ABSTRACT

Biological Trait Responses of River Macroinvertebrate Assemblages to a Phosphorus Gradient

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Phosphorus is the most important nutrient driving anthropogenic eutrophication of inland fresh waters. Several river basins in the Ozark Highlands and Boston Mountains ecoregions of central North America have elevated concentrations of total phosphorus, due to both point-source discharges and nonpoint source runoff in their catchments. Benthic macroinvertebrate responses, expressed as density and biomass of biological trait groups (functional feeding group, voltinism), were modeled across a steep phosphorus gradient spanning 35 river locations. Biomass and density increased across the gradient, and communities shifted from diverse, insect-dominated communities to communities dominated by small, multivoltine taxa (such as Chironomidae) and benthic algal grazers, particularly pleurocerid snails. These shifts are likely related to increased benthic primary production and supply of phosphorus to small-bodied consumers with high phosphorus demand (under the growth rate hypothesis). These results imply that phosphorus enrichment can have significant effects at multiple trophic levels in river ecosystems.

Biological Trait Response of River Macroinvertebrate Assemblages to a Phosphorus Gradient

by

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A Thesis

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CHAPTER ONE

Introduction

Nutrient loading, common in both agricultural and urban watersheds, poses a significant problem to river health. Fertilizer runoff and wastewater treatment plant effluent both contain higher levels of nitrogen and phosphorus than would normally be present in such systems (Carpenter et al. 1998). While nutrient enrichment may not cause substantial increases in algal or aquatic macrophyte production in low-order, shaded streams due to light limitation (Hill 1996; Cashman et al. 2013), larger rivers, or those where natural riparian cover has been removed, may experience blooms of algae, particularly fast-growing filamentous algae such as *Cladophora* (DeNicola 1996; Stevenson 1996; Wang et al. 2007). High biomass of filamentous algae may contribute to poor water quality by lowering dissolved oxygen overnight and raising pH during the day, particularly in warm summer months (Biggs 1996; Rosemond et al. 2000). The dominance of fast-growing attached algae over smaller, slower-growing types may also cause a shift in the benthic macroinvertebrate community by altering the trophic structure due to a disproportionate increase in grazing taxa (Steinman 1996; Merritt et al. 2009) and taxa that prefer to live in mats of filamentous algae (Stevenson 1996). Further, blooms of filamentous green algae can reduce or eliminate taxa that cannot tolerate the loss of interstitial habitat associated with high algal biomass (Miltner and Rankin 1998; Wang et al. 2007).

Phosphorus is essential to life. It forms part of several necessary biomolecules, including energy molecules (ATP), enzymes, phospholipids, and nucleic acids (Sterner and Elser 2002; Sardans et al. 2012), but of these, nucleic acids, particularly ribosomal RNA, collectively contain the most phosphorus in an organism (Sterner and Elser 2002). As ribosomal RNA is essential to protein synthesis and thus to growth, phosphorus demand can also be related to growth rate. The Growth Rate Hypothesis (GRH) states that "differences in organismal C:N:P ratios are caused by differential allocations to RNA necessary to meet the protein synthesis demands of rapid rates of biomass growth and development" (Sterner and Elser 2002). Phosphorus content has been demonstrated to change during development, with higher body % P observed in early, rapid-growth instars of several aquatic insects (Back and King 2013). In studies of other invertebrates, polyploidic snails in New Zealand demonstrate more growth in phosphorus-enriched conditions (Neiman et al. 2013), indicating that their reproduction is generally phosphorus-limited. Thus, it may be expected that many taxa will grow faster when more phosphorus is present, and that higher phosphorus levels may favor smaller bodied taxa (with higher phosphorus requirements) than slower-growing or larger-bodied taxa that have a more stable phosphorus content over the majority of their aquatic life cycle (Back and King 2013).

In the absence of anthropogenic nutrient inputs, mid-order rivers are typically low in dissolved nitrogen and phosphorus. Phosphorus, in particular, is often the limiting nutrient in river systems because unlike nitrogen, it is not present as a gas in the atmosphere, it has a propensity to adsorb to sediment, and its primary pathway to bioavailability is via internal recycling within an ecosystem (Newbold and O'Neill 1982; Mulholland 1996; Evans-White et al. 2009; Small et al. 2009; Sardans et al. 2012). Nitrogen, on the other hand, can be brought into the aquatic ecosystem biologically by nitrogen-fixing cyanobacteria in the periphyton (Mulholland 1996) or by inputs from N-fixing terrestrial vegetation (Shaftel et al. 2012).

An increase in primary production, in this case benthic algae, often will cause an increase in secondary production, particularly primary consumer biomass. Studies in the Florida Everglades (King and Richardson 2007), in tropical ponds (Sarnelle et al. 1998), and in coastal estuaries (Nixon and Buckley 2002) demonstrated increases in biomass of primary consumers with nutrient enrichment, at least up to a certain point (a subsidy or subsidy-stress response, as per Odum (1979)). A stress response to excess phosphorus may occur for several reasons. It may be due to the growth of large quantities of relatively low-quality algae such as *Cladophora* (Dodds and Gudder 1992), causing growth to be restricted by low food quality. Furthermore, insect scrapers may have difficulty consuming filamentous algae that has grown into long strands, because of the difference between the size of those algae and the mouthparts of the insect (Steinman 1996; Merritt et al. 2009). Finally, a stress response may occur at high phosphorus levels because of other factors generally associated with anthropogenic phosphorus inputs, such as increased sedimentation and changes in the flow regime (Richards et al. 1993).

Studies examining the effects of nutrient enrichment on primary production must consider multiple components of a food web to better understand potential alterations to structure and function of a stream ecosystem. A trait-based analysis of the stream macroinvertebrate community may be particularly useful in assessing the effects of nutrient enrichment on ecosystem structure and function (Díaz et al. 2008; Zuellig and Schmidt 2012; Dézerald et al. 2015). Macroinvertebrate functional feeding groups are probably the best-known of the biological traits used in ecological studies, and comparisons between types can assess several whole-river characteristics, including degree of heterotrophy, the function of the riparian zone, and the presence of multivoltine taxa that may not be immediately captured (Cummins et al. 2005; Merritt and Cummins 2007; Merritt et al. 2009). Macroinvertebrate generation length is another trait that can be used to describe response in nutrient-enriched systems, as taxa which vary in generation length may develop differently under the GRH (Townsend and Thompson 2007), or may respond differently to the increase in food availability caused by nutrient enrichment.

This study examines the response of biological traits of macroinvertebrate assemblages to a steep phosphorus gradient across rivers in south-central North America. For the purpose of forming hypotheses, it was assumed that the relationship between total phosphorus (TP) and algal growth, and the relationship between benthic macroinvertebrate density and biomass, would be approximately linear. While this was unlikely to be true in practice, analyzing the differences between the expected and realized results allow for a more detailed understanding of the effects of TP and algal biomass on macroinvertebrate trait groups. Several hypotheses were formed regarding the response of macroinvertebrate communities to an increase in TP or to the associated increase in benthic algal biomass, expressed as benthic chlorophyll-a:

1. Total biomass and total density of benthic macroinvertebrates will increase as phosphorus and benthic algal biomass increase.
2. The response of various macroinvertebrate trait groups will vary in a manner consistent with their life histories. Table 1.1 outlines the expected responses for various trait groups.

Table 1.1 Macroinvertebrate trait groups and predicted response to P enrichment.

Trait	Expected Response	Rationale
Scrapers	Increase	Increased food supply and P content
Gathering Collectors	Increase; possible stress response at highest P	Increased food supply, potential competition from more efficient or more common grazers in other groups.
Filtering Collectors	Increase	Increased food supply (suspended organic matter)
Predators	Increase or subsidy-stress	Increased food supply; possible loss of food if community shifts at high P levels
Shredders	Decrease	Sensitive to anthropogenic effects correlated with high P
Multivoltine	Increase	Increased food supply and P for rRNA
Bivoltine	Increase	Increased food supply and P for rRNA
Univoltine	Increase or subsidy-stress	Majority of insect taxa fall into this category; thus it is likely to be the pattern of most taxa.
Semivoltine	Increase or subsidy-stress	May benefit from increase in resources (particularly semivoltine predators benefiting from more multivoltine taxa), or may be outcompeted by faster-growing taxa that can better take advantage of increased resources. .

CHAPTER TWO

Methods

Study Area

The study area includes portions of the Ozark Highlands and Boston Mountains ecoregions located in northeastern Oklahoma and northwestern Arkansas; the Ozark Highlands also extend into southeastern Kansas and southwestern Missouri (Omernik 1987), but the study was restricted to locations within Oklahoma and Arkansas. Streams in the Ozark Highlands are wide and sometimes braided, with substrate composed primarily of Mississippian-aged limestone (calcite) gravel and cobble, with some embedded chert (Splinter 2006; Splinter et al. 2010). Streams in the Boston Mountain ecoregion have similar gradients to streams in the Ozark Highlands, but slightly wider bankfull channel widths (Splinter et al. 2011). Stream substrate is slightly larger in the Boston Mountains, and is composed of Pennsylvanian-aged limestone, with some Pennsylvanian and Mississippian-aged limestone and shale (Splinter et al. 2010; Splinter et al. 2011). In the Ozark Highlands, the underlying geology is primarily fractured limestone: streams in the region are highly connected to groundwater; lower-order streams may go dry or intermittent during dry periods; and spring-fed streams are common (Omernik 1987; Wiken et al. 2011). The Boston Mountains are also underlain by limestone, providing some groundwater connection, but low-streams do not dry up as often and springs are less common (Splinter 2006; Wiken et al. 2011).

Site Selection

Thirty-five sites were sampled (Figure 2.1): 30 sites located in the Ozark Highlands, four in the Boston Mountains, and one on the border between the two ecoregions. Sites were located on rivers in the Arkansas River watershed in northeast Oklahoma and northwest Arkansas, and selected to span the full range of phosphorus concentrations in the region. Sites were located in areas where riparian cover was low or absent, in riffles composed primarily of cobble (10-20 cm).

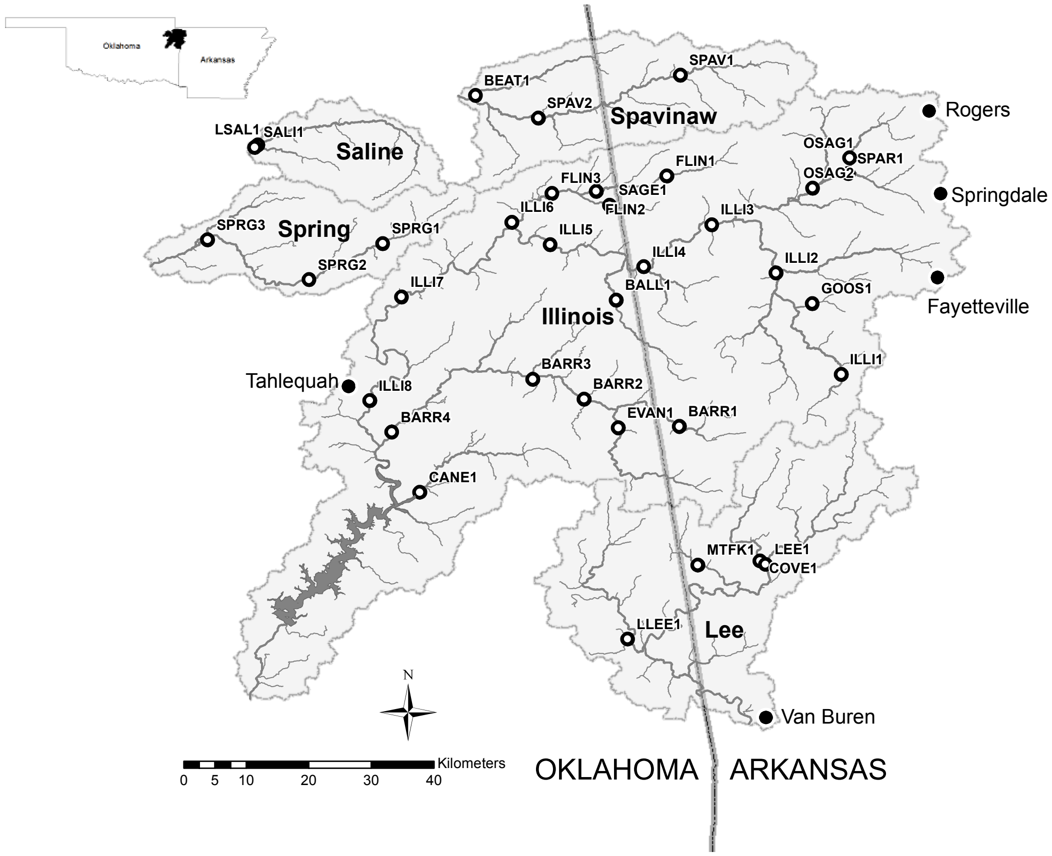

Figure 2.1: Site locations

Sampling

Samples were taken at each site every two months, from June 2014 to April 2015, for a total of six sampling events. Sites were sampled over a period of 5-10 days, with the exception of the event in August 2014, when one site (ILLI8) was sampled two weeks later as a result of significant rainfall that caused the river to rise to conditions that were unsafe for wading.

