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Measurements of cnidae from sea anemones (Cnidaria: Actiniaria), II: further studies of differences amongst sample means and their taxonomic relevance*

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SUMMARY: Lengths of cnidae, sampled from fresh tissue (tentacles, acontia or column) from the sea anemones *Metridium senile* (Linnaeus), *Cereus pedunculatus* (Pennant), *Sagartia elegans* (Dalyell), *Sagartia troglodytes* (Price), *Anthopleura thallia* (Gosse) and *Urticina eques* (Gosse), were measured. The mean, mode, median, standard deviation, variance, coefficient of variation, minimum, maximum and range of each sample were calculated. Lengths of nematocysts (basitrichs and microbasic *p*-mastigophores) and spirocysts had normal (Gaussian) frequency distributions, and there was no important within-sample variability. A between-sample (same specimen) difference was detected between mean lengths of microbasic *p*-mastigophores from acontia in *Sagartia elegans*. Intraspecific (between-specimen) differences amongst mean lengths of basitrichs from column ectoderm were detected amongst four specimens of *Urticina eques*, and a difference occurred between the mean lengths of microbasic *p*-mastigophores from the acontia of two specimens of *Sagartia elegans*. However, for each of the species *U. eques* and *S. elegans*, there was no direct correlation between the sizes of the anemones examined and the mean lengths of their nematocysts. Overall, the results confirm that cnida size, when taken in isolation, is not a reliable taxonomic character for sea anemones. For other purposes, mean cnida measurements may be useful and a rapid sample range test is suggested for analysing differences between them. Methods are also presented for estimating means and standard deviations from data in publications that provide only ranges of cnida sizes and the number of cnidae measured. The present results and conclusions supplement those of Williams (1996, *Scientia Marina*, 60: 339-351).

Key words: Actiniaria, cnidae, measurements, protocol, samples, statistics, taxonomy.

INTRODUCTION

The Cnidaria possess tiny intracellular capsules called cnidae (Mariscal, 1974). They serve a variety of purposes in the lives of cnidarians and comprise three main types: nematocysts, spirocysts and ptychocysts (Williams, 1996). Nematocysts are further subdivided into about 25 types (Mariscal, 1974). An inventory of all the cnida types present in the tissues of a cnidarian is known as its cnidome (Weill, 1926), but no information on cnida sizes is implicit in this definition. Long before the concept of a cnidome and a comprehensive classification of cnidae were first described by Weill (1926, 1934a, 1934b), it was suggested by Carlgren (1900) that any description of a sea anemone (order Actiniaria) should not be considered to be complete without the inclusion of measurements of its cnidae. Most authors have subsequently followed this convention, generally in uncritical fashion. However, the originator of the first cnida classification system considered cnida sizes to be of little taxonomic value (Weill, 1934b: 637).

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Admittedly, since cnidae are diagnostic of the Cnidaria, it would seem that descriptions of them should be useful in the identification and classification of species in this phylum; but whether these descriptions should include sizes as well as types of cnidae is still a moot point. The taxonomic relevance both of cnidomes and cnida measurements, particularly for Actiniaria, was challenged by Fautin (1988), who pointed out that prominence seems to have been ascribed to cnidae by default rather than through any demonstrated utility. Whilst it is possible that quantitative treatment of cnidae might improve their value as a taxonomic character, it has been difficult to test this hypothesis because methods of gathering appropriate data have generally been inadequately described and, until recently, very little was known of the statistical parameters of cnida measurements. In retrospect, it is surprising that so little emphasis has been placed upon the methods for gathering cnida measurements by authors who have used such data for taxonomic work.

A statistical study of actiniarian cnidae (Williams, 1996) has now revealed that the frequency distributions of random samples of lengths of cnidae of a single type are normal (Gaussian), facilitating the valid use of parametric significance tests to compare mean lengths of samples of cnidae. The methods were described fully and the crucial requirements of a protocol for biometric studies of cnidae were proposed. It was concluded that mean cnida sizes cannot be used in isolation from other taxonomic characters to differentiate species, because statistically significant differences may occur between the means of replicate samples from the same specimen, and of samples from different specimens of the same species.

Further work on actiniarian cnidae using a standard protocol is reported here, since the relevance of cnida sizes to taxonomy is so controversial (Fautin, 1988) and because validly collected data are still so sparse (Williams, 1996). The objectives of the present studies were: 1) to amass more data from species of sea anemones exhibiting a variety of reproductive methods, in order to supplement the evidence for statistically normal frequency distributions of cnida measurements, 2) to examine further the statistical parameters of cnida lengths within single tissue samples, 3) to examine further the differences between mean lengths of cnidae in samples from the same specimen, and 4) to examine further the differences between mean lengths of cnidae in samples from conspecific specimens of various sizes. The results expand existing knowledge of cnida biometrics, providing further frequency distributions for more cnida types from additional anemone species, and thus increase confidence in previous conclusions (Williams, 1996).

MATERIAL AND METHODS

Localities whence anemones were collected were recorded. The oral disk diameter (ODD) or pedal disk diameter (PDD) of each expanded anemone was measured to the nearest millimetre. Details of specimens examined and their sources are given with the relevant results. The methods of obtaining independent, random, homogeneous samples of cnida measurements from standard tissue samples taken from live anemones, and the various statistical analyses used, followed those established by Williams (1996).

The lengths of usually at least 40 cnidae of a predetermined type were measured in each tissue sample, as recommended by Williams (1996). Only undischarged cnidae were measured, because the sizes of capsules are reduced after discharge (e.g., Godknecht and Tardent, 1988). Measurements were recorded, in subsets of five, in the random order in which they were obtained. All results were recorded as microscope eyepiece-graticule divisions to simplify data handling.

Statistical analyses were carried out on the raw data, converting the means and other parameters to μm only after analysis, in order to avoid possible multiple errors of transcription or conversion. The analyses were carried out using MINITAB Statistical Software, Release 8. The particular statistical tests used are described and justified in the appropriate places in the Results section. The preselected significance level for rejection of null hypotheses for inferential statistical tests was 5% (P=0.05).

