

## Sample Size Determination for the Estimation of Population Density of Marine Benthos on a Tidal Flat and a Subtidal Area, Korea

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The requisite numbers of sample replicates for the population study of soft-bottom benthos were estimated from survey data on the Songdo tidal flat and subtidal zone in Youngil Bay, Korea. Large numbers of samples were taken; two-hundred-fifty 0.02 m<sup>2</sup> box corers and fifty 0.1 m<sup>2</sup> van Veen grabs were taken on the Songdo tidal flat and in Youngil Bay, respectively. The effect of sampler size on sampling efforts was investigated by pooling the unit samples in pairs, fours, eights, *etc.* The requisite number of sample replicates ( $n_r$ ) was determined by sample variance ( $s^2$ ) and mean ( $m$ ) function ( $n_r = s^2/P^2m^2$ ), at  $P=0.2$  level, in which  $s^2$  and  $m$  were calculated from the counts of individuals collected. For example, seven samples of 0.02 m<sup>2</sup> corer for the intertidal and two samples of 0.1 m<sup>2</sup> van Veen grab for subtidal fauna were required to estimate the total density of community. The smaller sampler size was more efficient than larger ones when sampling costs were compared on the basis of the total sampling area. The requisite number of sample replicates was also predicted ( $\hat{n}_r$ ) by substituting  $\hat{s}^2$  obtained from the regression of  $s^2$  against  $m$  using the Taylor's power law ( $\hat{s}^2 = am^b$ ). The regression line of survey data on  $s^2$  and  $m$  plotted on log scale was well fitted to the Taylor's power law ( $r^2 \geq 0.95$ ,  $p < 0.001$ ) over the whole range of  $m$ . The exponent  $b$  was, however, varied when it was estimated from  $m$  which was categorized into classes by its scale. The fitted exponent  $b$  was large when both density class and the sampler size were large. The number of sample replicates, therefore, could be more significantly estimated, if regression coefficients ( $a$  and  $b$ ) would be calculated from sample variance and mean categorized into density classes.

### INTRODUCTION

The estimation of abundance has often been used for population studies. Parameters describing population changes, *i.e.*, rates of growth and birth, survivorship, mortality, *etc.*, are based largely on abundance. Abundance estimation has also been applied to the estimation of ecological indices to describe community structures. In order to provide useful estimates of abundance, a predetermination of the number of sample replicates ( $n_r$ ) and sampler size is essential. This also insures a cost effective sampling design.

The selection of  $n_r$  is a simple procedure if one has *a priori* estimate of the mean and variance of samples. Using a standard technique (Elliot, 1977):

$$n_r = s^2/P^2m^2 \quad (1)$$

where  $s^2$  is the sample variance,  $P$  is the desired precision and  $m$  is the sample mean. The precision,  $P$ , is the desired ratio of the standard error to the sample mean. In many field surveys, however,

preliminary samples to determine  $m$  and  $s^2$  needed for the estimation of  $n_r$ , are often not collected for a variety of reasons. A number of empirical algorithms to predict  $s^2$  from a known  $m$  have, therefore, appeared in the literature (Downing, 1979; Morin, 1985; Vezina, 1988).

An approximation of  $s^2$  can be obtained by assuming that  $s^2$  of a set of replicates is predictable from  $m$  using the equation:

$$\hat{s}^2 = am^b \quad (2)$$

(Taylor, 1961; Elliot, 1977) where  $a$  and  $b$  are coefficients of regression. Since then, this trend has been observed for many benthic populations. Pooling the large body of data from the literature, this general variance function was used to examine the sample number requirements for epiphytic, lacustrine and marine benthos (Downing, 1979; Downing and Anderson, 1985; Downing and Cyr, 1985; Morin, 1985; Vezina, 1988). Using regression analysis based on large sets of data, certain values of  $b$  were proposed: Downing (1979) estimated the

constant  $b$  in Equation 2 as 1.392 for freshwater fauna and Vezina (1988) as 1.219 for marine benthos. Despite the success in predicting  $b$  and its importance to sampling design, this approach has not been fully tested with actual data obtained from systematic field surveys.

The most debated aspect of empirical  $s^2:m$  equation applied to estimate  $n_r$ , would be the accuracy of the predicted  $\hat{s}^2$  obtained from the general model with fitted constant of  $b$  ( $s^2$  and  $n_r$  are denoted as  $\hat{s}^2$  and  $\hat{n}_r$  in this paper, if they resulted from a regression analysis). The constant  $b$  may greatly affect  $\hat{s}^2$  in Equation 2, because  $b$  is used as an exponent. The predicted number of replicates,  $\hat{n}_r$ , obtained by substituting  $\hat{s}^2$  into Equation 1 was also supposed to be varied to some extent. The predicted  $\hat{n}_r$  should, therefore, be evaluated, especially since obtaining even a few sample replicates is expensive for marine benthos.

We suppose that the accuracy of the parameter  $b$  is central in determining  $\hat{n}_r$ , if  $s^2$  has to be predicted by Equation 2. However, little effort has been made to evaluate the accuracy of  $b$  through systematic sampling on a large enough scale. In most cases,  $b$  was determined from a regression curve based on a large body of published data (Downing, 1979; Morin, 1985; Vezina, 1988). Individual data on  $s^2$  and  $m$  obtained from different origins were combined to yield a composite data set and were applied to regression analysis. Different sizes of sampler were included and  $s^2$  and  $m$  were, therefore, normalized to a  $m^2$  area (Downing, 1979; Downing and Anderson, 1985; Downing and Cyr, 1985; Morin, 1985). Vezina (1988) stated that the effect of sampler size on  $s^2$  is very small for marine benthos.

Yamamura (1990), who had analyzed actual data, indicated that  $b$  became larger as the sampler size increased. Originally, the exponent  $b$  has been considered as a constant value indicating the degree of aggregation (Elliot, 1977; Downing, 1979), but the degree of aggregation depends largely on the density and areal extent of distribution (Downing, 1986). We tried to evaluate the effect of the density and sampler size on  $b$  using large survey data. The variation of  $b$  has not been fully examined by field investigation, especially when it deals with marine benthos. Riddle (1989) analyzed more than two hundred macrofauna samples collected near Belhaven Bay, Scotland. However, his investigation concentrated on the evaluation of the cost efficiency of the sampling area and the sampler size applied

was extremely small ( $0.0018 \text{ m}^2$ ).

