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A novel statistical analysis of cnidocysts in acontiarian sea anemones (Cnidaria, Actiniaria) using generalized linear models with gamma errors

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Abstract

Comparative studies on cnidocysts, involving adequate statistical treatment, are very scarce. Classical statistical tests are frequently used assuming normal frequency distributions of capsule lengths, but many distributions are non-normal in acontiarian sea anemones. A traditional choice in these situations are non-parametric tests, although they are not as powerful as parametric tests. An extension of classical methods was developed by some authors; these models, called Generalized Linear Models (GLM), can be used under certain conditions with non-normal data. In view of the properties of our data, that are positive, skewed and with constant coefficient of variation, a GLM with gamma distribution and inverse link function was chosen to analyse the cnidae of acontia from the species *Haliplanella lineata*, *Tricnidactis errans* and *Anthothoe chilensis*. Graphical analysis of residuals showed that these assumptions were reasonable. This method allowed us to avoid transformation of data set and controversial cases in the limit of significance level. For this task, appropriate subroutines in GLIM language were written. In all cases highly significant differences were found between the specimens considered for every species and nematocyst type (b-rhabdoids, p-rhabdoids B1b and p-rhabdoids B2a).

Keywords: Cnidae length; Statistics; Gamma distribution; Acontiaria

Introduction

Cnidarians are characterized by the presence of subcellular structures called cnidocysts (nematocysts, spirocysts and ptychocysts). In the order Actiniaria cnidocysts study is advanced and the description of their size and distribution is commonplace in taxonomic studies (Fautin 1988). In acontiarian sea anemones they are used to define families, in accordance with the criteria introduced by Carlgren (1949). However, authors such as Schmidt (1972) discredit the study of nematocysts, since they consider that their high variability in several orders of Anthozoa lessens their taxonomic and phylogenetic value. Other authors have carried out studies dealing with nematocyst sizes and have reported on their variation, distribution, allometry and statistical parameters (Thomason 1988; Zamponi and Acuña 1991, 1994; Williams 1996, 1998, 2000; Acuña and Zamponi 1997; Chintiroglou et al. 1996, 1997), also suggesting

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appropriate methodologies. Many papers describe the cnidae from different species, but few comparative studies have been carried out involving significant amounts of data, combined with adequate statistical treatment.

Frequently used statistical tests assume a normal distribution of capsule lengths, but lately this assumption has been proved to be wrong for acontiarian sea anemones (Acuña et al. 2003). A traditional choice in these situations is to use less powerful non-parametric tests. An extension of classical methods called Generalized Linear Models (GLM) was developed by Nelder and Wedderburn (1972). These models are mathematical extensions of linear models that do not force data into unnatural scales via transformations, and thereby allow non-linearity and non-constant variance structures in the data (Hastie and Tibshirani 1990). They are based on an assumed relationship (called a link function; see below) between the mean of the response variable and the linear combination of the explanatory variables. Data may be assumed to be from several families of probability distributions, including the normal, binomial, Poisson, negative binomial, or gamma distributions, many of which better fit the non-normal error structures of most biological data. Thus, GLMs are more flexible and better suited for analyzing biological relationships. Available software to apply them is still scarce, and this is perhaps the reason why their use is not as extended as it could be.

To our knowledge only 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) when data were non-normal. The aim of the present paper is to carry out a comparative study of cnidae of acontia from the species *Haliplanella lineata* (Verrill, 1869), *Tricnidactis errans* Pires, 1988 (Haliplanellidae) and *Anthothoe chilensis* (Lesson, 1830) (Sagartiidae), using a GLM with gamma distribution, in order to test the suitability of this model in nematocysts' studies.

