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Abstract

This study aims to examine the relationship between e-cigarettes (both with nicotine and without) and the effects on the standard metabolic rates of crayfish. In this experiment, 42 crayfish were divided into three treatment groups and exposed to a designated solution of either a control solution or a solution containing e-cigarette (nicotine or non-nicotine) vapor. Their standard metabolic rates were then measured by using oxygen uptake as a proxy. The results of this experiment indicate that there is no statistically significant relationship between the use of electronic cigarettes and the standard metabolic rate of crayfish. However, this study can be adjusted slightly to improve results, which would be meaningful in the growing research on e-cigarettes and their overall effects. The sample size was too small to warrant any meaningful results, the measurements were not direct measurements of standard metabolic rate, and there were too many extra variables that were not accounted for. It was unknown whether or not the environment became hypoxic during the trials. This could have been alleviated by implementing an intermittent-flow respirometry system (Svendsen et al., 2016) to constantly oxygenate the water during the experimental trials.

Introduction

The purpose of the experiment was to examine the relationship between electronic cigarette vapor (nicotine & non-nicotine) and the standard metabolic rate of *Procambarus clarkii* (crayfish). Standard metabolic rate is a quantified value that represents how efficiently an ectotherm (at rest) uses energy stores to carry out bodily functions that are vital for survival. To

quantify the efficiency of an ectothermic organism's energy usage at rest (standard metabolic rate), a multitude of factors are examined to deliver a value with the unit "calories". Since crayfish are ectotherms and do not have the capacity to internally regulate their body temperature, their metabolic rate is dependent on the temperature of the environment. Temperature must be indexed throughout the experiment because the standard metabolic rate of ectotherms increases as temperatures increase (Auer et al., 2018).

Metabolic rate studies have obvious importance in nature as animals with lower metabolic rates (per unit mass) can survive longer without food than their counterparts with higher metabolic rates (per unit mass). This is because animals with lower metabolic rates require fewer calories to maintain successful bodily functions, like breathing, heart rate, etc. in contrast with animals with higher metabolic rates ("Metabolism: What It Is, How It Works and Disorders," n.d.). Metabolic Rate studies are also of keen interest in humans because metabolic rate is a proxy for weight loss, gain, and management. However, there is a lack of research targeting a potential link between metabolic rate and e-cigarette smoking, due to the recent arrival of the artificial smoking devices.

Most studies are centered around the harmful causes of nicotine-containing e-cigarettes due to the highly addictive nature of nicotine. The majority of research regarding e-cigarettes and their physiological effects doesn't focus on the comparison between nicotine-containing and nicotine-free devices. As a result, it's commonly believed that nicotine-free devices are a less-harmful alternative. This experiment examined the relationship between nicotine-containing e-cigarettes, nicotine-free e-cigarettes and their subsequent effects on the standard metabolic rate (SMR) of crayfish. Oxygen consumption was used as a proxy for the standard metabolic rate of the crayfish. There are many ways to measure standard metabolic rate, but for this particular

experiment, it makes most sense to use oxygen consumption as the basis for measurement because it is most practical given the situation. Using oxygen consumption as a proxy for standard metabolic rate has many advantages over more direct methods. Oxygen consumption measurements are much easier to complete, they don't require the calculations for work that are involved with direct calorimetry, and they don't include the contributions to metabolism from the gut's microbiome, which uses anaerobic respiration as its' main source of energy (Hill et al., 2016). The more oxygen consumed indicates a higher standard metabolic rate and the less oxygen consumed indicates a lower standard metabolic rate.

