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ORIGINAL PAPER

A comparison of some population density sampling techniques for biodiversity, conservation, and environmental impact studies

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Abstract Twelve terrestrial and marine studies were conducted at various sites in Malaysia, Brazil, and the United States between April 1999 and February 2004. These data were analyzed using five density estimate techniques for stationary (non-motile) organisms including Stratified Random Sampling, Point-Center Quarter, Third Nearest Object, Weinberg, and Strong. The Strong method gave the most accurate density estimates of stationary animals and plants. Stratified Random Sampling ranked second best and the Third Nearest Object the third best. Belt or strip transects may be preferable but can be restrictive in some situations because of logistics and associated time constraints. Straight line measurements on reefs were 3–27% more accurate than reef slack line and reef contour measurements. Most study areas measured with the standardized Morisita index of dispersion were moderately aggregated. Results from the Third Nearest Object and Point-Center

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Quarter techniques indicate that the addition of more data to establish a density correction factor does not necessarily give more accurate estimates of density.

Keywords Abundance · Biodiversity · Community analysis · Conservation · Density · Environmental impacts · Statistical sampling

Abbreviations

SRS	Stratified Random Sampling
PCQ	Point-Center Quarter or Point-Quarter
3NO	Point to Third Nearest Object

Introduction

One of the critical requirements of biodiversity, conservation, and environmental impact studies in terrestrial, freshwater, or marine habitats is the need to detect changes in population densities of selected species. The objective of the present study was to compare simple and common density estimation techniques in order to find a technique for stationary (i.e., non-motile) organisms that was easy to use, rapid, and relatively accurate. We compared the direct count or census in a study area with density estimates from Stratified Random Sampling, Point-Center Quarter, and Point to Third Nearest Object techniques as well as the line-intercept methods of Strong (equivalent to a modified Eberhardt method—see Krebs 1999) and Weinberg. Although most of these techniques assume a random distribution of organisms (whereas most organisms are aggregated), the objective of the study was to find the easiest, most rapid, and most accurate method of estimating densities based in large part on several popular methods that have been used for many decades in the U.S. The notable exception is the Weinberg method which was buried in the marine literature and consequently generally unavailable to terrestrial, freshwater, and even many marine biologists. Detailed discussions of these techniques and equations are presented in Weinberg (1981), Sutherland (1996), Barbour et al. (1999), Krebs (1999), Buckland et al. (2001), Mitchell (2001), and Bakus et al. (2004, 2006). Computer programs written in C++ for density estimations are available from the first author.

Materials and methods

Marine studies were conducted subtidally in Malaysia and Brazil and in the intertidal (littoral) zone in southern California. Terrestrial projects were conducted in southern California and Oregon (see Appendix for details). Transect lines were placed haphazardly over coral reefs in Malaysia and arranged equidistantly in other studies and were either selected randomly or we used the inner transect lines (e.g., PCQ). The number of random samples taken (n) generally varied with the size of the study area. For SRS $n = 6–50$, for PCQ $n = 44–360$, and for 3NO $n = 18–300$. Sample size was variable for the Strong and Weinberg techniques ($n = 4–51$), depending on the number of organisms encountered along the transect line.

Density comparisons were made between straight transect lines, slack transect lines (i.e., the line or tape is allowed to rest by its own weight on the reef), and reef contour lines (i.e., the line is pressed against the contour of the substratum) in Malaysia (subtidal) and southern California (intertidal) to indicate the degree of error in density estimations by those methods when compared with straight line measurements.

A simulation project was conducted during 2002 and 2003. Visual displays of points with different distributions (e.g., random, uniform, aggregated) were photocopied from Krebs (1999, p.115,124) and enlarged. Eleven transect lines were drawn lengthwise from the top of each rectangular box to the bottom, and 7 transects were randomly selected for counts and measurements of points with a millimeter rule. Using a table of random numbers, 35 random points were selected from the randomly selected transect lines for PCQ; 42 random points from the randomly selected transect lines were used for 3NO. This technique is the same as those used in the field studies but on a much smaller scale. For the SRS method the area was subdivided into 8 blocks of four cells, and one cell was randomly sampled from each of the 8 blocks.

