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# Functional characteristics of nematocysts found on the scyphomedusa *Cyanea capillata*

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**Abstract**

Although prey capture by cnidarians is mediated through nematocysts, their influence on prey selection by cnidarians remains poorly documented. The difficulty in visualizing nematocyst–prey interactions remains the chief obstacle to understanding how the wide variety of nematocyst types influences the mechanics of prey capture. One solution to this limitation has been to assign functional roles to nematocysts based on morphological characters of discharged cnidae. Here we report results of an alternative approach based upon dynamic traits of nematocyst discharge. We examined tubule lengths, tubule discharge velocities and net-to-gross displacement ratios of tubules of discharging nematocysts possessed by the cosmopolitan scyphomedusa, *Cyanea capillata.* This nematocyst assemblage consisted of euryteles, birhopaloids and three different isorhizas — a-isorhizas, A-isorhizas and O-isorhizas. Dynamic traits varied little within each nematocyst type but there were significant differences between the different types. Most importantly, dynamic traits varied significantly within a broad category of nematocyst – the isorhizas – indicating that conventional classification schemes that infer function based on broad nematocyst categories may not appropriately describe the functional roles of these nematocysts. The dynamic properties of discharging nematocysts were consistent with physical results described in studies using scanning electron microscopy images of nematocyst–prey interactions. These data suggest that nematocysts vary significantly in their roles during predation, but that inferences relating prey selection with broad nematocyst categories merit careful examination.


*Keywords:* Cnidae; Discharge; Feeding mechanisms; Morphology; Prey capture

## 1. Introduction

Predation by medusae plays an important role in the trophic ecology of many pelagic ecosystems (Purcell, 1989; Matsakis and Conover, 1991; Olesen, 1995; Costello and Colin, 2002). Despite its importance, many of the mechanisms that determine predation rates and prey selection are poorly defined. Prior to prey ingestion, medusae must encounter, capture and successfully transfer the prey to their mouths for ingestion. Consequently, prey selection is determined by the outcome of these events (Costello and Colin, 1994; Colin et al., 2005, 2006; Hansson and Kiørboe, 2006). Following encounter with prey, nematocysts presumably play a critical role in determining prey selection by controlling the success with which a medusa is able to capture and retain prey for ingestion (Purcell and Mills,

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1988). Due to the inherent difficulties of observing prey capture by nematocysts, their precise functions during prey capture are not well understood.

Nematocysts are diverse in structure and interactions with target tissues (Mariscal, 1974; Williamson et al., 1996). The nematocyst itself consists of an intracellular capsule containing a tightly coiled and folded tubule. Upon receipt of appropriate stimuli, a nematocyst discharges by everting the folded tubule. The discharged tubules are distinguished by a variety of morphologies. Tubule dimensions and patterns of spine location are the basis of a formidable nomenclature describing the various nematocyst types (Weill, 1934; Östman, 2000). The functional significance of the great differences in size, shape, and spination of the nematocyst capsules, tubules and shafts is not understood. A substantial amount of progress has been made on understanding the nature of nematocyst toxins (summarized in Williamson et al., 1996) and the stimuli involved in initiating nematocyst discharge (e.g. Thorington and Hessinger, 1998, reviewed in Kass-Simon and Scappaticci, 2002). Less is understood about how nematocysts function in capturing prey.

The 25 or more known types of nematocysts are typically categorized into four functional groups: those that pierce (penetrants), entangle (volvents), or adhere to prey (glutinants), and those that adhere to the substrate. The majority of described nematocyst types are assumed to be penetrants because the tip of their tubules are open and are inferred to be capable of delivering the toxins characterizing cnidarian stings. Beyond this, most of our current knowledge on prey capture by nematocysts is based upon studies which either relate the nematocyst assemblage found on a medusan species to its diet (Purcell and Mills, 1988; Carrette et al., 2002; Peach and Pitt, 2005), or studies using microscopy to examine preserved samples of nematocysts and their captured prey (Purcell, 1984; Heeger and Möller, 1987; Heeger et al., 1992; Östman and Hydman, 1997; Peach and Pitt, 2005). Due to the inherent difficulty in observing the fastest cellular process identified, few studies have directly observed and described the discharge properties of different types of nematocysts (Holstein and Tardent, 1984; Nuchter et al., 2006). Besides the limited number of studies that have documented discharge velocities of stenotele nematocysts (Holstein and Tardent, 1984;

