RESEARCH ARTICLE

Larval mannitol diets increase mortality, prolong development and decrease adult body sizes in fruit flies (Drosophila melanogaster)

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ABSTRACT

The ability of polyols to disrupt holometabolous insect development has not been studied and identifying compounds in food that affect insect development can further our understanding of the pathways that connect growth rate, developmental timing and body size in insects. High-sugar diets prolong development and generate smaller adult body sizes in Drosophila melanogaster. We tested for concentration-dependent effects on development when D. melanogaster larvae are fed mannitol, a polyalcohol sweetener. We also tested for amelioration of developmental effects if introduction to mannitol media is delayed past the third instar, as expected if there is a developmental sensitive period for mannitol effects. Both male and female larvae had prolonged development and smaller adult body sizes when fed increasing concentrations of mannitol. Mannitol-induced increases in mortality were concentration dependent in 0 M to 0.8 M treatments with mortality effects beginning as early as 48 h post-hatching. Larval survival, pupariation and eclosion times were unaffected in 0.4 M mannitol treatments when larvae were first introduced to mannitol 72 h post-hatching (the beginning of the third instar); 72 h delay of 0.8 M mannitol introduction reduced the adverse mannitol effects. The developmental effects of a larval mannitol diet closely resemble those of high-sugar larval diets.

This article has an associated First Person interview with the first author of the paper.

KEY WORDS: Nutrition, Polyol, Body size, Development

INTRODUCTION

Duration of development and adult body size are controlled by three related variables in holometabolous insects: growth rate, critical weight (the point at which the developmental period is no longer affected by resource levels), and the interval to the cessation of growth (Davidowitz and Nijhout, 2004; De Moed et al., 1999). Because size and development time are controlled by the same three parameters, a direct, positive relationship is expected and typically observed between developmental duration and adult body size (Blanckenhorn, 1998; Blueweiss et al., 2013; Nijhout et al., 2010; Roff, 2000; Thomas, 1993; Zwaan et al., 1992). However, some environmental variables can differently affect growth rate, critical weight and interval to the cessation of growth, causing neutral or even negative relationships between body size and development time (De Moed et al., 1999; Nijhout et al., 2010). High-carbohydrate larval diets, specifically sucrose and glucose, lead to delays in adult eclosion (due to delayed onset of pupation, but not prolonged pupation periods), reduced survival and lower adult body mass (Chen et al., 1996; Liourea et al., 2016; Matzkin et al., 2011; Musselman et al., 2011; Reis, 2016).

We asked whether larval diets, including the sugar alcohol mannitol, had developmental effects similar to high-sugar diets. We previously reported that mannitol, a non-sugar polyol carbohydrate, prolonged development when fed to Drosophila melanogaster larvae (Fiocca et al., 2019), and larvae fed mannitol were smaller than control larvae of the same age (Barrett and Fiocca, personal observation). Mannitol is a sugar alcohol and isomer of sorbitol. It is produced naturally as a product of fermentation and is found commonly in plants, bacteria and fungi (Jamieson et al., 2001; Lewis and Smith, 1967; Onishi and Suzuki, 1968). Mannitol is used as a low-calorie sweetener, sweetening foods without increasing blood glucose levels or insulin in humans (Saha and Racine, 2011; Yao et al., 2014). However, ingestion and breakdown of mannitol by Tribolium castaneum beetles increased hemolymph trehalose levels, indicating mannitol may be a nutritive source of dietary carbohydrates in some insect taxa at certain life stages (Kikut, 2018; Takada et al., 2017).

In adult D. melanogaster, mannitol ingestion generated concentration-dependent, female-biased mortality (Fiocca et al., 2019). However, this effect may not carry across all developmental stages. In the only study of mannitol’s effects across multiple life stages, mannitol increased mortality in sweet potato whitefly (Bemisia tabaci) adults while nymphs saw no lethal effect (Hu et al., 2010). Mannitol can be found in both fresh and rotting fruits (where Drosophila larvae often feed) due to microbial fermentation, but the impact of mannitol on D. melanogaster larvae has not been explored (Makinen and Soderling, 1980; Onishi and Suzuki, 1968). We hypothesized that mannitol ingestion during D. melanogaster development would generate phenotypes similar to those produced by high-sugar diets (Matzkin et al., 2011; Musselman et al., 2011; Reis, 2016). The ability of polyols to disrupt holometabolous development has not been studied, and identifying additional compounds that affect insect development can further our understanding of the pathways that connect growth rate, developmental timing and body size in insects.

