Spring 2023

Effects of Nicotine vs Non-nicotine E-cigarettes on the Aggression Behavior of Crayfish

Alex Kimble
ajk199@uakron.edu

Introduction

Smoking cigarettes cause 1 out of 5 deaths in America each year, from cardiovascular disease, cancer, and pulmonary disease (Benowitz M.D., 2010). In addition, smoking is the leading cause of preventable disease (Benowitz M.D., 2010). This led to the marketing of alternatives to smoking cigarettes. E-cigarette use has been drastically increasing over the last decade especially in the middle and high school aged youth (Jebai et al., 2022). Many studies have been done over e-cigarettes. In a study done in 2021 overviewing the effects of e-cigarette use on the physiology of the human body, e-cigarettes were deemed to have increased respiratory compilations and increased cardiovascular risk (Marques, et al., 2021). The study also compared the harmful effects of e-cigarettes and conventional cigarettes, and it was determined that the e-cigarettes cause the same physiological effects to a lesser degree suggesting it is a “safer choice” (Marques, et al., 2021).

Nicotine has a chemical formula $C_{10}H_{14}N_2$ and is classified as a stimulant (DrugBank, 2005). One of nicotine’s functions is acting as an agonist at nicotinic acetylcholine receptors meaning that when nicotine binds to these receptors in the brain positive cations (such as sodium, potassium, and calcium) flow through the channel causing a depolarization at the membrane of the cell (DrugBank, 2005). This leads to more calcium ion channels opening allowing more calcium into the cell. One role calcium plays in the cell involves stimulating vesicle trafficking towards the cell membrane releasing dopamine into the synaptic cleft (DrugBank, 2005). Dopamine release has been linked to causing nicotine to be so addictive as dopamine allows someone to feel pleasure, satisfaction, and motivation (HealthDirect, 2021).

Nicotine’s effect on behavior has contradicting results in different animal models (Picciotto et al., 2015). Picciotto describes three previous studies that use different model organisms including cats, mice and rats. The results from the three different studies were different for each species. The study involving cats examined nicotine’s effect on aggression

behavior of biting mice. The study found that a higher administered dose of nicotine causes a significant decrease in the behavior involving biting mice (Picciotto et al., 2015). Instead, the cats who received the high nicotine dose were more likely to paw at the mice than they were to bite them (Picciotto et al., 2015). The findings in the cat study differ with a later foot-shock aggression study with mice where increased dosage of nicotine led to increased aggression (Picciotto et al., 2015). In an article outlining some factors that affect aggressive behavior in the model organism crayfish, the author, Richard Bovbjerg, directly speaks on the role of size differences and the role of sex on aggressive behavior. Bovbjerg's findings on the role of size differences indicate that dominance order in crayfish is directly related to size (Bovbjerg, 1956). The results on the role of sex as described by Bovbjerg indicated that males are significantly more aggressive when compared to females (Bovbjerg, 1956). These results are vital for the design and development of the research project when determining which sex of crayfish to use and whether or not it is vital to use both sexes, and if one is particularly better for the experiment than the other. Sizing is specifically important as it is less desirable to have crayfish that significantly differ in mass as it skews the results. The claw size and number also fit into the discussion of size differences as they can dramatically affect the results as well. This is the case because when a crayfish is missing a claw or if one of their claws is deformed (tiny), it is similar to humans missing a hand. They are disadvantaged when undergoing hand to hand combat against other crayfish. Crayfish claws are the weapon of choice as the most aggressive actions labeled in **Table 1** involve using claws. Therefore, having a deformed claw or not having a second claw at all really gives the opponent the upper hand and is why crayfish with a deformed or missing claw will not be used.

The purpose of our study is to produce data that will further clarify how nicotine affects aggression behavior in order to determine whether nicotine significantly affects aggression levels of crayfish. The benefits of this study are determining whether or not e-cigarettes have an

effect on aggressive behavior: it is important to gain more knowledge as to whether the substitution of e-cigarettes is safe at all. This study can give a general idea on whether or not e-cigarettes can affect behavior. In addition, more data will allow a determination whether addiction should be our only concern when it comes to e-cigarettes.

The goal of this research project will be to identify how and/or if e-cigarette use causes changes in behavior, specifically aggression. For this study, nicotine vs nicotine free e-cigarettes are the independent variables. The model organism is the male crustacean crayfish (*Procambarus clarkii*). Crayfish are being used for this study in particular because they are non-social organisms who are very territorial when placed in an environment with other crayfish. The research question would then be, if crayfish of similar size and gender are in different habitats containing nicotine or a nicotine-free environment, then will the crayfish exposed to nicotine have a significantly higher level of aggression in comparison to the crayfish exposed to the nicotine-free environment.

Materials and Methods

For this experiment, it was critical for the crayfish to be paired with opponents of similar size and sex. This study originally consisted of 30 male crayfish randomly assigned into three different treatment groups (control, non-nicotine, and nicotine) each containing ten crayfish. **Appendix 1, 2, and 3** include all the mass (g), sex, and description of appendage condition of the crayfish in the three treatment groups; control, non-nicotine, and nicotine. The control treatment group had crayfish that were not exposed to the e-cigarette vapor. Instead, the crayfish were in a regular dechlorinated water habitat for twenty-four hours with no e-cigarette vapor infused. The non-nicotine treatment group consisted of crayfish that were exposed to e-cigarette vapor however, that e-cigarette vapor did not contain nicotine. A vape juice without nicotine was used to create the vapor for the non-nicotine treatment group. The process as to how the e-cigarette vapor was infused into the dechlorinated water can be seen in **Figure 1**.

