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Composition, biometry and statistical relationships between the cnidom and body size in the sea anemone *Oulactis muscosa* (Cnidaria: Actiniaria)

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This study analyses the possible relationships between body size and length of cnidae from different tissues of the sea anemone *Oulactis muscosa*. We describe the cnidom, providing new qualitative and quantitative data. Our description adds spirocysts for tentacles and acrorhagi, and is more precise about the ranges and types of basitrichs, microbasic *b*-mastigophores, and holotrichs. We distinguish two types of holotrichs in the acrorhagi, and differentiate between microbasic *b*-mastigophores and basitrichs in the actinopharynx and mesenterial filaments. A relationship between cnida length and body weight was not demonstrated. The results are based on a complete account of cnida types from all tissues, and considering the great number of capsules measured (5400) and the modern statistical tools employed, we think that a normal distribution of cnida lengths is uncommon, perhaps refuted. This finding is very important when a quantitative analysis of cnidae is necessary and an adequate statistical tool must be used. We have shown that generalized linear models are an alternative and therefore analyses can be done with parametric methods despite the non-normal distribution of cnida size. The use of these statistical tools should be generalized since appropriate package for analyses (like the R package) are available from the web and the obtained results are robust and powerful.

INTRODUCTION

Cnidarians are characterized by the presence of subcellular structures called cnidocysts (nematocysts, spirocysts and ptychocysts); they have acquired various degrees of importance in the different cnidarian groups. In the Order Actiniaria, cnidae study is advanced and the description of their size and distribution is commonplace in taxonomic studies (Fautin, 1988). Cnidae are distinctive and morphologically variable microtools, secreted inside a single cell, the cnidocyte. They are extraordinarily complex collagenous, intracellular secretions serving a wide range of general and specialized functions, including aggression, feeding, defence or larval settlement. Mechanically, they are pressurized capsules with an attached eversible tubule that is inverted inside the capsule before firing. The capsule or its tubule may be filled with toxins, irritants, or adhesives that are released when the tubule everts during firing (Francis, 2004). Cnidae are discharged after a single use, and are abundant where present; thus their production and use must entail considerable energetic investment by the animals. The taxonomic value of statistical analyses of cnidae measurements in sea anemones has not been fully evaluated (Fautin, 1988; Williams, 2000; Acuña et al., 2003). Several authors have carried out studies dealing with nematocyst sizes and have reported on their distribution and biometry (Acuña & Zamponi, 1997; Chintiroglou et al., 1997), and have also suggested appropriate methodologies. In an effort to enhance the value of cnidae for distinguishing species, statistical analyses of nematocysts measurements have been used to study the taxonomy of sea anemones (Ardelean & Fautin, 2004 and references therein). Notwithstanding, few comparative studies have examined significant amounts of data, using adequate statistical treatment (Acuña et al., 2004). In spite of many quantitative studies of cnidocysts, changes in cnida size with body size appear to have been overlooked or discounted; and no studies have been conducted on cnida scaling *per se*. Moreover a possible relationship between developmental stage or polyp size and cnida size has occasionally been noted (discussed in Francis, 2004). Cnida scaling was described for the first time by the mentioned author, but based only on one adhesive cnida type (spirocyst) from two different tissues (ectoderm of the feeding tentacles and acrorhagi) in two sympatric anemones, *Anthopleura elegantissima* (Brandt, 1835) and *A. xanthogrammica* (Brandt, 1835).

Some authors assume a normal distribution of capsule lengths (Williams, 2000; Ardelean & Fautin, 2004), but this assumption has been proved to be wrong at least for acontiarian sea anemones (Acuña et al., 2003) and zoanthids (J. Ryland, personal communication). The lack of symmetry and the lack of independence between mean and variance, apparent in many of their size distributions, are major obstacles to comparative studies using classical statistical

Table 1. *Cnidae of* Oulactis muscosa.