Three transects were established at each site, located in riffle habitat with primarily cobble substrate, in areas representative of the productive habitat in the riffle. In smaller rivers, transects were located in separate riffle sections if more than one was available within an approximately 100-meter reach. In larger rivers, where riffle-pool sections are longer and each 100-meter reach often contains only one riffle area, all transects were established within that riffle.

Each transect was divided into five sampling points, for a total of fifteen points per site. At each sampling point, a macroinvertebrate sample was collecting using a Hess sampler (0.086 m^2) fitted with 250 µm mesh. Individual collections from each point were composited into a single sample with a total area of 1.29 m^2. Macroinvertebrate samples were placed on ice at the time of collection and preserved with 5% buffered formalin within twelve hours.

At each macroinvertebrate collection point, visual estimates were made of several stream characteristics: dominant substrate type, substrate embededness, fine sediment cover, and the density of grazing scars in the benthic algae caused by the central stoneroller (*Campostoma anomalum*, an abundant minnow that feeds primarily on periphyton (Power and Matthews 1983; Matthews et al. 1986)). Dominant substrate type was recorded and then expressed on the site level as a percentage of each type (silt, sand, gravel, cobble, boulder, or bedrock). Substrate embeddedness was expressed as a percentage (0-100); fine sediment cover was expressed on a scale of 1-20, and stoneroller grazing was expressed on a scale of 1-10. At the center of each transect, surface water velocity was measured with a Marsh-McBirney Flo-Mate 2000 (Marsh-McBirney, Inc.), and canopy cover was measured using a convex spherical densitometer (Platts and Nelson 1989).

Periphyton biomass was sampled by collecting one large (10-20 cm) cobble from each sampling point and brushing the upper surface free of periphyton using stainless steel wire brushes. Periphyton was rinsed from cobbles and brushes using squirt bottles filled with water from the site in which the periphyton was collected. Periphyton brushed from all 15 cobbles was composited and placed on ice. Within 24 hours, a subsample of slurry was extracted using a pipettor while homogenized vigorously on a stir plate. Triplicate subsamples were filtered onto separate 47 mm glass-fiber filters and frozen for chlorophyll-a extraction. Aluminum foil was used to determine the cumulative surface area of cobble sampled (cm^2) using an area-mass regression (Dudley et al. 2001).

Water samples were collected at the upstream end of each site for analysis of TP and total nitrogen (TN). Water temperature, specific conductance, dissolved oxygen, pH, and turbidity were measured using an EXO1 multiparameter data sonde (YSI Inc., Yellow Springs, OH).

Discharge at each site was measured either directly or by using real-time measurements from a USGS gauge station if located close to a site. Nineteen sites were located near gauge stations; at the remaining 16 sites, discharge was measured with a Marsh-McBirney Flo-Mate 2000 (Marsh-McBirney, Inc.). For most locations, depth and velocity were measured at 20 locations evenly spaced across the full width of the channel, and the cross-sectional area thus obtained was multiplied by the velocity thus obtained to calculate discharge (Gore 2007). At locations that were less than five meters across, only ten measurements were taken. During the first few events, discharge was also measured directly at several of the sites located near gauge stations, to ensure that measurements of discharge were similar between USGS data and on-site measurement.

Laboratory Methodology

Macroinvertebrate samples were rinsed through a series of nested sieves: 4.75 mm, 2 mm, and 0.25 mm. All organisms retained in the 4.75 mm sieve were counted and identified (King and Richardson 2002; King and Richardson 2007). For the first event (June 2014), the entire 2 mm fraction was also identified. For subsequent events, the 2 mm fraction was divided into 25% subsamples. Each subsample was then picked completely in a randomly assigned order until at least 300 organisms (within 10%) were found our all four subsamples had been picked. Organisms retained in the 0.25 mm sieve were stored in ethanol and retained for possible identification in the future.

Macroinvertebrates were identified to lowest practical taxonomic level. Insects were identified to genus when possible, with the exception of Chironomidae, which was left at the family level. Morphospecies were identified for some common genera, particularly the mayfly genus *Maccaffertium*. Bivalves and crustaceans were also identified to genus when possible; crayfish were identified to genus, as the high number of females and immature males precluded the accurate assignment of most crayfish to species, although *Orconectes neglectus* and at least one other *Orconectes* species were observed. Gastropods and leeches were identified at the family level, and segmented worms were identified at the class level (Oligochaeta). Flatworms and roundworms were identified at the phylum level (Turbellaria and Nematoda, respectively), and aquatic mites were identified as Acari.

TP and TN were analyzed on a Lacaht Quik-chem Flow Injection Autoanalyzer, using the molybdate and cadmium reduction method, respectively, following persulfate reduction (APHA 1998). Periphyton chlorophyll-a was extracted in ethanol and analyzed using spectrophotometric analysis (Biggs and Kilroy 2000; Steinman et al. 2007).

Biomass Calculations

Biomass, as dry mass, was estimated following the length-mass relationships established by Benke et al. (1999), with a few exceptions. Individuals were measured for total length and/or head capsule width, except in cases where the standard measurement for that class of organisms is otherwise (e.g. carapace length rather than full-body length is the standard measurement for Decapoda). In cases where organisms could not be measured, event averages or site averages for that taxon were used: generally, event averages were used for taxa with strong seasonal life cycles (e.g. most insects) and site averages were used for taxa without strongly-defined seasonal life cycles (e.g. Oligochaeta). Length-mass relationships expressed in ash-free dry mass in Benke et al. (1999) also included measurements of percent ash, which allowed those measurements to be converted to dry mass using the following equation:

$$DM = AFDM \times \frac{100}{100 - \%\,Ash}$$

For taxa which had no equations given in Benke et al. (1999), taxa with similar body size and growth rates (generally from the same family) were used as proxies, or family- or order-level equations were used. For taxa without an appropriate proxy in Benke et al. (1999), other sources were sought. Other sources used in biomass calculations were: Sample et al. (1993), Edwards et al. (2009), Baumgärtner and Rothhaput (2003), and Méthot et al. (2012). For the burrowing mayfly genus *Ephemera*, for which no head-capsule width equation could be found, complete organisms were measured and a regression developed to relate head-width to total length, allowing the total length equation from Benke et al. to be used to calculate biomass. Chironomidae individuals were not measured; rather, 20 Chironomidae were randomly selected, dried, and weighed, and the resulting mass per individual was used for all Chironomidae for that sample. Similarly, randomly sampled adults in the beetle family Elmidae were weighed directly to be used as proxies, as no beetle-specific equation included adults that small in its prediction range. *Stenelmis* and *Optioservus*, the two most common taxa, were weighed directly; for other adult elmids, the average of those two genera was used.

Furthermore, observations during initial site selection suggested that one important primary consumer, and scraper, would be snails of the family Pleuroceridae. Thus, a length-mass regression was established for the snails collected in this study. Aperture width was measured and related to dry mass of the whole snail (shell included) for 100 snails that had been hand-collected and frozen. Very small snails (AW $\leq$ 1.9 mm) were highly variable; for snails of that size, the average mass of all snails of that size and smaller was used for total snail mass. For larger snails (AW 2 – 6.2 mm), the following regression was established:

$$\textit{Whole Snail Dry Mass} = 0.0026(\textit{Aperture Width})^{2.815}$$

Macroinvertebrate Trait Groups

Traits selected were functional feeding groups (scrapers, gathering collectors, filtering collectors, predators, and shredders); insect voltinism (multivoltine, bivoltine, univoltine, and semivoltine); and, after initial sampling showed the likely high impact of pleurocerid snails at high phosphorus levels, Pleuroceridae, non-Pleuroceridae, and non-Pleuroceridae scrapers (the functional feeding group to which Pleuroceridae belong). Functional feeding group for each taxon was established based on the EPA Rapid Bioassessment Protocol (Barbour et al. 1999). Generation length (voltinism) of aquatic insects was determined from literature (Brigham et al. 1982; Wiggins 1996; Stewart and Stark 2002; Merritt et al. 2009). In cases where voltinism of a particular genus varied with region, the region closest to the study area was used. Taxa whose voltinism could not be established were excluded from this analysis. Taxa with life cycles longer than two years were not assessed because only two such taxa were found in the study.

Model Development

The relationship between TP and benthic algal biomass (expressed as mg chlorophyll-a per m^2) was analyzed using generalized linear modeling (GLM). The model was fitted using a gamma distribution with an identity link, to account for the variance in the data increasing with the mean (Crawley 2012). The model was fit using the *glm* function in R (R Core Team 2015).

Macroinvertebrate biomass and density were analyzed as response variables for the following categories: total, scrapers, filtering collectors, gathering collectors, predators, shredders, multivoltine insects, bivoltine insects, univoltine insects, and semivoltine insects. Because pleurocerid snails formed such a large portion of the biomass at many sites, additional analyses of biomass and density for pleurocerid snails, all non-pleurocerid macroinvertebrates, and all non-pleurocerid scrapers were performed. Non-Pleuroceridae snails (Physidae, Planorbidae, and Hydrobiidae) were included with these scrapers, as they were found only during particular conditions and times of the year rather than being present year-round as were the Pleuroceridae.

GLMs were used to fit the models. Due to overdispersion in the data when a Poisson distribution was specified, a negative binomial distribution was specified when fitting the models (Hilbe 2011; Crawley 2012). Models were fit using the *glm.nb* function in the MASS package for R (Venables and Ripley 2002). Models for both macroinvertebrate biomass and density were developed using ln(TP) (mg/L) and benthic chlorophyll-a (mg/m^2) as separate predictors. To test for subsidy-stress as well as unidirectional models, models were developed using both the predictor and the predictor squared, and the better model was selected by comparing Akaike's Information Criterion (AIC; Peruggia 2003) for use in further analysis. A Pearson correlation table was developed to assess inter-relation between explanatory variables, allowing stream variables which were strongly correlated to TP or benthic chlorophyll-a to be excluded from model development. Stream characteristic variables that were not strongly correlated to TP or benthic chlorophyll-a were then assessed using bubble plots (Zuur et al. 2010) to determine whether they should be tested for inclusion in the models. Optimal models were developed, using standard model reduction, which included either TP or benthic chlorophyll-a, and other potentially explanatory stream characteristic variables chosen during visualization. Comparisons between models were assessed by comparing AIC (Zuur et al. 2009). In cases where models were very similar ($AIC_c < 2$), the simpler model was preferred; models with fewer terms were preferred over more complex ones; and increasing or decreasing trends were preferred to a subsidy-stress response. The *predict.glm* function was used to visualize the response of macroinvertebrate trait groups to phosphorus or chlorophyll-a; other variables in the model, if any, were held constant at the median value of that variable when the expected response was plotted. Plots were created using the ggplot2 and gridExtra packages for R (Wickham 2009; Auguie 2016).

CHAPTER THREE

Results

The relationship between phosphorus and benthic algal biomass (expressed throughout as mg chlorophyll-a per m^2) (Figure 3.1), while generally increasing, also demonstrated an increase in variance as phosphorus increased. TP was a significant predictor of chlorophyll-a concentration ($p < 0.001$), and explained 56.4% of the variation in the model.

Quantiles of stream characteristic variables are presented in Table 3.1. Pearson correlations between these variables, used to assess which variables were sufficiently independent to be included in the same model, are presented in Table 3.2. TP and benthic chlorophyll-a were correlated with TN, specific conductance, pH, % impervious

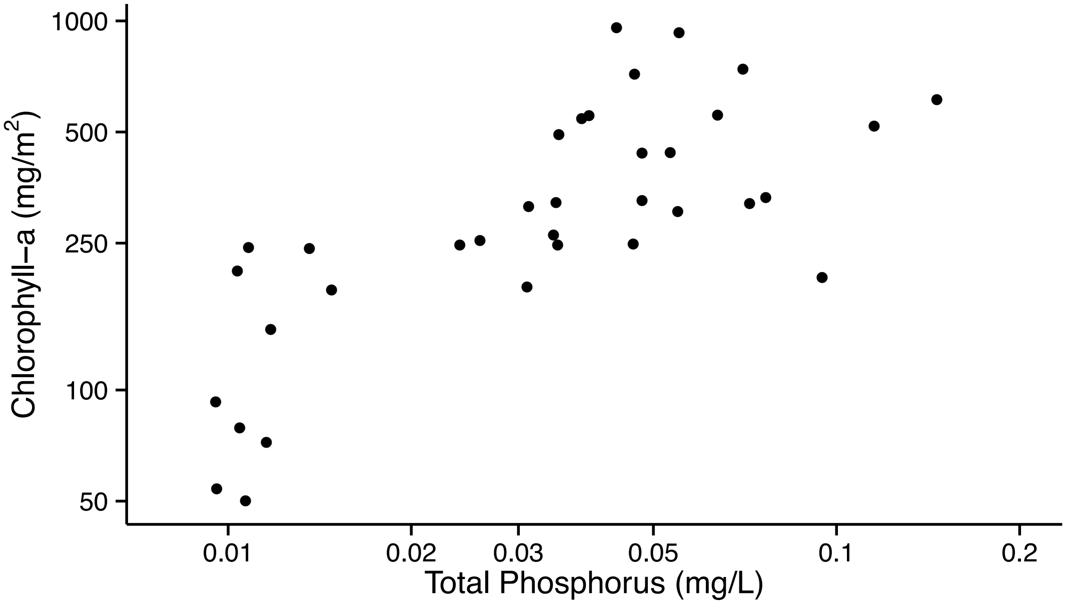

Figure 3.1: Algal biomass over the phosphorus gradient

cover, % forest, and % pasture; these were excluded from further analysis. Benthic chlorophyll-a was also correlated with minimum discharge, although not as strongly; minimum discharge was retained for further analysis, but eliminated when confounding effects with chlorophyll-a were observed.

