

**Quality of the nuisance diatom *Didymosphenia geminata* for macroinvertebrate nutrition in
a southeastern U.S. hypolimnetic tailwater river**

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Abstract

Didymosphenia geminata is a mat-like, colony forming diatom native to areas of western North America and found in high elevation oligotrophic rivers across the Northern and Southern Hemispheres. The alga has drawn concern in recent decades due to its negative impacts on the community makeup of benthic macroinvertebrates in streams where it is considered a nuisance or invasive species. Diatoms can serve as a major food source for aquatic macroinvertebrates, however colonies of *D. geminata* are often much larger and more abundant than populations of other diatoms. The C:N ratios of aquatic plant tissue can serve as an indicator of both the availability of nutrients to the plant and the capacity for transfer of available nutrients to primary consumers. In September 2016 we conducted a study in the South Fork Holston River tailwater in eastern TN, USA to determine the nutritional quality of *D. geminata* for benthic macroinvertebrates as it compares to native tailwater flora. Samples of nine aquatic macrophyte and submergent plant taxa including *D. geminata* were collected from three tailwater sites downstream of the South Holston Reservoir to better understand the impacts of cool, hypolimnetic releases on plant tissue quality. C:N ratios of all samples were measured using a Flash Elemental Analyzer (Thermo Fisher Scientific). Tissue quality of *D. geminata* did not differ significantly from any other study taxa. C:N ratios of two angiosperm and one bryophyte species decreased significantly with increasing distance from the dam, and the C:N ratios of two of these species were positively correlated with mean annual mean temperatures. The C:N ratio of a filamentous algae increased significantly moving away from the dam and was negatively correlated with annual mean temperature. Our results indicate that *D. geminata* may serve as a food source of comparable quality to native floral species for tolerant benthic macroinvertebrate taxa (e.g. Chironomidae), and our data suggest that the impacts of hypolimnetic releases on tissue quality of aquatic plant taxa are mitigated within a relatively short distance from the dam.

Introduction

The success of commercially valuable ecosystems depends on the maintenance of a network of trophic levels functioning and interacting reliably in the face of human exploitation. The flora at the base of any food web is of particular importance, allowing microscopic single-celled algae to exert influence on even the highest trophic levels in a community. Several studies cite nuisance blooms of the colony-forming diatom *Didymosphenia geminata* as a major cause of declines in the value of recreational fisheries (Bergey *et al.* 2009, Habera *et al.* 2009, Kumar *et al.* 2008). One such study by Beville *et al.* (2012) cited both a predictable behavioral response of fishermen and fishing water closures designed to prevent *D. geminata* spread as drivers of this decline. Yet, beyond creating an eyesore or hazard for recreational anglers, *D. geminata* exhibits a range of negative effects on the ecology of impacted river systems. One of the best-studied impacts of *D. geminata* is alteration of benthic macroinvertebrate community makeup. Studies have demonstrated clear reductions in species richness and diversity with a trend toward dominance by more tolerant invertebrate taxa in *D. geminata*-impacted waters (Saltveit *et al.* 1994).

While the primary consumers of these lotic systems are not eliminated entirely, secondary consumers and constituents of higher trophic levels that rely on sensitive taxa (e.g. Ephemeroptera, Plecoptera, Trichoptera – EPTs) may be excluded from areas impacted by *D. geminata*, whereas chironomid predators will benefit from a growing food resource (Gillis and Chalifour 2010). Current data is insufficient to predict the impacts of *D. geminata* on trophic levels beyond primary consumers, so it is important to further investigate the impact of *D. geminata* on macroinvertebrates in an effort to unravel its impacts on commercially valuable and ecologically vital species.

The C:N:P stoichiometric ratios of aquatic vascular plants and bryophytes as well as inter-taxon differences remain fairly constant across a gradient of nutrient supplies (Demars and Edwards 2007). Macrophytes, particularly macroalgae, are often depleted in N as compared to phytoplankton. Duarte (1992) explained this as a reflection of a greater degree of N-limitation to growth in macrophytes, however *D. geminata* is an unusual nuisance alga in its preference for oligotrophic conditions (Kirkwood *et al.* 2009). Additionally, low levels of dissolved inorganic phosphorus are often associated with *D. geminata* blooms. It is then reasonable to suggest that *D. geminata* C:N ratios may be more independent of nutrient supply than the macroalgal species considered in the existing literature. Furthermore, research by Bergey *et al.* (2009) demonstrates the importance of diatoms as a food source in both marine and freshwater food webs, citing a high nutritional value due to the storage of excess energy as nutrient-rich oils.