RESULTS

Descriptive statistics of samples of cnida lengths

Tests for normality

These results comprise lengths of spirocysts or nematocysts in either of two tissue types (tentacle or acontium) taken from live specimens of the five

species of anemone examined. The lengths of only one of the types of cnida in each tissue sample were measured. The datasets analysed were as follows:

a) Dataset METRID1: lengths of spirocysts from one tentacle tip of a specimen of *Metridium senile* (Linnaeus) with a 15 mm ODD from Newton Ferrers, Devon, UK (Table 1).

b) Dataset CEREUS1: lengths of microbasic *p*-mastigophore nematocysts from one tentacle tip of a specimen of *Cereus pedunculatus* (Pennant) with a 26 mm ODD from Newton Ferrers, Devon, UK (Table 2).

c) Dataset SAGART6: lengths of basitrich nematocysts from the free end of an acontium from a speci-

TABLE 1. – Statistical parameters of dataset METRID1, which comprises lengths of spirocysts from a tentacle tip of *Metridium senile*. SD= sample standard deviation; CoV = coefficient of variation (%). All parameters except *n* and CoV in μm (accuracy ± 0.83 μm). P = probability associated with normality test.

Subset	*n*	*Mean*	*Mode*	*Median*	*SD*	*Variance*	*CoV*	*Min*	*Max*	*Range*	*P*
1	5	16.4	16.7	16.7	0.75	0.56	4.6	15.1	16.7	1.6	<0.01
2	5	16.9	18.4	17.5	2.07	4.28	12.3	13.4	18.4	5.0	<0.01
3	5	12.7	-	13.4	2.54	6.45	20.0	10.0	15.1	5.1	<0.01
4	5	15.2	16.7	16.7	2.24	5.02	14.7	11.7	16.7	5.0	<0.01
5	5	14.0	15.0	15.0	2.54	6.45	18.1	10.0	16.7	6.7	<0.01
6	5	14.9	15.0	15.0	1.90	3.61	12.8	11.7	16.7	5.0	<0.01
7	5	18.4[a]	20.0	18.4	1.77	3.13	9.6	15.9	20.0	4.1	<0.01
8	5	14.9	-	15.0	2.79	7.78	18.8	11.7	18.4	6.7	<0.01
9	5	14.5	-	15.0	2.54	6.45	17.5	10.9	17.5	6.6	<0.01
10	5	16.0	16.7	16.7	3.04	9.24	19.0	11.7	20.0	8.3	<0.01
1	5	16.4	16.7	16.7	0.75	0.56	4.6	15.1	16.7	1.6	<0.01
1-2	10	16.7	16.7	16.7	1.50	2.25	9.0	13.4	18.4	5.0	<0.01
1-3	15	15.3	16.7	16.7	2.64	6.97	17.2	10.0	18.4	8.4	<0.01
1-4	20	15.3	16.7	16.7	2.49	6.20	16.3	10.0	18.4	8.4	<0.01
1-5	25	15.0	16.7	15.0	2.49	6.20	16.6	10.0	18.4	8.4	<0.01
1-6	30	15.0	16.7	15.0	2.37	5.62	15.8	10.0	18.4	8.4	<0.01
1-7	35	15.5	16.7	15.9	2.57	6.60	16.6	10.0	20.0	10.0	<0.01
1-8	40	15.4	16.7	15.0	2.57	6.60	16.7	10.0	20.0	10.0	<0.01
1-9	45	15.3	16.7	15.0	2.56	6.55	16.7	10.0	20.0	10.0	<0.01
1-10	50	15.4	16.7	15.4	2.59	6.71	16.8	10.0	20.0	10.0	<0.01

[a] Statistically significantly different (P<0.05) from overall mean (15.4 μm) of whole dataset.

TABLE 2. – Statistical parameters of dataset CEREUS1, which comprises lengths of microbasic *p*-mastigophore nematocysts from a tentacle tip of *Cereus pedunculatus*. SD = sample standard deviation; CoV = coefficient of variation (%). All parameters except *n* and CoV in μm (accuracy ± 0.83 μm). P = probability associated with normality test. There were no statistically significant differences between the means of the independent subsets of 5 and the overall mean (19.5 μm) of the whole dataset.

Subset	*n*	*Mean*	*Mode*	*Median*	*SD*	*Variance*	*CoV*	*Min*	*Max*	*Range*	*P*
1	5	19.0	20.0	20.0	1.49	2.22	7.9	16.7	20.0	3.3	<0.01
2	5	20.4	20.0	20.0	0.46	0.21	2.2	20.0	20.9	0.9	<0.01
3	5	20.2	20.0	20.0	0.92	0.85	4.5	19.2	21.7	2.5	<0.01
4	5	19.7	20.0	20.0	0.95	0.90	4.8	18.4	20.9	2.5	<0.01
5	5	19.4	20.0	20.0	1.37	1.88	7.1	17.5	20.9	3.4	<0.01
6	5	19.4	-	19.2	0.70	0.49	3.6	18.4	20.0	1.6	<0.01
7	5	19.5	19.2	19.2	0.46	0.21	2.4	19.2	20.0	0.8	<0.01
8	5	19.7	-	19.2	2.01	4.04	10.2	17.5	22.5	5.0	<0.01
9	5	19.2	-	19.2	1.67	2.79	8.7	17.5	20.9	3.4	<0.01
10	5	18.9	20.0	19.2	1.40	1.96	7.4	16.7	20.0	3.3	<0.01
1	5	19.0	20.0	20.0	1.49	2.22	7.9	16.7	20.0	3.3	<0.01
1-2	10	19.7	20.0	20.0	1.26	1.59	6.4	16.7	20.9	4.2	<0.01
1-3	15	19.9	20.0	20.0	1.15	1.32	5.8	16.7	21.7	5.0	<0.01
1-4	20	19.8	20.0	20.0	1.08	1.17	5.4	16.7	21.7	5.0	<0.01
1-5	25	19.7	20.0	20.0	1.13	1.28	5.7	16.7	21.7	5.0	<0.01
1-6	30	19.7	20.0	20.0	1.07	1.14	5.4	16.7	21.7	5.0	<0.01
1-7	35	19.7	20.0	20.0	1.00	1.00	5.1	16.7	21.7	5.0	<0.01
1-8	40	19.7	20.0	20.0	1.13	1.28	5.7	16.7	22.5	5.8	<0.01
1-9	45	19.6	20.0	20.0	1.19	1.42	6.1	16.7	22.5	5.8	<0.01
1-10	50	19.5	20.0	20.0	1.22	1.49	6.2	16.7	22.5	5.8	<0.01

TABLE 3. – Statistical parameters of dataset SAGART6, which comprises lengths of basitrich nematocysts from the end of an acontium of *Sagartia elegans*. SD = sample standard deviation; CoV = coefficient of variation (%). All parameters except *n* and CoV in μm (accuracy ± 0.46 μm). P = probability associated with normality test. There were no statistically significant differences between the means of any independent subsets of 5 and the overall mean (30.5 μm) of the whole dataset.

