Umbrella of *Mastigias papua* (Scyphozoa: Rhizostomeae: Mastigiidae): hardness and cytomorphology with remarks on colors

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**Abstract:** *Mastigias papua*, known as the golden (or spotted) jellyfish, is an epipelagic jellyfish widely distributed in the warm waters of the West Pacific. This jellyfish has a brownish body, owing to zooxanthellae, and white spots. We measured the maximum force to pierce the umbrella, which averaged 94–144 mm in diameter, to evaluate the hardness of *M. papua*, and returned a range of 0.14–0.45 N. Correlation analyses indicate that when the *M. papua* medusa grows (i.e., becomes heavier), the umbrella becomes larger in diameter, as well as thicker and harder within the size range we examined. However, a significant relationship between the hardness of the umbrellar apex and the thickness of the umbrella was not obtained. White spots are comprised of loose aggregates of mesogleal cells containing reflective granules. Since the white spots and the transparent parts were not significantly different in hardness, the spots were unlikely to strengthen the umbrella. The primary function of the spots may be the shading of solar radiation. Most of the zooxanthellae are located in mesogleal cells, and often beneath the exumbrellar epidermis. Therefore, light shading by white spots may be unnecessary for the zooxanthellae in mesogleal cells.

**Key words:** Force gauge, Mesoglea, Pigment cell, Transmission electron microscopy, Zooxanthellae

**Introduction**

Gelatinous zooplankton such as pelagic cnidarians, ctenophores, and pelagic tunicates, have soft and relatively large bodies. According to the ‘dense dwarfs versus gelatinous giants’ model (Dölger et al. 2019), having a large, low-energy (gelatinous) body is one of two equally successful strategies in planktonic filter feeders. While the low-density bodies of gelatinous plankton allow for near neutral buoyancy in the water column, they may sometimes be too soft to protect against physical attacks from their predators.

Few studies have quantified the hardness of gelatinous plankton. Sakai et al. (2018) reported that the maximum force was 0.4–0.5 N to pierce the integumentary tissue of the pinkish-brown salp *Pegea confoederata* (Forskål, 1775) with a steel rod (1 mm diameter). This value is similar to the force required to pierce the gelatinous integuments of colonial ascidians *Clavelina* spp. (Sakai et al. 2019), and approximately 1% of the force needed to pierce the leathery integument of the solitary ascidian *Halocynthia roretzi* (Drasche, 1884) (Hirose et al. 2018). Hardness can be an important feature of gelatinous plankton, and comparable measurements are required in more species.

Many gelatinous plankton distributed in the epipelagic layer have transparent or semi-transparent bodies, which help them avoid visual predators. However, in some epipelagic species the bodies are colored and/or have conspicuous color patterns. For instance, dendroid-shaped hemocytes in the mantle are pigment cells that give the body color in the pinkish-brown salp *P. confoederata* (Hirose & Nishikawa 2018). Although the cytomorphological basis of body color has not been well investigated in jellyfish, it is important to discuss the function and evolutionary traits of the colors/patterns.

The golden (or spotted) jellyfish, *Mastigias papua* (Lesson, 1830) is an epipelagic scyphozoan distributed in warm
waters throughout the Indo-Pacific. Similar to the name, the medusa of this species is entirely brownish due to symbiotic dinoflagellates (zooxanthellae), and it has white spots on its gelatinous body. In the present study, the maximum force to pierce the umbrella of *M. papua* was measured to evaluate its hardness. The umbrellas of young and adult medusae were also ultrastructurally examined to determine the cytomorphological basis of body color. We discuss the potential function of the spots and the process of acquisition of the algal symbionts.

**Materials and Methods**

**Animals**

Medusae of *Mastigias papua* were collected near the sea surface with a hand net off Hamahigajima Island (Uruma, Okinawa, Japan: 26°19′30″N, 127°57′00″E) on July 28, 2019. Sixteen individuals were used for the measurement of the umbrella hardness. Each of the individuals was put in a plastic cup and the total body weight (wet weight) was obtained with an electronic balance, subtracting the cup weight. The diameter of each umbrella was measured with a ruler to the nearest millimeter. Exumbrellar tissues of approximately 5 × 5 mm were cut from two specimens with umbrella diameters close to 2.5 cm, and were fixed in a solution of 2.5% glutaraldehyde, 0.45 M sucrose, and 0.1 M cacodylate (pH 7.4) for microscopic observations.

