

**Herbivory on Submersed and Floating Water Lily Leaves  
(*Nuphar variegatum* and *Nymphaea odorata*)  
In two lakes**

**BIOS 569: Practicum in Aquatic Biology**

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**Abstract:**

Herbivory on *Nuphar variegatum* and *Nymphaea odorata* floating and submersed leaves was quantified in two lakes on the University of Notre Dame Environmental Research Center property. Damage to marked leaves was quantified during 22 May to 9 July, 1994. In Kickapoo Lake, grazing damage to *Nymphaea* floating and submersed leaves was greater than damage to *Nuphar* floating and submersed leaves. Mean damage rates for *Nymphaea* floating leaves were 1.22% and 1.50% damage per day while those for *Nuphar* floating leaves were 0.14% and 0.28% damage per day. *Nymphaea* submersed leaves had damage rates of 0.55% and 0.85% damage per day and *Nuphar* submersed leaves had 0.11% and 0.53% damage per day. In West Long Lake, *Nuphar* submersed leaves had greater percent damage (1.81% damage per day) than *Nuphar* floating leaves (1.41% damage per day) earlier in the summer while the reverse was seen later in the summer. Grazer observations suggest that in Kickapoo Lake, many abundant grazers were specific to or preferred *Nymphaea*, including a leaf-mining chironomid and the beetle *Donacia proxima*. In West Long Lake, much of the damage appeared to be caused by caddisflies. Laboratory experiments on Limnephelid caddisfly grazing selectivity were performed by exposing 8 macrophytes of known weight and random placement to 3 caddisflies for 24 hours. Each of 14 replicates had a control experiment with the same random placement of macrophytes and no caddisflies present. Corrected proportional weight losses indicate that caddisflies do graze upon *Nuphar* submersed leaves, but may prefer other macrophytes, including *Potamogeton amplifolius* and *Myriophyllum alterniflorum*, which were more abundant in Kickapoo Lake than in West Long Lake. This could explain the lower grazing damage to submersed leaves of both species in Kickapoo Lake and suggests the complexity of macrophyte-herbivore interactions.

**Introduction:**

The potential role of freshwater macrophytes as important food web components in aquatic systems has been traditionally neglected in the scientific literature. Recent work in this area has focused on rooted, floating macrophytes such as the water lilies *Nuphar* and *Nymphaea* (Juliano 1988, Kouki 1991a, 1991b, 1993, Setälä 1991, Wallace & O'Hop 1985). Results from these studies indicate that herbivory on lilies does occur and, in fact, has a significant impact on the lilies. These findings contradict the conventional wisdom which holds that freshwater macrophytes are rarely consumed and invite further research into the potential role of macrophytes in freshwater systems.

The best studied macrophyte, as indicated, is the water lily. Water lilies are extremely abundant in many ponds and lakes and their floating leaves are easily observed. Various types of herbivores have been identified on the floating leaves of water lilies. These include aphids, fly larvae, lepidopteran larvae and several chrysomelid beetles (Kerfoot 1988). Of these, the chrysomelid beetle *Pyrrhalta nymphaeae* has been found to be the major specialist herbivore and thus the best studied (Kerfoot 1988, Lodge & Hay 1993). The entire life cycle of this insect, from egg development through three larval instar stages to adulthood, takes place on the floating *Nuphar* water lily leaf (Kerfoot 1988). Often these beetles are forced to migrate to new leaves in order to avoid drowning as the leaves sink, illustrating their dependence upon this "terrestrial" environment (Kouki 1991b).

The effects of herbivory on the water lily plant have only been examined in detail for *Pyrrhalta*. Grazing by these beetles has been observed to significantly reduce leaf longevity (Juliano 1988, Kouki 1991a, 1991b, Wallace & O'Hop 1985). Wallace and O'Hop (1985) found that grazed leaves had an average life of 17 days while ungrazed leaves appeared healthy at 47 days. Beetles were found to consume, on average, 17% of a single leaf's total surface area during a 20 day period (Kouki 1991b). Beetle grazing created damaged areas that were subsequently invaded by microorganisms including bacteria and fungi (Kouki 1991a, 1991b, Wallace & O'Hop 1985). The decomposition and eventual sinking of the grazed leaves were accelerated by this microbial activity (Wallace & O'Hop 1985).