In this study, we quantified the effect of mannitol feeding as a larva on adult body size as measured by thorax length (Bergland et al., 2008; Chechi et al., 2017; Santos et al., 1994). We assessed the effects of increasing concentrations of dietary mannitol on D. melanogaster larval survival, and pupariation and eclosion times. We analyzed if developmental delays were due to a delay in the onset of pupariation,
and/or prolonged time in the pupal stage. We also evaluated if delaying mannitol introduction to larvae by 72 h, to approximately the early third instar (Tyler, 2000), could reduce or eliminate the decreased survival and prolonged developmental duration, as would be expected if there were a sensitive period for developmental effects of mannitol ingestion.

RESULTS

Effects of larval ingestion of mannitol on adult body size

Adult female body size decreased as mannitol concentration increased, with 0.8 M emerging adults having smaller body sizes than 0 M or 0.4 M emerging adults (Fig. 1, Dunn’s: 0–0.8 M, Z=4.44, P<0.0001; 0.4–0.8 M, Z=2.59, P=0.029; 0–0.4 M, Z=2.12, P=0.10). Male body size also decreased as mannitol concentration increased, with 0.8 M and 0.4 M emerging adults having smaller body sizes than 0 M emerging adults (Fig. 1, Dunn’s: 0–0.8 M, Z=4.77, P<0.0001; 0–0.4 M, Z=4.12, P<0.0001; 0.4–0.8 M, Z=0.88, P=0.99). For females, the linear regression of mannitol concentration on body size was \( y=-0.04930x+1.022 \) (F=21.7, P<0.0001, R²=0.12); for males, \( y=-0.04644x+0.8992 \) (F=26.90, P<0.0001, R²=0.14). The slopes did not differ between males and females (F=0.04, P=0.84) indicating increasing mannitol concentration did not affect one sex’s body size differently than the other (two-way ANOVA: interaction effect, F=1.07, d.f.=2, P=0.34). The intercepts were significantly different (F=792.6, P<0.0001) indicating females had larger body sizes than males at all concentrations (two-way ANOVA: sex, F=769.2, d.f.=1, P<0.0001).

Concentration-dependent developmental delay prior to the onset of pupariation and reductions in survival

Developmental delay

Time to pupariation was significantly increased in the 0.4 M, 0.6 M and 0.8 M conditions as compared to controls (Fig. 2, ANOVA with Tukey’s: 0.4 M, q=8.61, P<0.0001; 0.6 M, q=14.35, P<0.0001; 0.8 M, q=8.97, P<0.0001), but not the 0.2 M condition (q=3.15, P=0.18). Time to adult eclosion was significantly increased in all the treatment conditions relative to controls (ANOVA with Tukey’s: 0.2 M, q=4.11, P<0.04; 0.4 M, q=8.96, P<0.0001; 0.6 M, q=14.85, P<0.0001; 0.8 M, q=11.52, P<0.0001). However, the time between pupariation and eclosion was not significantly different from controls in any mannitol treatment (Fig. S2, ANOVA: F=1.04, P=0.39), indicating the major cause of eclosion delay was a delay in the onset of pupariation caused by mannitol’s effects during larval development.

Reduced survival

We next assessed the effect of mannitol on D. melanogaster larval and pupal mortality. Mortality was concentration dependent for D. melanogaster larvae and pupae when assessed prior to eclosion, with 0.4 M, 0.6 M and 0.8 M treatments showing a significant difference from the control (Fig. S3, Mantel-Cox: 0.2 M, \( x^2=0.28, P=0.60 \); 0.4 M, \( x^2=9.40, P=0.002 \); 0.6 M, \( x^2=23.53, P<0.001 \); 0.8 M, \( x^2=19.41, P<0.001 \)). Highly significant differences in larval mortality occurred as early as 48 h after the eggs were laid in the 0.6 M and 0.8 M (Fig. 3, Mantel-Cox: 0.6 M, \( x^2=5.24, P=0.022 \); 0.8 M, \( x^2=10.39, P=0.001 \)) and 72 h after the eggs were laid in the 0.4 M, 0.6 M and 0.8 M (Mantel-Cox: 0.4 M, \( x^2=4.47, P=0.035 \); 0.6 M, \( x^2=11.81, P<0.001 \); 0.8 M, \( x^2=11.88, P<0.001 \)). The best-fit sigmoidal curve for pre-eclosion LC₅₀ data was:

\[
Pr(\text{mortality}) = 0.78 \left(1 + e^{\left(-\frac{\text{mannitol (mg/L)}}{0.30}\right)}\right)
\]

