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Behavioral Choices of Apple Snails, Pomacea Maculata, Under Varied Chemical Landscapes

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BEHAVIORAL CHOICES of APPLE SNAILS, *POMACEA MACULATA*, UNDER VARIED CHEMICAL LANDSCAPES

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By

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ABSTRACT

Habitat choice is a critical behavior for organisms to successfully survive and reproduce. These choices are dictated by available environmental information about potential predation risks or food patches that form the organism's sensory landscape. This study specifically focused on the behavioral choices of two invasive apple snail (*Pomacea maculate*) populations exposed to varying predation threats. We collected snails from Florida and Alabama which were used in laboratory experiments with varied sensory landscapes. Trials consisted of controls with no cues (FL: n = 7, AL: n= 7), an attractive treatment with introduced food cues (FL: n = 4, AL: n = 6), and an aversive treatment with introduced alarm cues (FL: n = 5, AL: N = 8). All trials were analyzed for zone choice and behavioral responses. Chi squared analyses revealed differences in initial and final arm choice based on treatment group (Initial: χ^2 = 10.834, df = 4, p = 0.029, Final: χ^2 = 16.648, df = 4, p = 0.00226). However, generalized linear models did not demonstrate any difference in the amount of time snails spent in the neutral zone (Treatment: Dev = 42.5, p = 0.97; State: Dev = 1167.1, p = 0.20; Treatment x State: Dev = 1407.4, p = 0.36) and a square-root transformed linear model did not demonstrate any difference in the amount of time the snails spent in the odor arm (Treatment: F = 0.79, p = 0.38; State: F = 0.038, p = 0.85; Treatment x State: F = 2.63; p = 0.12.) Finally, Chi squared analyses showed no difference in active versus inactive behaviors based on treatment (χ^2=0.36, df = 2, p = 0.84) or by state (χ^2= 3.02, df = 1, p = 0.08). More studies on these snails' chemical landscape and associated behaviors could inform population management for this formidable aquatic invader.

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Andrea Nicole Adams

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INTRODUCTION

Habitat choice is a critical behavior for organisms to successfully survive and reproduce. Specifically, choosing a more valuable (e.g., resource rich) habitat can dictate which organisms will successfully survive and/or reproduce. These choices are dictated by gathering environmental information such as chemical, visual, and/or mechanical information from biotic and/or abiotic sources (Pavlov et al. 2008; Shartau et al. 2010; Clay and Helms 2017; Mogdan 2019) hence their sensory landscape. To make these types of decisions using the sensory landscape, organisms must gather information from their surrounding habitat to pick good habitats. Hence predator/ prey cue within the environment that organisms cue in on based on sensory mechanisms within their evolution. One study examined habitat choice by quantifying behavioral choices of crayfish when provided with chemical information through food and predator cues (Jurcak and Moore 2014). They found that non-consumptive effects often occurs when prey alter their behavior in response to sensory signals that the predators release into the environment verses the food abundance within the area. Jurcak and Moore’s work suggests that the organisms’ habitat selection might be context dependent; the presence of certain cues can influence decision making for selecting a suitable habitat. This study demonstrates the importance of non-consumptive effects (e.g., behavioral, or physiological changes in prey due to perceived predation threats) play a role in habitat selection. However, these types of behaviors are not unique to crayfish. There have been multiple studies done on the non-consumptive effects on aquatic organisms and respected bird species (Brevilglieri et al., 2017, Mitchel and Harborne, 2020, Sheriff., et al., 2020). Non-consumptive effects has been very well defined in many aquatic systems like fishes (Mitchel and Harborne, 2020, Sheriff., et al., 2020), apple snails whether it be native or invasive (Siegfried et al., 2022, Yoshie and Yusa, 2011), insect larvae (Hermann and

Thaler, 2014, Thaler and Griffin, 2008), and marine systems whether it be native or invasive (Burgin and Hardimen, 2015, Kindinger and Albin, 2017) depending on certain behaviors that where looked at with the respected predator within the respected environment. This information can be important in understanding the behavior and invasion of introduced species in aquatic habitats.

Introduced species, such as invasive species, have had global impacts on the occurrence of distribution events in natural communities through these introductions to ecosystem's integrity and functioning (Sole et al. 2021). Invasive species are a large contributor to biodiversity loss, and they are a major cause of species extinction in ecosystems that are geographically and evolutionary isolated (e.g., small islands) (Clark and Johnson 1984; Savidge 1987). Invasive species can specifically decrease biodiversity within complex communities by interrupting species interactions, out completing native species for resources, and predation avoidance invasive species are already accustomed to (Galiana et al., 2014, Monette et al., 2016, Ueshima and Yusa, 2015). This can interrupt a food web by decreasing native species richness and the number of links per species (Galiana et al. 2014) due to the invasive species taking over certain niches within the environment at a more advanced rate than the natives. This can be anything from the invasive species consuming more food than the native species (Morrison and Hay 2011; Monette et al. 2016) to the invasive species outcompeting the natives by simply out-surviving them. For instance, some invasives may have adaptations that better allow them to detect certain predation cues that are within the environment due to anti-predator behaviors (Ueshima & Yusa, 2015). Ueshima and Yusa (2015) found that when predators are present, antipredator behavior is not necessarily directly linked to the chemical odors the predators emit into the environment, but realistically to crushed conspecifics in hatchlings *Pomacea canaliculata*. The behavior associated to the conspecific odor were found to have a greater proportion of responses than the single feeding predators. The hatchlings responded to the single feeding predators (turtle) odors by going above the water line, and another single feeding predator (carp) odor by going above the water line. All these findings show they have a fine-tuned innate antipredator behavior (Ueshima and Yusa 2015). Invasive species can also have the upper hand ecologically if they do not have natural predators within the new environment that can manage the population (Guo et al., 2017). This means that there are no known predators they have been catalogued that could reduce the population by consuming them beside human interaction through conservational means. These interactions are important for understanding how and why these species are successful in taking over areas in which they are not normally found through their behavioral choices in the respected sensory landscape. One species that has been listed as a formidable aquatic invader is the apple snail, genus *Pomacae* (family *Ampullariidae*, order *Architaenioglossa*) (Lowe et al. 2000).