219 benthic taxa were collected and divided into trait groups. Both biomass and density of each macroinvertebrate category were dominated by a single taxon. Table 3.3 lists the top three taxa, by biomass and density, in each category. For the most part, the same taxon dominated both biomass and density, except in cases where the dominant organism by biomass includes very large individuals such as the crayfish *Orconectes*.

Table 3.1: Quantiles of mean site characterization variables

	Quantiles				
Site Variable	0	0.25	0.5	0.75	1.0
TP (mg/L)	0.010	0.014	0.035	0.054	0.146
TN (mg/L)	0.11	0.78	1.56	2.46	5.02
Chlorophyll-a (mg/m^2)	50	206	304	506	959
Specific Conductance (µS/cm)	104	221	305	356	566
Minimum Discharge (m^3/S)	0	0.14	0.37	0.88	5.01
Mean Discharge (m^3/S)	0.20	0.51	1.05	2.63	13.20
pH	7.4	7.6	7.8	7.9	8.4
Turbidity	0.268	0.831	1.17	2.871	5.624
% Cobble	45.6	77.2	83.3	86.7	97.8
% Embededness	29.2	33.6	36.7	39.8	48.4
Fine Sediment (Scale 1-20)	4	7	9	10	12
Depth (m)	0.14	0.15	0.18	0.22	0.26
Velocity (m/s)	0.23	0.35	0.52	0.58	0.77
Canopy Cover	0	1	4	14	48
Stoneroller Grazing (Scale 1-10)	0	1	1	2	3
Area (km^2)	35.5	96.3	232.9	421.0	2465.6
% Impervious Cover	0.1	0.5	1.2	4.4	21.5
% Forest	7.3	27.8	36.7	49.9	84.7
% Pasture	7.0	39.1	48.3	52.3	67.7

Details of the biomass and density responses of macroinvertebrate trait groups to TP and benthic chlorophyll-a are presented in Table 3.4 (TP) and Table 3.5 (benthic chlorophyll-a), indicating both the direction of the response and any additional variables which were included in the optimal model. Models for total biomass and density are presented in Figure 3.2; similar figures for macroinvertebrate trait groups are presented in the Appendix.

Table 3.2: Pearson Correlation Table of Environmental and Landscape Variables. Phosphorus, nitrogen, and area were log-transformed before analysis.

Variable	TP	TN	CHLA	SPC	QMIN	QMN	PH	TURB	COB	EMB	SED	DEP	VEL	CAN	SR	AREA	IMP	FOR
TP	1.00																	
TN	0.83	1.00																
Chlorophyll-a	0.63	0.63	1.00															
Specific Conductance	0.89	0.84	0.71	1.00														
Minimum Discharge	0.28	0.29	0.69	0.29	1.00													
Mean Discharge	0.09	0.03	0.46	0.04	0.89	1.00												
pH	0.70	0.70	0.81	0.74	0.51	0.32	1.00											
Mean Turbidity	-0.14	-0.39	0.11	-0.21	0.29	0.49	0.07	1.00										
% Cobble	-0.25	-0.31	-0.05	-0.21	0.09	0.18	-0.08	0.36	1.00									
% Embededness	0.41	0.24	0.57	0.44	0.38	0.30	0.61	0.29	0.27	1.00								
Fine Sediment	0.09	-0.07	0.32	0.16	0.20	0.25	0.26	0.70	0.30	0.51	1.00							
Depth	0.07	-0.03	0.33	0.01	0.59	0.57	0.29	0.18	-0.01	0.19	-0.04	1.00						
Velocity	0.44	0.45	0.55	0.38	0.64	0.51	0.58	-0.07	-0.08	0.27	-0.19	0.57	1.00					
Canopy Cover	0.04	0.23	-0.09	0.06	-0.32	-0.39	-0.07	-0.19	-0.24	-0.16	-0.10	-0.50	-0.28	1.00				
Stoneroller Grazing	0.18	0.30	0.16	0.07	0.21	0.11	0.15	-0.50	-0.20	0.03	-0.37	0.35	0.41	-0.36	1.00			
Area	0.07	0.07	0.54	0.11	0.94	0.94	0.39	0.35	0.05	0.31	0.18	0.58	0.59	-0.35	0.24	1.00		
% Impervious Cover	0.66	0.55	0.52	0.84	0.27	0.09	0.57	-0.10	0.02	0.42	0.19	0.07	0.22	-0.11	-0.07	-0.05	1.00	
% Forest	-0.87	-0.92	-0.64	-0.88	-0.30	-0.03	-0.64	0.40	0.33	-0.27	0.11	-0.05	-0.41	-0.17	-0.28	-0.03	-0.69	1.00
% Pasture	0.68	0.82	0.48	0.54	0.19	-0.03	0.43	-0.43	-0.44	0.07	-0.23	-0.04	0.38	0.30	0.44	0.08	0.16	-0.81

Table 3.3 Most common taxa in each trait group. Parenthetical numbers indicate the number of taxa in the group.

Biomass		Density	
Taxon	%	Taxon	%
Filtering Collectors (16)			
Cheumatopsyche	57.0%	*Cheumatopsyche*	65.1%
Corbicula	16.7%	*Anthopotamus*	9.0%
Isonychia	7.8%	*Isonychia*	8.9%
Gathering Collectors (38)			
Orconectes	90.3%	Chironomidae	39.9%
Ephemerella	5.6%	*Ephemerella*	26.6%
Chironomidae	2.7%	*Tricorythodes*	11.3%
Predators (96)			
Corydalus	48.3%	Turbellaria	22.5%
Argia	8.1%	*Isoperla*	13.6%
Isoperla	6.6%	*Argia*	10.9%
Scrapers (38)			
Pleuroceridae	83.4%	Pleuroceridae	27.6%
Physidae	12.0%	*Psephenus*	22.2%
Psephenus	1.2%	*Stenelmis*	10.9%
Shredders (25)			
Tipula	44.9%	*Allocapnia*	29.2%
Pycnopsyche	21.5%	*Marilia*	17.1%
Strophopteryx	9.0%	*Strophopteryx*	13.8%
Multivoltine (8)			
Chironomidae	93.0%	Chironomidae	96.3%
Petrophila	6.2%	*Petrophila*	2.3%
Simulium	0.4%	*Acerpenna*	0.5%
Bivoltine (13)			
Cheumatopsyche	74.8%	*Cheumatopsyche*	49.7%
Isonychia	10.3%	*Tricorythodes*	23.1%
Helicopsyche	9.4%	*Caenis*	8.2%
Univoltine (74)			
Ephemerella	29.0%	*Ephemerella*	39.3%
Corydalus	28.8%	*Maccaffertium*	17.1%
Maccaffertium	12.4%	*Isoperla*	5.3%
Semivoltine (10)			
Agnetina	78.2%	*Agnetina*	56.2%
Optioservus	9.2%	*Optioservus*	29.1%
Gomphus	8.5%	*Gomphus*	11.0%

Table 3.4: Macroinvertebrate trait group modeled responses to TP. “Subsidy-stress” indicates a relationship with $\ln(TP)^2$; “Increasing” or “decreasing” indicates a relationship with ln(TP). .

		P-values (if significant)						
Model	Response	TP	QMIN	SED	CAN	SR	AREA	% Explained
Biomass								
Total	Increase	0.003	-	-	-	-	-	20.1%
Non-Pleuroceridae	-	-	-	-	-	-	-	-
Pleuroceridae	Increase	0.003	-	-	-	-	-	19.5%
Scrapers	Increase	0.006	-	-	-	-	-	15.2%
Non-Pleuroceridae Scrapers	Subsidy-Stress	<0.001	-	<0.001	0.001	-	<0.001	53.8%
Gathering Collectors	-	-	-	-	-	-	-	-
Filtering Collectors	Increase	<0.001	-	-	0.031	-	-	35.1%
Predators	Subsidy-Stress	<0.001	-	0.001	-	-	-	41.6%
Shredders	Decrease	0.001	-	-	-	0.030	-	36.7%
Multivoltine	Increase	<0.001	-	-	-	-	-	30.9%
Bivoltine	Increase	<0.001	-	-	0.009	-	-	42.2%
Univoltine	Subsidy-Stress	<0.001	-	-	-	-	-	31.7%
Semivoltine	Subsidy-Stress	0.006	-	-	-	-	-	19.0%
Density								
Total	Increase	<0.001	-	-	-	-	-	42.1%
Non-Pleuroceridae	Subsidy-Stress	0.003	-	-	-	-	-	50.6%
Pleuroceridae	Increase	0.022	-	-	-	-	-	13.2%
Scrapers	Increase	<0.001	-	-	-	-	-	27.3%
Non-Pleuroceridae Scrapers	Increase	0.004	-	-	-	-	-	17.9%
Gathering Collectors	Increase	<0.001	-	-	0.021	-	<0.001	60.1%
Filtering Collectors	Increase	<0.001	-	-	-	-	-	27.0%
Predators	-	-	-	-	-	-	-	-
Shredders	Decrease	0.001	0.010	-	-	-	-	25.5%
Multivoltine	Increase	<0.001	-	-	-	-	-	40.0%
Bivoltine	Increase	<0.001	-	-	0.039	0.018	-	36.3%
Univoltine	Subsidy-Stress	0.002	-	-	-	-	0.001	49.9%
Semivoltine	Increase	0.004	0.016	-	-	-	-	28.6%

Table 3.4: Macroinvertebrate trait group modeled responses to benthic chlorophyll-a. Subsidy-stress” indicates a relationship with CHLA^2; “Increasing” or “decreasing” indicates a relationship with CHLA .

		P-values (if significant)						
Model	Response	CHLA	QMIN	SED	CAN	SR	AREA	% Explained
Biomass								
Total	Increase	0.018	-	-	-	-	-	12.56%
Non-Pleuroceridae	-	-	-	-	-	-	-	-
Pleuroceridae	Increase	0.006	-	-	-	-	-	15.18%
Scrapers	Increase	0.016	-	-	-	-	-	10.82%
Non-Pleuroceridae Scrapers	Subsidy-Stress	0.010	-	<0.001	-	-	-	40.12%
Gathering Collectors	Decrease	0.049	-	-	-	-	-	9.09%
Filtering Collectors	Increase	0.001	-	-	-	-	-	17.36%
Predators	-	-	-	-	-	-	-	-
Shredders	Stress-Subsidy	0.001	-	-	-	-	-	31.09%
Multivoltine	Increase	<0.001	-	-	-	-	-	53.63%
Bivoltine	Subsidy-Stress	0.001	0.007	-	-	-	-	37.78%
Univoltine	-	-	-	-	-	-	-	-
Semivoltine	Increase	-	-	-	-	-	-	25.74%
Density								
Total	Subsidy-Stress	0.030	-	-	-	-	-	45.97%
Non-Pleuroceridae	Increase	<0.001	-	-	-	-	-	40.27%
Pleuroceridae	Increase	0.014	-	-	-	-	-	9.60%
Scrapers	Subsidy-Stress	0.018	-	-	-	-	-	22.48%
Non-Pleuroceridae Scrapers	Subsidy-Stress	0.030	-	-	-	-	-	14.48%
Gathering Collectors	Increase	<0.001	-	-	-	-	-	45.36%
Filtering Collectors	Increase	0.002	-	-	-	-	-	18.87%
Predators	-	-	-	-	-	-	-	-
Shredders	Stress-Subsidy	0.003	-	-	-	-	-	20.05%
Multivoltine	Increase	<0.001	-	-	-	-	-	60.48%
Bivoltine	Increase	<0.001	-	-	-	0.005	0.004	33.66%
Univoltine	-	-	-	-	-	-	-	-
Semivoltine	Increase	<0.001	-	-	-	-	-	24.41%