Young medusae of *M. papua* were also obtained from laboratory-cultured polyps of the species. They were originally reared in the laboratory from planula larvae released by a female of the species collected in Minami-Izu, Japan (34°39′36″N 138°47′22″E) on August 31, 2017. Four young medusae of approximately 3 mm in umbrellar diameter were fixed with 2.5% glutaraldehyde, 0.45 M sucrose, and 0.1 M cacodylate (pH 7.4) for microscopic observations of the whole body.

Species identification was carried out based on Kramp (1961) and the original description (Lesson 1830). Recently, De Souza & Dawson (2018) re-described *M. papua* as endemic to the tropical western Pacific islands and identified the *Mastigias* specimens from Japan as *M. albipunctatus* Stiasny, 1920. The partial sequences of cytochrome c oxidase subunit 1 (COI) of the present specimens from Hamahigajima Island (LC594632) and Minami-Izu (LC594631), were included in the clade of *M. albipunctatus sensu* De Sousa & Dawson (unpublished data). The morphological characteristics were consistent with those of *M. papua*, rather than those of *M. albipunctatus*, such as the number of canal roots per octant (8, 9, 10), coloration of oral-arms (not “deep-blue”), and absence of a whitish accumulation of nematocysts at the apex. Moreover, Stiasny (1920) differentiated *M. albipunctatus* from *M. papua* collected in Japan in the original description of *M. albipunctatus*. For these reasons, we assigned the specimens as *M. papua sensu lato* in our present and previous reports (Hirose et al. 2021).

**Measurement of hardness**

We measured the hardness of the umbrella following Sakai et al. (2018). Umbrellar tissues (ca. 3 × 3 cm) including exumbrella and subumbrella were cut out at the apex and at the margin of the umbrella of each individual, and the thickness of each specimen was measured using Vernier calipers. After briefly blotting excess water using a paper towel, the specimen was placed between two acrylic plates (5 mm thick), each with a 3 mm diameter hole. A pin attachment (TP-20, IMADA Co., Ltd., Toyohashi, Japan) was mounted on a digital force gauge DST-2N (IMADA Co., Ltd.) that was fixed on a lever test stand FCA-50N (IMADA Co., Ltd.). The pin used was a steel rod (1 mm diameter) with a flat tip. Pulling the lever, the pin pierced the specimen through the hole of the acrylic plates, and the maximum force was recorded as the hardness. Measurements were carried out on both the transparent part and the white spot of the umbrella tissues 5 times for each individual, and the median value of the peak forces was used as the hardness of that individual. The measurement method applied here is the same as those in our previous studies (Hirose et al. 2018, Sakai et al. 2018, 2019); the measured values may vary depending on the method, such as the shape of the pin attachment of the force gauge.

Correlations among total weight, diameter, thickness, and hardness of the umbrellas were tested using Pearson’s correlation coefficient test following the Shapiro-Wilk test for normality in R version 3.5.1 (R Core Team 2018) and RStudio (RRID:SCR_000432). The hardness of the umbrella was compared between the apex and the edge of the umbrella by a paired t-test following the F-test of equality for two variances in R.

**Microscopy**

The specimens fixed with glutaraldehyde were briefly rinsed with 0.1 M cacodylate and 0.45 M sucrose, and post-fixed with 1% osmium tetroxide and 0.1 M cacodylate for 1.5–2 h on ice. They were then dehydrated through a graded ethanol series, cleared with n-butyl glycidyl ether, and embedded in epoxy resin. The specimens sectioned at a thickness of 0.5–1 μm were stained with 1% toluidine blue and examined under a light microscope (Olympus BX51). Thin sections were stained with uranyl acetate and lead citrate and examined under a transmission electron microscope (TEM; JEOL JEM-1011) at 80 kV.

In thick sections of young medusae, the number of zooxanthellae was counted to obtain the relative ratio of the abundance of zooxanthellae in each tissue.

