Veronika Lakhtikov, Adhya Shah, Sabrina Weiler

ANIM-004

Effect of Amputation on re-Symmetrization and Physical Behaviors of *Cassiopea xamachana*

Abstract

The purpose of this experiment was to investigate the physical behaviors and the re-symmetrization rate of the *Cassiopea xamachana* based on cutting ¼ through the manubrium (center of *Cassiopea)* versus ¼ amputation not including the manubrium. Jellyfish have four radial root canals, defined as tetramerous radial symmetry, all equally symmetrical. Re-Symmetrization is the process of reorganizing one's body parts to regain function after a loss of anatomical structure. This process takes approximately four days to complete to regain full function. *Cassiopea xamachana* swims upside down which is normal for the jellyfish, while swimming right side up and crashing into objects is abnormal. There were two groups: Group A was cut ¼ through the center, and Group B was cut ¼ off center. Each group had seven jellyfish, ensuring that each tank was not too crowded and each tank had similar environments. The jellyfish pre-amputation was used as a control for the *Cassiopea's* behaviors. The trial resulted with Group A dying sooner and having a slower pulsation rate than Group B. However, it had a faster re-Symmetrization rate compared to Group B. In conclusion, injury to the manubrium has an effect on the *Cassiopea.* Limitations of the study included the type of equipment used as the jellyfish were very delicate and finding a way to maintain the ammonia levels overnight and during the weekend. Future work that will be explored will be how different factors such as environmental changes affect their re-Symmetrization rate and physical behaviors.

Introduction

Cassiopea xamachana

Veronika Lakhtikov, Adhya Shah, Sabrina Weiler

ANIM-004

The Cassiopea xamachana, often referred to as the "upside-down jellyfish", is a cnidarian found in warm, brackish waters (Fujita *et al.* 2021). The mangrove jellyfish possesses distinctive characteristics, such as the ability to swim upside down, multiple mouths, and a remarkable capacity for re-symmetrization (Fujita et al. 2021; Ostendarp et al. 2022). Another feature of *Cassiopea* is the symbiotic relationship it shares with algae (Medina et al. 2021). The *Cassiopea* shares a mutualistic relationship with the dinoflagellate *Symbiodinium* (Colley et al. 1983). This mutualistic relationship provides the jellyfish with essential nutrients (Colley et al. 1983). On average, a mangrove jellyfish consumes approximately 9 grams of food daily (Becker et al. 2007). Mangrove jellyfish are generally composed of 5% protein and 95% water (Isenmann et al. 2019). The *Cassiopea* contains a firm, gelatinous structure, named the *mesogloea* that gives it the shape it has (Bigelow et al. 1900). The structure of the jellyfish also contains varied numbers of oral arms that contain buds of rhopalia (Ohdera et al. 2018). Rhopalia are sensory cells that sense light and with the help of statoliths, sense gravity. The manubrium of the *Cassiopea,* also known as the center of the *Cassiopea,* is the main structure of the *Cassiopea* containing the mouth. The *Cassiopea* contains a nervous system unlike most animals. The nervous system of this jellyfish is formed in a web-like structure. This "nerve net" covers the whole jellyfish body but is increased in the oral arms of the jellyfish (Amplatz et al. 2024). The *Cassiopea xamachana* also serves as a viable test subject for many experiments because it is easily tractable as well as cultured. It is used as a model species in certain studies concerning the status of the ocean, due to the wide variety of foods that the jellyfish eats. This leads the *Cassiopea* to be a bioindicator of the ocean (Templemen and Kingsford et al. 2012).

Veronika Lakhtikov, Adhya Shah, Sabrina Weiler

ANIM-004

Behaviors

The mangrove jellyfish exhibits both natural and unnatural behaviors, such as swimming upside down and entering a sleep-like state. Typically, the jellyfish swims upside down; conversely, swimming right side up and colliding with objects is considered abnormal behavior. (Albert et al. 2011; Becker et al. 2007). On average, a mangrove jellyfish consumes 9 grams of food per day (Becker et al. 2007). It takes about four days for the jellyfish to completely re-symmetrize, while anything longer than approximately 4 days is considered abnormal (Fujita et al. 2021). Re-Symmetrization will affect the physical behaviors of jellyfish, such as pulsation and movements. For example, higher-than-normal temperatures can cause the bleaching of jellyfish polyps (Yanik *et al.* 2018). The physical behaviors of jellyfish, including their sleep-like state, are also affected by their habitat (Nath *et al.* 2017).

