TOXICOKINETICS AND TIME-VARIABLE TOXICITY OF CADMIUM IN OPPIA NITENS KOCH (ACARI: ORIBATIDA)

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Abstract: The soil-living mite Oppia nitens Koch has recently been proposed as a promising test species for the ecotoxicological risk assessment of contaminated boreal soils. Adding oribatid mites to the assemblage of test species for soil is highly desirable given the enormous diversity and ecological significance of these microarthropods. The authors aimed at revealing how toxicity, lethal body concentration, and bioaccumulation of cadmium (Cd) changed over a period of 7 wk when mites were exposed to Cd-spiked natural soils. The estimated median lethal concentration (LC50) values showed a gradual decrease with time, but a steady state was not reached within 7 wk. Estimates for lethal body concentration varied from 44 μg Cd/g to 91 μg Cd/g dry body weight, with a tendency to increase with time. The estimated 50% effective concentration (EC50) for effects on reproduction after 7-wk exposure was 345 μg Cd/g dry soil. Accumulation of Cd in mites was extremely variable but overall showed a nonsaturating increase. A simple 1-compartment toxicokinetic model did not describe the data well. The analysis suggests that O. nitens has a storage-detoxification strategy that is not at equilibrium under chronic exposure. Considering the tiny body size of the animal, it is remarkable that long exposure times are necessary to reveal chronic toxicity. The use of oribatids provides a clear added value to soil risk assessment but trades off with exposure length. Environ Toxicol Chem 2017;36:408–413. © 2016 The Authors. Environmental Toxicology and Chemistry published by Wiley Periodicals, Inc. on behalf of SETAC.

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INTRODUCTION

Soil pollution is a serious worldwide problem in which metals play an important role. Soil contamination with cadmium (Cd) is believed to be a serious health risk, and negative effects may be found already at relatively low concentrations [1]. Metals are nondegradable and often accumulate in biological tissues [2–4]. Significant quantities of Cd are added to soils globally as a result of various anthropogenic activities, raising concerns about environmental health.

The ultimate effects of toxic chemicals on organisms depend on 3 factors: the extent of exposure to a toxicant, as influenced by environmental availability; the internal concentration, as determined by rates of uptake and elimination; and the interaction with receptors at the target site [5,6]. Ecotoxicity experiments may increase insight into these factors, and the results can be used to derive safe levels of chemicals in the environment [7].

Animals deploy a great variety of strategies to deal with potentially toxic loads of metals. One detoxification strategy is to fix metals in an insoluble form, often in designated tissues, thus preventing them from interacting with vital biochemical functions. In these cases metals can be retained within the body for a prolonged time. This means that under continuous exposure there is a tendency for the total internal body concentration to increase as long as the animal is exposed to the metal [5]. Such a response was found, for example, for Cd accumulation in pseudoscorpions [2] and isopods [3]. Species that are unable to eliminate Cd and do not reach a steady state for the body burden will have a 50% lethal concentration (LC50) that continuously decreases with exposure time [3]. This is observed for Cd in earthworms [8], oribatid mites, and pseudoscorpions [2].

Another strategy is to quickly eliminate any metal taken up; animals deploying this method reach a steady state after relatively short exposure times, in which the rate of elimination equals the rate of uptake, the body burden is low, and the LC50 approaches a stable value. This pattern is observed in Collembola and carabid beetles [2]. Reflecting the different toxicokinetic strategies, elimination rate constants estimated from kinetics experiments show a large variability among species [9].

In the present study, we focus on an enormously diverse but little investigated group of soil-living arthropods, the Oribatida. The use of oribatid mites in ecotoxicology studies was reviewed by Lebrun and van Straalen [10]; however, no oribatid species was presented as a good model because of the difficulties of laboratory culture. Most of the earlier experimental ecotoxicity work is on the camisiid species Platynothrus peltifer (Koch) [3,10–12], which, however, has a very long life cycle and is difficult to culture in the laboratory. Recently, Princz et al. [13] presented the oppiid mite Oppia nitens Koch as a candidate species for a standardized test in soil toxicity testing, with adult survival easily assessed in a relatively simple design.

