Spatial Aggregation, Precision, and Power in Surveys of Freshwater Mussel Populations[1]

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We studied aggregation in 76 populations of freshwater mussels from relatively homogeneous surroundings in a wide range of habitats. Chi-square tests for spatial aggregation found only 53% of mussel populations significantly ($p < 0.05$) aggregated. The variance of replicate mussel samples (s^2) varied with the mean number collected (m) as $1.49m^{1.17}$, but conformed to the general variance relation found for other aquatic taxa ($m^{1.5}$) at $m > 1$. The number of replicate samples ($\hat{n}$) required to estimate mussel abundance with a given level of precision (D = SE/m) is approximately $m^{-0.5}D^{-2}$. Sampling mussels with large quadrats requires between 5 and 25 samples for 20% precision. Sampling designs to determine significant impacts ($\alpha = \beta = 0.05$) require 7–50 samples of each population to detect doubling or halving of the population density, or three to nine to detect order-of-magnitude changes. Large sampling units are recommended to ensure acceptable sampling precision and accurate chi-square analyses of spatial aggregation and to permit ecologists to detect significant impacts on freshwater mussel populations.

Nous avons étudié l'agrégation chez 76 populations de moules d'eau douce vivant dans des milieux relativement homogènes et provenant d'une vaste gamme d'habitats. Des tests de khi carré de l'agrégation spatiale nous ont permis de trouver que seulement 53 % des populations de moules étaient agrégées de façon significative ($p < 0{,}05$). La variance des réplicats d'échantillons de moules (s^2) variait avec le nombre moyen de moules recoltées (m) soit : $1{,}49m^{1.17}$, mais suivait la relation générale de variance trouvée pour d'autres taxa aquatiques ($m^{1.5}$) lorsque $m > 1$. Le nombre d'échantillons nécessaires ($\hat{n}$) pour estimer l'abondance des moules avec un niveau de précision donné (D = SE/m) est d'environ $m^{-0.5}D^{-2}$. L'échantillonnage des moules à l'aide de grands quadrats nécessite la récolte de 5 à 25 échantillons afin d'obtenir une précision de 20 %. Il faut une plus grande réplication lorsqu'il est nécessaire de déterminer des impacts significatifs ($\alpha = \beta = 0{,}05$) : de 7 à 50 échantillons de chaque population pour détecter une densité de population qui double ou diminue de moitié, ou de 3 à 9 échantillons pour déterminer des changements d'ordre de grandeur. L'utilisation de grandes unités d'échantillonnage est recommandée afin d'assurer une précision d'échantillonnage acceptable, l'exactitude des analyses d'agrégation spatiale avec le khi carré et afin de permettre aux écologistes de détecter des impacts significatifs sur les populations de moules d'eau douce.

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The study of populations of freshwater mussels is of growing importance. Unionid mussels are gaining prominence in monitoring and detecting pollution (Day et al. 1990; Metcalfe and Charlton 1990) and acidification (Pynnönen 1990). They form the basis of the world's freshwater pearl industry (Coker et al. 1922; Kat 1982). Unionid glochidia can be fatal parasites of sport fishes (Lefevre and Curtis 1910; Matteson 1948), and juveniles are important food for fish (Negus 1966) and small mammals (Coker et al. 1922; Cvancara 1970). Freshwater mussels may have a major impact on phytoplankton dynamics and lake management because their feeding is unselective (Winter 1978; Tessier et al. 1984) and filtering rates are high (Price and Schiebe 1978). Mussels can make up much of the benthos biomass in lakes (Magnin and Stanczykowska 1971; Golightly and Kosinsky 1981), affect alkalinity budgets (Green 1980), and stimulate microinvertebrate production (Sephton et al. 1980). They are of paleontological (Green 1972) and archaeological (Kunz 1893; Hill 1983) interest. Many species are now endangered (Strayer 1980; DiStephano 1984; Miller et al. 1986) by habitat modification and the introduction of exotics like the Asian clam (*Corbicula fluminea*) (Lauritsen and Mozley 1989; Leff et al. 1990) and the zebra mussel (*Dreissena polymorpha*) (Hebert et al. 1989).

In spite of the broad importance of unionid population studies, sampling difficulties engendered by their spatial heterogeneity have made it difficult to make precise estimates of their abundance and study their population dynamics (Kat 1982). Although sampling programs for other benthic faunae can be planned and refined through the use of empirical sampling algorithms predicting natural spatial heterogeneity, no such treatment exists for freshwater mussels, and existing analyses of sampling design (e.g. Downing 1979) may not be applicable to them (see Riddle 1989). Ecologists thus have no systematic means of planning programs to sample freshwater mussels, and quantitative data on unionid population density remain scarce.