Materials and Methods

There were a total of 42 crayfish (33 males with an average mass of 22.92 ± 1.22 g and 9 females with an average mass of 25.28 ± 1.49 g) available for the experiment. Each crayfish was carefully analyzed and their sex, mass, and appendage numbers were recorded. They were then assigned to a test group and a number to serve as an identification code. They were split up into three equal groups of 14, with each group serving as a different treatment group. Each treatment group had the same male/female ratio, as each group contained 11 males and 3 females. Other than the separation based on sex, the crayfish were otherwise selected randomly. The average weight of all of the crayfish in the experiment was 23.4 g, with the average mass of the control, non-nicotine, and nicotine groups being 21.84, 23.34, and 25.09, respectively. Prior to experimentation, all of the crayfish were housed individually in shared dechlorinated tank water at room temperature (25 ± 1 C). They were fed the same type of food (Hikari Crab Cuisine (Kyorin, Japan)) twice per week and they were all of similar size and health. Each crayfish was placed in their own tank (18 x 11 x 13 cm) and submerged in 500 mL of their original tank water

overnight. The three different treatment solutions were a control solution of fresh water, a nicotine-based e-cigarette solution, and a non-nicotine-based e-cigarette solution. The e-cigarette solutions were produced by mixing vapor from an electronic cigarette into the dechlorinated water supply. To do this effectively and quantify the vapor concentration in the water, an apparatus was constructed that allowed for an efficient mixing of vapor into the water (Fig. 1). An erlenmeyer flask was filled with 1,000 mL of dechlorinated water, capped with a stopper with rubber tubing running through the stopper into the water. On the other side of the stopper, the rubber tubing was connected to a three-way valve, with the other two valves connected to the e-cigarette and the syringe. The e-cigarette produces vapor when air is sucked out of the mouthpiece, so the action of the syringe being pulled back was sufficient in producing vapor. When the valve was opened between the syringe and the e-cigarette, the syringe was pulled back for four seconds to produce approximately 55 mL of vapor. Once the vapor was in the syringe, the valve between the syringe and the flask was switched open. The syringe was emptied into the flask and the flask was inverted to effectively mix the vapor into the water. This was considered one puff. For each 1,000 mL erlenmeyer flask, 18 puffs were added to the flask. Since each puff was approximately 55 mL of vapor and there are 18 puffs per L, then there was roughly 990 mL of vapor per L of dechlorinated water. This process was repeated six additional times for a total of 7 L of sample. The original tank water was drained from each individual tank and each crayfish was submerged in their designated treatment solution. The crayfish were then exposed to the solution for 25 hours. After the exposure, the crayfish were individually placed into a 500 mL erlenmeyer flask full with their treatment solution. The flask was capped with a Vernier Optical Dissolved Oxygen probe placed in the water and connected to a computer. The computer documented the O2 levels of the water over a 30-minute time period.