Another aspect of herbivory on the water lily involves the heterophyllic nature of the plant. Kouki examined the relative production of floating and submersed leaves in *Nuphar* exposed to herbivory by *Pyrrhalta* (Kouki 1993). His results indicated that when floating leaves were consumed by herbivores the water lily began to produce more submersed leaves. The production of these leaves was thought to be advantageous to the plant because they encountered no herbivory in the experimental system.

The present study seeks to examine herbivory on *Nuphar variegatum* and *Nymphaea odorata* floating and submersed leaves by documenting percent damage over defined periods of time and observing and identifying grazers, with specific emphasis on those found on floating leaves of the two species. A third component of the study is an examination of the feeding preferences of a generalist grazer, larvae of the caddisfly *Limnephilus sp.*, observed to be an abundant grazer on *Nuphar* in one of the study lakes.

## Materials and Methods:

### 1. Study Sites

Field surveys of herbivory on water lilies were conducted in two lakes at the University of Notre Dame Environmental Research Center. Kickapoo Lake, a small lake with a well-developed littoral zone, supports populations of *Nuphar variegatum* Engelm, *Nymphaea odorata* Ait., and *Nuphar microphyllum* (Pers.) Fernald. West Long Lake, the western basin of an experimentally divided lake, is supplemented with nutrients and supports a manipulated piscivore population. The littoral zone is not well-developed and only one species of water lily, *Nuphar variegatum*, is found. *Myriophyllum alterniflorum* DC., *Nuphar variegatum*, *Nymphaea odorata*, *Pontedaria cordata* L., *Potamogeton amplifolius* Tuckerm. and *Zygnema* sp. used in the caddisfly grazing preference experiment were collected in Kickapoo Lake. *Isoetes braunii* Dur. was collected from West Long Lake and *Typha latifolia* L. was collected from a marsh area along Killarney Point road across from the maintenance building at UNDERC. *Lobelia dortmanna* L. was collected from Roach Lake on the UNDERC property.

### 2. Herbivory Damage Sampling

Floating and submersed leaves of *Nuphar variegatum* and *Nymphaea odorata* were analyzed for herbivory damage. *Nuphar* floating leaves are heart-shaped and large, with midrib lengths of collected leaves ranging from 8.9 to 28.7 cm, while *Nymphaea* floating leaves are circular, deeply notched and smaller, with midrib length ranging from 3.4 to 5.3 cm. *Nuphar* submersed leaves are fan-like in shape with midrib lengths of 7.5 to 4.2 cm.. *Nymphaea* submersed leaves are also fan-shaped but much smaller, with midrib lengths of 3 to 5.4 cm. The submersed leaves of both species are very thin and almost translucent and often reddish purple in color. Floating and submersed leaves of *Nuphar variegatum* were analyzed in West Long Lake while *Nuphar variegatum* and *Nymphaea odorata* floating and submersed leaves were analyzed in Kickapoo Lake.

In both lakes leaves were marked with forestry flagging tied around the petioles of submersed or floating leaves. Some *Nuphar* and *Nymphaea* floating leaves were marked while still furled and underwater while others were marked after surfacing. Submersed leaves of both species were marked while still furled or after unfurling. Only those leaves with no visible damage were marked and marking was accomplished by snorkeling the perimeter of each lake and marking all available leaves, thus providing an accurate distribution. The first set of leaves was marked on 22 May and collected 17 d later on 7 June. The second set was marked on 27 May and collected 22 d later on 17 June. The third set included only West Long Lake leaves and was marked on 8 June and collected 32 d later on 9 July. Each set differed in number and type of leaves marked because of leaf availability due to differential development and poor leaf condition as a result of herbivory (Tables 1).