Tissue/cnida type	Length range (μm)	Abundance
Tentacles		
Basitrich	11.00–29.00	abundant
Spirocyst	14.00–33.00	common
Acrorhagi		
Basitrich	12.00–29.00	scarce/present
Holotrich I	41.00–81.00	common
Holotrich II	42.00–73.00	abundant
Spirocyst	17.00–41.00	present
Column		
Basitrich I	15.00–26.00	abundant
Basitrich II	8.00–16.00	scarce
Holotrich	15.00–30.00	common
Actinopharynx		
Microbasic *b*-mastigophore	21.00–37.00	common
Basitrich	9.00–20.00	abundant
Microbasic *p*-mastigophore	12.00–27.00	scarce/present
Mesenterial filament		
Microbasic *b*-mastigophore	23.00–52.00	abundant
Basitrich	9.00–25.00	common
Microbasic *p*-mastigophore	14.00–28.00	scarce

methods. Non-parametric tests provide an alternative, since non-normality of data distribution can then be ignored, but these tests are less powerful. Generalized linear models (GLMs) provide another option, offering a broader set of distributions to choose from: the natural exponential family. With this in mind, analysis of cnida sizes can be done with parametric methods according with the non-normal distribution (Acuña et al., 2004). Allcock et al. (1998) and Watts et al. (2000) have applied GLMs to nematocysts of the sea anemones *Actinia equina* (L.) and *A. prasina* (Gosse), and Ardelean & Fautin (2004) used them to study the cnidocysts of a single specimen of *Actinodendrum arboreum* (Quoy & Gaimard, 1833).

The aim of this study is to use GLMs to analyse possible relationships between body size and length of cnidae from different tissues of the sea anemone *Oulactis muscosa* (Drayton in Dana, 1846). We complete the description of cnidae from *O. muscosa* published by Carlgren (1950), and examine all 15 types and sizes of cnida distributed in all different tissues from specimens of different sizes. Thus, this is the first analysis to explore the complete cnidom across individuals of different sizes.

MATERIALS AND METHODS

Sampling

Specimens of *Oulactis muscosa* were collected from the intertidal zone of Punta Cantera (38°05'S 57°32'W), Mar del Plata, Argentina. Individuals were kept in aquaria with aerated seawater, anesthetized by the addition of crystals of magnesium chloride, and preserved in 5% formalin. We selected individuals spanning the range of sizes found in the sampled zone. Since size and age are essentially decoupled, the weight was used as a size parameter due to the shrinkage

