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EPHYRAE RHOPALIUM NUMBERS AND OBSERVATIONS OF THE
UP-SIDE DOWN JELLYFISH *CASSIOPEA* (CNIDARIA: SCYPHOZOA)

A THESIS SUBMITTED TO THE GRADUATE DIVISION OF THE
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IN
ZOOLOGY
(ECOLOGY, EVOLUTION & CONSERVATION BIOLOGY)

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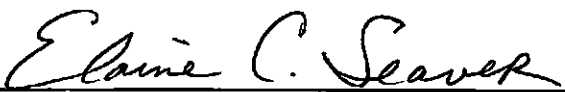
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ABSTRACT

Jellyfish rhopalia are gravity sensory organs located on the margin of the animal's bell. Rhopalia are therefore associated with jellyfish behaviors such as swimming, pulsing, and orientation. Often these rhopalium numbers are fixed in the medusa phase of its life cycle and vary among different species of jellyfish. Rhopalium development was documented in developing *Cassiopea* sp. jellyfish. These ephyrae were observed on a weekly basis until rhopalium numbers remained constant. It was observed that developing rhopalia can occur with natural structural precursors or *de novo*. Further results showed that there is high variation between clonal ephyrae in regards to the final rhopalium numbers. These observations support the research performed on clonal *Aurelia* ephyrae.

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CHAPTER 1 Introduction

Jellyfish are Cnidarians in the class Scyphozoa. In contrast to most actively swimming jellyfish, *Cassiopea* spp. (Order Rhizostomae) are commonly called “up-side down” jellyfish because adult medusae typically lie on the substratum of calm tropical water areas, waterways, and mangrove habitats with the oral side facing upwards (Fitt & Costley 1998). Instead of actively swimming in the water column, this jellyfish is benthic, spending most of its medusa phase in its up-side down orientation on the seafloor with only weak and sporadic pulsing movements of the bell (Bigelow 1900). Bigelow (1900) observed that if currents or waves pick the medusa up from the substrate it can regain its position by using the bell musculature to invert itself and return to the bottom. Additionally it is believed that the combination of *Cassiopea*'s concave bell, musculature, and pulsing behavior create a slight water suction that can assist the medusa in remaining on the bottom (Bigelow 1900).

While *Cassiopea* is heterotrophic, like many other animals of the Phylum Cnidaria, it also harbors symbiotic dinoflagellates belonging to the Genera *Symbiodinium* (Arai 1997). *Cassiopea*, as well as many other symbiotic Cnidarians, depends on “horizontal” transmission of *Symbiodinium* to form a symbiosis (Szmant 1986 & Richmond 1997). Horizontal transmission is when *Symbiodinium* is incorporated into host tissue and not maternally passed down. These dinoflagellates reside in the sediments and water column (Manning & Gates 2006, Santos et al. 2001) in the natural environment and are united with a Cnidarian host through chemical cues (Fitt 1985b). In contrast to “horizontal” acquisition of algal symbionts, “vertical” transmission of *Symbiodinium*

occurs in brooded Cnidarian larvae. These dinoflagellates are maternally derived (Fitt & Trench 1983, Colley & Trench 1983, Richmond 1997 & Thornhill et al. 2006).

Research on the molecular phylogeny of *Symbiodinium* from many Cnidarian hosts has been thoroughly investigated (Rowan & Powers 1991, Santos et al. 2001). Experiments have demonstrated that many non-symbiotic Cnidarians are capable of forming a symbiosis with different species or evolutionarily distinct molecular phylogenetic types of *Symbiodinium* (See Thornhill et al. 2006 for a review). However, most of these studies showed that following endocytosis of the algal symbiont, the host showed specificity toward homologous algae. Homologous algae are symbionts whose origin is from the same host species. Heterologous algae are symbionts derived from a different host, including different species (Thornhill 2006). Research by Fitt (1985a) and Belda-Ballie et al. (2002) showed that homologous algae have superior growth rates and competitive ability in many hosts.

The presence of *Symbiodinium* is essential for *Cassiopea* to mature (Figure 1) (Hofmann & Kremer 1981 & Hofmann et al. 1996). However, Hofmann & Kremer (1981) suggested that photosynthate released by the algal symbiont is not the driving factor of metamorphosis. Instead the physical presence of *Symbiodinium* in *Cassiopea* tissue will elevate the metabolism of the jellyfish. This surge in metabolism is believed to promote metamorphosis or strobilation (Rahat & Hofmann 1987). To date, a study by Rahat & Adar (1980) is the only record of non-symbiotic strobilation with *Cassiopea* (specifically with *Cassiopea andromeda*).

Alternation of generations is a process by which organisms alternate between asexual and sexual reproduction. Cnidarians, along with many other groups including

plants, exhibit this change from asexual to sexual reproduction with maturity. Unique to scyphozoan biology is an asexual fission process called strobilation (Figure 1-5). The life history of *Cassiopea* has been described by Bigelow (1900), Gohar & Eisawy (1960), Hofmann (1978), and Colley & Trench (1983). A brief summary of these findings are as follows: Most scyphozoans have two phases to their life cycle, a sessile polyp (Figure 1-3) and free-swimming adult form called the medusa (Figure 1-1). The free-swimming medusa stage (Figure 1-1) is sexual and dioecious. When sexual reproduction occurs, the larvae are called planulae (Figure 1-2). Planulae are free swimming and will migrate to benthic areas to settle and form a sessile polyp (Figure 1-3) (Müller & Leitz 2001).

The polyp can attach to various types of substrate, including the underside of ships and mangrove branches (Fleck & Fitt 1999, Müller & Leitz 2001). As mentioned previously, the symbiotic union of *Symbiodinium* with *Cassiopea* polyps (Figure 1-3) promotes strobilation in *Cassiopea*. Without this symbiotic relationship, the asexual phase of the life cycle will continue and will not progress to sexual reproduction (Hofmann & Kremer 1981, Hofmann et al. 1978). Along with budding (transverse fission), another form of budding is the production of non-symbiotic planula-like objects (Figure 1-4). Similar to a planula, these asexually produced planula-like forms can swim and settle to form more non-symbiotic polyps (Colley & Trench 1983).

When a symbiosis is formed with *Symbiodinium*, the polyp (Figure 1-3) will metamorphose into a monodiscous strobila (Figure 1-5). Over time (roughly 1 month in a lab setting (Hofmann et al. 1978)), the strobila (Figure 1-5) will begin to strobilate until it breaks free from its former polyp stalk and becomes a free swimming ephyra (Figure 1-6) (Spangenberg 1968a). The stalk left behind will regenerate another polyp head, and

the strobilation process will continue producing more ephyrae that are genetically identical to the first strobilated ephyra. For the purposes of this paper, a strobilation event refers to each time an ephyra or its clone mate is produced by strobilation. Strobilation events can continue as long as the symbiotic polyp or residual stalk are not subject to predation, are healthy and/or not overgrown with algae or other animals (personal obs). Similar life history observations have been documented by Spangenberg (1968a) for *Aurelia* sp. (Order Semaestome).

Eventually the ephyra (Figure 1-6) will mature into a medusa (Figure 1-1) and the life cycle will repeat (Bigelow 1900). Most scyphozoans exhibit the alternation of generations but may have different features at each stage. For example, *Cassiopea* has a monodiscous strobila (Bigelow 1900, Hofmann et al. 1978) whereas a Semaestome, *Aurelia* sp. (Moon jelly), has a multidiscous strobila (Spangenberg 1968a).