Subset	*n*	*Mean*	*Mode*	*Median*	*SD*	*Variance*	*CoV*	*Min*	*Max*	*Range*	*P*
1	5	30.0	-	31.3	2.22	4.93	7.4	27.6	32.2	4.6	<0.01
2	5	28.7	-	28.6	1.51	2.28	5.3	27.6	31.3	3.7	<0.01
3	5	29.1	26.7	26.7	3.30	10.89	11.3	26.7	33.2	6.5	<0.01
4	5	30.4	31.3	31.3	3.33	11.09	10.9	26.7	35.0	8.3	<0.01
5	5	30.9	30.4	30.4	1.40	1.96	4.5	29.5	33.2	3.7	<0.01
6	5	30.9	-	31.3	1.91	3.65	6.2	27.6	32.2	4.6	<0.01
7	5	32.1	-	32.2	0.77	0.59	2.4	31.3	33.2	1.9	<0.01
8	5	29.1	-	28.6	3.09	9.55	10.6	25.8	33.2	7.4	<0.01
9	5	31.9	32.2	32.2	2.49	6.20	7.8	27.6	34.1	6.5	<0.01
10	5	31.3	-	31.3	2.35	5.52	7.5	28.6	34.1	5.5	<0.01
1	5	30.0	-	31.3	2.22	4.93	7.4	27.6	32.2	4.6	<0.01
1-2	10	29.4	27.6	28.6	1.91	3.65	6.5	27.6	32.2	4.6	<0.01
1-3	15	29.3	27.6	28.6	2.34	5.48	8.0	26.7	33.2	6.5	<0.01
1-4	20	29.6	-	28.6	2.57	6.60	8.7	26.7	35.0	8.3	<0.01
1-5	25	29.8	31.3	30.4	2.42	5.86	8.1	26.7	35.0	8.3	<0.01
1-6	30	30.0	31.3	30.9	2.35	5.52	7.8	26.7	35.0	8.3	<0.01
1-7	35	30.3	31.3	31.3	2.30	5.29	7.6	26.7	35.0	8.3	<0.01
1-8	40	30.2	31.3	31.3	2.40	5.76	8.0	25.8	35.0	9.2	<0.01
1-9	45	30.4	31.3	31.3	2.44	5.95	8.0	25.8	35.0	9.2	<0.01
1-10	50	30.5	31.3	31.3	2.43	5.90	8.0	25.8	35.0	9.2	<0.01

men of *Sagartia elegans* (Dalyell) with a 9 mm PDD from Port Eynon, Gower Peninsula, Wales, UK (Table 3).

d) Dataset SAGART7: lengths of basitrich nematocysts from the free end of an acontium from a specimen of *Sagartia troglodytes* (Price) with a 15 mm PDD from Port Eynon, Gower Peninsula, Wales, UK (Table 4).

e) Dataset ANTHO1: lengths of basitrich nematocysts from one tentacle tip of a specimen of *Anthopleura thallia* (Gosse)* with a 4 mm PDD from Mijas Costa, Málaga, Spain (Table 5).

The following parameters were recorded for each dataset: mean, mode, median, standard deviation, variance, coefficient of variation, minimum, maximum and range. First they were calculated for each of the random, independent subsets of five lengths; then for the progressively accumulated subsets, viz., the first five lengths, the first ten, the first 15, and so on up to the whole sample of 40 or 50. Dotplots of the frequency distributions of all these subsets, both independent and accumulated, were prepared as described by Williams (1996): all appeared to represent approximately normal frequency distributions.

The normal scores (MINITAB command NSCORES) were then calculated, based on the distribution function of the standard normal, for each of the subsets of five and the progressively accumulated subsets as before. Then by linear regression of the raw data on their normal scores, a correlation test for normality of each of the subsets was carried out. A significance level of $P \leq 0.05$ indicates a normal frequency distribution (see Tables 1-5). This procedure is equivalent to the Shapiro-Wilk test, using the table of critical correlation values in Exhibit 5.1 (Chapter 4) of the MINITAB Reference Manual (Release 8). For a normal frequency distribution, the association should be linear and the normality plots were examined to confirm this because, when <15 points are involved, the correlation test may sometimes spuriously indicate a normal distribution even when the normality plot has a sigmoid shape and the dotplot indicates a bimodal distribution (Williams, 1996). The probabilities shown in Tables 1-5, and examination of the dotplots and normality plots (not shown), indicated that all the frequency distributions of the subsets and the cumulative data were normal (Gaussian).

Statistical parameters and within-sample homogeneity

The descriptive statistics of *Metridium senile* spirocysts, *Cereus pedunculatus* microbasic *p*-

* This appears to be the first authentic record of *Anthopleura thallia* from the Mediterranean Sea (36°31'N.4°44'W, 6 March 1992). Although Carlgren (1949) listed this species as having been recorded from the Mediterranean, he seems to have been in error, since none of the references that he cited mentions *A. thallia* from the Mediterranean. Neither do any other non-derivative publications, either before or after 1949, that I have consulted. Further details of this new record will be published elsewhere.

TABLE 4. – Statistical parameters of dataset SAGART7, which comprises lengths of basitrich nematocysts from the end of an acontium of *Sagartia troglodytes*. SD = sample standard deviation; CoV = coefficient of variation (%). All parameters except *n* and CoV in μm (accuracy ± 0.46 μm). P = probability associated with normality test. There were no statistically significant differences between the means of any independent subsets of 5 and the overall mean (15.4 μm) of the whole dataset.