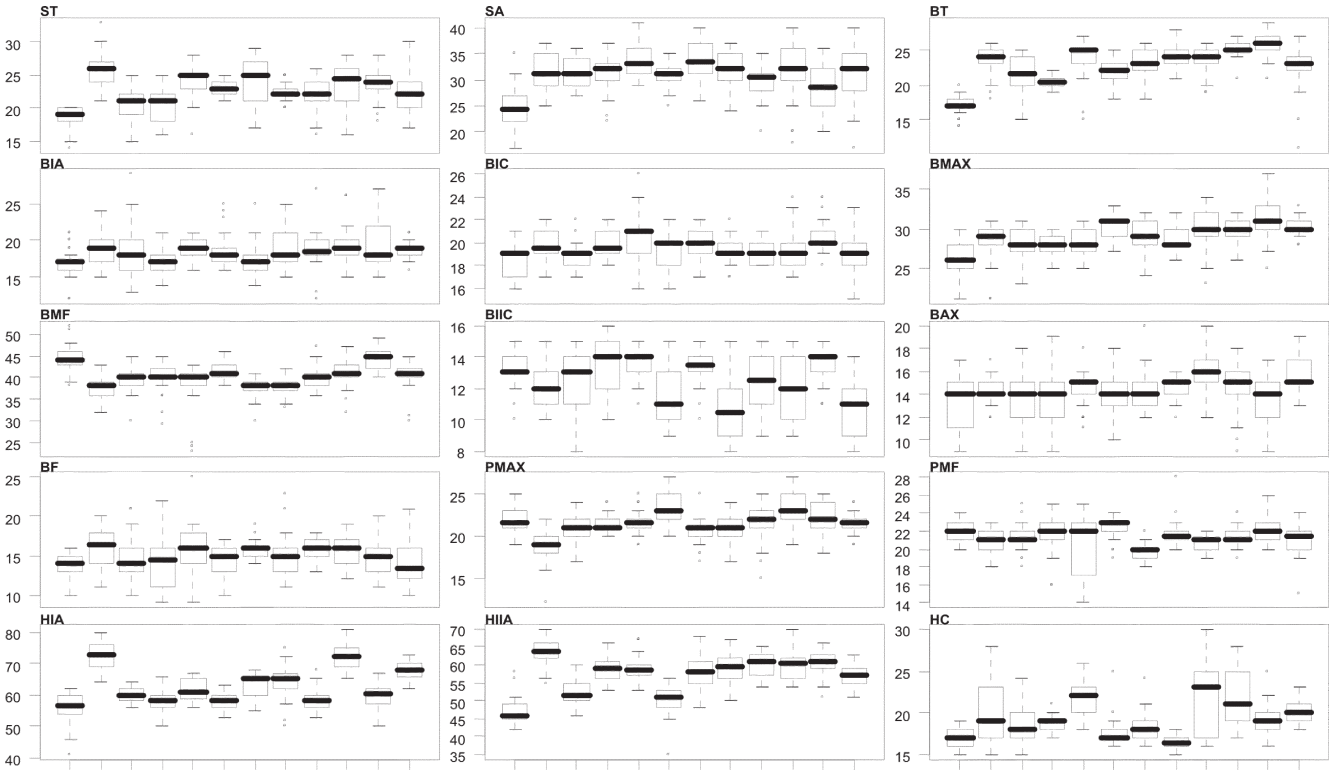

Figure 1. Boxplot of cnidae length (μm) of the 12 individuals ordered by body weight, for each combination of tissue and cnidocyst type. ST, spirocyst of tentacle; SA, spirocyst of acrorhagi; BT, basitrich of tentacle; BA, basitrich of acrorhagi; BIC, basitrich I of column; BMAX, microbasic b-mastigophore of actinopharynx; BMF, microbasic *b*-mastigophore of mesenterial filament; BIIC, basitrich II of column; BAX, basitrich of actinopharynx; BF, basitrich of mesenterial filament; PMAX, microbasic *p*-mastigophore of actinopharynx; PMF, microbasic *p*-mastigophore of mesenterial filament; HIA, holotrich I of acrorhagi; HIIA, holotrich II of acrorhagi; HC, holotrich of column.

