Predation Resistance and Nematocyst Scaling for 

Metridium senile and M. farcimen

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Abstract. Previous studies suggest that large body size reduces the risk of predation for acontiate sea anemones. For two species of Metridium, we found significant increases in the length of the acontial threads and in the mean lengths of the unfired acontial nematocyst capsules, with increasing body size. This supports the hypothesis that more damaging acontial defenses protect larger acontiate anemones from their predators. Metridium is planktivorous, and food size does not increase substantially with body size; so we expected smaller increases in nematocyst size for the feeding tentacles. In fact, scaling exponents were significantly smaller for the tentacle nematocysts than for acontial nematocysts of the same types in 3 out of 4 cases. This suggests that nematocyst scaling responds predictably to selection pressure. When specimens of the same size were compared, the non-clonal, subtidal species, M. farcimen, had significantly larger acontial nematocysts than did its clonal congener, M. senile, which lives at the upper tidal limits for major subtidal predators in the northeastern Pacific. Therefore, larger acontial nematocysts may be particularly advantageous where predation levels are high. These data demonstrate that closely related anemone species can be distinguished on the basis of ecologically and functionally relevant differences in nematocyst scaling.

Introduction

Predation is one of the strongest evolutionary pressures on many organisms, favoring a diverse suite of adaptations that reduce predation risk (Curio, 1976; Vermeij, 1982; Sih, 1985, 1987; Bronmark and Minin, 1992). Larger body size often decreases vulnerability to predation (Paine, 1976; Tripet and Perrin, 1994; Arsenault and Himelman, 1996; Lundvall et al., 1999) because predators are physically unable to consume larger prey (Tripet and Perrin, 1994; Arsenault and Himelman, 1996), or because larger prey are able to escape or to retaliate more effectively (Harris, 1986; Arsenault and Himelman, 1996; Christensen, 1996; Lundvall et al., 1999).

Acontiate sea anemones have specialized antipredator defenses. When attacked, the anemones contract and extrude many long, thread-like mesenterial filaments (the acontia) through the mouth and column wall (Hyman, 1940). The acontia are ciliated and motile, bearing densely packed tracts of large specialized nematocysts that fire on contact with predators. Damage from such contacts may dissuade a predator from continuing the attack (Harris, 1973; Edmunds et al., 1976; Shick, 1991). The three types of nematocysts found in acontia (basitrichs, microbasic b-mastigophores, and microbasic amastigophores) are also found in the feeding tentacles; but the b-mastigophore and amastigophore found in the acontia are more than twice the size of the tentacle cnidae (Hand, 1956).

Three acontiate anemones in the genus Metridium co-occur in the Pacific Northwest. The two studied here, M. senile (Linnaeus, 1761) and M. farcimen (Tilesius, 1809), differ in growth form and habitat preference. M. senile is smaller, and predominantly intertidal in the Pacific Northwest. M. senile replicates asexually by pedal laceration, and usually occurs in large, genetically identical clonal groups
(Bucklin, 1987b). *M. farcimen* grows much larger; is found only in subtidal habitats (Fautin et al., 1989); and does not replicate asexually, occurring as solitary individuals or as congregations of genetically distinct individuals (Bucklin, 1987a). Originally assumed to be an aclonal morph of *M. senile* (Hand, 1956), *M. farcimen* was shown through molecular analysis to be an independent species (Bucklin and Hedgecock, 1982) and its original name was reapplied by Fautin and Hand (2000). The third species, *M. exilis*, is a very small anemone that lives only in intertidal areas and forms clonal aggregations through binary fission (Hand, 1956).

Both laboratory studies and field observations suggest that *Metridium* becomes less vulnerable to predation with increasing body size. An important predator, the sea slug *Aeolidia papillosa*, shows a strong preference for small individuals of *M. senile* in choice experiments (Harris, 1976; Harris and Duffy, 1980). Furthermore, Harris (1973, 1991) found that *A. papillosa* is more likely to attack small individuals in an aggregation and, as a result, selects strongly for larger body size. Another key predator, the leather star *Dermasterias imbricata*, readily consumes small specimens of *M. senile* in the laboratory (Annett and Pierotti, 1984).

Body size of *Metridium* generally increases along a gradient from intertidal to subtidal habitats, mirroring an increase in density of both *A. papillosa* and leather stars. The presence of large individuals of *Metridium* sp. in the subtidal zone where predation pressure is more intense suggests a size refuge from predation (Annett and Pierotti, 1984; Harris, 1991).