Apple snails, *Pomacae*, exhibit an aquatic-terrestrial life history (i.e., spends most of the time in the water and only comes to land when they lay their eggs) (Hayes et al. 2009) that can reach larger body sizes than other snail species (Youens and Burks 2008). These snails also act as true herbivores instead of algal grazers that hold a veracious consumer of macrophytes unlike most snail species that are grazer or detritivores (Hayes et al. 2015). Consequently, these organisms can cause damage to habitats that consist of rice, filamentous algae, and macrophytes in eutrophic, shallow lakes (Joshi and Sabastian 2003; Yang et al. 2020). Due to their voracious appetite, these organisms eat more of the native food sources than other conspecifics within the area. Ampullariidae as a taxonomic group has a history of being successful invasive species (Cowie et al. 2006; Hayes et al. 2012; Burks et al. 2017). *P. maculata*, a species of

Ampullariidae snail, are known to invades wetlands to the point that they modify the structure and function of food webs directly by consuming aquatic plants (Morrison and Hay 2011a; Smith et al. 2015) and indirectly by competing with native species like *p. paludosa* (Connor et al. 2008; Posch et al. 2013). As they are also novel prey for native predators like the endangered snail kite (*Rostrhamus sociabilis*) they can directly increase the kite's population on a small timescale to help the endangered species increase, and indirectly cause shifts to competing species that consume the same food source (Cattau et al. 2016). These snails' invasions might lead to shifts in feeding patterns among other organisms or provide a new host for parasites, such as the rat lungworm, *Angiostrongylus cantonensis* (Kim et al. 2014), and a parasitic trematode (*Stomylotrema gratiousis*) (Pinto et al. 2015) through food webs due to their role as prey.

P. maculata can be seen as both predator and prey within the respected ecosystem. It is known that some *Pomacea* species, like *P. maculata*, eat smaller conspecifics when they have limited access to additional calcium (Burks et al. 2017). This can lead one to assume the younger snails have better adaptions to detect aversive cues (e.g., dead conspecifics) within the landscape to stay away from bigger conspecifics and predators like crayfish and turtles. *P. maculata* is also known to consume egg clutches of conspecifics and smaller individuals (Burks et al. 2017). Even though they are known to consume other conspecifics, *P. maculata* tends to primarily feed on herbivorous plants like wild taro (*Colocasia esculenta*) and water hyacinth (*Eichhornia crassipes*) (Bernatis 2014). Because they hold an intermediate consumer position in their local food web, *P. maculata* also serve as a food source to several predators. These predators include Swamp crayfish (*Procambarus clarkii*) and red-eared slider turtles (*Trachemys elegans scripta*) for smaller apple snails (Burks et al. 2017). Adult snails have been known to be consumed by bigger predators to include some avian species like Snail kites and Limpkins (Burks et al. 2017).

All this ties into the sensory landscape in how these organisms find food through chemical, visual, and mechanical stimuli. Specifically, these snails must find food, find mates, and stay away from areas where predators are located through various environmental cues. As primarily aquatic organisms, it is likely that these snails rely heavily on chemical information (Horgan 2018). Moreover, previous studies have found that young apple snails exhibit significant behavioral responses to aversive chemical stimuli such as dead conspecifics (Ueshima and Yusa 2015), yet no known literature is known to be on adult snails.

During the summer of 2021, observations during *P. maculata* field collections generated questions about the varied sensory landscape of populations under varied levels of predation threat. Specifically, researchers noted the ability to attract and trap snails using alfalfa plants (i.e., potential attractive stimulus) and they noted the absence of snails in areas more susceptible to predation events through the means of dead conspecifics (i.e., areas of potential aversive stimulus). Subsequently, *P. maculata* were collected from central Florida and a second, less predated population in south Alabama. In central Florida we observed that the population was hard to find considering the amount of vegetation within the area from a well-established population with heavy predation (per information on USGS ANS query). In the areas in which the Florida populations were found, the snails were very difficult to collect despite intensive multi-day sampling. This population also showed strong evidence of predation with large numbers of shell middens at 15 surveyed sites in the Lake Okeechobee and Lake Istokpoga areas of Central Florida. The observed behaviors of the populations in Florida were mostly avoidance behaviors like burrowing throughout the area due to possible heavy predation. There was evidence of high population density based on the presence of large snail kites and large numbers of apple snail egg clutches above the water line. This suggested a very strong predation presence that could cause the snails to burrow in the sediment to avoid predation and only surface for mating or laying egg clutches. Conversely, an invasive population of snails established in the 1990s in Mobile, Alabama, showed no evidence of predation as empty shells were rarely observed and no observations of predation (holes in shells, cracked shells, etc.) were observed during a two-year study from 2017-2018 (Slayton 2019). This site also had dense macrophyte cover and large sanil densities. Snail at this site were easily collected by hand in 2019 (~50 individuals/ 30-minute sampling effort) and hundreds were captured in a 2-day trapping effort conducted by the Alabama Department of Conservation and Natural Resources in Fall 2019 and Spring 2020. However, this population was eradicated in 2020 through intensive molluscicide efforts. Recently, (~3 years ago) a population of *P. maculata* was established in a residential retention pond in Montgomery, Alabama. These snails were easily collected by hand (~50/hour) and by using Van Dike snail traps resulting in collection of large numbers of individuals. However, at this location, macrophytes were limited and food was likely a limited resource for the snails. Like the population in Mobile there was little to no evidence of snail predation. These observations led the researchers to predict that the Alabama population in Montgomery were most likely food limited but experiencing no predation stress while snails in the Florida population are most likely not food limited with high predation stress. These contrasting populations provided a unique opportunity to evaluate the behavioral responses of *P. maculata* to positive (food stimulus) and negative (aversive) chemical cues. Thus, the goals of this study were to test if behavioral responses of adult apple snails to chemical cues are for this experiment and to assess whether those responses varied between populations under different environmental stressors. I had three working hypotheses: H_1: I hypothesized that the snails would have different behavioral responses to the attractive and aversive stimuli (i.e., treatment type). H_2: I hypothesized the snails would interact with the attractive stimuli by going towards the source, and the snails would avoid the aversive stimuli. H_3: I hypothesized that the snails from the Florida population would act differently and not come out to explore like the Alabama snails when presented with the attractive stimuli. I also hypothesized that the Florida snails would not come out at all during the aversive stimuli and the Alabama snails would still be active.

