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# Macroinvertebrates associated with submerged macrophytes: sample size and power to detect effects

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**Abstract**

When planning and conducting ecological experiments, it is important to consider how many samples are necessary to detect differences among treatments with acceptably high statistical power. An analysis of statistical power is especially important when studying epiphytic macroinvertebrate colonization of submerged plants because they exhibit large plant-to-plant variability. Despite this variability, many studies have suggested that epiphytic macroinvertebrates preferentially colonize plants based on plant architecture type (broad *versus* dissected leaves). In this study, we calculated the power and number of samples necessary to detect differences in epiphytic macroinvertebrate abundance (numbers and biomass) among five species and two architecture types of macrophytes in a lake in MI, U.S.A. Using power analysis, we found that we had very high power to detect the differences present between macroinvertebrate abundance by architecture type and by macrophyte species (power = 1.000 and 0.994; effect sizes = 0.872 and 0.646, respectively. However, to detect very small differences between the two architecture types and the five plant species, we determined that many more samples were necessary to achieve similar statistical power (effect size = 0.1–0.3, number of samples = 60–527 and 36–310, respectively; power = 0.9). Our results suggest that macroinvertebrate abundance does in fact vary predictably with plant architecture. Dissected-leaf plants harbored higher abundances of macroinvertebrates than broad-leaf plants (ANOVA, density $p = 0.001$, biomass $p < 0.001$). This knowledge should allow us to better design future studies of epiphytic macroinvertebrates.

## Introduction

When planning and conducting ecological experiments, it is important to consider how many samples are necessary to detect differences among treatments with acceptably high statistical power. Clearly, the goal is to maximize power (1 – Beta, the probability of correctly rejecting the null hypothesis) by minimizing beta (the probability of making a type II error or failing to reject a false null hypothesis) (Peterman, 1990a). Thus, for an experiment with low power, little confidence can be placed in a conclusion based on the failure to reject a null hypothesis. Power can be calculated for different assumed effect sizes (the magnitude of the change in the parameter of interest that can be detected by an experiment) (Cohen, 1988). The experiment may not be informative if the detectable effect size is larger than the effect size that is biologically or economically important (Rotenberry & Wiens, 1985). Through these calculations of power, a researcher can determine the feasibility of a study and anticipate how many samples are necessary to detect differences among treatments with various levels of power, thus facilitating better experimental design.

Although estimates of statistical power in ecological studies have been reported in some recent studies (e.g. Carpenter et al., 1995; Johnson, 1998), these important and biologically relevant statistics are still too seldom calculated and, in particular, have not been examined for studies of epiphytic macroinvertebrates. An analysis of statistical power is especially important when studying epiphytic macroinvertebrates because

these organisms exhibit large plant-to-plant variability due to predation, periodic macroinvertebrate emergence, fluctuations in macroinvertebrate food supply, appearance of new macroinvertebrate broods, natural mortality and the occurrence of macroinvertebrates of the same species but of different size (Gaufin et al., 1956; Mrachek, 1966; Soszka, 1975).

Epiphytic macroinvertebrates and the macrophytes they colonize are ecologically important components of many lake ecosystems. In particular, epiphytic macroinvertebrates are an important forage base for many species of juvenile fish that use macrophyte beds for cover and as a source for food (Diehl & Kornijow, 1998). However, macrophytes are diverse in shape and form, and the morphology of the plants may influence epiphytic macroinvertebrate colonization and abundance (Jackson, 1997). Submerged macrophytes can be grouped according to architecture based on plant morphology (the number, morphometry and arrangement of stems, branches and leaves) (Lillie & Budd, 1992). Macrophyte architecture type has been found to explain some of the variation in the abundance of macroinvertebrates, with plants having finely dissected leaves supporting more macroinvertebrates than plants with broader, undissected leaves (Krecker, 1939; Andrews & Hasler, 1943; Gerking, 1957; Mrachek, 1966; Gerrish & Bristow, 1979; Kershner & Lodge, 1990; Jeffries, 1993). It has been suggested that this pattern occurs because finely dissected leaves provide more habitat for colonization, more epiphyton for grazing macroinvertebrates or additional complexity which offers better refuge from predators. However, in a more recent study conducted on multiple lakes, macroinvertebrate abundance did not vary predictably with leaf dissection (Cyr & Downing, 1988).