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Smaller benthic invertebrates are known to be aggregated in space (Elliott 1977; Downing 1979). Usually, the variance (s^2) of replicate samples of benthic invertebrates is found to be significantly greater than the average of replicate counts (m). Downing (1979) found for lake benthos that the "variance was less than the mean in only 2.5% of 1500 sets of data examined." The biology of unionid molluscs might lead them to be more aggregated than other invertebrate groups. Among the most frequently and earliest cited causes of spatial aggregation is its possible value for reproduction (e.g. Anscombe 1950). The frequency of hermaphroditism in unionid populations (e.g. van der Schalie 1970) indicates that fertilization might be a difficult stage in their life cycle (Ghiselin 1974). Animals such as freshwater mussels, living at low densities and employing direct fertilization, might improve reproduction by clumping. On the other hand, low rates of predation in freshwater mussel populations might lead them to be randomly distributed (Rasmussen and Downing 1988). Observations made over the last 80 yr on the spatial heterogeneity of unionid molluscs (Lefevre and Curtis 1910; Little and Gentner 1969; Kessler and Miller 1978; Sephton et al. 1980; Mitchell and Collins 1984) have suggested that populations are aggregated, but the frequency of aggregation in this group is unknown.

Research on other groups of benthic invertebrates has not only shown that spatial distributions are aggregated, but that empirical observations of spatial s^2 of replicate samples rises with m in a predictable fashion (Downing 1979; Morin 1985; Vézina 1988). In general, s^2 of benthos samples varies as

$$(1) \quad s^2 = am^b$$

where a and b are fitted coefficients and b is usually between 1 and 2 (Downing 1979; Morin 1985; Vézina 1988). Further, a recent review of the literature on spatial variation in aquatic organisms (Downing 1991) suggests, on the basis of over 18 000 sets of replicate samples, that variance algorithms for aquatic taxa tend to

$$(2) \quad s^2 = m^{1.5}$$

on average. If eq. 1 or 2 holds for unionid mussels, then the number of requisite samples ($\hat{n}$) needed to obtain a given level of precision ($D = \mathrm{SE}/m$) could be estimated a priori (Downing 1979; Cyr et al. 1992):

$$(3) \quad \hat{n} = a \cdot m^{b-2} D^{-2} .$$

The effectiveness of such a strategy for the optimization of sampling programs is now well established (McIntyre et al. 1984; Morin 1985; Vézina 1988; Downing 1989), saving researchers wasted sampling effort and failed surveys, but is unavailable for unionid mussels.

If freshwater mussels are highly aggregated in space, the high variance of population samples may lead sampling surveys to be inconclusive (i.e. low power) (Forbes 1990; Peterman 1990). This will become increasingly important as sampling surveys are designed to monitor the effects of pollution or competition between exotic and indigenous species. If spatial heterogeneity of unionid populations is not accounted for in designing sampling surveys, we may be unable to conclude that there have been significant influences of exotic species like *Corbicula* or *Dreissena* until indigenous unionid populations have been completely replaced by exotics.

This article has two objectives. First, we test the hypothesis that unionid mussel populations are aggregated in space and that they are aggregated as frequently as other aquatic taxa. Second, we examine the predictability of spatial variance and show how this information can be used in planning precise and powerful surveys of freshwater mussel abundance.

Methods

The hypothesis that unionid mollusc populations are aggregated in space was tested in 76 populations (Table 1). Only populations that were found in relatively homogeneous surroundings were considered in order to avoid the biasing effects of sampling across gradients or among sampling strata (Downing 1991). Data on the mean number of mussels (m) collected per randomly or regularly placed sampling unit and the $n - 1$ weighted variance of these means (s^2) were derived from original collections or from the published literature. Because of the paucity of published quantitative data on unionid population density (cf. Vézina 1988), we made specific collections of mussel populations in northern Minnesota and southern Québec, covering ultraoligotrophic to eutrophic lakes across a representative range of habitat types. For each population, a chi-square test was applied to s^2/m to test for agreement with a Poisson distribution (Elliott 1977). A $s^2{:}m$ ratio significantly greater than 1 indicates that the population was significantly aggregated.

The hypothesis that spatial s^2 of unionid populations follows eq. 1 was tested by least squares regression analysis (Draper and Smith 1981) of the data in Table 1. Data were $\log_{10}$ transformed to linearize the response. The quantitative equivalence of the spatial heterogeneity of unionid molluscs and other aquatic taxa was tested by examining the fit of the spatial variances for unionids (Table 1) to the average variance function for all aquatic taxa (eq. 2) calculated from the data reviewed by Downing (1991). Variance functions were used to calculate the number of samples needed for a given level of precision ($\hat{n}$) following the protocol outlined by Downing and Anderson (1985) as modified by Downing (1989).