MATERIALS AND METHODS

Animal Collection & Housing:

P. maculata (50 individuals) were collected from two different sites from the peninsula of Florida, USA (hereon referred to as Florida snails). Florida snails were collected from Clay Lake, Lake Placid, FL, (27.31096020436958, -81.34198293599789) and Eagle Bay, Okeechobee, FL, (27.18871134721676, -80.83790612449653). All individuals were either hand caught or baited Van Dike snail traps (Van Dike Environmental Services Tallahassee, FL) were used. *P. maculata* were also collected from an urbanized drainage pond in central Alabama (hereon referred to as Alabama snails) located on Pike Road in Montgomery, Alabama (32.324119, -86.095551). Snails were caught in dipnets by hand and with Van Dike snail traps that were baited with alfalfa which were set out around a culvert and were allowed to sit for at a week. All snails were housed in a cooler with water from the collection site and labeled with a specific naming system for transportation to Jacksonville State University. All snails were marked using a unique identification system based on location of capture.

Once back in the lab, all animals were housed in a clear 12 oz container with 5-6 holes in the container for ventilation and 3-4 mL of water. All containers were also labeled with a naming system used on the snails. Snails were stored in a lab where conditions were in the lower optimal range of temperature for tropical aquatic organisms to allow movement but to decrease respiration. Food was given to the animals in the form of one spinach leaf and small doses of alfalfa until the trials three days out of the week. Food was given at the time of the water change within the containers which was changed once to twice a week to keep nitrogen levels down.

Experimental Design

I aimed to test the underlying behavioral choices of two populations of invasive apple snails from populations in Florida and Alabama by manipulating chemical stimuli to measure behavioral changes in *Pomacea maculata*. Treatment types included a control (de-chlorinated water), an aversive treatment (dead conspecifics), and an attractive treatment (alfalfa). All trials were conducted use a flow-through y-maze arena (Figure 1) and odors in the two treatments were delivered via one randomly chosen arm for each trial. Both Florida and Alabama snails were used in all three trial types. The arena that was used consisted of a 65.43 cm x 46.69 cm x 18.11 cm (LxWxH) blacked-out container and a gravity-fed delivery system (Figure 1; see arena set-up for more details). The blacked-out container was to ensure that the snails were not disturbed by external stimuli such as lighting or movement. A 43.58cm x 25.4cm length of black Plexiglas was placed in the arena to split the arena into two different halves of the respected arm choices. Water chemistry and water temperature were recorded before each trial to ensure optimal conditions for snails (Figure 2). All trials were recorded using the Live Streamer Cam 313, and trials were scored for initial and final zone choice, time spent in each zone, and behavioral responses determined using an ethogram.

Chemical Stimulus Preparation

Concentrations for aversive and attractive stimuli were based on experiments by Xu et al. (2014) and Ueshima and Yusa (2015). Attractive stimuli solutions were created by soaking 1 gram of alfalfa in 1 L of water for no more than 24 hours. The 1 L of stock solution was then introduced to 9L of water to create the 10L reservoir fill for an experiment. At the conclusion of the soaking period, the alfalfa was removed, and the odor was filtered to remove any remaining plant matter. Stock solutions were stored in glass mason jars and refrigerated. Jars were stored for no more than

24 hours the ensure that decay and subsequent odor changes did not occur. The aversive odor stock solution was created by soaking snail tissue in water for no more than 24 hours (1 gram of snail per 1 L of water). To obtain the soluble solution, we first weighed a combination snail in grams to the respected number of trails ran and multiplied the weight by 1000 to get the total water needed in mL. The solution used a combination of males and females to ensure that the sex of the animals would not bias the odor. Deceased snails were collected for odor generation after they were dissected for a separate parasite abundance study. The only internal organs we did not use in stock solution were the reproductive gland in the female snails due to toxicity (Giglio et al., 2016), and the foot. We did not use the foot organ due to the texture of the organ that could not be broken down into a soluble solution. From there, the solution was vortexed to make sure there was only small particles and filtered to ensure no excess larger body parts were used in the trials. This solution was stored in mason jars. No matter the treatment types the stored solutions were set out to room temperature before the solution was used in the trials.

Arena Set-up

The arena setup consisted of a 65.43 cm x 46.69 cm x 18.11 cm (LxWxH) blacked-out container and a gravity-fed delivery system (Figure 1). Each arm was randomized for the trial with the respected experimental procedures (see Experimental Procedures section below). The last part of the arena was an open space (no Plexiglas divider) (21.79cm x 27.94 cm) and was labeled the neutral zone. The delivery buckets were placed at the head of the arena and were elevated to a height of 25.4 cm to ensure a gravity fed system that achieved a flow rate of 10 L per 30-minute trial. A 4 mm diameter piece of tubing was connected to the 5-gallon treatment buckets that were attached to the arena at two input ports, one at the top of each arm. At the end of the arena, five output ports were drilled in the arena to ensure a flow-through system. The output water was then collected by two extra 5-gallon buckets located on the floor. The arena and buckets were completely emptied and refilled for each trial. The substrate in the arena was also rinsed multiple times between trials.