The patterns of epiphytic macroinvertebrate communities and their role in lentic food webs have been difficult to quantify, partly because sampling macroinvertebrates on submerged plants is difficult and past studies have not used comparable methods to sample, process, analyze and report data (Downing & Cyr, 1985; Jackson, 1997). In addition, power analyses have not been conducted in any study examining the patterns of epiphytic macroinvertebrates. Thus, questions remain about the relationship between epiphytic macroinvertebrates and macrophytes, and whether these organisms are too variable to discern patterns of abundance.

To address these questions, we designed a mesh bag sampler to sample macroinvertebrates associated with submerged plants. We assessed the sample size and statistical power to detect differences in macroinvertebrate abundance among species of plants from broad and dissected plant architecture types. We also examined patterns between macroinvertebrate abundance and plant species and architecture types. We hypothesized that broad-leaf plants would harbor fewer macroinvertebrates than dissected-leaf plants.

## Study site

We sampled epiphytic macroinvertebrates on August 4 and 5, 1998 in Heron Lake, located in Seven Lakes State Park in S.E. Michigan, U.S.A (42.81 N, 83.52 W). The lake has an extensive forested riparian zone and undergoes very little plant management, except for occasional mechanical harvesting in localized areas surrounding the public boat launch and beach. The surface area of Heron Lake is 53 ha and the mean depth is 3.5 m. Nearly 65% of the lake is littoral (littoral zone defined as average depth beyond which no plant growth is observed; $\sim$4.6 m). Nineteen plant species were recorded during macrophyte surveys performed in August, 1998 (Getsinger et al. 2000).

## Materials and methods

### *Sampling*

#### *Sampler description*

We sampled individual plant stems with a mesh bag sampler that is a modification of the folding quadrat sampler (Welch, 1948) (see Fig. 1). It is constructed of 200 and 500 $\mu$m mesh, thus the sampler collects organisms $>$500 $\mu$m. The sides are constructed of 200 $\mu$m mesh for flexibility, ease of construction and sampler deployment. Two steel rings provide structure to the mesh bag (the top ring is smaller than the bottom ring for easy inversion of the sampler). All seams are on the outside of the sampler, allowing for a smooth inner surface. The sampler is 65 cm long and 24 cm in diameter. It has a drawstring at the bottom to close the sampler and trap the sampled macrophyte and its associated macroinvertebrates. A crew of three people performs the sampling: one snorkeller collects samples and two people process the samples in a boat. The snorkeller positions the sampler above a plant and slowly (to limit disruption and subsequent

