J. Aquat. Plant Manage. 48: 96-102-

Estimating Lake-wide Watermilfoil Weevil (*Euhrychiopsis lecontei*) Density: The Roles of Quadrat Size, Sample Size, and Effort

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ABSTRACT

Eurasian watermilfoil (*Myriophyllum spicatum* L.), a nonindigenous macrophyte in North America, can reach nuisance levels in freshwater systems. The watermilfoil weevil (*Euhrychiopsis lecontei*) has been employed as a biological control agent for Eurasian watermilfoil. If lake-wide watermilfoil suppression is desired, then *E. lecontei* research needs to be focused at this spatial scale. However, previous studies and current management methods estimate *E. lecontei* density with limited sampling, and lake-wide studies are rare. In a single watermilfoil-infested lake previously stocked with *E. lecontei*, we (1) determined which of four quadrat sizes was most appropriate for sampling *E. lecontei*, (2) calculated the optimum number of samples required to estimate *E. lecontei* abundance at a lake-wide scale with varying precision, and (3) estimated the cost associated with each required sample size. Analysis of variance showed that differences between quadrat sizes were not significant ($p > 0.9775$), likely due to *E. lecontei's* highly variable distribution. Next, we collected lake-wide samples with a 0.1 m^2 quadrat to estimate *E. lecontei* density. Power analysis concluded that highly precise estimation (±20% of the true mean) of lake-wide *E. lecontei* abundance required a large sample size (>300) and substantial effort (261 h), and that reduced precision (±50% of the true mean) required 49 samples and 41.8 h. The patchy distribution of *E. lecontei* makes highly precise lake-wide density estimation difficult, implying that researchers and managers will need to either accept lower precision associated with their sampling or largely increase sample size and effort when estimating lake-wide *E. lecontei* density.

Key words: biological control, Michigan, *Myriophyllum spicatum*, optimum sample size, power, precision.

INTRODUCTION

Nonindigenous species (NIS) are considered one of the top threats to both native biodiversity and ecosystem function (Kolar and Lodge 2001). Controlling the spread and reducing the impacts of NIS is important for preserving biodiversity, protecting ecosystem function, and minimizing negative economic impacts (Lovell et al. 2005). The aquatic plant Eurasian watermilfoil (*Myriophyllum spicatum* L.), hereafter referred to as EWM, is not indigenous to North America and, once introduced, can reach nuisance levels in lakes, rivers, and reservoirs. Dense EWM growth can alter the physical and chemical conditions of water bodies and change fish and wildlife habitat (Keast 1984, Smith and Barko 1990, Madsen et al. 1991, Cheruvelil et al. 2001 Unmuth et al. 2000). Dense EWM growth can also negatively affect human uses by impeding swimming, fishing, and boating. When EWM accumulates at the water surface (i.e., forms a surface canopy) or broken EWM stems collect on shore, the aesthetic quality of the lake may also be reduced (Smith and Barko 1990). Therefore, there is much interest in effectively stopping the spread of this species into new aquatic ecosystems and for control in colonized systems.

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Biological control, the use of biological means (such as parasites, viruses, or predators) to suppress a pest population by reducing its numbers or the damage it causes (Eilenberg et al. 2001) is one EWM control option for infested aquatic systems. The benefits of this approach are the potential for long-term control and increased selectivity, and it is generally considered a more natural and sustainable method of control than alternatives such as chemical or mechanical control (Madsen et al. 2000). Although several biological control agents have been suggested for EWM control, this paper focuses on the watermilfoil weevil (*Euhrychiopsis lecontei*), which has shown biocontrol promise and is commercially available for stocking.

Euhrychiopsis lecontei is an aquatic beetle native to North America. All *E. lecontei* life stages are dependent on watermilfoil plants for food and habitat (Sheldon and O'Bryan 1996a). Research has found that although its natural host plant is a native variety of *Myriophyllum*, *E. lecontei* prefers EWM once exposed to it and is highly host-specific to EWM (Sheldon and Creed 1995, Solarz and Newman 2001, Sheldon and Creed 2003); therefore, *E. lecontei* has little negative impact on other aquatic plants (see Newman 2004 review of *E. lecontei*). Although the life history and host specificity of *E. lecontei* suggest promise as a biocontrol agent for EWM, effective EWM suppression is dependent on *E. lecontei* grazing rates, which are primarily determined by population density and EWM response to herbivory (Newman 2004).