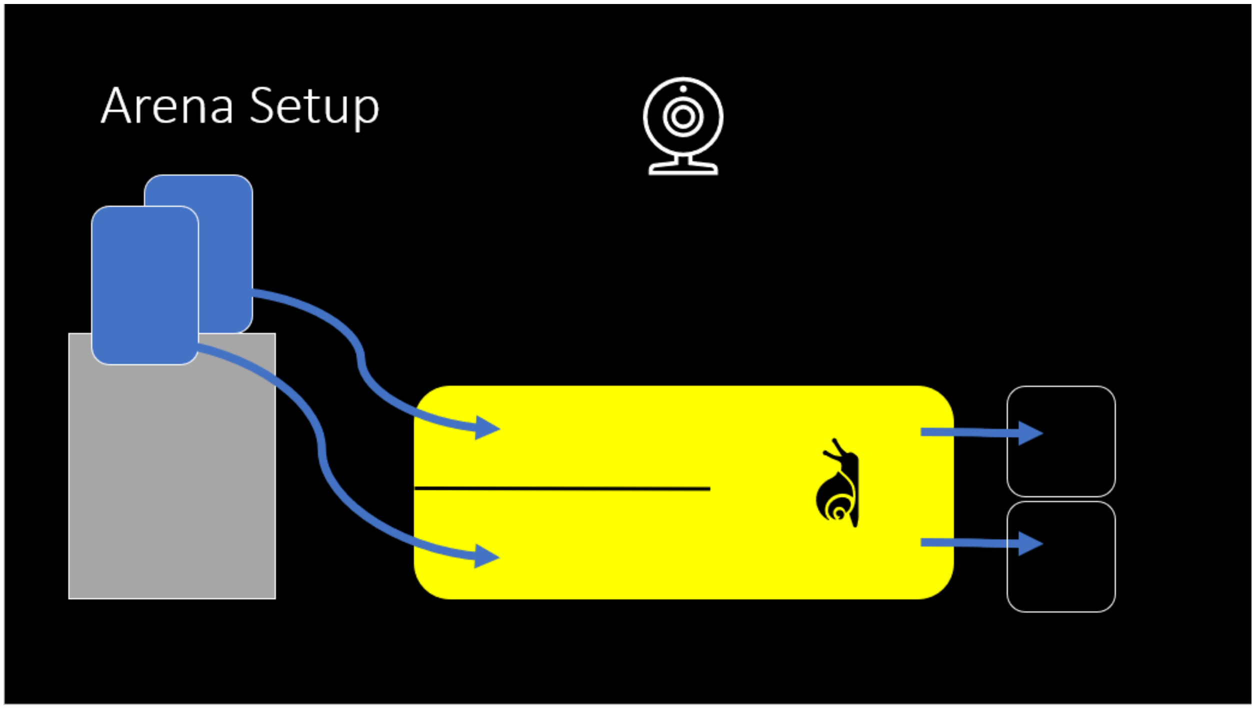

Figure 1: Arena set-up includes a gravitational flow-through system. The blue arrowing indicates tubing where the water flows into the arena and out of the arena by a 2cm tube to ensure movement. The arena is a 65.43X 46.69 X 18.11 cm (LxWxH). The black line located at the top to middle portion is a plexiglass divider that completely separated the respected arm choices on the left and right of the arena. The Live Streamer Cam 313 located above the arena to record the trials. The location of the arm choices was deemed left and right along with the portion where the snail is located the neutral arm choices. The snail that was placed in the neutral arm choice was giving an acclimation time frame between 5 to 10 minutes to get use to the change of water from the housing container to the arena water. All water that was inputted into the arena was gassed of the ensure that no chlorine was introduced

Water Storage and Chemistry

Water quality of the water stored in the bins prior to trial use was checked using a YSI meter after a 24h period of aeration to bubble off excess chlorine from the tap water. This meter checked for water quality (conductivity, pH, TDS, ORP, and temperature). This was to ensure that the testing water used in the arena was within adequate range of the natural settings in which the snails are normally found (Figure 2). The reasoning behind maintaining the water chemistry at certain parameters is that a low pH makes it extremely difficult for the snail to construct shells made of calcium carbonate (Batzer et al. 2005). These snails have also been reported to have a salinity tolerance when exposed in their younger life history stages up to 28 days (Bernatis et al. 2016) in the Floridian waters (Burks et al. 2017). Temperature limits the distributions of *Pomacae* that alter metabolic rates and influence behavior of many organisms including apple snails (Byers et al., 2013). Both low and high temperature influence the life history and distribution of these organisms through their distribution (Burks et al., 2017; Ramakrishnan 2007; Gettys et al. 2008; Darby et al. 2008; Hayes et al. 2015; Bernatis et al. 2016; Burks et al. 2017).

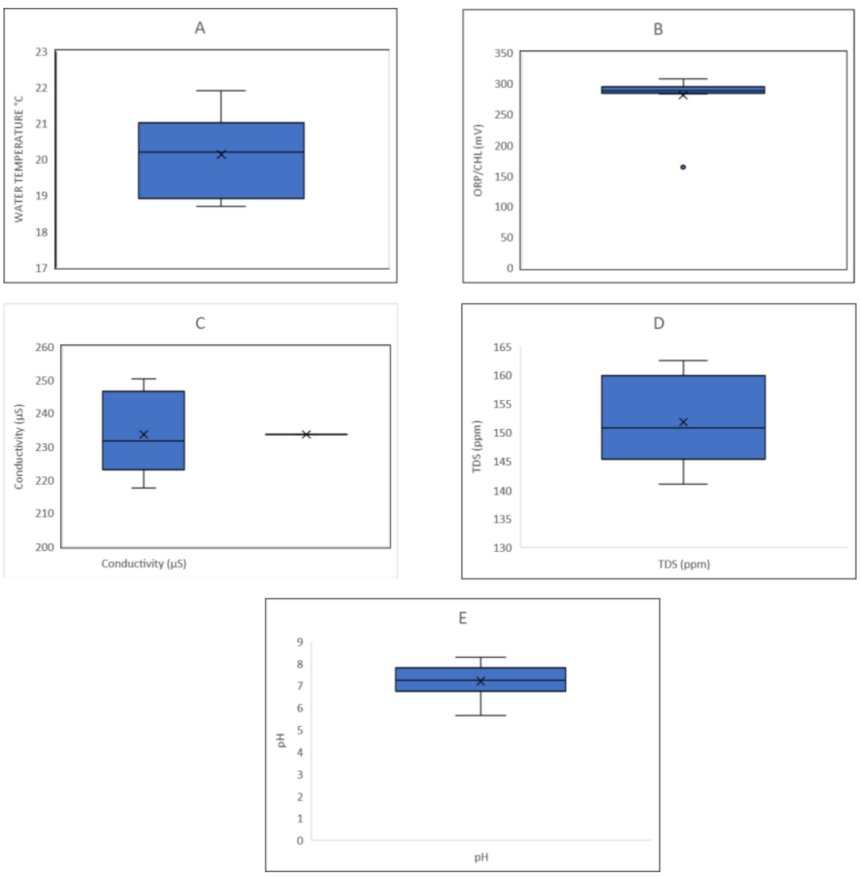

Figure 2: Above is the respected water chemistry throughout this experiment. The black lines above and below the box plot are the minimum and maximum of the respected graphs that occurred throughout this experiment. The line throughout the middle shows median, and the X located within the box shows the average between the respected graphs. Graph A) shows the average of the

water temperature °C (20.14°), Graph B) shows the average of the ORP/CHL mV (281 mV), Graph C) shows the average of conductivity μS (233.66 μS), Graph E shows on average of pH (7.21).