The presence of a dam, among other environmental variables, is an important predictor of *D. geminata* abundance (Kirkwood *et al.* 2009). Thus, the South Fork Holston River just below the TVA operated hydropower dam an ideal site for investigating the role of *D. geminata* in community ecology. Hypolimnetic-release dams are associated with cooler temperature regimes and lower levels of productivity than their epilimnetic-release counterparts because water is released from the colder, anoxic bottom layer of the reservoir. These artificially cooled, homogenized temperature regimes are in turn associated with proliferation of *D. geminata*. There has yet to be a study investigating the impact of the thermal gradient downstream of hypolimnetic-release dams on plant nutritional quality.

In this study we sought to further understand the relationship between *D. geminata* and benthic macroinvertebrate community structure in tailwater systems by investigating two questions: First, how does the nutritional quality of *D. geminata* for benthic macroinvertebrates

compare to that of native flora in the same system, and second how does the hypolimnetic-release dam impact the nutritional quality of river plants downstream?

Methods

Focal species

Didymosphenia geminata is a benthic, stalked diatom that creates colonies in the form of dense, tangled mats in cool, clear, oligotrophic river systems. These mats act as fine, mesh-like nets that collect detritus and loose sediment. Personal, extensive observation of *D. geminata* revealed its ability to entangle a diverse array of insects including EPTs and oligochaetes, though chironomids are found in an abundance that suggests they use the diatom as a food or habitat resource (Green *et al.* unpublished). *Didymosphenia. geminata* has yet to be reported above the South Holston Reservoir, but has been identified in tailwater rivers throughout the southeastern USA in areas where cool, hypolimnetic releases artificially cool thermal regimes.

Study area

The South Fork Holston River in eastern Tennessee is a fifth order tributary of the Tennessee and Holston Rivers. The Tennessee Valley Authority (TVA) manages the roughly 20 km tailwater of the South Holston Reservoir for recreation and hydroelectric power generation. The average base flow of $2.5 \text{ m}^3\text{s}^{-1}$ is increased to as high as $70.8 \text{ m}^3\text{s}^{-1}$ during hydropeaking via scheduled hypolimnetic (cold water) releases from the South Holston Dam. During the summer months, the TVA also uses hour long “pulses” to cool the warming waters and create conditions more suitable for recreationally fished trout (Habera *et al.* 2009). Operation of the dam creates the cool, relatively heterogeneous, low turbidity flow in which *D. geminata* thrives (Kirkwood *et al.* 2009).

In addition to *D. geminata*, the South Fork Holston River tailwater supports a diverse assemblage of native submergent plants and aquatic macrophytes including mosses belonging to

family Bryaceae, *Fontinalis antipyretica* (common river moss), *Chara* spp. (musk grasses), *Elodea canadensis* (water weed), *Potamogeton crispus* (curly leaf pondweed), and *Nasturium officinale* (watercress).

Harvesting and biochemical analyses

On September 22, 2016, we collected samples from three different tailwater sites downstream of the South Holston Reservoir [upper (A), middle (B) and lower (C)], with sites arranged in increasing in distance from the dam (Fig. 1, Table 1). For a comprehensive comparison, we harvested samples of four angiosperm (*P. crispus*, *Chara* spp., *E. canadensis*, and an unidentified taxa dubbed ‘river lettuce’), three bryophyte (*F. antipyretica* and two species of family Bryaceae, dubbed ‘flat’ and ‘round’ based on blade morphology), and two algal (*D. geminata* and *Rhizoclonium* spp.) species. We collected three replicates of each of nine study species present at each site and wherever possible we took our samples from locations spanning the width of the river. We placed harvested plants into individual sterile 7 oz. Whirl-Pak sample bags and transferred them to an ice-filled cooler for transport.

Once at the processing site (ASU) we transferred the samples to a freezer for preservation until processing. Samples were dehydrated for 72 hours inside opened Whirl-Paks placed inside a Console Freeze Dry System (Labconco). Freeze-dried samples of *P. crispus*, ‘river lettuce’, *E. canadensis*, and *Chara* spp. were pulverized to a fine power using individual 50 mL plastic grinding vials containing ten stainless steel beads, which were shaken by hand in three minute intervals until satisfactorily ground. The algae *D. geminata* and *Rhizoclonium* spp. were pulverized in an electric spice grinder run at three-minute intervals until samples were satisfactorily ground. The mosses, *F. antipyretica* and the two mosses of the family Bryaceae, were processed first by hand with a glass mortar and pestle, then with an electric Wiley mill until

satisfactorily ground. Carbon and nitrogen content of preweighed dry plant material were determined using a FLASH Elemental Analyzer (Thermo Fisher Scientific).