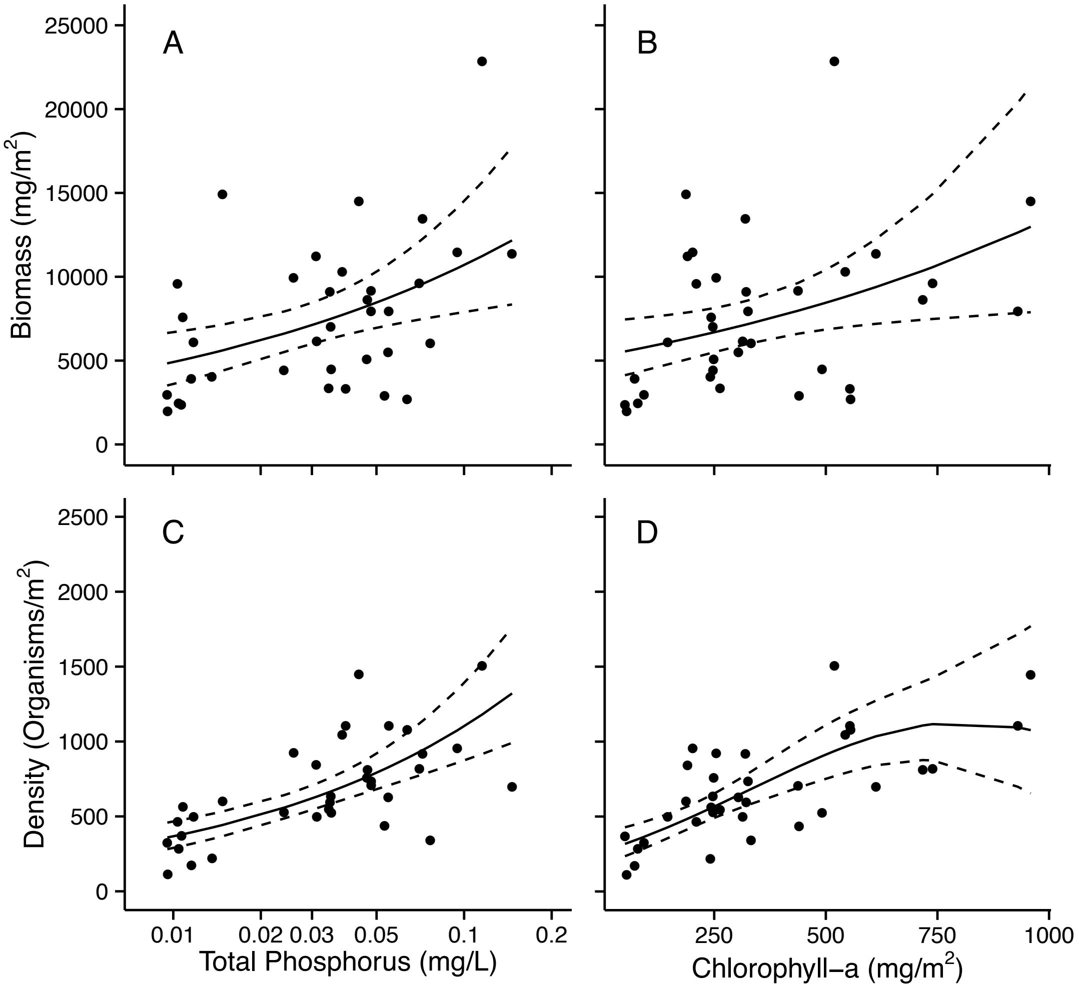

Figure 3.2: Total biomass and density data, against both TP and chlorophyll-a. Lines indicate a significant model result and 95% confidence interval. Deviance explained 20.11% (A); 12.56% (B); 42.08% (C); 45.97% (D).

CHAPTER FOUR

Discussion

Chlorophyll-a Relationship to Phosphorus

Phosphorus accounted for a little over half the variability in chlorophyll-a, showing that while it is important, it cannot be assumed to be a sole predictor. Previous studies on the impact of nutrients on benthic algal biomass have shown that the relationship is complex, depending on light, velocity, discharge, and other factors, as well as grazer presence/absence (Dodds and Gudder 1992; Hill 1992; Liess and Hillebrand 2006; Hillebrand et al. 2008; Evans-White et al. 2009; Liess et al. 2009; Ohta et al. 2011). In this study, benthic algal biomass exhibited a highly variable response to TP, beginning between 0.02 and 0.05 mg/L TP. While every effort was made during site selection to eliminate variability in physical factors that influence flow, particularly light availability, these factors may still have had an effect; additionally, variable grazing pressure likely accounts for much of the variation in response. This variation is particularly important to consider when comparing macroinvertebrate trait group response to TP as opposed to benthic chlorophyll-a, as the sites highest in chlorophyll-a occur around 0.05 mg/L TP.

Total Community Response

Previous studies of nutrient enrichment showed primary consumer density or biomass increasing along the nutrient gradient. When the community is considered as a whole, this study is no different: across both TP and chlorophyll-a concentrations, benthic macroinvertebrate biomass and density both increase. The relationship of total density with chlorophyll-a is the only one that is slightly at odds: it shows what may be the beginning of a stress response, tapering off at high levels of chlorophyll-a. This may indicate that organisms are using the increased food resources to grow larger, but not necessarily reproducing more; alternately, it may simply represent the need for more data at sites high in chlorophyll-a, to see if this pattern persists.

Pleurocerid Snail Response

The increase in macroinvertebrate biomass over the phosphorus gradient can be attributed almost entirely to the increase in pleurocerid snails; no significant model was found for non-pleurocerid biomass. Pleuroceridae were by far the most common organism collected by mass; 1,362 g DM Pleuroceridae were collected during the year-long study, nearly twice the total collected biomass of the next common (and individually largest) taxon, *Orconectes* (704 g). While there was no biomass response for non-pleurocerid macroinvertebrates, density of non-pleurocerid macroinvertebrates shows a broad subsidy-stress response to TP, decreasing as Pleuroceridae increase (Figure 4.1).

Functional Feeding Groups

Increasing phosphorus also prompted a shift in community structure (Figure 4.2), filtered by functional feeding group, except that Pleuroceridae (SC) are shown separately from non-Pleuroceridae scrapers. Each trait group has at least one significant model; when examined together, the four patterns of community response provide a picture of the changes brought about by enrichment. Non-Pleuroceridae scraper density increases along both TP and chlorophyll-a gradients, but biomass shows a subsidy-stress response; this indicates a shift, among the non-Pleuroceridae scrapers, towards smaller taxa at the highest end of the gradient. A similar relationship can be seen with gathering collectors: the community shifts towards higher numbers of smaller taxa (e.g. Chironomidae) as TP and chlorophyll-a increase. Shredders, as expected, decrease across the phosphorus gradient; predators show a subsidy-stress response, perhaps following the shift away from insect taxa—common pray of benthic macroinvertebrate predators—and towards pleurocerid snails, which are rarely subject to macroinvertebrate predation. Filtering collectors increased in both biomass and density; as they are subject to less pressure from resource competition than other feeding groups (Merritt et al. 2009), they, like the pleurocerid snails, are responding directly to the general increase in resources associated with eutrophication.

Overall, the pattern is one of increasing pleurocerid snails and collecting taxa, by density, and increase in pleurocerid snails and specifically filtering collectors by biomass. Grazing pressure from pleurocerid snails is one likely reason for the variability of the response of chlorophyll-a to phosphorus at higher levels of phosphorus; during sample

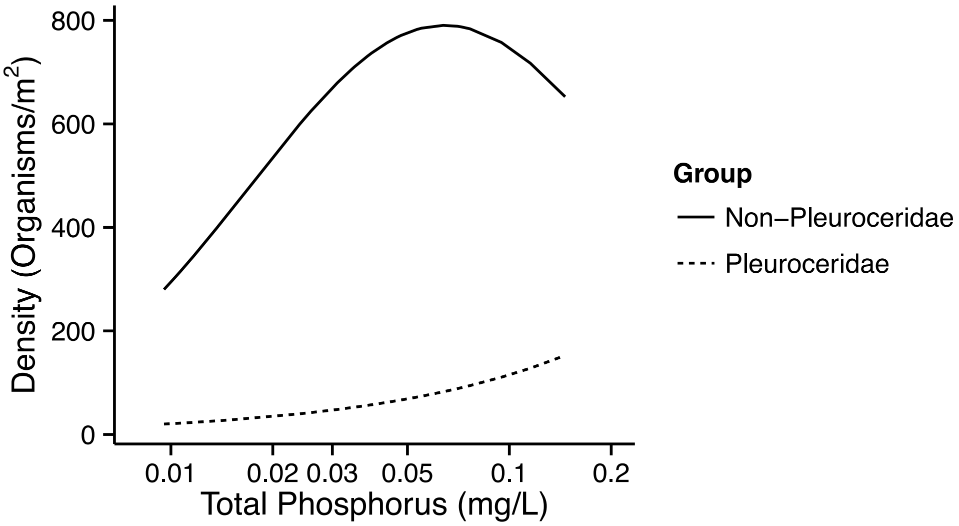

Figure 4.1. Modeled density response of Pleuroceridae and Non-Pleuroceridae to TP.

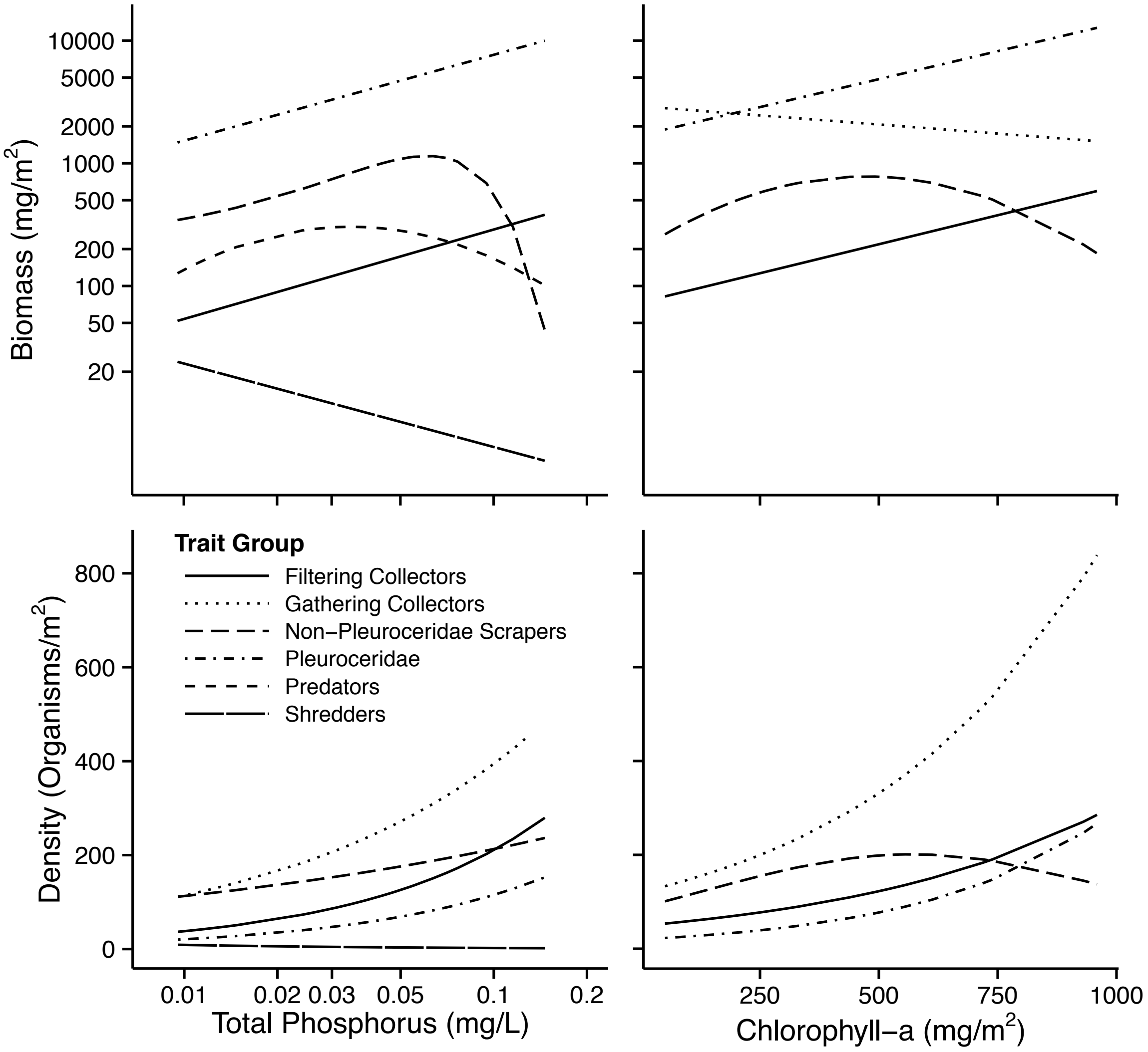

Figure 4.2. Modeled response of functional feeding groups to TP and chlorophyll-a. Note that in this figure, biomass is reported on a log scale, due to the difference in mass between Pleuroceridae and other taxa.

collection, lines of pleurocerid snails were sometimes observed, with filamentous algae visible on the cobbles on one side and the cobbles on the other side grazed clean. However, large filamentous blooms occurred even at sites where pleurocerid snails are abundant. This effect may be seasonal (reduced pleurocerid activity in colder weather), or it may result from changes in the pleurocerid population over time, as the snail population responds to the availability of benthic algae.

The lack of response of gathering collector biomass to TP is likely a result of the size range of members of the group: it includes the largest taxon (by individual size), *Orconectes*, and one of the smallest (but most numerous), Chironomidae. The second most common gathering collector taxon by both biomass and density is the mayfly *Ephemerella*, which competes directly with Pleuroceridae for *Cladophora* (McShaffrey and McCafferty 1991); this likely further confounds the issue.