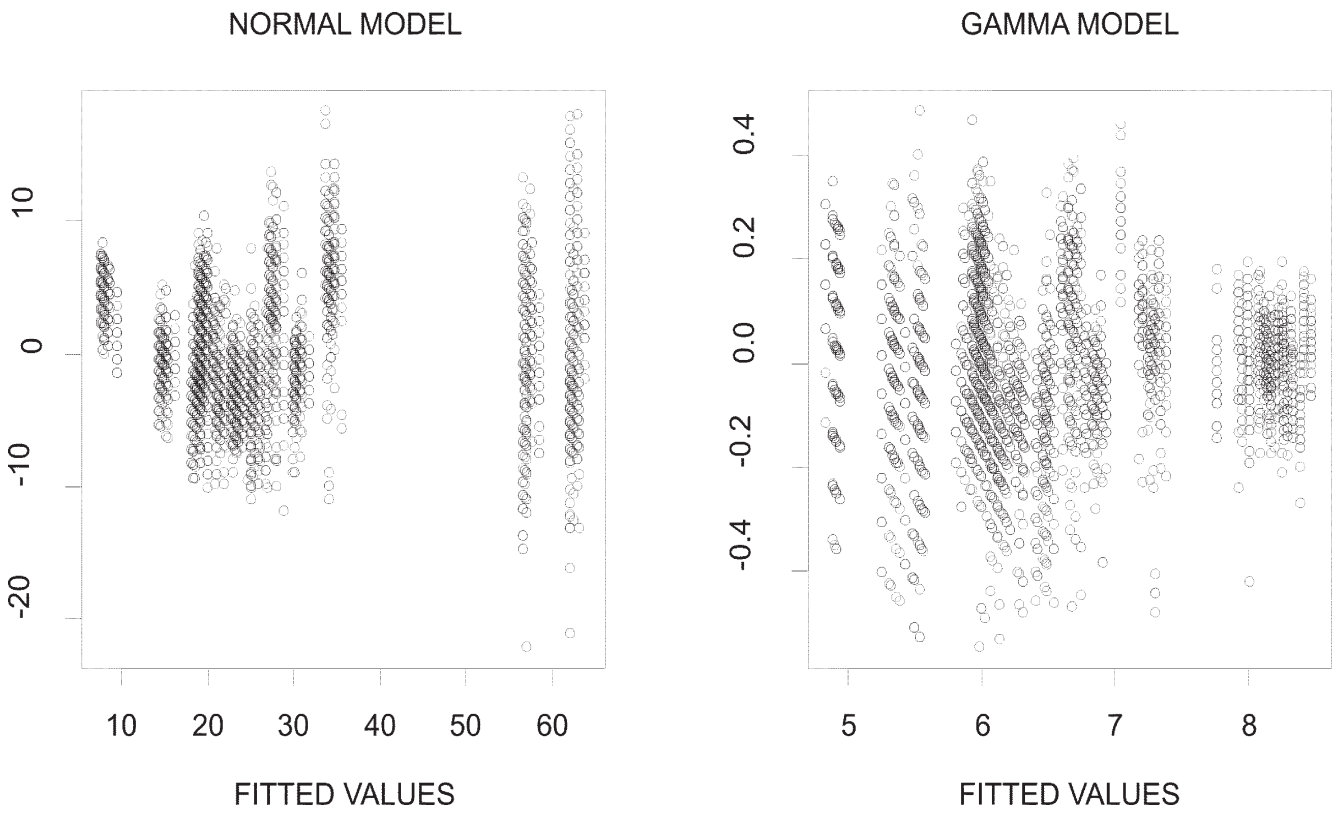

Figure 2. Fitted values versus deviance residuals for normal and gamma models.

of the body of these invertebrates. A total of 12 individuals of different weights was studied; their size rank and weight (g) are as follows: 1 (2.91), 2 (3.66), 3 (4.27), 4 (4.32), 5 (5.58), 6 (6.62), 7 (8.12), 8 (8.48), 9 (12.76), 10 (13.19), 11 (14.90), 12 (24.05). Gravel attached to the verrucae was removed prior to weighing.

For each individual, we measured the length of 30 unfired capsules, taken randomly, from each of the following tissues: tentacles, acrorhagi, column, actinopharynx and mesenterial filaments. In these tissues, we found spirocysts, basitrichs, microbasic *b*-mastigophores, microbasic *p*-mastigophores and holotrichs (the terminology follows that of England (1991)); thus, a total of 5400 measurements was done. All measurements were made using a Zeiss Axiolab microscope with a micrometric eyepiece at a magnification of 1000× (oil immersion). For tentacle tissue squash we used the tips, since the base is a cnidogenesis zone with cnidocysts in different stages of development and consequently with high variation in sizes.

Statistical analysis

Descriptive statistics (mean, standard deviation, coefficient of variation, minimum and maximum) were calculated for each possible combination of tissue and cnidocyst. Boxplots were drawn to display comparisons for each of the 12 specimens for each cnida and tissue.

Since most of these data (Acuña et al., 2003) are not normally distributed, the hypotheses of a functional relationship between capsule length and body weight was explored using GLMs with gamma distributed errors and log link. Gamma distribution was chosen because of the low variability of the coefficients of variation, and the lack of symmetry of these data. A classical normal model was also fitted for comparison.

