Exploring the Factors Affecting the Status of *Cassiopea andromeda* (Upside-down jellyfish) under Environmental Change

Cassiopea andromeda（Upside-down jellyfish）

Hsin-Ching Lin
Jo-Yu Chiu
Cian-jhen Senior High School, Taiwan(R.O.C.)

Abstract

We have seen many reports that Lin-yuan Wetland Park is the only lake in Taiwan where Upside-down jellyfish can be found. This sparked numerous questions in our minds, and we wanted to understand the relationship between the survival of Upside-down jellyfish and environmental changes. Our study designed with four sets of experiments. Observation of Upside-down jellyfish in its inverted state revealed that its tissue and mucus parts contain Zooxanthellae, and the cnidocytes in its tentacles are easily affected by the environment. The water quality investigation of the lake where Upside-down jellyfish lives weakly alkaline water and has a high tolerance to the environment. An experiment investigating the effect of light on Upside-down jellyfish's contraction behavior found that smaller Upside-down jellyfish contract more frequently than larger ones. In the experiment where Upside-down jellyfish was deprived of nutrients, we discovered that lightless module, Upside-down jellyfish consumes their own nutrients, resulting in significant shrinkage in its size. We found that the growth of Upside-down jellyfish is significantly affected by water quality and environmental changes.

Research purposes

1. Observation of Upside-down jellyfish Posture
2. Investigation of Water Quality in the Jellyfish lake
3. Effect of Light on the Contraction Behavior of Upside-down jellyfish
4. Effect of Nutrient Deprivation on Upside-down jellyfish

Research methods

1. Introduction to Upside-down jellyfish:

1-1 Scientific name:
Cassiopea andromeda. Also known as Upside-down jellyfish or Hanging jellyfish.
1-2 Classification:
Kingdom: *Animalia*
Phylum: *Cnidaria*
Class: *Scyphozoa*
Order: *Rhizostomeae*
Family: *Cassiopeidae*
Genus: *Cassiopea*
Cassiopea andromeda

1-3 Body structure:

Upside-down jellyfish is about 20-50 cm in length, with flattened bell and eight oral arms. The color of the body can be brown, light brown, white, or light blue, with spotted or striped patterns on the flattened bell. The body tissue is thin, allowing for gas exchange with the surrounding water directly through the cells. The body is made up of a gelatinous substance, with 95% of its composition being water, and without any hard structures such as bones or shells for protection. The oral arms contain cnidocytes, which are specialized cells that store and release venom to defend against predators or capture prey.

1-4 Habitat and behavior:

Upside-down jellyfish typically spends most of its time with its flattened bell facing downward and the oral arms facing upward to allow for symbiotic algae to photosynthesis and produce nutrients. It is not very active and moves by pulsing its flattened bell and opening/closing to propel itself forward. During high temperatures, its activity decreases, and during the winter when the temperature is cooler, it is more active. Due to its characteristics, it mainly inhabits shallow seas with abundant sunlight and without strong currents.

1-5 Preying and defense mechanisms:

Upside-down jellyfish obtains nutrients by relying on symbiotic Zooxanthellae in its oral arms and flattened bell to photosynthesis. It is a carnivorous animal and preys on planktonic organisms. Larger individuals may also consume small crustaceans or fish. When feeding, it releases much of mucous-containing bubbles with cnidocytes, which can sting or kill prey. It transports the food to its digestive cavity for absorption, and eliminates undigested residue through its mouth.

2. Observation of Upside-down jellyfish

2-1 Experiment on Separation of Symbiotic Algae Zooxanthellae from Upside-down jellyfish

From literature, it is known that Zooxanthellae are distributed throughout the tissue of Upside-down jellyfish and serve as a source of nutrients for the jellyfish. When Zooxanthellae reproduce and multiply in large numbers, they will flow through the channels in the jellyfish's tentacles into the digestive cavity for decomposition and absorption. Therefore, Zooxanthellae are embedded in the jellyfish tissue for symbiosis, they can still leave the tissue and enter the digestive cavity.

Objective: Use a microscope to observe the cell of Upside-down jellyfish , confirm the distribution of Zooxanthellae on Upside-down jellyfish, and attempt to separate Zooxanthellae.

Table 1, Comparison of Upside-down jellyfish and Zooxanthellae under the conditions of symbiosis and modulation.

	Upside-down jellyfish	Zooxanthellae
Under symbiosis	Upside-down jellyfish provides Zooxanthellae protection, shelter, nutrients (mainly waste containing nitrogen and phosphorus), and a constant supply of CO_2 required for photosynthesis	Zooxanthellae provides Upside-down jellyfish up to 90% of the energy needs (O_2 and carbohydrates)
Under modulation	Upside-down jellyfish can control the population of Zooxanthellae to prevent their overgrowth. This action is known as the expulsion of symbiotic algae, and some of the surrounding Upside-down jellyfish may continue to accept them	When the seawater temperature rises, the symbiotic Zooxanthellae do not feel threatened or run away. On the contrary, they become "greedy" and continue to stay with the jellyfish, but keep the sugars and proteins they produce for themselves, refusing to share with the jellyfish

2-2 Observation of mucus secretion after Upside-down jellyfish preyed on Mollyfish.