Subset	*n*	*Mean*	*Mode*	*Median*	*SD*	*Variance*	*CoV*	*Min*	*Max*	*Range*	*P*
1	5	14.9	14.7	14.7	1.37	1.88	9.2	12.9	16.6	3.7	<0.01
2	5	15.7	15.7	15.7	0.00	0.00	0.0	15.7	15.7	0.0	<0.01
3	5	15.2	14.7	14.7	0.80	0.64	5.2	14.7	16.6	1.9	<0.01
4	5	16.1	-	16.1	1.03	1.06	6.4	14.7	17.5	2.8	<0.01
5	5	14.7	13.8	14.3	1.16	1.35	7.9	13.8	16.6	2.8	<0.01
6	5	16.1	-	16.1	1.66	2.76	10.3	13.8	18.4	4.6	<0.01
7	5	15.7	14.7	14.7	1.84	3.39	11.7	13.8	18.4	4.6	<0.01
8	5	15.1	-	15.2	0.89	0.79	5.9	13.8	16.1	2.3	<0.01
1	5	14.9	14.7	14.7	1.37	1.88	9.2	12.9	16.6	3.7	<0.01
1-2	10	15.3	15.7	15.7	0.99	0.98	6.5	12.9	16.6	3.7	<0.01
1-3	15	15.3	15.7	15.7	0.90	0.81	5.9	12.9	16.6	3.7	<0.01
1-4	20	15.5	15.7	15.7	0.98	0.96	6.4	12.9	17.5	4.6	<0.01
1-5	25	15.3	15.7	15.7	1.04	1.08	6.8	12.9	17.5	4.6	<0.01
1-6	30	15.5	15.7	15.7	1.17	1.37	7.6	12.9	18.4	5.5	<0.01
1-7	35	15.5	-	15.7	1.25	1.56	8.1	12.9	18.4	5.5	<0.01
1-8	40	15.4	-	15.7	1.21	1.46	7.9	12.9	18.4	5.5	<0.01

TABLE 5. – Statistical parameters of dataset ANTHO1, which comprises lengths of basitrich nematocysts from a tentacle tip of *Anthopleura thallia*. SD = sample standard deviation; CoV = coefficient of variation (%). All parameters except *n* and CoV in μm (accuracy ± 0.46 μm). P = probability associated with normality test. There were no statistically significant differences between the means of any independent subsets of 5 and the overall mean (15.5 μm) of the whole dataset.

Subset	*n*	*Mean*	*Mode*	*Median*	*SD*	*Variance*	*CoV*	*Min*	*Max*	*Range*	*P*
1	5	15.5	15.7	15.7	0.41	0.17	2.7	14.7	15.7	1.0	<0.01
2	5	16.9	16.6	16.6	1.05	1.10	6.2	15.7	18.4	2.7	<0.01
3	5	15.3	14.7	14.7	1.58	2.50	10.3	13.8	18.0	4.2	<0.01
4	5	14.9	-	15.2	1.33	1.77	8.9	13.4	16.6	3.2	<0.01
5	5	14.6	14.7	14.7	0.53	0.28	3.6	13.8	15.2	1.4	<0.01
6	5	16.0	16.6	16.6	1.40	1.96	8.7	13.8	17.5	3.7	<0.01
7	5	15.0	15.7	15.2	0.77	0.59	5.1	13.8	15.7	1.9	<0.01
8	5	15.5	16.6	15.7	1.20	1.44	7.7	13.8	16.6	2.8	<0.01
1	5	15.5	15.7	15.7	0.41	0.17	2.7	14.7	15.7	1.0	<0.01
1-2	10	16.2	15.7	15.7	1.08	1.17	6.7	14.7	18.4	3.7	<0.01
1-3	15	15.9	15.7	15.7	1.29	1.66	8.1	13.8	18.4	4.6	<0.01
1-4	20	15.7	15.7	15.7	1.34	1.80	8.5	13.4	18.4	5.0	<0.01
1-5	25	15.4	15.7	15.2	1.29	1.66	8.4	13.4	18.4	5.0	<0.01
1-6	30	15.5	15.7	15.7	1.30	1.69	8.4	13.4	18.4	5.0	<0.01
1-7	35	15.5	15.7	15.7	1.25	1.56	8.0	13.4	18.4	5.0	<0.01
1-8	40	15.5	15.7	15.7	1.23	1.51	7.9	13.4	18.4	5.0	<0.01

mastigophores, and *Sagartia elegans*, *Sagartia troglodytes* and *Anthopleura thallia* basitrichs are shown in Tables 1-5, respectively. The data possess characteristics similar to those presented for other cnidae and anemone species by Williams (1996: Tables 1-3). Thus, the means and medians agreed fairly closely, but the mode was not generally a useful parameter, particularly in the small, independent subset samples. In the accumulated subsets, the means and the sample standard deviations stabilized within 10-15 observations, whilst the ranges stabilized within 20-40 observations (Tables 1-5). Hence, these results support the conclusion of Williams (1996) that measuring ≥40 cnidae provides reliable estimates of the mean and range of cnida lengths in a single tissue sample. Furthermore, this conclusion applies equally to spirocysts (Table 1) and nematocysts (Tables 2-5).

One-way analysis of means revealed no statistically significant differences between the mean nematocyst lengths in the independent subsets and the overall mean for each dataset (Tables 2-5),

TABLE 6. – Statistical parameters of datasets SAGART2 and SAGART3, which comprise lengths of microbasic *p*-mastigophore nematocysts from the ends of two acontia from the same specimen of *Sagartia elegans*. SD = sample standard deviation; CoV = coefficient of variation (%). All parameters except *n* and CoV in μm (accuracy ± 0.46 μm). P = probability associated with normality test.

Dataset	*n*	*Mean*	*Mode*	*Median*	*SD*	*Variance*	*CoV*	*Min*	*Max*	*Range*	*P*
SAGART2	50	57.7[a]	62.6	60.6	6.90	47.59	12.0	46.1	67.2	21.1	<0.01
SAGART3	50	52.8[a]	46.1	54.3	6.07	36.79	11.5	43.8	62.6	18.8	<0.01

[a] Statistically significantly different from each other by *t*-test (P=0.0003).

showing that these samples were homogeneous. Amongst the spirocysts of *Metridium senile* (Table 1), the mean of only one of the ten subsets was statistically significantly different at P<0.05 from the overall mean. The results of these analyses are in accord with the previous finding (Williams, 1996) that there tends to be no important within-sample heterogeneity of mean cnida lengths.

