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Surveying for Phylactolaemate Bryozoans by Sieving Lentic Sites for Their Statoblasts

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Abstract—Traditionally, colony collection has been the primary method for establishing the distribution patterns of phylactolaemate bryozoans. Often, however, intact colonies are absent due to seasonally changing environmental conditions. To help detect species that are present at a lentic site, we developed a sieving technique that isolates both the large and small statoblasts of freshwater bryozoans. Since statoblast morphology is species-specific, we can use statoblasts to establish more complete distribution information. We collect statoblasts by washing a bottom sediment or shore drift sample through a stack of standard sieves with mesh openings of 1.0 mm, 500 μm, and 150 μm. The 1.0 mm sieve separates large material from the sample. The 500 μm sieve isolates the large statoblasts from the small statoblasts which collect on the 150 μm mesh sieve. By sieving, we found the characteristic, large statoblasts of *Pectinatella magnifica* in 37% of 38 Illinois lakes. In comparison, when searching only for colonies, we found *P. magnifica* at only 21% of 135 lentic sites. Also, for the first time in Illinois, Wood and Marsh (1996) used sieves to detect the large statoblasts of *Lophopodella carteri* at two of these 135 sites. We only discovered this species once in the state by colony collection. Furthermore, by sieving, we found evidence that *Plumatella nitens*, which produces small statoblasts, occurs in 63% of 49 southeastern Wisconsin glacial lakes. Considering the abundant and consistent presence of statoblasts in nature, we propose that the method we describe will produce more reliable distribution data than colony collection alone.

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Introduction

Biologists surveying for freshwater bryozoans have traditionally looked for colonies on the undersides of aquatic plants, wood and rocks (Bushnell, 1965; Wood, 1989). Phylactolaemate bryozoans in temperate regions typically form colonies in the spring from asexually produced statoblasts that were formed by colonies during the summer and early fall months of a previous year. In tropical regions, bryozoans produce statoblasts to survive dry seasons. Thus, one is only able, in most parts of the world, to collect colonies in certain months of the year. Some bryozoan species, e.g. *Lophopodella carteri* and *Lophopus crystallinus*, produce colonies that are relatively small and translucent; and are thus easily overlooked in the field.

To counteract the difficulties associated with locating colonies, researchers can examine lake sediments or organic shore drift for the presence of statoblasts. Each species of phylactolaemate bryozoan produces its own characteristically sized, shaped and surface-sculpted statoblast. With increased use of scanning electron microscopy, researchers are relying more on statoblasts for species identification than on colony morphology (Wood, 2000). Floating white polystyrene foam discarded on the water provides an excellent background to which dark colored statoblasts adhere. Wood (1989) has noted the value of examining such substrates with a hand lens to determine the presence of bryozoan species in a given body of water. We are not convinced that this provides a large enough sample to accurately reflect the phylactolaemate assemblage present at a lentic locality.

Rieradevall and Busquets (1990) described the recovery of *L. crystallinus* statoblasts from lake sediments in Spain by filtering Ekman grab samples through a 150 μm net. Wood visited this site several years later and was able to recover statoblasts of *L. crystallinus* with the same technique and equipment. These successes led us to experiment with sieving lentic sediments in the Illinois survey, especially since it might reveal the presence of *L. crystallinus* which had not been found in Illinois since 1897 (Kofoid, 1908).

In this paper, we report on the success that

we have had with this method of sieving substrates from primarily lentic sites for both large and small statoblasts produced by phylactolaemate bryozoans.

Materials and Methods

During the course of three surveys, we developed a sieving method that separated statoblasts from sediment and organic debris. During the first two surveys we only isolated large statoblasts and, in the third survey, we isolated both large and small statoblasts. Bryozoan statoblasts are categorized as large or small based on their minimum dimensions. Members of the families Lophopodidae and Cristatellidae produce large statoblasts that have a minimum dimension of at least 650 μm. These statoblasts will not pass through a No. 30 sieve that has a mesh opening of 590 μm. However, members of the Plumatellidae and Fredericellidae produce small statoblasts that have a minimum dimension of at least 400 μm (Wood, 1991). These statoblasts will not pass through a No. 100 sieve that has a mesh opening of 150 μm.