From literature, it is known the jellyfish release a large amount of mucus bubbles when they prey, containing numerous cnidocytes that release nematocysts to sting or kill prey. Upon capturing Upside-down jellyfish and placing it in a glass tank for observation, we observed the jellyfish preying on the fish and releasing much of mucus.

Objective: To determine if cnidocytes and Zooxanthellae are found in the mucus of Upside-down jellyfish after they prey on Mollyfish.

3. Investigation of water quality in the habitat of Upside-down jellyfish

Through the analysis of water quality indicators in the habitat of Upside-down jellyfish, we aim to understand their living conditions and the changes in the environment around the jellyfish lake. Furthermore, by collecting data on the population of jellyfish, we can identify the environmental conditions that affect them, as shown in Figure 1.

Fig.1, Locations of the four stations（A, B, C, D）

4. Method for calculating jellyfish quantity:

To reduce errors, multiple sampling points are selected. Some points have more jellyfish, while others have fewer. A square meter floating box is placed under the water surface, and the number of jellyfish in the box is estimated. This process is repeated for several sampling points, and the average is calculated. Finally, the average is multiplied by the entire area of the jellyfish lake to obtain the total jellyfish quantity. The area in the middle of the jellyfish lake, which is not close to the shore, is sampled using drones. This operation is often performed in the morning, and the quality of the water, turbidity, wind and other factors have a significant impact. Only clear and identifiable photos will be included in the calculation.

5. Influence of light on Upside-down jellyfish contraction behavior

5-1 Influence of different light modules on Upside-down jellyfish contraction behavior

Objective: To investigate the contraction frequency of Upside-down jellyfish contraction behavior under different color light and different light intensity environments.
Hypothesis: According to literature, contraction behavior of Upside-down jellyfish is significantly lower when it is in the dark. Contraction frequency is related to substance exchange, predation, and providing resources for Zooxanthellae. The symbiotic Zooxanthellae have the highest absorption rate for blue-purple light during photosynthesis, followed by red light, and other colors have very low absorption rates. Therefore, theoretically, blue light and red light will have a greater help in the photosynthesis of Zooxanthellae. Therefore, it is assumed that Upside-down jellyfish will contract the most in magenta light (blue light combine with red light) with an intensity of 10 (the strongest).

5-2 Influence of cyclic light modules on contraction behavior

Objective: To investigate the changes in contraction frequency of Upside-down jellyfish at different time points under cyclic light modules.
Hypothesis: Due to the physiological clock of Upside-down jellyfish, noon has sufficient sunlight, which is the active time for Upside-down jellyfish, while night is the inactive time. By comparing different colors of light, it is assumed that the closer to noon, the higher the activity of the jellyfish.

6. The effect of no nutrient source on Upside-down jellyfish

Objective: To observe the variation of Upside-down jellyfish when shrinking due to a lack of nutrients when not being fed under both light and dark modules.
Hypothesis: According to literature, Zooxanthellae produces nutrients through photosynthesis and can provide up to 90% of the required nutrients for Upside-down jellyfish. Therefore, it is hypothesized that without light, the size of Upside-down jellyfish may gradually decrease due to the inability of Zooxanthellae to perform photosynthesis.

Result

1. Observation of Upside-down jellyfish state

   1-1 Experiment on the isolation of symbiotic Zooxanthellae from Upside-down jellyfish tissue. After centrifugation and separation, it was observed under a microscope that both the tissue and mucus parts contained Zooxanthellae, as shown in Figure 2.

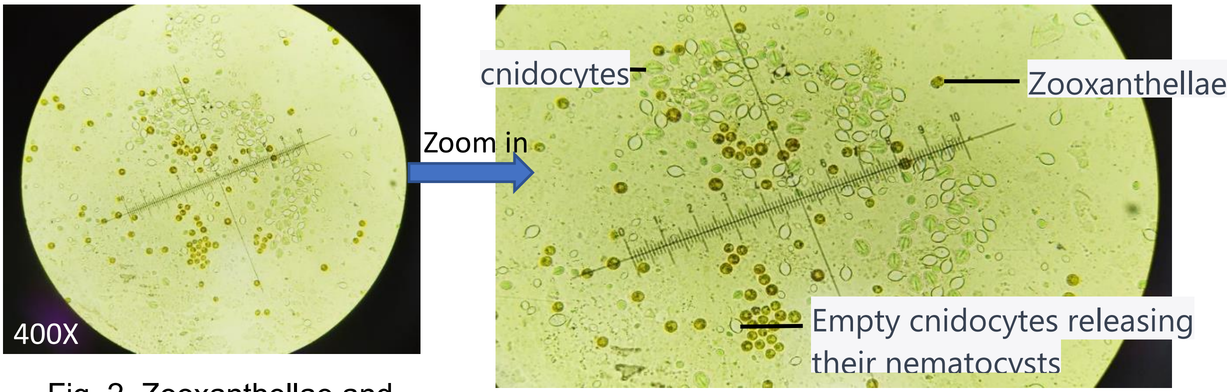


Fig. 2, Zooxanthellae and cnidocytes.

