Characterizing the role of phosphorus availability and periphytic algae in the food choice and performance of detritivorous caddisflies (Trichoptera:Limnephilidae)

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Abstract: Organisms that rely on detritus as their primary food source may face particularly strong nutritional constraints on growth and development, given the characteristically poor quality of detrital resources. In freshwater ecosystems, the low content of P in detritus often limits detritivore growth. Additionally, a growing body of evidence suggests the biochemical composition of algae, such as essential fatty acids, can limit aquatic detritivore growth. We investigated feeding preference and growth responses of common aquatic detritivores by performing paired feeding-preference and growth experiments on 4 species of larval caddisflies (Trichoptera) from the family Limnephilidae: *Asynarchus nigriculus*, *Anabolia bimaculata*, *Limnephilus externus*, and *Ecclisomyia* sp. We manipulated both the P content and epiphytic algal biomass of a common detrital food resource (decomposing sedge [*Carex* sp.]) by conditioning the detritus under 2 different light (ambient, shaded) and P (ambient [low], +P) regimes. We tested 3 hypotheses that describe feeding preferences and performance under different scenarios of P limitation, algal limitation, and co-limitation by P and algae. We observed evidence of preferential feeding behavior for each of the 4 taxa, with 2 species exhibiting preferences for conditioned detritus with high algal biomass and 2 for detritus from the +P treatments. We observed agreement between feeding preferences and performance (growth, growth efficiency, mortality) for only 2 taxa, with *A. nigriculus* exhibiting higher growth rates and growth efficiency on their preferred high-P detritus, and *L. externus* experiencing lower mortality when reared on their preferred high algal biomass detritus. These findings provide an initial step toward characterizing the feeding preferences and performance responses of aquatic detritivores to 2 potentially common nutritional constraints: detrital P and algal supply.
Key words: feeding preference, selective foraging, detritivore, detritus, phosphorus, Trichoptera

Many consumers are faced with the challenge of foraging among potential food sources that vary in their nutritional quality and which may not adequately provide the nutrients and biochemicals essential for growth, development, and reproductive success. Organisms that rely on detritus as their primary food source may face particularly strong nutritional constraints on growth and development, given the characteristically poor quality of detrital resources. For example, in freshwater systems, the high ratios of C to N and P in detrital biomass often exceed those of detritivores (Cross et al. 2003, Evans-White et al. 2005), suggesting that nutrient-limited growth of detritivores is common. Indeed, variation in the P content of leaf detritus modulates growth rates of leaf-shredding macroinvertebrates (Kendrick and Benstead 2013, Fuller et al. 2015, Halvorson et al. 2017) as well as patterns of consumer biomass and production in detritus-based stream ecosystems (Demi et al. 2018, 2019). Variation in detrital N can also affect individual growth rates and nutrient recycling by aquatic detritivores (Frainer et al. 2016, Halvorson et al. 2017, Fenoy et al. 2020). Moreover, a growing body of evidence suggests that, in addition to elemental (C, N, and P) content, resource biochemical composition may be an important indicator of resource quality in terrestrial and aquatic food webs (Guo et al. 2016b). For example, polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid and docosahexaenoic acid, have gained

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increasing attention as indicators of resource quality and have been linked to increased growth rates (Brett and Müller-Navarra 1997, Crenier et al. 2017) and reproductive success (Glencross 2009, Twining et al. 2018) across diverse animal taxa. PUFAs are frequently nondetectable in terrestrial plants, thereby reinforcing the notion that vascular plant detritus is a resource of poor nutritional quality in aquatic food webs (Hixson et al. 2015).

In freshwater ecosystems, colonization of detrital substrates by microbial decomposers (fungi and bacteria) enhances the palatability and nutritional quality of detritus, but the role of detritus-associated algae in modulating detritus quality has received less attention. Microbial decomposers immobilize inorganic N and P from the water column to support biomass production, thereby increasing the nutrient content of detritus, and they also degrade structural carbohydrates (e.g., cellulose) into compounds that are more digestible for aquatic detritivores (Cornut et al. 2015, Manning et al. 2015, Gulis et al. 2017). Thus, heterotrophic microorganisms are an important source of energy and nutrients for higher trophic levels in stream food webs (Cummins 1974). Historically, less attention has been directed toward the potential role of detritus-associated algae in these systems given that they tend to be consumed in very small quantities by many leaf-shredding macroinvertebrates. However, algae are a high-quality resource in aquatic food webs, typically higher in N and P than detritus and replete with essential PUFAs (Hixson et al. 2015), and can be an important nutritional source for aquatic detritivores (Kühmayer et al. 2020). Multiple studies now report that incorporation of algae into the diets of some leaf-shredding macroinvertebrates results in increased growth rates (Franken et al. 2005 Guo et al. 2016a, Crenier et al. 2017). As such, even small amounts of algae may play a more important role in detritivore nutrition and trophic dynamics in detritus-based ecosystems than previously recognized (Bumpers et al. 2017, Eckert et al. 2020).

Though nutritional constraints may be common for many aquatic detritivores, these animals can reduce nutritional imbalances in their diets by selectively consuming resources that are higher in potentially limiting elements and biochemicals. Selective feeding behavior is well documented in freshwater detritivores, which have been shown to discriminate among leaf litter of different species and at different stages of microbial colonization and decomposition as well as among different fungal species within a single leaf (see reviews by Cummins and Klug 1979, Graça 2001). Additionally, several studies have reported that some shredders preferentially consume algae over other available resources (Friberg and Jacobsen 1994, Leberfinger and Bohman 2010), suggesting that aquatic detritivores may overcome nutritional constraints imposed by low-quality vascular plant detritus by occasionally consuming higher quality algae. Such selective feeding behaviors are likely influenced by some combination of variation in the chemical (e.g., elemental and biochemical composition) and physical (e.g., leaf toughness) properties of different resources (Graça 2001). For example, multiple studies report selective consumption of leaf material with elevated N or P content (Hood et al. 2014, Cornut et al. 2015, Ohta et al. 2016), and other aspects of resource biochemical composition, including lipid content (Cargill et al. 1985), have also been linked to feeding preferences of aquatic shredders.

In this study, we tested the relative strength of feeding preferences for P and epiphytic algae by performing feeding-preference experiments with 4 species of detritivorous caddisflies (Trichoptera:Limnephilidae) that feed primarily on wetland sedge detritus (Wissinger et al. 1996). We focused on potential preferences for P rather than N, given that stoichiometric imbalances between consumers and detritus are generally larger for C:P than C:N, indicating a greater likelihood of P limitation in aquatic detritivores (Cross et al. 2003, though also see Evans-White et al. 2005). Additionally, we used paired growth experiments to assess whether caddisflies reared on their preferred diet exhibited increased performance (e.g., higher growth rate, lower mortality) relative to those reared on other diet treatments. We formulated 3 potential hypotheses that describe feeding preferences and performance under different scenarios of P limitation, algal limitation, and co-limitation by P and algae. We hypothesized that preference for high-P sedge detritus, regardless of algal biomass, would be an indicator of consumer P limitation, where species that preferred high-P sedge would show increased performance (e.g., higher growth and growth efficiency, reduced mortality) when reared on high-P sedge relative to low-P sedge. Additionally, we hypothesized that algal limitation would result in preferential consumption of high algal biomass sedge, regardless of P treatment, and performance would be greatest for individuals reared on high algal biomass sedge detritus. Finally, in a co-limitation scenario, we hypothesized that the greatest preference would be for sedge with high algal biomass and high P content, whereas sedge with low algal biomass and low P content would be the least preferred, and that individuals reared on the preferred high-P, high-algae diet would exhibit increased performance relative to other diets.