Statistical analysis

To determine differences between C:N ratios of *D. geminata* and the other focal taxa, we used SPSS software to performed a series of one-way ANOVAs with C:N ratio as the dependent variable and taxa as the fixed factor. We performed one-way ANOVAs to assess differences in C:N ratio of a particular taxa between sites. These tests were followed by Tukey HSD pairwise comparisons to determine where significant differences occurred. To assess the relationship between temperature (and thus thermal regime created by the dam) and C:N ratio of a given taxa, we used one year's worth of daily temperature data borrowed from unpublished research by Green *et al.*, which was gathered using temperature data loggers mounted to submerged dolomite boulders at each study site in the tailwater reach. A Shapiro-Wilk test was conducted to assess normality and detected significant right skewed data, indicating more data points in a cooler temperature range. We used nonparametric Spearman's Correlations to determine the relationship between annual mean temperature and average C:N ratio.

V. Results

Inter-taxon variation in C:N ratio

One-way ANOVAs found that the C:N ratio of *Didymosphenia geminata* was not significantly different than the C:N ratio of bryophyte or angiosperm taxa (Fig. 2).

Correlations among C:N ratio and site (distance from dam)

One-way ANOVAs revealed significant negative correlations between C:N ratio and site for *Chara* spp, 'river lettuce', and 'flat' bryaceae (Fig. 3a-c; $p=0.024$, $p=0.017$, $p=0.009$, respectively). The C:N ratio of *P. crispus* demonstrated a similar negative correlation, although not to a significant level ($p=0.054$). We also found a significant positive correlation between

C:N ratio and site for *Rhizoclonium* spp. (Fig. 4; $p=0.021$), although this species was only found at sites A and C. For each of the taxa with significantly variable C:N ratios, Tukey HSD pairwise comparisons found that the significant difference was driven by variation between sites A and C ($p=0.024$, $p=0.031$, $p=0.07$, $p=0.021$, respectively). Only the C:N ratio of ‘river lettuce’ was significantly different between sites B and C ($p=0.023$). *D. geminata* showed no significant variation in C:N ratio between sites ($p=0.078$).

Correlations among C:N ratio and annual mean temperature

Nonparametric Spearman’s Correlations revealed a significant correlation between C:N ratio and mean annual temperature for most study taxa that exhibited significant variation in C:N ratio between sites. We found significant positive correlations between plant quality (lower C:N ratio demonstrates higher tissue quality) and mean annual temperature for *Chara* spp. and ‘flat’ bryaceae (Table 2; $r_s=-0.843$, $p=0.004$; $r_s=-0.869$, $p=0.001$, respectively). ‘River lettuce’ exhibited a similar trend, but the correlation was not significant ($r_s=-0.632$, $p=0.068$). We found a significant negative correlation between tissue quality (lower C:N ratio indicates higher quality tissue) and annual mean temperature for *Rhizoclonium* spp. ($r_s=0.878$, $p=0.021$). *D. geminata* showed a similar trend of increasing C:N (decreasing quality) with temperature, but the correlation was not significant ($r_s=0.791$, $p=0.624$). This result was influenced by an analytical error that occurred in the Flash Elemental Analyzer that caused the C:N ratio of two site A samples of *D. geminata* to be recorded as nearly 1:1; an impossible elemental composition. Without this error it is reasonable to assume that *D. geminata* would not show a significant decrease in quality along an increasing temperature gradient.

VI. Discussion

Variation in cellular nutrient concentration within species could be influenced by age, developmental stage, and plant organ sampled, analytical error, and the chemistry of the

surrounding environment. In this study, we took care to reduce the impact of such factors. Only healthy, mature foliar plant tissues collected during one season from locales spanning the range of environmental conditions present at each site were used for elemental analysis.