Table 1. Statistical parameters of cnidocysts from acontia of studied species

Nematocyst	Indiv. No.	Mean	std. dev.	Skewness	CV
Haliplanella lineata					
b-rhabdoid	1	15.54	1.11	−1.33	0.07
	2	15.26	1.40	−0.68	0.09
	3	16.20	1.12	−0.72	0.07
	4	16.42	0.91	0.07	0.06
	5	15.90	1.22	−0.01	0.08
p-rhabdoid	1	14.54	1.30	0.33	0.09
B1b	2	14.38	1.10	0.30	0.08
	3	16.28	1.21	0.14	0.07
	4	15.60	1.11	−0.60	0.07
	5	15.52	1.49	0.18	0.09
p-rhabdoid	1	34.96	1.28	−0.04	0.04
B2a	2	37.58	1.98	−0.89	0.05
	3	39.00	2.41	−0.44	0.06
	4	37.98	2.36	0.00	0.06
	5	35.52	1.73	0.37	0.05
Tricnidactis errans					
b-rhabdoid	1	32.74	2.66	−0.68	0.08
	2	30.56	1.77	−0.77	0.06
	3	33.74	1.82	−0.46	0.05
	4	34.08	2.11	−0.59	0.06
	5	34.76	1.91	−0.49	0.06
p-rhabdoid	1	29.52	2.30	0.36	0.14
B1b	2	24.70	3.02	0.88	0.12
	3	26.84	3.47	−0.03	0.13
	4	30.54	3.94	0.41	0.13
	5	31.48	3.54	0.19	0.11
p-rhabdoid	1	65.34	7.28	−0.81	0.11
B2a	2	63.30	4.67	0.11	0.07
	3	64.78	5.12	−0.87	0.08
	4	69.16	3.45	−0.03	0.05
	5	69.20	3.87	−0.36	0.06
Anthothoe chilensis					
b-rhabdoid	1	26.58	2.01	0.20	0.07
	2	29.54	1.86	−0.72	0.06
	3	25.22	1.45	−0.42	0.06
	4	28.06	2.46	−0.78	0.09
	5	26.60	1.76	−0.56	0.07
p-rhabdoid	1	58.18	5.78	−0.34	0.10
B2a	2	61.10	4.81	−0.72	0.08
	3	61.28	7.13	−0.77	0.12
	4	57.10	7.01	0.08	0.12
	5	51.96	3.28	−0.01	0.06

std. dev., standard deviation; CV, coefficient of variation; Mean and std. dev. in μm.

Material and methods

Sampling

Specimens of *T. errans* and *A. chilensis* were collected from the intertidal of Mar del Plata (38°05′S, 57°32′W) (Buenos Aires, Argentina), and specimens of *H. lineata* from Las Grutas (40°48′S, 65°05′W) (Río Negro, Argentina). Live specimens were transported to the laboratory and kept in the aquaria with aerated seawater and fed *Artemia* nauplii twice a week. Five specimens of similar size of each species were selected for analysis of acontia cnidae, since, according to Allcock et al. (1998), it is essential for comparative purposes that nematocysts are measured from anemones of a limited size range. The length of 50 unfired capsules were measured and taken randomly from the following nematocyst classes, according to Schmidt (1969, 1972): b-rhabdoids, p-rhabdoids B1b, p-rhabdoids B2a from *H. lineata* and *T. errans* and b-rhabdoids and p-rhabdoids B2a in *A. chilensis*. A Zeiss Axiolab microscope with micrometric eyepiece at a magnification of