Oribatid mites are interesting because they belong to the most numerous and diverse group of soil organisms. However, except for P. peltifer, little is known about toxicokinetics and time-dependent toxicity in these animals, although such information is crucial in the design of toxicity experiments. Platynothrus peltifer seems to belong to the group of steady accumulators, but it is not known whether this is exemplary for Oribatida in general. The newly proposed model O. nitens
allowed us to explore this question in more detail than was possible before.

The main objective of the present study was to determine the uptake kinetics and time-variable toxicity of Cd in *O. nitens* exposed through a soil substrate, to obtain insight in an adequate exposure time for ecotoxicity experiments with this new and promising model species.

**MATERIALS AND METHODS**

*Test organism*

The test organism used for the present study was *O. nitens* Koch (Acarici: Oribatida: Oppiidae). The source culture was kindly provided by J. Prinz (Environment Canada) and had been propagated in the Ecological Science department of the Vrije Universiteit (Amsterdam, The Netherlands) for more than 2 yr. Animals were cultured on a substrate of plaster of Paris, mixed with charcoal (10:1:10 [w/w/w] plaster, charcoal, distilled water), on the bottom of plastic containers (18 cm × 13.5 cm, 4.5 cm height). The containers were covered with a perforated lid, and the substrates were moistened once a week with deionized water. Grains of baker’s yeast (Algist Bruggeman) were added as a food source. The containers were kept under laboratory conditions in a climate room set at 20 °C, 75% relative air humidity, and an 8:16-h dark:light regime. Using a stereomicroscope, the culture can be seen to consist of a multitude of larvae, nymphal stages, and adults, all crawling over the food and the plaster. For the experiments, newly emerged adults (recognized by their pink-brown color) were used. Often, *O. nitens* is believed to be wholly parthenogenetic; however, there is conflicting information on its reproductive mode [14]. In our experiments we observed only females, judged by the similarity of their ventral features with previous descriptions (cf. Keshavarz-Jamshidian et al. [15]).

*Spiking soils*

Test soil was a natural standard soil, LUFA 2.2 (Speyer), a loamy, sandy soil with pH 5.5 (in 0.01 M calcium chloride [CaCl2]), water holding capacity of 45.2%, cation exchange capacity of 10.0 cmol kg⁻¹, and total organic carbon content of 1.93%. Soils were gently oven-dried overnight at 50 °C before spiking. Soil samples were spiked with an aqueous solution of cadmium nitrate (Cd(NO₃)₂·4H₂O; Sigma-Aldrich; 99% pure) dissolved in deionized water in a concentration such that the desired final concentration of Cd was obtained when wetting the soil to 22.5% w/w, corresponding to 50% of water holding capacity. The selected nominal Cd concentrations were 12.5 µg Cd/g, 25 µg Cd/g, 50 µg Cd/g, 100 µg Cd/g, 200 µg Cd/g, and 400 µg Cd/g dry soil. Nonspiked soil was used as a control by adding sufficient deionized water to reach 50% of water holding capacity.

Portions of Cd-amended soils were kept for 2 wk at 20 °C in sealed plastic containers before distributing them over the experimental containers. Experimental containers were circular plastic pots of 4 cm diameter and 3 cm height, to which 20 g moist soil was added. The test containers had a gauze bottom (mesh 1 mm), which was sealed with wrapping foil to avoid moisture loss and mites escaping through the gauze. Each individual container was placed on a small plastic Petri dish to which some water was added to create a humid atmosphere around the container. Twenty-seven replicate containers were prepared for each of the 7 treatments. From these, 5 replicates per treatment were scheduled to be euthanized at 5 time points, and 2 additional containers per treatment were prepared for soil analyses and pH measurements at the end of the experiment.

Ten newly emerged adult mites were introduced into each container. The animals were fed weekly with baker’s yeast crumbs spread over the soil surface. Moisture loss of the soil was assessed weekly by weighing the containers, and the corresponding water loss was replenished. The containers (189 in total initially) were randomly arranged on a plate and incubated in a climate room using a 16:8-h light:dark cycle and a temperature of 20 °C.

