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Effects of anticoagulant rodenticide poisoning on spatial behavior of farm dwelling Norway rats

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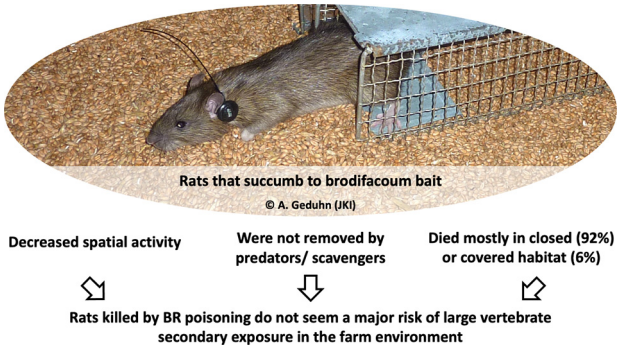
HIGHLIGHTS

- Brodifacoum (BR)-treated Norway rats moved rapidly & constantly less than controls.
- Some control, but no BR-treated rats were removed by predators.
- Liver BR concentration was higher during baiting versus oral delivery of $2xLD_{50}$.
- 92% of dead rats were inaccessible for large predators & scavengers.
- Rats killed by BR don't seem a major risk of large vertebrate secondary exposure.

GRAPHICAL ABSTRACT



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ABSTRACT

Commensal rodent species cause damage to crops and stored products, they transmit pathogens to people, livestock and pets and threaten native flora and fauna. To minimize such adverse effects, commensal rodents are predominantly managed with anticoagulant rodenticides (AR) that can be transferred along the food chain. We tested the effect of the uptake of the AR brodifacoum (BR) by Norway rats (*Rattus norvegicus*) on spatial behavior because this helps to assess the availability of dead rats and residual BR to predators and scavengers. BR was delivered by oral gavage or free-fed bait presented in bait stations. Rats were radio-collared to monitor spatial behavior. BR residues in rat liver tissue were analyzed using liquid chromatography coupled with tandem mass spectrometry. Norway rats that had consumed BR decreased distances moved and had reduced home range size. Treatment effects on spatial behavior seemed to set in rapidly. However, there was no effect on habitat preference. Ninety-two percent of rats that succumbed to BR died in well-hidden locations, where removal by scavenging birds and large mammalian scavengers is unlikely. Rats that ingested bait from bait stations had 65% higher residue concentrations than rats that died from dosing with two-fold LD_{50}. This suggests an overdosing in rats that are managed with 0.0025% BR. None of the 70 BR-loaded rats was caught/removed by wild predators/scavengers before collection of carcasses within 5–29 h. Therefore, and because almost all dead rats died in well-hidden locations, they do not seem to pose a significant risk of AR exposure to large predators/scavengers

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at livestock farms. Exposure of large predators may originate from AR-poisoned non-target small mammals. The few rats that died in the open are accessible and should be removed in routine searches during and after the application of AR bait to minimize transfer of AR into the wider environment.

1. Introduction

The Norway rat (*Rattus norvegicus*) is a commensal rodent species distributed throughout the world in a wide range of environments including rural habitats, such as agricultural land and farm structures, and urban habitats, such as sewer systems, parks, apartment blocks and home gardens (Wilson and Reeder, 2005). They pose a health risk because of the spread of several pathogens that can cause severe diseases in humans, livestock and pet animals (Meerburg et al., 2009). Furthermore, they cause losses to crops and stored products due to consumption and contamination and damage to infrastructure (Tobin and Fall, 2004) and invasive Norway rats can endanger native plant and animal species (Howald et al., 2015).

Similar to black rats (*Rattus rattus*) and house mice (*Mus musculus*, *M. domesticus*), Norway rats are usually managed with rodenticides, most often anticoagulant rodenticides (ARs) (Jacob and Buckle, 2018) because they effectively kill commensal rodents, are easy to use (Endepols et al., 2003; Endepols and Klemann, 2004) and there is an antidote available (Buckle and Eason, 2015). Anticoagulant compounds inhibit vitamin K epoxide reductase, an enzyme involved in the vitamin K cycle, which hampers blood clotting and eventually causes death several days after consumption of an effective dose (Buckle and Eason, 2015). For some rodent species including Norway rats, genetic resistance to some ARs has been reported (Rost et al., 2004; Pelz and Prescott, 2015) that renders affected compounds of all first (FGARs) and some second generation ARs (SGARs) ineffective for management (Endepols et al., 2012a; Endepols et al., 2012b; Buckle and Eason, 2015). The occurrence and spread of AR resistance has initiated the development of more potent second generation ARs (Rowe et al., 1985), such as brodifacoum (BR), that are suitable for rodent management even when resistant rodents are present (Buckle and Eason, 2015).

