# PLANT-HERBIVORE INTERACTIONS: CONSEQUENCES FOR THE STRUCTURE OF FRESHWATER COMMUNITIES AND EXOTIC PLANT INVASIONS

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# Plant-Herbivore Interactions: Consequences for the Structure of Freshwater Communities and Exotic Plant Invasions

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# SUMMARY

Invasive exotic species threaten native biodiversity, alter ecosystem structure and function, and annually cost over $100 billion in the US alone. Determining the ecological traits and interactions that affect invasion success are thus critical for predicting, preventing, and mitigating the negative effects of biological invasions. Native herbivores are widely assumed to facilitate exotic plant invasions by preferentially consuming native plants and avoiding exotic plants. Here, I use freshwater systems to show that herbivory is an important force driving the ecology and evolution of plant communities, but in contrast to expectation, selective herbivory on native plants does not appear to drive exotic plant invasions in freshwater, marine, or terrestrial systems.

In Chapter 1, I show that only the most chemically defended native plants can persist in the face of intense herbivory by non-native herbivorous grass carp (*Ctenopharyngodon idella)*, consistent with other studies showing that non-native herbivores often decimate native plant communities. In Chapter 2, I show that beaver (*Castor canadensis*) grazing has strong impacts on the abundance and species composition of herbaceous plant communities in beaver ponds, but these effects were context-dependent and depended on the identity of neighboring plants. In Chapter 3, I show that herbivory by Canada geese (*Branta canadensis*) and crayfish (*Procambarus spiculifer*) may drive the ecological specialization of plant-associated macro-invertebrates onto an aquatic moss that is chemically defended from consumption by these larger consumers. Thus, despite the long-standing assumption that herbivory is a weak ecological and evolutionary force in freshwater systems, herbivores appear to have strong impacts on the structure of freshwater communities.

In Chapter 4, I show that native crayfishes (*Procambarus spiculifer* and *P. acutus*) preferentially consume most exotic plants over phylogenetically-related and sympatric native plants. Analyses of 3 terrestrial datasets showed similar patterns, with native herbivores generally preferring exotic plants. Thus, exotic plants appear defensively naïve against the evolutionarily novel consumers in their new ranges, and exotic plants may escape their coevolved herbivores only to be preferentially consumed by native generalist herbivores. In further support of this hypothesis, Chapter 5 reports the results of a meta-analysis of 71 manipulative field studies including over 100 exotic plant species and 400 native plant species from terrestrial, aquatic, and marine systems revealing that native herbivores strongly suppress exotic plants, while exotic herbivores enhance the abundance and species richness of exotic plants by suppressing native plants. Both outcomes are consistent with the hypothesis that prey are susceptible to evolutionarily novel consumers. Thus, native herbivores provide biotic resistance to plant invasions, but the widespread replacement of native with exotic herbivores eliminates this ecosystem service, facilitates plant invasions, and triggers an invasional meltdown. Consequently, rather than thriving because they escape their co-evolved specialist herbivores, exotic plants may thrive because their co-evolved generalist herbivores have stronger negative effects on evolutionarily naïve, native plants.

# CHAPTER 1

# CHEMICAL DEFENSES PROMOTE PERSISTENCE OF THE AQUATIC PLANT *MICRANTHEMUM UMBROSUM*

## *Abstract*

Here, we show that five of the most common macrophytes from an aquaculture facility with high densities of the herbivorous Asian grass carp (*Ctenopharyngodon idella*) were commonly unpalatable to three generalist consumers – grass carp and the native North American crayfishes *Procambarus spiculifer* and *P. acutus*. The rooted vascular plant *Micranthemum umbrosum* comprised 89% of the total aboveground plant biomass and was unpalatable to all three consumers as fresh tissues, as homogenized pellets, and as crude extracts. Bioassay-guided fractionation of the crude extract from *M. umbrosum* led to four previously known compounds that each deterred feeding by at least one consumer: 3,4,5-trimethoxyallylbenzene (**1**) and three lignoids: β-apopicropodophyllin (**2**), (±)-(3*S*,4*R*,6*S*)-3-(3',4'-methylenedioxy-α-hydroxybenzyl)-4-(3",4"-dimethoxybenzyl)butyrolactone (**3**), and (-)-hibalactone (**4**). None of the remaining four macrophytes produced a chemically deterrent extract. A 16-month manipulative experiment showed that the aboveground biomass of *M. umbrosum* was unchanged when consumers were absent, but the biomass of *Ludwigia repens*, a plant that grass carp preferentially consumed over *M. umbrosum*, increased over 300-fold. Thus, selective feeding by grass carp effectively eliminates most palatable plants from this community and promotes the persistence of the chemically defended *M. umbrosum*, suggesting that plant defenses play critical yet understudied roles in the structure of freshwater plant communities

## *Introduction*

Herbivores were historically thought to have little impact on the ecology and evolution of freshwater plant communities (e.g., Shelford 1918, Hutchinson 1975). Recent reviews, however, show that herbivore impacts in freshwater systems rival those of marine and terrestrial systems, and aquatic herbivores often reduce the standing stock and alter the species composition of freshwater plants (Newman 1991, Cyr and Pace 1993, Lodge et al. 1998). Additionally, aquatic herbivores exhibit selective avoidance of chemically or structurally defended plants (Newman et al. 1996, Bolser et al. 1998, Cronin 1998, Cronin et al. 2002), yet there is surprisingly little direct evidence linking consumer feeding preferences to particular plant traits, or ultimately to shifts in plant community structure. For example, in Dorn and Wojdak (2004), the introduced crayfish *Orconectes virilis* selectively consumes the filamentous green alga *Cladophora* over the blue-green alga *Gleotrichia* and instigates a shift from green to blue-green algae in experimental ponds, but the mechanisms conferring resistance to herbivores in *Gleotrichia* were not investigated.

A number of studies, however, show that freshwater macrophytes are frequently unpalatable and contain a variety of secondary metabolites that could function as herbivore deterrents (Ostrofsky and Zettler 1986, Cronin et al. 2002). More than ½ of the crude extracts from 21 species of aquatic macrophytes that Prusak et al. (2005) surveyed, for example, deterred feeding by an omnivorous crayfish, although they did not identify the metabolites responsible for feeding deterrence. In fact, we know of only three freshwater plants with identified compounds that deter herbivores – watercress, *Rorippa nasturtium-aquaticum* (L.) Hayek (Newman et al. 1996), the waterspider bog orchid, *Habenaria repens* Nutt. (Bolser et al. 1998, Wilson et al. 1999), and lizard's tail, *Saururus cernuus* L. (Kubanek et al. 2000, Kubanek et al. 2001) – with a total of nine described secondary metabolites that influence herbivore feeding. In contrast, hundreds to thousands of secondary metabolites that deter consumers have been described from marine and terrestrial primary producers (Seigler 1998, Faulkner 2002 and references therein). These molecules can have strong cascading impacts on the ecology and evolution of plant-herbivore interactions in these systems (see Discussions in Hay and Fenical 1988, 1996, Hay 1996), suggesting that plant chemical defenses may play similar, but relatively uninvestigated roles in freshwater systems.

Here, we examined the feeding preferences of three generalist consumers among five species of macrophytes collected from an aquaculture facility stocked with high densities of the herbivorous Asian grass carp *Ctenopharyngodon idella.* To determine the traits promoting macrophyte persistence under intense herbivory, we assessed the palatability of (1) whole plants, (2) plant tissues ground and imbedded in an gel-like matrix to retain most of the chemical and nutritional traits but with normal morphological traits removed, (3) plant crude extracts, and (4) specific metabolites isolated using bioassay-guided fractionation. We also conducted a manipulative field experiment excluding herbivorous fishes and assessed the changes in the littoral plant community after 16 months to determine whether well-defended species were disadvantaged in the absence of herbivores.

## *Materials and Methods*

*Study Organisms*. We collected macrophytes from two 2000 $m^2$ ponds at the Owens and Williams fish hatchery in Hawkinsville, Georgia, USA. Each pond was stocked with >100,000 juvenile Asian grass carp *Ctenopharyngodon idella*, an exotic herbivorous fish introduced throughout the USA to reduce aquatic plant abundance (USGS 2005). On one occasion we observed one turtle (pond slider – *Trachemys scripta* Wied) and evidence of crayfish (e.g., a crayfish moult) in both ponds; these omnivores also consume macrophytes and could have further enhanced herbivore impact (Bjorndal 1991, Lodge et al. 1998), but their effects were likely small relative to large numbers of grass carp in each pond. The rooted, vascular plant *Micranthemum umbrosum* appeared to be the predominant plant species in one pond, while the floating green alga *Spirogyra* sp. appeared to be the predominant plant species in the other pond. The hatchery owner informed us that grass carp would frequently bite *M. umbrosum* but then forcibly reject it, and that *Spirogyra* sp. often persisted until all other macrophyte species had been consumed. Based on these observations, the high density of herbivores in these ponds, and the acrid taste of *M. umbrosum* (JP, personal observation), we hypothesized that these macrophytes possessed defensive traits promoting their persistence under intense herbivory.

On April 26, 2004, we determined the abundance of macrophytes in each pond by randomly locating five 0.25 $m^2$ quadrats on the littoral fringe (<1 m depth) of one side of each pond and determining the identity of macrophytes located beneath 36 points in each quadrat (we did not sample the remaining sides because they were disturbed by seine netting to capture fishes). The five most common macrophytes (the green alga

*Spirogyra* sp., and the vascular plants *Micranthemum umbrosum*, *Ludwigia repens*, *Juncus repens* Michx., and *J. effusus* L.) – were collected, transported to the laboratory in a chilled cooler, and fed to three consumer species within 24 hours of collection. We fed macrophytes to juvenile grass carp and to the native North American crayfishes *Procambarus spiculifer* and *P. acutus*. Both crayfishes have ranges across the southeastern United States (Hobbs 1981). We used crayfish as a bioassay organism because they can have strong impacts on aquatic macrophyte communities (Lodge and Lorman 1987, Creed 1994, Lodge et al. 1994, Dorn and Wojdak 2004), they are diverse and abundant foragers in aquatic habitats throughout North America (Lodge et al. 2000), they feed well in the laboratory (Bolser et al. 1998, Parker and Hay 2005), and we observed evidence (though limited) of crayfish in each pond.

*P. spiculifer* were collected from the Chattahoochee River, Atlanta, GA (33 ° 54'N, 84° 27'W); *P. acutus* were collected from an adjacent wetland. We housed each crayfish in a separate 12 x 12 x 10 cm cubicle with perforated walls receiving recirculating, filtered water. All animals were fed a maintenance diet of Bio-Blend Herbivore food 3-4 times week$^{-1}$. Grass carp would not feed when kept individually so we housed them in small groups of 3-6 animals in 3.5 L buckets with recirculating water.

*Feeding assays*. We determined the relative palatability of all five macrophyte species by offering 12-15 individuals of each consumer species a bite-sized portion of each macrophyte and recording whether each portion was eaten or rejected. If rejected, we fed consumers a piece of palatable aquatic macrophyte (*Egeria densa* Planch.) to assure they were not satiated. If the palatable macrophyte was rejected, that replicate animal was not included in the assay because it appeared satiated. Because grass carp were kept in small groups, we report results from the first fish that fed in each bucket as a replicate (*N* = 12-15 separate buckets). Order of macrophyte presentation was randomized separately for each replicate consumer. We then analyzed (using a Fisher's exact test) the proportion of animals that were willing to feed on each individual macrophyte species relative to the palatable control.

Low palatability of fresh macrophyte tissues could result from structural, morphological, nutritional, chemical, or other characteristics. To determine whether macrophyte morphology could account for feeding preferences, we destroyed morphological traits by incorporating freeze-dried and finely ground macrophyte tissues into gel-based foods constructed with sodium alginate (Hay et al. 1998). We added enough macrophyte powder to the paste to approximate the same dry mass per volume of macrophyte found in tissues from each species of macrophyte being assayed (see Methods-*Macrophyte Traits*). The gel was then coated onto the interior wall of a glass Petri dish and immersed in a hardening solution of 0.25M calcium chloride. After approximately one minute the gel was removed, rinsed in water, and cut into bite-sized portions. This method resulted in reconstituted macrophytes with similar morphologies and a soft, fleshy texture not unlike cooked pasta. Nutritional values and chemical defenses should have remained similar to those of intact macrophytes (however, freeze-drying can alter the activity of some metabolites, Cronin et al. 1995). These artificially softened macrophytes were then assayed against a palatable control food - a 1:1 mixture of freeze-dried and powdered broccoli and lettuce ('broc-let') that herbivores readily accept as food (Bolser et al. 1998). Broc-let content matched the dry mass per volume of each macrophyte being assayed. Feeding on pellets was recorded as the frequency of acceptance or rejection of treatment or control pellets, with these pellets being offered alternately. We then analyzed (via Fisher's exact test) the proportion of animals feeding on each individual reconstituted macrophyte species relative to the palatable control.

If gel-based treatments were unpalatable, this suggested a chemical basis for feeding rejection; we tested for chemical defenses by conducting feeding assays with crude extracts from each macrophyte incorporated into broc-let based sodium-alginate pellets as above (see above and Hay et al. 1998 for a general review). Extracts were acquired by macerating macrophyte tissues in a 1:1 mixture of water and methanol overnight, then successively extracting the macrophyte material for at least two hours in 1:1 and 1:2 methanol:dichloromethane. The extracts were combined and solvents removed under vacuum to yield a crude extract. For food preparation, each crude extract was dissolved in acetone, incorporated into broc-let powder and sodium alginate, and the solvent was evaporated by vigorous stirring in a fume hood. Control foods were treated identically (including addition of acetone) but without the addition of crude extracts. The dry mass content of treatment and control pellets matched the dry mass per volume content of each macrophyte being assayed. Pellets were fed to animals and the results were statistically analyzed as above.

*Macrophyte traits.* We measured selected macrophyte traits that are generally thought to be indicative of macrophyte nutritional quality or availability as a food, including: toughness, dry mass/volume, ash-free dry mass/volume, and soluble protein/volume and soluble protein/dry mass. Toughness was estimated by using a penetrometer (see Duffy and Hay 1991) to determine the mass of sand required to pierce a leaf with a needle. Two of the five macrophytes could not be adequately tested with this approach. The rush *Juncus effusus* was too tough to pierce with our penetrometer; the strands of the green alga *Spirogyra* were too thin to accept the needle.

Soluble protein content was estimated with the Bradford method. Triplicate composite samples of ground macrophyte material from each species (~5 mg) were digested in 1 ml of sodium hydroxide (1 mol/l) for 24 hr at 2.5°C, centrifuged, and 100 µl aliquots of the supernatant were added to 5 ml samples of Bradford reagent. After 10-15 min, absorbance of each sample at 595 nm was measured using a Spectronic 21D spectrophotometer against bovine serum albumin (BSA) standards.

Results were analyzed with ANOVA followed by Tukey multiple comparison tests, with transformations (log + 1) to correct heteroscedastic variances when necessary. Protein analyses were conducted on pooled samples of tissues from many individual plants. These data were not statistically analyzed because variances associated with the means were methodological rather than associated with difference among individual, replicate plants.

*Bioassay-guided fractionation. Micranthemum umbrosum* was the only macrophyte species with consistent evidence for a strong chemical defense. To separate and identify the defensive compounds, we used bioassay-guided fractionation of the total crude extract using the feeding response of the crayfish *Procambarus spiculifer*. We used crayfish rather than grass carp for these assays because grass carp had not yet acclimated to feeding in the laboratory when we began this fractionation. We did, however, test the deterrence of each isolated metabolite and also of the remaining crude extract minus the four deterrent compounds we isolated using all three consumer species. Extracts were initially tested at twice their extracted concentrations (by volume) to offset loss due to inefficient extractions and/or compound decomposition. Chromatographic fractions and pure compounds were tested by offering crayfish broc-let based pellets incorporated with fractions or compounds vs. control foods; results were statistically analyzed with Fisher's exact tests.

*Micranthemum umbrosum* was extracted successively with dichloromethane, acetone, and methanol, and these extracts combined to produce a crude extract. The deterrent crude extract was fractionated using silica gel flash chromatography (40-63 μm Aldrich silica gel eluting with increasing concentration of ethyl acetate in petroleum ether). The resulting 36 fractions were then grouped by similar thin layer chromatography (TLC) properties into seven fractions, of which two were deterrent. The deterrent component within the less polar deterrent fraction was purified by repeated flash chromatography as described above. The more polar deterrent fraction yielded two deterrent pure compounds via: 1) repeated silica gel flash chromatography eluting with toluene/ethyl acetate or toluene/petroleum ether/ethyl acetate; 2) recrystallization from hexanes/methanol/toluene (3:2:1); and 3) silica gel high performance liquid chromatography (HPLC) eluting with hexane/ethyl acetate, using a Zorbax RX-SIL HPLC column (9.4 x 250 mm; 5 micron) attached to a Waters Breeze HPLC system consisting of Waters 515 pump and Waters 2487 UV detector recording at 210 and 254 nm. A third fraction from the initial flash column separation did not initially deter crayfish feeding, but unusual $^1$H NMR signals motivated the purification of a fourth compound by flash column chromatography and HPLC as described above.

Pure compounds from each fraction were identified on the basis of $^1$H, $^{13}$C and 2D NMR spectroscopy and comparisons of NMR, IR, and mass spectral data with literature data. Optical rotations were obtained using a Jasco P-1010 polarimeter**.** IR data were acquired on a Nicolet 520 FTIR spectrophotometer with thin films on NaCl plates. $^1$H, $^{13}$C, and 2D NMR spectral data were obtained on a Bruker Avance DRX 500 MHz spectrometer using $CDCl_3$ referenced to residual $CHCl_3$ ($\delta$ 7.28).

*Quantification of isolated compounds.* To determine whether our isolated yields were comparable to the natural concentrations of these compounds in plant tissues, we quantified the concentrations of each of the four compounds from five separate individuals of *Micranthemum umbrosum* collected at the same time and under the same conditions as the bulk material used in this study. The plants were individually extracted with a 1:1 mixture of water and methanol, then successively for at least two hours in 1:1 and 1:2 methanol:dichloromethane.

Quantification of natural products was achieved by LC-MS/MS using a Micromass Quattro electrospray mass spectrometer attached to an Agilent 1100 HPLC with a reversed-phase Zorbax eclipse XDB-C8 column (1.0 x 150 mm, 3.5-micron) with a gradient system of water/acetonitrile (0.1 % formic acid) 19:1 (v:v) to 1:95 (v:v) over 31 minutes and monitored by UV at 254 nm. Three or four standard solutions (0.0001 – 0.10 mg/mL) of each of the four pure compounds were used to measure sample concentration by integration of the peaks for observed transitions from $[M+H]^+$ to a select daughter ion for each compound. These data were then used to establish standard curves ($R^2 > 0.99$ for each compound) for the quantification of these compounds in crude extracts of the five macrophyte samples. Once natural concentrations were known, we tested the effects of each compound at its natural concentration and at its isolated yield using each of our three consumers species.

*Experimental exclusion of herbivores and tests of herbivore preference among plants.* To determine whether the chemically-defended *Micranthemum umbrosum* was disadvantaged relative to less-defended plants in the absence of herbivory, we excluded grass carp and other potential consumers from caged areas of the pond for 16 months and measured the abundance of plants in caged vs. control areas. On April 26th, 2004 we established five blocks in the pond with three treatments in each block: 1) an uncaged treatment allowing full herbivore access, 2) a 3-sided cage control allowing herbivore access but controlling for cage artifacts, and 3) two 4-sided cages excluding herbivores. Each block had two 4-sided cages because we had originally intended to establish another treatment in one of the cages. We never imposed this treatment, thus both cages were considered replicates in the same block to calculate the cage effect. Each treatment area was 0.9 x 0.9 x 0.9 m, with the cage control and cage areas marked by 1.0 m tall steel rebar posts. Cages were constructed of 3.0 mm plastic mesh affixed to the rebar posts with cable ties. A 10-15 cm skirt was anchored around each cage to prevent consumers from burrowing under the mesh walls. On only one occasion did we encounter grass carp in the cages; both fishes were removed and were likely too small (<2 cm in length) to have begun feeding on macrophytes given that grass carp typically do not become herbivorous on macrophytes until they reach approximately 3 cm in length (Hickling 1966). Treatment blocks were established in linear arrays separated from each other by at least 4.0 m along the shoreline that was not used to seine fishes. Treatments were randomly assigned to each position in the block, with the restriction that the open treatment was on either end of the block. Watermarks on the cage walls suggested that the average treatment depth was approximately 15 cm, but we observed that cages were occasionally dry or up to 30 cm deep, consistent with the variability of water depth we observed in natural ponds in the area. Poor water clarity, however, prevented monitoring of plant cover when water depth exceeded approximately 10 cm.

We estimated initial plant cover in the treatments by determining the identity of macrophytes located beneath 36 points in a 0.25 $m^2$ quadrat placed directly in the center of each treatment cell. We intended to estimate plant cover throughout the experiment, but poor water clarity consistently prevented determination of cover after establishment of the cages. Therefore, we analyzed the initial total plant cover and the initial cover of the two species (*Micranthemum umbrosum* and *Ludwigia repens*) that were most abundant at the end of the experiment with a blocked one-way ANOVA, transforming (log + 1) to correct heteroscedastic variances (determined via Cochrans tests) when necessary.

On August 29, 2005, we harvested all of the aboveground plant material from each cage and weighed it to the nearest g. We analyzed the total aboveground biomass and the biomass of the two most common plant species in our treatments with a blocked one-way ANOVA, transforming (log + 1) to correct heteroscedastic variances (determined via Cochrans tests) when necessary. Significant ANOVA results were followed by multiple comparisons (Tukey tests) among treatment means.

Although our earlier laboratory feeding assays allowed us to determine which plant species were unpalatable, they are unsuitable for determining preference hierarchies among plant species. Thus, we conducted choice-feeding assays comparing grass carp preference for *Micranthemum umbrosum*, the plant that dominated cover in one of the grass carp ponds, with *Ludwigia repens*, a formerly rare plant that heavily recruited into our exclusion cage treatments (see Results). We also compared grass carp preference for *M. umbrosum* vs. *Najas guadalupensis*, and *L. repens* vs. *N. guadalupensis*; we chose *N. lupensis* because it dominated (>80% cover, N = 20 quadrats) the cover of a nearby (~ 300 m distant) pond of natural origin that did not have grass carp. We hypothesized that the dominant plants from the grass carp pond (*M. umbrosum* and *L. repens*) would be of lower preference than the dominant plant (*N. guadalupensis*) from a habitat that lacked grass carp, and that *M. umbrosum* would be of lower preference than a plant that recruited only to cages where we excluded grass carp.

Each replicate assay consisted of placing a binder clip with a 2.0 cm portion of each of two plant species into 18 buckets containing 2-5 grass carp. Each replicate was checked periodically to determine which plant had been eaten first, with all treatments harvested the following morning. We did not retain the replicates where both plants had been eaten as we could not determine which plant had been eaten first. Results were analyzed with Fisher’s exact tests.

## *Results*

*Macrophyte abundance*. In late April 2004, the littoral fringe of both grass carp ponds was largely unvegetated (Pond 1 = 76.7 ± 10.6% (SE) bare space, Pond 2 = 80.6 ± 10.3%, both $N = 5$), but macrophyte cover in each of the ponds was dominated by a single species (Pond 1: *Micranthemum umbrosum* = 86.5 ± 6.8% of total plant cover; Pond 2: *Spirogyra* = 94.4 ± 5.6% of total plant cover). Of the remaining four macrophyte species that we observed, *Juncus effusus* represented 9.8 ± 5.3% of total plant cover in Pond 1 and 2.8 ± 2.8% in Pond 2, *J. repens* comprised 2.8 ± 2.8% in Pond 2, and there were trace amounts of *Ludwigia repens* in Pond 1. A single individual of the sedge *Carex* sp. occurred in Pond 1 and comprised 3.7 ± 3.7% total plant cover in that pond; because this was only one individual, we did not include this species in our feeding assays. No other aquatic macrophytes were observed in the ponds.

*Feeding assays.* When offered as fresh macrophyte tissues, each of the five macrophyte species that we assayed was unpalatable relative to a control food to at least two of the three consumer species tested (Figure 1.1a). Of the two most common macrophytes, *Micranthemum umbrosum* was rejected as fresh tissue by all three consumers, whereas *Spirogyra* was rejected compared to a control food by *Procambarus acutus* and *Ctenopharyngodon idella,* but not by *P. spiculifer*. Although relatively uncommon in the ponds, *Ludwigia repens* was also significantly less palatable to all three consumers than was the control food (*Egeria densa*). After we destroyed plant morphological traits, palatability increased for some macrophytes, but feeding on *M. umbrosum*, *Spirogyra*, and the rush *Juncus repens* remained similar to that on intact plants (Figure 1.1b). When

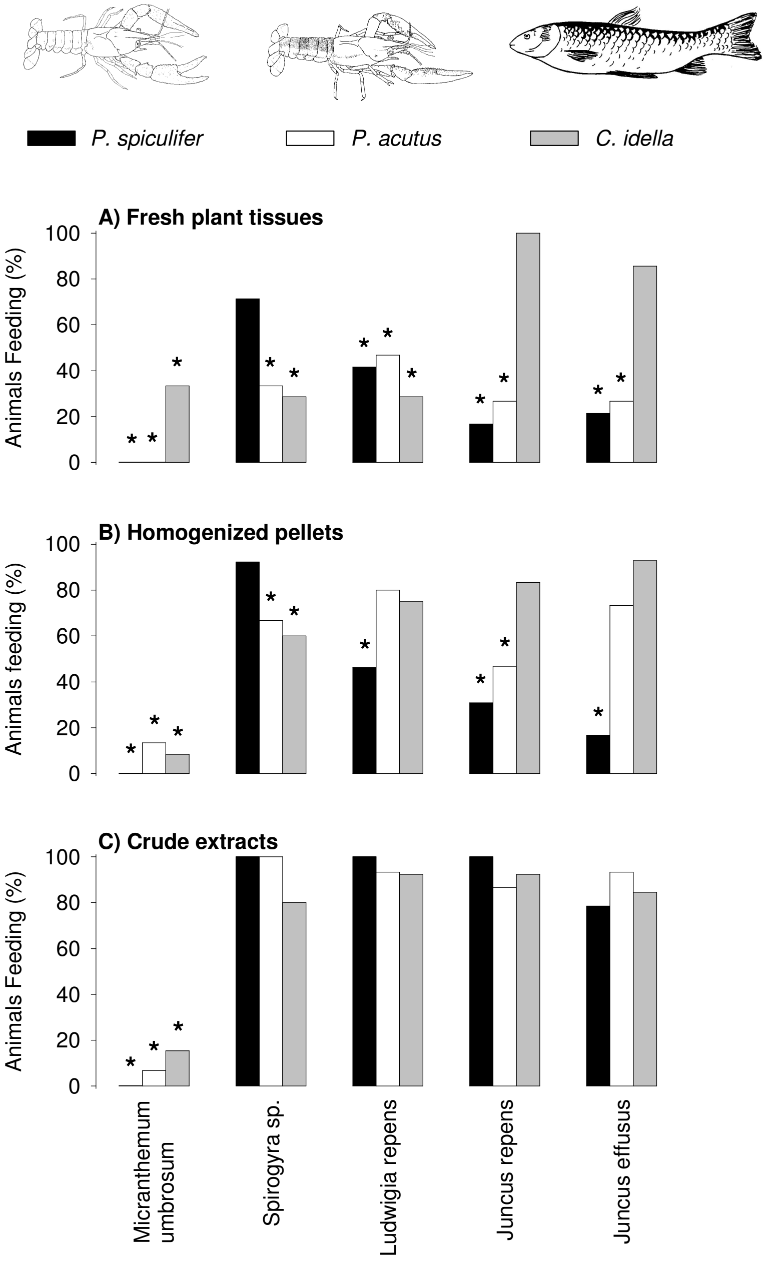


Figure 1.1. Percentage of 12-15 individual *Procambarus spiculifer* (filled bars), *P. acutus* (open bars), and *Ctenopharyngodon idella* (grey bars) feeding on A) fresh macrophyte tissues, B) homogenized macrophyte pellets at natural dry mass content, and C) crude extracts from five aquatic macrophyte species. Asterisks denote statistically significant reductions in feeding relative to a palatable control (*Egeria densa*) for each consumer species (Fisher's exact tests).

the crude extracts from macrophytes were incorporated into a palatable control food, only *M. umbrosum* remained unpalatable – suggesting a strong chemical deterrent to feeding by all three consumers (Figure 1.1c).