After taking pictures of the tissue observed (as shown in Figure 2), the glass slide was left on the microscope stage with the light on for 10-15 minutes. When returning to observe the same field of view, it was found that the proportion of intact cnidocytes was 33.9% (39 out of 115). However, after 10-15 minutes, the proportion of intact cnidocytes decreased to 13% with only 13 remaining, as shown in Figure 3. From literature, that environmental changes can trigger cnidocytes to fire nematocysts. The water evaporates and changes the environment, causing more cnidocytes to fire nematocysts, leaving only empty capsules.

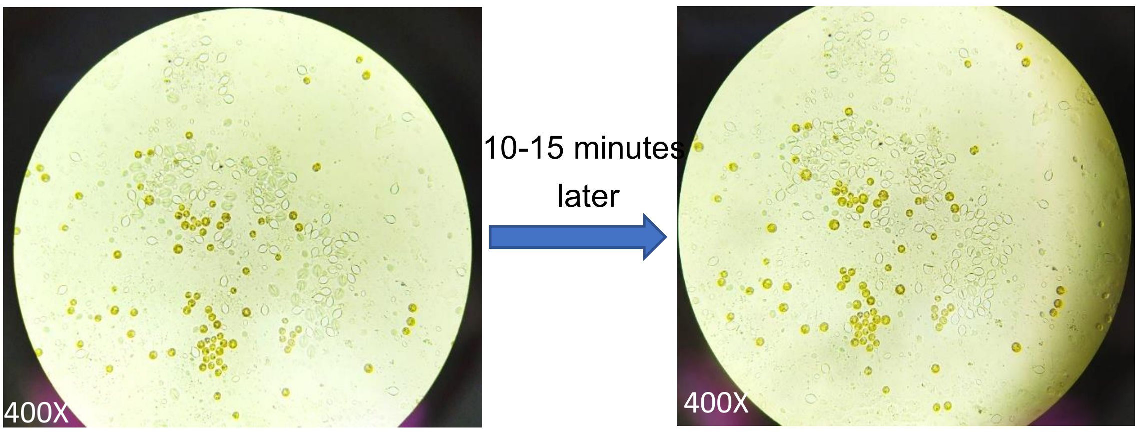


Fig 3, Observation after 10-15 minutes of continuous exposure to light on the microscope stage.

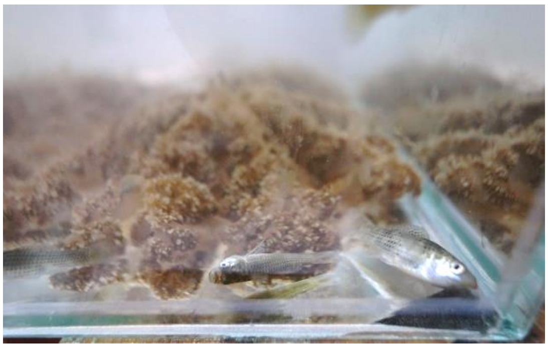
1-2 Results of Microscopic Measurements

Size of cnidocytes: approximately 5 to 10.5 micrometers

Size of Zooxanthellae: approximately 7.5 to 10 micrometers

1-3 Observation of mucus secretion after Upside-down jellyfish prey on Mollyfish

Upside-down jellyfish and Mollyfish are placed in a glass tank. After 10 minutes, it was observed that the Mollyfish suddenly became motionless and sank down. Upside-down jellyfish secrets a large amount of mucus that enveloped the Mollyfish, as shown in Figure 4. The mucus was observed under a microscope, as shown in Figure 5.