Research demonstrates that *D. geminata* and dam-altered thermal regimes in tailwaters exclude sensitive macroinvertebrate taxa (e.g. Ephemeroptera, Plecoptera, and Trichoptera [EPT]) in favor of species better adapted to cooler, relatively constant temperatures (Saltveit *et al.* 1994). Additionally, algal-dominated sites have higher macroinvertebrate mass and abundance, whereas vascular macrophyte communities have higher microinvertebrate diversity and species richness (Waters & San Giovanni 2002). A 2010 study by Gillis and Chalifour examining the impact of *D. geminata* abundance on benthic macroinvertebrate (BMI) abundance and species richness found that areas impacted by the diatom had macroinvertebrate densities nearly four times those of non-impacted areas. However, the proportion of Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies) (EPTs) was 30-50% lower in impacted vs. non-impacted sites, and impacted sites exhibited a shift toward chironomid and oligochaete dominance. A study in the South Fork Holston River by Green *et al.* (2017, unpublished) supported these assertions with the finding that greater *D. geminata* ash free dry mass (AFDM) was accompanied with higher macroinvertebrate abundance and lower diversity and richness.

Our results indicate that the dominance of Chironomidae and other more tolerant taxa in *D. geminata* impacted waters could be explained in part by the ability of these taxa to exploit the diatom as a food resource. Gut microscopy of chironomid samples found evidence of *D. geminata* consumption, whereas dissection of *Ephemerella* spp. and *Baetis* spp. individuals found no such evidence (Green and Blum, unpublished data). Our results do not indicate that *D. geminata* is a significantly lesser source of nutrients to macroinvertebrates than many other

surrounding aquatic macrophyte and submergent plant taxa (Fig. 2). Therefore we do not reject the hypothesis that macroinvertebrate taxa found thriving in *D. geminata*-impacted waters are able to use the diatom as a food resource.

An alternative, though not mutually exclusive hypothesis for the success of Chironomidae in *D. geminata*-impacted waters is that their small, elongate bauplan enables easy locomotion through the thick, netted mats of diatom stalks. EPTs are typically larger with more appendages and serration of the exoskeleton, which could make movement through *D. geminata* blooms difficult. Trichoptera (caddisflies) in particular require clean substrate for attachment of their cases, making substrate coated in *D. geminata* unsuitable habitat regardless of their ability to ingest the diatom.

Studies show that most primary production is not consumed by herbivores, but rather cycles back to the environment as detritus, a preferred food source for many benthic macroinvertebrates (Moore *et al.* 2004) (Green *et al.* 2017 unpublished data). Kornijow *et al.* (1995) found that freshwater invertebrates exhibit a clear feeding preference for decomposed algae and vascular plant tissue over living tissues of the same taxa. Detritus from aquatic macrophytes generally has higher quality tissues than terrestrial leaf detritus and serves as an important input of organic matter in autumn months (Hill and Webster 1983). The results of this study indicate that tissues of the algae *D. geminata* are not of significantly higher quality than those of angiosperm taxa (Fig. 2). These results are in line the findings of Kornijow *et al.* (1995) that the C:N ratios of angiosperms are lower than those of filamentous algae. However, stable isotope analyses of macroinvertebrates have shown that even in areas with abundant aquatic vascular plants harboring high densities of animal life, many species remained dependent on algal sources for organic carbon (Hamilton *et al.* 1991).

While the results of this study suggest that *D. geminata* C:N ratios are not highly variable between sites and water temperatures (Fig. 2), the amount of C and N transferred up the food chain by *D. geminata* may still vary by site as a result of dam-altered thermal regimes. The artificially cooler habitats may still have a negative impact on macroinvertebrate nutrition by reducing *D. geminata* decay, effectively trapping organic nutrients in the algal mats. A study by Green *et al.* (2017, unpublished data) found that seasonal variation in *D. geminata* ash-free dry mass (AFDM), an important variable for predicting macroinvertebrate richness and diversity, was much lower just below the South Holston Dam than at sites further downstream, where *D. geminata* AFDM was significantly lower in summer versus spring and winter months. Additionally, there is a general positive correlation between plant decomposition rate and N and P concentration (Enriquez *et al.* 1993), suggesting slow decomposition of *D. geminata* in its preferred oligotrophic conditions.