This study provides actual data on the number of sample replicates ( $n_r$ ) needed for the density estimation of marine benthos based on a large survey data. A total of 250 samples collected with a box corer ( $10 \times 20 \text{ cm}^2$  areal dimension) and 50 samples with van Veen grabs (covering  $0.1 \text{ m}^2$ ) were considered large enough to calculate the number of sample replicates. Taking samples from a contiguous series of quadrats and pairing the quadrats, the effect of sampler sizes on  $b$  and  $\hat{n}_r$  was also examined. Another concern was the behavior of  $b$  in Equation 2 used in Equation 1 for the estimation of  $n_r$ . We calculated  $b$  and  $\hat{n}_r$  for a range of mean and sampler sizes to demonstrate how much  $\hat{n}_r$  deviated from  $n_r$ , as determined from actual  $s^2$  and  $m$ .

## MATERIALS AND METHODS

The field survey for intertidal fauna was carried out on the Songdo tidal flat near Incheon, west coast of Korea ( $37^\circ 28' \text{N}$ ,  $126^\circ 36' \text{E}$ ) in April 1991. The composition of tidal flat substrate ranged from sand to silty sand. A  $0.1 \times 0.2 \text{ m}^2$  box corer (surface area:  $0.02 \text{ m}^2$ ) was used to take a contiguous series of 250 samples from five quadrats of  $1.0 \text{ m}^2$  areal dimension. The corer was pushed manually into the sediment down to 30-cm depth. Five quadrats of  $1 \text{ m}^2$  were laid side by side on the transect line set up parallel to the shoreline. Fifty  $0.1 \text{ m}^2$  grab samples were taken from a station at 10-m water depth in Youngil Bay ( $36^\circ 24' \text{N}$ ,  $129^\circ 31' \text{E}$ ) on one occasion in May 1991. The sediments were composed of clay- to silt-sized grains. The collected sediments were washed on a 1-mm mesh sized sieve, and the animals retained were fixed with 10% formalin solution in seawater. Individual numbers of each taxon were counted and total numbers per samples were calculated. Unless otherwise specified,  $s^2$  and  $m$  in this paper refer to the individual number per sample, not per  $\text{m}^2$ . Densities denoted by  $D$ , however, indicate the individual numbers per  $\text{m}^2$ .

Abundance per  $0.02$  and  $0.1 \text{ m}^2$  for each taxon and total abundance were subjected to numerical analysis. Statistics such as skewness and kurtosis as well as mean and variance were calculated to gain an overall picture of the frequency distribution of individual numbers among 250 or 50 samples. For a desired precision,  $P=0.2$ ,  $n_r$  was determined using Equation 1 on the bases of actual  $m$  and  $s^2$  of each taxon.

To examine the effect of sampler size on sampling effort, contiguous samples collected on the Songdo tidal flat were grouped to form several simulated series of samples of increasing sampler size. The sampler size was increased by blocking adjacent samples in pairs, fours, eights, *etc.* The shape of simulated sampler was regular square at 0.04 m<sup>2</sup>, 0.16 m<sup>2</sup> and 0.64 m<sup>2</sup> sampler size and rectangle (width:length=1:2) at 0.02 m<sup>2</sup>, 0.08 m<sup>2</sup> and 0.32 m<sup>2</sup> sampler size. It could be assumed that the species compositions in the simulated samples at each sampler size were independent because all 250 samples of 0.02 m<sup>2</sup> were collected from one station set up parallel to the shoreline under the same environmental condition. Values of  $m$  and  $s^2$  for the simulated samplers were then calculated as follows: sampler sizes of 0.02 m<sup>2</sup> ( $n=250$ ), 0.04 m<sup>2</sup> ( $n=125$ ), 0.08 m<sup>2</sup> ( $n=50$ ), 0.16 m<sup>2</sup> ( $n=20$ ), 0.32 m<sup>2</sup> ( $n=10$ ) and 0.64 m<sup>2</sup> ( $n=5$ ). Data on  $m$  and  $s^2$  calculated for single species and the total community per block size were then used in Equation 1 for the estimation of  $n_s$ .

Sampling costs of each combined block size were compared by the difference in total area calculated by the multiplication of  $n_s$  with the corresponding block size. The sampling area was produced by multiplying the estimated  $n_s$  of simulated sampler size of 0.02, 0.04, 0.08 m<sup>2</sup>, *etc.* with the corresponding sampler size for which  $m$ ,  $s^2$  and  $n_s$  were calculated. We assumed that the cost in collecting and processing the sediment increases linearly as the total area increases (Ferraro *et al.*, 1989). The study of the effect of sampler size on sampling effort was not extended to the van Veen grab samples taken from the subtidal zone of Youngil Bay since they were collected in an arbitrary fashion.

Taylor's power law (Equation 2) was applied to provide estimates of  $s^2$ , and then by substituting  $\hat{s}^2$  into Equation 1 we obtained the predicted  $\hat{n}_s$  value. The exponent  $b$  of Equation 2 was obtained from the regression of the observed  $s^2$  against  $m$  for 46 and 44 of the taxa collected on the Songdo tidal flat and the subtidal zone of Youngil Bay, respectively. All the simulated sampler sizes were used in calculating  $b$  in Equation 2. The exponent  $b$  was estimated again (denoted as  $b'$ ) based on  $s^2$  and  $m$  assigned into classes by its scale at every sampler sizes. The bias of  $\hat{n}_s$  from  $n_s$  at all sampler sizes and ranges of  $m$  was examined by a ratio of  $(\hat{n}_s - n_s)/n_s$ . The predicted  $\hat{n}_s$  at different ranges of  $m$  is denoted by ' ' in this paper.

## RESULTS

The abundance and dominant species of benthic organisms on the Songdo tidal flat differed from those of Youngil Bay. The subtidal area of Youngil Bay was more densely populated (3236 indiv./m<sup>2</sup>) than the tidal flat zone of Songdo (1230 indiv./m<sup>2</sup>), but higher diversity ( $H'$ : Shannon-Wiener's index) was shown on the tidal flat, although the numbers of species occurred in both study areas were similar. Macro-benthic animal collections from the sampling represented 56 taxa from the tidal flat and 60 taxa from Youngil Bay. Dominance, represented by McNaughton's index, was higher in Youngil Bay. Polychaetes were frequently found in both areas, but dominant species differed each other (Tables 1 and 2). A characteristic feature in species composition in the Songdo tidal flat was the dominance of a bivalve, *Mactra veneriformis*, which is considered as a typical suspension feeder in silty to sandy flats on the Korean coast.

Tables 1 and 2 give general information on the statistics describing the frequency distribution of abundance in the sample quadrats. Numbers of replicates required for the estimation of population density of each species occurring on the tidal flat and in the subtidal area are presented. On the Songdo tidal flat, seven corers of 0.02 m<sup>2</sup> areal coverage were appropriate to determine the total density of community, but greater replications are required to estimate the density of a single species (Table 1). The number of box corer samples required to estimate the population density of a polychaete, *e.g.* *Heteromastus* sp., common to the tidal flats was 48. However, more than a thousand replicates are required if abundance is extremely low.