Generation Length Response

There is a decided shift in life history strategy over the phosphorus gradient (Figure 4.3). Specifically, taxa which have two or more generations per year exhibit growth along the gradient, in both biomass and density, while those with generation lengths of a year exhibit a subsidy-stress response and those of two years a subsidy-stress biomass response to biomass and a slight increasing density response. Multivoltine and bivoltine taxa are likely taking advantage of the increase in food resources (particularly multivoltine taxa, as bivoltine taxa biomass begins to subside at high chlorophyll-a), and may also be freed from phosphorus limitation under the GRH. Univoltine taxa, many of which are fast-seasonal (Merritt et al. 2009) are outcompeted by taxa that are able to exploit available resources more quickly. Semivoltine taxa, most of which are predators

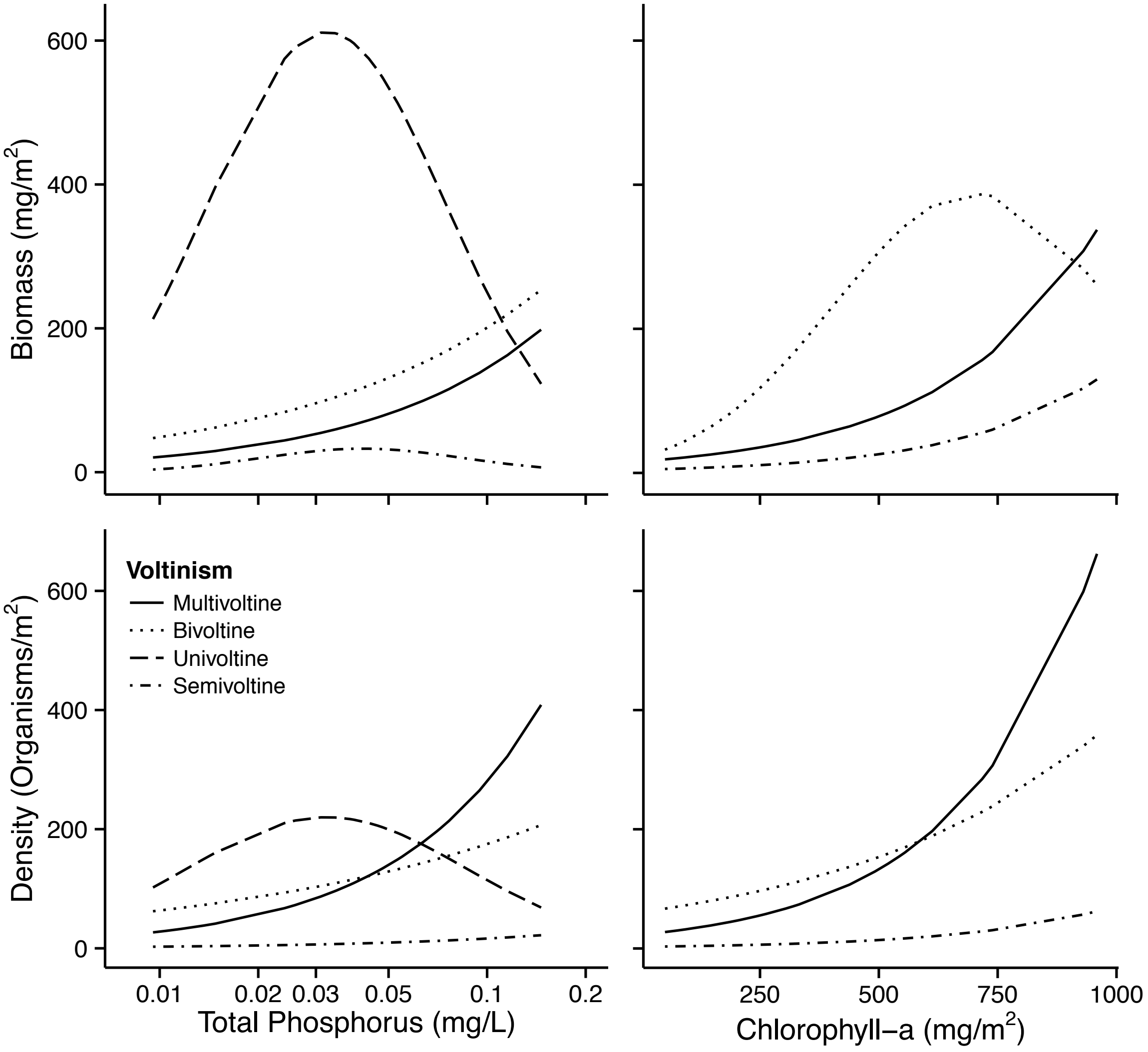

Figure 4.3. Modeled response of functional feeding groups to TP and chlorophyll-a.

(dominated by the stonefly *Agnetina*) may not be able to take full advantage of the increase in prey biomass because they are constrained by their longer life-history, or may decline at high levels of phosphorus because they are moderately sensitive to anthropogenic impacts associated with high TP.

Conclusion

Overall, the benthic community response to the phosphorus gradient demonstrates that while the interactions in the system are likely to be complex, the main driver of the response is the presence of pleurocerid snails and their interaction with the growth of filamentous algae. Pleurocerid snails are true perennials in the stream system, remaining in the stream throughout the year, with significant growth periods in spring and fall, and lifespans between two and eleven years (Huryn et al. 1994; Brown et al. 2008). They have been demonstrated to alter periphyton growth and community development (Tuchman and Stevenson 1991; Rosemond et al. 1993), and to compete directly with grazing insect taxa for food resources (Harvey and Hill 1991; Hill 1992). They are also highly resistant to predation in adulthood (Vermeij and Covich 1978), though juvenile pleurocerids form a large portion of the diet of river darters when they are available (Haag and Warren 2006). The shift in community away from aquatic insects and towards these snails may have implications further up the food chain (in insectivorous fishes), and impact fish in the system that are important for tourism.

Due to their ability to sheer off long filamentous strands, the relationship between eutrophication and the presence of nuisance blooms of filamentous is likely to be confounded by the presence of pleurocerid snails, particularly during periods of heavy snail growth. Specifically, levels of phosphorus which have produced blooms in other experiments or in a laboratory setting may instead produce a bloom of snails. While this may be preferred aesthetically, what it means fundamentally is that when Pleuroceridae are present, a lack of blooming filamentous algae cannot be definitively interpreted as a non-problematic level of TP in the water. The benthic community at those locations will still be impacted by the high level of phosphorus, even if filamentous algae is not blooming; this must be considered when undertaking to manage streams impacted by anthropogenic phosphorus inputs.

APPENDIX

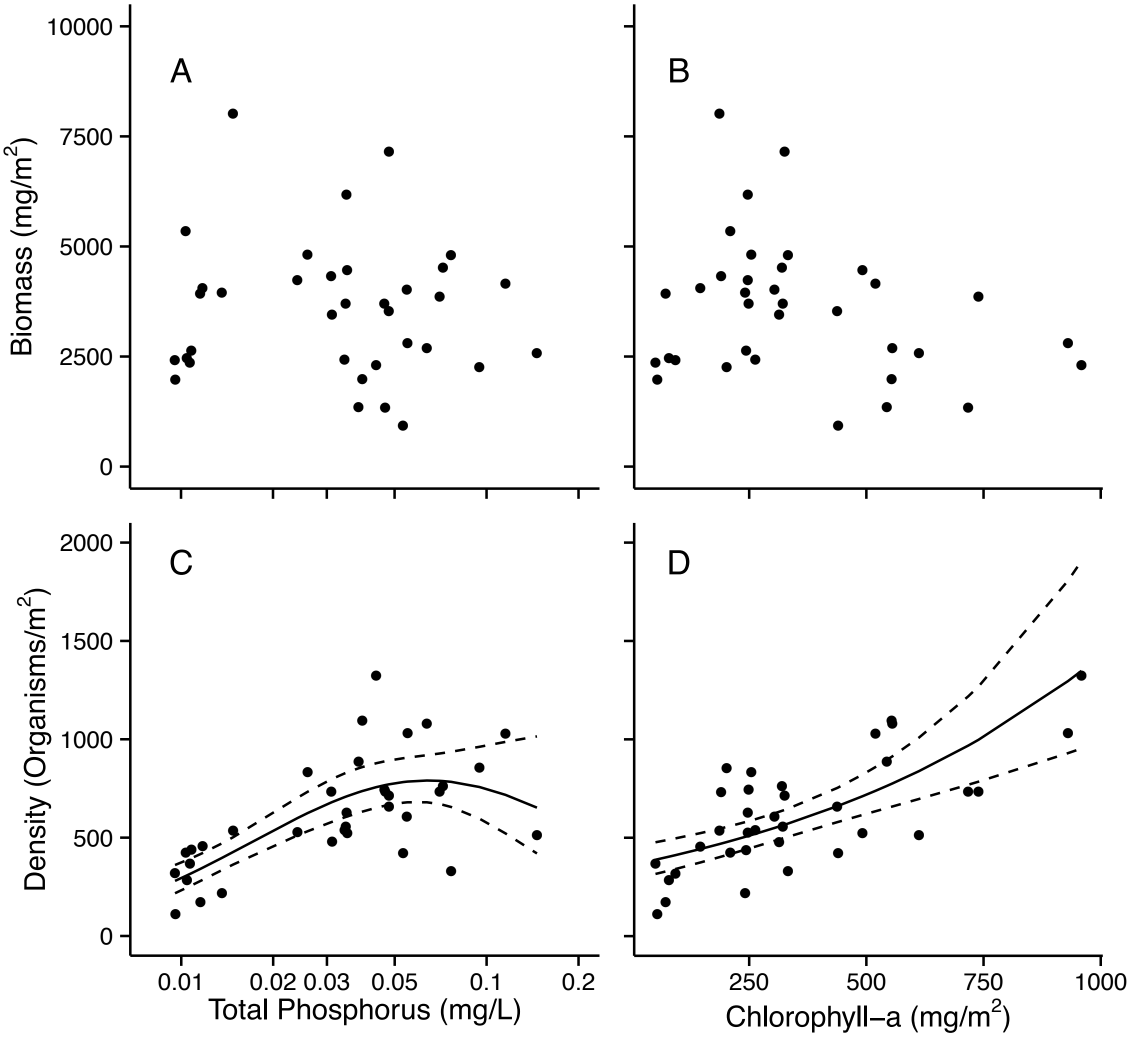

Figure A.1: Biomass and density data for non-pleurocerid macroinvertebrates, against both TP and chlorophyll-a. Lines indicate a significant model result and associated 95% confidence interval. Deviance explained 50.59% (C); 40.27% (D).

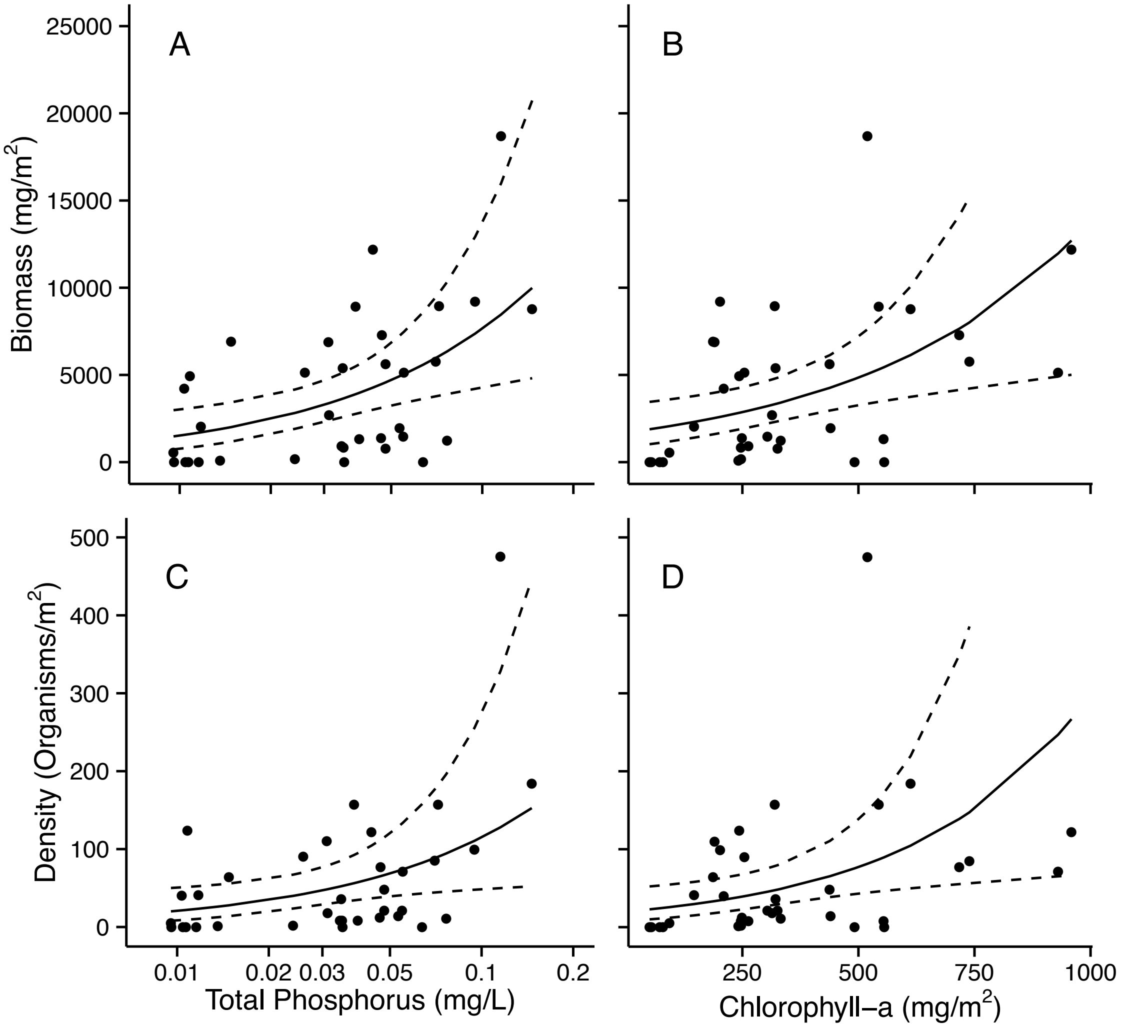

Figure A.2: Biomass and density data for Pleuroceridae, against both TP and chlorophyll-a. Lines indicate a significant model result and associated 95% confidence interval. Deviance explained 19.52% (A); 15.18% (B); 13.17% (C); 9.60% (D).

