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EFFECTS OF SAMPLE SIZE AND CONTAGION ON ESTIMATING FISH EGG ABUNDANCE

ANDREW E. JAHN
Los Angeles County Museum of Natural History
900 Exposition Boulevard
Los Angeles, California 90007

PAUL E. SMITH
National Marine Fisheries Service
Southwest Fisheries Center
P.O. Box 271
La Jolla, California 92038

ABSTRACT

Because pelagic fish eggs are usually distributed contagiously, the mean and variance estimated from egg surveys are often driven by a few samples of very high abundance. "Sampling" from simulated negative binomial data sets ($n = 20$ to $1,000$, $k = .1$ to $.4$) showed that the sample mean and variance were both highly dependent on the maximum observed value. As contagion (k^{-1}) increased, or n decreased, the chance of a sample including rare, high values decreased. In consequence, nominally 95% confidence limits excluded the population mean more than 5% of the time and tended to underestimate the mean more often than to overestimate it. Log-based parametric estimates were superior to those assuming a normal distribution of sample means, but only at $k = .4$ and $n \geq 500$ did the error rate approach 2.5% in both tails. Since contagion in pelagic fish egg distributions is often greater than this ($k < .4$), and affordable sample size usually small ($n < 1,000$), a method was sought that would improve accuracy by increasing the asymmetry of confidence bounds. One potential methodology is Easterling's "consonance regions," applied here to samples from a large set of *Engraulis mordax* egg data.

RESUMEN

Debido a la distribución contagiosa de los huevos de peces pelágicos, el promedio y la varianza estimados de recuentos de huevos son a menudo determinados por un bajo número de muestras con alta abundancia. Los "muestreos" de varios conjuntos de datos simulados de distribución binomial negativa ($n = 20$ a 1000, $k = 0.1$ a 0.4) indican que el promedio y la varianza de la muestra son ambas altamente dependientes del máximo valor observado. A medida que el grado de contagio (k^{-1}) aumenta o n disminuye, la probabilidad que una muestra contenga valores altos, de baja frecuencia, disminuye. Consecuentemente, los límites de confidencia del 95% excluyen el promedio de la población en un 5% de los casos y tienden, en general, a subestimar el promedio. Aun cuando las

estimaciones paramétricas con distribución logarítmica resultaron ser superiores a aquéllas para las cuales se supuso una distribución normal, el error alcanzó únicamente un 2.5% en cada cola cuando $k = .4$ y $n \geq 500$. Dado que el grado de contagio en las distribuciones de huevos de peces pelágicos es generalmente mayor ($k < .4$), ye el tamaño de muestra es generalmente pequeño ($n < 1000$), se buscó un método que mejorara la exactitud por medio de un aumento en la asimetría de los límites de confidencia. El método de Easterling o "método de regiones consonantes" ha sido usado en este trabajo con muestras provenientes de un alto número de datos de huevos de *Engraulis mordax*.

INTRODUCTION

The usual method of computing confidence intervals rests on the assumption that the distribution of (theoretical) sample means is normal (i.e., that the central-limit theorem applies). Robust as this assumption is, the patchy distribution of fish eggs and larvae can give rise to sufficient contagion in survey data to cause significant departures (e.g., the mean of northern anchovy egg samples of $n < 60$ tends to be skewed).

Although statistics texts treat the problem lightly, if at all, proposals for measuring precision in contagious data do appear in the fisheries literature (e.g., Taft 1960; Zweifel and Smith 1981; Pennington 1983; Pennington and Berrien 1984; Jahn, in press). All the proposed methods deal in some way with the asymmetric distribution of sample means, but little has been done to quantify the error rates inherent in each. This has moderate consequences in most fisheries applications, because sample size is typically held large to counteract the effects of contagion and achieve good precision. However, in research that enjoys less financial support, such as environmental impact studies, sample size is often set by factors external to the nature of the variability and is nearly always smaller than the investigator would wish for.

For a given level of abundance, the definition of "small" sample size depends on the desired precision and the degree of contagion. In this paper we demonstrate the interdependence of estimated

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mean and variance, and use simulation to quantify the actual precision obtained from such estimates over a range of sample sizes and degrees of contagion common in coastal ichthyoplankton work. The simulation results, based on completely specified negative binomial distributions, are compared with samples from large "populations" of real anchovy egg data. We also briefly explore an alternate method of estimating precision that shows promise for small samples.