Several possible explanations could account for the decreased vulnerability to predation of larger specimens of *Metridium*. Larger anemones may simply exceed the mechanical constraints of predator feeding mechanisms. This seems likely for the sea stars, which must settle on top of their prey (Annett and Pierotti, 1984). However, *A. papillosa* feeds by biting off pieces of the anemone in a manner not obviously limited by anemone size (Edmunds et al., 1976).

If larger anemones have better defense mechanisms, this might contribute to the increased resistance of larger anemones to predation. Edmunds et al. (1974) found that anemone species that lack acontia were preferred over acontiate species by *A. papillosa*, suggesting that the acontia may provide effective defense against this predator. Harris (1973, 1986) reported that acontial defenses could dissuade *A. papillosa* from feeding and could even result in the death of the sea slug. He noted that this defense mechanism appeared to be related to the size of the anemone (Harris, 1986).

In a companion study, Francis (2004) shows how scaling analysis can be used to detect evolutionary changes in cnida size and shape by comparing homologous cnida populations from different anemone tissues and species. Here we look at the scaling of acontium length, acontium diameter, and mean capsule size of the two large acontial nematocysts (amastigophores and b-mastigophores). Strong positive correlation of any or all of these variables with body size would support the argument made by Harris (1986) that large anemones are less vulnerable to predation because they defend themselves more effectively against predators. In addition, because the larger-bodied, non-clonal species, *M. farcimen*, lives in deeper water where predators are more abundant, we also expected stronger antipredator defenses in *M. farcimen* than in the smaller-bodied and clonal *M. senile*.

In designing the scaling study, we predicted relatively rapid increase in the size of the large acontial nematocysts with increasing body size, due to ongoing directional selection caused by predation. By contrast, we expected relatively little increase in the size of these same two nematocyst types in the feeding tentacles. Because prey size does not increase significantly with body size for *Metridium* (Sebens, 1981), nematocysts involved primarily in food capture and handling should be subjected to stabilizing selection.

**Materials and Methods**

**Specimen collection and handling**

Between 15 June and 1 August 1999, we collected specimens of *Metridium farcimen* on the boat dock at Skyline Marina, Anacortes, Washington (48°30′N, 122°41′W) and specimens from a single *M. senile* clone on the Schwarz boat dock, Garrison Bay, on San Juan Island (48°35′N, 123°10′W). A clonal sample is ideal for isolating the effects of body size independent of genetic variation. Anemones were deliberately chosen to provide an even distribution of body sizes spanning the available size range. At Shannon Point Marine Center, Anacortes, Washington, the anemones attached to the tank or to glass bowls in a continuous-flow sea table, where they were fed pieces of frozen cod once or twice a week.

**Body-size measurement**

Within 2 weeks, we weighed the living anemones on a Mettler P1200 balance after blotting them to remove as much excess water as possible. Because these soft-bodied animals are continually changing shape by taking in and expelling large quantities of seawater, blotted wet weight provides a nondestructive basis for comparing polyp sizes that is more reliable than linear dimensions. For example, the diameter of the expanded oral disc can vary greatly with the degree of expansion of the anemone; and the relationship between oral disc size and tissue volume may vary considerably between species. Daily measurements of 10
individuals (\textit{M. senile}) over a 3-day period indicated that wet weights were reproducible within ± 10%.

\textbf{Nematocyst size measurement}

Several extruded acontia or excised tentacles were fixed in 1–2 ml of 10% formalin to prevent nematocyst discharge. The tissues were disrupted using a glass tissue grinder to randomly mix and disperse the sample.

A drop of this nematocyst mixture was scanned systematically with non-overlapping fields (method of Williams, 1996), and maximum capsule lengths and widths of the first 20 each of the unfired b-mastigophore and amastigophore capsules were measured (method of Hand, 1954) using 400× magnification and a video image analysis system (Optimax software package). Means and their standard errors were calculated for each sample of 20 capsules. Duplicated measurements using 20 video images indicated that capsule dimensions were reproducible within ± 2% (length) and ± 5% (width).

\textbf{Extended acontia measurement}

To provide comparative estimates of acontial length for specimens of different size, we removed each anemone from the tank and held it upside down, which caused extrusion of the acontia. When the strand was fully extended under the pull of gravity, we measured the length from the oral sphincter to the most distal tip, which we called the extended acontia length.