Research of *E. lecontei* suggests that it is well-suited for EWM biocontrol, yet it does not always reach population densities high enough to control EWM. A number of studies have investigated factors that may be limiting *E. lecontei* density (Sheldon and O'Bryan 1996b, Sutter and Newman 1997, Newman and Biesboer 2000, Tamayo et al. 2000, Newman et al. 2001, Ward and Newman 2006); however, the reasons for incomplete control remain unclear. One problem with our current state of knowledge is that previous studies have sampled *E. lecontei* densities at a different spatial scale than EWM control is desired, often sampling a limited number of EWM beds in a lake or making lake-wide population inferences from a relatively small number of EWM stems. If we wish to control EWM at the whole-lake scale, we need to understand *E. lecontei* population dynamics and the factors that may limit *E. lecontei* effectiveness at that scale, and therefore must sample and estimate *E. lecontei* density at the whole-lake scale. Our knowledge of *E. lecontei,* however, is based mainly on small-scale studies. Therefore, *in situ* studies of *E. lecontei* at the lake-wide scale are essential to improve our understanding of lake-wide population dynamics and its use as a biocontrol agent.

When developing a sampling program, three main components need to be addressed: sample units (i.e., what will you collect [quadrats or individual stems]); sample strategy (i.e., how will you collect samples [such as transects, randomly determined points, and point intercept]); and statistical power (i.e., how many samples will you collect), which determines your ability to detect differences among treatments (Peterman 1990). When conducting scientific study, results must estimate the population density of the species of interest, with the desired level of precision, without incurring excessive costs (Hutchins 1994). Adequate statistical power can ensure that study results have the precision necessary to detect differences in population densities over time or between experimental manipulations. Therefore, regardless of the sample unit selected or the sampling strategy employed, accounting for power for *in situ* population studies must be addressed. Collecting preliminary samples and running power analysis can determine the number of samples required to reach the desired level of precision, known as optimal sample size, and sample collection and processing times can be used to estimate cost.

Our study selected the sample unit and sampling strategy we felt most appropriate to explore optimal sample size and the cost necessary to estimate lake-wide *E. lecontei* population density using a variety of desired precision levels. Although many previous studies collected individual stems and reported *E. lecontei* densities as number/EWM stem, we chose to use quadrats to collect and estimate *E. lecontei* and EWM densities. The use of quadrats allowed us to collect a large number of EWM stems quickly. By counting EWM stems in these samples, we were able to present *E. lecontei* density in both aerial and per stem basis. Our objectives were to determine:

1. The quadrat size that most minimizes cost while maximizing accuracy for estimating lake-wide *E. lecontei* abundance.

2. The number of quadrats required (using the chosen quadrat size from above) to estimate lake-wide *E. lecontei* density across a range of confidence intervals and detection levels.

3. The amount of effort needed to collect and process the required number of quadrats for each of the scenarios in objective 2.

Based on past research of epiphytic invertebrates (Downing and Anderson 1985), we expected that although using a smaller quadrat would require a larger number of samples to be collected, this scenario would more accurately estimate *E. lecontei* abundance than taking fewer samples with a larger quadrat. We also expected that smaller quadrats would minimize cost, measured as processing time in the lab (Downing and Anderson 1985). To meet our objectives, we conducted an intensive field study of one lake infested with EWM and previously stocked with *E. lecontei* in Michigan, USA. The information gained from this study will provide a useful tool for future scientific study of *E. lecontei* population ecology and inform whole-lake EWM management.