Partial rescue of developmental delays

Delivering introduction of mannitol to the larval diet by 72 h (72-h plates) significantly decreased pupariation and eclosion times in the 0.4 M treatment (Fig. 4A; ANOVA with Tukey’s, pupariation, q=12.71, P<0.0001; eclosion time, q=7.94, P<0.0001), and the 0.8 M treatment (pupariation time: q=7.02, P<0.0001; eclosion time: q=5.23, P=0.0047) relative to plates where larvae were fed the same concentration of mannitol from hour 0 after the eggs were laid. Pupariation and eclosion times were not significantly different from controls, but this may be an effect of small sample size due to relatively low survival through the larval stage (n=9 surviving pupae, Fisher’s: 0 M, P=0.08; 0.2 M, P=0.09).

Concentration-dependent reduction of mannitol’s developmental effects by delaying mannitol introduction to larvae for 72 hours

Partial rescue of larval survival

Waiting 72 h before introducing larvae to mannitol media also significantly increased survival to eclosion (relative to initiating mannitol feeding at day 0) at 0.4 M and 0.8 M mannitol concentrations (Fig. 5A, Mantel-Cox: 0.4 M, \( x^2=8.91, P=0.003 \); 0.8 M, \( x^2=11.88, P<0.001 \)).
flies fed mannitol diets showed developmental effects similar to those induced by high-sugar diets. We tested the effects of a mannitol diet on several aspects of larval phenotypes in D. melanogaster. Mannitol increased D. melanogaster developmental duration and decreased emerging adult body size in a concentration-dependent manner. While larval density was not controlled in our experiment for body size, increased density typically leads to decreased thorax lengths; in our least dense vials (0.8 M) we saw the smallest body sizes, indicating that larval density was not responsible for this trend (Santos et al., 1994). The phenotypic effects of a mannitol diet on the duration-size relationship in D. melanogaster were similar to the effects of high-sugar diets (Matzkin et al., 2011; Musselman et al., 2011; Reis, 2016; Rovenko et al., 2015).

Increased developmental duration due to mannitol ingestion was a result of delayed onset of pupariation, not prolonged pupal metamorphosis. Both the stage of larval development when mannitol was introduced (first or third larval instar), and the mannitol concentration in food, influenced the severity of mannitol’s phenotypic effects. Delaying 0.4 M mannitol introduction for 72 h eliminated the effects on development time and survival; 0.8 M mannitol still had significant, although lessened, effects when introduction was delayed for 72 h. These phenotypic effects are consistent with those of high-sugar diets that generate smaller adult body sizes and prolonged development prior to the onset of pupariation (Matzkin et al., 2011; Musselman et al., 2011; Reis, 2016); as with our experiments on mannitol, the high-concentration sugar diets had stronger effects earlier in larval development (prior to the third instar) (Musselman et al., 2011).

Models of the independent effects of growth rate, critical weight, and the interval to the cessation of growth on the duration-size relationship in Manduca sexta indicate that variation in growth rate can lead to a negative relationship, while variations in interval to the cessation of growth and critical weight generally lead to positive relationships (Davidowitz et al., 2005; Nijhout et al., 2010). Critical weight typically occurs directly after the second molt in D. melanogaster, approximately 72 h post-hatching (De Moed et al., 1999; Tyler, 2000). 72 h delays in introducing high-concentration dietary mannitol still led to increased D. melanogaster development times, it is thus unlikely that mannitol decouples the positive duration-size relationship via altering critical weight. Instead, mannitol may impact growth rate and/or the interval to cessation of growth, potentially by disrupting the insulin/TOR signaling pathway that regulates these variables in D. melanogaster (Mirth and Shingleton, 2012).

0.8 M, \( \chi^2=6.80, P=0.009 \). In the 0.4 M 72-h plates, survival was not significantly different from 0 M treatment (Fig. 5B; \( \chi^2=0.00, P=0.986 \)), while the 0.8 M 72-h plates treatments were significantly different from 0 M (\( \chi^2=8.03, P=0.005 \)).

