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Effects of short-term exposure to a pesticide mixture on free-swimming behavior in goldfish, *Carassius auratus*☆

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**A R T I C L E   I N F O**

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**A B S T R A C T**

The prevalence of pesticides in the aquatic environment continues to increase due to anthropogenic activities and poses a threat to aquatic organisms. Notably, the intensive use of pesticides can cause detrimental effects (i.e., chemical stressors) on animal behavior. The aim of this study was to determine the short-term exposure effects (5-day) of an environmentally relevant pesticide mixture (low- and high-dose: metolachlor 2.4 and 12 μg/L; linuron 2.0 and 10 μg/L; isoproturon 1.2 and 6.0 μg/L; tebucanazole 1.2 and 6.0 μg/L; alachlor 0.8 and 4.0 μg/L; atrazine 0.4 and 2.0 μg/L; pendimethalin 0.4 and 2.0 μg/L; azinphos-methyl 0.8 and 4.0 μg/L) on fish swimming behaviors. Results indicated that a low dose of pesticide mixture decreased the distance swam in goldfish. Spatial behavior use was also altered during short-term pesticide exposure, with increased time spent in the lowest horizontal region in aquariums in both low- and high-dose exposure groups. In vertical spatial use analysis, all exposure groups showed lowered amounts of time spent in the middle region in aquariums, especially in high-dose exposure groups. Collectively, these data indicate that short-term exposure to pesticide mixture significantly alters free-swimming behavior in teleost species.

1. Introduction

Pesticides are of major concern due to their widespread global distribution in terrestrial and aquatic environments (Singh et al., 2023). These compounds seep into the soil following rainfall events, causing them to infiltrate groundwater reservoirs years after their discontinuation, including the detection of atrazine up to 27 years after its last use (Ghirardelli et al., 2021; Singh et al., 2023). In addition to percolation, runoff and pesticide drift (i.e., pesticide movement through the air) have also contributed to increased pollutant levels within water bodies (Walklate 1992; Yan et al., 2022). Through these mechanisms, agricultural chemicals infiltrate bodies of water, introducing various chemicals, such as pesticides, organic compounds, heavy metals, and other pollutants (Donohue et al., 2005; Ullah and Zorrie-zahra, 2015). Studies have inferred that aquatic organisms may accumulate chemicals in water more readily than terrestrial organisms (Willis and McDowell, 1982; Clasen et al., 2018). For example, Coats et al. (1989) found that synthetic pyrethroids are more toxic to aquatic organisms, such as fish than birds and mammals. This brings to light another major concern of widespread environmental pollution and provides grounds for the careful consideration of their effects on aquatic ecosystems (Willis and McDowell, 1982; Clasen et al., 2018).

Although toxic chemicals found in natural ecosystems are generally sublethal, there are further deleterious effects that can be observed with prolonged exposure, such as alteration in reproductive, social, and predator avoidance behaviors (Scott and Sloman, 2004). Many studies have found that fish exhibit negative behavioral changes when exposed to chemical stressors (Jabeen et al., 2018; Pandit and Gupta, 2019; Lacy et al., 2023). Behavioral change can be induced by physiological stress or psychological stress including xenobiotic exposure (i.e., pesticides), high temperature, or exposure to hypoxic conditions (Israeli and Kimmel 1996; Cerquiera et al., 2021; Lacy et al., 2023). Contamination at sublethal levels may lead to 'ecological death' when complex behavior is disrupted, compromising individual survival, growth, and reproduction (Martin et al., 2007; Scott and Sloman, 2004; Gandar et al., 2016). Thus,
based on behavioral responses, an individual population can be evaluated for deleterious effects of chemical stressors (Ford et al., 2021).

There is a growing awareness that even low concentrations of environmental pollutants (e.g., pesticides, PCB, DDT, etc.) may cause sublethal changes in physiology and behavior important for the survival and reproduction of aquatic organisms (Filice et al., 2022; Lacy et al., 2023). Because the behavior of an organism responds to physical, chemical, and social interactions; it is, therefore, a critical aspect of understanding plausible destructive effects of environmental contamination on animal behavior (fish, shellfish, etc.; Connell and Miller, 1984; Kane et al., 2005; Gandar et al., 2016; Fitzgerald et al., 2021).

