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1. Describe the experimental setup and identify independent and dependent variables. For this experiment newly spawned egg clusters of the common cuttlefish were

collected and transported to a lab under controlled conditions. This experiment aimed to see how climate change (temperature increases) affects the development and survival rates of these eggs. To do this the eggs were evenly distributed in eighteen different rectangular tanks that were mechanically filtered and UV-sterilized. The independent variable in this experiment is the controlled change in temperature using a custom-made digital STC-3000, which would increase the temperature through a submerged digital thermostat upon demand. They also could lower the temperature using seawater chillers. During this experiment, the pH, salinity, dissolved oxygen, and temperature were monitored daily. The laboratory acclimation was done for 8 days, accounting for at least two-thirds of their embryogenesis. After this period the eggs were randomly distributed to six different treatments which fluctuated the immersion times in water. This was another independent variable in the experiment to see the effects of tidal extremes on the development and survival rates. The dependent variable in this

experiment is the development time/survival rates of the eggs as they differed depending on the different temperature levels of the independent variable.

2. What are ROS and why are they important?

ROS stands for Reactive Oxygen Species which are a group of unstable molecules produced by all kinds of cells. They are important and beneficial to biosystems as they act as signaling molecules and are involved in many basic mechanisms. For example, they play a role in metabolism, and immunity, and act as intermediates for the redox behavior of O<sub>2</sub>. Not only do they act as signaling molecules, but they are important for enhancing immunologic defenses. These are all very fundamental roles in biosystems and prove to be beneficial for growth.

3. Choose a figure and summarize the findings. Critique the figure. Why is it good/Bad? What could be improved? What do you like?

Figure one of this article shows the sampling location in the Sado estuary which is located on the west coast of Portugal. Not only does this figure provide a map but also shows a direct photograph of the newly spawned eggs during the low tide. I think this figure is good as it states the location and provides a picture for reference to ensure all aspects of the experiment for future studies. One thing that could improve this map is adding some color for the land or sea so it can be easily determined the difference between the two. I liked how they added a real photograph of the sampling site to provide some more background and conditions of where they were collected.

4. Using this paper as a guide, what are the ramifications of increased temperature

and tidal extremes on cuttlefish?

Using this article as a reference, the results show that both the temperature and tidal extremes had a direct effect on the cuttlefish. When it comes to temperature the cuttlefish showed significantly shorter embryonic development times when exposed to increased temperatures. This shows that the temperature had a direct effect on the development times of the eggs. However, when it comes to the tidal extremes there was no significant effect on the development time, but the hatching success significantly increased under immersion and had an opposite trend when exposed to tidal emersion. It is worthy to note that even with this opposite trend it still had no direct effect on the hatching success.

#### References

Yang, S., & Lian, G. (2020). ROS and diseases: role in metabolism and energy supply. *Molecular and cellular biochemistry*, 467(1-2), 1–12.

<https://doi.org/10.1007/s11010-019-03667-9>