Examining the Preference of Sodium Concentration in the Diets of *Nasutitermes corniger* (Blattodea:Termitidae)(Motschulsky)

Emile Fierro Morel, James McCain, Jose Torres

Edited by Erik Aguirre Cordero

Abstract: Sodium is an essential element for the nervous system of many animals, including termites. This experiment tested different sodium concentrations on rotting wood blocks and cellulose in a pure, powdered state. *Nasutitermes corniger* termites were collected from a nearby colony. Thirty-eight colonoids were created, which consisted of 10 soldiers and 50 workers each. Each colonoid was kept in a Petri dish with a damp filter. For the first part of the experiment, Seventy-six blocks of wood were cut into pieces of similar mass and dipped into sodium concentrations ranging from 0-10% for 10 seconds. The blocks were placed 1cm apart on the arenas and the termites were left to roam for a certain amount of time. In the second part of the experiment, sodium concentrations ranging from 2-8% were made by mixing with pure, powdered cellulose. The arenas contained all four concentrations this time. It was observed that *N. corniger* termites do not have a clear sodium preference, however some trends were apparent in both experiments. Further experimentation would be needed to explore these trends.

Keywords: termites, *Nasutitermes corniger,* sodium concentration

Nasute termites, including many other organisms with a nervous system, use sodium-ion gated voltage channels. Sodium is a crucial electrolyte for the nervous system to work properly, as well as for maintaining osmotic balance and muscle activity (Da Silva and Williams 2001). For termites, sodium is also important for their reproductive system (Kaspari et al. 2014). Usually, higher plants have about 1.0 mg * kg-1 sodium (Marschner 2011), but decomposers and herbivores that consume these plants have sodium concentrations that are 100-1000 fold higher (Council 2006), (Mattson 2012). When sodium levels are low in the ecosystem, it becomes more strenuous for decomposers and bacteria to thrive. The location of this experiment was at Texas A&M's Soltis Center in Costa Rica, which is approximately 40 kilometers from the Pacific coast. Sodium supplies in the ecosystem can vary geographically (Tan , Latysh et al. 2005). The element is transported as aerosols through rainfall coming from the coast, and the concentration of sodium drastically decreases as it moves further inland (Stallard and Edmond 1981). The effect of leaching can also contribute to sodium decrease in inland ecosystems (Vitousek et al. 1986). In a similar experiment done by Kaspari et al. (2014), it was observed that supplemented sodium in an inland lowland rainforest of Ecuador four times than the normal amount increased termite density and activity. In this experiment, the sodium preferences of *N. corniger* were tested on two different substrates, wood and pure cellulose. The wood used is what the termites would

typically find in the forest; damp and rotting. However, pure powdered cellulose has no environmental additives such as fungi or other possible chemical cues found in rotting wood. It is predicted that low to medium levels (2-4%) of salt (NaCl) would be most preferred by termites on both substrates.

Materials And Methods

Experiment One: Wood Blocks

Termite Collection

Termite colony was found near the research station and the species was identified. The nest was destroyed by using sticks and bare hands. The pieces of nest were placed on the ground and all termites localized were collected with an aspirator (Bioquip, Rancho Dominguez, CA). A total of 10 aspirator vials were used to capture all termites necessary. The termites collected were then separated into workers and soldiers. A total of 38 colonoids were created. The colonoids consisted of 50 workers and 10 soldiers. A total of 1900 workers and 380 soldiers.

Making colonoids

Each termite was picked up with featherweight forceps (Bioquip, Rancho Dominguez, CA) and its caste was identified. The colonoids were kept in Petri dishes (Bioquip, Rancho Dominguez, CA) with slightly damp filter paper (GE Healthcare, Chicago, IL) covering the entire bottom half of the dish. The Petri dish was secured by two rubber bands.

Food Preparation

A large log of rotting wood was collected from the forest and broken down into smaller blocks with masses of 5-10 grams using a knife. At total of 76 blocks were cut. The sodium chloride solutions ranging from 0, 0.5, 1, 2, 4, 7, 10% were made by mixing table salt and tap water. Two hundred and fifty ml were prepared of each solution concentration. The amount of table salt used to prepare the solutions were 25.082g, 17.502g, 10.093g, 5.066g, 2.502g, 1.248g, respectively. The table salt was weighed using a gram scale (American Weigh Scales, Cumming, GA). The blocks were submerged into corresponding concentrations for 10 seconds each with forceps.