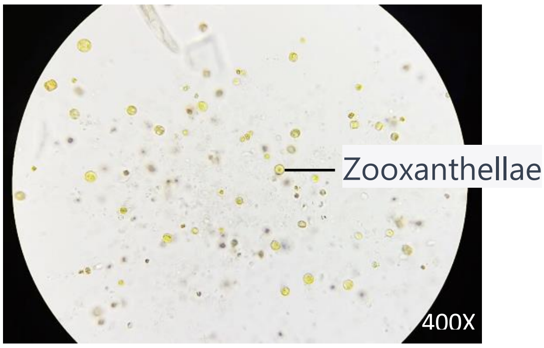
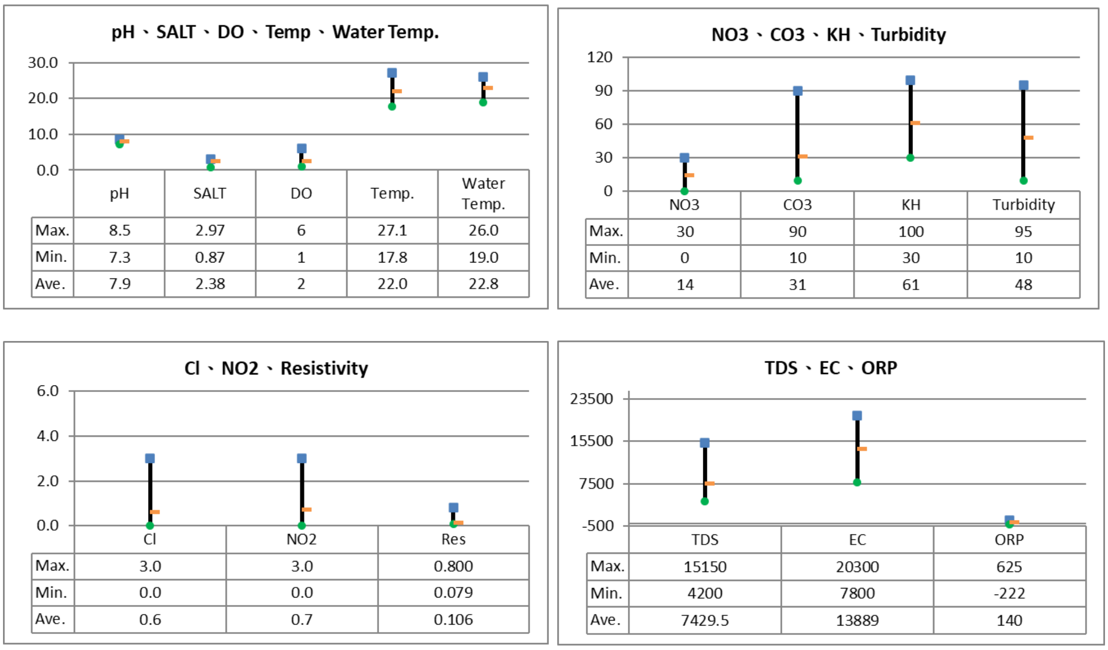


Fig. 4, Upside-down jellyfish causing the death of a Moorish fish

Fig. 5, Observation Zooxanthellae in the mucus

2. Investigation of Water Quality in jellyfish lake

Upside-down jellyfish thrive in different conditions, and even a small change in a single value may cause changes in the jellyfish population. Based on multiple field measurements, Figure 6 presents the results of our investigation from December 30th, 2022 to February 22nd, 2023.



	pH	SALT	DO	Temp.	Water Temp.
Max.	8.5	2.97	6	27.1	26.0
Min.	7.3	0.87	1	17.8	19.0
Ave.	7.9	2.38	2	22.0	22.8

	NO3	CO3	KH	Turbidity
Max.	30	90	100	95
Min.	0	10	30	10
Ave.	14	31	61	48

	Cl	NO2	Res
Max.	3.0	3.0	0.800
Min.	0.0	0.0	0.079
Ave.	0.6	0.7	0.106

	TDS	EC	ORP
Max.	15150	20300	625
Min.	4200	7800	-222
Ave.	7429.5	13889	140

Figure 6, Investigation of environmental factors in jellyfish lake

Upside-down jellyfish grow under different conditions, and even small changes in environmental factors may lead to changes in their bodies. From figure 6 ,jellyfish thrive in alkaline pH levels and a humid environment, with water temperature at around 20 degrees Celsius. While there is a signific difference between the highest and lowest turbidity values, we cannot determine whether it is a result of jellyfish-induced changes or other factors. ORP (oxidation-reduction potential) fluctuates between positive and negative values, but the average value is positive, indicating a lower concentration of organic pollutants and good water quality, and higher visibility.

3. Distribution and Characteristics of Upside-down jellyfish in Different Locations

The location of jellyfish growth is also essential for their survival. For example, a rough and uneven terrain place is not suitable for jellyfish as it may cause them injury or even death. Therefore, they tend to choose areas with smoother terrain and water flow.

To gain a deeper understanding of the ecology of jellyfish, we visited Mr. Chen Junqiang, Chairman of the Lin Yuan Love Township Association, who explained the impact of wetland ecology on jellyfish.

a. The original retention pond of the wetland did not have water. However, with the government's wetland cultivation plan, the retention pond was transformed into jellyfish lake.
b. In addition to Upside-down jellyfish morphology, suitable environments for planula larva and polyp morphology should also be considered when cultivating jellyfish.
c. Mangroves and sea hibiscus act as natural impurity filters and also facilitate the attachment and growth of planula larva and polyp.
d. If water flow is too fast, planula larva and polyp cannot attach.
e. Temperature primarily controls the growth of jellyfish. If the temperature is too high, not only will Zooxanthellae leave, but the jellyfish's body cells will also not be able to withstand it.
f. Different areas of Jellyfish lake receive different amounts of sunlight, and varying water flow speeds lead to different water temperatures.
g. Jellyfish quantity statistics are calculated by counting the number of Upside-down jellyfish in photos(Figure 7).