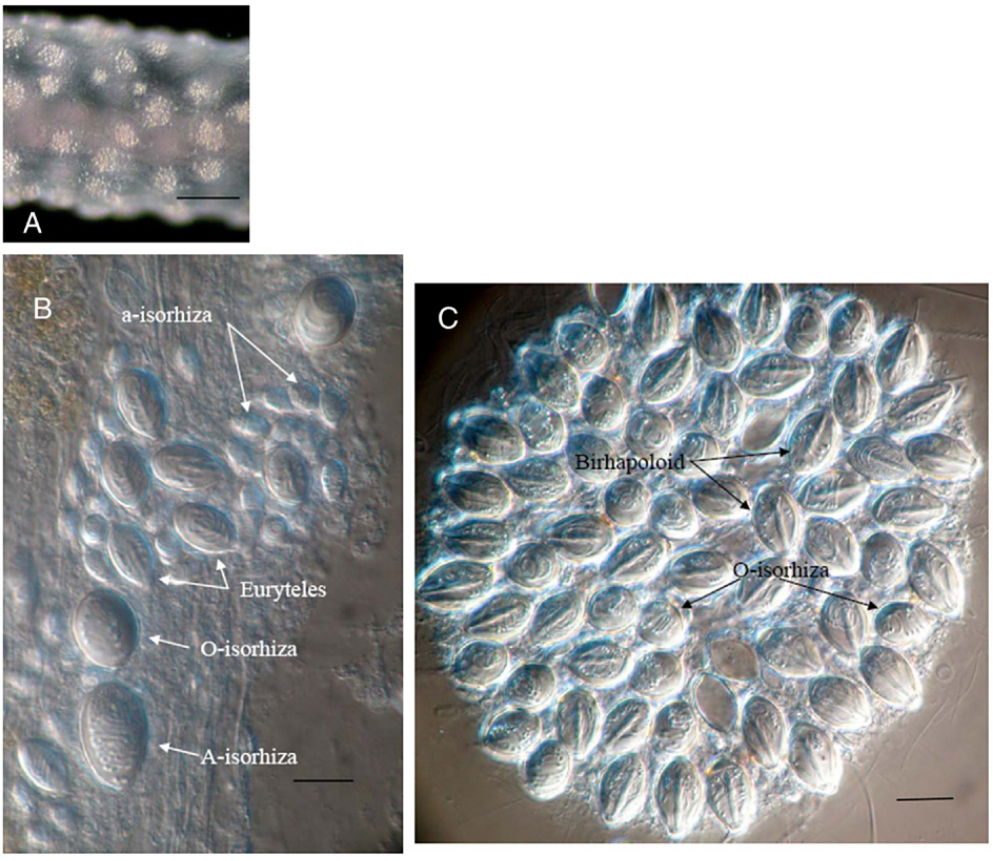


Fig. 1. Light-microscopic images of nematocyst batteries on *Cyanea capillata*. A) intact tentacle with multiple batteries; B) close-up of individual battery from the tentacle; C) close-up of individual battery from the oral arm. Scale bars are 100 μm long in A and 10 μm long in B and C.

