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EFFICIENCY OF SAMPLING TO DETERMINE POPULATION SIZE OF *CYRTOBAGOUS SALVINIAE* (COLEOPTERA: CURCULIONIDAE)

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ABSTRACT

Salvinia molesta D. S. Mitchell (Salviniales: Salviniaceae), a small floating fern introduced from South America, is causing an increasing number of problems in the US. Increased reliance on the biocontrol agent, *Cyrtobagous salviniae*, in the US is becoming more commonplace and several mass-rearing facilities have been developed. Because of differences in sampling protocols including sample size, reporting parameters, and numbers released, an investigation into sampling efficiency was initiated. A small pond in southern Louisiana was sampled in an effort to understand what constitutes an adequate sample size and methodologies needed to estimate numbers of weevils. A clumped distribution in the pond was identified, which required a large number of samples to be taken to minimize differences in means and variation. When randomly selecting 10 sets of samples where $n = 5$ for Sep, means varied from a high of 280 weevils/m^2 to a low of only 50 weevils /m^2, a difference of nearly 6-fold. However, when randomly selecting 10 sets of samples where $n = 20$, means were much more consistent and varied from a high of approximately 250 weevils/m^2 to a low of 125 weevils/m^2, a difference of only 2-fold. Sampling is expensive and to gain the most information based on the number of samples taken it is recommended that 1) the confidence interval be reported, especially when releasing weevils based on an estimation of population size; 2) understand spatial distribution and sample accordingly; and 3) when possible, initiate pilot sampling programs to acquire prior information on sampling biases, sampling errors, and differences in distribution.

Key Words: biological control, giant salvinia, sampling, mass-rearing

RESUMEN

Salvinia molesta D. S. Mitchell (Salviniales: Salviniaceae), un pequeño helecho flotante introducido de América del Sur, está causando un incremento en el número de problemas en los EE.UU. Un aumento en el uso confiable del agente de biocontrol, *Cyrtobagous salviniae*, es cada vez más común y varias instalaciones de producción masiva se han desarrollado. Debido a las diferencias en los protocolos de muestreo, incluyendo el tamaño de la muestra, los parámetros de reportados y cifras publicadas, se inició una investigación sobre la eficiencia del muestreo. Se tomaron muestras de una pequeña laguna en el sur de Louisiana en un esfuerzo por entender lo que constituye un tamaño adecuado de muestra y metodologías necesarios para estimar el número de gorgojos. Una distribución agrupada en la laguna fue identificada la cual necesitó la toma de un número grande de muestras para mínimizar las diferencias en los promedios y la variación. Al seleccionar al azar 10 grupos de muestras en donde $n = 5$ para Sep, el promedio varió de un máximo de 280 gorgojos/m^2 a un mínimo de sólo 50 gorgojos/m^2, una diferencia de casi 6 veces. Sin embargo, al seleccionar al azar 10 grupos de muestras en donde $n = 20$, el promedio fue mucho más consistente y varió desde un máximo de aproximadamente 250 gorgojos/m^2 a un mínimo de 125 gorgojos/m^2, una diferencia de sólo 2 veces. El muestreo es costoso y para sacar la mayoría de la información basada en el número de muestras tomadas se recomienda que: 1) se reporte el intervalo de confianza, especialmente cuando se liberan los gorgojos en base a una estimación del tamaño de la población, 2) entender la distribución espacial y mostrar en correspondencia y 3) cuando sea posible, iniciar programas pilotos de muestreo para adquirir información previa sobre los sesgos de muestreo, errores de muestreo y las diferencias en la distribución.

Palabras Clave: control biológico, salvinia gigante, muestreo, cría en masa

Giant salvinia (*Salvinia molesta* D. S. Mitchell; Salviniales: Salviniaceae), a native of Brazil, is a floating fern introduced into the US through the aquatic nursery trade (Julien et al. 2002; McFarland et al. 2004). Since its introduction in the middle to late 1990's, giant salvinia has dispersed both naturally and anthropogenically, and in less than 20 years can now be found as far west as the Hawaiian Islands, east into the peninsula of Florida, and north into Virginia (Jacono et al. 2001). It is one of the world's worst weeds causing manifold problems throughout the sub-tropical and tropical regions of the earth. Impacts are varied and include hindering navigation; disrupting water intake for municipal, agricultural and industrial purposes; degrading water quality; decreasing floral and faunal diversity; impacting threatened and endangered species; and increasing mosquito larval habitat for species that are known to transmit encephalitis, dengue fever, malaria, and rural filariasis or elephantiasis (Bennett 1966; Thomas & Room 1986).

Giant salvinia has caused significant problems in over 20 countries including Australia, New Zealand, Fiji, the Philippines, India, Indonesia, Malaysia, Singapore, Papua, New Guinea, the Ivory Republic, Ghana, Zambia, Kenya, Namibia, Botswana, South Africa, Madagascar, Columbia, Guyana, several Caribbean countries (including Cuba, Puerto Rico, and Trinidad) (Storrs & Julien 1996) and the list of affected areas increases yearly. In the US, it is now found in at least 90 localities in the southern states including Texas, North Carolina, South Carolina, Louisiana, Georgia, Florida, Alabama, Mississippi and west into Arizona, and California especially in its more northern distribution (Personal communication Alexander Perret, Louisiana Department of Wildlife and Fisheries, October 2013).

Giant salvinia reaches damaging infestation levels because of its tremendous growth rate. While it has been shown to only reproduce vegetatively (i.e., viable spores are not produced; Loyal and Grewal 1966) this is more than sufficient to form surface mats up to 1 m thick with plant numbers approaching 5000/m^2 and biomass production of upwards of 100 tons/ha/year (Mitchell & Tur 1975). Even greater production is possible under more favorable conditions; it has been known to double its biomass in 1 to 8 days, depending on prevailing environmental conditions (Mitchell & Tur 1975; Cary & Weerts 1984).