The crayfish in both non-nicotine and nicotine treatment groups were exposed to the e-cigarette vapor for twenty-four hours following the protocol from a previous study that also infused e-cigarette vapor into the water (Piechowski et al., 2021). The nicotine treatment group was similar to the non-nicotine treatment group, however, the vape juice used contained nicotine. The infusion of the e-cigarette vapor was done the same as the non-nicotine treatment group as seen in **Figure 1**. **Appendix 4** showed the crayfish assigned to each treatment group that were participants in the study. The purpose of this table was to make it easier to pair the crayfish together, finding opponents of relative size for the trials. Note the crayfish identification numbers that are red as those that are the same crayfish but were used twice in the experiment. The crayfish in the table are organized from largest mass (g) to smallest mass to express how the crayfish were paired together. This process differs from the random assignment of the crayfish into treatment groups as the pairings needed to be between crayfish of relative size (mass), sex, and use of appendages. Therefore, the crayfish were non-randomly assigned to the pairings. **Tables 2, 3, and 4** contain the final pairings of crayfish that were used.

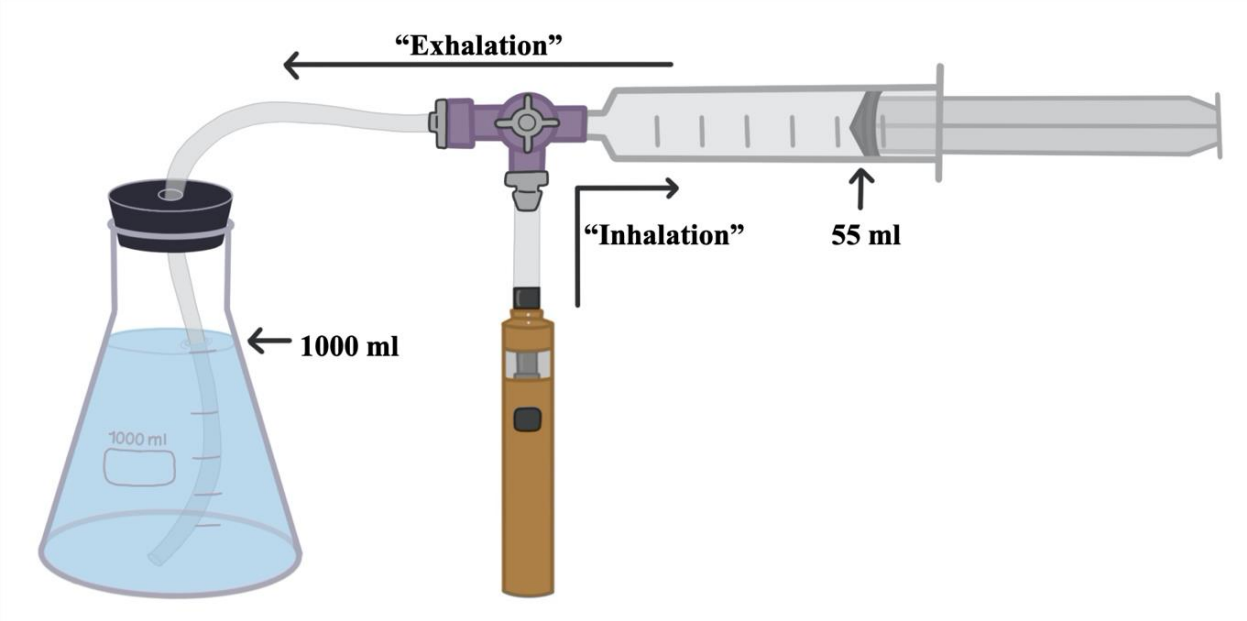

Figure 1).- This figure shows the general setup for infusing the e-cigarette vapor into the water. The setup was constant for both non-nicotine and nicotine groups. (Grismer et al., 2021).

Table 1 describes the actions of aggression the crayfish exhibited and the point score that each action was awarded (Mead, 2008). For example, during the matchup a crayfish turned from the other crayfish and quickly retreated to the opposite end of the enclosure, then the crayfish was given a -2 score. Since there are two crayfish who fought against each other, both crayfish were scored simultaneously while watching the recording of their interactions. All fifteen matchups were recorded. The scoring process occurred while watching a slow-motion replay of the recorded interaction between the two crayfish. There are two types of aggression; offensive and defensive. (Picciotto et al., 2015). Therefore, if crayfish number two attacked crayfish number one with unrestrained fighting, crayfish number two would be awarded a +5 score. However, if crayfish number one returned that same amount of aggression towards crayfish number two in a defensive manner, crayfish number one would also be given a +5 score for defending itself. **Table 1** was used as a standard to keep scoring the same across all 15 matchups. A photograph of all eight aggressive actions with their score can be seen in **Figure 2**.