Past studies of sampling design have been oriented toward improving the precision of population estimates, but environmental impact studies must not only be precise but powerful enough to detect specified levels of change in population density. In studying the potential impact of exotic species on indigenous ones, the ability to determine significant differences among average densities of unionid mussel populations does not depend only on the precision of each mean. A given level of precision may or may not be adequate, depending on the size of difference that one needs to be able to detect. In such cases, power analysis can be used to optimize the sampling design (Cohen 1988; Peterman 1990).

Measures of spatial aggregation can be used to calculate the number of samples required to detect a given difference between two mean population densities using a t-test (Cyr et al. 1992). The analysis sought the number of samples needed to provide t-tests for differences between hypothetical means (m_1, m_2),

$$(4) \quad t = \frac{m_1 - m_2}{s'_{m_1 - m_2}} ,$$

that would be statistically significant at a given level of α and β. The number of samples ($n = n_1 = n_2$) necessary to reach a given level of α and β (where β is $1 -$ power) was determined iteratively for pairs of m_1 and m_2 where m_1 and m_2 varied between 1 and ≈300 mussels per sample. The sample numbers n_1 and n_2 were set equal to each other in this analysis for com-

TABLE 1. Data used for the analysis of spatial aggregation in unionid mussels. Minnesota lake data were collected in Clearwater Creek (47°22′N, 93°25′W), Clearwater Lake (47°22′N, 93°25′W), Crooked Lake (47°23′N, 93°17′W), Leech Lake (47°04′N, 94°33′W), Purvis Lake (47°49′N, 92°01′W), Wabana Creek (47°19′N, 93°25′W), and Wabana Lake (47°21′N, 93°28′W). Québec data were collected in Fraser Lake (45°23′N, 72°10′W), lac Bonny (46°05′N, 74°05′W), lac Brôme (45°18′N, 72°30′W), lac Brompton (45°25′N, 72°10′W), Lake Champlain (45°04′N, 73°08′W), lac d'Argent (45°18′N, 72°19′W), lac de l'Achigan (45°57′N, 73°58′W), lac Hertel (45°33′N, 73°08′W), lac Magog (45°18′N, 72°03′W), and Lake Memphrémagog (45°N, 72°14′W). Data on the Mississippi River are from Johnson (1976), Lake St. Clair from Hiltunen (1971), and Lake Biwa from Mori (1976) and Higashi and Hayashi (1964). n is the number of replicate samples included in the mean, m is the arithmetic mean number of organisms per sample, s^2 is the $n - 1$ weighted variance of m, and $\chi^2 = s^2 (n - 1)/m$ (Elliott 1977). Asterisks indicate significant departures from a random spatial distribution: $*p < 0.05$; $**p < 0.01$; $***p < 0.001$; NS indicates that no significant departure from $m = s^2$ was found. Taxa indicated as "mixed species" contained primarily *Elliptio complanata* but a few *Anodonta grandis*.