Numerous control strategies have been implemented for the management of salvinia (McFarland et al. 2004). These include the use of more traditional methods including mechanical and chemical technologies. Mechanical control options are not particularly effective being expensive and often do not produce even partial control (Chikwenhere & Keswani 1997). However, in certain instances, especially in small isolated areas, mechanical control may be employed with some success. The use of chemical technologies can be effective but tend to produce only short-term control and can become expensive, especially when multiple treatments are needed over the course of a growing season (Chikwenhere & Keswani 1997). Other methods employed for salvinia control in the US include flushing and drawdowns (personal communication, Louisiana Department of Wildlife and Fisheries 2011). Increasing water flow to 'flush' plants out of a waterbody or drainage can reduce biomass locally but may increase the distribution of salvinia downstream. Drawdowns (which serve to desiccate and kill the plant) do reduce biomass and can isolate the plant into smaller areas allowing easier access for mechanical removal or chemical treatment. However, when water levels return to normal remaining plants can be scattered throughout the water body making treatment even more difficult.

The use of alternative control methods such as biological control has been shown to produce long-term sustainable control (Julien et al. 2002). One agent has been approved for release in the US, the salvinia weevil (*Cyrtobagous salviniae* Calder & Sands; (Coleoptera: Curculionidae)), and is the method of choice for management in many overseas locations. *Cyrtobagous salviniae* is a small weevil ranging in length from 1.5 to 2.0 mm (Julien et al. 2002). Adults are typically black but newly emerged individuals may often be brown. Legs are reddish-brown in coloration. The dorsal surface of the weevil is covered with numerous shallow depressions or punctures as well as yellow peltate scales. Adults typically reside on or beneath the leaves or fronds of *S. molesta*. A thin film of air adheres to the bottom of the weevil allowing for respiration during periods of submergence. Eggs are laid singly in cavities formed by the female's feeding activity. Hatching occurs in approximately 10 days. The larvae are white and attain lengths of only 3 mm. Total larval development requires 3 to 4 weeks and is dependent on temperature. Larvae construct cocoons on the "roots" (in reality submersed leaves). The pre-pupal and pupal periods last about 2 weeks.

While effective, biological control can take several years and there is some concern that it may not be particularly effective in the more northern extreme of the US distribution of salvinia. Even so, the use of biological control is gaining increased favor in the USA. Over the last 5 years, rearing operations for *C. salviniae* have been developed at the Federal, state, and local levels, allowing the release of large numbers of weevils in a variety of water bodies, particularly in Texas and Louisiana (Johnson et al. 2010, unpublished information). Such an active approach to the use of biocontrol is promising and allows the more widespread application of a technology that offers the possibility of longer and more sustainable control. However,

set-backs have occurred. First, releases of weevils from various rearing operations are not coordinated to any large extent between the various agencies and institutions; i.e., no central database is available, which would allow easy consultation and comparison. Also, in many cases, there is only minimal monitoring of release sites using sampling protocols designed to accurately document weevil populations and subsequent impact over the long term. Hence, information on current numbers of biocontrol agents and impact levels, which is essential to make informed decisions on the need for additional releases, is lacking. In addition, numbers of weevils released is often determined and reported differently by various agencies leading to erroneous information exchange on actual numbers introduced.

To address these issues, a study was initiated to explore changes in *C. salviniae* distribution over time in a small pond used for rearing in southern Louisiana in an effort to examine sampling efficiency and accuracy as an aid in developing appropriate sampling protocols.

Materials and Methods

Sampling was conducted in a small, man-made pond located at N 29° 33' 51.31" W 90° 45' 56.04" in southern Louisiana near Houma where salvinia completely covered the entire pond. The pond was constructed as a weevil nursery facility to provide weevils for releases in various salvinia infestations in the state of Louisiana. The pond was constructed in Feb 2010 and is approximately 112 m × 21 m and ranges in depth from 0.3 m to 0.6 m. The pond was pumped and fertilized until Mar 20, 2010 when giant salvinia plants from a nearby canal were added. Approximately, 11,000 weevils contained on plant material (estimated using Berlese funnels) from a rearing facility located at Golden Ranch near Gheens, Louisiana were added on 9 June 2010 and were applied to all areas of the pond. The first weevil harvest for field release occurred on 27 Jul 2011.

The pond was sampled 3 times during 2011 beginning 14 Apr 2011 with subsequent sampling dates on 9 Jun 2011, and 23 Sep 2011. An equally spaced grid of 30 points (i.e., 6 equally spaced transects across the length of the pond with 5 approximately equally spaced points on each transect) was assigned to the pond (Fig. 1) and each point sampled using a 0.05 m^2 strainer to allow drainage of accumulated water. After collection, the plants were transported via overnight shipping with as little free standing water as possible to the Lewisville Aquatic Ecosystem Research Facility (LAERF), Lewisville, Texas or the U. S. Army Engineer Research Facility (ERDC), Vicksburg,

Fig. 1. Google Earth satellite image of pond showing location of sampling points.

Mississippi for processing. Excess water from the plants was again removed, plants weighed and subsequently placed in a Berlese Funnel to extract the weevils into 70% ethanol. After complete drying, the number of adults and larvae were enumerated using a dissecting microscope at 7X to 10X magnification. Total numbers of adult weevils and larvae were calculated on a per m^2 and a per kg weight wet basis.

A variety of statistics were used to determine overall weevil population numbers, temporal and spatial weevil distribution, as well as efficiency of sampling at various sample sizes. The majority of the statistics were calculated using Statistica version 9 (Statsoft 2009). Significant differences were ascertained using ANOVA and Tukey HSD test with $P = 0.05$ unless otherwise indicated. To examine differences in means and associated variances at various sample sizes, points were selected at random using Statistica's random sample generator using the fast random number algorithm to form new sets of data. Points that formed each new generated set were selected randomly within each sampling date, within certain areas of the pond, and for various quantitative measurements; e.g., number of adults per m^2, number of larvae per m^2, etc. XLSTAT 2012 (www.xlstat.com) as an add-in to Excel was used to determine negative binomial distribution fits to count data with significance determined using Chi-square. Frequency of counts was determined as a density function (shown as density on the y-axis) expressed as the percentage of all counts divided by the size of the interval.

Spatial interpolation using natural neighbor interpolation was used to graphically portray distributional changes for *C. salviniae* for various quantitative measurements over time. Natural neighbor interpolation locates the closest (or local) subset of input samples to a query point and "applies weights" to them based on similar areas in order to interpolate a value (Sibson 1981). It does not surmise trends nor produce outliers that are not already represented by the input data, and it works similarly well with regularly and irregularly distributed data (Watson 1992). This technique was accomplished using software created by ESRI, ArcGIS v10 - ArcInfo & Spatial Analyst extension (ESRI, Redlands, California).

