the highest rates, while both dreissenids and gizzard shad excrete P at the highest rates. Mass-specific (a) P, and (b) P excretion rates relative to dreissenids and nine fish species. Box plots represent the median, first and third quartiles, and minimum and maximum values. Colours indicate sampling period and location (summer in Lake Erie for the first and fall in the Detroit River for the second) and symbols represent each dreissenid unit set and individual fish. Dotted lines indicate mean nutrient excretion rates and ratios while the red line is the $\log_{10}$ of the Redfield ratio ($\log_{10}(16) = 1.2$).

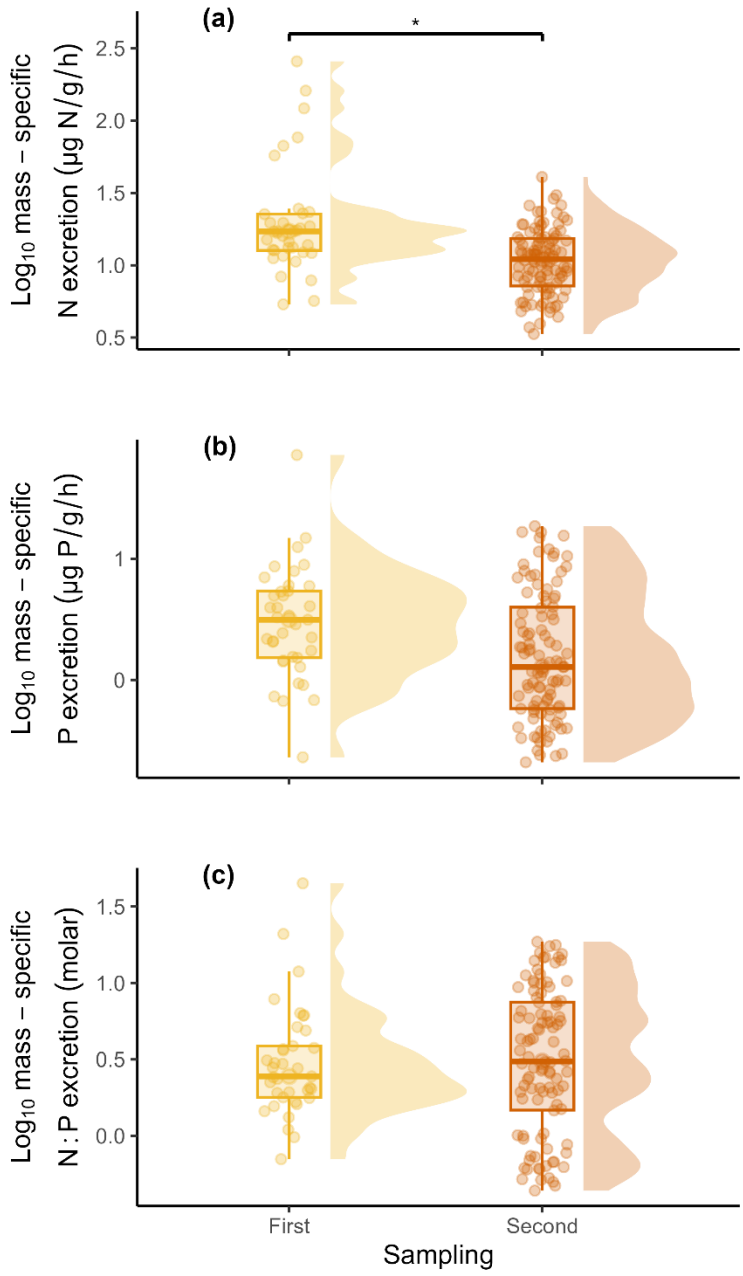

Figure 2. Animals excrete significantly more N in the first sampling period relative to the second (summer in Lake Erie vs. fall in the Detroit River). Mass-specific (a) N, (b) P, and (c) N:P excretion rates relative to the season for dreissenids and nine fish species. Box plots represent the median, first and third quartiles, and minimum and maximum values. Half-eye plots correspond to the density distribution of the raw data. Colours indicate sampling period and

location (summer in Lake Erie for the first and fall in the Detroit River for the second) and symbols represent each dreissenid unit set and individual fish.

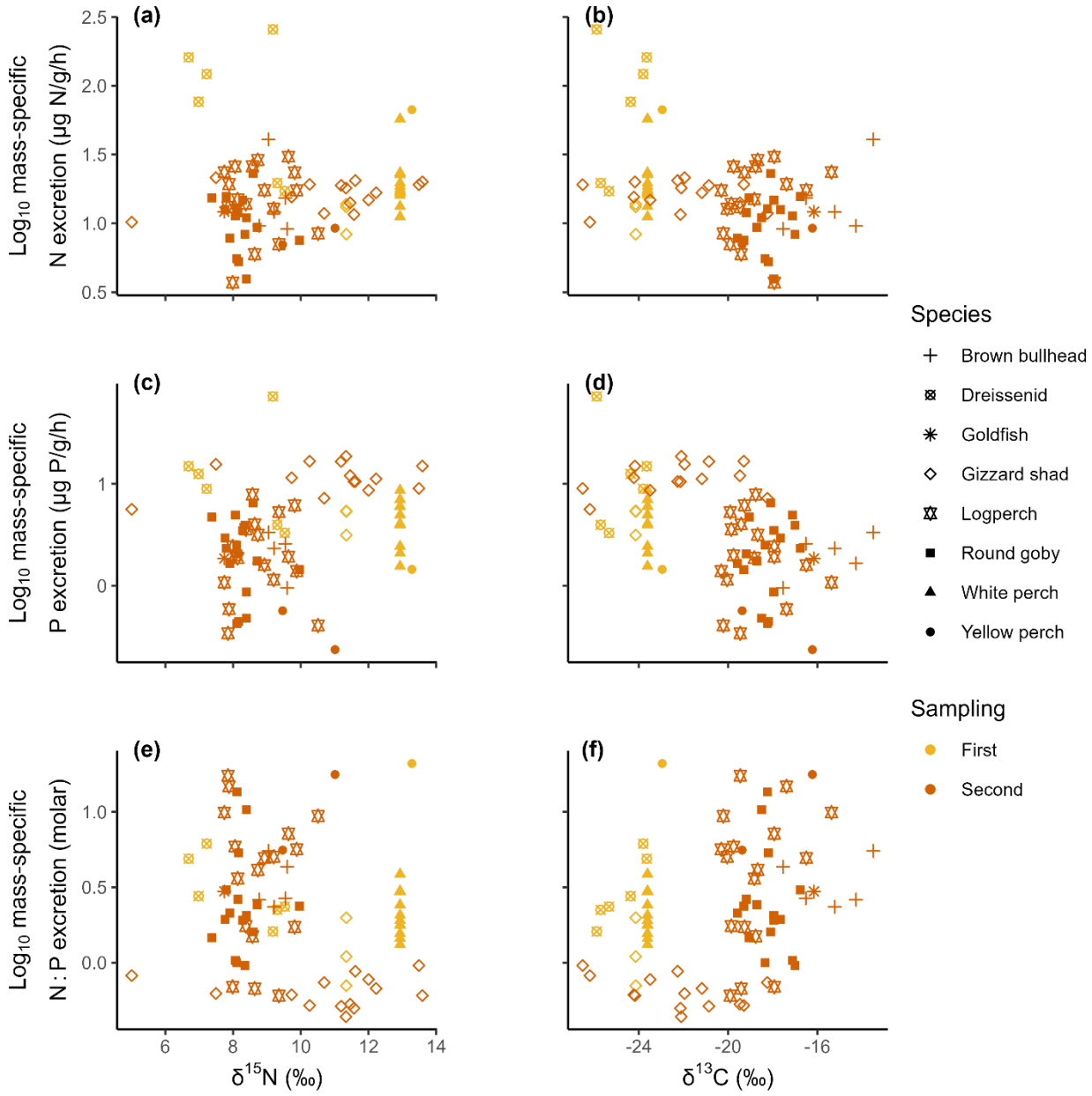

Figure 3. There is no significant relationship between animal mass-specific excretion and tissue $\delta^{15}N$ and $\delta^{13}C$. Mass-specific N, P, and N:P excretion rates relative to (a, c, e) tissue $\delta^{15}N$, and (b, d, f) $\delta^{13}C$ for dreissenids and seven fish species. Colours indicate sampling period and

location (summer in Lake Erie for the first and fall in the Detroit River for the second) and symbols represent each dreissenid unit set and individual fish.

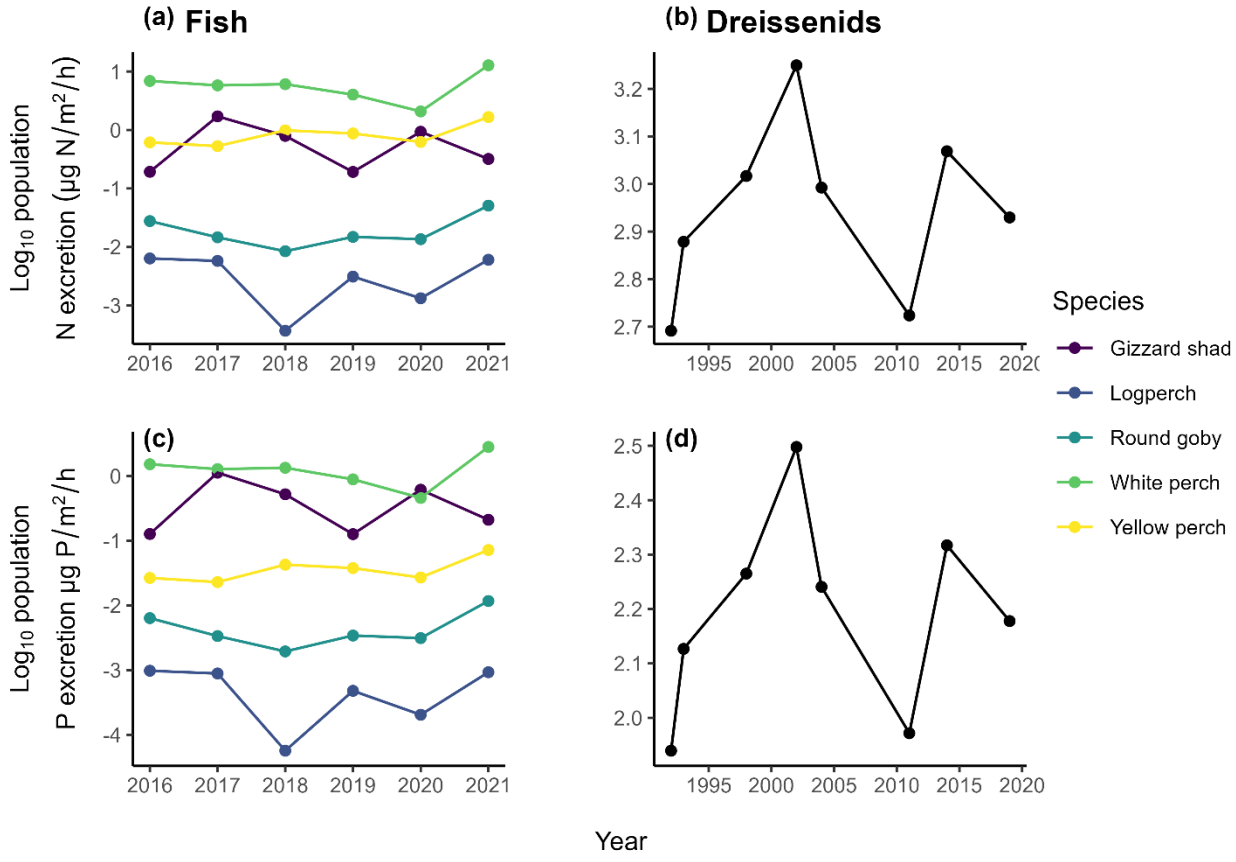

Figure 4. White perch and dreissenid population N and P excretion rates are the highest, while logperch population N and P excretion rates are the lowest. Population (a) N, and (b) P excretion rates relative to a six-year period (2016-2021) for five fish species and an 18-year period for dreissenids. Temporal variations in population N and P excretion are based solely on biomass variations, while mass-specific excretion rates are kept constant. Note scale differences between fish and dreissenids.

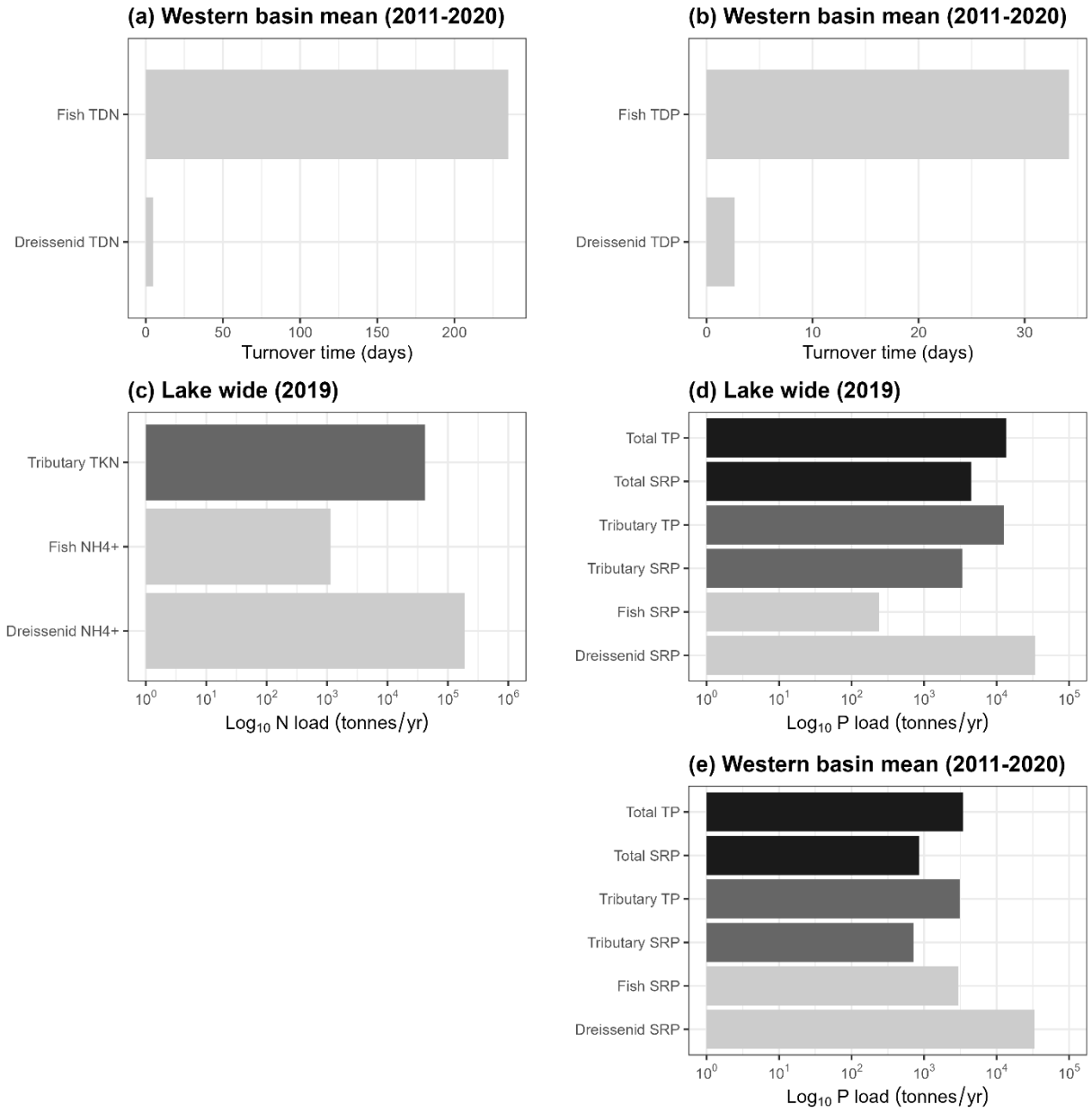

Figure 5. Fish and dreissenids can turnover the Western basin nutrients in less than 235 days and exceed SRP loads exceed external SRP and TP load by up to two orders of magnitude. Aggregate fish populations and dreissenid turnover times for (a) TDN, and (b) TDP using Western basin mean biomass from 2011 to 2020 for total fish species catch and dreissenids. N loads from (c) lake wide tributary TKN, fish and dreissenid NH_4^+. P loads from all external sources (total TP and SRP), main Canada and US tributaries (tributary TP and SRP) and

aggregate fish populations and dreissenid SRP excretion using (d) lake wide external P loads and biomass for five fish species and dreissenids in 2019, and (e) Western basin mean external P loads and biomass from 2011 to 2020 for total fish species catch and dreissenids.

Supplementary Information

Tables

Table S1. Summary of individual-level N, P, and N:P excretion rates, dry mass, stable isotopes, and elemental tissue composition for nine fish species and dreissenids in summer and fall. Nutrient excretion rates are based on NH_4^+ and SRP estimates.

Taxa	Sampling	response	n	mean (± SD)	min	max	CV
Fish	First	Mass-specific N excretion (µg N/g/h)	31	17.94 ± 12.82	5.37	66.97	0.71
		Mass-specific P excretion (µg P/g/h)	31	3.16 ± 2.31	0.23	8.66	0.73
		Mass-specific N:P excretion (molar)	31	5.06. ± 8.37	0.70	44.87	1.65
		Individual dry mass (g)	31	374.87 ± 284.71	40.00	1070.00	0.76
		δ15N (‰)	12	12.64 ± 0.68	11.34	13.28	0.05
		δ13C (‰)	12	-23.68 ± 0.29	-24.15	-22.96	-0.01
		Tissue C (%)	12	46.64 ± 1.83	44.85	51.79	0.04
		Tissue N (%)	12	13.21 ± 1.07	11.14	13.74	0.08
		Tissue C:N (molar)	12	3.87 ± 0.63	3.29	5.08	0.16
Dreissenids		Mass-specific N excretion (µg N/g/h)	6	108.86 ± 91.95	17.16	257.08	0.84
		Mass-specific P excretion (µg P/g/h)	6	19.28 ± 26.28	3.29	72.10	1.36
		Mass-specific N:P excretion (molar)	6	3.33 ± 1.78	1.61	6.15	0.53
		Individual dry mass (g)	6	0.021 ± 0.02	0.00	0.05	0.94
		δ15N (‰)	6	8.15 ± 1.32	6.69	9.53	0.16
		δ13C (‰)	6	-24.8 ± 0.98	-25.89	-23.65	-0.04
		Tissue C (%)	6	45.8 ± 1.38	43.39	47.42	0.03

		Tissue N (%)	6	10.86 ± 0.3	10.28	11.16	0.03
		Tissue P (%)	6	1.28 ± 0.13	1.10	1.42	0.10
		Tissue C:N (molar)	6	4.92 ± 0.11	4.75	5.06	0.02
Fish	Second	Mass-specific N excretion (µg N/g/h)	102	12.17 ± 6.53	3.35	40.74	0.54
		Mass-specific P excretion (µg P/g/h)	102	3.22 ± 4.20	0.21	18.63	1.30
		Mass-specific N:P excretion (molar)	102	5.10 ± 4.77	0.44	18.59	0.93
		Individual dry mass (g)	102	150.84 ± 208.78	1.00	887.00	1.38
		δ15N (‰)	55	9.23 ± 1.63	5.01	13.59	0.18
		δ13C (‰)	55	-19.13 ± 2.62	-26.52	-13.50	-0.14
		Tissue C (%)	55	45.96 ± 3.01	38.53	52.43	0.07
		Tissue N (%)	55	12.92 ± 1.09	10.66	16.33	0.08
		Tissue C:N (molar)	55	3.57 ± 0.28	3.21	4.69	0.08

standard deviation (SD), minimum (min), maximum (max), sample size (n), coefficient of variation (CV)

Table S2. Summary of population-level N, P, and N:P excretion rates and biomass for five fish species over a six-year period (2016-2020) and dreissenids over a 27-year period (1992-2019).

Taxa	**response**	**n**	**mean (± SD)**	**min**	**max**	**CV**
Fish	Population N excretion (µg N/m^2/h)	30	1.57 ± 2.86	3.70E-04	12.75	1.82
	Population P excretion (µg P/m^2/h)	30	0.38 ± 0.66	5.67E-05	2.83	1.74
	Population N:P excretion (molar)	30	0.28 ± 2.86	1.20E-04	1.33	1.40
	Biomass (g/m^2)	30	0.08 ± 0.13	2.15E-05	0.60	1.63
Dreissenids	Population N excretion (µg N/m^2/h)	8	949.67 ± 409.93	491.37	1776.49	0.43

Population P excretion (μg P/m^2/h)	8	168.2 ± 72.61	87.03	314.65	0.43
Population N:P excretion (molar)	8	29.08 ± 12.55	15.05	54.41	0.43
Biomass (g/m^2)	8	8.72 ± 3.77	4.51	16.32	0.43

Table S3. ANOVA model outputs using mass-specific N, P, and N:P excretion rates as response variables, and species as a predictor variable.

	df	**SS**	**MS**	**F**	***p***
Mass-specific N excretion					
Species	9	5.139	0.571	10.817	< 0.001
Residuals	129	6.809	0.053	NA	NA
Mass-specific P excretion					
Species	9	25.263	2.807	29.204	< 0.001
Residuals	129	12.399	0.096	NA	NA
Mass-specific N:P excretion					
Species	9	15.034	1.670	18.151	< 0.001
Residuals	129	11.872	0.092	NA	NA

Table S4. Summary emmeans outputs for mass-specific N, P, and N:P excretion rates across species including brown bullhead (BB), dreissenid mussels (DM), largemouth bass (LB), round goby (RG), yellow perch (YP), gizzard shad (GS), yellow bullhead (YB), logperch (LP), goldfish (GF), and white perch (WP).

contrast	ratio	SE	df	lower CI	upper CI	null	t ratio	*p*
Mass-normalized N excretion								
BB / DM	0.12	0.03	129	0.05	0.27	1	-8.54	0.00
DM / LB	7.91	1.96	129	3.56	17.57	1	8.35	0.00
DM / RG	7.11	1.80	129	3.14	16.07	1	7.74	0.00
DM / YP	6.78	1.70	129	3.02	15.23	1	7.62	0.00
DM / GS	4.96	1.21	129	2.27	10.88	1	6.58	0.00
DM / YB	6.00	1.72	129	2.39	15.06	1	6.27	0.00
DM / LP	4.78	1.20	129	2.13	10.72	1	6.22	0.00

contrast	ratio	SE	df	lower CI	upper CI	null	t ratio	*p*
DM / GF	5.86	1.88	129	2.09	16.43	1	5.52	0.00
DM / WP	3.70	0.99	129	1.56	8.79	1	4.88	0.00
BB / WP	0.44	0.09	129	0.23	0.85	1	-4.05	0.00
LB / WP	0.47	0.09	129	0.25	0.89	1	-3.79	0.01
BB / LP	0.57	0.10	129	0.32	1.01	1	-3.16	0.06
RG / WP	0.52	0.11	129	0.27	1.02	1	-3.15	0.06
BB / GS	0.59	0.10	129	0.34	1.02	1	-3.13	0.06
WP / YP	1.83	0.38	129	0.95	3.54	1	2.96	0.10
LB / LP	0.60	0.11	129	0.34	1.07	1	-2.86	0.13
GS / LB	1.59	0.26	129	0.94	2.72	1	2.82	0.14
LP / RG	1.49	0.27	129	0.82	2.69	1	2.16	0.49
GS / RG	1.43	0.25	129	0.82	2.51	1	2.07	0.56
WP / YB	1.62	0.40	129	0.73	3.58	1	1.97	0.62
LP / YP	1.42	0.26	129	0.79	2.55	1	1.93	0.65
GS / YP	1.37	0.23	129	0.79	2.37	1	1.83	0.72
GF / WP	0.63	0.18	129	0.25	1.58	1	-1.61	0.84
GS / WP	0.75	0.15	129	0.40	1.40	1	-1.50	0.89
BB / YB	0.71	0.16	129	0.35	1.47	1	-1.50	0.89
BB / GF	0.70	0.19	129	0.29	1.65	1	-1.35	0.94
LP / WP	0.78	0.16	129	0.40	1.50	1	-1.24	0.96
LB / YB	0.76	0.17	129	0.37	1.56	1	-1.24	0.96
BB / YP	0.81	0.14	129	0.45	1.44	1	-1.20	0.97
GF / LB	1.35	0.36	129	0.57	3.18	1	1.13	0.98
LP / YB	1.26	0.29	129	0.61	2.61	1	1.01	0.99
BB / RG	0.85	0.15	129	0.47	1.52	1	-0.92	1.00
LB / YP	0.86	0.15	129	0.49	1.51	1	-0.87	1.00
GS / YB	1.21	0.26	129	0.60	2.44	1	0.87	1.00
GF / LP	0.82	0.22	129	0.34	1.94	1	-0.76	1.00
RG / YB	0.84	0.19	129	0.40	1.77	1	-0.74	1.00
GF / RG	1.21	0.33	129	0.51	2.91	1	0.72	1.00
GF / GS	0.85	0.22	129	0.36	1.97	1	-0.63	1.00
LB / RG	0.90	0.16	129	0.50	1.60	1	-0.60	1.00
GF / YP	1.16	0.31	129	0.49	2.76	1	0.55	1.00
YB / YP	1.13	0.26	129	0.54	2.35	1	0.54	1.00
BB / LB	0.94	0.16	129	0.54	1.65	1	-0.34	1.00
RG / YP	0.95	0.18	129	0.53	1.73	1	-0.25	1.00
GS / LP	0.96	0.16	129	0.56	1.67	1	-0.23	1.00
GF / YB	1.03	0.31	129	0.39	2.71	1	0.08	1.00

contrast	ratio	SE	df	lower CI	upper CI	null	t ratio	*p*
Mass-normalized P excretion								
GS / YP	17.64	4.07	129	8.40	37.05	1	12.45	0.00
GS / LB	11.65	2.60	129	5.67	23.92	1	10.98	0.00
BB / GS	0.12	0.03	129	0.06	0.24	1	-9.52	0.00
DM / YP	21.24	7.20	129	7.13	63.27	1	9.02	0.00
DM / LB	14.03	4.69	129	4.78	41.16	1	7.90	0.00
GS / YB	9.83	2.90	129	3.80	25.38	1	7.75	0.00
WP / YP	8.25	2.28	129	3.39	20.07	1	7.64	0.00
BB / DM	0.10	0.03	129	0.03	0.28	1	-6.97	0.00
GS / LP	4.77	1.10	129	2.27	10.01	1	6.77	0.00
GS / RG	4.77	1.12	129	2.24	10.15	1	6.66	0.00
DM / YB	11.84	4.56	129	3.42	40.96	1	6.41	0.00
LB / WP	0.18	0.05	129	0.08	0.44	1	-6.27	0.00
LP / YP	3.70	0.91	129	1.68	8.14	1	5.34	0.00
RG / YP	3.70	0.92	129	1.66	8.24	1	5.26	0.00
DM / LP	5.74	1.95	129	1.93	17.10	1	5.16	0.00
BB / WP	0.25	0.07	129	0.10	0.59	1	-5.13	0.00
DM / RG	5.74	1.96	129	1.91	17.26	1	5.12	0.00
WP / YB	4.60	1.52	129	1.58	13.37	1	4.60	0.00
GF / GS	0.22	0.08	129	0.07	0.69	1	-4.25	0.00
DM / GF	5.42	2.34	129	1.35	21.81	1	3.91	0.01
GF / YP	3.92	1.42	129	1.22	12.61	1	3.76	0.01
LB / LP	0.41	0.10	129	0.19	0.88	1	-3.75	0.01
LB / RG	0.41	0.10	129	0.19	0.89	1	-3.69	0.01
BB / YP	2.03	0.49	129	0.93	4.42	1	2.94	0.11
LP / WP	0.45	0.12	129	0.18	1.09	1	-2.90	0.12
GS / WP	2.14	0.56	129	0.92	5.00	1	2.88	0.12
RG / WP	0.45	0.13	129	0.18	1.10	1	-2.87	0.13
GF / LB	2.59	0.93	129	0.81	8.21	1	2.65	0.21
DM / WP	2.58	0.93	129	0.80	8.27	1	2.61	0.22
BB / LP	0.55	0.13	129	0.25	1.20	1	-2.48	0.29
BB / RG	0.55	0.13	129	0.25	1.21	1	-2.44	0.31
LP / YB	2.06	0.63	129	0.77	5.52	1	2.36	0.36
RG / YB	2.06	0.64	129	0.76	5.58	1	2.34	0.37
GF / WP	0.47	0.18	129	0.14	1.64	1	-1.93	0.65
GF / YB	2.18	0.89	129	0.59	8.09	1	1.92	0.66
YB / YP	1.79	0.55	129	0.67	4.81	1	1.91	0.66
BB / GF	0.52	0.19	129	0.16	1.66	1	-1.82	0.72

contrast	ratio	SE	df	lower CI	upper CI	null	t ratio	*p*
LB / YP	1.51	0.36	129	0.70	3.26	1	1.74	0.77
BB / LB	1.34	0.32	129	0.63	2.86	1	1.26	0.96
DM / GS	1.20	0.40	129	0.42	3.47	1	0.57	1.00
LB / YB	0.84	0.25	129	0.32	2.22	1	-0.56	1.00
BB / YB	1.13	0.34	129	0.43	3.01	1	0.41	1.00
GF / RG	1.06	0.39	129	0.33	3.44	1	0.16	1.00
GF / LP	1.06	0.38	129	0.33	3.41	1	0.16	1.00
LP / RG	1.00	0.25	129	0.45	2.23	1	0.00	1.00
Mass-normalized N:P excretion								
GS / YP	0.08	0.02	129	0.04	0.16	1	-11.34	0.00
GS / LB	0.14	0.03	129	0.07	0.28	1	-9.09	0.00
BB / GS	5.12	1.14	129	2.51	10.47	1	7.36	0.00
GS / YB	0.12	0.04	129	0.05	0.31	1	-7.26	0.00
GS / LP	0.20	0.05	129	0.10	0.42	1	-7.09	0.00
RG / YP	0.26	0.06	129	0.12	0.56	1	-5.57	0.00
WP / YP	0.22	0.06	129	0.09	0.53	1	-5.57	0.00
GS / RG	0.30	0.07	129	0.14	0.63	1	-5.24	0.00
DM / GS	4.12	1.33	129	1.46	11.61	1	4.40	0.00
GS / WP	0.35	0.09	129	0.15	0.80	1	-4.08	0.00
LP / YP	0.38	0.09	129	0.18	0.83	1	-4.00	0.00
BB / YP	0.40	0.09	129	0.19	0.85	1	-3.91	0.01
GF / GS	3.82	1.32	129	1.25	11.63	1	3.87	0.01
LB / WP	2.55	0.67	129	1.09	5.97	1	3.53	0.02
DM / YP	0.32	0.11	129	0.11	0.93	1	-3.44	0.03
GF / YP	0.30	0.11	129	0.09	0.93	1	-3.43	0.03
LB / RG	2.19	0.52	129	1.02	4.71	1	3.31	0.04
WP / YB	0.35	0.11	129	0.12	1.00	1	-3.21	0.05
RG / YB	0.41	0.12	129	0.15	1.09	1	-2.95	0.10
LB / YP	0.57	0.13	129	0.27	1.20	1	-2.44	0.31
BB / WP	1.79	0.48	129	0.76	4.23	1	2.17	0.48
LP / WP	1.73	0.47	129	0.72	4.13	1	2.02	0.58
GF / YB	0.47	0.19	129	0.13	1.69	1	-1.90	0.67
GF / LB	0.52	0.18	129	0.17	1.62	1	-1.85	0.70
DM / YB	0.51	0.19	129	0.15	1.71	1	-1.80	0.73
BB / RG	1.54	0.37	129	0.71	3.33	1	1.80	0.74
DM / LB	0.56	0.18	129	0.20	1.62	1	-1.75	0.76
LB / LP	1.47	0.34	129	0.70	3.12	1	1.67	0.81
LP / YB	0.61	0.18	129	0.23	1.60	1	-1.65	0.82

contrast	ratio	SE	df	lower CI	upper CI	null	t ratio	*p*
LP / RG	1.49	0.36	129	0.68	3.26	1	1.63	0.83
BB / YB	0.63	0.19	129	0.24	1.64	1	-1.55	0.87
YB / YP	0.63	0.19	129	0.24	1.65	1	-1.54	0.87
BB / LB	0.70	0.16	129	0.33	1.47	1	-1.54	0.87
DM / WP	1.44	0.51	129	0.46	4.50	1	1.02	0.99
BB / GF	1.34	0.47	129	0.43	4.18	1	0.83	1.00
GF / WP	1.33	0.50	129	0.40	4.48	1	0.76	1.00
GF / LP	0.77	0.27	129	0.25	2.42	1	-0.73	1.00
BB / DM	1.24	0.41	129	0.43	3.59	1	0.66	1.00
DM / RG	1.24	0.41	129	0.42	3.63	1	0.64	1.00
DM / LP	0.83	0.28	129	0.29	2.42	1	-0.55	1.00
RG / WP	1.16	0.32	129	0.48	2.80	1	0.55	1.00
GF / RG	1.15	0.41	129	0.36	3.63	1	0.38	1.00
LB / YB	0.90	0.26	129	0.35	2.32	1	-0.36	1.00
DM / GF	1.08	0.46	129	0.28	4.21	1	0.18	1.00
BB / LP	1.03	0.24	129	0.48	2.21	1	0.14	1.00

Table S5. ANOVA model outputs using mass-specific N, P, and N:P excretion rates as response variables, sampling period and location (summer in Lake Erie for the first and fall in the Detroit River for the second) and species as predictor variables with an interaction effect.

	df	**SS**	**MS**	**F**	***p***
Mass-specific N excretion					
Sampling	1	1.928	1.928	40.546	< 0.001
Species	9	3.415	0.379	7.983	< 0.001
Sampling:Species	3	0.663	0.221	4.648	0.004
Residuals	125	5.942	0.048	NA	NA
Mass-specific P excretion					
Sampling	1	2.151	2.151	23.272	< 0.001
Species	9	23.143	2.571	27.822	< 0.001
Sampling:Species	3	0.816	0.272	2.942	0.036
Residuals	125	11.553	0.092	NA	NA
Mass-specific N:P excretion					
Sampling	1	0.006	0.006	0.066	0.797

	df	SS	MS	F	*p*
Species	9	15.105	1.678	18.181	< 0.001
Sampling:Species	3	0.256	0.085	0.924	0.431
Residuals	125	11.539	0.092	NA	NA

Table S6. ANOVA model outputs using mass-specific N, P, and N:P excretion rates as response variables, sampling period and location (summer in Lake Erie for the first and fall in the Detroit River for the second) and species as predictor variables with an interaction effect for the two species tested in both sampling periods and locations (i.e., gizzard shad and largemouth bass).

	SS	df	F	*p*
Mass-specific N excretion				
Sampling	0.204	1	4.295	0.040
Species	3.415	9	7.983	< 0.001
Sampling:Species	0.663	3	4.648	0.004
Residuals	5.942	125	NA	NA
Mass-specific P excretion				
Sampling	0.031	1	0.330	0.567
Species	23.143	9	27.822	< 0.001
Sampling:Species	0.816	3	2.942	0.036
Residuals	11.553	125	NA	NA
Mass-specific N:P excretion				
Sampling	0.077	1	0.832	0.363
Species	15.105	9	18.181	< 0.001
Sampling:Species	0.256	3	0.924	0.431
Residuals	11.539	125	NA	NA

Table S7. Lmer model outputs mass-specific N, P, and N:P excretion rates as response variables, temperature (temp) as a predictor variable and species as a random effect.

	SS	MS	Num df	Den df	F	*p*
Mass-specific N excretion						
Temp	0.390	0.390	1	91.93	7.594	0.007
Mass-specific P excretion						
Temp	0.139	0.139	1	127.70	1.445	0.232
Mass-specific N:P excretion						
Temp	0.032	0.032	1	102.16	0.345	0.558

Table S8. Lmer model outputs mass-specific N, P, and N:P excretion rates as response variables, $\delta^{15}N$, $\delta^{13}C$, %N, or C:N as a predictor variable and species as a random effect.

	SS	MS	Num df	Den df	F	*p*
Mass-specific N excretion						
$\delta^{15}N$	0.002	0.002	1	68.63	0.034	0.854
Mass-specific N excretion						
$\delta^{13}C$	0.036	0.036	1	39.66	0.554	0.461
Mass-specific N excretion						
Tissue %N	0.000	0.000	1	73.36	0.006	0.936
Mass-specific N excretion						
Tissue C:N	0.023	0.023	1	57.25	0.362	0.550
Mass-specific P excretion						
$\delta^{15}N$	0.009	0.009	1	72.87	0.077	0.782
Mass-specific P excretion						
$\delta^{13}C$	0.049	0.049	1	51.39	0.434	0.513
Mass-specific N:P excretion						
$\delta^{15}N$	0.002	0.002	1	70.90	0.022	0.881
Mass-specific N:P excretion						
$\delta^{13}C$	0.049	0.049	1	51.39	0.434	0.513

Table S9. AIC table comparing linear mixed effect model fits using mass-specific N, P, and N:P excretion rates as response variables, stable isotopes as a fixed variable, and species as a random variable.

Model	**df**	**AIC**	**ΔAIC**
Mass-specific N excr ~ δ^{15}N + (1|Species)	4	37	7
Mass-specific N excr ~ δ^{13}C + (1|Species)	4	38	8
Mass-specific N excr ~ %N + (1|Species)	4	37	7
Mass-specific N excr ~ C:N + (1|Species)	4	35	5
Mass-specific N excr ~ (1|Species)	3	30	0
Mass-specific P excr ~ δ^{15}N + (1|Species)	4	82	7
Mass-specific P excr ~ δ^{13}C + (1|Species)	4	82	7
Mass-specific P excr ~ (1|Species)	3	75	0
Mass-specific N:P excr ~ δ^{15}N + (1|Species)	4	66	7
Mass-specific N:P excr ~ δ^{13}C + (1|Species)	4	82	23
Mass-specific N:P excr ~ (1|Species)	3	59	0

Table S10. Summary of N and P loads by source including external total TP and SRP, main US and Canada tributaries TP and SRP, main US tributaries TKN, and community-level fish and dreissenid NH_4^+ and SRP excretion both lakewide and in the Western basin only except for TKN that was only reported lakewide (NCWQR 2022). Lakewide estimates are based on external P loads and biomass for five fish species and dreissenids in 2019 (Environment and Climate Change Canada 2021, Karatayev et al. 2021a, NCWQR 2022). Western basin estimates are based on mean external P loads and biomass for total fish species catch from 2011 to 2020 (Environment and Climate Change Canada 2021, Keretz et al. 2022, DuFour et al. 2023).

Source	Chemical form	Region	N load (tonnes/yr)	P load (tonnes/yr)
Total	TP	Lakewide	-	13544
	SRP		-	4470
Tributary	TP		-	12591
	SRP		-	3381
	TKN		41900	-
Total	TP	Western basin	-	3440
Total	SRP		-	860
Tributary	TP		-	3099
Tributary	SRP		-	713
Fish	NH_4^+ and SRP	Lakewide	1147.66	238.24
		Western basin	12439.76	2951.80
Dreissenids	NH_4^+ and SRP	Lakewide	191142.32	33855.01
		Western basin	187862.43	33274.07

Figures

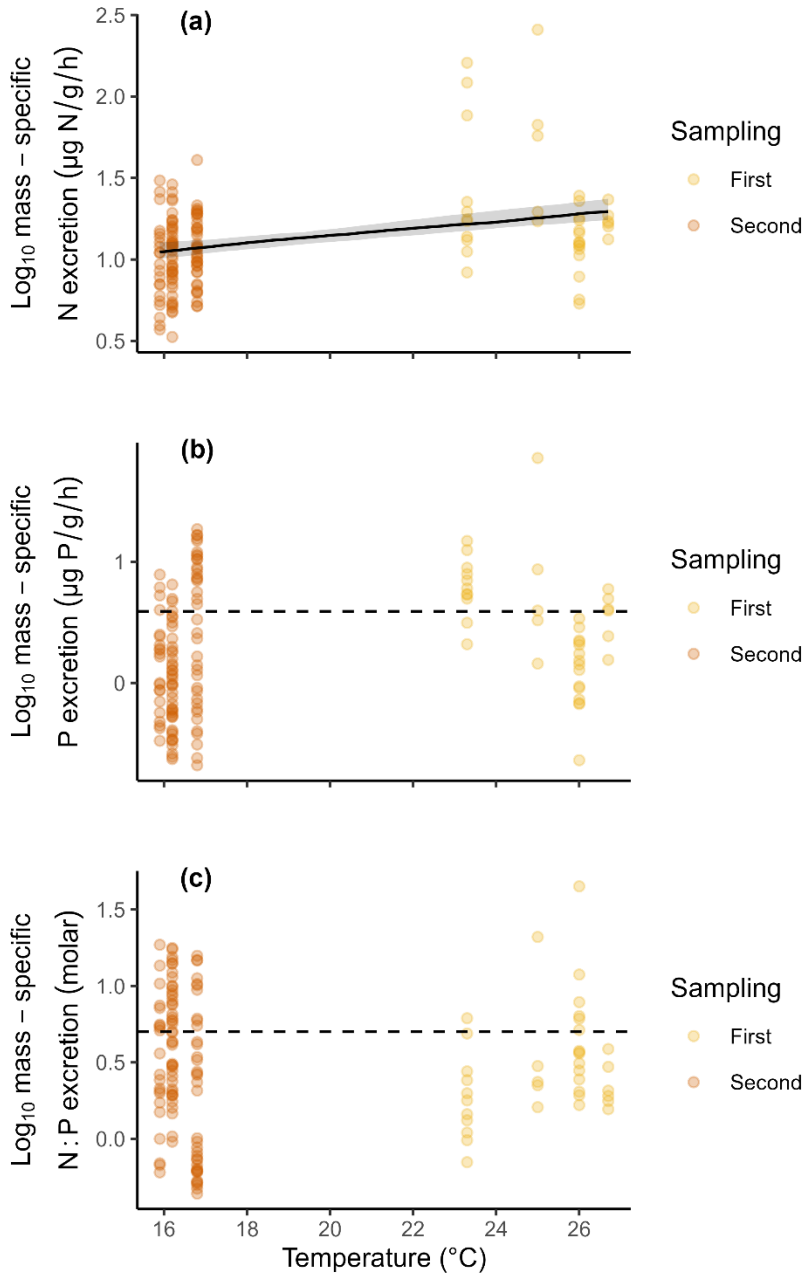

Figure S1. Animals excrete significantly more N at higher temperatures. Mass-specific (a) N, (b) P, and (c) N:P excretion rates relative to temperature for dreissenids and nine fish species. Box plots represent the median, first and third quartiles, and minimum and maximum values. Half-eye plots correspond to the density distribution of the raw data. Colours indicate sampling

period and location (summer in Lake Erie for the first and fall in the Detroit River for the second) and symbols represent each dreissenid unit set and individual fish.

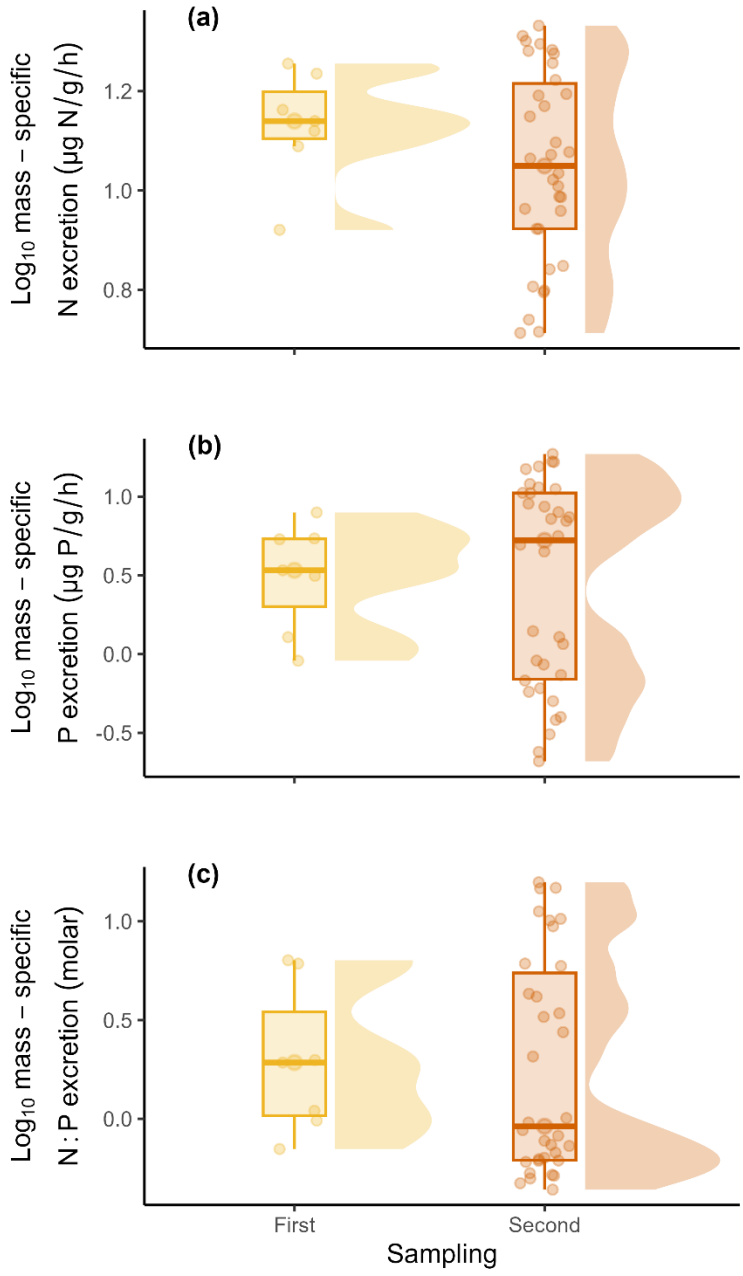

Figure S2. Largemouth bass and gizzard shad excrete significantly more N in the summer relative to the fall. Mass-specific (a) N, (b) P, and (c) N:P excretion rates relative to season for two fish species tested in both seasons (i.e., largemouth bass and gizzard shad). Box plots

represent the median, first and third quartiles, and minimum and maximum values. Half-eye plots correspond to the density distribution of the raw data. Colours indicate sampling period and location (summer in Lake Erie for the first and fall in the Detroit River for the second) and symbols represent each dreissenid unit set and individual fish.

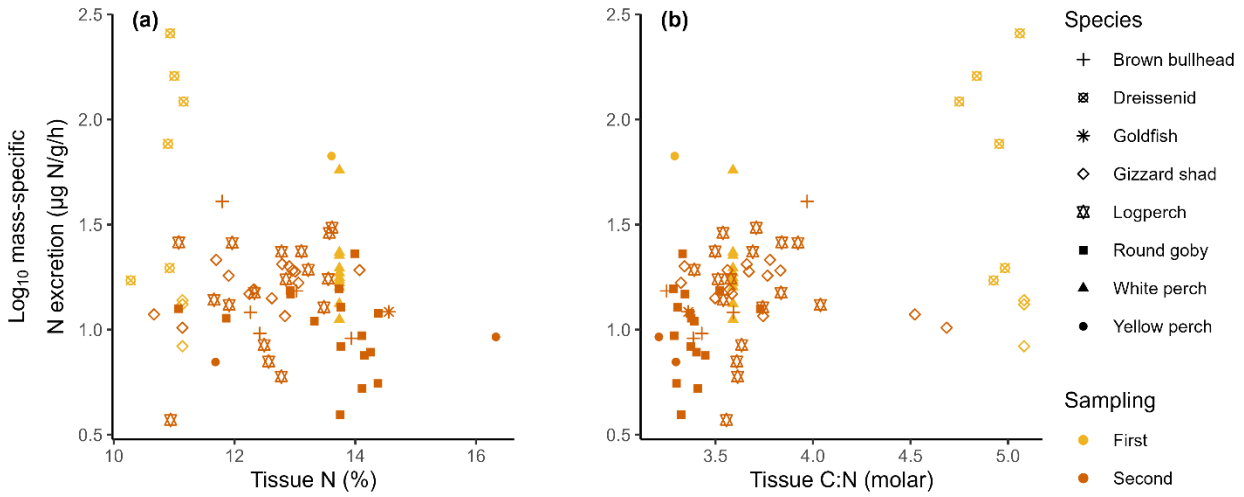

Figure S3. There is no significant relationship between animal tissue %N and C:N and mass-specific N excretion rates. Mass-specific N, excretion rates relative to (a) tissue N, and (b) tissue C:N for dreissenids and seven fish species. Colours indicate sampling period and location (summer in Lake Erie for the first and fall in the Detroit River for the second) and symbols represent each dreissenid unit set and individual fish. When $p > 0.05$, a dashed horizontal line was generated based on fish mean mass-specific nutrient excretion rates.