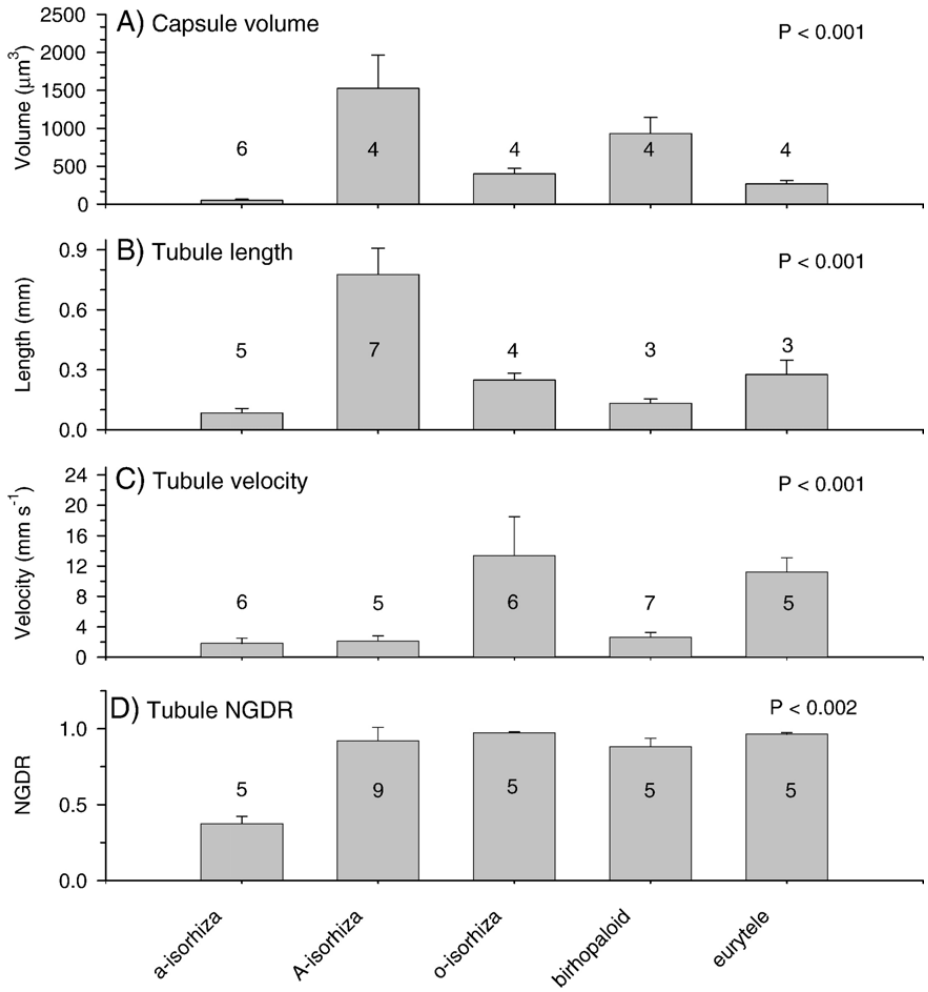


Fig. 2. Nematocyst morphological traits — A) capsule volume and B) filament length of discharged nematocyst tubule — and dynamic traits — C) tubule discharge velocity and D) tubule net-to-gross displacement ratio (NGDR). Error bars represent standard deviations from the mean. *P*-values represent results of Kruskal–Wallis Ranks Test with $df=4$ for each test. The numbers in and above each bar represent sample sizes ($n$ = number of medusae) for each nematocyst type. Each medusan value represents the mean of several nematocyst measurements (range = 3–13 nematocysts per medusae).

Nuchter et al., 2006), no studies have documented or compared the basic properties of nematocyst tubules during discharge, including tubule velocity, path and length. Therefore, we do not know whether the tubules of similar types of nematocysts discharge similarly or whether their discharge properties are related to the nematocyst categories identified by taxonomists. Since tubules are the part of nematocysts typically observed clinging to prey (Purcell, 1984; Heeger and Möller, 1987; Heeger et al., 1992; Östman and Hydman, 1997; Peach and Pitt, 2005), their properties likely determine how nematocysts capture prey, and hence, their functions.

Our goal was to describe and compare the static and dynamic traits of the tubules of several different types of nematocysts to determine whether the broad nematocyst classifications (e.g; isorhizas, euryteles) are related to their functional properties. Our approach utilized high-speed video and light-microscopy to quantify the discharge characteristics of nematocysts found on the scyphomedusa *Cyanea capillata*.