Table 1).- This table is important in scoring the interactions between the crayfish. Using this standard table helps standardize the scoring process (Mead, 2008).

Points	**Actions**
-2	Fast retreat
-1	Turns away slowly backs away from the opponent
0	No observed response to the opponent
1	Approaches the other crayfish without threat display
2	Approaches the other crayfish with a threat display (meral spread or antenna whip)
3	Boxing, pushing, or touching with closed claws
4	Active claw use with open claw
5	Unrestrained fighting

a) b) c) d)

e) f) g) h)

Figure 2).- Above is a cluster of images depicting scorable actions from **Table 1**. **Figure 2a** starts in the top left and goes from left to right where you will find **Figure 2h** in the bottom right corner. **Figure 2a** depicts a -2 point action and **Figure 2h** depicts the +5 point action both correlating to **Table 1** aggression behavior actions (Mead, 2008).

There were three different categorical matchups that were tested: Control versus Nicotine, Control versus Non-Nicotine, and Non-Nicotine versus Nicotine. Within each categorical matchup there were five trials. **Tables 2, 3, and 4** include the total scores that each crayfish were awarded at the end of their trial. The average score of the treatment group was calculated for each categorical matchup. A T-test assuming unequal variance was the statistical analysis used. A p-value below 0.05 indicates a significant difference in the groups tested. The score differential was helpful in checking for outliers in the data. The mass differential is key to keeping the experiments consistency between categorical matchups. This plays an important role in the selection process for matchups of crayfish.

The treatment groups were the following: control, nicotine, and non-nicotine. The mean masses of the treatment groups were the following: control: 21.17 g (+/- 1.61 g), nicotine 22.80 g (+/- 2.34 g), and non-nicotine 23.50 g (+/- 1.86 g). There were three crayfish that were used in two interactions and are specified in (**Appendix 4**). The control treatment group exposed the crayfish to regular dechlorinated water. The nicotine and non-nicotine treatment groups each add e-cigarette vapor that was infused into the water. **Figure 1** depicts an image of how the vapor was infused into the water. The vape, a Joyetech eGo AIO, was filled with Strawberry Pom flavored juice by the brand Naked. The vape was held in the on position for four seconds as the syringe was pulled back to the 55 mL mark. After four seconds, the stop switch was flipped, the syringe expelled the vapor gathered and pushed it into the 1000 mL Erlenmeyer flask through the tubing. The tubing was then pulled out and the flask was plugged and shook to infuse the vapor into the water. This process was repeated eighteen times per 1000 mL of water. The only difference between the nicotine and the non-nicotine treatment groups was the

juice used. The flavor was consistent; however, the nicotine's juice contains nicotine as an active ingredient whereas the nicotine is absent in the non-nicotine group.

The experiment consisted of introducing a pairing of crayfish into an enclosure where animal interaction occurred. Since crayfish are territorial animals this interaction was physical. The pairs can be seen in **Tables 2, 3, and 4**. The two crayfish were introduced into this enclosure with each other for a total of ten minutes and after the ten minutes the crayfish were separated. The enclosure was filled with regular dechlorinated water and placed in a Styrofoam box so that the observer's presence would not influence animal behavior. The crayfish's aggression behavior was recorded and scored using **Table 1** at a later date. The scores of the physical interactions are found in **Tables 6, 7, and 8**.

Results

The statistical analysis showed that the aggression behavior scores was not significantly different between the control, non-nicotine, and nicotine treatment groups. **Tables 2, 3, and 4** show the difference in the mean scores between the two treatment groups being compared in the respective table along with the p-value from the t-test. **Figures 3, 4, and 5** emphasize the information held within **Tables 2, 3, and 4**. They also highlight the points scored in their respective pairings to visualize how the crayfish in each treatment group performed in respect to the other treatment groups.

Table 2 shows an average score difference of 14 between the control and nicotine groups with a p-value of 0.26. The control group had an average score of 16.6 points and the nicotine group had a score of 30.6 points. The importance of these two numbers indicate that the nicotine group over the five trials exhibited a more aggressive behavior than the control group. This was the case in each of the five trials. However, the aggression behavior did not differ by a lot in some trials. This led to a decreased average score differential between the two treatment groups. Meaning the two opposing crayfish exhibited similar aggression levels rather

than one crayfish asserting its dominance over the other. The statistical analysis produced a p-value of 0.26 allowing a determination that the aggression behavior between the two groups is not statistically significant. It is important to note the average mass difference between the opponents of crayfish was less than a gram. This means that all the opponents were similar in size which was a key for the experiment because it indicates there is a level playing field and allows a deeper investigation into the factors affecting the study.

Table 3 shows an average score difference of 24.80 with a p-value of 0.16 between the control and non-nicotine treatment groups. The control group had an average score of 43.4 points. The Non-nicotine group had an average score of 28.6 points. The control group had a higher score than the non-nicotine group in four out of five trials. This means that the control group exhibited a higher aggression behavior than the non-nicotine group in almost every trial. The score difference shows a marker of how much more aggressive the control group was. It is higher than the preceding two treatment groups that were previously discussed; meaning, a greater difference in aggression was observed. The p-value is lower and closer to the 0.05 range than the previous p-value. However, at 0.16 it is still too high for the aggression behavior between the two treatment groups to be statistically significant. The mass differential is to see if the increased aggression behavior in the control group is related to a size difference. Furthermore, the mass difference is less than a gram and indicates a level playing field that is not giving one treatment group an advantage over the other.