*Macrophyte traits.* Table 1.1 shows toughness, dry mass, ash-free dry mass, and protein content of the macrophytes examined in this study. *Micranthemum umbrosum* was the softest macrophyte that we tested with the penetrometer, was of intermediate rank in dry mass and in soluble protein per volume of plant, but it had the highest ash-free dry mass per volume of macrophyte. *Spirogyra* could not be tested with the penetrometer because of its filamentous morphology, but it has no obvious structural barriers to grazing. *Spirogyra* was generally nutritionally poor relative to the other plants; it ranked lowest in dry mass, ash-free dry mass, and protein content per volume of plant (Table 1.1). *Ludwigia repens* was relatively soft and of intermediate to low rankings in mass and protein content. The prostrate rush *Juncus repens* was the toughest macrophyte that we could test, and it had the highest dry mass, second highest ash-free dry mass, and protein content when measured volumetrically, but the lowest protein content when expressed as a % of dry mass. The emergent rush *J. effusus* was too tough to test with the penetrometer, had intermediate dry mass per volume, relatively low ash-free dry mass per volume, and moderately low protein content. Our palatable control food, a 1:1 mixture of powdered broccoli and lettuce ('broc-let'), had relatively low dry mass, ash-free dry mass, and protein content per volume of plant, but it had the highest protein content of all the foods when expressed as a % of dry mass (Table 1.1).

Table 1.1. Mean (± SE) and sample sizes (in parentheses) for each analysis of selected macrophyte traits. Species that share a letter within a column are not significantly different from one another in unplanned comparisons following ANOVA; broc-let not included in statistical analysis.

| **Macrophyte** | **Mass to pierce (mg)** | **Dry mass/vol. (mg/ml)** | **AFDM/vol. (mg/ml)** | **Soluble protein (mg/ml)** | **Soluble protein (% dry mass)** |
|---|---|---|---|---|---|
| *Micranthemum umbrosum* | 5.40 ± 0.768 (5)**a** | 88.0 ± 7.57 (8)**b** | 25.6 ± 5.30 (8)**b** | 4.83 | 5.49 |
| *Spirogyra* sp. | Too thin to test | 45.2 ± 4.90 (4)**a** | 7.26 ± 1.95 (4)**a** | 3.06 | 6.77 |
| *Ludwigia repens* | 9.19 ± 1.31 (5)**a** | 73.3 ± 10.1 (5)**ab** | 13.7 ± 2.44 (5)**ab** | 4.77 | 6.51 |
| *Juncus repens* | 13.3 ± 1.04 (5)**b** | 142 ± 14.3 (5)**c** | 24.4 ± 4.46 (5)**b** | 6.96 | 4.90 |
| *Juncus effusus* | Too hard to test | 80.3 ± 9.97 (5)**b** | 10.5 ± 2.54 (5)**ab** | 4.81 | 5.99 |
| Broc-let control | N/A | 55.2 ± 1.41 (3) | 6.27 ± 0.475 (3) | 4.04 | 7.32 |
| ANOVA *P*-values | ***P* = 0.001** | ***P* < 0.0001** | ***P* = 0.004** | N/A | N/A |

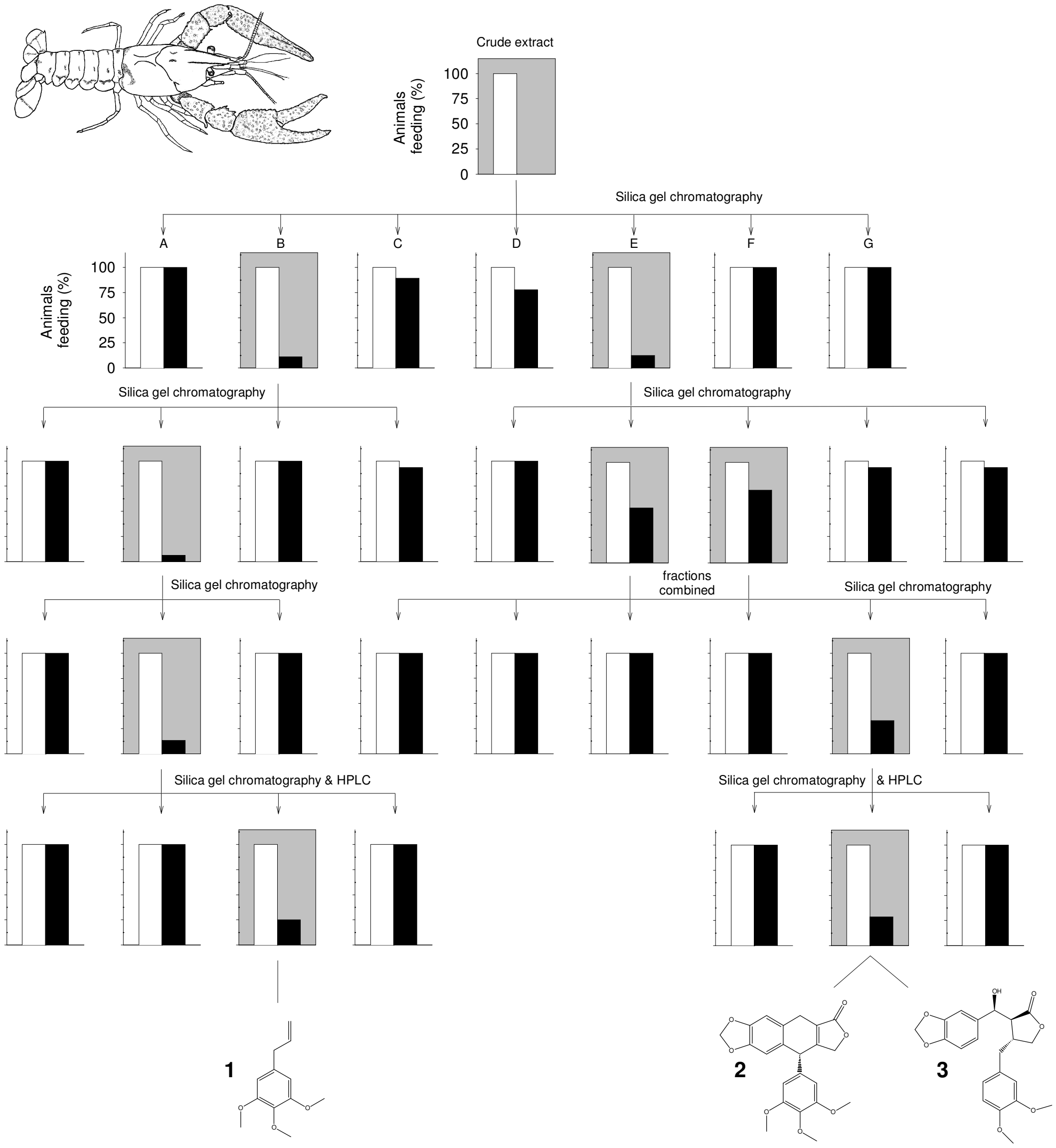


Figure 1.2. Bioassay-guided fractionation of crude extracts from *Micranthemum umbrosum*. Each graph shows the percentage of 12-15 individual *Procambarus spiculifer* feeding on a solvent-only control food (open bar) versus control food containing macrophyte extracts. Shaded graph panels denote statistically significant ($P < 0.05$) feeding reductions relative to the palatable control (Fisher's exact tests).

*Bioassay guided fractionation.* The crude extract of *Micranthemum umbrosum* strongly deterred feeding by *Procambarus spiculifer* (Figure 1.2). Two of the initial seven fractions from this extract strongly reduced crayfish feeding (fractions B and E, Figure 1.2). Purification of the active component in fraction B via three silica gel chromatographic columns revealed 3,4,5-trimethoxyallylbenzene (elemicin) (**1**) as the bioactive metabolite (Figure 1.2). Similar bioassay-guided separation of fraction E, followed by HPLC purification, led to identification of the deterrent compounds β-apopicropodophyllin (**2**) and (±)-(3*S*,4*R*,6*S*)-3-(3',4'-methylenedioxy-α-hydroxybenzyl)-4-(3",4"-dimethoxybenzyl)butyrolactone (**3**) (Figure 1.2). Although fraction C did not initially deter crayfish feeding (Figure 1.2), unusual $^1$H NMR signals motivated the purification of (-)-hibalactone (**4**), also known as (-)-savinin, from this fraction (Figure 1.3).

*Quantification of compounds **1–4***. We initially tested the deterrent fractions at twice their yield (by volume) to make up for assumed losses during purification, but compound quantification by LC-MS/MS showed that even doubling the presumed natural concentration did not approach the actual concentration occurring in the crude extract for each of the 4 compounds assayed (Table 1.2). The isolated yields of compounds **1** and **2** were 19% and 16% of their natural concentrations, respectively, while compounds **3** and **4** were isolated at only 1% and 8% of their natural concentrations, respectively (Table 1.2). When elevated to their natural concentrations, compounds **1** and **2** were both deterrent to all three consumers (Figure 1.3). In contrast, when we elevated compounds **3** and **4** to their natural concentrations, compound **3** was deterrent to *Procambarus*

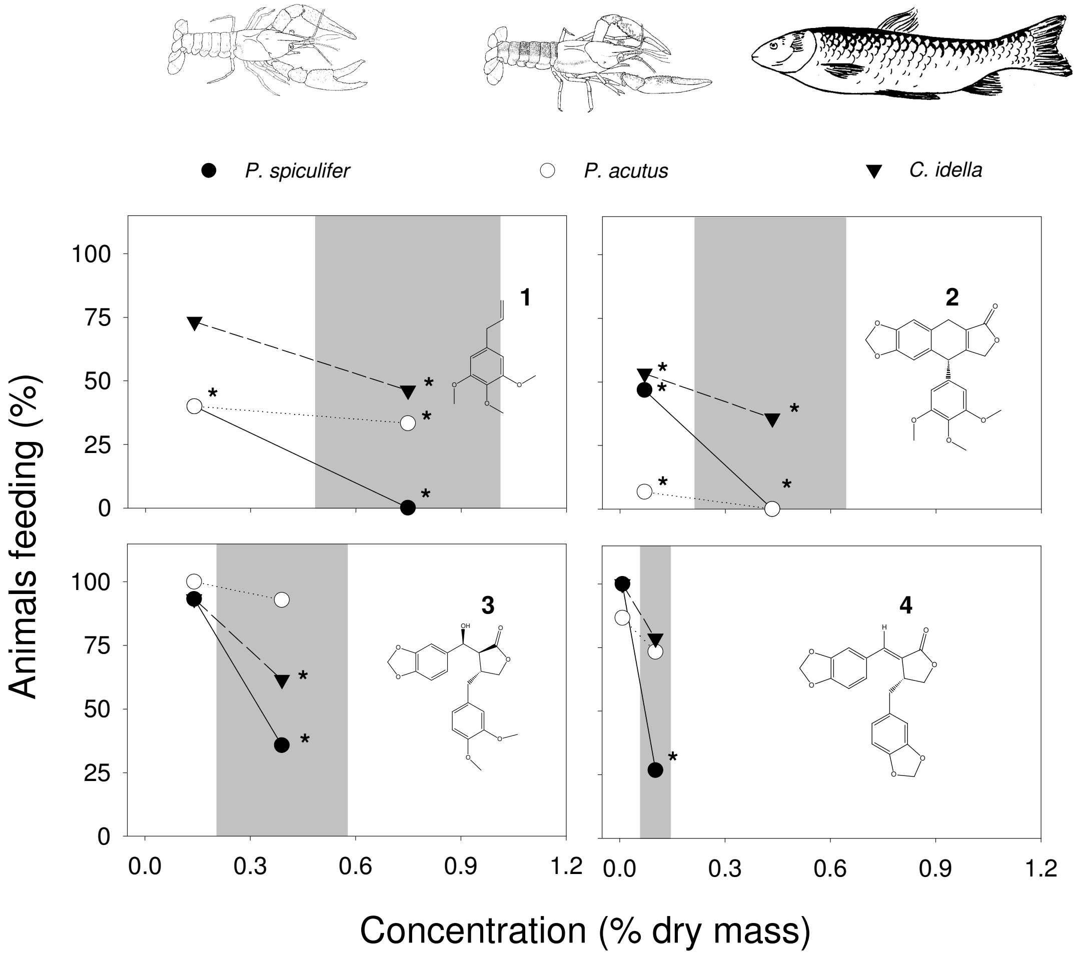


Figure 1.3. Percentage of 12-15 individual *Procambarus spiculifer* (filled circles), *P. acutus* (open circles), and *Ctenopharyngodon idella* (filled triangles) feeding on pellets containing A) 3,4,5-trimethoxyallylbenzene (1), B) β-apopicropodophyllin (2), C) (±)-(3S,4R,6S)-3-(3',4'-methylenedioxy-α-hydroxybenzyl)-4-(3",4"-dimethoxybenzyl)butyrolactone (3), and D) (-)-hibalactone (4). Asterisks denote statistically significant reductions in feeding relative to a palatable control for each consumer species ($P < 0.05$; Fisher's exact tests). The shaded area is the quantified natural concentration (by dry mass) ± 1 standard deviation (see Table 1.2); feeding assays to the left of this shaded area were conducted at the isolated yield of each metabolite.

Table 1.2. Isolated yield and quantitatively determined dry mass concentrations (% of dry mass ± SD) of four deterrent compounds isolated from the crude extract of *Micranthemum umbrosum*.

| **Quantification method** | **Concentration of compound (% of macrophyte dry mass)** | | | |
|---|---|---|---|---|
| | **1** | **2** | **3** | **4** |
| *Isolated yield (N=1)* | 0.14 | 0.07 | 0.0045 | 0.0078 |
| *LC-MS/MS (N=5) quantification* | 0.75 ± 0.26 | 0.43 ± 0.22 | 0.39 ± 0.19 | 0.10 ± 0.04 |

*spiculifer* and to grass carp, but not to *P. acutus*, whereas compound **4** was deterrent to *P. spiculifer* but not to the other consumers (Figure 1.3). For three of the four compounds tested, the magnitude of feeding depression appeared stronger for *P. spiculifer* than for the other consumers (Figure 1.3).

We also tested whether we had isolated all of the strongly deterrent compounds by assaying the crude extract minus the fractions containing the four isolated compounds (i.e., we used TLC to group fractions from the first silica gel column that lacked compounds **1–4**). None of our three test consumers were significantly deterred by this 'crude minus deterrent fractions' extract ($N = 13\text{-}15$ for each consumer species, % acceptance $\geq 86.7\%$, $P \geq 0.50$). However, given the significant compound degradation and/or inefficient yields that we observed (Table 1.2), it is possible that unknown, but potentially deterrent, compounds within this crude extract were tested at concentrations significantly lower than their natural levels.

*Experimental exclusion of herbivores*. At the initiation of the experiment, there was no difference in total plant cover ($P = 0.833$), the cover of *Micranthemum umbrosum* ($P = 0.089$), or the cover of *Ludwigia repens* ($P = 0.641$) among the open, cage control, and cage treatments (data not shown). After 16 months of excluding grass carp and other potential herbivores (e.g., crayfish, turtles), there was 2.4-fold more total plant biomass ($P = 0.004$, Figure 1.4a) and over 300-fold more *L. repens* ($P = 0.007$, Figure 1.4c) in the cage vs. open treatments. Biomass of the unpalatable macrophyte *M. umbrosum* was unaffected ($P = 0.774$, Figure 1.4b). Thus, herbivore exclusion allowed other species to

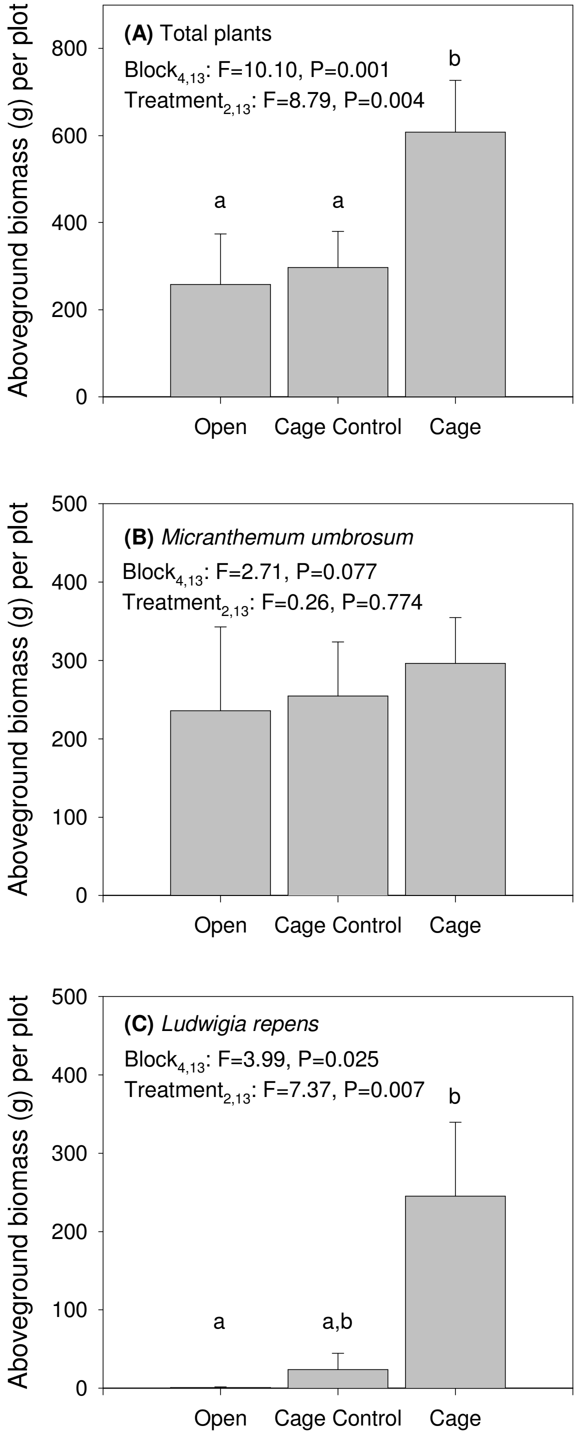


Figure 1.4. Final aboveground biomass per plot of A) all plants, B) *Micranthemum umbrosum*, and C) *Ludwigia repens* in open, cage control, and cage treatments after 16 months. Statistical results are from 1-way blocked ANOVAs. Bars that share the same letter were not statistically different from one another in Tukey tests.

increase in abundance but did not alter the abundance of the chemically defended *M. umbrosum*.

When offered a choice between two plant species, grass carp preferred *Ludwigia repens* over *Micranthemum umbrosum* by 14 to zero ($P < 0.001$), *Najas guadalupensis* over *M. umbrosum* by 11 to zero ($P < 0.001$), and *N. guadalupensis* over *L. repens* by 14 to zero ($P < 0.001$). The striking differences in preference for all contrasts clearly establishes a preference hierarchy of *N. guadalupensis* > *L. repens* > *M. umbrosum*.

## *Discussion*

It is a common pattern in marine and terrestrial habitats for selective feeding by herbivores to shift plant species composition towards chemically or structurally defended plants (Hay and Fenical 1988, Rosenthal and Berenbaum 1992, Hay 1997). In contrast, aquatic herbivores commonly reduce plant standing stock and alter species composition (Lodge 1991, Newman 1991, Cyr and Pace 1993, Lodge et al. 1998), yet experimental investigations linking herbivore feeding preferences to particular plant traits and ultimately to shifts in plant community structure are rare. Here, we show that five of the most common macrophytes collected from an aquaculture facility for herbivorous Asian grass carp, *Ctenopharyngodon idella,* were commonly unpalatable to three generalist consumers – non-native grass carp and the native North American crayfishes *Procambarus spiculifer* and *P. acutus*. The most common macrophytes - *Micranthemum umbrosum* and *Spirogyra* sp. - comprised 87% and 94%, respectively, of the total macrophyte cover in two grass carp ponds, and both were unpalatable to grass carp (Figure 1.1). *Spirogyra* appeared nutritionally inadequate to these consumers, and *M.*

*umbrosum* was chemically defended by at least four secondary metabolites (Figures 1.2, 2.3). When we excluded grass carp and other potential herbivores from experimental portions of one of the ponds, a plant that was preferred over *M. umbrosum - Ludwigia repens* - increased over 300-fold in the herbivore exclusion treatment. A nearby natural pond that lacked grass carp was dominated by *Najas guadalupensis,* a plant that grass carp preferentially consumed over both *M. umbrosum* and *L. repens*. Thus, selective feeding by grass carp effectively eliminates most palatable plants from this community and promotes the persistence of less palatable, chemically defended or nutritionally inadequate plants.

Grass carp and most crayfish species are generalist consumers that will eat a variety of plants (Parker and Hay 2005) but still selectively feed among species based on their structural, nutritional, and chemical traits (Cronin et al. 2002). However, knowledge of traits alone may not be predictive of feeding preferences among different consumer species (e.g., Hay et al. 1987b, Hay and Fenical 1996). For example, both the grass carp and crayfish *Procambarus acutus* rejected the filamentous green alga *Spirogyra* (Figure 1.1). The crayfish *P. spiculifer*, however, readily consumed *Spirogyra* (Figure 1.1), and in another study the crayfish *Pacifastacus leniusculus* preferred it over other aquatic plants (Warner and Green 1995). Among the five plant species that we tested, *Spirogyra* had the lowest protein content per volume of plant (Table 1.1), suggesting that nutritional inadequacy may explain its low palatability to *P. acutus* and to grass carp, but the variation among consumers (Figure 1.1, Warner and Green 1995) suggests that palatability depends on the palate of the consumer. Moreover, *Spirogyra* and other filamentous algae reportedly persist in these and other ponds only until submersed macrophytes have been selectively removed by grass carp (P. Williams, pers. obs., and reviewed in Van Dyke et al. 1984). This suggests that plants can delay or reduce herbivory by being nutritionally poor, but they may be unlikely to escape consumption once higher preference plants have been removed.

It is uncertain why *Ludwigia repens* was rejected in the fresh tissue assays (Figure 1.1). It was readily eaten over *Micranthemum umbrosum* in choice assays with fresh tissue, was readily eaten by all but one consumer as homogenized pellets (Figure 1.1b), and was eaten by all consumers when extracts were incorporated into a palatable control food (Figure 1.1c). The prostrate rush *Juncus repens* and the emergent rush *Juncus effusus* were both tough plants that may have been structurally defended from consumption by crayfishes (Table 1.1, Figure 1.1). However, at least one crayfish species rejected the softened, homogenized pellets of each species (Figure 1.1b), although no consumers rejected the chemical extracts (Figure 1.1c). Thus, it appears that both rushes could be structurally defended, but we cannot exclude the possibility that deterrent compounds in the softened foods were lost during the extraction process. Alternatively, the higher protein content of broc-let powder (Table 1.1) may have provided extra feeding incentives that counterbalanced deterrent chemistry. Other investigations have shown that consumers are more likely to feed on chemically defended but nutritionally-rich foods (Duffy and Paul 1992, Cruz-Rivera and Hay 2003).

Despite these ambiguities for some consumer and macrophyte species, *Micranthemum umbrosum* was clearly chemically distasteful to all three consumers (Figure 1.1), and we isolated four natural products that serve as chemical defenses against herbivory in this aquatic plant (Figures 1.2, 1.3). Each of the four compounds that we isolated from *M. umbrosum* has previously been described, but this is the first study to report on their ecological function as defenses against herbivory. Compound **1** is an essential oil commonly found in aromatic plants including nutmeg and parsley (De Vincenzi et al. 2004). It has been implicated as an antimicrobial compound (Marston et al. 1995), a growth inhibitor of green algae (Della-Greca et al. 1992), and as an insecticide (Miyazawa et al. 1992). β-Apopicropodophyllin (**2**) has previously been isolated from the Mexican medicinal plant *Hyptis verticillata* 'bushmint' and is from a class of lignoids active against several cancer cell lines (Schrecker and Hartwell 1952, Aiyar and Chang 1977, Buchardt et al. 1986, Novelo et al. 1993). Compound **3** had been synthesized before but was not previously known as a natural product (Ganeshpure and Stevenson 1981, Pelter et al. 1988). Lignan **4** occurs in juniper and several woody plant species (e.g., Hartwell et al. 1953); it inhibits prostaglandin E2 production (Ban et al. 2002), tumor necrosis factor -α production and T cell proliferation (Cho et al. 2001), and is a synergist for insecticides (Matsubara 1972). Despite isolating these four compounds that depressed herbivore feeding, we lost from 81% to 99% of the natural concentrations of these molecules during isolation procedures (Table 1.2). Given this poor yield, it is possible that additional deterrents were present but recovered at concentrations too low to be biologically active.

Prior to this study, there were only three freshwater plants with described herbivore feeding deterrents – watercress, *Rorippa nasturtium-aquaticum* (L.) Hayek (Newman et al. 1990, Newman et al. 1996), the waterspider bog orchid, *Habenaria repens* Nutt. (Bolser et al. 1998, Wilson et al. 1999), and lizard's tail, *Saururus cernuus* L. (Kubanek et al. 2000, Kubanek et al. 2001) – with a total of nine described secondary metabolites demonstrated to influence herbivore feeding. Our study brings the new total of described herbivore anti-feedants in freshwater plants to 13. Of these 13, 10 are lignoids, including three in this study and seven compounds isolated from *S. cernuus* (Kubanek et al. 2001). This general, though still preliminary, pattern suggests that lignoids – of which several thousand have been described from numerous plant taxa (Seigler 1998, Ward 1999) – are common, but often overlooked, defensive compounds warranting additional study.

Plant defense theory predicts that chemically-defended plants will have fewer resources for growth and thus will be competitively displaced by less defended plants when herbivore pressure is lessened (Herms and Mattson 1992). To test this hypothesis, we excluded grass carp for 16 months and documented a 300-fold increase in the abundance of *Ludwigia repens* (Figure 1.4), a plant that was preferred over *Micranthemum umbrosum* in a choice feeding assay. We did not see, however, a decrease in the abundance of *M. umbrosum* in the cage treatments (Figure 1.4). Thus, although chemical defenses in *M. umbrosum* appear to promote its persistence of in the face of intense herbivory, we saw little evidence to suggest competitive displacement of *M. umbrosum* by *L. repens* in the absence of herbivores. There are several potential explanations. Our experiments ran through two growing seasons, but the long history (>20 years) of grass carp herbivory in this habitat may have consistently excluded other species and reduced the potential pool of new colonists exhibiting high growth, low defense strategies. Additionally, the only species that did show a large increase in abundance - *L. repens* - is also relatively unpalatable (Figure 1.1), and thus may not be a much better competitor than *M. umbrosum*. Moreover, despite the long-standing view that constructing and storing defensive compounds is physiologically costly and detracts from growth and reproduction, empirical evidence is conflicting (Koricheva 2002), suggesting that investment in chemical defense need not necessarily restrain growth and competitive ability. Finally, grass carp will repeatedly sample foods even if they don't ingest the plants (P. Williams, pers. obs.); this chronic sampling may have depressed *M. umbrosum* abundance in the open and cage control treatments and obscured competitive effects in the cage treatments. Nevertheless, on a percentage basis, excluding herbivores led to dramatic increases in *L. repens* that reduced the relative abundance of *M. umbrosum* from 89% to 54% of the total plant community, indicative of chemical defenses promoting the dominance of *M. umbrosum* in this plant community.

Grass carp were introduced into North America in the 1960s to suppress invasive aquatic plants (e.g., hydrilla) that were displacing native vegetation (Van Dyke et al. 1984). However, grass carp feed on a variety of native and exotic aquatic plants (Parker and Hay 2005), and their use as biocontrol agents has been curtailed due to their strong negative effects on native plant communities (Bain 1993). They can eliminate all vegetation and have cascading effects on native food webs (Krzywosz et al. 1980, Van Dyke et al. 1984, Maceina et al. 1992, Bain 1993, McKnight and Hepp 1995, Hanlon et al. 2000, Bonar et al. 2002, Kirkagac and Demir 2004). In our study, grass carp ponds were populated only by the most distasteful, structurally defended, or nutritionally inadequate plants (Figure 1.1, 1.4), suggesting that grass carp herbivory drives plant communities towards species that may be poor foods for native consumers, consistent with other studies showing that exotic herbivores often have negative impacts on native species by negatively affecting native plants (Holmgren 2002, Parker et al. submitted).

Herbivory in freshwater systems is more important than previously thought (Lodge and Lorman 1987, Feminella and Resh 1989, Newman 1991, Cyr and Pace 1993, Lodge et al. 1994, McKnight and Hepp 1995, Lodge et al. 1998), and freshwater plants are frequently chemically or structurally defended from consumers (Newman et al. 1996, Bolser et al. 1998, Cronin 1998, Kubanek et al. 2001, Cronin et al. 2002, Prusak et al. 2005). Rarely, however, have the mechanisms of deterrence (e.g., structural or chemical defenses) been linked to the broader context of community structure. Here, we show that selective herbivory by grass carp shifts the species composition of freshwater plant communities towards plants that are distasteful, structurally defended, or nutritionally inadequate (Figures 1.1, 1.4), suggesting that plant defenses can play critical yet understudied roles in the structure of freshwater plant communities.