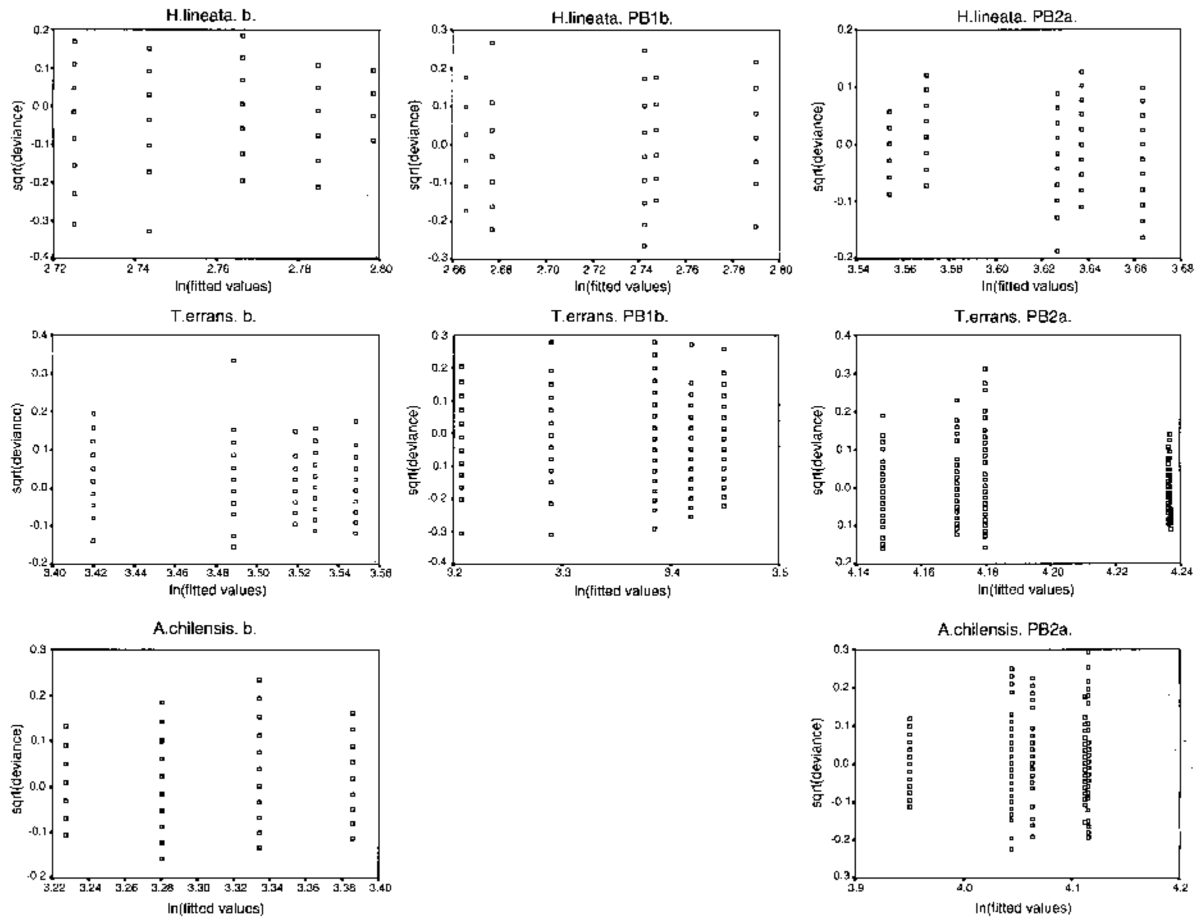

Fig. 1. Scatterplots of *sqrt*(deviance) vs. ln(fitted values).

1000 × was used for measuring purposes; a total of 2000 measurements were done.

Statistical analysis

In view of the non-normality of most of these data (Acuña et al. 2003), other continuous distributions were considered. The frame to do this were GLMs, that are statistical models given by

- A vector $y = [y_1, \ldots, y_n]$ of independent random variables with distribution in the natural exponential family and with a mean vector $\mu = [\mu_1, \ldots, \mu_n]$.
- A vector of parameters $\beta = [\beta_1, \ldots, \beta_n]$ whose relation with μ is given by $g(\mu) = X\beta$, where X is an $n \times p$ known matrix and g is a smooth one-to-one function called *link function*.

The main improvements of GLMs over classical linear models are hence:

- The ability to handle a larger class of distributions for the response variable Y. Apart from the Gaussian, other distributions are for example the binomial, Poisson and Gamma (Mccullagh and Nelder 1989).
- The relationship of the response variable Y to the linear predictor (LP) through the link function $g(\mu)$.