A terrestrial project was conducted on Joshua trees (*Yucca brevifolia* Engelm.) and Mojave yuccas (*Yucca schidigera* K.E. Ortgies) in February 2004 in Joshua Tree National Park, southern California. A preliminary study consisted of running measuring tapes in an X configuration from corner to corner of a 50 × 50 m plot. Third and fourth lines were positioned midway between the north-south and west-east borders. For PCQ, 13 numbers were selected from a table of random numbers for each line. A correction factor was developed, based on a comparison of the actual density with the estimated density, and applied to a second (adjacent) 50 × 50 m plot. Twenty-six random points were selected along two crossed diagonal transect lines for 3NO. Total (actual) counts of both plant species were determined in all study areas. A laser rangefinder was used to measure the majority of distances. The density and distribution of *Y. brevifolia* and *Y. schidigera* were measured in another two plots of the same size using the same techniques. The density of *Y. schidigera* was measured using the same techniques in a 100 × 100 m plot.

Results

A summary of the results of the most accurate density estimation techniques from our studies is presented in Table 1. The Strong method is often the most accurate technique followed closely by the SRS method with the 3NO, Weinberg, and PCQ techniques trailing. Straight transect lines in Malaysia all measured 15 m long; slack lengths varied from 15.3 to 16.9 m; contour lengths measured from 15.9 to 20.5 m. In southern California straight transect lines all measured 10 m long; slack lengths measured from 10.2 to 10.6 m; contour lengths ranged from 10.9 to 11.8 m. Standardized Morisita indices (Krebs, 2000) ranged from 0.44 to 0.52 (mean = 0.49) except for Alhambra Park (city park −0.39). The use of only one diagonal line in the 3NO study resulted in a relatively large error in the density estimation of Joshua trees; we rectified the error by the inclusion of a second diagonal line (Table 2). The density estimate error in the PCQ study showed a high degree of accuracy with one line but low degrees of accuracy when two, three, or four lines were used in distance measurements. Using four lines to develop a correction factor for the strongly aggregated Mojave yuccas yielded a highly inaccurate estimation of density.

Table 1 Summary of results of density estimation techniques[a]

Table	Most accurate method (MA)		Second most accurate method (SMA)
3	W (2) & S (2)		S (2) & W (2)
4	S (5) & W (3)		W (3) & S (5)
5	S (2) & W (1)		SRS (1) & S (1) & PCQ (1)
6	3 NO (1)		S (1)
7	W (1)		S (1)
8	S (1)		SRS (1) & PCQ (1)
9	3NO (2)		SRS(1) & W(1)
10	SRS (2)		PCQ (1) & S (1)
11	S (2) & W (2) & SRS (1) & 3NO (1)		S (3) & W (1) & SRS (1) & PCQ (1)
Summary for Tables 3 and 4 (2 techniques)			
Strong	7	+	7
Weinberg	5	+	5
Summary for Tables 5–10 (5 techniques):			
Strong	3	+	4
SRS	2	+	3
3NO	3	+	0
Weinberg	2	+	1
PCQ	0	+	3
Summary for simulation in Table 11 (5 techniques):			
Strong	2	+	3
Weinberg	2	+	1
SRS	1	+	1
3NO	1	+	0
PCQ	0	+	1

[a] Excluding Table 2, a density estimation correction study. PCQ = Point-Center Quarter; S = Strong; W = Weinberg; 3NO = Point to third Nearest Object; SRS = Stratified Random Sampling; () = No. most or second most accurate density estimations. These data represent a summary from Tables 3–11 in the Appendix

Table 2 Density of Joshua trees (*Yucca brevifolia* Engelm.) and Mojave yuccas (*Yucca schidigera* K.E. Ortgies) in Joshua Tree National Park, S. California, in February 2004[a]

Lines used	Density–Plot 1 (No. indiv./0.25 hectare)	Actual count	Correction factor	Density–Plot 2 (No. indiv./0.25 hectare)	Density with correction factor applied	Actual count	Density error
A. Point to Third Nearest Object comparison for Joshua trees (*Y. brevifolia*) in a 50 × 50 m (0.25 hectare) plot.							
1	17.75	14	0.79	34.52	27.27	16	11.27
1 & 2	18.75	14	0.75	22.00	16.50	16	0.50
B. Point Center Quarter comparison for Joshua trees (*Y. brevifolia*) in a 50 × 50 m (0.25 hectare) plot.							
1	16.75	17	0.99	15.75	15.59	15	0.41
1 & 2	19.00	17	1.12	21.50	24.08	15	9.08
1,2,3	17.95	17	1.06	23.02	24.40	15	9.40
1,2,3,4	19.42	17	1.14	21.72	24.76	15	9.76
C. Point Center Quarter comparison for Mojave yuccas (*Y. schidigera*) in a 100 × 100 m (1.0 hectare) plot.							
1–4 and 5–8	351	248	1.42	287	408	216	192