METHODS

Experimental design overview

To test for feeding preferences and performance responses relating to the periphytic algal biomass and P content of detritus, we fed larval caddisflies wetland sedge detritus (*Carex* sp.) conditioned under 2 different light and P treatments. Light and P treatments were crossed to create a total of 4 different sedge treatments: ambient light and ambient P (Light A), ambient light and added P (Light + P), shaded and ambient P (Shade A), and shaded and added P (Shade + P). A thorough description of the preparation of

sedge treatments is described below. We created 2 incubation tubs for each sedge treatment (8 total tubs) as sources of sedge for paired feeding-preference and growth experiments for each of the 4 caddisfly species. However, we did not use sedge from the 2nd incubation tubs for the Light A, Light + P, and Shade + P treatments for any experiments. We used sedge from the 2nd incubation tub for the Shade A treatment only during the final experiments, conducted with *Ecclisomyia* sp., because of depletion of sedge from the 1st incubation tub for that treatment. All other treatments for *Ecclisomyia* sp. used sedge from the same incubation tubs as those used for the experiments with the other 3 species. All experiments with those 3 species used sedge from the same incubation tubs for all 4 sedge treatments (Table 1).

We tested feeding preferences for periphytic algae and detrital P content with multiple-choice feeding experiments where caddisflies were placed in feeding chambers and provided sedge from each of the 4 treatments. A full description of the feeding-preference experiments is presented below. Briefly, for each species, we stocked 25 replicate feeding chambers with sedge detritus from each treatment (sedge from each treatment randomly placed in the 4 corners of the chamber) and a single larval caddisfly (Table 1). Caddisflies were allowed to feed for 4 to 5 d among the 4 treatments and preferences were determined based on differences in consumption among the 4 sedge treatments. We tested differences in performance of caddisflies from the focal species by conducting separate growth experiments in conjunction with the feeding-preference experiments. We started paired feeding-preference and growth experiments for each species within 24 h except for *L. externus* (see below and Table 1). For the growth experiments, we reared larvae for 9 to 15 d (depending on species) on each of the 4 different sedge treatments. We created 5 replicate feeding chambers for each of the 4 treatments, each containing 5 caddisflies at the beginning of the experiments (Table 1). As performance metrics, we determined growth rates and growth efficiency as well as mortality and pupation rates.

Study area and focal taxa

We conducted the feeding-preference and growth experiments at the Rocky Mountain Biological Laboratory (hereafter RMBL) in Gothic, Colorado, USA, during the summer of 2019. All feeding-preference and growth experiments were performed with 4 species of larval caddisflies (Trichoptera) from the family Limnephilidae: *Asynarchus nigriculus* (Banks, 1908), *Anabolia bimaculata* (Walker, 1852), *Limnephilus externus* (Hagen, 1861), and *Ecclisomyia* sp. Species identifications were based on prior knowledge of the local species pool by the authors and were confirmed by S. Wissinger (deceased; Allegheny College, Meadville, Pennsylvania, USA). These 4 focal species are found commonly in small ponds and beaver wetlands in the area around RMBL and are known to exhibit primarily detritivorous feeding habits, including the shredding of vascular plant detritus (Wiggins 1996, Wissinger et al. 1996), though gut-contents analysis of *Ecclisomyia* larvae suggests considerable reliance on diatoms (Wiggins 1996).

We collected animals by hand and with dip nets from ponds near RMBL during July and August of 2019. All individuals of a given species used in either the growth or feeding-preference experiments were collected from the same pond on the same day except for *L. externus*. We collected larvae of *L. externus* for the growth and feeding-preference experiments from 2 different ponds on separate dates (Table 1).

Table 1. Summary of the experimental design for the feeding-preference and growth experiments. Full details of each experiment are described in the Methods. Replicates indicates the number of experimental chambers used in each experiment. For the feeding-preference experiments, the 4 sedge types were included within each replicate, whereas for the growth experiments, the 4 sedge types were in 5 separate replicates. # individuals indicates the total number of caddisflies used in each experiment, and ind./replicate indicates how many caddisflies were contained within each replicated experimental unit. Sedge sources indicate the incubation tubs from which sedge was used for the experiment and to determine the chlorophyll *a* biomass and %P of sedge detritus for the 4 treatments during the feeding-preference and growth experiments for each taxon. LP_1 = the light ambient + P tub 1, LA_1 = light ambient tub 1, SP_1 = shade + P tub 1, SA_1 = shade ambient tub 1, SA_2 = shade ambient tub 2.

Taxon	Experiment	Start date	Duration (d)	Replicates	# ind.	Ind./replicate	Sedge sources
Anabolia bimaculata	Preference	7/22/2019	4	25	25	1	LP_1, LA_1, SP_1, SA_1
	Growth	7/23/2019	9	5	100	5	LP_1, LA_1, SP_1, SA_1
Asynarchus nigriculus	Preference	7/31/2019	5	25	25	1	LP_1, LA_1, SP_1, SA_1
	Growth	7/31/2019	12	5	100	5	LP_1, LA_1, SP_1, SA_1
Ecclisomyia sp.	Preference	8/28/2019	5	25	25	1	LP_1, LA_1, SP_1, SA_2
	Growth	8/28/2019	13	5	100	5	LP_1, LA_1, SP_1, SA_2
Limnephilus externus	Preference	8/12/2019	4	25	25	1	LP_1, LA_1, SP_1, SA_1
	Growth	7/16/2019	15	5	100	5	LP_1, LA_1, SP_1, SA_1

For the later feeding-preference experiment, we selected individuals from a higher elevation pond with delayed phenology to ensure that larvae collected there were at a similar developmental stage (3rd instar) to those used in the earlier growth experiment, which we had collected at a lower elevation pond. For each species, all individuals used in each experiment were selected to be visually similar in initial body size and development stage (from ~3rd- [*L. externus* and *A. nigriculus*] to 5th-larval [*A. bimaculata* and *Ecclisomyia* sp.] instar) to minimize the potential for confounding effects of ontogeny in dietary preferences and nutritional requirements (Cargill et al. 1985). All experiments for each species commenced within 48 h of animal collection. Animals were held in plastic wash basins filled with ~2.5 cm of unfiltered pond water prior to the start of the experiments.

Preparation of sedge detritus treatments

Animals in both the feeding-preference and growth experiments were fed sedge detritus (*Carex* sp.) that we collected from a single beaver pond at RMBL on 28 June 2019. Sedges are an important source of coarse particulate detritus to lentic systems around RMBL and are an important food resource to their macroinvertebrate assemblages (Wissinger et al. 1996, 1999). To create our different sedge treatments, we distributed the fresh sedge detritus among eight 0.83-m^2 plastic cattle tanks and then added ~80 L of unfiltered water (depth of ~5 cm) from a nearby pond to each incubation tank. We then evenly distributed the sedge in a single layer to minimize self-shading within each tank. We covered 4 of the 8 tanks with black woven polypropylene weed barrier fabric (Ultra Web 3000 Groundcover; DeWitt Company, Sikeston, Missouri), which blocked >95% of solar radiation, to create the shaded, low-algae sedge treatments (Shade A and Shade + P). The shade cloths remained in place until all experiments were completed in September 2019. To create +P treatments, on 29 June 2019, we added an aliquot of concentrated sodium phosphate (Na_2HPO_4) solution to 2 each of the shaded and unshaded tanks to raise the P concentration to ~100 μg/L above ambient. We repeated the P amendments 2 additional times prior to beginning the feeding-preference and growth experiments in separate tanks to ensure sufficient microbial modification of detrital-P concentration. We incubated the sedge under the 4 treatments (Light A, Light + P, Shade A, Shade + P) for 17 d prior to commencement of the feeding-preference and growth experiments on 15 July 2019.

