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# Nicotine and e-cigarette effect on maximal metabolic rate (MMR) in Procambarus clarkii

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## I. Abstract

The use of electronic cigarettes, commonly referred to as e-cigarettes or vapes, has increased exponentially in recent years despite the lack of research into potentially negative physiological effects. The rise in e-cigarette use is likely due to the overwhelming evidence regarding the addictive properties of nicotine and the damage caused by traditional tobacco cigarettes. Most e-cigarettes, however, still contain nicotine and are associated with a rise in nicotine-related intoxications (Belkoniene et al., 2019). There are nicotine-free e-cigarette options, which are gaining popularity, but it is unknown if they carry similar or other negative physiological effects. Studies involving e-cigarettes containing nicotine show that inhalation increases whole-body metabolic rate, yet studies including nicotine-free e-cigarettes are currently lacking (Olfert et al., 2018). This project intended to investigate any differences in maximal metabolic rate (MMR) following an acute exposure period to water with dissolved e-cigarette vapors; with the hypothesis that both nicotine and nicotine-free vapor will increase MMR. The Louisiana crayfish, *Procambrus clarkii*, was the organism studied due to availability and their low maintenance requirement. E-cigarette vapor did not have a statistically significant effect on MMR between treatment groups or with the control group. Future research could benefit from a prolonged exposure time, as well as a higher concentration of e-cigarette vapor at the time of exposure.

## II. Introduction

Electronic cigarettes, popularly known as e-cigarettes or vapes, have increased significantly within the past decade, especially within the youth and young adult population, with over three million middle and high school students reporting use in 2022, according to the CDC (2022). This corresponds to a greater than 20% increase since 2011 (Jones and Salzman, 2020). Vaping was initially viewed as a method to wean off traditional tobacco cigarettes since nicotine concentration can be controlled in e-cigarettes. However, many have transitioned to e-cigarette vaping as a primary source of nicotine because of its accessibility, variety of flavors, and lack of supposed carcinogens found in traditional tobacco cigarettes (Sapru et al., 2020). Additionally, peer pressure, curiosity, and the variety of flavors available are increasing the popularity of nicotine-free e-cigarette use. While this is viewed as a safer method of vaping, e-cigarette aerosol without nicotine still contains many chemicals, including acrolein, diacetyl, and acetaldehyde, that carry potential adverse health effects (Trifunovic et al., 2022). However, physiological effects of nicotine-free e-cigarette use and how these chemicals affect whole body metabolic rate is currently an area of study with few answers.

Maximal metabolic rate (MMR) is the maximum rate of aerobic metabolism and therefore the maximum rate at which adenosine triphosphate (ATP) can be produced as an energy source via the electron transport chain. Nicotine exposure is known to increase whole body metabolic rate (Bradley et al., 2010). While some may view increased metabolic rate and nicotine's appetite suppressant properties as an aid in losing weight, the intentions of this study were to demonstrate one of many ways that e-cigarette use can cause physiology to deviate from the standard, even in a non-nicotine sample (Bloom et al., 2018). Due to preliminary research findings of heavy metals in e-cigarette aerosols, as well as a rise in e-cigarette or vaping use-

associated lung injury, commonly known as EVALI, it is suspected that nicotine is not the sole chemical in e-cigarettes that can have negative effects (Marrocco et al., 2022). The purpose of this project was to demonstrate non-nicotine e-cigarettes can impact normal physiology, MMR was compared in crayfish exposed to nicotine and non-nicotine vaping chemicals. MMR was measured by inducing exhaustion, followed by a ten-minute period where oxygen consumption was measured. The Louisiana crayfish, *Procambrus clarkii*, was the organism studied, mainly due to availability at The University of Akron, their low maintenance requirement, and their increasing use as a model organism.

## III. Materials and Methods

Forty-two adult crayfish (*P. clarkii*), thirty-three males and nine females with an average mass of 23.40 ± 1.02 grams (g), were obtained from The University of Akron Principles of Biology laboratory. Crayfish were purchased in March 2020 and 2022 from Louisiana Crawfish Company. These crayfish were subject to multiple experiments over the course of the last several months, including alteration glucose concentrations via serotonin injections, GABA injections, as well as dopamine and caffeine-infused water; all of which were individual experiments. The crayfish were left undisturbed for eight months prior to nicotine exposure.

Crayfish were randomly (sex-balanced) divided into three groups, fourteen crayfish per group (n=14), with eleven males and three females per group. The first treatment group was exposed to e-cigarette vapor that did not contain nicotine, 0mg/mL. The second treatment group was exposed to e-cigarette vapor that did contain nicotine at 12mg/mL concentration. The third group, the control group, was not exposed to e-cigarette vapor and was exposed only to room temperature, 25±1°C dechlorinated water.