In addition, statistics to determine spatial distribution were calculated using ROOKS CASE (Sawada 1999), as an add-in to Excel to calculate Moran's I as a determinant of spatial clustering. Adjacency was accomplished using the Queen's case.

Results and Discussion

Measurements

When considering sampling procedures and techniques for estimating numbers of insect biocontrol agents released or present at field locations it is important to understand and recognize inherent errors, possible inaccuracies, and biases associated with each type of technique. Sampling for salvinia weevils has been accomplished by a variety of methods including the use of Berlese funnel extractions, submersing plant material to extract weevils, and/or direct examination of the plant material with reporting of numbers on a per area and/or a per weight basis (Tipping and Center 2005; Diop and Hill 2009; Tewari and Johnson 2011). Both measurements have inherent sampling biases and errors, so choosing an appropriate technique can depend on the purpose of the data collection and reporting. For example, sampling on a per weight basis using fresh plant weight can easily add variation based on the amount of free water remaining in the samples. The magnitude of these errors can be significant since water weight can add substantially to the sample. However, such errors due to excess free water can be reduced by carefully removing the water using blotting or swing drying techniques and using the same techniques consistently for each sampling. Another method to reduce such errors is to dry the plant material completely and calculate weevil numbers on a dry weight basis as was done in Tipping and Center (2005) for *S. minima* Calder and Sands. Tewari and Johnson (2011) found excellent correlations between the wet weight of *S. minima* and its dry weight though no details were provided on techniques used to remove excess water.

Similarly, obtaining numbers on a per area basis also has its own set of sampling biases. If the weight of salvinia in a sampling area changes significantly either temporally or spatially, then changes in weevil numbers can be pronounced. Such changes in salvinia weight per unit area were observed during this study (Fig. 2). For example, the weights of salvinia in transect 1 increased more than 2.5 fold from 7 kg/m^2 for the Apr sample to over 18 kg/m^2 for the Jun sample. Weight per unit area then decreased to about 9 kg/m^2 in Sep. Contrast this to transect 6 where a linearly decreasing trend in weight per unit area was observed to decrease 2 fold over the course of the growing season. Hence, even in a small area significant changes in weight per unit area occurred over time and throughout the pond; possibly influencing the estimated number of weevils especially in relation to number of weevils on a per area basis, where changes in weight per unit area does not influence weevil number.

However, even with such errors and biases, a highly significant positive correlation between numbers of weevils on a weight and area basis was identified with significance values of < 0.05 and r-values of over 0.90 for both adults and larvae (Fig. 3). This indicates that, at least for this dataset, either parameter would be suitable for

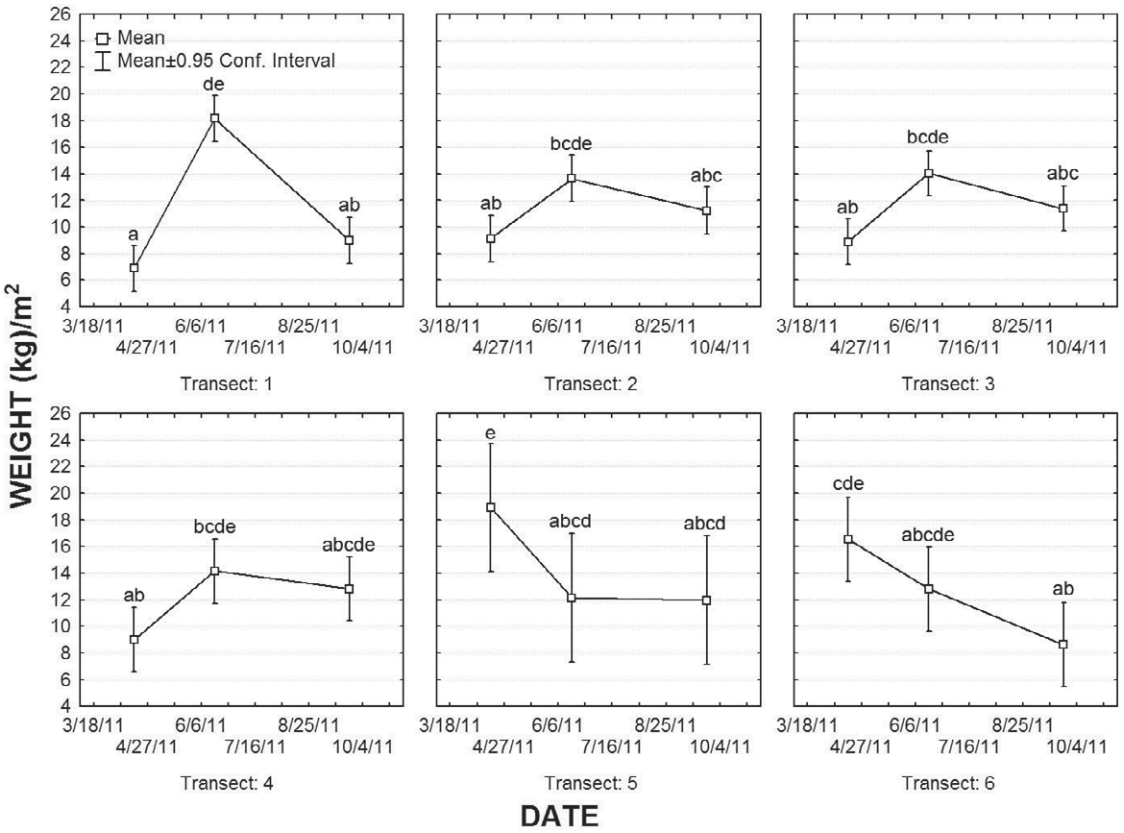

Fig. 2. Changes in salvinia wet weight per unit area for each transect. ANOVA detected significant differences for Date: df = 2, 72, F = 10.908, P = 0.000073, Transect: df = 5, 72, F – 20.63, P = 0.0417, and Date X Transect: df = 10, 72, F = 68.03, P = 0.000000.

characterizing population levels, since they are significantly positively correlated to each other. In addition, if such linear relationships hold for data collected at different locations and times of the year it may be possible to construct a generalized linear relationship to allow conversion from one parameter to the other. This would provide a straightforward comparison of weevil numbers reported by either method. However, before this type of conversion is utilized, it is important that this relationship be tested more thoroughly using data collected from different locations and times of the year. Since these two parameters are so closely related the following discussions will utilize weevil numbers on a per unit area basis only.