Metamorphosis from a sessile polyp to a free swimming ephyra is a critical time for the development of a jellyfish. Developing a new anatomy for a mobile lifestyle requires the development of new structures. Among these new structures are rhopalia which are sensory structures unique to jellyfish that develop during strobilation (Spangenberg 1968a). These sensory structures are located along the margin of the bell of all scyphozoans, and contain an eyespot and a statocyst containing statoliths (Hyman 1940). Rhopalia are associated with jellyfish behaviors such as swimming, pulsing and orientation in the water column (gravity reception) (Romanes 1876, Horridge 1956, Passano 1982, Spangenberg 1991).

Bigelow (1900) reported that statoliths (which Bigelow called otoliths) appear at the base of tentacles on the polyp before rhopalia are formed. “These statoliths increase

in number until they form a conspicuous mass” “While the distal part of the tentacle is still completely functional, the proximal part has become differentiated into a rhopalium” (Spangenberg 1968a). When an ephyra strobilates, there are no longer any polyp tentacles and rhopalia are seen along the margin of the ephyra’s bell (Figure 2). The number of rhopalia is often fixed in the medusa phase of the jellyfish life cycle, though numbers can vary among species. The number of rhopalia can range from 8 to 16 in Rhizostome species (Arai 1997). The number of rhopalia in adult *Cassiopea* is variable (Bigelow 1900) both within species and between species (Holland et al. 2004). Both Bigelow (1900) and Holland et al. (2004) reported a similar range in adult *Cassiopea* rhopalia number 15 – 23.

Observations of lab reared *Cassiopea* ephyrae revealed that rhopalia were not evenly distributed along the margin of the ephyrae. In fact, some rhopalia were located closer together than other rhopalia on the same ephyra (Figure 3). Since the strobilation process was complete, there were no polyp tentacles from which a rhopalium could develop. Therefore, it was unknown how these additional rhopalia developed on a growing ephyra after the strobilation process. I hypothesized that either:

- 1) additional rhopalia develop by budding off existing rhopalia or
- 2) additional rhopalia develop *de novo*, between other existing rhopalia, with no pre-existing gross anatomy structures or polyp tentacles.

Along with testing these hypotheses, I also document the difference in rhopalium numbers between strobilation events. Extensive research has been performed on rhopalium development and experimentation on the jellyfish *Aurelia* (Order

Semaeostome) at the ephyra and medusa stages (Spangenberg 1968b, 1991, Spangenberg et al. 1996, Horridge 1969). However, *Cassiopea* spp. has not been studied.

CHAPTER 2 Materials & Methods

Cassiopea - zooxanthellae (*Symbiodinium*) infection

Cassiopea are horizontal transmitters of *Symbiodinium*. Ephyrae for this study were created by introducing *Symbiodinium* to non-symbiotic *Cassiopea* polyps. These non-symbiotic *Cassiopea* polyps and the *Symbiodinium* culture used to create a symbiosis were both obtained from lab cultures at the Hawai'i Institute of Marine Biology, Kāne'ohe, Hawai'i.

The *Cassiopea* polyps had remained *Symbiodinium*-free for 10+ years and were collected from the north-east shore of O'ahu. The zooxanthellae culture of *Symbiodinium* (Clade A) was created by extracting zooxanthellae cells from a *Cassiopea* sp. jellyfish collected from Kāne'ohe Bay. A homologous symbiont was used to guarantee that a symbiosis was formed (Fitt & Trench 1983, Rowan & Powers 1991, Santos et al. 2001 & Thornhill et al. 2006).

Each non-symbiotic *Cassiopea* polyp was placed into 9ml filtered (0.22µl) seawater (FSW) in one well of a 6-well plastic culture plate. Each polyp remained isolated in its individual well with FSW and was fed brine shrimp (*Artemia* sp.) twice per week for 1 month prior to initiating symbiosis. Two days after each feeding, the polyps were cleaned with a Q-tip® and the FSW changed.

After a one week starvation period, approximately 60,000 *Symbiodinium* cells in 1 ml FSW were added to each well to initiate symbiosis. *Symbiodinium* density was

estimated using a hemacytometer. All polyps were inoculated with the same culture of *Symbiodinium*. Immediately after the algal symbiont was added to each well, the *Cassiopea* polyps displayed the same feeding behavior as when fed brine shrimp (*Artemia* spp.). The polyps were left in the algal suspension for 1 week, after which all polyps were rinsed with FSW and each container was thoroughly cleaned with cotton swabs. Polyps were fed, containers cleaned and the FSW was replaced twice a week thereafter. All polyps were held under the same conditions and under a 12:12 light/dark cycle at 0.69×10^{16} quanta $\text{sec}^{-1} \text{cm}^{-2}$ with a constant temperature of 25°C.

Data were taken from ephyrae that were produced from the first strobilation event and clone mates produced from the second strobilation events. Ephyrae that strobilated from each strobilation event that were damaged in handling or deformed naturally were not included in data analyses. Deformed ephyrae would either die or would have misshapen bells, oral arms or no oral arms, or lacked rhopalium development.

Microscopy

Weekly observations were performed to document the development of rhopalia. The observation period started one week after strobilation and ended when the number of rhopalia remained constant. Observations were made for 12 weeks for the first strobilation event. However, by eight weeks after strobilation, the number of rhopalia remained constant, even with continued growth in bell diameter. Therefore, eight weeks of observations were conducted for the second strobilation event.

Upon strobilation, free swimming ephyrae were removed and isolated into 50ml beakers with 40ml FSW. Ephyrae remained in the same beaker throughout the

observational period. Ephyrae were fed, cleaned, and the FSW replaced twice a week. Observations of rhopalia, rhopalium numbers and ephyra bell diameters were also recorded by analyzing digital photography. Ephyrae were temporarily transferred to a small Petri dish with 6ml FSW and 3ml 0.37g/ml MgCl solution (FSW) for observations. The ephyrae were left in the MgCl solution until completely sedated. Observations were recorded using digital photography and a dissecting microscope at 10x. Immediately after pictures were taken, ephyrae were removed from the MgCl solution and placed in a “recovery” beaker with 300ml FSW. When an ephyra resumed pulsing or swimming it was returned to its 50ml beaker.

Weekly observations were recorded and numbers of rhopalia were counted. Changes in number of rhopalia over time were noted. Attention was focused on each individual rhopalium to see if budding occurred and to examine how rhopalium development occurred. To assess the possibility of *de novo* development, weekly pictures of the same ephyra were oriented in the same positions to match up existing rhopalia. It was important to confirm that there were no pre-existing structures present on the bell margin of each ephyra. When no structures such as immature rhopalia or statocysts were observed, I could safely conclude that the rhopalium developed *de novo* (Figure 4 & Figure 6).

Field survey

Thirty-seven adult *Cassiopea* medusae, 10 – 27 cm in bell diameter, were collected from the south and windward side of O‘ahu and kept temporarily in sea tables or in the lagoon of the Hawai‘i Institute of Marine Biology. After rhopalia were counted

the jellyfish were returned to the collection site. The number of rhopalia varied from 16 to 19 with a mean of 17. These observations are slightly different than the observations made by Holland et al. (2004) who found that number of rhopalia varied from 17 to 23 with a mean of 19. Unlike Holland et al. (2004), I did not identify *Cassiopea* to the species level in my field survey. Holland et al. (2004) also collected *Cassiopea* that ranged in diameter from 5 to 23 cm.