Re-Symmetrization

The mangrove jellyfish (Cassiopea xamachana) exhibits regenerative capabilities known as re- Symmetrization. This enables them to restore any lost tissues and structures of their body by going back to a radially symmetrical state. This includes the re-Symmetrization of their oral arms as well as manubrium. Research has shown that fragments of umbrella tissue can regenerate into fully functional medusae. This highlights the role of their cellular energy used in facilitating this process (Fujita et al. 2021) (Ostendarp et al., 2022). Regeneration involves the reorganization of tissues to maintain radial symmetry and restore essential functions such as feeding and locomotion (Ostendarp et al. 2022). This not only enhanced survival following injury but also aided in highlighting their natural behaviors and what they used in their everyday life (Abrams et al. 2015). By having regenerative abilities, mangrove jellyfish are able to use it to their ability when in dangerous situations. This makes mangrove jellyfish an extremely adaptive species.

Materials and Methods

Set-up Table

Groups	Amputation Amount	Through the manubrium:	Sample Size
Group A A (Amputation through Center)	¼	Yes	7
Group B B (Amputation Off Center)	¼	No	7

Care of Cassiopea xamachana

The *Cassiopea xamachana* were in 10 gallon tanks using deionized water. Aquarium salt was added to the tanks as well. Approximately, 7 jellyfish for a 10 gallon tank and remained in a temperature range of 22-24 degrees celsius. The pH range was about 8.2. The optimal salinity range is 30-33 parts per thousand (ppt). The tanks remained at 1.023 specific gravity (SG).

Pre-Amputation

Place gloves on hands and begin to sanitize the amputation area with isopropyl alcohol and ensure no extraneous objects are in the way. Disinfect all tools and wash all equipment (scalpels included). While washing scalpel, ensure that thick gloves remain on to prevent harm. Place petri-dish in the disinfected area to prepare for amputation.

Amputation

While wearing gloves, retrieve the *Cassiopea* using extreme care with a mesh net and place using hands, within the petri dish. Make sure the *Cassiopea* is flat on the petri dish to insure the most accurate amputation. Place *Cassiopea* upside down with the arms facing upwards. Place the scalpel in the center of the jellyfish and slowly cut, cutting away from yourself.

Data Analysis

Different behaviors of the jellyfish were observed and recorded. All data was recorded in Excel. Angles were recorded through protractors and pulsation was recorded through a five minute video recording where the pulsation was counted for each minute (the pulsation is visual (a team member counted)). All legs of the *Cassiopea* were counted and recorded within a table as well. The formula for a percentage of the Group A re-Symmetrization rate was $\frac{angle\ re-Symmetrized\ in\ degrees}{angle\ cut\ (degrees)} \cdot 100$. This formula was applied to all the jellyfish in the group of A. Then the data was inputted into a line graph where the number of days was x and the percentage re-Symmetrized was y. The formula for a percentage of the Group B re-Symmetrization rate was $\frac{length\ of\ re-Symmetrization\ (cm)}{length\ of\ incision\ cut\ off\ center\ (cm)} \cdot 100$. The averages and standard deviation for all measured components was calculated by the functions of Excel. There was a video recording for 5 minutes to observe the pulsation rate of the jellyfish where for both Group A and Group B, the data was stored into line graphs as well. For both pulsation and re-Symmetrization, the data was stored into line graphs and bar graphs. The data was stored on an excel spreadsheet where the average for each minute was recorded. A paired t-test to compare the re-symmetrization rate and the pulsation rate of the jellyfish was used to compare both groups. The t-test was used from the functions of Excel.

