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Adaptations
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Exploratory 3

Salinity Stress and Osmoregulation in *Tigriopus californicus*

Introduction

Many adaptations exist in animals that allow them to live in extreme environments. There are bacteria that live in lakes of liquid carbon dioxide, archaea that live in hot springs, tubeworms that build their homes in frozen methane, and bacteria that use chemosynthesis as a form of primary production near deep sea vents. One does not need to go to the end of the earth to find organisms living in harsh or extreme environments. They are all over the place. Life can be found in supralittoral (splash) tidepools. The temperature and salinity fluctuations in these pools can cause a great amount of stress for organisms. High salinity environments are difficult to live in due to osmotic pressures and salt gradients altering the composition of body fluids of animals living there. Living in a highly saline environment does many things including osmotically drawing water from the body and increasing the levels of salts. Water is crucial to many life functions (respiration and metabolism not the least) and altered salt levels will affect nervous systems. To deal with these stressors, some organisms use osmoregulation. Osmoregulation is the active regulation of water concentrations in the body in efforts to maintain homeostasis. This is done mainly through regulating excretion.

One of the organisms that has adapted to living in the harsh conditions of the splash pools is *Tigriopus californicus*. It is a harpacticoid copepod that can be found in tidepools on the west coast of North and South America. It's high tolerance to temperature and salinity fluctuations

provide it with a large range. It is fairly large (1.2-1.4mm) (Animal Encyclopedia) and noticeably red in color. They are omnivores as they will eat anything from algal films on rocks to body tissues of sponges (Animal Encyclopedia). They have the amazing ability to withstand an incredible range of salinity from 0.26 to 1.45 M (McDonough 1981). They can also tolerate incredible levels of desiccation. They have even been known to be revived from a dried out splash pool when sea water is replaced (Powlik 1996).

In this study I explore the upper and lower limits of salinity for *Tigriopus californicus* as well as their ability to osmoregulate. Based upon personal observations and estimates I hypothesize that *T. californicus* does have the ability to osmoregulate and that they can survive salinity conditions ranging from 20ppt to 100ppt.

Methods/Materials

The first step in this study was making observations of the presence and absence of *T. californicus* in splash pools in the area. I went to Shore Acres, Coos county, OR and noted spatially which splash pools the copepods were living in. This gave me a baseline trend for making estimates of salinity tolerances. I then collected several hundred test subjects from a splash pool of intermediate size and a salinity of 34ppt. I then made up seven different concentrations of seawater using distilled water and sea salts. I made the following concentrations: 0ppt, 20ppt, 35ppt, 60ppt, 80ppt, 100ppt, and 120ppt. I calculated salinity concentrations using a refractometer. Using these solutions I subjected the test subjects to two separate studies. The first was to determine the upper and lower salinity limits *T. californicus* can survive. To do this I placed 50-60 individuals in each solution described above. I kept them

at roughly 20°c in small watch glasses. I allowed them to sit for a 24 hour period then determined the percentage that had survived at the end of the period. I counted the individuals using a dissecting microscope. For the other study I only used three concentrations of the saltwater. I used 35ppt, which acted as a control, 60ppt, and 80ppt. I placed 100+ individuals in each solution and stored them for 24 hours at 20°c. At the end of the 24 hour period I centrifuged the test subjects and extracted the supernatant. I then used a blunt probe to crush the bodies of the test subjects, releasing the body fluids. I centrifuged the crushed bodies and then extracted the supernatant which was their body fluids. I then tested the osmolarity of the body fluids and the saltwater using a vapor pressure osmometer. Unfortunately I was only able to test the osmolarity of the test subjects at 35ppt and 60ppt as most of the individuals in the 80ppt solution had died which limited the available body fluid to test.