The number of replicates for sampling the subtidal fauna in Youngil Bay is presented in Table 2. Two grabs of 0.1 m<sup>2</sup> surface area are needed to estimate the total density for the desired precision of  $P=0.2$ . However, the requisite number of samples was considerably greater for the density estimation of a single taxon at this precision level. Approximately ten to twenty grabs were adequate for the commonly encountered species, but there were species that require more than a thousand grabs for density estimation.

The skewness and kurtosis describing the degree of asymmetry and peakedness of the frequency curves obtained from the 250 box corers (Table 1) and 50 grab samples (Table 2) indicate a rela-

**Table 1.** The number of sample replicates ( $n_r$ ) needed for the density estimation of benthic fauna occurred on the Songdo tidal flat. Total number of individuals collected (total no. indiv.), sample mean ( $m$ : number of individuals per 0.02 m<sup>2</sup> box corer), sample variance ( $s^2$ ) and other statistics describing the frequency distribution of abundance in 250 samples are also included (Abbreviations in parentheses: B=bivalvia, D=decapoda, E=echinodermata, G=gastropoda, P=polychaeta)

Taxon		Total no. indiv.	Mean ( $m$ )	Variance ( $s^2$ )	Skewness	Kurtosis	Frequency (%)	$n_r$ ( $P=0.2$ )
<i>Heteromastus</i> sp.	(P)	1279	5.12	50.62	2.10	7.23	56.7	48
<i>Macraa veneriformis</i>	(B)	1117	4.47	43.12	1.56	1.76	56.3	54
<i>Aedicira</i> cf. <i>pacifica</i>	(P)	751	3.00	26.84	2.00	3.62	43.0	74
<i>Macoma incongrua</i>	(B)	498	1.99	6.26	1.35	1.33	57.0	39
<i>Hinia festiva</i>	(G)	446	1.78	15.63	7.03	69.55	53.6	123
<i>Mediomastus</i> sp.	(P)	261	1.05	3.24	2.48	8.76	39.2	74
<i>Macrophthalmus dilatatus</i>	(D)	215	0.86	2.33	2.59	9.48	36.5	79
<i>Nephtys longosetosa</i>	(P)	198	0.79	3.02	2.91	9.95	27.8	121
<i>Protankyra bidentata</i>	(E)	164	0.66	0.73	1.04	0.06	44.1	42
<i>Anaitides</i> sp.	(P)	128	0.51	0.61	1.45	1.41	36.1	58
<i>Nephtys polybranchia</i>	(P)	107	0.43	0.75	3.23	14.63	28.9	102
<i>Solen strictus</i>	(B)	97	0.39	0.49	1.95	4.07	28.5	81
<i>Ruditapes philippinarum</i>	(B)	72	0.29	0.32	2.18	5.52	24.3	96
<i>Glycera decipiens</i>	(P)	62	0.25	0.24	1.81	2.53	22.1	98
<i>Leonnates persica</i>	(P)	59	0.24	0.31	2.72	7.80	17.1	141
<i>Harmothoe forcipata</i>	(P)	57	0.23	0.25	2.53	6.65	17.1	120
<i>Glycinde</i> sp.	(P)	52	0.21	0.26	2.70	7.30	15.2	147
<i>Aricidea elongata</i>	(P)	49	0.20	0.31	2.64	7.56	18.3	198
<i>Aricidea</i> sp.	(P)	38	0.15	0.26	4.19	20.92	10.6	279
<i>Aricidea jeffreysii</i>	(P)	37	0.15	0.19	4.35	26.86	11.4	212
<i>Marphysa</i> sp.	(P)	34	0.14	0.22	3.80	16.80	11.4	290
<i>Haploscoloplos elongatus</i>	(P)	31	0.13	0.12	2.73	7.16	11.8	198
<i>Ancistrosyllis hanaokai</i>	(P)	28	0.11	0.27	7.08	60.60	7.2	546
<i>Bullacta exarata</i>	(G)	27	0.11	0.11	3.42	12.12	9.1	254
<i>Lumbrineris heteropoda</i>	(P)	26	0.10	0.12	3.32	11.38	9.5	280
<i>Nephtys californiensis</i>	(P)	19	0.08	0.08	4.35	20.41	6.1	341
<i>Marphysa sanguinea</i>	(P)	17	0.07	0.07	4.12	17.94	6.1	363
<i>Leonnates</i> sp.	(P)	16	0.06	0.08	3.67	13.82	7.2	466
<i>Nectoneanthes oxypoda</i>	(P)	11	0.05	0.05	5.24	30.26	4.2	614
<i>Cyclina sinensis</i>	(B)	10	0.04	0.05	6.52	46.30	3.0	896
<i>Notomastus</i> sp.	(P)	9	0.03	0.04	6.37	45.36	3.0	868
<i>Diogenes</i> sp.	(D)	9	0.03	0.04	6.37	45.36	3.0	868
<i>Eteone</i> sp.	(P)	8	0.03	0.24	15.94	258.00	0.4	6550
<i>Capitella capitata</i>	(P)	8	0.03	0.04	6.89	53.23	2.7	1002
<i>Phacosoma japonicum</i>	(B)	6	0.02	0.02	6.32	38.86	2.3	1071
<i>Periserrula leucophryna</i>	(P)	6	0.02	0.03	8.33	77.52	1.9	1436
<i>Diopatra sugokai</i>	(P)	6	0.02	0.02	6.96	47.62	1.9	896
<i>Nectoneanthes latipoda</i>	(P)	5	0.02	0.02	6.32	38.86	2.3	1542
<i>Magelona</i> sp.	(P)	5	0.02	0.02	6.96	47.62	1.9	1290
<i>Glycera subaenea</i>	(P)	2	0.01	0.01	11.21	126.51	0.8	3263
<i>Glycera rouxii</i>	(P)	2	0.01	0.01	11.21	126.51	0.8	3263
<i>Tharyx</i> sp.	(P)	2	0.01	0.02	15.94	258.00	0.4	6550
<i>Tritodynamia rathbuni</i>	(D)	2	0.01	0.02	15.94	258.00	0.4	6550
<i>Sternaspis scutata</i>	(P)	2	0.01	0.01	11.21	126.51	0.8	3263
<i>Goniada</i> sp.	(P)	2	0.01	0.01	11.21	126.51	0.8	3263
<i>Pilargis</i> sp.	(P)	2	0.01	0.01	11.21	126.51	0.8	3263
All species		6150	24.60	159.30	1.10	2.10	100.0	7

tionship with the sample mean. The frequency distribution was more skewed and peaked as the sample mean decreased. The large frequency of zero (absence) appeared to be responsible for the very skewed and peaked curve. Although not shown graphically, a log-linear relationship between the skewness, kurtosis and the number of replicates was observed.