METHODS

Statistics

Skewness ($g1$) and kurtosis ($g2$) were calculated according to procedures in Sokal and Rohlf (1969). Formulas used in computing parametric confidence limits were:

$$m \pm t_\alpha \cdot SE \qquad (1)$$

$$m \cdot \exp\{\pm t_\alpha \sqrt{[\ln(1 + SE^2/m^2)]}\} \qquad (2)$$

where m is the sample mean, t_α the standard normal deviate (here approximated as $= 2$), and SE the standard error of the mean. Formula 1 is the familiar method, which assumes a normal distribution of m. Formula 2, from Zweifel and Smith (1981), assumes the log-normal distribution of m.

Another method explored was the procedure of simultaneous model fitting and parameter estimation suggested by Easterling (1976), in which an array of parameters is employed in goodness-of-fit tests to define a two-dimensional region in which the data are consonant with the specified model. A full description of this procedure, as applied here using the negative binomial frequency distribution and x^2 goodness-of-fit tests, is given in Jahn (in press).

It should be said at the outset that Easterling's proposal was not specifically for making population inferences, but rather for obtaining an objective description of data. Our motivation for applying the technique to a problem of inference was that it produces an asymmetry of fiducial limits that has the desired properties for small samples from contagious distributions. Easterling (1976) has shown that, for a given probability level, consonance regions will tend to be wider than parametric confidence intervals, the difference depending on the nature of the data. We have found that, with small sets of ichthyoplankton data as treated here, a probability of 0.2, or an 80% consonance region, gives an interval of comparable size to a 95% con-

fidence interval, but with more appropriate asymmetry, as will be shown.

Simulations

To obtain an empirical estimate of the accuracy of parametric confidence limits, "sampling" was carried out on three simulated data sets, each distributed as a negative binomial completely specified by the parameters m (the mean, set $= 10$ in all cases) and k (an inverse contagion parameter, set $= 0.1, 0.2,$ and 0.4). The simulated populations, generated according to procedures given in Elliott (1971), consisted of 50,000 numbers each, sufficient to produce variances $> 99\%$ of asymptotic values ($s^2 = m + m^2/k$). From each population, 1,000 random samples of $n = 20, 50, 100, 200, 500,$ and 1,000 were taken, and their mean, variance, and maximum value recorded.

Egg Data

Real fish-egg abundance data came from surveys employing a 0.05 m^2 vertically towed net, the CalVET sampler (Smith et al. 1985). The six CalVET surveys for 1980-85 took 5,338 samples, of which 2,311 were positive for northern anchovy (*Engraulis mordax*) eggs. Ages of all eggs from each sample were estimated from stage of development and field temperature (Lo 1985). For subsampling purposes, the 3,027 (5,338 − 2,311) negative stations were considered outside the spawning area and omitted as "false" zeros[1]. The first (A) and second (B) whole days after spawning, and total eggs (T), were the three "populations" from which random subsamples of $n = 20, 46, 100, 200,$ and 500 were taken. The two smallest sample sizes correspond to the number of samples per cruise in a program of nearshore egg and larval surveys, wherein mean abundances have been reported with various measures of precision, including some methods used here (Brewer and Smith 1982; Lavenberg et al. 1986; Jahn, in press).

RESULTS AND DISCUSSION

Simulations

For a given level of contagion, the range and symmetry of the distribution of sample means were (as expected) strongly related to sample size (Figure 1). The width of the range of the central 95% of sample means was well predicted from population parameters as 4 standard errors of the mean