CHAPTER 3

Results

***Cassiopea* zooxanthellae (*Symbiodinium*) infection & ephyra strobilation**

After two weeks of exposure to the *Symbiodinium* algal symbiont, zooxanthellae were visible in concentrated regions on the upper portions of the polyps (Figure 5). Monodiscous strobilation occurred approximately one month (34.2 ± 4.5 days) after the onset of symbiosis. The second strobilation event occurred approximately 28.3 ± 3.2 days after the first strobilation event. These observations are similar to those of Hofmann et al. (1978). However, unlike the reports of Hofmann et al. (1978), in my study, with successful acquisition of *Symbiodinium* zooxanthellae there was a complete shut off of asexual reproduction.

The first strobilation event provided 73 animals, of which 12 were damaged or killed by handling, and 16 were naturally deformed. Therefore, 45 ephyrae were analyzed from the first strobilation event. The second strobilation event yielded 42 ephyrae clone mates of which 24 were analyzed and 18 were naturally deformed. To date, there have been no other records of the survival of *Cassiopea* ephyrae directly after strobilation.

Ephyra behaviors

Upon strobilation, ephyra would actively swim right-side up. This behavior, quite opposite of the adult medusa form, was observed both day and night and ceased after eight weeks of observations. All ephyrae were approximately 0.7 ± 0.3 cm in diameter when the swimming behavior stopped. After eight weeks, no new rhopalia developed. At

this time ephyrae would be seen resting at the bottom of the beaker (up-side down). If the beaker or the ephyra was perturbed, the ephyra would quickly resume active swimming behavior. When an adult *Cassiopea* is perturbed, it also swims right-side up for brief periods of time (personal obs).

Rhopalium development

My observations revealed that rhopalia did not bud off existing rhopalia. Instead, rhopalium development occurred *de novo* at a site between two existing rhopalia in some ephyrae (Figure 6). A few newly strobilated ephyrae had immature rhopalia that consisted of a rhopalium-like structure but which lacked an eyespot and statocyst containing statoliths (Figure 4). Weekly photomicrographs determined that these immature rhopalia would become fully developed over time with the onset of these features (Figure 4). Weekly photomicrography also revealed that out of the 69 ephyrae analyzed in this study, 14 displayed *de novo* development of rhopalia. All other ephyrae showed no *de novo* development.

Of all the ephyrae that developed new rhopalia, one animal developed two rhopalia while all other ephyrae produced only one. The time for a new rhopalium to develop was approximately 7 ± 2 days and the time for an immature rhopalium to finish developing was approximately 7 ± 1 day.

Rhopalium number & bell diameters of ephyrae

Rhopalia were counted on the photomicrographs and the bell diameters measured for each ephyra (Table 1). There was no correlation of rhopalium number and ephyra

bell diameter for medusae produced in the first (Pearson correlation = -0.111, $p = 0.466$) (Figure 7) or second strobilation events (Pearson correlation = -0.147, $p = 0.494$) (Figure 8). It should be noted that rhopalia were counted only if completely developed. A completely developed rhopalium had an eye spot, a statocyst, and statoliths.

Two trends were noted in rhopalium numbers. Fifty-two ephyrae strobilated with a number of rhopalia that remained constant throughout the observational period, and 17 ephyrae had an increase in the number of rhopalia over time by continued development of immature rhopalium (Figure 4) and/or *de novo* development (Figure 6). The first strobilation event had 14 out of 45 ephyrae that increased rhopalium number and the second strobilation event showed this trend in 3 out of 24 ephyrae. It should be noted that there was one instance where the ephyra lost a rhopalium then regained it at the same site on the bell margin within the 8 week observation time.

Final rhopalium numbers from the first strobilation event ranged from 8 to 17 rhopalia with a mean of 13 ± 2.8 (1 SD) and a mode of 16 (Figure 9). Final rhopalium numbers from the second strobilation event ranged from 6 to 16 rhopalia with a mean 11 ± 3.1 and a mode of 8 (Figure 10).

To compare the number of rhopalia between clone mates from the first and second events, a paired t-test was used ($n = 24$). This confirmed there was a significant difference in the final rhopalium numbers between strobilation events ($p = 0.003$).

The difference between starting and final number of rhopalia was also compared between the two strobilation events. Increased rhopalium numbers from the first strobilation event ranged from 1 to 5 (Figure 11). However, increased rhopalium

numbers were lower in the second strobilation event, with a range from 1 to 3 (Figure 12). The second strobilation event had fewer instances of rhopalia increases (Figure 12).

CHAPTER 4

Discussion

In this study, newly strobilated *Cassiopea* ephyrae showed *de novo* development of rhopalia. This is unusual as research has suggested that rhopalium development occurs at the base of a polyp's tentacle during the process of metamorphosis (Bigelow 1900 & Spangenberg 1968a). All ephyrae observed in this study had no remnants of polyp tentacles. Therefore, it is unknown how these rhopalia developed. However, research performed on a different jellyfish *Chrysaora* (Order Semaestome) showed the importance of the nutrient content of amoeboid cells as a contributing factor to delayed onset of rhopalium development (Spangenberg 1968a). In this study, *de novo* development as well as completed development of immature rhopalia occurred approximately one to two weeks after strobilation. However, it is unknown if amoeboid cells play a role in this delayed onset in *Cassiopea*.

Research has suggested that rhopalia are modified basal portions of polyp tentacles (Bigelow 1900). Therefore the number of rhopalia could be a function of the number of tentacles on a jellyfish polyp. Since the observational period in this study started one week post strobilation, with particular attention focused on ephyrae, I could not correlate polyp tentacle numbers to rhopalium numbers as the jellyfish matured. It is possible that the *de novo* developed rhopalia could have had a polyp tentacle derivative at some point in the life cycle. Future studies should incorporate data which documents polyp tentacle number and differentiation as rhopalium develop.

Final rhopalium numbers were different among ephyrae and clone mates. These observations were also noted in a similar study with *Aurelia* ephyrae (Gershwin 1999).

Unlike the monodiscous *Cassiopea strobila*, *Aurelia strobila* are stacked upon each other, and a strobilation event occurs when the distal strobila breaks free from the strobila stack and become a free swimming ephyra (Spangenberg 1968a). Gershwin (1999) showed that clonal *Aurelia* ephyrae from the same stack had different morphologies which included different rhopalium numbers.

Different rhopalium numbers are present in *Aurelia* ephyrae and also adult populations of wild *Aurelia* medusa (Gershwin 1999). The field survey of *Cassiopea* also found this variation of rhopalium numbers in adult *Cassiopea*. Gershwin (1999) describes clonal variation in symmetry of jellyfish as a plastic “bet-hedging strategy” in which a jellyfish with one particular symmetry is not at an advantage or disadvantage from another due to unpredictable changes in the environment. Romanes (1876) experimentally correlated more sense organs (rhopalia) with increased nervous activity (metabolic rate) and therefore Gershwin felt that jellyfish with more sense organs may be unable to meet food consumption demands when food availability is depleted. Although this may be a concern for a non-symbiotic jellyfish, *Cassiopea*'s symbiotic nature, could balance these elevated metabolic demands if necessary as well as house large numbers of rhopalia around their bell margins. *Cassiopea* and other Rhizostome jellyfish are known to have higher rhopalium numbers compared to other Scyphozoans (Arai 1997).