The effect of sampler size on sampling costs was compared on the basis of the sampling area. The number of replicates and sampling areas for each sampling size and for each taxon at a given precision,  $P=0.2$ , are shown in Table 3. Generally, the small sampler size was more efficient. The sampling area needed to estimate the population density increased with increasing sampler size. For

**Table 2.** The number of sample replicates ( $n_r$ ) required for a density estimation of species found in Youngil Bay. Mean indicates the number of individuals per van Veen grab of 0.1 m<sup>2</sup> area coverage (Abbreviations as in Table 1)

Taxon		Total no. indiv.	Mean ( $m$ )	Variance ( $s^2$ )	Skewness	Kurtosis	Frequency (%)	$n_r$ ( $P=0.2$ )
<i>Maldane cristata</i>	(P)	11805	231.47	4677.70	0.7	0.9	100.0	2
Amphipoda indet.	(A)	1655	33.10	338.38	0.6	-0.1	96.1	8
<i>Amphicteis guneri</i>	(P)	585	11.47	55.27	1.3	2.5	98.0	11
<i>Praxillella affinis</i>	(P)	481	9.43	40.01	1.9	6.1	96.1	11
<i>Notomastus</i> sp.	(P)	381	7.47	14.05	0.6	-0.0	98.0	6
<i>Nereis</i> sp.	(P)	262	5.14	8.75	0.5	-0.4	96.1	8
<i>Magelona japonica</i>	(P)	129	2.53	5.15	0.9	0.3	78.4	20
<i>Glycera chirori</i>	(P)	126	2.47	3.43	0.9	1.4	84.3	14
<i>Pista cristata</i>	(P)	110	2.16	3.35	3.0	13.9	88.2	18
<i>Glycinde</i> sp.	(P)	103	2.02	2.84	1.4	2.4	84.3	17
<i>Lumbrineris longifolia</i>	(P)	85	1.67	2.69	1.3	2.4	70.6	24
<i>Euchone</i> sp.	(P)	63	1.24	1.94	1.1	0.4	60.8	32
<i>Polydora ciliata</i>	(P)	63	1.24	4.30	2.4	5.7	49.0	70
<i>Prionospio cirrifera</i>	(P)	58	1.14	1.37	0.7	-0.8	60.8	27
<i>Aricidea</i> sp.	(P)	39	0.76	0.77	0.6	-0.9	49.0	33
<i>Lumbrineris heteropoda</i>	(P)	32	0.63	1.02	1.6	1.7	35.3	65
<i>Chone</i> sp.	(P)	32	0.63	0.94	1.7	2.3	39.2	60
<i>Lumbrineris nipponica</i>	(P)	30	0.59	1.50	2.7	8.1	27.5	108
<i>Cirratulus</i> sp.	(P)	28	0.55	0.76	1.7	3.0	35.3	63
<i>Tharyx</i> sp.	(P)	22	0.43	0.48	1.7	2.5	33.3	65
<i>Pseudopotamilla myriops</i>	(P)	21	0.41	1.61	3.7	13.8	13.7	238
<i>Eulalia</i> sp.	(P)	20	0.39	0.32	1.1	0.2	35.3	51
<i>Melina elisabethea</i>	(P)	20	0.39	0.51	2.8	10.3	31.4	83
<i>Spiophanes berkeleyorum</i>	(P)	18	0.35	1.09	5.1	29.3	21.6	219
<i>Micromaldane</i> sp.	(P)	18	0.35	0.70	2.7	7.0	19.6	140
<i>Callithaca adamsi</i>	(B)	16	0.31	0.37	1.8	1.9	23.5	95
<i>Siphonalia</i> cf. <i>fusoides</i>	(G)	14	0.27	0.32	1.9	2.7	21.6	105
<i>Prionospio pinnata</i>	(P)	11	0.22	0.25	2.3	4.4	17.6	133
Ophiuridea indet.	(E)	10	0.20	0.20	2.2	4.1	17.6	128
<i>Harmothoe imbricata</i>	(P)	9	0.18	0.22	2.7	6.6	13.7	180
<i>Haploscoloplos elongatus</i>	(P)	7	0.14	0.12	2.1	2.4	13.7	157
<i>Mediomastus</i> sp.	(P)	5	0.10	0.13	3.9	15.1	7.8	332
<i>Macoma incongrua</i>	(B)	4	0.08	0.11	4.5	20.8	5.9	453
<i>Magelona</i> cf. <i>californica</i>	(P)	4	0.08	0.11	4.5	20.8	5.9	453
<i>Drilonereis</i> sp.	(P)	4	0.08	0.07	3.1	7.8	7.8	294
<i>Periploma otohineae</i>	(B)	3	0.06	0.06	3.8	12.1	5.9	400
<i>Aphrodita aculeata</i>	(P)	3	0.06	0.06	3.8	12.1	5.9	400
<i>Capitella capitata</i>	(P)	2	0.04	0.04	4.7	20.5	3.9	612
<i>Ancistrosyllis hanokai</i>	(P)	2	0.04	0.04	4.7	20.5	3.9	612
<i>Diopatra sugokai</i>	(P)	1	0.02	0.02	6.9	46.0	2.0	1250
<i>Laonice</i> sp.	(P)	1	0.02	0.02	6.9	46.0	2.0	1250
<i>Nephtys ciliata</i>	(P)	1	0.02	0.02	6.9	46.0	2.0	1250
<i>Pectinaria</i> sp.	(P)	1	0.02	0.02	6.9	46.0	2.0	1250
<i>Pista</i> sp.	(P)	1	0.02	0.02	6.9	46.0	2.0	1250
All species		16487	329.70	6336.00	3.2	0.2	100.0	2

example, a dominant polychaete species, *Heteromastus* sp., needed more than 15 times greater sampling area at the sampler size of 0.64 m<sup>2</sup> than 0.02 m<sup>2</sup>. If the area to be sampled is small, as observed by the smaller sampler, less sediment will be processed and sampling costs are reduced.

