

**Flow-Related Fragmentation versus Herbivory on
Macrophyte Decomposition in Freshwater Streams**

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ABSTRACT

Processing of leaves of four macrophyte species (*Nuphar advena*, *Calla palustris*, *Potamogeton amplifolius*, and *Vallisneria americana*) was studied during the summer in a northern Michigan stream. In June 1997, plant material was secured onto cobble and placed in a riffle. Loss in mass and surface area was measured after 1, 4, 7, 14, 21 and 28 days. The order of decomposition (in declining rate) was: *N. advena* = *V. americana* > *C. palustris* > *P. amplifolius*. However due to confounding factors, *C. palustris* is really believed to have the fastest processing rate. These rates may be determined primarily from flow-related fragmentation and thus influenced by plant shape, structure, and toughness. Toughness measurements were determined to be (in descending order): *N. advena* > *P. amplifolius* = *V. americana* > *C. palustris*. The potential role of macroinvertebrates in fragmentation of the plants was explored through a selective herbivory experiment using the native crayfish *Orconectes propinquus*. With the exception of *V. americana*, *O. propinquus*' selectivity seemed to be based on toughness preferring (in descending order): *C. palustris* > *P. amplifolius* > *V. americana* and *N. advena*. Selectiveness between conditioned and unconditioned material was undetectable with all species except *V. americana*. These results suggest chemical defense in *V. americana*. The inconsistencies between processing rate order and macroinvertebrate preference order suggest flow-related fragmentation to be the dominant mechanism of macrophyte decomposition in this system.

INTRODUCTION

Detrital pathways are major sources of energy flow in all ecosystems. In the case of freshwater ecosystems, two different sources contribute to the pathways: 1) autochthonous and 2) allochthonous (Webster and Benfield 1986). In streams the major emphasis of study has been directed towards the allochthonous source (i.e. terrestrial leaf litter), despite the fact that in lakes macrophyte detritus has been found to be an important source of food for organisms common in both lentic and lotic habitats such as snails, crustaceans, and insects (Newman 1991). The conventional wisdom concerning macrophytes has been that live macrophytes are rarely consumed by herbivores and are only functionally important in aquatic food webs as detritus (Lodge 1991, Newman 1991, Mann 1976). One explanation for this observation could be that macrophytes in the form of detritus are actually more palatable to invertebrates. According to previous findings, selective herbivory is based on such non-nutritional characteristics as secondary compounds and toughness (Lodge 1991) which would favor detritus as the superior food choice. Furthermore, if invertebrates exhibit selective herbivory among various macrophyte detritus, then the rates at which macrophyte detritus is processed may differ. This type of selectivity may help to better define the role that invertebrates fill in decomposition and how their actions affect decay rates.

Autochthonous input may be the major contributor to the energy base in many streams depending on the season (Newman 1991) or in low-ordered streams that lack canopies or extensive riparian vegetation (Webster and Benfield 1986). Furthermore, macrophytes decay twice as fast as terrestrial leaf litter (Newman 1991) and so may provide a more immediate energy source for the stream at crucial times of the year. In order to increase our understanding and help to better characterize this important source of energy for stream ecosystems, we studied the decay rates of four macrophyte species in a lake fed northern Michigan stream that supported the growth of strong macrophyte communities in addition to receiving them from the lake's outflow. Our objectives were to: 1) quantify the decay rates of two submergent (*Vallisneria americana*, *Potamogeton amplifolius*) versus two emergent (*Nuphar advena*, *Calla palustris*) macrophyte species; and 2) determine if abiotic factors such as temperature, pH and structural characteristics influenced the processing rates. Data was also collected for future study to enable a quantification of macroinvertebrate colonization patterns and their influence on the rates. We expected the degree of structural support maintained by the plant to establish a spectrum of varying rates such that macrophyte decomposition rates would be faster than those reported for terrestrial leaf litter, and submergent species' rates would be faster than the emergent species' due to the reduction of structural support required by the aquatic environment. In future studies, we would also expect that following an initial leaching period in which the secondary compounds break down and microbes condition the plants, macrophyte decomposition rates would be positively correlated to macroinvertebrate abundance due to both consumptive and mechanical causes. In an additional study we attempted to quantify the effect of preferential feeding on the decay rates of the same four macrophyte species using *Orconectes propinquus* as a model organism. Our objectives were to quantify feeding behavior when presented with a

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paired-choice of conditioned versus unconditioned macrophyte biomass of the same as well as when presented with a choice of all four macrophyte species. Previous studies have shown the nutritional value to differ little among emergent, floating and submergent macrophytes (Lodge 1991), so in both experiments we expected *O. propinquus*' preferential selection to be based on two factors: 1) the presence of secondary compounds and 2) the leaf toughness. According to these parameters conditioned material would be preferred in the first experiment.