Species	Water body	Spatial density (no.·m^{-2})	Quadrat size (cm^2)	n	m	s^2	χ^2
Amblema costata	Mississippi River	2.8	1 800	4	0.50	1.00	6.00 NS
Anodonta grandis	Clearwater Lake	0.6	10 000	25	0.60	1.50	60.00***
Anodonta grandis	Crooked Lake	3.4	10 000	25	3.36	8.24	58.86***
Anodonta grandis	Lac Hertel	0.1	10 000	27	0.15	0.13	23.01 NS
Anodonta grandis	Lac d'Argent	0.4	10 000	11	0.36	0.45	12.63 NS
Anodonta grandis	Purvis Lake	0.9	10 000	15	0.87	0.70	11.23 NS
Anodonta grandis	Wabana Lake	0.2	10 000	21	0.19	0.16	17.00 NS
Anodonta grandis	Wabana Lake	0.6	10 000	26	0.58	0.49	21.40 NS
Anodonta grandis	Wabana Lake	0.8	10 000	26	0.85	1.90	56.00***
Anodonta grandis	Wabana Lake	1.6	10 000	25	1.56	3.17	48.82**
Elliptio complanata	Lac Brôme	32.0	1 000	5	3.20	5.20	6.50 NS
Elliptio complanata	Lac Brôme	89.0	1 000	10	8.90	18.54	18.75*
Elliptio complanata	Lac Brompton	15.0	1 000	10	1.50	19.39	116.33***
Elliptio complanata	Fraser Lake	6.1	10 000	33	6.12	6.73	35.21 NS
Elliptio complanata	Lac Hertel	4.8	10 000	27	4.81	5.23	28.29 NS
Elliptio complanata	Lac de l'Achigan	3.3	10 000	7	3.29	3.91	7.13 NS
Elliptio complanata	Lac de l'Achigan	18.1	10 000	7	18.14	44.14	14.60*
Elliptio complanata	Lac Bonny	21.0	10 000	15	21.00	55.57	37.05***
Elliptio complanata	Lac d'Argent	19.6	10 000	11	19.55	108.07	55.28***
Elliptio complanata	Lac de l'Achigan	1.4	10 000	7	1.43	0.95	4.00 NS
Elliptio complanata	Lac de l'Achigan	6.0	10 000	7	6.00	2.67	2.67 NS
Elliptio complanata	Lac de l'Achigan	6.0	10 000	7	6.00	7.00	7.00 NS
Elliptio complanata	Lac de l'Achigan	8.7	10 000	32	8.70	16.79	59.82***
Elliptio complanata	Lac de l'Achigan	9.6	10 000	7	9.57	24.29	15.22*
Elliptio complanata	Lac de l'Achigan	11.4	316	36	0.36	0.41	39.62 NS
Elliptio complanata	Lac de l'Achigan	13.7	10 000	7	13.71	14.57	6.38 NS
Elliptio complanata	Lac de l'Achigan	18.7	10 000	12	18.67	42.42	25.00**
Elliptio complanata	Lac de l'Achigan	24.2	10 000	12	24.17	115.06	52.37***
Elliptio complanata	Lac de l'Achigan	24.2	1 000	36	2.42	5.16	74.79***
Elliptio complanata	Lac de l'Achigan	26.6	10 000	36	26.56	150.20	197.96***
Elliptio complanata	Lac de l'Achigan	27.0	3 162	36	8.53	20.48	84.07***
Elliptio complanata	Lac de l'Achigan	27.8	100	36	0.28	0.21	26.00 NS
Elliptio complanata	Lake Champlain	39.7	10 000	3	39.67	81.33	4.10 NS
Elliptio complanata	Lake Memphrémagog	5.0	1 000	10	0.50	0.50	9.00 NS
Elliptio complanata	Lake Memphrémagog	5.0	1 000	10	0.50	0.94	17.00*
Elliptio complanata	Lake Memphrémagog	11.0	1 000	10	1.10	0.99	8.09 NS
Elliptio complanata	Lake Memphrémagog	42.0	1 000	15	4.20	9.17	30.57**
Elliptio complanata	Lake Memphrémagog	47.6	100	21	0.48	0.46	19.40 NS
Elliptio complanata	Lake Memphrémagog	53.0	10 000	8	53.00	549.71	72.60***
Elliptio complanata	Lake Memphrémagog	54.5	316	18	1.72	1.15	11.39 NS
Elliptio complanata	Lake Memphrémagog	56.5	3 162	14	17.86	12.75	9.28 NS
Lampsilis radiata	Clearwater Creek	3.3	10 000	3	3.33	4.33	2.60 NS
Lampsilis radiata	Clearwater Lake	0.8	10 000	25	0.84	1.22	34.94 NS
Lampsilis radiata	Crooked Lake	0.7	10 000	25	0.68	1.14	40.35*
Lampsilis radiata	Crooked Lake	0.8	10 000	25	0.80	3.00	90.00***
Lampsilis radiata	Crooked Lake	14.3	10 000	25	14.32	67.14	112.53***
Lampsilis radiata	Lake Champlain	29.3	10 000	3	29.33	158.33	10.80***
Lampsilis radiata	Leech Lake	0.1	10 000	22	0.09	0.09	20.00 NS
Lampsilis radiata	Wabana Creek	0.4	10 000	25	0.40	0.83	49.98**
Lampsilis radiata	Wabana Lake	0.4	10 000	26	0.38	0.25	16.00 NS
Lampsilis radiata	Wabana Lake	0.8	10 000	26	0.85	1.58	46.55**
Lampsilis radiata	Wabana Lake	1.2	10 000	33	1.24	1.63	41.90 NS
Lampsilis radiata	Wabana Lake	1.7	10 000	25	1.68	1.48	21.10 NS
Lampsilis radiata	Wabana Lake	1.9	10 000	21	1.86	1.13	12.15 NS
Lampsilis radiata	Wabana Lake	3.2	10 000	20	3.15	2.24	13.51 NS
Lampsilis radiata	Wabana Lake	3.9	10 000	30	3.87	4.53	34.00 NS

TABLE 1. (*Concluded*)