Characterization of Population

There was a significant increase in weevil population over time as measured by numbers per m^2 in the pond (Fig. 4). For example, numbers of adults per m^2 increased 10-fold from about 3 adults per m^2 in mid Apr to over 30 adults per m^2 by Sep. Similar increases were observed for larvae per m^2 where increases of over 7-fold occurred during the same time period. Variation during these periods was high with 95% confidence intervals using a pooled variance component for larvae per m^2 that ranged from about 40 to 160; a 4-fold difference.

Such high variation is evidently at least partially due to the spatial distribution of weevil populations in the pond. Even with a relatively small area, the number of adults and larvae on a m^2 basis, catches varied significantly across the pond (Fig. 5). For example, for larvae per m^2 there was a significant exponentially decreasing trend with smaller numbers associated with higher transect numbers (Fig. 6). Numbers varied from a high of over 400 larvae per m^2 for transect 1 to near zero for transect 6. Such a clumped distribution is evidently not unusual; even in small rearing boxes (1.2 × 2.4 m), 1/100 m^2 samples taken adjacent to each other can exhibit considerable variation in weevil number and produce highly different population estimates (personal communication, Ms. Julie Nachtrieb, LAERF).

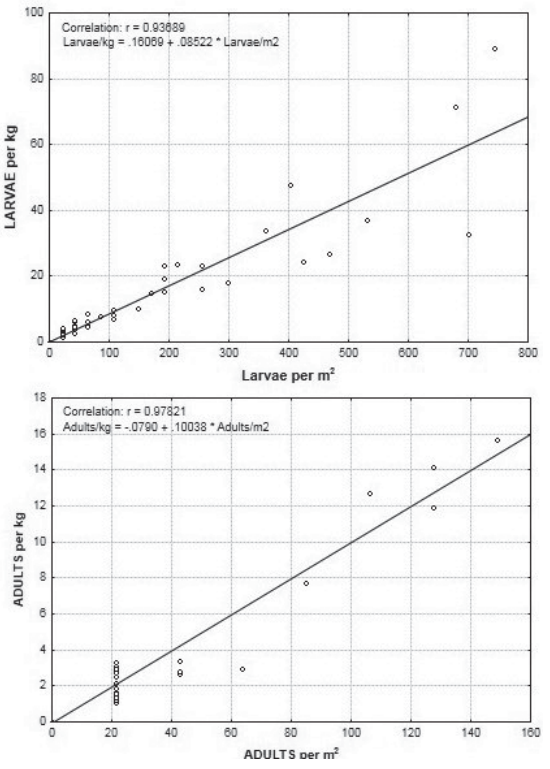

Fig. 3. Correlations between numbers of adults and larvae per kg and per m^2 for all sampling points and dates. Significant correlations were detected at $P < 0.05$, $n = 90$ for both adults and larvae.

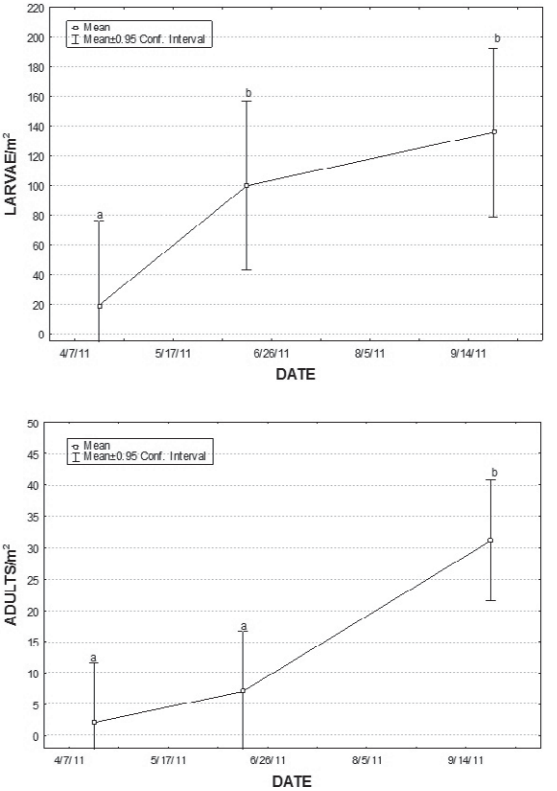

Fig. 4. Average number of adult and larvae *C. salviniae* per m^2 over time. ANOVA detected significant differences between dates with df = 2, 87 and $P < 0.05$ for adults/m^2 - $F = 23.29$ and larvae/m^2 - $F = 26.37$. Means with different letters are significantly different at $P < 0.05$ based on Newman-Keuls Test.

Distribution among field sites also exhibits considerable spatial variation (unpublished data - Grodowitz). This can be further illustrated graphically by using natural neighbor interpolation. Fig. 7 depicts changes in total weevil number per m^2 over time by using natural neighbor interpolation and then displaying it graphically. Note that this graphical representation closely coincides with data presented previously that demonstrated higher numbers per m^2 occurring on the western end (transect 1) of the pond with numbers falling off to near zero as one approached the eastern end (transect 6). Interestingly, graphically, even within those areas containing high numbers of weevils, there were differences in weevil numbers from within areas adjacent to one another. This also serves to illustrate the highly clumped nature of the weevil populations and the problems associated with deriving a true estimate of number of individuals.

Spatial clustering or a clumped distribution is noted statistically with Moran's I test. At least for the Sep sample (where the highest number of larvae and adults were observed) spatial clustering was significant with Moran's $I = 0.04769$, $n = 30$, $P < 0.05$, z-Normal = 3.8033. However, no significant clustering was observed for Apr (Moran's $I = 0.04779$, $n = 30$, $P > 0.05$, z-Normal =0.8965) or for Jun (Moran's $I = 0.0808$, $n = 30$, $P > 0.05$, z-Normal = 1.2482) samples. Lack of statistical significance in the Apr and Jun samples, though indicated visually in Fig. 7, is most likely due to smaller population sizes occurring on those dates.