Results

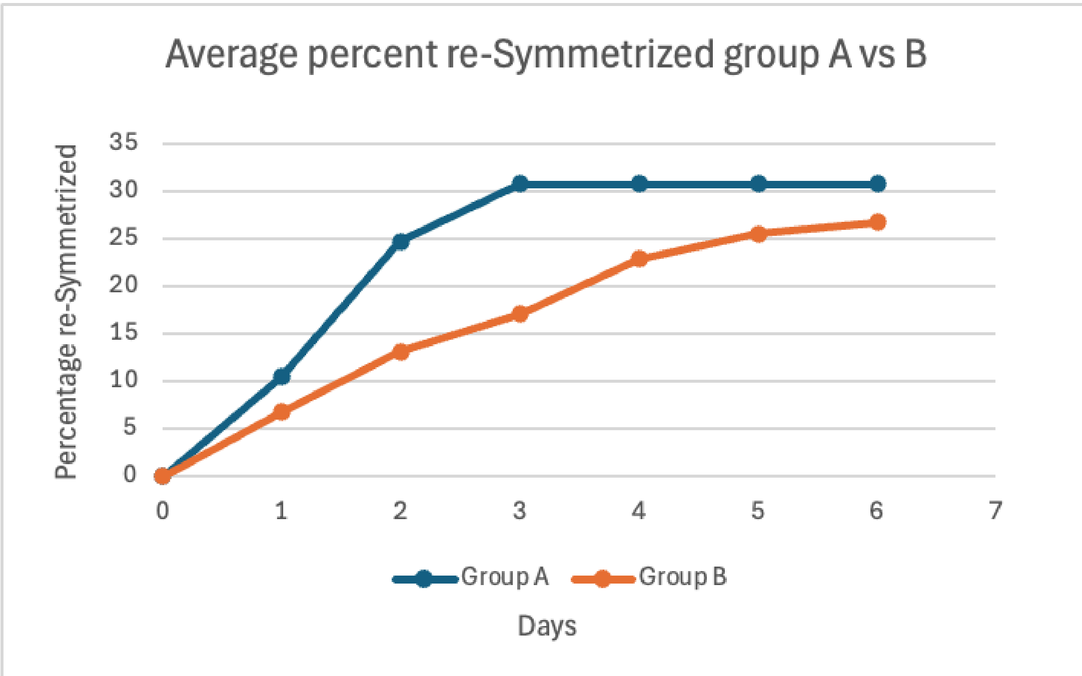

Figure 1. Trial 1 Average Percent re-Symmetrized Group A vs B in a line graph. Each line represents the percentage of *Cassiopea* re-Symmetrized in both groups. Group A represents the blue line and Group B represents the orange line. The x represents the number of days and the y represents the percentage re-Symmetrized. Group B re-Symmetrized at a slower rate compared to Group A.

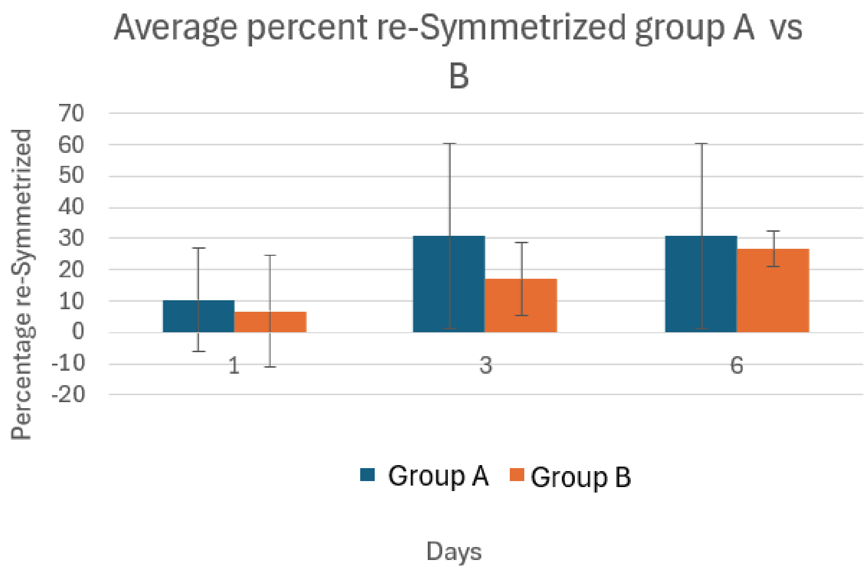

Figure 2. Trial 1 Average Percent re-Symmetrized Group A vs B in a bar graph. Each bar represents the percentage of *Cassiopea* re-Symmetrized in both groups. Group A represents the blue bars and Group B represents the orange bars. The x represents the number of days and the y represents the percentage re-Symmetrized. Group B re-Symmetrized at a slower rate compared to Group A. Standard deviation is shown in error bars for the groups. An unpaired t-test was conducted resulting in the finding that none of the values were statistically significant.

