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Concentration-response of an anthraquinone-based repellent for raccoons (*Procyon lotor*)☆

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ABSTRACT

Wildlife repellents can be part of non-lethal management strategies to reduce the negative impacts of wildlife to property, agricultural production, and human health and safety. Raccoons (*Procyon lotor*) are associated with negative impacts in all three of these areas. Anthraquinone is a useful avian repellent and its utility as a mammalian repellent is still being explored. Our objective was to evaluate laboratory efficacy of an anthraquinone-based repellent for raccoons using different concentrations. We fed captive raccoons whole corn treated at 0.5%, 1%, 1.5% and 2% anthraquinone and examined their behavioral response related to feeding repellency including consumption and change in duration related to approach, interaction and extended interaction with the feed bowl. Feeding repellency was 26–37% for whole corn treated with 0.5–1.5% anthraquinone and 71% for whole corn treated with 2% anthraquinone. Interaction duration among the treatments varied ($p = 0.005$) with a longer interaction duration with the food bowl at 2% anthraquinone compared to 0.5% anthraquinone. However, the addition of anthraquinone did not significantly alter behaviors of approach, interaction, or extended interaction between pretreatment and treatment for the raccoons within treatments. The decreases in consumption that we observed warrant development of further research and field evaluation regarding anthraquinone as a deterrent with raccoons or to repel vertebrate competitors from raccoon specific baits.

1. Introduction

The frequency and consequence of human-wildlife interactions are affected by continued increases in human populations and associated changes in land use, as well as the local abundance of some wildlife species in developed landscapes. Human-wildlife conflicts can occur when wildlife consume human-valued foods, when wildlife occupy human-valued places and when wildlife may be reservoirs of zoonotic diseases; raccoons (*Procyon lotor*) are a prime example of a species that are often targeted for nuisance behaviors and/or to mitigate disease risk in landscapes where conflicts may be greatest. Raccoons are secretive, nocturnal meso-carnivores native to North America (Prange et al., 2011). Like most carnivores, they are considered a primarily solitary species, though studies have shown that aggregations will form when resources are concentrated (Wehtje and Gompper, 2011). Raccoons are also considered a synanthropic species, with population densities consistently higher in urban and suburban areas compared to rural areas (Prange et al., 2003; Gross et al., 2012). This synanthropic lifestyle is accomplished chiefly due to their tendency to focus foraging on anthropogenic foods and higher annual recruitments in urban areas resulting in increased survival rates (Prange et al., 2003; Bozek et al., 2007). The combination of all these characteristics in raccoons can lead to a population overabundance in urban and suburban areas, and in turn, can cause human-wildlife conflicts (Barrett et al., 2019; Schell et al., 2021).

Raccoon-caused conflicts include damage to homes and buildings, along with damage to agriculture, as raccoons will consume commercial fruits, melons, and corn, peanut, and soybean crops (Riley, 1989; Kern, 2000; Beasley and Rhodes Jr, 2008). Perhaps most significantly, raccoons can also be responsible for transmission of zoonoses, such as rabies virus, to pets and humans (Beltrán-Beck et al., 2012). The negative impacts of raccoons to property, agricultural production, and human health and safety may be mitigated with non-lethal management strategies. Non-lethal methods that deter feeding behavior may reduce damage to agricultural production. Altering feeding behavior may coincide with changes in other behaviors, such as reduced aggregations

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at feeding sites.

Multiple studies concerning the development of non-lethal methods to deter feeding behaviors with raccoons have been reported. Conover (1989) evaluated 17 compounds as aversive conditioning agents in captive raccoons. Emetine dihydrochloride (CAS 316–42–7) and hyoscyamine (CAS 101–31–5) significantly reduced consumption of treated food in both choice and no-choice experiments. No other chemical was effective in either type of experiment. Only emetine dihydrochloride at a dose of 0.05 g caused a generalized aversion (but not general appetite suppression) for untreated food after repellent exposure. Kern (2000) suggested that Ro-Pel or Bitrex, an extremely bitter substance, is a very effective feeding deterrent in raccoons. However, it is unable to be used on plants grown for human consumption. Campbell and Long (2007) discovered that raccoons were not repelled by a commercially available raccoon repellent (Get Away®, Woodstream, Lititz, PA, USA). Martin (2007) observed reduced egg consumption (but not reduced food consumption) after exposing raccoons to estrogen-treated eggs.