Our results are in line with the predictions set forth by the serial discontinuity concept (SDC). The SDC attempts to adjust the river continuum concept (RCC) for regulated waters through determination of discontinuity distance (DD), or the longitudinal shift of a river parameter (e.g. temperature, species diversity) caused by management (Ward and Stanford 1983). Because the intensity of DD is influenced by the parameter in question and the position of the dam or regulatory entity along the river, it is important to consider that the South Fork Holston River tailwater is a lower reach of its Fork. As expected, damming of the lower reaches produced a diminished temperature range directly below the dam, but this altered thermal profile and its impacts on C:N ratio were mitigated quickly within roughly 20 km downstream (Fig. 3a-c, Fig. 4). These findings also support the conclusions of Green *et al.* (2017)

Direction for Future Study

Some literature suggests that the amount of structural C in aquatic bryophytes and vascular plants is unrelated to the concentration of biochemically functional N (Duarte, 1992) (Demars and Edwards 2008). C:N ratios, while relatively inexpensive and simple to measure, are not the most effective standalone indicators of trophic linkages between primary producers and primary consumers (Cloern *et al.* 2002). As a result, future studies would benefit by assessing a different parameter of plant tissue quality that is directly indicative of the nutritional contribution of a given plant taxa to higher trophic levels.

It is important to understand the role of *D. geminata* in the ecology of tailwater habitats because of its potential to disrupt food webs by changing the community structure of primary consumers. Spread of *D. geminata* to previously uncontaminated waterways requires introduction of only a single diatom. We must fully understand the role of *D. geminata* in nutrient cycling to help determine whether it should be considered native, a nuisance, or an invasive and to plan mitigation efforts in an informed manner. Future studies should focus on the relative contributions of healthy and decaying *D. geminata* to the nutrient supply available to benthic macroinvertebrates. Additionally, efforts should be made to determine the diversity species richness of macroinvertebrates required to maintain healthy populations of secondary consumers and beyond to assess the level of impact commercially valuable fish species can withstand to their food supply.

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Tables and Figures

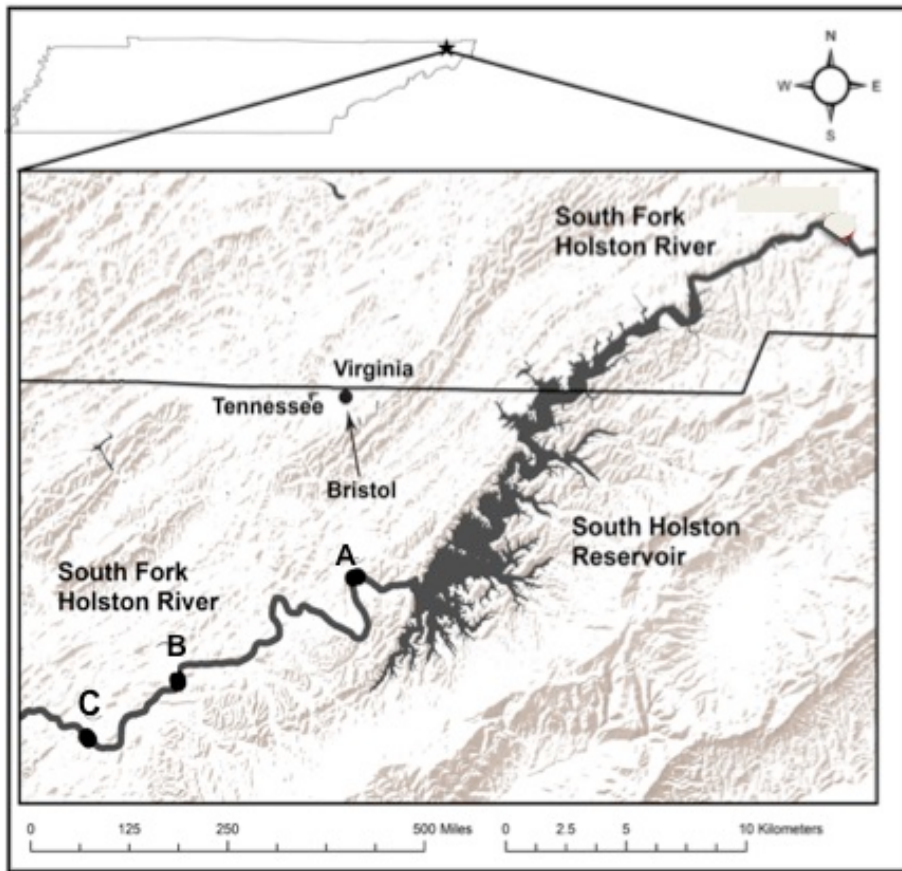


Figure 1. Topographic map showing the approximate locations of sites A, B, and C along the South Fork Holston River in eastern Tennessee. Note the location of the South Holston Reservoir, and that all sites fall in the tailwater.