We periodically added unfiltered pond water, or stream water from the adjacent East River, to the tanks to account for evaporative water loss and to maintain a volume of ~80 L within the tanks. We used natural water sources to approximate the chemical conditions under which sedge detritus would condition and decompose naturally in the study area. Replacing evaporative water loss with unfiltered water from natural sources may elevate nutrient concentrations and introduce algal cells to the incubation tanks, but the low ambient P and chlorophyll concentration in the water column of the East River and ponds in this area (<2 μg P/L and 5–10 μg P/L, respectively; BWT, unpublished data) indicate that any such effects on detrital-P concentration and algal biomass would be minimal relative to the effects of the experimental treatments.

We periodically drained and replaced water in the Shade A treatments to reduce the development of anaerobic conditions during incubation. We did not observe evidence of anaerobic conditions within the unshaded or +P-shaded tanks. We did not make direct measurements of dissolved oxygen concentrations during incubation. Additionally, we did not measure heterotrophic microbial biomass (bacteria and fungi) on decomposing sedge and therefore cannot rule out treatment effects on microbial decomposer assemblages.

For the feeding-preference and growth experiments, we collected sedge from 4 incubation tubs, 1 for each treatment, to stock the experimental chambers. We collected sedge from the incubation tubs on the same day that we set up the experiments for each taxon. We also collected multiple sedge clippings (~0.05 g dry mass, 6 total replicates) from the same 4 incubation tubs to measure periphytic algae (3 replicates) and detrital P concentration (3 replicates) within ~24 h of the commencement of experiments for each taxon. Upon collection, we immediately placed each of the 3 replicate sedge clippings from each treatment into 30-mL dark polypropylene bottles, returned them to the laboratory, and froze them at −20°C until analysis. We placed additional sedge clippings (3 replicates/treatment) in sterile polypropylene sample bags, returned them to the laboratory, and dried them at 60°C for a minimum of 48 h.

We inferred algal biomass from Chl *a* concentrations based upon a non-acidification method for fluorometric Chl *a* analysis described by Welschmeyer (1994). Briefly, periphytic Chl *a* was extracted from sedge with a buffered ethanol solution (90% ethanol, 10% supersaturated $MgCO_3$ solution), where enough ethanol was added (usually 7 mL) to submerge all sedge clippings. Samples were covered and placed in the dark at room temperature for 4 to 8 h to allow for chlorophyll extraction. We measured Chl *a* concentrations on a Turner Designs Trilogy Fluorometer (Sunnyvale, California) and standardized them to the dry mass of sedge used for each extraction by dividing total sample Chl *a* mass by sedge dry mass. Dry mass was determined by drying the chlorophyll extracted sedge at 60°C for a minimum of 48 h and weighing. We did not correct Chl *a* measurements to account for residual Chl *a* remaining in the sedge detritus. Differences in Chl *a* among treatments are likely a function of algal biomass, rather than differences in residual Chl *a* of the sedge detritus, although we cannot rule out heterotrophic microbial effects on residual Chl *a* biomass resulting from our experimental treatments. We analyzed all samples within 30 d of collection.

For P-concentration analysis of sedge, we homogenized the dried samples with a Spex® 8000 ball mill (Metuchen, New Jersey) and stored them in glass scintillation vials in a desiccator until analysis. We took a small subsample from each of the 3 homogenized samples, weighed it, and combusted it for 2 h at 500°C. Combusted samples were digested in a $K_2S_2O_8$ solution at 100°C for 1 h prior to colorimetric analysis of total P, which followed methods described by Ostrofsky and Rigler (1987) and was done using a GENESYS 10S UV-VIS Spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts). A summary of the Chl *a* and P concentration of the sedge detritus at the commencement of each set of feeding-preference and growth experiments is presented in Table 2.

Feeding-preference experiments

To conduct the feeding-preference experiments, we used paper clips to secure 1 g (±0.15) of wet sedge detritus from each of the 4 sedge treatments and randomly assigned each of these bundles to 1 of 4 quadrants of a 20 × 20-cm clear plastic feeding chamber. For each species, we conducted a feeding-preference experiment with 25 replicate feeding chambers, where we randomized the different sedge treatments among the 4 quadrants in each chamber. We then added unfiltered pond water to each feeding chamber to a depth of ~2 cm and stocked each chamber with a single larval caddisfly. Feeding-preference experiments ranged from 4 to 5 d, depending on the caddisfly species (Table 1). These experiments, along with the growth experiments (described below), were conducted in an enclosed outdoor shed constructed of a steel frame and white vinyl covering. Screened windows in the shed allowed ventilation and ensured that internal temperatures were similar to external air temperatures throughout the day. Water temperatures within the growth chambers used in the experiments were within the range of natural temperatures of ponds within the local area (BWT, unpublished data). Solar radiation was not measured within the shed but was likely reduced relative to outdoor conditions.

We assessed feeding preferences by determining the proportion of total mass loss from all sedge treatments in each chamber that was attributed to each of the 4 sedge treatments (Lockwood 1998). We determined mass loss for each sedge treatment by subtracting final dry mass from initial dry mass for each sedge treatment in each of the 25 chambers. Initial dry mass was estimated based on wet masses using a wet-to-dry mass conversion factor that was established prior to conducting the experiments (dry mass / wet mass = 0.107, SE ±0.003, $n = 40$, $r^2_{(\text{dry mass} \sim \text{wet mass})} = 0.78$). We determined dry mass from samples that were dried at 60°C for at least 24 h prior to weighing. To account for potential effects of autogenic changes (e.g., microbial decomposition, physical fragmentation) in mass loss during each experiment, we measured rates of mass loss in the absence of detritivores for each sedge treatment (see Growth and performance experiments below for a description of the controls). From these controls, we calculated the rate of autogenic mass loss as the % of initial dry mass lost/d for each sedge treatment where autogenic mass loss = ([initial mass – final mass] / initial mass) / elapsed d. We calculated caddisfly consumption of sedge from each treatment individually by 1st determining total sedge mass loss (autogenic + caddisflies) for each treatment in each of the 25 feeding chambers (initial mass – final mass), then correcting for autogenic (non-caddisfly consumption) mass loss by subtracting estimated autogenic mass loss (for each treatment) from total mass loss. The resulting value was assumed to reflect consumption of sedge from each treatment by caddisflies. We determined total caddisfly consumption (across all 4 treatments) by summing the adjusted mass loss values for each of the 4 sedge treatments in each chamber. We then calculated proportional

Table 2. Summary of mean (±SE, $n = 3$) chlorophyll *a* (Chl *a*) biomass (μg/g dry sedge) and %P (of dry mass) of conditioned sedge detritus from samples collected (3 from each of 4 incubation tubs) at the commencement of feeding-preference and growth experiments (i.e., sedge sources in Table 1) for each of the caddisfly taxa. Corresponding dates for the experiments for each taxon are presented in Table 1. Chl *a* and %P for *Limnephilus externus*, which is the only taxon where feeding-preference and growth experiments were not commenced within 24 h, were from 12 August 2019.