ARs inhibit blood clotting not only in target rodent species but also in non-target wildlife. Non-target animals may consume bait directly (primary exposure), which occurs when AR bait is applied in the open or non-targets might enter bait stations present in their habitat when their body size is similar to the target rodents. Furthermore, ARs can be transferred to non-target species indirectly when predators or scavengers consume target or non-target animals that have been exposed (secondary exposure) because second generation ARs are persistent (Fisher et al., 2003) and are bio-accumulative (Vein et al., 2013). Transfer of ARs occurs in taxa including mammals (McDonald et al., 1998; Fournier-Chambrillon et al., 2004; Geduhn et al., 2015), passerine birds (Walther et al., 2021) as well as predatory birds and owls (Christensen et al., 2012) (reviewed in Nakayama et al., 2019).

Due to the risk to the environment, there is no regular registration for the use of ARs in the biocide sector in the EU. However, because of the importance of rodent control for public health and lack of suitable alternatives, biocidal use of ARs can be approved for a shortened period of five years in the EU (2012). To minimize AR exposure of non-target animals during rodent management, registration authorities in the EU and in other regions of the world (Buckle and Prescott, 2018) impose risk mitigation measures (RMM). These measures include the use of bait stations (no surface broadcast of bait), the mandatory intervals of bait station inspection, the safe disposal of left-over bait as well as the search for and the removal of rats that have succumbed to the poison. The latter is intended to limit the number of prey items with AR residues available to terrestrial and avian predators and scavengers (Buckle and Prescott, 2018).

So far, it has been largely unclear whether wild rodents that have consumed AR bait change their spatial behavior. Changes in spatial activity and habitat use could make them more or less likely to be preyed on before they succumb to the effect of the rodenticide. To our knowledge, there is only one published field study of black rats living in a forest in New Zealand during brodifacoum (BR) baiting (Hooker and Innes, 1995). It indicates that there is no difference between movement patterns before and after administration of a lethal dose of BR, but sample size (2 males/2 females), and consequently, the statistical power was low. No field data are available for Norway rats or other wild rodents.

Little is known about key parameters of spatial behavior of wild Norway rats, such as home range size, distances moved and habitat selection. Telemetry work suggests that Norway rats select well covered habitat on farms (Taylor, 1978; Lambert et al., 2008) with minimum convex polygon (MCP) home ranges of 288 m^2 of males and 157 m^2 of females (Lambert et al., 2008). Similar home range sizes were reported from livestock farms in Argentina (Gómez Villafañe et al., 2008; Montes de Oca et al., 2017). However, anecdotal evidence suggests that large distances of >3 km can be covered within one night at decreased food availability (Hardy and Taylor, 1980).

There is almost no information available in which places poisoned rats succumb to ARs and if the habitat structures make it easy for predators or scavengers to find carcasses, but such data are highly relevant to assess the usefulness of RMMs or the risk for consumers of poisoned rats. The only published field data we are aware of show that the vast majority of California ground squirrels (*Spermophilus beecheyi*) exposed to a field application of diphacinone dies below ground (Baldwin et al., 2021). Rats may behave similarly. However, work in enclosures resulted in 12/18 Norway rats poisoned with BR (0.005%) dying away from cover and suggests a reduction in thigmotactic behavior and distorted day/night activity (Cox, 1991). Data of Gemmeke (1990) for Norway rats poisoned with AR compounds also collected in enclosures indicate lethargy, increased above ground activity and about similar numbers of dead rats being present above and below ground. However, rats in a natural environment may act differently to the behavior in an enclosure setting. Therefore, it is difficult to derive general patterns for risk assessment.

There is a plethora of studies considering AR residues in non-target species including rodents (Elliott et al., 2014; Geduhn et al., 2014; Elmeros et al., 2019), but little is known about AR residue levels in rats when ARs are used for rodent management. This is surprising as poisoned target species are considered a major avenue for the transfer of AR compounds to predators and scavengers via secondary exposure.

In a large-scale replicated field study, we aimed to assess key features of spatial behavior (home range size, distances moved, habitat selection) of Norway rats on livestock farms in relation to BR uptake. It was assumed that the consumption of BR leads to a reduction in spatial activity and preference for habitats that provide shelter. We also determined the habitat structure where rats died assuming that rats tend to use well covered structures when moribund and we measured residue levels of BR at the time of death. The findings can be used to assess the risk of secondary exposure posed by rats carrying AR residue to predators and scavengers due to behavioral patterns of rats.

2. Material and methods

2.1. Study area

The study area where farms were located is a mosaic of about 60% agricultural land and 15% forest patches. Farms produced mostly corn,

rapeseed and grain crops, and there was grassland used for silage to feed dairy cattle held on the farms, which is typical for the area. Farm size is mostly 10–50 ha (Destatis, 2019). Annual mean temperature is 8.8 °C, mean precipitation 782 mm/year and mean sunshine duration 1558 h/year (DWD, 2020). There were structures for housing farmer families, animals and to store machinery, animal feed and other equipment. Several farms were surveyed visually and using wildlife cameras (M-100, Moultrie Products LLC, USA) for signs of rats for two weeks prior to the study to identify suitable farms and locations on farms for trapping rats.

Based on the survey, farms were selected that were at least 2.3 km apart. Farmers did not use AR on their properties for at least eight months prior to the study.