[a] Elevation 704 m

Discussion

The Strong technique was the most accurate in estimating densities (Table 1). Stratified random sampling, the canon of field ecologists for many decades, fared well in density estimation techniques. Logistically, the method is more difficult to carry out than line transect and nearest neighbor techniques. This is particularly true for underwater sampling where the handling of tape measures and quadrat frames is awkward (especially if there is surge). The straight line density estimation was 3–27% more accurate than using slack line or reef contour methods. The greater the heterogeneity of the substratum, the higher the density error when using slack or contour lengths.

Standardized Morisita indices were indicative of a natural moderate aggregation. The tendency towards uniform dispersion in Alhambra Park (−0.39) is characteristic of city parks where trees are planted with a planned spatial distribution. The simulation study using small dots distributed within rectangular plots showed that the Strong method was best for small clumps and large clumps with individuals randomly distributed. The Weinberg method was most accurate for an aggregated distribution and for large clumps with individuals uniformly distributed. SRS was best for a uniform distribution whereas 3NO was most accurate for a random distribution. Caution must be taken when using correction factors in estimating densities because corrected estimated densities may or may not result in an increase in accuracy.

The following suggestion for an overall methodology that conducts density estimates for stationary (i.e., non-motile) organisms in a relatively rapid, easy and accurate manner is based on the present study and on information from the literature (see References).

Conduct a preliminary study

Be certain to sample each part of a heterogeneous study site. For intertidal studies, place a small number of transects either perpendicular or parallel to the shoreline, depending on the type of question asked. Alternatively, use random points with coordinates; or use random plots in a grid system superimposed over a map of the study region.

Conduct a definitive density study in the same area

Calculate a separate density estimate for each heterogeneous area, and proportion the sampling effort (i.e., number of transects) to the sizes of the heterogeneous parts (Krebs 1999). For example, if one-third of the substratum has boulders and two-thirds relatively flat rock, assuming 12 transects, sample four transects in the boulder region and eight transects on flat rock.

Consider using the Strong method for studying the densities of one to three species of stationary organisms. The Strong method works best with organisms about 0.3 m in size. To speed up the collection of data: (1) For trees—Two people walk along each side of the transect line. When encountering a tree, the people move out to the edges of the canopy then use a lazer rangefinder to measure the distance between them. (2) For shrubs and corals—Two people walk or swim along each side of the transect line and measure the orthogonal width of the organisms encountered with a measuring tape. Belt or line strip transects may be even better in many instances although this method may be unsuitable for some sites (e.g., dense mangroves of *Rhizophora mangle*, certain plants scattered within dense second growth tropical rain forests). Consider using the 3NO technique for randomly distributed forest trees since the method is simple and rapid, especially if using a laser rangefinder. Use the Stratified Random Sampling method if information is desired on both densities and spatial distribution or dispersion (i.e., degree of aggregation). If densities of several or all species in the area are desired, then use strip, belt, or line strip transects (e.g., 30 × 1 m) (Buckland et al. 2001). This can be subdivided into units of length for estimates of the degree of aggregation of organisms. For underwater studies, direct counts can be made by swimming along a tape holding a meter-long rod perpendicular to the tape and recording the results. Wider belts or strips are often necessary for adult fishes and for terrestrial studies.

For organisms that cannot be easily counted (e.g., coralline algae, blackberries) use quadrats to estimate percentage cover. Effective visual estimates of intertidal percentage cover have been made with 10 cm by 10 cm quadrats (Dethier et al. 1993). Percentage cover can also be obtained by determining total intercept lengths (line intercept technique) and dividing by the total transect length (Bakus and Nishiyama 1999).