Species	Water body	Spatial density (no.·m^{-2})	Quadrat size (cm^2)	n	m	s^2	χ^2
Lampsilis siliquoidea	Lake St. Clair	9.0	741	3	0.67	1.33	4.00 NS
Lampsilis siliquoidea	Lake St. Clair	27.0	741	3	2.00	11.98	11.98**
Lampsilis ventricosa	Crooked Lake	0.6	10 000	25	0.64	1.07	40.25*
Leptodea fragilis	Mississippi River	4.2	1 800	4	0.75	0.92	3.67 NS
Leptodea fragilis	Wabana Creek	0.1	10 000	25	0.12	0.36	72.00***
Ligumia sp.	Crooked Lake	2.5	10 000	25	2.52	5.18	49.30**
Mixed species	Lac Magog	5.0	1 000	10	0.50	0.50	9.00 NS
Mixed species	Lake Memphrémagog	4.0	1 000	10	0.40	4.89	110.00***
Mixed species	Lake Memphrémagog	21.1	1 000	9	2.11	4.86	18.42***
Mixed species	Lake Memphrémagog	22.2	1 000	9	2.22	2.94	10.60 NS
Mixed species	Lake Memphrémagog	30.0	100	10	0.30	2.33	70.00***
Mixed species	Lake Memphrémagog	30.7	10 000	6	30.67	141.87	23.13***
Mixed species	Lake Memphrémagog	36.0	10 000	5	36.00	226.50	25.17***
Obliquaria reflexa	Mississippi River	7.8	1 800	5	1.40	2.30	6.57 NS
Obliquaria reflexa	Mississippi River	9.7	1 800	4	1.75	2.25	3.86 NS
Truncilla donaciformis	Mississippi River	4.4	1 800	5	0.80	0.70	3.50 NS
Truncilla donaciformis	Mississippi River	11.1	1 800	4	2.00	3.33	5.00 NS
Unio biwae	Lake Biwa	44.0	225	3	0.99	0.98	1.98 NS
Unio biwae	Lake Biwa	118.0	225	3	2.66	0.33	0.25 NS
Villosa fabilis	Lake St. Clair	9.0	741	3	0.67	0.33	1.00 NS

putational simplicity. Scheffé (1959) and others have suggested that, among the assumptions of analyses based on the normal distribution, equality of variances is the most critical. Because the variances associated with the different means were expected to be unequal (eq. 1), the variance of the difference in means ($s^{2\prime}_{m_1-m_2}$) was calculated as (according to Steel and Torrie 1980)

$$(5)\quad s^{2\prime}_{m_1-m_2} = \frac{s_1^2 + s_2^2}{n}$$

and the degrees of freedom (df′) were approximated as

$$(6)\quad \mathrm{df}' = \frac{((s^2_1 + s^2_2)/n)^2}{((s_1^2/n)^2 + (s_2^2/n)^2)/(n-1)}$$

where s_1^2 and s_2^2 were estimated from eq. 1 and n is the number of samples used in the estimation of both m_1 and m_2. This analysis was repeated for a variety of combinations of m_1 and m_2 at $\alpha = \beta = 0.05$. α and β were set equal to each other because in impact studies, it may be just as important to minimize the probability of failing to infer a significant difference when it actually exists (type II error) as it is to minimize the probability of inferring a significant difference when there is not one (type I error) (Parkhurst 1990; Peterman 1990).

Results and Discussion

Spatial Aggregation

Unionid mollusc populations were frequently aggregated in space, even though samples were taken within relatively homogeneous sampling strata. Of 76 comparisons, 45% showed significant spatial aggregation (Table 1). Surprisingly, however, 55% of the s^2/m could not be discerned from a Poisson distribution. Unionid mussel populations had aggregated distributions much less frequently than other benthic taxa. Figure 1, however, shows that populations sampled with $n < 9$ and $m < 10$ yielded s^2/m that indicated no significant aggregation more frequently than populations sampled with

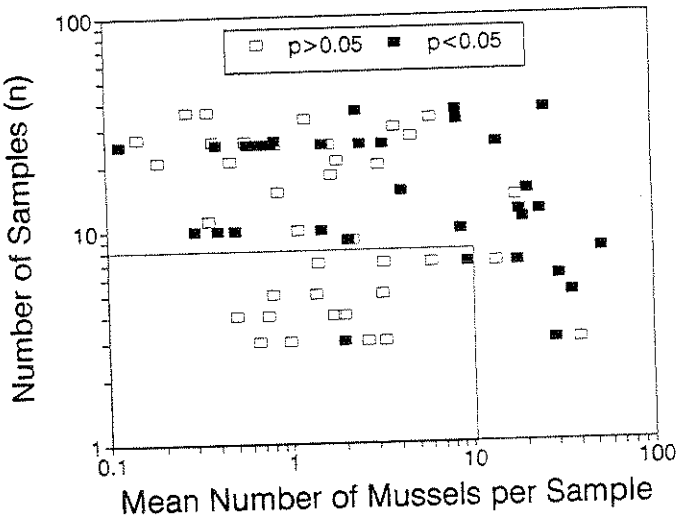

FIG. 1. Results of chi-square tests of s^2/m to test for significant spatial aggregation in populations of unionid mussels. Open squares indicate that no significant aggregation was seen, and closed squares indicate that chi-square values were statistically significant ($p < 0.05$). The rectangle encloses the region where $n < 9$ and $m < 10$.

more sampling units and in which larger numbers of mussels were collected. Our data suggest that either aggregation is rare or chi-square tests are unlikely to detect spatial aggregation at low n and m. Even ignoring populations with $n < 9$ and $m < 10$, however, we found that only 53% of mussel populations were significantly aggregated. The data with $n \geq 9$ and $m > 10$ suggested that mussel populations occurring at higher spatial densities were more frequently aggregated (up to 86% of populations) than those at lower densities (Fig. 2). Elliott's (1977) assertion that "The spatial dispersion of a population is seldom random or regular. . ." does not seem to apply to unionid molluscs. Many freshwater mussel populations are not significantly aggregated within their habitat.