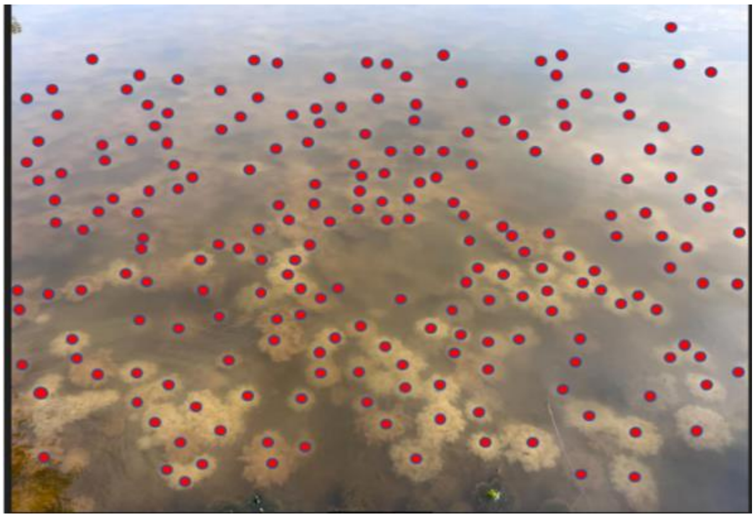


Fig. 7, Calculation Method of Jellyfish Abundance

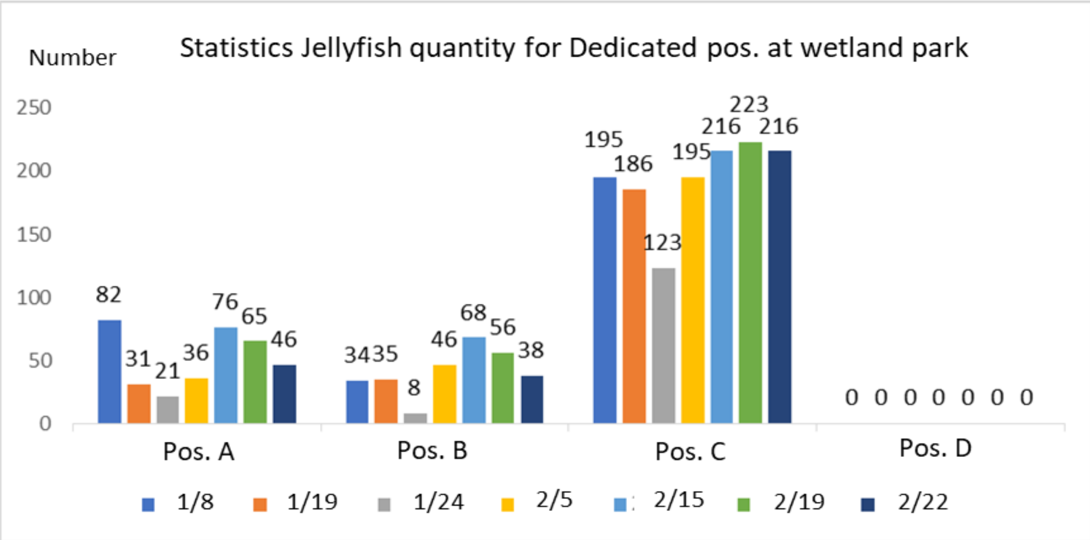


Fig. 8, the number of jellyfish at points A, B, C, and D recorded during the period from December 30, 2022, to February 22, 2023.

However, due to the reflection of light in different locations, it is impossible to count the number of jellyfish at location D, rendering its data unusable. Looking at locations A, B, and C, location C has a particularly high number of jellyfish(Figure 8). When taking photos of jellyfish, factors such as inadequate equipment, shooting angles, and the turbidity of the water on that day led to unclear images which made it difficult to accurately count the number of jellyfish.

4. Analysis of Spatial Distribution of Environmental Factors

There are many factors that affect the environment. For jellyfish, water quality is the most direct. We will first analyze the water quality at different locations.

4-1 pH Value

4-1-1 Average pH values at locations A, B, C, and D in Jellyfish lake range from 7.8 to 8.0 (Figure 9), with the maximum value during the measurement period being 8.5 and the minimum being 7.3, indicating an alkaline pH in Jellyfish lake.

4-1-2 Looking at locations A, B, C, and D individually, the pH values at locations A, B, and D are similar, while location C has a lower pH value than the other locations, suggesting that it is closest to the inflow point and has a faster water flow rate.

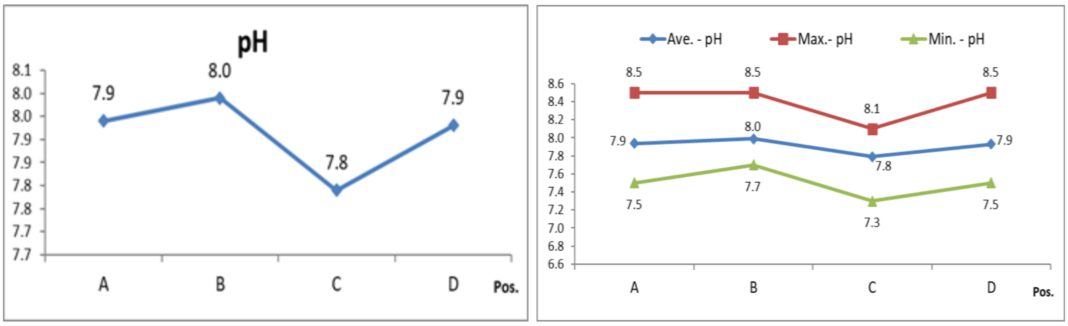


Fig. 9, Distribution of pH values at different locations.