METHODS

Leaf Processing

The study was conducted during the summer months of 1997 in Tenderfoot Creek, an outflow of Tenderfoot Lake located in the western half of the upper peninsula of Michigan (46° 13'N, 89° 32'E; elev.=500m) Maloney and Lamberti (1994). A riffle site about eight meters wide was chosen three km downstream from Tender foot Lake (Maloney and Lamberti 1994) where the substrate was dominated by equal proportions of cobble and gravel. To help establish the type of environment to which the macrophytes were exposed the pH of the stream was determined at two different times (beginning and end) during the experiment ranging from 7.2-7.8. Mid-day temperatures were taken periodically to estimate a range of temperatures for the stream (ranged between 20°-26°C). Twenty-four leaf packs were constructed for each species using either only the leaf (i.e. *N. advena*), a portion of the plant (i.e. *P. amplifolius*), or the entire plant de-rooted (i.e. *C. palustris* and *V. americana*), and then secured with rubber bands to the top of cobble of approximately the same size. The leaf packs were not contained by any enclosure so as not to introduce any additional microenvironment artifacts. Based on observation, these artifacts could have been quite significant since leaves enclosed in mesh bags appeared to have an accelerated decay rate, an altered distribution of decay, and an obvious increase in invertebrate density (especially *Trichoptera*s). Initial weight was determined by measuring wet weight after thorough blotting. Initial surface area was also determined using a scanner and computer digitizer. Following the protocol described by Maloney and Lamberti (1994), the cobble was placed in the stream according to a 4X24 randomized block design parallel to the current and off set from the mid-channel to help prevent major loss of plant material downstream. This design allowed four replicates of the four species at six different sampling periods. Sampling was done at 1, 4, 7, 14, 21 and 28 days by removing an entire block at a time. Each piece of cobble was removed into a sieve and the leaf packs removed for further processing in the laboratory. After the leaves were separated from other debris and invertebrates, their wet-weight (amount biomass remaining) and surface area was determined. The invertebrates were preserved for further study. A penetrometer similar to the one used in Maloney and Lamberti (1994) was used to measure the original leaf toughness (i.e. the force required to penetrate the leaf cuticle).

Data Analysis. Leaf processing rates were calculated as the rate coefficient for mass loss as well as for surface area loss. The relevancy of data in certain cases was disregarded for one of two reasons: 1) if the entire plant was missing (If the stem remained, the data was considered valid because the loss could be related to the

weakened plant tissue and not to broken rubber bands), or 2) if the plant re-rooted and the new growth could not be corrected for (8 cases for *C. palustris* percent mass measurements). An analysis of covariance (ANCOVA) with time as the covariate, tested the null hypothesis that the processing rates were not different among plant species. Further analysis using a Tukey's HSD test determined which species differed in their rates of decomposition. As in Maloney and Lamberti (1994) the leaf toughness, defined as the force required to penetrate the leaf cuticle, was determined for the four plant species using fresh (no signs of damage) leaf material from each. To determine the penetrance force (toughness) of each plant species, the mass (grams) required to penetrate the plant material (x) was fit into the equation of Gallardo and Merino (1993):

$$\text{Penetrance force in kPa} = \frac{x}{13.8 \text{ mm}} \times \frac{9.8 \text{ kPa}}{1 \text{ "gram force"/mm}^2}$$

Feeding Assay

The crayfish were caught from Tenderfoot lake using bait traps and maintained individually in covered circular enamel pans. Thus, the crayfish were isolated both from each other and from external stimuli so as to minimize possible behavior altering disturbances. Each container also contained a piece of cut PVC tubing to offer additional refuge. The crayfish were fed continuously so that their feeding behavior was not influenced by starvation stress. Flake fish food was used to help establish similar nutritional histories as well as to avoid preconditioning the crayfish to a specific macrophyte food source. Six hours before each trial, however, the food source was removed and their water replaced. Aerated well water was used in their pans but kept shallow enough to allow the crayfish to obtain their own oxygen. Crayfish of similar carapace length were used to help account for possible variation in feeding behavior which may be associated with different stages in their life cycle. Temperature was monitored for major differences at the beginning and at the end of the feeding period as well as among the replicates. A total of twenty replicates were maintained for each feeding trial.

For the paired-choice feeding assay, two equally sized leaf squares of known surface area were secured to the bottom of each pan using pieces of cut bath mat so that they stood erect. The surface area of the square was dictated by the plant type: 1) *N. advena*, 9 cm²; 2) *V. americana*, 2 cm²; 3) *P. amplifolius*, 9 cm²; and 4) *C. palustris*, 4 cm². The two squares (one of which was cut from fresh plant material and one from material which had been conditioned for one week in the stream) were placed next to each other and away from either opening of the PVC tube. The duration of the feeding period was held constant across the twenty replicates but varied among trials according to the behavior of the crayfish in an attempt to optimize the amount of leaf material consumed (*N. advena* and *P. amplifolius*, 20 hrs; *V. americana*, 24 hrs; *C. palustris*, 8 hrs). Each square's remaining surface area was then analyzed on a digitizer.

For the feeding assay including all four species, 1 cm² squares of each species were secured (as before) randomly in one of four positions evenly spaced around the perimeter of the pan. The feeding period was allowed to run for twenty-four hours after which the squares were collected and digitized to measure the difference in surface area.

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Data Analysis. To compare the amount of biomass consumed between the two treatments, a Wilcoxin signed rank test was used which eliminated the replicates where the crayfish consumed all or none of the two choices. Where the crayfish ate $\leq 5\%$, the sample was considered not to show consumption. These modifications resulted in a reduction of replicates from $n=20$ to : *N. advena* ($n=7$), *V. americana* ($n=10$), *P. amplifolius* ($n=18$), *C. palustris* ($n=10$). Each of the four plant species was analyzed independently in this manner. To analyze the crayfish' preference among the four different plant types when offered them simultaneously a Multivariate Analysis of Variance (MANOVA) was used.

