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The Combined Effects of *Hydrellia* spp. Herbivory and Native Plant Competition on the Biomass and Surface Coverage of Hydrilla

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PURPOSE: Hydrilla (*Hydrilla verticillata* (L.f.) Royle) was grown in earthen ponds with competitive pressure by four species of native plants with two levels of herbivory (*Hydrellia* spp. flies present or absent) to determine the combined effects of herbivory and native plant competition on the biomass and surface coverage of Hydrilla.

BACKGROUND: Biological control and native plant competition are methods used for the management of invasive or weedy plant species. For hydrilla, two species of leaf-mining flies, *Hydrellia pakistanae* Deonier and *H. balciunasi* Bock, have been used as biological control agents since 1987 (Buckingham and Grodowitz 2004). Leaf mining by the larvae of these species decreases the ability of hydrilla to photosynthesize. This results in reduced biomass, tuber and turion production, and fragment viability (Doyle et al. 2002, 2007; Grodowitz et al. 2003; Owens et al. 2006, 2008). Native plants, through resource competition, have also been identified as an effective management tool for invasive plants. In previous studies, native plant competition reduced both the biomass and tuber production of hydrilla (Doyle et al. 2007; Grodowitz et al. 2006). While research has been conducted for both biological control and native plant competition as single management methods, there is a lack of information regarding the interactions of these two strategies as combined management tools. To that end, we examined the combined effect of herbivory by *Hydrellia* spp. flies and plant competition on dioecious hydrilla biomass and surface coverage in three earthen research ponds.

MATERIALS AND METHODS: Dioecious hydrilla was initially cultured in two 1,845 L mesocosm tanks located outdoors at the Lewisville Aquatic Ecosystem Research Facility (LAERF) located in Lewisville, Texas, (33E04’45”N, 96E57’30”W). Each tank contained 150 – 1 L pots of sediment obtained from ponds at the LAERF. Pond sediments were submerged in 90 °C water for 25 minutes to reduce non-target species seed germination. On 10 April 2006, each pot was planted with one – 10 cm apical stem fragment of hydrilla. After planting, alum-treated water from Lake Lewisville was added gradually as plants reached the surface, to a final height of 0.6 m.

The study was conducted in three 0.3 ha earthen ponds (40m by 60m) at the LAERF. Preparation of the study ponds included draining, mowing, and rototilling. Each pond was separated lengthwise into two congruent treatment areas using a pond liner barrier that restricted water flow between sides (Nachtrieb et al. 2011). Pond water was gravity fed from Lake Lewisville, Lewisville, Texas and supplied evenly to both sides of each pond to maintain a depth of approximately 1m at planting sites.

All treatment areas included hydrilla with native plant competition. Native plants were previously introduced into the ponds during studies conducted during 2004 and 2005 (Nachtrieb et al. 2007 and

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Nachtrieb et al. 2011) and were allowed to grow naturally from the existing seed and tuber bank. Four native plant species were established in the ponds including: American pondweed (*Potamogeton nodosus* Poiret), Illinois pondweed (*P. illinoensis* Morong), white water lily (*Nymphaea odorata* Ait), and Mexican water lily (*N. mexicana* Zucc.). On 12 May 2006, each treatment area was planted with 12 replicates of hydrilla, from cultures described above. Each replicate was enclosed in a 91 cm diameter by 1.2 m tall cylinder (cage) constructed from 5 cm by 10 cm mesh, welded-wire fencing anchored with 1.2 m lengths of rebar. Cages provided protection from disturbances such as herbivory by turtles or ducks. Cages were placed at equidistant intervals and positioned at equal depths by following the pond's contour. Each cage was planted with three - 1 L pots of cultured hydrilla. Plants were removed from pots and planted directly into sediment.