# CHAPTER 2
# CONTEXT-DEPENDENT EFFECTS OF BEAVER GRAZING ON FRESHWATER MACROPHYTES

## *Abstract*

Beavers (*Castor canadensis*) eat both woody and non-woody plants, yet their impacts on non-woody plant populations and communities are often attributed to ecosystem engineering rather than herbivory. Here, we manipulated beaver feeding via cage exclusion experiments to show that beavers reduce the total mass of herbaceous aquatic macrophytes by about 50% and produce dramatic effects on macrophyte species composition. Although herbivory strongly affected the standing stock of these plant communities, impacts to particular species varied between wetlands and appeared to be context dependent. Bur-reed *Sparganium americanum* occurred at each of two wetlands investigated; it was strongly impacted by beavers at the site where it was the dominant plant but not at the site where it co-occurred with the more palatable and abundant *Saururus cernuus* (lizard's tail). By contrast, losses to grazing were moderated for lizard's tail where it co-occurred with woolgrass sedge tussocks (*Scirpus cyperinus*) that were generally avoided by beavers. Thus, lizard's tail may have served as attractant plant that reduced impact on bur-reed, whereas woolgrass tussocks provide lizard's tail with an associational escape from herbivory. Our results suggest that beavers can profoundly alter the community structure of aquatic macrophyte communities, but the strength of these effects for particular species depends on the identity and abundance of neighboring plants.

## *Introduction*

Herbivores were historically thought to have little impact on freshwater plant communities (e.g., Shelford 1918, Rosine 1955, Hutchinson 1975). Recent reviews, however, show that herbivores remove as much or more of the plant standing stock in freshwater systems as they do in marine and terrestrial systems (Newman 1991, Cyr and Pace 1993, Lodge et al. 1998). Early misconceptions about the importance of herbivory in freshwater communities arose from a misplaced focus on insects and snails as major herbivores rather than larger consumers (Lodge et al. 1998). Instead, the herbivores with the greatest impact in aquatic systems tend to be generalist consumers like waterfowl, crayfish, mammals, and fish (Lodge et al. 1994, Van Donk and Otte 1996, Santamaria 2002, Qvarnemark and Sheldon 2004). Despite the recognition of the importance of large consumers for aquatic plant communities, relatively few experimental studies have quantified the impact of these generalist consumers.

Beavers (*Castor canadensis*) are ecosystem engineers well-known for their abilities to alter plant communities by felling trees, constructing dams, and digging canals (Jones et al. 1994). The impacts of beavers on plant communities can be extensive. At the landscape scale, beavers can increase plant diversity by 33% compared to areas without beavers because beaver activity promotes the persistence of herbaceous wetland plants found only in association with beavers (Wright et al. 2002). Best known for eating woody plants and shrubs (Fryxell and Doucet 1993), beavers are thought to affect wetland plant communities indirectly though ecosystem engineering effects such as changes in water depth, sediment composition, and flood plain geomorphology (Naiman et al. 1988, Naiman et al. 1994, Pollock et al. 2003). However, beavers often spend the majority of their time foraging for herbaceous plants, particularly in the summer when new woody growth is limited, and occasionally even prefer herbaceous plants over most woody vegetation in cafeteria-style feeding assays (Jenkins 1980, Svendsen 1980a). However, these studies primarily assessed beaver diet choices, whereas few studies have examined how beaver foraging influences the structure of aquatic macrophyte communities.

Here, we experimentally evaluated the impacts of beavers on herbaceous wetland plants by excluding beavers and monitoring changes in plant species composition and above-ground biomass after 13-15 months. We also conducted a short-term experiment testing whether *Saururus cernuus*, a plant that was strongly impacted by beavers in our long-term experiment, gains an associational refuge from beavers by living within the thick foliage of sedge tussocks. We found that beavers had strong effects on the standing stock and species composition of wetland plant communities, but effects on particular species varied as a function of other plants in the community.

## *Materials and Methods*

*Site Description.* Fieldwork was conducted in three beaver wetlands in separate watersheds located along the Chattahoochee River in the Chattahoochee River National Recreation Area (CRNRA,) in Atlanta, Georgia, USA. Johnson Ferry wetland ($33^o$ 54.7' N, $84^o$ 24.3' W) is approximately 5 km north of the Gumby Swamp wetland ($33^o$ 54.6' N, $84^o$ 27.0' W), which is approximately 1 km north of the Cochran Shoals wetland ($33^o$ 54.3' N, $84^o$ 26.8' W). All three wetlands drain into the northern bank of the

Chattahoochee River. At the onset of the study, beaver activity at all three wetlands was conspicuous, including active lodge and dam building, uprooted macrophytes, torn and chewed woody vegetation, beaver tracks, and beaver canalization. We also occasionally observed other potential herbivores at sites, including one swamp rabbit (*Sylvilagus aquaticus* Bachman), mallards (*Anas platyrhynchos* L.), and two American snapping turtles (*Chelydra serpentina*), all of which are either herbivorous or omnivorous and known to include plants in their diet. Although our methods would also exclude these herbivores, there was no evidence (e.g., tracks in the vicinity of torn vegetation) to suggest their impact approached that of beavers. Consequently, we assumed that observed herbivory was by beavers, though we could not rule out the potential for minor herbivory by these or other species.

*Long-term exclusion cages.* We evaluated the impacts of beavers on wetland macrophytes by experimentally excluding beavers and monitoring changes in plant species composition and above-ground biomass over 13-15 months. In late March, 2003, we established six treatment blocks in each wetland with three treatments in each block: 1) an open treatment allowing herbivore access, 2) a 3-sided cage control allowing herbivore access, but controlling to some extent for the presence of caging materials, and 3) a 4-sided cage excluding herbivores. Each block consisted of three 122 x 122 cm plots arranged in an "L" with one plot at the corner of the L. Treatments were randomly assigned to the three plots, with the stipulation that the open treatment had to occupy either end of the L. Cage treatments were constructed of 91 cm tall wire fencing (5.1 x 7.6 cm mesh galvanized steel "rabbit fence") affixed to four 122 cm steel rebar posts.

The cage control was a similarly constructed three-sided cage that shared one wall with the cage treatment. The open treatment abutted one side of the cage or cage control treatment and was delineated with steel rebar posts but no mesh fencing. Plots were established at similar depths across treatments ($P = 0.882$) but were in significantly deeper water at Gumby Swamp (45 ± 2 cm, mean ± SE) than Cochran Shoals (31 ± 1 cm) and Johnson Ferry (25 ± 2 cm, $P < 0.001$, 2-way blocked ANOVA). To assess whether vegetation varied across treatments at the onset of the experiment, we placed a 1.0 $m^2$ quadrat atop each treatment and recorded the identity of plants present within each of 81 10 x 10 $cm^2$ gridded squares in the quadrat. This method resulted in more than one species per cell, thus we analyzed whether the frequency of the species comprising >90% of the total vegetation in our treatments varied. At Cochran Shoals, there were no differences in the initial frequency of *Sparganium americanum* ($P = 0.402$), *Saururus cernuus* ($P = 0.402$), or *Ludwigia palustris* ($P = 0.455$) across the cage, cage control, or open treatments. At Gumby Swamp, there were no differences in the initial frequency of *Sparganium americanum* ($P = 0.268$), *Ludwigia palustris* ($P = 0.263$), or *Polygonum densiflorum* ($P = 0.303$). At Johnson Ferry, there were no differences in the initial frequency of *Spirogyra* sp. ($P = 0.927$), *Potamogeton diversifolius* ($P = 0.467$), or *Ludwigia palustris* ($P = 0.643$).

In April, 2003, we noticed that beaver activity at Cochran Shoals was highly concentrated in particular areas of the marsh, whereas some areas where we had placed cages appeared to be in areas that were not visited. To increase the spatial coverage of our cages and capture these strong impacts, on April 16 we established four additional blocks (constructed as before) in areas of apparent beaver activity. As before, there were no differences in the initial frequency of the most abundant plants; *Sparganium americanum* (*P* = 0.499), *Saururus cernuus* (*P* = 0.958), *Ludwigia palustris* (*P* = 0.249), and *Polygonum* spp. (*P* = 0.095). Similarly, on June 23, 2003, we also established four additional blocks (constructed as before) in areas of apparent beaver activity at Gumby Swamp. There were no differences in the initial frequency of *Sparganium americanum* (*P* = 0.290), *Polygonum densiflorum* (*P* = 0.648), or *Juncus effusus* (*P* = 0.213).

In fall of 2003, the National Park Service informed us that local government officials had trapped and removed beavers from the Johnson Ferry wetland. In subsequent visits to this wetland, there were few signs of beaver activity. Thus, we considered the Johnson Ferry wetland a 'control' site where beavers had been experimentally removed but other herbivores may have been present (e.g., rabbits, waterfowl, turtles, deer, etc.). To determine whether these remaining herbivores impacted vegetation at this wetland in the absence of beavers, we analyzed the final frequency of the most abundant plant species in our treatments.

In late July, 2004, after 13-15 months of intact treatments, we harvested all above-ground plant biomass from one-half of each treatment at both Cochran Shoals and Gumby Swamp. We harvested only half of each plot to minimize disturbance to wetland plant communities, with the harvested side being randomly selected. Live plants were clipped at the sediment surface, sorted to species, and weighed to the nearest g. Approximately two weeks prior to harvest, both wetlands had naturally drained; thus drying plants to remove surface water weight was unnecessary. We also collected and weighed the accumulated leaf litter on the sediment surface in each treatment. We analyzed the final above-ground plant biomass for all plants combined, for those species that were abundant, and for the total leaf litter in each treatment with a blocked two-way ANOVA. Data were square root transformed where necessary to meet ANOVA assumptions.

*Indirect effects of sedge tussocks on lizard's tail.* In early June, 2003, we noticed that the emergent plant lizard's tail, *Saururus cernuus,* was abundant within the cages but not the open treatments at the Cochran Shoals wetland (this species was not present at Gumby Swamp, see Results). Additionally, lizard's tail was rare throughout the rest of the wetland except for where it grew intermixed with woolgrass sedge tussocks, *Scirpus cyperinus* (L.) Kunth. We hypothesized that sedge tussocks might impede beaver foraging and provide *S. cernuus* an associational refuge from herbivory (Hay 1986). To determine whether *S. cernuus* was positively correlated with the presence of sedge tussocks, we counted the number of *S. cernuus* shoots emerging from each of 50 individual sedges. We paired these observations with counts from 50 equal sized areas adjacent to each sedge tussock. To obtain equal sized areas, we used a telescoping ring that approximated the mean diameter of each tussock of interest. Control plots were selected using a random compass direction and were placed within 20 cm of the tussock edge. We analyzed whether the frequency or density of *S. cernuus* shoots differed within vs. outside of sedge tussocks with a Fisher's exact test and a paired t-test, respectively.

To determine if *Saururus cernuus* gained an associational defense from beaver herbivory by growing within sedge tussocks, we conducted a factorial experiment excluding beavers and manipulating the presence or absence of sedge foliage. We identified 60 sedges of roughly similar size and counted the total number of leaves on *S.*

*cernuus* shoots within the perimeter of each tussock as a measure of *S. cernuus* abundance. We then used shears to clip and remove the above-ground sedge foliage from 30 randomly selected sedge tussocks, leaving the *S. cernuus* shoots intact. We placed standard tomato-cages reinforced with 16-gauge galvanized wire (giving a mesh size of approximately 6.0 x 10.0 $cm^2$) around 15 of the clipped sedges and 15 of the unclipped sedges. We trimmed back re-grown sedges every few days. Thus, our design resulted in a crossed factorial with the following treatments: (1) no clipping, no cage = ambient herbivory or control, (2) no clipping, cage (3) clipping, no cage, (4) clipping, cage. This design allowed us to test the effects of beaver herbivory on *S. cernuus* in the presence and absence of sedge foliage.

After two weeks we counted the number of leaves remaining on *Saururus cernuus* shoots within each treatment. The % change in the total number of leaves over this time period for each treatment was analyzed with a 2-way ANOVA followed by Tukey tests. Four replicates (one from each treatment) were excluded from the analysis because two cages had been pushed over and grazed by beavers, and two open treatments appeared to have been trampled by humans.

## *Results*

*Long-term exclusion cages*. At Johnson Ferry where beavers had been removed but other potential herbivores remained, there were no differences in the final frequency of plants comprising >90% of the total vegetation: *Ludwigia palustris* (*P* = 0.462), *Polygonum* spp. (*P* = 0.146), *Sagittaria latifolia* (*P* = 0.117), *Leersia oryzoides* (*P* = 0.116), or *Juncus* *accuminuatus* (*P* = 0.121). There were also no visual indications that biomass differed across the treatments. By contrast, there were consistent signs of beaver activity at both Cochran Shoals and Gumby Swamp throughout the study (Figure 2.1a), and biomass was noticeably greater inside the cages (Figure 2.1b). Excluding beavers at Cochran Shoals and Gumby Swamp produced a 2.9X increase in above-ground plant biomass compared to open plots (Figure 2.2a). Excluding beavers had similarly large impacts on plant biomass at each wetland (Fig. 2.2b, c). Plant mass did not differ between open area and cage control treatments, suggesting that cages did not introduce substantial artifacts affecting plant growth. The lack of a significant site x cage interaction term (*P* = 0.702) indicated that grazing effects on total above-ground plant biomass were consistent between the two wetlands (Figure 2.2). Significant block effects occurred for all contrasts, indicating the spatial patchiness of plant mass and type throughout both wetlands. The significant site effect (Figure 2.2a) simply indicates the greater mass of plants across all treatments at Cochran Shoals versus Gumby Swamp.

Two abundant plant species were strongly affected by beavers. Bur-reed, *Sparganium americanum*, was the only plant that was present in our cages at both wetlands; considering data from both wetlands, it was 1.7X more abundant inside the cage vs. the open plots (Figure 2.3a). However, the significant site x cage interaction term (*P* = 0.025) indicates that this effect varied between wetlands. Bur-reed was

Figure 2.1. (a) Beaver foraging near one of our cages. (b) View of one experimental block showing 3-sided cage control, cage, and open treatment at Cochran Shoals wetland. Note presence of lizard's tail, *Saururus cernuus*, inside the cage treatment, but torn and uprooted vegetation outside of the protected area.

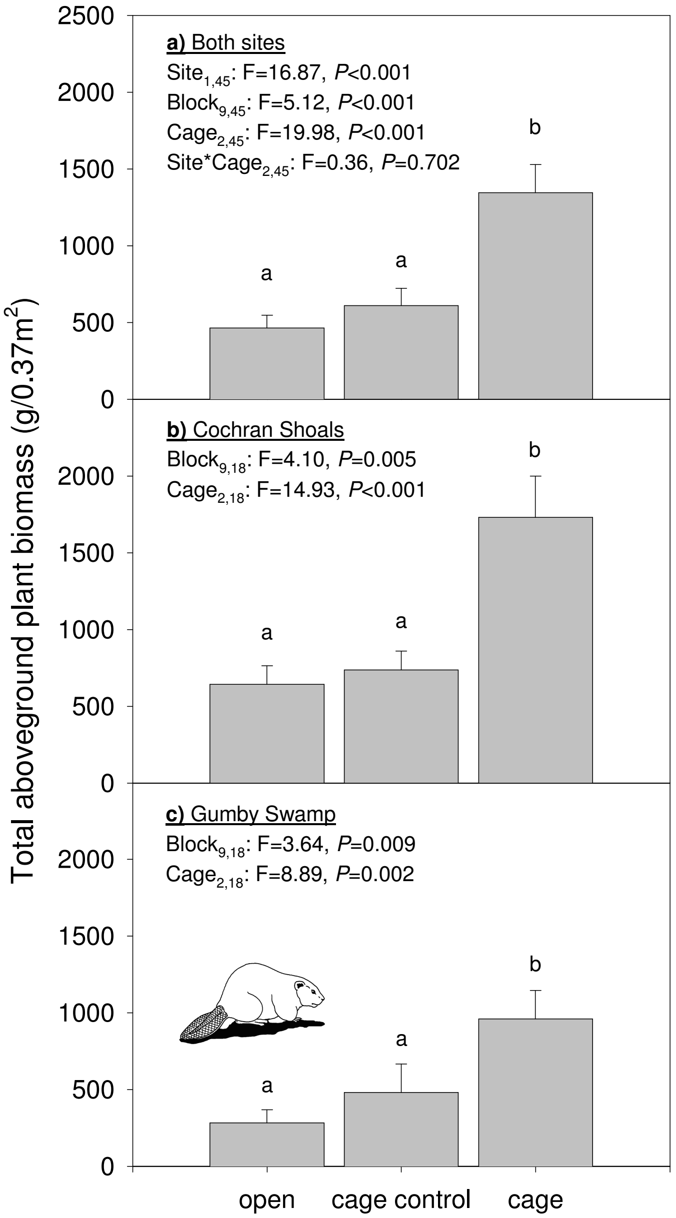


Figure 2.2. Final above-ground plant biomass per ½ plot for a) both sites, b) Cochran Shoals, and c) Gumby Swamp in each treatment type after 13-15 months. Statistical results are from 2-way (both sites) or 1-way (individual sites) blocked ANOVAs. Bars that share the same letter were not statistically different from one another in Tukey tests.

unaffected by grazing at Cochran Shoals (Figure 2.3b), but at Gumby Swamp it was 9X more abundant inside the cage plots than in the open plots (Figure 2.3c). Lizard's tail, *Saururus cernuus*, was not present at Gumby Swamp, but at Cochran Shoals it was 180X more abundant inside cages versus the open plots where it was nearly absent (Figure 2.4). The remaining species at each wetland were either too patchily distributed or too scarce to have their responses adequately captured by the cages we deployed.

Beaver activity also strongly affected litter accumulation. Across both wetlands, leaf litter accumulation was 5X greater inside the cages than in the open plots (Figure 2.5a). Caging increased litter by 4.6X at Cochran Shoals and by 6.4X at Gumby Swamp (Figure 2.5b,c). The intermediate value for litter accumulation in cage controls at Gumby Swamp (Figure 2.5c) suggests that cage artifacts could have contributed somewhat to the pattern at this location.

*Indirect effects of sedge tussocks on lizard's tail.* At the Cochran Shoals swamp, shoots of lizard's tail, *Saururus cernuus,* occurred about 3X more frequently inside sedge tussocks than in equal sized adjacent areas lacking tussocks (Table 2.1). Additionally, shoot densities inside sedge foliage were 10X higher than outside of sedge foliage (Table 2.1). Two-weeks after we clipped back sedge foliage in some plots, leaf abundance of *S. cernuus* had increased by 73-93% in areas that were clipped and protected by a cage, or areas where sedges were not clipped, but abundance of *S. cernuus* declined by 43% in areas where sedge was clipped but not caged and thus exposed to beaver feeding (Figure 2.6). Losses in the clipped and uncaged treatment differed significantly from the changes in all other treatments ($P<0.001$, Figure 2.6). Contrasts among the other three treatments

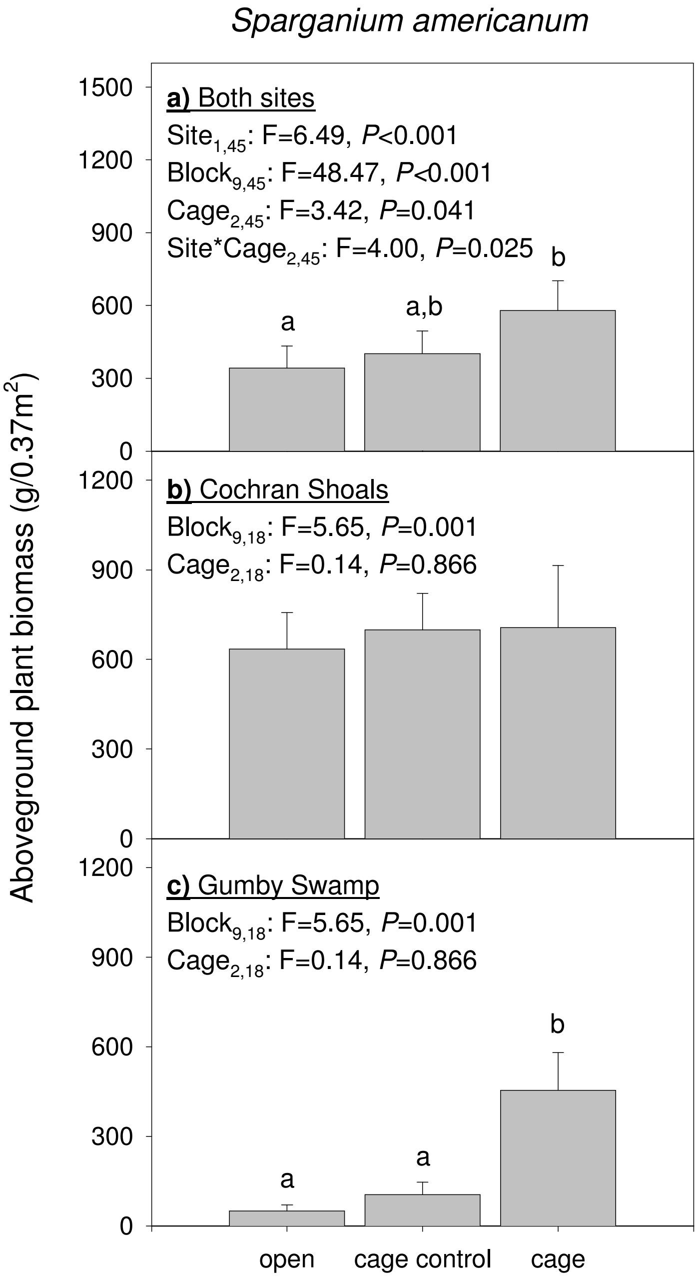


Figure 2.3. Final above-ground plant biomass for bur-reed *Sparganium americanum* per ½ plot for a) both sites, b) Cochran Shoals, and c) Gumby Swamp in each treatment type after 13-15 months. Statistical results are from 2-way (both sites) or 1-way (individual sites) blocked ANOVAs. Bars that share the same letter were not statistically different from one another in Tukey tests.

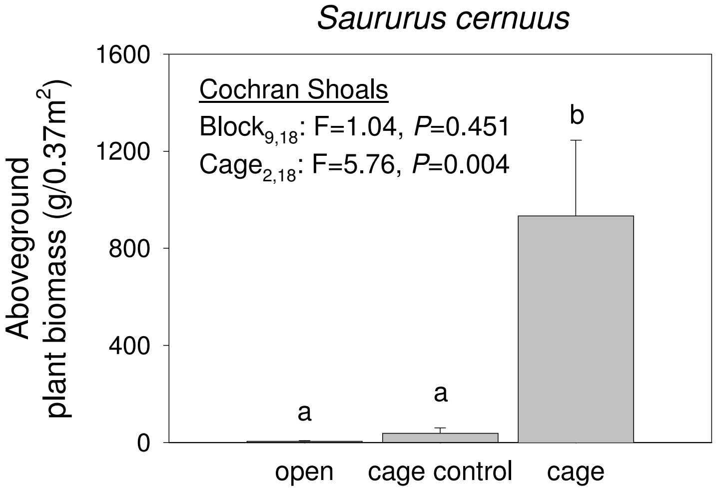


Figure 2.4. Final above-ground plant biomass for lizard's tail *Saururus cernuus* per ½ plot for Cochran Shoals in each treatment type after 13-15 months. Statistical results are from a 1-way blocked ANOVA. Bars that share the same letter were not statistically different from one another in Tukey tests.

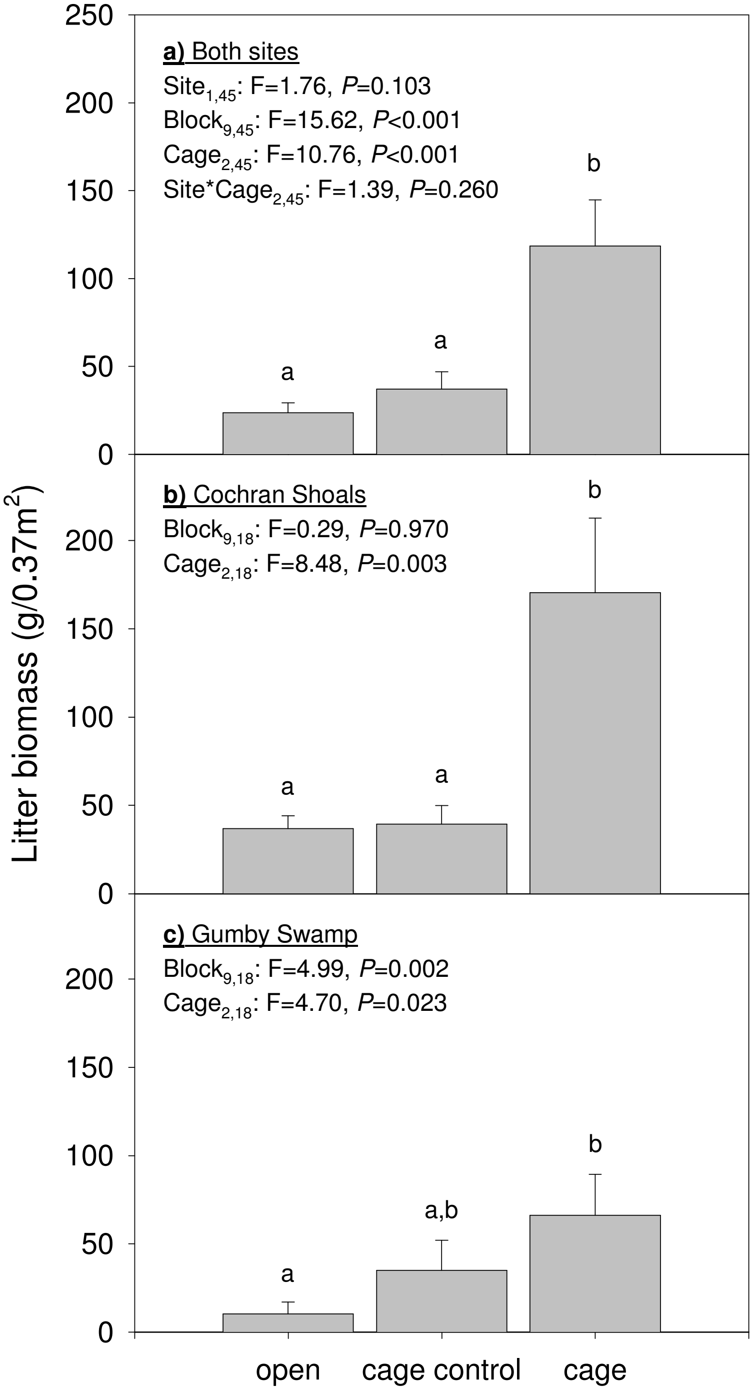


Figure 2.5. Final above-ground litter biomass per ½ plot for a) both sites, b) Cochran Shoals, and c) Gumby Swamp in each treatment type after 13-15 months. Statistical results are from 2-way (both sites) or 1-way (individual sites) blocked ANOVAs. Bars that share the same letter were not statistically different from one another in Tukey tests.

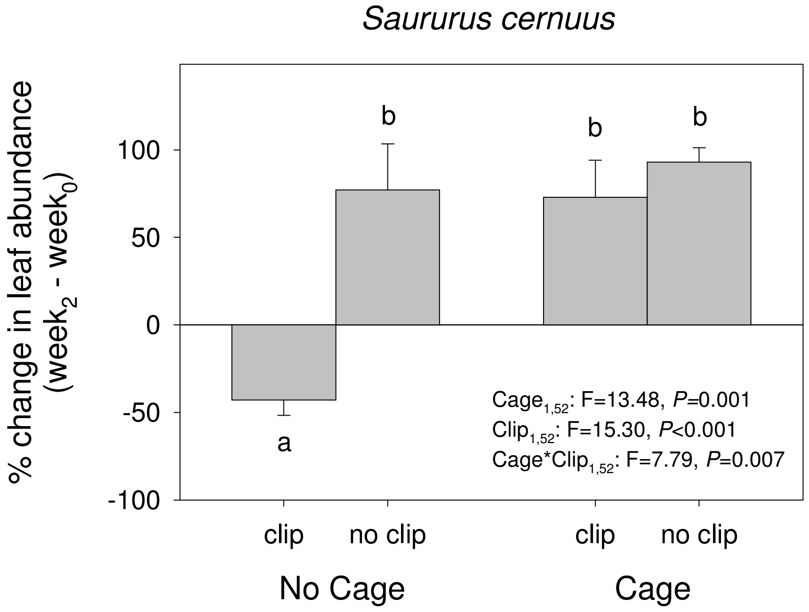


Figure 2.6. The percent change in leaf abundance for lizard's tail, *Saururus cernuus*, plants two weeks after we clipped back the surrounding foliage of the sedge *Scirpus cyperinus* and then left these plants either unprotected from beaver herbivory or protected with a cage. Statistical results are from a 2-way crossed ANOVA. Bars that share the same letter were not statistically different from one another in Tukey tests.