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[1] By "false" zeros we mean that these observations were taken outside the spawning area and not by chance from within it. (For a more thorough treatment see Smith 1973.) This oversimplification will have slight consequences for the biological character of our example data sets, but the allocation of zeros is a problem in fisheries practice that rivals that of precision.
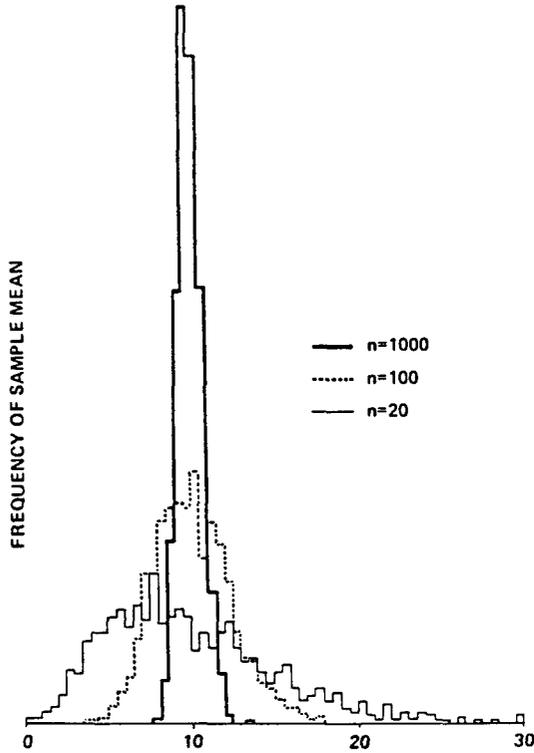
Figure 1. Superimposed histograms of 1,000 sample means for three sample sizes from a negative binomial population with $m = 10$, $k = .2$.

TABLE 1

Summary Statistics of Sample Means from Negative Binomial "Populations" of 50,000 Numbers with μ = 10 and Parameter k as Indicated

	n	m	LL	UL	w	$4\mu(kn)^{1/2}$	$\dfrac{UL - \mu}{\mu - LL}$
$k = .1$	20	9.9	1.15	26.25	25.1	28.3	1.84
	50	10.3	3.44	20.92	17.5	17.9	1.66
	100	9.9	4.90	16.66	11.8	12.6	1.31
	200	9.9	6.06	14.67	8.6	8.9	1.19
	500	10.0	7.51	13.07	5.6	5.7	1.23
	1000	10.0	8.06	12.01	4.0	4.0	1.04
$k = .2$	20	9.9	2.70	21.70	19.0	20.0	1.60
	50	9.9	4.72	16.64	11.9	12.6	1.26
	100	10.0	6.10	14.89	8.8	8.9	1.25
	200	10.0	7.075	13.475	6.4	6.3	1.19
	500	10.0	8.07	12.01	3.9	4.0	1.04
	1000	10.0	8.66	11.37	2.7	2.8	1.02
$k = .4$	20	9.9	4.05	18.70	14.65	14.1	1.46
	50	10.0	6.00	14.78	8.8	8.9	1.20
	100	9.9	7.02	13.10	6.1	6.3	1.19
	200	10.0	7.87	12.265	4.8	4.5	1.06
	500	10.0	8.58	11.56	3.0	2.8	1.10
	1000	10.0	9.01	11.03	2.0	2.0	1.04

LL = 2.5ᵗʰ percentile; UL = 97.5ᵗʰ percentile; w = UL − LL; n = sample size; m = average of sample means.

(approximated as $4\mu/\sqrt{kn}$, Table 1), but this interval was never precisely centered on the population mean. The asymmetry of the interval [(upper limit − μ)/(μ − lower limit)] varied inversely with both n and k (Table 1 and Figure 2). Although the distribution of sample means is interesting and informative in a theoretical context, the real problem in practice is estimating population parameters from the information contained in a single sample.

For all three simulated populations, as in the marine sampling environment, parameter estimates were highly dependent on relatively rare, high values. In small samples these extreme observations can dominate parameter estimates; on the other hand, their absence can lead to severe underestimates of the mean and variance. The dependence of these parameter estimates on the maximum observed value is shown in Figure 3 for the case $k = 0.2$, $n = 50$.

Overestimating the variance (and concomitantly, the mean) produces wide confidence inter-

vals which, though imprecise, tend to be accurate in that they include the population mean. Conversely, underestimating the variance often leads to confidence intervals that are too small and exclude the true mean. These trends account in principle for the distributions of samples producing confidence limits that were too low or too high (Table 2). For the same reason that the "curves" in Figure 2 are not smooth, the numbers in Table 2 yield only approximate probabilities, but these should serve as useful indicators of the effects of sample size and contagion on measuring precision.