RESULTS

Leaf Processing

Over a sampling period of 28 days obvious patterns evolved among the four plant species although the results were somewhat confounded by 1) biomass loss downstream and 2) regrowth. A regression analysis revealed significant processing rates for *N. advena* and *V. americana* both in percent surface area remaining ($p=0.001$ and $p<0.001$ respectfully) (fig. 1 and 2). *C. palustris* had a significant processing rate according to surface area measurements ($p=0.033$) but not for biomass measurements ($p=0.183$) probably due to *C. palustris*' tendency to re-root and grow (fig. 3). The new growth was obvious and was easily excluded from the surface area measurements without having to exclude the entire case. The new growth however could not be compensated for in the biomass measurements and so the replicate had to be disregarded altogether. *P. amplifolius* did not have a significant processing rate with either measuring method (surface area, $p=0.140$ and biomass, $p=0.241$) (fig. 4). An analysis of Covariance (ANCOVA) with time as the covariate only showed significant differences in plant processing rates among the four plant species when surface area data was analyzed ($p<0.001$) but not biomass ($p=0.106$). A Tukey's pairwise comparison test revealed that *C. palustris* was the only plant to be significantly different from the other three (*N. advena*, $p=0.003$; *P. amplifolius*, $p=0.021$ and *V. americana*, $p=0.045$). Thus, *C. palustris* was shown to have a faster processing rate than *P. amplifolius*, but slower than either *N. advena* or *V. americana* ($N,V>C>P$). Despite these results which may still be obscured by *C. palustris*' regrowth in spite of compensatory efforts during measurement, *C. palustris* may have actually been processed the fastest of the four plants. *C. palustris* began to root and produce new growth after the third sampling date (7 days). Prior to the regrowth, and within the first week of processing, *C. palustris*' percent surface area remaining declines drastically compared to *N. advena*'s more gradual loss (fig. 5).

Both emergent plant species showed a higher rate of mechanical loss downstream than the two submergent plant species. *N. advena* had 8 cases and *C. palustris* had 7 cases where either the entire plant was lost or extensive mechanical damage occurred (only stem remaining) whereas *P. amplifolius* only had two cases and *V. americana* had four towards the end. The majority of *C. palustris*' cases occurred within the first week in contrast to *N. advena*'s cases which mostly occurred after 3-4 weeks of processing. *N. advena*'s initial more gradual decline in biomass also appeared to be from mechanical damage as it occurred primarily around the edges and tips.

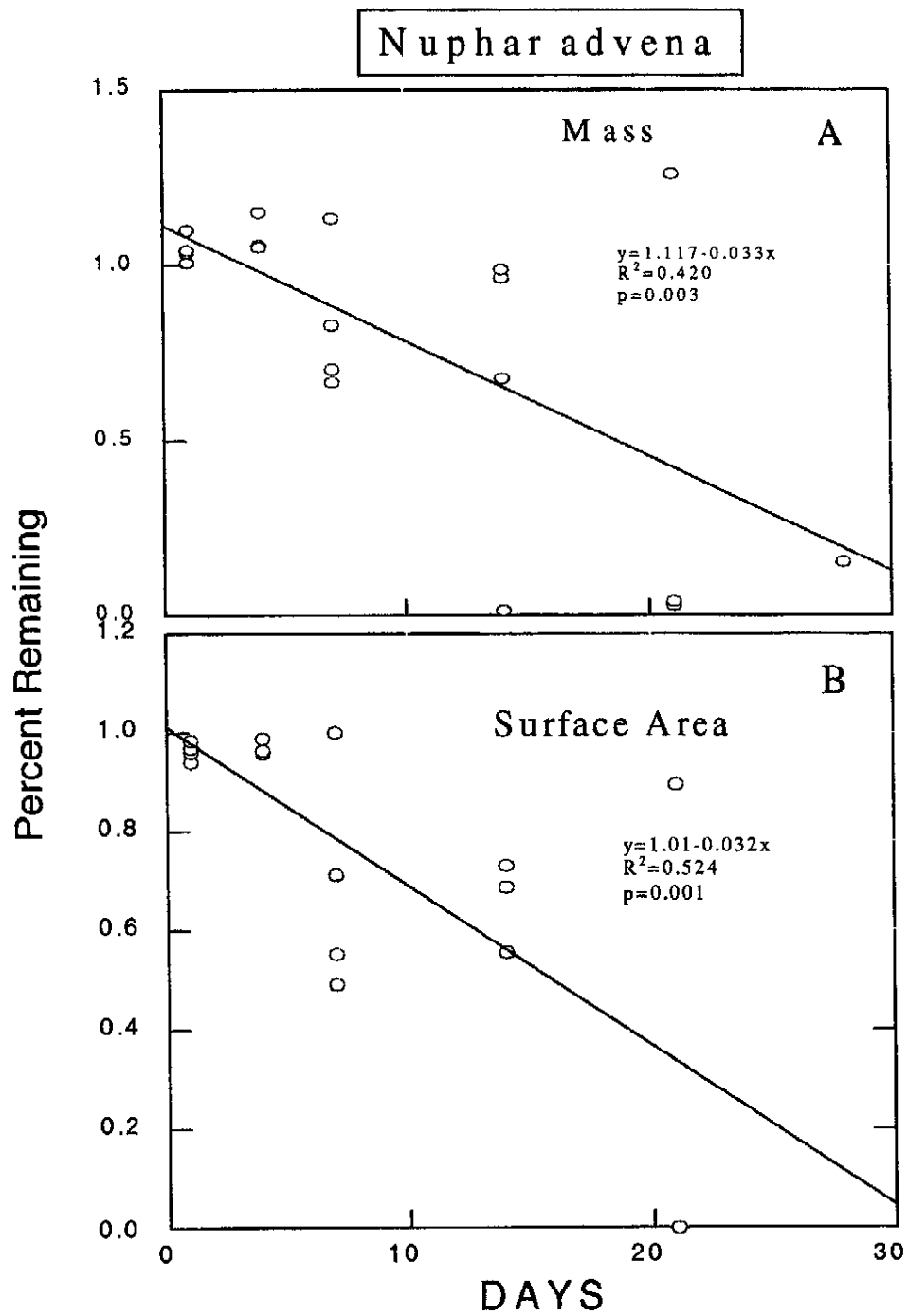


Fig. 1. The decomposition rate of *Nuphar advena* over 28 days in terms of percent remaining mass (a) and surface area (b).