Two levels of herbivory (*Hydrellia* spp. flies present or absent) were included in this study. The insecticide Abate® 4-E (Abate) (Clarke Mosquito Control Products, Inc. Roselle, IL) was used in one treatment area of each pond to eliminate *Hydrellia* spp. larvae. Abate was applied weekly as an emulsifiable concentrate at 0.047 lbs a.i./acre (1.5 fl. oz/acre) using a 1-gallon (nominal size) pressurized sprayer (Root-Lowell Manufacturing Co. Lowell, Michigan). Applications were restricted to monotypic stands of hydrilla to reduce impact to native insect/plant interactions. *Hydrellia* spp. introductions were planned for the remaining treatment area in each pond approximately one month after planting or upon hydrilla reaching the water's surface. However, introductions were deemed unnecessary as *Hydrellia* spp. fly colonization occurred naturally from nearby ponds.

Twelve – 10 cm stems of hydrilla (one per cage) were collected from each treatment area approximately every four weeks to enumerate *Hydrellia* spp. immatures / kg hydrilla fresh weight and associated percent damaged leaves. Counts were determined using a stereomicroscope at 8X to 80X magnification. Collections began approximately one month after planting. Following a biomass harvest of 6 cages per treatment area on 28 August 2006, only six – 10 cm stems of hydrilla (one per remaining cage) were collected for the remainder of the season until 28 November 2006.

On 28 August 2006, six cages were randomly selected from each treatment area and harvested for above-ground plant biomass. Plants were separated into two categories — natives or hydrilla — and dried to a constant weight in a forced-air drying oven at 55 °C.

Macrophyte surface coverage was recorded twice during the study. On 28 August 2006, prior to biomass harvesting, a photograph was taken of each cage from 0.5m above the water's surface. A second set of photographs, detailing a broader area, were taken on 19 October 2006 from 3.35m above the water's surface. Six pictures were taken per treatment area, three near the cages in approximately 1m of water and three in the deepest area of each pond in approximately 1.5m of water. All pictures were analyzed using Image-Pro® Express 5.1 (Media Cybernetics, Inc. Bethesda, Maryland). The total area of each image as well as the total areas of surface coverage of hydrilla, mixed beds (natives and hydrilla), or open water (areas devoid of vegetation) were calculated to determine percent surface coverage. Ground truthing revealed hydrilla growing among all native beds; therefore a category for natives alone was not included.

STATISTICAL ANALYSIS: Plant dry biomass between herbivore and non-herbivore treatments were analyzed with a one-way ANOVA for hydrilla and native plants separately. Experimental data were analyzed at a significance level of $p < 0.05$ using STATISTICA version 8.0 (StatSoft, Inc., 2008, Tulsa, OK).

RESULTS AND DISCUSSION: *Hydrellia* spp. flies inhabiting nearby hydrilla ponds at the LAERF served as starter colonies and enabled rapid natural fly colonization into the three study ponds. Within one month of planting, *Hydrellia* spp. reached approximately 3,900 and 2,700 immatures per kg, respectively, in herbivore and non-herbivore treatment areas (Figure 1) with approximately 30% damaged leaves in both treatments (Figure 2). In non-herbivore treatment areas, fly levels and respective percent damaged leaves decreased thereafter due to weekly insecticide treatments (Figure 1 & 3). In contrast, herbivore treatment area fly levels reached a maximum of greater than 5,000 immatures per kg in October (Figure 1) and approximately 50% damage in both September and the beginning of November (Figure 2). By the end of November, fly levels had decreased to approximately 2,000 immatures per kg with less than 10% damage (Figure 1 & 2). These same seasonal trends of population peaks towards the end of warm weather followed by decreases during winter months have been noted in previous studies with *Hydrellia* spp. (Grodowitz et al. 2009). Winter decreases of larvae in hydrilla leaves have been attributed to larvae moving into the stem of hydrilla to overwinter (Harms and Grodowitz 2011).