**Results**

**Umbrella hardness and size parameters**

The medusae used in the measurements ranged from 94 mm (umbrella diameter) and 103.4 g (total weight), to
144 mm and 354.6 g, respectively. Hardness at the transparent part of the umbrella ranged from 0.14 to 0.45 N. Figure 1 summarizes the correlations between total weight, umbrella diameter, thickness at the umbrellar apex and margin, and hardness at the umbrellar apex and margin. The total weight and umbrella diameter were strongly correlated (r = 0.946, P < 0.001; Pearson’s correlation coefficient test). The weight and diameter also had strong correlations with the thickness at both the apex and margin, and the hardness at the umbrellar margin, while the hardness at the umbrellar apex was only moderately correlated. A moderate positive correlation was found between the thickness at the umbrellar apex and margin. The hardness at the margin was strongly correlated with the hardness at the apex and moderately correlated with the thickness (both apex and margin). Small correlations between the hardness
at the apex and the thickness (both apex and margin) were not statistically supported.

The thickness at the umbrellar apex was significantly greater than the thickness at the umbrellar margin ($P < 0.05$, paired $t$-test) (Fig. 2A). The apex was significantly harder than that of the margin on the transparent part ($P < 0.001$) but not on the white spots ($P = 0.06$) (Fig. 2B). The paired $t$-test did not show significant differences in hardness on the transparent part or the spots at both the umbrellar apex ($P = 0.19$) and margin ($P = 0.31$) (Fig. 2B).

**Fine structures of the umbrella**

**Young medusae**

There were many dark dots throughout the body of young medusae fixed with osmium tetroxide, which were identified as the clusters of symbiotic algal cells (zooxanthellae) (Fig. 3A). In the cross section, the umbrella was comprised of four epithelial layers, two layers of mesoglea, and a gastrovascular cavity connecting to the mouth opening (Fig. 3B); both the upper and lower surfaces of the umbrella were entirely covered with exumbrellar epidermis and subumbrellar epidermis. Mesoglea filled the space between the epidermis and the gastrodermis lining the wall of the gastrovascular cavity. Free cells were sparsely distributed in the mesoglea. The clusters of zooxanthellae were mainly distributed in the mesoglea and occasionally found in the gastrodermis. Of the 374 zooxanthella cells examined in the histological sections, 315 (84.2%) were found in the mesoglea, 55 (14.7%) were in the gastrodermis, and four (1.07%) were found in the gastrovascular cavity.

The exumbrellar epidermis was mostly comprised of highly vacuolated cuboidal cells, and the epidermal cells had cellular bulges and processes on the apical membrane (Fig. 3C). Some epidermal cells contained an oval inclusion that consisted of a moderately electron-dense outer layer, and a heavily electron-dense inner core (arrow in Fig. 3C). The cells in the mesoglea were generally amoeb-
boid in shape and some contained vacuoles or granules (Fig. 3D). Multiple zooxanthella cells were intracellularly located in a single mesogleal cell, forming the clusters of zooxanthellae recognized in the histological sections (Fig. 3B, D). The gastrodermis was a simple epithelium comprised of squamous and cuboidal cells, and from these cells emerged cellular processes about 2 µm in length on the apical membrane facing the gastrovascular cavity (Fig. 3E, F). Zooxanthellae are occasionally found in gastrodermal cells. The subumbrellar epidermis was comprised of cuboidal cells that often had vacuoles and granules.

**Adult medusae**

The medusae had brownish bodies and white spots, and some individuals were partly blue or green (Fig. 4A). The spots appeared white under epi-illumination, and black under transmission illumination (Fig. 4B, C). In histological sections, roundish mesogleal cells were loosely aggregated to form the spots. As shown in Fig. 4D, the cell density was much higher on the right side of the broken line. The roundish mesoglea contained reflective granules of approximately 0.5 µm in diameter (Fig. 4E). In TEM, the granule content was sublimated under the electron beam and oval holes remained in the thin sections (Fig. 4F). The exumbrellar epidermis was comprised of highly vacuolated cuboidal cells that had dense microvilli on the apical side of the cell membrane (Fig. 4G, H).