Vallisneria americana

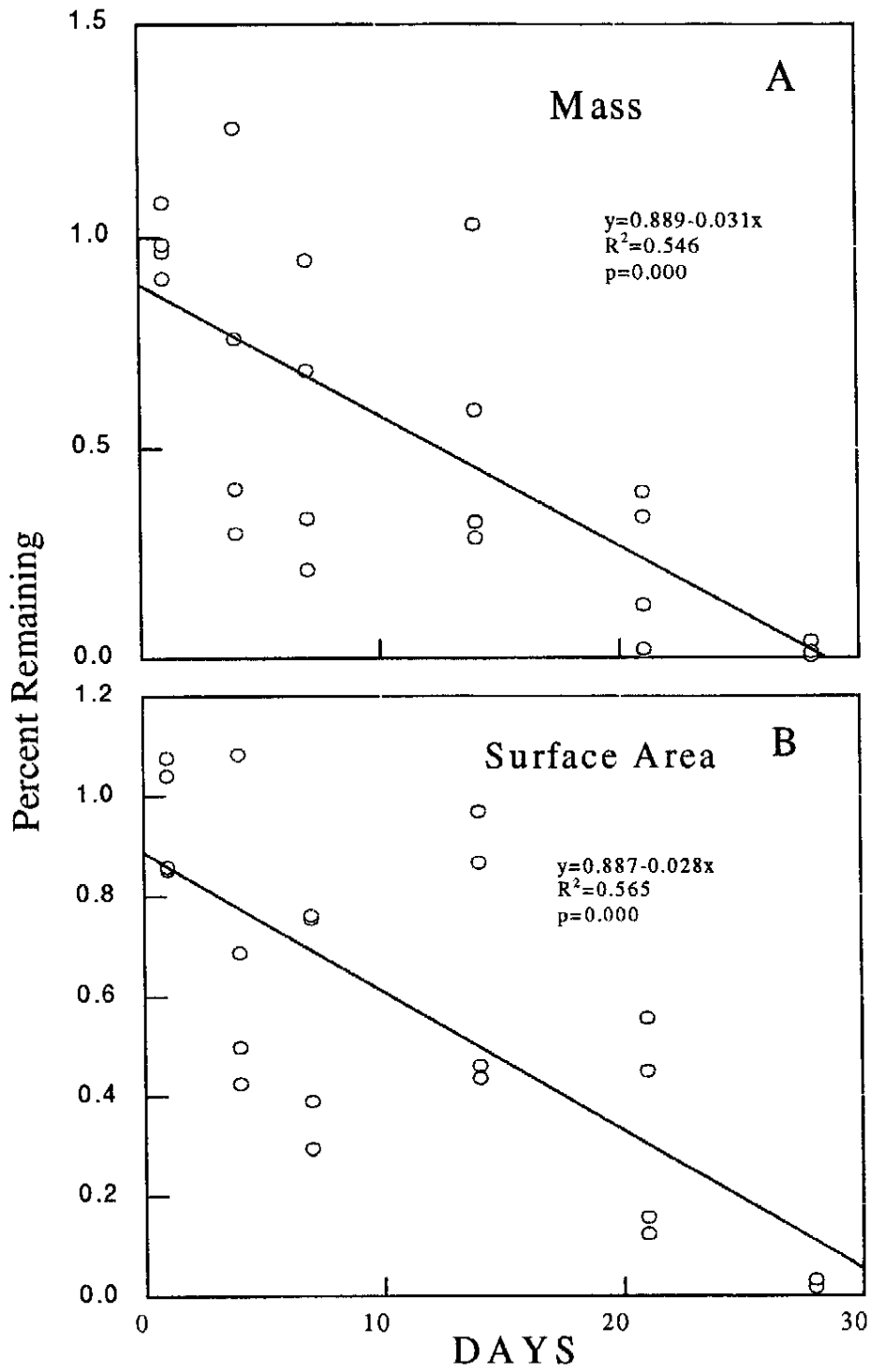


Fig. 2. The decomposition rate of *Vallisneria americana* over 28 days in terms of percent remaining mass (a) and surface area (b).