To measure acontium diameter, we gently removed a single extruded acontial thread from each individual, placed it on a slide with a drop of seawater and coverslip, and measured the diameter (width) of an undamaged portion at 100×. Daily measurements of 10 individuals (\textit{M. senile}) over a 3-day period indicated that acontium lengths and acontium diameters were reproducible within ± 31% and ± 13%, respectively.

\textbf{Statistical analyses}

Untransformed data were used to examine the relationship between body size and the other measurements obtained from each individual. For ease of interpretation, the graphs all use linear scale on the abscissa (mean nematocyst capsule dimensions, acontium diameter, and effective acontial length) and log scale on the ordinate (wet weight), as in Longley (1984) and Francis (2004). This makes it easier to display the standard errors of the means and to assess the magnitude and possible biological relevance of any between-sample differences (Smith, 1984).

Data were log-transformed (base 10) for statistical analyses, a common practice in scaling studies where the relationship is well represented by a power function (Huxley, 1932; Smith 1984) as would be predicted here by simple dimensional analysis (LaBarbera, 1989; Francis, 2004).

We calculated Spearman rank correlations for the relationship between anemone wet weight and each of the other metrics, using the NCSS software package, ver. 2000 (Hintze, 2001); and significance at \( P = 0.05 \) was determined using sequential Bonferroni adjustment within groups of related tests (Rice, 1989). A nonparametric test for correlation is most appropriate for determining the significance of these relationships because the wet weights are not normally distributed and there is error in both the dependent and independent variables. Thus the data do not meet the assumptions of ordinary least squares regression (OLS; Sokal and Rohlf, 1981; Rayner, 1985).

For describing functional relationships such as these, McArdle (1988) showed that reduced major axis regression (RMA) provides a more robust estimate of the slope (\( b_{\text{RMA}} \)) than does OLS, where values for Pearson’s product moment correlation (\( R \)) are less than 0.9 and the error for \( X \) is more than one-third that of the \( Y \) variable (probably true, here). OLS consistently underestimates these slopes. Note, also, that OLS and RMA estimates converge as \( R \) increases, and that the variance and standard error are numerically the same for RMA and OLS estimators (McArdle, 1988). \( R \) and the percent standard error of estimate (%SEE; Smith, 1984) provide measures for goodness of fit.

RMA estimates and 95 percent confidence intervals (95% CIs) for the slopes and intercepts were generated by bootstrapping each data set 10,000 times using SYSTAT, ver. 9 (SPSS, 1998), selecting the median values to estimate slopes and intercepts, and selecting the 250th and 9750th values as the upper and lower 95% confidence values. Bootstrapping provides estimates without assumptions about data distribution, and these estimates agree to three decimal places with coefficients calculated directly from sample statistics.

Because the data do not meet the assumptions for a Model I ANOVA (or ANCOVA), we use the bootstrapped confidence intervals to compare slopes and intercepts (M. LaBarbera, Univ. of Chicago, pers. comm.). By definition, two slopes are significantly different at the 0.05 level if each mean is outside the 95% CI of the other; however, it is necessary to be aware of the possibility for multiple testing error (Hess, 1993).

\textbf{Results}

\textbf{Scaling of acontia}

Extended acontia length increased significantly with body size for both \textit{Metridium} species (Fig. 1A, Table 1); and it increased more rapidly than would be predicted for isometric scaling (positive allometry). The predicted null value for isometric scaling of dimensions relative to mass (0.33) is
outside the 95% confidence intervals for both species (Table 1). An empirically measured scaling exponent for pedal disc diameter as a function of ash-free dry weight (mass) in *M. senile* (0.36) also lies outside the 95% CIs (Sebens, 1981).

By contrast, mean widths of the unfired capsules were not significantly correlated with body size for any of the acontial nematocysts (Fig. 3, Table 3).

For the tentacle nematocysts, mean length of the unfired capsules was significantly and positively correlated with body size for all cases except one. Change in capsule length was not correlated with body size for the tentacle b-mastigophores of *M. farcimen* (Fig. 2, Table 1).

**Tissue differences in nematocyst scaling**

Acontial nematocysts were consistently longer than tentacle nematocysts of the same type (Figs. 2 and 4), and scaling exponents were significantly higher for the acontial nematocysts in 3 cases out of 4 (Fig. 2; 95% CI, Table 2). The exception was *M. senile*, where 95% CIs for tentacle and acontial amastigophores overlapped (Fig. 2, Table 2).