Inferential statistics of samples of cnida lengths in replicate tissue samples from the same specimen

Samples of acontia from a single anemone were taken in order to assess the variability of mean cnida lengths within and between samples. The datasets were SAGART2 and SAGART3, the lengths of microbasic *p*-mastigophore nematocysts from the free ends of two different acontia from a specimen of *Sagartia elegans* with a 25 mm PDD, trawled from the Fairlie Roads off the Isle of Cumbrae, UK (Table 6).

First of all, the two datasets were analysed in the same way as the data in Tables 1-5. All the subsets and both of the full datasets had approximately normal frequency distributions, as revealed by the dot-plots and normality plots (not shown) and the correlation tests for normality (Table 6). When the differences between the means of the subsets within each dataset were analysed, no within-sample heterogeneity was detected. To confirm that there was no difference between the variabilities of the datasets, an F-test (two-tailed) for equality of two variances (Snedecor and Cochran, 1967) was applied, giving F=1.29 (49, 49 d.f.) so the null hypothesis was accepted. A subsequent comparison of the means of SAGART2 and SAGART3 by a *t*-test using a pooled standard deviation showed that they were significantly different at P=0.0003 (Table 6).

There was, therefore, significant between-sample heterogeneity within the same anemone, as previously shown for cnida samples from the tentacles of *Urticina eques* and the column ectoderm of *Sagartiogeton laceratus* by Williams (1996).

Inferential statistics of samples of cnida lengths in tissue samples from different specimens of the same species

Single tissue samples were taken from each of four anemones to assess any differences between mean cnida lengths amongst specimens of the same species, in this case *Urticina eques* (Gosse). The datasets were URTIC2, URTIC3, URTIC4 and URTIC5, comprising the lengths of basitrich nematocysts from the column ectoderm, just above the limbus, of four specimens with respective PDDs of 30, 150, 37 and 12 mm, trawled simultaneously from the Fairlie Roads off the Isle of Cumbrae, UK (Table 7).

The four datasets were initially analysed in the same way as the data from other species in Tables 1-6. All the subsets and the full datasets of URTIC3, URTIC4 and URTIC5 had approximately normal

TABLE 7. – Statistical parameters of datasets URTIC2, URTIC3, URTIC4, URTIC5, which comprise lengths of basitrich nematocysts from just above the limbus of four specimens of *Urticina eques*. PDD = pedal disk diameter (mm); SD = sample standard deviation; CoV = coefficient of variation (%) . All parameters except PPD, *n* and CoV in μm (accuracy ± 0.46 μm). P = probability associated with normality test.

Dataset	*PDD*	*n*	*Mean*	*Mode*	*Median*	*SD*	*Variance*	*CoV*	*Min*	*Max*	*Range*	*P*
URTIC2	30	50	16.7[a]	18.4	17.5	2.37	5.60	14.2	12.4	20.7	8.3	0.05
URTIC3	150	50	19.5[a]	19.3	19.3	0.81	0.66	4.2	18.0	22.1	4.1	<0.01
URTIC4	37	50	18.1	18.4	18.4	0.78	0.60	4.3	16.6	19.8	3.2	<0.01
URTIC5	12	50	17.9	18.4	18.0	1.14	1.29	6.4	15.2	20.3	5.1	<0.01

[a] Statistically significantly different (P<0.0001) from each other and from the overall mean (18.05 μm) of all four datasets.

frequency distributions, as revealed by the dotplots and normality plots (not shown), and the correlation tests for normality (Table 7). However, the dotplots of the accumulated subsets of URTIC2 were somewhat suggestive of a bimodal distribution, although the peaks were linked by intervening points. Furthermore, one of the ten independent subsets was not normal by the Shapiro-Wilk test approximation; and the last six of the accumulated subsets were also not normally distributed. The whole dataset just failed to show a normal distribution at P=0.05 (Table 7). Nevertheless, since these departures from normality were only just significant, analysis of means was still carried out on this dataset, as well as the other three. The test is robust and relatively insensitive to slight-to-moderate deviations of the data from normality (Ryan, 1989). No differences amongst the means of the subsets within any of the datasets were detected, confirming the lack of any important within-sample heterogeneity.

Subsequent analysis of means of the four complete datasets revealed some highly significant differences (Table 7). There was, therefore, considerable between-specimen heterogeneity amongst the mean cnida lengths from the column ectoderm of the four specimens of *Urticina eques*. However, no overall direct correlation existed between the sizes of the anemones and the mean lengths of their cnidae (Fig. 1). The largest (URTIC3; PDD 150 mm) and smallest (URTIC5; PDD 12 mm) specimens possessed the largest and smallest cnidae, respectively, with a statistically significant difference between mean lengths of 1.6 μm. However, the specimen URTIC2, with a PDD 2.5 times greater than that of URTIC5, possessed cnidae with a mean length 1.2 μm less than that of URTIC5. Moreover, the two specimens closest in size, with PDDs of 30 mm (URTIC2) and 37 mm (URTIC4), possessed cnidae with a statistically significant difference between mean lengths of 1.4 μm.

As a further example, single samples of acontia from each of two specimens of *Sagartia elegans* were

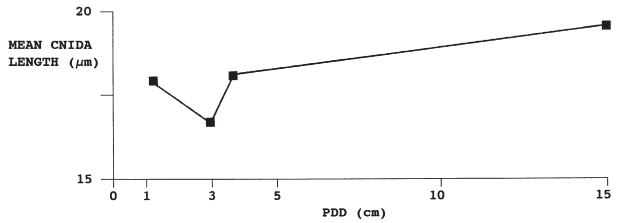

FIG. 1. – Mean lengths of basitrich nematocysts from the column ectoderm of four specimens of *Urticina eques* plotted against pedal disck diameter (PDD).

taken, in order to compare the mean lengths of one of the cnida types. The datasets were SAGART3 and SAGART4, comprising the lengths of microbasic *p*-mastigophore nematocysts from the free ends of acontia from two specimens of 25 and 9 mm PDD, respectively, trawled simultaneously from the Fairlie Roads off the Isle of Cumbrae, UK (Table 8). These data provide further evidence for the lack of direct correlation between the sizes of conspecific anemones and their cnida sizes. To test for any difference between the variabilities of the datasets, an F-test (two-tailed) for equality of two variances (Snedecor and Cochran, 1967) was carried out, giving F=2.41 (49, 24 d.f.), which is just statistically significant at P<0.05. The subsequent *t*-test was therefore modified for unequal variances, and revealed a statistically significant difference (P<0.0001) between the mean cnida lengths. The larger anemone possessed the smaller cnidae (Table 8).