Metric	Experiments	Light A	Light + P	Shade A	Shade + P
Chl *a*	*Anabolia bimaculata*	9.3 ± 1.2	20.8 ± 2.1	3.3 ± 2.3	1.2 ± 0.7
	Asynarchus nigriculus	20.0 ± 3.1	27.7 ± 7.2	10.5 ± 5.1	2.6 ± 2.7
	Ecclisomyia sp.	20.0 ± 1.5	24.6 ± 1.3	0.44 ± 0.15	5.3 ± 3.0
	Limnephilus externus	13.2 ± 3.2	11.2 ± 1.2	3.6 ± 0.8	2.8 ± 1.3
%P	*Anabolia bimaculata*	0.091 ± 0.006	0.092 ± 0.014	0.094 ± 0.009	0.12 ± 0.003
	Asynarchus nigriculus	0.087 ± 0.011	0.084 ± 0.010	0.083 ± 0.007	0.078 ± 0.004
	Ecclisomyia sp.	0.081 ± 0.005	0.103 ± 0.009	0.087 ± 0.008	0.102 ± 0.007
	Limnephilus externus	0.081 ± 0.001	0.114 ± 0.012	0.125 ± 0.003	0.110 ± 0.015

mass loss for each treatment by dividing, individually, consumption of each of the 4 sedge treatments by total consumption.

We assessed feeding preferences for each species, in part, by using Hotelling's 1-sample multivariate T^2 test, which accounts for the lack of statistical independence among multiple food types offered in the feeding chambers to individual consumers (Roa 1992, Lockwood 1998). Following procedures outlined by Lockwood (1998), we based our analyses on proportional consumption rather than total consumption to avoid potential bias caused by random variation in total consumption across chambers. When the null hypothesis of no preference was rejected, we performed pairwise comparisons to determine which food types were consumed preferentially over others. For these comparisons, we built 95% confidence intervals around the differences between proportional consumption estimates for each pair of resources (treatments) following the procedure presented by Lockwood (1998). We interpreted resulting intervals that contained 0 as evidence of a lack of differences among treatments (Lockwood 1998). However, inferences were not based solely on this analysis but rather on a more comprehensive weight of evidence approach. Hotelling's *t*-test was performed using the *Hotelling* package (version 1.0.8; Curran 2021) in R (version 3.10; R Project for Statistical Computing, Vienna, Austria).

Growth and performance experiments

We assessed growth and performance (growth efficiency, mortality, pupation) of each of the 4 focal caddisfly species on the 4 sedge treatments by rearing animals exclusively on each type of sedge for durations of 9 to 15 d, depending on species. We ran experiments for 13 to 15 d except when we observed pupation for individuals of 2 taxa (*A. bimaculata* and *Ecclisomyia* sp.), prompting the early termination of those experiments. The growth experiments were conducted in 36 × 20-cm clear plastic chambers, with 15 g (±0.15) of wet sedge randomly assigned to each growth chamber, such that each chamber contained only sedge from 1 of the 4 treatments. We created 5 replicate chambers for each of the 4 sedge treatments for a total of 20 chambers used for each species and control trial (see below). We filled each chamber with unfiltered pond water to a depth of ~2 cm and added 5 caddisflies to each chamber, a density selected to minimize intraspecific competition (Klemmer et al. 2012). Chambers with sedge from the 2 shaded treatments (Shade A and Shade + P) were covered with shade cloth to inhibit the growth of algae during the experiment.

At the beginning of each experiment, we estimated initial body mass of each focal taxon by sacrificing 20 caddisflies, drying them (60°C for >24 h), and separately weighing them to determine their dry mass. Initial body mass was 5.1 mg (SD = 2.6) for *A. bimaculata*, 6.8 mg (SD = 3.0) for *A. nigriculus*, 4.0 mg (SD = 1.6) for *Ecclisomyia* sp., and 5.1 mg (SD = 2.6) for *L. externus*. We used the mean body mass of those 20 individuals as the initial body mass of each taxon for growth rate calculations.

At the conclusion of each experiment, we collected all remaining caddisflies to determine individual daily instantaneous growth rates (IGRs), mortality, and pupation within all treatments. No deceased individuals were recovered, likely because of consumption by the remaining individuals given that cannibalism has been reported for at least some of these species (Wissinger et al. 1996), though empty larval cases remained. We dried each of the remaining individuals from each treatment at 60°C for >24 h and weighed them to determine final individual body mass. We used these data to calculate a daily IGR for individuals reared on each sedge treatment by using the following equation:

$$IGR = (\ln[W_{t+\Delta t} - W_t])/\Delta t, \qquad \text{(Eq. 1)}$$

where W_t is initial mass of the individual at time t, $W_{t+\Delta t}$ is the final mass of the individual at time $t + \Delta t$, and Δt is the length of the time interval (in d). We excluded pupating individuals from the IGR calculations. We assessed the effects of the different sedge treatments on mortality and pupation by calculating the percentage mortality and the percentage of individuals that had pupated for each treatment.

We estimated growth efficiencies for animals reared on each of the sedge treatments as an additional metric to assess the influence of diet on consumer performance. Growth efficiency describes the percentage of ingested material that is converted to consumer biomass. To calculate growth efficiency, we 1st determined the total biomass produced in each chamber during the growth experiments by summing the change in body mass of each individual (final dry mass – estimated initial dry mass) within each chamber. We then divided the total change in biomass from each chamber by the mass loss of sedge from each chamber after having corrected for autogenic mass loss.

We estimated autogenic mass loss from controls that were set up similarly to the growth experiments (e.g., similar chambers, 5 replicates for each treatment, 15 g [±0.15] of wet sedge/chamber), except that no caddisflies were added to the chambers. We conducted the controls twice during summer 2019 concurrent with the 1st and last growth experiments. We also determined estimates of autogenic mass loss for the feeding-preference experiments from these control experiments. To estimate sedge mass loss attributed to animal consumption in the growth experiments, we used a similar approach to that described above to adjust for autogenic losses.

To test for treatment effects on consumer performance metrics measured from the growth experiments (IGR, growth efficiency, mortality, pupation), we used a 2-factor analysis of variance (ANOVA) design, where light, P, and

the light × P interaction terms were interpreted as tests of the algal, P, and co-limitation hypotheses, respectively. ANOVAs were performed using the mean of each independent replicate (i.e., each chamber). Per the recommendations contained within Wasserstein et al. (2019), we report *p*-values and present summary statistics for the multiple ANOVA models but interpret our results and base our inferences on a more holistic weight of evidence approach that also considers effect sizes and our a-priori knowledge of the study system. We performed ANOVA analyses in R.