This difference in rhopalium number could be a function of hormone regulation in the strobilation process. Spangenberg (1971) and Silverstone et al. (1978) documented hormonal regulation in the strobilation process in *Aurelia*; however, there were no correlations to rhopalium development or the role of these hormones on jellyfish

symmetry (Gershwin 1999). This irregular developmental pattern could indicate a lack of tight developmental control.

Since developing ephyrae were reared in a constant volume of seawater, rates of growth of ephyrae could be a result of confinement in a small volume of water and insufficient nutrition. No study has correlated feeding with growth of ephyrae. It is possible that if fed more, the ephyrae in my study would have become larger in the observational period but it is unknown how more feeding would affect rhopalium development. Hofmann et al. (1978) noted that with more feeding there was an increase in polyp growth and asexual reproduction. He also found that more feeding, as well as the symbiosis with *Symbiodinium* increased strobilation rates. Hofmann and colleagues (1978), however, did not compare his strobilation rates to non-symbiotic strobilation rates.

Histology or confocal microscopy of rhopalium development would increase our understanding of these developmental events in *Cassiopea*. Although it is generally understood that tentacle degradation has a role in rhopalium development (Spangenberg 1968a), histology of each metamorphogenic step from polyp to ephyra could reveal more details involved with rhopalium development.

Rhopalium development has been thoroughly documented in the jellyfish *Aurelia* (Spangenberg 1968a, Spangenberg 1968b, Spangenberg 1971 & Spangenberg 1991). However, since *Aurelia* and *Cassiopea* have different developmental patterns (multidiscous and monodiscous respectively) and medusa lifestyles (eg. swimming, orientation and pulsing), rhopalium development could also be different between the two.

I found that *Cassiopea ephyrae* began actively swimming within the first eight weeks after strobilation. Bigelow (1900) correlates this change in orientation with the development and growth of ephyral oral tentacles that are similar to the medusa stage. Oral tentacle lengths on each ephyra were not measured in this study, although it would be very informative to document anatomical changes with rhopalium development in regards to the switch of *Cassiopea* behavior from juvenile to adult.

Early experiments performed by Cary (1915) involved cutting adult *Cassiopea xamachana* in halves to observe regeneration capability. He found that removal of rhopalia decreased jellyfish regeneration capabilities and pulse rate of adult *Cassiopea xamachana*. Similar observations have been made on *Aurelia* as well (Romanes 1876, Schwab 1977, and Passano 1982). Quick regeneration of loss or damaged body parts is vital for the survival of a jellyfish. If rhopalia are linked to the jellyfish's capability to regenerate lost parts, there may be an advantage to having large numbers of rhopalia as demonstrated in *Cassiopea* and other Rhizostomes.

	Initial Rhopalia	Final Rhopalia	Initial Diameter cm	Final Diameter cm
1st Strobilation				
Mean	12	13	0.61	1.28
Range	8-17	8-17	0.36-1.25	0.63-1.8
2nd Strobilation				
Mean	11	11	0.61	1.14
Range	6-16	6-16	0.40-0.98	0.80-1.9

Table 1. Summary of rhopalium numbers and ephyra bell diameters of the first ($n=45$) and the second ($n=24$) strobilation events. This table summarizes 8 weeks of data after each strobilation event.

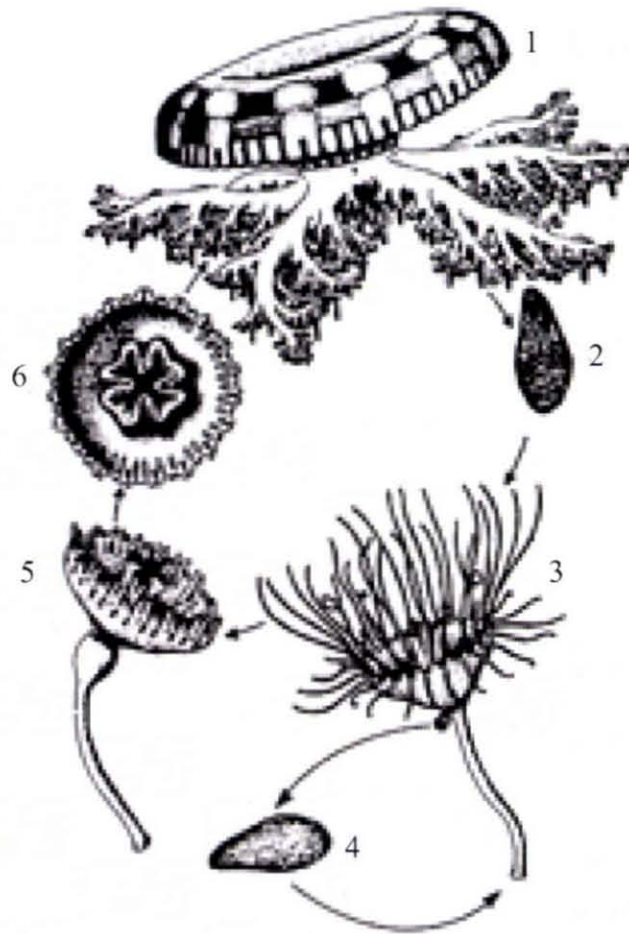


Figure 1. Life cycle of *Cassiopea* illustrated by Colley & Trench 1983.

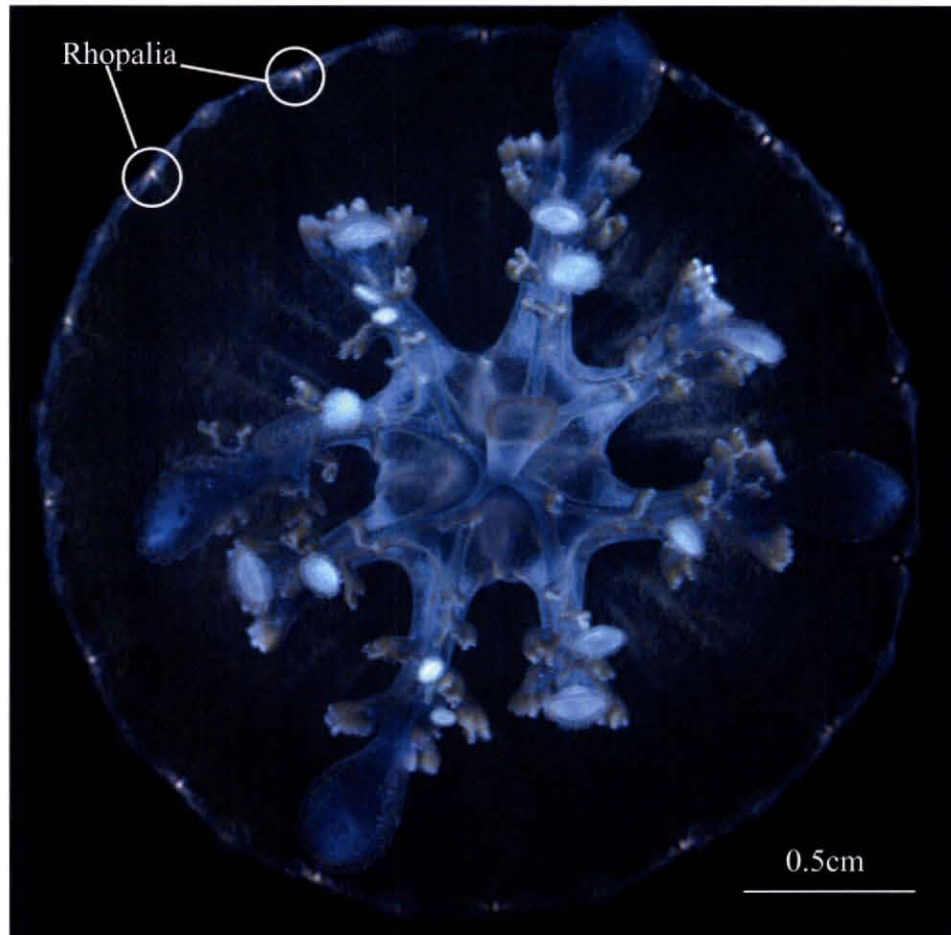


Figure 2. Rhopalia located around the margin of an ephyra's bell.