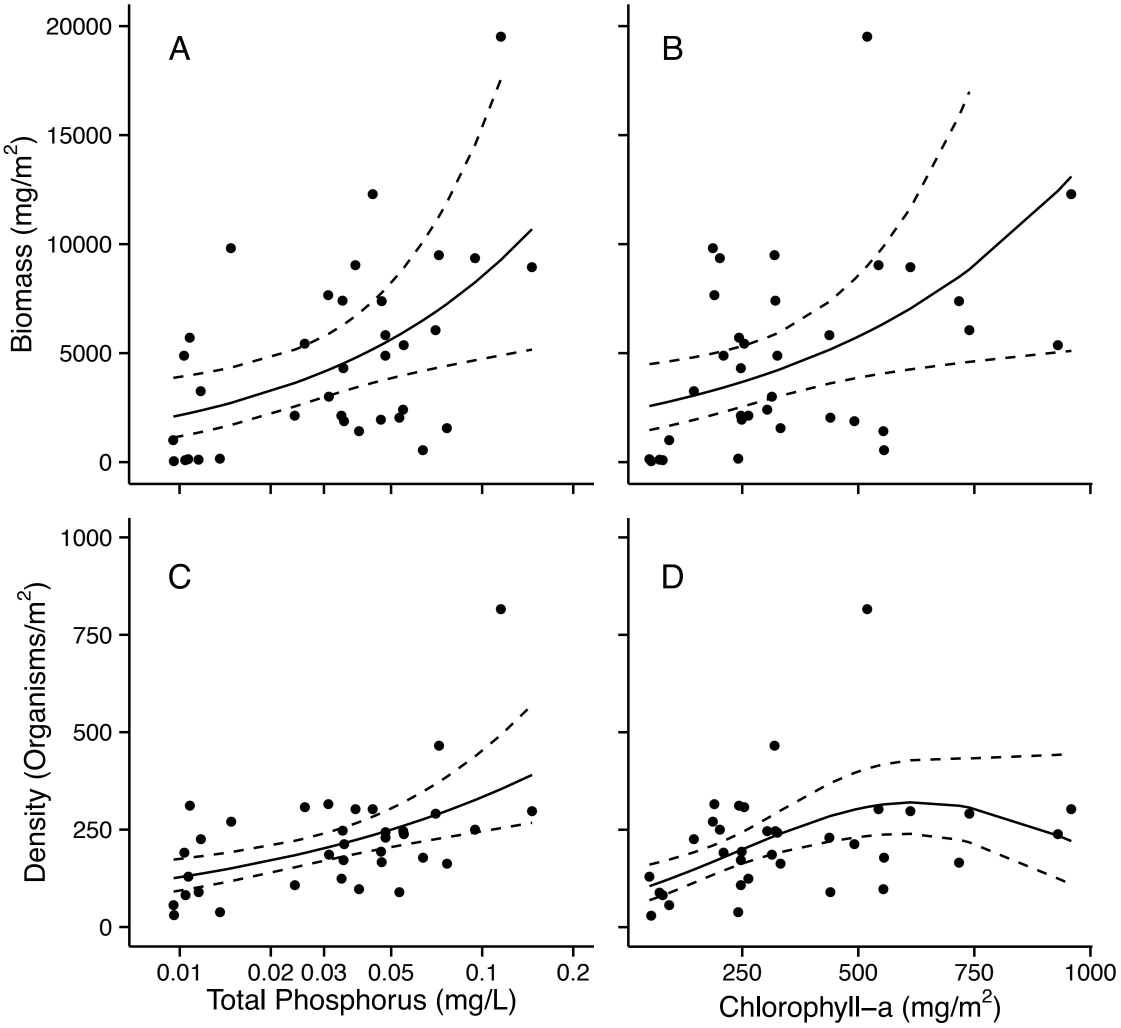

Figure A.3: Biomass and density data for scrapers, against both TP and chlorophyll-a. Lines indicate a significant model result and associated 95% confidence interval. Deviance explained 15.15% (A); 10.82% (B); 27.30% (C); 22.48% (D).

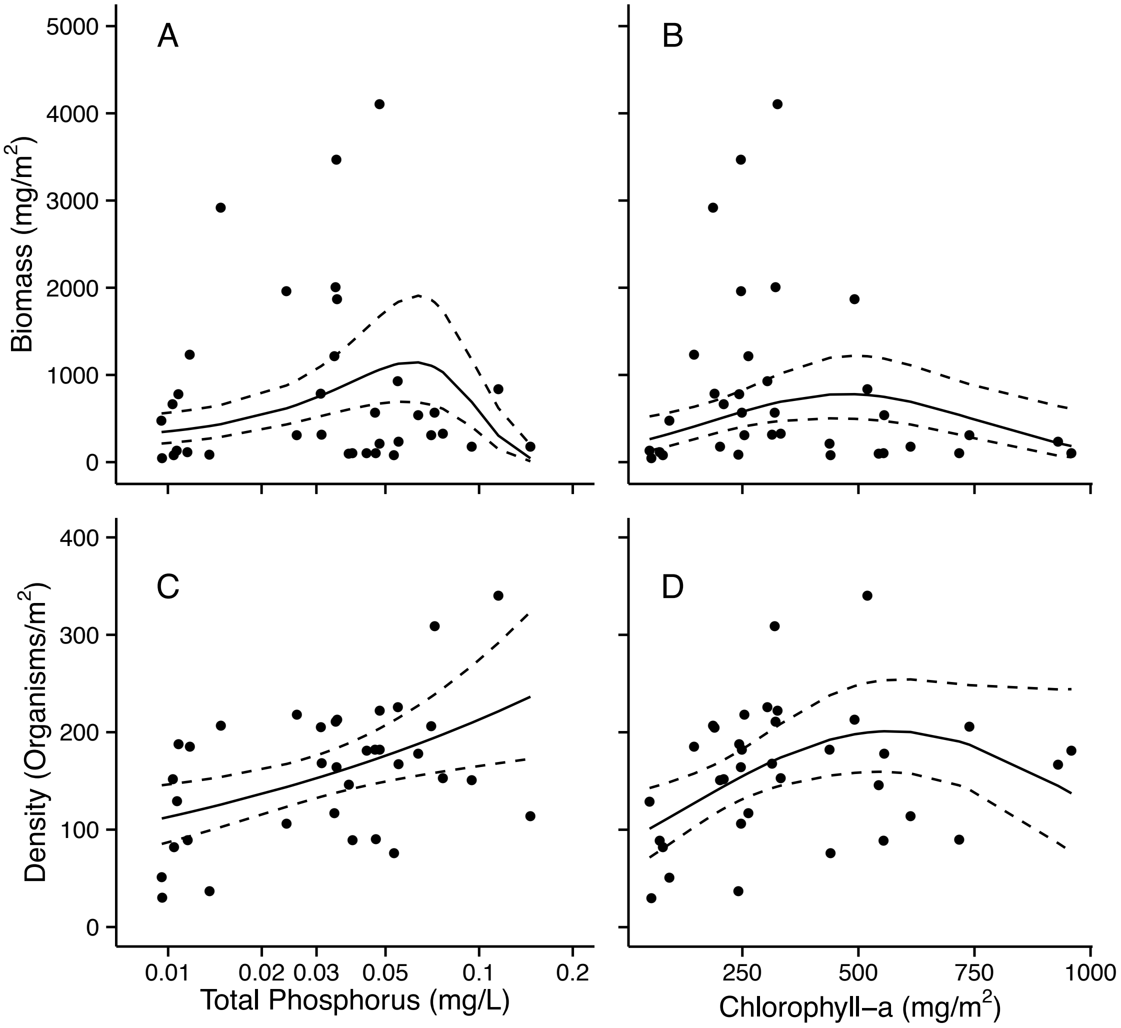

Figure A.4: Biomass and density data for non-pleurocerid scrapers, against both TP and chlorophyll-a. Lines indicate a significant model result and associated 95% confidence interval. Deviance explained 53.76% (A); 40.12% (B); 17.86% (C); 14.48% (D).

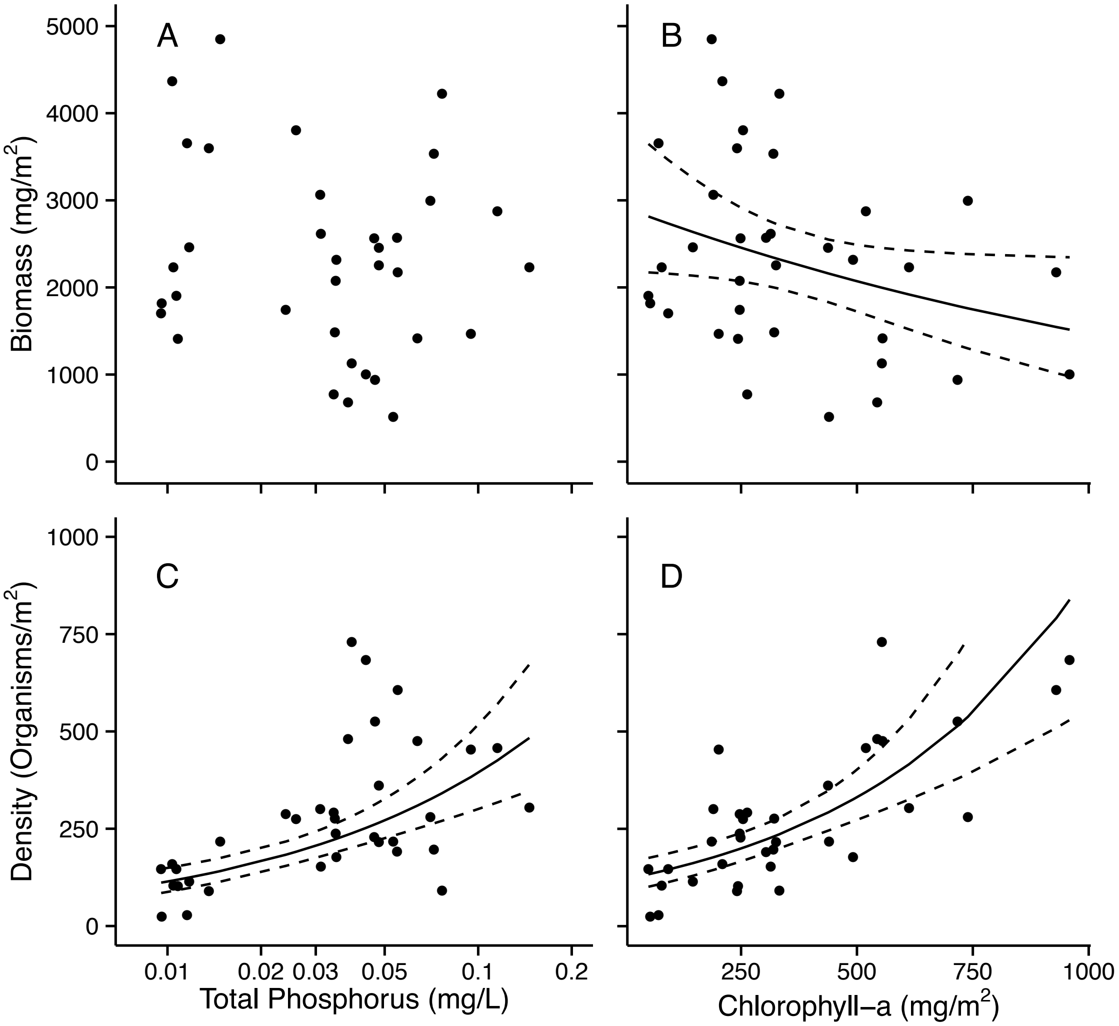

Figure A.5: Biomass and density data for gathering collectors, against both TP and chlorophyll-a. Lines indicate a significant model result and associated 95% confidence interval. Deviance explained 9.09% (B); 60.14% (C); 45.36% (D).