Following collection, each leaf was xeroxed and leaf type, date, and lake were noted. The leaves were analyzed for percent damage using a digitizing tablet and Sigma Scan. In the case of leaves with perimeters altered by grazing, reconstructions of the intact leaf were made using factors such as leaf diameter and midrib length. These two factors were set equal when necessary in order to estimate leaf size. Mean percent damage per day was determined and 95% confidence limits were calculated for these mean daily damage values. Tukey-Kramer tests were performed to measure the

significance of the raw data for each time period in Kickapoo Lake. T-tests were used to obtain the same information for West Long Lake.

### 3. Observation and identification of grazers

During the course of the experiment, observations were made concerning the most abundant types of grazers on the two water lily species as well as differences in grazer populations between lakes. Changes in grazer populations were also noted over the course of the summer. In Kickapoo Lake, changes in beetle populations were monitored by random selection of a floating leaf by tossing a float among a patch of water lilies and then counting the number of beetles on fifteen leaves in the area of the selected leaf. In West Long Lake, due to smaller water lily populations, beetle populations could be easily monitored by directly counting the number of beetles on each of several water lily patches in the lake. Six *Nymphaea* floating leaves and six *Nuphar* floating leaves from Kickapoo Lake were dissected and several grazers isolated and identified. In addition, 2 to 3 suspected leaf-mining midges were placed in each of two enclosures. Another two enclosures contained 1 individual lepidopteran larva of either *Pyralidae* or *Noctuidae*. Each enclosure was a .5 liter glass jar with intact water lily leaves of the type from which the grazer was isolated. The enclosures were placed in the laboratory and observed periodically for 24 hours.

### 4. Laboratory experiment on caddis fly grazing preferences

Caddisfly larvae (*Limnephilus sp.*) were offered samples of nine macrophytes in a controlled environment. Three caddisflies were placed in each of fourteen dishpans. Each dishpan was "gridded" and the macrophyte samples were randomly distributed on the grid in each pan. Another fourteen pans contained the same random distributions but had no caddisflies present and served as the control for weight change by the macrophytes during the experiment. The pans were set up such that each experimental pan was directly across from its corresponding control pan. The experiment was run for 24 hours and 45 minutes, from 6:15 PM 29 June to 7:00 PM 30 June.

The caddisflies were collected from West Long Lake while the macrophytes were obtained from several lakes on the UNDERC property (see Study Sites). The nine macrophytes used were *Zygnema sp.*, *Potamogeton ampifolius*, *Myriophyllum alterniflorum*, submersed *Nuphar variegatum*, *Pontedaria cordata*, *Lobelia dortmanna*, floating *Nuphar variegatum*, *Isoetes braunii*, and *Typha latifolia*.

An above ground portion, or where appropriate, a leaf of each macrophyte was selected such that all of the nine samples allowed what we estimated to provide an equal encounter rate. These samples were either surface-dried using paper towel or spun-dry with a two minute spinning cycle. They were then weighed and this weight, plus or minus 0.005g, served as the initial weight for all replicates. All subsequent macrophyte samples were dried and weighed in a manner consistent with this original procedure. The macrophyte samples weight ranges and drying method were as follows: *Isoetes* (0.311-0.321g, spun), *Lobelia* (0.338-0.348g, spun), *Myriophyllum* (0.357-0.367g, spun), *Nuphar* floating (0.174-0.181g, blotted), *Nuphar* submersed (0.105-0.113g, blotted), *Pontedaria* (0.200-0.207g, blotted), *Potamogeton* (0.052-0.060g, blotted) and *Typha* (0.171-0.180g, blotted).

The macrophyte samples were secured to the bottom of the dishpans using small suction cups with slits in them. Following the 24 hour experimental period, the

macrophyte samples were carefully removed from the suction cups and all loose or floating macrophyte parts were collected and sorted. The same drying and weighing procedures were used to obtain final weight.

The initial and final masses of each macrophyte for each replicate were compared to the initial and final masses of their respective controls. Corrected proportional weight change was calculated using the equation  $(I_e - F_e / I_e) - (I_c - F_c / I_c)$  where  $I_e$  stands for initial experimental weight,  $F_e$  stands for final experimental weight,  $I_c$  stands for initial control weight and  $F_c$  stands for final control weight.