CHAPTER 3

Fine ecological scales highlight the nonlinear relationships of animal nutrient excretion with dissolved organic matter

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Abstract

Ecosystems can exhibit nonlinear dynamics resulting from interactions and feedbacks across various ecological scales. For example, large-scale nutrient cycling incorporates small-scale biogeochemical processes that may vary locally. However, it remains uncertain whether

biogeochemical processes like animal-mediated nutrient recycling exhibit nonlinearities across space and levels of biological organization. Animal-mediated nutrient recycling may vary with animal taxonomic rank, trophic position and abiotic factors such as light and nutrient supply. In aquatic ecosystems, dissolved organic matter modulate light and nutrient supply which may indirectly affect animal-mediated nutrient recycling. In this study, we examined nitrogen and phosphorus excretion of fish and mayflies in 11 streams that varied in dissolved organic matter (DOM) composition and concentration measured in units of carbon (DOC). We analyzed animal nutrient excretion at three levels: individual, population, and community and two spatial scales (reach and watershed), using animal groups defined by taxonomic rank or trophic position. We found linear and nonlinear relationships between animal nutrient excretion and DOC and DOM composition that followed both unimodal and bimodal patterns and varied with levels of biological organization, spatial scale, and animal groups. We also identified two critical DOC thresholds (4.5-5.5 and 7-8 mg C/L) beyond which animal nutrient excretion and biomass shifted positively or negatively and that were within the range of previously identified thresholds with DOC. Animal-mediated nutrient recycling is responsive to changes in DOC and DOM at fine ecological scales, while the magnitude and direction of this change is taxon-specific. Overall, our study provides a new set of evidence that nonlinearity is inherent to dynamic ecological systems.

Key words: nonlinear dynamics; ecological threshold; scale; animal-mediated nutrient cycling; dissolved organic carbon; biogeochemical cycling

Introduction

Ecological systems are increasingly viewed as complex, characterized by nonlinear dynamics, threshold effects, critical transitions, and alternative stable states that can be observed on a range of temporal, spatial, and organizational scales (Sugihara and May 1990, Hagstrom and Levin

2017). Notably, the frequency of nonlinear relationships, and potentially ensuing regime shifts, are expected to increase with human-induced environmental change (Folke et al. 2004). Recognizing the prevalence of nonlinearity (e.g., D'Amario et al. 2019), there is a need to examine spatiotemporal patterns in order to capture meaningful nuances to improve our understanding of ecological systems. While nonlinearity is often studied on a time scale, many studies also explore this pattern on a spatial scale; an approach that can be particularly informative when applied across multiple ecologically-relevant scales (Hagstrom and Levin 2017). Indeed, large-scale processes such as trait biogeography and nutrient cycling emerge from small-scale interactions and the coevolution of organisms with their environment, which are in turn influenced by large-scale processes (Levin et al. 1999). One area where the multi-scale nonlinearity framework could be extended is on the role of animals in nutrient cycles, which, to our knowledge, remains untested.

Animals are an integral part of nutrient cycles. Through excretion and egestion, consumers enhance the recycling and translocation of nutrients, particularly in their inorganic forms (Bardgett and Wardle 2003, Halvorson and Atkinson 2019), and contribute a large proportion of the nutrients and organic carbon that supports primary production (McIntyre et al. 2008, Roman and McCarthy 2010, Masese et al. 2020). In aquatic ecosystems, nutrient recycling by animal consumers is continuous, thus typically stable and more bioavailable than nutrients derived externally from the watershed (Williamson et al. 2018). However, differences in excretion rates and stoichiometric ratios of nutrients recycled by animal consumers may cause high variation in the supply of nutrients within aquatic ecosystems at small spatial scales (Schindler and Eby 1997, Spooner et al. 2013, Hopper et al. 2021). Consumer nutrient excretion rates may differ with trophic position (Hopper et al. 2021), taxonomic rank (Rock et al. 2016,

Benelli et al. 2019), functional traits (Atkinson et al. 2017), and intraspecific trait variation (Moody et al. 2018,). Moreover, proximate factors such as surrounding land cover, light availability, and water chemistry impact habitat quality, thereby shaping food web structure and function, including animal-mediated nutrient recycling (Atkinson et al. 2017). The well-documented effects of human activities on these proximal factors may thus have far reaching consequences on the role of aquatic consumers in nutrient recycling (James et al. 2007, Wilson and Xenopoulos 2011, Spooner et al. 2013, Allgeier et al. 2017).

One proximal factor in aquatic ecosystems that presents a largely unknown role in animal-mediated nutrient recycling is dissolved organic matter (DOM). Typically measured in units of carbon (DOC), DOC in aquatic systems is one of the main regulators of ecosystem structure and metabolism while also playing an important role in global carbon cycles. DOC concentration and DOM composition vary widely among aquatic ecosystems (Williams et al. 2010, Fasching et al. 2019) and are expected to change as a consequence of global change (i.e., brownification; Solomon et al. 2015, Xenopoulos et al. 2021). Ecological relationships with DOC are also known to display nonlinear behaviour (e.g., D'Amario et al. 2019). Higher amounts of more labile, microbial-like, easily degradable DOM can stimulate microbial activity through enhanced resource supply, thereby increasing nutrient regeneration and the amount of carbon available to the food web through the microbial loop (Limberger et al. 2019) and promoting primary and secondary production (Johnson et al. 2012, Robbins et al. 2020). Terrestrial nutrients are also directly associated with DOM, increasing their availability to primary producers (Solomon et al. 2015). However, DOM can have complex and interactive effects within aquatic ecosystems. For instance, despite increased resource subsidies, elevated DOC can reduce light availability, thus limiting primary and secondary production at higher

concentrations (Karlsson et al. 2015, Benoît et al. 2016, Van Dorst et al. 2019). Other studies have reported the absence of a DOC effect on primary and/or secondary production (Koizumi et al. 2018) or the presence of positive or unimodal effects (Kominoski et al. 2007, Finstad et al. 2014, Kelly et al. 2018b) both within and across geographical regions (Holgerson et al. 2022). These opposing results are partly due to the tradeoff between light and nutrient availability along DOM gradients (Isles et al. 2021a), making it difficult to disentangle the effects of DOM on aquatic food webs (Vasconcelos et al. 2019).

The interplay between light and nutrient availability as mediated by DOM can alter the quantity and quality of basal resources and subsequently affect the rate and elemental ratios of nutrients excreted by animal consumers (Downs et al. 2016). For example, a high supply of C from increased light availability or microbial-like DOM relative to a low supply of nutrients would generate a high quantity of low-quality (high C:P and C:N ratios) primary production (Sterner et al. 1997, Frost et al. 2007). In contrast, decreased light at higher concentrations of DOC or humic-like DOM would limit primary productivity and generate a low quantity of high-quality (low C:P and C:N) primary production (Sterner et al. 1997, Frost et al. 2007). Consequently, high C:nutrient basal resources would foster increased growth and biomass production of consumers with elevated C:nutrient body composition and low nutrient excretion rates, whereas low C:nutrient basal resources would limit growth and biomass production of consumers with low C:nutrient body composition and high nutrient excretion rates (Frost and Elser 2002, Downs et al. 2016). Therefore, evaluating nonlinear patterns in animal-mediated nutrient recycling within the context of DOC- and DOM-mediated changes in light and nutrient availability could help unravel the multi-dimensional influence of DOM on the biogeochemistry

of aquatic ecosystems, as well as provide a useful framework to study the implications of environmental change on animal-mediated nutrient recycling.

Here, our goal was to use 11 streams that varied in concentration (DOC) and composition of DOM to determine their associative effects on N and P excretion rates of both aquatic vertebrates (fish) and invertebrates (mayflies) spanning four trophic positions. We used a gradient design to detect nonlinear relationships and critical thresholds (Kreyling et al. 2018) and evaluated animal-mediated nutrient recycling across three levels of biological organization (i.e., individual, population, and community; Fig. 1). We predicted that animal-mediated nutrient recycling would vary nonlinearly among streams due to expected nonlinear relationships between primary production, animal growth rates and ambient DOC and DOM composition (Fig. 1a), much like the ones observed in lakes (e.g., Kelly et al. 2018b). While some studies based in low to mid-sized streams reported a positive effect of DOC on stream periphyton biomass and chlorophyll-a (Frost et al. 2007), other studies noted that positive effects of DOC on bacterial biomass were largely driven by the labile fraction of DOC and that light limitation still constrained primary production (Kominoski et al. 2007, Robbins et al. 2017). Considering that both stream DOC and riparian vegetation can attenuate a significant – and even higher – proportion of ultraviolet B radiation reaching the benthos compared to lakes (Frost et al. 2005), we expected the response of primary production to DOC-induced light limitation in low to mid-sized streams to be similar to that of lakes.

Specifically, we predicted (1) an inverse unimodal pattern in mass-normalized N and P excretion rates, such that these rates would decrease from low DOC and labile, microbial-like DOM environments (high light, low nutrients) to intermediate DOC and more diverse DOM environments (high light, high nutrients) due to the associated increase in animal growth and

resource quality, then increase from these intermediate conditions to high DOC and more recalcitrant, humic-like DOM environments (low light, high nutrients) due to the associated decrease in animal growth and high resource quality (Fig. 1b). Similarly, we expected (2) that population and community nutrient excretion and biomass would follow a unimodal nonlinear pattern in association with DOC and DOM composition, but this pattern would be opposite to mass-normalized nutrient excretion rates as biomass would drive population-level nutrient excretion rates (Fig. 1c). We also predicted that (3) nutrient excretion maximum and magnitude would vary based on consumer taxonomic rank and trophic position, with fish nutrient excretion rate peaking at higher DOC and mixed DOM composition (i.e., mixture of microbial-like and humic-like components) levels but with a lower individual-level nutrient contribution compared to mayflies due to differences in metabolic rates (Fig. 1d). Lastly, we predicted that (4) regardless of the nonlinear pattern, animal nutrient excretionand biomass in high DOC and more recalcitrant, humic-like DOM environments (low light, high nutrients) would be lower than in low DOC and labile, microbial-like DOM environments (high light, low nutrients) due to the differential effect of light and nutrient availability on animal mass-normalized excretion rates and biomass (Fig. 1).

Methods

Study design

To investigate spatial patterns in animal nutrient excretion, we sampled 11 streams in southern Ontario, Canada (Table S1, Fig. 2). Streams were selected based on prior knowledge of this region (Wilson and Xenopoulos 2008, 2009) and to span a DOC gradient that ranged from 3 to 15 mg C/L and a DOM gradient from labile, microbial-like to more recalcitrant, humic-like (Fig. S1). At each stream, we sampled the most abundant fish at three trophic positions and the

most ubiquitous benthic macroinvertebrate. Taxa sampled included an order of grazer benthic macroinvertebrate, the mayfly (*Ephemeroptera*); three invertivores species, johnny darter (*Etheostoma nigrum*), eastern blacknose dace (*Rhinichthys atratulus*), and juvenile largemouth bass (*Micropterus salmoides*); a facultative piscivore species, rock bass (*Ambloplites rupestris*); and three omnivore species, creek chub (*Semotilus atromaculatus*), common shiner (*Luxilus cornutus*), and white sucker (*Catostomus commersonii*). Trophic positions were determined using the field guide developed by Holm et al. (2009). A total of nine taxa spanning four trophic positions were tested, including two to four trophic positions per site. All sampling was done between July 29th and August 15th, 2019 following the Canadian Council for Animal Care's guidelines for best practices (Trent U. AUP #25754).

Sampling and nutrient excretion experiment

Sampling methods used to collect individuals for our nutrient excretion experiments were also used to estimate the abundance of each taxon at each sampled stream. Fish were collected using three-pass electrofishing over 50 m along the stream, with upstream and downstream blocking seine nets when necessary and voltage adjusted for stream conditions to the minimum power required to direct fish towards the netters. Each captured fish was identified to species and was released if it did not belong to the species of interest. The total number of individuals per species was counted and the population biomass estimated using the equations described by Jones and Stockwell (1995) for one-pass electrofishing. Mayflies were only present at eight of the eleven streams (Table 1) and were collected by using a combination of kick-sweep on the stream bed and flushing overturned rocks. Sampling effort varied until enough mayflies were collected for the excretion experiment.). The total number of individuals collected in the sampler (0.0625 m^2) was then scaled to the sampling area (50 m x stream width).

Nutrient excretion rates were determined for seven to fifteen individuals of each fish species and ten sets of eight to seventeen mayflies in each stream to account for body size and species-specific variability. The sample size and number of individuals per sample for fish and mayflies were determined from comparable excretion experiments carried out in streams (James et al. 2007, Hopper et al. 2018). Following collection, each individual sample (eight to seventeen mayflies or one fish) was placed in a whirl-pak bag filled with 0.2 to 2L of base water collected from a stream with relatively low ambient ammonia (NH_4^+-N, µg N/L) and soluble reactive phosphorus (SRP, µg P/L) that was prefiltered through 1 µm borosilicate glass microfiber filter. Bags containing sampled individuals and two additional bags without individuals (controls) were incubated in the stream to promote excretion rates reflective of *in situ* conditions. Bags were incubated for 17 to 53 min for fish and for 1 hr to 1hr and 53 min for mayflies depending on the ambient temperature and the size of the organisms collected in the bag. Incubation time varied to ensure the detection of a nutrient excretion signal and to allow for animal recovery from handling stress while minimizing fasting effects (Whiles et al. 2009) and nutrient uptake by bacteria. After incubation, individuals were removed from the bags and weighed (fish only). Wet mass was then converted to dry mass using a conversion factor of 0.25 (Vanni and McIntyre 2016). All mayflies were placed on ice to be dried and weighed in the laboratory. Shortly after collection, water samples from both the incubation experiments and ambient stream water were filtered through pre-ashed 0.7 µm Whatman GF/F glass microfiber filters, then through 0.22 µm polycarbonate membrane filters and stored at 4 °C prior to analysis. Stream discharge and mean water velocity were measured using a flowmeter (SonTek FlowTracker 2).

Nutrients, DOC, and DOM composition

Nutrient excretion samples were quantified as NH_4^+ and SRP using a Varian Cary 50 Bio UV-Visible spectrophotometer and the phenate (Solórzano 1969) and molybdate blue methods (Murphy and Riley 1962), respectively. For each sampling location, we collected water samples to determine SRP, NH_4, total dissolved nitrogen (TDN, µg N/L), total dissolved phosphorus (TDP, µg P/L), DOC (mg C/L), and DOM composition of the stream water. TDN was measured using spectrophotometry via the second derivative method (Crumpton et al. 1992) following persulfate digestion, and TDP was quantified using the molybdate blue method (Murphy and Riley 1962) following persulfate digestion. DOC was assayed on a Shimadzu TOC-VWP Total Organic Carbon Analyzer using a persulfate digestion. DOM composition was examined using absorbance and fluorescence analyses based on methods described in Williams et al. (2010, 2016) and detailed in Appendix S1.

Statistical analyses

Hierarchical generalized additive models (HGAM, Pedersen et al. 2019), based on GAM (mgcv package, version 1.8-31; Wood 2011), were used to examine the effects of DOC and DOM composition on reach-scale animal nutrient excretion and biomass at the individual, population, and community levels, and watershed-scale animal nutrient excretion at the community level. HGAM are advantageous as they can account for potential nonlinear associations between independent and dependent variables and can isolate group-specific patterns (Pedersen et al. 2019). Our HGAM were based on group-specific smoothers that can differ in their level of wiggliness and followed the structure: y ~ s(x, by = factor, m = 2, bs = "tp") + s(factor, bs = "re") (Model I; Pedersen et al. 2019) using REML as the smoothing parameter. DOM absorbance and fluorescence measurements were visualized in a Principal

Component Analysis (PCA, *prcomp* function in R) to understand the variation in DOM composition among streams. PC1 explained 50.5% of the variance in DOM composition among streams and was positively associated with C_{humic} and HIX and negatively associated with ß:α and S_R (Fig. S1). The PC1 score was used as a measurement of DOM composition and served together with DOC as independent variables for all subsequent HGAM analyses. We also tested the correlation between DOC and DOM composition derived from PC1, as well as between DOC or DOM composition and TDN, TDN, and travel time using a Pearson's correlation test.

We first estimated nutrient excretion rates at the individual and population levels. Individual-level nutrient excretion rates were first calculated per capita (µg N or P/individual/h) by subtracting the NH_4 or SRP concentration in the prefiltered base water (i.e., initial concentration; NH_4 = 0 µg N/L, SRP = 3.4 µg P/L) from the NH_4 or SRP concentration in the water samples in the experimental bags post-incubation (i.e., final concentration) then correcting for sample water volume, number of individuals, and time. Nutrient excretion rates were then normalized for mass using species-specific size scaling coefficients generated from the $\log_{10}$-$\log_{10}$ relationship between per capita excretion rates (positive values only) for a given species and nutrient and species dry mass to obtain mass-normalized excretion rates (µg N or P/g/h; Fig. S2, S3). Population abundance was calculated using methods developed by Jones and Stockwell (1995) for fish populations or scaled to the sampling area for mayfly populations. Next, population abundance estimates were multiplied by the average fish population mass to generate areal biomass (g/m^2). Mass-normalized nutrient excretion rates were then combined with estimates of areal biomass at each sampled site to estimate population-level (by taxonomic rank or trophic position) areal excretion rates (µg N or $P/m^2/h$).

Subsequently, we used HGAM to test individual- and population-level associations between the dependent variables of mass-normalized nutrient (i.e., N, P, and N:P) excretion rates, dry mass, areal population nutrient excretion rates, biomass, and the independent variables of DOC and DOM composition for both fish and mayfly taxa. Animal groups based on broad taxonomic ranks (i.e., fish or mayfly) were included as a random factor. Since DOC and DOM composition were not correlated (Table S1), both were used as independent variables in any given model. To further investigate population-level nutrient excretion, we also ran HGAM using trophic position as a random factor. Mass-normalized and population nutrient excretion rates and ratios did not meet normality and homoscedasticity assumptions based on the histogram and QQ-plot of residuals (gratia package, version 0.6.0; Simpson 2021). Hence, we used a log-link Tweedie distribution for all response variables except for the individual- and population-level nutrient excretion ratios to allow for the variance function powers to be estimated between 1 and 2 during fitting (tw() function in mgcv; Wood 2011). Mass-normalized and population molar ratio N:P excretion rates were $\log_{10}$ transformed following the convention recommended by Isles et al. (2021a). Model structure was as follows: response variable ~ *f*(DOC, by = taxonomic rank, bs = "tp") + *f*(DOM, by = taxonomic rank, bs = "tp") + *f*(taxonomic rank, bs = "re"). Model fits were then compared with intercept-only and streams-only null models based on the AIC values. The streams-only models allowed us to test whether the patterns observed in animal nutrient excretion could be equally or better explained by another variable that varies among streams, assuming no correlation between DOC and DOM composition.

For the community-level nutrient excretion, we compared the relative contribution of nutrients by the aggregate animal community to the measured ambient SRP and NH_4 concentrations. To do this, we estimated aggregate areal population nutrient excretion rates by

summing areal population nutrient excretion rates across sampled taxa (n = 9). To bolster this community-level estimate, we used the areal biomass of additional fish species (n = 21) detected during sampling and their respective trophic positions to calculate a nutrient excretion rate based on the average trophic position specific rates we measured. Community-level nutrient excretion was calculated by converting aggregate areal population nutrient excretion rates (µg/m^2/h) to volumetric nutrient excretion (E_V; µg/L) using the equation developed by McIntyre et al. (2008): $E_V = (E_A \times A \times T)/V$, with areal aggregate population excretion rates (E_A, µg N or P/m^2/h), stream surface area (A, m^2), travel time or mean water velocity (T, h), and stream reach volume (V, L). This relationship describes the contribution of nutrients by animals via their excretion to the stream assuming complete mixing and no uptake. Travel time was calculated by dividing the stream reach length (50 m) by the reach mean velocity (m/h). Stream reach volume was estimated by multiplying the stream reach length by the cross-sectional area (m^2). As ambient nutrient concentrations and volumetric nutrient excretion did not meet normality and homoscedasticity assumptions, HGAM were run using a log-link Tweedie distribution to evaluate the effect of DOC and DOM composition on ambient and community-level excretion (nutrient source); model structure: response variable ~ *f*(DOC, by = Nutrient source, bs = "tp") + *f*(DOM, by = Nutrient source, bs = "tp") + *f*(Nutrient source, bs = "re").

To provide additional context on measured nutrient excretion rates at a larger scale, we used reach-scale aggregate areal population nutrient rates and scaled them to the estimated surface area of their corresponding stream network. While we recognize that aggregate areal population nutrient rates will vary by reach based on species assemblage composition and biomass, we assumed constant aggregate population nutrient rates across stream reaches. Watershed-scale nutrient excretion fluxes were then compared with the baseflow NH_4^+ and SRP

flux exported from the stream to generate a nutrient recycling ratio and correct for differences in stream network area across sites. Baseflow nutrient flux was estimated by multiplying ambient NH_4^+ and SRP by discharge. The stream network area calculation is detailed in Appendix S1. Given that our nutrient flux estimates at the watershed scale did not meet normality and homoscedasticity assumptions, we ran HGAM using $\log_{10}$ transformed ratios and a gaussian family distribution to test the effect of DOC and DOM composition on the estimated animal-mediated nutrient flux (model structure: $\log_{10}$(response variable) ~ *f*(DOC, bs = "tp") + *f*(DOM, bs = "tp"). All statistical analyses were done using R Statistical Software (v4.1.3; R Core Team 2023) and RStudio (v2023.6.1.524 RStudio Team 2023) on a Windows PC (v 22H2).

Results

Sampling and nutrient excretion experiment

DOC and DOM composition were positively correlated with TDP across streams, while DOC only was positively correlated with travel time but did not have a significant correlation with discharge, although it showed a negative relationship (Table S2. Animal N and P excretion rates, dry mass (g), and biomass (g/m^2) were highly variable among streams (Table S3). Population-level and community-level nutrient excretion exhibited the highest coefficients of variation (CV), varying from 1.5 to 2.2 for fish, 0.5 to 1.9 for mayfly, and 1.8 to 2.2 for fish and mayfly combined (Table S3). Mass-normalized N:P excretion rates were also variable, with a CV of 1.5 for fish and 4.8 for mayfly (Table S3). For all functional traits at the individual and population levels, there was lower variability within than among streams as most CV within streams were lower than among streams (Table S3, S4, Fig. S3). Overall, fish excreted nutrients at consistently higher rates than mayflies across all levels of biological organization (Table S3, Fig. 3a-f, Fig. 4a-f, Fig. 5a-f).

Individual-level nutrient excretion

Most fish and mayfly mass-normalized nutrient excretion rates and individual dry mass had nonlinear relationships with DOC and DOM, although the pattern of the association differed with animal taxonomic rank (Fig. 3). All tested models fit better than null models as determined by AIC scores (Table S4), where the highest deviance explained was in mass-normalized N excretion with 78.4% (Table S5). Mayfly mass-normalized N excretion increased with DOC, particularly past 7 mg C/L (Fig 2a) but did not significantly change with DOM composition, although these appeared higher with more humic-like DOM (Fig. 3b). Fish mass-normalized N excretion showed opposite patterns, with no significant relationship with DOC despite an observed subtle decrease with increasing DOC (Fig. 3a), and a unimodal increase from microbial-like to mixed DOM, followed by a decrease with more humic-like DOM (Fig. 3b).

Fish and mayfly mass-normalized P excretion displayed similar patterns in relation to DOC. Both fish and mayfly mass-normalized P excretion had a bimodal association with DOC, with two maxima at about 6.5 and 13 mg C/L, and two minima at 4.5 and 9 mg C/L (Fig. 3c). Conversely, fish mass-normalized P excretion decreased linearly with DOM, while mayfly mass-normalized P excretion did not have a significant relationship with DOM even if it appeared the highest in mixed DOM (Fig 3d). The mayfly unimodal and bimodal trends for mass-normalized N and P excretion resulted in opposite bimodal patterns in the association between the molar ratio of mayfly mass-normalized N:P excretion and DOC and DOM. For DOC, two mayfly mass-normalized N:P maxima were detected at 5 and 9 mg C/L, and two minima at 7 and 12 mg C/L (Fig. 3e). There was no significant relationship between mayfly mass-normalized P excretion and DOM although it appeared the lowest in mixed DOM (Fig. 3f). The relationship between fish N:P excretion and DOC was unimodal, decreasing down to 7 mg C/L, followed by

a subtle increase, whereas there was no significant change in fish N:P excretion across the DOM gradient (Table S5, Fig. 3f). Lastly, both fish and mayfly dry mass had marginal variations with DOC and DOM (Table S5, Fig. 3g-h), with a maximum at 12 mg C/L for fish, 8 mg C/L for mayflies, and in mixed DOM for both fish and mayflies.

Population-level nutrient excretion

Population-level nutrient excretion was tested using animal groups based on taxonomic rank (Table S5, Fig. 4) or trophic position (Table S6, Fig. 5). For models based on taxonomic rank, only those including areal population P excretion and biomass fit better than null models based on AIC scores (Table S4) and only fish were related to changes in DOC and DOM (Table S5, Fig. 4). However, all tested models based on trophic position fit better except for the one including population N:P excretion (Table S4). Variations in DOC and DOM explained up to 66.9 and 88.9% of deviance in population N excretion for models based on taxonomic rank and trophic position, respectively (Table S5, S6). For models based on taxonomic rank, only fish had a bimodal relationship between population P excretion, biomass, and DOC with two maxima at 5.5 and 11 mg C/L, and one minimum at 8 mg C/L; although there was uncertainty around the second maximum (Fig. 4a, c, g).

For models based on trophic positions, population P excretion had significant relationships with DOC for all four trophic positions and with DOM for all trophic positions except for omnivores (Table S6, Fig. 5c-d). Population P excretion relationships with DOC and DOM composition showed opposite patterns, with a decrease at high DOC and an increase in humic-like DOM for all trophic positions except for grazers (Fig. 5c-d). Facultative piscivores had a nonlinear decrease in population P excretion with DOC that accelerated past 8 mg C/L and in population N excretion and biomass with DOM that accelerated mixed DOM (Fig. 5b, c, h). In

contrast, facultative piscivore population P excretion increased linearly with DOM (Fig. 5d). Invertivores population P excretion decreased linearly with DOC, while population N and P excretion had a unimodal relationship between and DOM consisting of a decrease from microbial-like to mixed DOM and an increase from mixed DOM to humic-like DOM (Fig. 5b, c, d). Lastly, omnivore population N and P excretion and biomass had a bimodal relationship with DOC, with two maxima detected at 5.5 and 11.5 mg C/L, and one minimum at 8 mg C/L (Fig. 5a, c, g).

Community-level nutrient excretion

Community-level nutrient excretion was evaluated at the reach scale as a volumetric excretion rate expressed as a nutrient concentration. Both HGAMs including N and P concentrations of ambient stream water and the aggregate animal community nutrient excretion (i.e., volumetric nutrient excretion) fit better than null models based on AIC scores (Table S4). Additionally, variations in DOC and DOM explained between 87.7 and 75.9% of deviance in N and P concentration, respectively (Table S7). Reach-scale community-level associations with DOC and DOM indicated that nutrient concentrations in streams were often greater than animal contributions, but there was no relationship between stream nutrient concentrations and DOC and DOM (Table S7 and Fig. 6). Conversely, animal volumetric N and P excretion increased nonlinearly with variations in DOC with an acceleration of this trend past 8 mg C/L (Fig. 6a, c). There was no significant relationship between animal volumetric N and P excretion and DOM (Table S7 and Fig. 6b, d). As a result, the ratio of nutrients contributed by the aggregated animal community to ambient nutrient concentrations decreased with DOC, although there was greater uncertainty associated with animal volumetric nutrient excretion at higher DOC (Fig. 6a, c).

Community-level nutrient excretion at the watershed scale was assessed as the ratio of stream water nutrient export to the aggregate animal community flux scaled up to the entire stream network. Both HGAMs of nutrient recycling flux ratios fit better than null models, but the difference in AIC score between the model including P flux ratio and the null model was minimal (Table S4). Variations in DOC and DOM also explained up to 64.3% of variation in nutrient flux ratio (Table S8). In contrast to our reach-scale results, the watershed-scale aggregate animal community nutrient flux was much higher than the ambient nutrient flux by several orders of magnitude (Table S3, Fig. S2). A decrease in ambient N flux with DOC likely resulted in our observed negative N flux ratio in relation to DOC (Fig. S2a).

Discussion

The relationship between animal-mediated nutrient recycling and DOC, as well as DOM composition is complex and context-dependent due to the interplay between light and nutrient availability. We demonstrate in this study that spatial variation in DOM can explain differences in animal-mediated nutrient recycling among streams, most often through unimodal and bimodal patterns. We distinguished two recurring DOC thresholds (4.5-5.5 and 7-8 mg C/L) beyond which nutrient excretion and biomass shifted positively or negatively. We also found that the relationships between nutrient excretion and DOM depended on the level of biological organization. The control of DOM on nutrient excretion thus varies from the individual- through the population- to the community-level. The relationship between nutrient excretion and DOC and DOM also interacted with spatial scale and animal groups based on taxonomic rank or trophic position. Our analysis shows that at a fine ecological scale, animal nutrient excretion rates vary with DOC and DOM composition for specific taxa, while at a larger scale, nutrient

excretion by the aggregated animal community may constitute a large and reliable source of bioavailable nutrients regardless of DOC and DOM composition.

We detected nonlinear patterns in most relationships between nutrient excretion rates and DOC and DOM across levels of biological organization and spatial scales which reinforce the existence of nonlinearity in ecological responses to DOC (e.g., Finstad et al. 2014, Seekell et al. 2015, D'Amario et al. 2019). Furthermore, we identified several maxima and minima in the nonlinear relationships between nutrient excretion and DOC that occurred around 4.5-5.5, and 7-8 mg C/L beyond which nutrient excretion changed drastically. These DOC thresholds are similar to those previously found for DOC and lake primary production (Seekell et al. 2015), stream dissolved CO_2 (D'Amario and Xenopoulos 2015), and the structure and function of diatom and mussel communities in streams and rivers (D'Amario et al. 2019). Our results lend further support for the prevalence of nonlinearity in the effects of DOC on ecosystem function and, more generally, ecological patterns at multiple organizational and spatial scales. A fruitful avenue for empirical research lies in determining whether given thresholds capture natural variability or represent meaningful tipping points for regime shifts, particularly post-environmental perturbations; a question that could be tackled by recently developed frameworks on ecological dynamic regimes (Sánchez-Pinillos et al. 2023).

Mass-normalized nutrient excretion rates were related to variations in DOC and DOM both linearly and nonlinearly. The nonlinear increase of mayfly mass-normalized N excretion with DOC that accelerated past 7 mg C/L was partially consistent with our initial predictions of an inverse unimodal relationship at the individual level. The predicted relationship is most likely due to the trade-off between light and nutrient availability across the DOC and DOM gradients, which can differentially fuel or suppress animal growth (Van Dorst et al. 2020, Robbins et al.

2020). Although the predicted unimodal relationship between mayfly dry mass and DOC and DOM was weak, we propose that the accelerated increase of mayfly mass-normalized N excretion with DOC past 8 mg C/L could be the result of a negative (James et al. 2007, Benoît et al. 2016, Van Dorst et al. 2020) or nonlinear relationship between mayfly growth and DOC not captured by our measurements of dry mass. Additionally, mass-normalized N:P excretion rates relative to DOC were opposite for fish and mayflies, whereas mass-normalized P excretion rates were similar. These relationships could be driven by physiological differences in N requirements between different species of fish and mayfly and their degree of homeostasis (Sterner et al. 1997, Atkinson et al. 2017). Fish species may differ in their N retention based on whether growth is more lipid or protein-based (Downs et al. 2023) leading to N excretion rates stagnating along the DOC gradient or decreasing in humic-like DOM environments relative to microbial-like DOM. The bimodal relationship observed between mass-normalized P excretion and ambient DOC for both fish and mayflies was opposite to our predictions and may stem from nonlinear shifts in consumption rates, likely driven by changes in resource C:P availability fueled by elevated P supply in cropland-dominated streams and potential C limitation (James et al. 2007, Wilson and Xenopoulos 2011). Thus, high resource C:P would lead to lower mass-normalized P excretion rates and low C:P resource would lead to higher mass-normalized P excretion rates.

Fish areal population P excretion rates and biomass were related to DOC in nonlinear bimodal patterns, but these associations exhibited a high degree of statistical uncertainty. When we analyzed population-level excretion rates and biomass for animal groups based on trophic position, we found clearer linear and nonlinear signals in relation to DOC and DOM, especially for population P excretion. Our results in relation to DOC and DOM are consistent with parts of our initial predictions for some trophic positions, such that population nutrient excretion rates

increased at lower DOC for omnivores and grazers and in microbial-like DOM for facultative piscivores, and decreased at higher DOC for facultative piscivores, invertivores, and omnivores and in humic-like DOM for grazers. Evidently, there were discrepancies and sometimes lack of relationships between population nutrient excretion rates and biomass and DOC and DOM among trophic positions. These inconsistencies may be driven by both the limited number of populations observed across the gradients and group or species-based differences in ecology and sensitivity to DOC-induced changes in ambient light and nutrients (Benoît et al. 2016). The latter explanation highlights that the relationships between ecological responses and DOC and DOM are taxon-specific and underscores the importance of considering functional groups when evaluating multi-species responses and effects on ecosystem processes (de Bello et al. 2021a).

We found that the relationship between community-level nutrient excretion and variations in DOC and DOM changed with spatial scale. At the reach scale, the aggregate animal community supplied increasing amounts of nutrients relative to ambient nutrient concentrations in streams with higher DOC. The increase in the animal community volumetric nutrient excretion was likely driven by a higher travel time of water through the sampled 50 m reach. At the watershed scale, the aggregate animal community provided high and steady N flux across DOC and DOM gradients, but the ratio of ambient water to the aggregate animal community N flux was negatively associated with spatial variations in DOC and DOM, likely due to a decline in ambient N flux. The decline in ambient N flux was likely driven by the negative association between ambient DOC and discharge which is expected during baseflow conditions in the summer (Wilson and Xenopoulos 2008). Overall, we suggest that animal-mediated nutrient recycling at the reach scale makes a greater contribution to the nutrient pool as stream DOC increases. This could in turn fuel both the ‘green’ (algae-based) and ‘brown’ (detritus-based) food

webs by enhancing benthic algal biomass and stimulating heterotrophic microbial activity (Atkinson et al. 2021).

A few factors may explain the weaker or absent relationships between community-level excretion and DOC and DOM. While we provide a coarse estimate, our aggregated animal community volumetric nutrient excretion and fluxes are likely underestimated given that we only sampled one order of invertebrates and may have missed important contributors in some of the sampled streams such as crayfish and mussels (Spooner et al. 2013, Hopper et al. 2018, 2020). Likewise, our results could be biased by our assumption that animal community assemblage composition and is consistent across the watershed, although Wilson and Xenopoulos (2011) reported variable fish biomass for similar streams in the same region. Streams with elevated ambient nutrients had more microbial-like DOM (Wilson and Xenopoulos 2009; Williams et al. 2010) and higher fish biomass but streams with excessive nutrients were fishless (Wilson and Xenopoulos 2011). Another source of error could be our use of population nutrient excretion averages per trophic position to determine the population nutrient excretion of the untested fish species. Nevertheless, the relatively constant animal community nutrient flux regardless of differences in DOC and DOM is in agreement with the conclusion in Williamson et al. (2018) that nutrient excretion by animals at the ecosystem scale is a stable source of nutrients compared to other sources.

Our study provides insight on how changes to environmental factors (here, DOC and DOM composition) can affect animal nutrient excretion nonlinearly at fine ecological scale (i.e., based on space and the level of biological organization). At the reach-scale, nutrient excretion rates and biomass displayed dynamic, and sometimes, opposite relationships with DOC and DOM depending on taxonomy or trophic position, whereas the contribution of the aggregated

animal community to the in-stream nutrient pool increased with higher DOC and more humic-like DOM. Yet at the watershed scale, animal communities appeared to be a remarkably high and consistent source of nutrients that surpassed ambient nutrient levels, regardless of DOC and DOM. Collectively, our results revealed different relationships between nutrient excretion and stream DOC and DOM and thus emphasize the need to consider DOC in conjunction with DOM composition for a more accurate assessment of the combined effects of organic carbon on ecosystem function. More generally, we demonstrate the importance of accounting for both organizational and spatial scales when studying variation in ecosystem processes, particularly for the detection of nonlinear ecological patterns.

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Figures

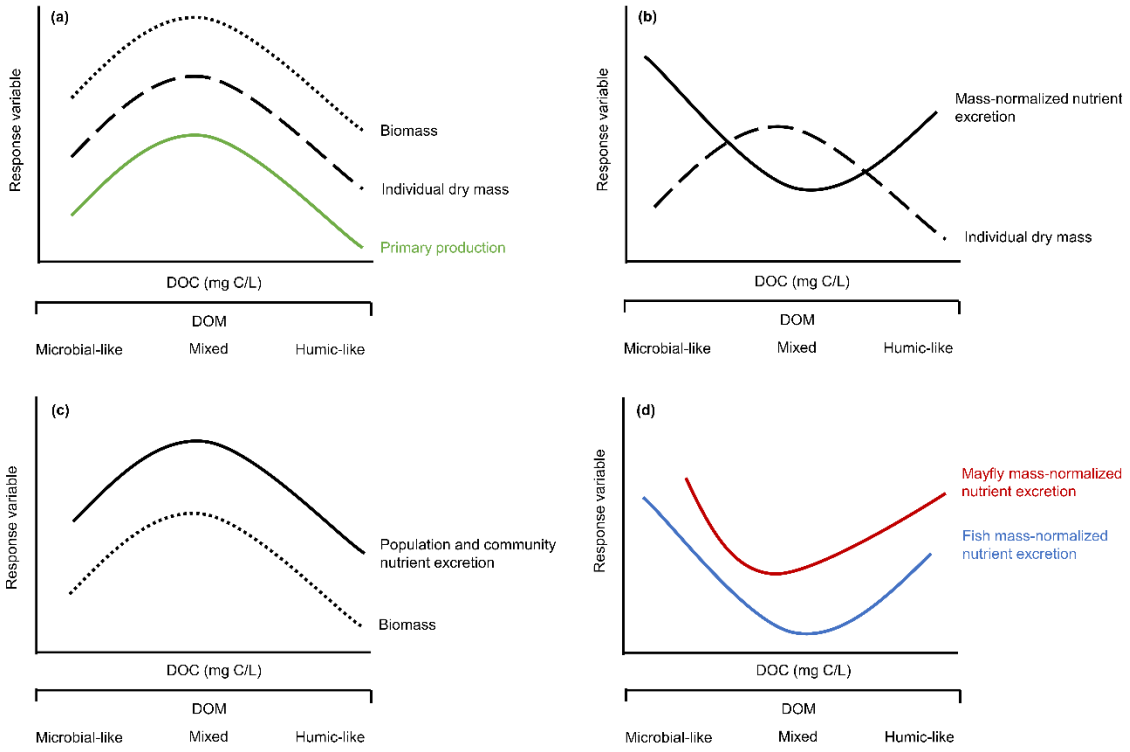

Figure 1. Conceptual diagram illustrating predicted unimodal relationships between animal nutrient excretion rates, individual dry mass, and biomass with variations in DOC concentration and DOM composition. Due to the light and nutrients availability tradeoff, (a) the quantity of primary production is expected to follow a unimodal relationship with DOC and DOM, which would affect animal individual dry mass and biomass. Consequently, we expect (b) mass-normalized nutrient excretion rates to follow an inverse unimodal relationship with DOC and DOM due to the unimodal changes in individual dry mass, and (c) population and community nutrient excretion rates to follow a unimodal relationship with DOC and DOM due to the unimodal changes in population biomass. We also expect (d) differences in mayfly and fish mass-normalized nutrient excretion rates maximum and magnitude along the DOC and DOM gradients.

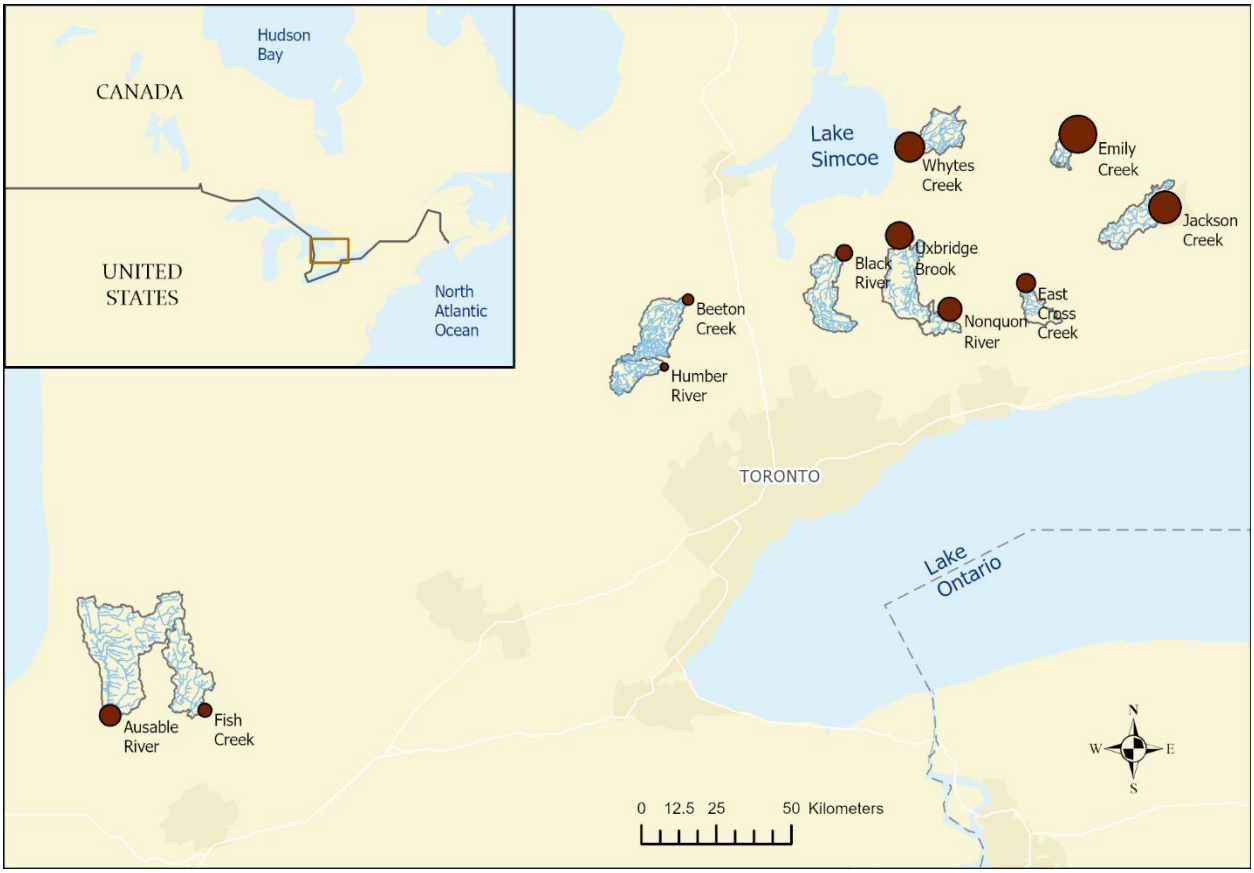

Figure 2. Map of study area with the focal watersheds of the 11 study sites in Ontario, Canada. Location data point size indicates relative DOC concentration (3-15 mg C/L).

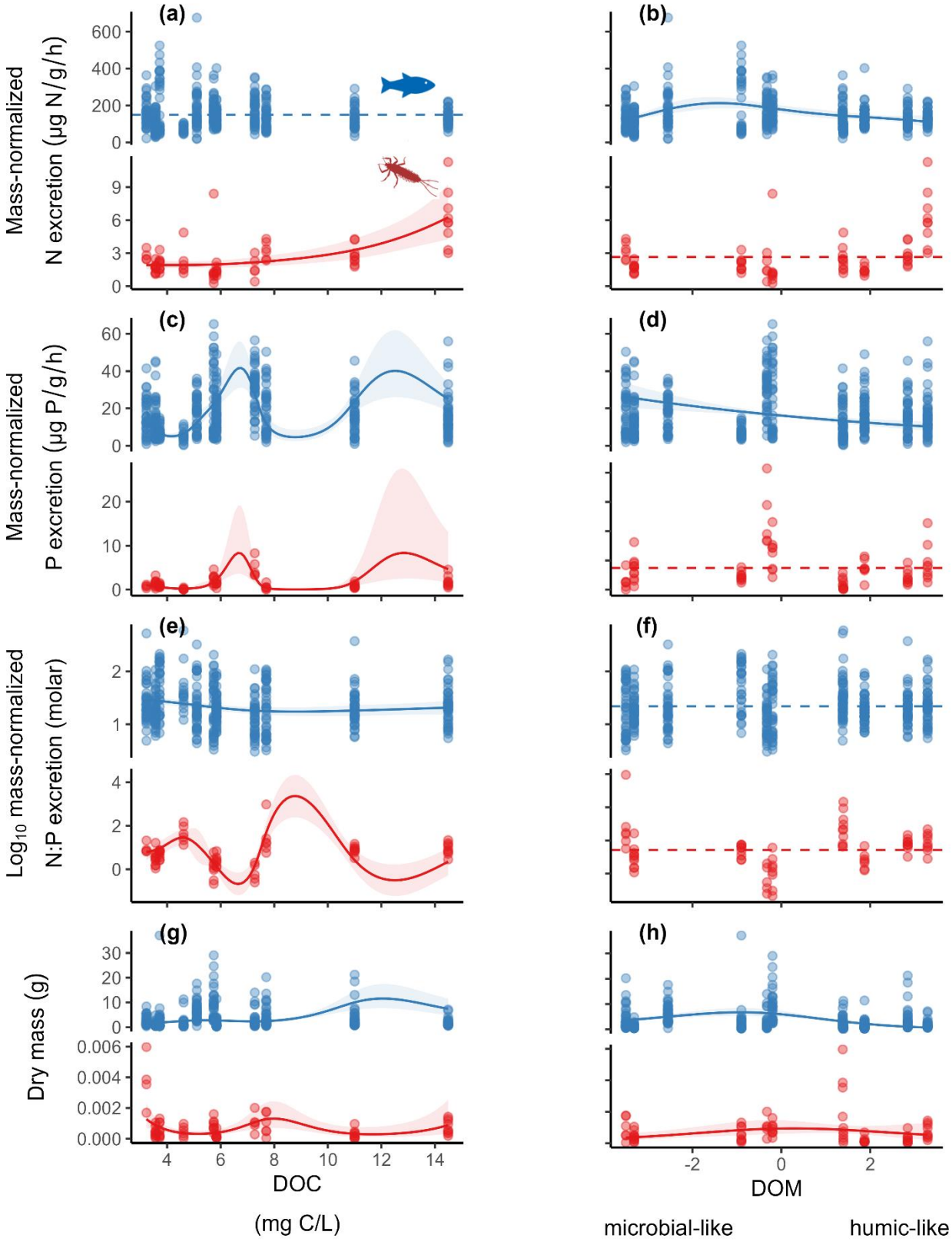
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
Mass-normalized N excretion (µg N/g/h)
Mass-normalized P excretion (µg P/g/h)
Log10 mass-normalized N:P excretion (molar)
Dry mass (g)
DOC
(mg C/L)
DOM
microbial-like
humic-like

Figure 3. Fish and mayfly show both unimodal and bimodal individual nutrient excretion rates and ratios and dry mass associations with DOC and DOM. Mass-normalized (a-b) N, (c-d) P, and (e-f) N:P excretion rates, and (g-h) individual dry mass relative to DOC or DOM and taxonomic rank for mayflies and seven fish species. Best-fit lines and 95% confidence intervals were generated using GAM based on DOC and DOM partial effects and were only included if $p < 0.05$. When $p > 0.05$, dashed horizontal lines were generated based on fish mean nutrient excretion rates or ratio. A summary of analytical results is given in Table S3.