RESULTS

Feeding preferences

Each of the 4 focal taxa exhibited some evidence of preferential feeding behavior among the 4 sedge treatments, though the strength of this evidence varied among species (Table 3, Fig. 1). We observed the strongest feeding preferences for *Ecclisomyia* sp. (Fig. 1), which showed greater preference for sedge grown in the 2 ambient light treatments, with each accounting for ~45% of total consumption, on average. Among the 2 shaded treatments, *Ecclisomyia* sp. showed slight preference for the Shade + P sedge over the Shade A sedge (Fig. 1), though consumption of each was small relative to total consumption. *Limnephilus externus* preferentially consumed sedge from the 2 ambient-light treatments, which each accounted for ~28 to 34% of total consumption, on average, whereas the 2 shade treatments each accounted for ~18% of consumption (Fig. 1). Furthermore, there was a difference in proportional consumption of 6% among the 2 ambient-light treatments, indicating that *L. externus* preferred the Light + P (34% of total consumption) to the Light A treatment (28% of consumption) (Fig. 1). For *A. nigriculus*, the mean proportion of sedge consumed from the 2 +P treatments was similar (~30–33% total consumption) and greater than that of either of the ambient-P treatments (~18% of total consumption, each) (Fig. 1). Consumption did not differ on the basis of light treatment for *A. nigriculus* (Fig. 1). Finally, for *A. bimaculata*, sedge from the +P treatments accounted for ~28 to 30% of total consumption, whereas sedge from each of the ambient-P treatments accounted for ~21% of total consumption, a difference of 7 to 9% between the 2 +P treatments and the ambient-P treatments (Fig. 1).

Table 3. Summary of Hotelling's T^2-test results for the feeding preference experiments, where n = 25 feeding chambers (1 caddisfly and all diet treatments present in each chamber). Tests were based on proportional consumption data, which included consumption of all 4 diet treatments in multiple food-choice experiments. Preference was assessed by comparing the proportion of total material consumed for each food type. df = degrees of freedom.

Taxon	T^2	df	F	p-value
Anabolia bimaculata	7.94	3,22	2.42	0.093
Asynarchus nigriculus	14.98	3,21	4.56	0.013
Ecclisomyia sp.	564.65	3,22	172.53	<0.001
Limnephilus externus	73.90	3,21	22.49	<0.001

Growth, growth efficiency, mortality, pupation

Growth responses to the various treatments varied considerably among the 4 taxa, with some species exhibiting negative growth rates on at least some treatments and other species exhibiting positive growth when reared on all treatments. For example, larvae of *A. bimaculata* lost mass, on average, on all sedge treatments (Table 4, Fig. 2). There was a small but variable effect of light treatment on the growth of *A. bimaculata*: individual mass loss was on average 2.4× greater (range: 1.3–3.7×) in the 2 shaded treatments than in the 2 ambient-light treatments. There was no evidence for P-treatment effects on the growth of *A. bimaculata* (Table 4). For larvae of *A. nigriculus*, the only individuals that added mass were those reared on the Light + P and Shade + P treatments (Fig. 2), indicating strong P effects on growth in this species (Table 4). However, growth did not differ substantially between the Light + P and Shade + P treatments or between the Light A and Shade A treatments, indicating a lack of effects of light treatment and the light × P-treatment interaction for *A. nigriculus*. Individuals of *Ecclisomyia* sp. and *L. externus* gained mass, on average, on each diet treatment (Fig. 2) and both appeared to grow slightly faster on Light + P-conditioned sedge than on the other sedge treatments, among which growth rates were similar for each taxon. However, the small differences in growth between the Light + P and other treatments (~1.4× in *Ecclisomyia* sp. and ~1.3–1.4× in *L. externus*) for these 2 taxa were not supported by all lines of evidence (Table 4).

We also measured growth efficiency for individuals reared on each diet treatment as an additional indicator of consumer performance. We measured negative growth efficiencies for larvae of both *A. bimaculata* and *A. nigriculus* on at least some diets (Table 5). Growth efficiency for *A. bimaculata* was between 1.3 and 7.9× lower, on average, on sedge from the 2 shaded treatments than on sedge from the 2 ambient-light treatments (Table 5). The least-negative growth efficiencies measured for *A. bimaculata* occurred on Light + P; growth efficiency was anywhere from 3.7 to 7.9× lower on the other 3 treatments, indicating a potential light × P-treatment interaction. However, these differences were not supported by all lines of evidence (Table 6). Larvae of *A. nigriculus* grew most efficiently on sedge from the 2 +P treatments (Table 5), whereas growth efficiency on the 2 ambient-P diets was frequently negative. There were no apparent effects of light or the light × P interaction on growth efficiency in *A. nigriculus* (Table 6). For larvae of *L. externus*, there was a small effect of light treatment on growth efficiency (Tables 5, 6), though this effect appears to be driven primarily by higher growth efficiency on the

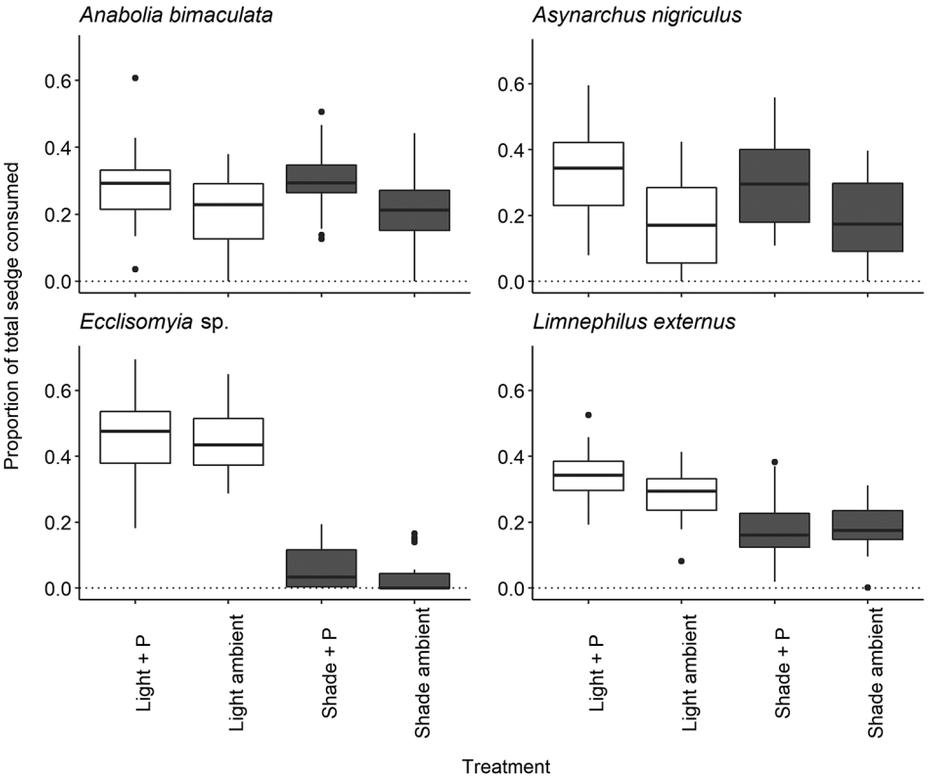

Figure 1. Boxplots showing proportional consumption of each of the 4 diet treatments during the multiple food-choice feeding-preference experiments for the 4 focal caddisflies (n = 25 ind./species). Proportional consumption of each food type is based on the total amount of sedge consumed from all treatments throughout the duration of the experiment. In the boxplots, the thick horizontal bar indicates the median, the upper and lower bounds of each box indicate the interquartile (25–75%) range, the horizontal whiskers indicate the minimum and maximum values within 1.5× the interquartile range, and dots indicate values that are >1.5× the interquartile range.

Light + P treatment, which was between 1.8 and 2.0× greater than on the other treatments, thus indicating potential interactive effects of light and P treatments. Larvae of *Ecclisomyia* sp. exhibited the highest growth efficiency on the Shade A treatment (4.1%), which was between 1.5 and 2.0× greater than that for any other treatment (range: 2.1–2.7%). However, these differences were not supported by all lines of evidence (Tables 5, 6).