4-2 Water temperature & Air temperature

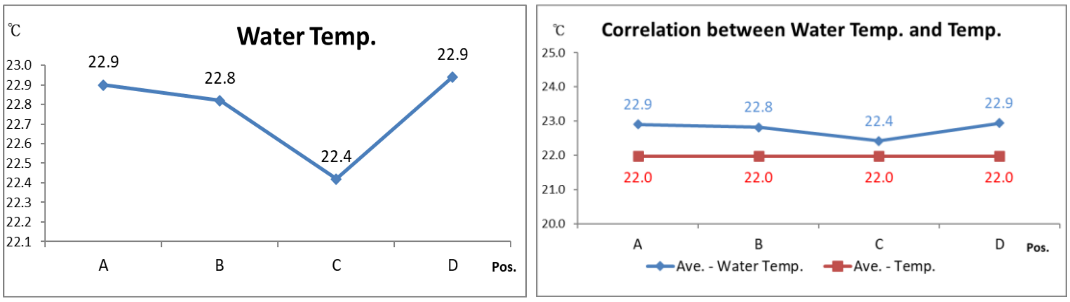


Fig. 10, Distribution of air and water temperature at different sampling sites.

In Figure 10, the average water temperature at location C is the lowest, but the correlation between air and water temperature at location C is not significant.

4-3 Other environmental factors.

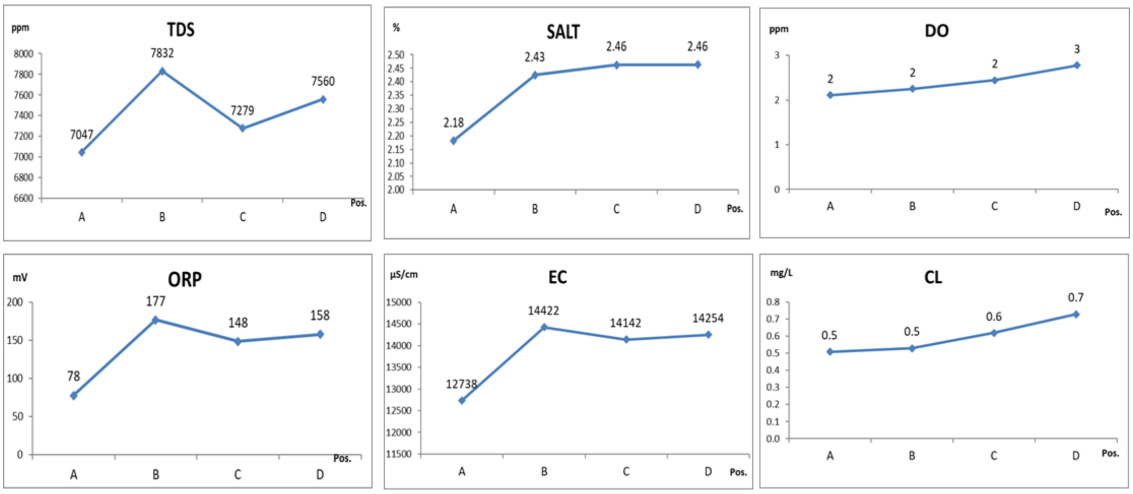


Fig. 11. Distribution of Other Environmental Factors at Each Sampling Point

For the water quality monitored at the four sampling points A, B, C, and D in the jellyfish lake, the average values of the environmental factors were calculated from December 30th, 2022 to February 22nd, 2023, and trend lines were drawn as shown in Figure 11.

- TDS, ORP, and EC values show similar trends across the sampling points.
- SALT and conductivity showed similar trends across the sampling points.
- Dissolved oxygen and CL showed similar trends across the sampling points.

5. Effects of Light on the Contraction Behavior of Upside-down jellyfish

5-1. Effects of Different Light Modules on the Contraction Behavior of Upside-down jellyfish.