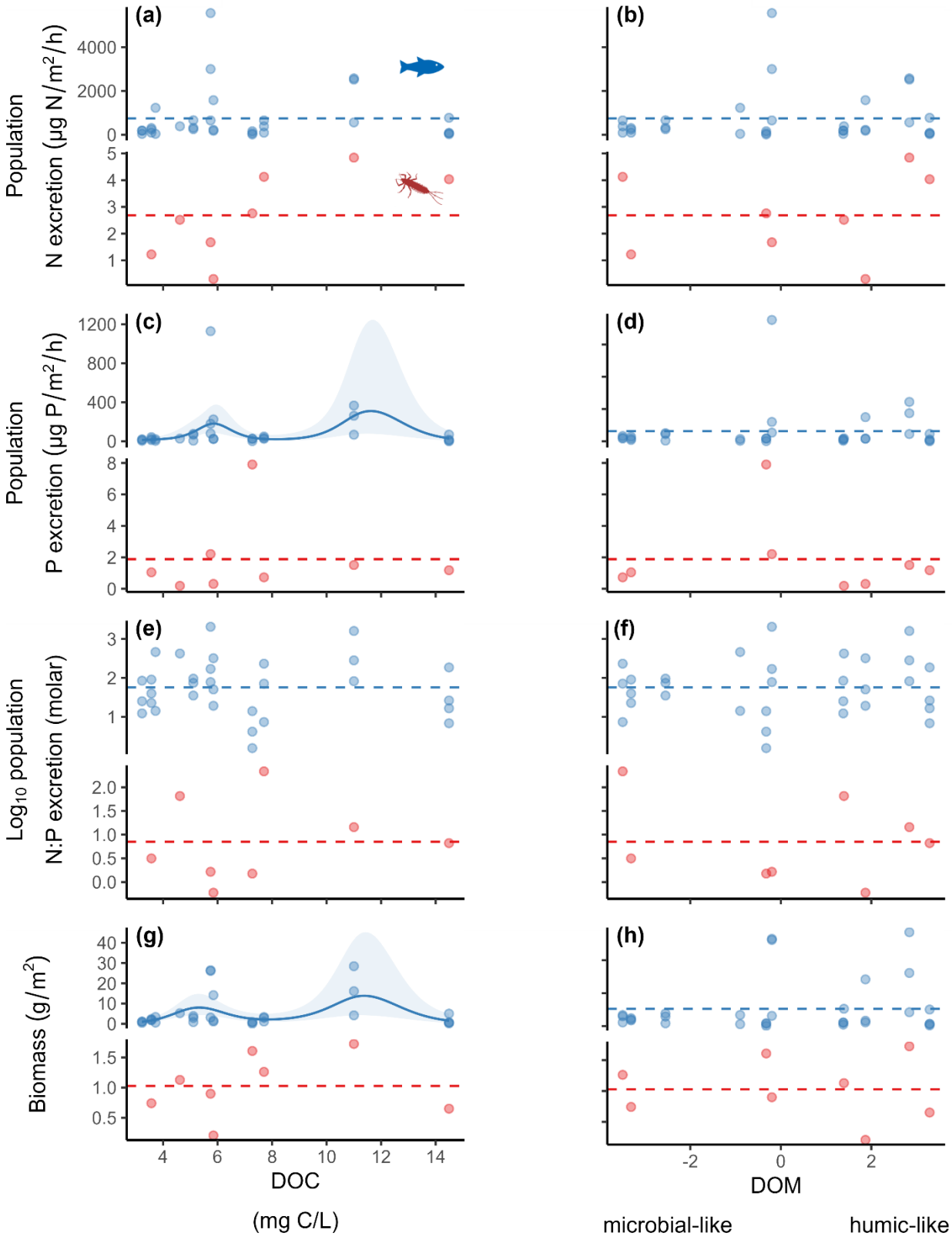
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
Population
N excretion (µg N/m²/h)
Population
P excretion (µg P/m²/h)
Log10 population
N:P excretion (molar)
Biomass (g/m²)
DOC
(mg C/L)
DOM
microbial-like
humic-like

Figure 4. Fish have bimodal population nutrient excretion rates and areal biomass associations with DOC. Population (a-b) N, (c-d) P, and (e-f) N:P excretion rates, and (g-h) areal biomass relative to DOC or DOM and taxonomic rank for mayflies and seven fish species. Best-fit lines and 95% confidence intervals were generated using GAM based on DOC and DOM partial effects and were only included if $p < 0.05$. When $p > 0.05$, dashed horizontal lines were generated based on fish mean nutrient excretion rates or ratio. A summary of analytical results is given in Table S3.

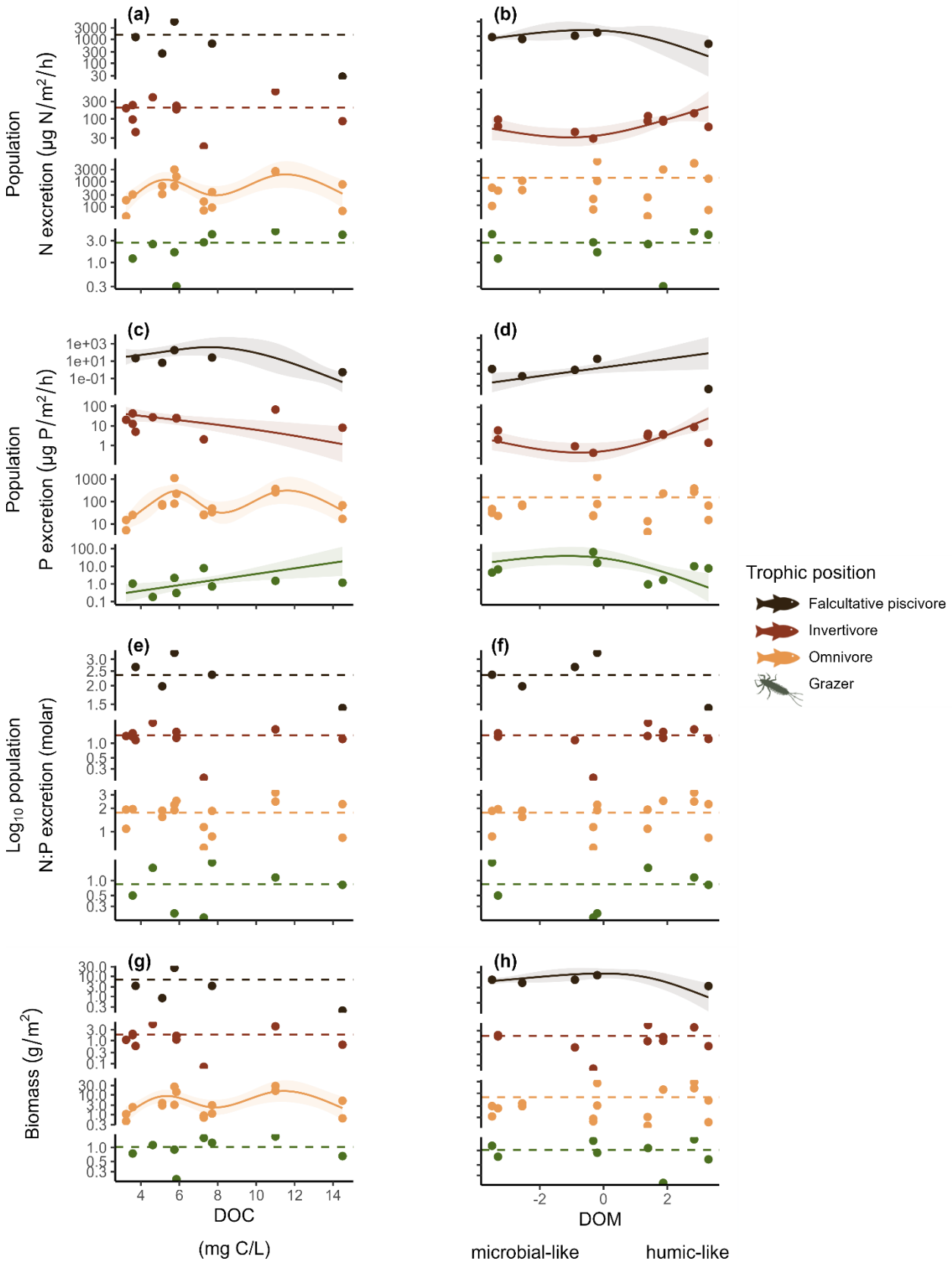
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
Population N excretion (µg N/m²/h)
Population P excretion (µg P/m²/h)
Log10 population N:P excretion (molar)
Biomass (g/m²)
DOC
(mg C/L)
DOM
microbial-like
humic-like
Trophic position
Falcultative piscivore
Invertivore
Omnivore
Grazer

Figure 5. Piscivore and omnivore areal population nutrient excretion rates and biomass displayed both linear and nonlinear relationships with DOC and DOM. Population (a-b) N, (c-d) P, and (e-f) N:P excretion rates, and (g-h) areal biomass relative to DOC or DOM and trophic positions for mayflies and seven fish species. Best-fit lines and 95% confidence intervals were generated using GAM based on DOC and DOM partial effects and were only included if $p < 0.05$. When $p > 0.05$, dashed horizontal lines were generated based on fish mean nutrient excretion rates or ratio. Model fit is shown on a $\log_{10}$ scale for better pattern visualization. A summary of analytical results is given in Table S4.

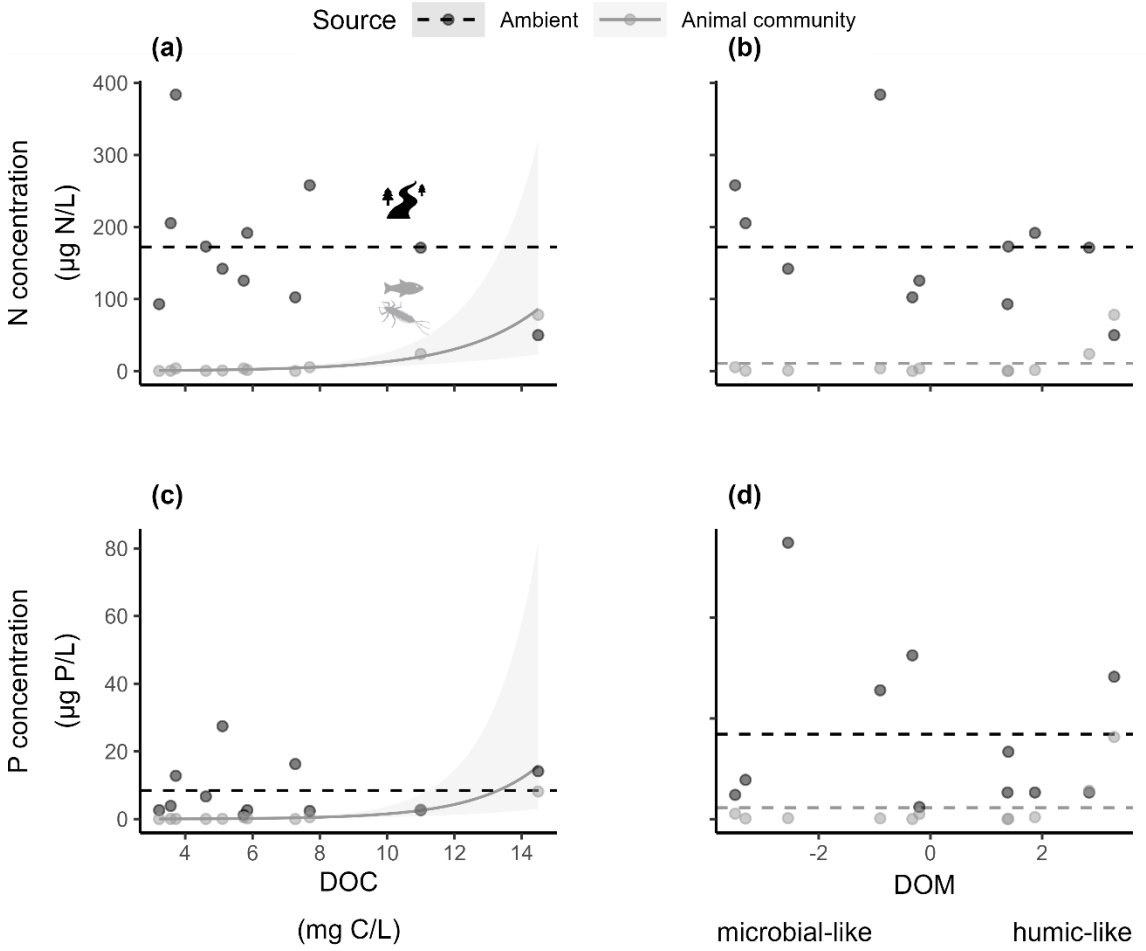

Figure 6. **Aggregate animal community volumetric N excretion makes a higher contribution to ambient nutrient concentrations as DOC increases and DOM becomes more humic-like**. (a-b) N and (c-d) P concentrations of stream ambient water and the aggregate animal community volumetric nutrient excretion rates relative to DOC and DOM. Best-fit lines and 95% confidence intervals were generated using GAM based on DOC and DOM partial effects and were only included if $p < 0.05$. When $p > 0.05$, dashed horizontal lines were generated based on fish mean nutrient excretion rates or ratio. A summary of analytical results is given in Table S5.

Supplementary Information

Methods

DOM composition analyses

A combination of spectrophotometric and spectrofluorimetric techniques were used to examine DOM composition. First, absorbance was measured from 800 to 230 nm using spectrophotometry and was corrected for water turbidity. We calculated the specific ultraviolet absorbance at 254 nm ($SUVA_{254}$; L/mg C/m), a measure of DOM aromaticity (Weishaar et al. 2003), and at 440 nm (α440), a measure of brown color. We also calculated the spectral slope ratio (S_R), an indicator of molecular weight (Helms et al. 2008). DOM fluorescence was measured on a Varian Cary Eclipse fluorescence spectrophotometer that emits wavelengths between 270 and 600 nm at 5 nm increments and measures excitation between 230 and 500 nm at 2 nm increments. This generated three-dimensional excitation emission matrices (EEMs) that were corrected for blanks and the inner filter effect using corresponding absorbance measurements (Mobed et al. 1996, McKnight et al. 2001). Raman peak of Milli-Q at 350 nm excitation was used as a reference value to express fluorescence intensities in Raman units (RU; Williams et al. 2010).

Based on the fluorescence measurements, three indices were calculated: the fluorescence index (FI) used as an indicator of DOM source (terrestrial vs. microbial; McKnight et al. 2001), the ß:α ratio which reflects the extent of DOM degradation (Parlanti et al. 2000, Wilson and Xenopoulos 2009), the humification index (HIX) which reflects the extent of humification (Ohno 2002). We then used fluorescence measurements to run a parallel factor (PARAFAC) analysis to reduce matrix data into discrete components (Murphy et al. 2013). EEMs were fit to an existing PARAFAC model generated using regional data (Williams et al. 2016) and following

recommendations by Stedmon and Bro (2008) using the DOMFluor toolbox in MA TLAB R2018b. Prior to analysis, EEM wavelength ranges were first trimmed to 250–500 nm excitation and 300–600 nm emission. Based on the PARAFAC model, seven fluorescent components were identified, including three as ubiquitous (C1) or terrestrial (C2-C3) humic-like combined as C_{humic}, one as soil, fulvic-like (C4), two as microbially-derived (C5-C6) combined as $C_{microbial}$, and one as protein-derived (C7). We calculated the mean fluorescence maxima (F_{max}) and the proportion of each component at each of the 11 sites.

Stream network area calculation

The stream network area was calculated in ArcGIS Pro 2.8.2, using the Ontario Hydro Network Enhanced Watercourse linear layer and the OIH Integrated Waterbodies polygon layer (OIH; OMNRF 2020). Stream width was estimated based on stream segment Strahler order and associated trapezoidal mean of median stream width as established by Downing (2012). Areas from both the OIH waterbodies and the estimated stream area were summed to determine the stream network area of each of the 11 study sites (Fig. 1). Given the data we had available, stream width estimates based on Strahler order was the most appropriate method to get a rough estimate of stream network area, although we acknowledge that this method has its limitations (Hughes et al. 2011) and that stream network area can vary with seasonality and region.

Tables

Table S1. Streams watershed, physical, and chemical characteristics. Watershed area and land cover characteristics were extracted from the Ontario Watershed Information Tool (OMNRF 2023).

	Strahler order	Watershed area (km²)	Discharge (m^3/s)	Land cover (%)				Temperature (°C)	DOC (mg C/L)	TDN (µg N/L)	TDP (µg P/L)
				Wetland	Woodland	Urban	Cropland				
Ausable River	4	351.9	0.2132	9.7	4.5	4.7	80.9	22.2	5.7	660	38.2
Beeton Creek	5	213.4	0.1315	7.4	17.4	6.1	68.7	20.9	3.6	2843	8.8
Black River	5	122.3	0.0949	17.6	25.4	6.7	50.1	19.7	4.6	930	12.6
East Cross Creek	3	47.9	0.2228	9.9	41.2	3.3	45.2	24	5.1	930	12.6
Emily Creek	4	30.1	0.0006	28.0	2.3	3.2	66.5	24.3	14.5	1080	50.9
Fish Creek	4	150.3	0.0829	3.1	4.3	2.5	90.0	22.6	3.7	1300	32.0
Humber River	5	252.1	0.4589	19.8	21.3	9.7	48.6	17.7	3.2	1130	23.4
Jackson Creek	5	107.1	0.0229	25.3	10.0	6.8	57.6	22.6	11.0	810	24.6
Nonquon River	4	25.9	0.0987	9.9	43.7	3.3	43.0	18.7	5.8	640	16.6
Uxbridge Brook	4	152.2	0.6155	13.2	20.5	7.7	58.3	20.7	7.3	860	18.4
Whytes Creek	4	86.6	0.0243	19.7	8.0	2.5	69.3	23.4	7.7	4590	35.0

Table S2. Summary from Pearson's correlation analysis for ambient water chemistry.

Variables	r	n	*p*
DOC ~ DOM	0.51	11	0.11
DOC ~ TDN	-0.05	11	0.87
DOC ~ TDP	0.63	11	0.04*
DOC ~ Discharge	-0.35	11	0.29
DOC ~ Travel time	0.76	11	< 0.01**
DOM ~ TDN	-0.68	11	0.37
DOM ~ TDP	0.3	11	0.02*
DOM ~ Discharge	-0.08	11	0.8
DOM ~ Travel time	0.43	11	0.43

*sample size (n); *p < 0.05; **p < 0.01; ***p < 0.001*

Table S3. Descriptive statistics summary of animal functional traits for fish and mayfly at the individual, population, and community levels.

Taxa	Functional trait	n	Mean (± SD)	Min	Max	CV
Fish	Mass-normalized N excretion (µg N/g/h)	411	149.554 ± 85.164	19.151	675.744	0.6
	Mass-normalized P excretion (µg P/g/h)	411	17.519 ± 12.661	0.239	65.213	0.7
	Mass-normalized N:P excretion (molar)	411	36.931 ± 56.122	3.020	598.787	1.5
	Individual dry mass (g)	418	3.305 ± 4.254	0.150	37.150	1.3
	Population N excretion (µg $N/m^2/h$)	31	748.460 ± 1191.836	17.756	5557.494	1.6
	Population P excretion (µg $P/m^2/h$)	31	94.978 ± 209.807	0.534	1129.620	2.2

	Population N:P excretion (molar)	31	213.156 ± 452.234	1.573	2055.749	2.1
	Population biomass (g/m^2)	31	5.242 ± 8.086	0.073	28.472	1.5
Mayfly	Mass-normalized N excretion (µg N/g/h)	73	2.654 ± 1.973	0.276	11.271	0.7
	Mass-normalized P excretion (µg P/g/h)	73	1.484 ± 1.426	0.007	8.287	1.0
	Mass-normalized N:P excretion (molar)	73	23.48 ± 112.408	0.212	954.940	4.8
	Individual dry mass (g)	73	0.001 ± 0.001	2.38E-05	0.006	1.2
	Population N excretion (µg N/m^2/h)	8	2.685 ± 1576	0.306	4.841	0.6
	Population P excretion (µg P/m^2/h)	8	1.881 ± 2.519	0.183	7.903	1.3
	Population N:P excretion (molar)	8	38.689 ± 74.958	0.597	216.274	1.9
	Population biomass (g/m^2)	8	1.028 ± 0.507	0.208	1.725	0.5
Fish and mayfly	Volumetric N excretion (µg N/L)	11	10.738 ± 23.373	0.067	78.128	2.2
	Volumetric P excretion (µg P/L)	11	1.142 ± 2.466	0.009	8.156	2.2
	Community N recycling (g/h)	11	1598.835 ± 2908.379	113.329	9802.419	1.8
	Community P recycling (g/h)	11	213.131 ± 423.422	6.695	1434.588	2.0

standard deviation (SD), minimum (min), maximum (max), sample size (n), coefficient of variation (CV)

Table S4. Coefficients of variation of animal functional traits for within stream replicates of 11 streams (Ausable River, Beeton Creek, Black River, East Cross Creek, Emily Creek, Fish Creek, Humber River, Jackson River, Nonquon River, Uxbridge River, Whytes Creek) for mayflies and seven fish species. No coefficient of variation was calculated for mayfly population-level nutrient excretion rates and biomass as these were considered as a single taxon.

		Mass-normalized N excretion (µg N/g/h)	Mass-normalized P excretion (µg P/g/h)	Mass-normalized N:P excretion (molar)	Individual dry mass (g)	Population N excretion (µg N/m^2/h)	Population P excretion (µg P/m^2/h)	Population biomass (g/m^2)
Fish	AUS	0.4	0.8	1.1	0.8	0.5	0.9	0.4
	BEE	0.6	0.8	0.9	0.7	0.4	0.5	0.1
	BLA	0.3	0.6	1.8	1.4	-	-	0.4
	ECC	0.6	0.5	1.5	0.6	0.4	0.5	0.4
	EMI	0.3	0.7	1.1	0.8	1.1	0.9	1.0
	FISH	0.7	0.4	0.8	2.0	0.9	0.6	0.7
	HUM	0.4	0.6	1.9	0.7	0.4	0.4	0.3
	JAC	0.4	0.6	1.8	1.3	0.5	0.5	0.6
	NON	0.4	0.6	0.8	1.2	1.0	1.0	1.1
	UXB	0.4	0.4	1.1	1.2	0.8	0.7	0.7
	WHY	0.5	0.7	1.0	1.1	0.6	0.2	0.4
Mayfly	AUS	1.4	0.5	1.0	0.4	-	-	-
	BEE	0.2	0.6	1.0	0.7	-	-	-
	BLA	0.6	1.1	0.9	0.7	-	-	-
	ECC	-	-	-	-	-	-	-
	EMI	0.4	0.7	0.6	0.6	-	-	-
	FISH	0.3	0.4	0.4	0.8	-	-	-
	HUM	0.2	0.5	0.7	0.5	-	-	-
	JAC	0.3	0.6	0.4	1.0	-	-	-
	NON	0.3	0.4	0.7	1.1	-	-	-
	UXB	0.6	0.4	0.8	0.6	-	-	-
	WHY	0.2	1.0	2.2	0.8	-	-	-

Table S4. AIC table comparing individual, population, and community levels Hierarchical General Additive Model (HGAM) model fits for the response variables *i)* mass-normalized N, P, and N:P excretion and individual dry mass using DOC and DOM as predictors and vertebrate classification as a random factor; *ii)* population N, P, and N:P excretion and areal biomass using DOC or DOM as predictors and taxonomic rank as a random factor; *ii)* population N, P, and N:P excretion and areal biomass using DOC and DOM as predictors and trophic position as a random factor*; iv)* N and P concentration using DOC and DOM as predictors and nutrient source as a random factor; and *v)* N and P flux ratio using DOC and DOM as predictors.

Model	**df**	**AIC**	**ΔAIC**
i) Individual level (reach-scale) – By taxonomic rank			
Mass-normalized N excr ~ *f*(DOC, by = Taxo. rank.) + *f*(DOM, by = Taxo. rank.) + *f*(Taxo. rank)	13	4873	0
Mass-normalized N excr ~ Stream + *f*(Taxo. rank)	14	4893	20
Mass-normalized N excr ~ *f*(Taxo. rank)	4	4949	76
Mass-normalized P excr ~ *f*(DOC, by = Taxo. rank.) + *f*(DOM, by = Taxo. rank.) + *f*(Taxo. rank)	21	3164	0
Mass-normalized P excr ~ Stream + *f*(Taxo. rank)	14	3176	12
Mass-normalized P excr ~ *f*(Taxo. rank)	4	3311	147
Mass-normalized N:P excr ~ *f*(DOC, by = Taxo. rank.) + *f*(DOM, by = Taxo. rank.) + *f*(Taxo. rank)	16	506	0
Mass-normalized N:P excr ~ Stream + *f*(Taxo. rank)	13	537	31
Mass-normalized N:P excr ~ *f*(Taxo. rank)	3	594	88
Individual dry mass ~ *f*(DOC, by = Taxo. rank.) + *f*(DOM, by = Taxo. rank.) + *f*(Taxo. rank)	16	830	0
Individual dry mass ~ Stream + *f*(Taxo. rank)	13	2644	1814
Individual dry mass ~ *f*(Taxo. rank)	4	943	113
ii) Population level (reach-scale) – By taxonomic rank			
Population N excr ~ *f*(DOC, by = Taxo. rank.) + *f*(DOM, by = Taxo. rank.) + *f*(Taxo. rank)	11	509	7
Population N excr ~ Stream + *f*(Taxo. rank)	14	502	0
Population N excr ~ *f*(Taxo. rank)	4	510	8

Population P excr ~ *f*(DOC, by = Taxo. rank.) + *f*(DOM, by = Taxo. rank.) + *f*(Taxo. rank)	13	354	0
Population P excr ~ Stream + *f*(Taxo. rank)	14	358	4
Population P excr ~ *f*(Taxo. rank)	4	371	17
Population N:P excr ~ *f*(DOC, by = Taxo. rank.) + *f*(DOM, by = Taxo. rank.) + *f*(Taxo. rank)	7	99	9
Population N:P excr ~ Stream + *f*(Taxo. rank)	13	90	0
Population N:P excr ~ *f*(Taxo. rank)	3	93	3
Population biomass ~ *f*(DOC, by = Taxo. rank.) + *f*(DOM, by = Taxo. rank.) + *f*(Taxo. rank)	11	176	0
Population biomass ~ Stream + *f*(Taxo. rank)	14	178	2
Population biomass ~ *f*(Taxo. rank)	4	188	12
iii) Population level (reach-scale) - By trophic position			
Population N excr ~ *f*(DOC, by = Troph. position) + *f*(DOM, by = Troph. position) + *f*(Troph. position)	19	482	0
Population N excr ~ Stream + *f*(Troph. position)	16	498	16
Population N excr ~ *f*(Troph. position)	6	502	20
Population P excr ~ *f*(DOC, by = Troph. position) + *f*(DOM, by = Troph. position) + *f*(Troph. position)	22	335	0
Population P excr ~ Stream + *f*(Troph. position)	16	355	20
Population P excr ~ *f*(Troph. position)	6	362	27
Population N:P excr ~ *f*(DOC, by = Troph. position) + *f*(DOM, by = Troph. position) + *f*(Troph. position)	15	99	14
Population N:P excr ~ Stream + *f*(Troph. position)	15	85	0
Population N:P excr ~ *f*(Troph. position)	5	92	7
Population biomass ~ *f*(DOC, by = Troph. position) + *f*(DOM, by = Troph. position) + *f*(Troph. position)	19	168	0
Population biomass ~ Stream + *f*(Troph. position)	16	176	8
Population biomass ~ *f*(Troph. position)	6	184	16
iv) Community level (reach-scale) - By nutrient source			
N concentration ~ *f*(DOC, by = Source) + *f*(DOM, by = Source) + *f*(Source)	8	186	0
N concentration ~ Stream + *f*(Source)	14	214	28
N concentration ~ *f*(Source)	4	210	24
P concentration ~ *f*(DOC, by = Source) + *f*(DOM, by = Source) + *f*(Source)	8	75	0
P concentration ~ Stream + *f*(Source)	14	97	22

P concentration ~ *f*(Source)	4	95	20
iv) Community level (watershed-scale)			
N flux ratio ~ *f*(DOC) + *f*(DOM)	4	25	0
N flux ratio ~ 1	2	32	7
P flux ratio ~ *f*(DOC) + *f*(DOM)	4	57	0
P flux ratio ~ 1	2	58	1

Table S5. Summary of Hierarchical General Additive Model (HGAM) results for analyses including fish and mayfly mass-normalized nutrient excretion rates and ratios, individual dry mass, population nutrient excretion rates and ratios, areal biomass, using DOC and DOM as predictors and taxonomic rank as a random factor.

Predictors	**Response variable**							
	Mass-normalized N excretion	**Mass-normalized P excretion**	**Mass-normalized N:P excretion**	**Individual dry mass**	**Population N excretion**	**Population P excretion**	**Population N:P excretion**	**Population biomass**
f(DOC):Fish								
edf	1	7.071	2.589	3.86	2.708	4.479	1	3.769
ref. df	1	7.521	3.107	3.987	3.192	5.009	1	3.965
p	0.26	<0.001***	<0.01**	<0.001***	0.09	<0.001***	0.56	<0.001***
f(DOC):Mayfly								
edf	2.196	6.378	6.562	3.766	1	1	1	1
ref. df	2.64	6.811	6.905	3.957	1	1	1	1
p	<0.001***	<0.001***	<0.001***	<0.01**	0.29	0.05	0.36	0.71
f(DOM):Fish								
edf	3.672	1.003	1	1.981	1.577	1	1	1
ref. df	4.303	1.005	1	1.999	1.802	1	1	1
p	<0.001***	<0.001***	0.3	<0.001***	0.23	0.5	0.49	0.9
f(DOM):Mayfly								
edf	1	1.723	1.747	1.792	1	1.686	1	1

ref. df	1	1.916	1.927	1.955	1	1.901	1	1
p	0.54	0.07	0.06	0.02	0.7	0.11	0.28	0.76
Intercept	2.932	1.477	1.017	-3.139	3.622	2.119	1.329	0.685
R^2 adj.	0.37	0.35	0.35	0.2	0.07	0.03	0.13	0.23
Deviance explained (%)	78.4	58.3	37.1	73.7	66.9	70.7	24	53.5

estimated degrees of freedom (edf), reference degrees of freedom (ref. df), R^2 adjusted (R^2 adj.)

**p < 0.05; **p < 0.01; ***p < 0.001*

Table S6. Summary of Hierarchical General Additive Model (HGAM) results for analyses including fish and mayfly using DOC and DOM as predictors and trophic position as a random factor.

Predictors	**Response variable**			
	Population N excretion	**Population P excretion**	**Population N:P excretion**	**Population biomass**
f(DOC):Fac. piscivore				
edf	1	2.386	1	1
ref. df	1	2.721	1	1
p	0.23	<0.01**	0.19	0.13
f(DOC):Invertivore				
edf	1	1.348	1	1
ref. df	1	1.608	1	1
p	0.1	0.04	0.27	0.06
f(DOC):Omnivore				
edf	3.768	4.111	1	3.754
ref. df	3.967	4.442	1	3.963
p	<0.01**	<0.001***	0.63	<0.01**
f(DOC):Grazer				
edf	1	1	1	1
ref. df	1	1	1	1
p	0.14	0.01*	0.33	0.64
f(DOM):Fac. piscivore				
edf	2.104	1	2.079	2.486
ref. df	2.237	1	2.398	2.678
p	0.03*	0.03*	0.26	0.03
f(DOM):Invertivore				
edf	2.128	1.915	1.595	2.127
ref. df	2.427	1.989	1.893	2.424
p	0.04*	<0.01**	0.4	0.05

f(DOM):Omnivore				
edf	1	1	1.277	1
ref. df	1	1	1.486	1
p	0.47	0.86	0.33	0.49
f(DOM):Grazer				
edf	1	1.835	1	1
ref. df	1	1.973	1	1
p	0.58	0.03	0.24	0.71
Intercept	4.277	2.688	1.406	0.45
R^2 adj.	0.79	-0.06	0.27	0.53
Deviance explained (%)	88.9	88.6	49.3	76.2

estimated degrees of freedom (edf), reference degrees of freedom (ref. df), R^2 adjusted (R^2 adj.)

**p < 0.05; **p < 0.01; ***p < 0.001*

Table S7. Summary of Hierarchical General Additive Model (HGAM) results for analyses including N and P concentrations of ambient streams water and the aggregate animal community nutrient excretion (i.e. volumetric nutrient excretion), using DOC and DOM as predictors and nutrient source as a random factor.

Predictors	**Response variable**	
	N concentration	**P concentration**
f(DOC):Ambient		
edf	1	1
ref. df	1	1
p	0.33	0.29
f(DOC):Animal		
edf	1	1
ref. df	1	1
p	<0.001***	<0.001***
f(DOM):Ambient		
edf	1	1.125
ref. df	1	1.237
p	0.39	0.21
f(DOM):Animal		
edf	1	1
ref. df	1.001	1
p	0.84	0.61
Intercept	3.121	0.355
R^2 adj.	0.6	0.09
Deviance explained (%)	87.7	75.9

estimated degrees of freedom (edf), reference degrees of freedom (ref. df), R^2 adjusted (R^2 adj.)
**p < 0.05; **p < 0.01; ***p < 0.001*

Table S8. Summary of Hierarchical General Additive Model (HGAM) results for analyses including nutrient flux ratio ambient water to the aggregate animal community at the watershed scale, using DOC and DOM as predictors.

Predictors	**Response variable**	
	N flux ratio	**P flux ratio**
f(DOC)		
edf	1	1
ref. df	1	1
p	0.02*	0.16
f(DOM)		
edf	1	1.046
ref. df	1	1.09
p	0.74	0.07
Intercept	-7.191	-5.908
R^2 adj.	0.6	0.23
Deviance explained (%)	64.3	38.7

estimated degrees of freedom (edf), reference degrees of freedom (ref. df), R^2 adjusted (R^2 adj.)

**p < 0.05; **p < 0.01; ***p < 0.001*

Figures

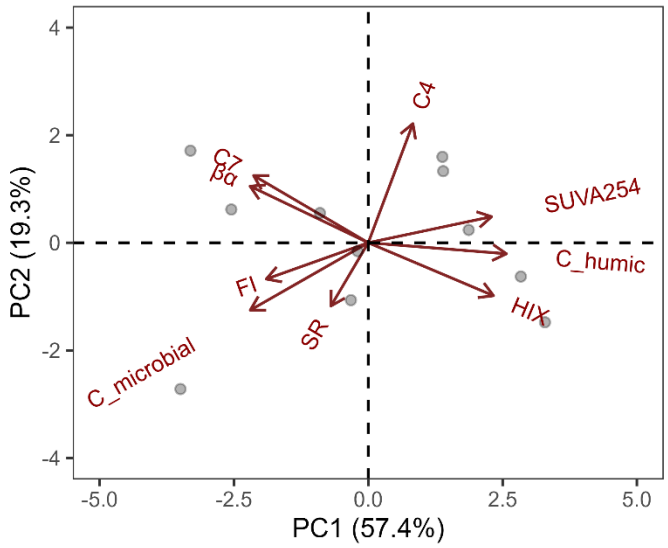

Figure S1. DOM based on PC1 ranges from labile, microbial-like to more recalcitrant, humic-like. Principal Components Analysis based on DOM absorbance and fluorescence measurements in 11 stream ambient waters: ubiquitous or terrestrial humic-like fluorescence (C_{humic}), soil, fulvic-like fluorescence (C4), microbially-derived fluorescence ($C_{microbial}$), protein-derived fluorescence (C7), specific absorbance at 254 nm ($SUVA_{254}$), fluorescence index (FI), freshness index (ß:α or ßa on the graph), and slope ratio (S_R; see methods). Eigenvectors and associated explanatory variables are in red and solid points represent the 11 streams. PC axis 1 explains 50.5%, while PC axis 2 explains 19.1% variation in the environmental data.

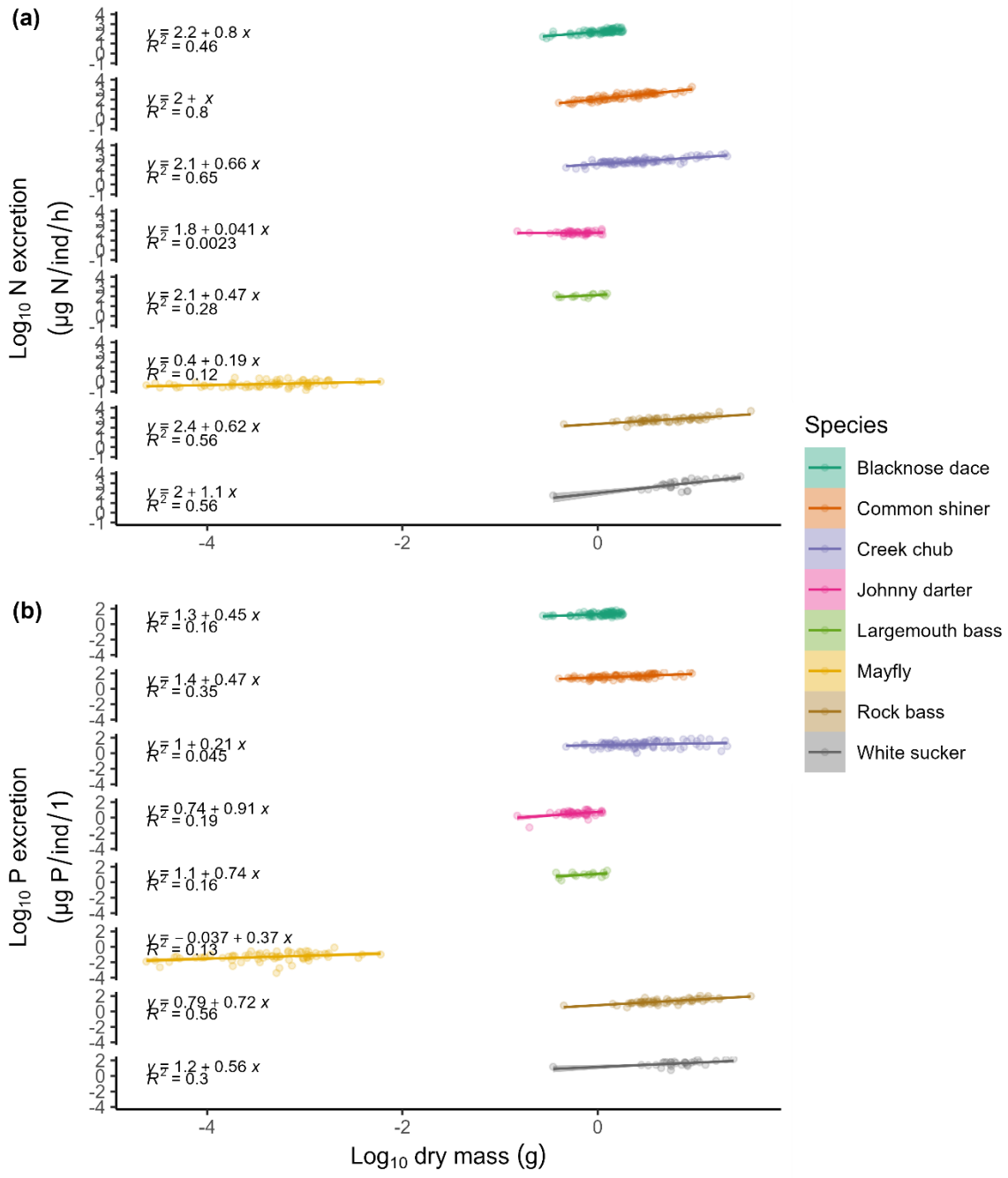

Figure S2. Regression analysis of $\log_{10}$ per capita N excretion rates relative to $\log_{10}$ dry mass for mayflies and seven fish species.

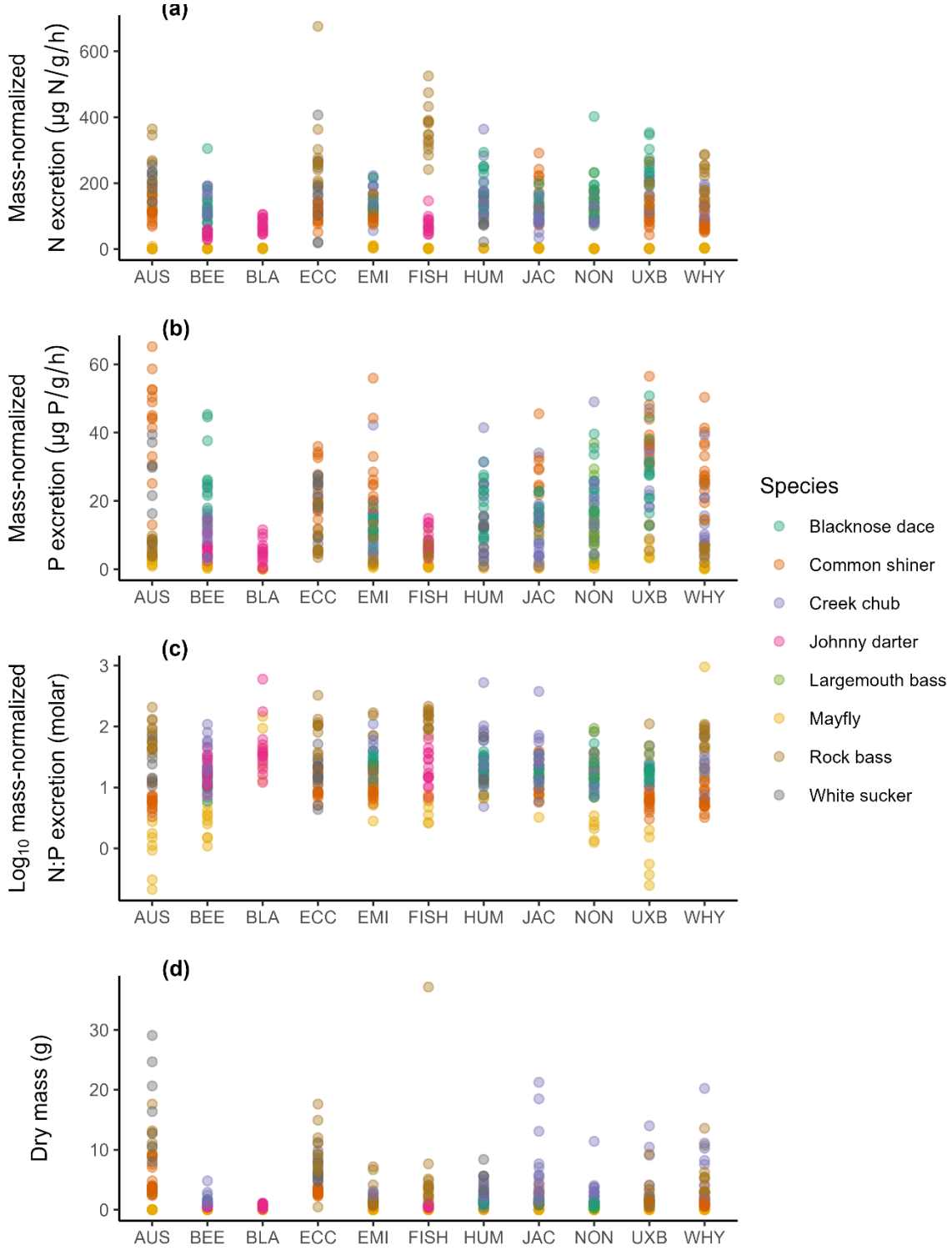

Figure S3. Mass-normalized (a-b) N, (c-d) P, and (e-f) N:P excretion rates, and (g-h) individual dry mass relative to 11 streams (Ausable River, Beeton Creek, Black River, East Cross Creek,

Emily Creek, Fish Creek, Humber River, Jackson River, Nonquon River, Uxbridge River, Whytes Creek) for mayflies and seven fish species.

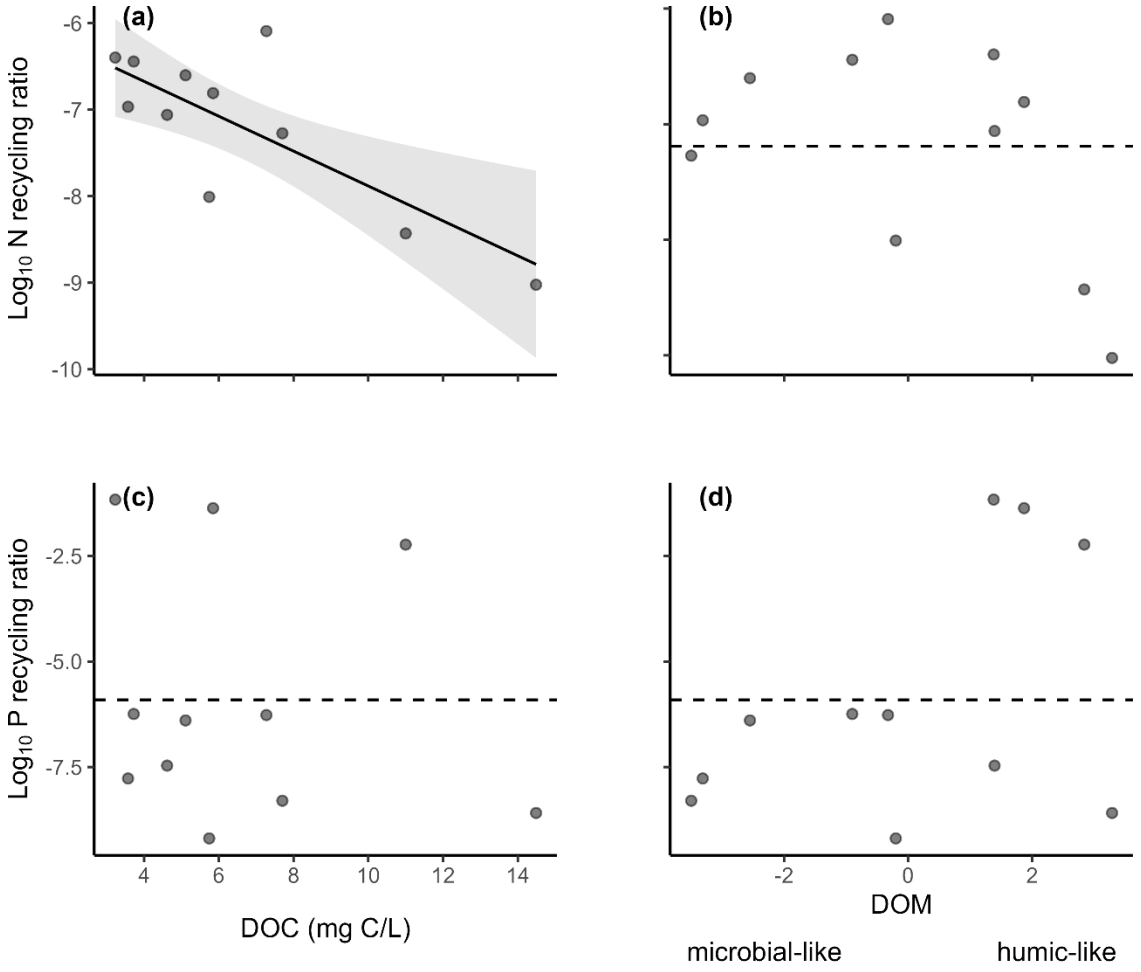

Figure S4. **The nutrient recycling ratio of ambient water to aggregate animal community at the watershed scale reveals both linear and nonlinear associations with variations in DOC and DOM**. (a-b) Log_{10} N flux ratio and (c-d) P flux ratio relative to DOC and DOM. Best-fit lines and 95% confidence intervals were generated using GAM and were only included if $p < 0.05$. When $p > 0.05$, dashed horizontal lines were generated based on fish mean nutrient excretion rates or ratio. A summary of analytical results is given in Table S7.

CHAPTER 4

Fish supply distinct nutrients and dissolved organic matter composition relative to ambient dissolved organic matter in northern lakes

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Abstract

Biogeochemical cycling in aquatic ecosystems is crucial, often facilitated by consumers through processes like excretion. While fish play established roles in nitrogen (N) and phosphorus (P) cycling, their impact on carbon (C) cycling remains underexplored. This role gains significance in ecosystems where dissolved organic matter (DOM) regulates light and nutrient availability, crucial for C cycling and a sentinel for global change. However, the influence of DOM on biological processes is complex, with organisms showing diverse responses, sometimes leading to nonlinear patterns. We investigated fish excretion of N, P, DOC, and DOM composition in 11 boreal lakes with varying DOM levels. Contrary to expectations, fish mass-normalized nutrient excretion rates varied nonlinearly with ambient DOC, with little evidence supporting an inverse unimodal relationship. Fish also exhibited lower DOC excretion rates in medium DOC environments and excreted more terrestrially- and microbially-derived DOM in low DOC environments. Overall, our findings underscore the dynamic role of fish as conduits of nutrients and carbon in aquatic ecosystems, shaped by ambient conditions and land cover composition.

Keywords: biogeochemical cycling, dissolved organic carbon, animal-mediated elemental cycling, consumer nutrient dynamics, browning, climate change, fish

Introduction

Animals are central to aquatic biogeochemical cycling, serving as key contributors of bioavailable nutrients and organic matter that can alleviate nutrient limitation, fulfill a large proportion of ecosystem demand, and alter food web structure and function (Atkinson et al. 2017, Higgins et al. 2023). These multifaceted impacts are realized through both direct mechanisms, such as egestion and excretion (Halvorson and Atkinson 2019), and indirect processes including animal carcass decomposition (Hood et al. 2007), egg laying (Jones and Mackereth 2016), and trophic interactions (Limberger et al. 2019, Parr et al. 2020). In the last two decades, there has been an increased interest in the effect of aquatic animal excretion, particularly invertebrates, on ambient dissolved organic matter (James et al. 2007, DOM; e.g., Parr et al. 2019, Johnston et al. 2022). Mussels, for instance, can contribute labile (easily degradable) DOM, constituting a substantial portion of the ambient labile DOM pool (Hopper et al. 2021), while aquatic insects fulfill high ambient carbon (C) demands (Parr et al. 2019). Zooplankton also release dissolved organic carbon (DOC; Frost et al. 2004, Frost and Tuchman 2005), particularly bioavailable DOM rich in protein-like fluorescence and low in aromaticity relative to other DOM excreted materials and to lake DOM (Johnston et al. 2022). However, the impact of consumers on biogeochemical cycling is not uniform and is influenced by various factors, including species traits such as taxonomic rank or trophic position (Atkinson et al. 2017, 2019, Hopper et al. 2021), and ambient conditions such as land use (Wilson and Xenopoulos 2011). Another factor that influences animal elemental excretion is ambient DOC and DOM (Klemet-N'Guessan et al., Chapter 3) both indirectly by influencing the light environment of basal resources or directly by influencing resource quality (Sterner et al. 1997).