The log-linear relationship between  $s^2$  and  $m$  is demonstrated for the corer size of 0.02 m<sup>2</sup> and the grab covering 0.1 m<sup>2</sup> (Fig. 1). The numbers of replicates plotted on Figs. 1c and 1d were computed using the actual  $s^2$  and  $m$  from taxa listed in Tables 1

and 2. Although both regression lines of  $n_r$  were well fitted by the least square method at the  $p=0.0001$  level ( $r^2 \geq 0.95$ ), the difference between  $n_r$  and  $\hat{n}_r$  estimated from the regression line increased for larger values of  $m$ . We supposed that the constant  $b$  largely affected  $\hat{s}^2$  and the  $\hat{n}_r$  because of its exponential power on  $m$  (Equation 2). We assumed also that  $b$  should be specified for a certain range of  $m$  especially when  $b$  was fitted on a log-scale axis. Therefore,  $m$  was assigned into classes according to the scale of  $m$  and the corresponding  $b$  and  $\hat{n}_r$ .

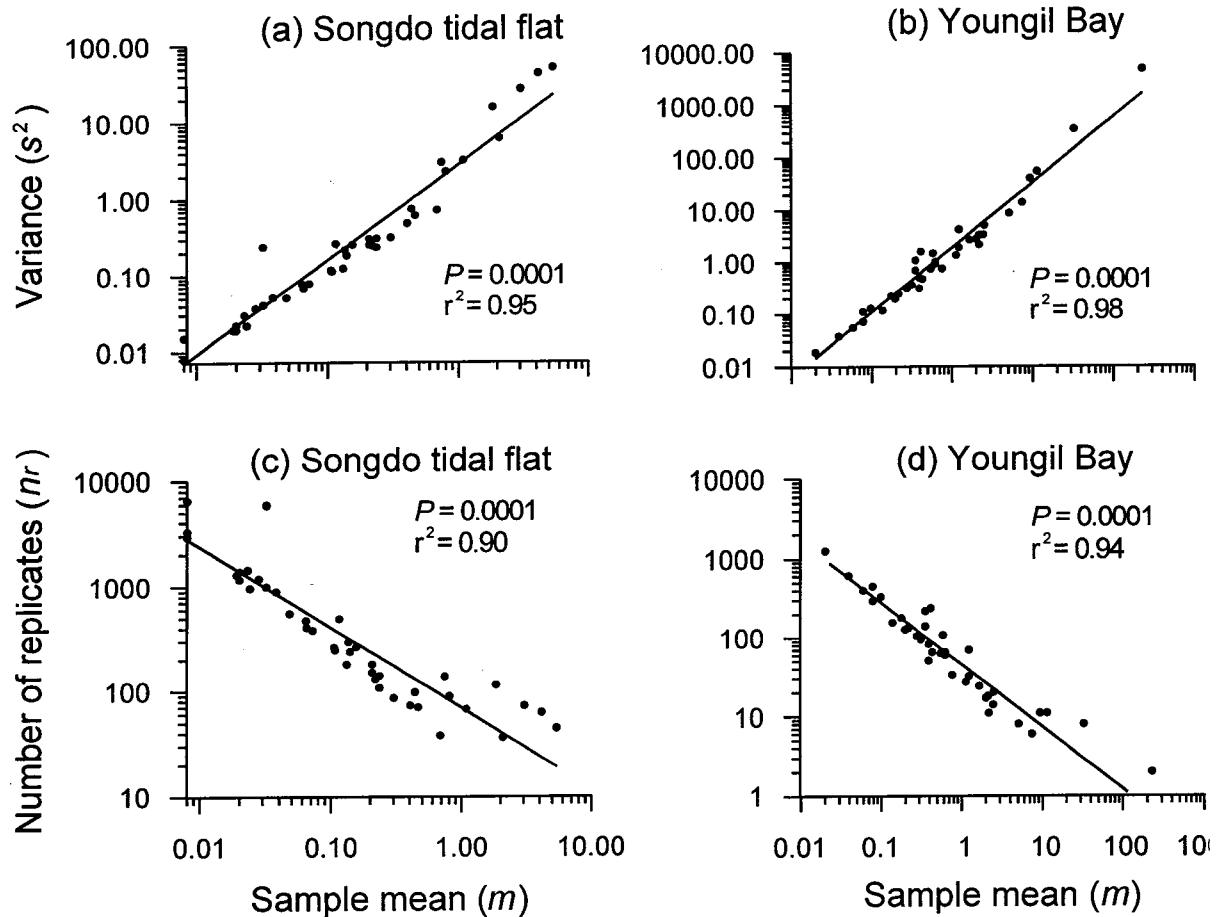
**Table 3.** The number of sample replicates ( $n_r$ ) at different simulated sampler sizes and total area ( $T_A$ ; in  $m^2$ ) to be sampled for the density estimation of individual taxa occurred on the Songdo tidal flat. The sampler size was simulated by combining the adjacent box corners in pairs ( $0.04 m^2$ ), fours ( $0.08 m^2$ ), eights ( $0.16 m^2$ ), etc. The total area ( $T_A$ ) to be sampled was calculated by multiplying  $n_r$  with the corresponding sampler size (All values of  $n_r$  were calculated at  $P=0.2$  level)