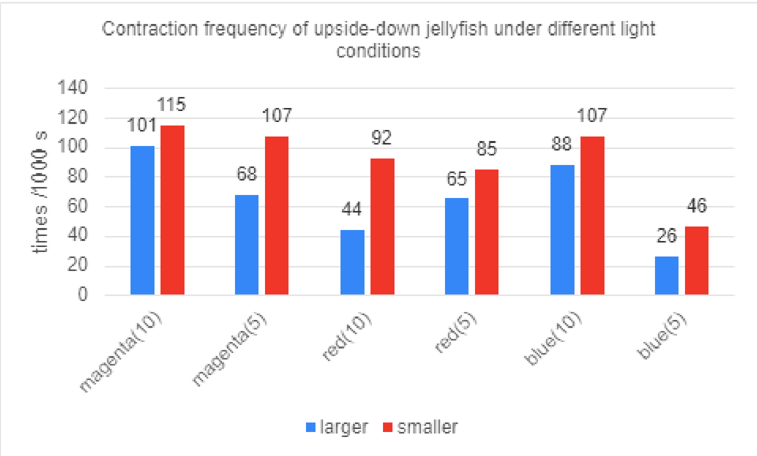


Fig. 12, Contraction frequency of Upside-down jellyfish under different colored lights

- Under different colored lights and intensities, smaller Upside-down jellyfish show a higher frequency of contractions than larger ones.
- The total contraction frequency of larger Upside-down jellyfish under different colored light intensities, in descending order, is: magenta (10) > blue (10) > magenta (5) > red (5) > red (10) > blue (5).
- The total contraction frequency of smaller Upside-down jellyfish under different colored light intensities, in descending order, is: magenta (10) > blue (10) = magenta (5) > red (10) > red (5) > blue (5).
- Regardless of size, the highest contraction frequency occurr under magenta light (10), while the lowest is under blue light (5).

5-2 The effect of time on Upside-down jellyfish contraction behavior

We found that the contraction frequency of the jellyfish varied at different times, as shown in Figure 13.

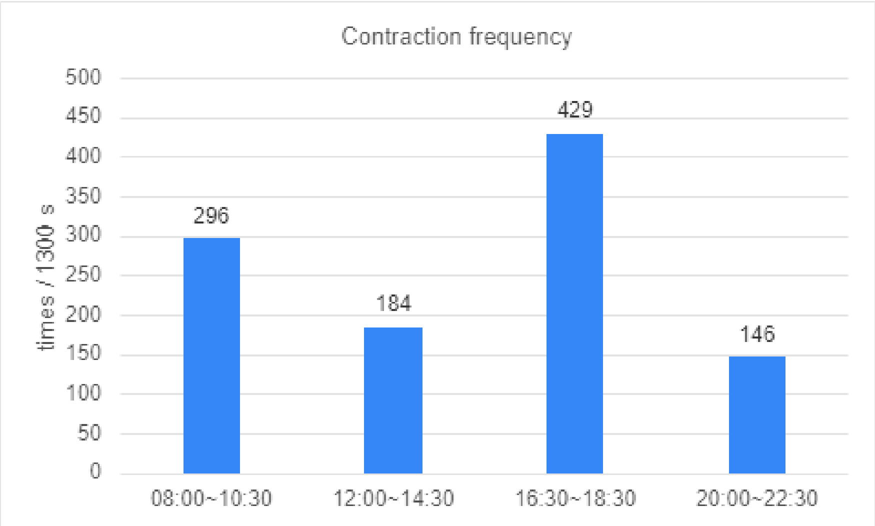


Fig. 13, the number of Upside-down jellyfish contractions at different times.

According to Figure 13, the order of single-cycle activity from high to low is 16:30~18:30, 08:00~10:30, 12:00~14:30, 20:00~10:30.

6. The impact of nutrient deprivation on Upside-down jellyfish

We wanted to know if Upside-down jellyfish would change their body size due to malnutrition, so we designed a lightless module and a light module.

6-1 Lightless Module

According to Table 2, Upside-down jellyfish gradually shrink over time under lightless module.

Table 2, Size of the flattened bell of Upside-down jellyfish under lightless module

	Initial size (cm)	Size after 6 days (cm)	Variation (cm)
jellyfish A (large)	12	10	-2
jellyfish A (medium)	9	7.5	-1.5
jellyfish B (large)	12	10	-2
jellyfish B (medium)	9	8	-1
jellyfish C1 (small)	5	4	-1
jellyfish C2 (small)	4.5	4.5	0

The larger Upside-down jellyfish showed more reduction in size than the smaller ones. According to the literature, when the nutrients in the water become scarce and when the ciliates cannot provide all the nutrients, Upside-down jellyfish will start to consume their own nutrients, resulting in a reduction in size.

6-2 magenta light module

Table 3, Size of the flattened bell of Upside-down jellyfish under magenta light module

	Initial size (cm)	Size after 6 days (cm)	Variation (cm)
jellyfish A (large)	10	9	-1
jellyfish A (medium)	7.5	6.5	-1
jellyfish B (large)	10	10	0
jellyfish B (medium)	8	7	-1

Compared the magenta light module (Figure 3) with the lightless module, the jellyfish in the magenta light module tend to have less shrinkage, perhaps because the module can support Zooxanthellae photosynthesis ,and provides nutrients for the jellyfish.

Discussion

1. When taking photos of jellyfish, factors such as inadequate equipment, shooting angles, and the turbidity of the water on that day led to unclear images which made it difficult to accurately count the number of jellyfish.