**Species differences**

Scaling exponents were indistinguishable for homologous nematocyst populations from the two *Metridium* species (Table 2, overlapping 95% CIs for RMA estimates of the slopes for capsule length vs. wet weight). When specimens of the same size were compared (narrow allometry, *sensu* Smith, 1984), mean capsule length was always greater for *M. farcimen* than for *M. senile* (Fig. 2); and these differences in elevation were significant (Table 2, non-overlapping 95% CIs for intercepts).

**Discussion**

Because predators prefer to eat smaller specimens of *Metridium*, Harris (1986) suggested that the effectiveness of specialized antipredator defenses may vary with anemone size. Our data support this hypothesis. The extended length and diameter of the acontial threads themselves and the capsule lengths of the large acontial nematocysts (b-mastigophores and amastigophores) were all positively correlated with body size.

Effectiveness of the acontia as predator deterrents presumably is related to the pain and damage caused to a potential predator and increases with the number and potency of nematocysts brought into contact with that predator. Thus, more acontial threads, longer acontia, more nematocysts, and larger acontial nematocysts should all improve deterrence.
Table 1

Acontium dimensions: scaling constants for Metridium farcimen and M. senile, from reduced major axis regression equations

| Species (n) | Scaling exponent† (95% CI) | Intercept† (95% CI) | $X$, $Y$‡ ($\%$SEE) | $R$¶ | $P$# |
|-------------|----------------------------|---------------------|----------------------|------|-----|
| **Log (effective acontia length, cm) ($Y$) vs. log (wet weight, g) ($X$)** | | | | | |
| *M. farcimen* (15) | 0.4498 (0.373–0.5165) | 3.612 (3.148–4.233) | 0.896, 0.961 (29%) | 0.94 | <0.0001* |
| *M. senile* (8) | 0.5969 (0.4738–0.7993) | 3.190 (2.872–3.401) | 0.226, 0.639 (28%) | 0.96 | <0.0001* |
| **Log (acontium diameter, nm) ($Y$) vs. log (wet weight, g) ($X$)** | | | | | |
| *M. farcimen* (23) | 0.1369 (0.1055–0.1788) | 253.51 (236.59–266.69) | 0.703, 2.500 (21%) | 0.75 | <0.0001* |
| *M. senile* (9) | 0.2501 (0.1718–0.3493) | 223.87 (209.41–236.05) | 0.291, 2.423 (21%) | 0.86 | 0.02 |

§ Number of anemones in the sample.
† Slope (scaling exponent, $b$) and intercept ($a$) of the RMA regression line ($\log(Y) = b \times \log(X) + a$); bootstrapped 95% confidence intervals (95% CI).
‡ $X$ and $Y$, mean values for $\log(X)$ and $\log(Y)$.
¶ Pearson correlation coefficient ($R$), and percent standard error of estimate ($\%$SEE).
# $P$ value calculated in Spearman rank correlation.
* $P < 0.05$, groupwise error adjusted using a sequential Bonferroni adjustment.

Table 2

Nematocyst lengths: scaling constants for Metridium farcimen and M. senile, from reduced major axis regression equations

| Nematocyst type: Species and tissue (n)§ | Scaling exponent† (95% CI) | Intercept† (95% CI) | $X$, $Y$‡ ($\%$SEE) | $R$¶ | $P$# |
|----------------------------------------|----------------------------|---------------------|----------------------|------|-----|
| **Log (mean capsule length, nm) ($Y$) vs. log (wet weight, g) ($X$)** | | | | | |
| *b-mastigophore* | | | | | |
| *M. farcimen* acontia (27) | 0.0353 (0.0297–0.0408) | 69.023 (68.234–69.823) | 0.843, 1.869 (4%) | 0.88 | <0.0001* |
| *M. farcimen* tentacles (10) | 0.0084 (0.0035–0.0169) | 25.003 (24.660–25.235) | 0.735, 1.405 (3%) | 0.11 | 0.6 |
| *M. senile* acontia (9) | 0.0505 (0.033–0.0696) | 60.117 (59.293–60.814) | 0.291, 1.794 (2%) | 0.90 | 0.0005* |
| *M. senile* tentacles (9) | 0.0181 (0.0134–0.0224) | 22.387 (22.336–22.454) | 0.16, 1.353 (1%) | 0.92 | 0.0065* |
| *amastigophore* | | | | | |
| *M. farcimen* acontia (21) | 0.0431 (0.0362–0.0506) | 71.614 (70.795–72.278) | 0.575, 1.879 (3%) | 0.91 | <0.0001* |
| *M. farcimen* tentacles (10) | 0.0112 (0.0075–0.0165) | 27.861 (27.606–27.990) | 0.735, 1.453 (2%) | 0.77 | 0.0075* |
| *M. senile* acontia (9) | 0.047 (0.0362–0.0651) | 63.241 (62.373–63.680) | 0.291, 1.814 (2%) | 0.95 | <0.0001* |
| *M. senile* tentacles (8) | 0.0308 (0.0149–0.0529) | 24.889 (24.604–25.177) | 0.268, 1.405 (3%) | 0.75 | 0.0053* |