Taxon	Density (D)	0.02 $m^2$		0.04 $m^2$		0.08 $m^2$		0.16 $m^2$		0.32 $m^2$		0.64 $m^2$	
		$n_r$	$T_A$	$n_r$	$T_A$	$n_r$	$T_A$	$n_r$	$T_A$	$n_r$	$T_A$	$n_r$	$T_A$
<i>Heteromastus</i> sp.	255.9	48	0.97	35	1.39	27	2.19	23	3.76	22	7.09	23	14.66
<i>Mactra veneriformis</i>	223.4	54	1.08	49	1.97	41	3.31	40	6.40	40	12.81	44	28.38
<i>Aedicira</i> cf. <i>pacifica</i>	150.2	74	1.49	64	2.57	60	4.83	61	9.70	61	19.61	68	43.75
<i>Macoma incongrua</i>	99.6	39	0.79	25	0.99	18	1.45	15	2.36	14	4.52	15	9.70
<i>Hinia festiva</i>	89.2	123	2.46	85	3.41	65	5.16	43	6.92	33	10.67	24	15.56
<i>Mediomastus</i> sp.	52.3	74	1.48	46	1.84	34	2.70	27	4.25	26	8.22	26	16.56
<i>Macrophthalmus dilatatus</i>	43.0	79	1.58	68	2.73	54	4.33	44	6.97	44	14.24	46	29.25
<i>Nephtys longosetosa</i>	39.5	121	2.42	88	3.52	66	5.26	47	7.49	44	14.20	49	31.14
<i>Protankyra bidentata</i>	32.9	42	0.84	29	1.16	24	1.94	23	3.62	22	6.97	23	14.43
<i>Anaitides</i> sp.	25.7	58	1.17	47	1.89	42	3.35	41	6.51	41	13.22	45	28.86
<i>Nephtys polybranchia</i>	21.5	102	2.04	61	2.43	26	2.05	16	2.57	11	3.48	9	5.59
<i>Solen stricatus</i>	19.4	81	1.62	40	1.61	29	2.33	25	4.06	24	7.60	26	16.81
<i>Ruditapes philippinarum</i>	14.4	96	1.91	57	2.26	39	3.11	34	5.40	27	8.63	29	18.47
<i>Glycera decipiens</i>	12.4	98	1.96	48	1.93	23	1.88	14	2.25	12	3.90	9	5.64
<i>Aricidea elongata</i>	11.8	139	2.78	94	3.74	47	3.73	33	5.26	26	8.34	23	14.43
<i>Leonnates persica</i>	11.4	150	3.01	104	4.17	59	4.72	42	6.64	36	11.60	38	24.14
<i>Harmothoe forcipata</i>	10.5	142	2.85	74	2.95	25	1.98	16	2.60	8	2.50	8	5.06
<i>Glycinde</i> sp.	9.9	165	3.29	89	3.56	53	4.21	40	6.38	23	7.52	21	13.70
<i>Aricidea</i> sp.	7.6	279	5.58	159	6.36	97	7.72	42	6.75	35	11.29	25	15.97
<i>Marphysa</i> sp.	7.4	247	4.95	166	6.62	85	6.82	57	9.13	57	18.28	41	26.56
<i>Aricidea jeffreysii</i>	6.8	249	4.98	127	5.06	88	7.02	44	7.09	34	10.77	27	17.24
<i>Haploscoloplos elongatus</i>	6.3	198	3.97	99	3.97	61	4.90	27	4.38	16	5.02	12	7.89
<i>Ancistrosyllis hanaokai</i>	5.5	546	10.91	263	10.53	122	9.76	50	8.00	33	10.67	23	15.00
<i>Lumbrineris heteropoda</i>	5.3	260	5.20	117	4.69	48	3.86	29	4.63	21	6.67	19	12.00
<i>Bullacta exarata</i>	5.1	273	5.45	126	5.04	94	7.48	32	5.15	18	5.73	17	10.66
<i>Leonnates</i> sp.	3.8	337	6.73	220	8.81	94	7.52	42	6.64	25	8.10	24	15.11
<i>Nephtys californiensis</i>	3.4	421	8.43	189	7.56	94	7.48	45	7.25	26	8.47	16	10.18
<i>Marphysa sanguinea</i>	3.2	407	8.15	270	10.82	118	9.41	68	10.95	33	10.67	22	14.00
<i>Nectoneanthes oxypoda</i>	2.3	614	12.28	281	11.25	128	10.21	71	11.39	58	18.55	48	30.62
<i>Cyclina sinensis</i>	1.9	896	17.91	415	16.61	261	20.87	163	26.11	72	23.11	25	16.00
<i>Diogenes</i> sp.	1.7	868	17.36	468	18.73	258	20.63	171	27.36	76	24.44	66	42.51
<i>Notomastus</i> sp.	1.7	868	17.36	564	22.56	261	20.87	92	14.69	57	18.32	48	31.01
<i>Eteone</i> sp.	1.5	6550	131.00	3129	125.20	—	—	—	—	—	—	—	—
<i>Capitella capitata</i>	1.5	1002	20.05	551	22.06	209	16.70	91	14.50	65	20.74	56	35.56
<i>Periserrula leucophryna</i>	1.1	1436	28.72	673	26.91	258	20.63	149	23.85	65	20.74	21	13.35
<i>Phacosoma japonicum</i>	1.1	1071	21.42	502	20.06	332	26.53	237	37.90	111	35.55	47	30.03
<i>Nectoneanthes latipoda</i>	1.1	1071	21.42	606	24.26	230	18.36	79	12.63	50	16.00	38	24.01
<i>Diopatra sugokai</i>	1.0	1290	25.80	606	24.26	230	18.36	121	19.37	94	30.22	38	24.01
<i>Magelona</i> sp.	1.0	1290	25.80	606	24.26	400	31.97	237	37.90	111	35.55	47	30.03
<i>Glycera subaenea</i>	0.4	3263	65.25	1550	62.02	612	48.96	237	37.90	111	35.55	47	30.03
<i>Glycera rouxii</i>	0.4	3263	65.25	1550	62.02	1250	99.97	500	80.00	250	79.99	125	79.92
<i>Tharyx</i> sp.	0.4	6550	131.00	3129	125.16	1250	99.97	—	—	—	—	—	—
<i>Tritodynamia rathbuni</i>	0.4	6550	131.00	3129	125.16	1250	99.97	500	80.00	250	80.01	125	79.92
<i>Sternaspis scutata</i>	0.4	3263	65.25	1550	62.02	612	48.96	500	80.00	250	79.99	125	79.92
<i>Goniada</i> sp.	0.4	3263	65.25	1550	62.02	1250	99.97	—	—	—	—	—	—
<i>Pilargis</i> sp.	0.4	3263	65.25	1550	62.02	612	48.96	500	80.00	250	80.01	125	79.92
All species	1230	7	0.14	4	0.16	3	0.24	2	0.32	2	0.64	1	0.64

(denoted by  $b'$  and  $\hat{n}'$  in Table 4) were calculated.

The exponent  $b$  at every simulated sampler sizes and  $b'$  at different ranges of  $m$  are shown in Table 4. The  $s^2:m$  function was well fitted for all sampler sizes ( $r^2 \geq 0.95$ ,  $p=0.0001$ ), but the exponent  $b$  differed. The estimated  $b$  was, for example, 1.237 at  $0.02 m^2$  and 1.842 at  $0.64 m^2$  sampler size. It was

found to increase with sampler size. The relationship between  $b$  and sampler size ( $A$ ) could be expressed by a linear function of  $b=1.32+0.91A$  ( $r^2=0.88$ ,  $p=0.006$ ). Differences among  $b$ 's were also observed when  $s^2$  was fitted to  $m$  belonging to a certain class. The exponent  $b'$  was large when  $m$  was large. This tendency was shown at all simulated



**Fig. 1.** The variance and number of sample replicates required for the density estimation of benthic fauna occurred on the Songdo tidal flat and Youngil Bay, Korea, are plotted against the mean. All values are based on the individual number per sampler ( $0.02 \text{ m}^2$  and  $0.1 \text{ m}^2$  area coverage on the Songdo tidal flat and in Youngil Bay, respectively). Regression lines were fitted to (a)  $s^2 = 2.781m^{1.236}$ , (b)  $s^2 = 1.907m^{1.238}$ , (c)  $n_r = 69.525m^{-0.764}$ , (d)  $n_r = 47.675m^{-0.762}$ .

sampler sizes on the Songdo tidal flat. With the increasing  $m$ , the  $b'$  increased from 0.770 to 1.748 at  $0.02 \text{ m}^2$  sampler size and from 1.257 to 2.224 at  $0.64 \text{ m}^2$  sampler size. Three classes of  $m$  in Table 4 were arbitrarily categorized by the densities converted from  $m$  (Tables 1 and 2). The assignment of  $m$  into classes by its scale is also biologically justified when we consider density as an intrinsic property of organisms.

