

**Grid challenge test to evaluate Safe Sea protection level against
Rhizostoma pulmo sting**

Purpose:

The purpose of this study was to test the protection levels of Safe Sea sunscreen against *Rhizostoma pulmo* sting (Fig. 1).

Envenomation by cnidarians is a worldwide problem. Cnidarians are equipped with stinging cells, each of which contains a stinging apparatus capable of delivering toxins into the victim when activated. Safe Sea sunscreen inhibits jellyfish, coral and hydroids stinging mechanism based on patented technology.

Safe Sea has been successfully tested on the Atlantic *Chrysaora* (sea nettle), the Atlantic *Chiropsalmus* (Box jellyfish) the Atlantic *Rhopilema*, the Atlantic *Cyanea* and several other Jellyfish species.

Rhizostoma pulmo is highly abundant along the eastern Mediterranean coast of Spain. The Medusae swarm heavily along the UK coasts. Several reports indicate that *Rhizostoma pulmo* is toxic, causing pain and erythema reaction to bathers and tourist beachgoers.

Rhizostoma pulmo does not have marginal or long tentacles. Beneath the bell is a large manubrium and its distal portion is divided into four pairs of oral arms (mark OA in Fig. 1) that consist of three winged portions. The manubrium oral arms contain high density of stinging cells.

The scope of this study was to monitor Safe Sea efficacy against *Rhizostoma pulmo* sting.

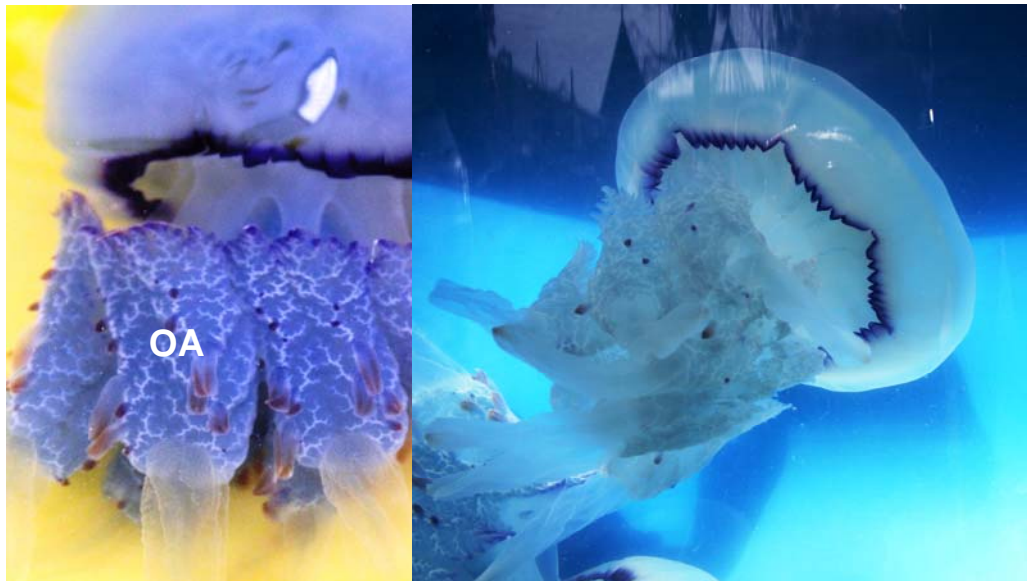


Figure 1. *Rhizostoma Pulmo*. OA, oral arms

Testing Protocol:

1. Grids of 2.5X4 cm were marked on inner arms to conduct 20 independent experiments.
2. About 30 sections of one square cm of *Rhizostoma pulmo* oral arms (Fig 1) were prepared from two fresh species that were collected in the Mediterranean and kept in large seawater tank for 3 hours prior to the test.
3. Eleven (11) out of the 20 grids were randomized selected to be treated with 20µl of Safe Sea lotion to achieve skin covered of 2mg/cm² by Safe Sea. Nine (9) grids were randomized to be untreated.
4. Lotion was applied 10-15 minutes before the test.
5. *Rhizostoma pulmo* oral arms were laid on the skin grids for 10 min (Fig. 2).
6. Five minutes after tentacle skin exposure, the tentacles were pressed to the skin using microscopic glass slide (Fig. 3).
7. At the end of the 10 minutes, tentacles were removed and inflammation was monitored for each grid after 30 min.



Figure 2. Skin exposed to *Rhizostoma pulmo* tentacles.



Figure 3. Pressing *Rhizostoma pulmo* tentacles on the skin.

Data collection and analysis:

The skin grids were monitored for inflammation and were scored using an inflammation index.

Inflammation index:

No inflammation was scored "0".

Inflammatory reaction was scored "1".

Table 1: Inflammatory (1,0) scoring of skin grids after contact with *Rhizostoma Pulmo* tentacles.

Safe Sea	0	0	0	0	0	0	0	0	0	0	0
Control	1	1	0	1	0	1	1	0	0		

Protected level: Skin protection level representing the percent of skin grids that did not developed any symptoms after contact with *Rhizostoma Pulmo* tentacles (Table 2).

Table 2: Skin protection level

	Protection Level
Safe Sea	100%*
Control	44%*

*T-test results $P < 0.01$

Digital photographs of control unprotected skin were taken 30 minutes after *Rhizostoma pulmo* tentacles application (Fig. 4).



Figure 4. *Rhizostoma pulmo* inflammation of unprotected skin.

Second stage: Safe Sea pre-lotion arm submersed into *Rhizostoma Pulmo* oral arms. No affects were monitored (Fig. 5)



Figure 5. Contact of Safe Sea protected hand with jellyfish

Summary:

To evaluate Safe Sea protection level against *Rhizostoma pulmo* stings a grid challenge test was conducted with live medusa and human subjects. A medical examination was performed by an expert and skin reaction was scored.

In the control 56% of the skin grids demonstrated erythema. The erythema was widely spread along the tentacle application area. Most of the reactions were visible up to 2 hours.

No visible signs of stinging were notable in skin grids treated with Safe Sea.

p-values were <0.01 demonstrating a statistically significant difference between control and Safe Sea groups.

Conclusions:

Exposure of the skin to *Rhizostoma pulmo* tentacles generated skin erythema. In contrast, under the same conditions, Safe Sea prevented *Rhizostoma pulmo* stinging.

Under the above protocol, Safe Sea significantly inhibited the development of pain and skin reaction resulting from contact with the *Rhizostoma pulmo* tentacles.