Two kinds of linear components were evaluated: one with two factors (tissue and cnidocyst type) and weight taken as a covariate and the second one taking weight as a third factor. The difference between them is that in the first case a correlation between length and weight is supposed but in the second one length is supposed to vary between specimens but without a numerical structure (order or distance) derived from specimen weight. The general form of this model is:

$$g(length) = \beta_0 + \beta_1 tissue + \beta_2 cnidatype + \beta_3 weight$$

where g(·) is the canonical link: inverse in the gamma case and identity in the normal case (McCullagh & Nelder, 1989). Plots of fitted values vs deviance residuals were obtained to be used as diagnostic tools. All the calculations were performed with R package, available at http://www.r-project.org/.

RESULTS

Cnidae composition

We examined the types, length and abundance of 360 capsules for each type and tissue, in 12 individuals (Table 1). Carlgren (1950) published the cnidom of *Oulactis muscosa* but did not mention some types that we found. His account naturally does not reflect all of the terminology and distinctions used by more recent authors; for example, he listed atrichs in column and acrorhagi, but this cnida is now correctly termed holotrich (Edmands & Fautin, 1991), because it posses small spines (Bigger, 1982). He

Table 2. *Measures of global fit for different models.*

Model	R-squared	AIC
Normal	0.9091	31612
Gamma (inverse link, weight as a covariate)	0.9133	28917
Gamma (log link, weight as a covariate)	0.8957	29918
Gamma (inverse link, weight as a factor)	0.9171	28691

AIC, Akaike's information criterion.

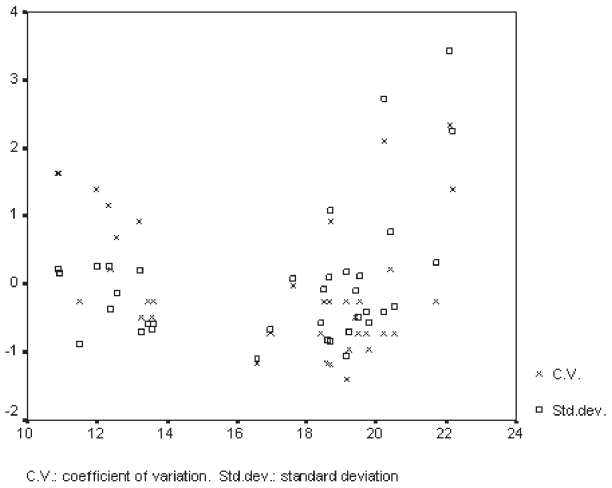

Figure 3. Scatterplot of mean versus standard deviation and mean versus CV.

also did not distinguish between basitrichs and microbasic *b*-mastigophores. In the tentacles, we also found spirocysts, which were not mentioned by Carlgren (1950). We found spirocysts in the acrorhagi, and distinguished two types of holotrichs: holotrich I (wider) and holotrich II (slender). We found a wider range in the sizes of basitrichs, probably due to the high number of measurements made. Carlgren (1950) recorded basitrichs as very abundant, but we only found them as 'present.' Concerning the column, the only differences between our study and that of Carlgren (1950) are in the range of basitrichs, with higher values found by Carlgren. In the actinopharynx, we distinguished between microbasic *b*-mastigophores and basitrichs, the latter being more abundant. Finally, in the mesenterial filaments, Carlgren (1950) identified two types of basitrichs, which we distinguished as basitrichs and microbasic *b*-mastigophores (Table 1).

Cnidae biometry

Mean capsule length varies between specimens, and there does not seem to be a relationship between cnida length and body weight (Figure 1); this illustrates and summarizes the descriptive parameters. Standard deviation (SD) tends to increase with mean, consequently coefficient of variation (CV) is homogeneous. From the scatterplot of mean vs standardized CV and vs SD (Figure 3), it can be seen there that SD increases with mean, whereas CV does not. This suggests that a GLM with gamma errors is appropriate.

All coefficients in the gamma model were highly significant, showing that cnida length varies with tissue, type, and specimen. Global fit was measured with deviance based R-squared (Cameron, 1996); its value was greater for the gamma model than for the normal model; AIC values agreed with R-squared (Table 2). Plots of fitted values vs deviance residuals can be seen in Figure 2; it is known that they should reveal a random pattern, since any discernible pattern or curvature is an indication of a poor choice of the model. As can be seen in Figure 2, for the normal model they show a curvature indicating a poor fit while for gamma model they are symmetrical over the horizontal line placed at zero. In the linear component, we took weight as a continuous variable and as a factor; we obtained better results with the second alternative (Table 2).