*Experimental procedures*

After 3 wk, 4 wk, 5 wk, 6 wk, and 7 wk of incubation, the containers were taken from their dish, the wrapping foil was removed, and the containers were placed in a Tullgren extractor [16]. The mites could escape from the drying soil through the gauze bottom and were caught in an empty plastic jar with no plaster. These collection vials were inspected daily. The absence of plaster in the collection vials was found to be crucial to facilitate counting. Nymphal stages were distinguished from the adults by their pale color and body size. Both juveniles and adults were counted. The recovered adult mites from each replicate were transferred separately in microtubes and freeze-dried for chemical analysis. During the experiment we noted that among the extracted *O. nitens* were individuals of the grain mite *Acarus siro* Linnaeus (Oribatida: Astigmata: Acaridae). This very small mite, most likely introduced as eggs in the yeast, can be mistaken for juvenile *O. nitens* if not inspected under the microscope. These mites were ignored when counting *Oppia* nymphs.

*Determining Cd in mites*

Animals were freeze-dried for 2 d and then weighed using a microbalance (UMT2; Mettler Toledo) to measure dry weights. Animals were subsequently transferred to Pyrex tubes, which had been ultra-cleaned by a special cleaning protocol involving detergent and ultrapure nitric acid (HNO₃). The mites were digested in a 7:1 mixture of ultrapure HNO₃ (Ultrex II Ultrapure Reagent 67–69%; J.T. Baker) and perchloric acid (HClO₄; Ultrex II Ultrapure Reagent 70%; J.T. Baker). The tubes were heated in a heating block until acids were completely evaporated. Shortly before analysis, the dry pellets were dissolved in 300 µL of 0.1 N ultrapure HNO₃. Cadmium concentrations were measured using a Zeeman graphite furnace atomic absorption spectrophotometer (AAS; Perkin Elmer; 5100 ZL). The detection limit in the digests was 0.3 pg/mL, which corresponds to a concentration in the animal of 9 ng/g (at an average dry body wt of 10 µg). Cadmium concentrations in the animals were much higher than this level. Reference material (National Research Council, Canada; Dolt-4, containing 24.3 ± 0.8 µg/g) was digested in parallel to ensure quality control over the analytical process, and blank tubes were included to check for possible contamination.

*Soil analysis*

Soil samples were dried at 50 °C for 24 h. Cadmium concentrations were measured in 2 replicates. A subsample of approximately 130 mg was digested in tightly closed Teflon bombs using 2 mL of a mixture of 4:1 HNO₃ (65% pro analysis) and hydrochloric acid (HCl, 37% pascal; Sigma-Aldrich). The bombs were placed in an incubator (Binder ED 53) and heated for 7 h at 140 °C. After digestion, the bombs were cooled down to room temperature, and 8 mL deionized water was added. Subsequently, samples were analyzed by flame AAS (Perkin
Elmer; AAnalyst 100) for total soil Cd concentrations. As a certified reference material, ISE sample 989 of river clay (Wageningen, The Netherlands) was analyzed in parallel to the samples. Cadmium concentrations measured in the reference material were within 13% of the certified value.

To measure exchangeable metal concentrations and pH, duplicate soil samples of 5 g were transferred to plastic jars; 25 mL of 0.01 M CaCl$_2$ was added, and the mixture was shaken overnight on a rotary shaker (250 rpm); after allowing the sediment to settle for a few minutes, pH was measured using a WTW Inolab 7110 instrument. Subsequently, a membrane filter (0.45 μm, 47 mm, cellulose nitrate; Sartorius) was used to filter the extracts. Extracts were kept at 4 °C for 5 d until analysis. Samples were analyzed using graphite furnace AAS (Perkin Elmer 5100 ZL). Blanks and reference material (ISE 989) were included to check for the accuracy of the procedures.