Table 2.1. The frequency and density of lizard’s tail, *Saururus cernuus*, plants located inside sedge tussocks or in equal-sized adjacent areas lacking sedges. Statistics are from a Fisher’s exact test (frequency) and paired t-test (density).

| | Inside sedge tussock | Outside Sedge tussock | |
|---|---|---|---|
| No. of plots (out of 50) with *Saururus cernuus* | 38 | 14 | $P < 0.001$ |
| Mean (± SE) no. of *S. cernuus* shoots | 6.7 ± 1.5 | 0.64 ± 0.18 | $P < 0.001$ |

did not differ, indicating that sedge tussocks were as effective as cages at excluding beaver foraging.

## *Discussion*

Beavers are well-known ecosystem engineers that can impact herbaceous plant communities via flooding and other changes to edaphic or hydrologic conditions (Naiman et al. 1988, Naiman et al. 1994, Pollock et al. 2003). Generally known for their tree-felling and strong effects on riparian vegetation, beavers also preferentially feed on herbaceous plants throughout much of the season in some locations (Jenkins 1980, Svendsen 1980b), and thus may directly impact herbaceous plants in beaver wetlands. In our study, plant biomass in treatments accessible to beavers was reduced to less than half the biomass occurring in areas that were caged to exclude beavers (Figure 2.2). Exclusion cages also caused dramatic shifts in species composition; the Cochran Shoals site switched from dominance by bur-reed *Sparganium americanum* to dominance by lizard's tail *Saururus cernuus* when cages were erected (compare Figure 2.2 with Figures 2.3 and 2.4). These effects appeared to result from beaver herbivory, as we commonly observed beaver tracks leading away from plants that were missing substantial portions of foliage (Figure 2.1a), and the one wetland where beavers were removed lacked such strong impacts. Other studies have also described beavers' affinity for herbaceous wetland plants (Jenkins 1980, Svendsen 1980b, Fryxell and Doucet 1993), suggesting that beaver feeding on aquatic macrophytes may be more common than currently recognized. These results also demonstrate the strong impacts that generalist, often vertebrate, herbivores have on freshwater macrophyte communities (reviewed in Lodge et al. 1998), despite traditional claims that herbivory is unimportant to the ecology and evolution of freshwater plants (e.g., Shelford 1918, Rosine 1955, Hutchinson 1975).

Bur-reed, *Sparganium americanum*, was the only plant species found in both wetlands and it was strongly suppressed by beavers at Gumby Swamp but not at Cochran Shoals (Figure 2.3). This variance could be due to the presence of more palatable, alternative macrophytes at the Cochran Shoals site. Beavers exhibit clear preferences for specific plant species when multiple choices are presented in cafeteria-style trials (e.g., Doucet and Fryxell 1993), but the choice of any particular species will be affected by the others that are available. The selective removal of lizard’s tail, *Saururus cernuus,* from nearly all open areas at Cochran Shoals (Figures 2.4 and 2.6) is consistent with beavers preferentially attacking lizard’s tail over bur-reed at this site. In fact, lizard’s tail may have served as an ‘attractant’ plant that reduced herbivory on the less-preferred bur-reed at this wetland (Atsatt and O'Dowd 1976). In contrast, lizard’s tail was absent from Gumby Swamp, thus the strong impacts of beavers on bur-reed at Gumby Swamp may have occurred because the alternative food sources at that wetland, primarily soft rush *Juncus effusus*, dense knotweed *Polygonum densiflorum*, and parrotfeather *Myriophyllum aquaticum*, were lower preference than bur-reed. These patterns suggest that the differential impacts to bur-reed may have arisen due to differences in the identity of its neighbors at the two wetlands, an interaction termed ‘associational susceptibility’ when a plant is selectively attacked where it co-occurs with less preferred neighbors (Brown and Ewel 1987, White and Whitham 2000). Whether the absence of lizard’s tail at Gumby Swamp was related to beaver or other factors is unknown, but the strong effects at

Cochran Shoals suggests that herbivory could play a role in either extirpating this species or preventing its colonization.

In contrast to associational susceptibility, plants that are highly susceptible to herbivores can gain associational resistance by living in close proximity to plants that are avoided by herbivores (Atsatt and O'Dowd 1976, McNaughton 1978, Hay 1986). In our study, lizard's tail was largely absent from open areas of Cochran Shoals that were available to beavers, but the plants appeared to escape detection or removal by associating with a sedge (*Scirpus cyperinus)* that rarely exhibited evidence of attack by beavers. Lizard's tail was 10x more abundant at Cochran Shoals when associated with sedge tussocks (Table 2.1). However, when we removed sedge foliage from some plots, lizard's tail plants lost about ½ of their foliage within two weeks, while plants protected by either sedge foliage or cages increased their foliage by 73-93% (Figure 2.6). Thus, lizard's tail gained a spatial refuge from herbivory by associating with the dense foliage of sedge tussocks. *Scirpus* sedges have stout, silica-rich stems that may have physically deterred beaver foraging on lizard's tail plants associated with these sedges. Our findings are similar to other studies where tussock-forming sedges facilitate plant diversity by impeding vertebrate herbivores (Levine 2000). Similar positive effects of sedge tussocks on wetland plant diversity have been noted previously (Ervin and Wetzel 2002, Ervin 2005). Interestingly, approximately one month after we terminated this relatively short-term experiment, beavers essentially clear-cut all of the sedges in the Cochran Shoals wetland, leaving behind the clipped sedge vegetation but removing lizard's tail plants. Thus, sedge plants provide a spatial refuge from herbivory, but this refuge was temporally limited to the time period when beavers were unwilling or unable to forage within the sedge foliage.

Although beavers had strong impacts on lizard's tail, this same plant is unpalatable and chemically noxious to crayfish (Kubanek et al. 2000, Kubanek et al. 2001). It is not uncommon for plant chemical defenses to be effective against one consumer but not another (e.g., Schupp and Paul 1994), suggesting that the lignoids that render lizard's tail repugnant to crayfish are inadequate defenses against beaver browsing, or alternatively that the populations of lizard's tail at our study site differed chemically from the populations studied by Kubanek et al. (2001). Additionally, we cannot rule out the possibility that some 'herbivory' may be related to ecosystem engineering rather than foraging. For example, beavers often clear vegetation and other debris to deepen water channels for predator escape routes and access to foraging areas (Muller-Schwarze and Sun 2003). Removing *S. cernuus* may be particularly useful given that its stout rhizomes form thick mats that might impede pond engineering. In support of this hypothesis, we often observed floating, uprooted lizard's tail rhizomes in areas where beavers were actively digging. However, the upright foliage from these rhizomes appeared to have been bitten, suggesting that beavers were still consuming lizard's tail foliage. Thus, we cannot definitively say that beaver impacts were due solely to herbivory, though the net impact of both herbivory and pond engineering clearly had strong impacts on the abundance of lizard's tail in this study (Figures 2.4 and 2.6).

In addition to their direct impacts on wetland plants, beavers could also indirectly influence wetland communities by altering detrital inputs and processing. Leaf litter from both allocthonous and autochthonous sources are important sources of organic matter in freshwater systems (Vannote et al. 1980), often fueling higher trophic levels (Wallace et al. 1999). In our study, beaver activity reduced the amount of leaf litter that accumulated inside the open treatments, generally because the dense stands of plants inside the cages died and senesced *in situ*, whereas treatments exposed to beavers had less standing vegetation to produce litter. Thus, by decreasing plant abundance, active beaver foraging may reduce the overall input of leaf litter from autochthonous sources into beaver ponds. Additionally, beaver swimming and crawling activities analogous to bioturbation may have increased leaf breakdown rates and contributed to the observed differences in leaf litter standing stock. Coupled with alterations in the species composition of macrophyte communities, beavers could indirectly influence carbon flow, detrital processing, nutrient availability, and ultimately production of higher trophic levels in beaver ponds by altering the quality and quantity of carbon sources, similar to results with other large herbivores (McInnes et al. 1992, Wardle et al. 2002).

Herbivory on freshwater macrophytes has historically been considered rare (e.g., Shelford 1918, Rosine 1955, Hutchinson 1975), but recent studies and reviews show that herbivory by large generalist consumes can profoundly affect freshwater communities (Lodge et al. 1998, and references therein). Beavers have long been recognized as important in creating wetlands and affecting stream and riparian community structure and function (Naiman et al. 1988, Naiman et al. 1994, Pollock et al. 2003). Our results demonstrate the potential importance of beavers in affecting aquatic macrophyte communities as well. When we excluded beavers for 13-15 months, wetland plant mass increased 2.9X and there were striking differences in species composition. Although herbivory strongly affected these plant communities, impacts to particular species appeared to be context dependent. The strong impacts of beavers on bur-reed were moderated where it co-occurred with lizard's tail (Figures 2.3c and 2.4), and strong impacts on lizard's tail were moderated where it co-occurred with sedge tussocks (Figure 2.6). Thus, beavers can profoundly alter the community structure of wetland plant communities, but the strength of these effects for particular species depends on the identity of neighboring plants.

# CHAPTER 3
# BRYOPHYTES AS CHEMICALLY DEFENDED REFUGIA FOR FRESHWATER MACROINVERTEBRATES

## *Abstract*

Herbivory is rarely considered an important process affecting the ecology and evolution of freshwater macrophyte communities. However, examination of a riverine plant community suggests that aquatic herbivores may drive patterns of plant abundance, the evolution of traits to minimize herbivory, and the ecological specialization of smaller herbivores onto host-plants that are avoided by large herbivores. The common stream plant riverweed, *Podostemum ceratophyllum,* was selectively consumed in feeding assays with both Canada geese, *Branta canadensis*, and crayfish, *Procambarus spiculifer*. At our study site, preferential consumption of riverweed appears to promote the abundance of an unpalatable moss, *Fontinalis novae-angliae,* that we show is chemically defended by a $C_{18}$ acetylenic acid (octadeca-9,12-dien-6-ynoic acid). Selective herbivory by geese and crayfish may also drive mesograzer specialization onto the unpalatable moss, possibly because it represents a host where mesograzers can seek safety from consumption by omnivorous geese and crayfish. Twice as many macroinvertebrates colonized the chemically-defended moss *Fontinalis* as riverweed, and in laboratory feeding assays, both the amphipod *Crangonyx gracilis* and the isopod *Asellus aquaticus* consumed significant amounts of moss but rejected riverweed. Neither amphipod nor isopod feeding was deterred by the crude organic extract of *Fontinalis*, suggesting that these mesograzers tolerate or circumvent the chemical defenses that deterred larger consumers. Despite the long-standing notion that herbivory is a weak ecological and

evolutionary force in freshwater systems, intense herbivory by geese and crayfish may drive plant community structure in this riverine system towards a chemically defended macrophyte, but smaller, less mobile herbivores may obtain enemy-free space by circumventing these chemical defenses to live and feed on a host-plant that is avoided by larger herbivores.

## *Introduction*

Herbivores were historically thought to have little impact on the ecology and evolution of freshwater plant communities (e.g., Shelford 1918, Rosine 1955, Hutchinson 1975). Recent reviews, however, show that herbivores remove as much or more of the plant standing stock in freshwater systems as they do in marine and terrestrial systems (Newman 1991, Cyr and Pace 1993, Lodge et al. 1998). Early misconceptions about the importance of herbivory in freshwater communities arose from a misplaced focus on insect herbivores and snails (see discussion in Lodge et al. 1998). Instead, the herbivores with the largest impacts in freshwater systems are often generalist consumers like waterfowl (Sondergaard et al. 1996, Van Donk and Otte 1996, Weisner et al. 1997, Santamaria 2002), crayfish (Lodge and Lorman 1987, Lodge 1991, Lodge et al. 1994), mammals (Qvarnemark and Sheldon 2004), and fish (Van Donk and Otte 1996).

In marine and terrestrial systems, large, generalist herbivores often alter plant communities by selectively consuming palatable species and avoiding chemically or structurally defended plants (Hay and Fenical 1988, Crawley 1989, Rosenthal and Berenbaum 1992, Hay 1997). Although freshwater macrophytes are frequently unpalatable and contain a variety of secondary metabolites that may function as herbivore deterrents (Cronin et al. 2002, Prusak et al. 2005), few studies have identified the molecules responsible for feeding deterrence and linked these defenses to feeding selectivity and, ultimately, plant community structure. Additionally, most studies of chemical defenses in freshwater plants have focused on their direct role as deterrents to generalist consumers (Newman et al. 1996, Bolser et al. 1998, Kubanek et al. 2001). However, patterns from marine and terrestrial systems show that plant chemical defenses often have cascading, indirect effects on community structure. For example, the same plant chemical defenses that are effective against the large, generalist herbivores that commonly drive patterns of plant abundance are often either ineffective or even stimulatory to smaller, more sedentary herbivores that use plants as both habitat and food (e.g., insects and marine mesograzers) (discussed in Hay et al. 1987a, Bernays and Graham 1988, Hay 1992). These smaller grazers can gain associational defenses from predation by living on host plants that are chemically noxious to large grazers (Hay et al. 1987a, Hay et al. 1988, Hay et al. 1990), and this enemy-free space (Jeffries and Lawton 1984) may have played a large role in the evolutionary radiation of specialist herbivores onto chemically-defended marine and terrestrial plants (Hay et al. 1987a, Bernays and Graham 1988, Hay 1992).

Freshwater macroinvertebrates are abundant on freshwater macrophytes (Brusven et al. 1990, Bowden 1999, Hutchens et al. 2004) and thus may be under strong selection to avoid plants that are consumed by generalist herbivores and omnivores. However, rather than choosing a host that is a spatial refuge from larger consumers, most aquatic macroinvertebrates are assumed to choose host plants that provide more habitable living space and trap more particulate organic matter (i.e., food) from the water column (e.g., Dudley 1988, Suren and Winterbourn 1992),. Despite this, freshwater invertebrates suffer intense predation from omnivorous crayfish (Lodge et al. 1994), fish (Sheldon 1987, Johansson 1991, Flecker and Townsend 1994), and waterfowl (Marklund et al. 2002), and this impact is often exacerbated when invertebrates live on plants that are eliminated by generalist consumers (Lodge et al. 1994). Thus, grazing by generalist consumers could potentially drive freshwater invertebrates to select host-plants that are avoided by larger grazers, much like it does for small herbivores in marine and terrestrial systems.

Here, we addressed whether small, sedentary herbivores in freshwater systems preferentially live and feed on plants that are chemically repugnant to the predominant large grazers along the rocky shoals in a southeastern US river. We asked the following questions: 1) How does plant abundance on riverine rocky shoals correlate with palatability to Canada geese, *Branta canadensis* L., and crayfish, *Procambarus spiculifer* LeConte? 2) Are small, plant-associated macroinvertebrates more abundant and more likely to feed on a plant that is unpalatable to geese and crayfish? 3) Does plant secondary chemistry differentially affect feeding preferences of large and small grazers?

## *Materials and Methods*

*Natural history of study site and organisms*. Work was conducted in the Chattahoochee River National Recreation Area (CRNRA) near Atlanta, Georgia, USA. Similar to other streams and rivers in the Piedmont region (Mulholland and Lenat 1992), the rocky shoals of the Chattahoochee are covered by dense stands of plants adapted to life in the torrents. Particularly common are riverweed *Podostemum ceratophyllum* (Michaux), a vascular plant that attaches directly to the rocky substratum via holdfasts, much like marine seaweeds, and *Fontinalis novae-angliae* (Sull.), an aquatic moss that also attaches directly to the rock surface. Other plants that were observed during our study were Brazilian waterweed *Egeria densa* (Planch.), the emergent bur-reed *Sparganium americanum* (Nutt.), the submersed milfoil *Myriophyllum pinnatum* (Walt.) B.S.P., and the green alga *Nitella flexilis* (L.), each referred to hereafter by genus.

Herbivory on riverine macrophytes in the CRNRA appears intense. We commonly observed Canada geese, mallards (*Anas platyrhynchos* L.), and crayfish (*Procambarus spiculifer*) grazing along the shoals and riverbanks of the CRNRA. There was also conspicuous evidence of grazing by beaver (*Castor canadensis* Kuhl) and muskrats (*Ondatra zibethicus* L.). Attempts to use manipulative methods to quantify the impact of grazing by these large herbivores were unsuccessful due to destruction of herbivore exclusion cages by rapid flow following heavy rains or nightly dam openings.

*Plant abundance*. To characterize the plant community in areas where herbivory appeared intense, we measured the percent cover and biomass of the common plants located on rocky shoals in an area where we frequently observed ~100 Canada geese feeding (the Jones Bridge Park Unit, 34’ 00.053N, 84’ 14.220W). The plant and herbivore community in this area appeared similar to rocky shoals throughout the CRNRA. To determine percent cover, we haphazardly placed 11 1.0 $m^2$ quadrats on the substratum and recorded the frequency and identity of plants located under 25 points within each quadrat. We then collected all of the plant biomass from three randomly selected 0.04 $m^2$ areas within each quadrat, taking the mean of these three sub-replicates to estimate mean plant biomass per 0.04 $m^2$. In the laboratory, these samples were sorted to species, spun in a salad spinner to remove excess water, and weighed to the nearest mg. Percent cover and biomass data were analyzed with one-way analysis of variance (ANOVA) following log transformation to correct heteroscedastic variances as detected with Cochran’s test.

*Feeding assays with Canada geese and crayfish*. To determine the feeding preferences of the common generalist grazers in this system, we fed six species of aquatic macrophytes to two species of generalist consumers that were common in the CRNRA. We fed Canada geese and crayfish the vascular plants *Podostemum, Egeria, Sparganium, Myriophyllum*, the green alga *Nitella*, and the bryophyte *Fontinalis*. To feed Canada geese, we collected plants and transported them in a chilled cooler to a nearby public boat-ramp where geese normally congregated. While sitting in a canoe in the middle of the river, we used bread to attract a gaggle of geese (~75 individuals) and to initiate goose feeding. After geese were sufficiently acclimated and feeding, we picked a random goose and threw a small handful of foliage from a single species of macrophyte directly in front of it. Geese that picked up and ate the plant were recorded as accepting the plant as food. Geese that picked up the plant and then rejected it were subsequently thrown a piece of bread to determine if they were satiated. If a goose rejected the plant and ate the bread, the plant was considered rejected. Geese that rejected both the plant and piece of bread were not counted in the feeding assay. Geese that did not pick up the macrophyte were not counted. Order of macrophyte presentation was randomized throughout the assay. We used plumage coloration to identify and feed separate geese during these assays, occasionally stopping feeding until new geese arrived and some of the initial geese departed. It is possible, however, that some geese were fed and counted more than once for the same plant species. We analyzed the proportion of animals that fed on each macrophyte species relative to the control level of 100% acceptance by bread-feeding, non-satiated geese using a Fisher's exact test.

We conducted similar feeding assays with crayfish in the laboratory. We used crayfish because they often have strong impacts on aquatic plant communities (Lodge and Lorman 1987, Creed 1994, Lodge et al. 1994, Dorn and Wojdak 2004), they feed well in the laboratory (Parker and Hay 2005), and we frequently observed crayfish foraging on rocky shoals at the study site. We collected approximately 50 *Procambarus spiculifer* from the Chattahoochee River and housed each crayfish in separate 12 x 12 x 10 cm cubicles with perforated walls receiving recirculating, filtered water. Crayfish were fed a maintenance diet of Bio-Blend Herbivore food 3-4 times per week. We determined the relative palatability of plants by offering 15-22 individual crayfish a bite-sized portion of each macrophyte species that we had fed to geese and recorded whether each portion of food was eaten or rejected. If rejected, we fed crayfish a piece of palatable aquatic macrophyte (*Ludwigia palustris* L.) to assure they were not satiated. If the palatable macrophyte was rejected, the replicate was not included as the animal appeared satiated. Order of macrophyte presentation was randomized separately for each replicate consumer. We then analyzed the proportion of animals that fed on each macrophyte species relative to the palatable control with a Fisher’s exact test.

*Macroinvertebrate distribution and feeding on plants*. To determine whether small, plant-associated macroinvertebrates were more abundant on plants that were unpalatable to geese and crayfish, we characterized the abundance and species composition of plant-associated macroinvertebrates on the two most abundant macrophytes (*Podostemum* and *Fontinalis*) in our study area. We collected 17 samples of the bryophyte *Fontinalis* and the vascular plant *Podostemum* from the Jones Bridge Unit by detaching macrophytes from the substratum and quickly placing them into downstream plastic bags while still underwater. Previous studies using similar methodologies resulted in fewer than 1% loss of moss-associated insects (Glime and Clemons 1972). In areas where Canada geese could feed, most of the *Podostemum* was heavily disturbed and grazed down to a turf of only about 2 cm in height. We avoided these disturbed areas by collecting *Podostemum* and *Fontinalis* plants that were of similar size and growth morphology from deeper (>0.75 m) areas of fast-flow in the same vicinity. We noted that Canada geese had difficulty feeding in these areas due to rapid flow and depth. In the laboratory, we rinsed each macrophyte over a 0.5mm sieve and enumerated all fauna retained on the sieve. Crustaceans were identified to species; insects were identified to order or family. Plants were then spun in a salad spinner to remove excess water and weighed to the nearest mg. We standardized the total abundance of all species and the abundance of selected, common species to the wet mass of each macrophyte; t-tests determined statistical differences between host plants in faunal abundance per g of wet plant mass.

To determine if plant-associated macroinvertebrates would feed on the most abundant macrophytes in our study area, we conducted no-choice feeding assays with the amphipod *Crangonyx gracilis* and the isopod *Asellus aquaticus* offered either *Fontinalis* or *Podostemum*. We used these consumers because they were abundant in our field samples and because preliminary assays indicated that their feeding was adequate to allow reasonable quantification. For these assays, we placed three amphipods or two isopods into one compartment of an ice cube tray containing 18-22 mg portions of either plant species and approximately 25 ml of water. Each plant portion was sonicated for 5-15 sec before use to remove particulate organic matter that could confound feeding on plant tissues; removal of particulates was confirmed by visual inspection prior to use. Sonicating did not appear to damage macrophyte tissues. Controls for changes in plant mass unrelated to herbivory consisted of identical portions from the same individual plants placed into the same ice cube tray but without herbivores. After three days we calculated the mass of plants eaten by consumers using the formula: $(T_i \times C_f/C_i) - T_f$, where $T_i$ and $T_f$ were initial and final wet masses of tissue exposed to herbivores, and $C_i$ and $C_f$ were initial and final wet masses of controls (as in Cronin and Hay 1996). Results were analyzed with paired t-tests.

*Plant traits that affect consumer feeding.* We measured macrophyte traits that are generally thought to be indicative of nutritional quality or availability as a food. These traits included: dry mass/volume, ash-free dry mass/volume, soluble protein/volume, and soluble protein/dry mass for all six macrophyte species. Soluble protein was estimated with the Bradford method. Triplicate composite samples of ground macrophyte material from each species (~5 mg) were digested in 1 ml of sodium hydroxide (1 mol) for 24 hr at $2.5^{o}C$, centrifuged, and 100-μl aliquots of the supernatant were added to 5 ml samples of Bradford reagent. After 10-15 min, absorbances of the samples at 595 nm were measured in a Spectronic 21D spectrophotometer against bovine serum albumin (BSA) standards. Results were analyzed with ANOVA followed by Tukey multiple comparison tests, with log transformations to correct heteroscedastic variances when necessary (Cochran's tests). Protein analyses were conducted on pooled samples of numerous individuals. Because variances associated with these means would be due to procedures and not variances among individual plants, these values were not statistically analyzed.

We also conducted feeding assays testing whether plant morphology or secondary chemistry could account for differences in plant palatability. For these assays, we focused on *Podostemum* and *Fontinalis* because these were the most abundant plants in our study area, they differed in their palatability to common herbivores, and they served as hosts to different densities and species of macroinvertebrates. To evaluate possible effects of macrophyte morphology on herbivore feeding, we destroyed plant structure by freeze-drying and grinding tissues of *Podostemum* and *Fontinalis* to a fine powder and imbedding these powders into gel-based foods that had similar morphologies and textures (Hay et al. 1998). For each artificial food, we added enough macrophyte powder to a 20% alginic acid paste to approximate the same dry mass per volume of macrophyte for each species of macrophyte being assayed. The gel was then coated onto the interior wall of a glass Petri dish and immersed in a hardening solution of 0.25M calcium chloride. After approximately one minute the gel was removed, rinsed in water, and cut into bite-sized portions. This method resulted in reconstituted macrophytes with similar morphologies and a soft, fleshy texture resembling cooked pasta. Chemical defenses and nutritional traits should have remained similar to those of intact macrophytes (however, freeze-drying can alter the activity of some metabolites, Cronin et al. 1995). These artificially softened macrophytes were then assayed against a palatable control food - a 1:1 mixture of freeze-dried and powdered broccoli and lettuce ('broc-let') that herbivores readily accept as food (Bolser et al. 1998). Broc-let content matched the dry mass per volume of each macrophyte being assayed. Feeding on pellets was recorded as the frequency of acceptance or rejection of treatment or control pellets, with treatment pellets always offered first. We then analyzed the proportion of animals feeding on each individual reconstituted macrophyte species relative to the palatable control using Fisher's exact test.

If gel-based treatments were unpalatable to herbivores, it suggested that the plant was chemically defended or nutritionally inadequate. We tested for chemical defenses by incorporating crude extracts from *Podostemum* or *Fontinalis* into a broc-let based sodium-alginate food as above and feeding pellets of these foods to crayfish (see above and Hay et al. 1998 for a general review). These extracts were acquired by macerating macrophyte tissues in a mixture of water and methanol (1:1/v:v) overnight, then successively extracting the macrophyte material for at least two hours in and methanol:dichloromethane (1:1/v:v and 1:2/v:v). The solvents were then removed under vacuum. For food preparation, the crude extract was dissolved in acetone, incorporated into broc-let powder and sodium alginate, and the solvent was evaporated by vigorous stirring in a fume hood. Control foods were treated identically, including addition of acetone, but without extracts. The dry mass content of treatment and control pellets matched the dry mass per volume content of each macrophyte being assayed. Pellets were fed to animals and results were statistically analyzed as above.

To determine whether the feeding of plant-associated macroinvertebrates was influenced by plant secondary chemistry, we offered amphipods and isopods similar artificial foods incorporated with the crude extracts from *Podostemum* or *Fontinalis*. Foods were constructed as above, but we spread control (broc-let) and treatment (broc-let treated with either *Podostemum* or *Fontinalis* extract) paste over window-screen mesh prior to hardening it in the calcium chloride solution (Hay et al. 1998). We then offered either three amphipods or two isopods a choice between a control and treatment window screen mesh with the embedded artificial food in 5.5 cm diameter petri dishes with 15 ml of water. Feeding was quantified as the number of mesh squares out of 25 from which food had been cleared, with assays harvested when half of either food had been eaten. The proportion of control and treatment squares eaten relative to the total squares eaten was analyzed with paired t-tests.

*Bioassay guided fractionation.* The crude extract of *Fontinalis* deterred crayfish feeding so we used bioassay-guided fractionation of this extract to isolate and identify the compounds responsible for deterrence. Wet moss (200 ml) was blended in 250 ml of water and then allowed to soak for 5-10 minutes to trigger any enzyme-activated reactions (Newman et al. 1996, Bolser et al. 1998) before adding an equal volume of methanol (e.g., Prusak et al. 2005). After three hours, this extract was decanted and solvents were removed via rotary evaporation. The remaining plant material was successively extracted in 400 ml of methanol:dichloromethane (1:1/v:v) for 12 hours and 400 ml of methanol:dichloromethane (1:2/v:v) for three hours, removing solvents by rotary evaporation after each step. These extracts were then combined and tested as before by offering crayfish broc-let based pellets incorporated with chemical extracts vs. control foods that were treated identically but without the addition of extracts. Extracts were tested at their isolated concentrations by volume (i.e., the extract from 1 ml of plant was incorporated into 1 ml of treatment food), but at an elevated concentration relative to plant dry mass basis to offset loss due to inefficient extractions and/or compound decomposition (i.e., we coated this extract onto 0.07 g of broc-let whereas 1 ml of *Fontinalis* was equivalent to 0.31 g of dry plant mass, Table 3.1).

The deterrent crude extract was then partitioned into five fractions of increasing polarity using a modified Kupchan liquid-liquid partitioning (Kupchan et al. 1975). These fractions were assayed for effects on crayfish feeding and deterrent fractions were further fractionated using silica gel flash column chromatography (40-63 μm Aldrich silica eluted with a gradient of hexane and ethyl acetate). Chromatographic fractions were grouped by common thin layer chromatographic (TLC) properties and tested for deterrence of crayfish feeding. Deterrent fractions were subjected to flash chromatography as before, and this was repeated as necessary until achieving a deterrent fraction that consisted of one major compound. This fraction was further separated using high performance liquid chromatography (HPLC - Zorbax RX-SIL silica column, 9.94 x 250 mm, 5-micron, attached to a Waters Breeze HPLC system, 515 pump, with a Waters 2487 UV detector at 210 and 254 nm, eluted with a gradient of hexane and acetone), resulting in one deterrent compound. At this step we attempted to obtain a structure by NMR spectroscopy, but there was insufficient quantity of material for analysis. Thus, we obtained and extracted new plant material as before and used repeated flash chromatography to obtain a fraction that displayed similar TLC characteristics to the deterrent fraction from the previous extraction. Fractions from the final separation were pooled according to common NMR spectral traits and tested for deterrence of crayfish feeding. The major compound from fractions that deterred crayfish feeding was then identified on the basis of $^{1}H$, $^{13}C$, and 2D NMR spectroscopy. All NMR spectral data were obtained on a Bruker Avance DRX 500 MHz spectrometer using $CDCl_3$ as solvent with TMS as internal standard referenced to residual $CHCl_3$ ($\delta$ 7.28).