DISCUSSION

Our description of the cnidom of *Oulactis muscosa* adds spirocysts for tentacles and acrorhagi, and is more precise about ranges and types of basitrichs, microbasic *b*-mastigophores, and holotrichs. For example, we distinguish two types of holotrichs in the acrorhagi, and differentiate between microbasic *b*-mastigophores and basitrichs in the actinopharynx and mesenterial filaments. A comparison of the cnidae of four *Oulactis* species: *O. concinnata* (Drayton in Dana, 1846), *O. magna* (Stuckey, 1909), *O. coliumensis* (Riemann-Zürneck & Gallardo, 1990) and *O. muscosa*, in Haüssermann (2003), mentions that many of the differences among these species may be attributed to the patchy distribution and low numbers of certain types of cnidae.

We did not assume a normal distribution of size for all cnidae of *O. muscosa*. Our results are based on a complete account of all types of cnidae from all tissues, we consider the greatest number of capsules measured (5400), and we employ modern statistical tools. Our data better fit a gamma distribution than a normal one (Figure 2). These finding agrees with results of Acuña et al. (2003, 2004) for the cnidae of acontiarian sea anemones, and with those of Ryland (personal communication) for zoanthids. Therefore, we think that a normal distribution of length is uncommon, perhaps refuted.

A common choice in non-normally distributed data is to use less powerful non-parametric tests. Instead, we used generalized linear models (GLMs) that do not force data into unnatural scales via transformations and thereby allow non-linearity and non-constant variance structures in the data (Hastie & Tibshirani, 1990). According to Acuña et al. (2004), GLMs are more flexible and better suited for analysing biological relationships.

All types of cnidae were highly variable in size between individuals. The holotrichs from acrorhagi showed the greatest absolute size and the most variability. Variability in these may be a consequence of the high turnover rate of these cnidae, which are implicated in defence and aggression. Spirocysts were highly variable in both tentacles and acrorhagi, these structures being involved in feeding and aggression, respectively. Williams (2000) has confirmed that spirocysts from *Metridium* showed higher than usual coefficients of variation, validating the general impression that variability is higher for spirocysts than for other cnidae (Francis, 2004).

All coefficients in the gamma model were highly significant, showing that cnida length varies with specimen, tissue, and type. Our results do not support a functional relationship between cnida length and body weight. This agrees with Edmands & Fautin (1991), who noted that nematocyst size did not appear to correlate with animal size in *Aulactinia veratra* (Drayton in Dana, 1846). Although Francis (2004) found that larger *Anthopleura* and *Tealia* (a junior synonym of *Urticina*) do produce larger spirocysts, she examined only 20 capsules from each individual and from two different tissues. Spirocysts have the highest coefficient of variation (Williams, 2000; Francis, 2004) and may thus be less appropriate for comparative studies than nematocysts. Such comparative studies should include, when possible, all types of cnidae to provide a broad and consistent scope for robust results and conclusions. On the other hand Chintiroglou et al. (1997) found that the lengths of the tentacle basitrichs and spirocysts, and acrorhagi holotrichs (mentioned as atrichs) in *Actinia equina mediterranea* Form II, are positively correlated with weight; meanwhile the basitrich from actinopharynx of *A. equina mediterranea* Form I is not influenced by individual body weight. In both cases non-parametric tests were used.

The lack of symmetry and the high variability apparent in many of the size distributions of cnidae are major obstacles to performing comparative studies. We have shown that GLMs provide an alternative model that offers a broader set of distributions to choose from, the natural exponential family. With GLMs, analyses can be done with parametric methods despite the non-normal distribution of cnida size (Acuña et al., 2004). The use of these statistical tools should be generalized since appropriate package for analyses (like the R package) are available from the web and the obtained results are robust and powerful. In this way we can estimate the limits of variability within species through a large number of samples and observations.

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