When sampled from cages, several trends in the biomass and percent surface coverage of hydrilla and native plants (mixed beds) were noted. Native plant biomass and percent coverage (mixed beds) did not differ between herbivore and non-herbivore treatments (Figure 3 & 5). This suggests that an equal amount of competitive pressure was exerted by native plants in each treatment area. The biomass of hydrilla was reduced by approximately 35% due to the combined effects of *Hydrellia* spp. herbivory and native plant competition (Figure 4). In contrast, the percent surface coverage of hydrilla did not differ between treatments (Figure 5). Hydrilla weakened by herbivory was not as robust as herbivore-free hydrilla, but in 1m of water is easily able to grow to the surface and produce a canopy. Monotypic stands of hydrilla occupied approximately 60% of the surface coverage while mixed beds covered 40% regardless of treatment area (Figure 5). Open water areas were not detected during cage sampling.

When percent macrophyte coverage was taken from a wider sampling area in October, a different trend was noted. Regardless of location (deep or shallow) or treatment (herbivore or non-herbivore), mixed plant beds were present in greater percentages than monotypic hydrilla beds (Figure 6). Furthermore, distinctly opposite trends were noted for mixed and hydrilla beds. Regardless of location, the percent coverage of mixed beds displayed an increasing trend when herbivores were removed while monotypic beds of hydrilla decreased (Figure 6). While hydrilla had increased growth in the presence of biocontrol agents, the trend was a result of decreased growth of native plants in the presence of invertebrate herbivory. Without herbivores present, the native plants were better able to compete with hydrilla for surface coverage. In a previous study, it was shown that the biomass of two native plants, American pondweed and Illinois pondweed, increased two to three fold when herbivores were removed by insecticide applications (Nachtrieb et al. 2011). Low insecticide dosages and specific application to hydrilla beds were employed to reduce the effect of Abate on non-target, native insects. While these methods may have helped until the first sampling event in August, by October the growth and subsequent surface coverage of native plants were aided by the apparent decrease in native herbivores. Surface coverage of mixed beds ranged from 68 to 89%, while the coverage of monotypic beds of hydrilla ranged from 10 to 32% (Figure 6). Open water areas were almost nonexistent, barely reaching 1% surface coverage in deep water areas (Figure 6).