DISCUSSION

The data on actiniarian cnida sizes presented herein, and by Williams (1996), constitute a large body of information that fills a previously existing gap in knowledge of the statistical parameters of cnidae (Fautin, 1988). Hence, the controversial issue of whether mean cnida length may be used validly as a taxonomic character may now be addressed on an objective basis.

TABLE 8. – Statistical parameters of datasets SAGART3 and SAGART4, which comprise lengths of microbasic *p*-mastigophore nematocysts from the free ends of acontia from two specimens of *Sagartia elegans*. PDD = pedal disk diameter (mm); SD = sample standard deviation; CoV = coefficient of variation (%). All parameters except PDD, *n* and CoV in μm (accuracy ± 0.46 μm).

Dataset	*PDD*	*n*	*Mean*	*SD*	*Variance*	*CoV*	*Min*	*Max*	*Range*
SAGART3	25	50	52.8[a]	6.07	36.79	11.5	43.8	62.6	18.8
SAGART4	9	25	61.2[a]	3.91	15.29	6.4	51.6	69.1	17.5

[a] Statistically significantly different from each other by *t*-test (P<0.0001).

The biometrics of cnidae from nine species of North-east Atlantic anemone have now been studied using the same protocol (Williams, 1996 and present results). The species were chosen to reflect several modes of reproduction which potentially exert different kinds of genotypic influences on the mean cnida sizes of individuals of the various species. Some of them reproduce by oviparity plus some asexual means, viz., *Nematostella vectensis* Stephenson, *Haliplanella lineata* (Verrill), *Sagartia elegans* (Dalyell), *Sagartiogeton laceratus* (Dalyell), *Metridium senile* (Linnaeus) and *Anthopleura thallia* (Gosse). Others reproduce only by viviparity, viz., *Sagartia troglodytes* (Price) and *Cereus pedunculatus* (Pennant), whilst *Urticina eques* (Gosse) reproduces only by oviparity. The tissues examined were tentacles, acontia or column ectoderm. The cnidae measured were spirocysts, basitrichs and microbasic *p*-mastigophores, which are the three types that most commonly comprise the cnidome of an actiniarian species (Carlgren, 1940, 1945, 1949), and also microbasic amastigophores.

Data presented in Tables 1-7 herein and by Williams (1996: Tables 1-4) from a total of eight species of anemone have shown that the statistical frequency distribution of random samples of cnida lengths is typically normal and that there tends to be no important within-sample heterogeneity. These results provide the basis for the valid use of parametric methods to test for statistically significant differences between means of cnida lengths. However, such inferential statistical treatment is shown to be inappropriate for taxonomic purposes because of between-sample (same specimen) and between-specimen heterogeneity within the same species. Furthermore, there is no predictable relationship between body weight and the mean cnida lengths of conspecific anemones of various sizes (Williams, 1996 and present results).

Between-sample (same specimen) heterogeneity amongst nematocyst lengths has been demonstrated wherever it has been sought, viz., in the acontia of *Sagartia elegans* (Table 6), in the column ectoderm of *Sagartiogeton laceratus* (see Williams, 1996) and in the tentacles of *Urticina eques* (see Williams, 1996).

Similarly, between-specimen heterogeneity amongst nematocyst lengths has been detected in all three species examined for it. In the case of *Urticina eques*, there was no overall direct correlation between cnida lengths and anemone sizes (Table 7; Fig. 1). All four specimens of *U. eques* were trawled simultaneously from the same locality, so they would be expected to have had access to similar food of the same nutritional value. As *U. eques* reproduces only by oviparity, the mean sizes of nematocysts in any individual are primarily controlled by the genotypes of its parents.

Sagartiogeton laceratus, on the other hand, reproduces not only sexually, but also by pedal laceration (see Williams, 1996: Fig. 1b). Therefore, a small, asexually produced individual and its much larger progenitor share the same genotype and may possess cnidae of about the same sizes, e.g., datasets 2 and 3 of Table 6 and datasets 2 and 3 of Table 7 in Williams (1996). However, it was also shown that the cnida sizes of *S. laceratus* of about the same size may be statistically significantly different (Williams, 1996: Tables 6 and 7 and Fig. 5). The specimens were collected at the same time from the same place, so all would have had access to the same food source.

Sagartia elegans also reproduces both sexually and by pedal laceration. One of the two sympatric specimens examined here (Table 8) had a PDD 2.8 times greater than that of the other, and yet its mean cnida length was statistically significantly less (by 14%) than that of the smaller specimen. Clearly then, in the cases of *Urticina eques*, *Sagartiogeton laceratus* and *Sagartia elegans* there is no consistent correlation between mean cnida lengths and anemone sizes, even in specimens collected at the same time and place, and whatever may be the mode of reproduction of each of the species. Furthermore, Stephenson (1929) found no correlations between the sizes of anemones and the mean lengths of nematocysts in the acontia of *Sagartia elegans* and *Cereus pedunculatus*. Chintiroglou (1996), who examined the mean lengths of eight cnida types in different-sized specimens of *Edwardsia claparedii* (Panceri), found statistically significant direct correlations for 4/8 types, but not for the remainder. Such a finding illustrates the danger of drawing a general conclusion regarding correlation between cnida lengths and anemone sizes based upon a single species, particularly when examination of several cnida types leads to different conclusions.

The foregoing facts support the conclusion that differences between mean cnida lengths constitute an unreliable taxonomic character for sea anemones when taken in isolation (Williams, 1996). This appears to be true from the standpoints both of inferential statistics and comparative morphology. For instance, statistically there is a demonstrable diffi-

culty in comparing the mean sizes of the cnidae from a single unidentified specimen of an anemone with the published cnida sizes of potential conspecifics, because of the various levels of intraspecific heterogeneity that may exist. In the worst case, only the holotype specimen of a species may be available with which to compare a single recently collected unidentified specimen. Such a problem is not uncommon when dealing with preserved museum material or comparing new results with inadequate published data.