The sampling variance obtained for unionid populations varied as a significant function of the average number of organisms collected:

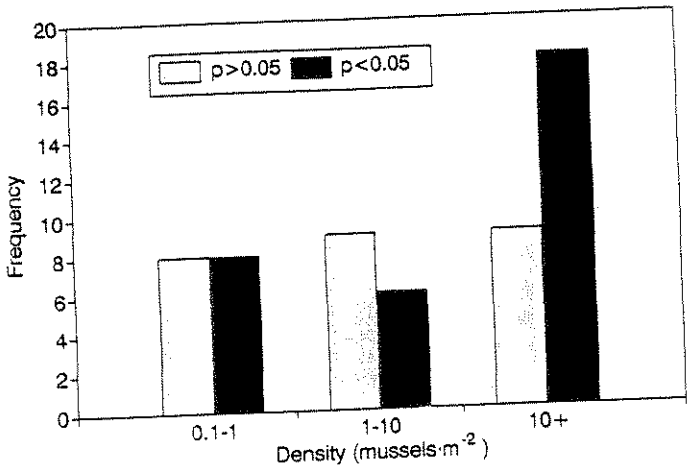

FIG. 2. Frequency of significant aggregation (chi-square test, $p < 0.05$) in populations of unionid mussels found at different spatial densities. Only populations sampled with at least $n = 9$ and $m = 10$ are included.

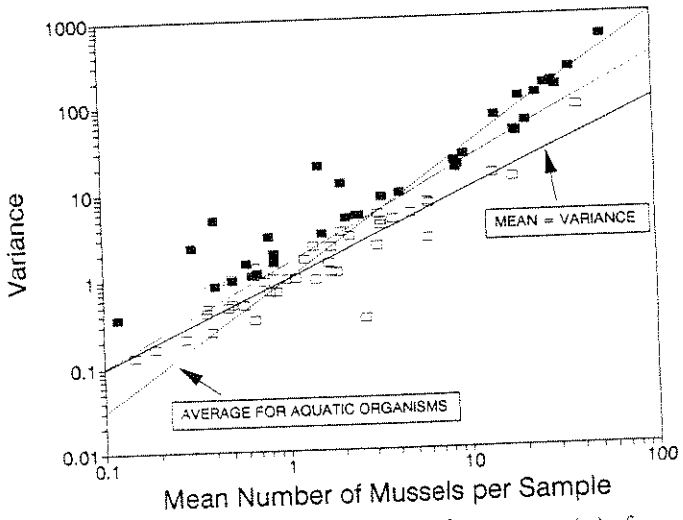

FIG. 3. Relationship between the variance (s^2) and mean (m) of replicate samples of freshwater mussel populations. The solid line indicates $s^2 = m$ (randomness) and the dotted line indicates $s^2 = m^{1.5}$ (eq. 2). Closed and open squares indicate that the chi-square test (Elliott 1977) for agreement with the Poisson distribution showed significant ($p < 0.05$) or lack of significant difference ($p > 0.05$), respectively.

(7) $s^2 = 1.49m^{1.168}$

(log–log regression: $n = 76$, $r^2 = 0.85$, RMS = 0.1121, $F = 413$, $p << 0.0001$). This relationship is illustrated in Fig. 3. Only 3 of the 28 such relationships for variance of freshwater organisms reviewed by Downing (1991) had lower exponents (Fig. 4), but this is probably due to the larger average number of replicate samples included in m and s^2 calculations for unionid molluscs. Downing (1986) has shown that these empirically derived exponents (b in eq. 1) vary approximately as $b = 2.057 - 0.11 \log_{10} n_v - 0.37 \log_{10} n_r$, where n_v is the number of replicate population estimates included in m and s^2, and n_r is the number of m:s^2 pairs included in each regression of s^2 on m. Given the average n_v of 16 (Table 1) and the 76 points included in the derivation of eq. 7, we would expect a priori an exponent of about 1.23. The low exponent probably results, in part, from the sampling designs used for unionid mussels.