2.2. Data sampling and editing

Norway rats were trapped from March to May and November to December 2016 on six livestock farms (farms 1–6) around Münster, Germany (52°N, 8°E). 12–44 live traps of various types (Schwengber, Jagdfallen Steingraf, Kortenbrede GmbH, Tomahawk live trap) were set at locations inside and outside buildings where rat activity was expected. Traps were pre-baited with a mix of rolled oats, peanut curls, chocolate spread and apple pieces for 2–6 weeks, checked every 3–4 days, and bait was replaced as necessary. All traps were covered with black plastic sheets to protect rats from environmental conditions. On each farm, rat trapping was conducted for 4–7 days, and traps were checked every 12 h. Traps were equipped with fresh bait and pieces of non-woven dust sheet for nesting. All rats were transferred from the trap to a veterinary anesthesia workstation (Trajan 808 Air, Vapor 19.3, Dräger AG, Germany) to anesthetize the animals by inhalation of an isoflurane-oxygen mix (2.5–5%) following the procedures stated in Imholt et al. (2018). Unconscious rats were weighed to the nearest gram with a laboratory scale (Kern 440-53, Kern & Sohn GmbH, Germany) to determine the appropriate volume for a twofold LD_{50} of BR (0.6 mg BR/kg body weight). Adult rats >200 g body weight were used for the trial. BR was dissolved in diethylene glycol and administered to 53 unconscious treatment animals via oral gavage at a volume of 330 μl/100 g. Sixteen experimental control rats were similarly anesthetized but did not receive BR solution (Table 1).

In September to November 2019, further rats were trapped and radio-collared using a similar protocol for collaring at two additional farms (farms 7–8, at least 2.2 km apart) in the study area at least three days before the farms were baited with BR-bait (Table 1). Bait used contained 0.0025% BR in bait based on cereals and vegetable fat (Bayer AG, Germany). Baiting followed best practice procedures for Norway rat management (UBA, 2014).

Rats were equipped with a radio collar (TXE-116CZ, 10 g, 150 MHz, 40 bpm, Telenax, Mexico,) that emitted a signal at an individual frequency changing in pulse rate when the internal movement sensor did not detect movement for 4–5 h. This enabled us to determine the approximate time of death and to search for dead animals without delay. After dosing and fitting the radio-collar, rats were kept under an infrared heat lamp (Philips, 150 W) and released after full recovery at the point of capture.

Radio tracking on farms 1–4 started the day following the tagging procedure at least 18 h after tagging to prevent tracking potentially biased behavior shortly after release. The position of each rat was determined with a three-element-Yagi antenna (Linflex, Biotrack Ltd., UK) and an VHF-receiver (Australis 26k, Titley Scientific, Australia) with the "homing-in" method (White and Garrott, 1990) to assess spatial behavior and habitat use. Rats on farms 1–4 were located twice per hour for 2 h before and 2 h after sunrise/sunset and for 1 h during the day, resulting in 18 radio fixes per 24 h-period (day). This was repeated for each rat until the signal indicated death of an animal, which was then recovered if possible. Rats on farms 5–8 were located once every day until the signal indicated death of an animal, which was then recovered if possible.

For each radio fix and for the location where an animal was recovered dead (see below), habitat characteristics were recorded and mapped (Google, 2013). Mapped locations of dead rats were transferred to digital geo-referenced maps (BKG, 2017) using ArcGIS (ESRI, 2012). UTM-coordinates of locations were used to calculate the minimum distance moved between consecutive locations on farms 1–4. Values were summed for the first and the last day of radio-tracking. Home ranges were calculated as 100% minimum convex polygons (100% MCP) (Mohr, 1947) with program *R* (RCoreTeam, 2018) package *adehabitatHR* (Calenge, 2006) on farms 1–4.

Three habitat categories were mapped: closed (e.g., inside buildings, walls, silos, stacks of wood or stone, burrows), covered (e.g., tall vegetation, hedges, tall crops etc.), open (e.g., lawns, driveways and other sealed surfaces). The Jacobs' index (Jacobs, 1974) was calculated for each animal on farms 1–4 to assess habitat preference. The available habitat was based on the habitat present in each animal's 100% MCP (third order selection, Johnson, 1980).

2.3. Chemical analyses for BR residues

Radio-collared rats recovered dead were stored at −80 °C for at least two weeks to inactivate zoonotic parasites. Rats were defrosted for removal of liver tissue that was stored at −20 °C until analyzing for all ARs registered for use in products in Germany at the time of the trials: chlorophacinone, coumatetralyl, brodifacoum, bromadiolone, difenacoum, difethialone, flocoumafen and warfarin, even though only BR was used in trials (Walther et al., 2020). For the verification of the analytical process, each thawed sample was first fortified with the surrogate substances acenocoumarol, coumachlor and diphacinone-d4. The analytes were extracted with a solution of methanol and water (2:1, *v*/v) by vigorously blending with an Ultra-Turrax. Following centrifugation, an aliquot of the supernatant was cleaned through a solvent exchange with dichloromethane over a diatomaceous earth solid phase column. An aliquot of the purified solution was evaporated to dryness. The residue was redissolved in methanol/water (1:1, v/v) with the internal standards warfarin-d5 and chlorophacinone-d4. The amounts of ARs in the liver samples were estimated using liquid

Table 1
Number of Norway rats (*Rattus norvegicus*) radio-tracked on eight farms in the Münsterland region in western Germany.