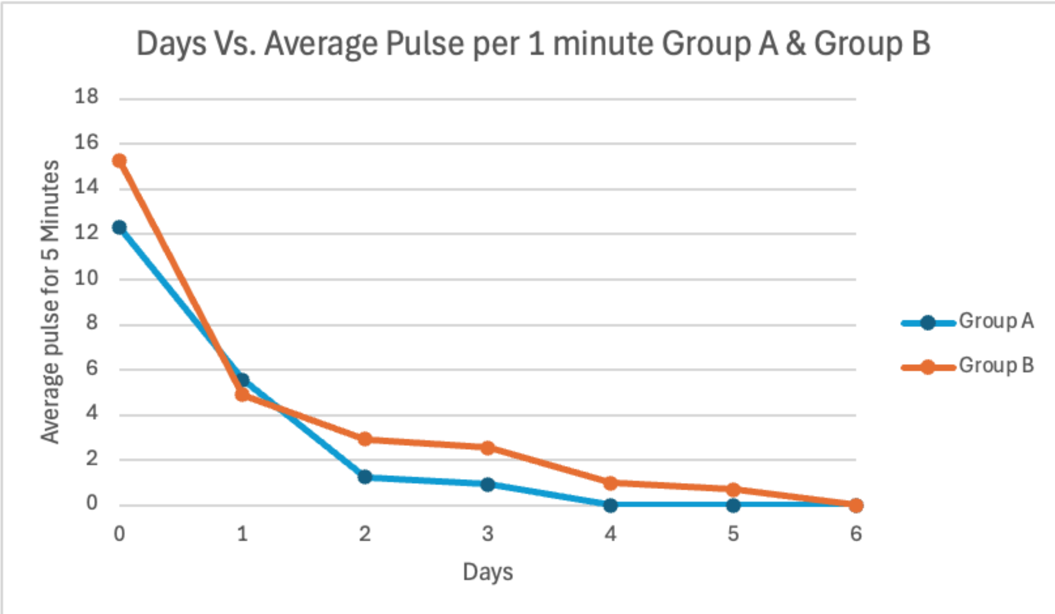

Figure 3. Trial 1 Average Pulse per 1 minute for Group A vs B in a line graph. Each dot on the line represents the average pulse for 1 minute in a day. Group A represents the blue line and Group B represents the orange line. The x represents the number of days and the y represents the average pulse for 1 minute. Group B pulsed at a higher rate compared to Group A. An unpaired t-test was conducted resulting in the finding that the values were not statistically significant.

A(Amputation Through Center)	Number of Legs Pre-Amputation	Number of Legs right after amputation	Number of legs 6 days after amputation
Jellyfish #1	4	3	3
Jellyfish #2	6	4	4
Jellyfish #3	3	2	2
Jellyfish #4	5	4	4
Jellyfish #5	4	3	3
Jellyfish #6	4	3	3
Jellyfish #7	5	4	4
Average:	4	3	3

Figure 4. Trial 1 Table of Legs for Cassiopea's in Group A. The first column shows the number of legs that each jellyfish had pre-amputation. The second column shows the number of legs each jellyfish had left right after amputation. The third column shows the number of legs left over 6 days after amputation. The last row shows the average number of legs pre-amputation, right after amputation, and 6 days after amputation. There is no change in the number of legs from right after amputation to 6 days after, which shows that the legs of the jellyfish did not grow back.

B (Amputation Off Center)	Number of Legs Pre-Amputation	Number of Legs right after amputation	Number of legs 6 days after amputation
Jellyfish #1	5	4	4
Jellyfish #2	5	4	4
Jellyfish #3	3	2	2
Jellyfish #4	4	3	3
Jellyfish #5	4	3	3
Jellyfish #6	6	4	4
Jellyfish #7	6	4	4
Average:	5	3	3

Figure 5. Trial 1 Table of Legs for Cassiopea's in Group B. The first column shows the number of legs that each jellyfish had pre-amputation. The second column shows the number of legs each jellyfish had left right after amputation. While the third column shows the number of legs left over 6 days after amputation. The last row shows the average number of legs pre-amputation, right after amputation, and 6 days after amputation. The consistency in the amount of legs throughout the trial after amputation shows that the legs of the jellyfish did not grow back.