**Discussion**

While the soft body is the most conspicuous feature of gelatinous plankton, the hardness level has rarely been
quantified and compared. Correlation analyses indicated that as the *Mastigias papua* medusa grows (i.e., becomes heavier), the umbrella becomes greater in diameter, thickness and hardness within the size range we examined (Fig. 1), although the correlation between the hardness of the umbrellar apex and the umbrella thickness was not statistically supported. While the thickness and hardness are larger at the umbrellar apex than at the edge, the umbrellar margin may need to be flexible for swimming (Fig. 2). In contrast, Iwatani et al. (2004) reported that the umbrellar margin is harder than the umbrellar apex in the giant medusae of *Nemopilema nomurai* Kishinouye, 1922, and the correlation between the diameter and hardness was not statistically supported in these medusae, ranging from 588 to 1230 mm in diameter. Therefore, the regional difference in hardness may depend on the proportions of the umbrella and/or the size range of the medusae examined. The hardness of the *M. papua* medusae investigated in the present study was 0.14–0.45 N depending on the medusa size, and these values are comparable to the hardness of the gelatinous integument of tunicates: 0.39–0.5 N in the salp *P. confoderata* and approximately 0.33 N in the colonial ascidian *Clavelina cycles* Tokioka & Nishikawa, 1975 (Sakai et al. 2018, 2019). These values may reflect the common hardness levels of gelatinous tissue in marine invertebrates. Unfortunately, we cannot compare these values with those of *N. nomurai* because the measurement method was not the same. Many more species must be measured using a standardized method to provide an overview of the hardness of gelatinous plankton.

The exumbrellar epidermis emerges as dense microvilli in the adult medusae, while the epidermis has some cellular bulges and processes in the young medusae (Fig. 3C, 4G, H). The exumbrellar microvilli are suggested to reduce surface reflection, when compared to a flat surface (Hirose et al. 2021); however, the white spots were shown to successfully reflect light. The spots are comprised of loose aggregates of mesogleal cells containing reflective granules that were sublimated under the electron beam. This may indicate that the granular content was in a crystalline state. The granular content may be similar to that of fish iridophores that contain crystalline platelets of guanine (e.g., Fujii 1993), but cytochemical analyses should be performed in the medusae. Although such white spots in *M. papua* have been suggested to enhance the structural robustness of the gelatinous tissue (e.g., Uchida 1925), the hardness between the white spots and the transparent parts of the umbrella was not significantly different in this study, indicating that white spots are unlikely to enhance the mechanical strength of the umbrella (Fig. 2B). The primary function of the spots may be shading and/or regulating solar radiation in the epipelagic habitat. White spots may not only protect the inner tissue from harmful radiation, but also provide suitable light conditions for the symbiotic zooxanthellae, by functioning as a shelter. However, many clusters of zooxanthellae are found beneath the exumbrellar epidermis over the spots (Fig. 4D), suggesting that light shading by the white spots may be unnecessary for the zooxanthellae in mesogleal cells. Alternatively, solar reflection on the white spots may provide more light for the zooxanthellae beneath the exumbrellar epidermis and/or serve to camouflage the contour of the body.

Djeghri et al. (2019) estimated that 20–25% of scyphozoan species are associated with dinoflagellate symbionts, that is, zooxanthellae. *M. papua* medusae owe their brownish body color to zooxanthellae distributed throughout the body, most of which are intracellularly located in the mesoglea (Fig. 3D), although some are in gastrodermal cells (Fig. 3E), and free zooxanthellae are occasionally found in the gastrovascular cavity. The planula of *M. papua* has no zooxanthellae, but the scyphistoma requires zooxanthellae for strobilation (Sugiura 1963, 1964); therefore, zooxanthellae need to be acquired from the environment. The distribution pattern of zooxanthellae implies that the zooxanthellae engulfed by the medusa are transported to the gastrovascular cavity, and endocytosed by the gastrodermal cells. The zooxanthellae-laden cells may then migrate into the mesoglea or transfer the algal cells to mesogleal cells (Fig. 5). The same process of zooxanthella acquisition has been proposed for *Cassiopea xamachana* Bigelow, 1892 (Fitt & Trench 1983, Colley & Trench 1985), *Mastigias* sp. (Muscatine et al. 1986), and other scyphozoans (reviewed in Djeghri et al. 2019).

The shape, size, hardness, and coloration of jellyfish have been under selective pressure for better survival in their habitat. While the body is so soft that it is easily damaged by the predators’ attack, tissue hardness is necessary to support the body shape for movement. Therefore, jellyfish umbrellas potentially differ in hardness depending on their habitats. The colors and patterns also differ among species; transparency is important in order to be invis-
ible at depths with a high light regime, while some colors/patterns may be functional at low light or aphotic depths. Additionally, photosymbionts in the jellyfish absorb photosynthetically active radiation. To better understand these invertebrates, it is necessary to conduct comprehensive comparative surveys on the physical and optical properties of jellyfish with various life styles and habitats.

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