Farm no.	BR application	Number of Norway rats used for data analyses										
		Total	Distances moved		Home range		Habitat preference		Location of dead rat		AR residues	
			T	C	T	C	T	C	T	C	T	C
1–4	Oral gavage	41	36	16	34	15	36	16	41	NA	17	NA
5–6	Oral gavage	2	Not studied		Not studied		Not studied		2	NA	2	NA
7–8	Free-fed	27	Not studied		Not studied		Not studied		27	NA	17	NA
Total		70	36	16	34	15	36	16	70	NA	36	NA

Rats were exposed to the anticoagulant rodenticide brodifacoum (BR) and spatial behavior, habitat preference, location of dead rats and anticoagulant rodenticide (AR) residues in liver tissue were assessed. On farms 1–6, rats received a twofold LD_{50} BR solution via oral gavage (treatment, T) before the radio-tracking period and control rats (C) remained untreated. On farms 7–8, radio-collared rats were free-fed BR bait (0.0025%) in bait stations (treatment, T). Baiting followed best practice procedures for Norway rat management (UBA, 2014). NA – not applicable.

chromatography coupled with tandem mass spectrometry in negative electrospray ionization mode (for details see supplementary material).

2.4. Statistics

Minimum distances moved (dependent variable) were compared between the first and the last day of observation, treatment and sex (explanatory variables) using a linear mixed model. Differences between 100% MCPs (dependent variable) of the first and last day of the observation period, treatment and sex (explanatory variables) were compared with a general linear mixed model for negative binomial distribution. In both models, individuals were nested in site (farm) as random factor (repeated measures).

The percentage of rats found dead in the three habitat categories (closed, covered, open) was calculated separately for farms 1–8 and compared with a Kruskal-Wallis test. Comparison of BR residues in liver tissue samples from rats dosed with twofold LD_{50} BR (farms 1–6) and rats that consumed free-fed bait (farms 7–8) was conducted with a Wilcoxon-Mann-Whitney test. The correlation between BR residue concentration and day of death was analyzed with the Pearson correlation coefficient (oral delivery) or Spearman's rho (free-feeding). For statistical analyses, Program *R* (RCoreTeam, 2018) in *RStudio* (RStudioTeam, 2016) and for modeling the R-package "*lme4*" (Bates et al., 2015) were used.

3. Results

3.1. Spatial behavior

During radio telemetry at farms 1–4, 4500 radio fixes were collected to calculate individual minimum distances moved and home range size (Table1). Of 50 rats dosed with brodifacoum and 16 untreated experimental control rats, minimum distances moved were calculated for 36 treated (23 males, 13 females) and 16 control rats (7 males, 9 females). For eight radio-collared rats, radio-tracking data were insufficient for calculations. Four treated rats survived until the end of the study, and two rats lost transmitters (data not used in analysis). These transmitters were found shortly after radio-collaring rats and close to the places where the rats were released. The transmitters were undamaged indicating that collars were not fitted properly.

At the end of the observation period, in treated rats, the minimum distances moved were about 50% shorter than at day 1 ($p = 0.002$; decrease in males from 111 ± 11 m to 49 ± 17 m and in females from 69 ± 13 m to 35 ± 8 m; Fig. 1a). Minimum distances moved of