As in the classical case, the choice of the appropriate distribution and the presence of outliers can often be tested through scatterplots of residuals. Although several types of residuals are available for GLMs, deviance residual plots are most suitable for this purpose (Breslow 1996). They are defined for a gamma distribution as follows:

$$d(y_i, \hat{\mu}_i) = -2\left(\ln \frac{y_i}{\hat{\mu}_i} + \frac{y_i - \hat{\mu}}{\hat{\mu}_i}\right)^{1/2},$$

where $\hat{\mu}_i$ is the estimator obtained from the current model. A plot of deviance residuals vs. ln(fitted values) reveals possible outliers, when isolated points appear, or unsatisfactory covariate scale or link function when a curvature exists; an approximately constant scatterplot is expected if the model is good. Another important tool is a Q–Q plot of deviance residuals vs. the correspondent

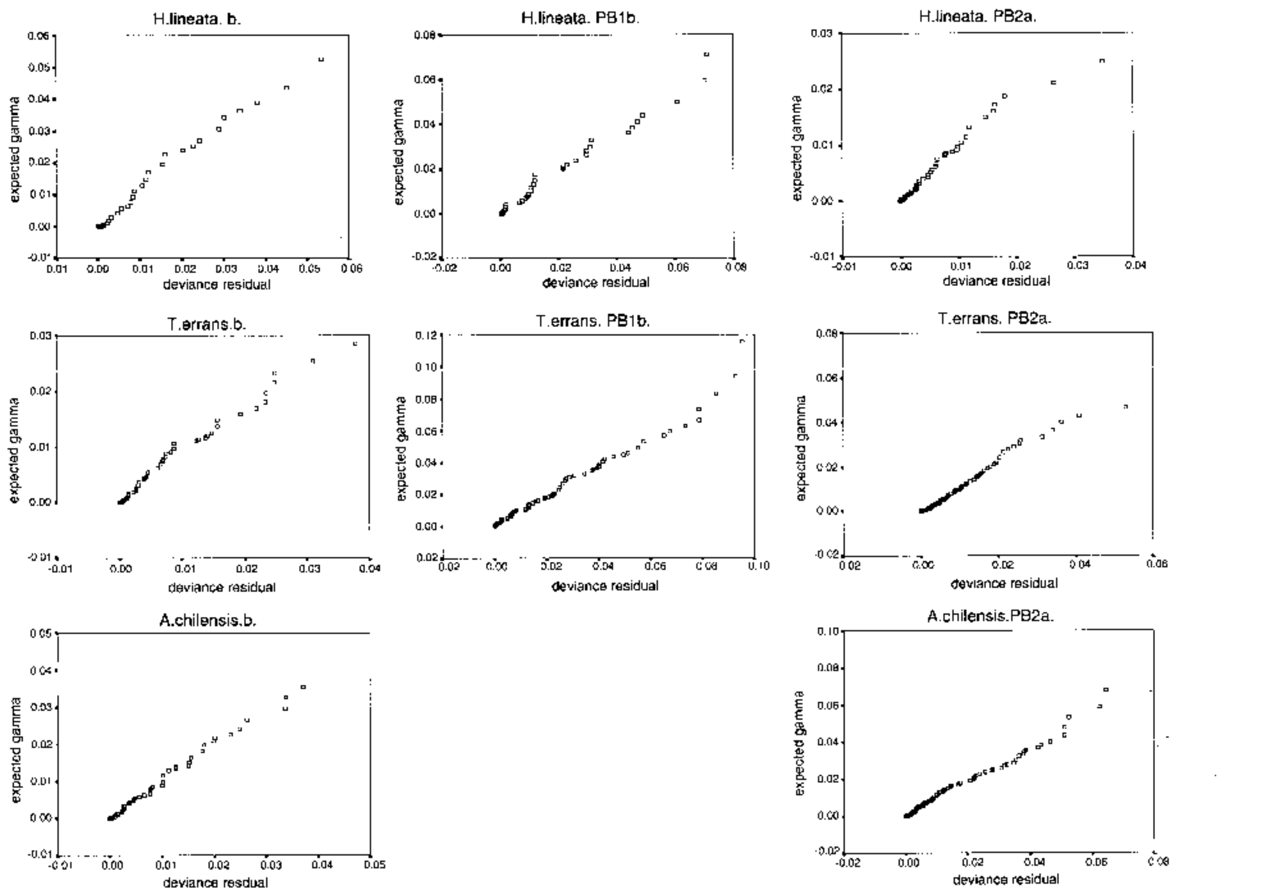

Fig. 2. Scatterplot of quantiles of gamma distribution vs. deviance residuals.

quantiles; if the chosen distribution is appropriate it will show linear pattern. These two kind of graphics were obtained for nematocyst data.