§ Number of anemones in the sample.
† Slope (scaling exponent, $b$) and intercept ($a$) of the RMA regression line ($\log(Y) = b \times \log(X) + a$); bootstrapped 95% confidence intervals (95% CI).
‡ $X$ and $Y$, mean values for $\log(X)$ and $\log(Y)$.
¶ Pearson correlation coefficient ($R$), and percent standard error of estimate ($\%$SEE).
# $P$ value calculated in Spearman rank correlation.
* $P < 0.05$, groupwise error adjusted using a sequential Bonferroni adjustment.
Larger anemones have more and bigger acontia than smaller conspecifics

Potential effectiveness of the acontia as deterrents should be a function of total nematocyst-bearing area, which is proportional to the length of the thread, number of threads, and nematocyst density. Part of the increase in total nematocyst-bearing area as anemones grow occurs because the acontia connect to the inner edge of the mesenteries, and larger anemones have more mesenteries (Stephenson, 1928).

Larger anemones also have longer acontia (Fig. 1, Table 1) that can be extended farther, and thus are more likely to contact a predator. Longer acontia also carry more nematocysts and have more surface to wrap around a prospective predator. The length of the acontial threads increases more rapidly with body size (scaling exponents 0.45 and 0.60) than the null expectation (scaling exponent 0.33). This positively allometric relationship suggests that relatively rapid increase in acontial length with increasing body size (and increasing energy supply; Sebens, 1981) has been favored by natural selection in both *Metridium* species (Fig. 1A).

Acontium diameter, another factor in total nematocyst-bearing area, also increases with body size, but this relationship is negatively allometric. That is, as body size increases, acontium thickness increases less rapidly (Fig. 1B, scaling exponents 0.14–0.25) than external body dimensions such as foot diameter (0.36; Sebens, 1981) or the null expectation of isometry (0.33), and much less rapidly than acontium length (0.45 and 0.60). A less rapid increase in this aspect of acontial structure seems to favor deployment advantages over structural strength or maximum in-

**Figure 2.** Mean length (± 1 SE) of unfired nematocyst capsules vs. anemone wet weight for (A) b-mastigophores and (B) amastigophores from the tentacles (triangles) and acontia (squares) of *Metridium farcimen* (unfilled shapes) and *M. senile* (filled shapes). Means and their standard errors are based on systematic samples of 20 unfired capsules from each tissue of each specimen. Correlations of each individual’s mean capsule length with wet weight are significant for all acontial nematocysts and tentacle nematocysts except b-mastigophores from *M. farcimen* (P < 0.05 for groupwide error; Spearman rank correlation with sequential Bonferroni correction).

**Figure 3.** Mean width (± 1 SE) of unfired nematocyst capsules vs. anemone wet weight for acontial nematocysts. Correlations with wet weight are not significant for any of the populations (P < 0.05 for groupwide error; Spearman rank correlation with sequential Bonferroni correction).
crease in contact area. A relatively narrow acontial thread can be more flexible than a very thick thread, bending more readily with water currents and wrapping more easily around a predator’s fine morphological features such as the cerata of a slug. While a relatively thin thread is likely to detach from the anemone more easily, the acontia appear to be disposable structures. Acontial nematocysts continue to fire after the acontial threads have become detached (pers. obs.).

Notably, only part of the acontial thread is nematocyst-bearing: the ciliated tract is dedicated to acontium mobility. It would be interesting to know whether these regions increase proportionally with increasing anemone size.