Importantly, fish behavior is a promising model and valuable study tool in toxicological research, as well as neuroscience research due to ‘quantifiable actions’ operating through the central and peripheral nervous system (Hong and Zhu, 2019; Fu et al., 2022). These lead to behavioral disruption of reproduction, feeding, and predator avoidance in fish, due to toxin accumulation and alteration of their biological processes (Kane et al., 2005; Melvin and Wilson, 2013; Sandoval-Herrera et al., 2019). Notably, behavioral patterns are systematic and somewhat predictable (designed to fulfill the organism’s needs for fitness, survival, and adaptation); therefore, modulations in the activities provide quantifiable endpoints for study (Little and Brewer, 2001; Halappa and David, 2009; Kane et al., 2005; Melvin and Wilson, 2013).

Recent publications have added to the growing body of evidence demonstrating that long-term and short-term exposure to pesticide mixtures alter fish behavior (Bonanza et al., 2016; Narra, 2016; Lacy et al., 2023). Behavioral changes have been observed in Walking Catfish (Clarias batrachus) exposed to endosulfan, carbofuran, methyl parathion, and cypermethrin, including inactivity, bursts of hyperactivity, and aberrant swimming behaviors (Narra, 2016). Similarly, in Common Goldfish (Carassius auratus) exposed to a pesticide cocktail reduced distance swim, increased cumulative actionless time, and variations in evolutionary development (Ota and Abe, 2016). Furthermore, in toxicological variations that can be used to understand domestication and evolutionary development (Ota and Abe, 2016). Additionally, they are a well-studied species with a wide array of genetic and morphological variations that can be used to understand domestication and evolutionary development (Ota and Abe, 2016). Furthermore, in toxicological settings, goldfish has been extensively studied, leading to our selection of it as our model organism for this research (Xia et al., 2013; Jacquin et al., 2019; Lacy et al., 2022; Lacy and Rahaman, 2022, 2023).

2.2. Experimental animals

One hundred fifty common goldfish (Carassius auratus) were obtained from local markets and transported to the laboratory facility at the University of Texas Rio Grande Valley (UTRGV) Brownsville campus. Fish (mixed-sex, average size, length: 4.5 ± 0.8 cm, body weight: 1.6 ± 0.08 g) were then stocked in six glass aquariums (25 fish/aquarium; capacity of 20-gallon each, approximately 75 L) with reverse osmosis freshwater. Reverse osmosis water was used due to known contamination by agricultural runoff, municipal waste, and factory discharges in local water sources (Sexton et al., 2013; Sexton and Salinas, 2014). The optimum temperature for goldfish (22 ◦C) was achieved using a submersible aquarium heater. Water quality parameters such as temperature, pH level, and dissolved oxygen concentrations were monitored with a YSI probe (YSI Professional Plus, Yellow Springs, OH, USA) daily. There were no significant changes in physio-chemical parameters during the experimental period. Fish were fed commercial goldfish pellet daily (~3% body weight/day) and acclimated for 4 weeks under controlled laboratory conditions (day-night cycle: 12:12 h) before pesticide exposure.

2.3. Pesticides exposure experiment

Pesticidal compounds for mixture toxicity were selected based on a literature review, where it was found that pesticidal compounds (metolachlor, linuron, isoproturon, tebucanozole, aclonifen, atrazine, pendimethalin, and azinphos-methyl) were commonly detected simultaneously in aquatic environments (Kreuger, 1998; Graymore et al., 2001; Battaglin and Fairchild, 2002; Dabrowski et al., 2006; Loewy et al., 2011; Polard et al., 2011; Taghavi et al., 2011; Debenest et al., 2010; Climent et al., 2019; Jacquin et al., 2019).

A total of six aquaria (25 fish/aquarium) were used for this experiment, two aquaria for control (no pesticides), two for low-dose, and another two for high-dose pesticide mixture. Fish were exposed 5 days to an environmentally relevant pesticide mixture (low dose: S-metolachlor 2.4 μg/L, linuron 2.0 μg/L, isoproturon 1.2 μg/L, tebucanozole 1.2 μg/L, aclonifen: 0.8 μg/L, atrazine: 0.4 μg/L, pendimethalin 0.4 μg/L, and azinphos-methyl 0.8 μg/L; and high dose: S-metolachlor 12 μg/L, linuron 10 μg/L, isoproturon 6.0 μg/L, tebucanozole 6.0 μg/L, aclonifen 4.0 μg/L, atrazine 2.0 μg/L, pendimethalin 2.0 μg/L, and azinphos-methyl 4.0 μg/L). Water changes (~25%) were conducted every other day and re-spiked with pesticides in the aquarium according to the methods described by Jacquin et al. (2019). All analytical-grade pesticides were purchased for MilliporeSigma (Burlington, MA, USA). The dose and pesticide mixtures were applied in this experiment according to Jacquin et al. (2019) and Lacy et al. (2023). All exposure experiments were conducted with the approval of the University of Texas Rio Grande Valley’s Institutional Animal Care and Use Committee (protocol# AUP-22-09).