We selected anthraquinone for our concentration-response experiments with raccoons because anthraquinone is commercially available as a chemical repellent and feeding deterrent for preplant seed treatments and foliar applications in the United States (DeLiberto and Werner, 2016). Since the early 1940 s, anthraquinone, a naturally occurring compound, has been identified as a useful avian repellent (Heckmanns and Meisenheimer, 1944; DeLiberto and Werner, 2016). However, anthraquinone's utility as a mammalian repellent is still being explored (Werner et al., 2016; Baldwin et al., 2018) and identifying anthraquinone efficacy in raccoons is a necessary step to for determining how the repellent may compare to other nonlethal methods, such as trapping, used in raccoon management. Recently, Snow et al. (2021) observed concentration-dependent feeding repellency in wild pigs (*Sus scrofa*) offered whole-kernel corn treated with an anthraquinone-based seed treatment. Though the study was focused on wild pigs, raccoons were also observed feeding at the bait sites and did not appear to be repelled by seed treatments including 0.5–3% anthraquinone under field conditions, which occurred in February and March in Texas and August in Alabama.

The next logical step for considering anthraquinone as a raccoon repellent was to conduct concentration-response feeding experiments in captivity. Subsequent applications of an anthraquinone-based repellent could be developed based upon the results of our captive experiments (e.g., foliar applications for the protection of ripening agricultural crops). Our purpose was to evaluate the laboratory efficacy of an anthraquinone-based repellent and the associated behavioral responses for raccoons, a species commonly associated with human-wildlife conflicts in the United States. We conducted controlled feeding experiments to evaluate the concentration-specific repellency of anthraquinone with raccoons in captivity.

2. Materials and methods

2.1. Facilities, subjects and diets

We conducted feeding experiments during October–November 2019 at the United States Department of Agriculture (USDA), National Wildlife Research Center's (NWRC) outdoor animal research facility in Fort Collins, Colorado (USA). We sourced adult raccoons (14 female, 18 male) from earlier and/or ongoing unrelated studies. Raccoons in the study consisted of 25 captive-born subjects received September 2018 (Ruby Fur Farm, Inc., New Sharon, Iowa), 2 captive-born subjects received July 2018 (Ruby Fur Farm, Inc., New Sharon, Iowa) and 5 wild-caught raccoons captured during June 2017 in Larimer County, Colorado. We provided water ad libitum to all raccoons throughout our experiments. The care and use of all raccoons associated with our feeding experiments was consistent with the guidelines of the NWRC Institutional Animal Care and Use Committee (QA-3142).

Raccoons were housed individually in outdoor enclosures (3 × 3 × 2.5 m) that contained a den box and enrichment structures during the study (e.g., quarantine, holding, acclimation, experiment). We provided maintenance diet (200 g of omnivore diet; Mazuri Ominvore-Zoo Feed "A", Richmond, Indiana, USA) to all raccoons during quarantine and holding, whereas the study diet consisted of whole corn. When raccoons were fed the study diet, their water bowl was placed along the back of the enclosure to increase the distance from the feed bowl. All feeding occurred at the same location within the enclosures (Fig. 1). We used Avipel® repellent (a.i. 50% 9,10-anthraquinone; Arkion Life Sciences, New Castle, DE, USA) for our raccoon feeding experiments and formulated seed treatments for our experiments by applying aqueous solutions (60 ml/kg) to whole corn using a rotating mixer and household spray equipment.

2.2. Anthraquinone concentration-response relationship

We conducted two feeding experiments to establish a concentration-response relationship of anthraquinone-treated seeds for raccoons. We used no-choice tests to establish concentration-response relationships for anthraquinone in raccoons (Werner et al., 2009). Daily corn consumption was measured throughout the concentration-response experiments. Unconsumed corn (remaining in food bowls) and spillage were collected (at 08:00–09:30 h, daily) and weighed (± 0.1 g). Weight change (e.g., desiccation) of whole corn was measured daily by weighing corn offered within a vacant enclosure throughout our experiments.