DOC-induced changes in light penetration and nutrient supply affect resource availability to aquatic primary producers that can, as a result, vary in quantity and quality (Rowland et al. 2015, Rock et al. 2016, Downs et al. 2016). The light:nutrient hypothesis predicts that primary producers and consumers tissue elemental composition should vary relative to ambient light (or C) and nutrients (nitrogen, N, and phosphorus, P), such as a high supply of C:nutrients stoichiometric ratios would promote high organismal C:nutrient, while a low supply of C:nutrients would promote low organismal C:nutrients (Sterner et al. 1997). For example, a field manipulation experiment carried out under varying light and nutrients regimes demonstrated that phytoplankton production was highest under high ambient light and P, while phytoplankton C:P content was lowest at low light and high P and highest at high light and low P (Downs et al. 2016). This resulted in an increase in carnivorous fish body C:N, C:P, and N:P, as well as N:P excretion with basal resource P and zooplankton production. Fish N:P excretion under low light and high P supply also specifically deviated from the expected Redfield ratio of 16:1, a threshold that reflects the transition from N to P limitation in phytoplankton (Ptacnik et al. 2010, Downs et al. 2016). Furthermore, fish growth was highest under high ambient light and N, and lowest under low light and N (Downs et al. 2016). Changes in both basal and prey elemental composition with DOC-induced variations in light and nutrient availability could thus lead to changes in the elemental composition and stoichiometry of consumer tissue and excretion.

DOC and DOM composition are important regulators of the biogeochemistry of aquatic ecosystems that can affect food webs in complex, and often unpredictable ways (Solomon et al. 2015, Bishop et al. 2022). This influence stems from ongoing changes in both DOC and DOM, shaped by the unique characteristics of each ecoregion and surrounding human activities (Xenopoulos et al. 2021). Significant gaps persist in our understanding of nutrient and energy

transfers across food webs along DOC and DOM gradients and at various ecological scales (Blanchet et al. 2022). In boreal surface waters, increases in DOC are usually associated with increases in total nutrients (Mattsson et al. 2005, Isles et al. 2021b), and yet are concurrent with a decline in inorganic nutrients (Mosquera et al. 2022). These divergent trends have implications for the supply of C, N, and P leading to changes in DOC:nutrient stoichiometry and affecting nutrient limitation and productivity during the growing season, as well as labile C limitation during the winter (Bergström et al. 2015, Isles et al. 2021b).

The overall impact of DOC inputs on lake biological processes hinges on the degree of coupling between increases in DOC and changes in DOM composition and the local dynamics of organic and inorganic nutrients. This dynamic is especially critical in nutrient-poor northern lakes, where DOM exerts a dual control on C (or light) and nutrient availability (Bergström and Karlsson 2019). Notably, the tradeoff between light and nutrient supply frequently results in a unimodal relationship between DOC and both pelagic primary production (Seekell et al. 2015, Holgerson et al. 2022) and fish biomass (Finstad et al. 2014). The specific DOC threshold, however, varies across ecosystems, modulated by factors such as lake morphometry, DOM aromaticity, and DOC:nutrient stoichiometry (Seekell et al. 2018a, Kelly et al. 2018b). Ultimately, the unimodal changes in the quality of basal resources along DOC and DOM gradients may translate into nonlinear resource use, processing, and release by consumers.

In this study, we sampled 11 boreal lakes that varied in DOC and DOM to determine relationships between ambient DOC and fish N, P, DOC, and DOM excretion rates across trophic positions and at both the individual and ecosystem levels. Specifically, fish N and P excretion rates and ratios were evaluated across the DOC and DOM gradient, while fish DOC and DOM composition excretion rates were tested in three lakes with different DOC levels (i.e., low,

medium, and high). Overall, we hypothesized that fish would be important nutrient and DOM contributors in oligotrophic boreal lakes and that their contribution would vary nonlinearly with ambient DOC and DOM. Considering that the supply of nutrients increases with ambient DOC and that recent research in our study region demonstrated a decrease in depth-integrated phytoplankton biomass and zooplankton biomass with ambient DOC (Tonin et al. 2022, Sherbo et al. 2023), we expected basal resources to decrease in quantity (biomass or C) but increase in quality (elemental composition) and subsequently affect fish growth and C:nutrient ingestion, assimilation, and release nonlinearly due to the tradeoff between C and nutrients availability. In a low DOC environment, fish are likely to be limited by N or P rather than C. Consequently, they may retain nutrients more efficiently to bolster growth (Sterner and George 2000), resulting in low mass-normalized nutrient excretion rates (Vanni and McIntyre 2016). Conversely, in a high DOC environment, fish may be C-limited, prompting them to consume more nutrients to acquire sufficient C for growth and resulting in higher mass-normalized nutrient excretion rates (Vanni and McIntyre 2016).

Specifically, we predicted that (1) mass-normalized N and P excretion rates would follow an inverse unimodal relationship with DOC and DOM, such that these rates would decrease from low DOC and labile, microbial-like DOM environments (high light, low nutrients) to a given DOC threshold and more diverse DOM environments (high light, high nutrients) due to the concomitant increase in fish growth and decrease in resource DOC:nutrient, and an increase from these intermediate conditions to high DOC and more recalcitrant, humic-like DOM environments (low light, high nutrients) due to the concomitant decrease in fish growth and low resource DOC:nutrient. As a result, mass-normalized N:P excretion rates would remain consistent across the DOC and DOM gradient. Furthermore, we predicted that (2) mass-normalized DOC

excretion rates would be lower at higher ambient DOC levels, whereas mass-normalized DOC:N and DOC:P excretion rates would follow an inverse unimodal pattern, with similar rates at low and high DOC levels and lower rates at medium DOC levels. Based on the evidence that invertebrates release bioavailable DOM, we also predicted that (3) fish would supply labile DOM dominated by protein-like materials and poor in aromatic compounds that would decrease with higher ambient DOC due to a decrease in the proportion of bioavailable DOM.

Methods

Study design

To evaluate spatial patterns in animal nutrient, DOC, and DOM excretion rates and ratios, we sampled 11 boreal lakes at the IISD-Experimental Lakes Area (IISD-ELA) in northwestern Ontario, Canada (49◦41'42.0"N; 93◦43'27.7"W, Table 1). Lakes at ELA are oligotrophic and drain a jack pine-dominated (*Pinus banksiana*) forest (Brunskill and Schindler 1971). The IISD-ELA lakes are within the same ecoregion, remote, and minimally impacted by human activity. DOC concentrations have also been relatively stable since 2001 and are temporally coherent among lakes (Imtiazy et al. 2020). DOM composition is typically mostly terrestrial-like and humic-like due to its absence from any human influence (Williams et al. 2016). The study lakes were selected to span a DOC gradient from 3 to 11 mg C L^{-1} based on epilimnetic measurements carried out in June-August 2018 to 2022 (Table 1) and a DOM gradient from protein, microbial-like to aromatic, humic-like composition (Fig. S1). Of the 11 lakes, three were selected to represent low, medium, and high DOC levels (L224 = 3.5, L239 = 7.2, and L222 = 10 mg C L^{-1}, respectively; Table 1) and undertake DOC and DOM composition excretion experiments. We sampled the four most abundant and widespread fish species across the 11 lakes. These species spanned three trophic positions, with one to three trophic positions tested at each site. Fish tested

included the omnivores fathead minnow (*Pimephales promelas*) and pearl dace (*Margariscus margarita*), and the carnivores (i.e., invertivore/piscivore) yellow perch (*Perca flavescens*) and white sucker (*Catostomus commersonii*). Trophic positions were determined using previous stable isotope analyses in the study area (Hecky and Hesslein 1995, Sterner and George 2000). All experiments were done between June 9th and 30th, 2022 following the Canadian Council for Animal Care's guidelines for best practices (Trent U. AUP #26239).

Elemental excretion experiment

Three to twenty individuals per species were sampled at each lake to account for body size and species-specific variability. Fish were collected using trap nets at most of the lakes (n = 8), as well as seine nets (n = 2), and minnow traps (n = 1), when necessary. Based on prior excretion experiments using fish in lakes (Klemet-N'Guessan et al., Chapter 3), we aimed for 15 to 20 individuals of varying sizes per species but were limited by fish availability on any given day and lake. Trap nets and minnow traps were set the day prior to fish collection for 17 to 24h. Shortly after collection, each individual fish (< 450 g wet mass) was placed in a whirl-pak bag filled with 0.2 to 6L of base water collected from one of the sampled lakes (L626) with low ambient dissolved nutrient concentrations (N = 8.7 µg N L^{-1}, P = 1.7 µg P L^{-1}) and prefiltered through pre-ashed 1 µm borosilicate glass microfiber filters. We incubated the bags holding tested fish and two control bags without individuals *in situ* (i.e., in the lake or in the lab with lake water) at ambient temperature for 20 to 41 minutes depending on ambient temperature and fish size and to account for both handling stress and fasting effects (Whiles et al. 2009). Generally, incubations at temperatures > 20 °C and fish > 20 g wet mass would vary between 20 and 30 minutes, while lower temperatures and smaller fish would lead to incubations ranging from 30 to 40 minutes. Following incubation, fish were removed from the bag and weighed. Both excretion

and ambient water samples were filtered through pre-ashed 0.7 µm Whatman GF/F glass microfiber filters, followed by 0.22 µm polycarbonate membrane filters, then kept on ice and stored at 4 °C until analysis.

Estimating individual-level excretion

Individual-level elemental excretion rates were calculated by subtracting the nutrient concentration, DOC concentration or DOM composition parameter in the prefiltered base water in the control bags (i.e., initial conditions) from the nutrient concentration, DOC concentration or DOM composition parameter in the experimental bags post-incubation (i.e., final conditions). Individual-level elemental excretion rates were then normalized for fish mass using size scaling coefficients generated from the $\log_{10}$-$\log_{10}$ relationship between per capita excretion rates by all species (values > 0 only) for a given element and fish dry mass to obtain mass-normalized excretion rates (unit of element g^{-1} h^{-1}; Fig. S2).

Water chemistry and physical measurements

Nutrient excretion samples were analyzed for ammonia (NH_4^+-N, µg N L^{-1}) and soluble reactive phosphorus (SRP, µg P L^{-1}) using the phenate (Solórzano 1969) and molybdate blue methods (Murphy and Riley 1962), respectively, on a spectrophotometer. For each sampled lake, we collected water samples to determine ambient SRP, NH_4, DOC, and DOM composition. DOC (mg C L^{-1}) was quantified on a Shimadzu TOC-VWP UV Analyzer using a persulfate digestion. DOM composition was examined using absorbance, fluorescence, and parallel factor (PARAFAC) analyses following methods detailed in Williams et al. (2013, 2016). Briefly, 11 DOM composition parameters were used in this study including specific ultraviolet absorbance at 254 nm (SUVA254; L mg^{-1} C m^{-1}) as a measure of aromaticity (Weishaar et al. 2003), spectral slope ratio (SR) as an indicator of molecular weight (Helms et al. 2008), the fluorescence index

(FI) as an indicator of DOM source (terrestrial vs. microbial; McKnight et al. 2001), the ß:α ratio which reflects the extent of DOM degradation (Parlanti et al. 2000, Wilson and Xenopoulos 2009), the humification index (HIX) which reflects the extent of humification. Six PARAFAC components were also identified, including three as ubiquitous (C1) or terrestrial (C2-C3) humic-like, one as soil, fulvic-like (C4), one as microbially-derived (C5), and one as protein-derived (C7) expressed as mean fluorescence maxima (Fmax) and the proportion of each component in each of the lakes.

Ambient lake light attenuation (K_d, m^{-1}), thermocline depth (m), conductivity (µS m^{-1}), epilimnetic total dissolved nitrogen (TDN, µg N L^{-1}), epilimnetic total dissolved phosphorus (TDP, µg P L^{-1}), pH, particulate C, N, P (µg L^{-1}), and epilimnetic chlorophyll-a (chl-a, µg L^{-1}), were collected as part of the IISD-ELA long-term monitoring program or ongoing projects in June 2022 for all lakes except for three which were based on data collected in June 2019 (L222), 2018 (L470), and August 2007 (L377, light attenuation and thermocline depth only). Light attenuation was calculated using measurements of photosynthetically active radiation profiles collected by a flat plate quantum sensor (LICOR LI-192, Lincoln, Nebraska, U.S.A.) above the lake surface (in air), then at a depth of 0.5 m and every metre interval until the measured light was <1% of the surface light measurement. Thermocline depth was determined as the depth with the maximum rate of temperature change per depth interval using temperature and depth data collected by an RBR (XRX-620 CTD). All lake bathymetry parameters including surface area (ha), mean depth (z_{mean}, m), and max depth (z_{max}, m) were extracted from an online repository (IISD Experimental Lakes Area 2022) or provided directly by IISD-ELA (watershed area, ha). Lake water retention time was also estimated using the equation developed by for an average year for IISD-ELA lakes linking lake watershed area to water volume. Given that L470 water

retention time was below detection, we used 0.1 yr as a proxy, as previously determined by Newbury and Beaty (1980; Table 1).

Statistical analyses

To evaluate individual- and ecosystem-level excretion relative to variations in ambient DOC and DOM, we used hierarchical generalized additive models (HGAM, Pedersen et al. 2019), based on GAM (mgcv package, version 1.8-42; Wood 2011). HGAM are useful to account for potential nonlinear relationships between the independent and dependent variables and for variations among grouping levels (Pedersen et al. 2019). Our HGAM were based on group-specific smoothers that can differ in their level of wiggliness and followed the structure: y ~ s(x, by = factor, m = 2, bs = "tp") + s(factor, bs = "re") (Model I; Pedersen et al. 2019). DOM absorbance and fluorescence measurements were visualized in a Principal Component Analysis (PCA, *prcomp* function in R) to examine the variation in DOM composition among lakes. PC1 explained 75.52% of the variance in DOM among lakes and was positively associated with more humic-like and terrestrial DOM (C1, C4, $SUVA_{254}$, and HIX) and negatively associated with microbial-like DOM (ß:α, SR, C7, and C3; Fig. S3).

The PC1 score was used as a composite variable for DOM composition and considering that it was strongly correlated with DOC ($r(9) = 0.9$, $P < 0.001$), only DOC was kept as the independent variable for subsequent analyses. At the individual-level, mass-normalized nutrient excretion rates were included as the response variable, ambient DOC as the fixed effect, and fish trophic position as the group-specific and random factor. Mass-normalized nutrient excretion rates and ratios did not meet normality and homoscedasticity assumptions based on the histogram and QQ-plot of residuals (gratia package, version 0.6.0; Simpson 2021). For that reason, we used a log-link Tweedie distribution for mass-normalized N and P excretion rates

only to allow for the variance function powers to be estimated between 1 and 2 during fitting (tw() function in mgcv; Wood 2011). Mass-normalized N:P excretion rates were $\log_{10}$ transformed as recommended by Isles et al (2020). Our final models were structured as: nutrient excretion rates and ratios ~ *f*(DOC, by = trophic position, bs = "tp") + *f*(trophic position, bs = "re"). Model fits were then evaluated relative to intercept-only and lake-only null models based on AIC. The lake-only models allowed us to test whether the patterns observed in animal nutrient excretion could be better explained by other variables that vary among lakes.

To evaluate whether DOC and DOC:nutrient excretion rates and ratios differed across ambient lake DOC levels, Analysis of Variance (ANOVA) tests were used to compare DOC, DOC:N, and DOC:P excretion rates across three DOC levels (i.e. low, medium, and high). A $\log_{10}$ transformation was also applied to both DOC and DOC:nutrient ratios given that normality assumptions were not met for DOC and that ratios should be log-transformed. Among groups differences were then assessed using a posthoc Tukey's test. Half-eye plots were generated to visualize the density distribution of observations (stat_halfeye function, ggdist package; Kay 2023).

We examined the signature of fish DOM excreta following two steps. First, t-tests were run on each one of the 11 mass-normalized DOM indices and components (RU) based on absorbance and fluorescence to determine whether the DOM optical parameters were greater than 0, which would indicate that fish excreted that DOM compound. ANOVAs were then run to assess whether the mass-normalized DOM optical parameters excretion changed across the three ambient lake DOC levels. Second, we used a non-metric multidimensional scaling ordination (NMDS) with Bray-Curtis distances using all DOM optical parameters to visualize both fish DOM excreta and ambient lake DOM composition (metaMDS function, vegan package; version

2.6-4 Oksanen et al. 2022). Permutational analysis of variance (PERMANOVA) was then used to test whether fish DOM excreta composition was significantly different from ambient lake DOM composition (adonis function, vegan package, 999 permutations; Oksanen et al. 2022). Multivariate homogeneity of groups dispersions were also assessed to test for group homogeneity of variances (betadisper function, vegan package, Oksanen et al. 2022). Final plots were generated (tidyverse package; Wickham et al. 2019) and arranged to visualize results coherently (ggarrange and fivz_pca_biplot functions, ggpubr package Kassambara 2023). All statistical analyses were done using R Statistical Software (v4.1.3; R Core Team 2023) and RStudio (v2022.2.1.461 RStudio Team 2022) on a Windows PC (v 22H2).

Results

Nutrient excretion rates

Fish mass-normalized N excretion rates were within the range of rates observed in fish in southern Ontario streams (Table S1; Klemet-N'Guessan et al., Chapter 3). However, mass-normalized P excretion rates were particularly high, which resulted in mass-normalized N:P excretion on average below the Redfield ratio (9.7 vs. 16, Table S1, Fig. 1c). Fish mass-normalized DOC, DOC:N, and DOC:P excretion rates, as well as individual dry mass were highly variable, with coefficient of variations (CV) ranging from 2.5 to 6.2 (Table S1).

Fish varied in N, P, and N:P excretion rates across the DOC gradient at varying magnitudes (Table S2, S3 and Fig. 1). Tested model fit was better than all intercept-only null models but none of the models including lake as a predictor (Table S2). Given that ambient DOC was associated with several other biochemical variables including DOM, TDP, TDN, and epilimnion chl-a (Table 1, Fig. S1, S2), these factors likely acted in concert with DOC in mediating the effects on fish nutrient excretion. Variations in DOC explained between 3 and 23%

of variations in mass-normalized nutrient excretion rates in carnivores and omnivores (Table S3). Mass-normalized N and P excretion rates had significant bimodal relationships with DOC, respectively, that varied differently by trophic position except for omnivore mass-normalized P excretion rates (Fig. 1a-b). Specifically, carnivore and omnivore mass-normalized N excretion rates displayed one minimum and one maximum at 5.8 and 8 mg C L^{-1}, and 6.3 and 10 mg C L^{-1}, respectively, although there was high uncertainty around the maximum (Fig. 1a). Variations in mass-normalized P excretion rates with DOC for carnivores were marginal despite being significant, with a subtle maximum at 5.3 mg C L^{-1} and an increase beyond 8 mg C L^{-1} (Fig. 1b). Given that variations in DOC only explained 3% of variations in mass-normalized P excretion rates, it is unlikely that DOC had a meaningful effect on mass-normalized P excretion rates. As a result, fish mass-normalized N:P excretion rates had a unimodal relationship with DOC with a minimum at 5.8 mg C L^{-1} and a maximum at 8.8 mg C L^{-1} for carnivores, and a minimum only at 6.8 mg C L^{-1} for omnivores (Fig. 1c).

DOC and DOM excretion rates

We found a significant relationship between ambient DOC and fish mass-normalized DOC ($F_{2,52}$ = 3.47, P = 0.04; Fig. 1a) and DOC:N excretion rates for individual fish ($F_{2,52}$ = 4.82, P = 0.01; Fig. 2a-b), but not on fish mass-normalized DOC:P excretion rates ($F_{2,52}$ = 0.62, P = 0.54; Fig. 2c). Specifically, mass-normalized DOC (P = 0.03, 95% CI [0.06, 1.41]; Fig. 3a) and DOC:N excretion rates (P < 0.01, 95% CI [0.89, 1.19]; Fig. 3b) were significantly higher in the lake with higher ambient lake DOC relative to the lake with medium ambient lake DOC levels. Moreover, mass-normalized DOC and DOC:nutrient excretion rates were normally distributed at low and medium ambient DOC levels, but appeared bimodal at the high ambient DOC level for DOC and DOC:P excretion rates only (Fig. 2). Variation in fish mass-normalized DOC and DOC:nutrient

excretion rates was relatively consistent across responses in the lake with low ambient DOC compared to the lakes with medium and high DOC levels, likely due to the higher sample size at low ambient DOC levels relative to the other two lakes (Fig. 2). For example, in the lake with medium ambient DOC levels, there was higher variability in fish mass-normalized DOC:N compared to DOC excretion rates (Fig 2a-b).

DOM excretion included both fish that excreted measurable (i.e. positive) and fish that did not excrete measurable (i.e., negative) DOM absorbance and fluorescent excretion rates to accurately quantify fish influence on each DOM optical parameter. Negative values typically arise from measurement errors; however, they may also suggest that fish excreted minimal to no amount of the element in question. Given that DOM absorbance and fluorescence are calculated using complex formulations, detection limits cannot be retrieved. Of the 11 DOM composition mass-normalized excretion rates tested, five were significantly higher than 0 across all lakes (Table S4, Fig. 3). These included, in increasing median order, C2 (terrestrial, humic-like), FI (source), C5 (microbial, humic-like), C7 (protein-like), and C4 (soil, fulvic-like; Fig. 3). While mass-normalized $SUVA_{254}$ (aromaticity) excretion rates were not significantly different from 0 due to the high variability, particularly below 0, it had the second highest median after C2 (terrestrial, humic-like; Fig. 3). In contrast, mass-normalized C4 (soil, fulvic-like) excretion rates were the least variable (Fig. 3). There were also significant differences in fish mass-normalized DOM excreta composition across ambient DOC (Table S6). Fish at low ambient DOC excreted DOM richer in C5 (microbial, humic-like), C2 (terrestrial, humic-like), FI (source), C4 (soil, fulvic-like), and $SUVA_{254}$ (aromaticity) relative to fish at medium and high ambient DOC that excreted more similar DOM (Table S6, Fig. 3). Fish at high ambient DOC only differed from fish at medium ambient DOC levels by excreting DOM richer in C5 (microbial, humic-like) and C3

(terrestrial, humic-like; Table S6, Fig. 3). Additionally, there was higher variability in fish mass-normalized DOM optical parameter excretion rates at low ambient DOC compared to those at medium and high ambient DOC (Fig. 3).

The NMDS and PERMANOVA revealed that the DOM composition of fish mass-normalized excretion rates at low, medium, and high ambient DOC separated distinctly from that of ambient water (Fig. 4), although this was not significant for low ($F_{2,43} = 1.66$, $P = 0.11$), medium ($F_{1,16} = 3.61$, $P = 0.05$), nor high ambient DOC ($F_{1,14} = 3.03$, $P = 0.07$). There was also high heterogeneity among mass-normalized DOM excretion rates for fish at low ($F_{2,43} = 3.7$, $P = 0.03$), medium ($F_{1,16} = 5.22$, $P = 0.04$), and high ambient DOC ($F_{1,14} = 9.02$, $P < 0.01$).

Discussion

The role of fish in both nutrient and carbon cycling relative to local conditions is an important but largely overlooked process in aquatic ecosystems. We evaluated N, P, DOC, and DOM composition excretion by fish in 11 boreal lakes that differed in ambient DOC and DOM. We found that ambient DOC had a nonlinear relationship with fish mass-normalized N excretion but was unlikely to affect fish mass-normalized P excretion. Patterns in carnivore and omnivore mass-normalized N and N:P excretion relative to DOC diverged the most beyond 6.3 mg C $L^{-1,}$ although there was high uncertainty associated with those patterns for mass-normalized N excretion rates. Additionally, most fish excreted labile DOM, although fish in low DOC environments excreted DOM that was more terrestrially- and microbially-derived and more variable than fish in medium and high DOC environments. Based on our findings, we conclude that fish act as dynamic conduits and modifiers of nutrients and carbon, both reflecting ambient conditions and the composition of the surrounding land cover, and transforming ingested materials.

We found that ambient DOC exhibited a bimodal relationship with fish mass-normalized N excretion rates, particularly pronounced before reaching a DOC concentration of 7.3 mg C L^{-1}. Yet, ambient DOC had a weak to absent relationship with mass-normalized P excretion rates. The initial decline in mass-normalized N excretion rates down to 5.8 - 6.3 mg C L^{-1} was likely due to a decrease in dietary N and was followed by an increase associated with higher N intake. This threshold closely aligns with the 7 mg C L^{-1} transition identified in prior research in our study region, marking a shift in zooplankton diet from P-rich sub-epilimnetic phytoplankton to lower quality terrestrial organic matter (Tonin et al. 2022). Patterns observed in both carnivore and omnivore mass-normalized N excretion rates beyond 7.3 mg C L^{-1} lack a clear biological explanation due to the observed high level of uncertainty. Additionally, given that the inverse unimodal pattern was only found in mass-normalized N excretion rates, it is unlikely that it was driven by changes in fish growth rates, contrary to our predictions. Lastly, the observed weak relationship between carnivore mass-normalized P excretion and ambient DOC may be attributed to an overall low P availability in our lakes, surpassing effects from light and potential C limitation at high DOC (Downs et al. 2016).

Two potential mechanisms could explain the discrepancy between mass-normalized N and P excretion rates along the DOC gradient. It is possible that phytoplankton and zooplankton P content did not vary with ambient DOC (Tonin et al. 2022) while N content did, leading to constant P but variable N intake for fish along the DOC gradient. Alternatively, selective feeding on P-rich resources before the 5.8 - 6.3 mg C L-1 threshold may have resulted in lower N intake given that invertebrate N and P content rankings tend to show opposite patterns (Frost et al. 2003), while increased consumption rates beyond this threshold, aimed at maintaining growth despite diminished resource quantity and quality (Sterner and George 2000), may have increased

both N and P intake. Considering that fish body N content tends to exhibit greater stability compared to P (Sterner and George 2000), and given the likelihood of fish being more P-limited in our lakes, fish may have preferentially excreted N relative to P with excess dietary N and P to both maintain homeostasis and meet fish higher body P requirements. Despite fish high P body requirements, fish mass-normalized P excretion rates remained high across ambient DOC.

Fish mass-normalized P excretion was high relative to mass-normalized N excretion rates, as well as previously reported P excretion rates for fish (e.g., Atkinson et al. 2019). As a result, fish N:P excretion rates averaged 9.7, much lower than the Redfield ratio of 1:16. This contrasts with previous conclusions in our study region using similar fish species (Sterner and George 2000), but consistent with several studies showing high variability in fish N:P excretion, with rates both approaching the Redfield ratio (Williamson et al. 2018) and diverging from it (McManamay et al. 2011, Hopper et al. 2020). Similarly, previous work in boreal lakes revealed that N-limited zooplankton recycled low N:P ratios at around 1.9, a trend that could further exacerbate phytoplankton N-limitation of in those lakes (Bergström et al. 2015, Isles et al. 2018). A plausible explanation for the observed high mass-normalized P excretion in boreal ecosystems, relative to ecosystems with more direct impacts from surrounding agricultural activities, is that animals in those ecosystems may differ in their dietary P requirements and assimilation efficiency due to differences in food quality and ingestion rates (Glaholt Jr and Vanni 2005, Czamanski et al. 2011). In P-rich systems where P is not limiting to primary producers nor consumers and organismal P content high (Frost and Elser 2002, James et al. 2007), animal dietary P requirements and assimilation efficiency may be lower compared to P-poor systems such as boreal lakes. This discrepancy could result in the preferential release of P as egesta rather

than excretion (Halvorson and Atkinson 2019). Future studies could investigate the underlying physiological mechanisms driving elevated P excretion rates in boreal lakes.

Fish exhibited comparable rates of DOC excretion in high and low DOC environments but higher rates in high compared to medium DOC environments. This outcome may reflect the physiochemical conditions under which fish DOC excretion was evaluated. The IISD-ELA lakes, draining uniform coniferous forests, show differences in DOC primarily due to variations in water retention time, often associated with lake size (Algesten et al. 2004), photodegradation, and biological processing of terrestrially-derived DOC (Curtis and Schindler 1997, Catalán et al. 2016) rather than diverse inputs from catchment runoffs. Thus, our spatial gradient is more influenced by temporal processes within each lake than interactions at the aquatic-terrestrial interface. Overall, fish mass-normalized DOC excretion rates were on average 0.83 mg g^{-1} h^{-1}, which are two orders of magnitude higher than measured mass-specific DOC excretion rates in streams insects (Parr et al. 2019).

Consistent with earlier findings in the same lakes, ambient DOC decreased with prolonged water retention time, and our low DOC lake had the longest retention time, while the high DOC lake had the shortest (Curtis and Schindler 1997). Despite these differences, the surprising similarity in fish DOC excretion rates between low and high DOC environments suggests that fish in low DOC lakes may process C similarly. Alternatively, it could be linked to varying degrees of carbon sequestration and mineralization in each lake (Hall et al. 2019), with fish preferentially cycling "fresh" DOC from spring runoffs in low DOC environments. Either way, this result may signify a disproportionate role for fish as a C source on a mass-normalized basis in low relative to high DOC lakes. Furthermore, the higher DOC and DOC:N excretion rates in high compared to medium DOC environments is consistent with the predicted ascending

limb of our predicted inverse unimodal relationship between ambient DOC and mass-normalized DOC:nutrient excretion rates. However, the observed higher mass-normalized DOC:N excretion rates in the high DOC lake was driven by higher DOC excretion rates and lower N excretion rates compared to medium DOC environments, contrary to our prediction that DOC:nutrient excretion rates would increase with both a decrease in growth rates and low resource DOC:nutrient content. No relationship was found between mass-normalized DOC:P excretion rates and DOC levels, suggesting once again that growth rates may not have influenced the increase in DOC:N excretion rates. Instead, fish may have ingested or assimilated more DOC and less N than expected.

Fish provided distinct DOM signatures that were consistently labile across all three lakes but differed in other DOM characteristics among ambient DOC environments and trophic position. In line with our prediction, fish excreted bioavailable, protein-like DOM across all lakes. However, fish also excreted DOM that was more aromatic and humic-like than previously reported (Hopper et al. 2021, Johnston et al. 2022), particularly in low DOC environments. This is consistent with the expectation that lakes with longer water retention time, like our low DOC lake, would exhibit relatively higher proportions of terrestrially-derived and microbial humic-like DOM components due to greater photo- and biodegradation compared to our medium and high DOC lakes with shorter water retention times (Benner and Kaiser 2011, Rodríguez-Cardona et al. 2022). Species-specific variations in DOM composition (Mangal et al. 2016), along with shifts in plankton and animal community composition across DOC environments, may result in differences in the composition of excreted dietary DOM. However, it is unclear whether fish DOM excretion reflects the DOM composition of their diet or results from DOM transformation post-digestion. Although studies have detected plankton-derived DOM components released

through exudation or leakage (Thornton 2014, Mangal et al. 2016), the composition and processing of DOM within organismal tissue, particularly in animals, remain poorly understood and require further investigation. Nonetheless, our findings suggest increased body retention of terrestrial DOM components by fish in medium and high DOC environments. In contrast, fish in low DOC environments may contribute to the resuspension of terrestrial, recalcitrant DOM components owing to their elevated excretion rates.

We found that fish mass-normalized DOM excretion in low DOC environments was also more microbially transformed and variable compared to medium and high DOC environments. This variability is likely due to the higher diversity of fish species and associated diets tested in low DOC environments relative to medium and high DOC environments, which exclusively harbored carnivorous yellow perch for the excretion experiment. Greater variability in fish mass-normalized DOM excretion in low DOC environments may thus not be related to differences in ambient DOC. Moreover, omnivore DOM excretion differed from that of carnivores and ambient DOC in the low DOC environment. The difference between omnivorous white sucker and fathead minnows and carnivorous pearl dace was presumably caused by pearl dace distinctive DOM excretion signal, which could not be fully captured by our PARAFAC model. Pearl dace feed on both aquatic and terrestrial invertebrates and may thus integrate a unique terrestrial resource base into the DOM assimilated and released (Parr et al. 2019). An alternative explanation may involve the secretion of organic chemicals, such as alarm cues due to stress induced by the experiment, by pearl dace through their skin, characterized by a composition distinct from that of other species.

We contend that as DOC continues to rise and DOM change in northern aquatic ecosystems, fish excretion will continue to support P ecosystem demands but may have variable

or negligible effects on N and C demands, respectively. More broadly, we anticipate as northern regions witness longer growing seasons and increased precipitation, aquatic ecosystems may face dual challenges of decreased nutrient loads associated with heightened nutrient uptake by expanded terrestrial vegetation (Piao et al. 2020) and increased nutrient loads caused by land-use changes and fertilizer use aimed at enhancing crop production for the ever growing food demands (Sala 2000). These changes could further alter DOC-nutrient stoichiometry, aquatic primary production, and ultimately animal-mediated nutrient and carbon cycling.

Acknowledgements

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Table 1. Physical and chemical characteristics of the 11 study lakes, including lake area, mean depth (Z_{mean}), maximum depth (Z_{max}), water retention time (WRT, calculated using Newbury and Beaty's (1980) lake watershed area and volume equation for an average year), light attenuation (K_d), thermocline depth, conductivity, pH, epilimnetic dissolved organic carbon (DOC), total dissolved nitrogen (TDN), total dissolved phosphorus (TDP), and chlorophyll a (chl-a). All data starting from K_d onwards was collected on a sampling day different from the excretion experiments in June 2022 for all lakes except for three which were based on data collected in June 2019 (L222), 2018 (L470), and August 2007 (L377, light attenuation and thermocline depth only). Fish include fathead minnows (FM), yellow perch (YP), white sucker (WS), and pearl dace (PD).

Lake	Area (ha)	Z_{mean} (m)	Z_{max} (m)	WRT (yr)	K_d (m^{-1})	Thermocline depth (m)	Conductivity ($\mu S\ cm^{-1}$)	pH	DOC (mg C L^{-1})	TDN (µg N L^{-1})	TDP (µg P L^{-1})	Chl-a (µg L^{-1})	Fish
L114	12.02	1.50	4.26	1.24	0.807	3.38	11.08	6.08	6.34	283	2.7	3.79	FM
L222	16.39	3.66	6.30	1.16	1.296	2.25	21.88	6.60	10.04	349	5.8	3.93	YP
L224	26.16	11.72	27.29	13.42	0.433	4.38	12.02	5.85	3.46	184	1.4	1.17	FM, WS, PD
L239	54.14	11.40	31.46	6.65	0.826	4.38	17.22	6.61	7.16	290	5.4	2.96	YP
L373	27.38	11.35	21.23	16.03	0.453	3.63	18.31	6.05	3.96	217	1.8	1.15	WS, PD
L375	23.07	11.57	25.89	5.13	0.529	3.63	30.53	6.07	5.69	205	4.5	0.87	FM, WS, PD
L377	28.24	9.21	18.15	0.43	0.555	6.01	17.57	6.49	5.26	234	3.5	1.3	FM, WS, PD, YP
L378	25.16	7.94	16.62	6.22	0.655	3.38	14.98	5.91	7.24	256	3.8	2.01	WS, PD, YP
L442	15.44	8.76	17.93	3.51	0.643	2.88	15.97	6.49	6.81	258	0.7	1.45	FM, WS
L470	4.24	0.79	1.70	0.10	0.840	n/a	12.40	7.21	10.91	407	5.6	3.23	FM
L626	26.13	7.27	13.11	2.09	0.503	3.13	14.55	6.51	5.06	296	4.7	1.37	PD, YP

Figures

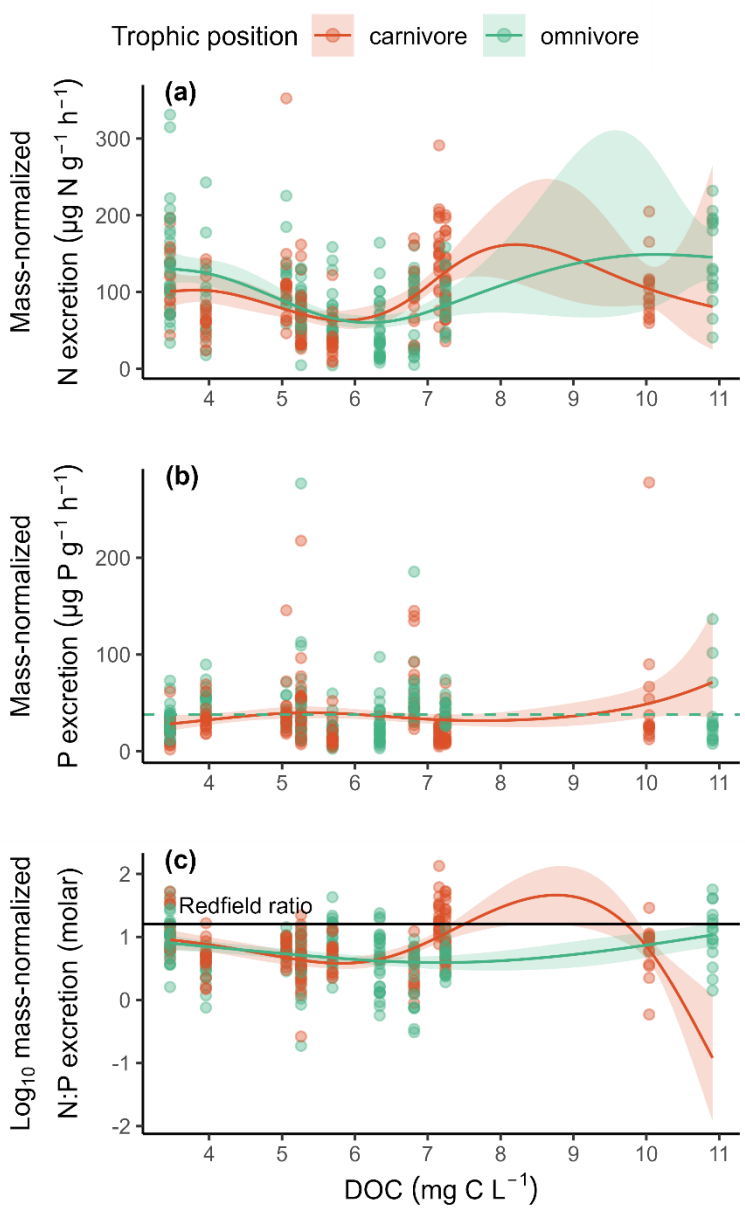

Figure 1. Carnivore and omnivore fish N excretion rates follow similar bimodal relationships with DOC. Mass-normalized (a) N, (b) P, and (c) N:P excretion rates relative to ambient DOC and trophic position for four fish species. Best-fit lines and 95% confidence intervals were generated using GAM and were only included if $P < 0.05$. Dashed horizontal line is the $\log_{10}$ of the Redfield ratio ($\log_{10}(16) = 1.2$).

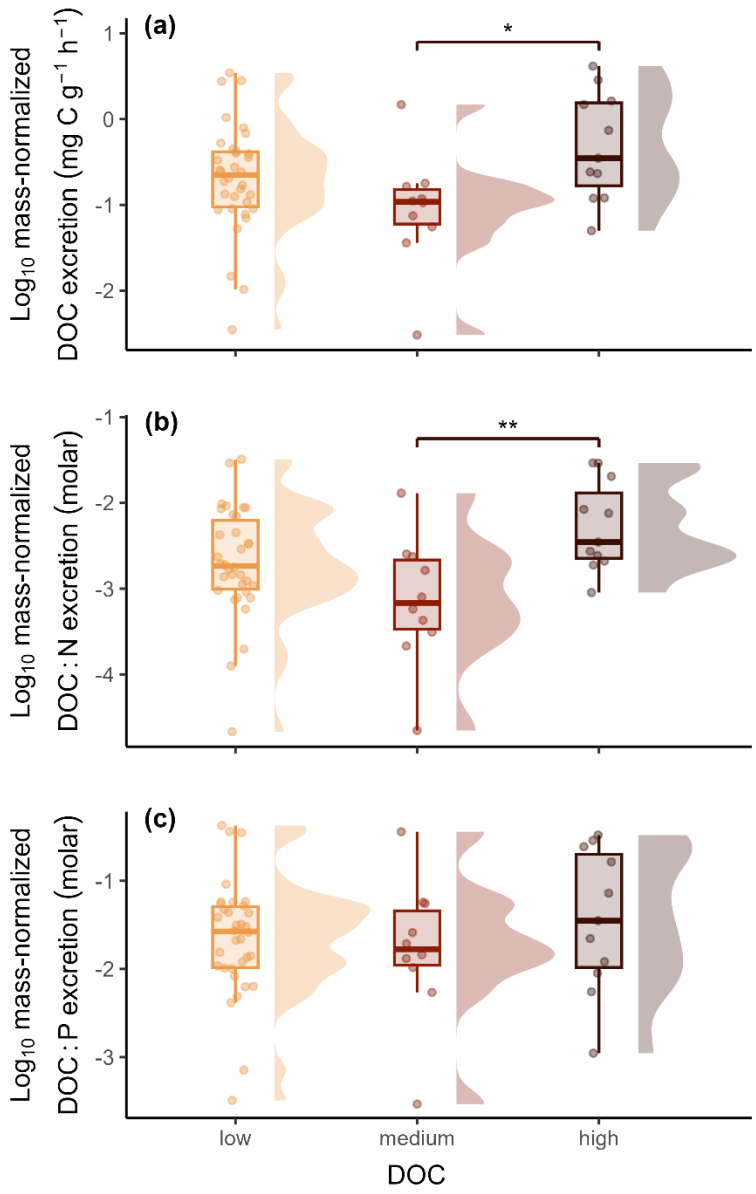

Figure 2. Fish DOC and DOC:N excretion rates were significantly greater at high ambient DOC levels relative to medium DOC levels. Mass-normalized (a) DOC, (b) DOC:N, and (c) DOC:P excretion rates and ratios for four fish species. Box plots represent the median, first and third quartiles, and minimum and maximum values. Half-eye plots correspond to the density distribution of the raw data. Colours indicate DOC level and symbols represent individual fish in

each lake. Asterisks denote significant pairwise group differences with (*) < 0.05 and (**) < 0.01.

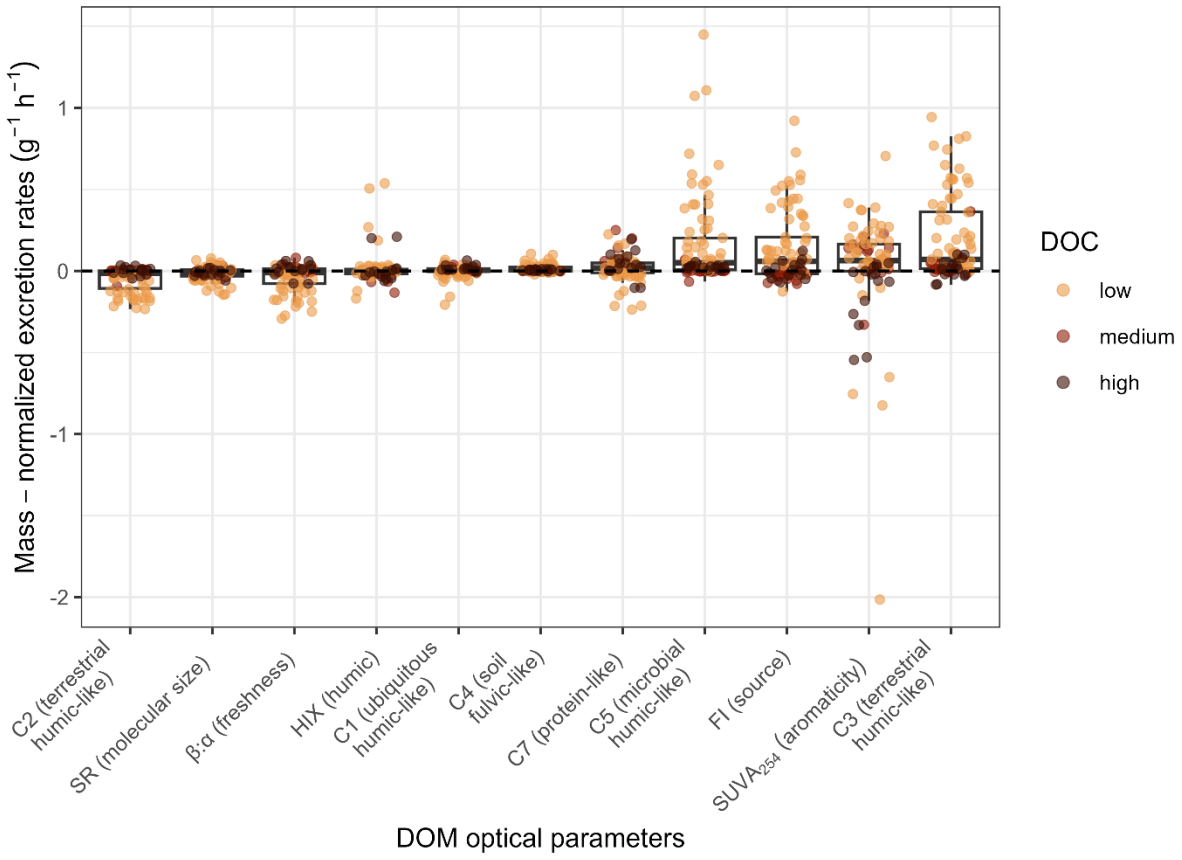

Figure 3. Fish mass-normalized DOM excretion rates in low DOC lakes are significantly enriched in terrestrial, humic-like, aromatic, and microbial DOM, while those in medium and high DOC lakes are higher in protein-like DOM. Mass-normalized excretion rates for 11 DOM absorbance and fluorescent indices and components. Box plots represent the median, first and third quartiles, and minimum and maximum values and are ordered in increasing median value. Colours indicate DOC level and symbols represent individual fish in each lake.

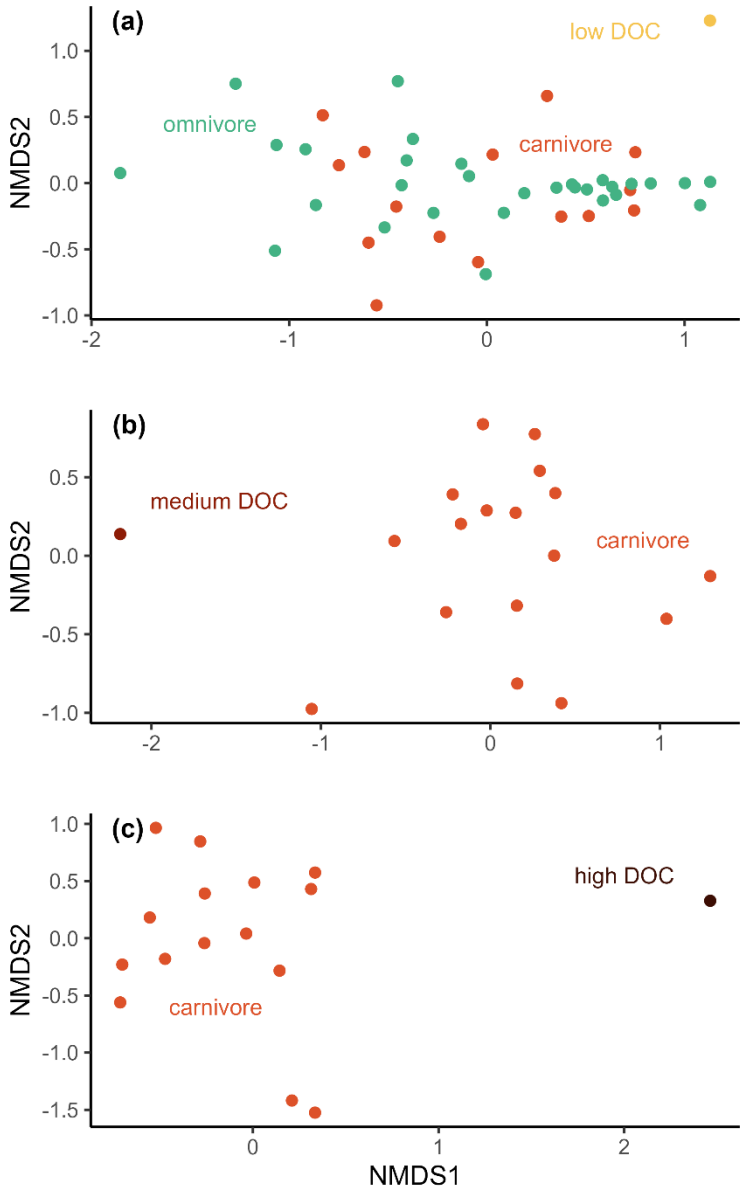

Figure 4. The DOM composition of omnivore mass-normalized excretion rates at low DOC is significantly different from that of carnivores and low ambient lake DOC levels. Non-metric multidimensional scaling ordination (NMDS) of DOM composition of fish excretion and ambient lake water at (a) low, (b) medium, and (c) high ambient DOC levels. Symbol colours and shape indicate ambient lake DOC level and individual fish trophic position in each lake.

Supplementary Information

Table S1. Descriptive statistics of fish nutrient and DOC excretion rates and ratios and individual dry mass.

response	n	mean (± SD)	min	max	CV
Mass-normalized N excretion (µg N/g/h)	353	94.07 ± 56.02	4.26	352.57	0.6
Mass-normalized P excretion (µg P/g/h)	353	36.58 ± 32.57	1.85	277.89	0.9
Mass-normalized N:P excretion	353	9.73 ± 12.12	0.19	133.64	1.2
Mass-normalized DOC excretion (mg C/g/h)	77	0.83 ± 3.76	0.00	32.57	4.5
Mass-normalized DOC:N excretion (molar)	77	0.01 ± 0.08	0.00	0.72	6.2
Mass-normalized DOC:P excretion (molar)	77	0.06 ± 0.16	0.00	1.15	2.5
Individual dry mass (g)	353	6.734 ± 14.03	0.17	104.00	2.1

standard deviation (SD), minimum (min), maximum (max), sample size (n), coefficient of variation (CV)

Table S2. AIC table comparing Hierarchical General Additive Model (HGAM) model fits for the response variables mass-normalized N, P, and N:P excretion rates using DOC as a predictor and trophic position as a random factor. Null models included lake and intercept-only models.