MATERIALS AND METHODS

We collected all EWM and *E. lecontei* from Lake Ovid. This lake, with a mean depth of 2.3 m and a maximum depth of 6 m, is located within Sleepy Hollow State Park in Clinton County, Michigan, USA. The 147 ha lake is a man-made reservoir created by a dam on the Little Maple River. Lake Ovid, with a Secchi depth of <1.5 m, is hypereutrophic (Kalff 2002), with a natural shoreline except for a public camping area, boat launch, and beach. During July 2006, a team from the Michigan Department of Natural Resources stocked 23,000 weevils at six locations in Lake Ovid. An additional 14,000 weevils were added in 2007 (Tim Machowicz, pers.

comm.). Using the point intercept method (Madsen 1999), we determined lake-wide EWM cover to be 39.5% during July 2008. Only two other macrophyte species were found, and they were uncommon.

Prior to quadrat collection, we visited Lake Ovid to determine extent and location of EWM beds. For the purposes of this study, EWM beds are defined as any area where EWM growth is within 50 cm of the water surface and thus could impede recreation. We collected waypoints using a handheld GPS unit (Garmin GPSmap 76S) around the perimeter of EWM beds. The GPS data were uploaded to ArcMap (ESRI v9.2) to digitize and map EWM beds using a MNDNR Garmin extension (Figure 1a) (www.dnr.state.mn.us, last accessed June 2008).

Determining Quadrat Size

We constructed a composite quadrat that consisted of four different quadrat sizes within the outer frame (Figure 2). We used 1.5 inch PVC pipe for the outer frame and steel wire for the inner quadrats. A steel wire was connected from the bottom left to the upper right corner of the PVC frame to stabilize the upper right corner of each inner quadrat. We used this quadrat to gather EWM samples of each quadrat size. These samples were collected from a single bed selected for this composite quadrat study (Figure 1b inset).

We visited Lake Ovid on 17 June 2008 to collect composite quadrat samples. We established five evenly-spaced transects perpendicular to shore from west to east along the bed. Three samples, each containing the four quadrat sizes, were taken per transect, providing us with 15 EWM composite quadrats and 60 total samples (Figure 1b inset). When taking samples, we submerged the quadrat a minimum of 0.5 m below the water surface, and to the substrate when possible, and used the wires and visual estimates to separate and clip the EWM stems. We did not record field collection time for composite quadrats because summing smaller quadrat times to estimate the effort of larger quadrats would not accurately represent collecting the larger individual quadrat. Minimum stem length was decided based on three factors: (1) our working definition of an EWM bed, (2) *E. lecontei* inhabit the upper portions EWM (Creed and Sheldon 1993), and (3) use of this stem length in previous *E. lecontei* research (e.g., Sheldon 1997, Jester et al. 2000 and Tamayo et al. 2000). Individual quadrat samples were placed in separate prelabeled, resealable 3.8 L bags while underwater and stored on ice until returned to the lab where they were kept refrigerated until processed.

We processed each sample individually using 37.9 L tubs and white dissecting trays. We placed one to two stems of EWM into a tray and carefully inspected them for all life stages of *E. lecontei*. We also recorded the number of EWM strands and meristems from transects four and five. We separated EWM strands into two categories: stems (strands that contained an apical meristem and were at least 10 cm long), and fragments (strands that had two broken ends or were less than 10 cm long). Because individual samples larger than 0.05 m^2 represented a portion of the total area for a quadrat, we summed values of the smaller sections to get values for the larger desired quadrat sizes. We kept track of processing time in minutes as a proxy for sampling cost.