Ideally, 95% confidence limits should be higher than the true mean 2.5% of the time and lower
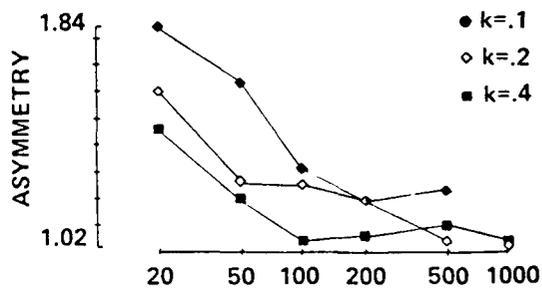

Figure 2. Asymmetry (see Table 1) of the distribution of sample means as a function of sample size (n) for three levels of contagion, k.
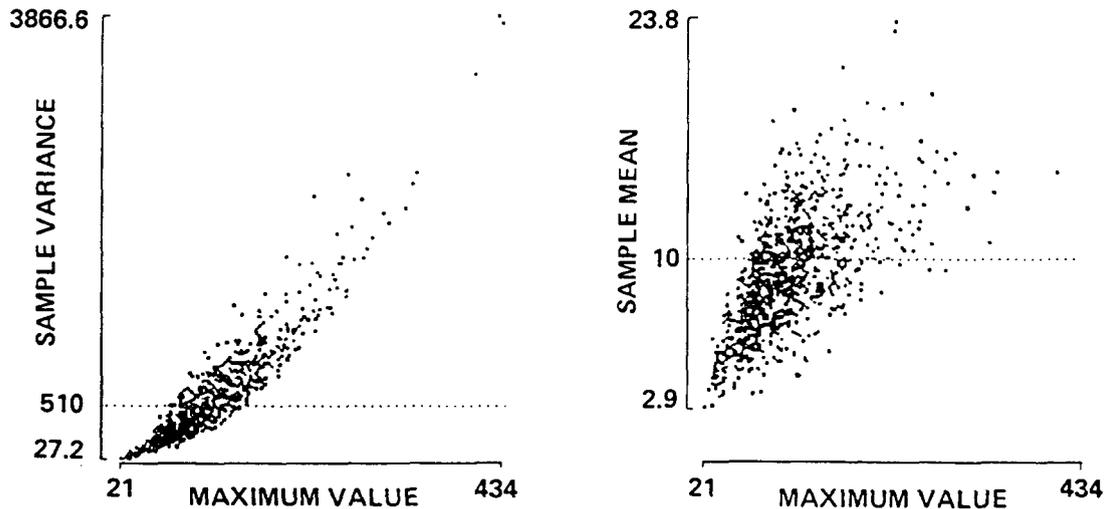
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Figure 3. Dependence of estimates of the variance and mean on maximum observed value. All samples were of n = 50 from a negative binomial population with mean = 10, variance = 510 (k = .2).

TABLE 2
Summary of Samples (from 1000 Iterations) Giving Computed 95% Confidence Limits (CL) That Excluded the Population Mean (μ).

	k	$n=20$		$n=50$		$n=100$		$n=200$		$n=500$		$n=1000$	
		L	H	L	H	L	H	L	H	L	H	L	H
Formula 1	.1	249	1	151	3	110	1	86	4	60	7	50	5
	.2	188	4	119	5	71	3	57	6	59	9	34	12
	.4	144	4	82	8	62	3	50	7	37	20	33	16
Formula 2	.1	162	22	90	22	66	14	54	14	42	15	36	20
	.2	117	26	66	19	42	23	35	27	42	21	30	18
	.4	89	25	50	19	41	11	38	22	29	28	28	22

Formula 1: $m \pm t_a \cdot SE$

Formula 2: $m \cdot \exp\{\pm t_a \sqrt{[\ln(1 + SE^2/m^2)]}\}$
where t_a was approximated as = 2.

L = number of upper CL's that were lower than μ; H = number of lower CL's higher than μ.

than the mean 2.5% of the time; i.e., the "tails" should be equal and add to 5%. For the simulations presented here (1,000 samples each), the tails should each average 25 samples. As shown in Table 2, the low tail (L) was always > 25, and the high

tail (H) usually < 25. The log-based method (formula 2) was superior to the conventional symmetrical limits (formula 1), but approached equal tails only at high n ($\geq$ 500) and k (.4). Because the simulated values of k are realistic, and larger values of n are often not, it is desirable to find a method that will further increase the asymmetry (shorten the low tail and lengthen the high) of computed confidence intervals. One promising approach is applied to real data, below.