## 2. Methods

In order to compare the firing and tubule properties of different types of nematocysts, we visualized the discharge of nematocysts that were both attached to and isolated from *C. capillata* tentacles and oral arms. Most nematocysts used in the study were isolated nematocysts except some observations of A-isorhiza. Medusae were individually hand collected from the surface waters surrounding the Marine Biological Laboratory in Woods Hole, Massachusetts. They were immediately transferred to a large 20 L bucket that was placed in a water bath at ambient temperatures.

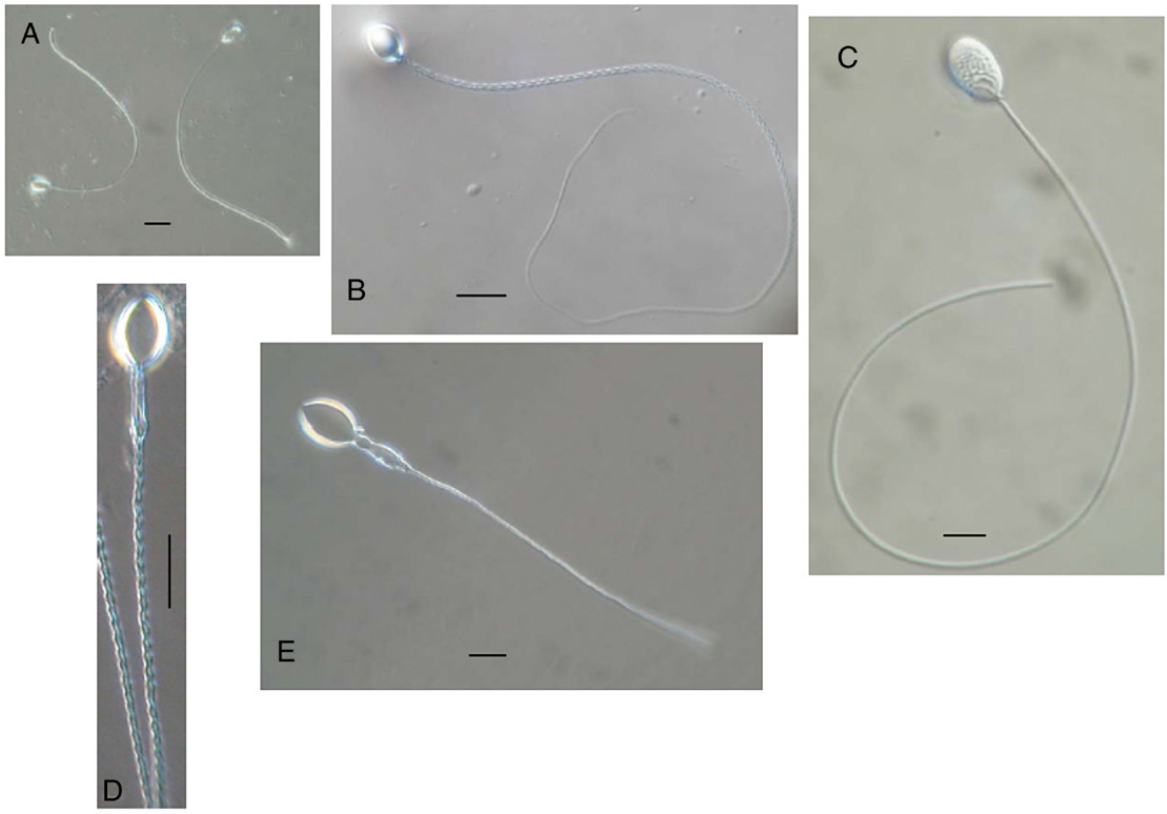


Fig. 3. Light-microscopic images of discharged nematocysts. A) a-isorhiza, B) O-isorhiza, C) A-isorhiza, D) eurytele, E) birhopaloid. Scale bars are 10 μm long.

New medusae were collected as necessary and only fresh tentacles and nematocysts were examined. Images of whole tentacle sections or isolated nematocysts were collected using a Nikon TE2000-U inverted microscope with phase and interference contrast optics. Nematocysts were isolated by scraping the epidermal layer from the tentacle. This isolated individual (e.g.; Fig. 3) and clusters (e.g.; Fig. 1B) of nematocysts. In order to visualize the firing of nematocysts, a digital image was taken before and after nematocyst discharge and discharge was video recorded at 250 frames per second (fps) using a Fastcam Super 10 K high-speed video camera. Nematocysts were discharged by adding a small drop of a 5% acetic acid solution to the seawater on the slide.