Rates of mortality averaged between 0 and 20% across all taxa and sedge treatments (Table 5). For both *A. bimaculata* and *Ecclisomyia* sp., mortality did not vary systematically among treatments, indicating minimal treatment effects in these taxa (Tables 5, 6). For *A. nigriculus*, mortality was greatest on the Shade + P treatment and 0 in the Shade A and Light + P treatments (Table 5). Mortality rates for *L. externus* were greatest on the 2 shade treatments compared with the ambient-light treatments (differences ranging from 8–20% mortality) and did not appear to be substantially affected by P treatment (Tables 5, 6).

We observed pupation during the growth experiments in only 2 of the 4 focal taxa: *A. bimaculata* and *Ecclisomyia* sp. Rates of pupation in *A. bimaculata* were nearly 2 to 3× greater for larvae reared on the 2 +P treatments than on the ambient-P treatments (Table 5). Rates of pupation in *A. bimacaulata* did not appear to respond to light treatment (Table 5). For *Ecclisomyia* sp., rates of pupation did not vary in a way that was indicative of strong treatment effects or treatment-interaction effects (Tables 5, 6).

DISCUSSION

We set out to characterize the feeding preferences and performance responses of 4 species of primarily detritivorous caddisflies in relation to 2 potential mechanisms of nutritional limitation, detrital-P concentration, and epiphytic algal biomass. We observed varying degrees of evidence for preferential feeding behavior for all 4 species of caddisfly. Specifically, *A. bimaculata* and *A. nigriculus* each showed patterns consistent with feeding preference for detritus from the +P treatments, and both *L. externus* and *Ecclisomyia* sp. demonstrated patterns consistent with feeding preferences for detritus with high algal biomass. However, we observed agreement between feeding preferences and performance for only *A. nigriculus* (growth and growth efficiency) and

Table 4. Summary of analysis of variance output analyzing treatment effects on instantaneous growth rates from growth experiments for the 4 focal larval caddisfly taxa reared on each of the 4 sedge treatments, $n = 5$ chambers/treatment, each with 5 caddisflies. df = degrees of freedom.

Taxon	Factor	df	Sum of squares	F	p-value
Anabolia bimaculata	Light	1,16	0.00062	5.50	0.032
	P	1,16	0.00001	0.11	0.743
	Light × P	1,16	0.00024	2.04	0.157
Aynarchus nigriculus	Light	1,15	0.00020	0.94	0.347
	P	1,15	0.00134	6.17	0.025
	Light × P	1,15	0.00003	0.16	0.699
Ecclisomyia sp.	Light	1,15	0.00237	1.70	0.212
	P	1,15	0.00237	1.71	0.211
	Light × P	1,15	0.00301	2.17	0.162
Limnephilus externus	Light	1,16	0.00013	0.82	0.380
	P	1,16	0.00008	0.55	0.469
	Light × P	1,16	0.00033	2.13	0.164

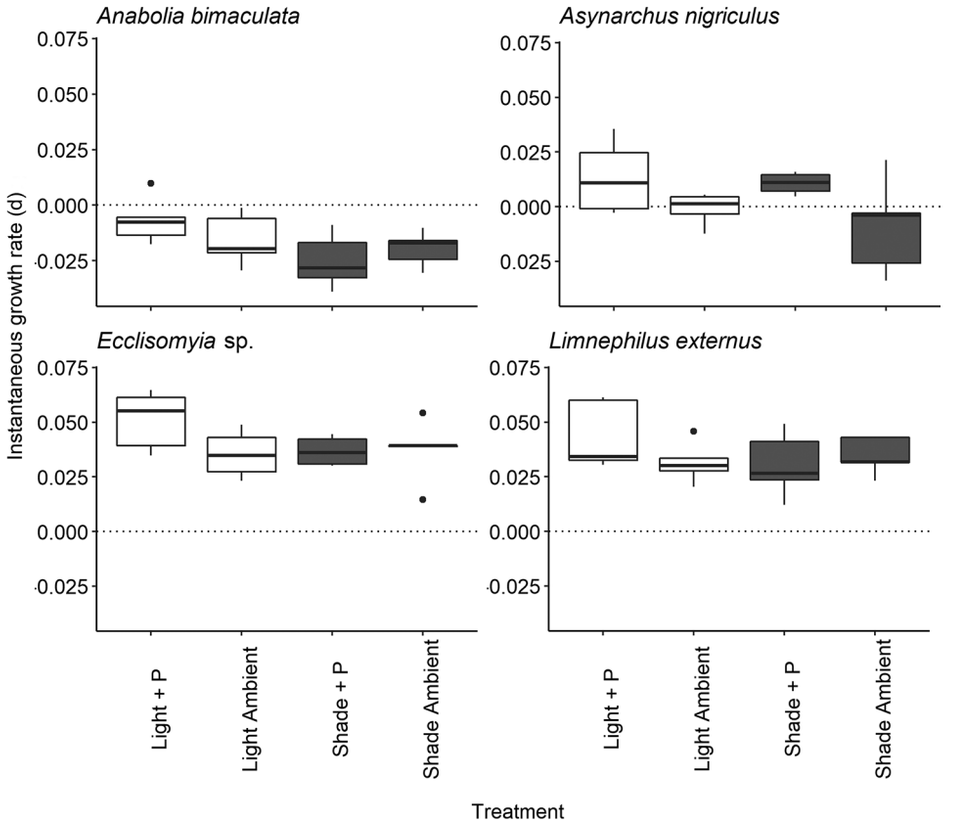

Figure 2. Boxplots summarizing daily instantaneous growth rates of the 4 focal caddisfly taxa when reared on each of the 4 sedge treatments ($n = 5$ replicates/treatment; each replicate chamber contained 5 individuals). Pupating individuals were excluded from instantaneous growth rate calculations. In the boxplots, the thick horizontal bar indicates the median, the upper lower and bounds of each box indicate the interquartile (25–75%) range, the horizontal whiskers indicate the minimum and maximum values within 1.5× the interquartile range, and dots indicate values that are >1.5× the interquartile range.

Table 5. Mean (±SE, $n = 5$ chambers/treatment, each with 5 caddisflies) growth efficiency (GE, % of ingested food converted to animal biomass), mortality (%), and frequency of pupating individuals (%) during the growth experiments for each of the 4 focal species.

Taxon	Treatment	GE (%)	Mortality (%)	Pupated (%)
Anabolia bimaculata	Light A	−1.6 ± 0.80	8 ± 11.0	24 ± 32.9
	Light + P	−0.43 ± 0.78	0 ± 0	40 ± 40.0
	Shade A	−2.1 ± 0.48	16 ± 16.7	20 ± 14.1
	Shade + P	−3.4 ± 1.1	12 ± 17.9	60 ± 31.6
Asynarchus nigriculus	Light A	0.09 ± 0.18	4 ± 8.9	0 ± 0
	Light + P	0.82 ± 0.41	0 ± 0	0 ± 0
	Shade A	−0.53 ± 0.76	0 ± 0	0 ± 0
	Shade + P	1.0 ± 0.26	20 ± 16.3	0 ± 0
Ecclisomyia sp.	Light A	2.1 ± 0.61	12 ± 11.0	16 ± 16.7
	Light + P	2.7 ± 0.28	8 ± 11.0	28 ± 17.9
	Shade A	4.1 ± 1.2	8 ± 11.0	32 ± 22.8
	Shade + P	2.2 ± 0.54	8 ± 11.0	16 ± 8.9
Limnephilus externus	Light A	2.0 ± 0.27	0 ± 0	0 ± 0
	Light + P	3.1 ± 0.49	4 ± 8.9	0 ± 0
	Shade A	1.8 ± 0.20	20 ± 14.1	0 ± 0
	Shade + P	1.9 ± 0.41	12 ± 11.0	0 ± 0

L. externus (mortality), indicating that feeding preferences may not always translate to improved performance for consumers, at least over relatively short (e.g., 1–2 wk) time scales.