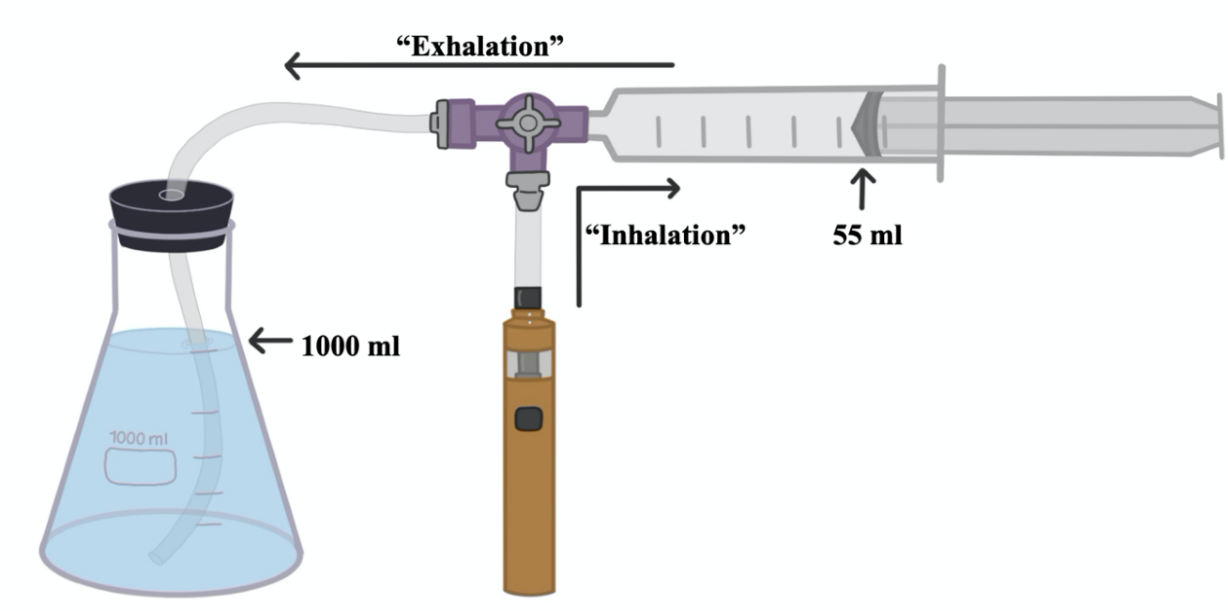

Figure 1. Graphic of the apparatus used in the methods of the experiment (Grismer et al., 2021)

Statistics

The data from the experiment was used in an analysis of variance (ANOVA) statistical test to determine whether there was a significant difference between any of the test groups. Tukey's HSD test was run to analyze the statistical relationship between the individual groups.

Results

The data of the experiment indicates that the average standard metabolic rate of the crayfish in the control group is **0.0629** with a standard error of **0.00907**. The average standard metabolic rate of the crayfish in the non-nicotine group is **0.0738** with a standard error of **0.0194**. The average standard metabolic rate of the crayfish in the nicotine group is **0.0548** with a standard error of **0.00853**.

An analysis of variance (ANOVA) test determines whether or not there is any statistically significant difference between any of the treatment groups by assigning a p-value. The p-value given from the ANOVA test is **0.605**. Since the p-value assigned by the ANOVA test is far greater than 0.05, the null hypothesis is rejected and it is concluded that there is no statistically significant difference between the groups.

Additional analysis of the data by Tukey's HSD tests confirm that there is no statistical significance between the groups. Tukey's HSD test between the control and non-nicotine groups gives a p-value of **0.619**, which is greater than 0.005, indicating no statistical significance between the control and non-nicotine groups. Tukey's HSD test between the control and nicotine groups gives a p-value of **0.522**, which is greater than 0.005, indicating no statistical significance between the control and nicotine groups. Tukey's HSD test between the nicotine and non-nicotine groups gives a p-value of **0.384**, which is greater than 0.005, indicating no statistical significance between the nicotine and non-nicotine groups.

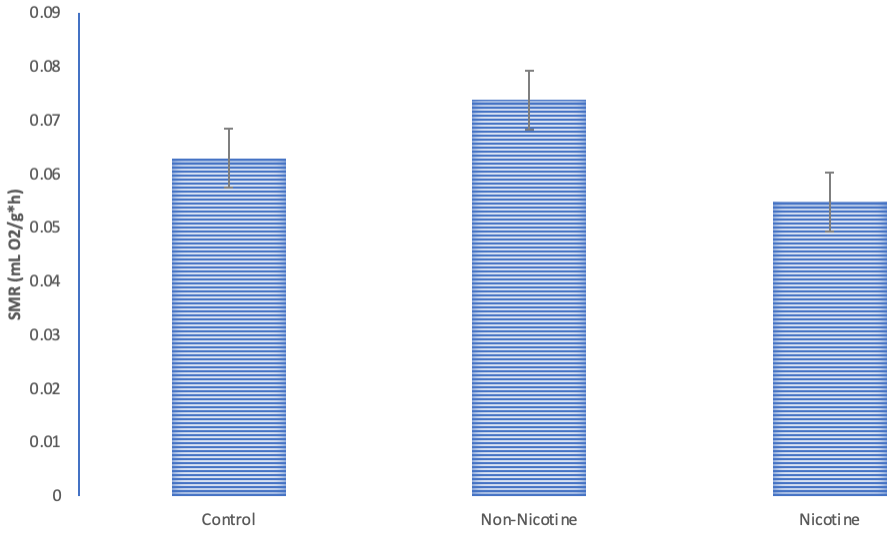

Figure 2. Chart consisting of the three treatment groups and the average and standard error of their standard metabolic rates.