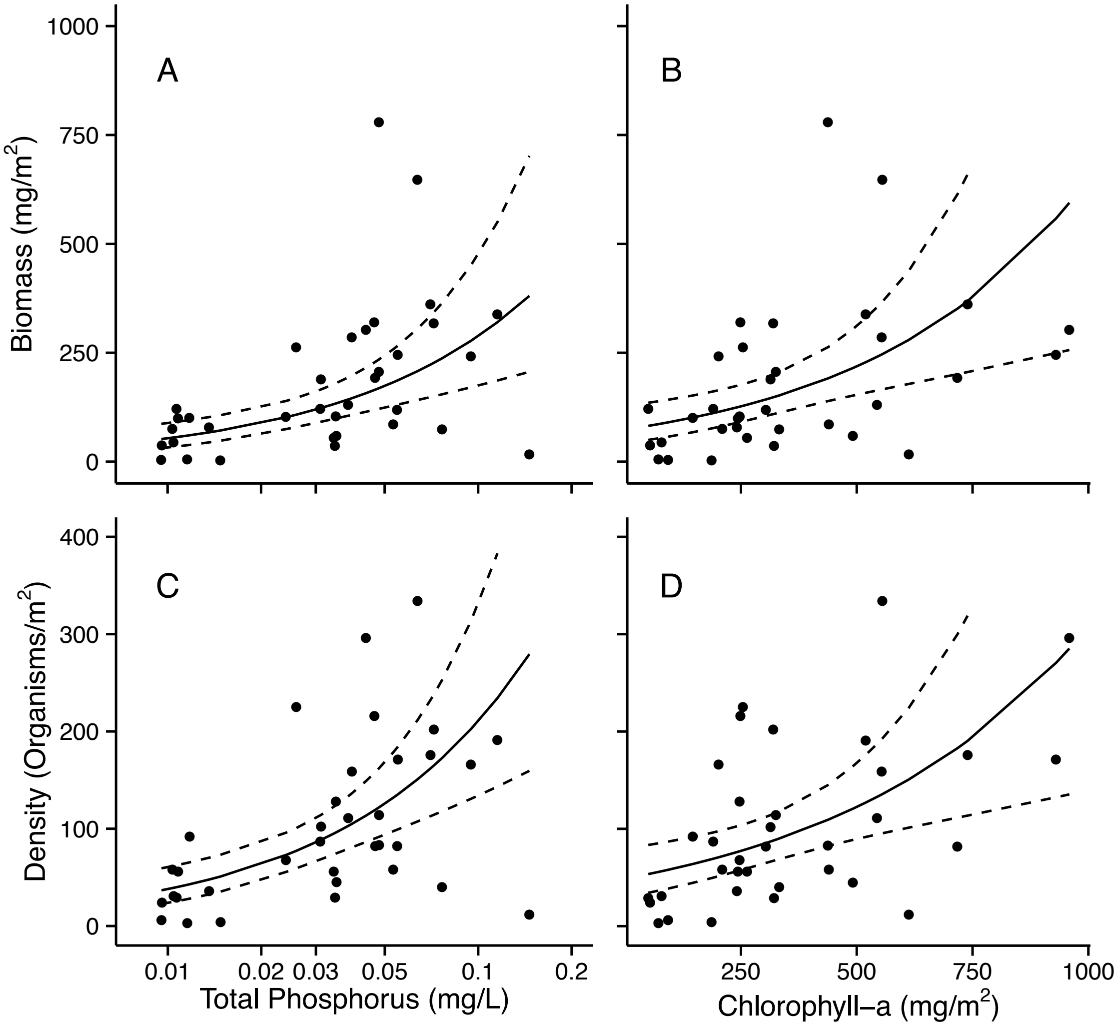

Figure A.6: Biomass and density data for filtering collectors, against both TP and chlorophyll-a. Lines indicate a significant model result and associated 95% confidence interval. Deviance explained 35.12% (A); 17.36% (B); 26.96% (C);1 8.87% (D).

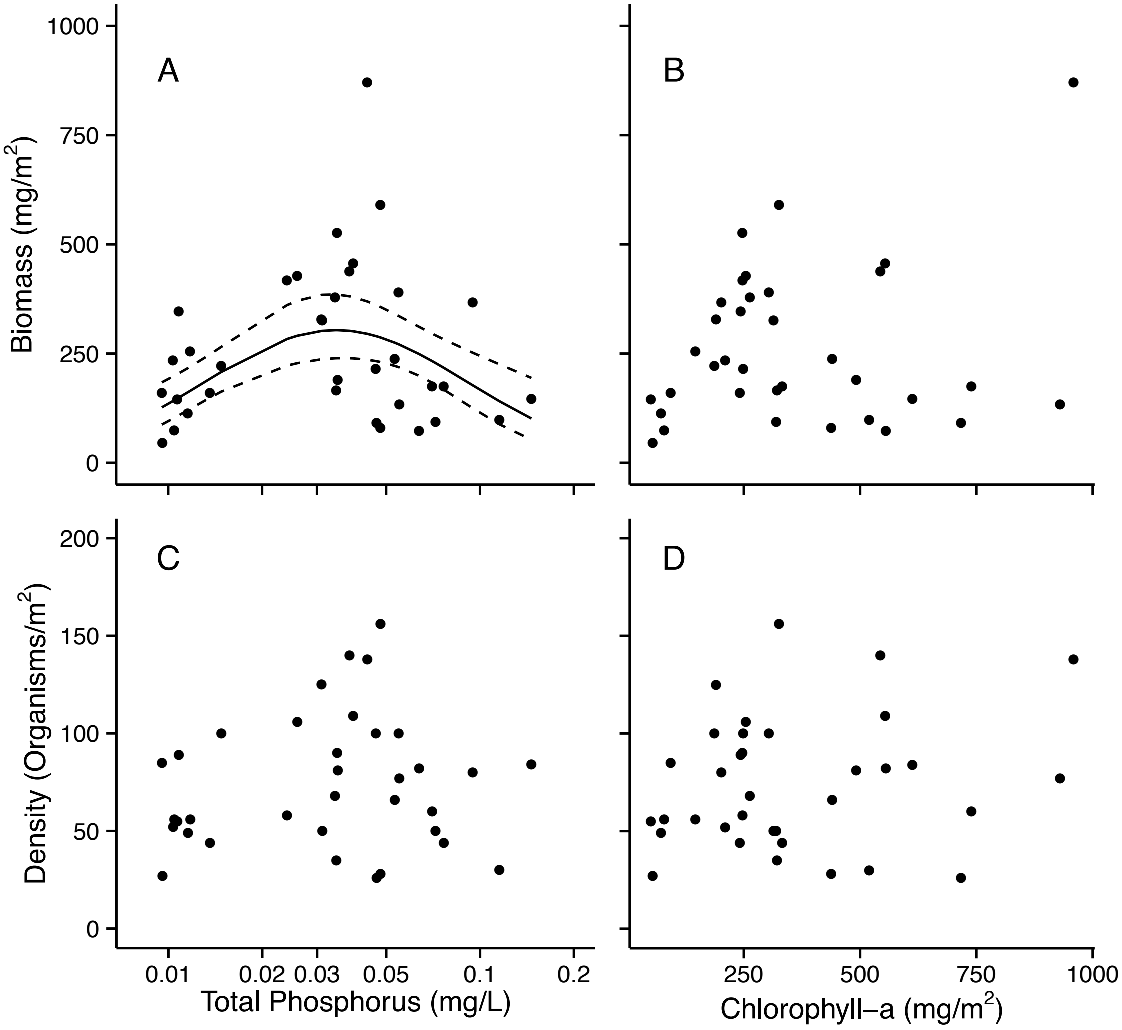

Figure A.7: Biomass and density data for predators, against both TP and chlorophyll-a. Lines indicate a significant model result and associated 95% confidence interval. Deviance explained 41.63% (A).

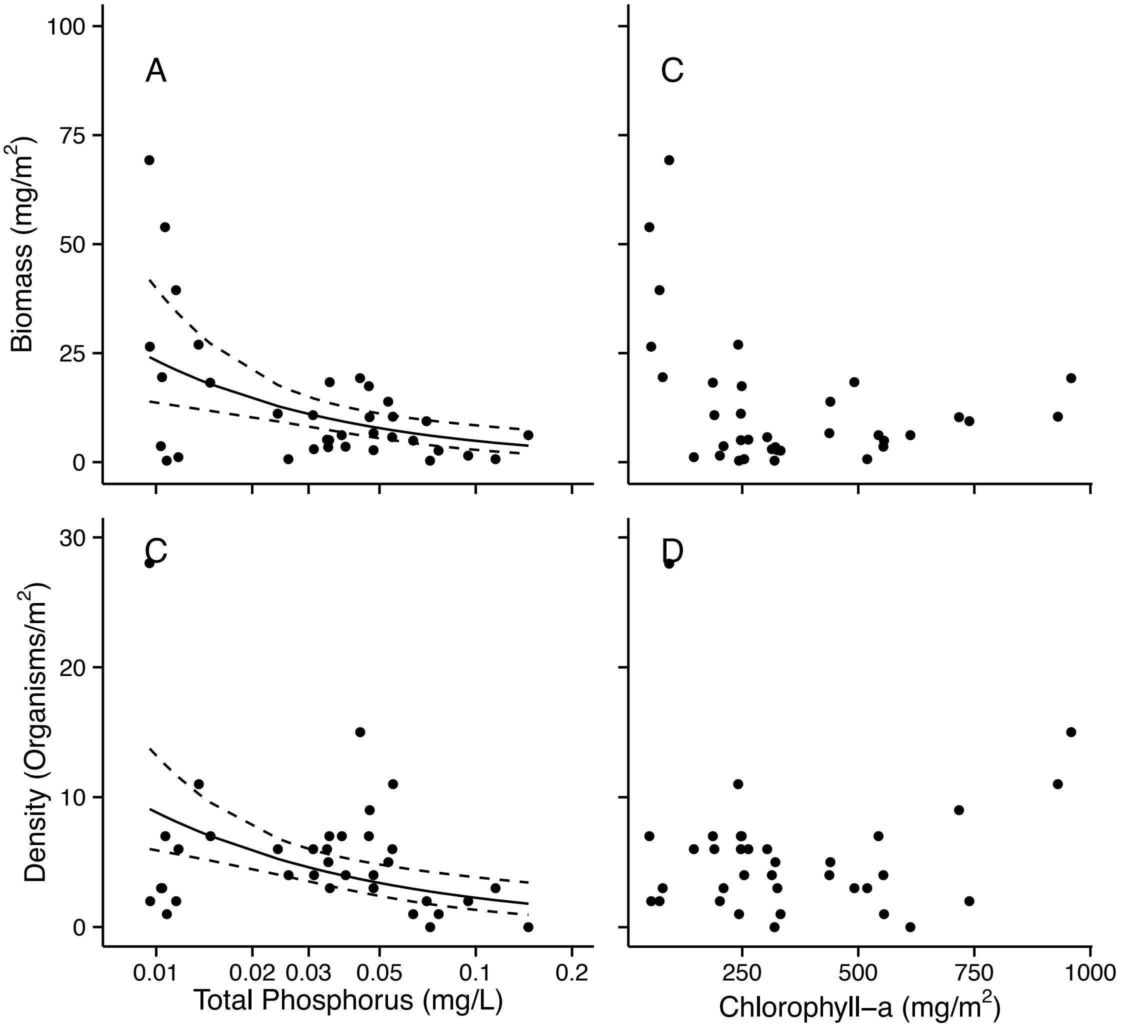

Figure A.8: Biomass and density data for shredders, against both TP and chlorophyll-a. Lines indicate a significant model result and associated 95% confidence interval. Deviance explained 36.73% (A); 25.55% (C).

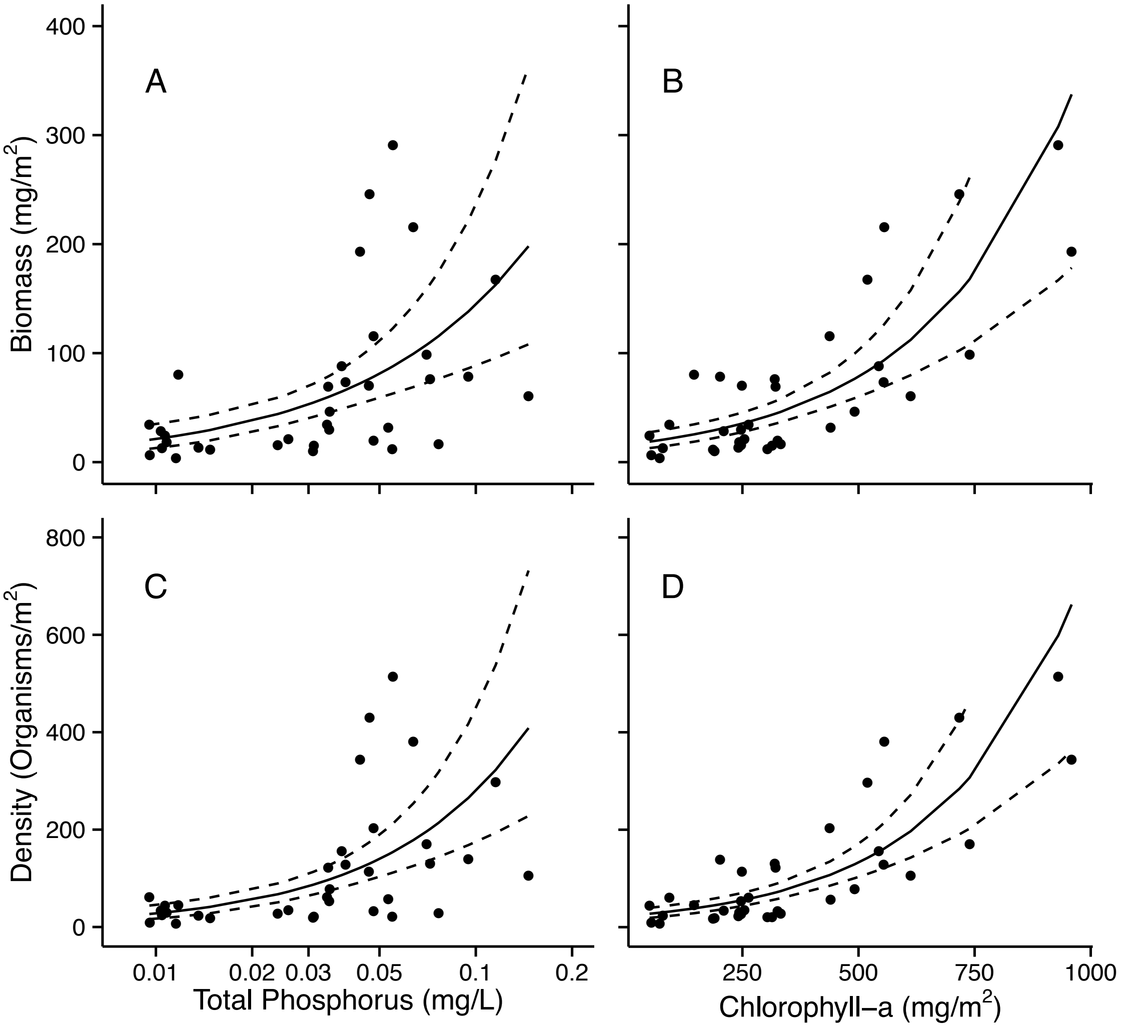

Figure A.9: Biomass and density data for multivoltine insects, against both TP and chlorophyll-a. Lines indicate a significant model result and associated 95% confidence interval. Deviance explained 30.90% (A); 53.63% (B); 39.95% (C); 60.48% (D).