When we initially tested this method, during the survey of the Bryozoa of Illinois and a survey of glacial lakes in northeastern Illinois, we were only interested in the large statoblasts produced by the Lophopodidae and Cristatellidae. More specifically, we were interested in rediscovering *L. crystallinus* (Kofoid, 1908) and *Cristatella mucedo* (Davenport, 1904) in Illinois. We also suspected that *Lophopodella carteri* might be present in Illinois, given its occasional occurrence in nearby states (Wood, 1989). We used two U.S. Standard Sieves, No. 25 (mesh opening 710 μm) and No. 30 (mesh opening 590 μm), stacked with the larger mesh on top. The No. 25 sieve stopped larger particles, primarily organic debris, but allowed any statoblasts present to pass through. The No. 30 sieve stopped the larger statoblasts as well as organic and inorganic particles in this size range. Using a dip net or Ekman grab, we collected a lentic substrate sample that usually filled the No. 25 mesh sieve. The sediment varied from mud to gravel, often containing organic material. Then we washed lake water through the sieves until the effluent was clear. Statoblasts of *C. mucedo*, *L. carteri* and *L. crystallinus* each possess processes or hooks which cause them to stick to one another and to organic debris in the No. 25 sieve, therefore it is important to wash the sample thoroughly so that at least some of these statoblasts, if present, pass into the No. 30 sieve.

We used three methods to facilitate water flow through the sieves: 1) we stirred the sediment around the edge of the upper sieve with a finger, 2) we forced water back through the sieves by immersing the stack of sieves, and 3) we tapped the bottom sieve from below with our fingers. The latter two methods force air into the sieve which suspends the sediment and permits water flow. We discarded the contents of the upper sieve and washed the contents of the No. 30 sieve into a 21 cm diameter specimen dish. By swirling the water and the sediment fraction in the dish, we were able to suspend the organic portion of the contents and decant it into a smaller No. 60 sieve (mesh opening 250 μm, thereby eliminating the heavier inorganic material. We finished the sample preparation by storing the organic fraction in a 500 ml straight-side, wide-mouth polycarbonate jar (Nalgene). We were able to remove any large statoblasts in the sample from the jar with forceps during examination with a 10–20 × dissecting microscope. There was a relatively small amount of organic material left in the sample at this stage of the process, so separation of statoblasts was easily accomplished; the clear polycarbonate jar allows microscope light to illuminate the material in the jar, and the straight-side feature allows examination of the contents of the entire jar.

Prior to the third survey of the glacial lakes of southeastern Wisconsin, we modified the sieving method to facilitate the capture of small statoblasts. This involved changes in sieve sizes, sample collection, rinsing method, and sample inspection. We experimented with samples from Lake Shabbona in DeKalb County, IL, which contains a diverse population of bryozoan species with which we could test our various methods.

To achieve maximum separation from the sieves, we needed to find a suitable combination of sieve sizes. We combined three sieves to perform the following duties: the top sieve

let statoblasts pass through yet stopped larger organic debris, the middle sieve stopped the large statoblasts, and the bottom sieve stopped the small statoblasts but still let water flow through. After experimenting with different sieve combinations with samples from Lake Shabbona, we found that the best separation was obtained by a No. 18 sieve (mesh opening of 1mm), followed by a No. 35 (mesh opening of 500 μm) and then a No. 100 (mesh opening of 150 μm) on the bottom. We also changed the manner of sample collection after finding that all statoblasts occur at least occasionally in shore drift where they are attracted to other organic material.

During June, July and August of 1996, we examined the glacial lakes of southeastern Wisconsin by employing this three-sieve combination. As the survey progressed, we began to collect shore drift and sediment samples and to put them into sealing plastic bags instead of rinsing them at the lake as we had before. Back at the laboratory, we washed the samples through the sieve set with a hose. We changed this step to speed up sample collection in the field, but we also found that the samples were easier to inspect under the microscope since the water in which the samples were suspended was clearer.

While microscopically examining the samples, we found that the small floatoblasts would aggregate around the edge of the polycarbonate jars making collection and viewing difficult (see Figure 1). Therefore, we began filtering the material using Fisher brand qualitative P8 Coarse/Fast filter paper. After the sample had settled in the polycarbonate jar, we gently poured-off the top of the solution into the filter paper. We would repeat this step until the water level reached the settled organic material remaining in the polycarbonate jar. Then, with a dissecting microscope, we could systematically scan the flattened filter paper for floatoblasts.