Crayfish were housed in individual tanks measuring 18x11x13 centimeters (cm), and approximately twelve inches were placed between tanks to avoid any agonistic behaviors. Crayfish tend to exhibit changes in behavior to prepare for an agonistic interaction if another crayfish is within viewing distance, actions such as climbing up the aquarium wall, turning away from other crayfish, and roaming their tank (Drozdz et al., 2006). Tanks were filled with 25±1°C dechlorinated water several inches above the height of the crayfish, approximately 500 mL, thus allowing the crayfish to aerate the water and have access to the surrounding atmosphere (Pierce, 2010). Ability to self-aerate, along with the short duration of the experiment, the water was not aerated by an air stone or other external source. Also due to short experiment duration and the potential to influence the concentration of e-cigarette vapor, a water filter was not used. Crayfish were acclimated to dechlorinated water for twenty-four hours prior to e-cigarette vapor exposure. Crayfish were fed Hikari Crab Cuisine twice weekly prior to experimentation, but food was withheld for the three days prior to oxygen consumption measurements (Compher et al., 2006).

E-cigarette vapor was collected via a three-way valve system connected to a 60 mL syringe, Joyetech eGo AIO vaping device, and a 1000 mL Erlenmeyer flask filled with 1000 mL of 25±1°C dechlorinated water, shown in **Figure 1**. The vaping device was fully charged and filled to the maximum fill line with Naked brand Strawberry Pom flavored vaping juice; with nicotine concentrations of either 0mg/mL or 12mg/mL. The 12mg/mL concentration was chosen because it is recommended as a concentration sufficient to cause cigarette cravings to subside and is used to quit tobacco cigarette smoking (Palmer and Brandon, 2018). This vaping juice contained a 65 vegetable glycerine (VG) to 35 propylene glycol (PG) ratio; an increasingly popular ratio that provides a medium flavor puff and a large cloud production, according to Vaping360 (2019). To mimic typical vaping habits, each puff consisted of 55 mL of vapor and

lasted three to four seconds. Following inhalation, the three-way valve was turned, and vapor was pushed from the syringe to the Erlenmeyer flask. The vapor and water were mixed by vigorously shaking the flask. It was determined that 18-55 mL puffs per 1 L of dechlorinated water was a physiologically relevant dosage for crayfish (Matta et al., 2006). These numbers also coordinated with standard vaping habits, according to RELX (2021). 7 L of vapor infused water was collected for each treatment group and each crayfish was housed in 500 mL of vapor infused water for 24 hours.

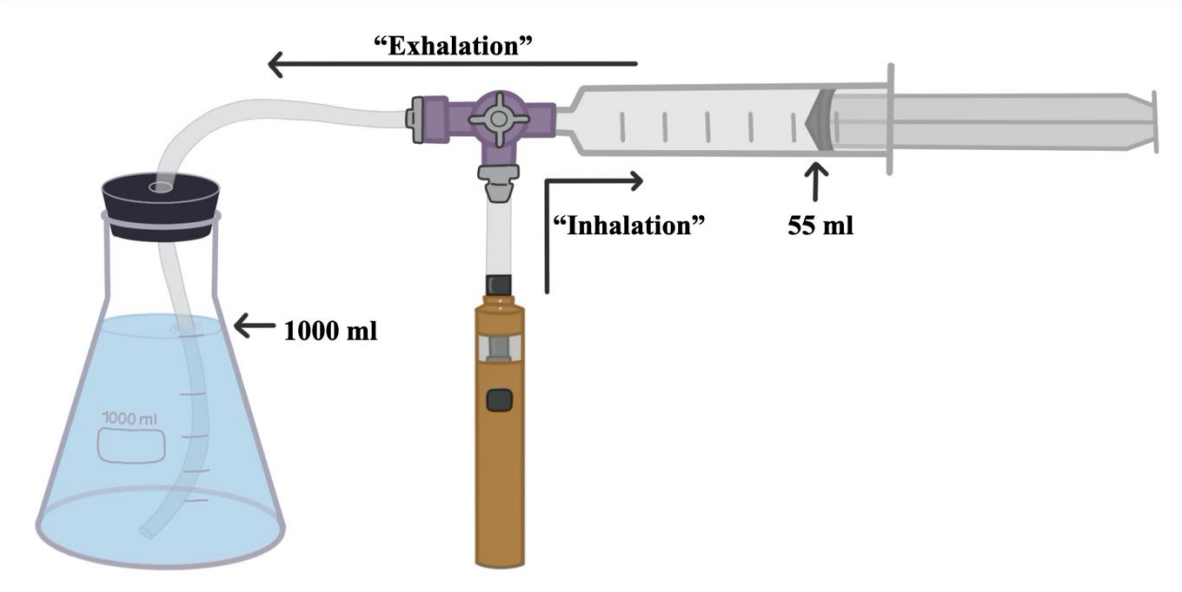


**Figure 1.** Three-way valve system used to create vapor-infused water (Grismer et al., 2021)

After a 24-hour exposure to either room temperature dechlorinated water, 0mg/mL nicotine vapor-infused water, or 12mg/mL nicotine vapor-infused water, the crayfish were prepared for MMR measurement via oxygen consumption levels.