Table 4 shows an average score difference of 15.20 and a p-value of 0.27 between the nicotine and non-nicotine treatment groups. Nicotine's average score was 29.6 points and non-nicotine's average score was 39.2 points. Based off of these scores alone, the scores contradict what would be predicted based on the previous trials of nicotine versus control and control versus non-nicotine alone. Non-nicotine treatment group showed more aggression than the nicotine group over three of the five trials and the average score overall. The average score

differential was brought down due to close trials with opponents that exhibited similar aggression behavior. Two trials had a score differential of 6 or less. The p-value of 0.27 is still very high and shows the aggression behavior between the treatment groups is not statistically significant. The mass differential was slightly over a gram with a difference of 1.12. This is still a very close reading and indicates a level playing field.

Table 2).- This table includes the matchups between crayfish and also their respective scores, average score per treatment group, score differential, mass differential, and finally T-test p-value. The p-value is 0.26 showing statistically non-significant data.

	Matchups						
	Control	**Score**	**Versus**	**Nicotine**	**Scores**	**Score Differential**	**Mass Differential (g)**
Crayfish Identification Number	6	43		6	45	2	2.17
	1	5		14	33	28	1.21
	12	31		7	49	18	0.04
	9	3		4	17	14	0.21
	5	1		11	9	8	0.86
Average Score		16.6			30.6		
					Average Score Differential	14	
					Standard Error	4.43	
T-test P-value two-tail	0.26					Average Mass Differential	0.90
						Standard Error	0.38

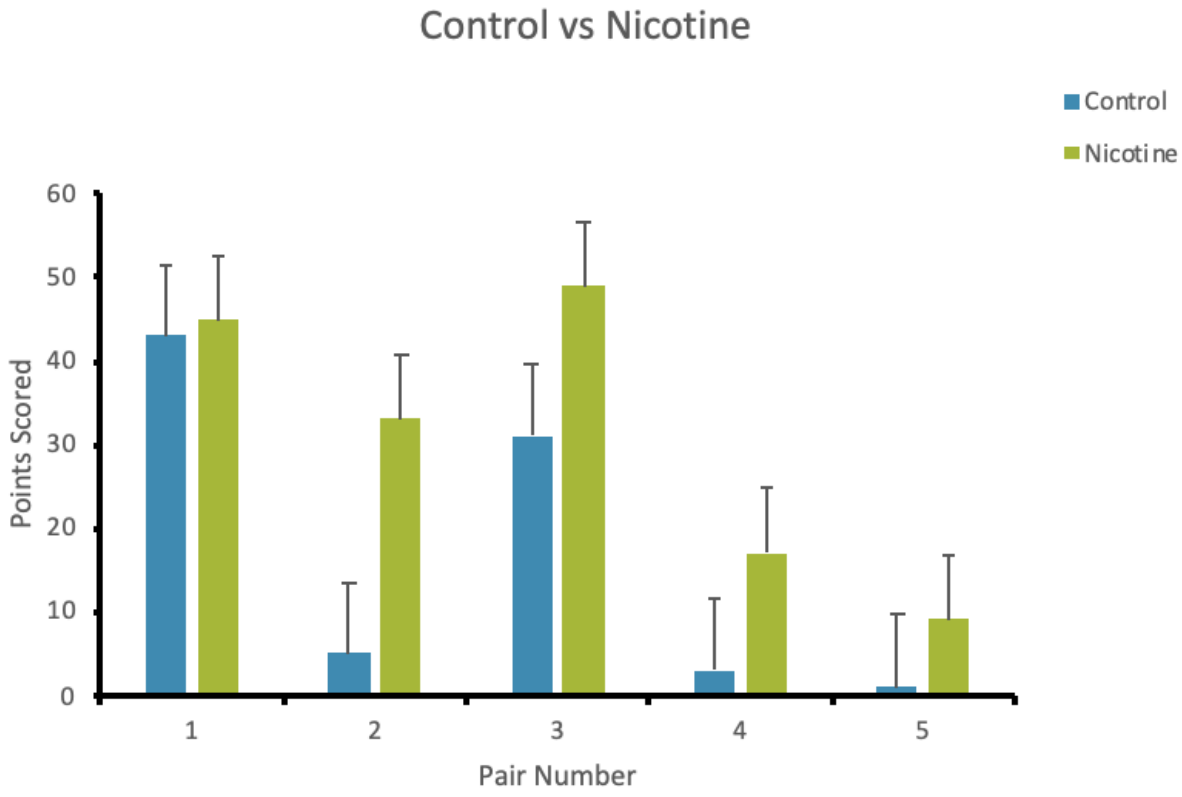

Figure 3 shows that the nicotine group routinely outperformed the control group. The average score of the control group was 16.6. The average score of the nicotine group was 30.6 leaving an average score difference of 14 (+/- 4.43). The average mass difference was 0.90 grams (+/- 0.38). The p-value was 0.26 showing statistically non-significant data.