Table 1. Data table containing the results from an analysis of variance (ANOVA) statistical test of significance.

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.00253	2	0.00126	0.509	**0.605**	3.24
Within Groups	0.0968	39	0.002487			
Total	0.0994	41				

Table 2. Data table consisting of the results of three different Tukey's HSD tests, each measuring the statistical significance between each of the three treatment groups.

Tukey's HSD Test	
	p-value
Control & Non-Nicotine	**0.619**
Control & Nicotine	**0.522**
Non-Nicotine & Nicotine	**0.384**

Discussion

As the use of electronic cigarettes, otherwise known as e-cigarettes, continues to grow throughout the country, it is important to analyze how these artificial smoking devices might affect the health (both long-term and short-term) of users. Much is known about the adverse effects of smoking traditional tobacco cigarettes, yet there is very little known about the potential effects of e-cigarettes, due to their recent arrival. The lack of concrete evidence against e-cigarettes coupled with the common public belief that they are harmless has led to a culture that is completely dependent on them. Throughout the past few years, cigarette smoking has been gradually getting replaced by e-cigarette smoking, which appeals to a younger audience, mainly in part because of the attractive flavors and convenience. Consistent and deliberate marketing of e-cigarette products directed towards a particular demographic was proven to significantly increase the usage of these products (Pokhrel et al., 2022). E-cigarette use is going to continue to

grow, and it is important to fully understand how these devices can affect the health of future populations.

Oxygen consumption was used as a proxy for standard metabolic rate because oxygen is needed to break down food into nutrients that are used as the body's fuel source. In biochemical terms, oxygen serves as the final electron acceptor in the electron transport chain that produces adenosine triphosphate (ATP), which is the body's main source of energy ("Why is breathing oxygen necessary," 2015). Since oxygen is needed to effectively convert food to energy, it is possible to use oxygen consumption as a proxy for how efficient the organism is turning food into energy. The more oxygen consumed indicates a higher standard metabolic rate and the less oxygen consumed indicates a lower standard metabolic rate.

It was expected that the crayfish exposed to the nicotine solution would have higher standard metabolic rates than the crayfish of the other two test groups. Nicotine is implicated in weight loss in many models because of its ability to bind to nicotinic cholinergic receptors in the brain and lead to upregulation of neurotransmitters that ultimately decrease appetite (Audrain-McGovern and Benowitz, 2017). Nicotine also serves to boost metabolic rate by stimulating the production of catecholamines like epinephrine, which stimulates thermogenesis in fatty tissues. There is also evidence to suggest that nicotine plays a role in converting white adipose tissue into brown adipose tissue by causing upregulation of uncoupling proteins (Seoane-Collazo et al., 2019). Brown adipose tissue increases metabolic rate because its higher levels of uncoupling protein use calories to produce heat instead of ATP. For these reasons, it was expected that the nicotine treatment group would, on average, have higher standard metabolic rates than the others.

There were a multitude of limitations involved in the experiment that might prevent any significant results. For starters, the time that the crayfish spent exposed in their designated treatment solution could have been too short, which could have prevented any physiological changes from taking place. The sample size might just have been too small to indicate any real changes. The measurements of the vapor concentrations are suspect at best, as it is improbable that each inhalation process generated an equal amount of vapor. This would lead to unequal vapor concentrations between groups, which would alter the results. As mentioned previously, the measurements are not direct measurements of metabolic rate, rather they are approximations of metabolic rate using oxygen as a proxy. For a better indication, carbon dioxide excretion could have been monitored and recorded along with the oxygen consumption rates. An additional aspect that wasn't considered is that the crayfish used in the experiment had previously been used in separate experiments. Those crayfish could have experienced long-term physiological effects that affected the results of this experiment.

All in all, while there are no statistically significant results from the study, it does serve as a building block for others to slightly revise and conduct an investigation of their own. The scientific community needs to explore deeply the potential impacts of electronic cigarettes before the populations fall deeper into their addictions.

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