Results

We examined thirty-eight lentic sites in Illinois for large statoblasts using the initial sieving method above. By using this method we were able to collect statoblasts of *Pectinatella magnifica* at 26 sites and *L. carteri* at 2 sites (Table 1). Our success rate at finding *P. magnifica* statoblasts by sieving in lentic sites was 14 of 38 sites or 37%. By comparison, we examined 135 lentic sites for colonies during the Illinois survey but only found *P. magnifica* at 29 of these sites or 21%. *L. carteri* occurs in three sites in Illinois. We discovered it by first sieving statoblasts at two of the 135 lentic sites. At the third Illinois site we only found colonies. At this last site we did not subsequently find statoblasts when we sieved a lake bottom sample that we had collected several hundred meters from the site where we found the colonies.

If a site has a bryozoan species, will the sieving method consistently reveal its presence? In the course of studying *L. carteri* in Lake Shabbona in DeKalb County, IL (Wood and Marsh, 1996) we sieved 38 samples for large bryozoan statoblasts from different locations in the lake. This lake contains both *P. magnifica* and *L. carteri*. We recovered at least one fragment of a statoblast of *L. carteri* in 37 of the 38 samples and at least one fragment of a statoblast of *P. magnifica* in 23 of the 24 samples for which we recorded evidence of this species. A number of these samples contained 50 or more valves or statoblasts of *L. carteri* and as many as 1000 in one sample. We collected the single sample that did not contain statoblast evidence of either of these species on our first trip to the lake during which we were still experimenting with the method.

In October of 1995, using the initial method, Jones, Hardy and Waclaw (unpublished) examined the glacial lakes of northeastern Illinois for large statoblasts. Of the seventeen lakes studied, only Cedar Lake, located in Lake Villa, Illinois, contained any of the large statoblasts. The bryozoan species observed there, *C. mucedo*, had not been reported from Illinois in over one hundred years (Davenport, 1904; Kofoid, 1908). On three return trips to this lake during the ensuing two years, we consistently found *C. mucedo* statoblasts, but no colonies.

During the summer of 1996, in southeastern Wisconsin, Jones and Hoekstra (unpublished) employed the modified sieving technique that separated small and large statoblasts. At first, Jones only tried to distinguish *Stephanella hina* from among the small statoblasts. Because of

FIGURE 1. We found the majority of the large statoblasts from the No. 35 mesh sieve in the bottom organic material in the polycarbonate jar, as seen here on the left, whereas, we found the majority of the small statoblasts from the No. 100 mesh sieve on the surface in the polycarbonate jar, as seen here on the right.

their translucent bodies, *S. hina* colonies can be difficult to locate (Smith, 1988). Therefore, we were of the opinion that if *S. hina* were in Wisconsin, we would be able to better locate it using the sieving method.

After employing this method during our first two trips to Wisconsin, we realized that we could undertake a more holistic study of each lake using this sieving method. After looking only for *S. hina* in the first 19 of 63 lakes, we began to identify all statoblasts in the remaining 44 lakes. In these 44 lakes, we found evidence of 8 different bryozoan species by locating their small statoblasts. The species identified were: *Plumatella nitens, Plumatella repens, Plumatella reticulata, Plumatella emarginata, Plumatella vaihiriae, Plumatella fungosa, Plumatella casmiana,* and *Hyalinella punctata.* In Comus Lake, Delavan, Wisconsin, we identified 5 species from one sample site. We returned to this lake but found colonies of only two of the five species. This provides further evidence that the sieving method is necessary for a more thorough survey. In addition, we found statoblasts of *P. nitens* at 29 of 44 lakes in southeastern Wisconsin. We sorted through material collected earlier in the study and found it at 2 additional sites. Therefore, in total, it was found in 31 of 49 lakes. These data indicate that *P. nitens* frequently occurs within its known distribution, at least in Wisconsin.

TABLE 1. Sieve method results for bryozoan species forming large statoblasts in Illinois, 1992–94.

	Species	
	P. magnifica	*L. carteri*
Only known by sieving statoblasts	14	1
Sieved statoblasts first, then found colonies	1	1
Colonies found first, then sieved statoblasts	11*	1

* There were no sites at which we found colonies without also finding statoblasts with the sieving method.