Nematocyst types were identified according to the characteristics described by Weill (1934) and terminology according to Östman (2000). Nematocyst capsule length and width and tubule length were measured from the still images. Capsule volumes ($V$) were calculated according to Purcell and Mills (1988) where the capsule is approximated as an ellipsoid:

$$V = 4/3\pi ab^2 \quad (1)$$

where $a$ is the radius of the length and $b$ is the radius of the width. Maximum discharge velocities, $v$, were measured from the high-speed video by tracking the leading point of the tubule every 0.004 s as it was fired out of the capsule. The path traveled by discharged tubules was quantified using their net-to-gross displacement ratios (NGDR). NGDR is the shortest distance between the starting and end position of the tubule tip divided by the total distance the tip traveled during discharge. This ratio describes how straight (NGDR = 1) or meandering (NGDR < 1) the tubule travel during discharge. All NGDR measurements were based on the path traveled by each tubule throughout the entire discharge event and each was based a minimum of four consecutive video frames.

Statistical analysis was performed using SigmaStat® Software (by Systat®). The non-parametric Kruskal–Wallis Rank test was employed to compare dynamic traits of nematocysts since most of the data did not conform to the assumption of normality. The statistical tests compared the nematocyst characteristics among different replicate medusae. Each medusan value represents the mean value from several of its nematocysts (range = 3–13 nematocysts per medusae).

## 3. Results

Nematocysts were arranged in batteries on the tentacles and oral arms of *C. capillata* (Fig. 1A) and each battery contained multiple types of nematocysts. Based on conventional nomenclature (Weill, 1934; Östman, 2000) we identified five types of nematocysts: three types of homotrichous isorhiza haplonemes (termed a-isorhiza, O-isorhiza, A-isorhiza), one type of microbasic eurytele heteroneme (termed a eurytele) and one type of heterotrichous microbasic birhopaloid heteroneme (termed a birhopaloid; Fig. 2A–E, respectively). Tentacle nematocyst batteries commonly contained all of the nematocysts except birhopaloids (Fig. 1B). Alternatively, the oral arm nematocyst batteries contained primarily birhopaloids and O-isorhizas (Fig. 1C). The capsule sizes and tubule lengths varied among the different types of nematocysts (Kruskal–Wallis Rank Test; $df=4$; $P<0.001$; Fig. 2A and B; Fig. 3A and B). A-isorhizas had the longest tubules followed respectively by O-isorhizas, euryteles, birhopaloids and a-isorhizas. As a result of different tubule lengths, discharged tentacle nematocysts formed three distinct spatial horizons along the tentacles (Fig. 4). Our findings on the types, distributions and sizes of nematocysts were similar to those described by Östman and Hydman (1997) for *C. capillata* collected from Gullmar Fjord, Sweden. Östman and Hydman (1997) provide a more detailed description of the different nematocyst morphologies.

In addition to their static traits, we examined several dynamic traits. The discharge velocities varied among the nematocysts (Kruskal–Wallis Rank Test; $df=4$; $P<0.001$), where euryteles and O-isorhizas discharged at faster rates than A-isorhizas and a-isorhizas (Dunn's post-hoc method; $P<0.05$; Fig 3C). Maximum velocities were observed immediately following discharge and subsequently decreased throughout discharge. Tubule velocity was not related to capsule size or tubule length (linear regression; $p>0.05$). For the slower discharging nematocysts, A- and a-isorhizas, 250 fields per second (fps) was a sufficient frame rate to resolve the full discharge event and we were able to observe the tubule emerging from the capsule. However, the shafts (i.e.; wide, barbed section of the discharged tubule adjacent to the nematocyst capsule) of the euryteles and birhopaloids were fully discharged within one video frame (0.004 s) and more than half of the tubules of O-isorhizas were also fully discharged within a frame and, therefore, for these nematocysts, our velocities represent the average velocity over the initial 4 milliseconds after discharge and are likely to be gross underestimates of initial velocities (Nuchter et al., 2006).