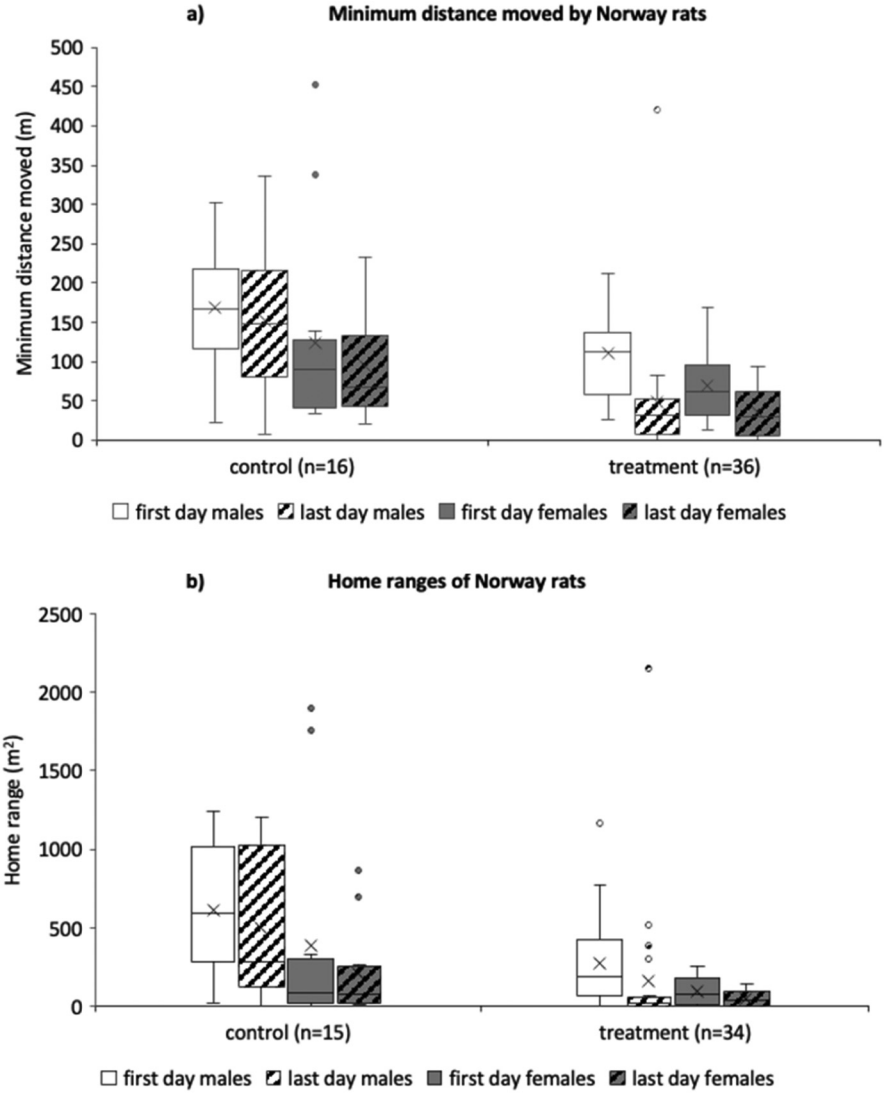

Fig. 1. a) Minimum distance and b) home range size (100% minimum convex polygons) moved of Norway rat (*Rattus norvegicus*) males and females in livestock farms 1–4 at the first and the last day of the radio-tracking period. Control rats remained untreated (control). Treatment rats received a twofold LD_{50} brodifacoum (treatment) before the radio-tracking period. Boxes - 25/75% quartile; X - mean; horizontal line - median; whiskers - ≤1.5-fold interquartile range; dots - ≥1.5-fold interquartile range.

treatment rats were 38% longer in males than females at the first day of the observation period and 29% longer at the last day of the observation period ($p = 0.035$). They were about two thirds shorter than in control rats ($p = 0.001$; Fig. 1a). There was no difference in control rats between the minimum distance moved on the first and last day of tracking ($p = 0.502$; Fig. 1a). Overall, male control rats (160 ± 19 m) moved more than female control rats (109 ± 19 m; p = 0.035).

Home range sizes (100% MCPs) could be calculated for 34 treatment rats (23 males, 11 females) and 15 control rats (7 males, 8 females; Table 1). Home ranges of males (control: 553 ± 92 m^2; treatment: 216 ± 56 m^2) were generally larger than home ranges of females (control: 300 ± 103 m^2; treatment: 70 ± 16 m^2) ($p = 0.003$). Treatment rats had smaller home ranges than control rats at first and last day of the observation period ($p < 0.001$; Fig. 1b). Depending on sex and treatment, there was a trend in treatment rats ($p = 0.093$) and control rats (p = 0.093) for 18–53% smaller home range size at the end of the observation period compared to day one. This reduction was about 50% larger in treatment than in control rats (Fig. 1b).

3.2. Habitat preference

3153 (76%) telemetry locations of 16 control and 36 treatment rats were in closed habitat structures, 344 locations (8%) in covered habitat and 686 locations (16%) in open habitat (Table 1). There was no difference in the relative number of observations in the three habitat categories between control and treatment rats ($p = 0.174$). Jacobs' index values < 0.5 suggest that there was no particularly strong preference for any of the three habitat categories (Fig. 2). However, control (p < 0.001) and treatment rats ($p = 0.036$) used closed structures more intensely than open habitat.

3.3. Location of dead rats

Three treated rats were killed by farm dogs on farm 3 soon after release (data not used in analyses; dogs leashed during subsequent work), and three control rats were caught by naturally occurring predators (buzzard (*Buteo buteo*) and eagle owl (*Bubo bubo*)) at farms 3 and 4.

All dead rats at farms 1–6 (oral gavage) were located at the property where they had been captured. They had died 3–6 days after dosing and free-feeding rats at farms 7–8 showed a peak mortality at days 5–7 after the start of baiting. A total of 70 (37 males, 33 females; Table 1) radio-collared BR-dosed rats were located within 5–29 h after death at farms 1–8 (farms 1–6, oral gavage, $n = 43$; farms 7–8, baiting, $n = 27$). There was no difference in the number of observations of rats in the three habitat categories between rats at farms 1–6 and farms 7–8 ($\chi^2 = 0.08$, $p = 0.96$), and hence, data were pooled for the assessment in what habitat structures poisoned rats died.

There was a difference among the percentage of rats found dead in the three habitat categories (H = 11, $p < 0.0036$; Fig. 3). 60 dead rats (91.5 ± 3.4% per farm) were found in closed habitats, 33 (51.7 ± 12.3% per farm) of them were located within buildings in walls, ceilings, under floors as well as other closed structures and 27 (47.6 ± 12.2% per farm) outside buildings in burrows, piles of stone, timber or other material. Seven carcasses (6.5 ± 2.6% per farm) were recovered from covered habitat in dense vegetation (Fig. 3) that consisted mostly of stinging nettle (*Urtica dioica*). Three dead rats (2.0 ± 1.3% per farm) were found in the open on gravel roads.