The hypotheses of equal means were tested using an Analysis of Deviance (ANODEV). This method is a generalization of ANOVA used under normality. One factor ANODEV can be seen as an univariate GLM with a single categorical covariate. GLIM package was used to perform the calculations (Francis et al. 1994).

Results

The descriptive statistics of data set used in this study was detailed by Acuña et al. (2003). Since the observations were positive, asymmetric and had constant coefficient of variation (Table 1), a gamma distribution was chosen to fit the data. An inverse link function $g(\mu) = 1/\mu$ was used, since this is the canonical link for gamma errors.

The scatterplots of deviance residuals vs. ln(fitted values) are shown in Fig. 1. As can be seen, the shape is approximately constant for all types of nematocysts in the three studied species. We detected a few big residuals, which were considered outliers and consequently eliminated. The fit of the observations to a gamma distribution was explored graphically, it was done via a *Q–Q* plot of residuals vs. the quantiles of the inverse cumulative gamma function. As can be seen in Fig. 2, the scatterplots look linear, so we accepted the gamma distribution as a proper model.

In the next step an ANODEV was performed, with errors distributed according to a gamma model and inverse link function. The hypothesis of equal means was always rejected (Table 2).

Discussion

The intense interest that cnidae have stimulated derives from their being the most complex intracellular secretion known, from the unique ability of cnidarians to secrete them, from their microstructural beauty, and increasingly from their biomedical importance

Table 2. Results of analyses of deviance

Species	b-rhabdoids		p-rhabdoids B1b		p-rhabdoids B2a	
	Statistic	*P*	Statistic	*P*	Statistic	*P*
Haliplanella lineata	7.816	0.00	19.978	0.00	37.259	0.00
Tricnidactis errans	30.369	0.00	30.289	0.00	13.082	0.00
Anthothoe chilensis	35.163	0.00	—		22.154	0.00

(Shick 1991). The cnidocysts are very important in taxonomic studies, but its value is discussed (Zamponi and Acuña 1991). Many authors have studied quantitative aspects of nematocysts belonging to different cnidarian taxa (Acuña et al. 2003), and most of them had applied traditional statistical tools, such as parametric and non-parametric tests. However, the lack of symmetry and the high variability apparent in many of their size distributions are major difficulties to perform comparative studies. Non-parametric tests provide an alternative, since non-normality of data distribution can be ignored, but the tests will be less powerful. GLMs provide an alternative that offers a broader set of distributions to choose from, the natural exponential family, and so the analysis can be done with parametric methods despite the non-normal distribution.

In this way we decided to use GLMs on the cnidae of acontia of sea anemones *H. lineata*, *T. errans* and *A. chilensis* and compare the results with those of previous papers using classical statistical methods. Although Acuña et al. (2003) analyzed the nematocysts of these actiniarians, this was possible only after transformation of many non-normal data sets. As a consequence some statistical tests used were less powerful and conclusions were not so solid as we could expect. We applied the GLM with Gamma Errors to our data and found this model to provide a good quantitative comparison of the acontian cnidae of the three species, obtaining the same statistical differences as found by Acuña et al. (2003). Although cnidae are taxonomically important characters within the sea anemones (Doumenc and Foubert 1984; Fautin 1988), both in defining higher taxa (Schmidt 1974) and in identifying species within a difficult group (Manuel 1988); our results suggest that cnidocysts are not quantitatively important to resolve taxonomical concerns, due to the observed significant statistical differences between the means of data set compared.

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