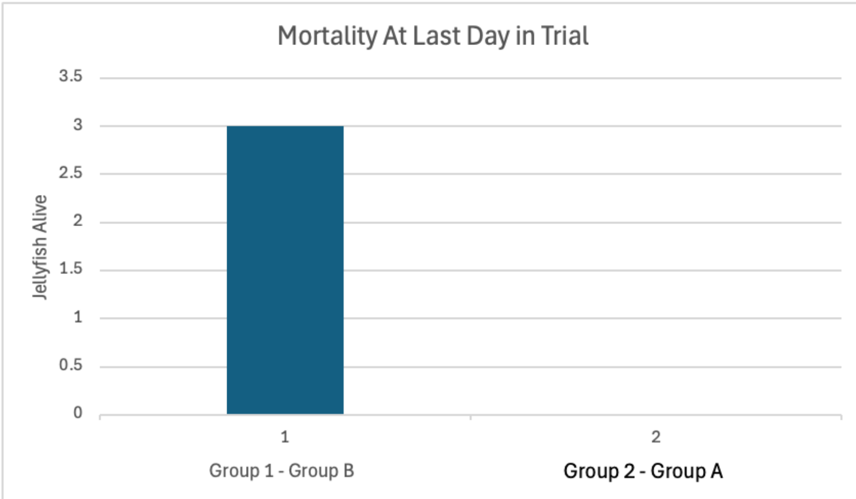

Figure 6. Trial 1 Mortality At Last Day In Trial in a bar graph. The bar graph overall shows the amount of *Cassiopea* that were alive at the end of the trial for each group. For the first bar, Group B is shown to have only 3 jellyfish that were alive. The second bar (not present due to value being 0) represents Group A showing that no jellyfish remained at the end of the experiment. The x value represents the groups and the y value represents the number of jellyfish alive.

Conclusions and Discussion

Overall, this experiment came with unexpected difficulties. There were several issues before we started the trial that arose. These issues may have resulted in the failure of the previous trials. One of the issues may have been human error in the amount being cut. This could have infiltrated a part of the *Cassiopea xamachana's* nervous system that triggered the jellyfish to not re-Symmetrize. Another margin of error may have been the amount of salt that was added into the tank. To measure the amount of salt needed, we were using a salinity checker, however, we later found that it was unreliable as it wasn't giving out the correct number. Therefore, we decided to use the volume of the water that was in the tank to calculate the amount of salt for

35ppm. We found this method to be much more accurate and reliable. Although this was an experiment that was left overnight with the correct conditions, there was no guarantee that the surrounding temperature did not fluctuate, resulting in the water cooling or making it too warm. Another factor that we changed from the first experiment was the amount of groups we had. Originally, we had 3 groups; control, cut through center, and cut off center. For the most recent trial, we had 2 groups and used the pre-cut data as if it were our control. We felt that this was more accurate because it showed the data for each *Cassiopea* in that specific group.

Most recent trial

Due to inadequate jellyfish material and tank setup (due to cost), unfortunately, many of the jellyfish did not make it through the extent of the trial. However, Group B survived longer than Group A, as all organisms in Group A had died by day 4. 3 jellyfish remained in Group B at the end of the trial, while no jellyfish remained in Group A (Fig. 6). Before the *Cassiopea's* had died in Group B, the number of legs that the *Cassiopea* had were recorded for both groups (Fig. 4 & 5). Group B had an overall longer survival rate. During this trial, the pulsation rate of Group B (Fig. 3 & 4), the group that was cut ¼ off the manubrium, showed higher pulsation rates compared to Group A (Fig. 3). However, as the trial went on, the Cassiopea showed decreased pulsation activity in both Group A and B. However, in Group B, where the jellyfish were amputated ¼ off the manubrium, the pulsation decreased at a slower rate compared to Group A (Fig. 3), amputation through the manubrium. In contrast, it was also observed that Group A, amputation ¼ in the manubrium/center, re-Symmetrized more than Group B, ¼ off the center (Fig. 1 & 2). To continue, although parts of the manubrium were taken when cutting through the center, Group A showed greater results when it came to angle closing. The fact that Group A had better re-Symmetrization results was interesting as there was a level of uncertainty when taking a part of their only major system (their nervous system) as well as the fact that they died sooner than Group B. Another limitation was that the conditions in which they were in could have caused a different rate of re-Symmetrization. Due to the environment and surrounding factors that were out of our control, we were unable to monitor them throughout the entire day. Overall, much was learned during this experiment. There were limitations but there was also a level of success that was achieved. Although during the first two trials we failed to collect data, the third trial was a viable and successful trial that helped us better understand the mechanics of taking care of jellyfish and especially their re-Symmetrization mechanism.

Past Trial

In one past trial, we were able to retain data for one jellyfish. One *Cassiopea,* a relatively large one, when amputated ¼ through the manubrium, re-symmetrized almost completely. However, it died one day after amputation.