Another way to examine spatial distribution of the weevils across the pond is to examine the frequency of weevil counts. When examining the distribution of counts for the entire pond for the Jun and Sep samples combined for adults and larvae, the shape of the distribution was highly skewed to the low end (Fig. 8). In fact, 65% percent of the samples taken from the entire pond for Jun and Sep had counts less than 100 with 40% containing no individuals. A similar shaped distribution was revealed for only the first 3 transects for the Jun and Sep sampling periods; i.e., those areas with the highest and most consistent numbers of weevils (Fig. 8). In this case over 30% of the samples had counts with less than 100 adults and larvae and over 20% had no in-

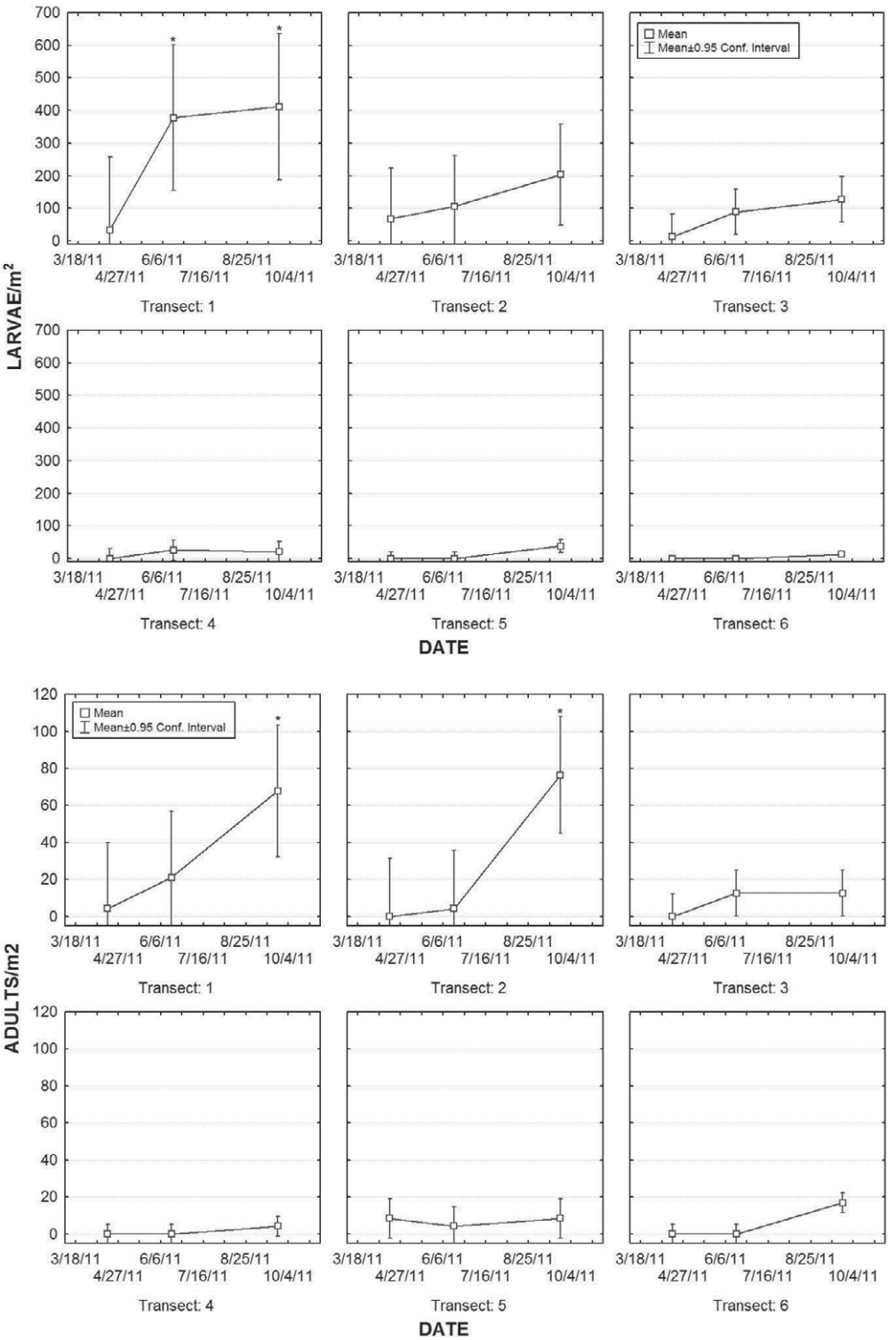

Fig. 5. Mean number of larvae and adult *C. salvinia* per m^2 over time and transect. ANOVA detected significant differences for larvae/m^2 for Date: df = 2, 72, $F = 7.6128$, $P = 0.00100$, Transect: df = 5, 72, $F = 11.5560$, $P = 0.000000$, Date X Transect = df = 10, 72, $F = 2.22273$, $P = 0.025613$ and for adults/m^2 for Date: df = 2, 72, $F = 15.857$, $P = 0.000002$, Transect: df = 5, 72, $F = 5.031$, $P = 0.000522$, Date X Transect: df = 10, 72, $F = 3.62001$, $P = 0.000614$.

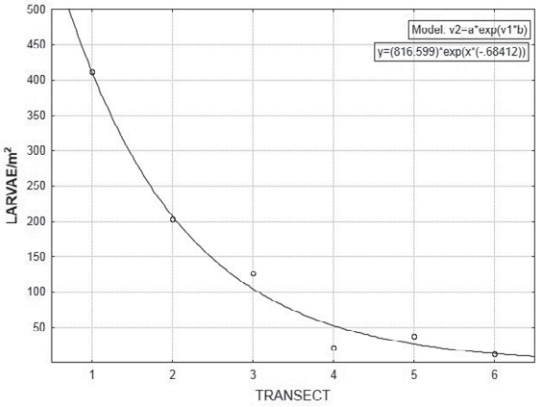

Fig. 6. Mean number of larvae per m^2 for all transects for Sep fitted with a significant $P < 0.05$ exponential curve where $R = 0.993$.

dividuals. In both of these cases, the counts fit a negative binomial distribution. When fitting a negative binomial distribution to the entire pond using a Chi-Square test, the distribution was not statistically different from a negative binomial distribution at $P = 0.226$. Similarly, when examining just the first 3 transects, the distribution did fit a negative distribution using a Chi-Square test at $P = 0.376$. It is interesting that a negative binomial distribution was commonly associated with biological organisms; this distribution has been cited as among the reasons why it is difficult to accurately estimate numbers of organisms on a spatial scale without taking very high number of random samples (Fisher 1941; Bliss & Fisher 1953; Goodell & Ferris 1980; Wiles et al. 1992; White & Bennetts 1996).