Experimental Procedures

All trials consisted of the same arena set-up (Figure 1) except for the type of solution used in the delivery buckets. The controls consisted of 10L of aerated water in each gravitational bucket. In total, 20L of water (combination of the gravitational delivery buckets) delivered into the arena through the gravitational flow-through system. This setup was used to ensure the snail had no preference between the arms of the arena. The snails were placed into the neutral zone for the acclimation time of 5 to 10 minutes. Acclimation time was used to ensure that the animal had time to acclimate to the new water and to ensure the snail would come out of its shell during the trial. From there the buckets were filled with the appropriate water per the trial type and the camera was set-up over the arena. After the acclimation period the camera was turned on so that the trial could be recorded along with the behaviors. After the trials ended the arena was emptied, rinsed, and refilled between each trial. Each bucket was rinsed at least three times to ensure that there was chemical odor left within the gravitational buckets. When cleaning the arena, all water was dumped into the sink and the sand was washed at least three times in between treatment trails with water. The first treatment group used the attractive stimulus of alfalfa (see Chemical Stimulus Preparation section above) in a single arm to test snail response. The second treatment group used the aversive stimulus of dead conspecifics in a single arm to test snail response. In both treatment groups, the other arm was 10L of aerated water. Arm selection for the stimulus introduction was randomized in every trial to ensure no side bias in the arena. All trials were run for 30-minutes, and recordings were stored in three different places to ensure that videos were not lost.

Ethogram and Data Analysis

An ethogram is a shorthand table of scoring behaviors used in behavioral studies in which behaviors are assigned a code or number for identification. Within the ethogram used in this study I used codes (Table 2). I used ethograms from previous studies to create the ethogram used in this study I pulled stationary, immobile (fixed), immobile (loose), burrowing, controlled floating, and crawling from two peer reviewed papers by Ekschmitt &Albrecht, 2008 and Watanabe et al., 2015. Active and Inactive behaviors are defined as being in an active state in using an identifiable anatomical feature(s) for movement, and inactive meaning that they are not using an identifiable anatomical feature(s) for movement. I added “floating with closed operculum” to indicate that the snail is inactive, as the snail’s operculum is slightly open, with the siphon out and slightly away from the body. I also added a better definition to “controlled floating” to the ethogram to explain the snail actively investigating the arena while being detached from the substrate. The behavior depicts that the snail’s operculum is open, siphon out around the body when the snail is floating and moving in directions within the water column. Finally, I added “exploring with siphon” to the ethogram to capture that the snail is completely outside of the operculum, the siphon is completely out, antenna is out, head is out of the shell, and they are actively floating within the water column in directions.

The first type of data collected was choice data. Specifically, we measured the first arm choice (hereon, “Initial Arm Choice”) and where the snail was when the trial ended (hereon, “Final Arm Choice”). Initial arm choice was determined when half of the shell had passed the edge of the Plexiglas divider within the first 5 minutes of the trial. If the snail had not that parameter within the first 5 minutes, their initial choice was coded as “neutral”. Final arm choice was determined by the final location of the snail at the end of the 15-minute trial. Four different chi-square analyses were run on these data: an initial and final arm choice each based on treatment type and state (population). Secondly, I collected data about the amount of time each animal spent in the neutral zone and the odor arm. For time spent in the neutral arm, I used a generalized linear model (hereon, GLM) was used due to the non-normal data distribution. Specifically, I used a GLM that assumed a quasi-Poisson distribution with a log link (R Core Team, 2021). For time spent in the odor arm, I used a square root transformation on the data due to an abundance of zeroes and fit those data to a linear model. Control trials were eliminated from the odor arm analysis as these trials did not have an odor arm. I also collected data on the amount of time spent at each behavior found in the ethogram (Table 1). Due to the limited number of behaviors exhibited by the snails in this study, we decided to instead assess behavior simply based on "active" or "inactive" behaviors (Table 1). For this data, we compiled the total time spent at active behaviors by adding the total time of each behavior coded as "active" in the ethogram (Table 1). We did the same for inactive behaviors. Next, we compiled these terms into a single term called "movement" to capture the relative amount of time spent at active versus inactive behaviors. If the individual in the trial has spent more time performing inactive behaviors, the movement variable was coded as "inactive". If the individual spent more time performing active behaviors, the movement variable was coded as "active". We then ran two Chi Square tests on these data to assess the role of treatment and population on the movement variable. These analyses were performed in R Statistical Software (R Core Team, 2021).

Table 1: Ethogram used within this study with the left side involving the behavioral definition, the middle is the abbreviation used in coding, and the right side is the rating of inactive to active status rating of the behavior.

Behavioral Definition	Abbreviation	Rating	Source
Stationary: when the snail is sunk in the sand, not floating, with closed operculum, and the siphon is not out.	ST	Inactive	Wantanabe et al. 2015
Immobile (fixed): Inactive and fixed on the substrate.	IBF	Inactive	Wantanabe et al. 2015
Immobile (loose): Inactive and not fixed on the substrate.	IBL	Inactive	Wantanabe et al. 2015
Floating with closed operculum: Animal is floating in the water column and the operculum is closed and the siphon is out.	FCO	Inactive	Andrea Adams
Borrowing: When the snail shifts the sand to bury the opening in the sand	BW	Active	Ekschmitt &Albrecht, 2008
Controlled Floating: When the snail is not all the way out, but operculum is open and not closed, the siphon comes out of the shell, when the snail is floating, the siphon is out, and moving in directions.	CF	Active	Ekschmitt &Albrecht, 2008 & Andrea Adams
Exploring with siphon: When is snail is completely out, when the snail is floating, the siphon is out, and moving in directions.	ES	Active	Andrea Adams
Crawling: when snail is completely out and is moving on the substrate.	CW	Active	Ekschmitt &Albrecht, 2008

RESULTS

Initial Arm Choice

Initial choice based on treatment alone was significant across treatments $\chi^2 = 10.834$, df = 4, p = 0.029. However, I was not able to complete pairwise comparisons due to the number of zeroes in the dataset (Table 2). The dataset suggests that the Neutral Zone was the initial choice across all treatments compared to either arm. Initial arm choice based on state (population) yielded no significance $\chi^2 = 0.626$, df = 2, p = 0.731 (Table 3).

Table 2: Shows the number of selected arm choices from the individuals within the trials for the Initial arm choice across all treatment type of analysis. I found that there was significance throughout the dataset.

Treatment	**Neutral**	**No odor**	**Odor**
Attractive	11	0	2
Aversive	10	0	0
Control	10	4	0

Table 3: Shows the number of selected arm choices from the individuals within the trials for the Initial arm choice across all treatment types for state (population) analysis. I found that there was no significance between the two populations.

State/Population	**Neutral**	**No Odor**	**Odor**
AL	17	3	1
FL	14	1	1

Final Arm Choice

Final choice based on treatment alone was also significant ($\chi^2 = 16.648$, df = 4, p = 0.00226). However, I was not able to complete pairwise comparisons due to the number of zeroes in the dataset (Table 4). Final choice based on state (population) also yielded a significant difference (χ^2 = 7.2163, df = 2, p = 0.0271) (Table 5). A follow-up pairwise comparison revealed that Florida snails chose the neutral arm significantly more often than the non-odor arm for final zone choice (p = 0.03).