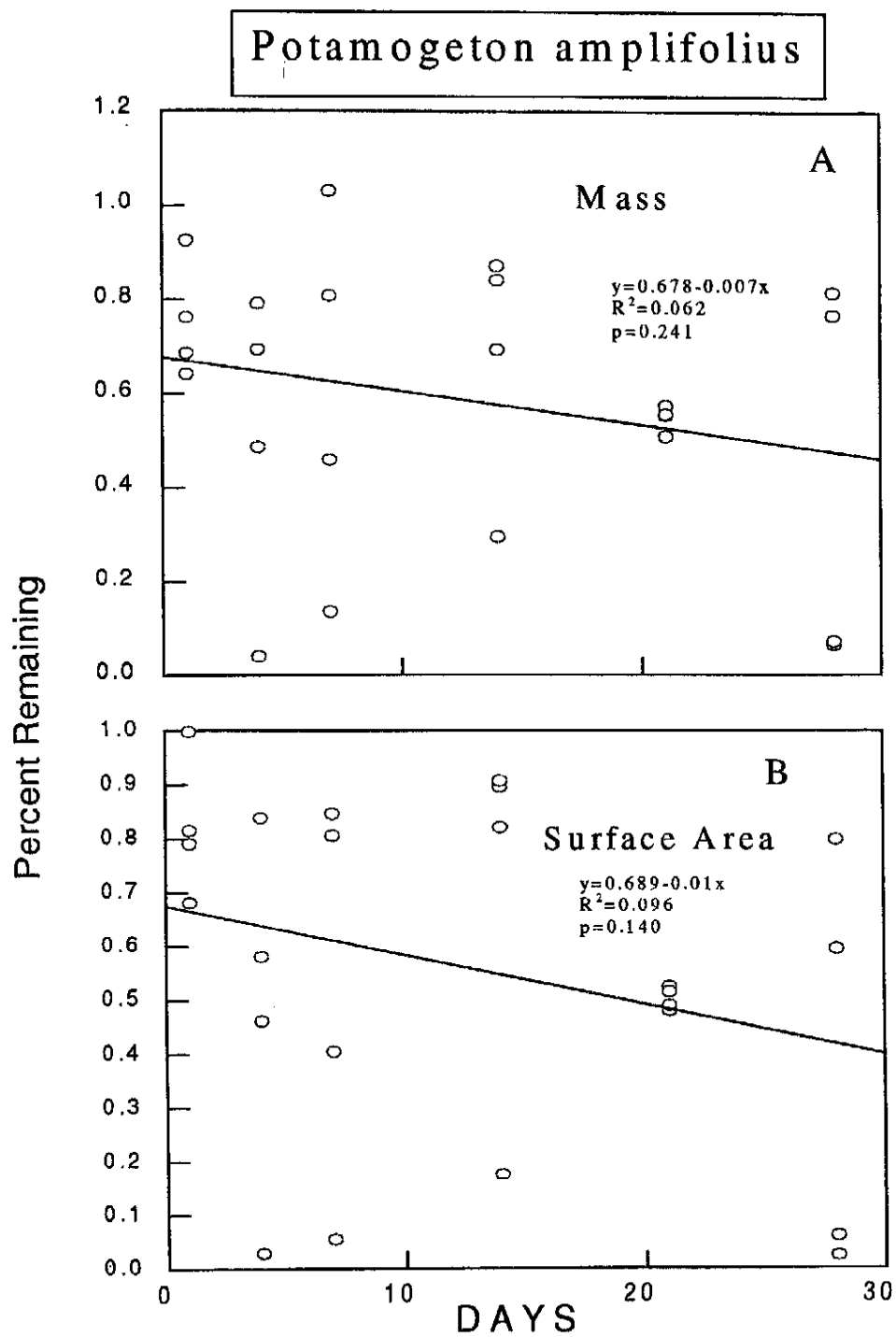


Fig. 4. The decomposition rate of *Potamogeton amplifolius* over 28 days in terms of percent remaining mass (a) and surface area (b). Neither one shows significant decomposition.

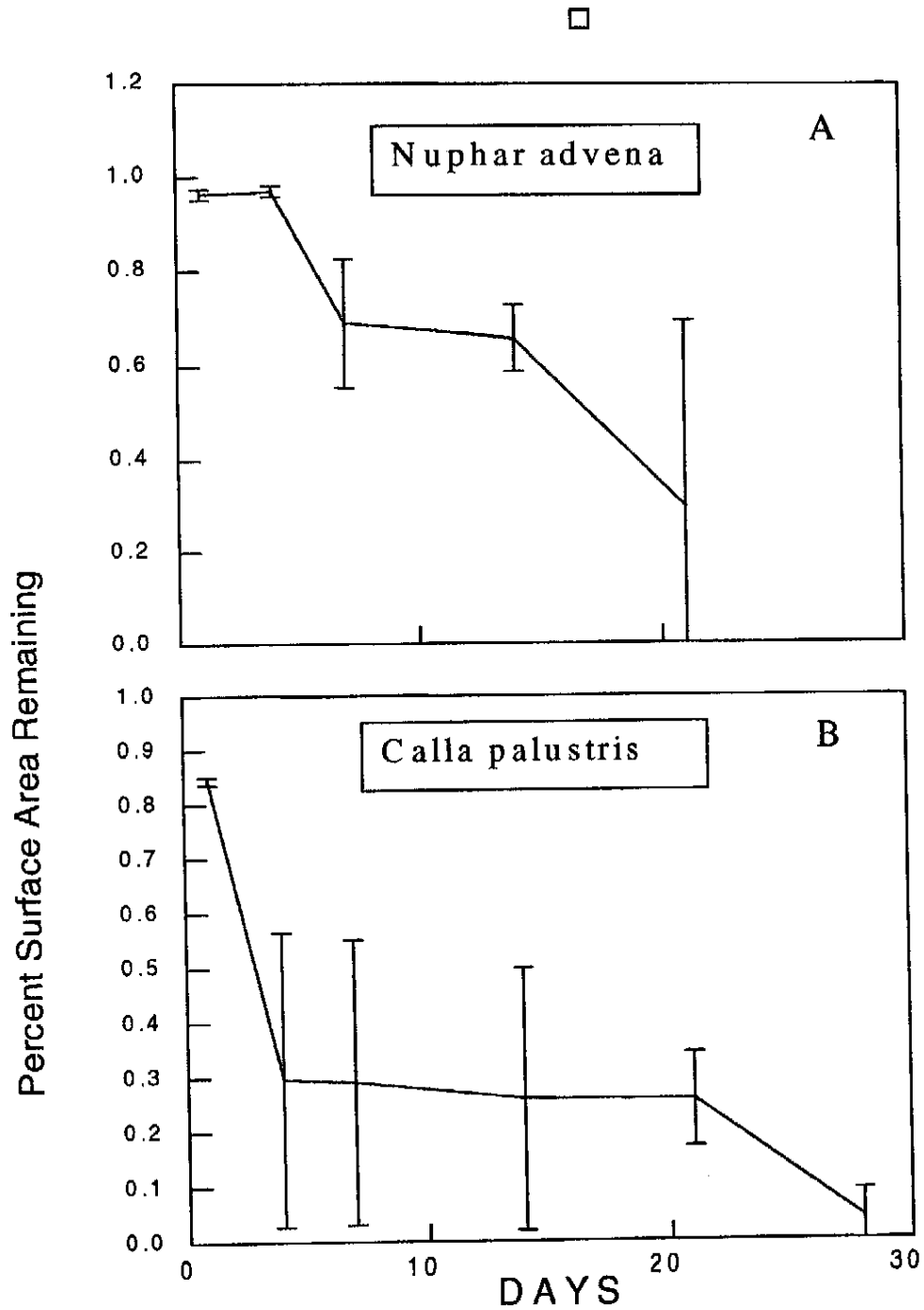


Fig. 5. Decomposition dynamics in terms of percent surface area remaining over 28 days for *Nuphar advena* (a) and *Calla palustris* (b). Vertical bars indicate 1 SE. All replicates were invalid at 28 days for *Nuphar advena*.