Real Data
The three "populations" of real anchovy-egg data (summarized in Table 3) were all positively skewed and peaked (leptokurtic), with large differences between mean and median values. In all cases the variance exceeded the mean squared, implying a high degree of contagion with values of the negative binomial parameter k < 1. For various reasons, including the truncation of zeros and the composite nature of the data sets (amalgamation of several years' sampling), the negative binomial distribution is only an approximate model for these

TABLE 3
Characteristics of Three Populations of Anchovy Egg Data Compiled from 2,311 Positive CalVET Tows from 6 Surveys, 1980–85

Population	Mean	Median	Maximum	χ^2	Skewness	Kurtosis	k	Freq(0)
A	9.88	2	261	426	5.36	42.45	.343	678
B	7.93	2	468	296	10.84	235.32	.394	632
T	24.22	10	605	1448	4.34	35.92	.656	0

A, B = first and second whole days after spawning. T = total eggs.

populations. This can be verified, for instance, by comparing the maximum likelihood estimates of k (Table 3) with the asymptotic moments relationship, $k' = m^2/(s^2 - m)$. However, as will be seen below, small samples from these distributions will tend to be negative-binomial-like enough that the null hypothesis in goodness-of-fit tests will seldom be rejected.

Ten random samples at each of five sample sizes (20 to 500, Table 4) were drawn from each of the three populations. At sample sizes < 500, the sample mean bore an approximately linear relationship to the maximum observations, shown for the A samples in Figure 4. Only one T sample (#7 at $n = 20$) produced a confidence interval that excluded the mean, but 16% of samples from the more positively skewed A and B populations (which also had $> 25\%$ zeros, Table 3) at $n \leq 200$ produced estimates of the mean that were more than 2 standard errors below the true value. The

TABLE 4
Mean (m), Median (md), Maximum (max), and Standard Error (SE) of Samples of *Engraulis mordax* Eggs