## *Results*

*Plant abundance*. Two macrophytes, *Podostemum* and the aquatic moss *Fontinalis*, covered 84% of the available area and comprised 98% of the total plant cover on rocky shoals in the Jones Bridge unit of the CRNRA (Figure 3.1a). The remaining plant cover was predominantly the benthic green alga *Vaucheria*. The cover of *Podostemum* (about 49%) and *Fontinalis* (about 35%) did not differ significantly (Figure 3.1a), but biomass was strongly dominated by *Fontinalis* (Figure 3.1b). Its mass was ~10X greater than that of *Podostemum* because *Fontinalis* commonly grew as large (~30 cm long), bush-like macrophytes while *Podostemum* occurred primarily as a short (~2 cm), turf-like layer in most places (Figure 3.1b). However, we frequently observed large clumps of *Podostemum* (~30 cm long) growing in deeper areas of rapid flow where Canada geese attempting to 'tip up' to feed were rapidly swept downstream.

*Feeding assays with Canada geese and crayfish*. Of the six macrophyte species surveyed for palatability, *Podostemum* was among the most palatable macrophytes to both Canada geese and crayfish, whereas *Fontinalis* was among the least palatable to both consumers (Figure 3.2a, b). Geese and crayfish feeding preferences were crudely similar. Geese and crayfish both fed significantly on the invasive exotic plant *Egeria*, but feeding for each consumer was significantly reduced for *Sparganium*, *Nitella*, and *Myriophyllum* (Figure 3.2a, b).

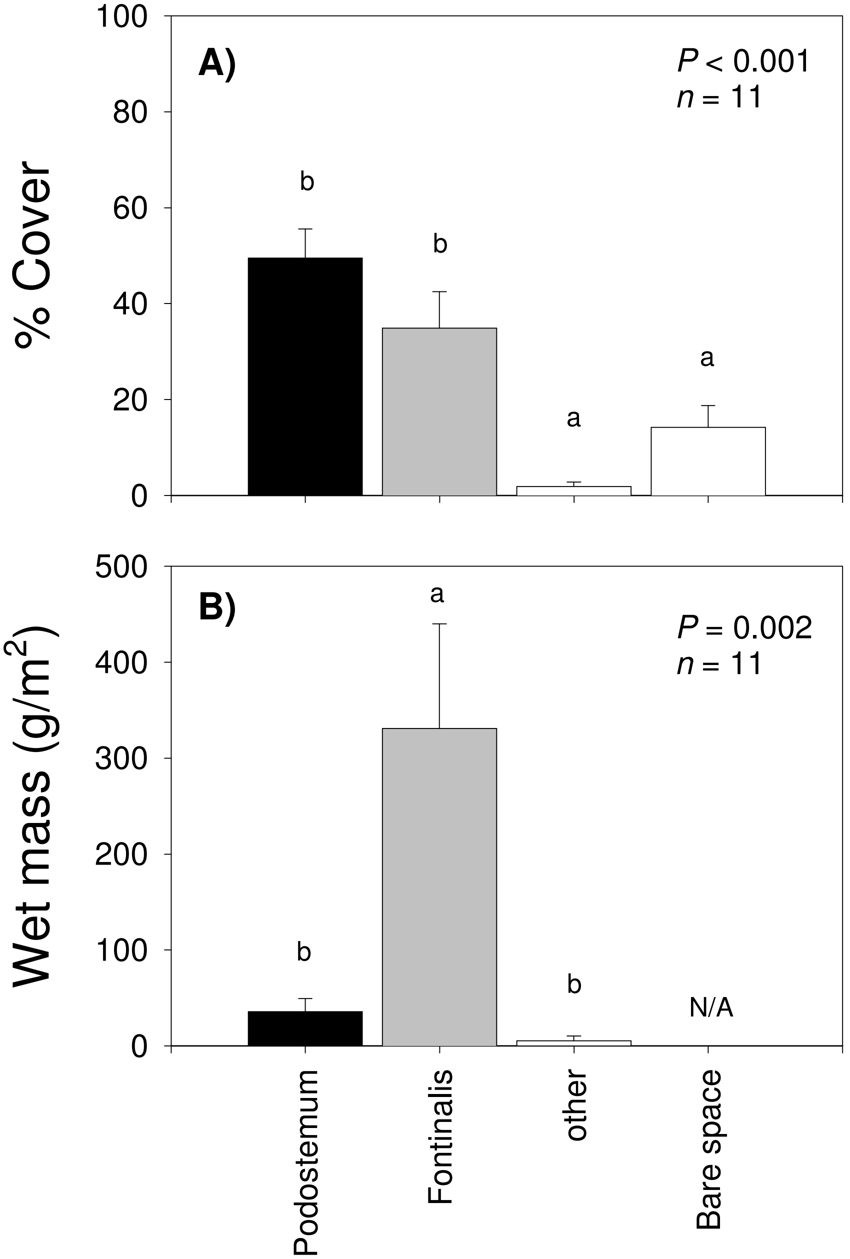


Figure 3.1. Percent cover A) and wet mass B) of plants located on the rocky shoals of the Chattahoochee River (34’ 00.053N, 84’ 14.220W). Statistics are from ANOVAs and letters above indicate significant groupings by Tukey post-hoc tests.

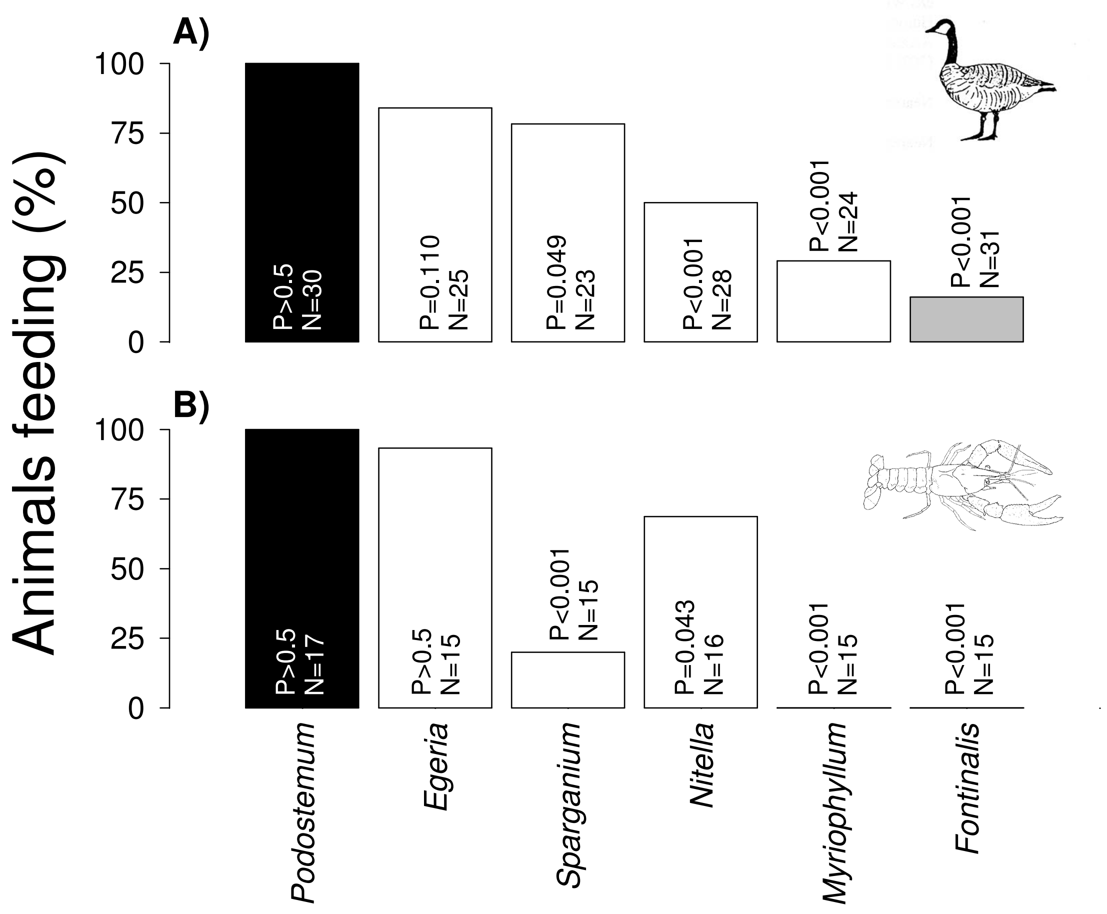


Figure 3.2. Percentage of A) Canada geese, *Branta canadensis*, and B) crayfish, *Procambarus spiculifer*, feeding on fresh macrophyte tissues from six aquatic macrophyte species collected from the Chattahoochee River. Statistics are from Fisher's exact tests and test feeding relative to a palatable control food (bread for geese, *Ludwigia palustris* for crayfish) for each consumer species.

*Macroinvertebrate distribution and feeding on plants*. The density of plant-associated fauna on *Fontinalis* was roughly twice that of *Podostemum* ($P = 0.002$, Figure 3.3). Additionally, mayflies (Ephemeroptera), midge larvae (Chironomidae: Diptera), the amphipod *Crangonyx gracilis* (Crustacea), and the isopod *Asellus aquaticus* (Crustacea), were all more abundant on *Fontinalis* than on *Podostemum* (Figure 3.3). In contrast, blackflies (Simuliidae: Diptera) and caddisflies (Hydropsychidae: Trichoptera) were both more abundant on *Podostemum*, significantly so for blackflies and trending in this direction for caddisflies (Figure 3.3).

In contrast to Canada geese and crayfish, the amphipod *Crangonyx gracilis* and the isopod *Asellus aquaticus* both consumed significant amounts of *Fontinalis* but not *Podostemum* (Figure 3.4). Amphipods and isopods both ate the green leaves from *Fontinalis*, leaving behind only the fibrous stems (JP and DB, personal observations).

*Plant traits that affect consumer feeding*. The least nutritious plant we assayed was *Sparganium*; it had the lowest dry mass, ash-free dry mass, and protein content among the six species of macrophyte we analyzed (Table 3.1). *Egeria*, *Nitella*, and *Myriophyllum* were of moderate values for these traits. *Podostemum* had the second highest dry mass, ash-free dry mass, and protein content (by % dry mass) among these six species, topped only by *Fontinalis* for each trait (Table 3.1). Thus, the two most abundant plants at our study site, *Podostemum* and *Fontinalis* (Figure 3.1), were also potentially the most nutritious. In contrast to its apparent high nutritional value, *Fontinalis* was among the least palatable species of macrophyte to both Canada geese and crayfish (Figure 3.2).

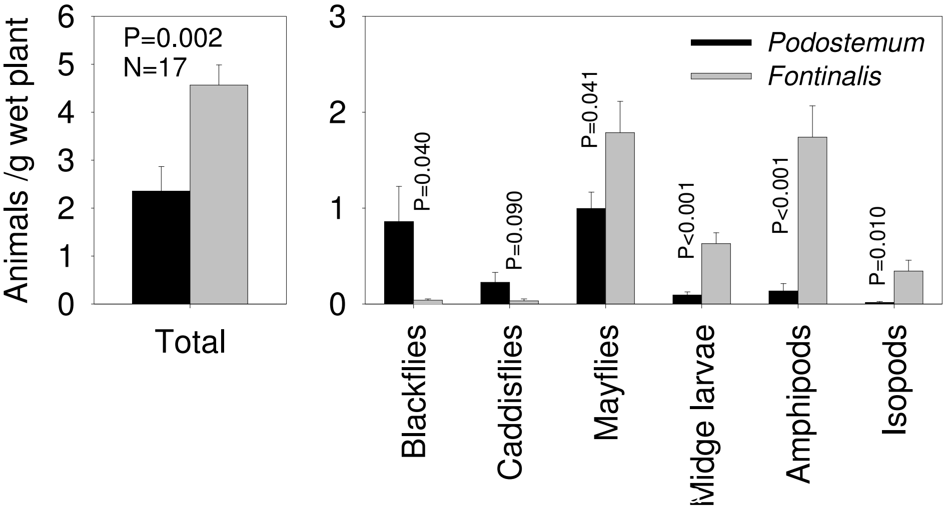


Figure 3.3. Abundance of plant-associated macroinvertebrates per g of wet plant for the vascular plant *Podostemum ceratophyllum* and the bryophyte *Fontinalis novae-angliae* collected from the Chattahoochee River. Statistics are from t-tests.

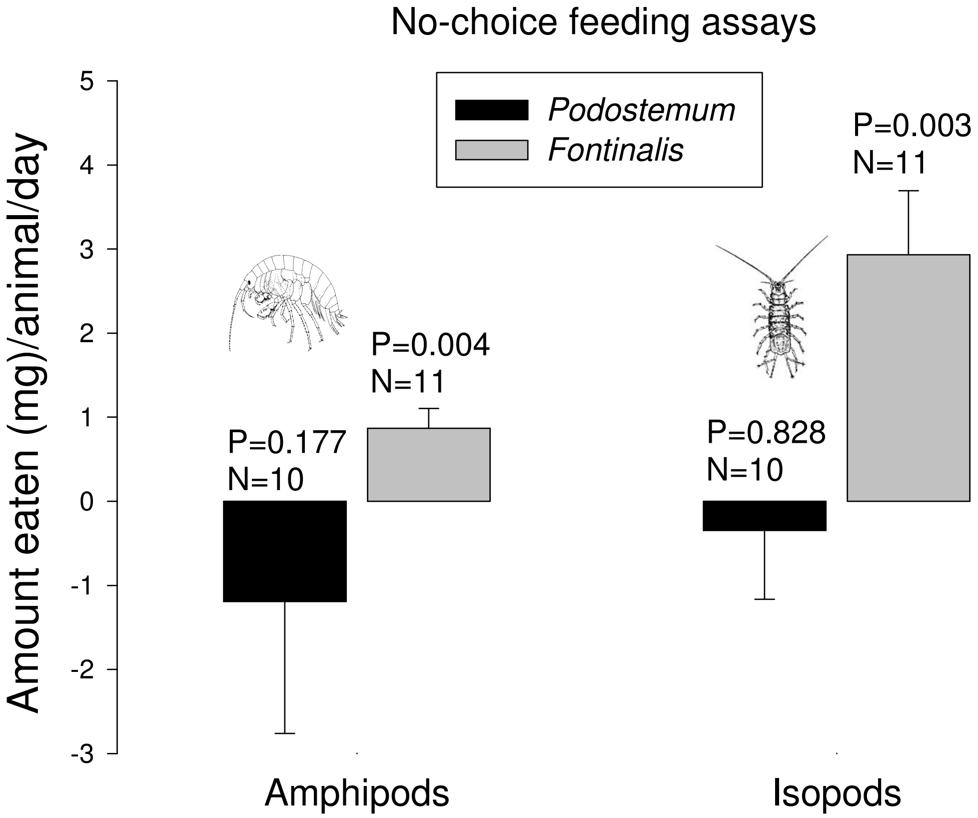


Figure 3.4. Difference in macrophyte mass (mean + SE) due to consumption by the amphipod *Crangonyx gracilis* and the isopod *Asellus aquaticus* when offered fresh tissues of either the vascular plant *Podostemum ceratophyllum* or the bryophyte *Fontinalis novae-angliae*. Statistics are from paired t-tests against control plants that were not exposed to herbivores.

Table 3.1. Mean (± SE) and sample sizes (in parentheses) for each analysis of selected macrophyte traits. Species that share a letter within a column are not significantly different from one another in unplanned comparisons following ANOVA (broc-let not analyzed).

| **Macrophyte** | **Dry mass/vol. (mg/ml)** | **AFDM/vol. (mg/ml)** | **Soluble protein (mg/ml)** | **Soluble protein (% dry mass)** |
|---|---|---|---|---|
| *Podostemum ceratophyllum* | 175 ± 22.5 (10)**c** | 132 ± 17.4 (10)**c** | 5.53 | 9.68 |
| *Egeria densa* | 78.9 ± 5.74 (10)**b** | 66.0 ± 3.29 (10)**b** | 4.23 | 3.34 |
| *Sparganium americanum* | 19.0 ± 3.09 (10)**a** | 12.63 ± 0.74 (10)**a** | 2.71 | 0.515 |
| *Nitella flexilis* | 109 ± 7.66 (10)**b** | 80.5 ± 5.29 (10)**b** | 6.38 | 6.95 |
| *Myriophyllum pinnatum* | 65.1 ± 2.44 (10)**b** | 51.6 ± 1.81 (10)**b** | 7.74 | 5.03 |
| *Fontinalis novae-angliae* | 314 ± 30.7 (10)**d** | 274 ± 27.9 (10)**d** | 3.22 | 10.1 |
| Broc-let control | 55 ± 1.41 (3) | 6.27 ± 0.47 (3) | 4.03 | 7.34 |
| ANOVA *P*-values | **$P < 0.0001$** | **$P < 0.0001$** | N/A (Composite samples) | N/A (Composite samples) |

When plant morphology was destroyed and the plant tissues from *Fontinalis* and *Podostemum* were incorporated into homogenized pellets, crayfish consumed pellets constructed from *Podostemum* as readily as they consumed pellets made from broc-let (Figure 3.5a), but feeding on pellets constructed from *Fontinalis* was significantly depressed relative to the broc-let control pellets. When crude extracts from each plant were incorporated into otherwise palatable foods, crayfish readily consumed foods treated with *Podostemum* extract, but feeding was suppressed by incorporation of *Fontinalis* extract (Figure 3.5b). Thus, crayfish rejected *Fontinalis* not because it was morphologically tough, but because it was chemically distasteful.

In contrast to crayfish, neither amphipods nor isopods were deterred by the crude extract from *Fontinalis* (Figure 3.6a, b). Amphipod feeding was stimulated by *Podostemum* extract (Figure 3.6a), whereas isopods were not affected (Figure 3.6b). However, the duration of these assays ranged from 2.5-8.0 days for amphipods feeding on *Fontinalis* extracts. A duration of 2-4 days is within the range for similar assays with marine mesograzers (Duffy and Hay 1991, Duffy and Hay 1994), yet the longer duration of the *Fontinalis* assay with amphipods makes it possible that feeding deterrents or stimulants had degraded or leached out of our artificial foods during this time. To evaluate changes in *Fontinalis* extract over time, we incorporated extract into broc-let as above and qualitatively analyzed TLC plates for compound degradation after 8 days. We also fed control and treatment foods that had soaked in water for 8-days to crayfish in order to determine whether *Fontinalis* extracts were still deterrent. There were no changes in the appearance of 8-day old *Fontinalis* extracts by TLC, suggesting that the presence major secondary metabolites was stable over this time period, though we did

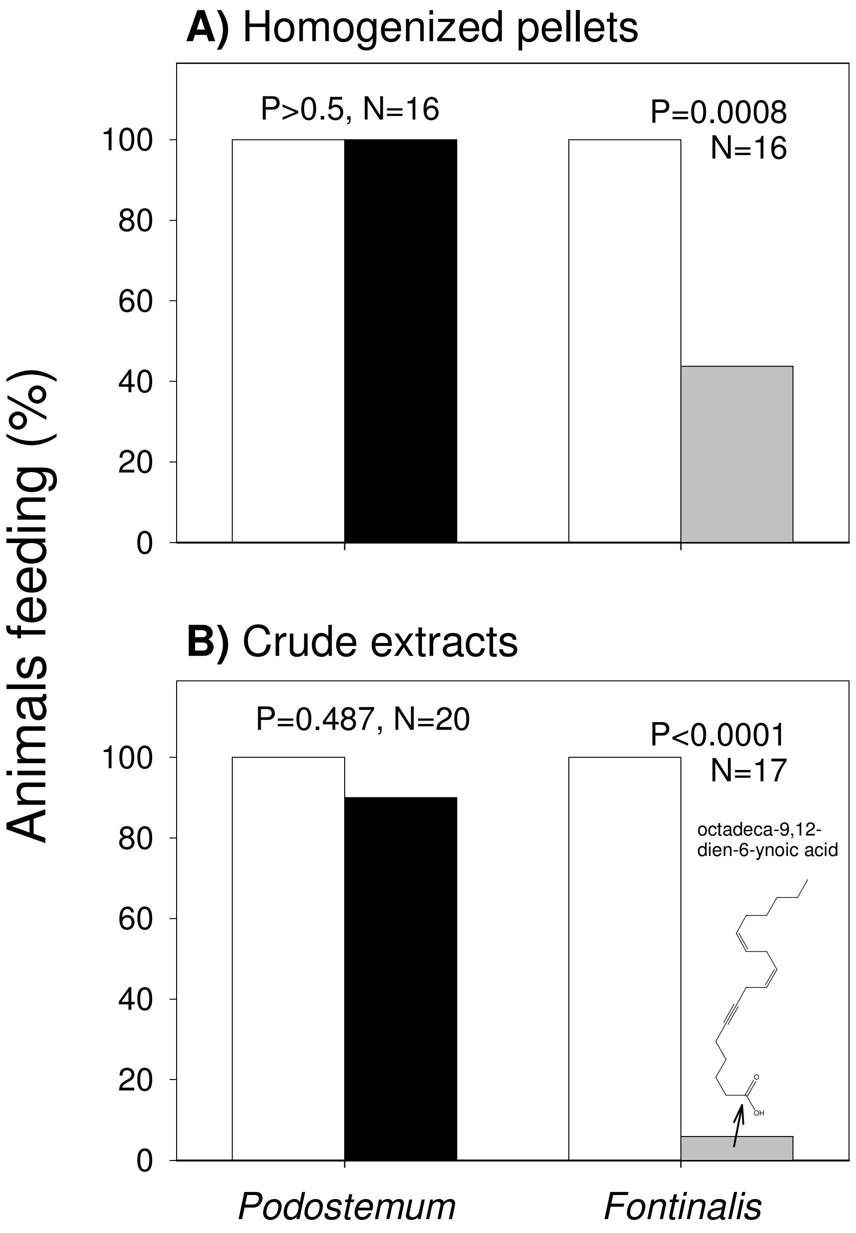


Figure 3.5. Percentage of crayfish, *Procambarus spiculifer*, feeding on A) homogenized tissue incorporated into pellets and B) pellets treated with crude extracts of either *Podostemum ceratophyllum* (black bars) or *Fontinalis novae-angliae* (grey bars). Statistics are from Fisher's exact tests comparing feeding on treatment foods relative to control foods made from broc-let (open bars). A $C_{18}$ acetylenic fatty acid, octadeca-9,12-dien-6-ynoic acid, was isolated from the crude extract of *F. novae-angliae* using bioassay-guided-fractionation, see Results.

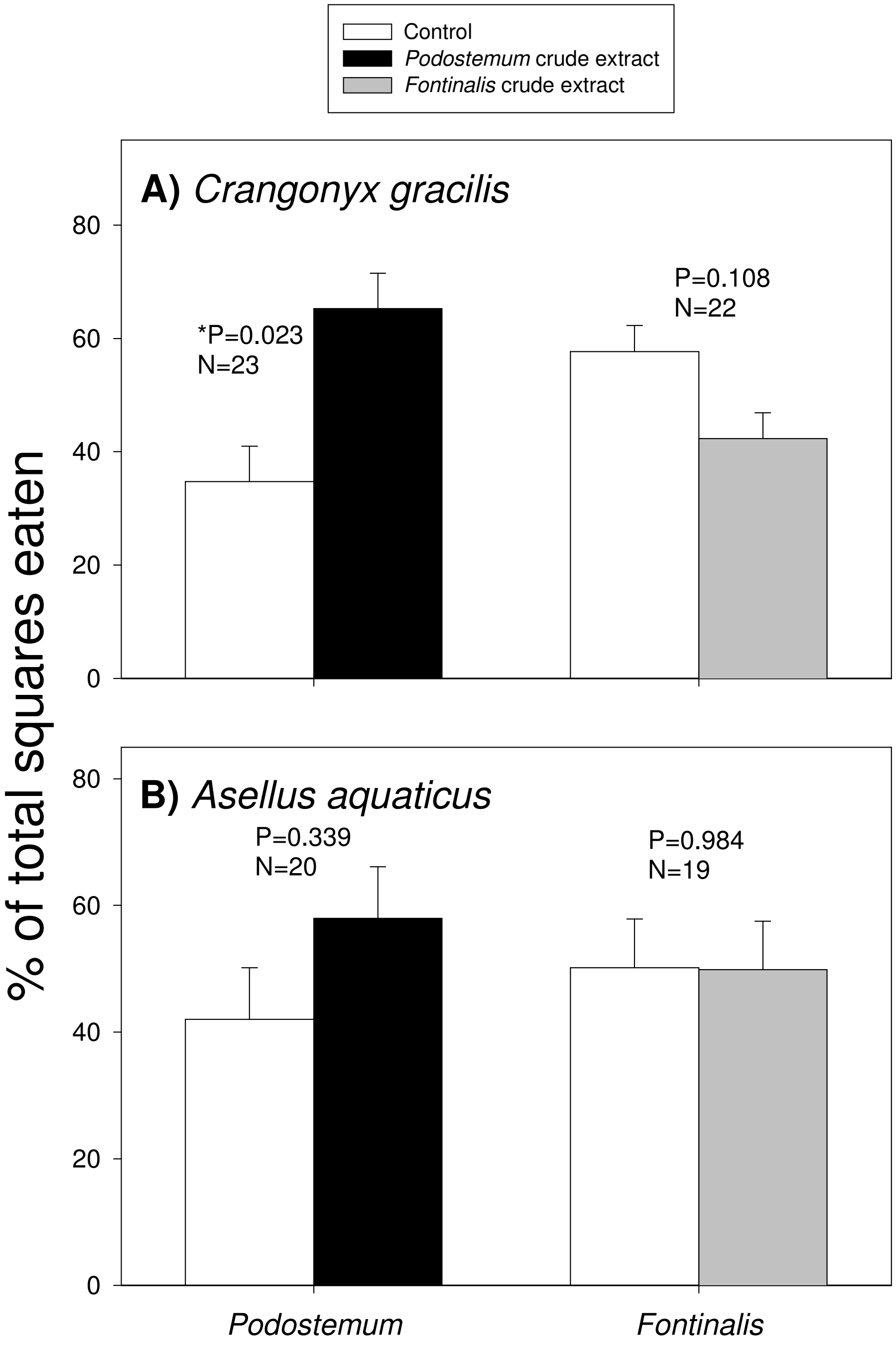


Figure 3.6. The effects of *Podostemum ceratophyllum* or *Fontinalis novae-angliae* crude extracts on feeding by A) the amphipod *Crangonyx gracilis* or B) the isopod *Asellus aquaticus*. Each pair of bars represents the mean (+ SE) amount of control and treatment food consumed relative to the total amount of food consumed in each replicate. Statistics are from paired t-tests.

not assess whether the quantity of active compounds had changed. However, deterrence of the *Fontinalis* extracts did not degrade over the 8 day period; for the 10 crayfish tested, all fed on the control food while only one fed from the treatment food ($P = 0.001$, Fisher's exact test).

*Bioassay guided fractionation.* The crude extract from the initial extraction of *Fontinalis* was deterrent to crayfish (1 of 17 animals ate the treatment food, $P < 0.001$, Fisher's exact test), as were the hexane and chloroform soluble portions of this crude extract (11 of 17 animals feeding, $P = 0.018$, and 4 of 17 animals feeding on fractions, $P < 0.001$, respectively). Silica gel flash column chromatography of the combined hexane- and chloroform-soluble fractions resulted in one fraction that was deterrent to crayfish (0 of 20 animals feeding, $P < 0.001$). Further separation of this fraction via flash chromatography and HPLC resulted in one fraction that deterred crayfish feeding (12 of 19 animals feeding, $P = 0.007$), and this appeared to be a single metabolite. However, once purified, the compound decomposed before we could obtain a structure by NMR spectroscopy. A second extraction was performed and, using repetitive flash chromatography and HPLC, we obtained a fraction that had TLC characteristics similar to the previous deterrent fraction; this new fraction also deterred crayfish feeding (12 of 19 animals feeding, $P = 0.004$). NMR spectroscopy followed by comparison of spectral data with previous accounts (Jamieson and Reid 1976) revealed the major component in this fraction to be a $C_{18}$ acetylenic fatty acid, octadeca-9,12-dien-6-ynoic acid (Fig. 5B). We estimated the isolated yield of this compound to be only 0.0036% of dry mass. By contrast, the same acetylenic acid was isolated at 0.29% in another aquatic moss, *F.*

*antipyretica* (Jamieson and Reid 1976). Given that acetylenic compounds are highly unstable when exposed to light, heat, and oxygen (Seigler 1998), this low yield could have been due to compound decomposition.