Experiment Setup

Experiment was conducted at 8pm at low light levels using only red lights to see. The extremes were done first. Eight colonoids were used to test the extremes. Eight aluminum foil containers (Great Value Walmart) with 6" x 4.9" x 1.77" dimensions were laid out on a table and the control blocks (0%) were placed on the left of each of the containers and the right and the treated block (10%) on the right. The blocks were 1 cm apart from each other and placed on the center of the container. Each container had one colony for it. The colonies were released at same time and left to roam the arena for 10 minutes. At the 10 minute mark the blocks were all removed and the termites counted on each block as well as the remaining individuals on each arena. The blocks used were disposed to prevent any sort of chemical communication as well as the arenas cleaned. For the concentrations 0.5, 1, 2, 4, 7%, the experiment was also done at 8pm and conducted in the same manner. Thirty colonoids were used for each intermediate concentration. The colonoids were reused throughout the experiments. Four blocks of each concentration were used total with corresponding control blocks. A total of 30 trials were completed for the intermediate concentrations. The placement of arenas for both the extremes and intermediates were randomized.

Experiment Two: Cellulose Powder

Food Preparation

Twenty grams of cellulose powder were used for each of the sodium concentration, which were 0% (control), 2%, 4%, 8%. The concentrations were made by mixing 30mL of tap water with 0.396g of salt for 2%, 0.81g for 4%, and 1.596g for 8%. The solutions were mixed with the cellulose powder to create a termite "cake" and then placed in an oven (Lab Genome, Houston, TX) for three hours at 60°C.

Arena Creation

The arenas were created by placing a circular filter paper that its diameter was as long as the width of the terrarium (Carolina Biological, Burlington, NC). A Sharpie pen (Sharpie, Oak Brook, IL) was used to draw a cross on the filter paper to separate the salt cellulose concentrations and labeled correspondingly. A pinch of each concentration was placed on each quarter of the filter paper.

Experiment Setup

Two trials were done starting at 8pm, with six arenas per trial. A total of 12 colonoids were used. These colonoids were also used in experiment one. The placement of arenas and salt concentration on filter papers were randomized. Both trials were run for one hour each at low lighting. Pictures of each arena were taken with an iPhone (Apple, Cupertino, CA) using no flash at 10, 20, 30 minutes and at the hour mark. Red lights were used to view the arenas in low lighting. After trials were complete, all termites in the one-hour picture were counted form each quadrant. The ones outside of the filter paper were not counted.

Data Analysis

The data of both experiments was analyzed using a Kruskal-Wallis test to determine a p-value and detect significant differences among treatments.

Results

The termite sodium preferences in Figure 1 show a slight trend between 2% and 7% concentration sodium,