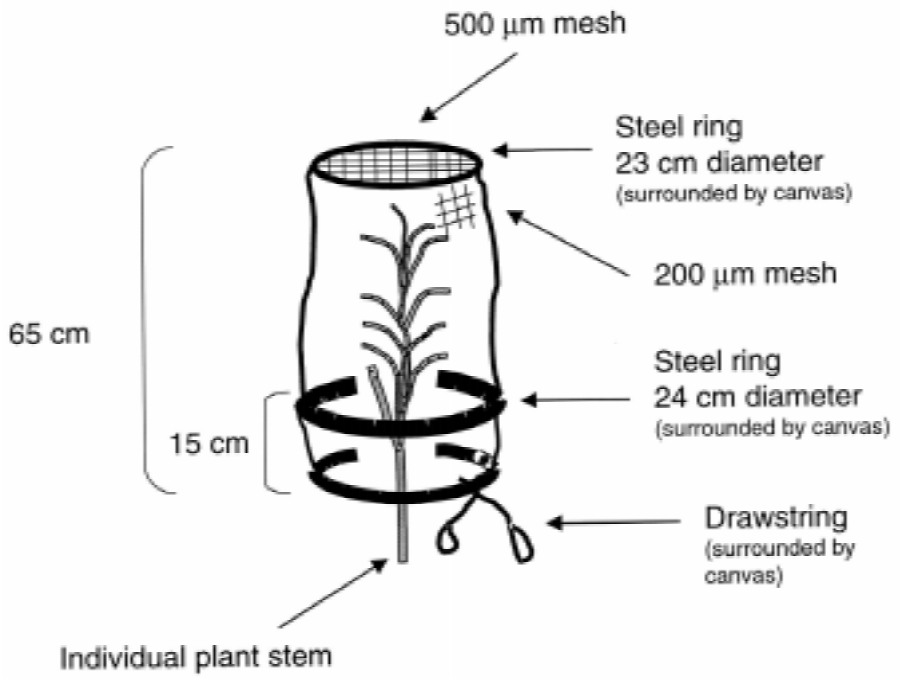


*Figure 1.* Epiphytic macroinvertebrate mesh bag sampler that is a modification of the folding quadrat sampler (Welch, 1948). The sampler has the dimensions of 65 × 24 cm and is constructed from 200 $\mu$m and 500 $\mu$m mesh, 2 steel rings and canvas. It is closed at the bottom by a drawstring.

loss of organisms) lowers it down until approximately 30–60 cm of plant is inside the sampler. Then the drawstring is pulled taut, the plant stem is broken off at its base and the sampler is brought to the surface. The processors in the boat cut off any additional plant material extending beyond the sampler. The sampler is then inverted and rinsed, and the contents (macrophyte, macroinvertebrates and water) are stored in a sealed plastic bag. Samples are kept in a dark refrigerator for up to 72 h, at which time further processing occurs.

*Sample protocol*

We sampled five common plant species that fit into the two plant architecture groups. Two plant species were classified as broad-leafed: *Potamogeton richardsonii* Benn. (clasping-leaf pondweed) and *Potamogeton illinoensis* Morong. (Illinois pondweed); and three as dissected-leafed: *Ranunculus* sp. (water crowfoot), *Potamogeton pectinatus* L. (sago pondweed) and *Myriophyllum spicatum* L. (Eurasian water milfoil) (see Fig. 2). We sampled epiphytic macroinvertebrates at three sites separated by greater than 100 m. Each site was approximately 2 m deep (average depth of littoral zone) and contained each of the five plant

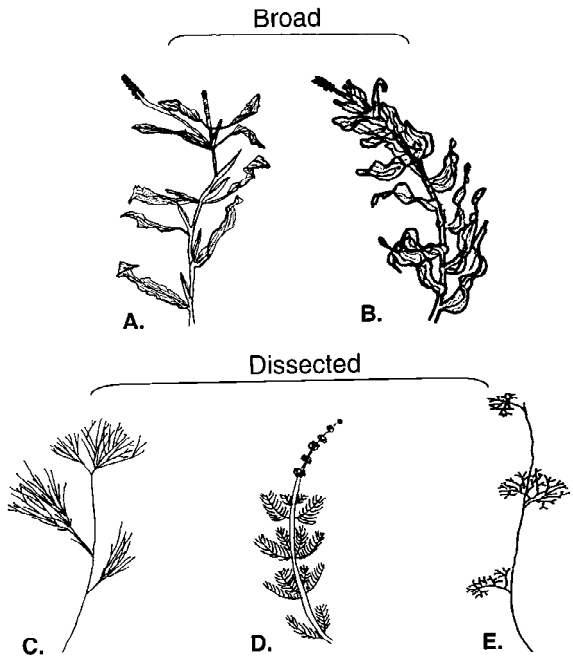


*Figure 2.* Five common macrophyte species of Heron Lake, MI, U.S.A. Broad-leaf: (a) *P. illinoensis* and (b) *P. richardsonii*; Dissected-leaf: (c) *P. pectinatus*, (d) *M. spicatum*, and (e) *Ranunculus* sp. Adapted from Fassett (1957).

*Table 1.* Examples of previous studies examining epiphytic macroinvertebrates in individual lakes

| Citation | Number of plant species | Number of replicates taken per plant species[a] |
|---|---|---|
| Andrews & Hasler (1943) | 8 | 17 |
| Gerking (1957) | 3 | 2 |
| Gerrish & Bristow (1979) | 3 | 10 |
| Krecker (1939) | 7 | Variable[b] |
| Mrachek (1966) | 8 | Variable[c] |
| This study | 5 | 15 |

[a] Number of replicates taken per plant species for a single sampling period.
[b] Number of plants sampled not reported, expressed as length of plant sampled.
[c] Number of samples reported as total for entire summer only (25–28 per plant species, number of times sampled not specified).

species. We sampled five macrophytes of each species haphazardly from approximately a 10 m radius around an anchored boat at each site, resulting in 15 individuals of each plant species totaling 75 samples. We chose these numbers of plant species and replicates based on comparisons with previous studies (see Table 1).