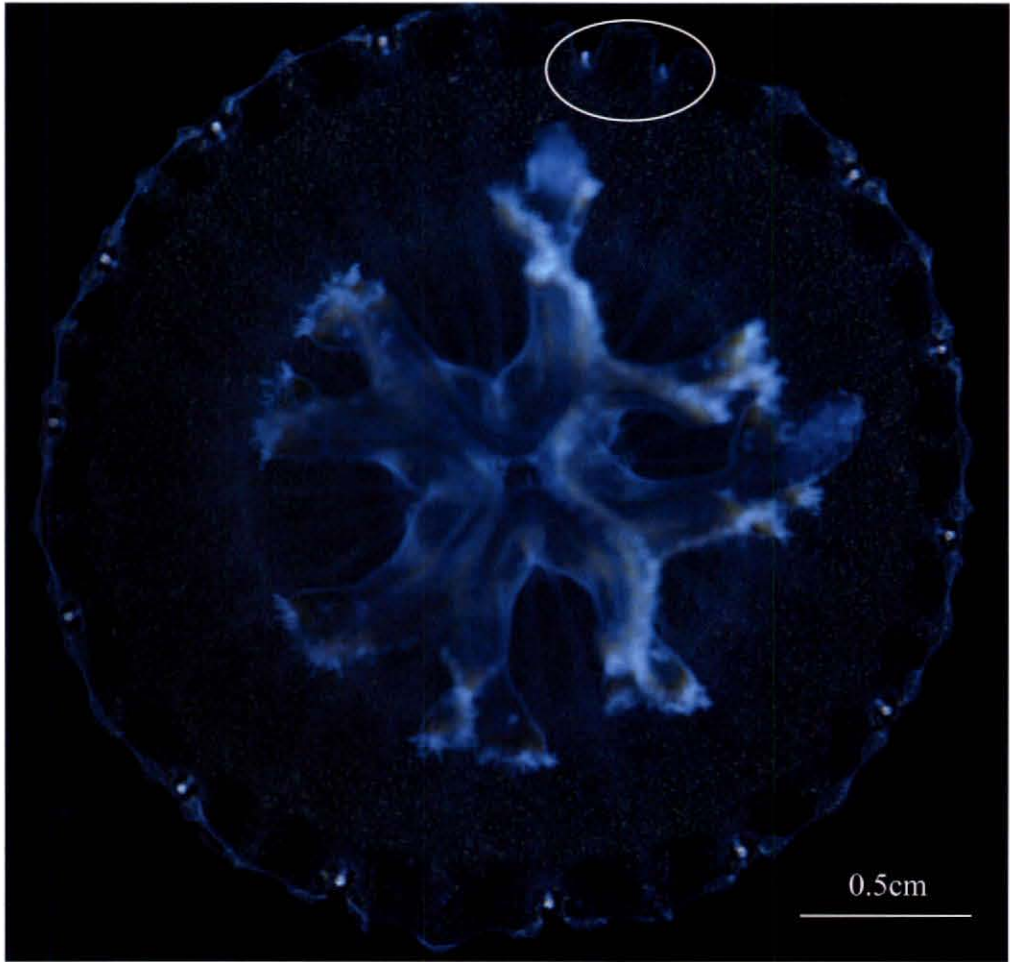


Figure 3. Circled are two rhopalia that are closer in proximity than other rhopalia.

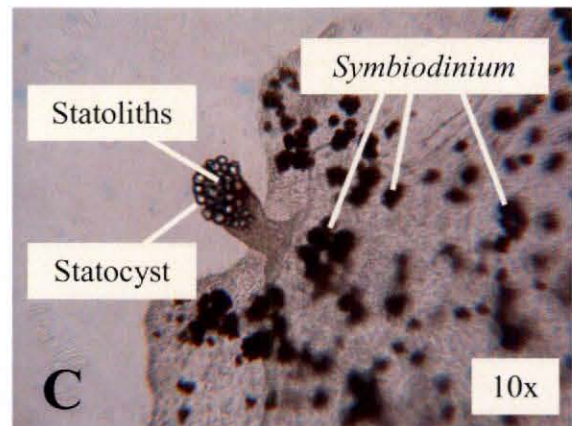
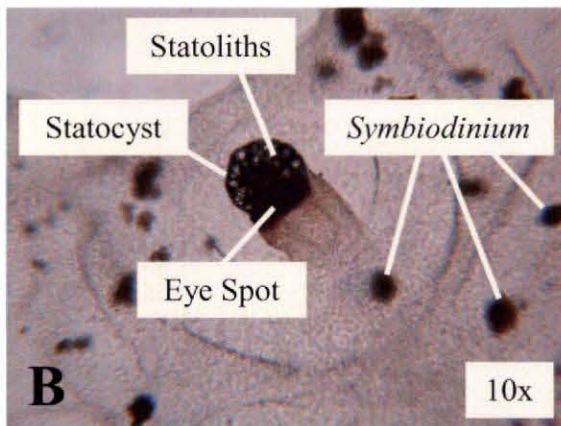
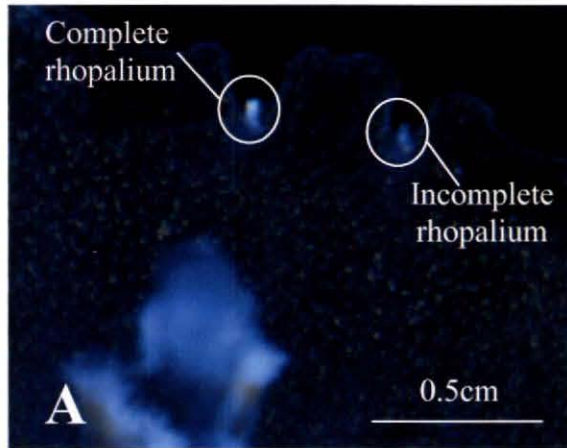


Figure 4. A: Diagram showing a complete and an incomplete rhopalium. B: Oral view of complete rhopalium. C: Aboral view of complete rhopalium illustrated in B.