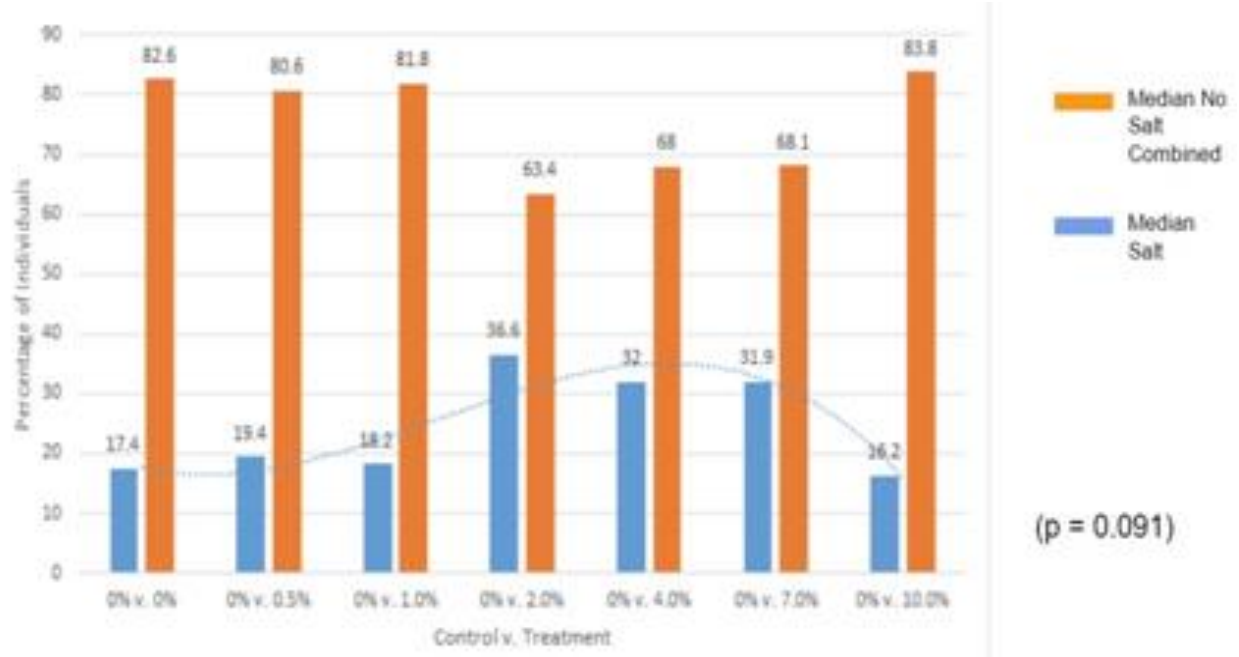

Figure 1: Sodium concentration preference by termites presented with different salt treatments on wood blocks. Preference expressed as percentage of total termites and comparing each concentration with a control

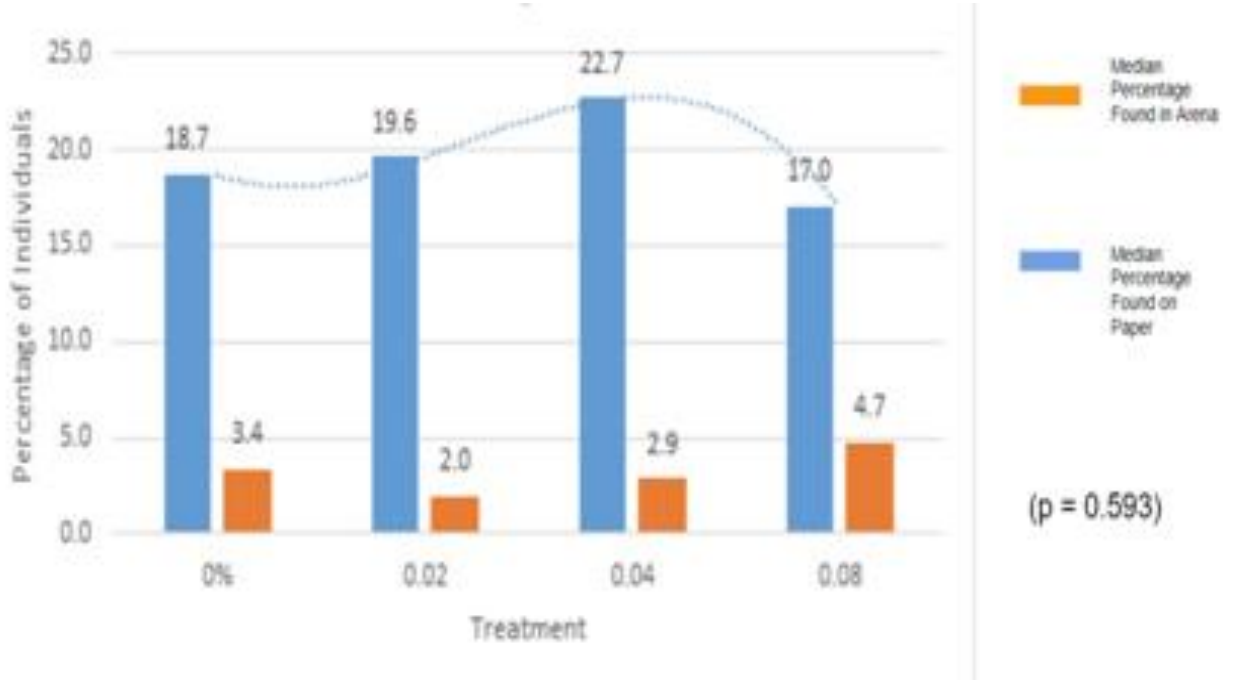

Figure 2: Sodium concentration preference by termites presented with different salt treatments on cellulose powder. Preference expressed as percentage of total termites and comparing each concentration with a control.