Effect of Sample Size

The influence of sample size on the estimation of the mean number of weevils as well as the variation is substantial. For example, when randomly selecting 10 sets of samples where $n = 5$ for Sep, means varied from a high of 280 weevils per m^2 to a low of only 50 weevils per m^2, a difference of nearly 6-fold (Fig. 9). However, when randomly selecting 10 sets of samples where n = 20, means were much more consistent and varied from a high of approximately 250 weevils per m^2 to a low of 125 weevils per m^2, a difference of only 2-fold.

These differences in population estimates could be important when making field releases or determining field populations of agents. For example, the total area of the study pond was roughly 2400 m^2. Assuming that the pond was completely covered with salvinia and the total biomass was harvested for release, the numbers of weevils released would be estimated at 672,000 weevils based on the high mean with only $n = 5$.

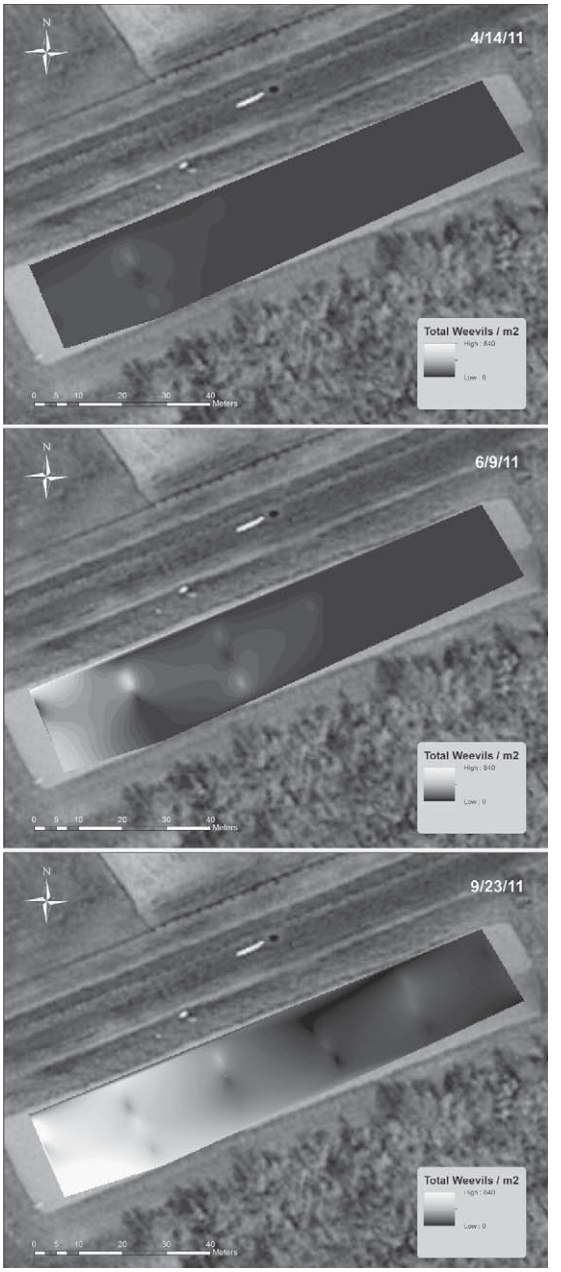

Fig. 7. Spatial analysis showing total weevil number per m^2 through time.

However, estimating number of weevils released with the low end of $n = 5$ would produce an estimate of only 120,000. With a larger number of replicates, there was still discrepancy in the estimated number of weevils released, but the difference between the means was much reduced (Fig. 9). For the Sep sample, means varied from a high of 250 weevils per m^2 at $n = 20$, and total number released would be estimated at 600,000. With the