MMR is the maximum rate of oxygen consumption, signifying the maximum rate of aerobic respiration (Norin and Clark, 2015). Therefore, measuring the maximal rate of oxygen consumption represents the maximal rate at which the body uses oxygen to produce energy in the

form of adenosine triphosphate (ATP). MMR is typically measured when the organism is in a state of exhaustion. Crayfish were exercised to exhaustion according to a protocol demonstrated in previous research (Rosewarne et al., 2016). Within the vapor-infused water, or the untreated water for CG, crayfish were worked to exhaustion by inducing a tail flip using the blunt end of a pencil. When the crayfish were unable to tail flip, they were placed onto their dorsal side and this process was continued until they were unable to turn over, ensuring exhaustion. The crayfish along with its treated water, depending on treatment group, were immediately transferred to a 500 mL Erlenmeyer flask.

The dissolved oxygen content of the water in the flask was measured using the Vernier Optical DO Probe, which works via a luminescence mechanism. The Vernier LabPro interface was used in conjunction with the Logger Pro app for data collection. Dissolved oxygen content was recorded for 10 minutes. The difference between initial and final dissolved oxygen content of the flask was used to calculate oxygen uptake by the crayfish. Following data collection, all animals were euthanized by freezing.

## IV. Results

An analysis of variance (ANOVA) was performed, and it was indicated that there was no statistically significant difference in the MMR between the control, non-nicotine, and nicotine groups, as shown in **Table 1**.

**Table 1.** ANOVA test performed demonstrated no statistical significance in mean MMR between control, nicotine, and non-nicotine exposed crayfish.

| *Source of Variation* | *Sum of Squares* | *df* | *Mean Square* | *F* | *P-value* |
|---|---|---|---|---|---|
| Between Groups | 0.007143 | 2 | 0.0035719 | 0.9791154 | 0.3846862 |
| Within Groups | 0.142276 | 39 | 0.0036481 | | |
| Total | 0.149420 | 41 | | | |

No significant difference was observed (p=0.385), however, a Tukey's post-hoc analysis was performed to examine the variation between treatment groups, as demonstrated in **Table 2**.

**Table 2.** Tukey's post-hoc analysis comparing variation in mean MMR between control, nicotine, and non-nicotine exposed crayfish.

| *Groups* | *P-value* |
|---|---|
| CG vs. Non-Nicotine | 0.432436 |
| CG vs. Nicotine | 0.204555 |
| Non-Nicotine vs. Nicotine | 0.518556 |

The Tukey's post-hoc analysis demonstrated that there was not a statistically significant difference between any of the specific comparisons between groups.