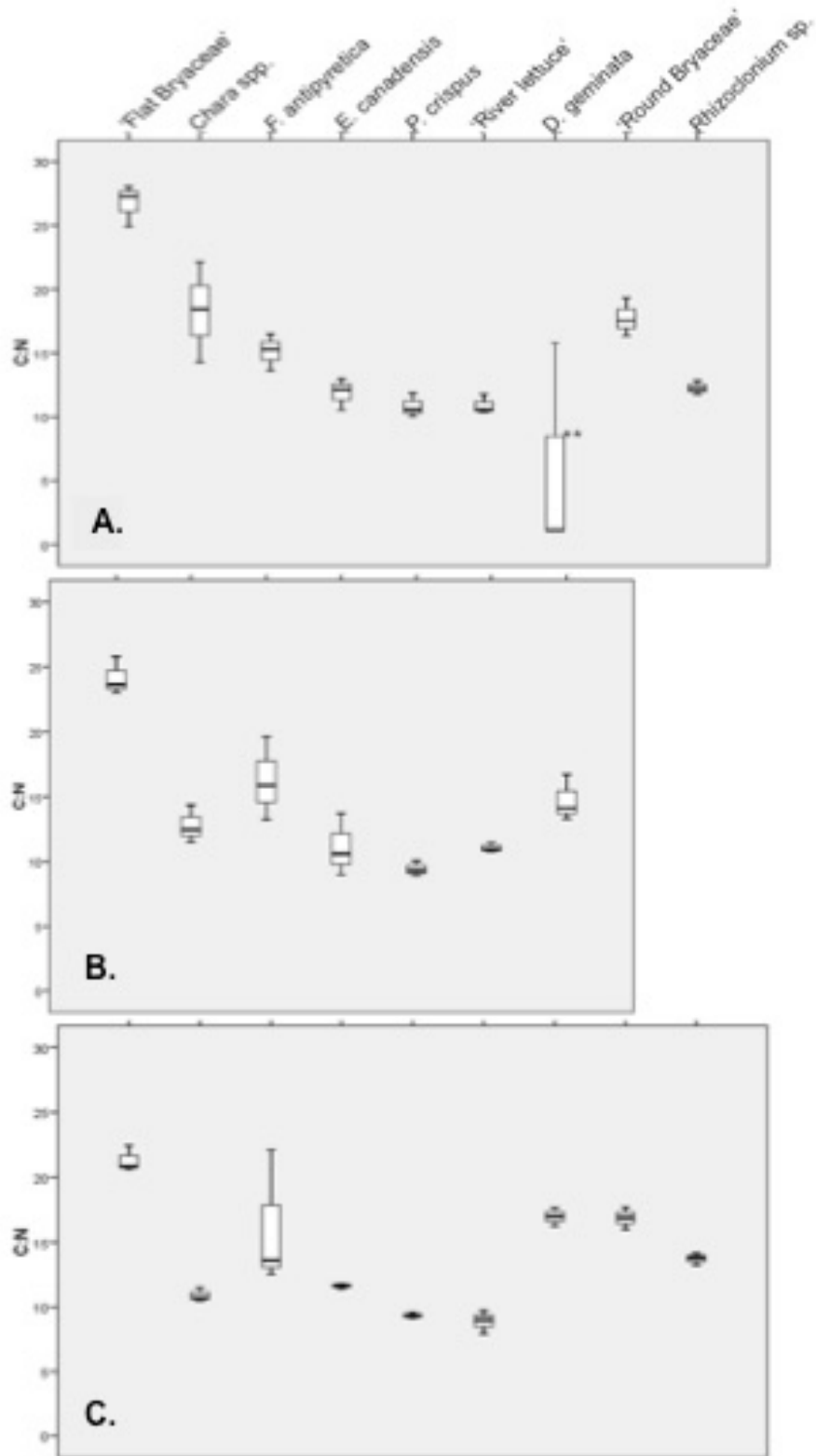


Figure 2. Inter-taxon differences in C:N ratios of the nine aquatic macrophyte and submergent plant taxa studied at sites A (top), B (middle), and C (bottom). Note the boxplot of *D. geminata*

at site A is made erroneous due to a faulty reading from the Elemental Analyzer that recorded the C:N ratio of two site A replicates as nearly 1:1. In the absence of the erroneous data points, there would likely be no significant variation in *D. geminata* C:N ratio between sites. Also note that ‘round’ bryaceae and *Rhizoclonium* spp. were not found at site B.