### Results:

#### 1. Herbivory Damage Sampling

In Kickapoo Lake *Nymphaea* floating leaves had the highest mean daily percent damage and *Nymphaea* submersed leaves the second highest mean daily percent damage during time periods 1 and 2 (Fig. 1 A&B). A Tukey-Kramer test indicated that *Nuphar* floating and submersed leaves had significantly less damage than *Nymphaea* floating leaves between 22 May and 7 June (Fig. 1 A). The only significant difference in damage between 27 May and 17 June was seen in *Nuphar* floating and submersed leaves and *Nymphaea* floating leaves. In West Long Lake, *Nuphar* submersed leaves showed greater mean daily percent damage during time periods 1 and 2 while *Nuphar* floating leaf mean daily percent damage was greater in time period 3 (Fig. 1 A-C). T-tests revealed that no significant differences in damage existed.

Tukey-Kramer tests were also used to compare damage across lakes. Between 22 May and 7 June, *Nuphar* submersed leaves from Kickapoo experienced significantly less damage than *Nuphar* floating and submersed leaves from West Long. *Nuphar* floating and *Nymphaea* submersed leaves from Kickapoo had significantly less damage than *Nuphar* submersed leaves from West Long. Between 27 May and 17 June, no significantly different damages were seen across lakes (Fig. 1 A&B).

#### 2. Observation and identification of grazers

In West Long Lake submersed *Nuphar variegatum* leaves were heavily grazed on 21 May and few floating *Nuphar variegatum* leaves had reached the surface yet. Large populations of caddisfly larvae (*Limnephilus sp.*) were observed in the lake in late May and many were observed feeding upon the submersed and developing floating *Nuphar* leaves. By mid-June the caddisfly population was reduced and small numbers of *Donacia proxima* (1-2 beetles per 15 leaves) were seen feeding on floating *Nuphar* leaves. In mid-July, *Pyrrhalta* larvae were found on floating *Nuphar* leaves (3-4 per beetle larvae per 15 leaves) and *Donacia proxima* populations were absent.

In Kickapoo Lake *Nuphar variegatum* and *Nymphaea odorata* submersed and unsurfaced floating leaves were present on 21 May. Little herbivory was seen at this time. During June, floating *Nymphaea* leaves were heavily grazed by *Hyporhygma sp.*, a leaf-mining chironomid larvae. Leaf dissections of *Nymphaea* floating leaves revealed the presence of 5-10 of these midge larvae per leaf. The midges were often found in cocoon-like cases at the ends of tracks of damage running throughout the leaves. In laboratory enclosure experiments, this leaf-mining damage pattern was confirmed. Floating *Nuphar* experienced only slight midge damage. *Pyralidae* larvae caused damage to both *Nuphar* and *Nymphaea* while another Lepidopteran, *Noctuidae* (*Bellura sp.*), was isolated from *Nuphar*. *Pyralidae* were observed in lab enclosure experiments

to cause localized damage by cutting away a section of the leaf and cementing it to another area of the leaf surface for use as a shelter. *Noctuidae* burrowed in the petiole of *Nuphar* leaves in lab experiments. By mid-June *Donacia proxima* was seen in large numbers on floating *Nymphaea* but few were observed on *Nuphar*. *Donacia proxima* had a major effect on floating *Nymphaea* due to its destructive grazing pattern. By 6 July the *Donacia proxima* population was very small, averaging 1 or 2 individuals per 15 leaves. In mid-July several *Pyrrhalta* larvae were found on floating *Nuphar* leaves.

### 3. Laboratory experiment on caddisfly grazing preferences

*Potamogeton* and *Nuphar* submersed leaves were consumed by caddisfly larvae (Figure 3). *Myriophyllum*, *Pontedaria* and *Lobelia* appear to have been consumed but this cannot be stated with certainty due to the overlap of the 95% confidence limit with zero weight change. Similarly, *Nuphar* floating leaves and *Isoetes* appear not to have been consumed. *Typha* showed a corrected proportional weight gain (Figure 3). Consumption of *Zygnema* was also observed but, due to physical dispersion, a final mass could not be taken.