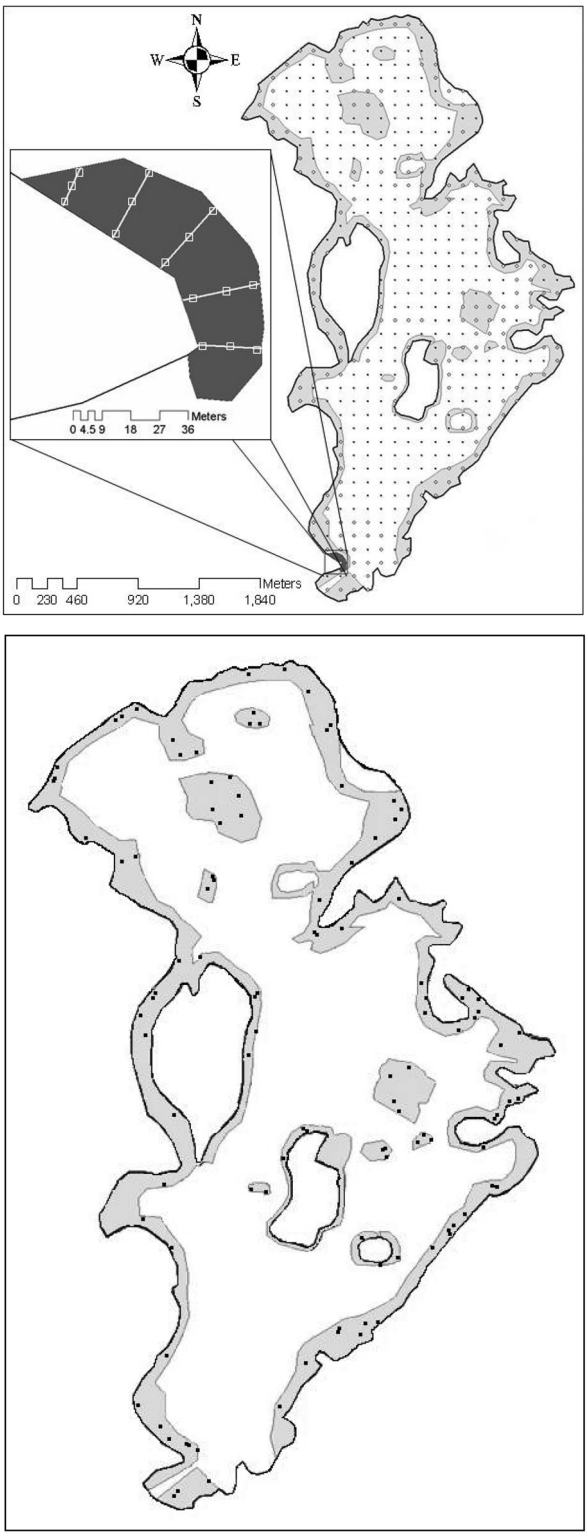

Figure 1. Lake Ovid with point intercept sample locations and mapped EWM beds for determining lake-wide weevil density (a) and lake-wide quadrat sample site locations (b). Point intercept sample locations used to determine lake-wide EWM cover. ● = no EWM found, ○ = EWM found. ■ = mapped EWM beds. ■ = bed sampled for quadrat size testing. Inset 1a depicts the EWM beds sampled during June to determine quadrat size. Composite quadrats were collected at three points along five transects.

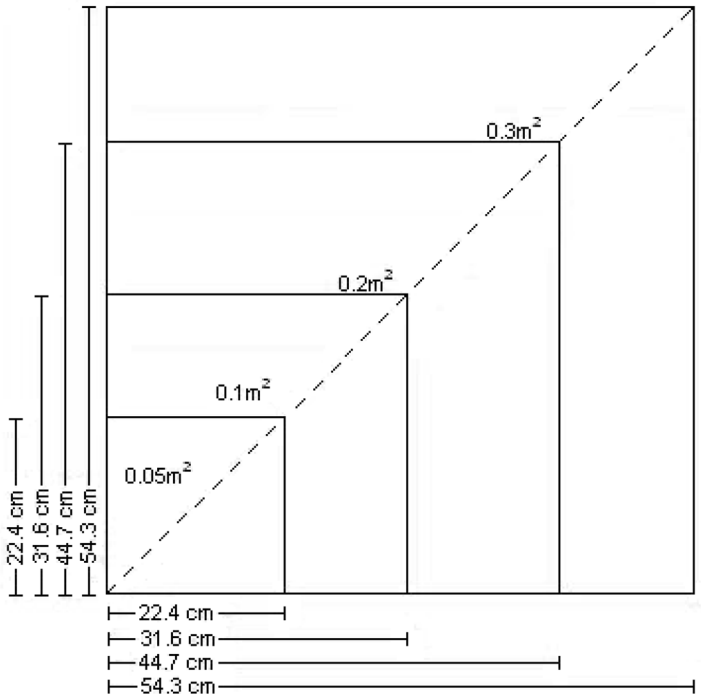

Figure 2. Composite quadrat containing four quadrat sizes and depicting diagonal support wire.