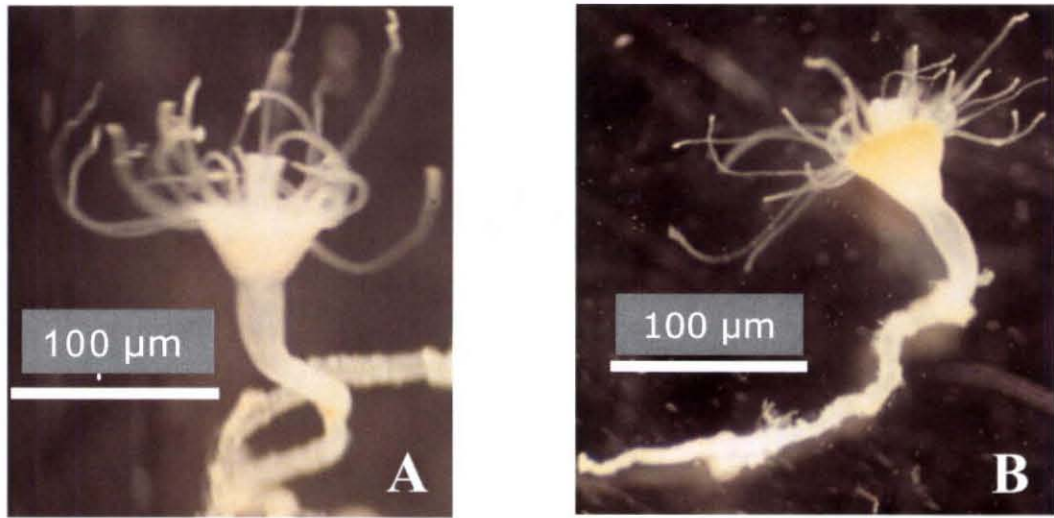


Figure 5. A: Polyp before *Symbiodinium* infection. B: Same polyp two weeks later with concentrated *Symbiodinium* band near tentacles.

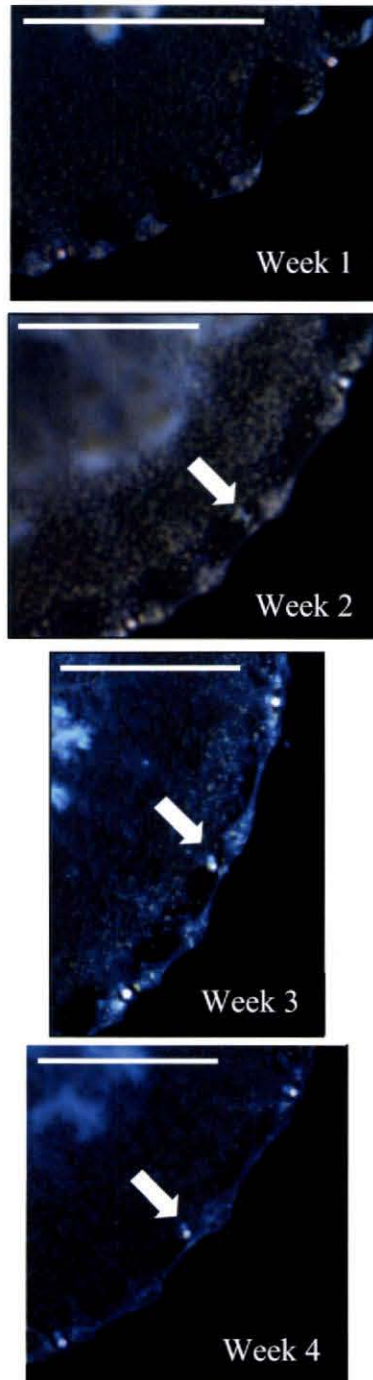


Figure 6. Rhopalium development *de novo* in ephyra. Week 1: No signs of pre-existing structures or immature rhopalium . Week 2: Arrow indicates development of rhopalium structure. Week 3: First view of statocyst with statoliths. Week 4: Fully developed rhopalium . Scale bars located on the top left of all images. Scale bars = 0.2cm

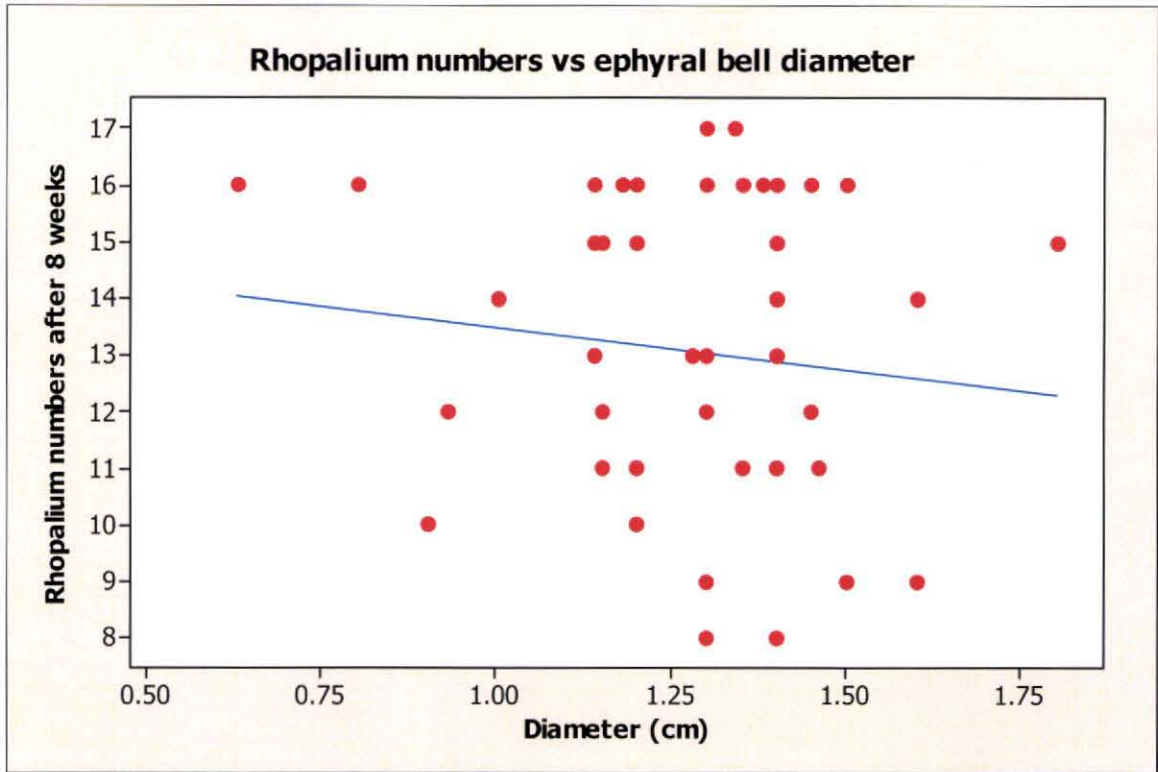


Figure 7. No correlation was observed when comparing rhopalia number to the bell diameters of all ephyrae strobilated from the first strobilation event. Pearson correlation = -0.111, p-Value = 0.466.