Data analysis

From the number of surviving mites as a function of the measured exposure concentration, LC50 values were estimated for weeks 3 to 7 using the trimmed Spearman-Karber method [17]. The same procedure was applied to survival as a function of the average Cd concentration in the mites, to obtain lethal body concentration values (internal LC50s). The effective concentrations causing 50% reduction of reproductive output compared to the control (EC50) after 6 wk and 7 wk were estimated with a logistic dose–response model [18].

Cadmium concentrations in the mites were also used to estimate toxicokinetic parameters. Measurements in control mites showed that there was a relatively high background concentration (C$_0$ = 1.1 μg/g dry body wt), which apparently reflects accumulation from the food in the culture. The background level was added as a constant to the regular 1-compartment kinetics model.

The data as a function of time showed that neither the Cd body concentrations in the mites nor the LC50 reached a steady state within 7 wk of exposure. Therefore, we modeled the uptake of Cd using a 1-compartment model with 0 elimination, which simply reads

$$C_{int}(t) = C_0 + C_{exp}k_1t$$

(1)

where C$_{int}$(t) is the Cd concentration in the animals at time t (micrograms per gram dry body wt), C$_0$ is the background internal Cd concentration (micrograms per gram dry body wt), $k_1$ is the Cd uptake rate constant (grams dry soil per gram dry animal per week), and C$_{exp}$ is the measured Cd exposure concentration (micrograms per gram dry soil).

This model predicts a linear increase of body burden with time, while in the same model the LC50 should decrease according to

$$LC50(t) = \frac{LBC}{k_1t}$$

(2)

where LBC is the (constant) lethal body concentration (micrograms per gram dry body wt) [19]. Estimates for $k_1$ were obtained for each exposure concentration by regressing Equation 1 on the accumulation data. Another estimate of $k_1$, as well as an estimate of lethal body concentration, was obtained from the toxicity data by nonlinear (least squares) regression of Equation 2. Regression and statistics were done using Excel and SPSS Statistics, Ver 21.

RESULTS

Measured Cd and pH changes in the soils

Nominal and measured Cd concentrations in LUFA 2.2 soil as well as measured Cd concentrations and pH in 0.01 M CaCl$_2$ extractable fractions during the experiment are given in Table 1. The actual Cd concentrations in all treatments were higher than the nominal ones. We used the measured concentrations in all subsequent analyses.

Cadmium concentrations in 0.01 M CaCl$_2$ extractable fractions increased from 0.001 μg/g dry soil in the control to 4.01 μg/g dry soil in the highest concentration. At the end of the experiment (after 7 wk) the extractable Cd concentrations had increased from 0.138 μg/g dry soil in the lowest to 5.27 μg/g dry soil in the highest concentration (Table 1).

With increasing Cd concentrations, pH$_{CaCl_2}$ of the test soil decreased. Spiking the soil with the highest Cd concentrations reduced mean pH from 5.35 to 4.39. The pH$_{CaCl_2}$ of the control soil was 5.46. However, at the end of the experiment, pH in all treatments had increased slightly by 0.13 units in the control to 0.76 units in the highest concentration. This increase equalized to a certain extent the spike-induced differences in soil pH.

Effects on survival and reproduction

Dose–response relationships for the effect of Cd on the survival of O. nitens at different exposure times are presented in Figure 1. The LC50 values estimated separately for each exposure time (see Table 2) showed a gradual decrease with