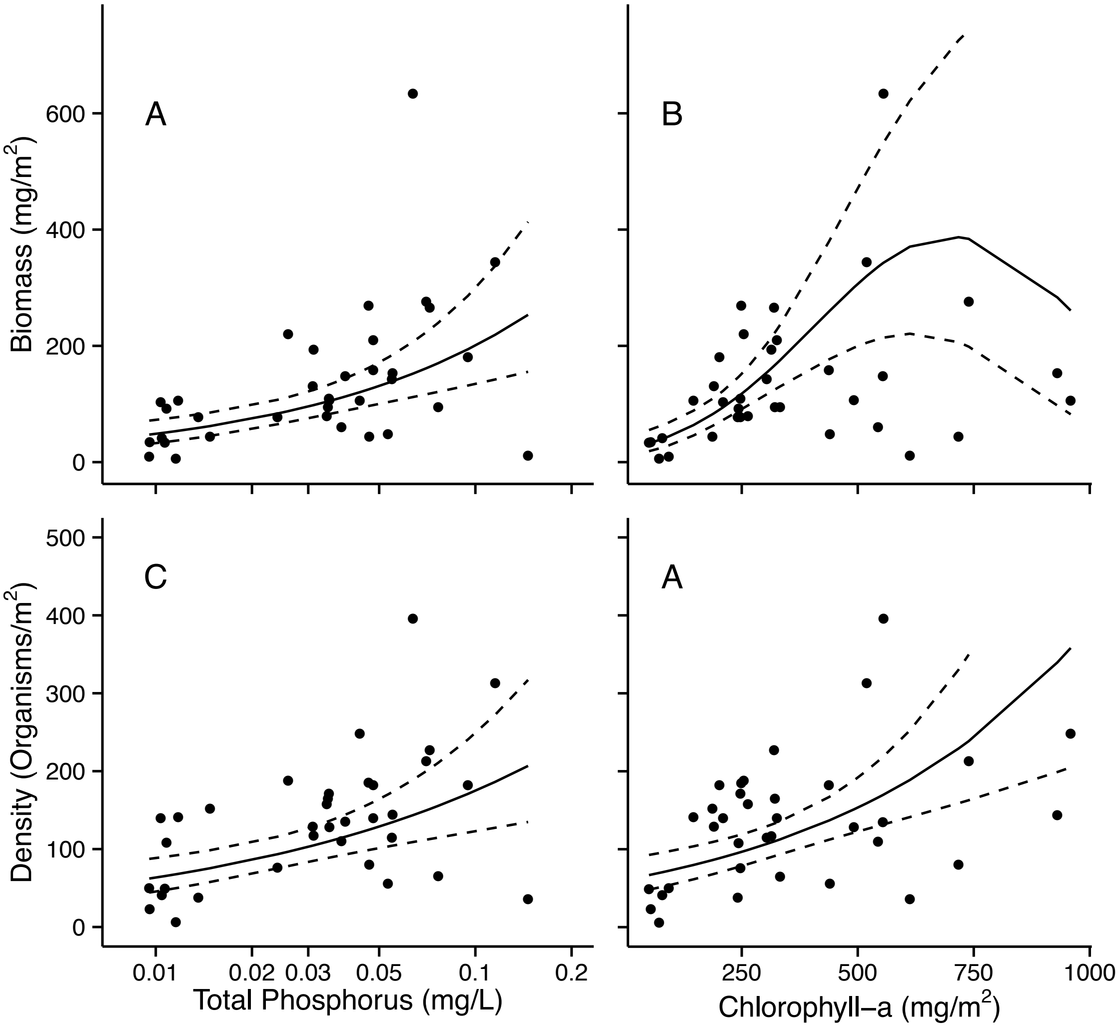

Figure A.10: Biomass and density data for bivoltine insects, against both TP and chlorophyll-a. Lines indicate a significant model result and associated 95% confidence interval. Deviance explained 42.17% (A); 37.78% (B); 36.35% (C); 33.66% (D).

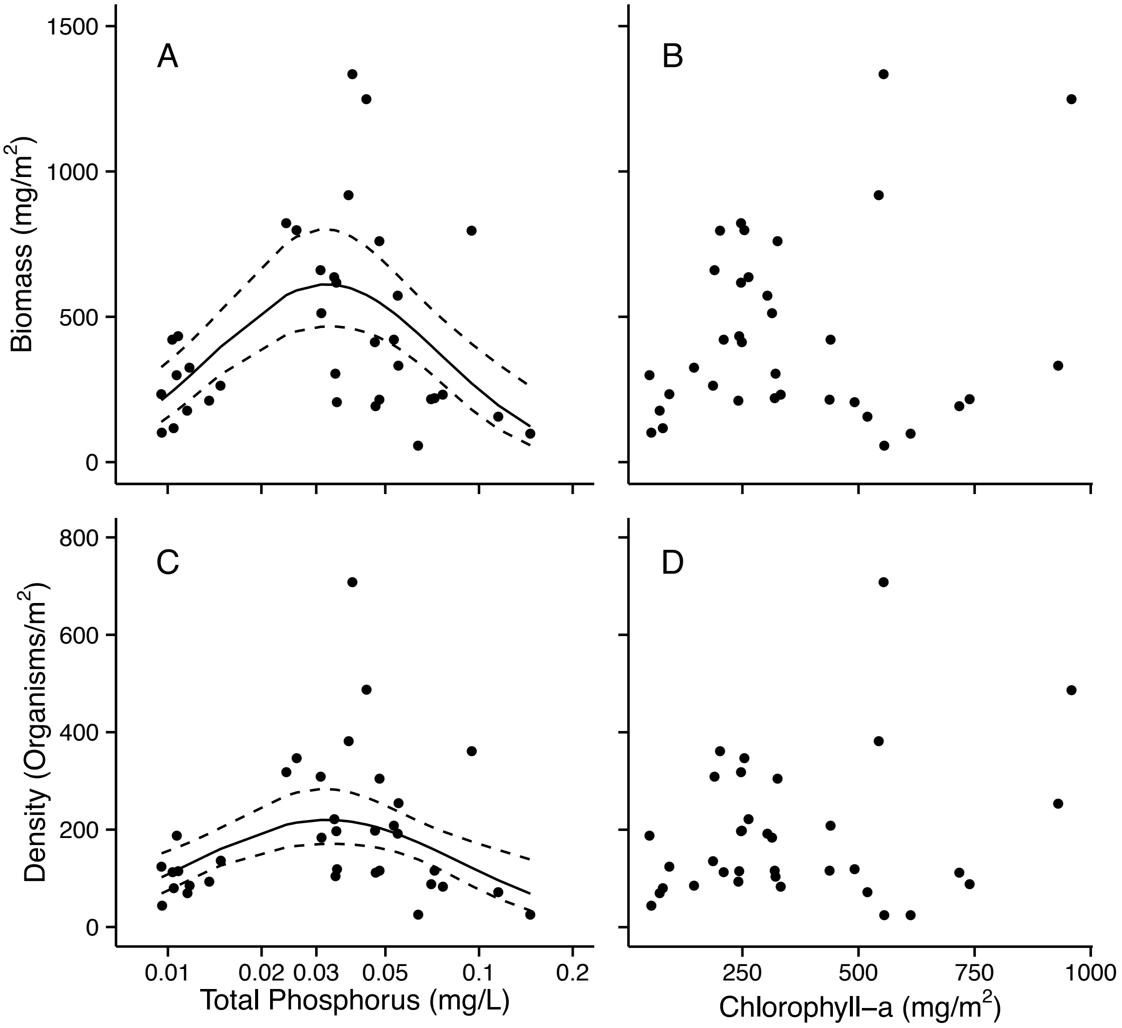

Figure A.11: Biomass and density data for univoltine insects, against both TP and chlorophyll-a. Lines indicate a significant model result and associated 95% confidence interval. Deviance explained 31.73% (A); 49.94% (C).

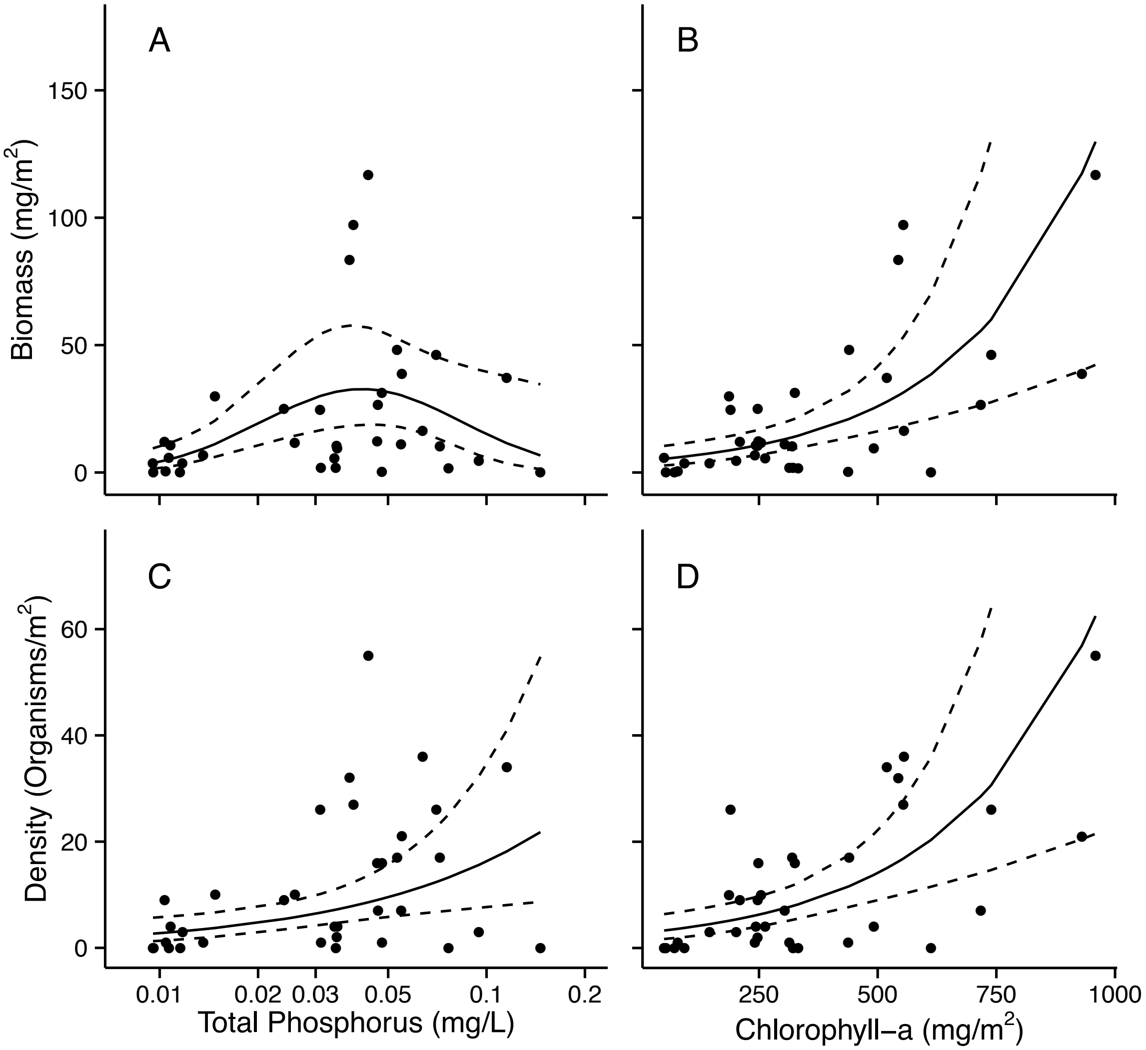

Figure A.12: Biomass and density data for semivoltine insects, against both TP and chlorophyll-a. Lines indicate a significant model result and associated 95% confidence interval. Deviance explained 18.98% (A); 25.74% (B); 28.60% (C); 24.41% (D).

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