**Discussion:**

The data as presented point to the importance of herbivory for *N. variegatum* and *N. odorata* floating and submersed leaves. These damage values, however, must be taken as conservative estimates due to the nature of the study. Only undamaged leaves were marked and this resulted in the exclusion of leaves subjected to grazing prior to the beginning of the experiment. This is an especially important consideration in West Long Lake, where most submersed and emerging floating *Nuphar* leaves were already heavily grazed on 22 May.

In Kickapoo Lake, grazer observations were limited to floating leaves. The significant differences between mean daily percent damage of *Nuphar* and *Nymphaea* floating leaves correlate with observational data. The larvae of the leaf-mining chironomid *Hyporhygma* sp. and the adult beetle *Donacia proxima*, both the source of substantial leaf damage, were observed mostly on *Nymphaea* leaves while *Nuphar* leaves, which were much larger in size, showed minor chironomid larvae damage and evidence of lepidopteran presence. Between 22 May and 7 June, *Nymphaea* submersed leaves showed significantly more damage than *Nuphar* floating leaves as well, suggesting that the entire *Nymphaea* plant was more subject to grazing during this period than *Nuphar* floating leaves, perhaps due to location in deeper water or structural or nutritional advantages.

In West Long Lake, no significant differences in damage between leaf types were found. However, large caddisfly larvae populations in May, as previously stated, led to an underestimation of herbivory damage occurring in the lake at this time. While not significantly different, a trend is seen in which submersed *Nuphar* encountered heavier grazing during the two sample periods encompassing 22 May through 17 June while floating *Nuphar* experience heavier grazing from 8 June through 9 July. Observational data suggest that *Donacia proxima* were found in the lake by mid-June and by July, *Pyrrhalta* larvae were present. The presence of these grazers provided a substantial grazing force on floating leaves which was absent earlier in the summer. At this same time, caddisfly populations dropped due to emergence, suggesting that if observations and leaf monitoring had continued, grazing damage on floating leaves would have been observed to increase or at least persist.

Damage rates for *Nuphar* and *Nymphaea* floating and submersed leaves underwent seasonal changes. In early summer, *Nymphaea* was grazed by *D. proxima* and *Hyporhygma* sp. in Kickapoo Lake. In West Long Lake, which lacked substantial macrophyte cover, submersed *Nuphar* leaves received the brunt of grazing during this period. The appearance of *Pyrrhalta* closely followed the disappearance of *D. proxima* in both lakes toward mid-summer and suggests the potential for a reversal in grazing trends, in which *Nuphar* floating leaves would encounter more grazing. These observations suggest some sort of timing of grazer emergence, perhaps dependent upon weather or other cues, such that over-grazing is prevented or previous damage aids the feeding of later grazers.

The grazing preference experiment involving *Limnephilus* sp. larvae can be used to explain field observations. In Kickapoo Lake, little caddisfly grazing of *Nuphar* or *Nymphaea* leaves was seen while extreme grazing was seen on *Nuphar* submersed in West Long Lake. The experimental results indicate that while *Nuphar* submersed is consumed by caddisflies and *Nuphar* floating may also be, other macrophytes, all of which are found in Kickapoo Lake, might be preferred. This suggests that caddisfly

grazing in Kickapoo Lake may focus on other macrophytes while in West Long Lake, few other food sources are available.

