Effect of Increasing Salinity Levels on the Foraging Behavior of *Orconectes virilis* in a Controlled Environment

Megan E. Lung
Sam Saravolatz
Brian Stark
Greg Hanafin

University of Michigan Biological Station
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Prof. Bob Pillsbury

Abstract.-
The use of road salt during the winter has been known to cause salinity changes in freshwater bodies both near and far from the application site. Increased salinity levels are known to damage plants and animals in various ways. Crayfish rely heavily on their sense of smell for many daily activities, including foraging for food. To test the effect of salinity on crayfish foraging ability, crayfish were placed in tanks of varying salinity. There is a negative relationship between increasing salinity levels and the amount of food consumed by crayfish, suggesting that road salt pollution has the potential to disrupt freshwater ecosystems. To prevent further damage to the environment, the Environmental Protection Agency should regulate the amount of road salt that is applied and its distribution.

Road salts are a popular form of keeping highways and roads free of ice during the winter in the northeastern United States, however the consequences of increasing salinity levels in surface and ground water due to road salts have not been completely explored (Kaushal *et al.* 2005). Increasing baseline summer salinity levels are believed to be caused by an increase in the use of road salts during the winter. Excess chloride ions enter water sources due to runoff and may influence salinity levels far from the highway (Kaushal *et al.* 2005). Chloride concentrations as low as 30mg/liter have been proven to damage terrestrial plants found at roadsides. Freshwater chloride concentrations of 0.25 ppt (parts per thousand) are not fit for human consumption and are able to harm freshwater plants and animals (Kaushal *et al.* 2005). Within the Great Lakes Region, chloride concentrations as high as 10 ppt have been recorded (Transportation Research Board 1991).

Many aquatic organisms are extremely sensitive to changes in water chemistry. Aquatic animals rely largely on chemoreception in locating food, avoiding predators and recognizing mates (Carr *et al.* 1987). Chemoreception is strongly tied with the olfactory system, in order for molecules and pheromones to be detected by an animal contact must be made with the molecule (Mead 2008). Increased chloride concentrations interfere with this ability and cause several other problems for the animal. Chemoreception is especially important in crayfish; they rely on their sense of smell to locate food, mates and habitats. Any interference in this system could result in an animal starving or being killed by a predator or in an encounter with another crayfish (Mead 2008). Crayfish interpret chemical signals both in both the spatial and temporal sense. Based on these signals crayfish adjust their speed, direction and orientation in order to reach food (Moore and Grills 1999).

Freshwater crayfish are able to tolerate many different kinds of environments and water conditions, including some tolerance for salinity (Holdich et al. 1997). Varying salinity levels influence have various consequences on crayfish life and development. Adults and juveniles handle varying salinity concentrations with different levels of success. Young juvenile crayfish are more sensitive to elevated salinity levels than older juveniles and adults and may die when exposed to even moderate salinity levels due to young juveniles having reduced osmoregulatory abilities. Optimal crayfish growth occurs at low salinity levels, possibly due to less energy being expended in osmoregulation (Holdrich et al. 1997). Between salinities of 12-18 ppt the threat and escape responses of *Cherax destructor*, an Australian species of freshwater crayfish, are impaired and death occurs at salinity levels about 25 ppt (Mills and Geddes 1980). *Orconectes rusticus* that are exposed to elevated salinity levels have shorter heart contractions than animals in waters with no salinity (Barkman 1970). Crayfish eggs are also capable of tolerating salinities of up to 14 ppt but will not hatch above 7 ppt (Holdich et al. 1997). Optimal weight gain and growth for most species of freshwater crayfish occurs at 0 ppt (Meade et al. 2002).

Like other fresh water species of crayfish, Orconectes virilis is also capable of tolerating a wide range of habitats and environmental conditions. These crayfish have been found in all kinds of habitats in the Great Lakes Region from lakes to rivers. The adaptive nature of O. virilis makes them an ideal species to study, they are tolerant to various temperatures, oxygen contents and salinity levels (Mead 2008). The effect of salinity on the foraging behavior of O. virilis has never been studied. Like all crayfish, O. virilis locates food by using chemorecptors and increasing levels of salinity should interfere with its ability to successfully forage. We expect that there is a negative relationship between salinity and foraging ability of O. virilis.

Materials and Methods

Orconectes virilis werecolled from Burt Lake, near the University of Michigan Biological Station in Pellston, Michigan. Crayfish were then stored in an articficial stream at the UMBS Lakeside Lab using water from Douglas Lake (salinity 0 ppt) and were later isolated and starved for two-three days before being used in atrial. As salt damages the chemoreceptors of crayfish, an animal was only used in a trial once. Control animals were also released after one trial.