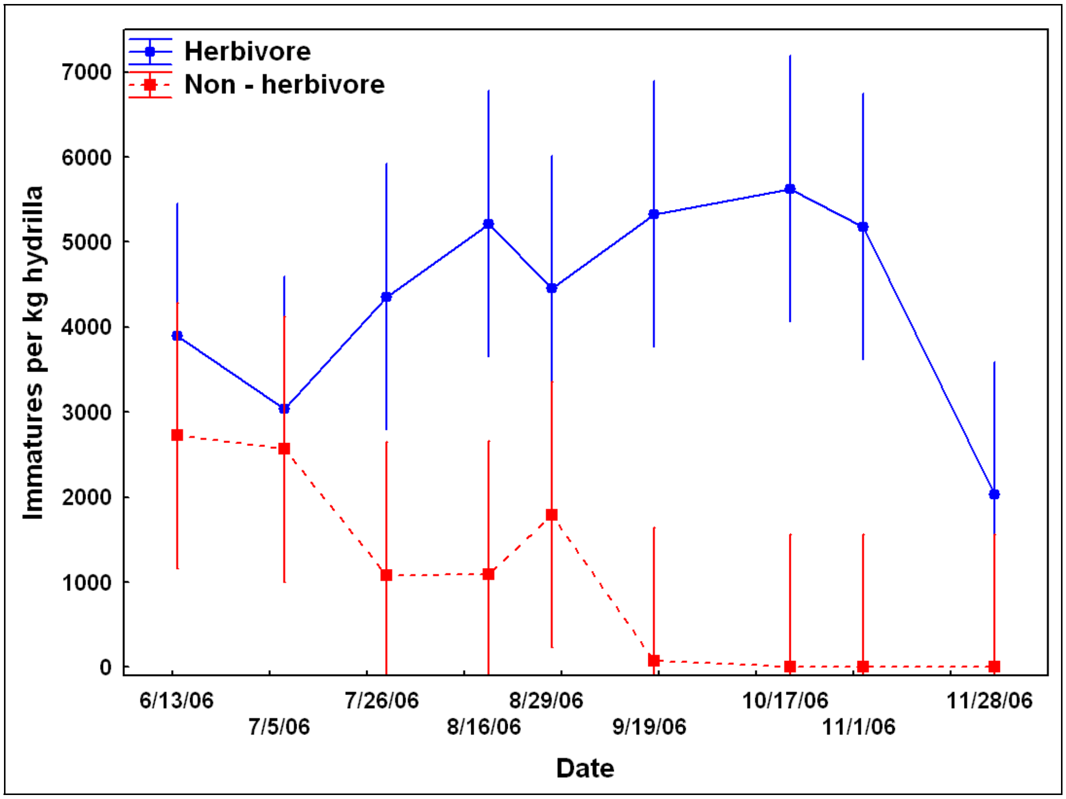

Figure 1. Mean (± 0.95 confidence interval) *Hydrellia* spp. immatures per kg hydrilla in each herbivory treatment and each sampling date in 2006.

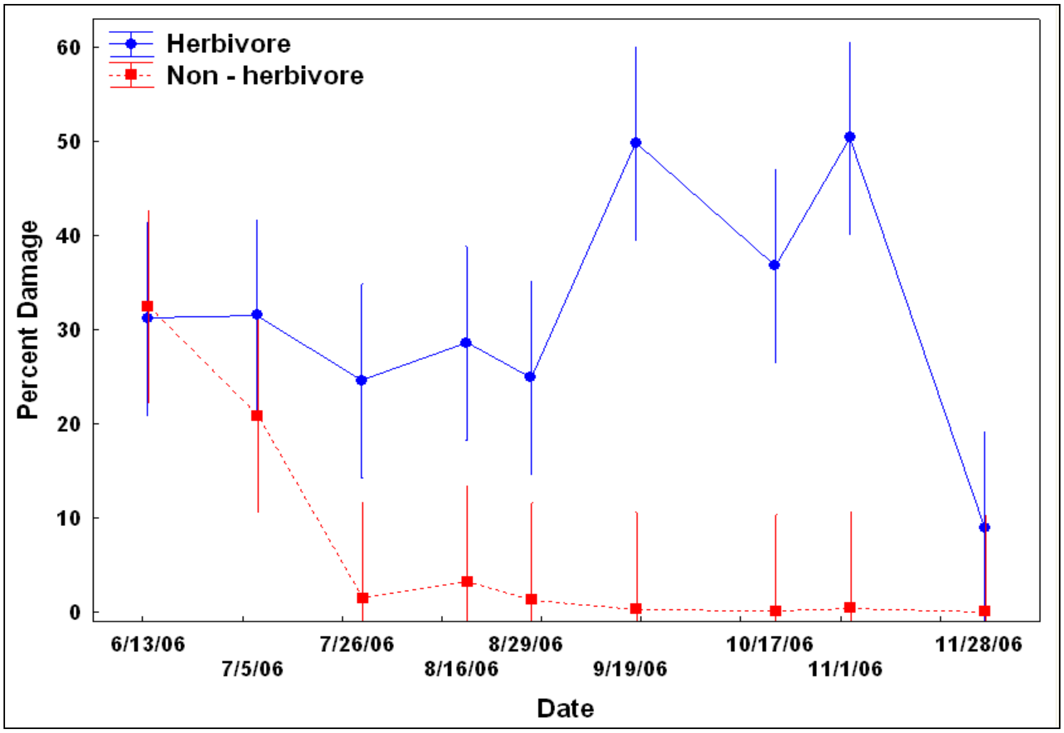

Figure 2. Mean (± 0.95 confidence interval) percent hydrilla leaf damage in each herbivory treatment and each sampling date in 2006.