Rapid methods of estimating densities include taking still photos of quadrats or swimming along a transect line using a digital videocamera. Species of interest can be marked then automatically counted in randomly selected digital frames using the computer program PointCount 99 (available from Dr. Phil Dustan, Department of Biology, College of Charleston, Charleston, SC 29424. Email: dustanp@cofc.edu). This method usually assumes that selected species can be identified in all random frames and that no organisms are covered by algae or corals, or live in cracks and crevices. The percentage cover in still photos or videos can be measured automatically with image processing software such as Sigmascan (see Wright et al. 1991 for a different example).

Average the densities for the entire study area

If an overall density is desired, calculate the average of the separately estimated densities.

Further information can be found in Bouchon (1981), Engeman and Sugihara (1998), Southwood and Henderson (2000), Strong (1966), Thompson (2002), Underwood (1976), and Warde and Petranka (1981).

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Appendix

Table 3 Density of subtidal hard corals at Pulau Pemanggil, Malaysia in April 1999[a]

Sampling technique	Pulau Pemanggil No.1 Density (No./m^2) *A. hyacinthus** (Dana, 1848) *n* = 7**	Pulau Pemanggil No.2 Density (No./m^2) *A. millepora* (Ehrenberg, 1834) *n* = 6	Pulau Pemanggil No. 3 Density (No./m^2) *E. lammelosa* (Esper, 1795) *n* = 6	Pulau Pemanggil No. 4 Density (No./m^2) *A. millepora* (Ehrenberg, 1834) *n* = 14
Actual Count (15 m^2 study area each)	**2.9*****	**6.5**	**1.4**	**16.9**
Weinberg:				
S	**0.70**	1.3	0.82	**4.3**
SL	0.64	1.4	0.93	4.2
C	0.59	1.3	0.69	3.9
Strong:				
S	0.50	**2.1**	**0.93**	4.1
SL	0.46	2.0	0.93	4.1
C	0.42	1.8	0.69	3.8

[a] Depth 2–9 m. * A. = *Acropora*; *E.* = *Echinopora*; ***n* = No. measurements; *** Boldface type indicates the actual count and density values closest to the actual count; S = straight line measurement; SL = slack line measurement; C = contour measurement

Table 4 Density of subtidal corals at Pulau Pemanggil (April 1999) and Pulau Langkawi (October 1999), Malaysia[a]

Sampling method	Pulau Pemanggil No. 1 Density (No./m^2) *Lobophyton* sp.* *n* = 15**	Pulau Pemanggil No. 2. Density (No./m^2) *Porites* sp. *n* = 12	Pulau Langkawi No. 1 Density (No./m^2) *Porites* sp. *n* = 14	Pulau Langkawi No. 2 Density (No./m^2) *Favia* sp. *n* = 15	Pulau Langkawi No. 3 Density (No./m^2) *Favia* sp. *n* = 14	Pulau Langkawi No. 4 Density (No./m^2) *Porites* sp. *n* = 14	Pulau Langkawi No. 5 Density (No./m^2) *Porites* sp. *n* = 14	Pulau Langkawi No. 6 Density (No./m^2) *Favia* sp. *n* = 14
Actual count:								
Straight	**3.13*****	**2.27**	**3.27**	**3.53**	**5.27**	**2.80**	**4.27**	**5.27**
Slack	**2.87**	**2.22**	**3.11**	**3.37**	**4.68**	**2.59**	**4.13**	**5.10**
Contour	**2.63**	**2.14**	**2.74**	**2.96**	**4.34**	**2.31**	**3.66**	**4.51**
Weinberg:								
Straight	**3.18***	**2.16**	1.96	2.33	2.23	**2.07**	1.94	2.51
Slack	2.91	2.12	1.87	2.22	1.98	1.92	1.87	2.43
Contour	2.67	2.04	1.65	1.95	1.84	1.71	1.66	2.15
Strong:								
Straight	7.15	2.69	**3.14**	**3.56**	**3.11**	3.90	**3.86**	**3.25**
Slack	6.54	2.64	2.99	3.39	2.76	3.61	3.74	3.12
Contour	5.99	2.54	2.63	2.98	2.56	3.21	3.31	2.78

[a] Depth 0.5–9 m. * *Lobophyton* sp. is a soft coral. *Porites* and *Favia* are hard corals. ***n* = No. measurements; ***Boldface type indicates the actual count and density values closest to the actual count. (15 m^2 study area each)