*Sample processing*

In the laboratory, we rinsed all individual macrophyte samples with water to detach macroinvertebrates, then dried the plants at 105 °C for 48 h and weighed them to estimate plant biomass. Macroinvertebrates were preserved in 95% ethanol, counted and identified to the lowest taxonomic level possible (usually genus). Length-weight equations from the literature were used to estimate macroinvertebrate biomass from body lengths measured using an ocular micrometer (Rogers et al., 1977; Smock, 1980; Meyer, 1989; Burgherr & Meyer, 1997; G.G. Mittelbach, unpublished data).

*Data analysis*

For all analyses, we standardized macroinvertebrate abundance by plant dry weight, which allows for the comparison of macroinvertebrate abundance among different plant species and architecture types. We conducted sample size and power analyses using PASS 6.0 software (NCSS Statistical Software; http://www.ncss.com/pass.html). We calculated the power to detect differences in macroinvertebrate abundance among the five plant species and two architecture types given the number of samples taken. Using one-way ANOVAs and setting alpha = 0.05, we estimated the number of samples necessary to detect differences in macroinvertebrate abundance between the five plant species and two architecture types at different levels of power and a fixed effect size. We also calculated the number of samples necessary to detect differences in macroinvertebrate abundance among the five plant species and two architecture types with a fixed power level and various effect sizes. Finally, we performed ANOVA tests to determine if macroinvertebrate abundance (expressed as numbers and biomass (mg) of animals per gram dry plant biomass) varied predictably by plant species or architecture.

## Results

Using power analysis, we found that by taking an average of 36 samples per architecture type, we had a power of 1.000 to detect the difference present between the two plant types (effect size = 0.872). In fact, it would have taken just 7–14 samples within each architecture type to detect this large difference with a power of 0.85–0.99 (see Fig. 3). However, to detect very small differences between the two architecture types (effect sizes = 0.3–0.1), we determined that 60–527 samples were necessary to achieve similar power (see Fig. 4a). However, intermediate effect sizes (0.6–0.4) could be reasonably achieved with 16–34 samples (power = 0.9) (see Fig. 4a).

For the same analysis of macroinvertebrate abundance by plant species, we found that with our sample protocol, we had a power of 0.994 to detect the differences present between the five plant species (effect size = 0.646). We could have taken just 7–14 samples within each plant species to detect these differences with a power of 0.820–0.994 (see Fig. 3). Similar to the analysis for plant architecture, we determined that 36–310 samples were necessary to detect very small differences between plant species (effect sizes = 0.3–0.1) and intermediate effect sizes (0.6–0.4) could be reasonably achieved with 10–21 samples (see Fig. 4b).

Our results suggest that macroinvertebrate abundance is significantly related to leaf dissection (see Fig. 5). Dissected-leaf plants (*M. spicatum*, *P. pectinatus* and *Ranunculus* sp.) harbored higher densities and biomass of macroinvertebrates than broad-leaf plants (*P. illinoensis* and *P. richardsonii*) (ANOVA, density $p = 0.001$, biomass $p < 0.001$). There were no significant differences in macroinvertebrate abundance among plant species within the same architecture type.

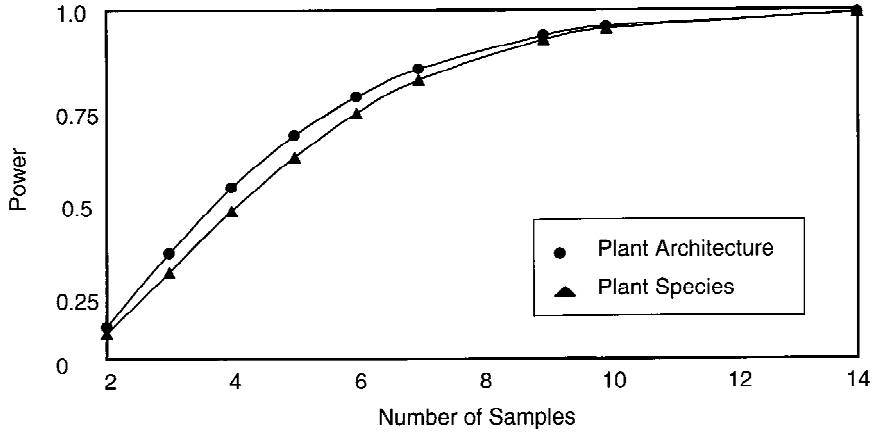


*Figure 3.* The relationship between the number of samples and power at alpha = 0.05 and effect size = 0.646. The number of samples necessary are indicated by circles for plant architecture and triangles are for plant species.