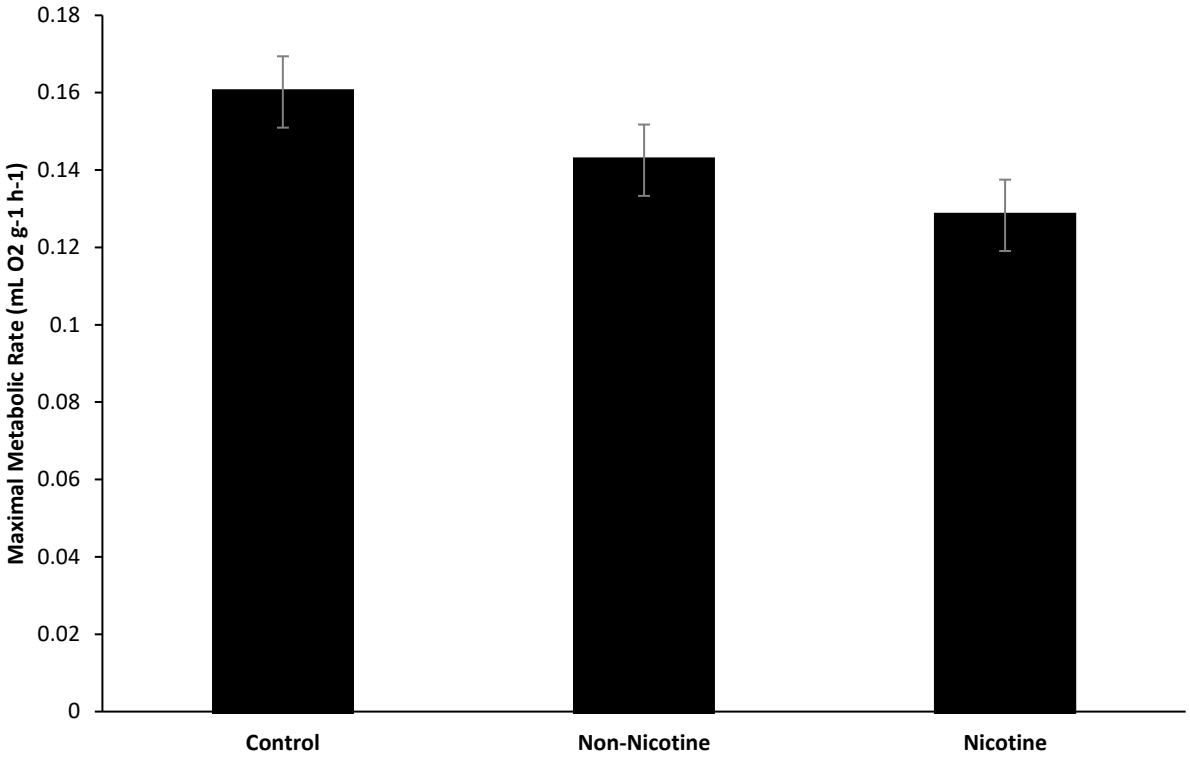


**Figure 2**. Mean maximal metabolic rate in milliliters of dissolved $O_2$ consumed (mL $O_2$) per gram of body weight (g) per hour of recording time (h). p = 0.385. Error bars represent standard error (SE).

## V. Discussion

The results of this study indicated that there was not a statistically significant difference in MMR between crayfish that were exposed to e-cigarette vapor with nicotine, e-cigarette vapor without nicotine, and a control group with no exposure (p=0.385). These results were unexpected. Research exploring nicotine's effect on MMR is limited, however, ample studies demonstrate nicotine increasing whole body metabolic rate and resting metabolic rate (RMR); hence similar results were hypothesized in terms of MMR (Perkins et al., 1989). While non-significant, the difference in mean MMR between control and nicotine groups showed a potential

trend when compared to the other two groups (p=0.205). Indicating that exposing crayfish to nicotine may have influenced MMR, however, further research is needed.

It has been demonstrated that standard metabolic rate (SMR) in *Cherax quadricarinatus*, a similar but larger species of crayfish, was 0.0312 mL $O_2 \bullet g^{-1} \bullet h^{-1}$ at 23°C (Aji, 2012). A similar SMR value of 0.03445 mL $O_2 \bullet g^{-1} \bullet h^{-1}$ was measured in *Austropotamobius pallipes* (Sutcliffe et al., 1975). Estimating that MMR is approximately four times the SMR, as demonstrated by Rosewarne et al., a value of 0.1248 mL $O_2 \bullet g^{-1} \bullet h^{-1}$ was calculated and is comparable to the control group MMR indicating that the crayfish used in this study were relatively healthy and had no initial deficits in SMR (2016).

It is unknown if crayfish became hypoxic in this experiment, which could severely alter results. Crayfish were able to self-aerate during standard housing between tests, however, were unable to during metabolic rate testing. Flow through respirometry would eliminate potential hypoxia. It is unknown if the optical probes used to measure oxygen concentration were subject to signal drift. Calibrating before each run or using non-optical oxygen sensors should have been performed to avoid signal drift (Badocco et al., 2012)

In order to redesign this experiment, the following protocol changes would be advisable. Crayfish used were purchased in March of 2020 and March 2022. It could be beneficial to use crayfish that hatched at similar times, it is possible that nicotine or e-cigarette vapors may have a different effect on crayfish that are drastically different in age. Additionally, the crayfish used in this study were subjects of multiple experiments prior. Although crayfish were unbothered for eight months prior to MMR testing, it is important to include that they were subjects to a wide array of exposures to different neurotransmitters and that a long-term effect was possible.

Another area of improvement would be in length of exposure to e-cigarette vapor. This experiment was aimed at effects following acute exposure; however, thirty-six or forty-eight hours of exposure could induce differences in MMR when compared to twenty-four hours. It would be interesting to see if a higher concentration of nicotine would produce expected results and produce a lower and significant p-value in the nicotine group. Since nicotine studies in crayfish are limited, nicotine concentration calculations were based on other organisms; 12mg/mL may have been too low a concentration to elicit a change in metabolic rate. Since some difference was observed in the nicotine group, higher nicotine concentrations, longer exposure, and a larger sample size could enhance results.

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