Differences in feeding preferences among taxa

Given that terrestrial plant detritus is characteristically low in P relative to consumer demands (Cross et al. 2003, Martinson et al. 2008) and that algae may be considered a higher quality resource (higher relative N and P concentrations, lacking structural carbohydrates, replete with PUFAs; i.e., Hixson et al. 2015), we expected that feeding preferences for the 4 focal taxa would be greatest for sedge conditioned under added P and ambient light (Light + P). However, only *L. externus* preferred sedge from the Light + P treatment over other treatments, though they also showed a strong secondary preference for the Light A sedge. The stronger preference for the Light + P treatment over the Light A treatment may be driven by the higher algal biomass on the former, potentially resulting from reduced autotroph P limitation rather than by differences in P concentration between the 2 ambient-light treatments. Further, *L. externus* showed no apparent preference for sedge from the Shade + P treatment, providing additional support for the role of algal biomass in driving the observed feeding preferences of this species.

Stoichiometric imbalances between consumers and their food may influence consumer food choices (Demi et al. 2021). For example, based on principles of ecological stoichiometry (Sterner and Elser 2002), one might predict a stronger preference for sedge from the 2 +P treatments for *L. externus* compared with *A. nigriculus*, given the higher body P concentration (and presumably greater P demand) of *L. externus* in ponds near RMBL (Balik et al. 2018). However, *A. nigriculus* equally preferred sedge from the +P treatments to sedge from either of the ambient-P treatments, whereas *L. externus* preferred sedge from the 2 ambient-light treatments to sedge from either shaded treatment, including shade + P. Thus, stoichiometric traits, such as body P concentration, do not appear to correspond to the interspecific variation in feeding preference we observed in this study (Balik et al. 2018), indicating stoichiometric imbalances may not be the primary driver of food choice.

Preferences for detritus from the +P treatments reported here for *A. nigriculus* and *A. bimaculata* may not be driven by nutrient concentration per se, but rather by differences in microbial conditioning resulting from P limitation of microbial decomposers. In this study, our P amendments appeared to have relatively little effect on the P concentration of sedge detritus used during some experiments (Table 1). The differences we report in P concentration among treatments during some experiments were likely a function of increased microbial activity resulting from reduced P limitation of microbial production, or by uptake and storage of excess P by microbial decomposers (Gulis et al. 2017), rather than from differences in the initial chemical composition of the sedge itself. Even though P amendments did not consistently result in higher detrital P in the +P treatments, added P may have modified other characteristics of detritus related to microbial conditioning, such as toughness, that have been

Table 6. Summary of analysis of variance (ANOVA) results testing for treatment effects on various performance (growth efficiency [GE], mortality, and pupation) metrics from the growth experiments conducted for each taxon (n = 5 chambers/treatment, each with 5 caddisflies). df = degrees of freedom.

		ANOVA summary				
Metric	Taxon	Factor	df	Sum of squares	F	p-value
GE (%)						
	Anabolia bimaculata	Light	1,16	0.00157	4.48	0.050
		P	1,16	0.00000	0.00	0.951
		Light × P	1,16	0.00073	2.08	0.169
	Asynarchus nigriculus	Light	1,15	0.00004	0.38	0.547
		P	1,15	0.00058	5.36	0.035
		Light × P	1,15	0.00008	0.73	0.405
	Ecclisomyia sp.	Light	1,16	0.00027	1.00	0.332
		P	1,16	0.00018	0.67	0.425
		Light × P	1,16	0.00083	3.06	0.099
	Limnephilus externus	Light	1,14	0.00029	4.41	0.054
		P	1,14	0.00010	1.51	0.239
		Light × P	1,14	0.00011	1.60	0.226
Mortality (%)						
	Anabolia bimaculata	Light	1,16	500	2.78	0.115
		P	1,16	180	1.00	0.332
		Light × P	1,16	20	0.11	0.743
	Asynarchus nigriculus	Light	1,15	225	3.01	0.103
		P	1,15	628	8.41	0.011
		Light × P	1,15	301	4.03	0.063
	Ecclisomyia sp.	Light	1,16	20	0.17	0.689
		P	1,16	20	0.17	0.689
		Light × P	1,16	20	0.17	0.689
	Limnephilus externus	Light	1,14	810	7.09	0.019
		P	1,14	36	0.31	0.585
		Light × P	1,14	154	1.35	0.265
Pupation (%)						
	Anabolia bimaculata	Light	1,16	320	0.07	0.574
		P	1,16	3920	0.07	0.062
		Light × P	1,16	720	3.27	0.402
	Ecclisomyia sp.	Light	1,16	20	0.33	0.800
		P	1,16	20	4.04	0.800
		Light × P	1,16	980	0.74	0.090

shown to influence detritivore feeding preferences (Nolen and Pearson 1993, Graça 2001). Moreover, we cannot rule out the possibility of P enrichment effects on sedge N concentration, which we did not measure during the experiments. Increases in microbial biomass resulting from reduced P limitation may increase detrital N as well as P concentration. We expected caddisflies to experience greater P than N limitation based on previous studies of stoichiometric imbalances among detritus and detritivores (Cross et al. 2003), but we acknowledge that variation in sedge N concentration among the 4 treatments may be at least partially responsible for the observed patterns of feeding preference and performance among the 4 caddisfly species (Frainer et al. 2016, Halvorson et al. 2017, Fenoy et al. 2020). The role of N may be especially true for *A. nigriculus*, which preferentially consumed and grew faster on sedge from the +P treatments, despite little difference in measured P content among the 4 treatments at the outset of those experiments (Table 1). P enrichment also may have modified microbial assemblage composition, which can affect detritivore feeding preferences because

some leaf-shredding aquatic insects feed selectively on leaves colonized by different fungal species (Arsuffi and Suberkropp 1986).

Our results suggest that periphytic algal biomass, in addition to detrital nutrient concentration or species identity, can influence how detritivores choose among detrital food resources. Preferences for conditioned detritus with high algal biomass may be driven by the higher quality of algae, which typically have higher N and P concentrations, are replete with essential biochemicals (such as PUFAs; Hixson et al. 2015), and are lacking in recalcitrant carbohydrates (lignin and cellulose) relative to vascular plant detritus. Thus, the preferences by *L. externus* and *Ecclisomyia* sp. for sedge with high algal biomass may be driven by multiple aspects of resource quality associated with algae. Preference by *Ecclisomyia* sp. for conditioned detritus from the ambient-light treatments is not surprising given that both diatoms and leaf detritus have been described as a major food source for species in this genus (Irons 1988, Wiggins 1996). Conversely, previous studies of gut contents of *L. externus* indicate that algae typically represent a very small fraction of their diet (Wissinger et al. 1996).