2.4. Collection of behavioral data

Behavioral data endpoints for 5 individual goldfish per aquarium were collected according to Xia et al. (2013) and Lacy et al. (2023) with minor modifications. Open-field testing is one of the most common analyses for animal behavior (Choleris et al., 2001; Thore et al., 2021). This testing was not used, however, due to the daily measurement of fish behavior within the experimental design, and concerns that daily handling stress and habituation to testing could interfere with the results (Walsh and Cummins, 1976; Sijisimondi and Weber, 1988). Briefly, fish were filmed daily over the 5-day experimental period using GoPro App (GoPro Inc., San Mateo, CA, USA) with a GoPro Hero 4 Silver camera (GoPro Inc.) connected via Bluetooth to an iPhone 7 (Apple Inc.,...
Cupertino, CA, USA). Filming was conducted over a 5-min period for each aquarium; between each recording, there was a 15-min period to wait for the normalization of fish behavior after entering the experimental lab to change camera orientation (Xia et al., 2013). The camera was mounted on a stand 28 cm from each aquarium, oriented towards one side. All aquariums were divided into 9 sections of 10.16 cm each with markings on each side to provide landmarks for observations. For the observer to have additional frames of reference while viewing the film, standard rulers were attached to the top and sides of all aquariums (Fig. 1A–C).

Two observers independently calculated the total distance swam for 5 uniquely identified fish per aquarium in the 5-min video using Windows Photos App (Microsoft, Redmond, WA, USA), slow-motion videos were created to ease the collection of behavioral data. Distance measurements were collected using a 10.16 cm grid to determine an individual fish’s movement (distance in cm) based on reviewing footage and following its path over the duration of the five-minute video. For the purpose of this experiment, swimming is defined as a state of intentional motion, characterized by movement of the fins (Sfakiotakis et al., 1999). During the experiment, five individual fish per tank were strictly observed for behavioral parameters, while the remaining fish remained in the tank to mirror a natural environment. Photo identification cards were created for each fish (identified based on physical features such as coloration, patterns, and spots) to enable observers to correctly identify and follow the same fish frame by frame throughout the videoed observation periods. This method was used to facilitate fish ID, as seen in previous literature (Germanov and Marshall 2014; Dala-Corte et al., 2016; Cerutti-Pereyra et al., 2018). The distance swam (measured in cm) for each experimental day was determined by recording the number of crossovers of the fish to new zones in the aquarium. Crossovers were defined as greater than 50% of the fish’s body entering a new zone of the aquarium (Xia et al., 2013; Lacy et al., 2023). Obtaining the average distance swam data over observation periods helps to determine the exposure time and the dose of contamination that influences the fish’s movement.

Spatial behavioral use (SBU, e.g., horizontal SBU and vertical SBU) was calculated by monitoring each of the 5 individual fish selected for the study over 1 min and recording how much time (sec) they spent in each of the nine regions (Lacy et al., 2023). The time spent in the regions was measured by calculating the time spent within that region starting from a crossover into the region (with at least 50% of the body) and ending when the fish exited the region (with at least 50% of the body) (Lacy et al., 2023). These measurements were repeated across replicates and compared between exposure groups and controls. These zones were further categorized into six regions as illustrated in Figs. 2A–C and 3A–C. Time spent in each region was analyzed by converting the time spent in each zone into the percentage of total observation time and evaluating the movement between horizontal sections and movement between vertical sections separately.