Size and potential effectiveness of acontial nematocysts increases consistently with body size

In the acontia, mean unfired capsule length of both nematocyst types increases as a function of wet weight, continuing to increase for the entire range of individuals sampled (Fig. 2). Capsule length is highly correlated with body size for both acontial nematocyst types in both Metridium species (Table 2). This increase in mean capsule lengths is substantial over the size ranges of anemones sampled and is likely to be biologically relevant. For this sample of M. farcimen, mean length of the acontial nematocyst capsules increases by 25%–29% for a 750-fold increase in body weight. For M. senile, a 70× increase in weight results in a 14%–23% increase in the mean length of the acontial nematocysts.

Longer nematocyst capsules can carry more toxin and a longer barbed tubule. The longer the barbed tubule, the more likely it is to succeed in penetrating the tissue of a prospective predator, and the deeper it can penetrate, increasing the potential for mechanical damage during penetration and deep damage by toxins.

Larger anemones do not have substantially wider acontial nematocysts. For M. farcimen, the actual increase in mean capsule width for the acontial b-mastigophores is only 2.6% for a 750-fold increase in body weight. For M. senile, there is no net increase in the mean width of the acontial nematocysts over the 70-fold increase in body size for this sample.

Differences between the two Metridium species reinforce the case for a causal relationship linking predation pressure to anemone size and nematocyst size

The acontial nematocysts of M. farcimen are consistently larger than those of M. senile for specimens of similar size across the entire range of overlapping body size (Fig. 2A). This can be seen as an extension of the pattern observed within each species individually.

Since M. farcimen is exclusively subtidal, it presumably is subjected to higher predation from subtidal predator species than is M. senile, especially at small body size. In the Pacific Northwest, M. senile lives primarily in the intertidal and on pilings, floats, and docks at protected sites where the threat of predation, especially by the leather star, probably is much less. If larger acontial nematocysts increase survival

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### Table 3

| Nematocyst type: Species and tissue (n)§ | Scaling exponent† (95% CI) | Intercept† (95% CI) | X̄, Ȳ‡ | R¶ (%SEE) | P# |
|----------------------------------------|---------------------------|---------------------|--------|-----------|---|
| Log (mean capsule width, nm) (Y) vs. log (wet weight, g) (X) | | | | | |
| b-mastigophore | | | | | |
| M. farcimen acontia (27) | 0.019 | 4.928 | 0.843, 0.709 | 0.37 | 0.0158 |
| | (0.0124–0.0286) | (4.837–4.991) | | (4%) | |
| M. senile acontia (9) | 0.0216 | 4.933 | 0.291, 0.699 | 0.21 | 0.61 |
| | (0.0138–0.0361) | (4.885–4.959) | | (3%) | |
| amastigophore | | | | | |
| M. farcimen acontia (21) | 0.0252 | 6.445 | 0.575, 0.824 | 0.41 | 0.619 |
| | (0.0175–0.0363) | (6.350–6.510) | | (5%) | |
| M. senile acontia (9) | 0.0159 | 6.358 | 0.291, 0.808 | 0.73 | 0.067 |
| | (0.0069–0.0231) | (6.327–6.396) | | (2%) | |

§ Number of anemones in the sample.
† Slope (scaling exponent, b) and intercept (a) of the RMA regression line (log (Y) = b * log (X) + log (a)); bootstrapped 95% confidence intervals (95% CI).
‡ Mean values for log (X) and log (Y).
¶ Pearson correlation coefficient (R), and percent standard error of estimate (%SEE).
# P value calculated in Spearman rank correlation.
* P < 0.05, groupwide error adjusted using a sequential Bonferroni adjustment.
and fitness in the presence of such predators, selection will usually be stronger for *M. farcimen* than for *M. senile*. This may explain why the acontial nematocysts of *M. farcimen* are much larger than those of *M. senile*, for specimens of the same size. Sampling additional *M. senile* clones is necessary to determine whether the size difference is consistent across different habitats and genotypes.