Model	df	AIC	ΔAIC
Mass-normalized N excr ~ *f*(DOC, by = Troph. position) + *f*(Troph. position)	12	3697	36
Mass-normalized N excr ~ Lake + *f*(Troph. position)	14	3661	0
Mass-normalized N excr ~ *f*(Troph. position)	4	3778	117
Mass-normalized P excr ~ *f*(DOC, by = Troph. position) + *f*(Troph. position)	9	3172	103

Mass-normalized P excr ~ Lake + *f*(Troph. position)	14	3069	0
Mass-normalized P excr ~ *f*(Troph. position)	3	3175	106
Mass-normalized N:P excr ~ *f*(DOC, by = Troph. position) + *f*(Troph. position)	9	355	64
Mass-normalized N:P excr ~ Lake + *f*(Troph. position)	12	291	0
Mass-normalized N:P excr ~ *f*(Troph. position)	3	399	108

Table S3. Summary of Hierarchical General Additive Model (HGAM) results using mass-normalized N, P, and N:P excretion as a response, DOC as a predictor, and trophic position as a random factor. Significant p values are indicated in bold.

Predictor	**Response variable**		
	Mass-normalized N excretion	**Mass-normalized P excretion**	**Mass-normalized N:P excretion**
f(DOC)*:carnivores*			
edf	3.823	2.799	3.722
ref. df	4.206	3.06	3.925
p	**<0.001**	**0.03**	**<0.001**
f(DOC)*:omnivores*			
edf	3.66	1	2.528
ref. df	4	1	2.86
p	**<0.001**	0.84	**<0.001**
Intercept	4.48	3.6	0.73
R^2 adj.	0.21	0.01	0.15
Deviance explained (%)	22.9	2.8	16.2

estimated degrees of freedom (edf), reference degrees of freedom (ref. df), R^2 adjusted (R^2 adj.)

Table S4. Results from t-test using mass-normalized DOM optical parameter excretion rates as the response variables. Significant p values are indicated in bold.

response	group 1	group 2	n	statistic	df	p
Mass-normalized C2 excr	1	null model	77	6.91892	76	**6.24E-10**
Mass-normalized C4 excr	1	null model	77	6.08542	76	**2.19E-08**
Mass-normalized FI excr	1	null model	77	5.75731	76	**8.56E-08**
Mass-normalized C5 excr	1	null model	77	5.315	76	**5.16E-07**
Mass-normalized C7 excr	1	null model	77	1.88058	76	**0.0319**
Mass-normalized HIX excr	1	null model	77	1.18572	76	0.12
Mass-normalized SUVA excr	1	null model	77	0.48532	76	0.314
Mass-normalized C1 excr	1	null model	77	-0.0626	76	0.525
Mass-normalized SR excr	1	null model	77	-3.2761	76	0.999
Mass-normalized BA excr	1	null model	77	-4.5582	76	1
Mass-normalized C3 excr	1	null model	77	-6.1089	76	1

Table S5. Results from Tukey's test using mass-normalized DOM optical parameter excretion rates as the response and ambient DOC (low, medium, high) as the predictor variables. Significant p values are indicated in bold.

response	group 1	group 2	null value	estimate	conf low	conf high	p adj
Mass-normalized C5 excr	low	med	0	-1.6591	-2.117	-1.2011	**1.54E-11**
Mass-normalized C2 excr	low	med	0	-0.9846	-1.3361	-0.6331	**2.02E-08**
Mass-normalized C5 excr	low	high	0	-0.8967	-1.2713	-0.522	**8.26E-07**
Mass-normalized FI excr	low	high	0	-0.9123	-1.3006	-0.5239	**0.000002 17**
Mass-normalized C4 excr	low	med	0	-0.7215	-1.0821	-0.3608	**0.000035 4**

Mass-normalized C2 excr	low	high	0	-0.7583	-1.1651	-0.3515	**9.87E-05**
Mass-normalized C4 excr	low	high	0	-0.5459	-0.8531	-0.2386	**2.23E-04**
Mass-normalized FI excr	low	med	0	-1.1394	-1.9034	-0.3754	**2.08E-03**
Mass-normalized C5 excr	med	high	0	0.7623	0.2356	1.28914	**2.64E-03**
Mass-normalized C3 excr	med	high	0	0.6469	0.0272	1.26667	**4.00E-02**
Mass-normalized SUVA excr	low	high	0	-0.5524	-1.0874	-0.0173	**4.16E-02**
Mass-normalized C3 excr	low	high	0	0.7933	0.0269	1.55981	**4.18E-02**
Mass-normalized SR excr	low	high	0	-0.5177	-1.071	0.03565	0.0701
Mass-normalized C1 excr	med	high	0	0.5507	-0.1178	1.21926	0.125
Mass-normalized C1 excr	low	med	0	-0.4784	-1.0612	0.10445	0.127
Mass-normalized HIX excr	med	high	0	1.1875	-0.4297	2.80469	0.184
Mass-normalized SUVA excr	med	high	0	-0.3665	-0.9393	0.20629	0.28
Mass-normalized HIX excr	low	med	0	-0.89	-2.3047	0.52477	0.284
Mass-normalized SUVA excr	low	med	0	-0.1859	-0.4963	0.12447	0.326
Mass-normalized C7 excr	low	med	0	0.2682	-0.2164	0.75291	0.382
Mass-normalized SR excr	med	high	0	-0.3558	-1.038	0.32638	0.412
Mass-normalized C2 excr	med	high	0	0.2262	-0.254	0.70648	0.498
Mass-normalized C4 excr	med	high	0	0.1756	-0.2465	0.59778	0.578
Mass-normalized C7 excr	low	high	0	0.1930	-0.3327	0.71885	0.651
Mass-normalized HIX excr	low	high	0	0.2975	-0.6409	1.23596	0.718
Mass-normalized SR excr	low	med	0	-0.1619	-0.7152	0.39147	0.752
Mass-normalized FI excr	med	high	0	0.2271	-0.5977	1.05187	0.784
Mass-normalized C3 excr	low	med	0	0.1464	-0.6346	0.92747	0.882

Mass-normalized BA excr	low	high	0	0.0808 7	-0.421	0.58273	0.913
Mass-normalized BA excr	low	med	0	0.0683 8	-0.454	0.59074	0.941
Mass-normalized C7 excr	med	high	0	- 0.0752	- 0.6334	0.48307	0.943
Mass-normalized C1 excr	low	high	0	0.0723 3	- 0.5105	0.65516	0.952
Mass-normalized BA excr	med	high	0	0.0124 8	- 0.4221	0.44711	0.997

Figures

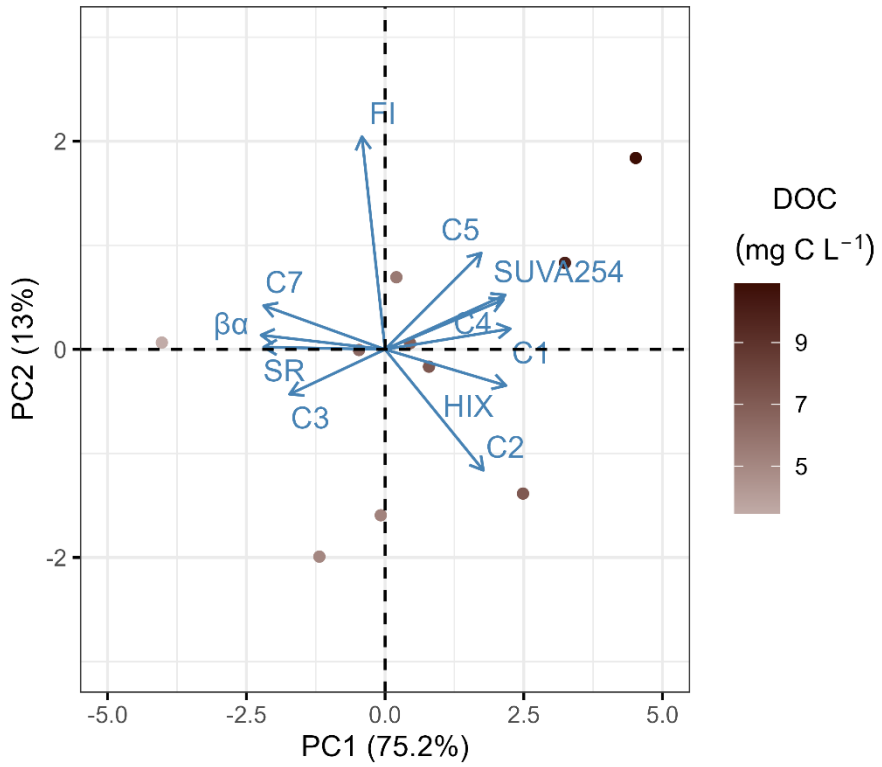

Figure S1. DOC and DOM based on PC1 ranges from low DOC, labile and microbial-like DOM to high DOC, more recalcitrant and humic-like DOM. Principal Components Analysis based on DOM absorbance and fluorescence measurements in 11 lakes waters: ubiquitous humic-like (C1), terrestrial humic-like (C2 and C3), soil, fulvic-like (C4), microbial, humic-like (C5), and protein-derived (C7), specific absorbance at 254 nm (SUVA254), fluorescence index (FI), freshness index (ß:α or ßa on the graph), humification index (HIX), and slope ratio (SR; see methods). Eigenvectors and associated explanatory variables are in blue and solid points represent the 11 lakes and their associated DOC value using a color gradient. PC axis 1 explains 75.2%, while PC axis 2 explains 13% variation in the environmental data.

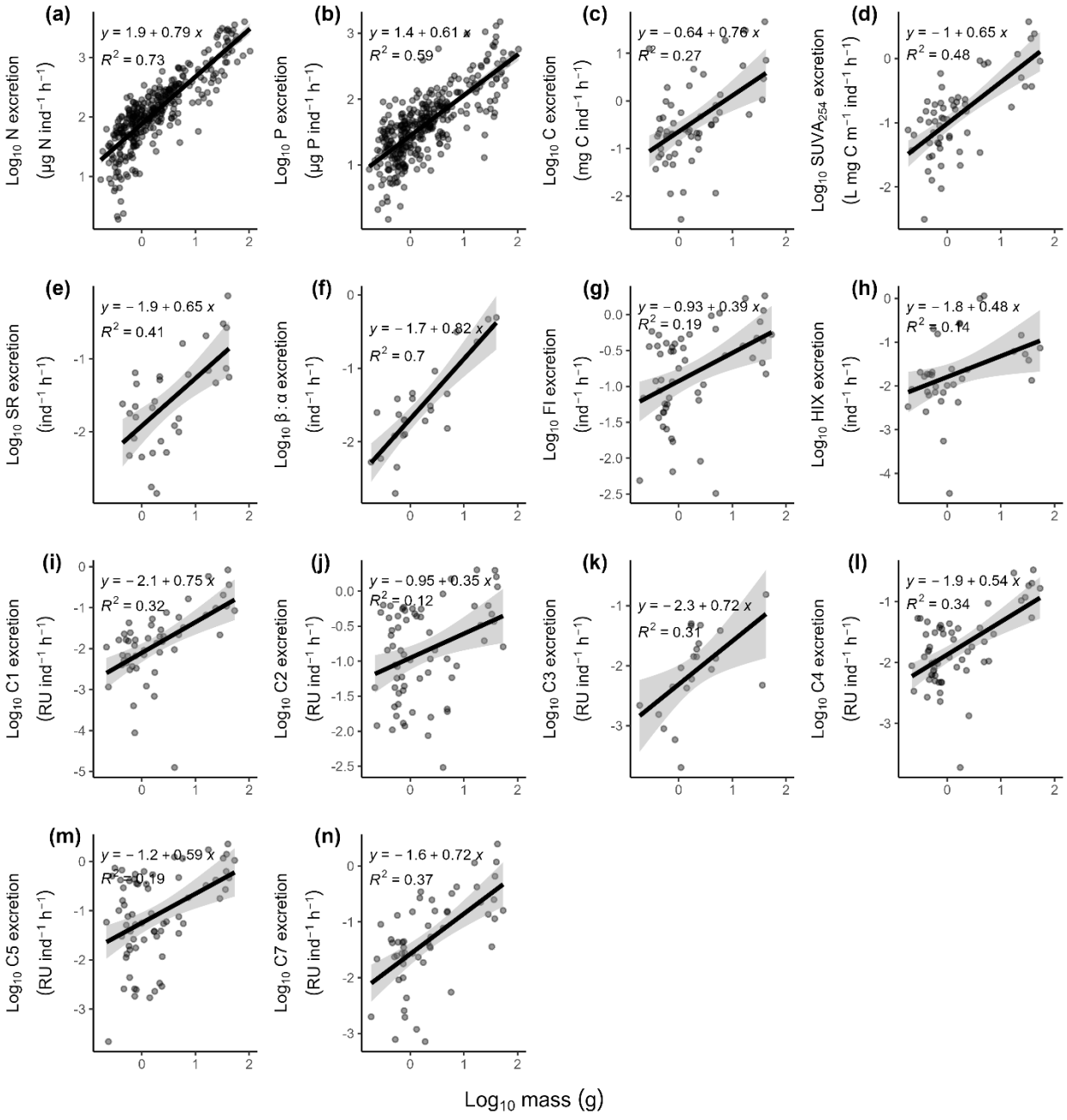

Figure S2. Regression analysis of $\log_{10}$ per capita elemental excretion rates for (a) N, (b) P, (c) DOC, (d) specific absorbance at 254 nm (SUVA254), (e) slope ratio (SR), (f) freshness index (ß:α), (g) fluorescence index (FI), (h) humification index (HIX), (i) ubiquitous humic-like (C1), (j) terrestrial humic-like (C2), (k) terrestrial humic-like (C3), (l) soil, fulvic-like (C4), (m) microbial, humic-like (C5), (n) and protein-derived (C7) relative to $\log_{10}$ mass.

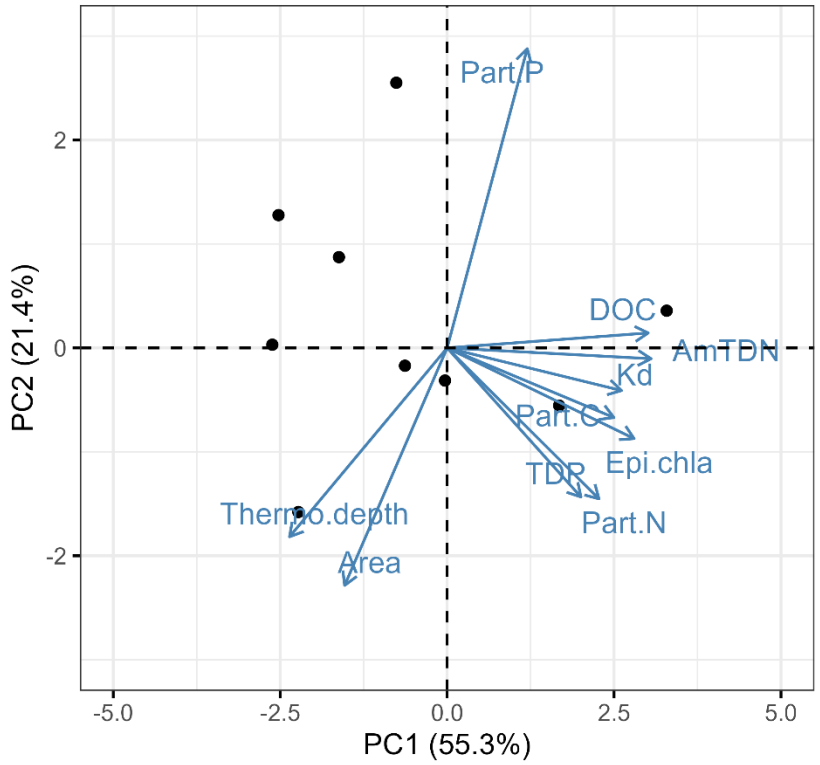

Figure S3. Lakes range from low to high TDP, DOC, K_d and epilimnetic chl-a (PC1) and from high conductivity and particulate P to high surface area and thermocline depth (PC2). Principal Components Analysis of sampled lakes physiochemical parameters parameters measured in each lake including lake surface area (ha), light attenuation (K_d, m^{-1}), thermocline depth (m), conductivity (µS m^{-1}), DOC (mg C L^{-1}), TDP (µg P L^{-1}), particulate C, N, P (µg L^{-1}), epilimnetic chlorophyll-a (chl-a, µg L^{-1}). Eigenvectors and associated explanatory variables are in blue and solid points represent the 11 lakes. PC axis 1 explains 39.1%, while PC axis 2 explains 30% variation in the environmental data.

CHAPTER 5

Whole-lake silver nanoparticles addition promotes phosphorus and silver excretion by yellow perch (*Perca flavescens*)

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Abstract

Fish excretion supports primary production by supplying essential nutrients. However, our understanding of how contaminants affect fish nutrient excretion rates and their persistence in aquatic environments is limited. This is relevant for contaminants like silver nanoparticles (AgNP), increasingly found in aquatic environments due to industrial use and runoff. We

investigated the effect of chronic exposure to AgNP under environmentally relevant conditions on yellow perch (*Perca flavescens*) nutrients and silver (Ag) excretion. Fifteen kg of AgNP were added over two ice-free seasons to a lake at IISD-Experimental Lakes Area in Canada. We measured the nitrogen, phosphorus, and Ag excretion rates and ratios by perch pre-, during, and post the AgNP addition phases in both the experimental and the reference lakes four times over a 10-year period. We found that in Year 2 of AgNP addition, exposed perch P excretion rates and P:Ag excretion ratios increased. However, our empirical P excretion rates in both lakes diverged from model-based predictions of perch P excretion rates, leaving the reasons for the increase in P excretion rates speculative. Nonetheless, Ag release by perch indicates that fish may contribute to legacy Ag contamination in aquatic ecosystems, thereby extending Ag exposure and uptake by aquatic organisms.

Keywords: nanomaterials, animal-mediated nutrient cycling, fish, nitrogen, whole-lake experiment, ecological stoichiometry

Introduction

Fish play an important role in nutrient cycles by excreting nutrients in aquatic ecosystems (Williamson et al. 2018). These recycled nutrients can constitute a significant, albeit variable, proportion of nutrient demand by primary producers in productive ecosystems (Vanni et al. 2006, Sharitt et al. 2021). The nutrient released sometimes exceeds that from watersheds nutrient loading (Williamson et al. 2018) and can create biogeochemical hotspots in lakes and rivers (McIntyre et al. 2008). The contribution of fish excretion to the nutrient pools varies, in part, due

to abiotic factors such as temperature and ambient nutrient concentrations (Wilson and Xenopoulos 2011). Animal nutrient excretion can also be affected by imbalances between their body and diet nutrient composition (Frost et al. 2004), as posited by the ecological stoichiometry theory (ES; Sterner and Elser 2002) and by the bioenergetics arising from their body size and temperature based on predictions from the metabolic theory of ecology (MTE; Brown et al. 2004). However, our understanding of how contaminants impact animal nutrient and contaminant excretion, particularly in higher trophic levels like fish, remains limited (Peace et al. 2021). Studies have mainly focused on invertebrates and controlled conditions, revealing that under contaminant exposure, excretion rates for both nutrients and contaminants increase (Yu and Wang 2002, Taylor et al. 2016, Perrotta et al. 2020). It is therefore needed to assess whether widespread contaminants like silver nanoparticles (AgNP) affect nutrient and contaminant excretion by fish under environmentally relevant conditions to better understand the impacts of contaminant exposure on food webs and biogeochemical processes.

AgNP are antimicrobial nanomaterials commonly used in a wide variety of consumer products, including medical, clothing, home and personal care products (Cohen et al. 2007, Nowack et al. 2012, Tulve et al. 2015, Vance et al. 2015, Zhang et al. 2016). AgNP can enter ecosystems via treated or untreated wastewater discharge or through unusual events such as a spill (Furtado et al. 2015). The concentrations of AgNP in effluents typically range from 0.0032−0.2 μg/L (Mitrano et al. 2012, Li et al. 2013, Cervantes-Avilés et al. 2019). However, given that global AgNP production has surged by two to fourfold over the last decade (Temizel-Sekeryan and Hicks 2020), it is possible that AgNP concentrations will rise in effluents and the environment. Within aquatic ecosystems, total Ag concentrations vary between 1 μg/L and 505 μg/L in Skudai River, Johor, Malaysia (Mat Lazim et al. 2023), while AgNP concentrations are

predicted to range from 0.00004 to 0.619 µg/L (Peters et al. 2018). In lakes, AgNP persist and spread rapidly across the surface area and water column post discharge, even during stratification, and maintain minimal levels of agglomeration and dissolution (Rearick et al. 2018, Martin et al. 2018). The stability and ubiquity of AgNP in natural conditions imply potential risks for detrimental effects on biota (Rearick et al. 2018). Yet, the time scale at which organisms experience AgNP toxic effects and their sensitivity to AgNP can vary relative to ambient conditions and taxonomy (Das et al. 2014, Norman et al. 2015, Conine et al. 2018).

AgNP accumulate in aquatic organisms from microbes and aquatic plants to upper-level consumers such as zooplankton and fish (Das et al. 2014, Norman et al. 2015, Vincent et al. 2017, Martin et al. 2018). Fish in particular can show signs of AgNP exposure with numerous physiological effects reported such as altered gene expression and metabolism (Bruneau et al. 2016, Qiang et al. 2020, Bao et al. 2020). For example, chronic AgNP exposure promotes silver (Ag) accumulation in the gill and liver tissues of yellow perch (*Perca flavescens*) (Martin et al. 2018). AgNP exposed perch also experience oxidative stress and reduced size-at-age in older individuals (Martin et al. 2017, Hayhurst et al. 2020), shift their diet from zooplanktivory to benthivory at a smaller fork length (Hayhurst 2018), and undergo considerable decline in population densities (Hayhurst et al. 2020). These AgNP effects on yellow perch could be accompanied by changes in their nutrient ingestion, assimilation, and release rates and ratios that may diverge from predictions based on ES and MTE (Elser and Urabe 1999). Additionally, although not yet quantified, chronic AgNP exposure could promote Ag release and return to the environment.

In this study, we used a whole-lake addition of AgNP to examine nitrogen, phosphorus, and Ag excretion from yellow perch. We compared fish nutrients and Ag release rates and ratios,

pre-, during (Year 1 and Year 2), and post-AgNP addition in an experimental (Lake 222) and a reference lake (Lake 239) at the IISD-Experimental Lakes Area in northwestern Ontario, Canada. We asked two key questions: *Q1)* Does chronic exposure to AgNP affect fish N, P, N:P, Ag, N:Ag, P:Ag excretion rates and stoichiometric ratios? and *Q2)* Do empirical fish nutrient excretion rates match modelled predictions based on ES and MTE? We predicted that: *Q1)* if AgNP exposure decreases fish size-at-age (Hayhurst et al. 2020) and overall contaminant exposure increases consumer N, P, and contaminant excretion rates (Tsui and Wang 2004, Taylor et al. 2016, Perrotta et al. 2020), then fish N, P, and Ag excretion rates during AgNP addition will increase relative to those pre-AgNP addition, in the reference lake and from Year 1 to Year 2 of AgNP addition, while fish N:P, N:Ag, and P:Ag will remain stable provided that N and P excretion rates increase at similar rates. It then follows that fish N and P excretion rates will decrease post-AgNP addition. We also predicted that *Q2)* if fish nutrient excretion rates fit a mass-balance framework under uncontaminated conditions, then only nutrient excretion rates by fish not exposed to AgNP would match predictions based on ES and MTE. To our knowledge, no study has examined Ag release by fish and here we report the first rates.

Materials and methods

Study design

We added AgNP into Lake 222 (L222) at the IISD-Experimental Lakes Area in northwestern Ontario, Canada (49°41'42.0"N; 93°43'27.7"W) during the 2014 and 2015 ice-free seasons (Year 1 and Year 2 of AgNP addition, respectively) and used nearby Lake 239 (L239) as a reference lake. L222 is an oligotrophic lake on the Canadian Shield with a surface area of approximately 163,900 m^2, a volume of 7.2 x 10^5 m^3, and a maximum depth of 6.3 m. L239 is also an oligotrophic lake with a surface area of 542,800 m^2, a volume of 5.9 x 10^6 m^3, and a maximum

depth of 30.4 m. Both lakes share similar conductivity and pH but L222 has on average higher phosphorus and DOC concentrations. We sampled twenty yellow perch in each lake and tested them for nutrient and Ag excretion during four sampling periods before, during, and after AgNP impact. Our four experiments included August 6th-7th 2012 (pre-AgNP addition), August 13th-15th 2014 (Year 1 of AgNP addition), August 12th 2015 (Year 2 of AgNP addition), and June 10th and 28th 2022 (post-AgNP addition) and followed the Canadian Council for Animal Care's guidelines for best practices (Trent U. AUP #12017-22503 and #26239). Yellow perch was selected because it is the primary fish species in both L222 and L239. They are an important prey for the northern pike and are widespread in North America. At the time of sampling in Year 1 of AgNP addition, perch had been exposed to AgNP for 59 days, while in Year 2 of AgNP addition, perch had been exposed for 493 days, with our tested fish having been exposed for less than a year given that these young-of-the-year.

Whole-lake AgNP addition

A total of 15 kg of polyvinylpyrrolidone (PVP)-capped AgNP with additional gum arabic stabilization were added in L222 from a point source near the lake in-flow over two field seasons in 2014 and 2015. In 2014, a total of 9 kg were added over 18 weeks from mid-June to late October, while in 2015, a total of 6 kg were added over 14 weeks from early May to late August. This addition approach simulated point source inputs from municipal wastewater treatment plants. AgNP were prepared and characterized (Martin et al. 2017) to create AgNP suspensions (5.2 g/L) consisting of 30–50 nm diameter particles which, when released into the lakes, shifted to mean diameters in the 20 nm range (Martin et al. 2018). These suspensions were pumped into the lake every 6 h using a peristaltic pump on a timer. AgNP dispersed quickly throughout the entire lake within 24 h of the first dose (Rearick et al. 2018). Total Ag (TAg) was collected using

carbon nanotube integrative samplers (CNIS) deployed in the water column, while dissolved Ag and AgNP were sampled directly from surface water. Detailed methods are described in Shen et al. (Shen et al. 2016) and Martin et al. (Martin et al. 2018). Total Ag concentrations varied between 1 and 11.5 µg/L, with a method detection limit calculated as 3 times the standard deviation of the concentrations in each batch of the procedural blanks (< 0.06 µg/L), positioning them at the lower end of natural levels in freshwater ecosystems (Mat Lazim et al. 2023), but surpassing typical effluent concentrations (Li et al. 2013, Cervantes-Avilés et al. 2019). Dissolved Ag remained in low concentrations (<0.34 µg/L) and was an overall low contributor to Ag (Rearick et al. 2018, Martin et al. 2018). Total Ag concentrations observed in AgNP were overall relatively stable and persistent in the lake likely due to the lake low ionic strength and high DOC concentrations (Furtado et al. 2015).

Excretion experiment and elemental analyses

Twenty juvenile yellow perch (indicated by fish fork length) were collected from each lake by seine net and kept for nutrient and Ag release studies. Sample size was determined from prior excretion experiments using fish in lakes (Higgins et al. 2006). Fish tested pre-, in Year 1, and post AgNP addition were between ages 0 and 2 (38 to 104 mm in fork length), while those tested in Year 2 of AgNP addition were of age 0 (32 to 44 mm in fork length) (Hayhurst et al. 2020). All of those fish would be considered zoobenthivorous, feeding primarily on zooplankton and small insects, with a switch from zooplanktivory to benthivory for fish between 75 and 100 mm (DeBoer 2017, Hayhurst et al. 2020). Following collection, each fish was placed in a whirl-pak bag with 0.2-0.75 L of base water collected from each source lake (experimental or reference lake) and prefiltered through 0.7 µm Whatman GF/F glass microfiber filters. Only fish post-AgNP addition in both the experimental and reference lakes were incubated using prefiltered

base water from another lake in the study area (L626) with low nutrient concentrations (N = 8.7 µg N/L, P = 1.7 µg P/L). Bags containing sampled individuals and five additional bags without individuals (controls) were incubated for 30 minutes in a tub filled with lake water collected at the time of the experiment and kept in the shade to maintain water temperature at ambient lake temperature. After incubation, individuals were removed from the bags, placed on ice, and weighed (wet mass) on site. Wet mass was then converted to dry mass using a conversion factor of 0.25 (Vanni and McIntyre 2016).

Excretion water samples from each incubation were filtered through a 0.22 µm polycarbonate membrane filter kept on ice until analysis. Samples were then quantified as total dissolved nitrogen (TDN, µg N/L) and total dissolved phosphorus (TDP, µg P/L) for samples pre- to Year 2 of AgNP addition and as ammonia (NH_4^+-N, µg N/L) and soluble reactive phosphorus (SRP, µg P/L) for samples post-AgNP addition. Sub-samples of unfiltered excretion water samples from the experimental lake were kept to quantify TAg. TDN was measured after persulfate digestion using a spectrophotometer and TDP was quantified using the molybdate blue method after digestion with persulfate (Murphy and Riley 1962). NH_4^+ and SRP were analyzed using the phenate (Solórzano 1969) and molybdate blue methods (Murphy and Riley 1962), respectively, without any persulfate digestion. TAg was analyzed by first acidifying the samples to 4% nitric acid and storing them at 4 °C, then heating the samples for 6 h at 70 °C to digest all organic matter, followed by inductively coupled plasma mass spectrometry (ICP-MS) (Furtado et al. 2015). Dissolved Ag concentrations and AgNP particle size in ambient water was quantified using single-particle ICP-MS (Rearick et al. 2018, Martin et al. 2018). Due to logistical constraints, TDN in water samples from the reference lake in Year 1 of AgNP addition could not be determined.

Nutrient and TAg excretion rates were calculated by subtracting the N, P, or TAg concentration in the prefiltered base water in the control bags (i.e., initial concentration) from the N, P, or TAg concentration in the water samples in the experimental bags post-incubation (i.e., final concentration). Mass-specific N, P, or TAg excretion rates were then calculated by dividing individual nutrient excretion rates by mass (µg N or P or Ag/g/h), and only positive values were kept for statistical analysis. Some mass-specific N and P excretion rates were below detection limit due to measurement error, while all mass-specific TAg excretion rates were above detection limit. This resulted in a sample size that ranged from four to twenty individuals depending on the lake, year, and element analysed (table S1).

Statistical analyses

Q1) Experimentally tested perch nutrient & total silver excretion rates and ratios

We first ran a two-way analysis of variance (ANOVA) to test whether fish dry mass differed between lakes and across years, then ran post-hoc tests (emmeans package, v1.8.6; Lenth 2021) to compare lakes across years. Second, we ran an analysis of covariance (ANCOVA) to test whether there was an interactive effect between dry mass and lake or year on perch nutrient excretion rates. Only year had an interactive effect with fish dry mass for perch P excretion rates ($F_{7,121} = 5.4$, $P < 0.001$), although fish dry mass in the experimental and reference lakes did not differ pre- and post-AgNP addition (P = 0.06 and 0.99, 95% CI [0.33, 1.01] and [0.4, 1.74] respectively; figure S1). To account for the temporal autocorrelation in each lake over the study period, we ran a repeated measure ANOVA using site class (i.e., treatment and control) and period (i.e., before, during, and after) as fixed factors with an interaction effect, and lake and year as random factors (lmer, lmerTest package, v3.1-3; Kuznetsova et al. 2017). However, due to the lack of replication and low sample size in some years, none of the models converged.

Although it cannot account for the temporal autocorrelation, a two-way ANOVA appeared to be a better approach to quantify differences in fish nutrient excretion rates between lakes and across years.

Accordingly, we ran two-way ANOVAs to test the effect of AgNP addition on perch nutrient excretion by comparing mass-specific N, P, and the molar ratio of N:P excretion rates in both lakes pre- during, and post-AgNP addition (model structure: *nutrient excretion ~ Lake*Year*). Given that nutrient excretion rates were significantly different across lakes and years, we ran post-hoc tests to compare lakes across years and to determine the effect size (i.e., the magnitude of difference between each lake) pre-, during, and post-AgNP addition at $p < 0.05$. Lastly, we ran non-parametric two-sample Wilcoxon rank sum tests to assess the effect of the year of AgNP addition (Year 1 or Year 2) in the experimental lake on Ag and nutrient excretion rates and ratios (model structure: *Ag or nutrient:Ag excretion ~ Year).* Dry mass and all nutrient and silver excretion rates and ratios were $\log_{10}$-transformed as the data did not meet normality assumptions.

Q2) Modelled perch nutrient ingestion and excretion rates

To further explain the unexpected results for P excretion rates pre-, during and post-AgNP addition, N, P, and C fluxes were simulated using two models based on ES and MTE. The first model was chosen for its flexibility, enabling parameter specification based on both empirically measured data from this study and literature sources (Schiettekatte et al. 2020). The second model was selected for its broad applicability, having been developed using a comprehensive global database of aquatic animal nutrient excretion rates (Vanni and McIntyre 2016). Both models were used to simulate nutrient excretion rates by unexposed fish in any given lake at the Experimental Lakes Area, with the second model serving to compare and

validate the outcomes of the first model. Thus, model parameters were assumed to be the same in the two lakes, and pre- and during AgNP addition.

The first model was built from a mass-balance framework (fishflux package, v0.0.1.6; Schiettekatte et al. 2020) and used as our main model. Model inputs included element-specific assimilation efficiencies, diet (i.e., benthic invertebrates) stoichiometry (Frost et al. 2003), fish body stoichiometry (Tanner et al. 2000), growth and minimal inorganic flux parameters estimated by Schiettekatte et al. (2020) and found in Fishbase (Froese and Pauly 2022) using fishflux functions, and activity scope calculated based on the related species European perch (*Perca fluviatilis*; Jensen et al. 2017). Detailed methods of the model are described by Schiettekatte et al. (2020) and in the supplementary information. Briefly, the elements (C, N, and P) needed for growth and minimal inorganic flux are coupled with the element-specific assimilation efficiency to estimate the required ingestion of C, N, and P. The limiting element, calculated based on the imbalance between required and diet C:N:P ratios, then informs the C ingestion rate. Following ingestion, elements are either egested or assimilated (body mass growth and inorganic flux). Model outputs include C ingestion rates (I_c), N and P excretion rates (Ex_N and Ex_P), growth rates (G_k), and limiting CNP element for fish of a given size range. These were used to determine whether our experimental results matched those predicted by the model.

Ingestion was estimated following two steps (Schiettekatte et al. 2020). First, the minimal required ingestion of the element k (C, N, and P) was defined as the sum of C, N, and P needed to maintain growth and body stoichiometry. This ingestion rate was set as a fixed value calculated by summing element-specific minimal supply rates ($S_{k;}$ Supporting Information$_{)}$. Second, ingestion rates were approximated using ES by estimating the limiting element using S_k and diet stoichiometry (D_c, D_N, and D_P in % of body content).

$$\textbf{Limiting element} = \begin{cases} \textbf{C if } \frac{S_C}{S_N} > \frac{D_C}{D_N} \textbf{ and } \frac{S_C}{S_P} > \frac{D_C}{D_P} \\ \textbf{N if } \frac{S_N}{S_P} > \frac{D_N}{D_P} \textbf{ and } \frac{S_C}{S_N} < \frac{D_C}{D_N} \\ \textbf{P, otherwise} \end{cases} \quad (1)$$

Following Liebig's minimum law, we assume that there is enough food to meet fish minimum needs (S_k). For example, if P is limiting, element-specific ingestion rates, I_k, (µg/day) are:

$$I_P = S_P \quad (2)$$

$$I_N = I_P \frac{D_N}{D_P} \quad (3)$$

$$I_C = I_N \frac{D_C}{D_P} \quad (4)$$

Once I_k is estimated, the ingested material can be partitioned between egestion (Eg_k) and excretion (Ex_k; i.e. total inorganic flux). Excretion can be further partitioned between minimal excretion rate (Ex_{0k}) and residual excretion rate (Ex_{rk}). Minimal excretion rate can either be due to metabolic costs (Ex_{0C}) or N or P minimal release (Ex_{0N} or Ex_{0P}), even if the element is limiting (Sterner and Elser 2002, Anderson et al. 2005). Yet due to element limitation, other elements may be consumed in excess in order to meet the individual's needs for that limiting element. As a

consequence, the exceeding 'residual' element will be subject to post-absorptive release via excretion (i.e. residual excretion rate Ex_{rk}) to maintain body homeostasis (Anderson et al. 2005). We can thus estimate element-specific excretion rates E_{xk} (µg/day) as:

$$\boldsymbol{Ex_k = Ex_{0k} + Ex_{rk},} \quad \textbf{(5)}$$

where

$$\boldsymbol{Ex_{rk} = I_k - G_k - Ex_{0k} - Eg_k} \quad \textbf{(6)}$$

We also simulated the effect of variations in diet stoichiometry (varying levels of %P and %N with %C kept at 45%) on the element limitation and nutrient fluxes of an individual perch of 0.3 g.

The second model was based on the "universal" models generated from more than 10,000 N and P excretion observations collected from the world's freshwater and marine vertebrates and invertebrates (Vanni and McIntyre 2016). We used this model to compare its calculated estimates to the outputs based on the Schiettekatte et al. (2020) model to evaluate whether these predicted our nutrient empirical rates better. Fish were assumed to feed on invertebrates only. N excretion rates were estimated:

$$\begin{aligned} &\boldsymbol{log_{10} N} \\ &\boldsymbol{= 1.461} \\ &\boldsymbol{+ 0.684 \times log_{10}(dry\ mass)} \\ &\boldsymbol{+ 0.0246 \times log_{10}(temperature)} \\ &\boldsymbol{- 0.2013 + 0.7804} \end{aligned} \quad \textbf{(7)}$$

P excretion rates were estimated following:

$$\begin{aligned} & \boldsymbol{log_{10} P} \\ & \boldsymbol{= 0.6757} \\ & \boldsymbol{+ 0.5656 \times log_{10}(dry\ mass)} \\ & \boldsymbol{+ 0.0194 \times log_{10}(temperature)} \\ & \boldsymbol{- 0.2480 + 0.7504} \end{aligned} \quad \textbf{(8)}$$

Mean fish dry mass and experimental temperature were used for each lake and year. Considering that the experimental temperatures were missing pre-AgNP addition, a random number between 16 and 20 (°C) was generated for each lake. All statistical analyses were done using R Statistical Software (v4.3.0; R Core Team 2023) and RStudio (v2023.06.1+524; RStudio Team 2023) on a Windows PC (v 21H2).

Results

Q1) Empirical perch nutrient & total silver excretion rates and ratios

Perch dry mass significantly varied between lakes and across years (ANOVA, $F_{7, 124} = 38.36$, $P < 0.001$; Figure S1). Specifically, perch in the experimental lake were significantly bigger than those in the reference lake pre-, in Year 1, and post-AgNP addition *(*$P < 0.001$; table S2 and figure S1). These differences in perch mass were accounted for by correcting nutrient excretion rates and ratios by mass and testing fish pre- and post-AgNP addition of similar size range.

Mass-specific nutrient excretion pre- and post AgNP addition

In the pre-AgNP addition phase, mass-specific N excretion rates were similar between lakes ($P = 0.94$; tables S1 & S2, figure 1a). Conversely, mass-specific P excretion rates were higher in the reference lake than in the experimental lake ($P < 0.001$; table S3 and figure 1c). Post-AgNP addition, both mass-specific N and P excretion rates were similar between lakes (N excretion: *P*

= 0.91, table S2 and figure 1a; P excretion: $P = 0.99$; table S3 and figure 1c). Additionally, mass-specific N and P excretion rates were comparable for any given lake pre- and post-AgNP addition, except for mass-specific P excretion rates in the reference lake which decreased from pre- to post-AgNP addition (tables S2 & S3), although this could be due to the difference in P form (TDP vs SRP).

Mass-specific nutrient excretion during AgNP addition

During the AgNP addition years, mass-specific N excretion rates increased in both lakes (experimental lake: $P < 0.001$; reference lake: $P = 0.02$, table S3) and while these changes were not statistically different between lakes ($P = 0.29$; table S3 and figure 1a), the effect size of mass-specific N excretion rates between lakes was almost four times higher in Year 2 of AgNP addition compared to pre-AgNP addition ($d = 2.58$ and $d = 0.73$, respectively; figure 1b). Mass-specific P excretion rates only increased in the experimental lake in Year 2 of AgNP addition relative to pre-AgNP addition ($P < 0.001$, table S2, figure 1c) but did not differ significantly from those in the reference lake ($P = 0.91$, table S2, figure 1c). Further, the effect sizes of mass-specific P excretion rates between lakes pre- and during Year 1 of AgNP additions were particularly high ($d = 15.33$ and $d = 8.9$, respectively) and substantially decreased in Year 2 of AgNP additions ($d = 1.49$; figure 1d). As a result, mass-specific N:P excretion rates increased from pre- to Year 1 of AgNP addition in both lakes, although it was only significant in the experimental lake (experimental lake: $P = 0.04$; reference lake: $P = 0.05$; table S2, figure 1e). However, mass-specific N:P excretion rates did not significantly differ between lakes in Year 2 of AgNP addition ($P = 0.4$; table S2), a result further supported by the overlapping effect sizes pre- and during Year 2 of AgNP addition ($d = 3.32$ and $d = 2.32$, respectively; figure 1f).

Additionally, a few fish in both the experimental and reference lakes excreted at particularly high N and N:P rates (figures 1a-c-e).

Fish exposed to AgNP released Ag, however, the rates of Ag excretion rates were similar between years (Wilcoxon, $P = 0.14$; Figure 2a). Moreover, N:Ag and P:Ag excretion rates increased in the second year, although this was only significant for P:Ag excretion rates (Wilcoxon, $P < 0.01$; Figure 2b-c).

Q2) Modelled perch nutrient ingestion and excretion rates

The model developed by Schiettekatte et al. (2020) accurately predicted N excretion rates from both lakes pre- and post-AgNP addition and most of N excretion rates from the reference lake in Year 2 of AgNP addition with almost all tested individuals falling within the 50-95% confidence intervals (Figure 3a). However, most of the tested N excretion rates from the experimental lake in Year 2 of AgNP addition fell outside of the predicted range, with excretion rates more than 20x higher than predicted rates at comparatively low dry mass (figure 3a). Conversely, the model did not predict P excretion rates accurately with almost all tested individuals falling outside of the 50-95% confidence interval (figure 3b). Surprisingly, the two individuals that excreted P at considerably high rates at a comparatively low dry mass were from the reference lake (figure 3b). The poor prediction strength of our empirical P excretion rates was corroborated with a predicted P-limitation for perch up to 2.1 g (figure S2) which, in theory, should lead to lower P excretion rates than those simulated by the model and observed in our empirical results. Moreover, model simulations showed that an N-limited diet with higher %P and lower %N content than our estimated perch diet and high C ingestion rates would yield the highest P excretion rates (figure 4a-d), although predicted C ingestion rates and N and P excretion rates

were an order of magnitude lower than those predicted using our estimated perch diet (figure 4b-c-d, figure 1a-c, figure S3). It is therefore unclear whether our tested perch were P- or N-limited.

Simulations based on the model developed by Vanni and McIntyre (2016) predicted higher nutrient excretion rates than those based on the model built by Schiettekatte et al. (2020); however these predictions did not match our empirical nutrient excretion rates (figure 3c-d). While simulated N excretion rates fell within the 50% confidence interval range predicted by the Schiettekatte et al. (2020) model, these overestimated empirical N excretion from both lakes pre- and post-AgNP addition, but underestimated rates from both lakes during AgNP addition (figure 3c). Similarly, simulated P excretion rates based on the second model were much higher than those based on the model by Schiettekatte et al. (2020). While simulations overestimated most empirical P excretion except for a few, especially from the reference lake (figure 3d), these showed a better fit to empirical P excretion compared to those based on the model built by Schiettekatte et al. (2020).

Discussion

We measured nutrients release in yellow perch before, during, and after a two-year whole-lake AgNP addition experiment four times over a 10-year period. We also measured Ag release during the exposure years. To our knowledge, this study is the first to document the effects of AgNP on nutrient release and to report Ag release rates from fish exposed to chronic levels of environmentally relevant AgNP. While recycling of nutrients is one important ecosystem process that is directly influenced by fish, our results demonstrate that they can also return contaminants to the water column, potentially contributing to legacy contamination in aquatic environments.

Overall, our results only partially supported our initial predictions. Our first prediction was that AgNP exposure would increase N and P excretion rates and maintain N:P excretion

rates. We found that P release in the exposed fish increased in Year 2 of AgNP addition compared to the fish pre-AgNP addition and those in the reference lake but did not differ from those in the reference lake in Year 2 of AgNP addition and seven years post-AgNP addition. Conversely, fish N release increased in both the experimental and reference lakes relative to pre-AgNP addition, then decreased post-AgNP addition. As a result, perch N:P excretion rates increased in both lakes but did not show any significant effect from AgNP exposure. Additionally, most N, P, and N:P excretion seven years post-AgNP addition were consistent with those pre-AgNP addition. Overall, the presence of temporal autocorrelation in our ANOVA models might have constrained our capacity to establish a causal relationship between AgNP exposure and the observed nutrient excretion rates and ratios. Our prediction that Ag excretion rates would increase from Year 1 to Year 2 of AgNP addition while nutrient:Ag would remain stable was countered with an observed absence of change in Ag excretion rates and a significant increase in P:Ag excretion ratios with AgNP exposure. Lastly, our prediction that only nutrient excretion rates by fish unexposed to AgNP would match predictions based on ES and MTE was partially supported. Model-based estimates indicated that although our empirical N excretion rates fit predictions for most fish unexposed to AgNP, our empirical P excretion rates diverged from P excretion rates expected for the fish size range we tested. Given the low ambient P levels in our studied lakes, the high P excretion by fish could represent an important internal source of bioavailable P for primary production, even in the presence of contaminants.

Empirical individual P excretion rates by perch increased in Year 2 of AgNP addition, although this increase may not be directly caused by AgNP exposure and could be dampened at the population level given the considerable decline in perch density in the experimental lake (Hayhurst et al. 2020). The decline in perch density in the experimental lake was attributed to

oxidative stress at the cellular level leading to a reduction in fish size-at-age, prey consumption, and metabolism at the individual level (Hayhurst et al. 2020), much like other studies have reported (e.g., Bilberg et al. 2010, Bruneau et al. 2016, Valerio-García et al. 2017, Qiang et al. 2020, Bao et al. 2020). Reduced fish size-at-age, activation of detoxification pathways, and heightened energy demands due to AgNP exposure might have disrupted metabolic processes, caused elemental imbalances, and altered elemental limitation (Peace et al. 2021). Elemental imbalances may have led to lower body P requirements and the release of excess dietary P (Showalter et al. 2016), thereby explaining our observed increase in P excretion rates in exposed perch. Alternatively, diminished growth and C:P ratio in phytoplankton could have translated into lower C:P in zooplankton and excess dietary P for perch (Das et al. 2014, Peace et al. 2021), potentially causing the observed high P excretion rates. Furthermore, we found significantly higher P excretion rates in the reference lake than in the experimental lake in both pre- and Year 1 of AgNP addition. This difference is likely due to the high abundance of P-rich amphipods in the reference lake and their rarity/potential absence from the experimental lake based on the benthic invertebrates community structure of nearby lake L223 (Frost et al. 2003, Tonin 2019). Yet at the ecosystem scale, perch declining density in the experimental lake from pre- to Year 2 of AgNP addition could hinder the increase in individual P excretion rates and even decrease perch contribution to the P pool as animal biomass often drives population nutrient excretion rates (Frauendorf et al. 2020, Hopper et al. 2020). These changes could also be enhanced by high individual N:P excretion rates during AgNP addition.

AgNP exposure did not affect fish N:P excretion rates. Nonetheless, N and N:P excretion rates by fish in both lakes were variable and high, with values often much higher than the Redfield N:P ratio of 16:1 (Redfield 1958, Ptacnik et al. 2010). Fish diet and body stoichiometry

change significantly throughout ontogeny (Showalter et al. 2016). Our sampled fish ranged from age 0 to 2; thus, the observed variations in N and N:P excretion rates in Year 2 of AgNP addition may be driven by age-specific changes in diet and body stoichiometry. Furthermore, high N:P excretion rates suggest that there was a relative excess dietary N (Showalter et al. 2016). Considering that fish in Year 2 of AgNP addition were smaller than fish pre-AgNP addition, it is possible that a higher proportion of tested fish mostly consumed zooplanktons such as copepods which could be more N-rich than benthic invertebrates (Frost et al. 2003, Showalter et al. 2016, Tonin et al. 2022). High N:P excretion rates have also been observed in mottled sculpin and can be the result of P limitation and strict homeostasis (McManamay et al. 2011), which would support our model predicted P limitation, but challenge our elevated empirical P excretion rates. Whether this is the case for the yellow perch remains speculative and would require measurements on perch body and diet N:P content. At the ecosystem scale, high N:P excretion rates could also dampen the observed increased P excretion rates and contribute to a higher lake ambient N:P. However, these results should be extrapolated with care given the absence of data on N excretion rates for Year 1 of AgNP addition and population-level excretion rates..