Table 4 also shows the bias of  $\hat{n}_r$  from  $n_r$ , which was expressed by the ratio (%) of  $[(\hat{n}_r - n_r)/n_r] \times 100$  (depicted by  $ER$  in the following) at various sampler sizes. This ratio indicates an error in percentage of  $\hat{n}_r$  to  $n_r$ , because  $\hat{n}_r$  was estimated based on the regression, whereas  $n_r$  was calculated from actual survey data of  $s^2$  and  $m$ . The error ratio of  $\hat{n}_r$  was 11.0% of  $n_r$  at  $0.02 \text{ m}^2$  sampler size and 21.2% at  $0.64 \text{ m}^2$  sampler size. The error ratio of  $\hat{n}_r$  to  $n_r$  ( $ER'$ ) using the  $b'$  value was lower than  $ER$  at smaller

sampler sizes (Table 4). Even at same sampler size, error ratio ( $ER'$ ) was largely reduced when  $a'$  and  $b'$  calculated from each density range were used. For example, at the density range of  $0.4\text{--}1.0 \text{ indiv./m}^2$  and  $0.02 \text{ m}^2$  sampler size,  $ER'$  using  $a'$  and  $b'$  was  $0.4 \pm 24.5\%$ , whereas  $ER$  using  $a$  and  $b$  was  $-30.3 \pm 21.3\%$ . In this case, the  $\hat{n}_r$  using  $a$  and  $b$  provided underestimates of about 30% while  $\hat{n}_r'$  was a precise estimate. This means that the number of sample replicates could be more unbiasedly estimated if the regression coefficients from sample variance and mean are calculated at each density class of organisms.

## DISCUSSION

Benthic biologists have employed a wide variety of sample replication. Early investigations proposed 5-haul with a  $0.1 \text{ m}^2$  grab as a minimum requirement

**Table 4.** On the basis of abundance data from the Songdo tidal flat and Youngil Bay, the exponent  $b$  at different simulated sampler sizes and  $b'$  at different ranges of  $m$  are compared. The comparison of  $ER$  ( $[(\hat{n}_r - n_r)/n_r] \times 100$ ) with  $ER'$  ( $[(\hat{n}_r' - n_r)/n_r] \times 100$ ) was made by employing  $b$  and  $b'$  in the calculation of  $\hat{n}_r$  and  $\hat{n}_r'$ , respectively (Abbreviations:  $n$ =number of sample replicates,  $N_{sp}$ =number of species)

Sampler size (m <sup>2</sup> )	$n$	Density range		$\hat{s}^2 = a \times m^b$			$\frac{(\hat{n}_r - n_r)}{n_r} \times 100$	$\hat{s}'^2 = a' \times m^{b'}$			$\frac{(\hat{n}_r' - n_r)}{n_r} \times 100$
		(indiv./m <sup>2</sup> )	$N_{sp}$	$a$	$b$	$r^2$		$a'$	$b'$	$r^2$	
<i>Songdo tidal flat (0.02 m<sup>2</sup> box corer)</i>											
0.02	250	0.4—255.8	46	2.787	1.237	0.95	11.0 ± 46.7				
		0.4—1.0	9	"	"		-30.3 ± 21.3	0.394	0.770	0.53	0.4 ± 24.5
		1.0—10.0	20	"	"		17.4 ± 33.1	1.269	0.977	0.70	6.2 ± 29.9
		10.0—255.8	17	"	"		25.3 ± 21.3	3.069	1.748	0.97	6.3 ± 40.9
0.04	125	0.4—255.8	46	3.023	1.316	0.95	14.2 ± 52.7				
		0.4—1.0	9	"	"		-16.8 ± 24.9	0.443	0.757	0.51	3.0 ± 23.3
		1.0—10.0	20	"	"		17.8 ± 39.5	1.514	1.032	0.72	6.6 ± 30.6
		10.0—255.8	17	"	"		26.4 ± 65.7	2.350	1.850	0.96	5.5 ± 40.4
0.08	50	0.4—255.8	45	2.874	1.422	0.95	17.9 ± 64.0				
		0.4—1.0	9	"	"		-22.7 ± 34.4	0.443	0.757	0.51	-6.6 ± 30.2
		1.0—10.0	19	"	"		25.2 ± 39.1	1.392	1.206	0.92	-18.0 ± 22.1
		10.0—255.8	17	"	"		33.2 ± 84.1	1.434	2.028	0.97	5.8 ± 42.6
0.16	20	0.4—255.8	43	2.530	1.564	0.95	21.7 ± 71.2				
		0.4—1.0	7	"	"		-23.8 ± 46.2	1.780	1.155	0.78	35.2 ± 62.8
		1.0—10.0	19	"	"		38.2 ± 53.8	1.679	1.301	0.94	6.6 ± 34.7
		10.0—255.8	17	"	"		26.3 ± 85.1	0.960	2.106	0.96	6.8 ± 48.5
0.32	10	0.4—255.8	43	2.136	1.692	0.96	23.9 ± 85.0				
		0.4—1.0	7	"	"		-27.6 ± 35.9	2.513	1.397	0.84	29.6 ± 57.4
		1.0—10.0	19	"	"		41.9 ± 61.7	1.655	1.482	0.92	7.2 ± 39.6
		10.0—255.8	17	"	"		31.3 ± 106.6	0.628	2.191	0.96	12.3 ± 61.1
0.64	5	0.4—255.8	43	1.597	1.842	0.97	21.2 ± 78.1				
		0.4—1.0	7	"	"		-27.0 ± 34.0	1.532	1.257	0.74	20.1 ± 46.2
		1.0—10.0	19	"	"		38.5 ± 56.7	1.403	1.707	0.92	9.6 ± 43.1
		10.0—255.8	17	"	"		26.3 ± 98.4	0.489	2.224	0.95	15.8 ± 67.9
<i>Youngil Bay (0.1 m<sup>2</sup> van Veen grab)</i>											
0.1	50	0.2—2314.7	44	1.955	1.245	0.98	8.0 ± 41.2				
		0.2—1.0	13	"	"		-15.4 ± 13.0	1.525	1.114	0.97	0.2 ± 12.7
		1.0—10.0	17	"	"		14.6 ± 39.5	1.909	1.286	0.69	7.3 ± 37.4
		10.0—2314.7	14	"	"		21.5 ± 50.4	1.224	1.502	0.97	7.7 ± 37.4