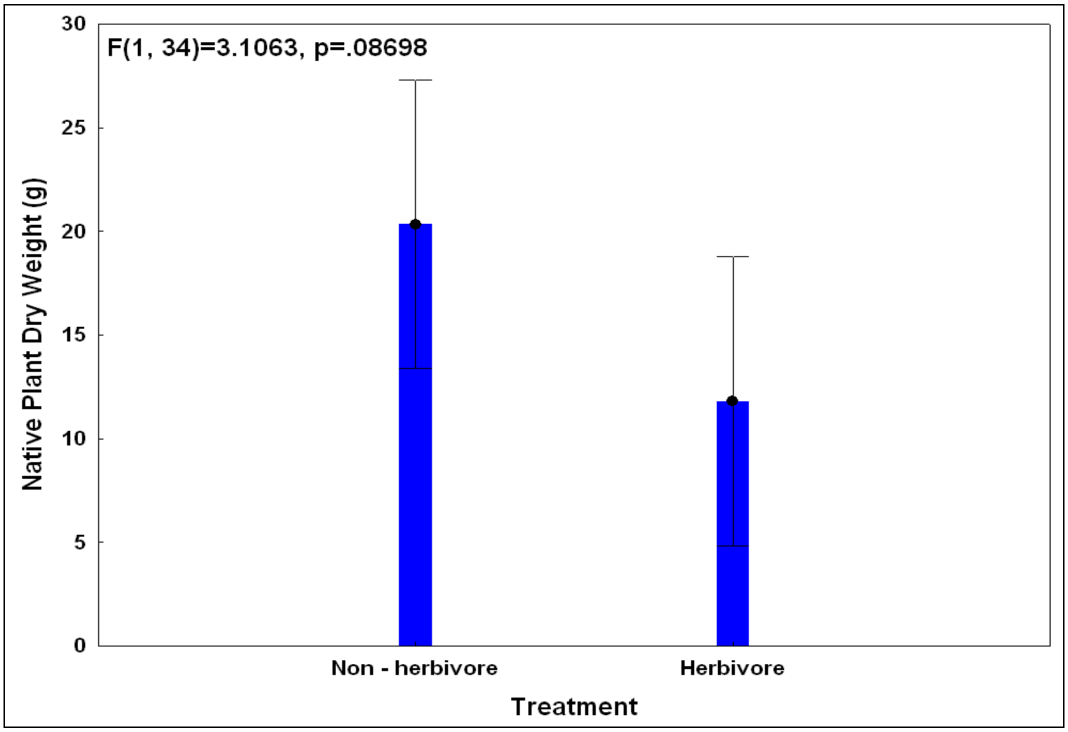

Figure 3. Mean (± 0.95 confidence interval) dry biomass (g) of native plants collected on 28 August 2006 per herbivore treatment area and analyzed with a one-way ANOVA.