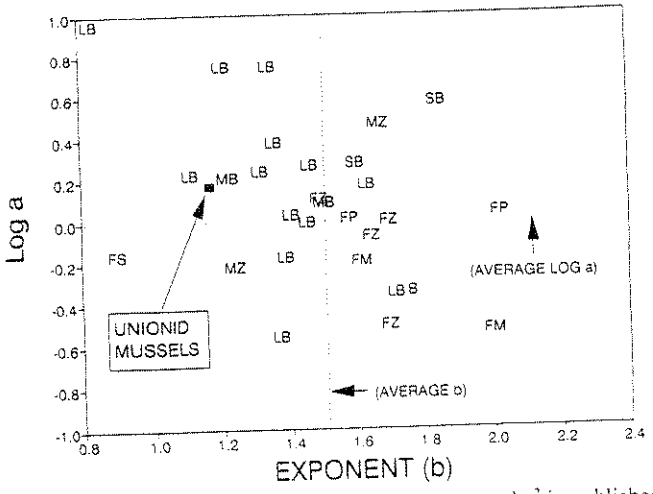

FIG. 4. Coefficients of the relationship between m and s^2 in published studies reviewed by Downing (1991). All m are expressed on a per sample basis, not normalized to common units of volume or surface area. Abbreviations for organism type: LB, lake benthos; SB, stream benthos; MB, marine benthos; FZ, freshwater zooplankton; MZ, marine zooplankton; FP, freshwater phytoplankton; B, bacteria; FS, freshwater sediment; FM, freshwater macrophytes. Literature sources are listed in Downing (1991).

Unionid mussel spatial distributions seem to approach randomness (i.e. $m = s^2$) when few organisms were collected in samples but follow the average relationship between m and s^2 found for other aquatic organisms (eq. 2) when many mussels were collected (Fig. 3). The variances of mussel samples tend toward the $s^2 = m$ line, indicating randomness at $m < 1$, but mussel variances depart from a $s^2 = m$ trend and follow eq. 2 more closely when $m > 1$. Apparent randomness at low m is a sampling artifact that arises because the minimum and maximum possible variances for population count data converge on $m = s^2 = 1/n$ at low m (Downing 1989, 1991). Sets of samples with $m < 1$ must approach randomness by mathematical necessity, and thus, their capacity to infer spatial pattern is greatly impaired. This also explains the low power of chi-square tests for spatial aggregation at low n and m (Fig. 1 and 2). For $m > 1$, ANOVA of the residuals of the data in Table 1 from eq. 2 shows that the degree of spatial heterogeneity of unionid mussel populations does not depart significantly from that seen in many other aquatic taxa ($p = 0.18$); thus, $s^2 = m^{1.5}$ may be considered a general rule followed closely by unionid mussels.

Prediction of Sampling Requirements

Equation 2 allows provisional estimates of requisite sample number for unionid mussels and other taxa to be made. The number of requisite samples for a specified degree of precision ($D = \text{SE}/m$) of mean densities of $m \geq 1$ can be estimated by substituting the coefficients of eq. 2 into eq. 3:

(8) $\hat{n} = 1 \cdot m^{-0.5} D^{-2}$.

Calculations of the number of required replicate samples for various spatial densities (number per square metre as opposed to number per sample) can be made by substituting [(number per square metre)/(10 000/A)] for m in eq. 8, where A is the area (square centimetres) covered by each replicate sample. Equation 7 is not used because it is biased by artifactually low s^2 at $m \leq 1$. Table 2 shows that many replicate samples must

TABLE 2. Predicted requisite number of samples of unionid molluscs to obtain a precision of 20% (i.e. SE/m = 0.2). Predictions of n from eq. 3 are shown for the range of spatial densities and sampler sizes encountered in the literature. Where $m < 1$, the sample numbers are the minimum number that could theoretically yield $D = 0.2$ (Downing 1989) or $[(1 - m)/(mD^2)] - 1$.

Density (no.·m^{-2})	Size of sampler (cm^2)				
	100	300	1000	3000	10 000
1	2474	807	224	57	25
3	807	252	57	26	15
10	224	57	25	15	6
30	57	26	14	8	5
100	25	14	8	5	2

be taken to obtain a precision of SE/m = 0.2. As with most other faunae, Table 2 shows that the number of replicate samples needed decreases with increased spatial density and sampler size. Even when very large numbers of mussels are collected in samples, however, precise population estimates usually require more than 10 replicate samples to be taken. This is especially sobering considering that the most frequent number of benthos samples taken by aquatic ecologists is three (Downing 1979). In fact, more than 85% of published estimates of mean density of benthic invertebrates are based on three samples or less (J. A. Downing, unpubl. obs.). In contrast with other benthic faunae, most of the cost of unionid mollusc population estimation is probably associated with the taking of the samples themselves (they are easy to separate from sediment and count); therefore, larger sampling units are probably best for sampling freshwater mussels (Table 2). This stategy will ensure large m and acceptable sampling precision, but will also permit robust chi-square tests for spatial aggregation.