We natural-log transformed the quadrat size data to achieve more normal distributions. We used these transformed data to run ANOVA and Tukey post-hoc tests using SAS 9.1 to determine whether or not the four quadrat sizes produced significantly different estimates of *E. lecontei* density (alpha < 0.05).

Determining Number of Quadrats

Based on the results of our sample size analyses, we used one quadrat size to collect 118 lake-wide *E. lecontei* samples on 22 July 2008. Quadrat samples were collected from EWM beds previously mapped (see previous section). To determine the appropriate number of samples per bed, we divided the individual bed areas by the sum of all bed areas and multiplied these proportions by 100. All beds comprising less than 3% of the total sampling area were assigned a minimum sampling value of three for two reasons: (1) *E. lecontei* density and bed size were not correlated (unpublished data) and (2) three is the minimum number that allows statistical analysis. We randomly generated sample points for each EWM bed using Hawth's Tools Extension (ArcMap 9.2; Figure 1a). We used GIS maps and GPS coordinates to locate our sample points in the field. The same techniques were used to collect, store, and process the EWM and *E. lecontei* samples as were described for the quadrat size study. For this stage of sampling we kept track of both sample collection and processing time as a proxy for cost.

We used Power analysis (equation 1) on the data collected from the lake-wide sampling to calculate the optimum sample size (N) required to reliably estimate lake-wide *E. lecontei* density using the selected quadrat:

$$N = (t_{\alpha/2}/d)^2(s^2/m^2), \quad (1)$$

with $t_2 = t$ value for a given probability (α), d = desired fixed proportion of the mean, s^2 = sample variance, and m = mean density (Buntin 1994). Sample variance and mean density were determined from the lake-wide samples collected. We calculated optimal sample size for a range of α and d to represent common confidence intervals and levels for pest management sampling programs (Buntin 1994).

RESULTS AND DISCUSSION

Quadrat Size

There were no differences in estimated *E. lecontei* density ($p = 0.9775$), EWM stem densities per square meter (0.0864), or estimated *E. lecontei* per EWM stem ($p = 0.8525$) between the four quadrat sizes collected from Lake Ovid during June 2008 (Table 1). We noted that the variation among samples was quite high, which likely attributed to the lack of difference among the four different sizes of quadrat. Previous foodweb studies of the littoral zone have found that epiphytic invertebrates are highly spatially variable, and this may account for our high sample variation (Downing and Cyr 1985, Cheruvelil et al. 2000). In fact, when we later ran power analysis to detect differences among the 0.05, 0.1, 0.2, and 0.3 m² quadrat sizes, we found that our power was low (0.82, 0.75, 0.69, and 0.70, respectively). Therefore, compared to the 15 samples we collected, we would have needed to take 39, 33, 29, and 30 samples, respectively, to determine a 50% difference in *E. lecontei* density between the quadrat sizes. Some other research has shown that a large number of epiphytic invertebrate samples are likely needed to achieve the high

TABLE 1. RESULTS OF COMPOSITE QUADRAT ANALYSIS. COSTS (LAB PROCESSING TIME) FOR ESTIMATING *E. LECONTEI* (N = 15) AND EWM (N = 6) DENSITY. ESTIMATED *E. LECONTEI* AND EWM DENSITIES WITH STANDARD ERROR IN PARENTHESIS AND RESULTS OF *POST HOC* TUKEY TEST FOR SIGNIFICANCE. MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT FROM EACH OTHER (TUKEY-KRAMER TEST, $\alpha \leq 0.05$). * = QUADRAT SELECTED FOR FURTHER USE.

Quadrat size (m²)	Average lab time (minutes)	Estimated *E. lecontei* density m² (standard error)	Estimated EWM stem density m² (standard error)	Estimated *E. lecontei* per EWM stem
0.05	46	135 (51.72) A	427 (163.30) B	0.32 C
0.1*	88	95 (33.19) A	362 (106.47) B	0.26 C
0.2	135	73 (23.75) A	283 (80.66) B	0.26 C
0.3	210	64 (21.02) A	261 (93.08) B	0.24 C

Table 2. Projected time needed to collect and process 0.10 m² quadrats to reach optimal sample size (N) for lake-wide *E. lecontei* population density estimation with varying desired fixed portion of the mean (*D*) and alpha values.