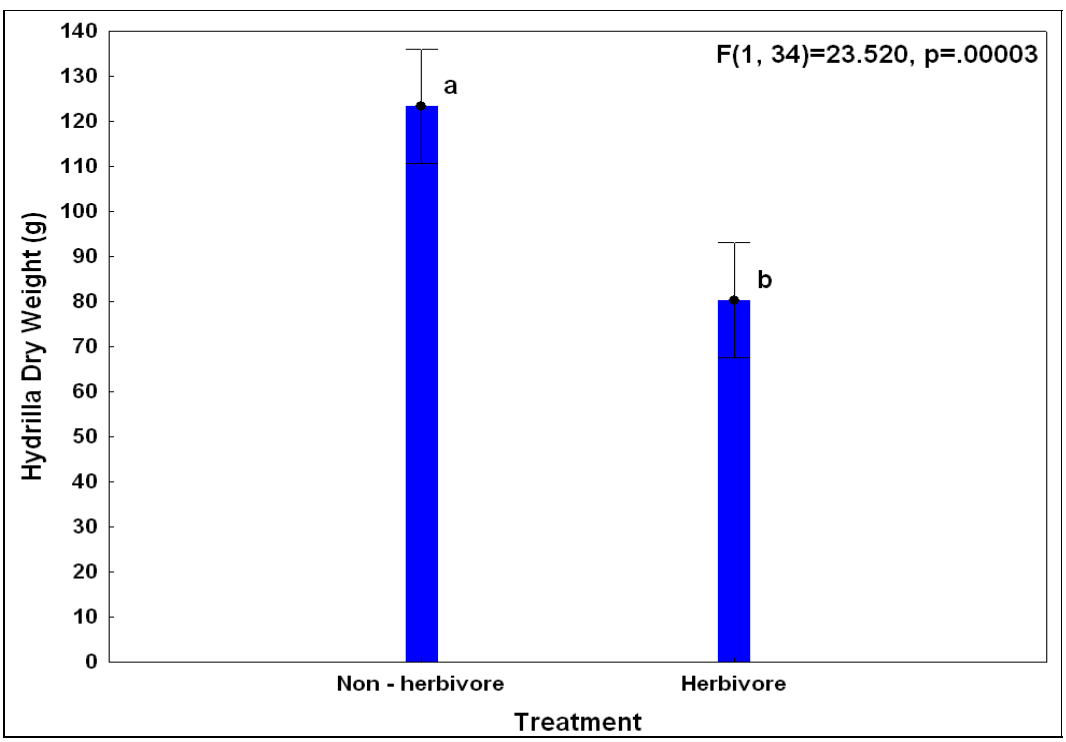

Figure 4. Mean (± 0.95 confidence interval) dry biomass (g) of hydrilla collected on 28 August 2006 per herbivore treatment area and analyzed with a one-way ANOVA. Means with the same letter are not significantly different.