We offered 32 raccoons untreated whole corn ad libitum in one food bowl for five days of acclimation within individual enclosures. We subsequently offered each raccoon 500 g of untreated whole corn in one bowl during each of treatment days 1–3. We ranked raccoons based upon average pretreatment consumption and assigned them to one of four treatment groups ($n = 8$ raccoons/group) such that each group was evenly populated with raccoons exhibiting variation in daily consumption levels. We randomly assigned treatments among treatment groups (0.5%, 1%, 1.5% and 2% anthraquinone; targeted concentrations, wt/wt). We pre-selected three concentrations (0.5%, 1% and 2%) to offer to raccoons in Groups 1–3, respectively, and tested these three groups at the same time. We did not pre-determine the fourth concentration tested and instead used results from Groups 1–3 to guide selection of a treatment concentration for Group 4, to estimate a concentration-response feeding curve to anthraquinone. We offered 500 g of whole corn treated with 0.5%, 1% or 2% anthraquinone in one bowl to 24 raccoons in Groups 1–3, respectively, during trial treatment day 4 (23 October 2019), and determined the mass (± 0.1 g) of uneaten corn and corn spillage at 08:00–09:30 h during treatment day 5. We repeated acclimation, pretreatment, and treatment, experimental trials with the remaining eight naïve raccoons by offering 1.5% anthraquinone-treated whole corn during treatment day 4 (i.e., Group 4, 31 October 2019).

2.3. Anthraquinone residue analyses

We used reversed-phase, high performance liquid chromatography

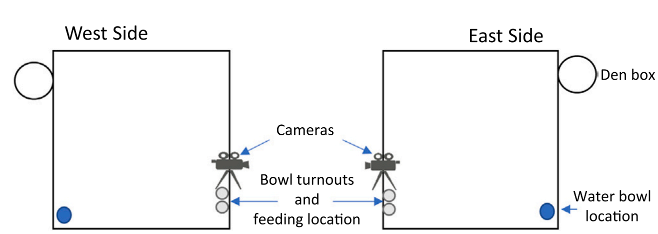

Fig. 1. Raccoon (*Procyon lotor*) enclosure setup used for testing anthraquinone repellency at the National Wildlife Research Center in Fort Collins, Colorado (USA), during autumn (October–November) 2019. All food, including the untreated and treated study diet, was offered at the bowl turnouts. Cameras were mounted 2.2 m high on the exterior of the opposite enclosure.

(HPLC) with ultraviolet detection to quantify anthraquinone residues (i.e., actual concentrations) among corn seed treatments (± 1 ppm anthraquinone). We collected a 200 g sample of each seed treatment associated with our concentration-response experiments. All samples were labeled and shipped to Arkion Life Sciences for residue analyses, where they were transferred to 4 °C and stored for the duration of the analysis period.

We used a five-point external calibration curve to calibrate the HPLC instrument. Samples were run in duplicate each day, and we checked single calibration points for each 10 injections. The average response was plotted against anthraquinone concentrations. We used linear regression to calculate sample concentrations. The limit of detection in the experiment was 5 ppm.

2.4. Raccoon behavioral responses

A fixed video monitoring system (cameras: 2.0 C-H4SL-BO1-IR and 2.0 W-H3-BO1-IR Avigilon Corporation, Vancouver, British Columbia, Canada) was set to record during the pretreatment and treatment days. The cameras were positioned so that the entire floor of an individual raccoon's enclosure along with the den box opening in the back and food bowl in the front was in the field of view. We scored the video data for each raccoon from the treatment days 3 and 4 and documented three behavior metrics: approach, interaction, and extended interaction. Approach was the duration in seconds between the first documented approach (adjacent and oriented to the food bowl) and first bowl interaction (assumed start of consumption). Interaction was the duration in seconds of the raccoon being within the plane of the food bowl and ending when the raccoon was no longer over and/or in the food bowl. Extended interaction was the total duration in seconds of multiple interactions that contained short breaks (<10 min). Scoring was recorded in Access (Microsoft Office) and time was recorded to the nearest second.