Our nutrient release simulations based on the Schiettekatte et al. (2020) model supported our empirical N excretion rates for most fish unexposed to AgNP but failed to predict our observed P excretion rates for both lakes and across years. The overlap between most of our empirical and modelled N excretion rates suggests that this model can predict perch nutrient fluxes. To compare and validate the results from the Schiettekatte et al. (2020), model we used the “universal” model developed by Vanni and McIntyre (2016) based on N and P excretion rates of aquatic animals worldwide. This model predicted higher N and P excretion rates than the Schiettekatte et al. (2020) model, although the predicted N excretion rates from both models

were more similar than the predicted P excretion rates. Additionally, the predicted P excretion rates based on the Vanni and McIntyre model (2016) better matched our empirical P excretion rates across lakes and years than those based on the Schiettekatte et al. model (2020), but still overestimated most rates by fish unexposed to AgNP. Overall, we demonstrate that it may be harder to predict P relative to N excretion rates using theoretical models, likely due to the high variability in body P requirements across taxonomic groups and life stages, which is itself driven by the diversity of body forms and P investment in bony structures (May and El-Sabaawi 2022, 2024).

One limitation of the Schiettekatte et al. (2020) model compared to the Vanni and McIntyre model (2016) is its dependence on a wider array of input parameters for predicting nutrient excretion rates. Most of our model input parameters for the Schiettekatte et al. (2020) model were retrieved from the literature, occasionally based on experiments done at different geographical locations, times or species. It is thus possible that these parameters did not adequately fit the specific context in which our empirical P excretion rates were measured. For example, our extracted von Bertalanffy growth parameters were mostly calculated from scale annual rings analysis instead of otolith analysis and may not be appropriate for juveniles. Furthermore, our P turnover rate may not be appropriate for yellow perch at the low temperatures we tested them (Schiettekatte et al. 2020). The model also assumes strict homeostasis, although juvenile fish are likely to vary in body stoichiometry, especially following a diet shift. These inadequacies may have affected the predicted limiting element and C ingestion rates, thereby underestimating P excretion rates. It is thus unclear whether our tested perch are P- or N-limited. Future studies could measure more growth and minimal inorganic flux parameters empirically and potentially expand this model to predict other types of fluxes such as contaminants.

These are the first measurements of Ag excretion from fish, though in our study, chronic AgNP exposure did not change perch Ag excretion rates. Other studies have demonstrated that zooplanktons and snails release mercury, metals, and antimicrobial agents following exposure to contaminants (Yu and Wang 2002, Tsui and Wang 2004, Taylor et al. 2016). Fish can detoxify the blood using three main excretion mechanisms: the branchial (via the gills), hepatic (via the liver), and renal (via the kidney) (Handy et al. 2008). While Ag concentrations were almost as high in perch gills as in their liver (Martin et al. 2018), branchial excretion is unlikely in our study as solute diffusion would have required the fish to be in an environment where Ag concentrations are lower than those in the gills (Handy et al. 2008). An alternative hypothesis is that the absorption of AgNP into the liver would have likely resulted in the dissolution of AgNP to Ag and its redistribution to other organs such as bones, via the blood (Al-Sid-Cheikh et al. 2019). Given that Ag has been found in fish bones, hepatic excretion could be a route for Ag spread in the whole body. Lastly, filtration through the kidney is only possible for small AgNP given that the molecular weight cutoff for the glomerular filter is 60 kDa and thus fits the average size of AgNP of 20 nm found in the experimental lake. We did not measure Ag in the kidneys of these perch but they do accumulate in fish kidneys (Pannetier et al. 2016, Martin et al. 2018, Al-Sid-Cheikh et al. 2019), suggesting that renal excretion is a likely route for Ag release. Additionally, both dietary and waterborne uptake could have contributed to Ag accumulation in perch tissue (Martin et al. 2018).

The uptake and release of Ag by perch may have important implications on perch metabolism and on Ag persistence in the environment. Chronic AgNP exposure increased P:Ag excretion rates and could indicate stress or damage from Ag presence in perch tissue. As previously noted, dissolved Ag can accumulate in fish bones and could later precipitate in salts

and alter bone cell function (Pounds et al. 1991, Al-Sid-Cheikh et al. 2019). Similarly, it is possible that Ag presence in perch tissues impaired P deposition into perch bones and increased P release via the kidney, while Ag release via the kidney remained stable due to its partial translocation to other fish organs via the hepatic route. This would have led to the observed higher P excretion rates per Ag unit. If bone formation was compromised, this could also explain perch reduced growth under AgNP exposure (Hayhurst et al. 2020). Lastly, perch high Ag excretion rates indicate that Ag release in the environment promotes Ag recirculation in the water column thus prolonging Ag exposure and uptake by fish and other organisms.

In our whole-lake AgNP addition experiment, we found that yellow perch under chronic AgNP exposure excreted P at higher rates than pre-AgNP addition but maintained similar rates post-AgNP addition. Perch also released Ag at a consistent rate between Year 1 and Year 2 of AgNP addition. We further showed that while AgNP exposure did not affect fish N and N:P excretion rates, fish displayed a higher variability in N and N:P excretion rates than their counterparts in the reference lake. Additionally, results from two mathematical models indicated that P excretion rates were harder to predict in both the experimental and reference lakes than N excretion rates. Thus, the extrapolation of empirical P excretion rates by exposed perch warrants caution. The reference and experimental lakes had similar physical and chemical characteristics except for their size, ambient dissolved organic carbon (DOC) and total dissolved P, with the experimental lake being smaller (16.39 vs. 54. ha) and showing higher DOC (12.1 vs. 6.8 mg/L) and TDP (9.8 vs. 6.3 mg/L) from 2012 to 2016 (Hayhurst 2018). While the effects of these variables on perch P excretion rates were not directly examined, they may have contributed to the observed variations in P excretion rates between the two lakes pre- and in Year 1 of AgNP addition. Overall, given that most N and P excretion rates were consistent post-AgNP addition

between lakes and relative to pre-AgNP addition, we argue that AgNP are unlikely to affect perch nutrient excretion rates and ratios long-term (i.e., seven years after exposure) once external AgNP inputs cease.

Our findings warrant further investigation to clearly determine whether AgNP directly or indirectly affect fish nutrient excretion rates and ratios. Most importantly, our study provides the first quantitative assessment of fish nutrient and Ag release when exposed to AgNP under environmentally relevant conditions and adds another layer to studies assessing the effect of AgNP in a natural setting on fish overall health and on aquatic ecosystems functioning (Rearick et al. 2018, Martin et al. 2018, Hayhurst et al. 2020). While we did not test fish Ag excretion post-AgNP addition, recent findings allow us to infer on potential Ag excretion rates post-AgNP addition. For example, yellow perch growth and population density decreased steadily during and post-AgNP addition (Hayhurst et al. 2020, Slongo et al. 2022). Similarly, the concentration of Ag in perch liver declined rapidly from Spring to Fall of the first year post-AgNP addition: from half of the levels in the second year of AgNP addition to null (Martin et al. 2018). Because of these changes and our observed lower N excretion rates post-AgNP addition relative to the AgNP addition phase, we suspect that Ag excretion rates post-AgNP addition were much lower than the levels observed during AgNP addition. Future work could involve measuring fish age class-specific nutrient excretion rates and ratio and Ag release post-AgNP addition and scale it to the lake perch population, while monitoring fish responses at the cellular, individual, and population levels. Additionally, evaluating the ecotoxicological effects of AgNP exposure on the relative role that fish populations play in nutrient cycling of P-poor lakes compared to other sources of nutrients would be key to assess the impact of AgNP contamination.

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Figures

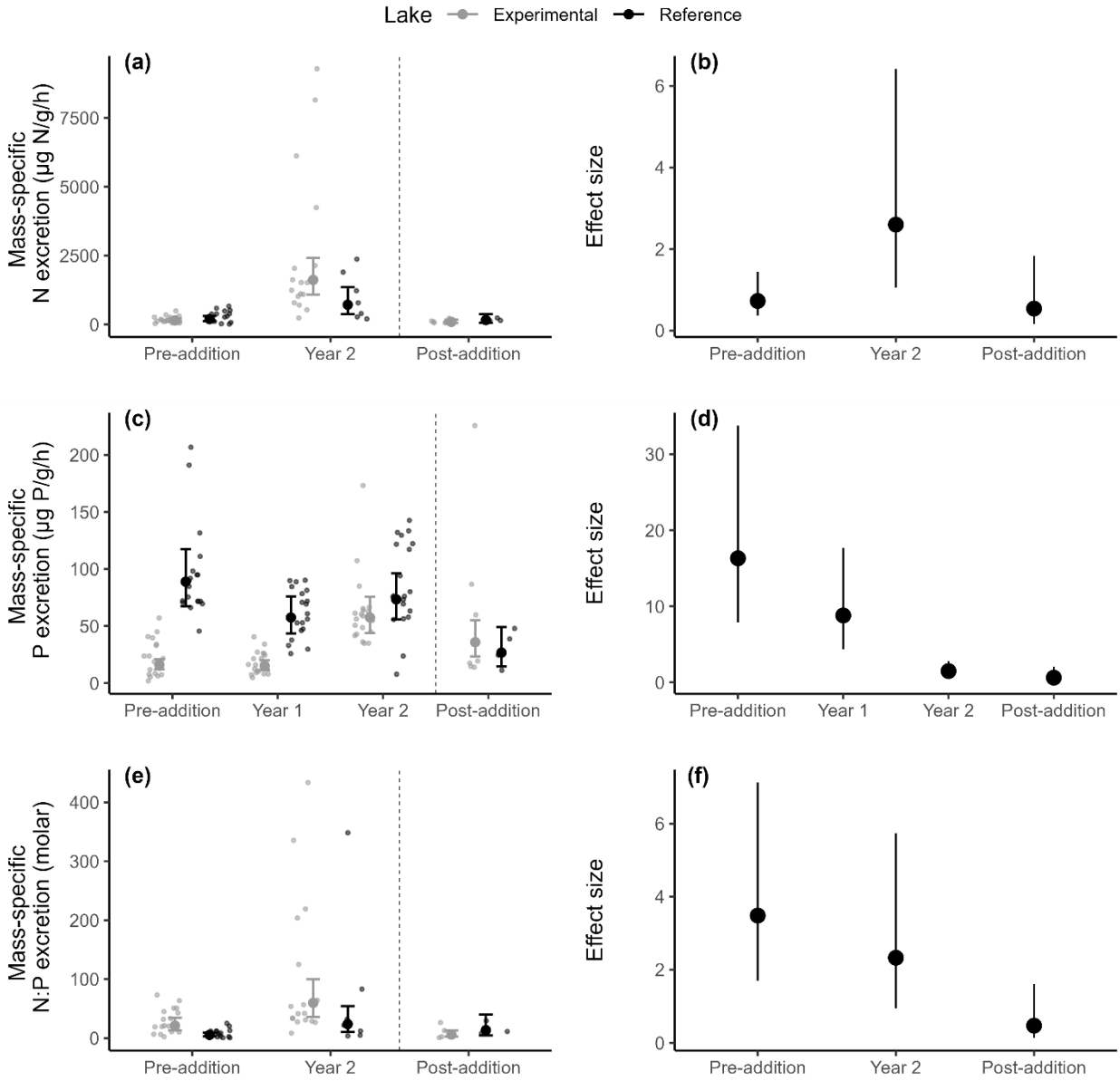

Figure 1. AgNP may affect perch P excretion rates in Year 2 of AgNP addition. Empirical mass-specific (a, c, e) N, P, and N:P excretion rates, respectively, and (b, d, f) their respective effect sizes pre-, during, and post-AgNP addition in the experimental and reference lakes. Effect sizes were back-transformed from the original $\log_{10}$ transformation. Large symbols represent mean fitted values, and small symbols represent individuals by lake. Error bars are 95%

confidence intervals and contrasting letters indicate significant differences. The dashed vertical line demarcates mass-specific N and P excretion rates pre- to Year 2 of AgNP addition from those post-AgNP addition due to the difference in N and P form quantified (TDN and TDP vs. NH_4^+ and SRP, see methods).

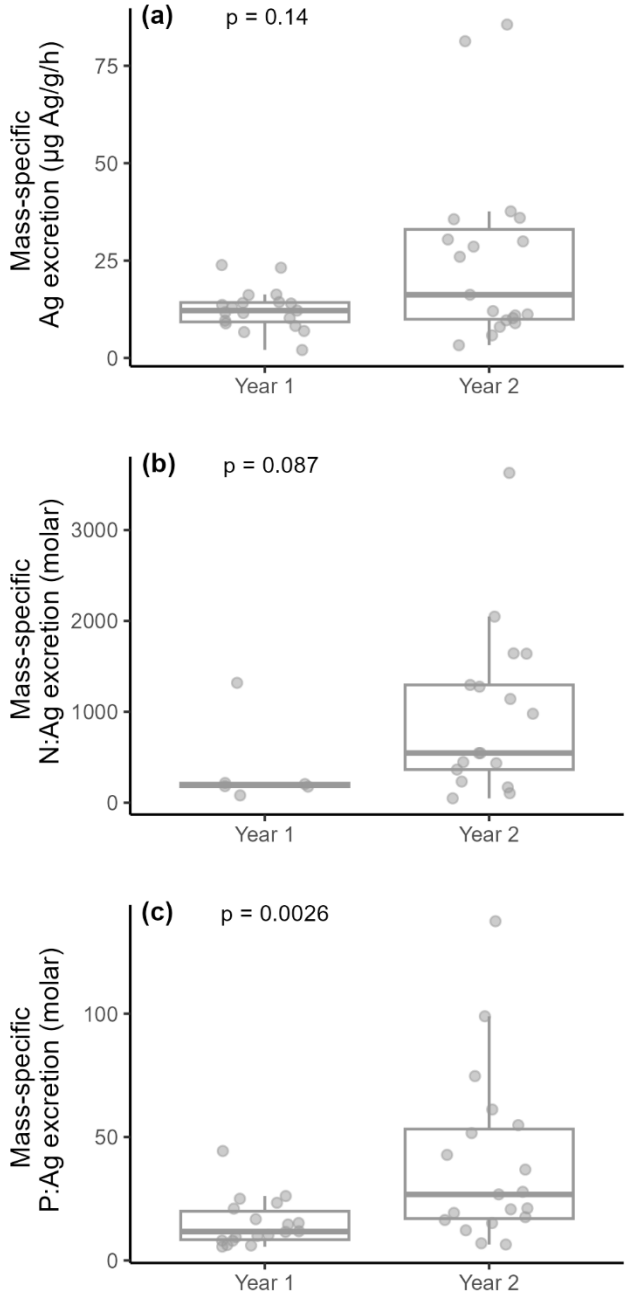

Figure 2. **Chronic AgNP exposure increases P:Ag excretion rates in the experimental lake**. Median, range, and standard errors of (a, b, c) mass-specific total Ag, N:Ag, and P:Ag excretion rates relative to year of AgNP addition. P-values are from Wilcoxon signed-rank tests. Symbols represent individuals by year.

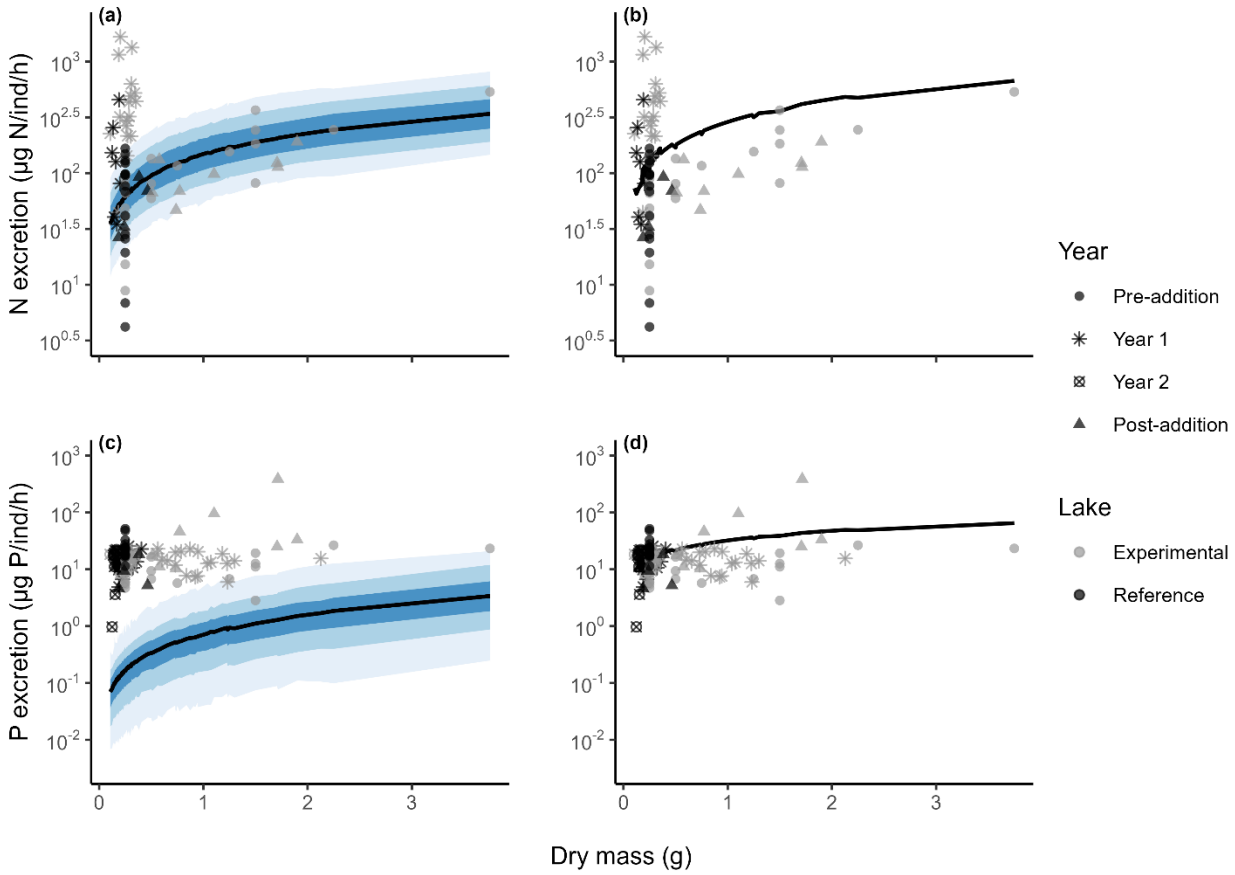

Figure 3. Predicted N excretion rates match empirical N excretion rates for most unexposed perch while predicted P excretion rates do not match experimental P excretion rates. Predicted individual N and P excretion rates relative to perch dry mass based on (a, b) Schiettekatte et al. model (2020), and (c, d) Vanni and McIntyre model (2016). Solid lines represent predictions based on model simulations. Blue gradient indicates 50%, 80% and 95% confidence intervals and grey fill 95% confidence interval around the fit. Symbols represent

individuals by lake (colour) and year of AgNP addition (shape) in the experimental and reference lakes. Predictions and observations are displayed on a $\log_{10}$ scale.

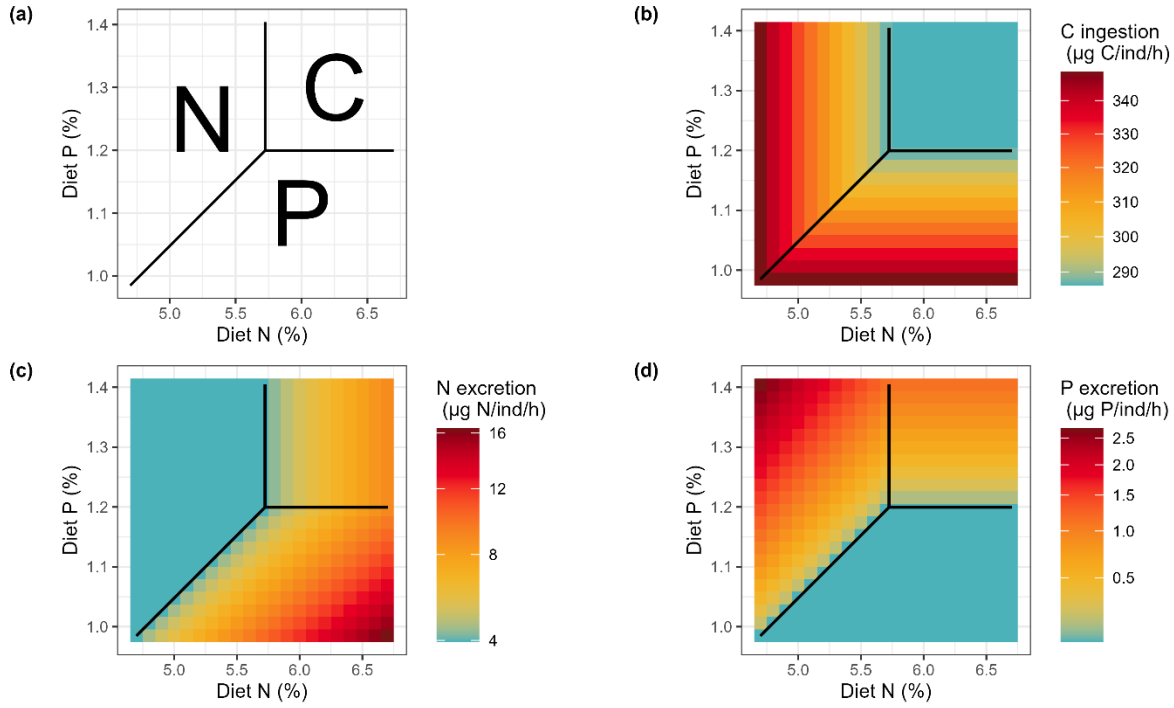

Figure 4. N-limited diet (high %P and low %N) and high C ingestion rates would yield the highest P excretion rates. Model simulations showing the effects of variations in diet stoichiometry (varying levels of %P and %N with %C kept constant at 45) for a perch of 0.3 g on (a) element limitation, (b) C ingestion rates, (c) N excretion rates, and (d) P excretion rates. Lines demarcate regions of C, N, or P limitation (Frost et al. 2004).

Supplementary Information

Minimal supply rate

The initial step of the Schiettekatte et al.(Schiettekatte et al. 2020) model is to estimate the minimal supply rate of C, N, or P elements needed per day for *(i)* body mass growth and *(ii)* minimal inorganic flux associated with metabolic (Ex_{0C}) and maintenance overhead costs (Ex_{0N} and Ex_{0P}) in a fish of a given size. The minimal supply rate S_k (g/day) of the element k (C, N, or P) is:

$$\mathbf{S_k} = \frac{(\mathbf{G_k} + \boldsymbol{Ex_{0k}})}{\mathbf{a_k}} \quad \textbf{(1)}$$

where G_k, Ex_{0k}, and a_k are element-specific growth rate (g/day), minimal inorganic flux (g/day), and assimilation efficiency (%), respectively.

i) Growth

Growth is calculated using growth rate parameters estimated based on otolith or annual rings analysis and the von Bertalanffy growth curve (VBGC) to describe the growth pattern.(von Bertalanffy 1957) Body length, l_t (cm in total length) at age t (year) is estimated as follows:

$$\mathbf{l_t} = \boldsymbol{l_\infty}(\mathbf{1} - \boldsymbol{e^{-\kappa(t-t0)}}) \quad \textbf{(2)}$$

where t_0 is age at settlement, l_∞ is the asymptotic adult length (i.e. length when growth rate is 0), and κ is a growth rate parameter ($year^{-1}$).(von Bertalanffy 1957) This equation allows us determine the age of a fish of a given body length. By adding one day to that age, we can estimate fish growth increment per day. Total growth rate in dry mass (G) can then be calculated using length-weight relationships and wet-to-dry mass conversion constants from the literature and FishBase.(Froese and Pauly 2022) Element-specific growth can then be expressed using element-specific body content percentage (B_k):

$$\mathbf{G_k} = \frac{\mathbf{B_k}}{\mathbf{100}}\boldsymbol{G} \quad \textbf{(3)}$$

ii) Minimal inorganic flux

Metabolic overhead cost (or C minimal excretion rate; Ex_{0c}) predictably scales with individual wet body mass and is the sum of the resting metabolic rate (Ex_{0Cr}) and the rate of energy expenditure for locomotion, feeding, and other activities (Ex_{0Ca}). This can be simply expressed as:

$$\mathbf{Ex_{0C}} = \boldsymbol{Ex_{0Ca}} + \boldsymbol{Ex_{0Cr}} = \boldsymbol{\theta Ex_{0Cr}} \quad \textbf{(4)}$$

Where θ is a dimensionless parameter called "activity scope" which should be >1 and less than the ratio between maximum metabolic rate and resting metabolic rate.(Hou et al. 2008, Barneche and Allen 2018)

Maintenance overhead costs (or N and P minimal excretion rate; Ex_{0N} and Ex_{0P}) can be quantified experimentally during starvation.(Mayor et al. 2011) These measurements include N and P turnover rates:(Anderson et al. 2005)

$$\mathbf{Ex_{0N}} = \boldsymbol{Ex_{0Nz}} \frac{\mathbf{B_N}}{\mathbf{100}} \boldsymbol{m_d}, \quad \textbf{(5)}$$

$$\mathbf{Ex_{0P}} = \boldsymbol{Ex_{0Pz}} \frac{\mathbf{B_P}}{\mathbf{100}} \boldsymbol{m_d}, \quad \textbf{(6)}$$

Where Ex_{0Nz} and Ex_{0Pz} are nutrient-specific and dry mass-specific turnover rate for N (g N/g/day) and P (g P/g/day), respectively, and m_d is the fish dry mass (g). Ex_{0Nz} and Ex_{0Pz} are assumed to remain constant throughout ontogeny.

Tables

Table S1. Summary of yellow perch dry mass, mass-specific N, P, Ag, N:P, N:Ag, and P:Ag excretion rates pre-AgNP addition, during Year 1 and Year 2 of AgNP addition, and post-AgNP addition in both the experimental and reference lakes.

Experimental condition	Lake	Response	n	mean (± SD)	min	max	CV
Pre-addition	Experimental	Dry mass (g)	20	0.89 ± 0.9	0.3	3.8	1.0
		Mass-specific N excretion (µg N/g/h)	20	183.0 ± 112.4	35.4	495.8	0.6
		Mass-specific P excretion (µg P/g/h)	20	21.6. ± 15.4	1.9	57.2	0.7
		Mass-specific N:P excretion (molar)	20	28.3 ± 20.0	2.3	73.2	0.7
	Reference	Dry mass (g)	20	0.25 ± 0	0.3	0.3	0.0
		Mass-specific N excretion (µg N/g/h)	15	301.1 ± 211.0	16.8	665.7	0.7
		Mass-specific P excretion (µg P/g/h)	19	95.3. ± 41.2	45.6	206.8	0.4
		Mass-specific N:P excretion (molar)	15	8.7 ± 7.2	0.5	25.1	0.8
Year 1	Experimental	Dry mass (g)	20	1.0 ± 0.4	0.6	2.1	0.4
		Mass-specific N excretion (µg N/g/h)	6	544.8 ± 505.7	144.1	1536.9	0.9
		Mass-specific P excretion (µg P/g/h)	19	17.7 ± 10.0	4.8	40.6	0.6
		Mass-specific N:P excretion (molar)	6	66 ± 43.5	26.2	130.6	0.7
		Mass-specific TAg excretion (µg Ag/g/h)	19	12.5± 5.3	2.1	23.9	0.4

		Mass-specific N:Ag excretion (molar)	6	363.8 ± 470.3	81.0	1318.8	1.3
		Mass-specific P:Ag excretion (molar)	18	15.2. ± 9.8	5.6	44.3	0.6
	Reference	Dry mass (g)	20	0.25 ± 0.06	0.2	0.4	0.2
		Mass-specific N excretion (µg N/g/h)	-	-	-	-	-
		Mass-specific P excretion (µg P/g/h)	19	61.5 ± 21.2	25.9	90.2	0.3
		Mass-specific N:P excretion (molar)	-	-	-	-	-
Year 2	Experimental	Dry mass (g)	20	0.3 ± 0.06	0.1	0.4	0.2
		Mass-specific N excretion (µg N/g/h)	18	2501.5 ± 2663.7	240.6	9283.1	1.1
		Mass-specific P excretion (µg P/g/h)	20	62.5 ± 31.3	34.9	173.2	0.5
		Mass-specific N:P excretion (molar)	18	101.1 ± 119.9	8.4	433.7	1.2
		Mass-specific TAg excretion (µg Ag/g/h)	19	25.7 ± 23.3	3.3	85.6	0.9
		Mass-specific N:Ag excretion (molar)	17	973.4 ± 915.8	48.9	3627.9	0.9
		Mass-specific P:Ag excretion (molar)	19	39.4 ± 34.3	6.4	137.5	0.9
	Reference	Dry mass (g)	20	0.2 ± 0.02	0.1	0.2	0.1
		Mass-specific N excretion (µg N/g/h)	7	1025.4 ± 847.9	205.5	2375.0	0.8

		Mass-specific P excretion (µg P/g/h)	20	85.5 ± 37.7	7.8	142.7	0.4
		Mass-specific N:P excretion (molar)	7	72.3 ± 124.8	3.7	348.4	1.7
Post-addition	Experimental	Dry mass (g)	8	1.1 ± 0.6	0.5	1.9	0.5
		Mass-specific N excretion (µg N/g/h)	8	105.1 ± 54.8	63.4	229.5	0.5
		Mass-specific P excretion (µg P/g/h)	8	59.0 ± 72.2	13.9	225.7	1.2
		Mass-specific N:P excretion (molar)	8	9.3 ± 8.1	0.6	26.2	0.9
	Reference	Dry mass (g)	4	0.3 ± 0.1	0.2	0.5	0.4
		Mass-specific N excretion (µg N/g/h)	4	167.6 ± 49.7	138.1	241.9	0.3
		Mass-specific P excretion (µg P/g/h)	4	30.6 ± 16.1	11.2	47.9	0.5
		Mass-specific N:P excretion (molar)	4	15.3 ± 9.6	7.9	29.4	0.6

Table S2. Summary outputs from ANOVAs for fish mass, and mass-normalized N, P, and N:P excretion, using lake and year and their interaction as the explanatory variables.

	df	**SS**	**MS**	**F**	***p***
Mass					
Lake	1	5.233	5.233	147.781	< 0.001
Year	3	3.420	1.140	32.192	< 0.001
Lake:Year	3	0.855	0.285	8.044	< 0.001
Residuals	124	4.391	0.035	NA	NA
Mass-normalized N excretion					
Lake	1	0.210	0.210	1.540	0.22
Year	2	13.914	6.957	51.012	< 0.001
Lake:Year	2	0.886	0.443	3.248	0.05
Residuals	66	9.001	0.136	NA	NA
Mass-normalized P excretion					
Lake	1	5.815	5.815	81.860	< 0.001
Year	3	2.446	0.815	11.477	< 0.001
Lake:Year	3	3.058	1.019	14.351	< 0.001
Residuals	121	8.595	0.071	NA	NA
Mass-normalized N:P excretion					
Lake	1	3.183	3.183	14.206	< 0.001
Year	2	6.079	3.040	13.564	< 0.001
Lake:Year	2	1.832	0.916	4.089	0.02
Residuals	66	14.790	0.224	NA	NA

Table S3. Summary outputs from emmeans contrasts for mass, and mass-normalized N, P, and N:P excretion, using lake and year and their interaction as the explanatory variables.

contrast	ratio	SE	df	lower CI	upper CI	null	t ratio	*p*
Mass								
Lake222 Year2014 / Lake239 Year2015	5.439	0.745	124.000	3.565	8.299	1.000	12.360	0.000
Lake222 Year2014 / Lake239 Year2014	3.763	0.516	124.000	2.466	5.741	1.000	9.671	0.000
Lake239 Year2015 / Lake222 Year2022	0.167	0.030	124.000	0.095	0.292	1.000	-9.874	0.000
Lake239 Year2012 / Lake222 Year2014	0.274	0.038	124.000	0.180	0.418	1.000	-9.441	0.000
Lake222 Year2014 / Lake222 Year2015	3.624	0.497	124.000	2.375	5.529	1.000	9.396	0.000
Lake222 Year2012 / Lake239 Year2015	3.466	0.475	124.000	2.271	5.288	1.000	9.071	0.000
Lake239 Year2014 / Lake222 Year2022	0.241	0.044	124.000	0.138	0.422	1.000	-7.841	0.000
Lake239 Year2012 / Lake222 Year2022	0.249	0.045	124.000	0.142	0.436	1.000	-7.668	0.000
Lake222 Year2015 / Lake222 Year2022	0.251	0.045	124.000	0.143	0.438	1.000	-7.633	0.000
Lake222 Year2012 / Lake239 Year2014	2.398	0.329	124.000	1.571	3.658	1.000	6.382	0.000
Lake222 Year2012 / Lake239 Year2012	2.323	0.318	124.000	1.523	3.545	1.000	6.152	0.000
Lake222 Year2012 / Lake222 Year2015	2.309	0.316	124.000	1.513	3.523	1.000	6.107	0.000
Lake222 Year2014 / Lake239 Year2022	3.048	0.723	124.000	1.466	6.336	1.000	4.695	0.000
Lake222 Year2022 / Lake239 Year2022	3.355	0.890	124.000	1.481	7.604	1.000	4.562	0.000
Lake222 Year2012 / Lake222 Year2014	0.637	0.087	124.000	0.418	0.972	1.000	-3.289	0.028
Lake222 Year2012 / Lake222 Year2022	0.579	0.105	124.000	0.331	1.012	1.000	-3.017	0.060
Lake222 Year2015 / Lake239 Year2015	1.501	0.206	124.000	0.984	2.290	1.000	2.964	0.069
Lake239 Year2012 / Lake239 Year2015	1.492	0.204	124.000	0.978	2.276	1.000	2.918	0.078
Lake222 Year2012 / Lake239 Year2022	1.942	0.461	124.000	0.934	4.037	1.000	2.796	0.105

contrast	ratio	SE	df	lower CI	upper CI	null	t ratio	*p*
Lake239 Year2014 / Lake239 Year2015	1.445	0.198	124.000	0.947	2.205	1.000	2.689	0.136
Lake239 Year2015 / Lake239 Year2022	0.560	0.133	124.000	0.270	1.165	1.000	-2.441	0.232
Lake239 Year2014 / Lake239 Year2022	0.810	0.192	124.000	0.390	1.684	1.000	-0.888	0.987
Lake239 Year2012 / Lake239 Year2022	0.836	0.198	124.000	0.402	1.738	1.000	-0.756	0.995
Lake222 Year2015 / Lake239 Year2022	0.841	0.200	124.000	0.405	1.748	1.000	-0.729	0.996
Lake222 Year2014 / Lake222 Year2022	0.908	0.165	124.000	0.519	1.588	1.000	-0.531	0.999
Lake239 Year2014 / Lake222 Year2015	0.963	0.132	124.000	0.631	1.469	1.000	-0.275	1.000
Lake239 Year2012 / Lake239 Year2014	1.032	0.141	124.000	0.676	1.575	1.000	0.230	1.000
Lake239 Year2012 / Lake222 Year2015	0.994	0.136	124.000	0.651	1.516	1.000	-0.045	1.000
Mass-normalized N excretion								
Lake222 Year2012 / Lake222 Year2015	0.094	0.026	66.000	0.042	0.212	1.000	-8.551	0.000
Lake222 Year2015 / Lake222 Year2022	16.860	6.092	66.000	5.838	48.689	1.000	7.818	0.000
Lake239 Year2012 / Lake222 Year2015	0.124	0.037	66.000	0.052	0.296	1.000	-7.028	0.000
Lake222 Year2015 / Lake239 Year2022	9.946	4.675	66.000	2.503	39.518	1.000	4.887	0.000
Lake239 Year2015 / Lake222 Year2022	7.487	3.295	66.000	2.057	27.244	1.000	4.574	0.000
Lake222 Year2012 / Lake239 Year2015	0.212	0.079	66.000	0.071	0.635	1.000	-4.152	0.001
Lake239 Year2012 / Lake239 Year2015	0.279	0.109	66.000	0.089	0.874	1.000	-3.282	0.020
Lake239 Year2015 / Lake239 Year2022	4.416	2.354	66.000	0.924	21.108	1.000	2.787	0.072
Lake222 Year2015 / Lake239 Year2015	2.252	0.853	66.000	0.741	6.845	1.000	2.143	0.278
Lake239 Year2012 / Lake222 Year2022	2.087	0.777	66.000	0.700	6.224	1.000	1.976	0.367

contrast	ratio	SE	df	lower CI	upper CI	null	t ratio	*p*
Lake222 Year2012 / Lake222 Year2022	1.588	0.565	66.000	0.559	4.512	1.000	1.300	0.784
Lake222 Year2022 / Lake239 Year2022	0.590	0.307	66.000	0.128	2.720	1.000	-1.014	0.912
Lake222 Year2012 / Lake239 Year2012	0.761	0.221	66.000	0.324	1.785	1.000	-0.941	0.934
Lake239 Year2012 / Lake239 Year2022	1.231	0.589	66.000	0.302	5.015	1.000	0.435	0.998
Lake222 Year2012 / Lake239 Year2022	0.937	0.436	66.000	0.239	3.676	1.000	-0.140	1.000
Mass-normalized P excretion								
Lake239 Year2012 / Lake222 Year2014	5.868	1.168	121.000	3.175	10.844	1.000	8.887	0.000
Lake222 Year2012 / Lake239 Year2012	0.180	0.035	121.000	0.098	0.330	1.000	-8.716	0.000
Lake222 Year2014 / Lake239 Year2015	0.206	0.041	121.000	0.113	0.379	1.000	-8.025	0.000
Lake222 Year2012 / Lake239 Year2015	0.218	0.042	121.000	0.120	0.397	1.000	-7.842	0.000
Lake222 Year2014 / Lake222 Year2015	0.263	0.052	121.000	0.143	0.482	1.000	-6.793	0.000
Lake222 Year2014 / Lake239 Year2014	0.263	0.052	121.000	0.142	0.487	1.000	-6.703	0.000
Lake222 Year2012 / Lake222 Year2015	0.278	0.054	121.000	0.153	0.506	1.000	-6.594	0.000
Lake222 Year2012 / Lake239 Year2014	0.278	0.055	121.000	0.152	0.511	1.000	-6.504	0.000
Lake239 Year2012 / Lake239 Year2022	3.323	1.122	121.000	1.173	9.414	1.000	3.557	0.012
Lake239 Year2012 / Lake222 Year2022	2.474	0.640	121.000	1.114	5.494	1.000	3.502	0.015
Lake222 Year2014 / Lake222 Year2022	0.422	0.109	121.000	0.190	0.936	1.000	-3.339	0.024
Lake222 Year2012 / Lake222 Year2022	0.446	0.114	121.000	0.202	0.984	1.000	-3.147	0.042
Lake239 Year2015 / Lake239 Year2022	2.743	0.922	121.000	0.973	7.736	1.000	3.002	0.063
Lake239 Year2015 / Lake222 Year2022	2.042	0.524	121.000	0.925	4.509	1.000	2.782	0.109

contrast	ratio	SE	df	lower CI	upper CI	null	t ratio	*p*
Lake222 Year2015 / Lake239 Year2022	2.153	0.724	121.000	0.763	6.072	1.000	2.281	0.313
Lake239 Year2014 / Lake239 Year2022	2.151	0.726	121.000	0.759	6.094	1.000	2.269	0.319
Lake239 Year2012 / Lake222 Year2015	1.543	0.303	121.000	0.842	2.831	1.000	2.208	0.355
Lake239 Year2012 / Lake239 Year2014	1.545	0.308	121.000	0.836	2.855	1.000	2.184	0.369
Lake222 Year2015 / Lake222 Year2022	1.603	0.412	121.000	0.726	3.539	1.000	1.838	0.596
Lake239 Year2014 / Lake222 Year2022	1.602	0.414	121.000	0.721	3.557	1.000	1.821	0.607
Lake222 Year2014 / Lake239 Year2022	0.566	0.191	121.000	0.200	1.604	1.000	-1.684	0.697
Lake222 Year2012 / Lake239 Year2022	0.599	0.201	121.000	0.212	1.689	1.000	-1.526	0.792
Lake222 Year2015 / Lake239 Year2015	0.785	0.152	121.000	0.431	1.428	1.000	-1.249	0.915
Lake239 Year2014 / Lake239 Year2015	0.784	0.154	121.000	0.428	1.438	1.000	-1.237	0.919
Lake239 Year2012 / Lake239 Year2015	1.211	0.238	121.000	0.661	2.221	1.000	0.975	0.977
Lake222 Year2022 / Lake239 Year2022	1.343	0.505	121.000	0.421	4.281	1.000	0.785	0.994
Lake222 Year2012 / Lake222 Year2014	1.057	0.208	121.000	0.577	1.939	1.000	0.284	1.000
Lake239 Year2014 / Lake222 Year2015	0.999	0.196	121.000	0.545	1.832	1.000	-0.004	1.000
Mass-normalized N:P excretion								
Lake239 Year2012 / Lake222 Year2015	0.091	0.035	66.000	0.030	0.277	1.000	-6.303	0.000
Lake222 Year2015 / Lake222 Year2022	10.092	4.674	66.000	2.592	39.298	1.000	4.991	0.000
Lake222 Year2012 / Lake239 Year2012	3.898	1.451	66.000	1.307	11.625	1.000	3.654	0.007
Lake239 Year2012 / Lake239 Year2015	0.228	0.114	66.000	0.053	0.984	1.000	-2.967	0.046
Lake222 Year2012 / Lake222 Year2015	0.353	0.125	66.000	0.125	0.998	1.000	-2.941	0.049

contrast	ratio	SE	df	lower CI	upper CI	null	t ratio	*p*
Lake222 Year2012 / Lake222 Year2022	3.562	1.624	66.000	0.934	13.582	1.000	2.786	0.072
Lake222 Year2015 / Lake239 Year2022	4.433	2.671	66.000	0.756	25.984	1.000	2.471	0.148
Lake239 Year2015 / Lake222 Year2022	4.016	2.265	66.000	0.767	21.031	1.000	2.464	0.150
Lake222 Year2015 / Lake239 Year2015	2.513	1.220	66.000	0.604	10.450	1.000	1.898	0.413
Lake239 Year2012 / Lake239 Year2022	0.401	0.246	66.000	0.066	2.429	1.000	-1.488	0.673
Lake222 Year2022 / Lake239 Year2022	0.439	0.293	66.000	0.062	3.116	1.000	-1.233	0.819
Lake239 Year2015 / Lake239 Year2022	1.764	1.205	66.000	0.237	13.101	1.000	0.831	0.961
Lake222 Year2012 / Lake239 Year2022	1.565	0.934	66.000	0.271	9.025	1.000	0.750	0.975
Lake222 Year2012 / Lake239 Year2015	0.887	0.425	66.000	0.218	3.615	1.000	-0.250	1.000
Lake239 Year2012 / Lake222 Year2022	0.914	0.436	66.000	0.225	3.709	1.000	-0.189	1.000

Figures

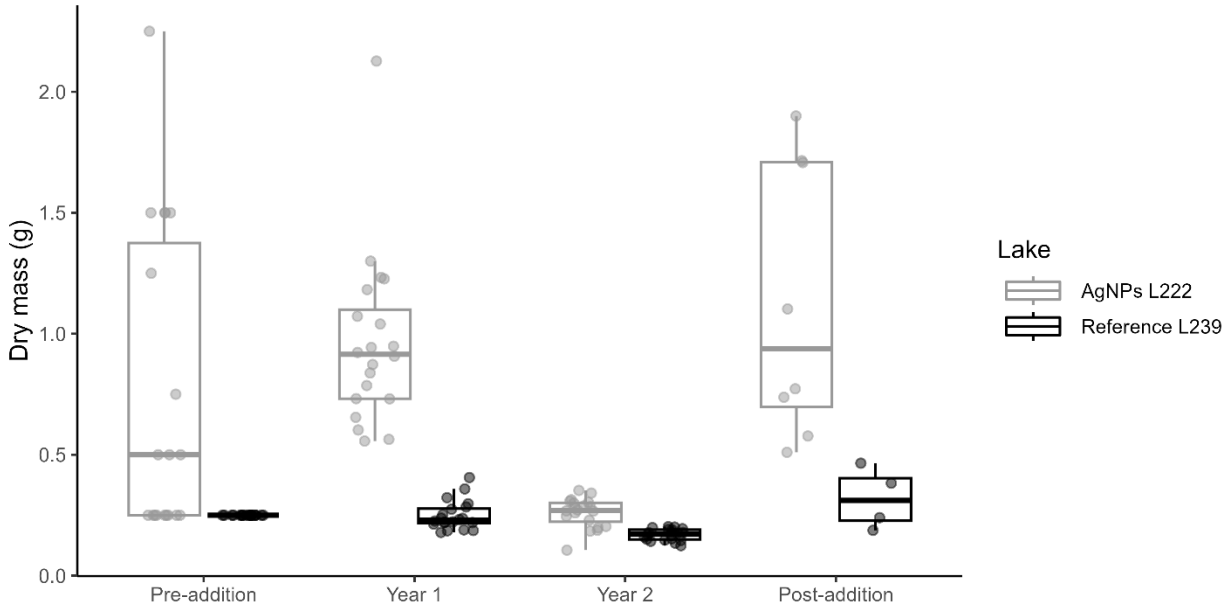

Figure S1. Empirical perch mass differs between lakes pre-, in Year 1, and post-AgNPs addition. Individual dry mass pre-, during AgNPs addition in the AgNPs 222 and reference 239 lakes. Symbols represent individuals by year and contrasting letters indicate significant differences.

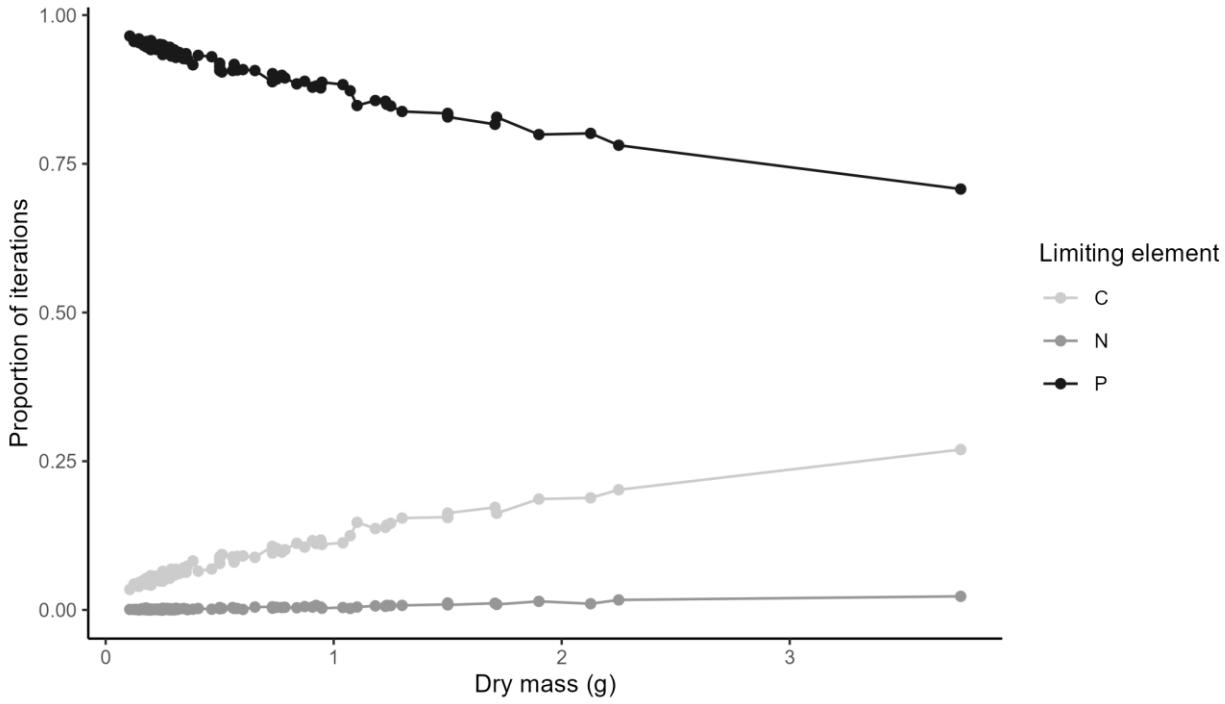

Figure S2. Model mainly predicts P-limitation for perch up to 3.75 g. Proportion of simulations iterations yielding C, N, or P as the limiting element for a given fish dry mass.