## Discussion

When planning and conducting ecological experiments, power analysis can lend insight into how many samples will be necessary to detect differences among treatments with acceptably high power. We performed these analyses on lentic, epiphytic macroinvertebrates collected with a mesh bag sampler. We found that we had extremely high power to detect the large differences in macroinvertebrate abundance between the five plant species and two plant architectures (power = 1.000 and 0.994, respectively). A 'conservative' estimate of the number of samples necessary to detect effects would allow alpha and beta to be set at a level of 0.05, whereas a more 'liberal' estimate would allow alpha to equal 0.05 and beta to equal 0.20 (Peterman, 1990b). Choosing an intermediate of these two (alpha = 0.05, beta $<0.1$, resulting in power $>0.9$), we determined that far fewer samples could have been taken within each species or architecture (9–14), thus allowing time for sampling additional species. We also found that we could reasonably take sufficient samples to detect intermediate differences among species or between architectures (10–21 and 16–34 samples, respectively, effect sizes 0.6–0.4). Although we suggest extrapolating our results to other sites, times or substrates cautiously because of high natural variability associated with epiphytic macroinvertebrates (Cheruvelil, 2000), ultimately, we are interested in the ecological significance of differences among macroinvertebrate populations. Studies such as this should help us to better design future research to assess this significance.

Epiphytic macroinvertebrates and the macrophytes they colonize are ecologically important components of lake ecosystems. Our results indicate that dissected-leaf plants harbored a higher abundance of macroinvertebrates than broad-leaf plants. In Table 1, we summarize some of the past research studying epiphytic macroinvertebrates in single lakes. These studies sampled from 3 to 8 plant species, took 2–85 replicates of each species, and, similar to this study, found that dissected-leaf plants harbored more macroinvertebrates than other plant types. We had enough information to calculate power for the study by Gerrish & Bristow (1979). With an alpha of 0.05 and an N of 10, they had a power of 1.000 to detect the very large differences found between the three plant species sampled on June 18, 1974 (effect size = 1.87). In fact, the authors could have detected smaller differences (effect size = 0.5) by taking only 18 samples of each plant species and they could have taken just three samples to detect the differences present (power $>0.9$). Knowing this, more time could have been spent sampling additional plant species rather than replicates within plant species, resulting in more information about the relationship between macroinvertebrate abundance and plant architecture.

## Conclusions

The management of aquatic plants typically involves the removal of plant biomass either selectively by species or nonselectively. Thus, plant management affects the abundance and community composition of macrophytes and, consequently, epiphytic macroin-