The penetrance force differed significantly ($p < 0.001$) among the four plant species. *N. advena* was the toughest requiring 23.65 kPa for penetration, followed by *P. amplifolius* and *V. americana* which both required 11.93 kPa with *C. palustris* being the easiest to penetrate with 8.31 kPa ($N > P$, $V > C$).

Feeding Assays

No generalizations could be drawn regarding *O. propinquus*' feeding preference between fresh and conditioned plant material. The crayfish did not significantly prefer one choice over the other except in the case of *V. americana* where they seemed to prefer the conditioned plant material over the fresh ($p = 0.012$). This lack of selectivity may be partly explained by the penetrometer results which indicated no significant difference in leaf toughness between the two options. On closer analysis of the feeding behavior on each plant species, a subtle pattern may be evident (fig. 6). The crayfish displayed their generalist behavior most with *C. palustris* which was also the most tender of the plants. The number of crayfish choosing each of their four options (conditioned, fresh, consume both, avoid both) was about even. *N. advena*, with the toughest cuticle, seemed to be left uneaten most of the time. The majority of the crayfish (90%) ate the *P. amplifolius* squares with only a slight preference for conditioned material. Many (about one-third) of the crayfish did not eat *V. americana* but the ones which did, strongly preferred the conditioned over the fresh material.

When the crayfish were offered their choice between the four plant species they fed selectively ($p = 0.001$). The MANOVA test recognized three significantly different groups based on percent consumption: *C. palustris* was preferred (98.7% \pm 19.2) over *P. amplifolius* (63.6% \pm 18.99) which was preferred over *N. advena* (47.1% \pm 2.74) and *V. americana* (28.3% \pm 2.74) (fig. 7).

DISCUSSION

Only four mechanisms directly determine the rate of leaf decomposition: (1) leaching; (2) microbial degradation; (3) macroinvertebrate fragmentation; and (4) flow-related fragmentation (Rader et al 1994). Based on our data and observations, flow-related fragmentation seems to be the driving mechanism of processing rates within our system. The initial focus of our study was to determine the effect of structure on: 1) the rate of microbial degradation, 2) the rate of microbial degradation, and 3) the rate of macroinvertebrate fragmentation. However, it soon became apparent that mechanical fragmentation was occurring at such a rate that made microbial degradation almost irrelevant at this stage of processing. Identification of the dominant mechanism controlling the decomposition is important because it is that mechanism which determines the transfer rates of energy and nutrients to the higher trophic levels (Rader et al 1991). Thus we suppose that physical fragmentation of the plant material functions as the initiating step allowing macrophytes to enter the food web as coarse particulate matter (CPOM). Whereas our projections for decay rates were based primarily on structure, the rate of mechanical loss may be a more complex function of the interaction among multiple variables such as shape, structure and plant material toughness.