Differences in the feeding preferences observed among the focal taxa may also result from intraspecific shifts in selectivity related to ontogeny (Cargill et al. 1985, Chung and Suberkropp 2009). For example, Cargill et al. (1985) observed that 5^{th}-instar larvae of several caddisfly species preferentially consumed leaf litter treated with lipid solutions containing unsaturated long-chain (18–20 C) fatty acids over untreated litter of the same species (red alder), whereas 3^{rd}-instar larvae did not. The author attributed this pattern to the need for final-instar larvae to accumulate lipid reserves that are necessary for metamorphosis and reproduction, a pattern that is consistent among holometabolous insects (Nestel et al. 2016). The 4 species used in the present study differed in their developmental stage from ~3^{rd} to 5^{th} instar at the time of the experiments, though individuals within a species were all of a similar developmental stage. As such, we cannot rule out the possibility that individuals from the 4 focal species of this study may modify their feeding preferences throughout their ontogeny.

This study is the first to simultaneously test feeding preferences of detritivores based on detrital P content and detritus-associated algae using multiple-choice feeding experiments with a single source of detritus conditioned under different experimental conditions, and thus better characterizes detritivore food choice relative to other experimental approaches. Numerous studies of feeding behavior in aquatic detritivores have reported patterns consistent with preferential consumption of detrital resources with elevated N or P concentration (Hood et al. 2014, Cornut et al. 2015, Frainer et al. 2016, Ohta et al. 2016, Halvorson et al. 2018) as well as preferential consumption of algae over nutritionally poor detritus (Friberg and Jacobsen 1994, Leberfinger and Bohman 2010). However, feeding preferences for high N or P detritus were typically inferred from differences in consumption rates from single resource treatments, rather than from experiments where consumers were simultaneously offered multiple resources of varying elemental composition. Moreover, differences in resource elemental composition were frequently achieved by altering detrital species identity (e.g., Ohta et al. 2016, though see Halvorson et al. 2018), which may introduce additional variation in nutritional quality (e.g., % lignin/cellulose) that affects consumer feeding behavior (Graça 2001, though see Vonk et al. 2016 and Hunting et al. 2016). Additionally, inferring feeding preferences from consumption rates in single resource treatments may be confounded by patterns of compensatory feeding on low-quality resources because individuals may increase consumption of low-quality foods to offset lower growth efficiencies (Fenoy et al. 2020). Most studies that have reported preferential consumption of algae by aquatic detritivores present consumers directly with algal food resources that are not associated with detrital substrates (Friberg and Jacobsen 1994, Leberfinger and Bohman 2010), even though periphytic algae growing on decomposing leaves may be the primary dietary source of algae for some leaf-shredding aquatic invertebrates.

Feeding preferences and consumer performance

Previous studies of feeding preference by aquatic detritivores report agreement between feeding preferences and multiple performance metrics, including growth (Bärlocher and Kendrick 1973, Arsuffi and Suberkropp 1986, Graç;a et al. 1993). We show caddisfly performance did not always vary between diets, and the various performance metrics measured mostly did not consistently vary in accordance with feeding preferences of each species. However, for *A. nigriculus*, both growth and growth efficiency were greatest on the preferred +P diets, and *L. externus* larvae experienced lower mortality when reared solely on their preferred high-algae diets. One potential explanation for the lack of diet effects on some performance metrics, and absence of consistent agreement between feeding preference and the various performance metrics among taxa, could be the duration of the growth experiments. The growth experiments ranged in length from 9 to 15 d and may not have been long enough for subtle effects of the dietary treatments to manifest. However, other authors report measurable differences in growth rates of aquatic insects from experimental food manipulations on shorter time scales (e.g., 6 d; Frost and Elser 2002), though other authors report differences from longer duration feeding experiments (e.g., 14 to >30 d; Fuller et al. 2015, Halvorson et al. 2018).

The onset of pupation may explain the negative growth of *A. bimaculata* individuals on all diets. We excluded pupae from the IGR calculations, but larval individuals may

have reduced feeding in advance of pupation, resulting in loss of body mass (Wallace et al. 1992). Nevertheless, *Ecclisomyia* sp. did not experience mass loss during the growth experiment, despite the onset of pupation for some individuals. Moreover, the negative growth rates observed for some larvae of *A. nigriculus* reared on ambient-P diet treatments suggest that other factors (nutritional or behavioral) may be the primary drivers of negative growth for some individuals. Indeed, negative growth rates from controlled feeding and growth experiments have been reported by multiple authors for other caddisfly species (Kendrick and Benstead 2013, Halvorson et al. 2017).

Increased growth rates and growth efficiency for larvae of *A. nigriculus* reared on +P diets are consistent with other studies that have reported P limitation of growth in various aquatic invertebrate taxa (Frost and Elser 2002, Kendrick and Benstead 2013, Halvorson et al. 2017). Additionally, increased dietary P supply and, more generally, reduced nutritional constraints have been linked to reduced development time and earlier pupation in some insect taxa, including other caddisfly species (Anderson and Cummins 1979, Perkins et al. 2004). However, our expectation of P limitation for each of the 4 focal taxa, based on the characteristically large imbalances between detritivore and detritus P concentration (Cross et al. 2003), was not supported. Stoichiometric analysis of leaf-shredding aquatic invertebrates and leaf detritus generally suggests consumer P limitation (Evans-White et al. 2005, Demi et al. 2018), yet other aspects of litter chemistry, including N concentration and biochemical composition (Raubenheimer and Simpson 2004), may impose greater constraints on consumer growth for these taxa. For example, Frainer et al. (2016) reported N limitation of growth of 3 aquatic detritivores despite stoichiometric traits that were suggestive of potential P limitation. The authors implicate N demand for chitin production during molting, as well as for silk production by larval caddisflies, as mechanisms that may explain the stronger than expected response of some aquatic detritivores to detrital N compared with P concentration.

Occasional consumption of algae can increase the growth of aquatic detritivores (Franken et al. 2005), and dietary supply of algal PUFAs has been suggested as a mechanism for growth limitation in a variety of consumers (Guo et al. 2016b, Crenier et al. 2017). Nevertheless, we did not observe increases in larval growth on high algae diets for any of the 4 taxa. Aquatic detritivores may avoid nutritional constraints posed by limited supply of high-quality algae via preferential assimilation and retention of essential algal PUFAs (Kühmayer et al. 2020). As such, consumers may be less susceptible to short-term supply deficiencies if they are able to preferentially retain essential PUFAs and turnover rates of these compounds are sufficiently slow. However, our understanding of how temporal variation in PUFA supply to aquatic consumers affects development throughout ontogeny is currently lacking.

Broader implications

There is increasing recognition that consumers can be simultaneously limited by multiple aspects of resource nutritional quality (Raubenheimer and Simpson 2004, Kaspari and Powers 2016). Detritivores are likely to be particularly susceptible to multiple dietary deficiencies given the low nutrient and biochemical quality of vascular plant detritus, though they may avoid such constraints by selectively feeding on high quality resources, when available. This study represents the 1st attempt to simultaneously characterize the feeding preferences and performance responses of aquatic detritivores to 2 purportedly common nutritional constraints: dietary P and periphytic algal supply. We demonstrate that several species of closely related caddisflies with similar feeding ecology vary in their feeding preferences with respect to these 2 potentially limiting food characteristics. Differences in selective feeding behavior may affect the functional role of closely related species in ecosystems by modifying the flow pathways of energy and nutrients in food webs. Characterizing such variation will become increasingly important as we seek to understand how animal communities respond to multiple drivers of environmental change, such as cultural eutrophication and changes in land use that modify riparian vegetation and light availability, that are acting simultaneously to influence the composition and quality of basal food resources in freshwater ecosystems.

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