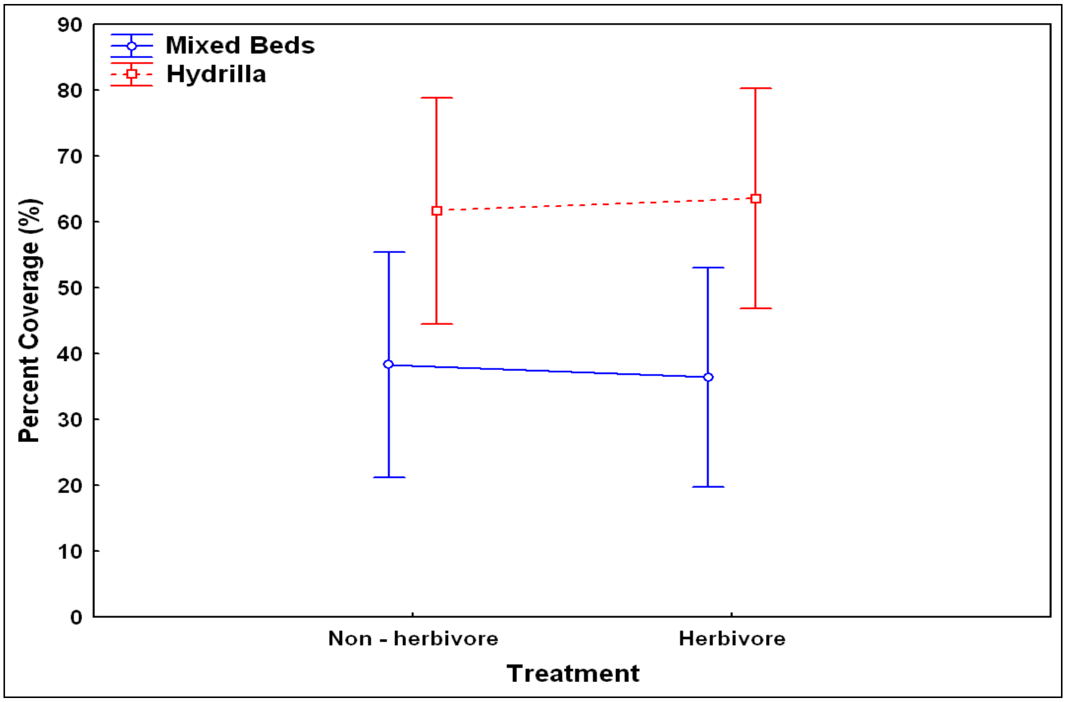

Figure 5. Percent of macrophyte surface coverage (± 0.95 confidence interval), per herbivore treatment area and plant community, recorded from individual cages on 28 August 2006.

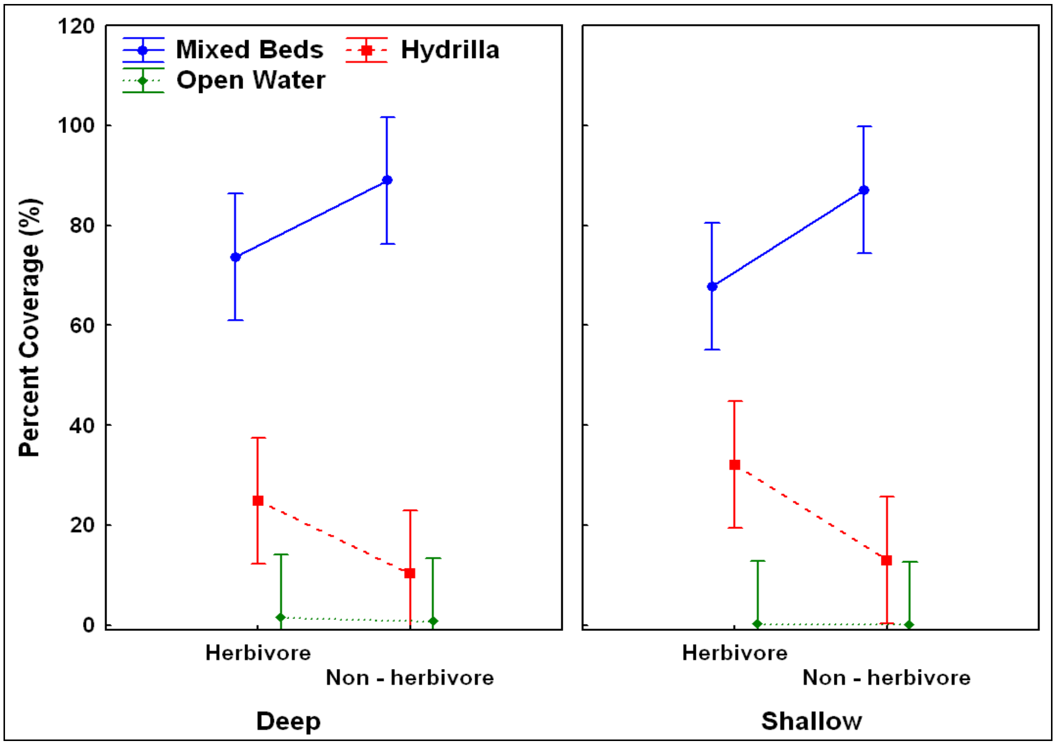

Figure 6. Percent of macrophyte surface coverage (± 0.95 confidence interval), per herbivore treatment area, pond location, and plant community, recorded 19 October 2006.

While the biomass of hydrilla was decreased by 35% as a result of biological control in combination with native plant competition, the ability of hydrilla to dominate surface coverage was not impacted. By the later sampling date, the competitive ability of native plants was artificially increased by accidental removal of native insect herbivores. In order to gain a better understanding of "real world" competition amongst hydrilla and native plants, native insect herbivores must be present. This makes designing a study to quantify the effects of biological control and competition difficult. Future studies should focus on multiple, separate field sites with and without biological control agents where an insecticide would not be necessary.

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