SBU analysis was used to identify changes in fish swimming behavior induced by low- and high-dose of pesticide mixture. Notably, SBU studies also focus on where contaminants may be present and the response of populations to those chemicals (Aranjio and Blasco, 2019). This method used in our study specifically seeks to elucidate whether the distribution of fish within the water column is altered, as opposed to measuring whether an individual fish’s swimming behavior is disrupted (Lacy et al., 2023). In this study, SBU was divided into horizontal and vertical sections in accordance with Hong and Zha (2019) and Lacy et al. (2023). Horizontal sections divided aquariums between upper, middle, and lower water columns (regions 1, 2, and 3, respectively), measuring how much time fish spent in each area of the aquarium determined how they used the vertical space/depth of water. Vertical sections divided the aquarium between left, middle, and right, (regions 4, 5, and 6, respectively) determining how they made use of the horizontal space. The baseline was determined by using the control groups.

**Fig. 1.** Effects of short-term pesticides exposure on distance swam of goldfish. Representative pictures of aquarium setup: control (A, no pesticides), low dose (B) and high dose (C) of pesticides mixture. (D-H) Distance swam of goldfish during day-1 (D), day-2 (E), day-3 (F), day-4 (G), and day-5 (H). Each value represents the mean ± SEM (N = 10). Different letters above the bar graph represent a significant difference (one-way ANOVA with Tukey’s test, P < 0.05). Student t-test further identified significant differences, shown as an asterisk (* P < 0.05).
2.5. Statistical analysis

To determine significant differences, a one-way ANOVA was performed followed by Tukey’s test for multiple comparisons. Student t-test was also performed for unpaired comparisons. A statistical significance was recognized when \( P < 0.05 \). All statistical analyses were conducted in GraphPad Prism (GraphPad, San Diego, CA, USA). All data are presented as mean ± standard error of the mean (SEM).

3. Results

3.1. Effects of pesticide mixture on distance swam

In the control group (CTL), no significant changes were observed in distance swam (DS) throughout the experimental period (Fig. 1D-H). On day-1, there was no significant difference in DS between CTL and LD groups; however, fish in HD exposure groups, the DS was significantly (\( P < 0.05 \)) shorter than CTL (\(~2.22\)-fold) or LD (\(~1.93\)-fold) exposure groups (Fig. 1D). On day-2, CTL and LD (\(~2.32\)- and \(~1.8\)-fold) groups were not significantly different; however, HD group demonstrated significant (\( P < 0.05 \)) reduction in DS (Fig. 1E). The DS on day-3 demonstrated a decrease around 1.54 and \(~1.5\) fold in LD and HD exposure groups, respectively, compared with CTL (Fig. 1F). On day-4, there were no significant differences in DS in CTL, LD, and HD exposure groups (Tukey’s test); however, Student’s t-test demonstrated that both LD and HD exposure groups were significantly (\( P < 0.05 \)) decreased in DS (\(~1.44\)-fold in LD and \(~1.45\)-fold in HD) compared with CTL (Fig. 1G). On day-5, DS significantly decreased (\( P < 0.05 \)) around 1.29-fold in LD and \(~1.49\)-fold in HD exposure groups compared with CTL (Fig. 1H).

3.2. Effects of pesticide mixture on horizontal spatial behavioral use

The horizontal spatial behavioral use (SBU) as illustrated in Fig. 2 helps to measure the difference in movement/SBU of fish moving vertically (up/down) in the water column (Fig. 2A-C). This metric determines whether fish spent the most time in the bottom, central, or upper section within the aquarium, as illustrated in Fig. 2A-C. In CTL groups, the time spent in region 3 (lower region) increased significantly (\( P < 0.05 \)) from days 1–3, day-3 had the highest time spent in region 3 (Fig. 2D). Over time, it demonstrated a decrease in days 3–4 and was stable from days 4–5. In CTL, time spent in region 2 (central region) decreased from days 1–3 with the least amount of time spent in region 2 on day 3. There was a subsequent increase recorded from days 3–5. Region 1 (upper region) decreased from days 1–3 and 4–5 but increased from days 3–4 (Fig. 2D).

In the LD exposure groups, time spent in region 3 was significantly (\( P < 0.05 \)) higher compared to the CTL group in days 1–2 (Fig. 2E). Day-1 had the highest time spent in region 3 compared to regions 1 and 2. Time spent in region 3 demonstrated a decline between days 1–2 and 3–4, while there was an increase from days 2–3. In regions 1 and 2, there was a similar trend with more time being spent in region 2 than region 1. In the LD exposure group, there was an increase in time spent in region 2 between days 1–2 and 3–4 and a decrease in time spent in days 2–3 and 4–5. Furthermore, in LD exposure groups, time spent in region 1 increased between days 1–2 and 3–5 but decreased from days 2–3 (Fig. 2E).