## *Discussion*

Despite the long-standing notion that herbivores have minimal impact on freshwater plant communities (see Lodge et al. 1998 for the history of this argument and for data negating it), our results suggest that herbivory by large, generalist consumers in freshwater environments could be driving plant community structure, the evolution of traits in freshwater plants, and thus indirectly influencing the abundance of plant-associated herbivores. By selectively consuming riverweed, *Podostemum ceratophyllum,* and avoiding the chemically repugnant aquatic moss *Fontinalis novae-angliae*, Canada geese appear to create chemically-defended refugia that are selectively colonized by small, macroinvertebrates that use these plants as both food and habitat, consistent with marine and terrestrial studies showing that animals often colonize chemically-defended plants to escape their own enemies (Hay et al. 1987a, Bernays and Graham 1988, Hay 1992).

Most previous examples of herbivory on freshwater macrophytes are limited to ponds and lakes. In contrast, our study was conducted in a river, where food webs are widely thought to be based on terrestrial litter input or *in situ* production by micro-algae, rarely on large macrophytes (Vannote et al. 1980). However, we observed intense grazing by waterfowl; and the signs of other grazers such as beavers, muskrats, and crayfishes that we observed all suggest that herbivory on macrophytes could play a significant role in riverine food-webs. Canada geese were, however, the most visually obvious consumers. Large groups of geese were frequently observed foraging on the vascular plant *Podostemum* on rocky shoals throughout the Chattahoochee River, whereas we never saw geese eat the aquatic moss *Fontinalis*. This pattern of feeding preference was confirmed in a field feeding assay (Figure 3.2). Additionally, in deeper or more turbulent areas where geese could not feed effectively, *Podostemum* plants were commonly as large as *Fontinalis*. These observations suggest that Canada geese were driving the patterns of plant abundance in this system. Similarly, waterfowl in other systems have dramatic top-down influences on aquatic plant communities (Sondergaard et al. 1996, Van Donk and Otte 1996, Weisner et al. 1997, Santamaria 2002, Abraham et al. 2005, Sponberg and Lodge 2005), suggesting that herbivory by waterfowl can be a dominant selective force on aquatic macrophyte communities.

Herbivory on living macrophytes by stream and riverine invertebrates has traditionally been considered rare (reviewed in Newman 1991). However, both the amphipod *Crangonyx gracilis* and the isopod *Asellus aquaticus* consumed significant amounts of *Fontinalis* but not *Podostemum* in the laboratory (Figure 3.4). Similarly, aquatic mosses and other plants have been found in the guts of aquatic insects (Jones 1950, Pritchard and Berte 1987, Suren and Winterbourn 1991), isopods (LaCroix 1996), and amphipods (Minckley and Cole 1963), suggesting that herbivory by invertebrates on aquatic macrophytes may be more common than previously considered. These findings parallel the results of research with marine mesograzers, which also were historically thought to be incapable of feeding on large macrophytes but can cause considerable damage to marine seaweeds (Brawley and Adey 1981, Duffy and Hay 2000), including the nearly complete elimination of kelps by amphipods along areas of the southern California coastline when conditions allow amphipods to escape control by predatory fishes (Tegner and Dayton 1991). Thus, our study adds to growing evidence that aquatic, plant-associated invertebrates are not merely using plants as habitat but also directly as a food resource (Soszka 1975, Urban 1975, Sheldon 1987, Lodge et al. 1998, Dorn et al. 2001), though their impacts on plant distribution are generally unknown.

The most abundant plants in our system both appeared to have traits that allowed them to either resist or tolerate herbivory by waterfowl. We isolated a $C_{18}$ acetylenic acid (octadeca-9,12-dien-6-ynoic acid) from the moss *Fontinalis novae-angliae,* and this compound appeared to chemically deter feeding by the crayfish *Procambarus spiculifer* (Figure 3.5). Previous work had suggested that reduced feeding on the aquatic moss *F. antipyretica* was associated with elevated phenolic concentrations (Liao and Glime 1996), though bioassay guided fractionation was not used to isolate the compounds responsible for feeding deterrence. The acetylenic acid that we identified, and other acetylenic fatty acids, are common to aquatic and terrestrial mosses (Anderson et al. 1975, Jamieson and Reid 1976, Kohn et al. 1987, Zinsmeister et al. 1991) but are uncommon in vascular plants (Seigler 1998) and have not previously been identified as deterrents to herbivore feeding. However, some acetylenic fatty acids have pronounced antimicrobial and antifungal properties (Borel et al. 1993, Fusetani et al. 1993, Li et al. 1994), and acetylenic alcohols in water hemlock (*Cicuta virosa*) are toxic to domestic livestock and humans (Seigler 1998). Rather than resisting herbivory, riverweed *Podostemum ceratophyllum* appears to tolerate herbivory. *Podostemum* has unique basal ‘roots’ that tightly adhere to rock surfaces and prevent plants from being swept downstream in rapid flow (Everitt and Burkholder 1991). Goose herbivory on *Podostemum* removed all but these roots and left a short, though living, carpet of *Podostemum* stems that still covered much of the areal space in this system (Figure 3.1a). Thus, in addition to providing a stable attachment point in rapid flow, rock-adhering roots may also allow riverweed to tolerate chronic herbivory by retaining both the basal meristems and a starchy root that persists even when the upright foliage has been removed, similar to rhizomatous terrestrial grasses (McNaughton 1983).

Marine and terrestrial studies commonly show that small, sedentary herbivores that spend large portions of their lives on one or a few plant species should be under strong selection to colonize chemically noxious plants to avoid both incidental and direct consumption (Hay et al. 1987a, Bernays and Graham 1988, Hay et al. 1988, Hay et al. 1990, Hay 1992). In support of this hypothesis, we found that chemically-defended aquatic moss *Fontinalis novae-angliae* supported twice the density of invertebrates as did *Podostemum ceratophyllum,* the plant that was most attractive to Canada geese and crayfish, (Figure 3.3). Additionally, the amphipod *Crangonyx gracilis* and the isopod *Asellus aquaticus* both consumed *Fontinalis* fresh tissues and crude extracts in laboratory feeding assays while avoiding *Podostemum* when presented whole, but were stimulated or unaffected by its extract (Figures 3.4 and 3.5). This suggests that these mesograzers may have been selected to tolerate or circumvent the chemical defenses that deterred larger consumers as a way of gaining both food and enemy-free space. Previous studies have also found dense communities of invertebrates on aquatic mosses (Brusven et al. 1990, Suren 1991, Suren and Winterbourn 1992, Suren 1993, Bowden 1999, Linhart et al. 2002, Habdija et al. 2004) and on *Podostemum* (Grubaugh and Wallace 1995, Hutchens et al. 2004), yet host-plant selection in freshwater systems has often been ascribed to differences among macrophytes in the provision of habitable living spaces, entrainment of particulate organic matter, surfaces for epiphytic algal growth, and shelter from turbulent flow (Lodge 1985, Brusven et al. 1990, Suren 1991, Linhart et al. 2002). These plant characteristics may partially explain the patterns in macroinvertebrate abundance that we observed. For example, *Podostemum* appeared relatively clean and free of debris, whereas *Fontinalis* appeared to be laden with significantly more organic debris (JP and DB, personal observations). Moreover, the complex and finely branched thallus of *Fontinalis* may simply have retained more animals in this turbulent flow environment than the simpler architecture of *Podostemum*. Thus, our results could be confounded by these other factors that covaried with macrophyte species. However, plants that are heavily settled by epiphytes, bacteria, particulate organic matter, and particularly small mesograzers, may represent better food sources for geese and crayfish as well as these small consumers, yet *Fontinalis* was clearly a low preference food for Canada geese and crayfish (Figure 3.2). Thus, amphipods and isopods that colonize *Fontinalis* may be subject to less predation from Canada geese and crayfish than if they had colonized *Podostemum*. Similarly, aquatic caterpillars of the pyralid moth *Munroessa gyralis* (which have generally limited mobility among plants) preferentially feed on two water lilies, *Nymphaea odorata* and *Brasenia shreberi* (Dorn et al. 2001), that are repugnant to omnivorous crayfish (Cronin et al. 2002, Parker and Hay 2005), whereas grazing insects avoid associating with a palatable green alga *Cladophora glomerata* that crayfish virtually exclude from streams (Creed 1994). Thus, ecological specialization on plants that are avoided by large herbivores may be a common, but often overlooked, feature in freshwater habitats.

Here, despite the historical argument that herbivory has little impact on freshwater plant communities (Hutchinson 1975), we show that herbivory by Canada geese and crayfish could be driving a riverine plant community from the palatable riverweed *Podostemum ceratophyllum* to the chemically defended aquatic moss *Fontinalis novae-angliae*. Although moss was avoided by geese and crayfish, it was colonized by twice as many invertebrates and was selectively consumed by both amphipods and isopods in laboratory feeding assays. These results are consistent with the hypothesis that small herbivores are under strong selection to choose host-plants that are avoided by larger consumers (Hay et al. 1987a, Duffy and Hay 1994), and suggests that herbivory can have strong direct and indirect effects on the ecology and evolution of freshwater plant communities.

# CHAPTER 4
# BIOTIC RESISTANCE TO PLANT INVASIONS? NATIVE HERBIVORES PREFER NON-NATIVE PLANTS

## *Abstract*

In contrast to expectations of the enemy release hypothesis, but consistent with the notion of biotic resistance, we found that native generalist crayfishes preferred exotic over native freshwater plants by a 3 to 1 ratio when plants were paired by taxonomic relatedness. Native crayfishes also preferred exotic over native plants when tested across 57 native and 15 exotic plants found growing sympatrically at 11 sites throughout the southeastern USA. Exotic grass carp that share little evolutionary history with most of these plants exhibited no preference for native vs. exotic species. Analyses of 3 terrestrial datasets showed similar patterns, with native herbivores generally preferring exotic plants, while exotic herbivores rarely exhibited a preference. Thus, exotic plants may escape their coevolved herbivores only to be preferentially consumed by the native generalist herbivores in their new ranges, suggesting that native herbivores may provide biotic resistance to plant invasions.

## *Introduction*

The enemy release hypothesis is one of the most commonly accepted theories to explain the success of invasive species. It posits that exotic species become invasive because they are liberated from consumer control in their new ranges (Darwin 1859, Elton 1958). The absence of coevolved specialist enemies and the preferential consumption of native species by native generalists putatively gives exotic species a competitive advantage over their native counterparts (Maron and Vila 2001, Keane and Crawley 2002, Shea and Chesson 2002). Native consumers are predicted to prefer native prey because evolutionarily novel, exotic species may be inadequate or distasteful prey (Siemann and Rogers 2003, Lankau et al. 2004). Recent studies confirm that exotic species commonly harbor fewer species of specialized parasites, pathogens, and consumers in their introduced vs. their native ranges (Wolfe 2002, Mitchell and Power 2003, Torchin et al. 2003). However, a lower diversity of enemies need not translate into less damage (Maron and Vila 2001).

Although enemy release has traditionally assumed that native consumers will be better adapted to consuming native than exotic prey, this perspective ignores the alternative possibility that exotic prey may be poorly adapted for deterring native consumers. Because exotic prey share no evolutionary history with native enemies, they will not have experienced selection from these consumers and may therefore lack effective defenses, i.e., the ‘new associations’ or ‘increased susceptibility’ hypotheses (Hokkanen and Pimentel 1989, Colautti et al. 2004). If native consumers prefer exotic over native prey, this suggests that native consumers could limit invasions, as suggested by the biotic resistance hypothesis (Elton 1958).

Despite these competing predictions, we know little about preferences of native consumers for native vs. exotic prey, leading to little predictive power for the likelihood of native consumers to influence biological invasions (Maron and Vila 2001, Keane and Crawley 2002, Shea and Chesson 2002, Colautti et al. 2004). However, in contrast to expectations from the enemy release hypothesis, one recent field test found that exotic plants in an experimental meadow suffered greater, rather than lesser, damage from naturally-recruited herbivores than did related native plants (Agrawal and Kotanen 2003).

Here, we report an experimental study of the feeding preferences of three generalist aquatic consumers (two native and one non-native species) for native vs. exotic freshwater plants from throughout the southeastern USA. Exotic plants are particularly common in freshwater habitats, and although herbivory on freshwater macrophytes was previously assumed to be insignificant, numerous reviews show that herbivory in freshwater systems rivals or exceeds herbivory in marine and terrestrial ecosystems (Lodge 1991, Newman 1991, Cyr and Pace 1993, Lodge et al. 1998). We focus on generalist rather than specialist herbivores because generalists: 1) commonly have stronger impacts on plant community structure in marine (Hay and Steinberg 1992, Hay 1997), freshwater (Lodge *et al.* 1998), and terrestrial systems (Crawley 1989), 2) are often the relevant consumers in freshwater ecosystems, where specialist herbivores are rare (Lodge *et al.* 1998, but see Solarz and Newman 1996, 2001), and 3) are more likely than specialists to feed on and thus impact exotic plants. In laboratory feeding assays we use both a phylogenetic approach, pairing 10 exotic plants with related natives to control for some evolutionary variance in morphology, chemistry, and other traits that might differ between unrelated taxa (Mack 1996), and a community approach, comparing consumer feeding preferences for 57 native and 15 exotic plant species that we found growing in sympatry at 11 sites scattered throughout South Carolina, Georgia, and Florida in the southeastern USA. Additionally, we extended our study to other ecosystems by re-analyzing data from the literature on the feeding preferences of four native (three grasshoppers and one slug) and four exotic (slugs) terrestrial herbivores when presented with subsets of 234 species of native and 51 species of exotic plants. These datasets were not phylogenetically-controlled but were comparable to our community-level analyses of freshwater systems. We hypothesized that if the evolutionary history of the plant-herbivore interaction was important, then native consumers would prefer, rather than avoid, exotic plants because these plants would not have been selected to resist these herbivores. In contrast, exotic consumers that share little evolutionary history with either native or exotic plants should have no preference as a function of plant origin.

## *Materials and Methods*

*Phylogenetically-paired assays.* We tested whether native consumers preferred to feed on native or exotic plants by conducting choice feeding assays in the laboratory with 10 phylogenetically-paired native and exotic plants (Table 1). To find suitable pairings, we examined over 50 separate wetlands, ponds, lakes, streams, and rivers in several southeastern states of the USA for co-occurring, taxonomically-related native and exotic species. In most cases, we selected pairings based on the first closely related taxa that we encountered, resulting in four congeneric comparisons and six confamilial comparisons (for exotic taxa that lacked native congeners). We found two exotic and two native

Table 4.1. Species used in the phylogenetically paired feeding assays. The designation numbers refer to the taxonomic pairs shown in Figure 1; location of collection is beneath each species. Results are shown for individual paired t-tests on each species pair for each consumer species (n = 15 for all pairings).

| | **Plant Origin** | | **Consumer species** | |
|---|---|---|---|---|
| | **Native** | **Exotic** | ***P. spiculifer*** | ***P. acutus*** |
| 1) | *Vallisneria americana* | *Egeria densa* | $t = -9.15$, | $t = -9.51$, |
| | Lake Juliette, GA | Lake Juliette, GA | $P < 0.0001$ | $P < 0.0001$ |
| 2) | *Vallisneria americana* | *Hydrilla verticillata* | $t = -4.59$, | $t = -4.91$, |
| | Lake Juliette, GA | Lake Juliette, GA | $P < 0.0001$ | $P < 0.0001$ |
| 3) | *Myriophyllum pinnatum* | *Myriophyllum spicatum* | $t = -2.86$, | $t = -2.82$, |
| | Chattahoochee River, GA | Lake Juliette, GA | $P = 0.013$ | $P = 0.014$ |
| 4) | *Pontederia cordata* | *Eichhornia crassipes* | $t = -2.65$, | $t = -10.12$, |
| | Lake Jackson, FL | Lake Blackshear, GA | $P = 0.019$ | $P < 0.0001$ |
| 5) | *Ludwigia palustris* | *Ludwigia hexapetala* | $t = 0.21$, | $t = 2.13$, |
| | Johnson Ferry, GA | Lake Blackshear, GA | $P = 0.839$ | $P = 0.051$ |
| 6) | *Myriophyllum heterophyllum* | *Myriophyllum aquaticum* | $t = -0.63$, | $t = -2.21$, |
| | Lake Jackson, FL | Lake Blackshear, GA | $P = 0.536$ | $P = 0.044$ |
| 7) | *Peltandra virginica* | *Colocasia esculenta* | $t = -5.04$, | $t = -4.67$, |
| | Lake Griffin, FL | Lake Griffin, FL | $P < 0.0001$ | $P < 0.0001$ |
| 8) | *Peltandra virginica* | *Pistia stratiotes* | $t = -4.54$, | $t = -3.33$, |
| | Lake Griffin, FL | Lake Griffin, FL | $P < 0.0001$ | $P = 0.005$ |
| 9) | *Panicum hemitomon* | *Panicum repens* | $t = -3.28$, | $t = -1.41$, |
| | Little Lake Harris, FL | Little Lake Harris, FL | $P = 0.005$ | $P = 0.179$ |
| 10) | *Commelina virginica* | *Murdannia keisak* | $t = -8.15$, | $t = -7.29$, |
| | Chattahoochee River, GA | Chattahoochee River, GA | $P < 0.0001$ | $P < 0.0001$ |

species in the genus *Myriophyllum*; we paired these so that the native and exotic plants most closely resembled each other morphologically. Twice we had to use one native species in two different contrasts. This occurred because: 1) we could find only one native plant (*Peltandra virginica*) that was confamilial with *Colocasia esculenta* and *Pistia stratiotes*, and 2) we could find only one native plant (*Vallisneria americana*) that was a submersed species similar to the exotic species in the same family (*Hydrilla verticillata* and *Egeria densa*). Although all of these plants are commonly found growing in sympatry across the southeastern US (USDA 2004), in the time-frame of this study we were only able to find exotic and related natives co-occurring at the same location for 6 of our 10 contrasts; in the other 4 contrasts, the paired relatives could only be found at different locations, potentially confounding site effects with plant origin effects for these four pairs. To be sure that we were not confounding plant origin effects with site of collection effects, and that we were not biasing our findings by using two native species in two paired assays each, we analyzed not only the entire data-set, but also sub-sets of the data after excluding contrasts that could have confounded our results.

To assess consumer preference, we fed plants to the native North American crayfishes *Procambarus spiculifer* and *P. acutus,* both of which have native ranges that overlap the sites and types of habitats that we sampled (Hobbs 1981). Crayfishes are diverse and abundant foragers in aquatic habitats throughout North America, and they can dramatically reduce aquatic macrophyte biomass and fundamentally alter plant community composition (Lodge *et al.* 1998 and references therein). *P. spiculifer* were collected from the Chattahoochee River, Atlanta, Georgia, USA; *P. acutus* were collected from an adjacent wetland. We housed each crayfish in a separate 12 x 12 x 10 cm cubicle with perforated walls receiving recirculating, filtered water and fed them a diet of Bio-Blend Herbivore food 3-4 times week$^{-1}$.

For each native vs. exotic contrast, we offered 15 individuals of each crayfish species a portion (500 ± 100 mg) from a native and a related exotic macrophyte bound side-by-side in a binder clip. We ended each replicate when a consumer had eaten approximately half of either species. Controls for changes in plant mass unrelated to herbivory consisted of identical portions from the same individual plants (when permitted by the size of the plant), or portions of plants from the same collection, placed into the same water table but without herbivores. We calculated the mass of native and exotic plants eaten by individual consumers with: $(T_i \times C_f/C_i) - T_f$, where $T_i$ and $T_f$ were initial and final wet masses of tissue exposed to herbivores, and $C_i$ and $C_f$ were initial and final wet masses of controls (as in Cronin and Hay 1996). Data were analyzed with a mixed model ANOVA using each replicate consumer as a randomized block nested within each species pair, followed by planned, paired contrasts.

*Feeding assays with regionally co-occurring macrophytes.* The phylogenetic assays provide a powerful contrast because we could pair all plants by taxonomy and six of the 10 pairs by location of collection as well. However, the requirement for finding pairs of congeneric or confamilial species constrained our sample size to 10 pairs. To determine the robustness of the pattern found in the above assay, we also evaluated the willingness of each crayfish species to feed on each of 57 native and 15 exotic aquatic macrophytes that we found growing in sympatry across the southeastern USA. This approach allowed us to increase our sample size considerably but included many sources of variance (site, time of collection, phylogenetic history) that we largely avoided in our paired assays. Thus, our question here was whether we could still detect preference patterns for native vs. exotic prey despite these additional sources of variance. In addition to assays with crayfishes, we also conducted assays with the exotic herbivorous grass carp *Ctenopharyngodon idella*, a generalist herbivore from Asia that has been introduced throughout the USA (USGS 2005). This contrast included only the 33 native and 14 exotic macrophytes that we encountered after acquiring grass carp. Grass carp were not used in the previous, phylogenetically-controlled assays because in pilot assays these consumers commonly took more than a week to acclimate and begin feeding in such trials. In contrast, crayfish trials were typically completed within a few hours.

For these assays, we collected the common native or exotic macrophyte species found growing sympatrically at 11 sites in South Carolina, Georgia, and Florida, USA (see legend in Figure 2 for sites). Plants were transported from the field to the lab in chilled coolers and kept chilled until fed to consumers, generally within 48 h. Because of the large number of species assayed (72 for crayfish and 47 for grass carp), we did not conduct cafeteria-style choice feeding assays. We instead determined acceptability as food by offering 15-22 individuals of each crayfish species a bite-sized portion of each macrophyte species and recorded whether each portion of food was eaten or rejected. If rejected, we fed consumers a piece of palatable aquatic macrophyte (generally *Ludwigia palustris*) to assure they were not satiated and rejecting all foods. If the palatable macrophyte was rejected, that replicate crayfish was not included in the assay because it appeared satiated. Grass carp would not feed when kept individually, so we housed 3-5 juveniles together in perforated 3.5 L buckets with recirculating water and report results from the first fish that fed in each bucket as a replicate ($n$ = 13-16 separate buckets). Order of macrophyte presentation was randomized separately for each replicate consumer. We then contrasted (using the Mann-Whitney test) the mean proportion of animals that were willing to feed on native vs. exotic plant species.

*Re-analyses of previous literature.* To provide a broader assessment of the relative palatability of exotic plants, we used the online database Web of Science (1945-present) to find three studies that had fed numerous species of native and exotic plants to native and exotic generalist herbivores (Cates and Orians 1975, Otte 1975, Rathcke 1985). This approach added 285 plant species and eight consumer species to our analysis. Our intent here was to test whether the patterns we found in aquatic systems also held for terrestrial plants and their consumers.

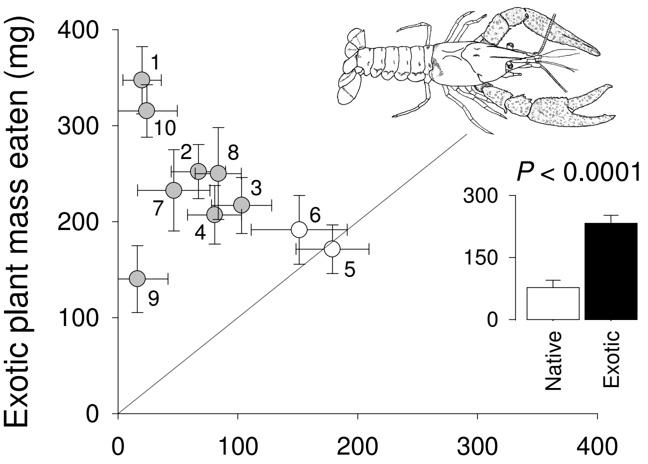
None of these studies had focused on the palatability of native vs. exotic species. Instead, all had assessed the relationship between plant successional status and palatability, and all had found opposing patterns. Cates and Orians (1975) found that one native and one exotic slug in the Pacific Northwest preferred early successional species, Otte (1975) found that three native grasshoppers in Texas preferred later successional species, and Rathcke (1985) found no preference as a function of plant successional stage among three exotic slugs in the American Northeast. We analyzed their data as a function of plant origin (native vs. exotic species) rather than as a function of successional status. To determine whether plants were native or exotic in each particular study, we used the authors' own designations in Cates and Orians (1975) and Otte (1975), though we changed one species (*Rumex crispus*) in Otte (1975) from native to exotic based on its current classification status (USDA 2004). For Rathcke (1985), we assigned plant origin using the PLANTS database (USDA 2004) Feeding assays in all three studies were conducted by offering each consumer a choice between a treatment plant and a standardized control plant species, resulting in a metric of relative amount eaten (amount of treatment plant eaten relative to control plant). We analyzed (Mann-Whitney tests) the relative amount eaten of all native and exotic plant species for each consumer in each study.

Although these studies found opposing influences of successional status on plant palatability, most of the exotic plants in each study were labeled early successional plants – 10 out of 14 species in Otte (1975), all 27 species in Cates and Orians (1975), and all 13 species in Rathcke (1985), potentially confounding plant origin with successional status. Thus, we also analyzed (Mann-Whitney tests) whether early successional exotic plants were more or less palatable than early successional native plants. We did not analyze these data with factorial ANOVAs (origin x successional status) because the near absence of late successional, exotic plants would render factorial analyses non-orthogonal and thus difficult to interpret.

### *Results*

In the 10 phylogenetically-controlled feeding assays, both native crayfish showed a significant, 3-fold preference for exotic over native species (Figure 4.1 insets; *P. spiculifer*: $F_{1,140} = 136.91$, $P < 0.0001$; *P. acutus*: $F_{1,140} = 147.51$, $P < 0.0001$). Each consumer significantly preferred exotic over native plants in 8 of the 10 taxonomic pairs (Figure 4.1, Table 1). Neither consumer ever preferred a native plant over its exotic relative, though one contrast trended in this direction (*Ludwigia* spp. comparisons for *P.*

**(a)** *P. spiculifer*



**(b)** *P. acutus*

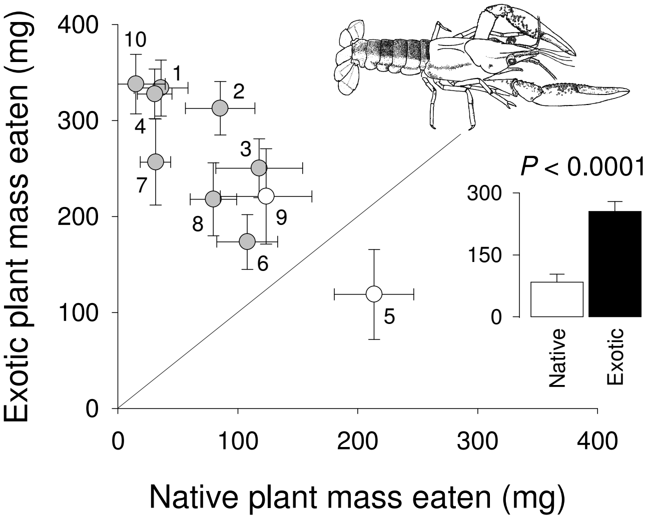


Figure 4.1. Mean (± SE) plant biomass consumed by the native crayfishes (a) *Procambarus spiculifer* and (b) *P. acutus* when offered a choice between phylogenetically-paired (either congeneric or confamilial) native and exotic freshwater plants. Grey circles were statistically significant individual feeding assays ($P < 0.05$, paired t-tests, Table 4.1). Insets are the overall means between native and exotic plants, with standard errors corrected for the nesting factor. Numbers refer to taxonomic pairs in Table 1.

*acutus*, Figure 4.1, Table 1). If the analyses excluded the non-sympatric species pairs or the contrasts with duplicative native species, we still found the same strong patterns. Native herbivores still strongly preferred exotic over native species ($P < 0.0001$ in all cases).

The above contrasts minimize uncontrolled variance due to taxonomy and, for six of the 10 paired contrasts, location. This allows a clear contrast of herbivore preference for similar native versus exotic plants but constrains our sample size due to the difficulty of finding related native and exotic species growing sympatrically. We considerably improved our sample size by comparing the palatability of native versus exotic species that we found growing at 11 locations across South Carolina, Georgia, and Florida. Despite the potential for large uncontrolled variance due to taxonomic and site-specific effects on palatability, exotic species ($n = 15$) were significantly more palatable than native taxa ($n = 57$) to both species of native consumer (*P. spiculifer:* $P = 0.034$; *P. acutus:* $P = 0.033$, Mann-Whitney tests, Figure 4.2), but not to the exotic grass carp ($P = 0.464$, $n = 14$ exotic and 33 native plants, Figure 4.2). Although the 10 exotic species from the phylogenetic pairings were also included among the 15 species in the community comparisons, these 10 species did not appear to drive the community relationship as several of the plants not included in the phylogenetic pairings were highly palatable to both crayfishes (Figure 4.2).