Most of the nematocyst tubules traveled with a relatively straight trajectory as they discharged and were characterized by net-to-gross displacement ratios (NGDRs) close to 1, though birhopaloid tubules spiraled slightly as it discharged. The tubules of a-isorhizas displayed a wide cork-screw trajectory as it discharged resulting in a lower NGDR than the other nematocysts (Dunn's post-hoc method; $P<0.05$; Fig. 4E).

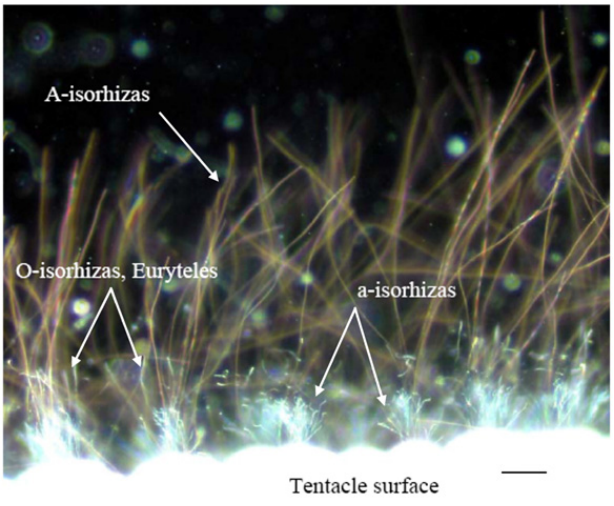


Fig. 4. Light-microscope image of discharged nematocyst tubules on the tentacle of *Cyanea capillata*. Scale bar is 100 μm long.

## 4. Discussion

The dynamic characteristics of nematocysts found on the scyphomedusa *C. capillata* do not correspond directly with the static traits used in conventional nematocyst classifications – i.e., capsule morphology, tubule and shaft diameters and spine patterns. For example, isorhizas, the most common nematocyst type within the phylum Cnidaria, are identified by their uniform tubule diameter (Östman, 2000) and are generally described as penetrating nematocysts (Purcell and Mills, 1988; Östman and Hydman, 1997; Heeger and Möller, 1987; Heeger et al., 1992). Although *C. capillata*'s three isorhizas were characterized by similar tubule diameters, their dynamic traits differed dramatically. The three types of isorhizas possessed different nematocyst tubule lengths, discharged at different velocities and were characterized by different tubule trajectory patterns (Fig. 3). The largest isorhiza type, A-isorhizas, had the longest tubule but the slowest discharge velocities. In contrast, the medium sized O-isorhiza was characterized by the most rapid discharge velocities. In fact, among the nematocysts found on *C. capillata*, the euryteles and O-isorhizas, despite

different tubule spine morphology (Fig. 2) and nomenclatural classifications, had the most similar velocities and lengths and, therefore, are most likely to function similarly. The lack of correspondence between a nematocyst's nomenclatural classification and its function suggests that caution is appropriate when using medusan nematocyst assemblages as indicators of prey selection and trophic role (Purcell and Mills, 1988; Carrette et al., 2002; Peach and Pitt, 2005). Further, the lack of correspondence between capsule size, discharge velocity and kinetic energy may confound the use of nematocyst sizes to infer prey size (Purcell, 1984; Carrette et al., 2002).

How might tubule properties influence nematocyst interactions with prey? Upon discharge, dynamic traits of the nematocyst, in conjunction with prey surface characteristics, determine whether the tubule penetrates the prey. Tubule velocity may be one of the most critical properties that determines whether nematocysts penetrate their prey because the energy used to penetrate prey, its kinetic energy ($KE = 1/2\ mass \times velocity^2$), is a squared function of velocity. We can obtain first order approximations of the kinetic energies of the different nematocysts if we assume that the capsule of an undischarged nematocyst is completely filled with the tubule and, therefore, the mass of the tubule can be estimated based on the volume of the capsule and the density of the nematocyst tissue ($1.24 \times 10^6$ kg/m$^3$; Weber et al., 1987). As expected, the relationship of kinetic energies among the nematocyst types is similar to that of the velocities, where O-isorhizas and euryteles discharged with more energy than A- and a-isorhizas and birhopaloids discharged with more energy than a-isorhizas (Fig. 5; Dunn's post-hoc method, $p < 0.05$).