Table 4: Shows the number of selected arm choices from the individuals within the trials for the Final arm choice across all treatment type of analysis. I found that there was significance throughout the dataset.

Treatment	Neutral	No odor	Odor
Attractive	7	0	6
Aversive	6	1	3
Control	6	8	0

Table 5: Shows the number of selected arm choices from the individuals within the trials for the Final arm choice across all treatment types for state (population) analysis. I found that there was no significance between the two populations.

State/Population	Neutral	No Odor	Odor
AL	7	8	6
FL	12	1	3

Time Spent in Arm Choices

The time spent in the neutral arm was not significant based on treatment type (Deviance = 42.50, df = 2; df Residuals = 34; p = 0.97), state (population) (Deviance = 1167.10, df = 1; df Residuals = 33; p = 0.1956), or the interaction of treatment and population (Deviance = 1407.40, df = 2; df Residuals = 31; p = 0.3642; Figure 3).

Time spent in the odor arm was also not significant based on treatment type ($F = 0.7913$; $df = 1, 19$; $p = 0.3848$), state (population) ($F = 0.0378$; $df = 1, 19$;; $p = 0.8478$), or the interaction of treatment and population ($F = 2.6273$; $df = 1,19$;; $p = 0.1215$; Figure 4).

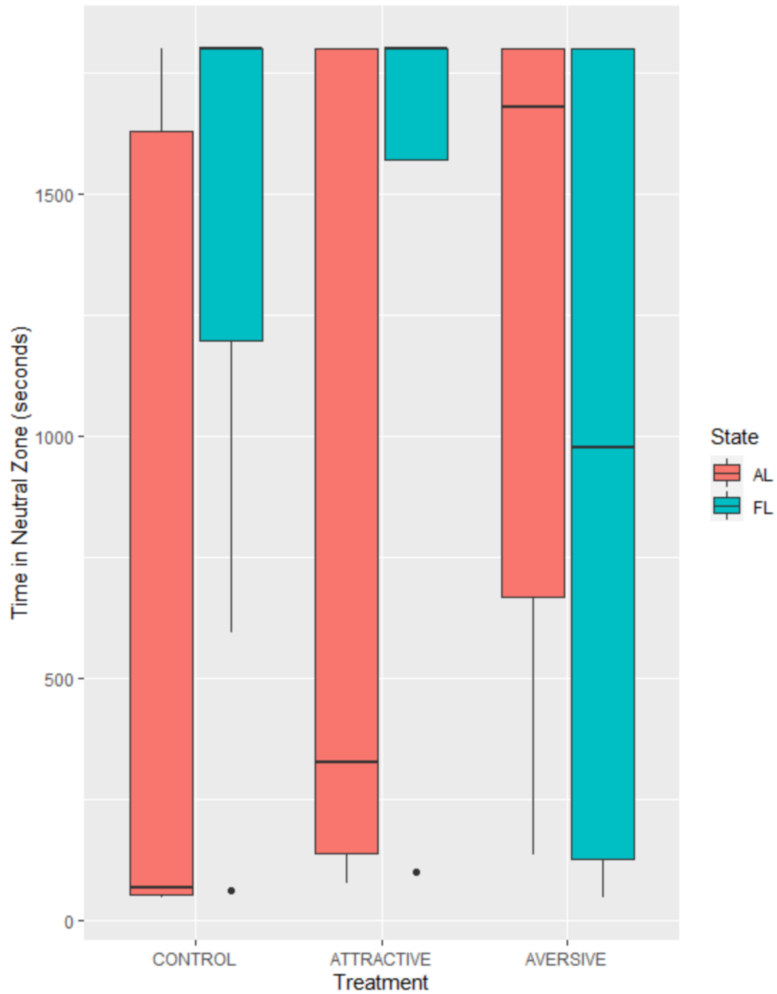

Figure 3: This box plot shows the differences in the amount of time each organism spent in the Neutral zone across treatment types. The x-axis shows the types of treatments whereas, the y-axis shows the total time the individual spent within the neutral arm choice. The red color shows the 1st and 3rd quartile of the amount of time the Alabama population spent in the neutral arm choice. The blue color shows the 1st and 3rd quartile of the amount of time the Florida population spent in the neutral arm choice. The lines coming off the 1st and 3rd quartile are the error bars within the analysis. Here we see that there is no significant between the amount of time spent in the neutral arm choice throughout the treatment types.