2.5. Statistical analysis

We hypothesized that anthraquinone is an effective feeding repellent with raccoons. We predicted that observed repellency would be positively correlated with tested anthraquinone concentrations. We previously established ≥ 80% repellency as efficacious during related captive feeding experiments with anthraquinone (Werner et al., 2009). The dependent variable was test consumption of anthraquinone-treated corn relative to average pretreatment consumption of untreated corn (i.e., percent repellency for each treatment group = (1 − (test consumption × average pretreatment consumption^{-1})) × 100). We used descriptive statistics ($\bar{x}$ ± S.E.M.) to summarize consumption (mg anthraquinone/kg body mass [BM]) of treated corn during concentration-response experiments.

We calculated summary statistics (minimum, first quartile, median, mean, third quartile, and maximum) for pretreatment, treatment, and difference between treatment and pretreatment for each behavioral metric for each treatment group in R (v 3.6.3; R Core Team, 2020). We recorded the start and stop time for each metric in hours:minutes:seconds and converted this to seconds using the package tidyr (v 1.1.2). We conducted a Cox proportional-hazards regression in R using the package survival (v 3.2–13) to determine if the duration of raccoon behaviors varied for each behavioral metric scored among treatment groups. We first tested whether the assumptions of equal proportional hazards were met before running the regression for each behavioral metric. Treatment time was the response variable and fixed effects were treatment group and time prior to treatment, the latter of which was included to account for individual variability. To evaluate for an increase or decrease in duration for any metric within a treatment group, we conducted a paired Wilcoxon signed rank test between pretreatment and treatment behavioral metrics in R. We considered α = 0.05 to indicate statistical differences in raccoon behaviors.

3. Results

3.1. Anthraquinone concentration-response relationship

We observed 26–71% feeding repellency among raccoons offered whole corn treated with 0.5%, 1%, 1.5% and 2% anthraquinone (target concentrations; Fig. 2). The actual concentrations for these corn seed treatments were 5,046 ppm, 9,842 ppm, 14,228 ppm and 20,377 ppm anthraquinone, respectively. The greatest repellency we observed (71%) was for raccoons offered whole corn treated with 2% anthraquinone and lowest repellency (26%) was associated with the 1% anthraquinone. There was no evidence of a correlation (p = 0.75) between repellency and anthraquinone concentration. Repellency within the 0.5%, 1%, 1.5% and 2% treatment groups ranged from 2%88%, 4–45%, 10–82% and 0–100%, respectively. On average, raccoons in the 0.5%, 1%, 1.5% and 2% anthraquinone treatment groups consumed 105 mg anthraquinone/kg BM (± 15 mg/kg, S.E.M), 277 ± 31 mg/kg, 423 ± 64 mg/kg and 218 ± 92 mg/kg during the test, respectively.

3.2. Raccoon behavioral responses

The range for approach times, interaction times, and extended interactions times were 0–24,890 s (6.9 h), 0–2,757 s (46 min), and 225 (3.8 min) - 37,918 s (10.5 h), respectively. The Cox regression assumption of equal proportional hazards was met for approach and interaction, yet violated for extend interaction. For the two behavioral metrics compared using survival analysis, the model results did not indicate any differences in approach duration times among treatments but there were differences in the duration of interaction behaviors among treatments. The duration of raccoon interactions with their food bowl in Group 1 (0.5% AQ) was significantly different from Group 2 (1.0% AQ) and Group 3 (2.0% AQ), but not Group 4 (1.5% AQ) (Table 1). The hazard ratio for Groups 2–4 was less than 1 indicating that each of these groups were associated with increased interaction times compared to Group 1. Within any of the treatment groups we were unable to identify any consistent change in duration for any of the behavior metrics we measured (Table 2). The most consistent change between pretreatment and treatment interaction behaviors was observed with Group 1 (0.5% AQ [p = 0.1]).