However, when dealing with common, easily obtained anemones, large numbers of live specimens may be collected from various field populations which might be suspected to comprise different species. Between-specimen and between-sample heterogeneities are then quantifiable, and the cnida sizes of the populations may be validly compared by a multifactorial analysis of variance. Unfortunately, if statistical significance is not achieved by any between-population difference, it does not necessarily mean that the populations compared are conspecific. This is where comparative morphology is of importance, because congeneric actiniarian species may possess the same cnidomes with only minor differences between the sizes of the corresponding cnida types in each species (e.g., Schmidt, 1972; Fautin and Chia, 1986; England, 1987). In such cases, other factors (e.g., morphological, physiological, behavioural, genetic or ecological) must be considered in addition to cnidomes and cnida sizes for the purpose of identifying species.

Statistical analysis of cnida sizes may, nevertheless, be useful for work other than taxonomic studies. However, the significance tests used by Williams (1996) and herein to compare means, although necessary to demonstrate adequately the fundamental data characteristics, are somewhat tedious to execute, even with the assistance of computer statistical software. A simpler, faster method of comparing means of samples of cnida measurements is desirable for routine analyses. A convenient method, which requires only simple arithmetical manipulation of means and ranges, is described in Appendix I.

The knowledge now gained of the statistical parameters of cnida length frequency distributions may be used in order to derive statistical information from older publications in which inadequate data on cnida sizes have been provided. For instance, given the range, the number of observations, and details of sampling, the essential parameters of a normal frequency distribution, viz., mean and standard deviation, may be estimated, even though the individual data are not provided (Appendix II).

CONCLUSIONS

1) The normal (Gaussian) frequency distribution of actiniarian nematocyst lengths is confirmed.

2) Spirocysts have the same statistical characteristics as nematocysts.

3) The lack of important within-sample heterogeneity of cnida lengths is confirmed.

4) The frequent occurrence of significant between-sample heterogeneity of mean cnida lengths within the same anemone specimen is confirmed.

5) Significant differences between mean cnida lengths from different specimens of the same species may occur, but these cnida lengths are not directly correlated with anemone size, even in specimens collected simultaneously from the same locality, and therefore subject to the same ecological conditions.

6) A rapid sample range test using the statistic D' (Appendix I) is appropriate for analysis of the differences between mean cnida measurements from any set of equal-sized samples.

7) The mean and standard deviation of a sample of cnida sizes may, with certain provisos, be estimated from data in publications that provide only ranges of cnida sizes and the number of cnidae measured (Appendix II).

RECOMMENDATIONS

A) Because of possibly significant between-sample and between-specimen heterogeneity within the same species, cnida measurements from such varied sources should not be pooled unless an appropriate statistical test, such as a multifactorial analysis of variance, has been used to compartmentalize the sources of heterogeneity. Uncritically combining data from different samples and specimens to calculate a standard deviation of the resulting heterogeneous statistical population gives a misleading result, and has no validity.

B) It should not be assumed that cnida sizes are correlated in any consistent way with the size, age, nutritional status or sexual maturity of individual conspecific anemones. Neither should it be assumed

that conspecific anemones of the same size possess cnidae of equal mean sizes.

C) Sampling and analysis of cnida sizes should follow a predetermined protocol (e.g., Williams, 1996). Depending on the experimental design, the statistical analysis should take into account the different levels (viz., within samples, between samples (same specimen) and between conspecific specimens) of potential heterogeneity in the results to be obtained.

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APPENDIX I

A range test for significance testing amongst equal-sized samples of mean cnida lengths

A simple method of testing all comparisons amongst means (a sample range test) was suggested by Snedecor and Cochran (1967, Table 10.9.1) and makes use only of the mean and range of each equal-sized sample. Note that the range is the difference between the largest and the smallest measurements; the actual values of the largest and smallest measurements in the distribution are referred to herein as the extreme values (extremes). Using the data in Table 1 herein as an example, the ranges of the 10 subsets of 5 cnida lengths are 1.6, 5.0, 5.1, 5.0, 6.7, 5.0, 4.1, 6.7, 6.6 and 8.3 μm; the sum of these ranges is 54.1 μm. This sum is then substituted in the following equation:

$$D' = \frac{CF \times \text{sum of ranges}}{n}$$

where D' = least sig[illegible] difference (P=0.05) between any pair of m[illegible]gths;

CF = Critical [illegible] (P=0.05) taken from Snedecor and Coch[illegible]7, Table 10.9.1);

n = sample siz[illegible]bsets, which must all be equal.

Hence for this [illegible]e, the substitutions ar[illegible]

[illegible] μm

5

Thus, any differences between means of subsets in Table 1 that exceed 4.869 μm are statistically significant at P<0.05. The only such difference is that between the subsets 3 and 7, which equals 5.7 μm; no other difference exceeds D'. This confirms the conclusion reached by using analysis of means, that the mean of subset 7 is significantly different from the overall mean of the whole dataset at P<0.05 (Table 1).

The sample range test applied also to Tables 2-5 herein, and to Tables 1-3 of Williams (1996), revealed no difference that exceeds D' between the means of

any pair of the 10 subsets of 5, confirming conclusions drawn from the original computations using the much more complex analysis of means. Comparing full datasets of 50 in sample range tests, there were similar confirmations of the conclusions previously drawn when statistically significant differences were obtained by analysis of means, viz., for Tables 6 and 7 herein, and Table 4 of Williams (1996).

This rapid sample range test is therefore deemed to be appropriate for analysing the differences between mean cnida measurements in datasets of equal-sized samples. Extensive tables were published by Kurtz *et al*. (1965: Table 4 for P=0.05 and Table 5 for P=0.01) for numbers of datasets of 2-20, 30, 40, 50, 100, 200, 500 and 1,000; and for the same numbers of observations in each dataset. The range test would be useful for routine analyses in studies other than taxonomic work, e.g., morphological, physiological, behavioural, genetic or ecological.