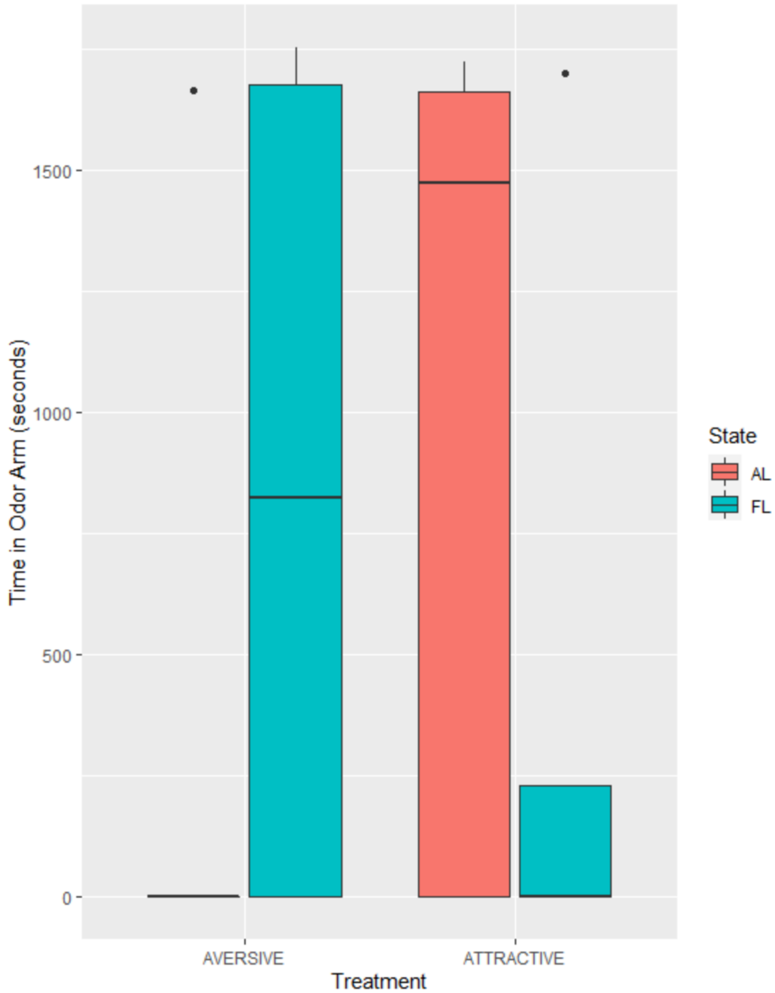

Figure 4: This box plot shows the differences in the amount of time each organism spent in the Odor zone across treatment types. The x-axis shows the types of treatments whereas, the y-axis shows the total time the individual spent within the neutral arm choice. The red color shows the 1st and 3rd quartile of the amount of time the Alabama population spent in the neutral arm choice. The blue color shows the 1st and 3rd quartile of the amount of time the Florida population spent in the neutral arm choice. The lines coming off the 1st and 3rd quartile are the error bars within the analysis. Here we see that there is no significant between the amount of time spent in the neutral arm choice throughout the treatment types. Though there are trends one can make assumptions about the odor treatments.

Movement Analyses

Movement was not significantly impacted by treatment type (χ^2=0.36, df = 2, p = 0.84; Table 6) or by state (population) (χ^2= 3.02, df = 1, p = 0.08; Table 7).

Table 6: Shows the number of selected individuals of movement within the treatment types. There was no significance found throughout this experiment analysis.

Treatment	Active	Inactive
Attractive	4	9
Aversive	3	7
Control	3	11

Table 7: Shows the number of selected individuals of movement within the state (population) analysis. There was no significance found throughout this experiment analysis.

State/Population	Active	Inactive
AL	8	13
FL	2	14

DISCUSSION

Although field observations suggested a behavioral difference between two populations of *P. maculata* this study found very little significant difference in the way they interact with a similar sensory landscape. I have found that initial arm choice had some significance based on treatment type though no follow up tests were done because of so many zeros within the dataset that contributed to no snail movement at any time throughout the trials. Initial arm choice for the Alabama population held no significance even though the population seemed to be more active that the Florida population. Final arm choice yielded the same as initial arm choice in comparison to the populations. Time spent in the different arm choices yielded not significant to the explanation of changes in time spent in the neutral arms and odor arms choice. Though trends could be seen throughout the experiment. Yet, there was no significance within the movement in treatment and state (population) analysis. You could see that the Florida population went towards the aversive odor and the Alabama population with some of the Florida population went towards the attractive stimulus (Figure 3).

First, the lack of difference across the two snail populations could be due to lack of sensitivity to the odor concentrations presented in this study. These behaviors and findings could potentially mean that the Florida population does not do much in the sense of using chemical smells like the odor treatments throughout this experiment to assist in their survival and fitness in their adult lifestyles when most of the literature mainly focuses on hatchlings (Burks et al., 2017, Ueshima and Yusa, 2015). Hence, the fact they stayed within the neutral zone throughout all treatments. It could potentially be that the odors induce an inactive response to the odors through means of experience this has also been seen in other aquatic species like the marine mud snail (Atema and Stenzler, 1977). They found that the mud snail shows a dramatic self-burial response in the presence of crushed conspecifics. Whereas this Florida population could have an inactive response to rely heavily on the shells protection they create. There could also be a potential possibility that this population has selected an inactive response in their life history throughout evolution this has been seen through other species of apple snails like *P. bridgesii* selected to have a behavioral response to different water levels (Watanabe et al., 2015). More studies need to be done in this area to see if it holds any significance. When looking at the Alabama population, you can see trends in the fact that they do respond to chemical stimuli by inactive movements to the inversive stimulus and more of an active response to the attractive stimulus (Figure 4) even though no significance was found. This could potentially be due to a life history shift due to them out growing their respected predators within the means of a newer population of Alabama (Burks et al., 2017). This has been documented in Burk et al. (2017) where the red-eared slider and red swap crayfish primarily feed on smaller apple snails whereas, Lumpkins and the snail kite feed on the larger snails. For the Alabama population sample site, we did not encounter lumpkins nor snail kites and only saw evidence of borrowing crayfishes for susceptible predators. More research needs to be done to see the population structure of different invasive species to see if this could potentially expose evidence of different life history traits seen throughout the same species but in different areas (Thomaz et al., 2015).

Another explanation for these unexpected findings could be varied behavioral reactions to odors we assumed to be aversive to all *Pomacae* snails. For instance, it has been seen that different life stage within a population has caused shifts in what the organisms perceive as their diet due to invasive species (Wood et al., 2017) Within my study, the snails of the Florida population were significantly bigger than the Alabama population. This size difference could correspond to variation in age and, consequently, diet for these two populations. This could explain why the Florida population went towards the aversive stimulus. The larger snails may perceive dead snail odors as a potential food source (i.e., an attractive stimulus) rather than an odor to avoid (i.e., an aversive stimulus). Another possible explanation as to why the Florida population did not do much in the sense of movement/behavior could be due to a shift in the way the apple snails perceive the different odors we selected. Meaning the risk of being seen cost too much, in the sense of predation threats around the area, to be able to expand energy to search for food sources when the area is so rich in the source. Hence, the fact that they normally stayed within the neutral area when introduced with the food odor and predation odor. There have been studies that suggest/observed that the bigger snails within populations have consumed hatchlings (Burks et al., 2017, Fang et al., 2010). Thus, movement towards the predation odor within my study could help with this assumption. Burks et al. (2017) observed that a larger snail would eat other conspecifics when the calcium carbonate available for shell building is low. This could potentially explain the weird trends in choices that the Florida population chose between the odor arm in the aversive treatments. Those used in this study that went towards the predation odor were those of bigger snails that were found. Yet, no study has been done on this to give it any significance. There is also a size difference to potentially consider through these observations, findings in this study, and within the known literature. This can be seen in many different aquatic species like crayfish where they consume each other or become more aggressive towards one another (Vetter et al., 2021). It seems that the larger snails tend to be inactive and not mobile when presented with predation odor, yet they fixated on inactive behaviors but still moved around with the siphon out. The smaller snails tend to exhibit going above the water line and staying there until the predation odor has stopped (Burk et al., 2017).