Table 5 Density of intertidal invertebrates at Royal Palms State Beach, Palos Verdes Peninsula, southern California in February and March 2001[a]

Sampling method	*Mytilus californianus* Conrad, 1837 mussel (No./ m^2)	*Collisella scabra* (Gould, 1846) limpet (No./m^2)	*Collisella strigitella* (Carpenter, 1864) limpet (No./m^2)
Actual count (32 m^2 study area)	**53.8***	**23.1**	**17.9**
Stratified random sampling	81.3 ± 18.8**	36.2 ± 8.3	29.0 ± 4.5
	n = 15	n = 15	n = 15
3rd Nearest Object	6.2 ± 4.2	11.0 ± 2.2	7.5 ± 0.6
	n = 300	n = 300	n = 300
Point-Center Quarter	6.6 ± 3.9	13.9 ± 2.8	10.7 ± 0.89
	n = 300	n = 300	n = 300
Weinberg			
S	25.1 ± 5.7	**20.8 ± 3.0**	10.2 ± 1.7
SL	24.7	20.5	9.8
C	22.0	18.4	9.1
	n = 30	n = 12	n = 10
Strong			
S	**43.7 ± 10.0**	31.0 ± 6.4	**18.5 ± 5.4**
SL	41.7	28.9	18.2
C	40.0	27.4	16.4
	n = 30	n = 12	n = 10
	M. californianus	*C. scabra*	*C. strigitella*
Morisita index of dispersion =	3.31	1.75	2.41
Standardized Morisita index of dispersion =	0.52	0.51	0.51

[a] *Boldface type indicates the actual count and density values closest to the actual count. **± = standard error of mean density values; n = No. measurements; S = straight line measurement; SL = slack line measurement; C = contour measurement

Table 6 Density of the subtidal sponge *Polymastia janeirensis* (Boury-Esnault, 1993) from Praia Brava, Buzios, Brazil in March 2001[a]

Sampling method	No. measurements	Density (No./m^2)
Actual Count	100 m^2 study area	**1.7***
Stratified random sampling	50 (0.25 m^2 quadrats)	5.8 ± 0.2**
Point-Center quarter	200 (50 random points × 4)	0.7 ± 0.1
3rd Nearest object	50	**1.6 ± 0.5**
Weinberg	30	1.2 ± 0.2
Strong	30	2.0 ± 0.6

[a] Depth 3 m. Source: Bakus et al. (2004). *Boldface type indicates actual count and density values closest to the actual count; **± = standard error of mean density values

Morisita index of dispersion = 1.29

Standardized Morisita index of dispersion = 0.49

Table 7 Density of Ponderosa Pine (*Pinus ponderosa* Laws.) and Coulter Pine (*Pinus coulteri D. Don*) trees at Charleton Flats, Angeles National Forest, San Gabriel Mountains, southern California, in April and May 2001[a]

Sampling method	No. measurements	Pine tree density (No./500 m^2)
Actual count	10000^2 m study area (100 × 100 m)	**4.8***
Stratified random sampling	50 (10 × 10 m quadrats)	7.5 ± 1.4**
Third nearest object	90	2.1 ± 0.47
Point-Center quarter	360 (90 random points × 4)	2.4 ± 0.39
Weinberg	42	**3.1 ± 0.32**
Strong	42	2.6 ± 0.32

[a] Elevation 1616 m.*Boldface type indicates the actual count and density values closest to the actual count. **± = standard error of mean density values

Morisita index of dispersion = 1.61

Standardized Morisita index of dispersion = 0.45

Table 8 Density of trees in Alhambra Park, Alhambra, California in May 2001[a]

Sampling method	No. measurements	Tree density (No./500 m^2)
Actual count	2601 m^2 study area (51 × 51 m)	**2.9***
Stratified random sampling	12	3.2 ± 0.18**
Third nearest object	18	2.1 ± 0.37
Point-Center quarter	44	2.6 ± 0.18
Weinberg	15	2.2 ± 0.42
Strong	15	**2.7 ± 0.51**

[a] City park—Elevation 152 m. *Boldface type indicates the actual count and density values closest to the actual count. **± = standard error of mean density values