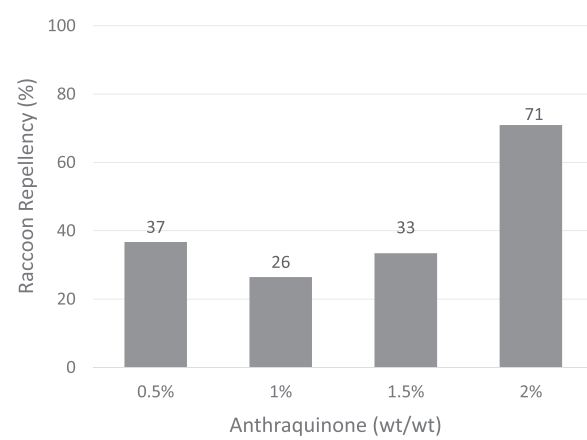

Fig. 2. Mean feeding repellency associated with four concentrations of Avipel® seed treatments (a.i. 50% 9,10-anthraquinone; Arkion® Life Sciences, New Castle, DE, USA) offered to raccoons (*Procyon lotor*) at the National Wildlife Research Center in Fort Collins, Colorado (USA), during autumn (October–November) 2019. The actual concentrations of the 0.5%, 1%, 1.5% and 2% corn seed treatments were 5,046 ppm, 9,842 ppm, 14,228 ppm and 20,377 ppm anthraquinone, respectively. Repellency represents test consumption relative to average, pretreatment corn consumption (*n* = 8 raccoons per concentration).

Table 1
The hazard ratio and p value generated from the Cox proportional-hazards regression. Three behavioral metrics, Approach, Interaction, and Extended Interaction, were tested for assumption of equal proportional hazards. Only results from the two behavioral metrics, Approach and Interaction, that met the assumption are reported. The hazard ratio and p value of the three higher concentrations of anthraquinone (AQ) are relative to the lowest concentration (0.5%) tested in Group 1. Eight individuals were in each treatment group and Group 4 was tested a week after Groups 1–3. Significance at $\alpha \leq 0.05$ indicated with an asterisk.

		Concentration (Group)		
Behavior	Parameter	1.0% AQ (2)	1.5% AQ (4)	2.0% AQ (3)
Approach	Hazard ratio	0.9946	0.7702	0.7142
	P value	0.9920	0.6350	0.5040
Interaction	Hazard ratio	0.2208	0.8154	0.1569
	P value	0.01483 *	0.7017	0.00492 *

Table 2
Number (#) of individual raccoons at the different concentrations of anthraquinone (AQ) that decreased, increased, or had no change in duration from pretreatment to treatment for the three behavior metrics examined: Approach, Interaction and Extended Interaction. The resulting p value from the Wilcoxon signed rank test is also provided. Eight individuals were in each treatment group and Group 4 was tested a week after Groups 1–3.

		Concentration (Group)			
Behavior	Parameter	0.5% AQ (1)	1.0% AQ (2)	1.5% AQ (4)	2.0% AQ (3)
Approach	# decrease	5	4	4	2
	# increase	3	3	3	6
	no change	NA	1	1	NA
	P value*	1	0.271	0.6726	0.5745
Interaction	# decrease	6	5	3	3
	# increase	2	3	2	5
	no change	NA	NA	3	NA
	P value*	0.1065	0.4406	0.2785	0.3828
Extended Interaction	# decrease	5	5	5	5
	# increase	3	3	3	3
	no change	NA	NA	NA	NA
	P value	0.4609	0.5469	0.4609	0.1953

= number
* R unable to calculate exact p-value or confidence interval when ties or zeros exist

4. Discussion

Anthraquinone reduced corn consumption by captive raccoons at all concentrations evaluated. The greatest reduction in feeding (i.e., 71% feeding repellency) was observed at the highest anthraquinone concentration (2%), but feeding repellency was not positively related to tested concentrations at 0.5–1.5% anthraquinone. The lowest concentration (0.5%) had greater repellency (37%) than the 1.0% and 1.5% concentrations, which had repellencies of 26% and 33%, respectively. Since repellency to anthraquinone is a learned behavior (Werner and Provenza, 2011), factors that impact the animals developing a negative association with anthraquinone can impact repellent efficacy; sensitivity to anthraquinone, behavior, and environmental conditions may be such factors (e.g., no-choice conditions in captive experiments versus various choices under field conditions).