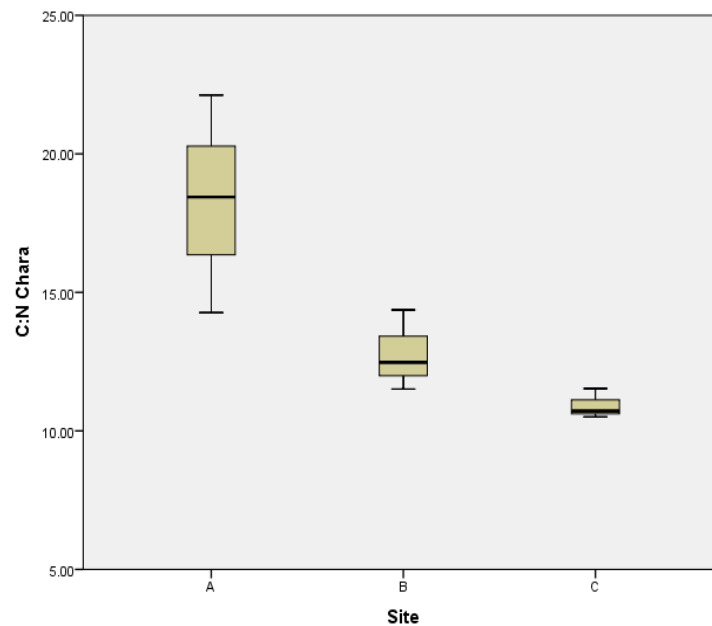


Figure 3a. Differences in C:N ratio of the angiosperm *Chara* spp. (common musk grass) between tailwater sites A, B, and C. Note that the y-axis scale is different than other parts of Fig. 3 with the intention of increasing readability.

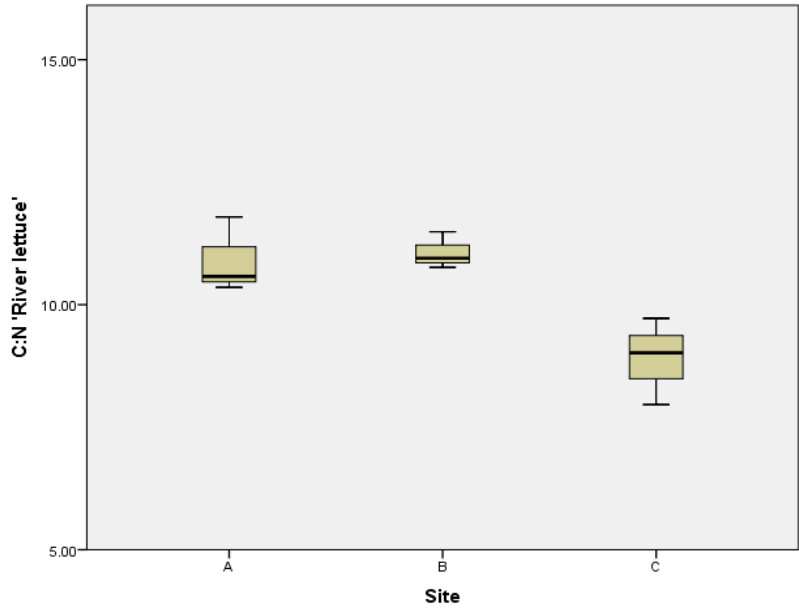


Figure 3b. Differences in C:N ratio of the angiosperm termed ‘river lettuce’ between sites A, B, and C. Note that the y-axis scale is different than other parts of Fig. 3 with the intention of increasing readability.