Although our analysis is based on limited data, we believe that it is representative of samples that will be encountered in the field. Our data bracket the range of habitats and densities in which unionids are found (0.1–118·m^{-2}; Table 1). For example, Coker et al. (1922) examined many populations of different species of freshwater mussels and indicated that the densest populations had 31 organisms·m^{-2}. These authors cited others who found densities as high as 178·m^{-2}, suggesting that any spatial density >43·m^{-2} is "unusual." Negus (1966) found mean densities as high as 42·m^{-2}, Cvancara (1970) as high as 54·m^{-2}, and Green (1980) as high as 36·m^{-2}. Small exotic bivalves like the Asian Clam, however, have been observed at densities up to 1475·m^{-2} (Miller et al. 1986), and zebra mussel densities may be even higher. The lack of significant departure of our data on s^2 and m from a general relationship based on more than 18 000 sets of samples (eq. 2) lends support to the generality of this m:s^2 rule and suggests that our findings may be extrapolated to bracket the high densities observed in exotic mussels. Because spatial heterogeneity may vary somewhat among species or in extreme habitats (e.g. Downing 1979), however, it would be prudent to temper predictions from eq. 8 with site-specific sampling experience.

Detecting Differences among Population Means

Many future surveys of unionid mussel abundance will be performed to detect the influence of exotic species or pollution on mussel communities. In such cases, it is not simply the precision associated with each population estimate that is important (e.g. Downing 1979; Morin 1985; Vézina 1988; Riddle 1989), but rather the ability to discern or detect changes in population levels, before and after changes in the environment. We can use our analysis of mussel spatial variation to determine the number of samples that must be taken to differentiate between two hypothetical mean densities.

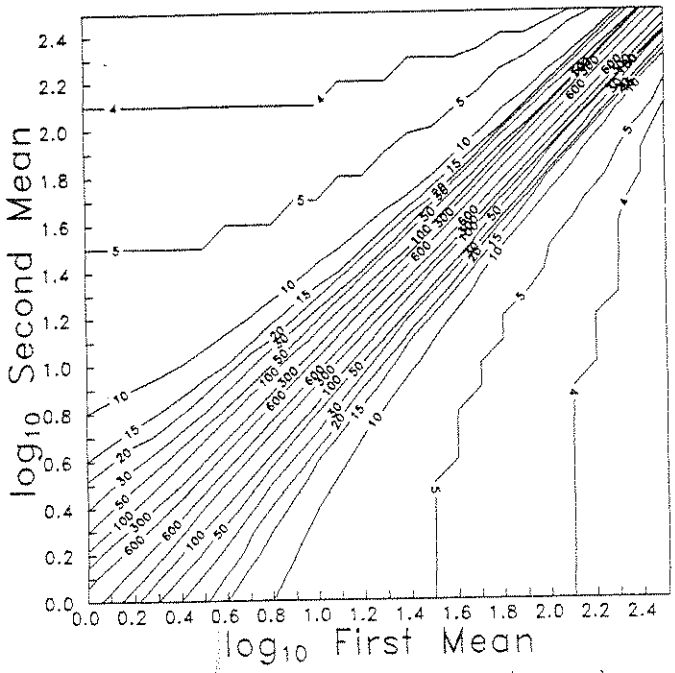

FIG. 5. Minimum number of samples required to estimate each mean mussel density in order to find a significant difference ($\alpha = \beta = 0.05$) between two freshwater mussel densities ($\log_{10}$). Calculations were made by substituting eq. 2 into eq. 4–6 and iterating n until $\alpha = \beta = 0.05$ (Cyr et al. 1992).

The number of replicate samples needed to detect significant differences decreases as the means and the differences among means increase (Fig. 5). Sampling surveys based on the median number of replicate samples taken in benthos studies (n = 3) could not even detect significant ($\alpha = \beta = 0.05$) differences of two orders of magnitude. Detection of doubling or halving of the population density would take about 50 samples to derive each mean at m = 1, around 15–20 samples at m = 10, and about five samples at m = 100. Even the detection of order-of-magnitude changes in population density would require between three and nine samples for the derivation of each of the estimated means involved in the comparison. Again, the use of large sampling units is indicated because mussel densities are rarely >100·m^{-2} and will decline when impacted, requiring greater numbers of samples. Our calculations show that most sampling designs currently used for sampling benthic organisms are inadequate for detecting changes in the abundance of unionid mussels.

This research has shown that unionid molluscs are frequently aggregated in space. A sufficient fraction of this spatial variability can be predicted based on estimates of the spatial density so that sampling programs can be planned without population-specific knowledge of the spatial pattern. Current approaches to sampling must be revised radically if we wish to monitor changes in unionid communities faced with pollution and introduced exotic species.

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