3.4. Anticoagulant rodenticide residues

Nineteen treated rats (12 males, 7 females) were recovered within hours after death from farms 1–6 (BR application via oral gavage) and were screened for AR residues (Table 1). BR was present in liver tissue of all rats. The mean BR residue concentration in liver tissue was 2115 ± 224 ng/g. There were traces of difenacoum (range 16–84 ng/g) detected in 42% of rats, and bromadiolone (178 ng/g) and coumatetralyl (95 ng/g) in one rat each. No other ARs were present. In 17 rats recovered shortly after death from farms 7–8 (7 males, 10 females; BR application via free-feed at bait stations), BR was present in all liver tissue samples at a mean concentration of 6011 ± 1181 ng/g. There were traces of difenacoum (range 4–120 ng/g) detected in 53% of rats fed on bait, but no other ARs were present. BR concentration was about 65% lower when BR was delivered via oral gavage versus baiting ($p = 0.015$, $r = 0.41$). The duration from dosing to death was not correlated to the BR residue concentration detected when rats received a two-fold LD_{50} BR via oral gavage ($n = 19$; $R^2 = 0.002$; $p = 0.85$). However, there was a considerable negative correlation in rats free-feeding on bait between the duration from BR bait application until death of rats and BR liver concentration ($n = 17$; $R^2 = 0.45$; $p = 0.003$; Fig. 4).

4. Discussion

The spatial behavior of Norway rats that have consumed a twofold LD_{50} of BR was different between day 1 after BR dosing and at the day of death and compared to untreated control rats. BR causes physiological effects within days of consumption leading to internal bleeding (Watt et al., 2005) that is likely to affect movements. As expected, there was a considerable decrease in minimum distances moved in

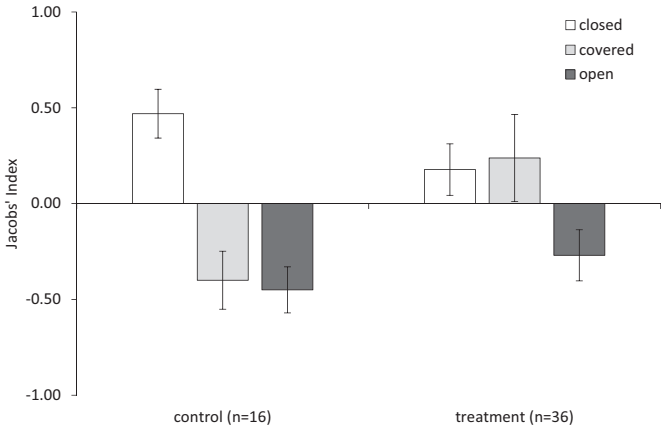

Fig. 2. Jacobs' index (Jacobs, 1974) for habitat preference of Norway rats (*Rattus norvegicus*) for closed, covered and open structures in livestock farms 1–4. Rats remained untreated (control) or received a twofold LD_{50} brodifacoum (treatment). Values are means of individuals ± standard error.

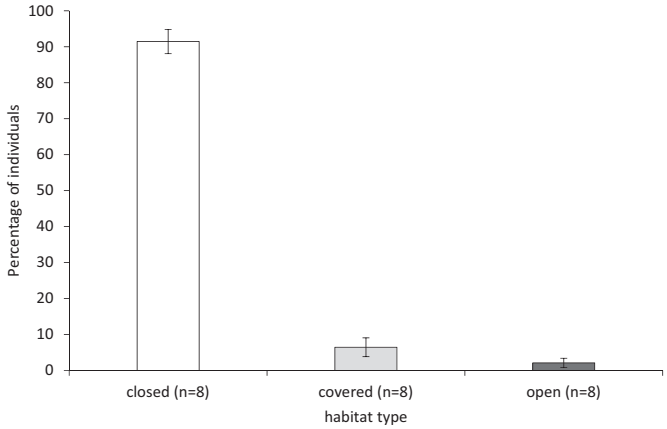

Fig. 3. Mean percentage of habitat where 70 Norway rats (*Rattus norvegicus*) across farms 1–8 succumbed to brodifacoum (twofold LD_{50}) delivered via oral gavage (farms 1–6) or via free-fed brodifacoum bait (farms 7–8). Rats were radio-collared and recovered within 5–29 h after death. Locations of dead rats were a) closed indoor and outdoor locations b) covered with dense vegetation and c) open habitats. Values are means of farms ± standard error.