APPENDIX II

How to derive statistical information from inadequate published data on cnida sizes

Now that it is known that the statistical frequency distribution of a sample of cnida lengths is typically normal (Williams, 1996), established mathematical relationships between parameters may be used to derive information from incomplete published data. Thus, if at least the extreme values of an observed range are given, then provided that the number of measurements is supplied, further information may, with certain provisos, be derived from the data. Ranges, rather than standard deviations, are often used as estimators of variability in industrial quality control work, where large numbers of small samples have to be dealt with in a short time. Hence there are well-established statistical procedures utilizing ranges, such as Lord's modification of the *t*-test, in which the range replaces the sample standard deviation in the denominator of *t* (Snedecor and Cochran, 1967: 120-122). A table is also available from which ranges may be converted to estimated standard deviations (see Snedecor and Cochran, 1967: Table 2.4.1).

When an author has given only the extremes of cnida lengths and the number of observations, how is it possible to retrieve as much information as possible from such incomplete data? The original sampling method is crucial. We might be told; or we might be able to deduce; or we might assume (albeit with considerable caution) that all the measurements were from one specimen. Making further cautious assumptions, that the sampling was independent and random, and knowing the number of observations, a sample standard deviation might be estimated from the range using Snedecor and Cochran's (1967) Table 2.4.1; the factor for the appropriate value of *n*, multiplied by the observed range gives the estimated standard deviation. Then, assuming the sample distribution to be normal, the mean might be estimated by calculating the median, the midpoint of the extreme values of the range (in a normal frequency distribution, the mean and median are equal). This would provide cautious estimates of the two fundamental parameters (mean and standard deviation) necessary for carrying out tests of significance (Snedecor and Cochran, 1967).

Table 9 shows the results of estimating means and standard deviations by the methods described above, using 11 randomly collected datasets, each of 40 or 50 values, from Tables 1-7. The estimated means did not deviate from the actual means by more than ± 2.8%. However, the estimated standard deviations, whilst occasionally in reasonable agreement with the calculated sample standard deviations, were up to 32.2% different. Nevertheless, when the estimated and actual variances (squares of standard deviations) of each dataset were compared by an F-test (two-tailed) for equality of two variances (Snedecor and Cochran, 1967) the null hypothesis was, in most cases, accepted. This was so even when a difference between the estimated and actual sample standard deviation was as great as about 22%. In 2/11 cases, however, when the difference exceeded 30% (SAGART2 and SAGART3), the estimated variance was significantly different from the actual variance. Overall though, these tests indicated that the estimated standard deviations were usually not statistically significantly different from the actual sample standard deviations for each dataset when the data resulted from random sampling. Nevertheless, it is clear, not only from the sig-

TABLE 9. – Means and standard deviations (SD) calculated from the 40 or 50 values in each of the datasets METRID1, CEREUS1, SAGART6, SAGART7, ANTHO1, SAGART2, SAGART3, URTIC2, URTIC3, URTIC4 and URTIC5, and the means and standard deviations estimated from only the minimum and maximum values in each dataset (using the methods described in Appendix II). Also, the estimated parameters are expressed as percentage differences from the calculated parameters.

Dataset	*n*	*Calculated parameters*		*Estimated parameters*		*Percentage differences*	
		Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*
METRID1	50	15.4	2.59	15.0	2.22	-2.6	-14.3[a]
CEREUS1	50	19.5	1.22	19.6	1.29	+0.5	+5.7[b]
SAGART6	50	30.5	2.43	30.4	2.04	-0.3	-16.0[c]
SAGART7	40	15.4	1.21	15.7	1.22	+1.9	+0.8[d]
ANTHO1	40	15.5	1.23	15.9	1.11	+2.6	-9.8[e]
SAGART2	50	57.7	6.90	56.7	4.68	-1.7	-32.2[f]
SAGART3	50	52.8	6.07	53.2	4.17	+0.8	-31.3[g]
URTIC2	50	16.7	2.37	16.6	1.84	-0.6	-22.4[h]
URTIC3	50	19.5	0.81	20.1	0.91	+2.8	+12.3[i]
URTIC4	50	18.1	0.78	18.2	0.71	+0.6	-9.0 [j]
URTIC5	50	17.9	1.14	17.8	1.13	-0.6	-0.9[k]

[a] Percentage difference between SDs not significant: F=1.36 (P>0.05 for 49, 49 d.f.)
[b] Percentage difference between SDs not significant: F=1.12 (P>0.05 for 49, 49 d.f.)
[c] Percentage difference between SDs not significant: F=1.42 (P>0.05 for 49, 49 d.f.)
[d] Percentage difference between SDs not significant: F=1.02 (P>0.05 for 39, 39 d.f.)
[e] Percentage difference between SDs not significant: F=1.23 (P>0.05 for 39, 39 d.f.)
[f] Percentage difference between SDs significant: F=2.17 (P<0.01 for 49, 49 d.f.)
[g] Percentage difference between SDs significant: F=2.12 (P<0.02 for 49, 49 d.f.)
[h] Percentage difference between SDs not significant: F=1.66 (P>0.05 for 49, 49 d.f.)
[i] Percentage difference between SDs not significant: F=1.26 (P>0.05 for 49, 49 d.f.)
[j] Percentage difference between SDs not significant: F=1.21 (P>0.05 for 49, 49 d.f.)
[k] Percentage difference between SDs not significant: F=1.02 (P>0.05 for 49, 49 d.f.)

nificance tests but also from the percentage differences, that the estimation of a standard deviation is rather less reliable than the estimation of a mean from the same data, even when random sampling was employed (Table 9).

Until quite recently, rather few publications that included cnida measurements could be found in which authors had provided the parameters of means and standard deviations, with adequate details of sampling. Only such detailed information would completely describe the statistical frequency distribution, assuming the sampling method to be valid and the distribution of the data to be normal. Unfortunately, some recent papers that include means and standard deviations reveal in the description of sampling that the descriptive statistics are invalidated by the sampling methodology. In fact, the parameters of the cnida measurements are frequently based on pooled data from multiple samples of tissue and different specimens. Thus, such samples are combined from several statistical populations. Invariably, no evidence of random sampling is provided, and the standard deviations given are, therefore, meaningless.