We demonstrate for the first time that scaling studies using data on cnida size and anemone body size can be useful for distinguishing between closely related species, even when the size ranges for homologous cnida populations are overlapping. Fautin et al. (1989) stated that "nematocysts do not appear to differ consistently between the two [*M. farcimen* and *M. senile*], their size

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**Figure 4.** Microbasic b-mastigophores and microbasic amastigophores from the acontia and tentacles of two anemone species. Average-sized nematocysts were selected from 2.5-g specimens of *Metridium senile* (to the left; A, B, E, and F) and *M. farcimen* (to the right; C, D, G, and H). Acontial nematocysts (A, B, C, and D) are much larger than tentacle nematocysts from the same specimens (E, F, G, and H). Amastigophores (A, C, E, and G) are shown to the left of b-mastigophores (B, D, F, and H) from the same tissues. In life, the tubule end of the capsule (pointing upward in these photographs) is close to the outer cell surface of the acontial gastrodermis. Photographs were taken using differential interference contrast at 1000× (oil immersion). Scale bar: 10 μm.
seemingly correlated more with size than species of the individual.” In fact, when specimens of the same size are compared, there are large and significant differences in the mean sizes of the acontial nematocysts from the two *Metridium* species. The cnida size data for the *M. senile* clone are well outside the expected range of *M. farcimen* for all four nematocyst populations. Under any reasonable assumption about the position of the clonal data within the *M. farcimen* population data (based on the 95% CI for the intercept of the *M. farcimen* data on capsule length), *M. senile* has shorter mean nematocyst lengths for 4 out of 4 sampled cnida populations. Thus this is a robust result that we predict will be confirmed when a wider sample of genotypes from *M. senile* is examined.

**Acontial nematocysts in other species scale similarly**

Stephenson (1929, his tables I and II) reported mean lengths and widths for two nematocyst types, “penicilli” (probably amastigophores) and “spirulae” (probably b-mastigophores), from the acontia of *Sagartia elegans* (30 capsules per type for each of 12 specimens) and *Cereus pedunculatus* (30 capsules per type for each of 7 anemones—2 newly released, brooded young and 5 adults). Stephenson stated that his data showed no consistent relationship between body size and nematocyst size. In fact, mean cnida lengths are significantly correlated with body size for all four of his acontial cnida populations; and mean capsule widths are significantly correlated with body size for 1 of the 4 (Spearman rank correlation, our Table 4). We also fit lines to the log-log plots of mean capsule dimension versus polyp diameter cubed. RMA slopes, intercepts, and $R^2$ values (Table 4) are very close to those reported here and by Francis (2004). Polyp diameters were cubed to make these plots dimensionally compatible with our data, and with most of the scaling literature, where body size typically is represented by a weight ($Wt \approx Vol. \times L^3$).

### Table 4

Lengths and widths of acontial nematocysts: scaling constants for *Sagartia elegans* and *Cereus pedunculatus*, from reduced major axis regression equations, computed using data from Stephenson (1929)

| Nematocyst type          | Species (n) | Scaling exponent† (95% CI) | Intercept† (95% CI) | $\bar{X}, \bar{Y}$‡ | $R^2$ (95% CI) | %SEE | $P$# |
|--------------------------|-------------|---------------------------|---------------------|-------------------|---------------|------|------|
| Log (mean capsule length, nm) (Y) vs. log (body diameter³, cm) (X) | b-mastigophore | | | | | | |
| S. elegans (12) | 0.0463 | 29.992 | 0.902, 1.519 | 0.60 | 0.0074* |
| C. pedunculatus (7) | 0.0361 | 29.512 | 0.400, 1.485 | 0.95 | 0.0137 |
| amastigophore | | | | | | | |
| S. elegans (12) | 0.0568 | 47.863 | 0.902, 1.731 | 0.67 | 0.0061* |
| C. pedunculatus (7) | 0.0569 | 35.318 | 0.400, 1.5706 | 0.95 | 0.0025* |
| Log (mean capsule width, nm) (Y) vs. log (body diameter³, cm) (X) | b-mastigophore | | | | | | |
| S. elegans (12) | 0.0629 | 2.941 | 0.902, 0.525 | 0.30 | 0.1397 |
| C. pedunculatus (7) | 0.0363 | 2.463 | 0.400, 0.460 | 0.82 | 0.0897 |
| amastigophore | | | | | | | |
| S. elegans (12) | 0.0465 | 4.761 | 0.902, 0.720 | 0.65 | 0.0213 |
| C. pedunculatus (7) | 0.0311 | 3.917 | 0.400, 0.605 | 0.98 | 0.0008* |

§ Number of anemones in the sample.
† Slope (scaling exponent, $b$) and intercept ($a$) of the RMA regression line ($\log(Y) = b \log(X) + \log(a)$); bootstrapped 95% confidence intervals (95% CI).
‡ Mean values for $\log(X)$ and $\log(Y)$.
¶ Pearson correlation coefficient ($R$), and percent standard error of estimate (%SEE).
# $P$ value calculated in Spearman rank correlation.
* $P < 0.05$, groupwide error adjusted using a sequential Bonferroni adjustment.
Natural selection can affect both nematocyst size and nematocyst scaling