Our re-analyses of published datasets from terrestrial systems showed similar feeding preference patterns. Cates and Orians' data (1975; their Tables 1-6) showed that both the native slug *Ariolimax columbianus* and the exotic slug *Arion ater* significantly preferred exotic ($n = 27$) over native plants ($n = 73$, $P \leq 0.0007$, Figure 4.3A, B). Otte's

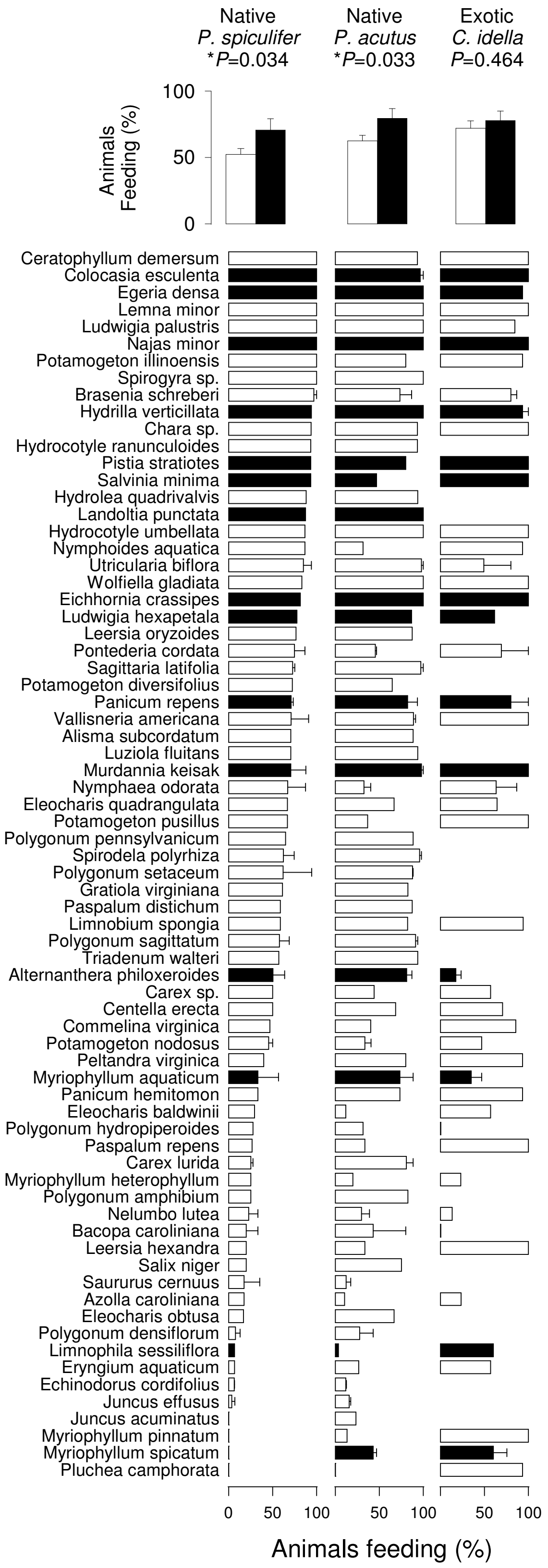
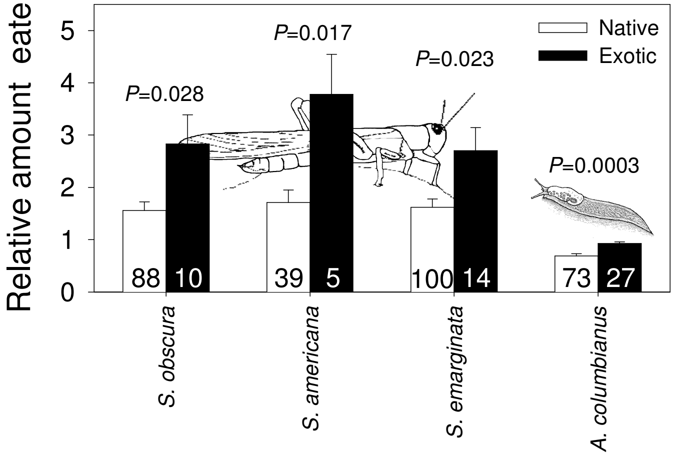


Figure 4.2. Mean (+ SE) percentage of crayfish (*Procambarus spiculifer* and *P. acutus*) and grass carp (*Ctenopharyngodon idella*) feeding on all native (open bars) and exotic (filled bars) macrophyte species that were encountered during this study (statistics from Mann-Whitney tests). For a species at a given site, we averaged results from separate feeding assays if this plant had both submersed and emergent leaves; we then used this mean value to compute a mean palatability across sites. Standard errors represent the variance across sites, not among leaf types. The summary data presented at the top of the figure are based on 57 native and 15 exotic plant species for crayfishes; 33 native and 14 exotic species for grass carp. Sites of collection were: Rum Creek, GA; east & west Lake Juliette, GA; Lake Blackshear, GA, Johnson Ferry, GA, Lake Seminole, GA; Priests Landing, GA; Par Pond, SC; Pond 4, SC; Lake Bradshaw, FL; Little Lake Harris, FL.

**(a)** Native herbivores



**(b)** Exotic herbivores

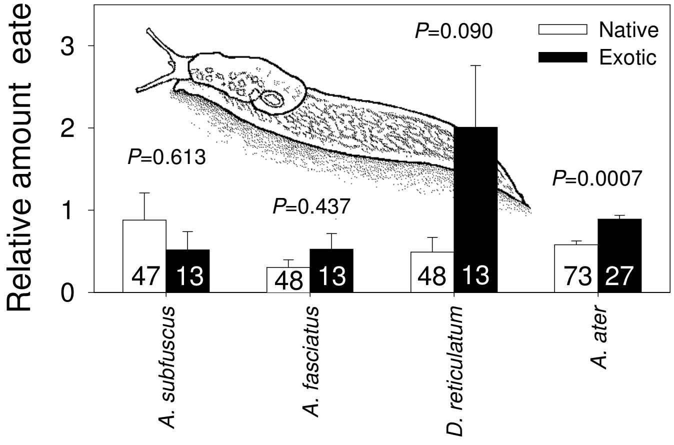


Figure 4.3. Mean (+ SE) relative amount eaten of native (open bars) and exotic (filled bars) plant species by (a) native and (b) exotic consumers when offered a choice between an individual plant species and a standardized control plant species; see individual papers for methodological details. Statistics are from Mann-Whitney tests and numbers at bottom of bars are sample sizes.

study (1975; his Table 1) showed that all three native grasshoppers significantly preferred exotic over native plants (*Schistocerca obscura:* $n = 10$ exotic and 88 native plants, $P = 0.028$, Figure 4.3A; *S. americana:* $n = 5$ exotic and 39 native plants, $P = 0.017$, Figure 4.3A; *S. emarginata:* $n = 14$ exotic and 100 native plants, $P = 0.023$, Figure 4.3A). Rathcke's study (1985; her Appendix Tables 1-6) indicated that all three exotic slugs had no preference between exotic and native plants (*Arion subfuscus:* $n = 13$ exotic and 47 native plants, $P = 0.613$, Figure 4.3B; *A. fasciatus:* $n = 13$ exotic and 48 native plants, $P = 0.437$, Figure 4.3B; *Deroceras reticulatum:* $n = 13$ exotic and 48 native plants $P = 0.090$, Figure 4.3B).

Because many of the exotic species in the above studies were early successional plants, the native vs. exotic contrasts could be confounded by traits of early successional vs. late successional species. When restricting the analyses to early successional species only, the native slug in Cates and Orians (1975) still strongly preferred early successional exotic plants ($n = 27$) over early successional native plants ($n = 36$, $P = 0.008$), whereas the exotic slug no longer showed a significant preference towards exotic plants, though it still trended in this direction ($P = 0.053$). The native grasshopper *Schistocerca emarginata* in Otte (1975) still preferred early successional exotic plants ($n = 10$) over early successional native plants ($n = 52$, $P = 0.007$), *S. obscura* trended in this direction ($n = 7$ exotic and 48 native plants, $P = 0.070$), and the remaining native grasshopper showed no significant preference ($n = 3$ exotic and 20 native plants, $P = 0.157$). However, the sample size in the latter contrast was too limited to be useful. By contrast, none of the exotic slugs in Rathcke (1985) distinguished between early successional exotic ($n = 13$) and native ($n = 8$) plants ($P \geq 0.171$ for *Arion subfuscus* and *A. fasciatus*), though *Deroceras reticulatum* trended towards preference of non-natives ($P = 0.054$). Thus, even though these comparisons did not directly compare herbivore preferences as a choice between early successional exotic and native plants, two of the native consumers still found exotic plants more palatable than native plants unconfounded by successional status. None of the exotic consumers exhibited this pattern of preference.

## *Discussion*

Our results directly oppose the prediction of the enemy release hypothesis that native generalists will prefer native prey (Keane and Crawley 2002, Colautti et al. 2004). Instead, both species of native crayfishes significantly preferred exotic over native plants. This was true in both the phylogenetic pairings and in the region-wide assays of plant palatability (Figures 4.1, 4.2). Results of analyses with terrestrial native consumers were similar. When we included all available data from our study and the terrestrial studies, 6 of 6 native generalist herbivores preferred exotic over native plants, while 4 of 5 exotic generalists did not (Figures 4.2, 4.3). When we constrained the terrestrial datasets and evaluated only early successional native vs. exotic species, the patterns were less dramatic, but similar in direction. Two of 4 native species significantly preferred exotic plants while none of the 4 exotic herbivores demonstrated any significant preference. Thus, exotic plants are more palatable than native plants to native generalist herbivores, suggesting the potential for native herbivores to provide biotic resistance to plant invasions.

Because our native herbivores were two crayfishes, three grasshoppers, and one slug, while our exotic herbivores were one fish and four slugs, our analyses potentially confound consumer origin (native vs. exotic) with consumer type (e.g., grasshoppers vs. slugs). Contrasts with native vs. exotic grasshoppers, native vs. exotic crayfishes, etc. would have been preferable but impossible in this case because: 1) the exotic crayfishes in North America are all range expansions (USGS 2005) that would have had a long evolutionary history with nearly all native flora, 2) there are no native carp in North America (Nelson 1994), and 3) there are no exotic grasshoppers in North America (Lockwood 1993). We did have one comparison among consumers of the same type. In Cates and Orians' study (1975), both the native and the exotic slug preferred exotic over native plants (Figure 4.3), but this pattern weakened and the exotic slug no longer significantly preferred exotic plants once we restricted the analysis to early successional plants (see Results). Despite limitations on the herbivores we could use, our analyses of the published terrestrial studies represent our best efforts to challenge our findings for freshwater systems, and they consistently support our finding that exotic plants are, on average, palatable and selectively consumed by native herbivores.

While our results were unanticipated within the framework of the enemy release hypothesis (Keane and Crawley 2002), they support a classic evolutionary hypothesis: exotic prey may be selectively attacked in their new ranges because they are defensively naïve against native consumers (Darwin 1859, pg. 44), i.e., the 'new associations' principle (Hokkanen and Pimentel 1989). This hypothesis is predicated on the notion that native plants have evolved under selection from native herbivores, whereas exotic plants have not and thus are unlikely to be better adapted than native plants to resist native herbivores (Hokkanen and Pimentel 1989, Colautti et al. 2004). In fact, similar to our results for generalists, several papers have examined preferences of specialists for smaller groups of species and often found that native specialists can also prefer exotic plants over their native hosts (e.g., Thomas et al. 1987, Hokkanen and Pimentel 1989, Solarz and Newman 1996, 2001, Trowbridge and Todd 2001, Trowbridge 2004). These patterns suggest that prey are often vulnerable to novel consumers that they have not been selected to deter. Analogously, it is well documented that exotic generalist consumers such as ungulates, grass carp, gypsy moths, Nile perch, mongooses, cats, rats, and snakes, following their introduction into new systems, often decimate naïve, native prey communities (e.g., Elton 1958).

All of the exotic freshwater plants that we studied are considered pestiferous or noxious by at least one state in the USA (USDA 2004), and most of these plants were high preference prey to native consumers (Figures 4.1, 4.2). Because it is the successful exotic plants that might be expected to best resist native consumers, our results are likely conservative (i.e., the most highly-preferred exotic plants may have been eaten to local extinction and simply not been available for us to collect and study). Despite this potential bias, our findings clearly show that exotic plants are *on average* more palatable than native species (Figures 4.1, 4.2, 4.3). There were, however, notable exceptions. Alligatorweed *Alternanthera philoxeroides* and the watermilfoils *Myriophyllum* spp. were relatively low preference prey (Figure 4.2), suggesting that invasiveness in these species could result in part from their low palatability to native generalists. In other studies, however, Eurasian watermilfoil *Myriophyllum spicatum* is preferred over native milfoils and unrelated native macrophytes by both a native specialist weevil and an exotic generalist moth larva (Solarz and Newman 1996, 2001, Gross et al. 2001), perhaps leading to declines in the abundance of this aquatic weed (Creed 2000).

Our data address herbivore feeding preferences rather than impacts in the field. In some cases, laboratory-derived feeding preferences may be tempered, or not expressed, under more complex field conditions (e.g., Schmitz 1998). Despite these exceptions, feeding preferences commonly translate to impacts on plant community structure (Lubchenco and Gaines 1981, Hay 1997), and such impacts have been observed experimentally with crayfish (Dorn and Wojdak 2004), grass carp (McKnight and Hepp 1995), grasshoppers (Schmitz 1994), and slugs (Hanley *et al.* 1995). Moreover, when impacts to exotic plants in the field have been measured, the overall pattern has been equivocal, with some exotic plants heavily damaged by native herbivores and others apparently not (Maron and Vila 2001, Keane and Crawley 2002, and references therein). In most of these cases, however, only a single exotic species was studied, and it may be misleading to extrapolate the results from a few, potentially unusual, exotic species to exotic species *in general*, particularly when the underlying assumption of enemy release is that exotic plants are avoided by native herbivores by virtue of their evolutionary novelty. In fact, in the only study that we know of examining herbivore damage to a broad sample of native and exotic plants in a field setting, Agrawal and Kotanen (2003) found greater insect damage to 15 exotic vs. 15 related native plants, indicative of selective feeding on exotic plants by naturally recruited herbivores.

We examined a taxonomically diverse and environmentally disparate group of plants - 291 native and 66 exotic species - and 11 herbivore species, thus presumably avoiding the bias of studying any particular exotic species, and we found that native herbivores generally preferred exotic over native plants. Nevertheless, this general trend does not preclude the possibility that a few unusual invaders will have novel defenses that are fortuitous pre-adaptations against local consumer species (analogous to Callaway and Aschehoug 2000). In these cases, evolutionary novelty may in fact impart a strong selective advantage for exotic over native species. Still, it remains to be tested whether the feeding preference patterns that we observed will translate to negative impacts on the distribution and abundance of exotic plants under field conditions.

Our results suggest that native, generalist consumers may suppress plant invasions via selective feeding on exotic plants. In some freshwater systems of Europe, native waterfowl and fishes nearly eliminate the North American invader *Elodea nuttallii* (Van Donk and Otte 1996), and numerous other studies document that generalist consumers limit the establishment and spread of exotic plants in a variety of systems (Maron and Vila 2001, Keane and Crawley 2002, and references therein). It appears rare, however, for generalist herbivores to preclude invasions entirely (Levine *et al.* 2004), suggesting that exotic plants may proliferate despite being vulnerable to the evolutionarily novel consumers in their new range. This could occur for several reasons. It may be rare to find intact, native herbivore assemblages or ecosystems unaltered by anthropogenic influences (Byers 2002), or the increased vulnerability of exotic plants to generalist herbivores may be offset by some other plant attribute, perhaps high growth rates, fecundity, or consumer tolerance (e.g., Schierenbeck et al. 1994, Rejmanek and Richardson 1996). Additionally, in some cases escape from particularly damaging specialists could outweigh the effects of gaining new generalist enemies. These hypotheses remain largely untested but will be critical to determining the net impacts of native enemies on exotic plants.

The influence of herbivores on plant invasions is uncertain (Maron and Vila 2001), but the proposed mechanisms and the importance of enemy release have often been accepted without critical evaluation (see discussions in Keane and Crawley 2002, Shea and Chesson 2002, Colautti et al. 2004). In contrast to the common assumption that exotic plants are advantaged because native generalist herbivores will prefer native plants, we found no evidence to support this hypothesis. Whether we considered freshwater or terrestrial plants, we found that: (1) a diverse group of native consumers (including crayfish, grasshoppers, and slugs) consistently preferred exotic over native plants (Figures 4.1, 4.2, 4.3, Table 1), whereas (2) exotic consumers (including fish and slugs) rarely demonstrated a preference between native vs. exotic plants (Figures 4.2, 4.3). Given that generalists typically have a larger impact on plant community structure than specialists (Crawley 1989, Hay and Steinberg 1992, Hay 1997, Lodge et al. 1998), the selective preference that we observed for exotic plants could negate the benefit of losing coevolved specialists, and native generalists could provide biotic resistance to plant invasions.

# CHAPTER 5
# INVASIONAL MELTDOWN: OPPOSING EFFECTS OF NATIVE VS. EXOTIC HERBIVORES ON PLANT INVASIONS

## *Abstract*

Exotic species are widely assumed to thrive because they lack natural enemies in their new ranges. However, a meta-analysis of 71 manipulative field studies including over 100 exotic plant species from terrestrial, aquatic, and marine systems revealed that native herbivores strongly suppressed exotic plants, while exotic herbivores enhanced the abundance and species richness of exotic plants. Both outcomes are consistent with the hypothesis that prey are susceptible to evolutionarily novel consumers. Thus, native herbivores provide biotic resistance to plant invasions, but the widespread replacement of native with exotic herbivores eliminates this ecosystem service, facilitates plant invasions, and triggers an invasional meltdown.

## *Introduction*

Invasive exotic species threaten native biodiversity (Wilcove et al. 1998), alter ecosystem structure and function (Mack et al. 2000), and annually cost up to $138 billion in the US alone (Pimentel et al. 2000). Determining the ecological traits and interactions that affect invasion success are thus critical for predicting, preventing, and mitigating the negative effects of biological invasions. A rich intellectual history of hypotheses has sought to explain why some exotic species become superabundant in their new ranges, including: (i) the Enemy Release Hypothesis, proposing that exotic species thrive because they lack co-evolved, natural enemies in their new ranges (Darwin 1859, Elton 1958, Maron and Vila 2001, Keane and Crawley 2002, Colautti et al. 2004), (ii) the Biotic Resistance Hypothesis, suggesting that native competitors and consumers naturally resist invasions, thus invasions are more prevalent when native species are removed (Darwin 1859, Elton 1958, Maron and Vila 2001, Stachowicz et al. 2002, Levine et al. 2004), and (iii) the Invasional Meltdown Hypothesis, proposing that positive interactions among exotic species promote invasions (Simberloff and Von Holle 1999). Despite the prominence of these ideas, most tests have focused on response variables that may not translate into demographic impacts (e.g., the number of native enemy species attacking an exotic species rather than the damage inflicted) (Mitchell and Power 2003, Torchin et al. 2003), or on a few "model" species (Wolfe 2002, DeWalt et al. 2004) that may not be typical of exotic species in general.

Invasional Meltdown is unique among hypotheses regarding invasion in that it focuses on positive rather than negative interactions, arguing that some exotic species become invasive because the introduction of a second exotic species increases the fitness of the first (Simberloff and Von Holle 1999). We reasoned that even enemies (exotic plants and exotic herbivores) could produce indirect positive interactions (Hay et al. 2004) if exotic herbivores had stronger impacts on native plants and thus indirectly facilitated exotic plants, a result that might be expected given that native plants are unlikely to be pre-adapted for defense against non-native herbivores. Via the same reasoning, native herbivores might selectively attack evolutionarily naïve exotic plants and suppress their ability to become invasive (Hokkanen and Pimentel 1989, Colautti et al. 2004, Parker and Hay 2005). Under this scenario, native herbivores could provide biotic resistance to invasions by exotic plants, but exotic herbivores could produce an invasional meltdown by suppressing native plant communities and freeing resources for use by exotic invaders.

## *Materials and Methods*

We tested the effects of native and exotic herbivores on exotic plant invasions using meta-analysis to examine 71 published studies that experimentally excluded herbivores and monitored the success of over 100 exotic and 400 native plant species. We focused on generalist herbivores because they often have large impacts on plant community structure (Crawley 1989, Hay 1997, Lodge et al. 1998), they are more likely than specialists to consume and thus influence exotic plants, yet their effects on plant invasions have been historically overlooked (Keane and Crawley 2002). Nearly half (N = 35) of the studies we found monitored herbivore effects on entire plant communities and thus avoided potential bias due to focusing on particular species that may not be indicative of exotic species in general. For these studies (community-wide studies, Table

5.1a), we evaluated how herbivores affected the relative abundance of exotic plants (the fraction of the total plant community that was non-native) and the absolute abundance (e.g., plant biomass and cover) and species richness of both native and exotic plants. An additional 36 studies monitored herbivore effects only for selected species (selected-species studies, Table 5.1b). These studies could not be used to assess community-wide changes in abundance of native versus exotic species as a whole, but they did provide valuable information on how herbivores affected native and exotic plant abundance and survival for an additional 51 exotic plant species not included in the community-wide investigations. We did not include studies reporting enemy diversity or leaf damage alone because these indirect estimates may not translate to impacts on plant demography (Siemann and Rogers 2003). Studies were drawn from a range of biomes (grasslands, scrublands, forests, deserts, freshwater and saltwater marshes, and lake bottoms) and herbivores (native bison, elk, deer, antelope, waterfowl, wallabies, rabbits, rodents, land crabs, fishes, mollusks, insects; and exotic cattle, horses, deer, sheep, goats, rabbits, rodents, fishes, mollusks, and insects).

We used multiple search criteria (e.g., herbivor* and (exclu* or cage* or insecticid*)) in the online database Web of Science (1945 - August, 2005) to compile studies that manipulatively excluded herbivores and examined at least one exotic plant species. Additional studies were found by searching the reference lists of empirical studies and review articles. Plant origin was commonly not identified by the authors because most studies addressed herbivore impacts to plant species composition rather than to exotic plant success *per se*. For these studies, we determined plant origin using online databases (e.g., the United States Department of Agriculture (USDA) PLANTS

Table 5.1. Studies examined in meta-analysis of herbivore control over plant invasions. a) studies that reported enough data to determine the relative abundance of plants that were non-native, and b) studies that reported herbivore impacts only to selected exotic species.

| Citation | Exotic plant | Study site | Manipulation | Response variable | Herbivore effect size(s) |
|---|---|---|---|---|---|
| **a) Community-wide studies** | | | | | |
| (Barry et al. 2004) | *Lythrum salicaria* | Wetlands, Ohio, USA | Cages (native deer & waterfowl) | Plant cover | -0.25 |
| | *Phragmites australis* | | | | -1.40 |
| | *Typha angustifolia* | | | | -0.77 |
| (Bell et al. 1987) | *Asparagus asparagoides* | Scrub-shrub and forests, Garden Island, Australia | Cages (native wallabies) | Plant cover | -3.35, -2.44, 0.18 |
| | *Asphodelus fistulosus* | | | | 0.88, 1.57, 0.59 |
| | *Carduus pycnocephalus* | | | | 0.74 |
| | *Galium murale* | | | | 2.19, 1.32, 0.18 |
| | *Geranium molle* | | | | 0.34, -3.11, -2.84, -0.11 |
| | *Oxalis pes-caprae* | | | | -0.10 |
| | *Sonchus oleraceus* | | | | -1.34, -0.41, -0.26 |
| | *Trachyandra divaricata* | | | | 0.41, 0.83 |
| | *Zantedeschia aethiopica* | | | | -1.10, -0.97 |
| (Bock et al. 1984) | *Convolvulus incanus* | Grassland, Arizona, USA | Cages (exotic cattle) | Plant cover | -0.47 |
| (Bork et al. 1998) | *Bromus tectorum* | Grassland, Idaho, USA | Cages (exotic sheep) | Plant cover | -0.29, 0.93 |

| | | | | | |
|---|---|---|---|---|---|
| (Bowers 1993) | *Carduus acanthoides* | Old field, Virginia, USA | Cages (native deer, woodchucks, rabbits, rodents) | Plant cover | 1.16, 0.06, -0.15, 1.06 |
| | *Carduus nutans* | | | | 0.79, -0.32, -0.43, 0.04 |
| | *Cerastium vulgatum* | | | | -0.26, 0.44, -0.63, -0.45 |
| | *Daucus carota* | | | | 0.30, -0.08, -0.29, -0.07 |
| | *Lactuca scariola* | | | | 0.53, -0.19, -0.47, -0.14 |
| | *Taraxacum officinale* | | | | -0.65, 0.04, 0.06, -0.55 |
| | *Trifolium pratense* | | | | -0.87, -0.20, 0.83, -0.24 |
| | *Verbascum thapsus* | | | | -0.18, 0.19, 0.84, 0.85 |
| (Brady et al. 1989) | *Eragrostis lehmanniana* | Desert grassland and oak woodland, Arizona, USA | Cages (exotic cattle) | Plant cover | 0.47 |
| (Cabin et al. 2000) | *Lantana camara* | Dry-forest, Hawaii, USA | Cages (exotic cattle & goats) | Plant cover | 0.08 |
| | *Pennisetum setaceum* | | | | -0.02 |
| (Cadenasso et al. 2002) | *Achillea millefolium* | Old fields, New Jersey, USA | Cages (native deer, rabbits, voles) | Plant cover | -0.48, 0.62 |
| | *Agropyron repens* | | | | -0.58, -1.36 |
| | *Agrostis gigantea* | | | | 0.49 |

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|---|---|---|---|---|---|
| | *Ailanthus altissima* | | | | 0.20 |
| | *Alliaria petiolata* | | | | 0.12 |
| | *Barbarea vulgaris* | | | | -0.02, 0.05 |
| | *Celastrus orbiculatus* | | | | 0.12 |
| | *Cerastium vulgatum* | | | | 0.10, 0.14 |
| | *Chrysanthemum leucanthemum* | | | | -0.22, -0.53 |
| | *Dactylis glomerata* | | | | 0.04 |
| | *Daucus carota* | | | | -1.62, -0.41 |
| | *Dianthus armeria* | | | | 0.51, 1.18 |
| | *Hieracium pratense* | | | | -0.04, -1.32 |
| | *Lactuca serriola* | | | | -0.34 |
| | *Lepidium campestre* | | | | 0.12 |
| | *Linaria vulgaris* | | | | 0.15 |
| | *Lonicera japonica* | | | | -0.16, 0.03 |
| | *Plantago lanceolata* | | | | 0.41 |
| | *Poa compressa* | | | | -2.08, 0.08 |
| | *Poa pratensis* | | | | -3.30 |
| | *Prunus avium* | | | | -0.18 |
| | *Pyrus malus* | | | | -0.12 |
| | *Rosa multiflora* | | | | -1.23, -1.56 |
| | *Rumex acetosella* | | | | -0.64, -0.73 |
| | *Silene latifolia* | | | | 0.31 |
| | *Stellaria media* | | | | 0.20 |
| | *Taraxacum officinale* | | | | -0.41 |
| (Chaneton et al. 2002) | Unspecified forbs | | | | |
| (Detling 1998) | *Poa pratensis* | Grasslands, South Dakota, Wyoming, and Montana, USA | Cages (native bison, elk, deer, antelope) | Plant cover | -0.04 |

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|---|---|---|---|---|---|
| | *Tragopogon dubius* | | | | -0.41 |
| (Donlan et al. 2003) | Miscellaneous exotics | Pacific Islands, California, USA | Herbivore removal (exotic goats) | Plant cover | |
| (Dormaar et al. 1994) | *Agropyron cristatum* | Grassland, Alberta, Canada | Cages (exotic livestock) | Plant cover | 0, -1.10 |
| | *Descurainia pinnata* | | | | 0.15, 2.34 |
| | *Lappula squarrosa* | | | | 1.18, 2.50 |
| | *Lepidium densiflorum* | | | | 1.07, 2.40 |
| (Evers et al. 1998) | *Alternanthera philoxeroides* | Salt marsh, Louisiana, USA | Cages (native waterfowl or exotic nutria) | Plant biomass per plot | 4.79, -7.29, -7.29, -2.47 |
| (Facelli et al. 1989) | *Panicum repens* | Grassland, Flooding Pampa, Argentina | Cages (exotic cattle) | Plant cover | 0.92 |
| (Fahnestock and Detling 2002) | *Bromus tectorum* | Grassland, South Dakota, USA | Cages (native bison) | Plant cover | -0.73 |
| | *Melilotus officinalis* | | | | 0.37, 0.66 |
| | *Convolvulus arvensis* | | | | 0.077 |
| (Goldson and Trought 1980) | *Trifolium repens* | Grassland, Canterbury, New Zealand | Insecticide (exotic insects) | biomass | 0.07 |
| (Hayes and Holl 2003) | *Holcus lanatus* | Grasslands, California, USA | Cages (exotic cattle) | Plant cover | -1.38 |
| (Holmgren et al. 2000) | *Erodium cicutarium* | Shrubland, Santiago, Chile | Cages (exotic cattle & horses) | Plant cover | 0.26, 0.89, -0.46 |
| (Keeley et al. 2003) | Unspecified annual grasses | Oak savanna, California, USA | Cages (exotic cattle & horses) | Plant cover | |
| (Kimball and Schiffman 2003) | Unspecified | Grasslands, California, USA | Cages (exotic cattle) | Plant cover | |
| (Laycock 1967) | *Bromus tectorum* | Grasslands, Idaho, USA | Cages (exotic sheep) | Plant biomass per plot | -0.10, 1.59, 1.45, 0.49 |