Table 3).- This table includes the matchups between crayfish and also their respective scores, average score per treatment group, score differential, mass differential, and finally T-test p-value. The p-value 0.16 showing statistically non-significant data.

	Matchups						
	Control	**Score**	**Versus**	**Non-Nicotine**	**Scores**	**Score Differential**	**Mass Differential (g)**
Crayfish Identification Number	14	35		9	6	29	1.02
	8	28		8	53	25	0.08
	7	45		1	21	24	1.68
	9	54		14	25	29	0.54
	14	55		12	38	17	1.26
Average Score		43.4			28.6		
					Average Score Differential	24.80	
					Standard Error	2.20	
T-test P-value two-tail	0.16					Average Mass Differential	0.92
						Standard Error	0.28

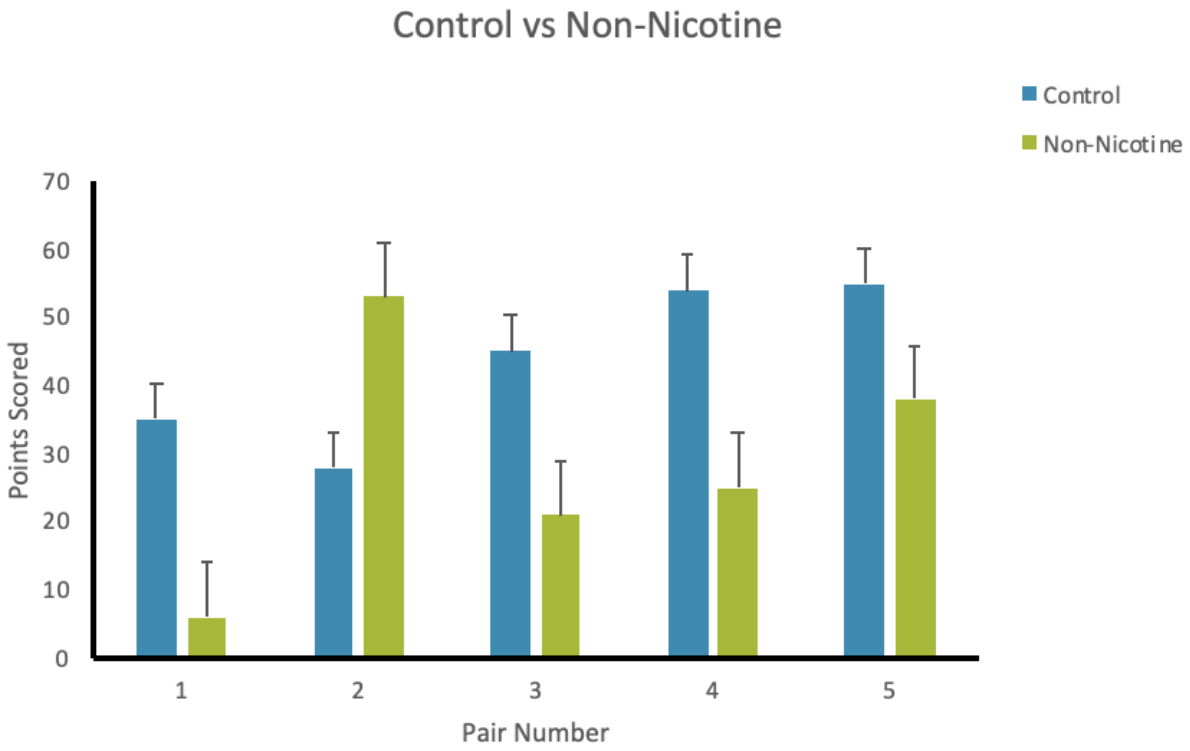

Figure 4 shows that the control group routinely outperformed the non-nicotine group, however, in pair number two non-nicotine outperformed the control. The average score of the control group was 43.4. The average score of the non-nicotine group was 28.6 leaving an average score difference of 24.80(+/- 2.20). The average mass difference was 0.92 grams (+/- 0.28). The p-value was 0.16 showing statistically non-significant data.

Table 4).- This table includes the matchups between crayfish and also their respective scores, average score per treatment group, score differential, mass differential, and finally T-test p-value. The p-value 0.27 showing statistically non-significant data.

	Matchups						
	Nicotine	**Score**	**Versus**	**Non-Nicotine**	**Scores**	**Score Differential**	**Mass Differential (g)**
Crayfish Identification Number	9	17		2	23	6	0.55
	14	38		6	35	3	1.41
	8	17		5	49	32	0.91
	12	32		10	56	24	0.41
	13	44		7	33	11	2.30
Average Score		29.6			39.2		
					Average Score Differential	15.2	
					Standard Error	5.53	
T-test P-value two-tail	0.27					Average Mass Differential	1.12
						Standard Error	0.34