	m	md	max	SE		m	md	max	SE		m	md	max	SE
A20	11.25	1	71	4.57	B20	4.55	1	27	1.59	T20	19.30	8	127	6.85
	11.85	3	71	4.09		4.25	1	47	2.33		15.55	7	83	4.31
	10.30	4	48	3.43		4.00	2	15	0.99		29.10	17.5	173	8.63
	7.25	1	37	2.67		7.05	1	34	2.22		24.95	9.5	144	8.09
	7.90	4.5	39	2.29		9.80	1.5	38	2.98		19.00	3	177	10.81
	7.45	3.5	47	2.89		9.95	5.5	61	3.32		25.10	15	114	6.55
	10.80	5	46	3.17		11.05	3	46	3.41		11.45	6	61	3.12
	10.30	7	39	2.35		6.70	1	34	2.40		46.70	19	252	14.58
	9.65	1.5	79	4.35		8.45	3	36	2.42		18.45	13.5	60	3.97
	7.10	1	55	3.31		3.75	1	27	1.44		23.40	11	106	7.14
A46	10.30	1	99	3.16	B46	10.09	3	86	2.57	T46	31.89	15	201	6.10
	5.80	1	71	1.86		4.44	1	37	1.20		21.52	11.5	97	3.84
	4.85	2	26	1.01		9.13	2.5	74	2.36		32.13	16	166	6.20
	12.15	6	71	2.57		15.91	2.5	468	10.15		25.54	8.5	140	4.91
	17.54	3	247	6.52		8.17	1	55	2.00		25.67	14.5	242	6.06
	11.35	4	94	2.53		10.74	3	119	3.03		19.96	8.5	117	3.65
	10.67	3	127	3.25		10.59	2	142	3.62		20.63	9.5	167	5.17
	7.96	3	109	2.54		7.78	1.5	88	2.23		30.67	15.5	177	6.07
	5.35	1.5	53	1.37		10.48	2.5	83	2.77		31.13	19	157	5.49
	10.80	3.5	84	2.78		6.37	2.5	35	1.28		28.94	16	248	6.27
A100	10.20	2	102	1.91	B100	8.19	2	128	1.73	T100	22.35	6.5	174	3.59
	9.38	3	119	1.69		5.71	2	39	0.88		24.04	13	147	2.92
	11.83	3	170	2.42		6.15	3	45	0.83		29.03	12	342	5.08
	11.93	3	244	2.94		12.35	1	468	4.94		25.27	7	382	4.70
	10.44	3	201	2.45		8.94	3	87	1.63		21.66	7.5	168	3.35
	7.46	2	87	1.38		10.71	3	201	2.61		24.34	9	217	3.82
	13.50	2	229	3.22		5.85	2	59	0.94		23.43	8.5	252	3.59
	9.54	3	118	1.76		14.15	3	468	4.89		22.52	11	171	2.83
	10.69	4.5	170	2.02		10.24	2.5	201	2.64		21.27	11	273	3.47
	10.12	4	65	1.35		6.86	2	69	1.15		27.46	10.5	173	3.80
A200	9.80	2	126	1.34	B200	8.60	3	131	1.10	T200	26.72	10.5	605	3.86
	12.28	3	245	1.88		7.95	1.5	142	1.20		22.05	9	174	2.22
	11.35	3	157	1.47		7.41	3	119	0.94		22.11	7	168	2.24
	13.43	3	261	2.12		9.94	4	140	1.24		22.49	9	273	2.42
	7.52	3	64	0.78		7.29	2	131	1.09		21.27	9	174	2.09
	9.26	3.5	157	1.28		5.98	2	42	0.60		25.77	9	273	2.82
	10.15	3	157	1.40		7.71	2	88	1.00		21.25	7	217	2.40
	7.96	2	94	1.05		7.23	3	64	0.81		23.05	10	252	2.42
	9.52	3	132	1.27		7.65	2	75	0.92		23.61	8	211	2.46
	10.50	2.5	261	1.69		7.62	2	142	1.08		21.33	10	195	2.06
A500	9.48	2	229	0.95	B500	7.79	3	120	0.61	T500	23.52	10.5	249	1.49
	9.52	2	229	0.91		8.49	2	468	1.13		24.26	9	251	1.70
	10.10	2	261	0.97		7.89	2	142	0.66		23.16	10	342	1.60
	10.40	3	261	0.97		8.49	2	128	0.67		22.20	10	342	1.45
	10.28	2	245	1.03		8.31	2	201	0.72		21.36	9	382	1.55
	8.88	2	245	0.83		8.19	3	140	0.64		26.23	11	605	2.06
	10.79	2	170	0.95		9.81	2	468	1.23		22.11	9	215	1.39
	10.82	3	261	1.04		8.11	3	201	0.68		23.51	10	251	1.54
	11.38	3	247	1.18		8.15	2	468	1.15		24.90	9	605	1.96
	10.60	3	201	0.98		7.86	2	142	0.67		23.35	11	342	1.59

A = first whole day, B = second whole day after spawning; T = total eggs. Number after age designation is sample size.
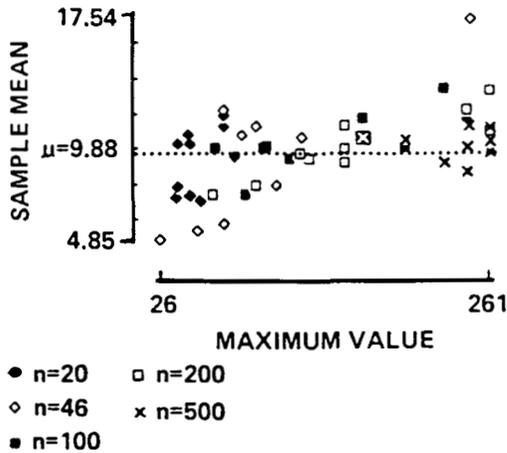
Figure 4. Dependence of the sample mean on the maximum observed value of samples drawn from a "population" of northern anchovy eggs (population A, Table 3).

worst case was sample A46#3, with sample mean = 4.85 and standard error = 1.01. The 95% confidence limits by formula 2 for this sample are 3.2–7.3, well below the true mean value of 9.9. However, the adaptation of Easterling's "consonance region" produced an interval estimate for the mean (3.5–10.5) that included the true value (Table 5). This method thus shows promise of reducing the frequency of samples in the "low tail,"

found above to be several times too high at sample sizes < 500.