While not addressed in the experimental design, it is important to question why caddisflies exhibited these feeding preferences. The top three preferred macrophytes, *Potamogeton*, *Myriophyllum* and *Nuphar* submersed leaves, all have a thin, easily torn leaf. Caddisflies, then, might prefer these three due to the ease of consumption. *Typha* samples, which were thick and tough, gained weight during the experiment and appeared to be the least preferred macrophyte. The weight gain is attributed to water uptake by the samples and uneven surface drying. Since no feeding was directly observed, we assume that this water gain did not mask any consumption but this cannot be stated with 100% certainty. Other factors beyond physical structure of macrophytes, such as nutritional content and relative location in the lake, may also affect caddisfly feeding

Herbivory was found to be an important interaction taking place among water lily populations in two UNDERC lakes. In addition, the caddisfly preference experiment suggests that herbivory may be important for other freshwater macrophytes, both emergent and submersed. The data and observations indicate that a wide variety of grazers, not just the larvae of beetles, may derive nutrition from macrophytes. In order to better understand the results, the caddisfly experiment needs to be redesigned to negate structural differences. Nutritional content of macrophytes species, long assumed to be low, must also be determined. Both field and laboratory portions of the study should be repeated for a variety of macrophytes and grazers. While the present study confirms the presence of herbivory on water lilies and indicates it in other macrophytes, the information generated by these additional studies could be used to compare and contrast its importance between macrophyte species and aquatic systems.

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Kickapoo Lake

West Long Lake

	<i>Nuphar</i> floating	<i>Nuphar</i> submersed	<i>Nymphaea</i> floating	<i>Nymphaea</i> submersed		<i>Nuphar</i> floating	<i>Nuphar</i> submersed
22 May-7 June	9	12	8	5		2	4
27 May-17 June	5	5	7	8		14	10
8 June-9 July						7	1

Table 1: Sample sizes for all leaf types collected in Kickapoo and West Long Lakes