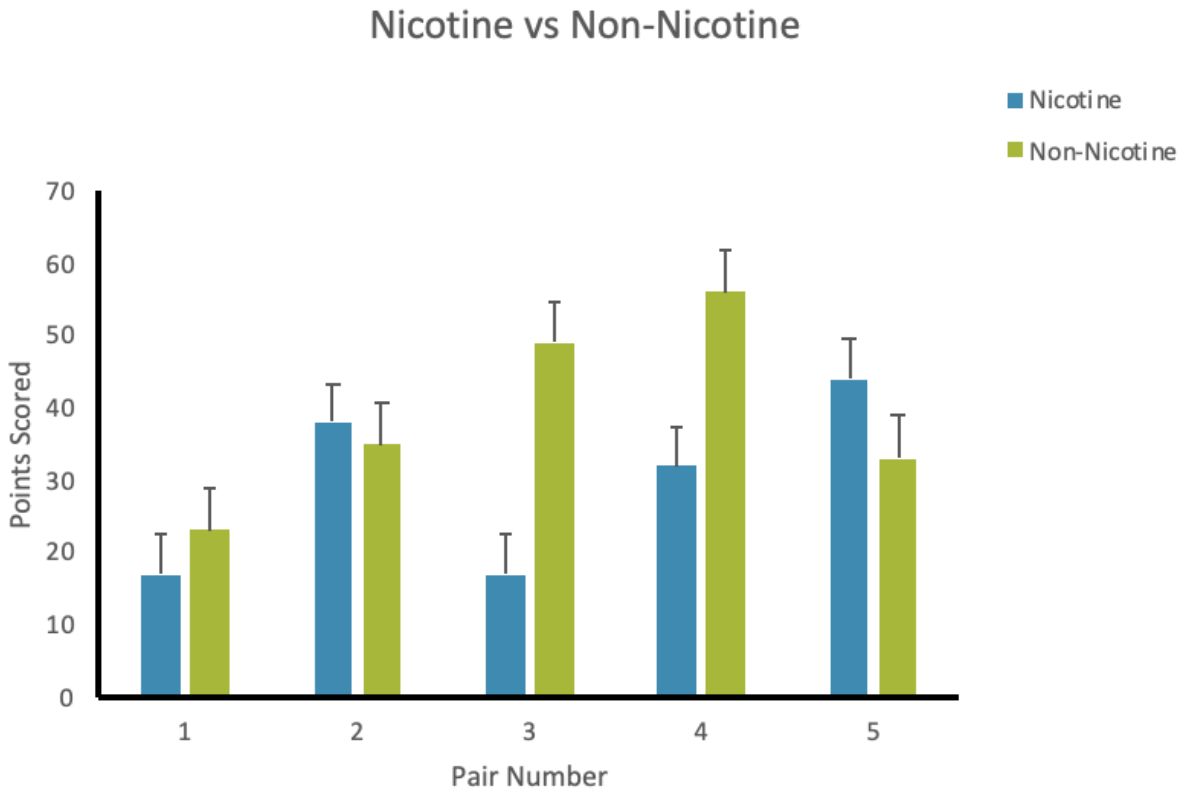

Figure 5 shows that the control group mainly outperformed the non-nicotine group. The average score of the control group was 43.4. The average score of the non-nicotine group was 28.6 leaving an average score difference of 24.80(+/- 2.20). The average mass difference was 0.92 grams (+/- 0.28). The p-value was 0.16 showing statistically non-significant data.

Discussion

The first step to understanding this experiment is to understand how the crayfish breathe. Crayfish use their gills to breathe dissolved oxygen within the water (Voshell Jr., 2002). This is the reason why it is important to infuse the e-cigarette vapor into the water because the vapor would not get into the respiratory system of the crayfish otherwise. Therefore, infusing the vapor into the water allowed the nicotine to be absorbed by the crayfish and then nicotine's effect on behavior can be observed. This study was aimed at discovering whether or not nicotine or non-nicotine e-cigarette vapor affects aggression behavior of crayfish.

Overall in the experiment, there was no statistically significant difference in the aggression behavior between the three treatment groups (control, non-nicotine, nicotine). In all

cases the p-value was greater than the alpha value of 0.05 indicating a non-significant difference. The reason that the p-value indicated a non-significant difference could be attributed to a few reasons. For example, the sample size might not be large enough, and the small sample size might have affected the statistical test. Also, the exposure time could have been insignificant and not long enough for the nicotine and non-nicotine to exert their effects. Potentially, a longer more chronic exposure to nicotine and non-nicotine e-cigarette vapor could have made a bigger difference on the aggressiveness of the crayfish.

Another plausible explanation is that nicotine does not increase aggressive behavior. One study from 1976 looked at effects of a pretreatment of nicotine on the aggressive behavior of cats biting/displaying other aggressive behaviors toward dead mice (Berntson et al., 1976). The study took 10 cats that previously did not spontaneously attack the dead mouse and ran different tests. One test gave the cats muscarinic compounds and observed the cat's aggressive behavior. Every time the cat demonstrated very aggressive behavior towards the dead mouse. However, when the cats were pretreated with nicotine in a separate test, this aggressive behavior went away. The study suggests that nicotine suppresses the aggressive display (Berntson et al., 1976). According to another study done in 2015 investigating nicotine's effect on aggression in three different mouse strains, nicotine produced a serenic (anti-aggressive) effect in three different mouse strains using social isolation induced aggression and resident-intruder assays (Lewis et al., 2015). The 1976 and 2015 studies along with the results we found seem to suggest that nicotine may in fact be a serenic agent.