The percent of pupae that did not eclose significantly decreased in 0.4 M treatments when mannitol introduction was delayed by 72 h, but no significant difference was found between 0 h and 72 h mannitol introduction in 0.8 M treatments (Fig. 6, Fisher’s: 0.4 M, \( P=0.017 \); 0.8 M, \( P=0.99 \)). The percent of pupae that did not eclose in 0.4 M 72-h plates was not significantly different from 0 M controls (Fisher’s: \( P>0.99 \)).

**DISCUSSION**

The typical positive relationship between holometabolous insect body size and developmental duration can be reversed under some dietary conditions (Danielsen et al., 2013; Huang et al., 2018; Reis, 2016; Xiao et al., 2005). In this study, we asked whether larval fruit...
In *D. melanogaster*, a carbohydrate-rich diet led to delays in eclosion and smaller pupal case sizes (Reis, 2016). Extremely high-sugar (e.g. 1 M sucrose) diets produced insulin resistance, leading to smaller wandering third instar larvae and adults irrespective of protein availability, the sugar used, or osmolarity of the food medium during development (Musselman et al., 2011). In addition, high-sugar feeding led to dramatic delays in pupariation (Musselman et al., 2011; Rovenko et al., 2015), similar to what we saw in our 0.4–0.8 M mannitol treatments. Delays in eclosion, due to high-sugar diets affecting the insulin-signaling pathway, caused delayed onset of pupariation but not prolonged metamorphosis (Matzkin et al., 2011); again, this is the same phenotype we induced when larvae were fed mannitol diets.

Because concentrations of all non-mannitol carbohydrates were kept the same in larval foods, *D. melanogaster* would need to be able to metabolize mannitol in order for it to increase levels of trehalose in the hemolymph like metabolizable sugars (glucose and sucrose). No studies have examined if *D. melanogaster*, or its common gut microbes, can metabolize mannitol, but female red flour beetles (*Tribolium castaneum*) have higher trehalose levels in the hemolymph after feeding on mannitol (Kikuta, 2018). Circulating trehalose is responsible for TOR activation in *D. melanogaster* fat bodies, contributing to cell growth during development; mannitol’s catalysis to trehalose may be responsible for mediating its effects via the insulin/TOR signaling pathway, similar to other carbohydrates (Kim and Neufeld, 2015; Morris et al., 2012).

Proper growth during development can also influence survival to, and in, adulthood (Mirth and Riddiford, 2007). High-sugar diets cause mortality in both *D. melanogaster* and *Drosophila mojavensis* (Matzkin et al., 2011; Rovenko et al., 2015). We found that mannitol causes mortality in *D. melanogaster* larvae after 48 h in a concentration-dependent manner, with an LC50 of 0.36 M. In addition, of the larvae that pupated in the 0.4 M and 0.6 M treatments, a significant number of them failed to eclose.

Starvation is a potential mechanism for mannitol’s effects on larval survival; however, this is unlikely due to mismatches between starvation phenotypes and our results. Post-critical weight starvation causes accelerated emergence (our 72 h plates saw normal or delayed emergence) while pre-critical weight starvation causes developmental delay but normal adult body sizes (unlike our smaller adults) (Beadle et al., 1938). Simply reducing nutritional availability throughout development generates smaller adult body sizes, but no change in survival through eclosion (Ormerod et al., 2017). Additionally, all mannitol-fed larvae received the same basic nutrients as were fed to...
the controls. Mannitol may also be acting as an osmotic stressor to larvae, despite their excellent osmoregulatory ability, as mannitol is known for its diuretic effects (Croghan and Lockwood, 1959; Nicolson, 1994; Diaz-Fleischer et al., 2019; Elhassen and Schrier, 2015; Grembecka, 2015). Other species exhibit longer development times, decreased body size, reduced feeding, and/or reduced survival in osmotically stressful environments (Chinathamby et al., 2006; Clark et al., 2004; Matthews, 1985; Wu et al., 2012; Nievalda et al., 2008; Rovenko et al., 2015), which matches some of our results.