Nematocyst size appears to vary in an adaptive manner among species and as a function of body size. This conclusion is based on differences in the sizes and scaling exponents for tentacle and acantial nematocysts of the same types from the two Metridium species (Figs. 2, 4; Table 2). These differences are linked to function-related differences in selection pressures. While feeding structures typically are subject to stabilizing selection, favoring optimal cost-effectiveness, structures used in antipredator defense are subject to directional selection, favoring maximum affordable effect (cf. energetic analysis of jumping vs. hovering; Alexander, 2000).

Metridium species are primarily filter feeders; and the size of captured prey is similar for anemones of different sizes, both in subtidal and in intertidal habitats (Purcell, 1977; Sebens, 1981; Sebens and Koehl, 1984). Hence there is little or no advantage to increased size in the tentacle nematocysts with increasing body size.

Actual increases in the mean size of tentacle nematocysts are relatively small. For M. farcimen, mean capsule lengths increase 0%–8% for a 750-fold increase in body weight; and for M. senile, capsule lengths increase 4%–6% for a 30- to 70-fold increase in body weight. Nonetheless, correlations with body size are significant in 3 cases out of the 4. There are at least three plausible explanations for this correlation, and they could act individually or in combination. (1) Food size, food type, or both may actually change somewhat with body size, favoring this slight increase in the size of some tentacle nematocyst types with increasing body size. (2) Scaling of the tentacle nematocysts may be advantageous in some context other than feeding. (3) Minimal scaling of these tentacle cnidae could also occur incidentally if the size of collagen-secreting cells typically does increase with body size (discussed by Francis, 2004).

For the acantial nematocysts, predation pressure favoring maximum affordable size is reflected in the larger size of the acantial nematocysts (Fig. 4) and the significantly larger RMA scaling exponents for acantial capsule lengths (0.035–0.051), as compared with the smaller scaling exponents (0.008–0.018) for the same nematocyst types in the tentacles (Fig. 2 and Table 2). There is one exception: for M. senile, the scaling exponents for capsule lengths of the acantial amastigophores (0.047) and the tentacle amastigophores (0.031) are not distinguishable at these sample sizes (Table 2).

Energy scaling analysis (Sebens, 1981) and mechanical analysis (Francis, 2004) both predict that larger anemones will be able to support larger defensive nematocysts: that is, the size of the maximum affordable and supportable defense structure should increase with body size. Sebens (1981) has shown that in some cases, larger anemone body size reflects higher net energy intake, suggesting that larger anemones may be able to afford relatively larger acantia and larger acantial nematocysts. Larger anemones also have thicker mesoglea (Shick, 1991; Francis, 2004), which can support a thicker attached layer of tissue and larger nematocysts (i.e., larger acantia and acantial nematocysts, Figs. 1 and 2; Tables 1 and 2).

Significant differences in nematocyst scaling exponents have never before been reported. Both the larger size (Fig. 4) and larger scaling exponents of the acantial nematocysts, as compared with their tentacle homologs (Fig. 2), reflect the difference in selective regimes. This suggests both that nematocyst scaling is responsive to selection and that the size of acantial nematocysts plays an important role in antipredator defense.

Summary

The strong and consistent pattern of positive correlations between the size of defensive structures and body size for these two Metridium species supports the hypothesis that better acantial defenses provide a refuge from predation for larger acantial anemones. These findings, combined with function-related differences between tissues and habitat-related differences between species, make a compelling argument for the value of larger acantial nematocysts in deterring potential predators. Differences in the scaling of tentacle and acantial nematocysts illustrate a novel pathway by which nematocysts may become specialized for particular functions.

Acknowledgments

Thanks to Richard Palmer, Mike LaBarbera, and an anonymous reviewer for valuable comments and suggestions. The work was supported in part by NSF 9731144, REU support through Western Washington University, Shannon Point Marine Center. For access to facilities and helpful discussion, we thank the directors, staff, and colleagues at SPMC (WWU); for the image analysis system at Shannon Point, we thank the NSF Instrumentation and Laboratory Improvement Program and Texaco Foundation. Without this support, the work would not have been possible.

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