There are a few things that I would do differently to try and expand on this topic and to be certain that e-cigarette vapor including nicotine and non-nicotine products does not influence the aggression behavior of crayfish. The first thing is ordering fresh crayfish that are in better shape than the crayfish that were obtained from the freshman biology class. Truthfully, I do not know how big of a difference it made, or if it made a difference at all but it is a factor that I would

try differently next time. Additionally, I would increase the sample size for all three treatment groups including an experiment that is testing both males and females not necessarily between the sexes but between males and females independently. There certainly may be a difference between the two sexes but due to the lack of a number of females they were excluded from the study. Another thing that I would do differently is increase the exposure time the crayfish had to the nicotine and the non-nicotine vapor chemicals. This time they were tested for 24 hours and next time I would do different lengths of time including a 48-hour, 96-hour, and even 168-hour exposure times. Nicotine or non-nicotine might take a longer time to exert its effect on the crayfish than what was given, and it is possible that giving the crayfish a longer exposure time to the chemicals could affect the results. Finally, I would have two to three judges judging the crayfishes' behavior and scoring them and the scores would then be averaged. This would weed out any subconscious biases that may be exacerbated with only having one-person score.

Appendix

Appendix 1).- This table depicts the mass, sex, and condition of appendages of the crayfish for the Control treatment group.

Treatment Group	Sex	Mass (g)	Appendage Condition
Control 1	Male	28.66	2 Claws
Control 2	Female	18.89	2 Claws
Control 3	Male	23.06	2 Claws
Control 4	Female	27.27	2 Claws
Control 5	Male	28.06	2 Claws
Control 6	Male	14.73	2 Claws
Control 7	Male	18.42	2 Claws
Control 8	Male	20.92	2 Claws
Control 9	Male	16.96	2 Claws
Control 10	Female	23.02	1 Claw and 1 Deformed Claw
Control 11	Male	16.67	1 Claw and 1 Deformed Claw
Control 12	Male	17.13	2 Claws
Control 13	Male	26.94	1 Claw and 1 Deformed Claw
Control 14	Male	24.94	2 Claws

Appendix 2).- This table depicts the mass, sex, and condition of appendages of the crayfish for the Nicotine treatment group.

Treatment Group	**Sex**	**Mass (g)**	**Appendage Condition**
Nicotine 1	Male	41.60	2 Claws
Nicotine 2	Female	24.41	2 Claws
Nicotine 3	Female	28.52	2 Claws
Nicotine 4	Male	16.75	2 Claws
Nicotine 5	Male	30.08	1 Claw and 1 Deformed
Nicotine 6	Male	12.56	2 Claws
Nicotine 7	Male	17.09	2 Claws
Nicotine 8	Male	26.11	2 Claws
Nicotine 9	Male	37.04	2 Claws
Nicotine 10	Female	26.14	2 Claws
Nicotine 11	Male	27.20	2 Claws
Nicotine 12	Male	18.43	2 Claws
Nicotine 13	Male	17.87	2 Claws
Nicotine 14	Male	27.45	2 Claws

Appendix 3).- This table depicts the mass, sex, and condition of appendages of the crayfish for the Non-nicotine treatment group.

Treatment Group	**Sex**	**Mass (g)**	**Appendage Condition**
Non-Nicotine 1	Male	20.10	2 Claws
Non-Nicotine 2	Male	37.59	2 Claws
Non-Nicotine 3	Female	33.30	2 Claws
Non-Nicotine 4	Female	19.61	1 Claw and 1 Deformed Claw
Non-Nicotine 5	Male	27.02	2 Claws
Non-Nicotine 6	Male	26.04	2 Claws
Non-Nicotine 7	Male	20.17	2 Claws
Non-Nicotine 8	Male	21.00	2 Claws
Non-Nicotine 9	Male	23.92	2 Claws
Non-Nicotine 10	Male	18.02	2 Claws
Non-Nicotine 11	Female	26.34	2 Claws
Non-Nicotine 12	Male	23.68	2 Claws
Non-Nicotine 13	Male	12.49	2 Claws
Non-Nicotine 14	Male	17.50	2 Claws

Appendix 4).- This table represents the assortment of crayfish in the three treatment groups organized from largest to smallest mass (g). This table made it easier to pair the crayfish with their opponents in the three categorical matchups.

	Control	**Crayfish Identification Number**	**Nicotine**	**Crayfish Identification Number**	**Non-Nicotine**	**Crayfish Identification Number**
	28.66 g	1	37.04 g	9	37.59 g	2
	28.06 g	5	27.45 g	14	27.02 g	5
	24.94 g	14	27.45 g	14	26.04 g	6
	24.94 g	14	27.2 g	11	23.92 g	9
	20.92 g	8	26.11 g	8	23.68 g	12
	18.42 g	7	18.43 g	12	21.00 g	8
	17.13 g	12	17.87 g	13	20.17 g	7
	16.96 g	9	17.09 g	7	20.10 g	1
	16.96 g	9	16.75 g	4	18.02 g	10
	14.73 g	6	12.56 g	6	17.50 g	14
Mean	21.17 g		22.80 g		23.50 g	
Standard Error	1.61 g	2 Crayfish used twice	2.34 g		1.86 g	