A single genetically variable insulin signaling pathway regulates growth, reproduction, longevity and metabolism in all insects, and contains conserved elements across all animals (Das and Anrr, 2017; Wu and Brown, 2006). This pathway is involved in numerous environmentally-driven polyphenisms generated during development, including caste differentiation in social insects, as well as geographically- and nutritionally-driven morphological variation (De Jong and Bochdanovits, 2003; Lin et al., 2016; Lu and Pietrantonio, 2011; Wheeler et al., 2006; Xu et al., 2015). Mannitol’s developmental effects provide an opportunity to compare phenotypic variation in response to the nutritional environment, generated by the evolution of insulin signaling genes across species. This study joins a growing body of work indicating that the frequently-cited positive relationship between duration of development and body size in insects can be altered by environmental variation, particularly via dietary influences. Our work also suggests that the importance of this variation, and its influence on specific developmental parameters, may change as development progresses past various sensitive periods. Mannitol’s effects on development provide a novel paradigm for exploring the environmentally-cued regulation of developmental-physiological relationships in insects.

**MATERIALS AND METHODS**

**Culturing Drosophila**

Wild-type (Canton S) *D. melanogaster* (Bloomington *Drosophila* Stock Center) were raised to adulthood on standard *Drosophila* media for laboratory culturing and reared in an insect growth chamber at 27.5°C, 50% relative humidity, with a 12-h:12-h photoperiod (Chakraborty et al., 2011). These conditions were used to rear adults and for all larval experiments. Standard media was prepared in 100 ml batches as follows: 9.4 g cornmeal, 3.77 g yeast, 0.71 g agar, 0.746 ml Propionic acid, 1.884 ml Tegosept (10% w/v methyl p-hydroxybenzoate in 95% ethanol), and 9.42 ml molasses (Genesee Scientific). The appropriate amount of mannitol (HiMedia; GRM024-500G, Lot 000249743) was added, and beakers were filled with distilled water to a final volume of 100 ml. After heating the mixed ingredients to set the agar, media was poured into vials and cooled until consistency was firm and uniform. An excess of media was provided, with 10 ml in each vial.

**Testing effect of larval mannitol feeding on adult body size**

Groups of 15 male and 15 female wild-type flies were raised on standard media were placed in vials containing 0 M, 0.4 M, or 0.8 M mannitol adult media (standard media recipe with no molasses) and allowed to lay for 24 h (at which time they were removed). Nine vials were used per concentration, with a total of 405 flies of each sex. Vials were checked for newly emerged adults every 12 h from day 10 to day 15, and every 24 h from day 15 to day 24 (the last day that a larva pupariated in the larval plate trials). Adult flies were removed from the vials and sexed; two males and two females were randomly selected every 24 h from each vial with selected adults. Selected adults were euthanized and...
photographed for body size measurements (0M: n=52 females, n=56 males; 0.4M: n=66 females, n=61 males; 0.8M: n=50 females, n=49 males). Photographs of the thorax were taken from a dorsal view at 4X magnification using a digital camera mounted on (0.7 X) on a dissecting scope. Measurements of thorax length were taken from the tip of the scutellum to the most anterior part of the mesothorax (Bergland et al., 2008; Chechi et al., 2017) in ImageJ using the ruler tool (Schneider et al., 2012), and photographs of a stage micrometer were used to convert pixels to mm.

**Testing effects of dietary mannitol on larval mortality and developmental delay**

Translucent media was produced by omitting the cornmeal from the standard media recipe and lowering the amount of agar to 0.52 g/100 ml (O’Donnell et al., 2017). Food was poured to a depth of 3 mm in 50 mm diameter petri dishes, allowing for the observation of the larvae in the food. Groups of over 100 mixed male and female wild-type flies raised on standard media were placed in each of ten egg laying chambers. At the end of 4 h, eggs were collected and five eggs were plated per petri dish, with mannitol concentrations from 0–0.8 M, at 0.2 M increments. Six petri dishes were used per concentration (n=30 eggs/concentration). Egg hatching, mortality, pupariation and eclosion were assessed every 24 h for 27 days using the methods detailed in (O’Donnell et al., 2017). Mean pr (mortality), days to pupariation, and days to eclosion were calculated for each concentration and a three-parameter sigmoid curve was fitted to the data to assess LC50 prior to eclosion.