| Total cadmium (μg/g dry soil) | 0.01 M CaCl$_2$ extractable cadmium (μg/g dry soil) | pH$_{CaCl_2}$ |
|------------------------------|---------------------------------------------------|---------------|
| Nominal                      | Measured                                          | Start of experiment | After 7 wk | Start of experiment | After 7 wk |
| Control                      | 0.38                                              | <0.001          | <0.001      | 5.46 ± 0.06         | 5.59 ± 0.34 |
| 12.5                         | 15.5 ± 0.40                                      | 0.100 ± 0.002  | 0.138 ± 0.031 | 5.35 ± 0.37         | 5.48 ± 0.37 |
| 25                           | 29.8 ± 1.12                                      | 0.215 ± 0.007  | 0.345 ± 0.006 | 5.29 ± 0.07         | 5.46 ± 0.21 |
| 50                           | 57.0 ± 1.75                                      | 0.502 ± 0.008  | 0.693 ± 0.257 | 5.15 ± 0.19         | 5.38 ± 0.22 |
| 100                          | 117 ± 2.95                                       | 0.958 ± 0.033  | 1.37 ± 0.44  | 5.01 ± 0.16         | 5.33 ± 0.37 |
| 200                          | 258 ± 5.39                                       | 1.46 ± 0.01    | 2.06 ± 0.05  | 4.74 ± 0.09         | 5.21 ± 0.19 |
| 400                          | 506 ± 19.2                                       | 4.01 ± 0.1     | 5.27 ± 0.44  | 4.39 ± 0.07         | 5.15 ± 0.23 |

*All values are the mean of 2 replicates ± standard deviation. CaCl$_2$ = calcium chloride.
time, continuing up to week 7. A steady state was not reached within 7 wk. Toxicity was also estimated as a function of the concentrations measured in the animals at each exposure. This produced estimates for the lethal body concentration varying from 44 \( \mu \text{g/g} \) to 91 \( \mu \text{g/g} \) dry body weight (Table 2). The lethal body concentration fluctuated somewhat with time and was highest after 7 wk of exposure.

Juveniles were observed only after 5 wk. Their number varied considerably per replicate (e.g., from 11 to 23 in the controls after 7 wk). It is not uncommon in experiments with small invertebrates to observe such (unexplained) variability [3].

At the intermediate Cd concentrations juvenile production seemed to increase (up to 58), but at the highest concentration no reproduction took place (Figure 2). The EC50 values for the effect of Cd on reproduction after 6-wk and 7-wk exposure were estimated as 301 \( \mu \text{g/g} \) and 345 \( \mu \text{g/g} \) dry soil, respectively.

Cd accumulation in mites

The Cd concentrations measured in the recovered adults after 3 wk, 4 wk, 5 wk, 6 wk, and 7 wk of exposure are shown in Figure 3. The data at the highest exposure concentration, causing severe toxicity and few surviving mites, were omitted from this graph. There was considerable variation in Cd body concentrations between individual mites, with outliers at the 2 highest exposure concentrations. The body concentrations obviously did not reach a steady state within 7 wk of exposure. Assuming a linear increase, rather than a classical saturation curve, the uptake rate constant \( (k_u) \) was estimated from the slope of the line (see Materials and Methods). Estimates varied between 0.021 g soil/g animal/wk and 0.037 g soil/g animal/wk (Table 3).

Temporal changes of toxicity

Estimates for LC50 showed an ongoing decrease with exposure time (Figure 4). This is in qualitative agreement with the nonsaturating linear increase of the body burden because the toxicokinetic model with 0 elimination (see Materials and Methods) predicts an ongoing decrease of LC50 (see Materials and Methods). Fitting this equation to the data produced estimates for the lethal body concentration (41.2 \( \mu \text{g/g} \) dry body wt) and the uptake rate constant \( (0.024 \mu \text{g/g dry body wt}) \). These values are in the same range as those estimated from the Cd accumulation data in Tables 2 and 3. However, the model did not fit the data well. The actual LC50 values (Figure 4) decreased more slowly than predicted by the toxicokinetic model. Thus, there is qualitative, but not quantitative, agreement between the accumulation of Cd and the increase of toxicity (decrease of LC50).