			Time hours (weeks)		
d	α	N	Collection	Processing	Total
0.2	0.05	309	18.3	242.8	261.0 (6.5)
0.2	0.10	214	12.7	168.6	181.3 (4.5)
0.3	0.05	137	8.1	107.9	116.0 (2.9)
0.3	0.10	95	5.6	74.9	80.6 (2.0)
0.4	0.05	77	4.6	60.7	65.3 (1.6)
0.4	0.10	54	3.2	42.1	45.3 (1.1)
0.5	0.05	49	2.9	38.8	41.8 (1.0)
0.5	0.10	34	2.0	27.0	29.0 (0.7)

levels of precision (Downing and Cyr 1985). In addition, edge effects of the composite quadrat may have introduced some bias in our results and deserves further investigation.

Average lab processing time increased as quadrat size increased (Table 2). Processing time would have been reduced most using a 0.05 m² quadrat. This increase in samples would have, however, required more sample transport space and increased total sample collection time by increasing the amount of time spent entering—exiting the water and locating additional sample sites. Therefore, based on the costs associated with each quadrat size, the increased number of samples determined by the *post hoc* power analysis for the 0.05 quadrat, and because there was no difference in weevil densities among quadrat sizes, we chose the size that we thought would be most practical for the next step of our study. We used a 0.1 m² quadrat for four reasons: (1) to minimize cost (processing time), (2) to build on previous *E. lecontei* scientific studies using 0.1 m² quadrats (Newman and Biesboer 2000), (3) because extrapolating weevil densities to represent abundance per square meter would be mathematically simple when beginning with a 0.1m² quadrat, and (4) because collecting a larger number of samples using smaller quadrats is often better than a smaller number of large quadrats (Green and Young 1993).

Required Sample Size

We estimated that the lake-wide *E. lecontei* density was 36.3/m² with a sample variance (s²) of 4145, and the average number of EWM stems per sample was 33.4 with a variance of 262, resulting in an estimated average of 1.09 *E. lecontei* per stem. Based on our estimated EWM bed area, we would estimate there were 264,000 *E. lecontei* in Lake Ovid at the time of sampling, suggesting a substantial increase from the 37,000 *E. lecontei* stocked since 2007. Several factors should be considered, however, when comparing stocking numbers to our estimate: (1) Lake Ovid likely had an existing population of *E. lecontei* prior to stocking, (2) since 2007, some *E. lecontei* have been removed for culturing purposes (with similar numbers being returned at the end of each season), and (3) using equation 1 and solving for *d*, our estimated number per m² is within 32% of the true mean; therefore, the true lake-wide *E. lecontei* population lies somewhere between 179,520 and 348,480 individuals. In fact, power analyses of the 22 July 2008 samples demonstrated that with an alpha of 0.05 and *d* value (desired fixed proportion of the mean) of 0.2, the optimal sample size is 309 quadrats or 10,321 stems. At the other end of the spectrum, using a *d* value of 0.5 would require 49 quadrat samples or 1651 stems. Additional analysis with the same *d* values and an alpha of 0.10 resulted in optimum samples needed ranging from 34 to 214 quadrats and 1147 to 7167 stems (Figure 3). Note that although we cannot determine whether EWM biocontrol is being achieved from our single-season of sampling, according to previous studies our estimate of *E. lecontei* density is within the range needed to bring about significant EWM decline and/or suppression (Newman 2004).

An additional factor that needs to be considered is our differentiation between EWM stems and fragments. We used only EWM stem data for data analysis. This has two main implications: (1) underestimation of EWM stem densities because stems broken during collection increased fragment counts and decreased stem counts, and (2) overestimation of *E. lecontei* per stem results. However, the fragment information could not be accurately transformed into a stem count. Future studies could overcome this by measuring individual stem and fragment lengths and dividing the total fragments lengths by a predefined minimum strand length.