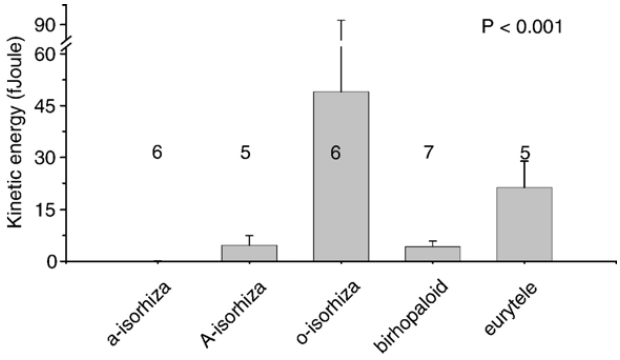


Fig. 5. Estimated kinetic energy of discharged tubules. Error bars represent standard deviations from the mean. *P*-value represents results of Kruskal–Wallis Ranks Test with $df = 4$. The numbers in and above each bar represent sample sizes ($n$ = number of medusae) for each type of nematocyst. Each medusan value represents the mean of several nematocyst measurements (range = 3–13 nematocysts per medusae).

The kinetic energies estimated in this study were orders of magnitude less than the kinetic energy of discharged stenotele nematocysts (on the order of femto- versus microJoules; Nuchter et al., 2006). For the O-isorhizas, euryteles and birhopaloids, this is due to our large underestimates of initial velocities and, therefore, the tubule kinetic energies of these nematocysts are likely to be more similar to stenoteles than our data suggests. However, we believe the kinematics of initial discharge by A- and a-isorhizas indicate that our initial velocities are not substantially underestimated for those nematocysts. Consequently, the discharge tubules of those nematocysts possess kinetic energies that are likely to be several orders of magnitude less than the O-isorhizas, euryteles and birhopaloids. These differences in velocities and kinetic energies among the nematocysts suggest very different functional properties.

Dynamic properties of tubule discharge directly affect the mechanistic basis of nematocyst function during prey capture. The rapid discharge velocities, high kinetic energies and strait discharge paths of the euryteles and O-isorhizas (and probably birhopaloids as well) enable these nematocysts to penetrate prey. The long tubules, slow discharge velocities and meandering paths of the A-isorhizas suggest they act to entangle prey. Previous studies using scanning electron micrographs of preserved samples of nematocysts on captured prey have repeatedly found euryteles and O-isorhizas of scyphomedusa to penetrate both hard and soft bodied prey for the full length of their tubule (Heeger and Möller, 1987; Heeger et al., 1992; Östman and Hydman, 1997; Peach and Pitt, 2005). In contrast, a-isorhizas are less effective penetrants (Heeger and Möller, 1987; Peach and Pitt, 2005) and have been found to only partially penetrate their prey, leaving the rest of their tubule to stick to the surface of the prey (Heeger and Möller, 1987). This observation is consistent with their lower discharge velocities and kinetic energies. In addition, A-isorhizas have only been observed to entangle prey and have not been documented to penetrate prey (Östman and Hydman, 1997).

The agreement between nematocyst dynamic traits and observed prey surface interactions indicates that dynamic traits of nematocysts may provide more insight into nematocyst roles during the predation process than do static morphological nematocyst features. Classification of nematocysts based solely on static morphological features, such as the category isorhiza, can mask significant differences in performance and functional traits of individual nematocyst variants. This becomes an important consideration when inferring prey selection based upon generalizations about the role of a general

category of nematocysts, such as the isorhizas, in the predation process because nematocysts that are categorically united can have significantly different functions. Further documentation of dynamic traits over a wider range of nematocyst types will be important to broaden understanding of the role different nematocysts play in the predation process.

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