Four tanks were set up containing 40 liters of water from Douglas Lake. The salinity levels of the tanks were as follows: 0 ppt, 2 ppt, 7.5 ppt and 30 ppt. Road salt (NaCl and $CaCl_2$) was dissolved in water in 10 liter increments before being added to the tank. Each tank contained two trials, with a mesh net and plastic divider splitting the tank in half. Each trial contained two shelters made of PVC pipe and a food container made of PVC pipe. A brick was placed between the shelters and the food container to reinforce the role of chemoreception and foraging.

Crayfish were measured for both length and width after being starved before being placed into trial tanks in the evening. Two animals were placed in each trial to deter cannibalism. Animals were given an hour to acclimate to the salinity level of the tank before a food cube was added. Food was constructed of a fish gelatin (see below) molded into a measureable cube that was weighed before being placed in the food container. Trials took place over 12 hours and the remaining food was measured at the end of the trial. Water in each tank was changed weekly after approximately seven trials. Prior to each trial, salinity was measured using a conductivity meter. Although there was slight daily variation, the differences were reliable. Sample tanks at each salinity were set up without crayfish to measure the change in mass of the food cubes during a trial. ANOVA and T-tests were used to compare the amount of food consumed by the crayfish at different salinity levels.

Construction of Fish Gelatin- 28 grams of sardines were blended with 500 mL of warm water and 43.2 grams of unflavored gelatin. This was refrigerated for 24 hours to solidify.

Results

The absorption weight for each salinity level was calculated and applied to the end weight results. These absorption rates are represented in Table 1.

Table 1: Absorption rates based on salinity level.

Salinity Level	Absorption Rate (grams/trial)
Control (0 ppt)	.107942075
2 ppt	.063054807
7.5 ppt	.0600577
30 ppt	.056608

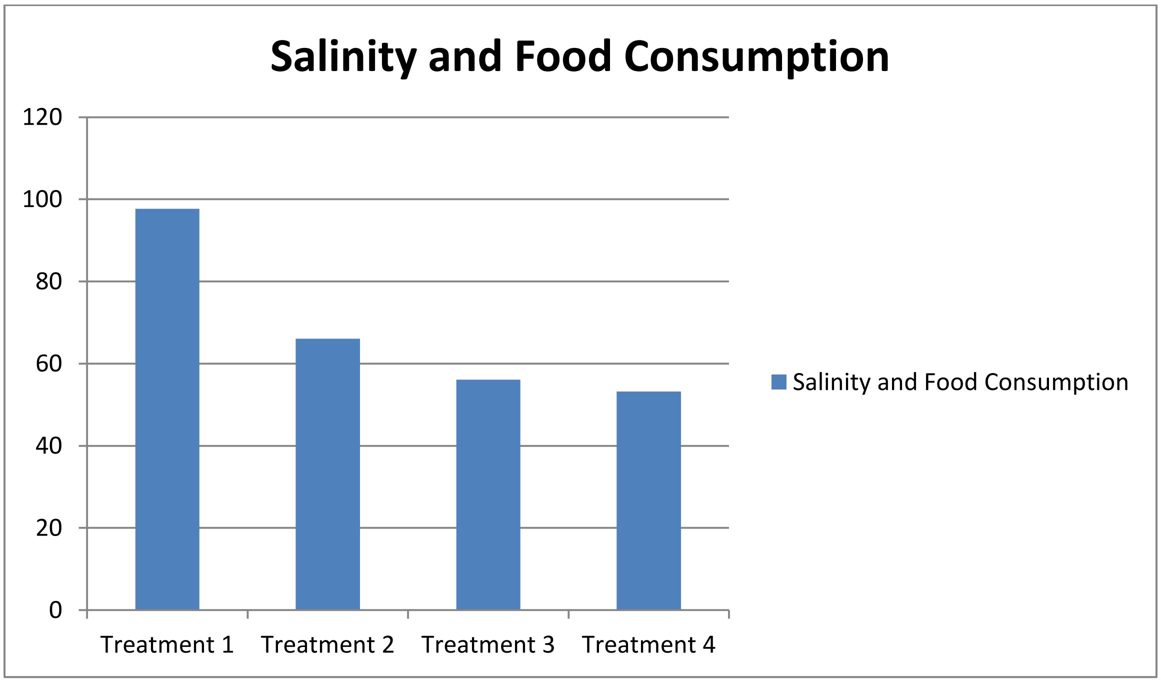
A Oneway ANOVA was used to determine if a relationship existed between salinity and the amount of food consumed by crayfish (Table 2). The ANOVA results were insignificant ($p=.187$) however a negative correlation does exist between salinity and food consumed (Table 3). The total consumption of the various salinity levels were: treatment 1 (control) was 97.66 grams, treatment 2 (2 ppt) was 66.04 grams, treatment 3 (7.5 ppt) 56.09 grams and treatment 4 (30 ppt) 53.18 grams.