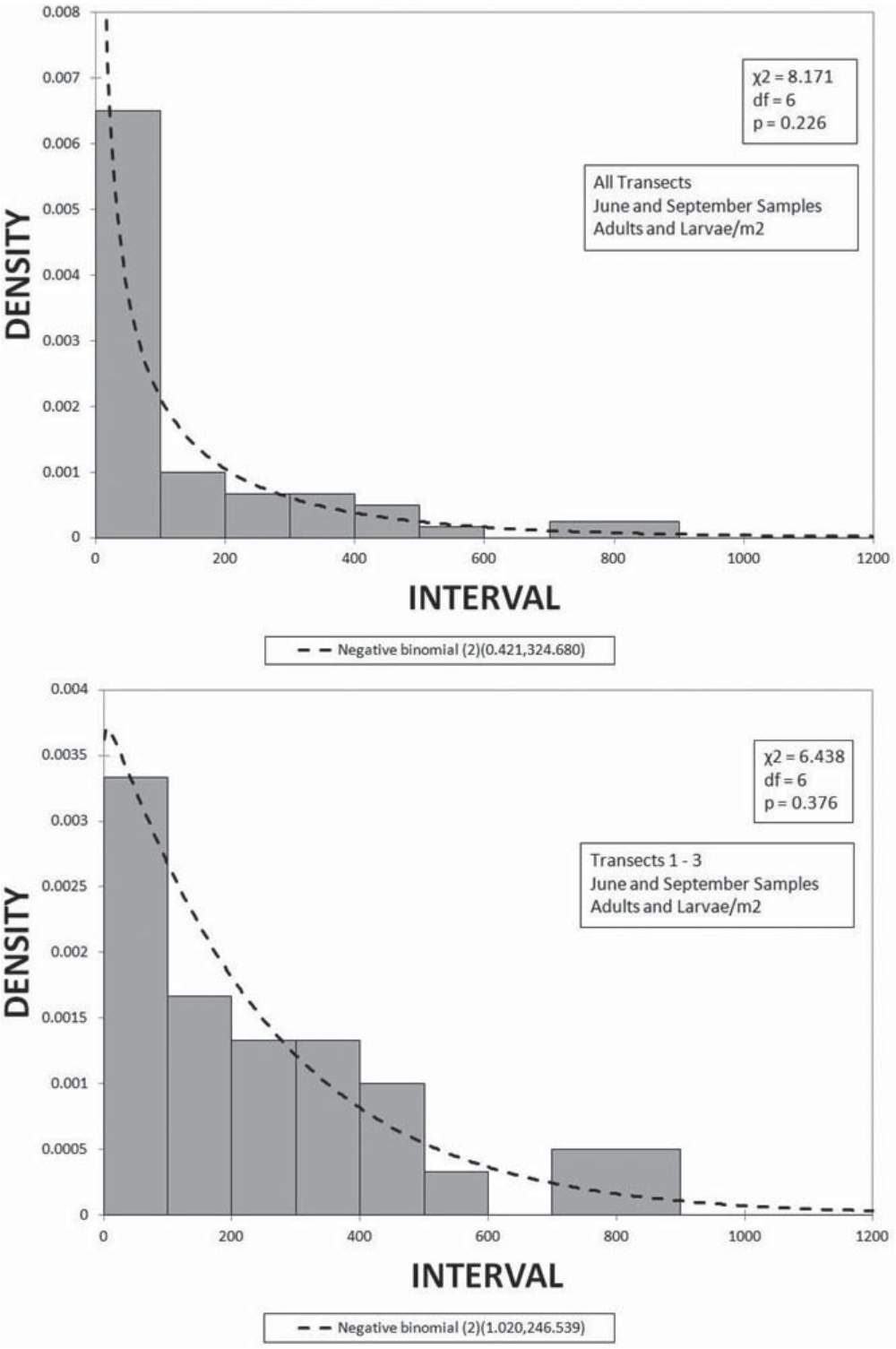

Fig. 8. Distribution of total weevil (larvae and adults) counts as expressed as a density of the interval length for the active growing season (i.e., Jun and Sep) for the entire pond (top) and for the first 3 transects only (bottom). The dashed line represents the fitted negative binomial distribution while the columns correspond to the actual frequency density.

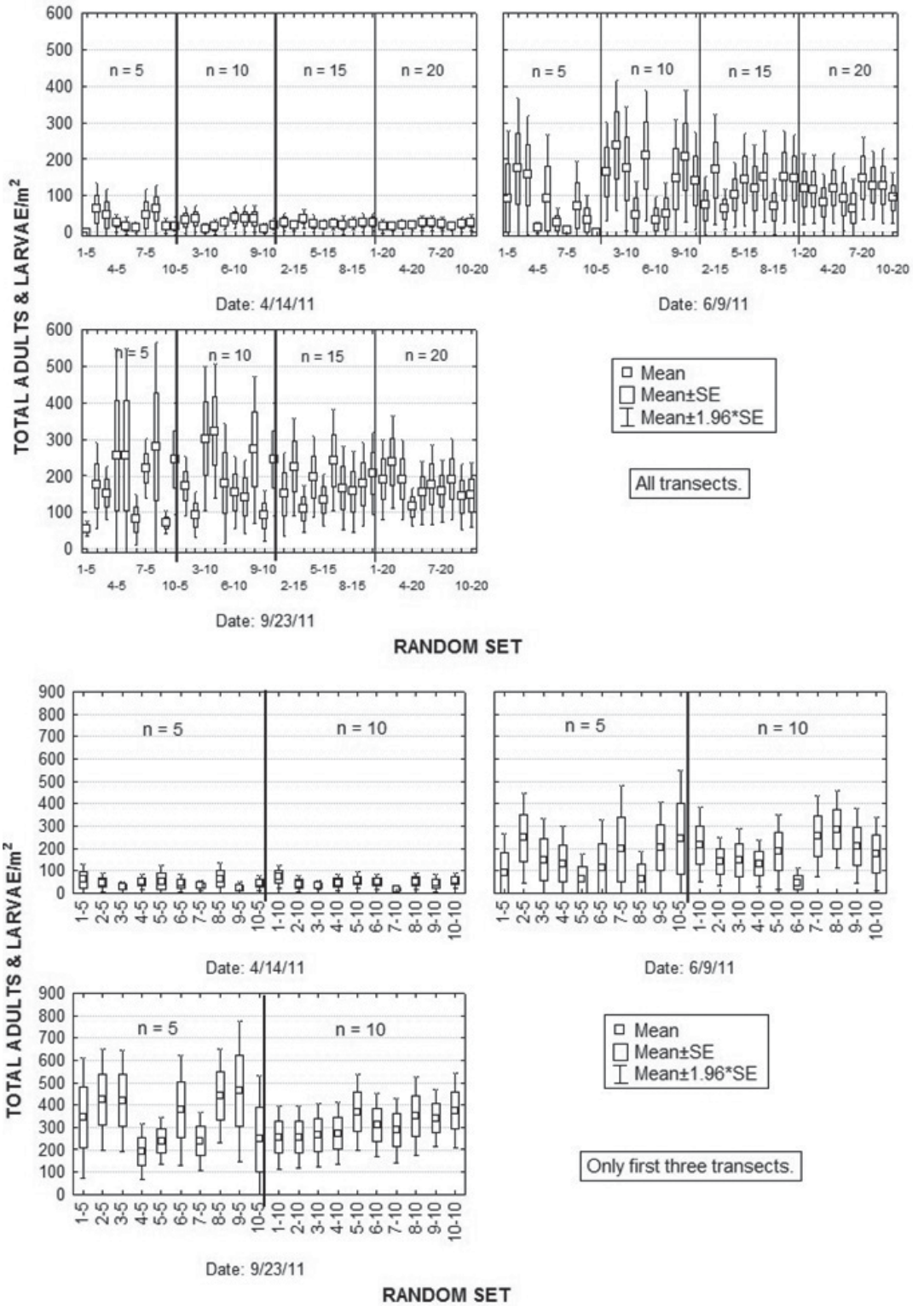

Fig. 9. Random sets of mean total number of weevils (i.e., both adults and larvae) for samples selected from the entire pond for each sampling date and for the first three transects. Number of samples taken varies from $n = 5$ to $n = 20$ for all transects and from $n = 5$ to $n = 10$ for the first three transects.

lower end of the range, where weevil number per m^2 was 125 weevils and $n = 20$, numbers released would be 300,000. This represents only a 2-fold difference compared to a 6-fold difference when using $n = 5$.