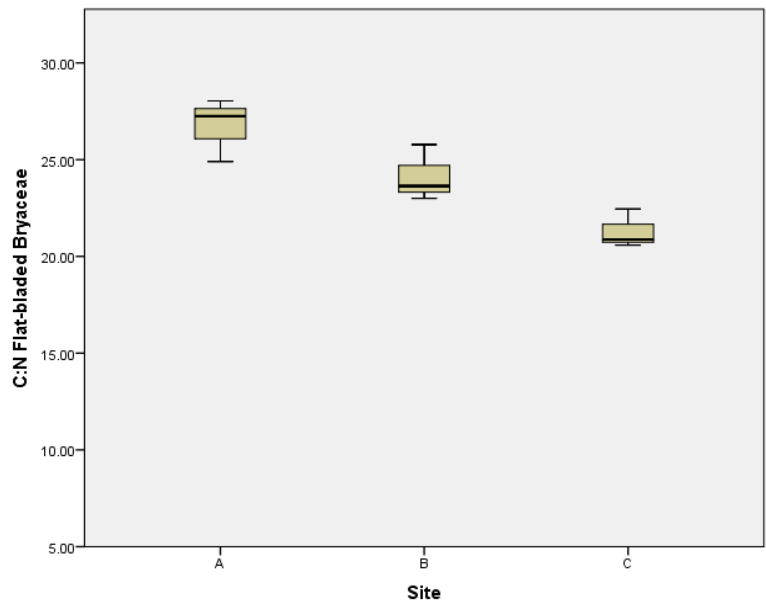


Figure 3c. Differences in C:N ratio of ‘flat-bladed’ bryaceae between sites A, B, and C. Note the y-axis scale is different than in other parts of Fig. 3 with the intention of increasing readability.

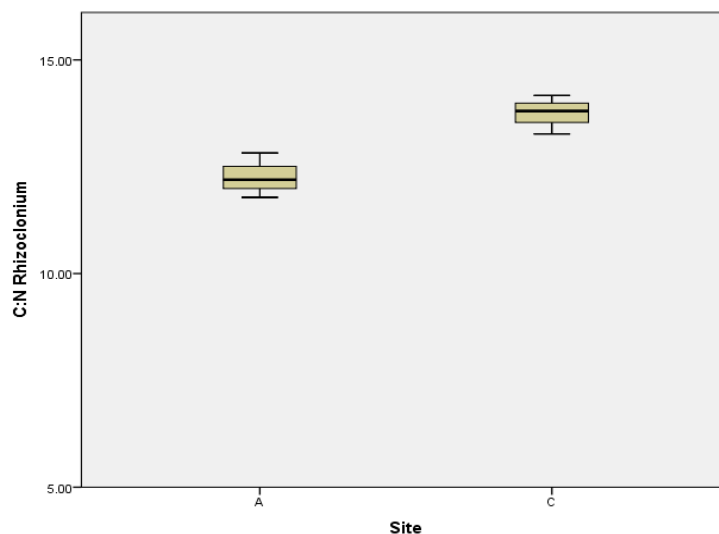


Figure 4. Difference in C:N ratio of the filamentous algae *Rhizoclonium* spp. Between sites A and C. Note that this species was not found at site B. Additionally note that the y-axis scale is different than in the similar Fig. 3 graph with the intention of increasing readability.

Site	Coordinates	Elevation	River Mile Marker	Distance from Dam
Site A	36°31'33.60", -082°06'50.40"	450.50 m	48.0	1.2 km
Site B	36°29'16.80", -082°11'42.00"	436.17 m	39.5	14.9 km
Site C	36°28'04.00", -082°14'13.20"	427.94 m	36.2	20.2 km

Table 1. Study site summary. We obtained site data from the study by Green *et al.* (unpublished) from which the current study was derived. Note that the distance between sites A and B is greater than between sites B and C.

		Table 2. Spearman's Correlations - Temperature vs. C:N Ratio								
		<i>D. geminata</i>	<i>F. antipyretica</i>	"Flat Bryaceae"	"Round Bryaceae"	<i>Chara</i> spp.	<i>Rhizoclonium</i> sp.	<i>E. canadensis</i>	<i>P. crispus</i>	"River lettuce"
CV of Temp	r_s	0.316	0.000	-0.369	-0.293	-0.422	0.878	-0.211	-0.632	0.158
	p	0.487	1.000	0.329	0.573	0.258	0.021*	0.586	0.068	0.685
	N	7	9	9	6	9	6	9	9	9
Annual Mean Temp (°C)	r_s	0.791	-0.158	-0.896	-0.632	-0.843	0.878	-0.105	-0.632	-0.632
	p	0.624	0.685	0.001**	0.573	0.004**	0.021*	0.787	0.068	0.068
	N	7	9	9	6	9	6	9	9	9

* $p < 0.05$ – significant correlation

** $p < 0.01$ – highly significant correlation

Table 2. Correlations between C:N ratios of studied plant taxa and annual mean temperature.