Future Studies

Future implications of research include seeing how different temperatures affect the re-Symmetrization rate and physical behaviors of the Mangrove Jellyfish. We could also extend the amount of trials done. Another thing we can do is test different foods on them. Also comparing how a vegetable and meat diet will affect their rate of re- Symmetrization.

Acknowledgements

Thank you, Dr. D. Marmor, Ms. N. Babbar, Dr. L. Wang, Ms. Zhu, Mrs. Macleod, Dr. J. Cohen, Dr. S. Lin, Mr. Z. Liang, and Ms. DePietro.

References

Abrams, M. J., Basinger, T., Yuan, W., Guo, C. L., & Goentoro, L. (2015).

Self-repairing symmetry in jellyfish through mechanically driven reorganization. Proceedings of the National Academy of Sciences, 112(26), E3365-E3373.

Albert, D. J. (2011).

What's on the mind of a jellyfish? A review of behavioural observations on Aurelia sp. jellyfish. Neuroscience & Biobehavioral Reviews, 35(3), 474-482.

Becker, E. W. (2007).

Micro-algae as a source of protein. Biotechnology advances, 25(2), 207-210.

Brierley, A. S. (2017). Plankton. *Current Biology*, *27*(11), R478-R483.

Colley, N. J., & Trench, R. K. (1983).

Selectivity in phagocytosis and persistence of symbiotic algae by the scyphistoma stage of the jellyfish Cassiopeia xamachana. *Proceedings of the Royal Society of London. Series B. Biological Sciences*, *219*(1214), 61-82.

Fujita, S., Kuranaga, E., & Nakajima, Y. I. (2021).

Regeneration potential of jellyfish: cellular mechanisms and molecular insights. Genes, 12(5), 758.

Godos, J., Grosso, G., Castellano, S., Galvano, F., Caraci, F., & Ferri, R. (2021).

Association between diet and sleep quality: A systematic review. Sleep medicine reviews, 57, 101430.

Hays, G. C., Richardson, A. J., & Robinson, C. (2005).

Climate change and marine plankton. *Trends in ecology & evolution*, *20*(6), 337-344.

Isenmann, E., Blume, F., Bizjak, D. A., Hundsdörfer, V., Pagano, S., Schibrowski, S., ... & Diel, P. (2019).

Comparison of pro-regenerative effects of carbohydrates and protein administered by shake and non-macro-nutrient matched food items on the skeletal muscle after acute endurance exercise. Nutrients, 11(4), 744. Association between diet and sleep quality: A systematic review. Sleep medicine reviews, 57, 101430.

Katsuki, T., & Greenspan, R. J. (2013).

Jellyfish nervous systems. Current Biology, 23(14), R592-R594.

Martínez, I., Herrera, A., Tames-Espinosa, M., Bondyale-Juez, D. R., Romero-Kutzner, V., Packard, T. T., & Gómez, M. (2020).

Protein in marine plankton: A comparison of spectrophotometric methods. *Journal of Experimental Marine Biology and Ecology*, *526*, 151357.

Medina, M., Sharp, V., Ohdera, A., Bellantuono, A., Dalrymple, J., Gamero-Mora, E., ... & Fitt, W. K. (2021).

The upside-down jellyfish Cassiopea xamachana as an emerging model system to study cnidarian–algal symbiosis. In *Handbook of marine model organisms in experimental biology* (pp. 149-171). CRC Press.

Nath, R. D., Bedbrook, C. N., Abrams, M. J., Basinger, T., Bois, J. S., Prober, D. A., ... & Goentoro, L. (2017).

The jellyfish Cassiopea exhibits a sleep-like state. Current Biology, 27(19), 2984-2990.

Ostendarp, M., Plewka, J., Flathmann, J., Tilstra, A., El-Khaled, Y. C., & Wild, C. (2022). Complete and rapid regeneration of fragments from the upside-down jellyfish Cassiopea. Jellyfish nervous systems. Current Biology, 23(14), R592-R594.

Schäfer, S., Gueroun, S. Kj., Andrade, C., & Canning-Clode, J. (2021). Combined effects of temperature and salinity on polyps and ephyrae of Aurelia solida (Cnidaria: Scyphozoa). Diversity, 13(11), 573.

Yanik, T., & Aslan, I. (2018). Impact of global warming on aquatic animals. Pakistan Journal of Zoology, 50(1).