In the HD exposure groups, the greatest percentage of time was spent in region 3 (Fig. 2F). In days 1–3, time spent in region 3 was stable, and the vast majority of time was spent within this region. Time spent within region 3 decreased between days 3–5 in the HD exposure groups. In the HD groups, time spent in region 2 was stable within days 1–3 and increased between days 3–5. Similarly, region 1 followed the same trend, with consistently low time spent in days 1–3, but increased between days 3–5 (Fig. 2F).
3.3. Effects of pesticides mixture on vertical spatial behavioral use

The vertical SBU illustrated in Fig. 3 helps quantify how fish move and spend time vertically (left to right) in the water column (Fig. 3A–C). On CTL day-1, fish spent most the of time in region 4; by day-5, the fish spent most of the time in region 6 (Fig. 3D). Time spent in region 4 remained stable from days 1–2 but fell from days 2–5. In all exposure groups, the time spent in region 5 was low in comparison with other regions. This effect was more pronounced in HD exposure groups (Fig. 3D).

In the LD exposure groups, time spent in region 4 was highest on day-1, but decreased between days 1–2 and 4–5; however, it subsequently increased from days 2–4 (Fig. 3E). Region 5 had the lowest overall time spent and lowered from days 1–2 and 3–4 with increased in days 2–3. In LD exposure groups, time spent in region 6 increased sharply from days 1–2 and increased from days 3–5; however, experienced a decrease from days 2–3 (Fig. 3E).

In the HD exposure groups, the time spent in region 4 increased from days 1–2 and 3–5; whereas it decreased from days 2–3 (Fig. 3F). In this group, fish occupied region 5 the lowest percentage of time consistently over the course of the study period. During the exposure period, time spent in region 5 HD exposure groups oscillated, increasing from days 1–2 and 3–4, and decreasing from 2 to 3 and 4–5; however, none of the changes in time spent in region 5 over the course of this study were significant (Fig. 3F).

4. Discussion

Behavioral parameters are an essential tool to determine detrimental effects toxicants may induce within organisms, such as teleost species (Chagas et al., 2021; da Luz et al., 2023). Fish behaviors can be very sensitive indicators of sublethal chemical exposure (Atchison et al., 1987; Little and Finger, 1990; Sharma et al., 2019). Studies have developed tests to evaluate changes in predator avoidance, feeding behavior, learning, social interactions, emotional, and locomotory behaviors in fish as a response to changes in the environment (Adelman et al., 2019; Atchison et al., 1987; Cobcroft and Battaglene, 2009; Xia et al., 2013; Fitzgerald et al., 2021; Fu et al., 2022). In this study, swimming behaviors were analyzed to quantify the changes that environmentally relevant concentrations of pesticide mixture induced in goldfish (a model teleost species) during short-term exposure (5 days). There was an observed dose-dependent effect of pesticide mixture on free-swimming behavior. Swimming behaviors, including distance swam (DS), are classified as one of the most important behaviors for survival (Little and Finger, 1990; Castro-Santos, 2003). An important finding of this study is that the DS in goldfish was significantly altered by pesticide exposure (5 days) and decreased in a dose-dependent manner with increasing pesticide concentrations. These findings are in agreement with Lacy et al. (2023) who found decreased DS in goldfish chronically exposed to pesticides mixture (e.g., metolachlor, linuron, isoproturon, tebucanozole, aclonifen, atrazine, pendimethalin, and azinphos-methyl) at 1, 2-, 3-, and 4-week exposure times in both low- and high-dose of pesticides. Our results expand upon this research and demonstrate that even under acute exposure DS can be altered, with goldfish showing a significant reduction in DS on day-1 of exposure in HD exposure groups. A previous study by Pereira et al. (2012) also recorded a decrease in DS in zebrafish exposed to 4 days of endosulfan. Furthermore, Beauvais et al. (2000) demonstrated that rainbow trout exposed to 1 and 4 days of malathion and diazinon pesticides both exhibited decreased DS in correlation with increasing pesticide concentration. Overall, these studies support our results; both short- and long-term exposures to environmentally relevant concentrations of pesticide mixture drastically reduce DS in teleost fishes.