Results

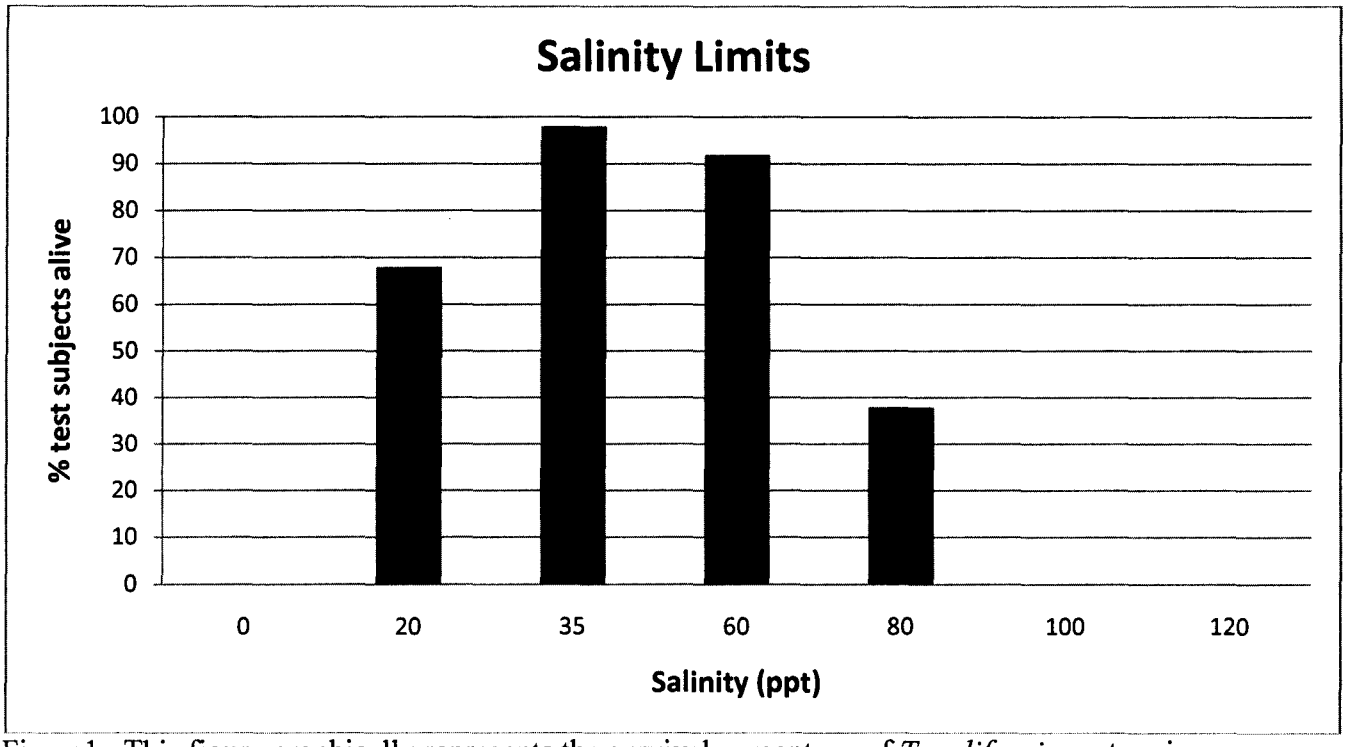

Figure1. This figure graphically represents the survival percentage of *T. californicus* at various salinity levels.

Osmoregulation

Salinity (mOsm/L)	Body Fluid Salinity (mOsm/L)
1293	1210
1782	1692

Table 1. This table shows the salinity of the solution the test subjects were exposed to for the 24 hour test period on the left. It also shows the salinity level of the body fluids of the test subjects after the test period.

When looking at figure 1, one should notice that based upon this series of tests the lower limit for the survival of *T. californicus* is a salinity level between 20ppt and 0ppt. The upper limit is between 80ppt and 100ppt. One should also notice, from looking at table 1, that the body fluid has a lower osmolarity than does the test solutions.

Discussion

The first aspect of this study yielded fairly clear results. There was a distinct range of tested salinity levels that allowed *T. californicus* to survive. The lowest salinity that had surviving test subjects was 20ppt. This does not mean that they cannot live below 20ppt, but that they cannot live at some concentration between 20ppt and 0ppt. A similar trend follows for the upper limit to salinity. The highest salinity tested with surviving test subjects was 80ppt, meaning their true upper limit must be between 80ppt and 100ppt. The peak survivability was at 35ppt which makes sense because it was the control for seawater. Most oceanic water is roughly 35ppt so it makes sense that it would be most easy to survive under those conditions. The second part of this study was a little less easy to understand. This is mostly due to a lack of data. Only the control (35ppt) and one test concentration (60ppt) set of data was available for study. It should be noted that some osmoregulation was exhibited when *T. californicus* was exposed to the increased salinity. The body fluid was lower in salinity (hyposmotic) than the external fluid. Both aspects of this study partially supported my hypothesis. I hypothesized that *T. californicus* could survive in salinities between 20-100ppt. My hypothesized lower limit may be correct, but it was shown that the test subjects died in 100ppt saline solution, refuting my hypothesized upper limit. I also hypothesized that *T. californicus* could use osmoregulation and it has been shown that they do.

There are a few sources of error in this set of studies. The first comes from inaccuracies in measuring the osmolarity of the body fluids. The test solutions and test subjects were centrifuged, but this did not completely separate the two. When the bodies were crushed there would be some leftover test solution which would contaminate the body fluids. This would

cause an overestimation of the osmolarity of the body fluids. Another source of error is the low degree of accuracy achieved using a refractometer. The device used was old and difficult to read.

There are a few things that could be done for further research using these studies. The first would be to use more than one test solution in the osmoregulation tests. This could be accommodated by using larger numbers of test subjects and smaller intervals of salinity levels such as 35ppt, 45ppt, 55ppt, and 65ppt. Another thing that could be done would be to more accurately describe the upper and lower salinity limits. This could also be done by using smaller increments of salinity levels. Survival could be monitored in solutions of 0ppt, 5ppt, 10ppt, 15ppt and 20ppt for the lower limits. Survival could also be noted for solutions of 80ppt, 85ppt, 90ppt, 95ppt, and 100ppt to determine the upper limit. I would also recommend attempting to determine the method used by *T. californicus* in regulating salt levels.

References

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