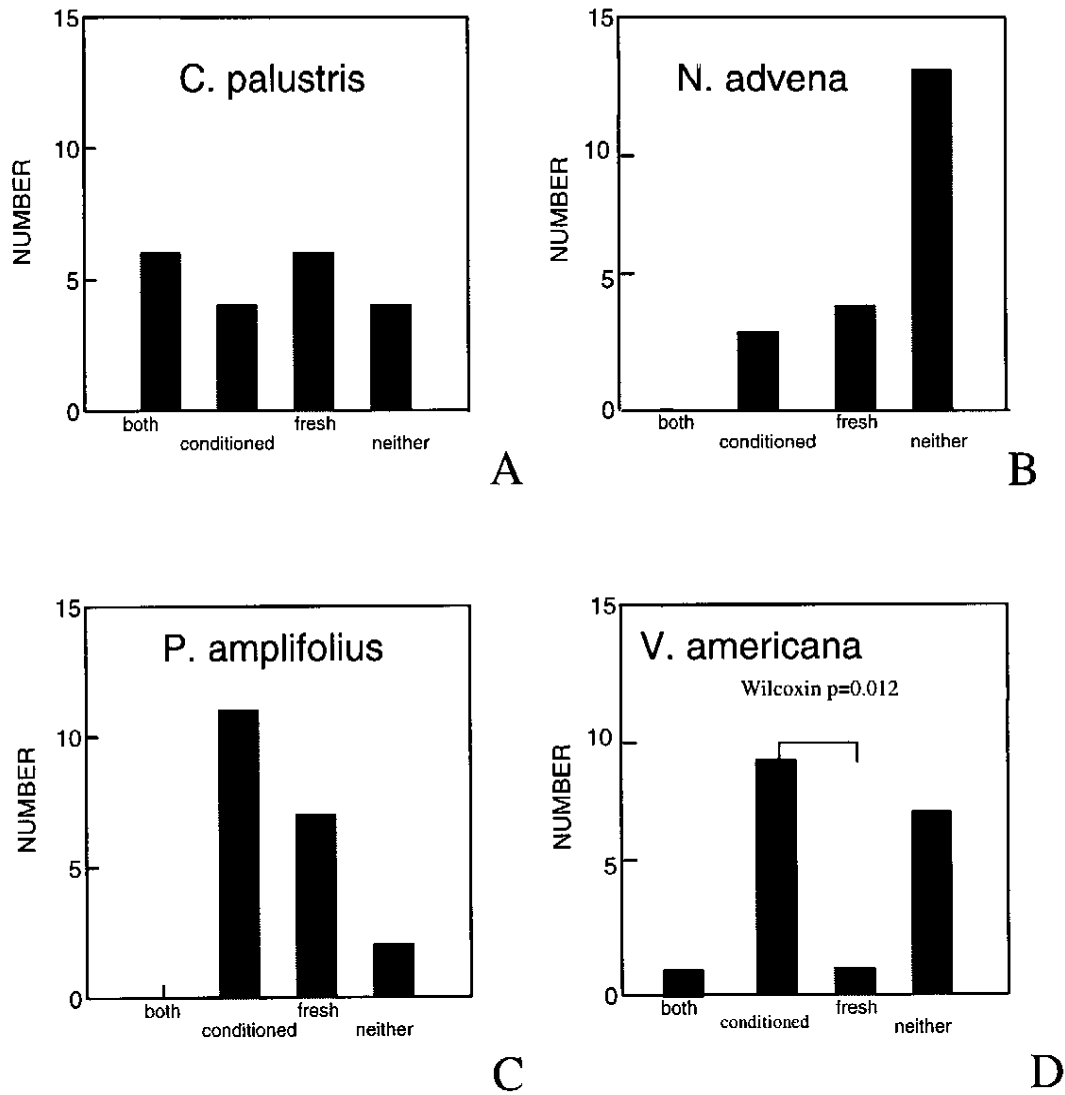


Fig. 6. The feeding preferences of *O. propinquus* when offered both conditioned and unconditioned macrophyte material. Bars represent the number (out of 18) of crayfish which fed on both treatments, only the conditioned, only the unconditioned or neither treatment for *C. palustris* (a), *N. advena* (b), *P. amplifolius* (c), *V. americana* (d). A wilcoxin-sign rank test on treatments (conditioned and fresh) recognized significance for *V. americana*.

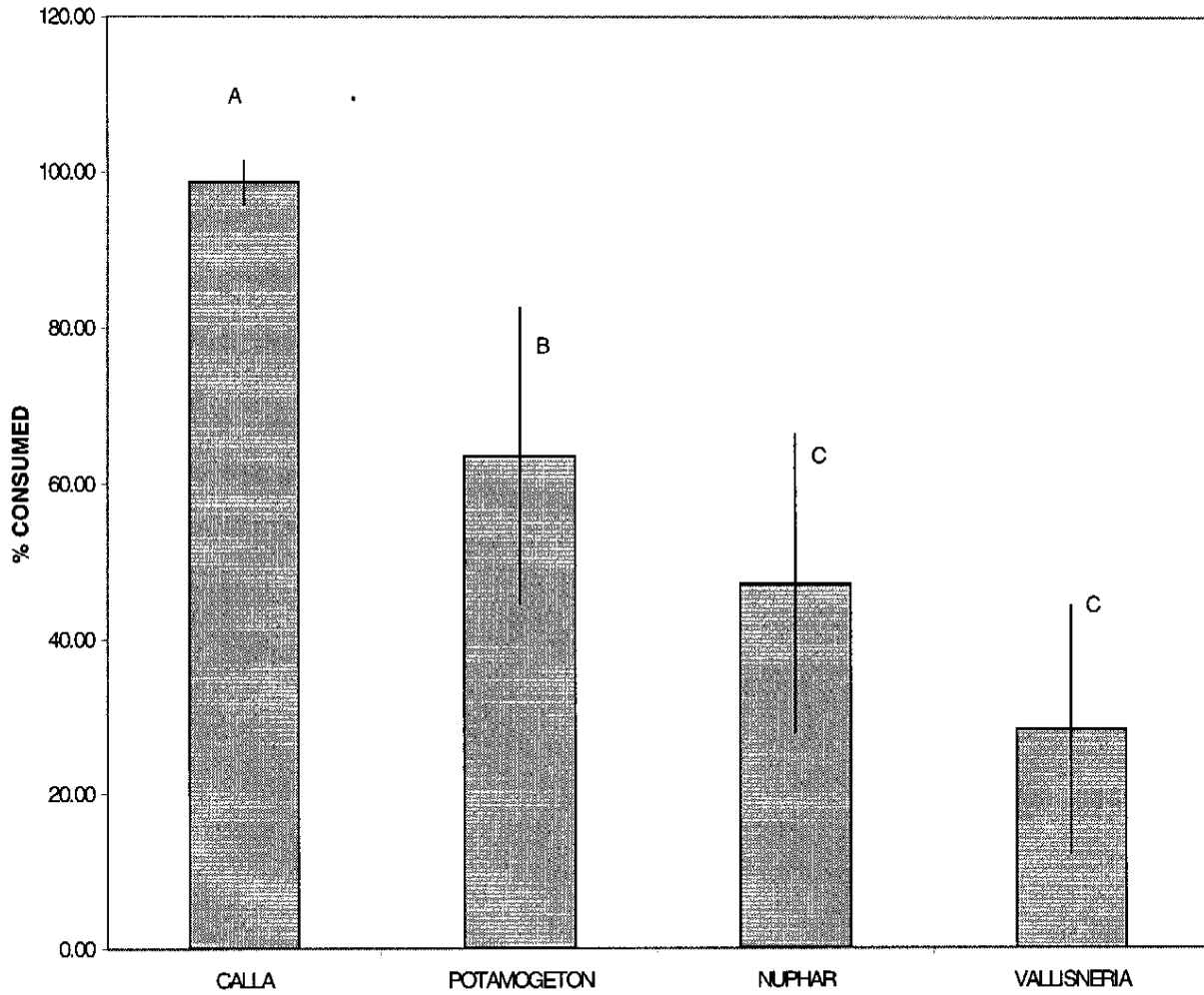


Fig. 7. The feeding preferences of *O. propinquus* when given the choice of 1cm x 1 cm squares of unconditioned *C. palustris*, *P. amplifolius*, *N. advena* and *V. americana*. Bars represent the percentage of surface area consumed of the initial plant square. Vertical lines represent 95% confidence intervals. Different capital letters above the bars indicate significantly different preferences (wilk's ; $p=0.001$)