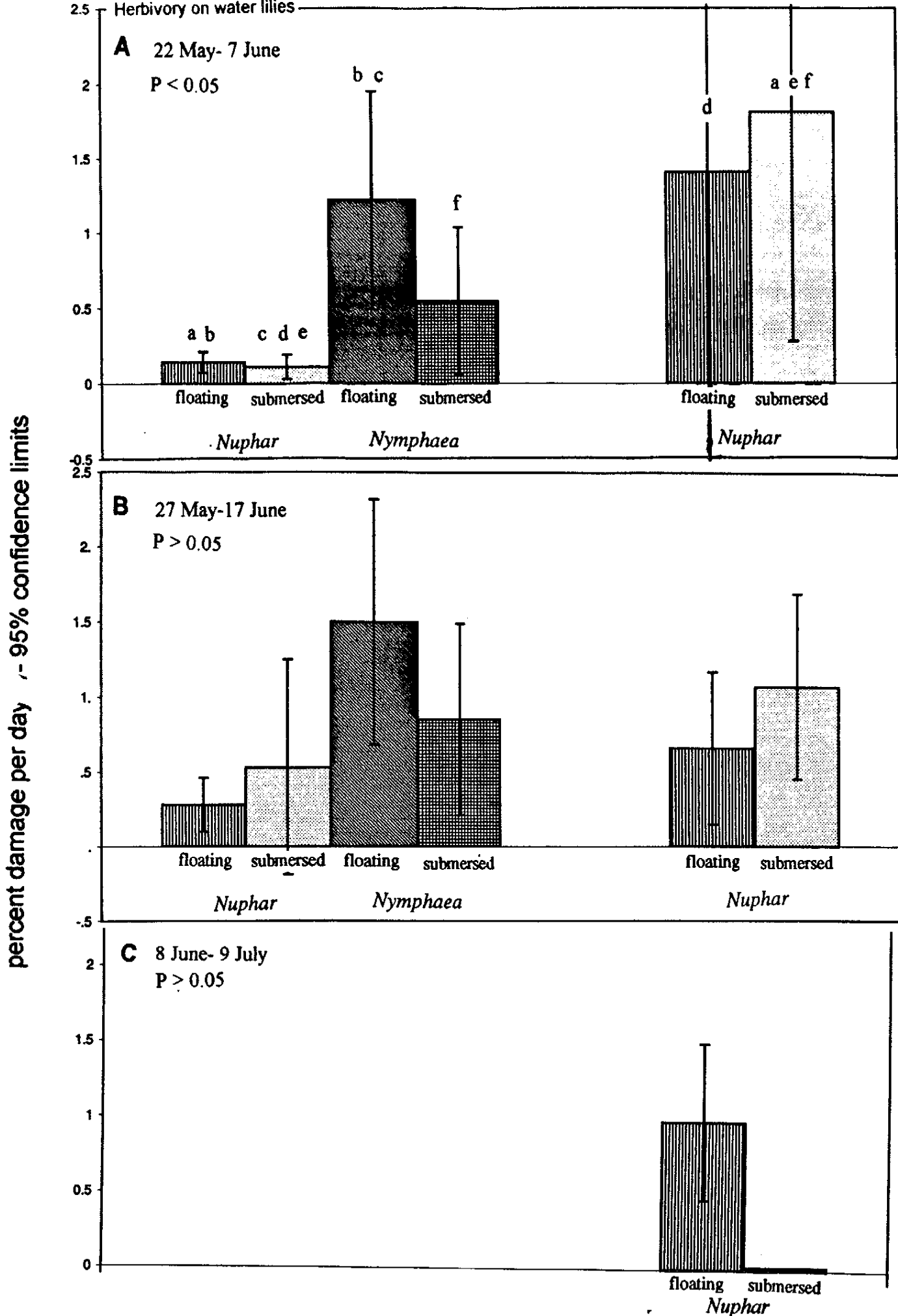


Figure 1: Mean daily percent damage for *Nuphar variegatum* and *Nymphaea odorata* floating and submersed leaves in Kickapoo and West Long Lakes