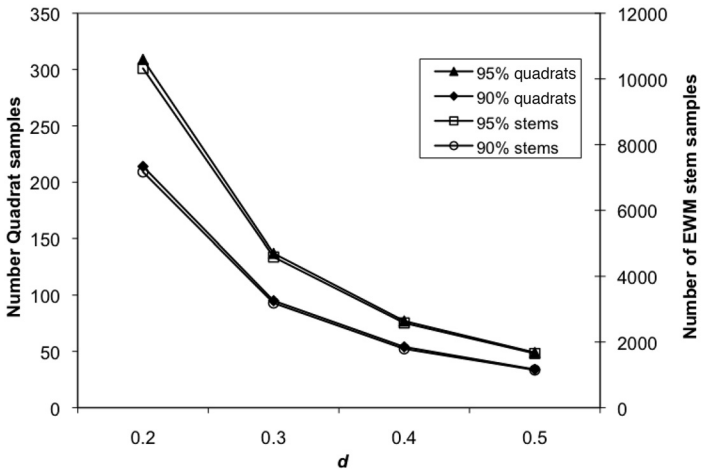

Figure 3. Optimal sample size (*N*) of quadrats and EWM stems to estimate lake-wide *E. lecontei* population density using equation 1. Calculated using a range of precision (*d*) and both 90 and 95% confidence intervals.

The estimated time to collect and process a 0.10 m^2 quadrat from Lake Ovid was 50.2 min. Using this estimate, the total time needed to process the optimum number of samples for each of the previous scenarios ranged from 29 to 261 h (Table 2). These results demonstrate that achieving high precision lake-wide *E. lecontei* estimates requires a large number of samples and considerable cost, but that by looking for just large differences between treatments (i.e., requiring lower power) it is possible to reduce samples and costs. For example, if we were interested in being 95% confident of our estimates of lake-wide *E. lecontei* density in Lake Ovid during summer 2008 within 50% of the true mean, we would need to spend one week to collect and process forty-nine 0.01 m^2 quadrats (or 1637 stems).

The decision of minimum acceptable power is dependent on the questions being asked and the resources available. In addition, power will be highly variable among lakes and years. For example, Lake Ovid has a relatively high *E. lecontei* density, which influences the optimum sample size calculations. A lake with low *E. lecontei* population density would require a larger number of samples to achieve similar levels of power. Therefore, power should be calculated for each project on an individual lake basis and likely reevaluated at the beginning of each sampling season. Although the optimum sample size values we calculated for Lake Ovid should not be directly applied to other lakes, they can provide general guidance on the likely range of samples needed when sampling *E. lecontei.*

Our results suggest that previous research that used fewer samples (quadrats or stems) from fewer EWM beds likely did not achieve adequate power to precisely estimate lake-wide *E. lecontei* density or detect differences in *E. lecontei* densities across lakes with a high level of confidence. Some of these studies may not have attempted to estimate lake-wide *E. lecontei* populations, and we are not suggesting that all *in situ* research of *E. lecontei* needs to be performed at the whole-lake scale. The issue of statistical power is important, however, when estimating *E. lecontei* population densities regardless of spatial scale and should be considered and reported. In addition, if we wish to use *E. lecontei* to manage EWM at the whole-lake scale, we need to understand *E. lecontei* population dynamics at that scale; therefore, we must sample, estimate, and study *E. lecontei* density at the whole-lake scale. Future research should conduct *a priori* power analysis when investigating *in situ E. lecontei* density and use the results to ensure sufficient power is realized.

ACKNOWLEDGMENTS

We would like to thank EnviroScience Inc., especially Marty Hilovsky and Cortney Marquette, for helping fund this research and providing information on *E. lecontei* stocking. Thanks to Emily Jacobson for her assistance in collecting and processing samples and Dr. Patricia Sorrano for allowing us to use her boat and other field equipment. We would like to thank Tim Machowicz for allowing us access to Lake Ovid and providing us with background information about the lake and its EWM and *E. lecontei* history. We would also like to thank Ray Newman for providing us with insights on *E. lecontei* sampling methods. We are also grateful to the anonymous reviewers, whose evaluation and comments served to strengthened the manuscript.

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