While fluctuations in mean number of individuals were decreased when sampling in areas containing higher number of weevils (i.e., transects 1 to 3), differences in means did occur and their magnitude was related to sample size (Fig. 9.). With $n = 5$ for the Sep sample, means varied from a high of over 460 adults and larvae per m^2 to a low of close to 180 individuals per m^2. This represented a difference of over 2.5 fold or over 330,000 weevils for the first 3 transects. However, such differences were again dampened when increasing sample size to $n = 10$. In this case, the differences amounted to only a 1.5 fold difference or about 160,000 individuals.

While some authors have indicated that increasing sample size did not lower variability for the salvinia weevil on common salvinia to any great extent with 5 samples being adequate (Tipping & Center 2005), this study shows otherwise for giant salvinia. For example, examining variation for $n = 5$ random samples for Sep for the entire pond (Fig. 9) standard errors ranged from a low of 10.8 to a high of 150.4, a difference of almost 14 fold. Contrast this to random samples of $n = 20$ for the Sep sample for the entire pond where standard errors ranged only 2.4 fold or from 27.0 to 64.5. Variation as influenced by sample size is well documented and is an important consideration when developing sampling protocols (Green 1979). For this pond, large amounts of variation can be accounted for, to some extent, by the highly clumped distribution exhibited by the weevils. Most samples contained very low numbers of individuals with a few containing relatively large numbers, thus increasing variation and reducing accurate estimation of the mean significantly.

The influence of sample size on estimating numbers of salvinia weevils is especially important for release programs since it has been shown that the introduction of higher numbers of individuals for biological control programs facilitates establishment and ultimately impact (Grevstad 1999). However, accurately estimating numbers of salvinia weevils on giant salvinia is difficult at best. In this pond situation, such difficulties were mainly due to extreme differences in spatial distribution of the weevils across a relatively small total area. Such clumped distribution, common for most organisms, makes it necessary to take large number of samples to minimize differences in mean numbers and also to minimize extremes in variation around the mean. Clumped distributions are likely the norm at most field sites and other mass-rearing locations. The number of samples taken in field sampling situations or especially for release programs should reflect the need for accurately estimating numbers.

However, the number of samples taken is typically related to the accessibility of sampling equipment, as well as time and money available. Sampling and associated processing and data summarization are expensive, so care needs to be taken to balance the need for accurately estimating numbers versus budgetary constraints. A good rule of thumb is to take as many samples as possible based on the available resources with the understanding that smaller sample sizes can easily provide means that differ significantly from those determined from higher number of samples. Based on the large variation observed in this study a recommended practice is to provide the confidence interval along with the mean in an effort to express the possible ranges of the mean. For the Sep sampling date, mean number of adults and larvae was 167 per m^2 but could be as low as 86 per m^2 or as high as 248 per m^2 based on the 95% confidence interval with $n = 30$. However, by randomly selecting only five samples for this same date range for the entire pond, the mean number of adults and larvae was 255 per m^2 with a potential range of 162 per m^2 to 672 per m^2 (Fig. 9).

It is important to realize that there is a diminishing return when taking higher number of samples in an effort to reduce sampling error. Green (1979) points out that standard error is decreased in proportion to the square root of n. Hence, standard error is reduced by a third by increasing n from 4 to 9. To reduce the standard error by another third would require increasing n to 21 and for another third to 46.

Understanding spatial distribution is also an important consideration for determining sample size since higher numbers of samples are required to reduce variation and provide a more accurate estimation of mean population size when populations are highly clumped. In this dataset, 15 to 20 samples are required before the confidence intervals and associated means tend to level and become somewhat consistent when sampling the entire pond. This includes areas of high clustering and areas with only minimal number of weevils (Fig. 9). However, when sampling only the first 3 transects during Sep (i.e., area of highest number of weevils and less total clustering), the number of samples required for the mean and error components to begin leveling was reduced to only $n = 10$.

It may be prudent to develop pilot sampling programs to determine the spatial distribution as well as error components for the sampling site (Green 1979). While such preliminary sampling may seem needless and an unnecessary expenditure of time, money and resources, such a program can potentially provide cost savings later when accurate sampling is essential. For example, for this pond situation, the development of a grid sampling program allowed the determi-

nation that the highest number of weevils with less overall clustering occurred in the west end of the pond. Thus the collection of this information before actual harvesting would have allowed better estimation of numbers of weevils, and thereby better accuracy regarding numbers of weevils harvested and released from the pond. This could be accomplished by designing a sampling program that stratified the sampling toward each end of the pond and concentrated harvesting from the area of the pond containing higher numbers of weevils. A less expensive but a somewhat reliable method is to visually assess the condition of the salvinia mat and sample those areas where obvious signs of weevil damage are exhibited (Harms et al. 2009). However, such visual signs can be misleading and, if possible, it would be more beneficial to actually take samples to determine weevil numbers and ultimately weevil distribution.

In summary, the importance of accurately estimating release numbers cannot be overstated. Time and time again, establishment success and subsequent impacts were correlated to numbers introduced with higher probability of success related to larger numbers released (Grevstad 1999). While rearing of weevils is relatively easy and cost effective (Harms et al. (2005) estimated costs of $8.20 per 1000 adults and larvae using pond rearing techniques and Nachtrieb (2012) estimated $0.05 per adults and larvae using above ground box rearing) and depending on the method chosen it can become costly and the release of higher than necessary numbers just adds to the expense when the excess weevils could be introduced into other areas where needed. It is recognized that sampling is also expensive and number of samples taken is often related to sampling equipment access, funding, and time constraints. In efforts to gain the most information based on the number of samples taken it is recommended that 1) the confidence interval be reported, especially when releasing weevils based on an estimation of population size; 2) understand the weevils spatial distribution to aid in reducing sampling variation; and 3) when possible, initiate pilot sampling programs prior to actual implementation of the study to acquire prior information on sampling biases, sampling errors, and differences in distribution based on environmental considerations.

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