with the highest total percent of termites at 2%, followed by 4% and 7% respectively. The p-value calculated was 0.091, meaning there was no significant difference between the sodium concentration choices presented, although there is a slight trend. The termite sodium preferences in Figure 2 for cellulose powder substrate appears to be at 4% sodium concentration. The p-value calculated was 0.593, confirms that the results here are insignificant. However, there appears to be a slight trend favoring 2-4% sodium concentration as well

Discussion

According to Figure 1, the observed differences in sodium concentration preferences by termites are not statistically significant. However, it is close to the significance threshold ($p<0.05$) and there appears to be an increase of termite preference between 2-7% sodium concentration. In Figure 2, the calculated p-value also points at differences not statistically significant, but a trend can also be seen between 2-8% sodium concentration. The observed trend ranges for both experiments overlap, which could indicate a basis for further experimentation. In a similar ant study in the Amazon rainforest (Kaspari et al. 2008), it was determined that as concentration of sodium in the environment decreased when moving further inland, ants became increasingly attracted to baits that had 1% sodium concentration. Even though this study used a concentration range of 0.01-1%, it was clear that the ants preferred higher concentrations when this element becomes scarcer. The trend seen in both figures also suggests that termites have a preference of sodium content in their diet as opposed to having none. It is very probable that the wood blocks used had virtually little to no sodium, since this experiment was conducted far inland. It was also noted that the preference

in both figures appears to decrease at the highest concentration in the experiment, showing that termites might have an aversion to higher concentrations which could make their food non palatable or even toxic for them. Overall, these trends in sodium concentration preference and needs by termites should be further explored in future studies. With more information on termites and other social insects' need for sodium and other minerals, which are critical for sustaining large groups, could be used for lab rearing and changes in soil content. For example, high levels of sodium negatively affect plants, especially those not adapted for coastal soil (Council 2006). However, low sodium contents can decrease the activity of microbial decomposers (Mattson 2012). Some challenges encountered to carry on this experiment was the need of more manpower to effectively count the termites necessary for the colonoids. It is probably that some colonoids had slightly more or less termites than originally planned. Also, the materials available to create and maintain the colonoids were not the most effective. It was noticed that the colonoids would suffer casualties from time to time, either from stress or by having too much humidity inside the Petri dish. However any time there was a casualty observed, it was replaced by another termite of similar caste status. The filter used could also have been eaten by the termites and therefore less reluctant to feed on the tested materials, since the filter was made of cellulose

References

Council, N. R. 2006. Mineral Tolerance of Animals: 2005, National Academies Press.

Da Silva, J. F., and R. J. P. Williams. 2001. The biological chemistry of the elements: the inorganic chemistry of life, Oxford University Press.

Kaspari, M., S. P. Yanoviak, and R. J. P. o. t. N. A. o. S. Dudley. 2008. On the biogeography of salt limitation: a study of ant communities. 105: 17848-17851.

Kaspari, M., N. A. Clay, D. A. Donoso, and S. P. J. E. Yanoviak. 2014. Sodium fertilization increases termites and enhances decomposition in an Amazonian forest. 95: 795-800.

Latysh, N. E., G. A. J. E. m. Wetherbee, and assessment. 2012. Improved mapping of National Atmospheric Deposition Program wet-deposition in complex terrain using PRISM-gridded data sets. 184: 913-928.

Marschner, H. 2011. Marschner's mineral nutrition of higher plants, Academic press.

Mattson, W. J. 2012. The role of arthropods in forest ecosystems, Springer Science & Business Media.

Stallard, R., and J. J. J. o. G. R. O. Edmond. 1981. Geochemistry of the Amazon: 1. Precipitation chemistry and the marine contribution to the dissolved load at the time of peak discharge. 86: 9844-9858.

Tan, K. H. 2005. Soil sampling, preparation, and analysis, CRC press.

Vitousek, P. M., R. L. J. A. r. o. E. Sanford Jr, and Systematics. 1986. Nutrient cycling in moist tropical forest. 17: 137-167.