**Testing for a change in severity of mannitol’s developmental effects when delaying introduction to larvae by 72 h**

Groups of approximately 100 mixed male and female wild-type flies raised on standard media were placed in each of ten egg laying chambers. At the end of 4 h, eggs were collected and plated on 0 M control translucent media where they were raised for 72 h. After 72 h, five larvae were plated per treatment petri dish (using translucent media), with the mannitol concentrations from 0–0.8 M, at 0.4 M increments. Six petri dishes were used per concentration (n=30 eggs/concentration). Larval mortality, pupariation, and eclosion were assessed every 24 h for another 22 days. Mean percent mortality, days to pupariation and days to eclosion were calculated for each concentration.

**Statistical analyses**

Analyses were performed using SPSS v. 24, SIGMAPLOT v.12.5, and Graphpad v. 8.0.0 (Graphpad Prism for Windows, 2018; IBM SPSS Statistics for Windows, 2016; SIGMAPLOT for Windows, 2013). The effects of mannitol introduction to larvae on adult body size were analyzed using Kruskal–Wallis test with Dunn’s multiple corrections for each sex. A two-way ANOVA was used to look for an interaction effect between sex and mannitol concentration on body size. A linear regression was fitted to the data for each sex across concentrations, and the slopes and intercepts were calculated for each concentration.

**Results**

Effects of eclosion day on male or female body size within a concentration were assessed using linear regressions in GraphPad, to understand the effects of mannitol in individuals that are more or less delayed in their development within a concentration and sex. This allowed us to look for any effect of day-based sampling bias, as we did not measure every emerging adult’s body size, but only two per day of each sex in each vial. There was no significant trend within each pair of concentration and sex (e.g. 0 M females) of emergence day on body size, except in 0.4 M males, indicating that flies emerging earlier and later within a concentration were not differently affected by mannitol and reducing the likelihood of day-based sampling bias on our results (Fig. S1: 0 M-female, F=0.47, P=0.50; 0 M-male, F=3.52, P=0.07; 0.4 M-f, F=0.36, P=0.67; 0.4 M-m, F=10.51, P=0.002; 0.8 M-f, F=0.16, P=0.69; 0.8 M-m, F=2.00, P=0.16). The slopes of the regressions across all six concentration-sex pairs were not significantly different from one another (F=0.53, P=0.75).

Larval mortality data across mannitol concentrations at 48 h, 72 h and pre-eclosion was assessed using survival analyses in SPSS (Bewick et al., 2004), with subjects living to the end of the trial or eclosed included in the analysis as right-censored values on the final day of that test (48 h, 72 h and the last day of the trial, respectively). Pupae that had not eclosed after at least 6 days at the end of the trial were marked as ‘dead’ on the final day of the trial (day 27). Differences in survival distributions across concentrations were tested using pairwise log-rank Mantel Cox tests. Three-parameter, best-fit sigmoidal function LC50 curves for larvae at 72 h, pre-pupariation, and pre-eclosion were generated in SIGMAPLOT. To analyze any effects on survival of delaying the introduction of mannitol to larvae by 72 h, we used a pairwise log-rank Mantel Cox test (with subjects eclosed before the end of the trial included as right-censored values on day 25, and pupae that had not eclosed marked as ‘dead’ on the final day).

To analyze developmental delays across concentrations, we used a one-way ANOVA with Tukey’s multiple comparisons test in GraphPad. To analyze differences in time from pupariation to eclosion, a one-way ANOVA with Tukey’s multiple comparisons was used. To analyze any phenotypic effects on pupariation/eclosion time across replicates (n=6/concentration) by delaying the introduction of mannitol to larvae by 72 h, we used a two-way ANOVA and Tukey’s multiple comparisons Tests in GraphPad. Differences in the number of larvae that pupated, but did not eclose, across concentrations in the delayed-introduction treatments were analyzed using Fisher’s exact tests in GraphPad.

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**Competing interests**

The authors declare no competing or financial interests.

**Author contributions**

Conceptualization: M.B., K.F., E.A.W., S.O., D.R.M.; Methodology: M.B., K.F., E.A.W., S.O., D.R.M.; Validation: M.B., K.F.; Formal analysis: M.B., K.F., E.A.W., S.O., D.R.M.; Investigation: M.B., K.F., E.A.W., C.M.; Writing - original draft: M.B., K.F., E.A.W.; Writing - review & editing: M.B., K.F., E.A.W., C.M., S.O., D.R.M.; Supervision: S.O., D.R.M.; Project administration: S.O., D.R.M.; Funding acquisition: S.O., D.R.M.

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**Supplementary information**

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