At the other extreme among A samples was A46#5, with mean = 17.54 and standard error = 6.52. The high mean and variance of this small sample were strongly affected by the maximum value of 247 (Table 4; Figure 4), giving a wide confidence interval by formula 2 of 8.54–36.02. The consonance region for this sample gives credence to a narrower range of values for the population mean (approximately 7–14, Table 6), excluding the sample mean but still containing values above and below 9.9, the true mean. The effect of the very high maximum value on estimates of central tendency and dispersion was therefore moderated by the shape of the rest of the data in the sample. Besides the maximum value, sample A46#5 also chanced to have two other values > 100 but a median of only 3, characteristics that contribute to the bilobed nature of the consonance region as computed here.

The goodness-of-fit results of Table 6 also suggest that the negative binomial may be a poor general model for the data of sample A46#5, as no $p \geq .5$ region was found. If these data were all that we knew about the population, with what confidence could we make statements about its parameters? Before such questions can be answered, work must be done to quantify the distribution of consonance regions for contagiously distributed data, and to work out the robustness of the method to departures from completely specified distribution

TABLE 5
Chi-Square Probabilities of Goodness of Fit to Sample A46 #3 (See Table 4) of Negative Binomial Models with Parameters m and k

m	.2	.3	.4	.5	.6	.7	.8	.9
12.5	.1							
12.0	.1	.1						
11.5	.1	.1	.1					
11.0	.1	.1	.1					
10.5	.2	.2	.1	.1				
10.0	.2	.2	.2	.1	.1			
9.5	.1	.2	.2	.2	.2	.1		
9.0	.2	.2	.2	.2	.2	.2	.1	
8.5	.2	.2	.2	.2	.2	.1		
8.0	.2	.2	.5	.2	.2	.2	.1	.1
7.5	.2	.2	.5	.5	.2	.2	.1	.1
7.0	.2	.2	.5	.5	.5	.2	.2	.2
6.5	.2	.2	.5	.5	.5	.2	.2	.2
6.0	.1	.2	.2	.5	.5	.5	.5	.2
5.5	.1	.2	.5	.5	.5	.5	.5	.5
5.0	.1	.2	.5	.5	.5	.5	.5	.2
4.5	.1	.2	.5	.5	.5	.5	.5	.2
4.0		.2	.2	.5	.5	.5	.2	.5
3.5		.1	.2	.1	.2	.2	.2	.2
3.0			.1	.1			.1	.1

TABLE 6
Chi-Square Probabilities of Goodness of Fit to Sample A46 #5 of Negative Binomial Models with Parameters m and k

m	.25	.35	.45	.55	.65	.75
15.0		.1				
14.5		.1				
14.0	.2	.1				
13.5	.2	.1				
13.0	.2	.1				
12.5	.2	.1				
12.0	.2	.1				
11.5	.2	.1				
11.0	.2	.1				
10.5	.1	.1	.1			
10.0	.1	.1				
9.5	.2	.1			.1	
9.0	.1	.1	.2	.1		
8.5			.2	.2		
8.0	.1	.2	.2	.2	.1	.1
7.5	.1		.2	.2	.2	.2
7.0		.1	.2	.2	.1	.1
6.5			.1	.1	.1	
6.0						

models. Because of the astronomical number of computations required, it is very unlikely that generally applicable tables will be forthcoming. However, solutions to specific situations, with a few models over a limited range of parameters, should be producible for a given research application.

One obvious limitation of the consonance region approach is that as sample size increases, the statistical power to reject the null hypothesis (i.e., no difference between sample and specified frequency distribution) increases as well. The consonance region will become correspondingly small until some practical limit is reached. At such a point it may be plausible to use models with more parameters, as suggested by Easterling (1976), but as sample size increases, so does the suitability of simpler fiducial methods, such as formula 2.

When, as in pelagic fish-egg and larval census work, the potential exists for a few observations to dominate parameter estimates, the best insurance against wrong estimates is large sample size. In some applications, the costs of increasing sample size may seem too high, and the lower precision of small samples may be acceptable. We have emphasized here that special methods are needed in these cases in order to make correct probability statements about the population. We are not advocating the use of small samples. Rather, it is hoped that the above examples and the data of Table 2 will be helpful to planners who must weigh the costs and benefits of various approaches to sampling contagiously distributed organisms.

ACKNOWLEDGMENTS

We thank James Petersen for his helpful comments on several versions of the manuscript. Two anonymous reviewers also made useful comments. Helga Schwarz helped prepare the manuscript. The first author thanks the Southern California Edison Company for financial support.

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