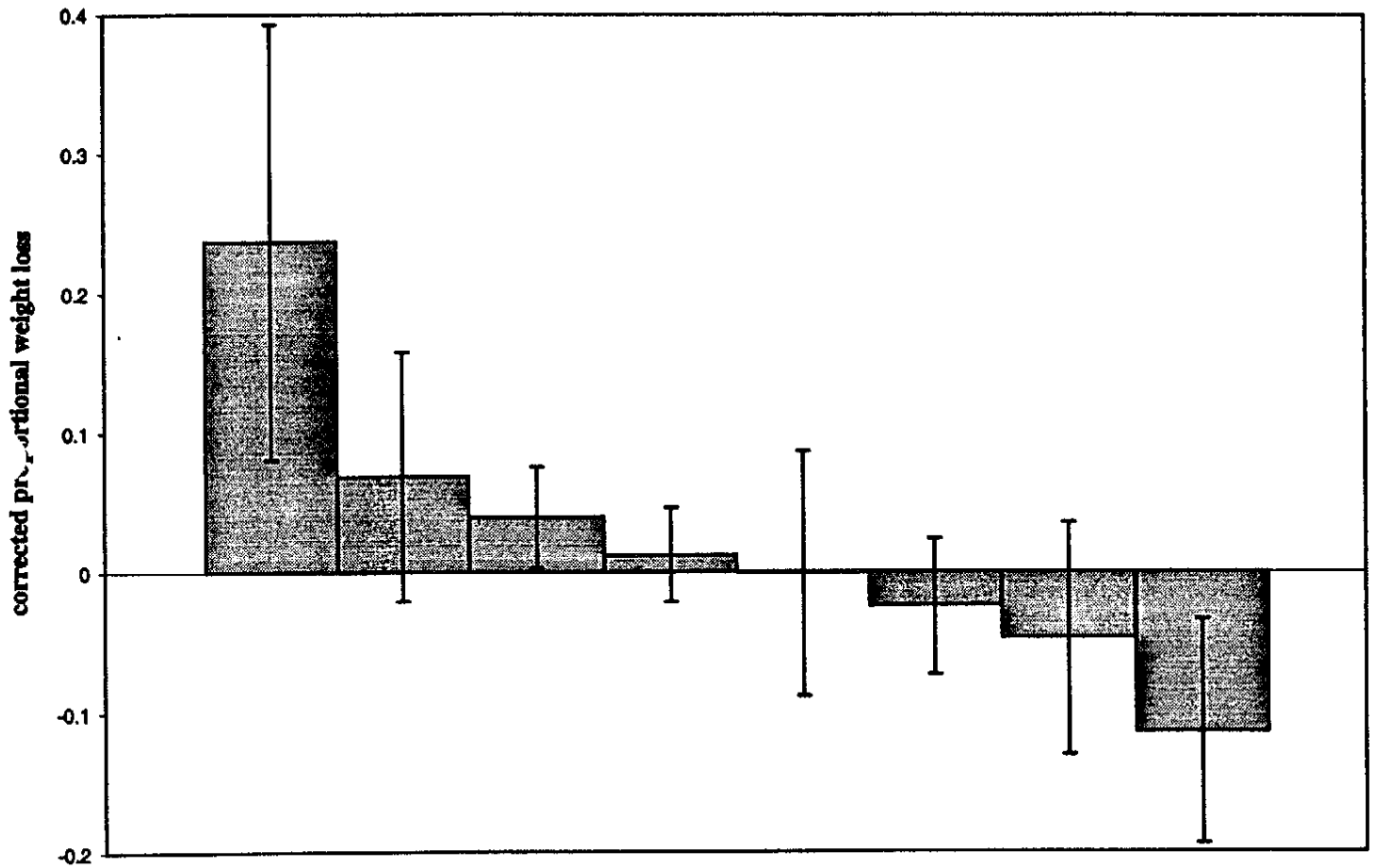


Figure 2: Corrected proportional weight loss for 8 macrophytes offered to *Limnephilus sp.* during grazing preference experiment

Herbivory on water lilies

Leaf Type	5/22-6/7	5/27-6/17
Nuphar floating	5.7	3.4
Nuphar floating	1.7	3.3
Nuphar floating	1.6	4.9
Nuphar floating	3.6	8.5
Nuphar floating	1.7	10.2
Nuphar floating	1.2	
Nuphar floating	1.5	
Nuphar floating	2	
Nuphar floating	1.8	
Nuphar submersed	2.7	0.7
Nuphar submersed	0.1	27.7
Nuphar submersed	2.1	1.6
Nuphar submersed	0.2	4.7
Nuphar submersed	4.4	23.2
Nuphar submersed	0.1	
Nuphar submersed	2.9	
Nuphar submersed	0.2	
Nuphar submersed	0.6	
Nuphar submersed	0.2	
Nuphar submersed	0.6	
Nuphar submersed	1.9	
Nymphaea floating	9.2	45.1
Nymphaea floating	9.5	43.6
Nymphaea floating	2.8	42.3
Nymphaea floating	50.7	4.3
Nymphaea floating	25	7.4
Nymphaea floating	16.8	57.7
Nymphaea floating	24.6	30.4
Nymphaea floating	26.8	
Nymphaea submersed	11.2	5.8
Nymphaea submersed	5.6	23.6
Nymphaea submersed	9.3	37.5
Nymphaea submersed	1.3	3.1
Nymphaea submersed	19	9
Nymphaea submersed		48.6
Nymphaea submersed		16.9
Nymphaea submersed		4.8

Appendix A: Raw data for Kickapoo Lake damage experiment

Herbivory on water lilies

Leaf type	5/22-6/7	5/27-6/17	6/8-7/9
Nuphar floating	9.7	2	12.8
Nuphar floating	38.2	9.4	12.8
Nuphar floating		6.4	59.5
Nuphar floating		9.7	48.9
Nuphar floating		68.9	20.9
Nuphar floating		23	37.9
Nuphar floating		1.1	26.8
Nuphar floating		10.7	
Nuphar floating		5	
Nuphar floating		45.8	
Nuphar floating		2.7	
Nuphar floating		4.3	
Nuphar floating		2.6	
Nuphar floating		12.1	
Nuphar submersed	20	42.7	0.5
Nuphar submersed	25	25.3	
Nuphar submersed	22.5	1.85	
Nuphar submersed	55	5.8	
Nuphar submersed		8.3	
Nuphar submersed		13.8	
Nuphar submersed		10.6	
Nuphar submersed		50	
Nuphar submersed		53.6	
Nuphar submersed		23.9	

Appendix B: Raw data for West Long Lake damage experiment