More studies that interpret the snail chemical landscape could potentially help conservationists understand how to further manage these snails through the means of their life history. If conservationists can find a way to interrupt or manipulate the important chemical stimuli these snails find as a “big motivator”, perhaps they can reduce invasive snail populations without harming the macrophytes and surrounding species within the same habitat. For example, conservationists have found a management concept in using “Bayluscide” by Bayer (17.1% niclosamide) are toxic to the snails, but it did not appear to harm the red swamp crayfish (*P. clarkii*) that live in the same habitat as the snails. Meaning that there is a way to get rid of the invasive apple snails without harming other species within the environment in which they inhabit (Burk et al., 2017). More studies on these snails’ chemical landscape could also tell us how to manage the population through the means of tropic interactions and education of different populations could potentially help with understanding the “how” and “why” certain habitats get over run quickly by these organisms. This could potentially lead us to a faster means of getting rid of stable populations as well as starting populations.

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APPENDIX A

Appendix A contains the raw data generated from this project.

State	Treatment	Treatment Code	Animal ID	Initial Zone Choice	Final Zone Choice	Total time in Neutral	Total time No odor	Total time Odor	Total time ST	Total time IBF	Total time IBL	Total time FCO	Total time Inactive	Total time CF	Total time CW	Total time Active	Movement
FL	ATTRACTIVE	FLT2	CL22	Neutral	Neutral	1571	0	229	0	829	0	360	1189	567	0	567	Inactive
FL	ATTRACTIVE	FLT2	CL24	Odor	Odor	99	0	1701	0	1541	0	0	1541	1109	12	1121	Inactive
FL	ATTRACTIVE	FLT2	CL26	Neutral	Neutral	1800	0	0	1800	0	0	0	1800	0	0	0	Inactive
FL	ATTRACTIVE	FLT2	CL29	Neutral	Neutral	1800	0	0	1773	27	0	0	1800	0	0	0	Inactive
FL	ATTRACTIVE	FLT2	CL32	Neutral	Neutral	1800	0	0	412	0	0	0	412	0	0	0	Inactive
FL	AVERSIVE	FLT1	CL31	Neutral	Neutral	1800	0	0	1800	0	0	0	1800	0	0	0	Inactive
FL	AVERSIVE	FLT1	EB11	Neutral	Neutral	1800	0	0	0	0	0	0	0	1800	0	1800	Active
FL	AVERSIVE	FLT1	EB14	Neutral	Odor	46	0	1754	0	0	604	0	604	343	0	343	Inactive
FL	AVERSIVE	FLT1	EB5	Neutral	Odor	151	0	1649	0	0	1173	0	1173	627	0	627	Inactive
FL	CONTROL	FLC	CL30	Neutral	Neutral	1800	0	0	0	1800	0	0	1800	0	0	0	Inactive
FL	CONTROL	FLC	EB5	No odor	No odor	60	1691	0	0	42	28	0	70	1730	0	1730	Active
FL	CONTROL	FLC	EB10	Neutral	Neutral	1800	0	0	0	0	1800	0	1800	0	0	0	Inactive
FL	CONTROL	FLC	EB11	Neutral	Neutral	1800	0	0	0	0	1800	0	1800	0	0	0	Inactive
FL	CONTROL	FLC	EB14	Neutral	Neutral	1800	0	0	0	0	1800	0	1800	0	853	853	Inactive
FL	CONTROL	FLC	EB8	Neutral	Neutral	592	0	0	62	0	1024	0	1086	0	78	78	Inactive
FL	CONTROL	FLC	EB16	Neutral	Neutral	1800	0	0	71	1729	0	0	1800	0	0	0	Inactive
AL	CONTROL	MTC	MT29	No odor	No odor	53	1747	0	673	1127	0	0	1800	0	0	0	Inactive
AL	CONTROL	MTC	MT4	Neutral	No odor	1800	0	0	1800	0	0	0	1800	0	0	0	Inactive
AL	CONTROL	MTC	MT47	Neutral	No odor	1800	0	0	129	0	0	111	240	0	1560	1560	Active
AL	CONTROL	MTC	MT5	No odor	No odor	46	1754	0	135	0	1665	0	1800	0	0	0	Inactive
AL	CONTROL	MTC	MT1	Neutral	No odor	66	1734	0	0	0	0	0	0	0	1562	1562	Active
AL	CONTROL	MTC	MT36	No odor	No odor	52	1748	0	0	1717	83	0	1800	0	0	0	Inactive
AL	CONTROL	MTC	MT49	Neutral	No odor	1456	344	0	0	560	656	0	1216	0	0	0	Inactive
AL	AVERSIVE	MTT1	MT34	Neutral	No odor	373	1427	0	0	0	52	361	413	23	1364	1387	Active
AL	AVERSIVE	MTT1	MT2	Neutral	Odor	136	0	1664	0	0	395	136	531	0	1269	1269	Active
AL	AVERSIVE	MTT1	MT23	Neutral	Neutral	1800	0	0	1501	0	0	299	1800	0	0	0	Inactive

AL	AVERSIVE	MTT1	MT17	Neutral	Neutral	1800	0	0	495	0	0	1305	1800	0	0	0	Inactive
AL	AVERSIVE	MTT1	MT38	Neutral	Neutral	1554	246	0	0	1209	260	63	1532	0	155	155	Inactive
AL	AVERSIVE	MTT1	MT45	Neutral	Neutral	1800	0	0	0	1233	0	56	1289	0	511	511	Inactive
AL	ATTRACTIVE	MTT2	MT2	Neutral	Odor	136	0	1664	0	0	197	314	511	0	1289	1289	Active
AL	ATTRACTIVE	MTT2	MT31	Neutral	Odor	373	0	1427	0	0	72	341	413	23	1364	1387	Active
AL	ATTRACTIVE	MTT2	MT13	Neutral	Neutral	1800	0	0	0	0	601	100	701	1099	0	1099	Active
AL	ATTRACTIVE	MTT2	MT16	Neutral	Neutral	1800	0	0	1390	0	0	158	1548	0	252	252	Inactive
AL	ATTRACTIVE	MTT2	MT51	Neutral	Odor	281	0	1519	0	0	1800	0	1800	0	0	0	Inactive
AL	ATTRACTIVE	MTT2	MT49	Neutral	Odor	140	0	1660	0	295	1296	0	1591	150	59	209	Inactive
AL	ATTRACTIVE	MTT2	MT37	Odor	Odor	77	0	1723	0	0	142	429	571	0	1215	1215	Active
AL	ATTRACTIVE	MTT2	MT44	Neutral	Neutral	1800	0	0	0	1336	0	165	1501	0	219	219	Inactive