2. In water quality testing experiments, more sampling points should be added in the mangrove pond as it is the source of the water flow, which may reveal more information related to the jellyfish lake.

3. In the experiment, the top three average contractions of Upside-down jellyfish are red light (10), blue light (10), and no light. After comparing the time, we believe that red light (10) is the strongest, possibly because it was just past noon and jellyfish are more active during this time due to their circadian rhythm. However, if jellyfish are kept in the dark for an extended period of time to adjust their circadian rhythm before conducting experiments, autophagy and other phenomena may occur. The difference in contractions between blue light (5) and blue light (10) is significant, with the former having

the second highest number of contractions and the latter having the least. This may be due to the measurement of blue light (5) at 6 am, which is far from the time of the last exposure to sunlight. In addition, the temperature of 22.6 degrees Celsius, which is reported in the literature as the most suitable temperature for the survival of Upside-down jellyfish, is exactly 6 degrees Celsius different from the temperature during the measurement, which led to a decrease in the activity of Upside-down jellyfish and a large difference in the data between blue light (5) and blue light (10). Comparing the contraction frequencies based on body size, the contraction frequency of smaller Upside-down jellyfish is higher than that of larger ones, which is consistent with what is described in the literature that smaller organisms have higher activity levels than larger ones.

4. The mucus contains a large amount of Zooxanthellae, and there are abundant small organisms such as halteria, ciliates, amoebae, and diatoms. We were very surprised and decided to keep the mucus for cultivation, which may enable further experiments on Zooxanthellae. It is not necessary to destroy the jellyfish tissues to obtain the mucus.

5. The main reason for the abundant growth of jellyfish in Lin Yuan Wetland Park is due to human intervention, including:
   (1) Pumping water into the jellyfish lake to reduce the temperature: The government has planned to cultivate the wetland park and pumps wastewater into the jellyfish lake to lower the water temperature. Since the water in the jellyfish lake is rarely replaced, the surface temperature can reach over 35 degrees Celsius during Kaohsiung's hot summers, which results in massive jellyfish deaths. However, by regularly pumping the wastewater into the jellyfish lake, the temperature can be effectively lowered.
   (2) Pumping wastewater from aquaculture into the jellyfish lake: The nearby aquaculture industry mainly breeds groupers and shrimps. The farmers pump seawater into the breeding pools and discharge wastewater into the mangrove area, which eventually flows into the ocean. The wetland cultivation department of the government suggests pumping the wastewater into the jellyfish lake, which also contains the jellyfish's polyps. The roots of the Black Mangrove tree provide a suitable habitat for the jellyfish polyps to attach and grow. The ample sunlight in the area also facilitates their growth.

Conclusion

1. Upside-down jellyfish's tissues and mucus contain Zooxanthellae, and after 10-15 minutes, the proportion of intact cnidocytes decreases from 33.9% to 13%, indicating that cnidocytes can be triggered by environmental factors such as temperature and evaporation.

2. The size of the cnidocytes is about 5 to 10.5 micrometers, while the size of Zooxanthellae is about 7.5 to 10 micrometers.

3. Upside-down jellyfish thrive in alkaline water with higher humidity and a temperature of around 20 degrees Celsius. The water should have low organic pollution levels, good water quality, and high visibility.

4. Smaller Upside-down jellyfish contract more frequently than larger ones under different colors and intensities of light. The contraction frequency is highest under magenta light(10) and lowest under blue light (5).

5. Upside-down jellyfish contract most frequently between 16:30 to 18:30 and least frequently between 20:00 to 10:30.

6. Larger Upside-down jellyfish shrink more than smaller ones. In the absence of food and light sources, Upside-down jellyfish in the water with decreasing nutrients and without enough nutrition from Zooxanthellae will consume their own nutrients and shrink in size. The larger ones will contract more noticeably.

7. Based on Upside-down jellyfish population development throughout the year, the temperature in Kaohsiung, and the government's planned wetland cultivation projects, it can be preliminarily concluded that there is a certain correlation between jellyfish growth, climate, and project planning. This study focuses on exploring the environment's impact on jellyfish development, with water quality and environmental climate being the most direct factors.

References

Ravi D. Nath, Claire N. Bedbrook, Michael J. Abrams, Ty Basinger, Justin S. Bois, David A. Prober, Paul W. Sternberg, Viviana Gradinaru,and Lea Goentoro。(2017)。The Jellyfish Cassiopea Exhibits a Sleep-like State。Current Biology 27, P.2984–2990。

C 5.Jantzen, C., Wild, C., Rasheed, M., El-Zibdah, M. & C. Richter, 2008. Pumping of pore water nutrients by the upside-down jellyfish Cassiopea sp. In coral reefs. 11th International Coral Reef Symposium, 7-11 July 2008, Ft. Lauderdale, USA.

Matthew, J. M. & J. Jason, 2003. The ontogenetic scaling of hydrodynamics and swimming performance in jellyfish (Aurelia aurita), Journal of Experimental Biology 206: 4125-4137.