Table 2: Results of the Oneway ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.368	3	.123	1.646	.187

Within Groups	5.073	68	.075		
Total	5.441	71			

Table 3: Negative Correlation between Salinity and Food Consumption



A T-tests was then used to compare salinity treatments and treatments with no salt. The T-test proved to be significant (Table 4, p=0.046) which means that the mean consumptions of food are different between salinity and control tanks.

Table 4: T-test results of tanks with salt vs. tanks with no salt

Treatment (Salt vs. NO Salt)	N	Mean	Std. Deviation	Std. Error Mean

V8	1	18	5.42543250556	4.520811923711	1.065565589242
	2	54	3.24642675119	3.740507149538	.509018549756

A second T-test was then performed to determine if the average food consumed by treatments 1 and 2 was significantly different from treatments 3 and 4 (Table 5). These results were insignificant (p=0.157).

Table 5: T-test results of treatments 1 and 2 (low) vs. treatments 3 and 4 (high)

	Treatment (Low vs. High Salt)	N	Mean	Std. Deviation	Std. Error Mean
V6	1	36	4.54703111333	4.459387138039	.743231189673
	2	36	3.03532526622	3.447297441746	.574549573624

Discussion

Although the results of the ANOVA were not significant, we believe that with more repetition the p value would be regarded as statistically significant. More trials would also reinforce the trends we observed based on salinity and food consumption. There was a large drop in the total food consumed by the crayfish between the control and the 2 ppt tank, suggesting that even a small amount of salt has a huge impact on the chemoreceptors of crayfish and their ability to locate food. The fish gelatin cube was not allowed to freely float within the tank and an obstacle placed within the tank ensured that the crayfish would not be able to run into the food based on chance.

The statistical insignificance of the T-test between high salt concentrations and low salt concentrations could either be due to the low number of trials or the absence of a relationship between high and low salt concentrations and food consumption. Trials took place for 12 nights during this experiment and each tank held two trials. An repeat of this experiment that took place over 24 nights instead of 12 would be able to clarify if a there is a difference between high and low salt treatments.

Moore and Grills' (1999) experiment on hydrodynamics and crayfish chemical orientation to food found that crayfish were able to distinguish fish gelatin from plain gelatin in a variety of substrates. Crayfish in this experiment also varied their walking speed and were found to walk faster when the odor of the fish gelatin was present. Crayfish were also found to walk straighter paths when fish gelatin was present. Increasing salinity levels in freshwater bodies may entirely disrupt these abilities of crayfish. Crayfish also relay on chemical reception in mate recognition, given the disturbance that salinity has on feeding capabilities, it is likely that increasing salinity would also disrupt breeding. Eggs of certain species of freshwater crayfish are will not hatch in salinity levels above 7 ppt.

Breeding disruption for crayfish in the Great Lakes Region has far reaching consequences. Crayfish are heavily integrated within the food web of freshwater communities both in the diet of many animals such as raccoons, river otters and coyotes and various species of fish (Kurta 1995) and as keystone predators of many freshwater environments. Crayfish also play an important role as detritivores and the facilitation of nutrient cycling (Mormot 2008).

The use of road salt is currently unregulated in the United States and is available for purchase at local grocery stores for application in driveways and sidewalks. In 2007, 50 million tons of road salt was used in the United States, 37% of that amount was used to de-ice roads (Tenenbaum 2008). Studies conducted in Minneapolis lakes found that road salt application has resulted in a saline layer forming at the bottom of some of the lakes, resulting in interference with oxygen transfer (Tenenbaum 2008). While the use of road salt is necessary to keep roads safe for travel, the Environmental Protection Agency should seek to install some form of regulation on the amount of road salt applied due to the risk of exposing freshwater bodies to salt. Sweden has a similar climate to much of the Great Lakes Region and northeastern United States and has recognized the danger road salt poses to the environment. Roads in Sweden have been repaved and snow plowing blades have been reengineered to make snow removal more efficient and minimize the amount of road salt applied to an area (Johansson 2006). A similar plan of action combined with regulating road salt application would be an excellent way to conserve freshwater environments.

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