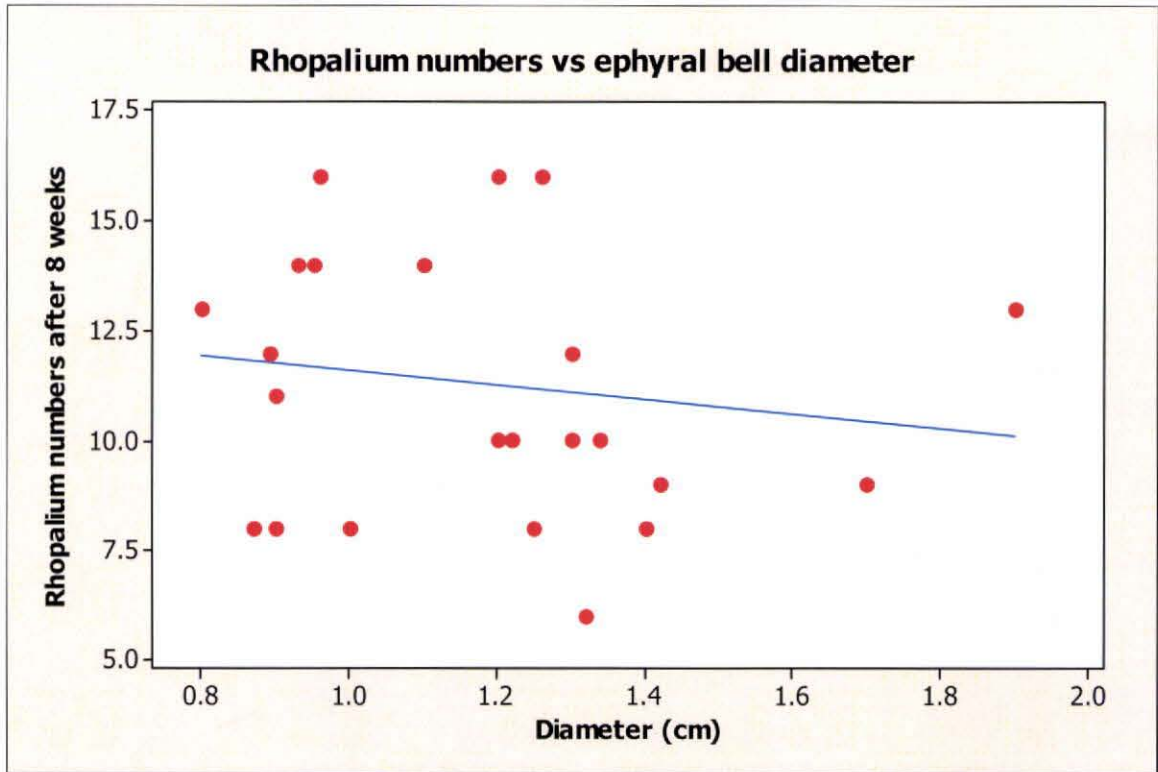


Figure 8. Similar to the first strobilation event there was no correlation of rhopalium numbers with the bell diameters of the ephyrae from the second strobilation event. Pearson correlation = -0.147, p-Value = 0.494.

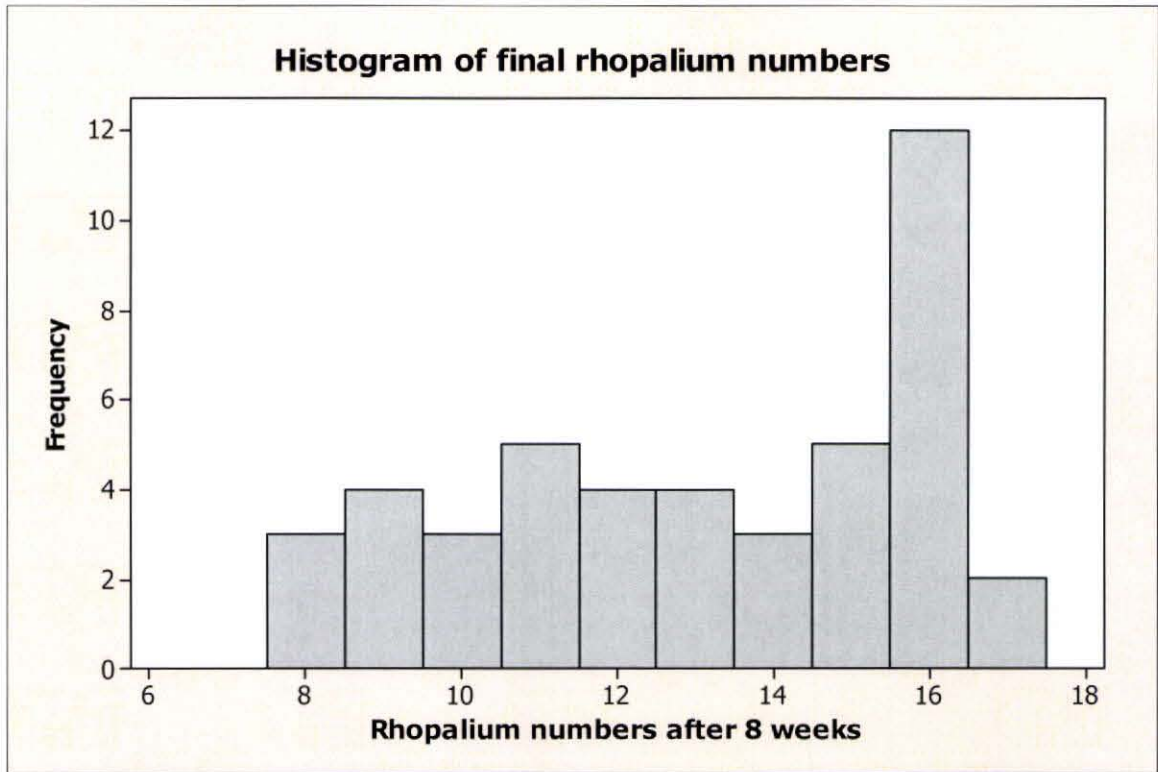


Figure 9. Distribution of final rhopalium numbers from the first strobilation event. The highest frequency noted was 16. $n = 45$.

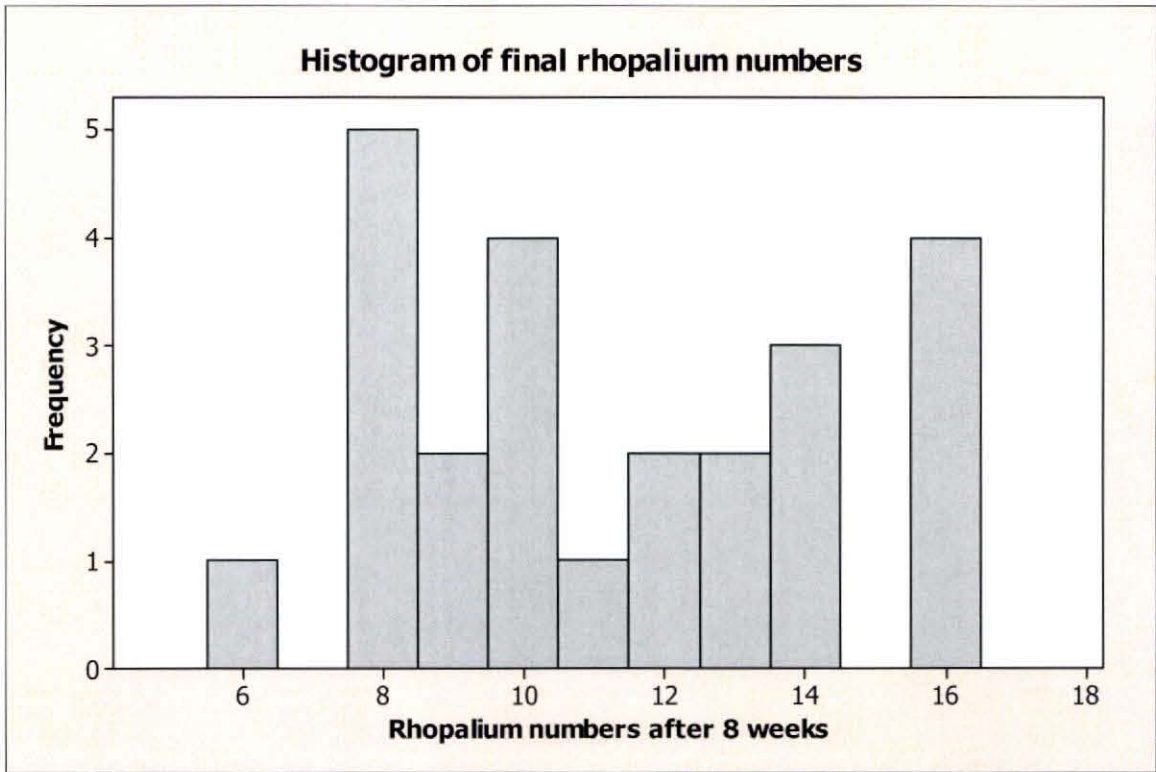


Figure 10. Distribution of final rhopalium numbers from the second strobilation event. Unlike the final rhopalium numbers indicated in the first strobilation event, the highest frequency noted was 8. $n = 24$.