Arguably, the most significant finding of this study is that fish exposed to both low and high concentrations of pesticide mixture spent a greater percentage of time in the lowest section of the aquarium,
especially within the bottom corners. Interestingly, in HD exposure, fish appeared to group more densely within the bottom left corner. In control groups, however, fish were more evenly distributed within the water column and spent a greater percentage of time in the middle and top areas of the tank. These results agree with previous spatial behavior studies (Araújo and Blasco, 2019; Lacy et al., 2023). Shinn et al. (2015) reported a similar spatial distribution in rainbow trout exposed (5-day) to atrazine, linuron, and metolachlor at low concentrations (4.5, 4.9, and 13.4 µg/L, respectively). Furthermore, Lacy et al. (2023) found that goldfish chronically exposed to the same pesticide mixture (i.e., metolachlor, linuron, isoproturon, tebuconazole, azolinfen, atrazine, pendimethalin, and azinphos-methyl) spent the highest percentage of time in the middle and lowest regions of the aquarium. Our results expand upon these previous studies by examining the effects of spatial use under short-term exposure regimens. Additionally, our results expand upon a relative lack of available information on the lateral preference of fish (movement left vs right) within the water column under exposure to pesticides.

Lateral preference of fish examines which region fish prefer to spend time in, generally whether fish prefer outside regions of aquaria or central regions of aquariums (Eissa et al., 2006). While preferences for time in, generally whether fish prefer outside regions of aquaria or pesticides. than 20% of the time on region 5 (middle column) with an even lower (movement left vs right) within the water column under exposure to relative lack of available information on the lateral preference of fish behaviors, known to negatively impact the profitability of aquacultured fish through increasing the instances of injuries and deformities in captive fish (Cobcroft and Battaglene, 2009; Noble et al., 2012). Our results demonstrate that fish in LD and HD exposure groups spent less than 20% of the time on region 5 (middle column) with an even lower percentage in the high-dose exposure group (<15%). This demonstrates that while goldfish do not prefer the center of the aquarium, their avoidance of that area increases under conditions of pesticide exposure. By contrast, Eissa et al. (2006) reported no significant differences in fish lateral preference when exposed to cadmium. Differences between these studies underscore the need for further research into the lateral spatial preference of fish. In our study, fish in all groups showed a preference for regions 4 and 6 (representing the left and right sides of the aquarium); however, the notable avoidance of the open center area of the tank could indicate a behavioral stress response. In a similar exposure study, salmonids exposed to the antiaspant fungicide 2(thiocyanomethylthio) benzothiazole (TCMTB) showed shelter-seeking behavior (Schreck et al., 1997). Goldfish preference for outside corners closest to the glass (regions 4 and 6) may be an indication of seeking shelter response to the pesticide mixture; however, more studies are required to confirm this observation (Schreck et al., 1997). Furthermore, in exposure groups due to the higher affinity for regions 3, 4, and 6, goldfish demonstrated more physical closeness (Fig. 2, Fig. 3, and graphical abstract). This indicates that further studies could be designed to evaluate whether exposure to pesticide mixtures affects sociability, another important behavioral endpoint (Cote et al., 2010). To our knowledge, no other previous studies provided data on short-term exposure to pesticides for lateral spatial preference.

5. Conclusion

This study provides the first clear evidence of short-term exposure to environmentally relevant pesticide mixture on free-swimming behavior in goldfish. Goldfish showed significant sensitivity to the exposure of the environmentally relevant pesticide mixture in a short period (5 days).

The authors have read and approved the submission of the final version of the manuscript.

Consent for publication

The authors have read and approved the submission of the final version of the manuscript.

Ethical statement

Laboratory experimental protocols including fish husbandry and euthanasia protocols were approved by the UTRGV Institutional Animal Care and Use Committee (protocol# AUP-22-09).

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CRediT authorship contribution statement

Esmirna Cantu: Conceptualization, Methodology, Validation, Formal analysis, Writing – original draft. Michelle Rivera: Conceptualization, Methodology, Validation, Formal analysis, Writing – review & editing. Brittney Lacy: Conceptualization, Methodology, Writing – review & editing. Md Saydur Rahman: Conceptualization, Methodology, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that there are no competing interests in the completion of this research work.

Data availability

Data will be made available on request.

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