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Although a regression analysis followed by an ANCOVA test suggested that the processing rates among the four plant species were (ranked in order for highest to lowest): *N. advena* and *V. americana*, *C. palustris*, and *P. amplifolius* (essentially zero). These results may not have been reliable for two reasons 1) *C. palustris*' tendency to root and grow after seven days, and 2) the loss, in some cases, of the entire plant downstream due to not being secured tightly enough to the rock. On closer analysis of the data we suggest that *C. palustris* was actually processed fastest due to its great rate of biomass loss within the first week in the stream.

The emergent plant species showed the greatest mechanical loss downstream. Their tendency to be fragmented by the current could be attributed to the fact that the leaves of emergent species are not built to withstand the force of the current. They have evolved to alternative terrestrial forces such as desiccation and gravity. Thus, the shape of the leaves do not flow with the current or promote laminar flow causing the edges and tips of the leaves to rip from the current's force. Additionally, the leaf to stem connection is stiffer and less pliable causing a greater tendency to break off. The penetrometer results indicate that the leaves of *N. advena* were tougher than the leaves of *C. palustris* which could explain why *C. palustris*' mechanical loss occurred within the first week while *N. advena* didn't suffer drastic losses until later in the study. The combination of proper shape, pliable structure and intermediate toughness of the submergent species may account for the relatively little mechanical damage to the plants. *V. americana*'s few cases of drastic loss appeared to result more from how they were secured to the rock than to inappropriate structure, shape or toughness. Thus we conclude that the elevated effects of the current on the emergent plant species allow these plants to enter the food web more rapidly. Further study should be done on tracking the physical processing of macrophytes, but we predict based on our data and observations that mechanical losses are the primary avenue for introducing macrophyte plant material into the food web.

Despite our belief that flow-related fragmentation acts as the primary mechanism driving decomposition in our system, we also tried to assess the potentially large contribution of macroinvertebrates in the fragmentation of macrophyte material. The macroinvertebrates were collected from each replicate as well as from controls (no macrophyte material) to determine any density or compositional differences which may exist. However, these collections were preserved and the analysis postponed for a future study. Instead we attempted to correlate the selective herbivory behavior of macroinvertebrates with the rate of macroinvertebrate fragmentation in the decomposition of macrophytes in streams using *O. propinquus* as a model organism. It has been suggested that *O. propinquus*' selectivity is strongly influenced by toughness and secondary compounds (Lodge 1991). Our paired-choice experiment offering unconditioned macrophyte material and conditioned material was designed to support this assertion as well as to test for potential deterrents in our chosen four macrophyte species. However, Nuphar which has previously been proven to contain secondary compounds (Lodge 1991) did not elicit a preference for the conditioned material as expected. This indicates that either the macrophytes were not conditioned for an adequate length of time to allow breakdown of the secondary compounds or that there were complicating factors. Furthermore, the penetrometer measurements did not detect a significant difference between conditioned and unconditioned leaf toughness. Again,

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these results are an indication in an inadequate conditioning period. Considering the lack of difference in the plant material, the resulting lack of selective herbivory among the crayfish (the preference for conditioned *V. americana* was an exception) is not surprising. Although this experiment does not test the effects of reduced toughness and chemical defenses directly, some interesting behaviors emerge of which are in agreement with previous observations (Lodge 1991, Newman 1991). The crayfish displayed generalist behavior with the most tender plant species *C. palustris*, avoidance behavior with the toughest species *N. advena*, preference in general for *P. amplifolius* and avoidance of *V. americana* unless conditioned.

Our findings that crayfish actually prefer an emergent macrophyte species over the submerged species may at first seem contradictory to the accepted belief that emergent species are usually selected against when given the choice because they are generally tougher and more heavily defended (Lodge 1991, Newman 1991). However the emergent *C. palustris* is actually the most tender of all the plants and so therefore would not be selected against. Additionally, *C. palustris* is obviously a fast growing macrophyte (as shown in the first experiment) and so, due to resource allocation, may not be as heavily defended (Newman 1991). As suggested by the literature (Lodge 1991, Newman 1991), leaf toughness appeared to be important in the crayfish's selection of food. Preference seems to be negatively correlated with leaf toughness such that they selected plants in the order of :1) *C. palustris* (least tough), 2) *P. amplifolius* (intermediate toughness), and 3) *N. advena* (toughest). Although *V. americana* is included in the least preferred group despite its intermediate toughness, this result is in keeping with the literature (Lodge 1991). Because conditioned *V. americana* is preferred over those plants which were unconditioned, and because it is apparently selected against despite its toughness suggests the possible presence of secondary compounds. Over all, crayfish did prove to be selective in their eating behavior and may offer a potential contribution to the decomposition rates of certain macrophyte species. Further study should be done on other macroinvertebrates as the relationship between plant and macroinvertebrate appears to be highly species specific. The inconsistencies between processing rate order and macroinvertebrate feeding preference order suggest flow-related fragmentation to be the dominate degradation force in this northern Michigan stream.

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