Discussion

Bryozoan species that form large, pigmented colonies are easier to locate than those that

produce small, translucent colonies. Sieving, however, makes determination of presence within a water body even easier. Bryozoans are able to produce tremendous numbers of statoblasts as the colonies mature and until unfavorable conditions slow down or terminate their growth. By sieving bottom sediments or shore drift, we are virtually assured recovery of at least one statoblast fragment for any bryozoan species present in that site by using this sieving method. The advantage of including this method during surveying is the detection of species that otherwise may be overlooked if colony collection is the primary focus of the survey. For instance, we have found *P. magnifica*, a species that produces a spherical colony that can be several decimeters in diameter, more frequently by sieving for its statoblasts than by only looking for colonies (Table 1). Species that form small translucent colonies, such as *L. carteri*, are also more likely to be discovered by sieving for statoblasts (Table 1) even if they occur less frequently in a region. *L. crystallinus* is a rare species that also forms small translucent colonies (Wood, 1991). We did not find this species in Illinois despite the fact that we sieved for their statoblasts at a number of sites in the area of their discovery a century ago near Havana, IL (Kofoid, 1908). However, statoblasts of this species have twice been reported elsewhere in recent years (Cooper and Burris, 1984; Rieradevall and Busquets, 1990).

We found that it is important to sieve lake bottom sediments for sinking floatoblasts and shore drift for floating floatoblasts. Wood and Marsh (1996) reported that *L. carteri*, and presumably *L. crystallinus*, produce floatoblasts that will only become buoyant after they have dried (see also Mukai and Oda, 1980). We recovered statoblasts of *L. carteri* more consistently from lake bottom sediments than from shore drift. *P. magnifica* and *C. mucedo* statoblasts, while they are buoyant, often appear in these samples as well. Shore drift, however, regularly yields more small statoblasts than lake bottom sediments.

In the past, species identification has been based upon colony morphology and statoblast identification with light microscopy. This has led to misidentifications. Bryozoologists have recently begun to study morphologically distinct statoblasts with SEM, have redescribed such species as *P. fungosa* (Geimer and Massard, 1987) and have distinguished species such as *P. nitens* (Wood, 1996). Therefore, to make an absolute identification of bryozoan species from statoblasts, SEM must be used since slight morphological differences can look almost identical using light microscopy.

Using only the sieving method for all sites has some limitations; colony searches must be performed in some instances. For example, we did not look for piptoblasts produced by the Fredericellidae in our sieve samples, nor did we look for the hibernaculae produced by the freshwater Gymnolaemata. Also, lotic localities are difficult to assess for statoblasts because the source may be far upstream or in a lentic site which feeds the lotic system. Therefore, to complete a distribution study which includes these sites, one must search for colonies at these sites, as only this procedure can provide a completely accurate reflection of the bryozoan species in these environments.

Another disadvantage results from our sieving procedure; we microscopically examine the sieved material at the end of the day when we have left the site(s) where we collected the sample(s). On those occasions that we have found statoblasts of a species that we did not collect as colonies, we have sometimes chosen to return to the site to try to collect colonies, a time-consuming procedure. To overcome this we would have to microscopically examine the sieved sample at the site so that we could search for colonies of any new species discovered based on statoblast identification.

If we recovered only one or two statoblasts during sieving, it is possible that they were introduced by waterfowl or animals and may never have produced colonies. Therefore, a return trip to sites of this nature must be done in order to establish reliable data. For instance, we thought the original *C. mucedo* statoblast discovered in Cedar Lake may have been such a statoblast. We have sampled Cedar Lake three times since the original survey and each time we have recovered several statoblasts from the sediment and/or shore drift. We have reasoned that by resampling and consis-

tently rediscovering statoblasts, the actual colonies had to have been present in the recent past in order to produce such results. Furthermore, statoblasts recovered from sediments may have been deposited by colonies that are no longer present, and the statoblasts themselves may not be viable any longer. Subsequent collection of colonies from the site is the only way to confirm whether the species whose statoblasts were previously uncovered are currently populating the site. In this case, it seems that *C. mucedo* may no longer be a viable species in colonial form.

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