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|---|---|---|---|---|---|
| (Leader-Williams et al. 1987) | *Poa annua* | Grasslands, South Georgia, Sub-Anarctic | Cages (exotic reindeer) | Plant cover | 1.98 |
| (Lord 1990) | Miscellaneous exotics | Grassland, South Island, New Zealand | Cages (exotic sheep) | | |
| (McKnight and Hepp 1995) | *Myriophyllum spicatum* | Lake, Alabama & Tennessee, USA | Cages (exotic grass carp) | biomass | -1.21, -0.85 |
| (Perrow et al. 1997) | *Elodea canadensis* | Lake, Norfolk Broads, UK | Cages (native waterfowl) | biomass | 0.69, -0.10 |
| (Popolizio et al. 1994) | *Alopecurus pratensis* | Riparian meadows, Colorado, USA | Cages (exotic livestock) | Plant cover | -0.26, 0.83 |
| | *Phleum pratense* | | | | -0.96, 0.13 |
| | *Poa pratensis* | | | | 0.29, -1.05 |
| | *Trifolium repens* | | | | 2.37, -0.36 |
| (Rose et al. 1995) | *Agrostis capillaris* | Grassland, Canterbury, New Zealand | Cages (exotic sheep, red deer, and chamois) | Plant cover | 3.33, 2.40, -0.62, -0.69 |
| | *Hieracium lepidulum* | | | | 2.89, 2.30, 0.62, 0.26 |
| | *Hieracium caespitosum* | | | | 1.95, 1.61, -0.34, 0.41 |
| | *Anthoxanthum odoratum* | | | | 0.14, 0.69, 0, 0.92 |
| | *Hieracium pilosella* | | | | -1.10, 1.39, 0, 0 |
| (Rusch and Oesterheld 1997) | *Unspecified* | Grassland, Flooding Pampa, Argentina | Cages (exotic cattle) | Plant cover | |
| (Schulz and Leininger 1990) | *Poa pratensis* | Riparian meadows, Colorado, USA | Cages (exotic cattle) | Plant cover | 1.25 |

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|---|---|---|---|---|---|
| (Sheldon 1987) | *Potamogeton crispus* | Lake, Minnesota, USA | Cages (native snails) | biomass | -3.97 |
| (Singer 1995) | *Bromus tectorum* | Grasslands, Montana, USA | Cages (native elk & bison) | Plant cover | -0.33 |
| | *Poa pratensis* | | | | 0.91 |
| | *Thlaspi arvense* | | | | -0.72 |
| (Stohlgren et al. 1999b) | Unspecified | Grasslands, Colorado, Wyoming, Montana, and South Dakota, USA | Cages (bison, elk , deer, moose, prairie dogs or exotic cattle, sheep, horses) | Plant cover | |
| (Van Donk and Otte 1996) | *Elodea nuttallii* | Lake Zwemlust, Holland | Cages (native waterfowl) | biomass | -3.92, -1.70 |
| (Walker et al. 2003) | *Anthoxanthum odoratum* | Grassland, South Island, New Zealand | Cages (exotic sheep and rabbits) | Plant cover | 0.11 |
| | *Hieracium pilosella* | | | | -0.96 |
| | *Trifolium arvense* | | | | 1.10 |
| | *Crepis capillaris* | | | | -0.09 |
| (Yates et al. 2000) | Unspecified | Eucalypt woodlands, Merredin, Australia | Cages (exotic sheep) | Plant cover | |

**b) Selected-species studies**

| | | | | | |
|---|---|---|---|---|---|
| (Bergelson 1990) | *Senecio vulgaris* | Grassland, Washington, USA | Molluscide (exotic slugs), seeds sown +/- standing dead grass | Number of seedlings | -1.23, -0.47, -0.28, -0.45 |
| (Biondini et al. 1998) | *Taraxacum officinale* | Prairie grassland, North Dakota, USA | Cages (exotic cattle) | Forb density | -0.98, -0.13 |

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|---|---|---|---|---|---|
| (Bossard and Rejmanek 1994) | *Cytisus scoparius* | Foothills & coastlands of California, USA | Cages (native elk, deer, gophers) | Number of plants | -0.26 |
| (Carson and Root 1999) | *Agrostis stolonifera* | Old field, New York, USA | Insecticide (exotic insects) | Mean biomass per plot or plant weight | -0.51 |
| | *Chrysanthemum leucanthemum* | | | | 0.06 |
| | *Phleum pratense* | | | | 0.65 |
| | *Daucus carota* | | | | 0.25 |
| | *Plantago major* | | | | -0.22 |
| | *Rumex crispus* | | | | -0.16 |
| (Case and Crawley 2000) | *Conyza sumatrensis* | Acid grassland, Silwood Park, UK | Insecticide, molluscide, cages (native insects, slugs, & rabbits) | Number of seedlings | -1.62, -0.64, -0.58, 0.40 |
| (D'Antonio 1993) | *Carpobrotus edulis* | Coastlands, California, USA | Cages (native rabbits, deer, gophers) | Seedling survival | -4.13, -0.28, -3.83, -4.17, -2.99, -4.33, -3.93, 0, -1.48, -3.42, -4.53, -4.34 |
| (D'Antonio et al. 1993) | *Carpobrotus edulis* | Coastlands, California, USA | Cages (native deer & rabbits) | Number of seedlings | 0.12, -0.45, -0.94, -0.79 |
| (DeWalt et al. 2004) | *Clidemia hirta* | Forest, Hawaii, USA | Insecticide (exotic insects) | Plant survival | -0.01, 0.01 |
| (Erneberg 1999) | *Anthemis cotula* | Old field, Paaskehojgaard, Denmark | Insecticide & molluscide (native insects and slugs) | Achenes per plant | -1.21, -1.50, -1.60 |
| (Esler 1989) | *Hydrilla verticillata* | Lake, Texas, USA | Cages (native waterfowl) | Plant biomass per plot | -0.86 |

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|---|---|---|---|---|---|
| (Fowler 2002) | *Bothriochloa ischaemum* | Grasslands, Texas, USA | Cages (exotic cattle) | Seedling biomass | -1.60, -0.86, -0.43, -0.21, -0.17, -0.16, -0.04, 0 |
| (Fuentes et al. 1983) | *Acacia caven* | Grasslands, Chile | Cages (exotic cattle, rabbits) | Seedling density | -1.33, 2.45 |
| (Green et al. 2004) | *Adenanthera pavonia* | Rainforest, Christmas Island | Cages (native land crabs) | Seedling survival | -0.36, -0.16 |
| | *Clausena excavata* | | | | -0.50, -0.19 |
| | *Leucaena leucocephala* | | | | -1.79, -0.40 |
| | *Muntingia calabura* | | | | 0, 0 |
| (Greiling and Kichanan 2002) | *Hypericum perforatum* | Grassland, Michigan, USA | Insecticide (native insects) | Seedling survival | -0.44, -0.34, 0.12 |
| (Julien et al. 1992) | *Alternanthera philoxeroides* | Pasture, Newcastle, Australia | Cages (exotic cattle) | Plant biomass per plot | -0.09 |
| (Kelt and Valone 1995) | *Mollugo cerviana* | Desert, Arizona, USA | Cages (exotic cattle) | Number of plants | -0.32 |
| (Kucera 1956) | *Bromus commutatus* | Grassland, Missouri, USA | Cages (exotic cattle) | Plant cover | 2.92 |
| | *Cerastium fontanum* | | | | 0.98 |
| (Kuijper et al. 2004) | *Elymus athericus* | Salt marsh, Schiermonnikoog, Holland | Cages (native rabbits & waterfowl) | Seedling survival | -1.14, -3.44, -0.06, -0.04, -0.09, -0.45, -0.07, -0.24 |
| (Lambrinos 2002) | *Cortaderia jubata* | Coastlands, California, USA | Cages (native rabbits, deer, gophers) | Seedling survival | -2.17, -3.53, -0.04, 0, -0.31 |
| | *Cortaderia selloana* | | | | -2.37, -2.66, -0.63, 0, -0.21, -1.66 |

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|---|---|---|---|---|---|
| (Lym et al. 1997) | *Euphorbia esula* | Grasslands, North Dakota, USA | Cages (exotic goats) | Stem density | -0.81, -1.07, 0.34, -0.99, -1.56, 0.56 |
| (McIntosh and Allen 1998) | *Anthoxanthum odoratum* | Grasslands, Australia | Cages (exotic rabbits) | Plant cover | 0.74, 0.12 |
|  | *Hieracium pilosella* |  |  |  | -3.07, 0.70 |
|  | *Hieracium praealtum* |  |  |  | -2.19, -2.43 |
| (Norbury and Norbury 1996) | *Festuca rubra* | Grasslands, Australia | Cages (exotic rabbits) | Plant biomass per plot | -4.80, -2.80 |
|  | *Agrostis capillaris* |  |  |  | -4.33, -3.07 |
|  | *Holcus lanatus* |  |  |  | -1.86, -4.54 |
|  | *Anthoxanthum odoratum* |  |  |  | -1.47, -2.80 |
|  | *Hieracium lepidulum* |  |  |  | -2.27 |
|  | *Linum catharticum* |  |  |  | 0.18, 0.69 |
|  | *Bulbinella angustifolia* |  |  |  | -0.05 |
| (O'Dowd and Lake 1990) | *Muntingia calabura* | Rainforest, Christmas Island | Cages (native land crabs) | Leaf residence time | -1.52 |
|  | *Leucaena leucocephala* |  |  |  | -0.65 |
| (Osborne and Sassic 1981) | *Egeria densa* | Ponds, Florida, USA | Cages (exotic grass carp) | Plant biomass per plot | -0.79 |
| (Peart 1989) | *Anthoxanthum odoratum* | Grasslands, California, USA | Cages (native deer, rabbits, voles, grasshoppers) | Number of plants | -0.21 |
| (Pierson and Mack 1990) | *Bromus tectorum* | Forests, Washington and Idaho, USA | Cages (native small mammals) | Number of plants | -0.11, -0.03, -0.07, -0.36, -0.31, -0.46, -0.08, -0.09 |

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|---|---|---|---|---|---|
| (Rachich and Reader 1999) | *Lythrum salicaria* | Wetland, Ontario, Canada | Insecticide, cages (native insects & deer) | Inflorescence mass | -1.57 |
| (Rice 1987) | *Erodium botrys* | Grassland, California, USA | Cages (native voles) | Seedling survival to reproduction | -1.64 |
| | *Erodium brachycarpum* | | | | -1.37 |
| (Schadler et al. 2003) | *Conyza canadensis* | Old field, Halle, Germany | Insecticide (native insects) | % change in plant cover | -0.46 |
| (Scherber et al. 2003) | *Senecio inaequidens* | Grassland, Silwood Park, UK | Cages (native rabbits) | Seedling survival | -2.59, -1.61 |
| (Schierenbeck et al. 1994) | *Lonicera japonica* | Old fields, South Carolina, USA | Insecticide, cages (native insects & mammals) | Plant biomass | 0.15, 0.18 |
| (Siemann and Rogers 2003) | *Sapium sebiferum* | Forest and prairies, Texas, USA | Insecticide (native insects) | Seedling survival | -3.59, -3.33, -0.69, -0.98 |
| (Stohlgren et al. 1999a) | *Bromus japonicus* | Grasslands, South Dakota, USA | Cages (native bison, elk, deer) | Plant cover | 1.01 |
| | *Poa pratensis* | | | | -0.16 |
| (Thebaud et al. 1996) | *Conyza canadensis* | Old fields, Montpellier, France | Insecticide, molluscide, (native insects& slugs) | Plant survival | 0, 0, 0.01, 0, 0.04, 0, -0.10, 0.03, 0.13, -0.03, 0, 0 |
| | *Conyza sumatrensis* | | | | 0.02, -0.03, 0.14, 0.09, 0.02, 0.03, 0.12, 0.08, 0.03, 0, 0, -0.01 |
| (Valone et al. 2002) | *Eragrostis lehmanniana* | Arid grassland, Arizona, USA | Cages (exotic cattle) | Plant cover | -0.09 |
| (Vazquez and Simberloff 2004) | *Rosa eglanteria* | Forests, Rio Negro and Neuquen, Argentina | Cages (exotic cattle) | Plant density | -0.64, -0.24, 0.06, 0.27 |
| (Vila and D'Antonio 1998) | *Carpobrotus edulis* | Coastlands, California, USA | Cages (native rabbits) | Mean survival time | -0.14, -0.48 |

Database http://plants.usda.gov/, the New Zealand Plants Database http://nzflora.landcareresearch.co.nz/, and the Germplasm Resources Information Network (GRIN) http://www.ars-grin.gov/cgi-bin/npgs/html/index.pl). Three species (*Phragmites australis, Poa pratensis,* and *Taraxacum officinale*) have obscure native ranges but were designated as exotics in their study region by the authors or by online databases. We considered these species to be exotic, but their exclusion from all analyses did not change our conclusions (data not shown). Herbivore origin was determined using the authors' own designations, and we considered cattle, sheep, and goats to be native to the Fertile Crescent of southwest Asia (Bruford et al. 2003). We considered horses to be non-native to North America given that they only recently been reintroduced to North America after an 11,000 year hiatus (Webb 1977). We used the same databases to determine whether exotic plants and exotic herbivores originated from the same region.

To be included in the analyses of herbivore effects on the relative abundance of exotic plants (community-wide studies, Table 5.1a), studies had to: 1) exclude herbivores and have a control site with herbivores, 2) conduct the experiment in a field setting, and 3) report enough data to determine the total proportion of exotic plants within the entire plant community, not just within a particular subset (e.g., summer annuals only). All studies were reported in terms of plant cover or biomass per area. We did not include studies utilizing herbivore inclusions because these types of studies were rare (two studies) and used artificially high consumer densities. We included three studies (N = 7 separate experiments) that reported only the abundance of 'major' species because these species always comprised greater than 80% of the total plant abundance. Their exclusion from analyses did not change our conclusions (data not shown). For experiments that used nested manipulations of several herbivore species, we used the appropriate treatment and control that differed only in the presence/absence of the herbivore of interest. A second group of studies (selected-species studies, Table 5.1b) fulfilled criteria 1 and 2 above but reported the response (plant abundance, survivorship, reproductive output, or plant size) of selected plant species rather than the entire plant community. We utilized the data from these experiments to analyze how herbivores influenced native and exotic plant survival, and in a broader analysis of both datasets (i.e., all known data examining herbivore impacts on exotic plants) asking whether herbivores have weaker impacts on more aggressive exotic plants.

Data from each paper were extracted from the text, tables, or digitized from figures using the software GrabIt! XP. If the relative abundance or species richness of plant communities was not explicitly calculated by the authors, we calculated the relative abundance by adding the absolute abundances of individual species, and by recording the presence or absence of species in species lists for species richness. When studies on individual exotic plant populations examined multiple variables of plant performance, we included only one of these variables in our analyses by prioritizing in order of the variables that were most commonly presented: i.e., plant abundance (e.g., plant cover, number of plants or biomass per area; N = 24 studies), survivorship (N = 14 studies), reproductive output (N = 11 studies), or plant size (N = 9 studies).

Data from each study were standardized using the unweighted log response ratio: $RR_u = \ln(\bar{X}_{+h})/(\bar{X}_{-h})$ (Hedges et al. 1999), where $\bar{X}_{+h}$ is the mean abundance, survival, or growth of plants in the presence of herbivores, and $\bar{X}_{-h}$ is the same metric in the absence of herbivores. We used the log response ratio because it does not require sample sizes or error measurements (Rosenberg et al. 2000) and because relatively few studies reported these data. The presence of zeros in the dataset indicates an ecologically meaningful outcome (e.g., 0 % survival in the presence of herbivores) but renders the log response ratio incalculable. We added a 1 to all data points to alleviate this problem. Using Meta-Win 2.0 (Rosenberg et al. 2000), we performed unweighted, mixed-effect model meta-analyses and calculated 95% confidence intervals for effect sizes using a bias-corrected bootstrapping technique with 9999 randomized re-calculations (Adams et al. 1997). Effects were considered significant ($P < 0.05$) when the 95% confidence intervals did not include zero. Differences between effects within an analysis were determined using a randomized re-sampling technique that tests for differences among groups in meta-analysis (Adams et al. 1997).

*Effect of herbivores on noxious exotic plants.* Following Mitchell and Power (2003), we used two proxies for noxiousness. We used state noxious weed lists maintained by the USDA Agricultural Research Service (http://www.ars-grin.gov/cgi-bin/npgs/html/taxweed.pl) to compile the number of US states listing each exotic plant species as noxious in agricultural systems. We also used a list compiled by the Alien Plant Working Group of the Plant Conservation Alliance (http://www.nps.gov/plants/alien/list/all.htm) to compile the number of agencies listing each exotic plant species as noxious in natural areas. This limited our analyses to studies conducted in the US. To determine whether herbivores had smaller effects on more widely noxious exotic plants, we calculated the mean herbivore effect size for each exotic species from all examined plant populations for all 71 studies, and then used linear least squares regression to look for a relationship between herbivore effect size and the number of states or agencies listing that species as noxious.

## *Results and Discussion*

In studies assessing herbivore effects on entire plant communities, native and exotic herbivores had strongly opposing effects on the relative abundance of exotic plants. Native herbivores suppressed, while exotic herbivores enhanced, the relative abundance of exotic plants (Figure 5.1). Native herbivores decreased the relative abundance of exotic plants by 28% (log response ratios correspond to a decrease in the relative abundance of exotic plants from 36.6 ± 5.5% in the exclusion treatments to 26.5 ± 5.2% in the grazed communities; mean ± SE, N = 25), consistent with the Biotic Resistance Hypothesis. In contrast, exotic herbivores increased the relative abundance of exotic plants by 68% (from 23.4 ± 3.8% in the exclusion treatments to 39.3 ± 4.9% in the grazed communities; N = 43). Overall, the relative abundance of exotic plants was 50% higher in communities grazed by exotic (39.3 ± 4.9%) than by native herbivores (26.5 ± 5.2%), consistent with the Invasional Meltdown Hypothesis.

An assessment of absolute, as opposed to relative, abundance also found that native and exotic herbivores had strongly opposing effects on native versus exotic plants. Native herbivores suppressed the abundance of exotic plants, whereas exotic herbivores suppressed the abundance of native plants (Figure 5.2A, B). Grazing by exotic herbivores also increased the species richness of exotic plants (Figure 5.2D) while native herbivores had no detectable effect on species richness of either native or exotic plants

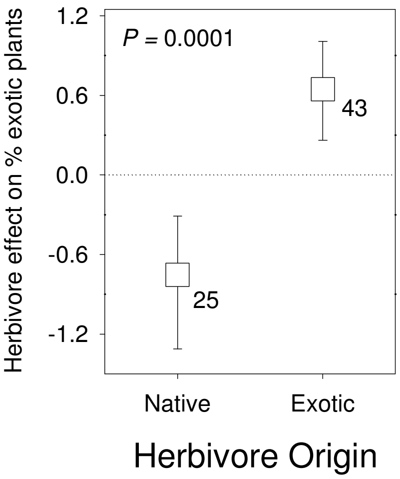

Figure 5.1. Effects of native and exotic herbivores on the relative abundance (% of total plant cover or biomass per area) of exotic plants from 35 studies conducting 68 experiments. Negative numbers indicate a decrease, and positive numbers an increase, in the relative abundance of exotic plants in the presence vs. absence of herbivores. Numbers to right of symbols are the number of experiments contributing to the mean. Points show means ± bias-corrected 95% confidence intervals. Effects are significant when the 95% confidence interval does not cross zero; P-value tests difference in effects of native vs. exotic herbivores.

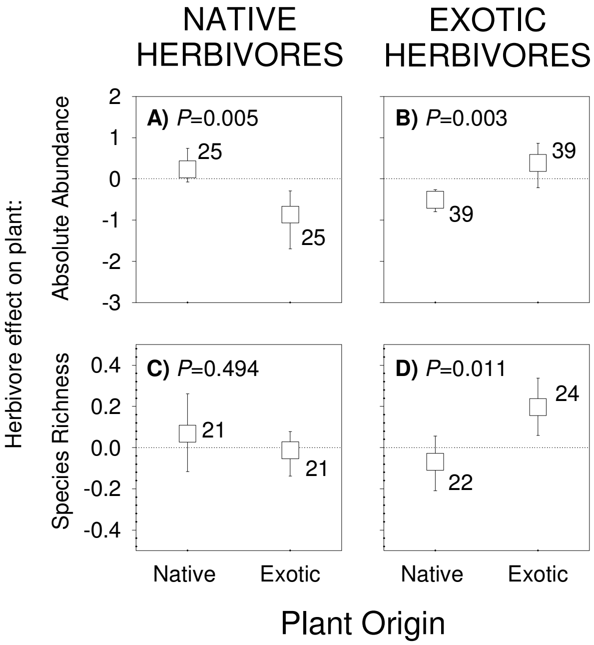

Figure 5.2. Effects of native (left panels) and exotic herbivores (right panels) on the absolute abundance (A and B) and the species richness (C and D) of native and exotic plants. P-values test differences between herbivore effects on native and exotic plants for each contrast. Other analyses as in Figure 5.1.

(Figure 5.2C). Thus, native herbivores limit the abundance but not the likelihood of invasions by new exotic plant species. In contrast, exotic herbivores indirectly and directly promote exotic plant dominance and richness by disproportionately reducing the abundance of native species.

Although these results could be confounded if experiments on native and exotic herbivores differed systematically in variables such as productivity, grazing rate, etc. that could affect herbivore impact (Stohlgren et al. 1999b, Chase et al. 2000), we found no evidence for such bias. We found no detectable differences in the size of exclusion plots or in annual precipitation (a proxy for productivity) between community-level experiments manipulating native vs. exotic herbivores (t-tests, $P = 0.945$ and $P = 0.494$, respectively, with precipitation analyzed for terrestrial experiments only). We also found no correlations between the impact of herbivores on the relative abundance of exotic plants and the size of exclusion plots ($P = 0.358$, $r^2 = 0.02$), annual precipitation ($P = 0.908$, $r^2 = 0.0$), or herbivore impacts on total plant abundance (a proxy for grazing intensity; $P = 0.873$, $r^2 = 0.0$). Thus, the strong differences that we found for the effects of native versus exotic herbivores were unlikely to be driven by other covariates.

Most previous assessments of herbivory on native versus exotic plants focused on invertebrate herbivore impact to plant size or growth (Maron and Vila 2001). In contrast, our analyses focused primarily on the impacts that vertebrate herbivores had on the abundance of exotic plants (24 of 25 experiments for the native herbivores). We hypothesized that vertebrate herbivores would have larger effects than invertebrate herbivores on plant invasions by virtue of their larger size, enhanced mobility, broader diets, and propensity to consume and kill plants rather than reduce their size or growth

(Crawley 1989, Hay and Steinberg 1992). To test this hypothesis, we analyzed the impacts that native vertebrate and invertebrate herbivores had on exotic plant survivorship from studies that focused only on the survival of individual species. Native vertebrate herbivores had a 4.5-fold larger impact on exotic plant survival than did native invertebrate herbivores (log response ratios were -1.65, CI = -2.17 to -1.14, N = 38 and -0.37, CI = -0.67 to -0.16, N = 42, respectively). This suggests that studies focused on native invertebrate herbivores alone may find small effects on plant invasions (Maron and Vila 2001, Keane and Crawley 2002, Colautti et al. 2004) because smaller and more specialized invertebrate herbivores generally have smaller impacts on plant survival than vertebrate herbivores (Crawley 1989, Hay and Steinberg 1992).

We were also concerned that the mean patterns shown in Figures 5.1 and 5.2 might hide important biotic signals if many exotic plants were strongly suppressed by native herbivores but the few that were avoided became aggressive invaders. We thus asked if herbivores suppress benign but not noxious invaders by determining whether herbivores had weaker impacts on plants that were more widely listed as noxious by natural resource agencies in the US (Mitchell and Power 2003). We did not find that herbivores were less effective against more invasive species. In agricultural systems, neither native nor exotic herbivores had weaker effects on plants more widely listed as invasive (Figure 5.3A,B). In natural areas, the same was true for native herbivores (Figure 5.3C), but exotic herbivores actually had stronger negative effects on plants more widely listed as invasive (Figure 5.3D), though this effect was modest. Thus, noxious exotic plants that are spreading most aggressively do not appear to experience greater escape from herbivores than benign exotic plants.

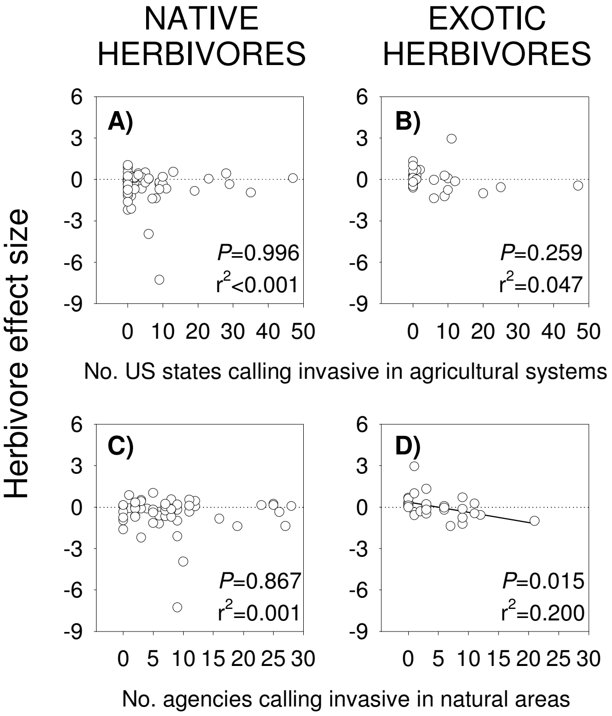

Figure 5.3. Mean effects of native herbivores (A and C) and exotic herbivores (B and D) on exotic plant species as a function of the number of states or natural resource agencies within the USA listing each species as noxious in agricultural (A and B) or natural areas (C and D), respectively. Statistical analysis was by linear least squares regression.

Historically, exotic species were thought to thrive by escaping their co-evolved natural enemies (Darwin 1859, Elton 1958), with little attention paid to the consequences of gaining evolutionarily novel enemies in their new ranges. However, accumulating evidence shows that native herbivores preferentially attack exotic over native plants (Parker and Hay 2005) and therefore suppress the absolute (Figure 5.2A) and the relative abundance (Figure 5.1) of exotic plants in native plant communities. Thus, exotic plants appear evolutionarily naïve and unable to repel native herbivores, and native herbivores suppress most exotic plants. Similarly, exotic herbivores are novel enemies to native plants. In the exotic herbivore studies we analyzed, 83% of the 41 exotic plant species for which ranges were known originated from the same region as the exotic herbivores. Thus, exotic plants tended to co-occur with exotic herbivores from that same region. By disproportionately affecting evolutionarily naïve, native plants, exotic herbivores may therefore promote the abundance and species richness of co-adapted exotic plants from their own native regions (Figure 5.2). These results have considerable implications for ecosystem conservation, suggesting that eradication of exotic herbivores and restoration of native generalist herbivores could mitigate exotic plant invasions, thus avoiding problems associated with introductions of non-native herbivores (Louda et al. 1997).

In recent centuries, Europeans have both extirpated native herbivores and introduced exotic herbivores as they colonized North and South America, Australia, and New Zealand (Crosby 1986, Simberloff and Von Holle 1999, Holmgren 2002). Our data suggest that these changes could promote invasional meltdowns and ecosystem collapse by replacing a source of biotic resistance (native herbivores, Figures 5.1, 5.2A) with species that promote further invasions (exotic herbivores, Figures 5.1, 5.2B, D).

Supporting this hypothesis, there have been large-scale invasions by exotic plants and profound transformations of native ecosystems following the replacement of native bison, elk, kangaroos, prairie dogs, moas, and tortoises with exotic cattle, pigs, horses, sheep, goats, and rabbits (Crosby 1986, Simberloff and Von Holle 1999, Holmgren 2002). Thus, exotic plants may become invasive not because they have escaped their co-evolved, specialist enemies, but because the decimation of naïve New World plants by Old World herbivores paved the way for invasions of plants that were adapted to these herbivores.

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