Morisita index of dispersion = 0.44

Standardized Morisita index of dispersion = −0.39

Table 9 Density of white fir trees (*Abies concolor* [Gordon & Glend.] Lindley) on Mt. Ashland, southern Oregon in June 2001 and June 2002[a]

Sampling method	No. measurements	Fir tree density (No./500 m^2)	
		2001	2002
Actual count 2001	900 m^2 study area (30 m × 30 m)	**52***	
Actual count 2002	600 m^2 study area (20 m × 30 m)		**27**
Stratified random sampling	6	70 ± 43**	17 ± 0.2
Third nearest object	49 (in 2001) 25 (in 2002)	**46 ± 1.9**	**18 ± 0.01**
Point-Center quarter	60	42 ± 9.6	38 ± 1.5
Weinberg	51	44 ± 3.7	13 ± 0.08
Strong	51	40 ± 1.4	13 ± 0.04
Morisita index of dispersion =		1.34	1.10
Standardized Morisita index of dispersion =		0.44	0.09

[a] Elevation 1890 m. *Boldface type indicates the actual count and density values closest to the actual count. **± = standard error of mean density values

Table 10 Density of Joshua trees (*Yucca brevifolia* Engelm.) and Mormon Tea (*Ephydra? nevadensis* S. Watson) in Joshua Tree National Park, southern California, in February and March 2002[a]

Sampling method	*Yucca brevifolia* Tree (No./500 m^2)	*Ephydra ?nevadensis* Shrub (No./500 m^2)
Actual count	**3.5****	**35.8**
Stratified random sampling	**3.6** ± **0.08****	**37.0** ± **0.28**
	n = 25	*n* = 25
Third nearest object	1.6± 0.4	21.7± 0.85
	n = 60	*n* = 60
Point-Center quarter	2.6 ± 0.66	26.7 ± 8.8
	n = 240	*n* = 240
Weinberg	6.4± ***	32.8 ± 17.5
	n = 4	*n* = 33
Strong	4.8± ***	38.2 ± 9.3
	n = 4	*n* = 33
	Yucca	*Ephydra*
Morisita index of dispersion =	1.96	1.10
Standardized Morisita index of dispersion =	0.50	0.50

[a] Elevation 680 m. *Boldface type indicates the actual count and density values closest to the actual count. **± = standard error of mean density values. ***standard error of the mean cannot be calculated because of either no data (i.e., plant absent) or of single measurements for each group. *n* = No. measurements

Table 11 A simulation study on the density of circular dots in different distribution patterns within rectangular sampling plots (from Krebs, 1999), December 2002[a]

Sampling method	Uniform (No./m^2)	Random (No. /m^2)	Aggregated (No./m^2)
Actual count	**3290****	**3300**	**3077**
Stratified random sampling	**2742** ± **17**	2376 ± 36	3746 ± 61
	n = 8	*n* = 8	*n* = 8
Third nearest object	4446 ± 132**	**3475** ± **478**	2452 ± 264
	n = 42	*n* = 42	*n* = 42
Point-Center quarter	4190 ± 1381	1998 ± 481	1242 ± 319
	n = 112	*n* = 112	*n* = 110
Weinberg	6116 ± 1018	4407 ± 303	**2691** ± **205**
	n = 48	*n* = 33	*n* = 20
Strong	5102 ± 850	3523 ± 222	2551 ± 153
	n = 48	*n* = 33	*n* = 20
Sampling method	Small clumps (No./m^2)	Large clumps individuals uniform (No./m^2)	Large clumps individuals random (No./m^2)
Actual count	1400	4690	2090
Stratified random sampling	2016 ± 150	4063 ± 117	1356 ± 107
	n = 8	*n* = 8	*n* = 8
Third nearest object	999 ± 522	3193 ± 895	758 ± 155
	n = 42	*n* = 42	*n* = 42
Point-Center quarter	445 ± 170	2602 ± 1166	5206 ± 276
	n = 101	*n* = 110	*n* = 106
Weinberg	1751 ± 334	**5071** ± **420**	3237 ± 445
	n = 17	*n* = 38	*n* = 28
Strong	**1494** ± **271**	4258 ± 395	**2584** ± **154**
	n = 17	*n* = 38	*n* = 28

[a] * Boldface type indicates actual count and density values closest to the actual count. **± = standard error of mean density values. *n* = No. measurements

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