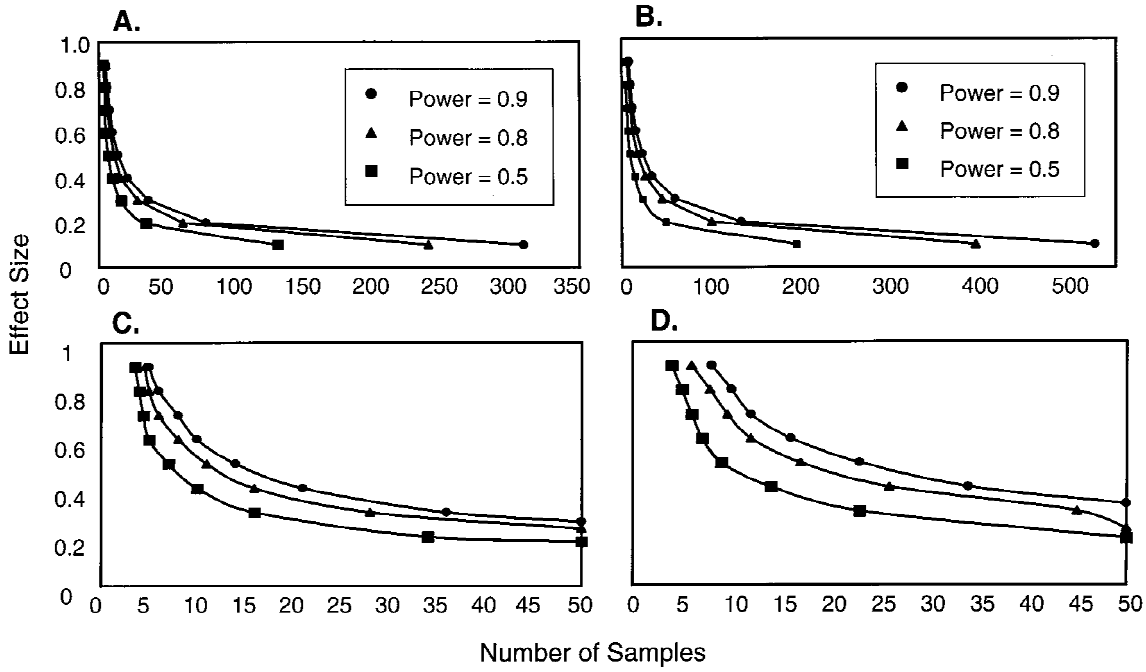


*Figure 4.* The relationship between the number of samples and effect size for (a) plant species and (b) plant architectures (alpha = 0.05). Power levels shown are 0.9 (circles), 0.8 (triangles) and 0.5 (squares). For comparison, (c) and (d) show the enlarged region of 0–50 samples.

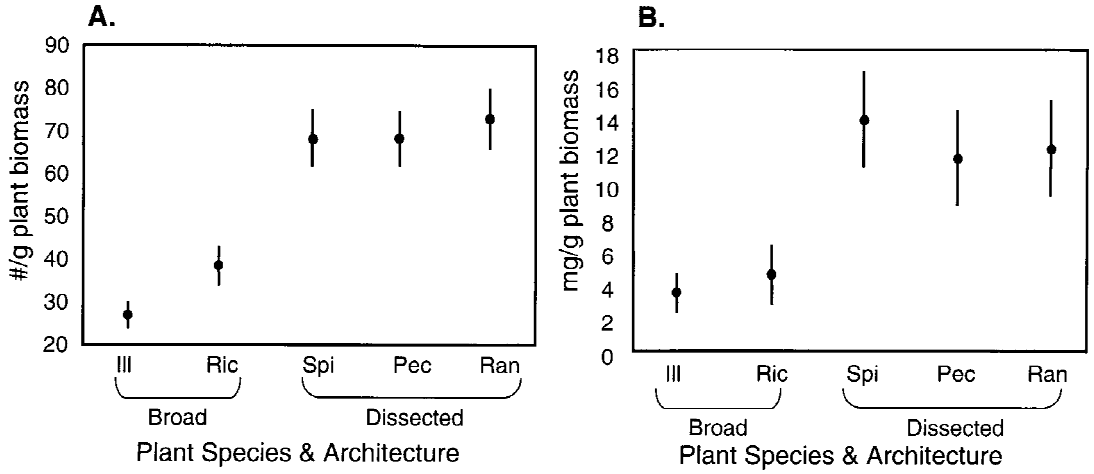


*Figure 5.* Macroinvertebrate (a) density and (b) biomass by plant species and architecture. Plant species are abbreviated as: Ill (*P. illinoensis)*, Ric (*P. richardsonii)*, Pec (*P. pectinatus)*, Spi (*M. spicatum)* and Ran (*Ranunculus* sp.). Bars represent the standard error for each plant species.

vertebrates. Because these macroinvertebrates are an important source of food for many species of juvenile fish, an important component of lake foodwebs, it is important that we understand the relationship between macrophytes and macroinvertebrates so that we may manage lakes better for both plants and fish. With the knowledge we have gained in this study, we are better prepared to answer questions such as: are the patterns seen here between macroinvertebrate abundance and plant architecture common among lakes? how do macroinvertebrates respond to changes in macrophyte communities? and, can epiphytic macroinvertebrates be used as indicators of lake water quality?

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