References

Benowitz, N. L. “Nicotine Addiction” 2010. N Engl J Med. https://www.nejm.org/doi/full/10.1056/nejmra0809890?casa_token=YmQdmdMFFLcAAAAA:rJRA4WauvYcMr67n9k_ut3y6ITV0dDWR_VKFVjeC8-PcBshGaeQ0uwpCGBP7XG3TFY0CbWn7HdOqA

Berntson, G., M. S. Beattie, J. Micheal Walker. “Effects of Nicotinic and Muscarinic Compounds on Biting Attack In the Cat”. September 1976. Pharm. Biochem. & Behav. Vol. 5 Iss. 3 pp. 235-239. https://www.sciencedirect.com/science/article/pii/0091305776900721

Bovbjerg, R. V., “Some Factors Affecting Aggressive Behavior in Crayfish” (April 1956). University of Chicago Press. Vol. 29 No. 2 pp. 127-136. https://www.jstor.org/stable/pdf/30152201.pdf?casa_token=HJjRqErzVwgAAAAA:Y8ZBW3nSKWtQWilJHPZvyyOqIffK6P5GfhB8CSBFZz7rs6cfw1zPxJsr5x3Kw2D-sk0gJSg7xw0EEJp8D442Ps4890yOx0gvSzCs7u4_kWxO-eEW_UE

Drug Bank, “Nicotine” (2005). Drug Bank Online. https://go.drugbank.com/drugs/DB00184

Fero, K. and Moore, P. A., “Social Spacing of Crayfish in Natural Habitats: What Role Does Dominance Play?” 08 January 2008. Behavioral Ecology and Sociobiology 62: 1119-1125. https://link.springer.com/article/10.1007/s00265-007-0540-x

Figler, M. H., Cheverton, H. M., G. S. Blank. “Shelter competition in juvenile red swamp crayfish (*Procambarus clarkii*): the influences of sex differences, relative size, and prior residence” (15 July 1999). Vol. 178 Iss. 1-2 pp. 63-75. https://www.sciencedirect.com/science/article/abs/pii/S0044848699001143

Grismer, Alyssa A.; Pumneo, Jenna E.; and Bagatto, Brian, "Effects of E-cigarette Vapor on Zebrafish Cell Regeneration and Wound Healing" (2021). *Williams Honors College, Honors Research Projects*. 1397. https://ideaexchange.uakron.edu/honors_research_projects/1397

Health Direct. “Dopamine” April 2021. https://www.healthdirect.gov.au/dopamine#:~:text=of%20the%20brain.-,What%20is%20the%20role%20of%20dopamine%3F,of%20opamine%20in%20the%20brain.

Lewis, A. S., Y. S. Mineur, P. H. Smith, E. Cahuzac, M. R. Picciotto. “Modulation of Aggressive Behavior in Mice by Nicotinic Receptor Subtypes”. 2015. Biochem. Pharm. Vol. 97 pp. 488-497. https://reader.elsevier.com/reader/sd/pii/S0006295215003895?token=EAF45F5035BCD40398EED24348A1CDCE7077EC4E4CD96C7599FAE268B0614E4340F13DABC916945C1F416E055E947FA3&originRegion=us-east-1&originCreation=20230414002102

Maine Department of Environmental Protection. “Crayfish and Shrimps (Decapoda)” 2023. Maine.gov. https://www.maine.gov/dep/water/monitoring/biomonitoring/sampling/bugs/crayfishandshrimps.html#:~:text=Both%20crayfish%20and%20shrimps20breathe,gills%20located%20beneath%20their%20carapace.

Marques, P., L. Piqueras, and M.-J. Sanz. “An Updated Overview of E-Cigarette Impact on Human Health”. 18 May 2021. Respiratory Research. https://respiratory-research.biomedcentral.com/articles/10.1186/s12931-021-01737-5.

Mead, K. S. “Crayfish Aggression and the Androgenic Gland in a Behavior Lab for Non-Majors” 15 June 2008. J. Undergrad. Neurosci. Educ. Vol. 6. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3592662/

Picciotto, M. R., Lewis, A. S., van Schalkwyk, G. I., Y. S. Mineur. “Mood and anxiety regulation by nicotinic acetylcholine receptors: A potential pathway to modulate aggression and related behavioral states” (25 June 2015). Neuropharmacology. Vol. 96 pp. 235-243. https://www.sciencedirect.com/science/article/pii/S0028390814004791?casa_token=c-HfpShZqsAAAAA:KNBdrZ4CrG6V9AM2PhLTteb3LWWAY0FsjGkVFgU48ezxZHSdklqOt_yNGHzKsuN-wKhGu94sg

Piechowski, Jennifer & Bagatto, Brian. (2021). Cardiovascular function during early development is suppressed by cinnamon flavored, nicotine-free, electronic cigarette vapor. Birth Defects Research. 113. 10.1002/bdr2.1951.

Rime, J., O. Osibogun, W. Li, P. Gautam, Z. Bursac, K.D. Ward, and Maziak, Wasim. “Temporal Trends in Tobacco Product Use Among US Middle and High School Students: National Youth Tobacco Survey, 2011-2020” (2022). Public Health Reports. https://doi.org/10.1177/00333549221103812