Mammals are variable in their sensitivity to anthraquinone with different levels needed to achieve repellency. Wild pigs had an 87% feeding repellency at 0.64% anthraquinone (wt/wt) (Santilli et al., 2005), while black-tailed prairie dogs (*Cynomys ludovicianus*) never exceeded a 37% feeding repellency even at 4.0% anthraquinone (wt/wt) (Werner et al., 2011). Anthraquinone repellency has also been tested in cottontail rabbits (*Sylvilagus audubonii*), California voles (*Microtus californicus*), Richardson's ground squirrels (*Urocitellus richardsonii*) and deer mice (*Peromyscus maniculatus*) at 0.25–2% anthraquinone (wt/wt) (Werner et al., 2016). Repellency ranged between 19% and 85% for these species overlapping with the raccoons (27–71%). However, the rabbits, voles, squirrels and mice had a more consistent relationship among concentration and feeding repellency than the raccoons. Sensitivity for raccoons may depend more on the individual raccoon and we observed a wide range of feeding repellency values within the different concentrations.

Raccoons are a behaviorally flexible species (Barrett et al., 2019; Daniels et al., 2019) and our behavioral metrics (approach, interaction, and extended interaction) indicated different behavioral responses by raccoons during the autumn experiment. We observed that 53% (17/32) of raccoons decreased interaction time, 38% (12/32) increased interaction time, and 9% (3/32) had no change in their interaction time. We observed similar trends with approach and extended interaction behaviors. Since anthraquinone did decrease consumption one interpretation is that some individuals reduced consumption by decreasing their time consuming the anthraquinone-treated corn and others reduced consumption by slowing down their consumption of the anthraquinone-treated corn increasing overall consumption time demonstrating the different behavioral flexibilities the raccoons might express (Table S1, Supplementary data). A hesitancy to consume might be a cause of the increase in interaction but reduction in feed consumption at 2% anthraquinone compared with 0.5% anthraquinone. These behavioral changes may have been an immediate response to changes in the texture and/or smell of the treated corn that were then followed by post-ingestion distress.

Environmental conditions may also impact repellency. Environmental conditions may include seasonal fluctuations, as well as local weather impacts or the availability of alternative food sources. Since we designed the study so that the concentration tested with group 4 would be based on the results from groups 1–3, Group 4 (1.5% AQ) was tested one week later, which coincided with colder temperatures; we recorded temperatures < 0 °C during that week to contrast with recorded temperatures of > 0 °C during the week prior when groups 1–3 were tested. The average pretreatment consumption was higher and with more variability during the colder week (249.9 g ± 26.7) compared to the warmer week (224.3 g ± 12.3) of testing.

Our primary interest in anthraquinone as a raccoon repellent was to reduce crop damage (e.g. use as a foliar application). For example, anthraquinone is commercially available in the United States for the protection of newly-planted corn (e.g., 0.5% anthraquinone seed treatment, wt/wt). At the concentrations we tested, we did not observe efficacious repellency defined as ≥ 80% (Werner et al., 2016) and thus, anthraquinone is unlikely to reduce crop damage by free-ranging raccoons. An alternative use of this repellent may be to reduce raccoon impact as a non-target species for oral baiting, yet this strategy may only be effective if the target species (e.g. wild pigs) are less sensitive to anthraquinone than raccoons. For example, Snow et al. (2021) observed 52% and 42% feeding repellency among wild pigs offered whole corn treated with 0.5% and 1.5% anthraquinone (wt/wt), respectively. Based upon the feeding repellency that we observed, anthraquinone may also have use as a feeding deterrent (i.e., when feeding is reduced <80% and associated behaviors, such as guarding a food resource, may be altered).

5. Conclusion

Avipel® seed treatments (a.i. 9,10-anthraquinone) on whole corn resulted in a feeding repellency of 27–37% with 5,046–14,228 ppm anthraquinone and a feeding repellency of 71% with 20,377 ppm anthraquinone with raccoons. These numbers can be used as a guide to develop further research and field trials to either using anthraquinone as a deterrent with raccoons or developing an application to repel other species and still have consumption by raccoons.

CRediT authorship contribution statement

Shylo R. Johnson: Conceptualization, Data acquisition, Data analysis and Interpretation, and Manuscript preparation. **Shelagh T. Deliberto:** Conceptualization, Data acquisition, Data analysis and Interpretation, and Manuscript preparation. **Urchek:** Data analysis and Interpretation, and Manuscript preparation. **Amy T. Gilbert:** Conceptualization, Data analysis and Interpretation, and Manuscript preparation. **Scott J. Werner:** Conceptualization, Data analysis and Interpretation, and Manuscript preparation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.applanim.2022.105628.

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