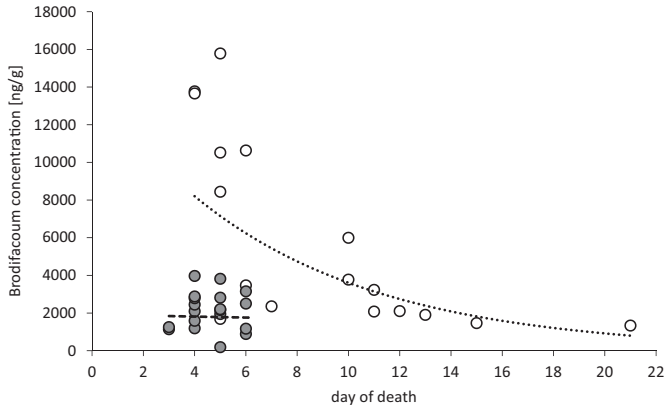

Fig. 4. Correlation of brodifacoum (BR) concentration in liver tissue of Norway rats (*Rattus norvegicus*) in livestock farms and 1) the duration from dosing with a twofold LD_{50} BR via oral gavage until death ($n = 19$; grey dots) and 2) the duration from the start of BR bait application until death of rats free-feeding on bait ($n = 17$, white dots).

treatment rats. This may reduce the risk of being caught and eaten by a predator and hence the risk of BR transfer along the food chain. Conversely, the speed of movement and escape behavior might have been impaired, which could potentially increase predation risk but this seems unlikely given none of the RB treated rats was caught by a predator.

Apart from changes in spatial behavior, other sublethal effects of ARs were observed in other studies, such as reduction of thigmotactic behavior in enclosures, disturbed temporal activity in cage trials and enclosures (Cox and Smith, 1992), lethargy in cage trials (Wilk, 1957) and enclosures (Gemmeke, 1990), and unnatural posture in cage trials (Littin et al., 2000). These effects may have occurred and might affect predation risk and associated secondary exposure but could not be detected by the indirect observation using radio-telemetry in this study. Such additional behavioral changes as well as reduced spatial activity may affect predation risk not only for Norway rats but also for non-target rodents that have accidentally consumed BR.

Minimum distances moved by control rats were about the distances moved by Norway rats in sewers (Heiberg et al., 2012) but smaller than the nightly maximum linear movements of farm rats (875 m) (Herden, 1992; MacDonald and Fenn, 1995). At the start of the telemetry period at farms 1–4, home range size in treatment rats was smaller compared to control rats. This could indicate a rapid onset of behavioral effects in rats dosed with BR. Both treated and control rats were anesthetized, but the latter were not gavaged. It seems unlikely that the gavage alone has caused altered spatial behavior of treatment rats because the volume delivered was small (330 μl/100 g), and animals were unconscious. In any case, the reduction in home range size from start to end of the telemetry period was more pronounced in treatment rats confirming our assumption that the consumption of BR leads to a reduction in home range size. Why there was also a decrease in home range size in control rats is unclear but might have been due to short-term environmental changes given the values were based on 1-day periods. Generally, home range sizes of rats were similar to the few published values, such as 288 m^2 in males and 157 m^2 in females (Lambert et al., 2008) and as reported elsewhere (Taylor and Quy, 1978; Cowan et al., 2003; Gómez Villafañe et al., 2008). The radio-collared rats generally preferred well sheltered habitats, such as buildings, silos, piles of stone and timber etc., where they were protected from predation. This is in accordance with the few earlier findings of telemetry studies with Norway rats on farms (Taylor, 1978; Lambert et al., 2008).

In the course of the study, there was no obvious shift of territory in the 43 treated rats at farms 1–6. They and all 27 rats found dead at farms 7–8 were discovered on the property where they were radio-collared. This suggests that poisoned rats do not leave the area. Norway rats seem reluctant to leave territories once established (Davis et al., 1948; Taylor and Quy, 1978; Lambert et al., 2008), as it is the case in other small rodent species (Jacob and Hempel, 2003; Jacob et al., 2004). This suggests that the transfer of ARs by Norway rats is similarly spatially restricted as in non-target small mammals where AR prevalence and concentration are the higher the closer to bait stations animals are trapped (Geduhn et al., 2014; Elmeros et al., 2019).

Interestingly, there was >20-fold inter-individual variation of residual BR concentrations in liver tissue in treatment rats despite the delivery of identical doses via oral gavage. Rats were recovered within hours after death making degradation of BR unlikely. Furthermore, the residual BR concentration did not correlate with the duration from dosing until death. Treated rats died mostly within 3–6 days after the BR dose was delivered, which is typical for BR poisoning in Norway rats (Littin et al., 2000). During this time, rats continue to consume rodenticidal bait (Gemmeke, 1990), and this was probably also the case for rats at farms 7–8 that had continuous access to bait. This was clearly reflected in BR residues that were almost threefold higher when rats free-fed on bait versus consumption of a twofold LD_{50} of BR via oral gavage. Continuous bait uptake of 0.0025% BR bait seemed to lead to considerable overdosing and may increase BR body loadings of rats. However, there seems to be an equilibrium reached after about four days of bait uptake at least for the AR chlorophacinone (Vein et al., 2013). Traces of difenacoum that were regularly present in rats at liver concentrations mostly near the detection limit may have originated from earlier baiting campaigns given the long half-life of the compound (Fisher et al., 2003).