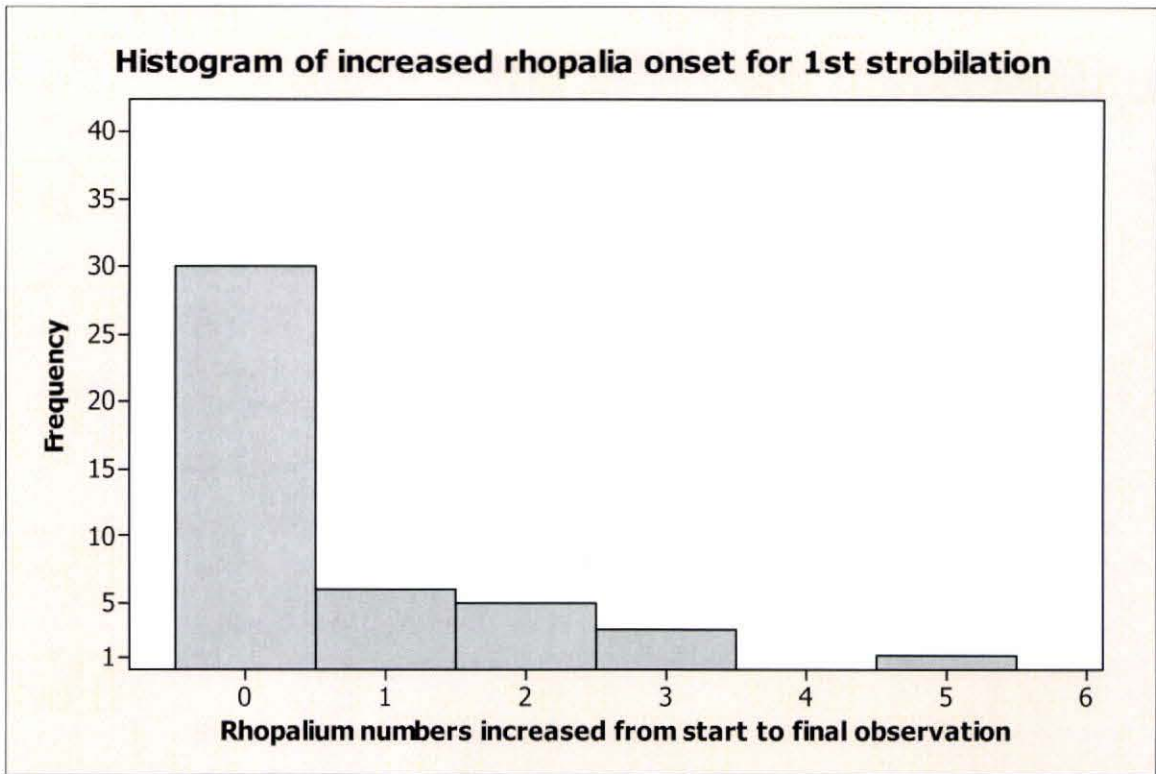


Figure 11. Total number of new rhopalia produced after strobilation from the start to last observation point (8 weeks). Zero means no addition of rhopalia. Except for zero, the highest frequency was the addition of 1 rhopalium. $n = 45$.

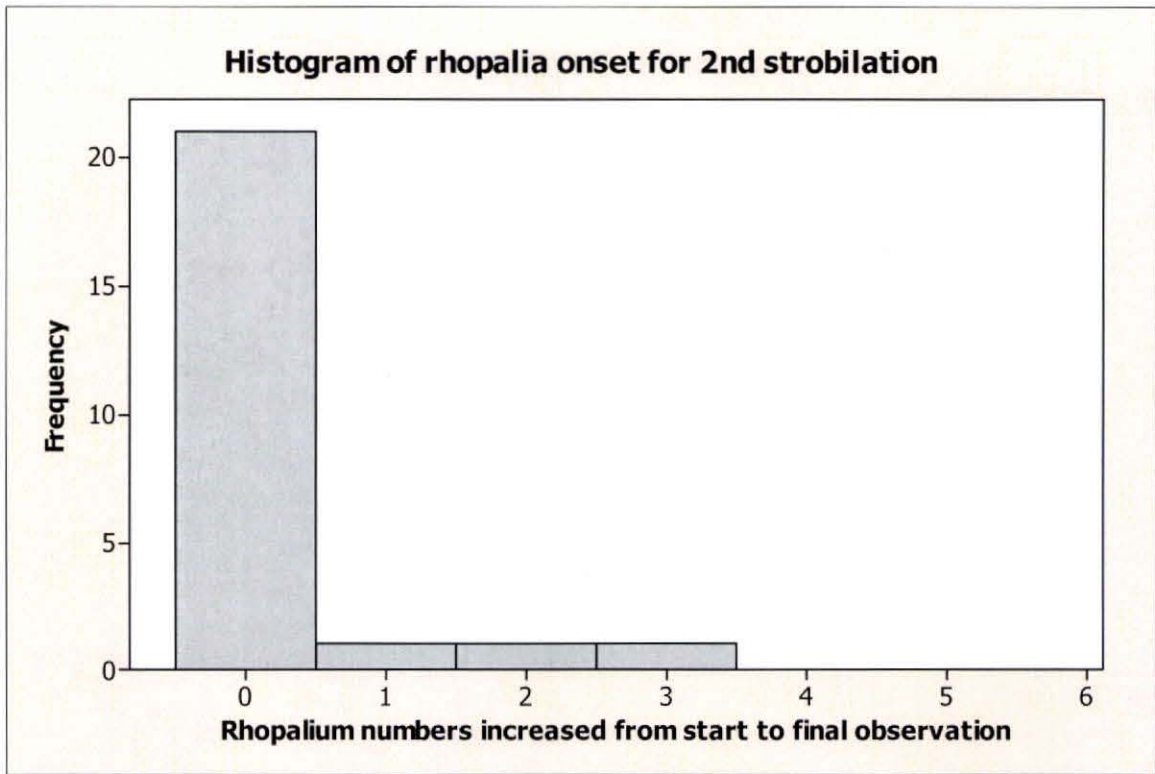


Figure 12. Number of rhopalia for ephyrae produced in the second strobilation event (8 weeks). Besides zero, which showed no addition of rhopalia, there was an even frequency onset of 1-3 rhopalium. $n = 24$.

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