(Longhurst, 1959). Saila *et al.* (1976) stated that one to three grabs per station were optimal for a number of selected species. Studies using the cumulative number of species have also tended to suggest 3 to 5 grab samples per station (Holme, 1964; Lie, 1968, see also the review of McIntyre *et al.*, 1984). These earlier works estimated the sample replicates based largely on the cumulative curve of species number and its mean (reviewed by Cuff and Coleman, 1979). Different from these works, Downing (1979) followed Taylor's power law to determine the number of sample replicates. Many efforts have been made to predict  $s^2$  (Pringle, 1984; Downing and Anderson, 1985; Morin, 1985; Vezina, 1988; see also the review of Andrew and Mapstone, 1987). Although all these works support Downing's

algorithm principally and our process is also not an exception, a lesson to be drawn from our survey data is that the number of replicates needed for an estimation of abundance of a taxon is too large to be applied in practice.

We found a log-linear relationship among  $s^2$ ,  $m$  and  $n_r$  as shown by a number of authors (Downing, 1979; Downing and Anderson, 1985; Vezina, 1988). The variance increased as population density increasing and the requisite number of replicates decreased with increasing population density and variance. A trend of lower values of  $n_r$  at lower skewness and kurtosis was also shown (Dale *et al.*, 1991). An evaluation of sampler size by pairing contiguous samples showed that the smaller sampler size had an advantage of reducing the total area to



be sampled. The use of large numbers of small-sized samples resulted in less costly density estimates (Downing, 1979, 1989; Downing and Anderson, 1985; Vezina, 1988).

Although all these results correspond well with those in Downing's and other works, there is a problem with the requirement of an excessive large number of sample replicates which is mostly impractical to apply even for a dominant species. We can, of course, neglect the numbers of replicates exceeded over several hundreds because of low abundance. Downing stated that the "best possible precision" is high if  $m$  is low, even when the species is distributed uniformly (Downing, 1989; Riddle, 1989). Another reason we may ignore the higher number of sample replicates demonstrated in Tables 1 and 2 can be found in the precision level of  $P=0.2$ . The value of  $P$  was given 0.2 as usual under the constraint that the error of 20% is tolerable (e.g. Elliot 1977). Although not drawn in figure, however, the  $n_r$  curves found by substituting different levels of  $P$  were sharply concave at  $P=0.2$  for only the dominant species. This level of  $P$  can not be applied to determining  $n_r$  for organisms distributed sparsely. The deeply concave curves were not found in rare species and, if they existed, values of  $P$  at these values of  $n_r$  were far larger than 0.2.

As shown in Tables 1 and 2, even the most dominant species, *Heteromastus* sp., on the Songdo tidal flat required 48 corer samples to determine the abundance. Common species in Youngil Bay required around 10 grabs except for the most dominant species, *Maldane cristata*. A choice of such a large number of replicates from the present study seems to be unrealistic. Even an operation with optimized ship and sampling gear will have limits on the number of samples obtained due to the allotted time for processing the sediments. Sample replicates applied in many field studies on the Korean coasts were, therefore, extremely small, mostly two to three hauls of grabs (Choi and Koh, 1984; Shin and Koh, 1990; Shin *et al.*, 1992). Difficulties still remain in adopting the theoretical  $n_r$  calculated from the relationship among  $s^2$ ,  $m$  and  $P$ .

Biases in  $b$  were observed when  $b$  values were estimated from various sampler sizes and ranges of  $m$ . It must be pointed out that our data set involves sample replicates in hundreds, not in thousands as treated in earlier works (Downing, 1986; Vezina, 1988). Downing (1986) demonstrated that  $b$  varied with the number of samples taken and the range of

$m$  considered. But Taylor *et al.* (1988) criticized Downing's (1986) results. Vezina (1988) stated that the variability of  $b$  was related, rather, to the number of sample replicates. Though these limits, Vezina (1988) proposed a general  $s^2:m$  function with a constant value of  $b=1.219$  ( $r^2=0.86$ ,  $n=3015$ ) for marine fauna covering a wide range of sample sizes and including meio- as well as macrofauna. The variability of  $b$  has long been debated. Morin (1985) compared  $s^2$  over a range of mean values and stated that  $b$  varied from 1.4 to 1.9 with increasing mean for stream benthos. Yamamura (1990) described the bias in  $b$  at different sampler sizes and found that the exponent  $b$  became larger as the sampling scale became larger.

The impressive correlation between  $s^2$  and  $m$  shown by plots of benthic fauna occurring on Korean coasts would lead to propose a general  $s^2:m$  function with determined  $b$ . Our survey data support that  $b$  fits very well over the whole range of  $m$  at a given sampler size (Table 4). However, error ratios of  $\hat{n}_r'$  to  $n_r$  ( $ER'$ ) have more or less lower values than those of  $\hat{n}_r$  to  $n_r$  ( $ER$ ) as shown in Table 4. Therefore, replicate number of samples can unbiasedly be estimated, if regression coefficients ( $a'$  and  $b'$ ) from sample variance and mean of each density class can be used.

The primary aim of our study was to present concrete numbers of sample replicates for taxa occurring on the Korean coasts. We followed the Taylor's power law to determine the number of sample replicates and a lesson to be drawn from the results was that even the dominant species requires a number of sample replicates which can hardly be taken. But taking and treating a few replicates are expensive for marine benthos samples. Ships for the field survey and labor for sample sorting are the most cost-demanding aspects. Especially, considering that the exponents ( $a$  and  $b$ ) of Taylor's law could be varied due to the difference in the distribution patterns of benthic animals with environmental conditions (Andrew and Mapstone, 1987), the approaches of Downing's algorithm for the determination of number of samples seem to be unrealistic.

In the revised IBP manual on the benthos study, McIntyre (1984) made extensive use of the general  $s^2:m$  function in determining the number of sample replicates. Our data support that the  $s^2:m$  function and Equation 1 are applicable and would be better than none as stated by Vezina (1988), but more sophisticated decision rule for the estimation of the

requisite number of sample replicates should be made. A possibility in reducing  $n_r$  would be found in setting  $P$  at higher level than 0.2, when we follow Downing's algorithm.

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