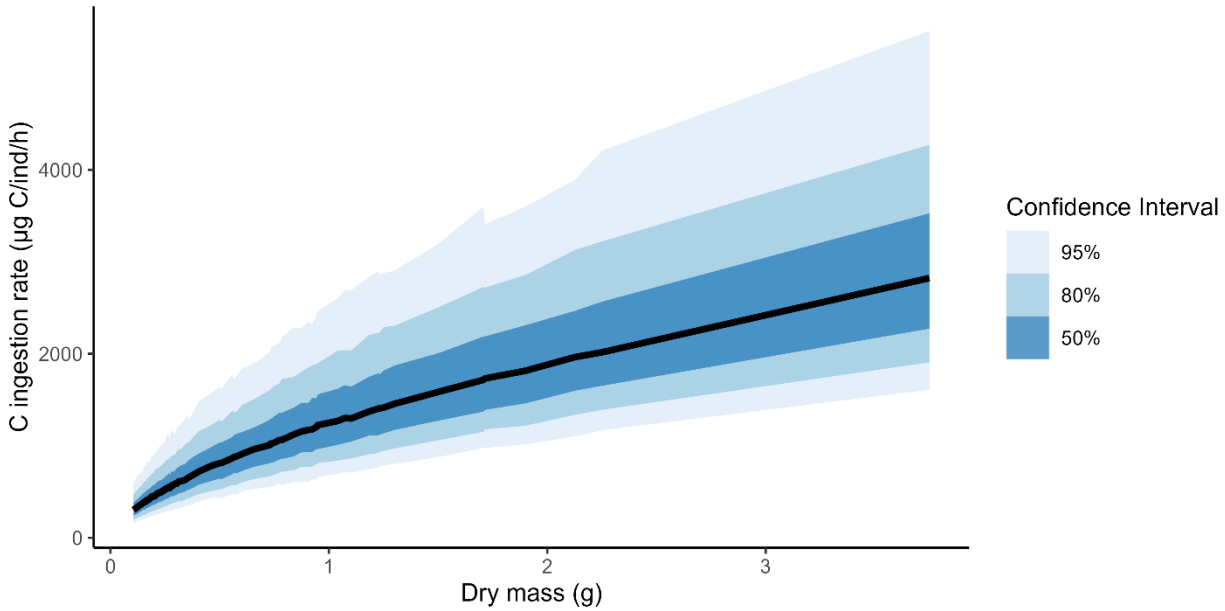

Figure S3. Predicted C ingestion rates increase about 20x with 3 g increase in perch dry mass. Blue gradient represents confidence intervals around the median in black.

CHAPTER 6

General Discussion

Animal-mediated elemental cycling is integral to the flow of essential nutrients and carbon both within ecosystems and at the interface of ecosystem boundaries. These elements derived from animals can act as life catalysts in desolate regions (Magnússon et al. 2020), sustain existing life, and pose potential threats when they harbor toxicity. In my thesis, I explored the interplay between three indirect consequences of global change; (1) high productivity of a large lake (Chapter 1), (2) change in dissolved organic matter (DOM) concentration and composition in streams and lakes (Chapters 2 and 3), and (3) increase in contaminants in aquatic ecosystems (Chapter 4), and animal nutrient, carbon, and contaminant excretion in environmentally relevant conditions.

In Lake Erie, a highly productive ecosystem, my research revealed that animals efficiently cycle substantial amounts of nutrients from external sources and sediments when their biomass is high. In streams and lakes exhibiting variations in ambient dissolved organic carbon (DOC) and DOM composition, I observed both linear and nonlinear shifts in animal nutrient and carbon excretion rates, particularly evident at the individual and local spatial scales. Notably, these shifts displayed contrasting trends between streams and lakes, indicating that the influence of ambient DOC and DOM composition depends on the ecosystem type and the element being excreted. Results from experiments involving the contamination of an entire lake with silver nanoparticles (AgNP) indicated that a two-year exposure to ambient total Ag concentrations ranging from 1 and 11.5 µg/L has minimal negative impact on fish nutrient excretion rates. Rather, ecosystem-based disparities in fish-mediated nutrient cycling may outweigh potential effects induced by contaminants.

Overall, I demonstrate that animal-mediated elemental cycling varies with space, time, and animal functional traits (i.e., body size and trophic position). Notably, individual-level nutrient excretion rates vary across ecosystems, ranging from relatively low rates in nutrient-rich large lake to relatively high rates in both nutrient-rich streams and nutrient-poor lakes. These findings extend our understanding of animal-mediated elemental cycling to large lakes, incorporating diverse species assemblages, novel abiotic factors such as ambient DOC and DOM composition, and underexplored excreted elements like DOC, DOM composition, and contaminants. Nonetheless, as with any field-based investigation, these findings present challenges and open new avenues for further research, which I address below.

Animal-mediated elemental cycling using trait-based approaches

The study of animal-mediated elemental cycling presents a formidable opportunity to bridge diverse disciplines including animal physiology, movement ecology, food web ecology, biogeochemistry, and even human nutrition and health, across ecological scales. Adopting a trait-based perspective can broaden the relevance of animal-mediated elemental cycling beyond a specific taxon. While I examined the modulating role of traits such as body size and trophic position on animal elemental excretion rates, it is important to recognize that animal elemental excretion rates themselves constitute a functional trait. This trait can be conceptualized both as an effect trait (de Bello et al. 2021b), influencing the movement and availability of elements within ecosystems, and as a response trait (de Bello et al. 2021b), fluctuating in response to both biotic and abiotic factors.

One challenge in examining animal-mediated elemental cycling across both levels of biological organization and environmental gradients is disentangling interspecific variability in nutrient excretion rates from the potential influence of environmental variables. For example, I

used a diverse species assemblage to estimate the relationships between ambient DOC and DOM composition and animal-mediated elemental cycling by taxonomic rank and trophic position. However, the absence of some study species at a given site introduces a potential source of error in identifying causal patterns between ambient DOC and animal elemental excretion. Future studies could leverage a functional approach to systematically quantify interspecific variability and incorporate a broader array of traits (Nock et al. 2016), such as consumer elemental ingestion rates, assimilation efficiencies, body elemental composition, and release through both excretion and egestion pathways. By integrating additional functional axes, we can enhance our capacity to detect changes in response traits, particularly at higher levels of biological organization.

Quantifying animal-mediated elemental cycling across ecosystems and ecoregions

I observed marked differences in individual-level nutrient excretion rates across the three study systems used in my experiments: Lake Erie, a network of 11 streams in southern Ontario draining both agricultural and forested land, and 11 boreal oligotrophic lakes located at the IISD-Experimental Lakes Area draining coniferous land. These ecosystems exhibited considerable variations in ambient total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP) levels, which undoubtedly influenced the quantity and quality of basal resources available to higher trophic levels. In analyzing individual-level nutrient excretion rates, I used various methods across the chapters of my thesis. Chapters 1 and 4 focused on mass-specific nutrient excretion rates, while Chapters 2 and 3 used mass-normalized nutrient excretion rates, characterizing them as either ammonium (NH_4^+) and soluble reactive phosphorus (SRP) in Chapters 1 to 3, or TDN and TDP in Chapters 1 and 4. Despite these methodological differences,

the comparisons made among my study systems, as well as between my study systems and those investigated in other studies, remain relevant and informative.

Lake Erie ambient TDN and TDP were relatively low, averaging 414.08 ± 40.76 µg TDN/L and 13.61 ± 1.5 µg TDP/L, despite the high external and internal nutrient loads (Singh et al. 2023, Bocaniov et al. 2023). This discrepancy is likely attributed to nutrient sequestration within biomass (Li et al. 2021) or sediments (Wang et al. 2021). Consequently, fish mass-specific N and P excretion rates averaged 31.01 ± 18.66 µg TDN/g/h and 10.02 ± 21.82 µg TDP/g/h and 17.94 ± 12.82 µg NH_4^+/g/h and 3.16 ± 2.31 µg SRP /g/h during the summer sampling period. The NH_4^+ and SRP excretion rates were however within the range observed in Lake Malawi/Nyasa/Nyassa, a larger volume Great Lake with comparatively lower external nutrient loading (André et al. 2003). This suggests a degree of consistency in nutrient cycling dynamics across lakes of differing sizes and nutrient regimes, underscoring the complexity of nutrient dynamics in aquatic ecosystems.

In contrast, the 11 streams in southern Ontario had very high ambient TDN and TDP, averaging 1433.88 ± 1209.83 µg TDN/L and 24.83 ± 1.3 µg TDP/L and displayed the highest animal mass-normalized N and P excretion rates for fish, averaging 149.55 ± 85.16 µg NH_4^+/g/h and 17.52 ± 12.66 µg SRP/g/h. Interestingly, the equivalent study in the oligotrophic boreal lakes at IISD-ELA (Chapter 4) revealed that despite low ambient TDN and TDP averaging 270.82 ± 65.05 µg TDN/L and 3.63 ± 1.77 µg TDP/L, fish mass-normalized nutrient excretion rates averaged 94.07 ± 56.02 µg NH_4^+/g/h and 36.58 ± 32.57 µg SRP/g/h. When accounting for the standard deviation, mass-normalized nutrient excretion rates at IISD-ELA are within the range of those observed in the more nutrient-rich streams and higher than rates observed in Lake Erie for P only.

Interestingly, dreissenid mass-specific N and P excretion rates in Lake Erie appeared more than five to six times higher than rates observed in fish, whereas mayfly mass-normalized N and P excretion rates in the 11 streams in southern Ontario were 50 to 18 times lower than rates observed in fish, contrary to findings in high-elevation tropical streams (Atkinson et al. 2019). This is likely related to dietary nutrients requirements, especially P-rich ribosomal RNA essential for growth (Elser et al. 2000). The highlighted disparities in animal nutrient excretion rates across my study systems demonstrate the value in localized studies using a variety of species.

Evaluating the effects of ambient DOM in streams and lakes

The relationship between animal-mediated nutrient cycling and ambient DOC and DOM composition differed markedly between streams and lakes. In streams, my findings indicated no discernible relationship between ambient DOC and DOM and fish mass-normalized N excretion rates and a multimodal relationship with mass-normalized P excretion rates, while patterns in lakes were inverted. These contrasting outcomes highlight the context-dependency of DOC and DOM impacts on animal-mediated elemental cycling and may be related to the physiography of the ecosystems studied (Creed et al. 2015, Seekell et al. 2018b), community composition (Van Dorst et al. 2022), differences in external N and P inputs into the system due to differences in land cover, and consumer elemental limitation. Whether my findings in streams and lakes could be applied to streams and lakes in other regions thus remains uncertain.

It is imperative to recognize that while I offer mechanistic insights into the plausible relationships between animal nutrient excretion and ambient DOC and DOM, my analyses are correlative. These relationships are undoubtedly intricate, serving more as a proxy for various underlying drivers, although direct effects of ambient DOC cannot be discounted. Future work

could delve deeper by testing the relationship between ambient DOC and animal-mediated elemental cycling on a temporal scale, perhaps by observing the response of consumer nutrient cycling following additions of DOC and DOM components in whole ecosystems or mesocosms (Zwart et al. 2016, Robbins et al. 2020). Incorporating additional traits such as consumer feeding ecology through stable isotopes analysis, consumer growth rates, and resource elemental ratios (Atkinson et al. 2017) might help in elucidating the underlying mechanisms driving the observed differences across gradients of DOC and DOM composition in my thesis chapters.

Evaluating the effects of contaminants in environmentally-relevant conditions

Evaluating the impact of contaminants in environmentally relevant conditions is critical for gaining a comprehensive understanding of their effects on entire food webs and ecosystem functions. However, achieving this requires a good understanding of the underlying mechanisms governing processes such as animal-mediated elemental cycling, particularly how animal nutrient excretion rates vary in a given species under uncontaminated conditions. Such deep mechanistic understanding is crucial if these mechanisms are to serve as endpoints for assessing stress, whether chemical or otherwise. Most physiological processes exhibit increased variability under stress, rendering highly variable and poorly understood processes unsuitable as endpoints for stress assessment. My research done at the IISD-ELA was the first on yellow perch nutrient excretion rates in oligotrophic boreal lakes. Consequently, interpreting the high P excretion rates observed across various experimental conditions posed a significant challenge when assessing the impacts of contaminant exposure on animal nutrient excretion rates. Selecting a species such as yellow perch as a potential model organism for field-based ecotoxicological studies may prove useful in establishing baseline knowledge about the response of a given organism under uncontaminated conditions.

Moreover, contaminants frequently co-occur with other pollutants, leading to potential additive or antagonistic effects (Li et al. 2023). While I could not establish a direct causal relationship between AgNP exposure and the observed higher mass-specific P excretion rates in the experimental lakes compared to the reference lake, it remains plausible that more pronounced effects could have been discerned if combined with other contaminants. Examining the impacts of multiple contaminants on animal-mediated nutrient cycling would introduce a higher degree of complexity to future research endeavors, but also enrich their realism and ecological relevance.

A call to standardize methods in animal-mediated elemental cycling studies

Comparing the elemental excretion rates I calculated with those from other studies poses a challenge due to the wide array of methods employed to estimate these rates across different levels of biological organization and spatial scales. For instance, individual-level nutrient excretion rates can be expressed as per capita (the unit of a given element per individual per unit time), mass-specific (the unit of a given element per unit of animal body mass per unit time), or mass-normalized (the unit of a given element per unit of animal body mass scaled using a species-specific or general scaling coefficient per unit time). Body mass can be further expressed as either wet (Schindler and Eby 1997) or dry mass (Downs et al. 2016), although the use of dry mass is generally preferred (Vanni and McIntyre 2016). The unit of the given element or body mass may also vary from micrograms to grams (e.g., Conroy et al. 2005, Hopper et al. 2021), micromoles (e.g., Atkinson and Forshay 2022), or parts per billion (e.g., Vaughn et al. 2022), while the unit of time could range from hours to years (Conroy et al. 2005, Vanni and McIntyre 2016). Additionally, it is often assumed that excreted NH_4^+ and SRP constitute a large proportion of excreted TDN and TDP, but this can vary. For example, our findings in Lake Erie revealed that

fish mass-specific excretion of NH_4^+ and SRP accounted for 58% and 32% of mass-specific TDN and TDP excretion, respectively. This contrasts with findings for cichlids in Lake Malawi, where NH_4^+ constituted 90% of excreted TDN and excreted SRP and TDP were equivalent (André et al., 2003).

Furthermore, a variety of methods can be used to scale individual-level nutrient excretion rates to the community or ecosystem level to underscore their significance, including volumetric nutrient excretion (McIntyre et al. 2008, Hopper et al. 2021), turnover times (Conroy et al. 2005 and this thesis), nutrient loads (Persson 1997), and the proportion of ecosystem demand met by aggregate animal nutrient excretion (Vanni et al. 2006, Atkinson et al. 2019, Balik et al. 2022). However, the abundance of approaches, coupled with the occasional absence of summary data, often complicates the contextualization of one's findings within the existing literature. While it is expected that the selection of approaches will vary based on the study's objectives, identified strengths and weaknesses of a given approach, and constraints of field and laboratory analyses, I advocate for the standardization of certain methods to establish a clearer foundation in this field of study. This standardization would be particularly beneficial as new excreted elements are explored (Atkinson et al. 2017).

A call to integrate animal-mediated nutrient cycling into models of elemental flux

Animal contributions to elemental cycling extend beyond ecosystem boundaries, particularly at the aquatic-terrestrial interface. For instance, the establishment of round goby (*Neogobius melanostomus*) in Lake Erie has significantly influenced the diet of watersnakes, with over 92% of their diet comprising round goby (King et al. 2006), suggesting that watersnakes likely contribute lake-derived nutrients to both terrestrial and aquatic ecosystems. Similarly, pearl dace

occasionally consume terrestrial insects (Tallman and Gee 1982), thus introducing terrestrially-derived nutrients and dissolved organic matter into the lake through their excretion and egestion.

More generally, one of the most famous examples of the role of animal-mediated elemental cycling across ecosystems is the annual migrations of salmons that provide substantial marine-derived nutrient subsidies through their excretion and senesced carcasses (Moore et al. 2007, Hood et al. 2007, Evans et al. 2020). Salmon-feeding bears further mediate these subsidies by redistributing nutrients across riparian landscapes through their excretion and egestion (Hilderbrand et al. 1999). While my thesis focused solely on animal elemental excretion, these examples underscore the complexity of animal-mediated elemental cycling, which encompasses elemental uptake, storage, and release through excreta, egesta, and ultimately carcass.

It is evident that animal-mediated nutrient and carbon cycling serves as a vital internal source, pool, and sink of elements within aquatic ecosystems, while also acting as an external source, pool, and sink of elements between aquatic and terrestrial ecosystems. Despite its significance, ecosystem-based nutrient budgets and global models of nutrient and carbon cycles often overlook this process, potentially leading to underestimations or biases in elemental flow (Schmitz et al. 2018). Therefore, I advocate for the systematic integration of animal-mediated elemental cycling into ecosystem nutrient budgets and models of nutrient and carbon cycles. Only through this integration can we achieve a truly comprehensive assessment of element flow within and across ecosystem boundaries.

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APPENDIX

CHAPTER 2 AUP #26601

Animal Use Protocol Application For Wildlife and Field Work Research

Project Info.

File No: 26601
Project Title: Animal-mediated nutrient recycling in the western basin of Lake Erie
Principal Investigator: Dr. Marguerite Xenopoulos (Natural Sciences\Biology)
Start Date: 2021/07/01
End Date: 2021/12/20
Keywords: fish, food web dynamics, aquatic biogeochemistry, nutrient cycling

Project Team Info.

Principal Investigator

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Other Project Team Members

Prefix	Last Name	First Name	Affiliation	Role In Project	Email

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Common Questions

1. Research Project Overview

#	Question	Answer
1.1	Title of Project (must be descriptive and unique)	Aquatic animal-mediated nutrient release in the western basin of Lake Erie
1.2	Please indicate either the research account number or the ROMEO project number that this protocol relates to:	PENDING
1.3	Lay Summary of project. Give the aims, and scientific significance of the work to be performed in terms understandable and meaningful to the general public. Please try to limit to 200 words or less.	Aquatic animals play important roles in nutrient cycles. Fish, in particular, can be important for the recycling of nutrients via their feeding activities. Once these nutrients are released from fish back into the environment, they become available again for primary producers and can fuel algal growth. In this project, we aim to measure nutrient release rates from fish in the western basin of Lake Erie across seasons and space. We will then combine individual excretion rates with population density data to calculate species nutrient release in the western basin of Lake Erie. Lastly we will compare the relative contribution of fish relative to other internal (via the sediments) and external sources of nutrients.
1.4	As principal investigator, please provide the date that the Animal Care course was completed.	2012/01/01
1.5	Please provide an emergency telephone number where you can be reached.	7055592433
1.6	Other Investigators. Are other investigators participating in this research? If yes, make sure to add all other investigators on this project by accessing the "Project Team" Tab.	Yes
1.7	Please provide the name(s) of the person(s) responsible for the care of the animals and	Sandra Klemet-N'Guessan - 514 701 1904 - Animal care course completed on 2019/02/27

	for the health monitoring program. Include and emergency number and the date that each of the individuals completed the Animcal Care Course	Aaron Fisk - 519-984-9931 - Aaron Fisk has completed training on animal use at the Universities of Georgia and Windsor more than 10 years ago, and has acted as the PI on many fish projects at the University of Windsor. He has extensive experience in field sampling and the application of tags to live animals that are then released and monitored over an extended period of time. He also has many ACC approved projects from the ACC at UWindsor (please contact them for further details). Emma Vokey - Animal care course completed on 2021/04/14
1.8	What are the specific objectives of this work?	(1) To quantify aquatic animal populations nutrient release and contribution to ecosystem recycling in one of the Laurentian Great Lakes across seasons and space, and (2) To compare this source of internal loads with other internal loads sources (via the sediments) and external loads based on data collected by our collaborators.
1.9	Purpose of Animal Study? (Check one) Note: Honours thesis projects are considered Research Projects If this is for an undergraduate course please complete the teaching protocol application.	Fundamental Research
1.10	Peer Review Has this project been peer reviewed for scientific merit through a research granting agency? If No, contact the Office of Research to arrange for two independent peer reviews. Note: External peer reviews may take some time to obtain; allow sufficient time between your submission and your proposed start date. Please refer to submission deadlines on the Animal Care website.	Yes
1.11	If yes, please indicate which funding agency. If No, contact the Office of Research to arrange for two independent peer reviews. Note: External peer reviews may take some time to obtain; allow sufficient time between your submission and your proposed start date. Please refer to submission deadlines on the Animal Care website.	NSERC

1.12	Replacement, Reduction and Refinement. For a review of the 3R's of animal use, please visit www.ccac.ca/en/alternatives/index.html, the "special topics" tab of that mage may be of use for your work. The P.I. has investigated and evaluated alternative methods which would accomplish the same scientific goals.	Yes
1.13	Support this statement with a brief description of the methods and sources used to determine that non-animal alternatives were not available, or an explanation of the aspects of the protocol that preclude using animals of a lower sentience. In addition, explain why the numbers of animals proposed cannot be further reduced. Finally, justify the invasive procedures (if any) proposed are at the lowest level possible and why they cannot be reduced further.	To quantify excretion rates of three species with different feeding strategies, real animals must be used to gather data about how the ambient nutrient conditions in such a large lake has affected them. This effect cannot be tested through modelling (and in fact, modelling these excretion rates has been highly criticized by experts in the field (Findlay et al. (2005)). Up to 120 fish per species will be used in three locations and at two sampling periods (August and October). These types of animal-mediated excretion experiments and incubations require many replicates (10-20) to account for body size and species-specific variability (Wilson and Xenopoulos 2011) and to be acceptable for peer-review and publication. The three species allow us to test the consistency of relationships in benthic and water column feeding species. The most effective ways to sample fishes in a large lake and minimize the fasting period between the moment the fish is caught and that it is tested (which would otherwise affect the excretion rates) is by using short term gill net sets and boat electrofishing. Our proposed sampling methods are based on existing protocols for this species (e.g., Wilson and Xenopoulos (2011) and our approved 2020 animal care protocol 20ACC6485).

2. Project Detail

#	Question	Answer
2.1	Proposed start date. (Note: If the application is approved the committee may assign a start date that differs from the proposed start date.)	2021/07/01

2.2	What is the duration of the project? (Even if the duration of the live animal work is only a few months, the protocol will be active for a minimum of one year from the date of approval.)	one year
2.3	Animals : Genus/Species	-Sander vitreus -Morone chrysops -Perca flavescens Any bycatch species will be recorded and reported to the ACC
2.4	Animals: Common Name	-Walleye -White bass -Yellow perch Any bycatch species will be recorded and reported to the ACC
2.5	Number of animals per species required for the duration of the study	120
2.6	If this is a multi-year project, estimate the number of animals per species to be used in year one, year two, and year three. (Note the sum of animals over all years must not exceed the total given	n/a
2.7	Provide a justification for the number of animals required.	The number of animals per species required is based on the three locations and two sampling periods planned (3 sites in the Western basin of Lake Erie will be sampled for each sampling period - once in August and one in October): 3 x 2 x 20 = 120 individuals per species. Metabolic and excretion rates are variable within individuals, and so more animals are needed for replicates to account for individual variation in respiration. In addition, metabolic and excretion rates are affected by body size, so different sizes of fish must be used to make a reasonable determination of scaling factor. The number selected is consistent with the number of fish used by Wilson and Xenopoulos (2011) in a similar fish study and our approved 2020 animal care protocol 20ACC6485
2.8	Location: Where will the study take place?	The project will take place at three different sites in the western basin of Lake Erie
2.9	If permits are required, please list them below. Upload scans of the permits to this form, or forward copies to the Office of Research or Manager of Animal Care.	MNRF scientific collection permit
2.10	If permits are required,indicate their status:	Pending

2.11	If obtained, provide the Permit Number	
2.12	Describe the precautions to be taken to avoid capturing non - target species or vulnerable animals.	All staff will be trained in the correct identification of fish and handling procedures. All staff will be trained in the correct identification of fish and handling procedures. This project requires live animals for the experiment, so methods of collection are designed to minimize mortality and stress. Given this, non-target species will be released alive. If target or non-target species experience significant mortality (>5% of the individuals collected) methods will be altered to reduce mortality (shorter gill net sets, lower frequencies on electrofishing) and this will be reported to the ACC. If vulnerable or threatened species are encountered, such as lake sturgeon (Acipenser fulvescens), collections will be stopped and moved to a new location.
2.13	Provide a detailed description of the procedures that will be used to trap/capture the animals.	Sampling methods need to capture live and healthy fish. We will use two methods to do this: short term gill net sets and boat electrofishing. Gill nets (multi panel, 1 to 3 inch mesh, 50 m long, 3 m high) will be set mid-water (2-3 m below surface) for < 30 min. Nets will be checked and study species will be transferred to coolers [SPECIFY VOLUME] with lake water and air stone maintained at the same temperatures as lake water. The electrofisher live wells are 200 L (with re-circulating lake water) or we will use 100 L coolers. The fish mass to water volume ratio depends on the fish species, DO concentrations and water turnover (i.e., build-up of CO2), among other things, but we have and will consider this during the experiment. We will use a conservative 1 cm of fish per L and will monitor dissolved oxygen and fish health when held in live wells to minimize stress and unintended deaths. If fish are overly stressed (rapid breathing, improper orientation) we will release them back into the lack. It is hard to predict how many fish we will have to catch to get our desired size classes. If catch rates are low for a certain size class, we will alter our methods

		(set nets at different depths, distance from shore) and if that does not work eliminate that size class from the experiment. Electrofishing will utilize standard frequencies for medium to large fish, only study species will be netted out of the water and placed into live wells (recirculate lake water) on the boat. Individuals between 5 and 20 cm total length will be kept for the experiment. Non-target species will be left in the water to recover, these will be monitored to ensure rapid recovery (normally this < 30 seconds after leaving the electrical field). We will monitor seabird activity to make sure embolized fish are not injured or killed.
2.14	Provide a detailed description of the procedures (e.g. medical, surgical, implants etc.) to be performed on live animals. Include, handling methods and restraint procedures and a description of how you will keep stress to as low a level as is reasonable. Reference to existing Trent SOPs is encouraged but please list any deviations if required.	Excretion experiments of field caught animals allow for the measurement of excretion rates on animals that have been feeding at 'natural' rates typical for that species in that particular ecosystem, and therefore may be more realistic and ecologically relevant (Vanni et al (2016)). A global database of field animal nutrient excretion experiments was established in 2016 and includes observation from 92 separate sources, encompassing 491 species of which > 200 are fish (Vanni et al. (2016)). This highlights that these experiments are commonplace and based on methods that follow standard principles. Lab controlled experiments require the fish capture, transportation to the lab facility, acclimation to the new environment, and controlled feeding which likely enhances stress on fish and the likelihood of generating unrealistic excretion rates results (Ikeda (1977)). In our field experiment, fish will be collected using gillnet sets. Following capture, fish will be brought on-deck and kept in a large holding tub filled with water from the lake. Fish will be placed as soon as possible in separate plastic bags (sterile whirl-pak bags purposely built for this kind of experiments) where they can recover from capture stress and excrete for 20 to 40 minutes depending on the fish size and the water temperature. Water volume in the bags will vary between 0.2 and 4L relative to the fish size. The

		aforementioned incubation duration and bags water volume are standard in excretion experiments (e.g. Atkinson et al. (2019)) and follow methods outlined in Whiles et al (2009) to minimize handling stress response and to avoid oxygen stress and waste buildup. Hence there should not be any oxygen depletion. Plastic bags will be placed in a second large holding tub filled with water from the lake before incubation to adjust to ambient lake temperatures and will remain in the tub during incubation, with the lid of the tub closed to reduce stress due to light exposure. The location of this project is in the western basin of Lake Erie so fish will be caught in water that is < 10 m. In this area the variation in the water temperatures is minimal from top to bottom (generally less than 2 or 3 C). The very surface of the lake, top 50 cm or so can be a few degrees warmer and we will avoid this layer for water collection (boat live well draws from under the boat). We will assess water temperatures at different depths during the fish capture and experiments, and monitor hold well temperatures. If these temperatures increase, we will adjust them by bringing cooler water from deeper layers of the water. Hence, temperature levels will be that of the ambient water. Plastic bags will be held in wine racks to keep them stable in the tub and reduce stress on fish. Identification and both weight and length measurements will be done by trained staff to ensure minimal handling and time removed from water. Lengths will be taken on a standard fish measuring board, which is wood with a ruler embedded and head board to push the front of the fish against for rapid measurement. For smaller fish (< 2 kg), fish will be placed in plastic bin with a small amount of lake water mounted on a scale. For larger fish, we will use spring loaded fish scale that holds the fish by the mouth. One person will handle the fish with gloves, a second person will record the measurements. Once measured, fish will be placed in the holding tank. Holding tanks will

		be dark and at lake temperatures. We will endeavor to only interact with the fish when handling upon capture and for experiments or tissue collection and will use gloves to protect the fish. We will monitor water conditions (temperature, dissolved oxygen) and the health and stress of the fish; Indicators include fish exhibiting obvious signs of lethargy, irregular swimming patterns, or orientation issues (on their side or upside down). If any signs of stress are obvious we will release all fish. Following the excretion experiment, three individuals per sampled species will be euthanized with blunt force to the head (an accepted alternative method (in the UK and U.S.) followed by cervical dislocation, and kept on ice for transport to the lab for tissue (stable isotopes and stoichiometric composition of carbon, nitrogen, and phosphorus). Based on stable isotope analysis of fish in Lake Erie, including these species, three individual samples will provide the required variation associated with these tracers. Blunt force trauma following by cervical dislocation will be used instead of MS-222 because there are logistical limitations on the boat for having a MS-222 kill tank or cooler, (there is not enough space on the boat). As well, because these experiments will be out on the water, we have no reasonable way of disposing of the used water with MS-222 (we cannot release it in the lake) and we do not have the space to hold this water (MS-222 would require changing the water regular to make it effective). If we waited to come to shore to kill the fish, some fish would be held in the holding tanks for 5 or 6 hours – which we can't accommodate and would be very stressful for the fish.
2.15	Provide details of agents to be administered to the captured animals. (include agent name, method of administration, dosage).	n/a
2.16	Provide details regarding how the animals will be monitored while captive?	Fish will be monitored every 10 min by visual observation (maintenance of equilibrium, opercular movements). Animals will not be released until they are upright and swimming

		normally. If an animal becomes injured during capture or does not recover after the experiment (never observed in all the excretion experiments done by PhD candidate Sandra Klemet-N'Guessan), fish will be euthanized.
2.17	Provide details of the intended fate of the animals used in the study. (If the animals are to be manipulated in a laboratory setting or husbandry is required at the university for more than 6 hours Section B. must be completed.	Most fish will be returned to the lake after measurements have been taken. Fish that have been euthanized at the site will be immediately returned to the University of Windsor GLIER and kept frozen until tissue and stomach content elements have been analyzed.
2.18	List the potential hazards (biohazard, chemical, physical) to staff.	The electrofishing boat will be captained by an electrofishing certified person who will ensure safety procedures are followed and all staff will be wearing life jackets. The risk to researchers (including drowning and electrocution) is extremely low, and the electrofishing vessel (built in 2020) has numerous built- in systems to reduce risk of electrocution
2.19	Qualification and Experience of Staff. List names, positions and relevant training and experience of all individuals who will be working directly with the animals. (Attach copies of relevant documents which corroborate your description of qualifications).	Sandra Klemet-N'Guessan - PhD candidate - has experience doing this kind of experiments from her month-long field sampling in summer 2019 19ACC4677 - is trained to netting using electrofishing boat, is certified to backpack electrofishing, and will be trained for using short term gill net sets Aaron Fisk - Professor - has more than 15 years experience doing this kind of experiments in the Great Lakes - is trained and certified for operating the electrofishing boat (see attached certificate) and for using short term gill net sets Emma Vokey - will be trained to net using electrofishing boat and for using short term gill net sets
2.20	Is surgery involved?	No
2.21	Will anaesthesia be used?	No
2.22	If yes, describe the specific anaesthetic doses, applications, techniques and recovery procedures. If this is an approved SOP, please indicate the SOP number.	n/a
2.23	Will analgesics be required?	No

2.24	If yes, give specific information on procedures, type and route of application of analgesics to be used in study	n/a
2.25	Agents and materials to be used in the study. (Check all that apply)	None
2.26	Specify for each agent a)Amount of agent and dosage; b)Route of administration; c) Frequency of administration, d)How agent is excreted by animal; e)Time period of excretion.	n/a
2.27	If you have selected an option other than “None”, Give details here. An MSDS will be provided to the Manager of Animal Care before the substance is permitted within Animal Care facilities. All work with radioactive and biohazardous materials require a radioactive work permit or a biosafety work permit respectively. Include the permit number here.	n/a
2.28	Potential health risks to humans or animals	See 2.18
2.29	Special animal care requirement(s) to deal with side effects on the animal.	See 2.16
2.30	Precautions to be taken by personnel (including animal care staff)	n/a
2.31	Special containment requirements (i.e. special storage, waste and animal disposal requirements, emergency procedures)	n/a
2.32	Describe the detailed dietary requirements of your animals	n/a
2.33	Will food deprivation occur?	No
2.34	If yes, give details.	n/a
2.35	Will water deprivation occur?	No
2.36	If yes, give details.	n/a
2.37	Please explain the specific experimental, surgical or other procedures to be used on the animals. Include the use of anaesthesia, analgesics or other pharmaceuticals. Include all the details of all procedures. If an SOP exists for a particular procedure it is not necessary to explain it here, simply reference	n/a

	the SOP number and any minor deviations to the SOP that may be necessary. Consult with the Animal Care Supervisor for applicable SOPs.	
2.38	Describe and justify the “point of intervention” that you will use and explain the resulting steps for work with the animals reaching these endpoints. The term “point of intervention” is defined as the point at which an experimental animal’s pain and/or distress is terminated, minimized or reduced, by taking actions such as killing the animal humanely, terminating a painful procedure, giving treatment to relieve pain and/or distress or removing it from the project. Endpoints, should be objective and quantifiable an	Period of collection and capture of fish will be the minimum necessary to carry out study objectives. If a captured fish is particularly distressed, as evident by our difficulty in handling the fish, it will be released back into the lake. If the fish exhibits obvious signs of lethargy, irregular swimming patterns, or orientation issues (on their side or upside down), the fish will be released. If the fish looks injured, it will be euthanized and used for lab analysis. Fish held in plastic bags will experience some distress from capture and captivity, but they will be kept at ambient temperature to minimize the distress. Plastic bags will be stabilized using wine racks. Fish density in holding tubs will be kept to minimal relative to fish size and number and oxygen levels will be be maintained by regularly refreshing the water; ambient water will be regularly replaced.
2.39	What is to be the final disposition of the animals? If euthanized, how will this be accomplished?	See 2.14
2.40	What is the anticipated level of invasiveness?	D)Procedures that cause moderate to severe distress or discomfort
2.41	While the study is active, what is the expected mortality rate associated with the procedures being used?	Less than 5%. If mortality is higher than 5% shorter gill sets will be used and lower frequencies for electrofishing, and any mortality would be reported in the ACC report.

3. Declaration

#	Question	Answer
3.1	As the Principal Investigator, I agree that no changes to the work as described above will be made without instruction from the ACC or without ACC approval of desired changes. I agree that no animal work will be performed on this project without ACC approval.	Yes
3.2	Declaration Date:	2021/04/13

Animal Care Info

Purpose of Animal Use(PAU):	**1. Studies of a fundamental nature in sciences rel ...**
Category of Invasiveness(CI):	**D**
Classification:	
Protocol Description:	

Walleye (Sander vitreus):

Animal Use General:Species 1 of 3

Species:	**Walleye (Sander vitreus)**
Species Keywords:	**Fundamental science**
Strain:	
Weight:	
Gender:	
Source:	
Source Contact:	
Transportation:	
Housing Building & Room:	
Experimental/Procedure Building & Room:	
PAU:	**1. Studies of a fundamental nature in sciences rel ...**
CI:	**D**
Comments:	
# Animals Requested:	**120**
# Animals Approved:	**0**

Yellow Perch (Perca flavescens):

Animal Use General:Species 2 of 3

Species:	**Yellow Perch (Perca flavescens)**
Species Keywords:	
Strain:	
Weight:	
Gender:	
Source:	
Source Contact:	
Transportation:	
Housing Building & Room:	
Experimental/Procedure Building & Room:	
PAU:	**1. Studies of a fundamental nature in sciences rel ...**
CI:	**D**
Comments:	
# Animals Requested:	**120**
# Animals Approved:	**0**

White bass (Morone chrysops):

Animal Use General:Species 3 of 3

Species:	**White bass (Morone chrysops)**
Species Keywords:	
Strain:	
Weight:	
Gender:	
Source:	
Source Contact:	
Transportation:	
Housing Building & Room:	
Experimental/Procedure Building & Room:	
PAU:	**1. Studies of a fundamental nature in sciences rel ...**
CI:	**D**

Comments:
 # Animals Requested: **120**
 # Animals Approved: **0**

Attachments

Doc / Agreement	Version Date	File Name	Description
		21 06 01 response letter to ACC comments.docx	Response letter to ACC comments
		IMG_3313.JPG	Aaron Fisk Class 1 boat electrofishing certificate

CHAPTER 3 AUP #25754

Animal Use Protocol Application For Wildlife and Field Work Research

Project Info.

File No: 25754
Project Title: The effect of dissolved organic matter on aquatic animals-mediated nutrient recycling
Principal Investigator: Dr. Marguerite Xenopoulos (Natural Sciences\Biology)
Start Date: 2019/05/01
End Date: 2019/12/31
Keywords: fish, food web dynamics, biogeochemistry, nutrient cycling

Project Team Info.

Principal Investigator

Prefix: Dr.
Last Name: Xenopoulos
First Name: Marguerite
Affiliation: Natural Sciences\Biology
Rank: Professor
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Phone1: 748-1011 ext. 7699
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Primary Address: DNA Building
Institution: Trent University
Country: Canada
Comments:

Other Project Team Members

Prefix	Last Name	First Name	Affiliation	Role In Project	Email

Miss.	D'Amario	Sarah	Natural Sciences\Biology	Co-Investigator	sarahcdamario@trentu.ca
Ms.	Klemet-n'guessan	Sandra	Natural Sciences\Biology	Person Responsible for Animal Monitoring	sandraklemet@trentu.ca

Common Questions

1. Research Project Overview

#	Question	Answer
1.1	Title of Project (must be descriptive and unique)	The effect of dissolved organic matter (DOM) on aquatic animals-mediated nutrient recycling
1.2	Please indicate either the research account number or the ROMEO project number that this protocol relates to:	55-52525
1.3	Lay Summary of project. Give the aims, and scientific significance of the work to be performed in terms understandable and meaningful to the general public. Please try to limit to 200 words or less.	CHANGED TO LAY TERMS AS REQUESTED: Aquatic animals play important roles in nutrient cycles. Fish, in particular, can be important for the recycling of nutrients via their feeding activities. Once these nutrients are released from fish back into the environment, they become available again for primary producers and can fuel algal growth. In this project we aim to measure nutrient release rates from fish found in rivers and streams that vary in their ambient nutrient and carbon conditions. Carbon quality may influence animal nutrient release rates directly and indirectly through its influence on microbial activity, changes to the fish food quality, and changes to the water temperature and metabolism. How carbon quality affects nutrient release rates from fish has not yet been studied. In addition to measuring nutrient release rates we will also estimate population density of each species

		and combined with individuals' weight to calculate species, nutrient release in each stream.
1.4	As principal investigator, please provide the date that the Animal Care course was completed.	2012/01/01
1.5	Please provide an emergency telephone number where you can be reached.	x7699
1.6	Other Investigators. Are other investigators participating in this research? If yes, make sure to add all other investigators on this project by accessing the "Project Team" Tab.	Yes
1.7	Please provide the name(s) of the person(s) responsible for the care of the animals and for the health monitoring program. Include and emergency number and the date that each of the individuals completed the Animcal Care Course	Sandra Klemet-N'Guessan - 514 701 1904 - Animal care course completed on 2019/02/27 Sarah D'Amario -705 933 7263 - Animal care course completed on 2019/02/21
1.8	What are the specific objectives of this work?	THIS WAS CHANGED AS REQUESTED: To examine animal-mediated nutrient pathways in streams we will: 1) Test the indirect effects of high light and low nutrients availability in low dissolved organic carbon (DOC) environments on animal-mediated nutrient release (i.e. the quantity of nutrients released by different fish species) and ecosystem recycling (i.e. the contribution of fish to the cycling of nutrients in the stream); 2) Test the indirect of effects of low light and high nutrients availability in high DOC environments on animal-mediated nutrient release; 3) test the hypothesis that animal-mediated nutrient release rates and ratios increase with low DOC and more microbial-like DOM quality (note DOM = dissolved organic matter) but decreases at high DOC, refractory DOM quality (note: the relationship between DOM and excretion rates is predicted to be nonlinear).
1.9	Purpose of Animal Study? (Check one) Note: Honours thesis projects are considered Research Projects If this is for an undergraduate course please complete the teaching protocol application.	Fundamental Research

1.10	Peer Review Has this project been peer reviewed for scientific merit through a research granting agency? If No, contact the Office of Research to arrange for two independent peer reviews. Note: External peer reviews may take some time to obtain; allow sufficient time between your submission and your proposed start date. Please refer to submission deadlines on the Animal Care website.	Yes
1.11	If yes, please indicate which funding agency. If No, contact the Office of Research to arrange for two independent peer reviews. Note: External peer reviews may take some time to obtain; allow sufficient time between your submission and your proposed start date. Please refer to submission deadlines on the Animal Care website.	NSERC
1.12	Replacement, Reduction and Refinement. For a review of the 3R's of animal use, please visit www.ccac.ca/en/alternatives/index.html, the "special topics" tab of that mage may be of use for your work. The P.I. has investigated and evaluated alternative methods which would accomplish the same scientific goals.	Yes
1.13	Support this statement with a brief description of the methods and sources used to determine that non-animal alternatives were not available, or an explanation of the aspects of the protocol that preclude using animals of a lower sentience. In addition, explain why the numbers of animals proposed cannot be further reduced. Finally, justify the invasive procedures (if any) proposed are at the lowest level possible and why they cannot be reduced further.	To measure the effects of dissolved organic matter (DOM; usually measured in units of carbon or DOC) on excretion rates of three species with different feeding strategies, real animals must be used to gather data about how the ambient nutrient and light environment (as mediated by dissolved organic matter) has affected them. This effect cannot be tested through modelling (and in fact, modelling these excretion rates has been highly criticized by experts in the field (Findlay et al. (2005)). Up to 15 fish per species will be used per stream. These types of animal-mediated excretion experiments and incubations require many replicates (10-15) to account for body size and species-specific variability (Wilson and Xenopoulos 2011) and to be acceptable for peer-review and publication. The three species allow us to test the consistency of relationships in benthic and water column feeding species.

		The most effective way to sample fishes in a small, shallow creek is by electrofishing; traps and seines are of limited use in this environment. Our proposed sampling methods are based on existing protocols for this species (e.g., Wilson and Xenopoulos (2011) and Blair et al (2018)).

2. Project Detail

#	Question	Answer
2.1	Proposed start date. (Note: If the application is approved the committee may assign a start date that differs from the proposed start date.)	2019/05/01
2.2	What is the duration of the project? (Even if the duration of the live animal work is only a few months, the protocol will be active for a minimum of one year from the date of approval.)	one year
2.3	Animals : Genus/Species	-Semotilus atromaculatus -Micropterus salmoides -Luxilus cornutus
2.4	Animals: Common Name	-Creek shub -Largemouth bass -Common shiner
2.5	Number of animals per species required for the duration of the study	180 creek shub, 180 largemouth bass, 180 common shiner
2.6	If this is a multi-year project, estimate the number of animals per species to be used in year one, year two, and year three. (Note the sum of animals over all years must not exceed the total given	n/a
2.7	Provide a justification for the number of animals required.	12 sites x 15 individuals per site = 180 individuals per species 180 individuals x 3 species = 540 fish total Metabolic and excretion rates are variable within individuals, and so more animals are needed for replicates to account for individual variation in respiration. In addition, metabolic and excretion rates are affected by body size, so different sizes of fish must be used to make a reasonable determination of scaling factor. The number selected is consistent with the number of crayfish used by Wilson and Xenopoulos (2011) in a similar fish study.

2.8	Location: Where will the study take place?	This project will take place in 12 Southern Ontario streams
2.9	If permits are required, please list them below. Upload scans of the permits to this form, or forward copies to the Office of Research or Manager of Animal Care.	MNRF scientific collection permit
2.10	If permits are required,indicate their status:	Pending
2.11	If obtained, provide the Permit Number	
2.12	Describe the precautions to be taken to avoid capturing non - target species or vulnerable animals.	All staff will be trained in the correct identification of fish and handling procedures. Areas where vulnerable species are known to exist will be avoided. All staff will be trained to identify species listed on the Species At Risk Act (e.g., black redhorse, channel darter, cutlip minnow, deepwater sculpin, eastern sand darter, lake chubsucker and lake whitefish).
2.13	Provide a detailed description of the procedures that will be used to trap/capture the animals.	Fish will be collected using a backpack electroshocker set to a frequency and voltage that will force fish to swim towards an anode for collection, but that will physcially harm the fish as little as possible. Note that a backpack electroshocker was used for Lisa Graham's project (ACC12008). In some cases, the electroshocker will only be used to direct fish into a beach seine net or where possible, a seine net alone will be used. Fish will be caught by a netter and placed in a reservoir (holding unit) in the river, so as to allow fresh water flow through the reservoir.
2.14	Provide a detailed description of the procedures (e.g. medical, surgical, implants etc.) to be performed on live animals. Include, handling methods and restraint procedures and a description of how you will keep stress to as low a level as is reasonable. Reference to existing Trent SOPs is encouraged but please list any deviations if required.	Fish will be collected using an electroshocker and nets. They will be placed in separate plastic bags where they can recover from capture stress and excrete for 20 to 40 minutes. Plastic bags will be incubated in the stream at ambient temperature placed in the stream before incubation to adjust to ambient stream temperatures and will remain in the stream during incubation. Hence, temperature levels will be that of the ambient water. Identification and both weight and length measurements will be done by trained staff to ensure minimal handling and time removed from water.

		Twelve individuals from each of three abundant species will be held briefly in the field for excretion rate measurements. This will include keeping them in plastic bags for up to 1 hour to let them recover from capture stress and let them excrete. Following the excretion experiment, 6 individuals of 3 common species abundant fish species (present > 10 individuals) will be euthanized with blunt force to the head (an accepted alternative method (in the UK and U.S.), and kept on ice for transport to the lab for tissue (stable isotopes and stoichiometric composition of carbon, nitrogen, and phosphorus) and gut content analyses. Blunt force trauma will be used instead of ms-222 in order to avoid effects of the chemical contamination on fish tissues.
2.15	Provide details of agents to be administered to the captured animals. (include agent name, method of administration, dosage).	n/a
2.16	Provide details regarding how the animals will be monitored while captive?	Fish will be monitored every 30 min by visual observation. Animals will not be released until they are upright and swimming normally. If there are signs of deformity or inability to maintain a normal swimming position, fish will be euthanized.
2.17	Provide details of the intended fate of the animals used in the study. (If the animals are to be manipulated in a laboratory setting or husbandry is required at the university for more than 6 hours Section B. must be completed.	Most fish will be returned to the stream after measurements have been taken. Fish that have been euthanized at the site will be immediately returned to Trent and kept frozen until tissue and stomach content elements have been analyzed.
2.18	List the potential hazards (biohazard, chemical, physical) to staff.	In addition to the normal risks of river stream sampling, there is risk of electrical shock through improper use of electrofishing equipment.
2.19	Qualification and Experience of Staff. List names, positions and relevant training and experience of all individuals who will be working directly with the animals. (Attach copies of relevant documents which corroborate your description of qualifications).	Sandra Klemet-N'Guessan - will be training as electrofishing crew leader on May 27th/28th Sarah D'Amario - will be training as electrofishing crew leader on May 27th/28th
2.20	Is surgery involved?	No

2.21	Will anaesthesia be used?	No
2.22	If yes, describe the specific anaesthetic doses, applications, techniques and recovery procedures. If this is an approved SOP, please indicate the SOP number.	n/a
2.23	Will analgesics be required?	No
2.24	If yes, give specific information on procedures, type and route of application of analgesics to be used in study	n/a
2.25	Agents and materials to be used in the study. (Check all that apply)	None
2.26	Specify for each agent a)Amount of agent and dosage; b)Route of administration; c) Frequency of administration, d)How agent is excreted by animal; e)Time period of excretion.	n/a
2.27	If you have selected an option other than “None”, Give details here. An MSDS will be provided to the Manager of Animal Care before the substance is permitted within Animal Care facilities. All work with radioactive and biohazardous materials require a radioactive work permit or a biosafety work permit respectively. Include the permit number here.	n/a
2.28	Potential health risks to humans or animals	See 2.18
2.29	Special animal care requirement(s) to deal with side effects on the animal.	See 2.16
2.30	Precautions to be taken by personnel (including animal care staff)	Precautions against being shocked include wearing of chest waders and rubber gloves at all times when shocking, having personnel with first aid training present at all times, having mobile phones in case of emergency.
2.31	Special containment requirements (i.e. special storage, waste and animal disposal requirements, emergency procedures)	n/a
2.32	Describe the detailed dietary requirements of your animals	n/a
2.33	Will food deprivation occur?	No
2.34	If yes, give details.	n/a

2.35	Will water deprivation occur?	No
2.36	If yes, give details.	n/a
2.37	Please explain the specific experimental, surgical or other procedures to be used on the animals. Include the use of anaesthesia, analgesics or other pharmaceuticals. Include all the details of all procedures. If an SOP exists for a particular procedure it is not necessary to explain it here, simply reference the SOP number and any minor deviations to the SOP that may be necessary. Consult with the Animal Care Supervisor for applicable SOPs.	n/a
2.38	Describe and justify the “endpoint(s)” that you will use and explain the resulting steps for work with the animals reaching these endpoints. The term “endpoint” is defined as the point at which an experimental animal’s pain and/or distress is terminated, minimized or reduced, by taking actions such as killing the animal humanely, terminating a painful procedure, giving treatment to relieve pain and/or distress or removing it from the project. Endpoints, should be objective and quantifiable an	Period and intensity of electroshocking will be the minimum necessary to carry out study objectives. Fish held in plastic bags will experience some distress from capture and captivity, but they will be kept at ambient temperature and in shade to minimize the distress. The period of captivity will be the minimum required to obtain reasonable estimates of excretion rates of phosphorus and nitrogen. Following brief handling in order to identify, measure the length and weigh the fish, fish kept for lab analysis, while all other fish will be released. If a captured fish is particularly distressed, as evident by our difficulty in handling it, it will be released back into the stream. Occasionally, injury or mortality as a result of electrofishing can occur. In this situation the fish will be euthanized (if not dead) and kept for lab analysis.
2.39	What is to be the final disposition of the animals? If euthanized, how will this be accomplished?	See 2.14
2.40	What is the anticipated level of invasiveness?	C)Procedures that cause minor stress or pain of short duration
2.41	While the study is active, what is the expected mortality rate associated with the procedures being used?	Less than 5%.