Rats that received BR via oral gavage died within a short period (3–6 days), which may have been too short to result in a detectable correlation between this period and BR residue concentration. In rats that free-fed on BR bait on farms 7–8, the period from bait placement to death was the shorter the higher the BR liver concentration was. This could indicate that large BR doses lead to rapid death of rats during field application of bait, resulting in rapid management success. However, it is unclear whether this is biased by the timing of consumption. Some rats may have been behaviourally suited to feed on bait early and in larger amounts than more cautious individuals that started to feed late and consumed less, which would result in a similar pattern. This should be tested in future field trials where timing and the amount of individual bait uptake are traced.

The likelihood of secondary exposure of large predators and scavengers depends on several factors including how easily they can find poisoned rodents. As assumed, almost all Norway rats died of BR poisoning at well-hidden locations inaccessible to avian or large mammalian predators and scavengers or in dense vegetation with limited access for them. Only 2.0 ± 1.3% of rats died in accessible areas. For the first time, these findings quantitatively support anecdotal evidence that after rodent control most rat carcasses are located in protective structures such as haystacks (Fenn et al., 1987).

Stoats (*Mustela erminea*) and least weasels (*Mustela nivalis*) could access rat burrows (King et al., 2007), and they as well as other terrestrial predators can enter dense vegetation. This is certainly more likely when dead rats are present in closed/covered outdoor locations (48% of rats in our study) than in closed indoor locations (48% of rats in our study). A high proportion of stoats and least weasels can have AR residues in liver tissue (Murphy et al., 1998; Elmeros et al., 2011) suggesting particularly high exposure frequency in this taxon. However, these predators only very exceptionally consume rats (McDonald et al., 2000; Elmeros, 2006; Piontek et al., 2015). This and our results seem to indicate that such predators are not so much exposed via dead rats but via alive rats active on the surface or via other sources such as non-target small mammals that have consumed BR. The latter seems likely (Elliott et al., 2014; Geduhn et al., 2014; Elmeros et al., 2019).

In this study, according to best practice (UBA, 2014; CRRU, 2015), searches were regularly conducted to locate and remove dead rats. All radio-collared rats that succumbed to BR could be retrieved within 5–29 h. None of these rats was captured alive or removed dead by predators before collection. This indicates a lower removal rate than for dead

water voles (*Arvicola terrestris*) from grassland where the scavenging rate is 87.5% within 0.5–1.5 days (Montaz et al., 2014). In contrast, predatory birds killed three of 16 control rats at farms 1–4. This may have been due to larger minimum distances moved by control rats, but this conclusion must remain vague because of the small sample size.

A small fraction of poisoned rats died in the open, and these individuals can and should be removed and disposed of to prevent secondary exposure of scavengers. Removal of carcasses is also sensible for hygiene reasons. The three dead rats (2.0% of all dead rats) encountered in the open were plainly visible so that searching and removing required little effort and time. Locating the seven rats in dense vegetation was possible only because they were radio-tagged and would have remained undetected in a routine search. At farms 1–6, a small fraction of the on-farm rat populations was dosed with BR but all rats at farms 7–8 had access to BR bait. In either scenario, the percentage of carcasses present in the open was small.

5. Conclusions

In a field situation, Norway rats reduced spatial activity after the consumption of BR and did not leave the area. Almost all rats that succumbed to BR died in well-hidden locations where removal is unlikely for scavenging birds and most terrestrial scavengers and consequently, none of 70 radio-collared BR-loaded rats was caught or removed by wild predators or scavengers. This indicates a minimal risk of secondary BR exposure for large predators and scavengers in the farm environment. However, smaller predators and scavengers may still have access to poisoned rats. Rats killed with BR on farms do not seem to pose a significant risk of BR exposure to large predators and scavengers. However, secondary exposure of such species is evident and may be also due to the uptake of non-target small mammals that have consumed BR (Brakes and Smith, 2005; Geduhn et al., 2014). BR residue concentrations were present in all rats and considerably higher when rats free-fed on BR bait than when BR was delivered via oral gavage indicating overdosing in a field situation. Attempts should be made to minimize the risk of transfer of ARs via overdosed target rodents, possibly by using pulse baiting techniques (Greaves et al., 1988; Buckle et al., 2012) or by inducing a stop-feeding effect (Endepols et al., 2017). All AR hydroxycoumarins belong to one mode of action and it is likely that results obtained with BR in this study similarly apply to all ARs.

CRediT authorship contribution statement

Bernd Walther: Conceptualization, Methodology, Investigation, Formal Analysis, Writing – Original draft preparation.

Hendrik Ennen: Investigation, Formal Analysis.

Anke Geduhn: Conceptualization, Methodology, Investigation, Writing – Review & Editing.

Annika Schlötelburg: Formal Analysis, Writing – Review & Editing.

Nicole Klemann: Conceptualization, Methodology, Investigation, Writing – Review & Editing.

Stefan Endepols: Conceptualization, Methodology, Writing – Review & Editing.

Detlef Schenke: Methodology, Investigation, Writing – Review & Editing, Writing – Supplementary Information.

Jens Jacob: Conceptualization, Methodology, Writing – Original draft preparation, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Stefan Endepols is affiliated with Bayer AG that manufactures anticoagulant rodenticidal products and funded the baiting trials. He did not contribute to the discussion and interpretation of results from these trials.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.147520.

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