3. Declaration

#	Question	Answer
3.1	As the Principal Investigator, I agree that no changes to the work as described above will be made without instruction from the ACC or without ACC approval of desired changes. I agree that no animal work will be performed on this project without ACC approval.	Yes
3.2	Declaration Date:	2019/02/28

Animal Care Info

Purpose of Animal Use(PAU): **1. Studies of a fundamental nature in sciences rel ...**
Category of Invasiveness(CI): **C**
Classification:
Protocol Description:

Creek Chub (Semotilus atromaculatus):

Animal Use General:Species 1 of 3

Species: **Creek Chub (Semotilus atromaculatus)**
Species Keywords:
Strain:
Weight:
Gender:
Source:
Source Contact:
Transportation:
Housing Building & Room:
Experimental/Procedure Building & Room:
PAU: **1. Studies of a fundamental nature in sciences rel ...**
CI: **C**
Comments:
 # Animals Requested: **180**
 # Animals Approved: **0**

Common shiner (Luxilus cornutus):

Animal Use General:Species 2 of 3

Species:	**Common shiner (Luxilus cornutus)**
Species Keywords:	
Strain:	
Weight:	
Gender:	
Source:	
Source Contact:	
Transportation:	
Housing Building & Room:	
Experimental/Procedure Building & Room:	
PAU:	**1. Studies of a fundamental nature in sciences rel ...**
CI:	**C**
Comments:	
# Animals Requested:	**180**
# Animals Approved:	**0**

Large Mouth Bass (Micropterus salmoides):

Animal Use General:Species 3 of 3

Species:	**Large Mouth Bass (Micropterus salmoides)**
Species Keywords:	**Fundamental science**
Strain:	
Weight:	

Gender:
Source:
Source Contact:
Transportation:
Housing Building & Room:
Experimental/Procedure Building & Room:
PAU: **1. Studies of a fundamental nature in sciences rel ...**
CI: **C**
Comments:
 # Animals Requested: **180**
 # Animals Approved: **0**

Attachments

Doc / Agreement	Version Date	File Name	Description
		xenopoulos 25754 aprvl email.msg	N/A

CHAPTERS 3 & 4 AUP #26239

Animal Use Protocol Application For Wildlife and Field Work Research

Project Info.

File No: 26239
Project Title: Linking carbon to structure and function in aquatic ecosystems
Principal Investigator: Dr. Marguerite Xenopoulos (Natural Sciences\Biology)
Start Date: 2020/05/01
End Date: 2022/09/30
Keywords: fish, food web dynamics, biogeochemistry, nutrient cycling

Related Awards:

Award File No	Principal Investigator	Project Title	Funding Snapshot	Notes
25455	Marguerite Xenopoulos	Linking carbon to structure and function in aquatic ecosystems	**NSERC** Program: Discovery Grants Program - Individual Type: Research Grant Account#: 55-52525 Requested: CAD 556,500.00 Awarded: CAD 330,000.00 **PROJECT TOTALS:** Requested: CAD 556,500.00 Awarded : CAD 330,000.00	N/A

Project Team Info.

Principal Investigator

Prefix: Dr.

Last Name: Xenopoulos
First Name: Marguerite
Affiliation: Natural Sciences\Biology
Position: Professor
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Phone2:
Fax: 748-1139
Primary Address: LHS Building
Institution: Trent University
Country: Canada
Comments:

Other Project Team Members

Prefix	Last Name	First Name	Affiliation	Role In Project	Email
Miss.	Hayhurst	Lauren	Animal Care	Co-Investigator	lauren.hayhurst@hotmail.com
	Hrenchuk	Lee		Co-Investigator	lhrenchuk@iisd-ela.org
	Yeung	Emily		Co-Investigator	emilyyeung@trentu.ca
Ms.	Klemet-n'guessan	Sandra	Natural Sciences\Biology	Co-Investigator	sandraklemet@trentu.ca

Common Questions

1. Research Project Overview

#	Question	Answer
1.1	Title of Project (must be descriptive and unique)	The effect of dissolved organic matter (DOM) on aquatic animals-mediated nutrient recycling
1.2	Please indicate either the research account number or the ROMEO project number that this protocol relates to:	55-52525
1.3	Lay Summary of project. Give the aims, and scientific significance of the work to be performed in terms understandable and meaningful to the general public. Please try to limit to 200 words or less.	Aquatic animals play important roles in nutrient cycles. Fish, in particular, can be important for the recycling of nutrients via their feeding activities. Once these nutrients are released from fish back into the environment, they become available again for primary producers and can fuel algal growth. In this project we aim to measure nutrient release rates from fish found in lakes that vary in their ambient nutrient and carbon conditions. Carbon quality may influence animal nutrient release rates directly and indirectly through its influence on microbial activity, changes to the fish food quality, and changes to the water temperature and metabolism. How carbon quality affects nutrient release rates from fish has not yet been studied. In addition to measuring nutrient release rates we will also use historical estimates population density of each species and combine them with individuals' weight to calculate species nutrient release in each lake.
1.4	As principal investigator, please provide the date that the Animal Care course was completed.	2012/01/01
1.5	Please provide an emergency telephone number where you can be reached.	x7699
1.6	Other Investigators. Are other investigators participating in this research? If yes, make sure to add all other investigators on this project by accessing the "Project Team" Tab.	Yes
1.7	Please provide the name(s) of the person(s) responsible for the care of the animals and for the health monitoring program. Include and emergency number and the date that	Sandra Klemet-N'Guessan - 514 701 1904 - Animal care course completed on 2019/02/27 Lee Hrenchuk - 204 291-7644 - Animal care course completed on 2014/05/09 Lauren Hayhurst - Animal care course completed on

	each of the individuals completed the Animcal Care Course	2014/08/25 Emily Yeung - 519 497 8189 - Animal care course completed on 2019/04/10
1.8	What are the specific objectives of this work?	To examine animal-mediated nutrient pathways in lakes we will: 1) Test the effects of high light and low nutrients availability in low dissolved organic carbon (DOC) environments on animal-mediated nutrient release (i.e. the quantity of nutrients released by different fish species) and ecosystem recycling (i.e. the contribution of fish to the cycling of nutrients in the lake); 2) Test the effects of low light and high nutrients availability in high DOC environments on animal-mediated nutrient release and ecosystem recycling; 3) test the hypothesis that animal-mediated nutrient release rates and ratios increase with low DOC and more microbial-like DOM quality (note DOM = dissolved organic matter) but decreases at high DOC, refractory DOM quality (note: the relationship between DOM and excretion rates is predicted to be nonlinear).
1.9	Purpose of Animal Study? (Check one) Note: Honours thesis projects are considered Research Projects If this is for an undergraduate course please complete the teaching protocol application.	Fundamental Research
1.10	Peer Review Has this project been peer reviewed for scientific merit through a research granting agency? If No, contact the Office of Research to arrange for two independent peer reviews. Note: External peer reviews may take some time to obtain; allow sufficient time between your submission and your proposed start date. Please refer to submission deadlines on the Animal Care website.	Yes
1.11	If yes, please indicate which funding agency. If No, contact the Office of Research to arrange for two independent peer reviews. Note: External peer reviews may take some time to obtain; allow sufficient time between your submission and your proposed start date. Please refer to submission deadlines on the Animal Care website.	NSERC

1.12	Replacement, Reduction and Refinement. For a review of the 3R's of animal use, please visit www.ccac.ca/en/alternatives/index.html, the "special topics" tab of that mage may be of use for your work. The P.I. has investigated and evaluated alternative methods which would accomplish the same scientific goals.	Yes
1.13	Support this statement with a brief description of the methods and sources used to determine that non-animal alternatives were not available, or an explanation of the aspects of the protocol that preclude using animals of a lower sentience. In addition, explain why the numbers of animals proposed cannot be further reduced. Finally, justify the invasive procedures (if any) proposed are at the lowest level possible and why they cannot be reduced further.	To measure the effects of dissolved organic matter (DOM; usually measured in units of carbon or DOC) on excretion rates of eight species with different feeding strategies, real animals must be used to gather data about how the ambient nutrient and light environment (as mediated by dissolved organic matter) has affected them. This effect cannot be tested through modelling (and in fact, modelling these excretion rates has been highly criticized by experts in the field (Findlay et al. (2005)). Up to 20 fish per species will be used per lake. These types of animal-mediated excretion experiments and incubations require many replicates (10-20) to account for body size and species-specific variability (Wilson and Xenopoulos 2011) and to be acceptable for peer-review and publication. The eight species allow us to test the consistency of relationships in benthic and water column feeding species. The most effective ways to sample fishes in small to medium-sized lakes and minimize the fasting period between the moment the fish is caught and that it is tested (which would otherwise affect the excretion rates) is by minnow trapping, small fyke netting, angling, and seine netting; trap nets can hold fish up to 3 days with little to no feeding. Our proposed sampling methods are based on existing protocols for this species (e.g., Wilson and Xenopoulos (2011) and our approved 2019 animal care protocol 19ACC4677).

2. Project Detail

#	Question	Answer
2.1	Proposed start date. (Note: If the application is approved the committee may assign a start	2020/05/01

	date that differs from the proposed start date.)	
2.2	What is the duration of the project? (Even if the duration of the live animal work is only a few months, the protocol will be active for a minimum of one year from the date of approval.)	one year
2.3	Animals : Genus/Species	-Margariscus margarita -Cottus cognatus -Notropis heterolepis -Esox lucius -Perca flavescensone -Catostomus commersonii -Pimephales promelas -Phoxinus eos
2.4	Animals: Common Name	-pearl dace -slimy sculpin -blacknose shiner -northern pike -yellow perch -white sucker -fathead minnow -northern redbelly dace
2.5	Number of animals per species required for the duration of the study	40 - 200 per species depending species presence
2.6	If this is a multi-year project, estimate the number of animals per species to be used in year one, year two, and year three. (Note the sum of animals over all years must not exceed the total given	n/a
2.7	Provide a justification for the number of animals required.	The number of individuals per species required will depend on the number of lakes the species is present. -pearl dace: 8 sites x 20 individuals = 160 -slimy sculpin: 7 sites x 20 individuals = 140 -blacknose shiner: 2 sites x 20 individuals = 40 -northern pike: 3 sites x 20 individuals = 60 -yellow perch: 4 sites x 20 individuals = 80 -white sucker: 8 sites x 20 individuals = 160 -fathead minnow: 6 sites x 20 individuals = 120 -northern redbelly dace: 5 sites x 20 individuals = 120 160+140+40+60+80+160+120+120 = 880 fish total Up to four species in each lake will be sampled, but given that species assemblage differ from a lake to another, a total of 8 species will be sampled. Metabolic and excretion rates are variable within individuals, and so more animals are needed for replicates to account for individual variation in respiration. In addition, metabolic and excretion rates are affected by body size, so different sizes of fish must be used to make a reasonable determination of scaling factor. The number selected is consistent with the

		number of fish used by Wilson and Xenopoulos (2011) in a similar fish study and our approved 2019 animal care protocol 19ACC4677
2.8	Location: Where will the study take place?	This project will take place in 10 lakes at the Experimental Lakes Area in Northwestern Ontario
2.9	If permits are required, please list them below. Upload scans of the permits to this form, or forward copies to the Office of Research or Manager of Animal Care.	MNRF scientific collection permit
2.10	If permits are required,indicate their status:	Pending
2.11	If obtained, provide the Permit Number	
2.12	Describe the precautions to be taken to avoid capturing non - target species or vulnerable animals.	All staff will be trained in the correct identification of fish and handling procedures. Areas where vulnerable species such as the lake trout are known to exist will be avoided.
2.13	Provide a detailed description of the procedures that will be used to trap/capture the animals.	Fish will be collected using a combination of minnow traps/small fyke nets, seine nets, and angling. Minnow traps and small fyke nets (< 1 m diameter) are used to capture small fish species such as minnows and yellow perch. Minnow traps are baited with a “bait ball” made of oatmeal and flour held within a PVC canister with mesh ends and are allowed to sit overnight but never for more than 24 h. Fyke nets are not baited and are deployed for <24 hours. Fish are removed from the trap/net and placed in tubs of fresh lake water covered in wetted mesh covers to prevent the fish from jumping out of the tubs. These fish are transported to shore for processing. Northern pike may be targeted by angling with barbless hooks. Care is taken to set the hook gently such that the fish does not swallow the hook too deeply. A rubber mesh landing net is used to bring the fish from the water into a large cooler in the boat filled with fresh lake water where the hook is removed with long pliers and (if needed) jaw spreaders (northern pike only). The fish is then transported by boat to the shore where it is placed in a large mesh holding pen (2-4 m3) and held until processing (<2 hours after

		capture) Beach seine nets (~2 m depth x ~70 m length; some nets have a bag ~2x2x2 m in the middle of the net while others are flat) are used to capture small-bodied fish species. The seine is deployed from the boat in a crescent shape with one end remaining on shore such that it encircles a half-circle shaped area of the lake. Researchers pull the net toward shore from both ends, making sure the weighted bottom line doesn't lift off the bottom of the lake. The fish are funneled toward the "bag" in the middle of the net (or the middle of the net if there is no bag) as the net is pulled to shore. Once the seine has been pulled to shore fish are removed from the net with small mesh dip nets and placed in coolers or tubs filled with fresh lake water. Non-target species are immediately released back into the lake. Adverse responses to capture are possible, including: 1. occasional injury or mortality due to entanglement in nets or gear; 2. occasional mortality of very small minnows; 3. occasional angling mortality (e.g., if hooked in gills). To minimize the potential for adverse effects, nets and traps are checked in a timely manner and fish are gently removed from traps/nets and immediately placed in tubs of fresh lake water. For angling, we only use barbless hooks, care is taken not to set the hook very strongly, a rubber landing net is used to bring the fish into the boat, and hooks are removed quickly from fish using long pliers and jaw spreaders (northern pike only). Young-of-year (YOY) fishes are fragile and easily damaged, meaning that much of the mortality we see is in this age class. We take care to handle all fish gently, but some mortality of the very smallest individuals is not uncommon
2.14	Provide a detailed description of the procedures (e.g. medical, surgical, implants etc.) to be performed on live animals. Include, handling methods and restraint procedures and a description of how you will keep stress to as low a level as is reasonable. Reference to existing Trent SOPs is encouraged but please list any deviations if required.	Fish will be collected using one or a combination of the following methods: minnow traps/small fyke nets, angling, and seine nets. Following capture, fish are transported to shore for sampling. Fresh lake water is added frequently to tubs to keep temperatures and oxygen at appropriate levels. In all cases, fish are handled as gently

		as possible, including the use of rubber or fabric mesh landing nets. They will be placed in separate plastic bags or large plastic containers (for fish >25cm) where they can recover from capture stress and excrete for 20 to 40 minutes. Bags and containers will be incubated in the lake so that temperature levels are be that of the ambient water. Following the excretion experiment, all individuals except for three individuals per sampled species will be anesthesized using MS-222, weighted, then released back to the lake. We are using MS-222 to anesthetize fish before weighing them to prevent fish from moving while they are being weighted. This is particularly important for big fish to minimize risk of injury, but this is not a required precaution for smaller fish. The three individuals per sampled species that will not be anesthetized prior to being weighed will thus be in the small size range. These will be used for lab analyses. This is a preferred method to weighing fish in a tared bucket of water for several reasons including 1) buckets of water will require high quantity of water, particularly for big fish which may exceed the maximum weight that can be measured on the balance, 2) mass results may be less accurate and harder to convert to dry mass later on for analysis purposes. The three individuals per sampled species that will be kept will be euthanized with blunt force to the head (an accepted alternative method (in the UK and U.S.) or by severing the spine behind the head, and kept on ice for transport to the lab for tissue (stable isotopes and stoichiometric composition of carbon, nitrogen, and phosphorus) and gut content analyses. A cranial blow followed by pithing of the brain will be used instead of MS-222 in order to avoid effects of the chemical contamination on fish tissues. This is an accepted method for fish euthenasia in the UK and USA that will be used only for those fish used for stoichiometric analysis.MS-222 is an organic C-rich compound (Ethyl 3-aminobenzoate methanesulfonate) that we

		inject into fish. This compound contaminates fish tissue with excess organic carbon. Our endpoint is to measure organic carbon composition of the fish tissue. Thus we cannot use MS-222 for these fish. Following fish release or euthanasia, the excretion water sample in the sampling bag or container will be filtered then analyzed back in the lab.
2.15	Provide details of agents to be administered to the captured animals. (include agent name, method of administration, dosage).	n/a
2.16	Provide details regarding how the animals will be monitored while captive?	Fish will be monitored every 10 min by visual observation (maintenance of equilibrium, opercular movements). Animals will not be released until they are upright and swimming normally. If an animal becomes injured during capture or does not recover after the procedure (rare), fish will be euthanized.
2.17	Provide details of the intended fate of the animals used in the study. (If the animals are to be manipulated in a laboratory setting or husbandry is required at the university for more than 6 hours Section B. must be completed.	Most fish will be returned to the lake after measurements have been taken. Fish that have been euthanized at the site will be immediately returned to the ELA field camp and kept frozen until tissue and stomach content elements have been analyzed.
2.18	List the potential hazards (biohazard, chemical, physical) to staff.	Deer ticks carrying Lyme disease are a potential concern at IISD-ELA. Rodents can be a source of exposure for humans to Hantavirus. Tricaine methanesulfonate (MS-222) is used as an anesthetic in this protocol. The powder (dry) form of MS-222 may cause irritation if exposed to skin, eyes, or if inhaled. All-terrain vehicles (ATVs) will be operated to commute from and to field sites and boats will be piloted on lakes; these motorized vehicles could be a potential physical hazard.
2.19	Qualification and Experience of Staff. List names, positions and relevant training and experience of all individuals who will be working directly with the animals. (Attach copies of relevant documents which corroborate your description of qualifications).	Sandra Klemet-N'Guessan - PhD candidate - has experience doing this kind of experiments from her month-long field sampling last summer 2019 under the protocol 19ACC4677. She will be trained for minnow trapping/angling in the first two weeks of May by Lee Hrenchuk and Lauren Hayhurst Emily Yeung - Undergraduate student - has

		experience catching and handling fish from her month-long field and lab work last Fall under the protocol 19ACC24353. She will be trained for minnow trapping/angling in the first two weeks of May by Lee Hrenchuk and Lauren Hayhurst Lee Hrenchuk - Senior Biologist at ELA - has 13 years of experience conducting and training others in a variety of fisheries-related procedures, including: • Diverse capture techniques for small- and large-bodied fish species (trap nets, gill nets, fyke nets, seine nets, minnow traps, and angling) • Basic techniques for collecting biological information from fish (weight, length, sex) • Safe and ethical practices for euthanizing fish using an overdose of MS-222 and severing of the spine • Lee has extensive experience training others in safe, ethical fisheries practices • Lee completed CCAC online training (including Fish module) from the University of Manitoba (2014) and "The Experimental Fish" online training from the Canadian Aquaculture Institute (2008). Lauren Hayhurst - Fisheries Research Biologist - has 7 years of experience conducting and training others in a variety of fisheries-related procedures, including: • Diverse capture techniques for small- and large-bodied fish species (trap nets, gill nets, fyke nets, seine nets, minnow traps, and angling) • Basic techniques for collecting biological information from fish (weight, length, sex) • Lauren has extensive experience training others in safe, ethical fisheries practices • Safe and ethical practices for euthanizing fish using an overdose of MS-222 and severing of the spine • Lauren completed Animal Care/Ethics online training (including Fish module) from the University of Manitoba (2014)
2.20	Is surgery involved?	No
2.21	Will anaesthesia be used?	Yes
2.22	If yes, describe the specific anaesthetic doses, applications, techniques and recovery	Fish are anesthetized with tricaine methanesulfonate (MS-222) buffered to a pH of ~7 with sodium bicarbonate (60 ug/L MS-

	procedures. If this is an approved SOP, please indicate the SOP number.	222; 120 ug/L sodium bicarbonate) prior to weighing them. The MS-222 and sodium bicarbonate are dissolved in lake water and the fish are immersed in the bath until an appropriate level of sedation is reached. Small-bodied fish take 1-2 minutes to reach an appropriate level of sedation, and large-bodied fish take 2-5 minutes. Level of sedation is assessed through visual observation of activity, opercular movements, change in equilibrium, and response to squeezing of caudal peduncle. After processing, fish are placed in a tub of fresh lake water which is regularly refreshed. The fish is monitored (maintenance of equilibrium, opercular movements, response to squeeze of caudal peduncle) while in the tub and is released back into the lake when deemed to be sufficiently recovered (generally takes just a few minutes). If a fish does not recover (rare), it is euthanized in an overdose bath of tricaine methanesulfonate (TMS; 400 mg/L) buffered to a neutral pH with sodium bicarbonate. The fish will be left in the overdose bath until 10 minutes after cessation of opercular movements, after which we will sever the spine just posterior to the head.
2.23	Will analgesics be required?	Yes
2.24	If yes, give specific information on procedures, type and route of application of analgesics to be used in study	MS-222 is an analgesic as it is a muscle relaxant which works by dampening nerve impulses. It thus works both to sedare the animal (reduce movement) and to relieve pain (Ramlochansingh et al. 2014)
2.25	Agents and materials to be used in the study. (Check all that apply)	None
2.26	Specify for each agent a)Amount of agent and dosage; b)Route of administration; c) Frequency of administration, d)How agent is excreted by animal; e)Time period of excretion.	n/a
2.27	If you have selected an option other than “None”, Give details here. An MSDS will be provided to the Manager of Animal Care before the substance is permitted within	n/a

	Animal Care facilities. All work with radioactive and biohazardous materials require a radioactive work permit or a biosafety work permit respectively. Include the permit number here.	
2.28	Potential health risks to humans or animals	See 2.18
2.29	Special animal care requirement(s) to deal with side effects on the animal.	See 2.16
2.30	Precautions to be taken by personnel (including animal care staff)	Regarding the use of MS-222, precaution will be taken when handling the powdered substance, including wearing appropriate PPE (e.g., gloves), and avoiding inhaling any of the substance. The 58 designated research lakes at IISD-ELA are closed to the public for fishing by the Ontario Ministry of Natural Resources and Forestry. This means that no fish are ever harvested from the lakes for consumption, such that the public is not at risk of consuming fish that have been recently exposed to MS-222 All personnel operating motorized vehicles will be required to possess the appropriate license and Pleasure Craft Operator's Card (PCOC). Personnel will also be trained prior to operating these vehicles. The following rescue equipment shall be carried in all watercraft when workers are present: floating rescue rope that is a minimum of 15 m in length, 2-way radio and/or another form of emergency communication such as a satellite phone, first aid kit, a minimum of 2 oars or paddles, bailing bucket of at least 4 L capacity, signalling device (e.g., whistle) and fire extinguisher (boats only). Workers will at all times work in pairs.
2.31	Special containment requirements (i.e. special storage, waste and animal disposal requirements, emergency procedures)	n/a
2.32	Describe the detailed dietary requirements of your animals	n/a
2.33	Will food deprivation occur?	No
2.34	If yes, give details.	n/a
2.35	Will water deprivation occur?	No

2.36	If yes, give details.	n/a
2.37	Please explain the specific experimental, surgical or other procedures to be used on the animals. Include the use of anaesthesia, analgesics or other pharmaceuticals. Include all the details of all procedures. If an SOP exists for a particular procedure it is not necessary to explain it here, simply reference the SOP number and any minor deviations to the SOP that may be necessary. Consult with the Animal Care Supervisor for applicable SOPs.	Fish are anesthetized with tricaine methanesulfonate (MS-222) buffered to a pH of ~7 with sodium bicarbonate (60 ug/L MS-222; 120 ug/L sodium bicarbonate) prior to weighing them. The MS-222 and sodium bicarbonate are dissolved in lake water and the fish are immersed in the bath until an appropriate level of sedation is reached. Small-bodied fish take 1-2 minutes to reach an appropriate level of sedation, and large-bodied fish take 2-5 minutes. Level of sedation is assessed through visual observation of activity, opercular movements, change in equilibrium, and response to squeezing of caudal peduncle. After processing, fish are placed in a tub of fresh lake water which is regularly refreshed. The fish is monitored (maintenance of equilibrium, opercular movements, response to squeeze of caudal peduncle) while in the tub and is released back into the lake when deemed to be sufficiently recovered (generally takes just a few minutes). If a fish does not recover (rare), it is euthanized in an overdose bath of tricaine methanesulfonate (TMS; 400 mg/L) buffered to a neutral pH with sodium bicarbonate. The fish will be left in the overdose bath until 10 minutes after cessation of opercular movements, after which we will sever the spine just posterior to the head.
2.38	Describe and justify the “point of intervention” that you will use and explain the resulting steps for work with the animals reaching these endpoints. The term “point of intervention” is defined as the point at which an experimental animal’s pain and/or distress is terminated, minimized or reduced, by taking actions such as killing the animal humanely, terminating a painful procedure, giving treatment to relieve pain and/or distress or removing it from the project. Endpoints, should be objective and quantifiable an	Period of collection and capture of fish will be the minimum necessary to carry out study objectives. If a captured fish is particularly distressed, as evident by our difficulty in handling it, it will be released back into the lake. Fish held in plastic bags will experience some distress from capture and captivity, but they will be kept at ambient temperature to minimize the distress. Plastic bags will be stabilized using rocks or wine racks. Fish density in holding tubs will be kept to minimal relative to fish size and number and oxygen levels will be be maintained by regularly refreshing the water; ambient water will be

		regularly replaced. Occasionally, injury or mortality as a result of fishing can occur. In this situation the fish will be euthanized (if not dead) by cranial blow and severing of the spine and kept for lab analysis.
2.39	What is to be the final disposition of the animals? If euthanized, how will this be accomplished?	See 2.14
2.40	What is the anticipated level of invasiveness?	C)Procedures that cause minor stress or pain of short duration
2.41	While the study is active, what is the expected mortality rate associated with the procedures being used?	Less than 10%.

3. Declaration

#	Question	Answer
3.1	As the Principal Investigator, I agree that no changes to the work as described above will be made without instruction from the ACC or without ACC approval of desired changes. I agree that no animal work will be performed on this project without ACC approval.	Yes
3.2	Declaration Date:	2020/03/13

Animal Care Info

Purpose of Animal Use(PAU): **1. Studies of a fundamental nature in sciences rel ...**
Category of Invasiveness(CI): **C**
Classification:
Protocol Description:

Slimy Sculpin (Cottus cognatus):

Animal Use General:Species 1 of 8

Species: **Slimy Sculpin (Cottus cognatus)**
Species Keywords:

Strain:	
Weight:	
Gender:	
Source:	
Source Contact:	
Transportation:	
Housing Building & Room:	
Experimental/Procedure Building & Room:	
PAU:	**1. Studies of a fundamental nature in sciences rel ...**
CI:	**C**
Comments:	
# Animals Requested:	**140**
# Animals Approved:	**0**

Fathead Minnow (Pimephales promelas):

Animal Use General:Species 2 of 8

Species:	**Fathead Minnow (Pimephales promelas)**
Species Keywords:	
Strain:	
Weight:	
Gender:	
Source:	
Source Contact:	
Transportation:	
Housing Building & Room:	
Experimental/Procedure Building & Room:	
PAU:	**1. Studies of a fundamental nature in sciences rel ...**
CI:	**C**
Comments:	
# Animals Requested:	**120**
# Animals Approved:	**0**

Northern Redbelly Dace (Chrosomus eos):

Animal Use General:Species 3 of 8

Species:	**Northern Redbelly Dace (Chrosomus eos)**
Species Keywords:	
Strain:	
Weight:	
Gender:	
Source:	
Source Contact:	
Transportation:	
Housing Building & Room:	
Experimental/Procedure Building & Room:	
PAU:	**1. Studies of a fundamental nature in sciences rel ...**
CI:	**C**
Comments:	
# Animals Requested:	**120**
# Animals Approved:	**0**

Animal Use Per Year:Species 3 of 8

Year	# Animals Used	# Re-Used from Previous Year	Number of Animals Re-Used	Protocol Number of First Use	Comments
2020	0		0		

Blacknose Shiner (Notropis heterolepis):

Animal Use General:Species 4 of 8

Species:	**Blacknose Shiner (Notropis heterolepis)**
Species Keywords:	
Strain:	
Weight:	
Gender:	
Source:	
Source Contact:	
Transportation:	
Housing Building & Room:	
Experimental/Procedure Building & Room:	
PAU:	**1. Studies of a fundamental nature in sciences rel ...**
CI:	**C**
Comments:	
# Animals Requested:	**40**
# Animals Approved:	**0**

Animal Use Per Year:Species 4 of 8

Year	# Animals Used	# Re-Used from Previous Year	Number of Animals Re-Used	Protocol Number of First Use	Comments
2020	**0**		**0**		

White sucker (Catostomus commersonii):

Animal Use General:Species 5 of 8

Species:	**White sucker (Catostomus commersonii)**
Species Keywords:	
Strain:	
Weight:	
Gender:	
Source:	
Source Contact:	
Transportation:	
Housing Building & Room:	

Experimental/Procedure Building & Room:
PAU: 1. Studies of a fundamental nature in sciences rel ...
CI: C
Comments:
 # Animals Requested: 160
 # Animals Approved: 0

Animal Use Per Year:Species 5 of 8

Year	# Animals Used	# Re-Used from Previous Year	Number of Animals Re-Used	Protocol Number of First Use	Comments
2019	0		0		

Pearl Dace (Margariscus margarita):

Animal Use General:Species 6 of 8

Species: Pearl Dace (Margariscus margarita)
Species Keywords:
Strain:
Weight:
Gender:
Source:
Source Contact:
Transportation:
Housing Building & Room:
Experimental/Procedure Building & Room:
PAU: 1. Studies of a fundamental nature in sciences rel ...
CI: C
Comments:
 # Animals Requested: 160
 # Animals Approved: 0

Northern Pike (Esox lucius):

Animal Use General:Species 7 of 8

Species:	**Northern Pike (Esox lucius)**
Species Keywords:	
Strain:	
Weight:	
Gender:	
Source:	
Source Contact:	
Transportation:	
Housing Building & Room:	
Experimental/Procedure Building & Room:	
PAU:	**1. Studies of a fundamental nature in sciences rel ...**
CI:	**C**
Comments:	
# Animals Requested:	**60**
# Animals Approved:	**0**

Yellow Perch (Perca flavescens):

Animal Use General:Species 8 of 8

Species:	**Yellow Perch (Perca flavescens)**
Species Keywords:	
Strain:	
Weight:	
Gender:	
Source:	
Source Contact:	
Transportation:	

Housing Building & Room:	
Experimental/Procedure Building & Room:	
PAU:	**1. Studies of a fundamental nature in sciences rel ...**
CI:	**C**
Comments:	
# Animals Requested:	**80**
# Animals Approved:	**0**

Attachments

Doc / Agreement	Version Date	File Name	Description
		IISD-ELA OMNRF LTCFSP approval_Klemet-Nguessan.pdf	N/A
		Schedule A_1095536_signed.pdf	N/A
		SKlemet-N'Guessan_LTCFSP_1095536_signed.pdf	N/A

CHAPTER 4 AUP #12017-22503

Trent University Animal Care Committee

Animal Care Protocol Application

Wildlife and Field Work Research

- Use this form for a new Animal Use Protocol application for wildlife and field based animal research. Research projects which require capturing animals in the wild and bringing them into the ACF for laboratory research or husbandry must complete Section B. of this form as well.
- All personnel (PI, faculty, staff and students) must have completed the Animal Care Course or equivalent within the last five years.
- This application is for research only. For animal use in undergraduate courses (not including honour's thesis projects), please complete a teaching Protocol Application.
- Complete each section in detail according to the instructions on the form.
- Providing detailed information will reduce the likelihood that an application will be delayed in the review process.
- When completed, Supervisors must submit the application by attaching it to an email to ACCProtocolsubmit@trentu.ca who will acknowledge receipt and ensure its distribution to Animal Care Committee members.
- Where Standard Operating Procedures (SOPs) exist for specific tasks, referencing the SOP title and number in the appropriate part of the application is all that is required. Minor variances from the SOP may be explained. Consult with the Manager of Animal Care for a list of SOPs.
- For assistance with medical, animal health status and animal health questions please contact Dr. Jennifer Laing (vet@trentu.ca). For assistance with other parts of this protocol please contact the Manager of Animal Care Jason Allen (animalcare@trentu.ca).
- Confirmation of protocol receipt does not constitute approval by the Animal Care Committee for the proposed work. You will receive notification of the Committee's decision as soon as possible after the protocol review.

Section A. (To be completed by all applicants) ***Date Completed 05/04/12 (dd/mm/yy)***

1. Title of Project (must be descriptive and unique)

The effects of nanosilver on fish stress and on nutrient cycling.

2. Lay Summary of project. Give the aims, and scientific significance of the work to be performed in terms understandable and meaningful to the general public.

The use of nanotechnology is becoming ever more prominent in consumer products, agriculture, energy and medical applications. With the increased use of nanomaterials there is an increase in the risk of nanomaterials entering freshwater systems in concentrations that are hazardous to the environment. This study will look into the impact of nanosilver on ecological services through a whole lake addition

experiment at the Experimental Lakes Area in northwestern Ontario. Fish will be sampled in this study to determine the direct impact of nanosilver on fish function and changes in feeding patterns.

3a. Principal Investigator (PI) (Faculty or Approved Research Personnel)

Name	Office	Dept	Phone	Email	Emergency no.	Date Animal Care Course completed
Chris Metcalfe	SC 205	Environmental Resource Studies	x7272	cmetcalfe@trentu.ca	705-772-2767	

3b. Name of Person submitting this protocol (all applications must be submitted to and approved by, the Principal Investigator prior to submission to the ACC)

Name	Office	Phone	Field Contact	Email	Emergency no.	Date Animal Care Course completed
Chris Metcalfe	SC 205	Environmental Resource Studies	x7272	cmetcalfe@trentu.ca	705-772-2767	

4. Other Investigators

Name	Office	Phone	Field Contact	Email	Emergency no.	Date Animal Care Course completed
Maggie Xenopoulos	D238	7699				

5. Person(s) responsible for the care of the animals and for the health monitoring program.

Name	Office	Phone	Field Contact	Email	Emergency no.	Date Animal Care Course completed
Jonathan Martin						Will complete

Beth Cheever						Will complete
Daniel Braun						Will Complete
Andrew Scott	D220	6126		andrewscott@trentu.ca	705 559 9558	March 31, 2008

6. What are the specific objectives of this work?

This study has three specific objectives:

1- To look at the impact of nanomaterials on fish stress.
   a. To look at the direct impacts of nanosilver on fish stress by determining and evaluating the stress indicators on fish such as induction of metallothionein, release of heat shock proteins, cytochrome P450 enzyme activity and circulating steroids.
2- To look at the impact of nanomaterials on nutrient recycling using fish as a vector for animals
   a. Animals play a major role in nutrient cycling. Excretion rates from animals can be equivalent to other major nutrient inputs. We will be looking at excretion rates (N,P, Ag) as well as the composition of the fish (C,N,P,Ag)
3- To look at the impact of nanomaterials on the trophic position of fish.
   a. Stable isotopes will also be measured on fish muscle tissue. Isotopes 13C and 15N from fish tissue will be used to determine changes in trophic levels and feeding rates from benthic invertebrate and zooplankton to benthic and pelagic fish.

7. Purpose of Animal Study? (Check one)

Breeding Colony/Stock ☐

Fundamental Research ☒

Medical and Veterinary Research ☐

Regulatory Testing ☐

Drug Development ☐

Education and Training (other than honours thesis projects) ☐

8. Peer Review

Has this project been peer reviewed for scientific merit through a research granting agency? ☒ **Yes** ☐ **No**

9. List the specific source(s) of funding for this project. Refer directly to the grant number.

NSERC Strategic Grants Program - Impacts of nanosilver on a lake ecosystem

If No, contact the Office of Research to arrange for two independent peer reviews.

Note: External peer reviews may take some time to obtain; allow sufficient time between your submission and your proposed start date. Please refer to submission deadlines on the Animal Care website.

10. Replacement, Reduction and Refinement.

For a review of the 3R's of animal use, please visit www.ccac.ca/en/alternatives/index.html, the "special topics" tab of that mage may be of use for your work.

The P.I. has investigated and evaluated alternative methods which would accomplish the same scientific goals.

☒ **Yes**

Support this statement with a brief description of the methods and sources used to determine that non-animal alternatives were not available or an explanation of the aspects of the protocol that preclude using animals of a lower sentience. In addition, explain why the numbers of animals proposed cannot be further reduced. Finally, justify the invasive procedures (if any) proposed are at the lowest level possible and why they cannot be reduced further.

11. Proposed start date: 10/05/12 **(dd/mm/yy)**

(Note: If the application is approved, the committee will assign a start date that may differ from the proposed start date)

12. What is the duration of the project? 3 Years

Notes: No work may be carried out before approval of the protocol by the ACC.

Protocols that are longer than one calendar year will require an annual summary to be submitted to the ACC (See Annual Summary form) regardless of the start or approval date. If an annual summary is not supplied, the project will be assumed to be complete at the end of a year and no further work will be permitted on it.

The maximum duration of a protocol is three (3) years. Projects that need to run beyond three (3) years must undergo a full protocol reassessment by the Animal Care Committee in the third year. Projects that are approved for one year but, as a result of circumstances, must run longer than a year may be extended through the submission of an Amendment.

13. Animals

Genus/Species Esox lucius, Perca flavescens, Margariscus margaria, Notropis hetrodon, Pimephales promelas, Catostomus commersoni, Lota lota

Common Name Northern Pike, Yellow Perch, Pearl Dace, Blackchin Shiner, Fathead Minnow, White Sucker, Burbot

14a. Number of animals per species required for the duration of the study.

Northern Pike – 80

Yellow Perch – 180

Pearl dace – 60

Blackchin Shiner – 60

Fathead Minnow – 60

White Sucker – 60

Burbot – 60

14b. The number of animals to be used in year one will be 180, year two 180 , and year three 180.

15. Provide a justification for the number of animals required

*****Tissue from euthanized fish will be shared by researchers to minimize individuals needed.

The calculations below represent a maximum number of individuals needed for each researcher but we anticipate the final numbers to be less.

Fish Stress Reponses

Northern Pike and Yellow Perch will be used in this study

Year 1- 2 lakes X (5 Pike, 15 Perch) X 2 sampling events X 1 year = 80 individuals

Year 2&3 -2 lake X 2 species X (5 Pike, 15 Perch) X 2 sampling events X 2 years = 160 individuals = 80/year

Nutrient Recyling

Northern Pike, Yellow Perch, Pearl Dace, Blackchin Shiner, Fathead Minnow, White Sucker and Burbot will be used in this study.

Year 1 – 2 lakes X (1 lake 3 species, 1 lake 6 species) X 10 individuals X 2 sampling events = 180

Year 2 &3 – 2 lake X (1 lake 3 species, 1 lake 6 species) X 10 individuals X 2 sampling events X 2 years = 360

* 10 individuals are needed for replication and multiple analyses for nutrient cycling excretion.

**6 of the 10 individuals will be euthanized for stable isotope analysis and tissue analysis with the remaining 4 individuals being returned to the lake. The euthanized individual numbers are calculated below.

Stable Isotopes (The same fish will be used from the nutrient recycling project)

Stable isotopes, stoichiometry analysis

Northern Pike, Yellow Perch, Pearl Dace, Blackchin Shiner, Fathead Minnow, White Sucker and Burbot will be used in this study.

Year 1 – 2 lakes X (1 lake 3 species, 1 lake 6 species) X 6 individuals X 2 sampling events = 108

Year 2 &3 – 2 lake X (1 lake 3 species, 1 lake 6 species) X 6 individuals X 2 sampling events X 2 years = 216 = 108/year

16. Location: Where will the study take place?

Experimental Lakes Area (lakes occurring within 49° 30' to 49° 50' N, 94° 15' to 93° 15' W)

Lakes 221 and 222

17. If permits are required, list them below and indicate their status (obtained or pending).

Permits applied for. Permits Status Permit number

Collecting fish for scientific purposes DFO hold permit that we will work under

(Attach copies of the permits to this form, or forward copies to ACC Chair or Animal Care Supervisor)

18. Describe the precautions to be taken to avoid capturing non - target species or vulnerable animals.

All staff will be trained in the correct identification of fish and handling procedures. The ELA is extensively sampled by the DFO and species in the lakes are well known.

There are no SARA species present in the lakes.

Below is a list of species in each lake:

Lake 221: Pearl dace (Margariscus margarita), yellow perch (Perca flavascens), northern pike (Esox lucius).
Lake 222: blackchin shiner (Notropis heterodon), fathead minnow (Pimephales promelas), white sucker (Catostomus commersoni), burbot (Lota lota), northern pike and yellow perch.

19. Provide a detailed description of the procedures that will be used to trap/capture the animals.

Fish will be collected by using trap nets, seine netting and angling if required.

Trap nets to be deployed in the lake have a holding area of 24 to 36 square feet. Traps will be checked every 1-2 days to minimize fish stress.

A large seine net will be deployed with the aid of a boat. The net will be pulled to shore where the target fish will be placed in coolers filled with fresh lake water. Non target species will be directly returned to the lake.

Angling is sometimes required to capture larger fish such as the Northern Pike. In this case, target species will be caught by angling. The fish will be brought into the boat using a rubber mesh net and placed directly into a cooler filled with fresh lake water.

20. Provide a detailed description of the procedures (e.g. medical, surgical, implants etc.) to be performed on live animals. Include, handling methods and restraint procedures and a description of how you will keep stress to as low a level as is reasonable.

Captured fish will be held at the lake site in coolers with lake water. The lake water will be refreshed every few minutes by bucket to minimize stress. At the collection site, fish will be euthanized by an overdose of MS-222 anesthetic or by a blow to the head to collect tissues for analysis of biomarkers, stable isotopes and stoichiometric composition. Fish will be weighed and measured, then immediately dissected and tissues removed (i.e. muscle, gill, blood, brain, liver, kidney, spleen). These tissues will be flash frozen on dry ice and transported to the lab for storage in a low termperature or ultra-low temperature freezer. In cases where the chemical composition, and especially the carbon content of the fish tissue will be analyzed, MS-222 cannot be used for fish euthanizia. MS-222 is an organic compound with sulfur functional groups, that will contaminate the fish (especially small fish) with excess carbon and sulfur. A cranial blow followed by pithing of the brain is an accepted method for fish euthenasia in the UK and USA that will be used only for those fish used for stoichiometric analysis.

Nutrient Recycling Excretion – 10 individuals/species/lake

Fish will be placed into bags of fresh lake water for 3-5 minutes to adjust.

Fish less than 0.5g will be placed as a group into a bag with 1 litre of filtered water.

Fish less than 50g will be individually placed into a bag with 1 litre of filtered water. Fish will be placed in the bags for approximately 1 hour.

Fish greater than 50g will be placed in larger bags/or containers containing 3 litres of water. Larger fish will have shorter incubation times (15 mins) to avoid oxygen stress and waste accumulation.

*Small Pike will be targeted with larger Pike returned to the lake

21. Provide details of agents to be administered to the captured animals.

(include agent name, method of administration, dosage).

22. Provide details regarding how the animals will be monitored while captive?

The oxygen content of the water used to hold the fish at the lakeside will be monitored with a portable oxygen meter and will not be allowed to fall below 8 mg/L.

23. Provide details of the intended fate of the animals used in the study. (If the animals are to be manipulated in a laboratory setting or husbandry is required at the university for more than 6 hours Section B. must be completed.

When possible fish euthanized will be divided between the group members to minimize the numbers of animals required to conduct the research.

Biomarker responses:

Fish that have been euthanized at the sampling site for biomarker responses will be placed on dry ice until they are brought back to the field lab. Fish tissues will be taken such as muscle, gill, liver, brain ect. The samples will then be placed in liquid nitrogen. The samples will then be shipped to Trent University.

Nutrient Recycling Excretion (Please refere to details in section 20):

Fish used for excretion will be placed in water for a short amount of time to determine excretion rates.

Stable Isoptopes + Elemental Composition: (Please refer to details in section 20)

60% of the fish used for excretion will be euthanized at the sampling station. The euthanized fish will then be returned to the field lab on dry ice where they will be frozen until further dissection of the fish.

24. List the potential hazards (biohazard, chemical, physical) to staff.

With any type of field sampling, there are risks associated to working outdoors on the water such as heat stress in hot weather, exposure in cold weather and physical hazards. However, all staff will be equipped with radios in order to contact personnel at the field camp, and they will carry first aid kits with them at all times.

25. Qualification and Experience of Staff. List names, positions and relevant training and experience of all individuals who will be working directly with the animals. (Attach copies of relevant documents which corroborate your description of qualifications).

Jonathan Martin – PhD Student starting May 2012

Beth Cheever – Post Doctoral Fellow starting May 2012

Andrew Scott – Lab Manager/Technician – Completed Electrofishing Crew Leader Course in 2008, Level 1 Fish Identification Course completed, Crew Leader or Member for 10+ sampling events.

Daniel Braun – Undergraduate Research Assistant

All personnel listed above will receive certification in Wilderness First Aid (April and May, 2012), and will have an Ontario Boaters Card. The sampling will take place in collaboration with a trained fish sampling crew from Fisheries and Oceans Canada.

Section B. (To be completed if animals to be housed at Trent or manipulated for research purposes)

26. Room number where animals will be housed. N/A

27. Is surgery involved? **X Yes** ☐ **No**

28 a. Will anaesthesia be used? **X Yes** ☐ **No**

28 b. If yes, describe the specific anaesthetic doses, applications, techniques and recovery procedures. If this is an approved SOP, please indicate the SOP number.

MS-222 overdose (i.e. >100 mg/L).

29 a. Will analgesics be required? ☐ **Yes X No**

29 b. If yes, give specific information on procedures, type and route of application of analgesics to be used in study.

30. Agents and materials to be used in the study. (Check all that apply)

Chemicals **X Nanosilver**

Radioactive materials ☐

Biohazardous materials ☐

Physical **X Nanosilver**

None ☐

If you have selected an option other than "None", Give details here.

All work with radioactive and biohazardous materials require a radioactive work permit or a biosafety work permit respectively. Include the permit number here as well as a detailed description of the agent and material to be used.

31. Describe the detailed dietary requirements of your animals?

Not applicable

32 a. Will food deprivation occur? ☐ **Yes X No.**

32 b. If yes, give details?

33 a. Will water deprivation occur? ☐ **Yes X No**

33 b. If yes, give details.

34. Please explain the specific experimental, surgical or other procedures to be used on the animals. Include the use of anaesthesia, analgesics or other pharmaceuticals. Include all the details of all procedures. If an SOP exists for a particular procedure it is not necessary to explain it here, simply reference the SOP number and any minor deviations to the SOP that may be necessary. Consult with the Animal Care Supervisor for applicable SOPs.

Section C: To be completed by all applicants.

35. Is pain or distress likely to be associated with the procedures or manipulations?

(Check one only) **X Yes** ☐ **No**

If Yes, describe how pain or distress will be alleviated or minimized?

Fish will be euthanized with an overdose of anaesthetic

36. Describe and justify the "endpoint(s)" that you will use and explain the resulting steps for work with the animals reaching these endpoints.

Wild fish may experience changes in diet or reduced foraging as a result of ecosystem level responses in the dosed lake. These changes are expected to be relatively subtle (i.e. reduced body weight), but not lethal to the fish.

Endpoints, should be objective and quantifiable and may include weight changes, specific behavioural changes, loss of appetite etc.

Endpoints may include weight changes, and relatively subtle effects on circulating hormones and enzyme activity.

37. What is the expected mortality rate associated with procedures being used?

Concentrations of silver in the lake (i.e. <10 ppb) are expected to be below the lethal concentrations for fish (i.e. 10-50 ppm), so mortalities are not expected from direct exposure to the test compound added to the lake.

38. What is to be the final disposition of the animals? If euthanized, how will this be accomplished?

Dissected fish will be bagged and returned to the ELA camp for disposal.

39. Indicate the category of invasiveness which best describes the protocol:

☐ **A Procedures used on most invertebrates or on live isolates**

☐ **B Procedures that cause little or no discomfort or stress**

X C Procedures that cause minor stress or pain of short duration

☐ **D Procedures that cause moderate to severe pain or discomfort**

☐ **E Procedures that cause severe pain near, at or above the pain tolerance threshold of un-anaesthetized animals.**

40. Declaration

As the Principal Investigator, I agree that no changes to the work as described above will be made without instruction from the ACC or ACC approval of desired changes. I agree that no animal work will be performed on this proposed project without ACC approval.

x I agree **April 5, 2012 Date (dd/mm/yy)**

Note: Submission of this form must be by the Supervisor or designate only. Submission of protocol by others will not be reviewed by the committee.