DISCUSSION

The present data reveal that the accumulation of Cd in \( \text{O. nitens} \) shows several peculiarities. First, accumulation

Table 2. Estimated median lethal toxicity for the mite \( \text{Oppia nitens} \) after 3 wk to 7 wk of exposure to cadmium in LUFA2.2 soil

| Time (wk) | LC50 \( (\mu \text{g/g dry soil}) \) | CI | Lethal body concentration \( (\mu \text{g/g dry body wt}) \) | CI |
|-----------|-----------------|-----|---------------------|-----|
| 3         | 490             | 394–610 | 44.3               | 27–74 |
| 4         | 419             | 382–460 | 37.2               | 32–42 |
| 5         | 399             | 369–429 | 67.5               | 53–87 |
| 6         | 381             | 353–411 | 45.0               | 41–50 |
| 7         | 265             | 225–315 | 90.6               | 71–116 |

*Median lethal toxicity was also estimated based on internal concentrations in surviving mites, providing estimated lethal body concentrations. Lethal body concentration and median lethal concentration were estimated using the trimmed Spearman-Karber method.

CI = confidence interval; LC50 = 50% lethal concentration (refers to the total measured cadmium concentration in soil).
continued for more than 7 wk without reaching equilibrium in the animal. Second, accumulation seemed to be linear, maybe even accelerating at high exposures, but not saturating as is commonly observed, implying absence of elimination. Third, in accordance with the ongoing accumulation, LC50 decreased continuously and did not reach equilibrium either. Fourth, however, the rate at which LC50 decreased was lower than expected according to the linear accumulation model.

The accumulation strategy of *O. nitens* is similar to that observed for pseudoscorpions and some isopods [2]. The pseudoscorpion *Neobodium muscorum* (Leach) accumulated Cd in a linear fashion and did not show any excretion during a 100-d elimination period. Also, for the isopods *Porcellio scaber* Lateille and *Oniscus asellus* Latreille and *Platynothrus peltifer* Aoki, and *Xenillus tegeocranus* (Hermann) localize heavy metals in electron-dense granules in the midgut epithelial cells [28–32]. In addition, adults of some oribatid species, such as *Nothrus silvestris* Nicolet, *Rhysotritia duplicata* (Grandjean) [28], and *Steganacarus magnus* [33], can survive a 1000 μg/g Cd concentration in soil over 36-wk and 4-wk exposure, respectively. Owojori and Siciliano [34] demonstrated that *O. nitens* accumulated Cd with 75% efficiency after 4-wk exposure. Handling of high amounts of Cd by oribatids may relate to their fungivorous feeding habits; fungi have been shown to accumulate heavy metals, so oribatids might be naturally selected for efficient metal-detoxification strategies [35]. Also, the accumulation of Cd may relate to the high demand of essential elements in some species, such as zinc, manganese, and calcium [11,36]. There are no data yet on mineral nutrition in *O. nitens*.

The ongoing accumulation of Cd by *O. nitens*, as seen in our experiment, would imply that LC50 would decrease (theoretically to 0) and not reach an ultimate constant value. Although an ongoing decrease was indeed observed, this was not in quantitative agreement with the linear accumulation model (Figures 3 and 4). The LC50 decreased more slowly than expected. This can be explained by assuming that an increasing (nonconstant) fraction of the accumulated Cd is not contributing to toxicity. For example, the fraction stored in a metabolically inactive compartment could increase during exposure. Hopkin [37] reported that isopods can store large amounts of metals in their hepatopancreas. Several terrestrial invertebrates can detoxify Cd by binding the free metal ion in cysteine-rich proteins (metallothioneins), which reduces its toxicity [38–40]. A nonconstant redistribution of internal Cd, involving binding to metallothioneins and storage in detoxified form, would imply that the internal distribution is not at equilibrium and that a single-compartment model does not hold. If the storage-detoxification process accelerates relative to uptake kinetics, this could explain why the LC50 decreases more slowly with time than expected from the total-body concentration of Cd. In addition, the observed acceleration of Cd accumulation at high exposure concentrations could be caused by failure of the detoxification system.

That *O. nitens* does not reach an equilibrium when exposed to Cd, not even after 7 wk, implies that exposure time in experiments with *O. nitens* should be rather long. This may be specific for Cd but could also hold for other metals. Tests shorter than 4 wk may seriously underestimate toxicity. In fact, tests with *O. nitens* seem to require an exposure period of at least 6 wk, maybe even longer, which is quite remarkable for such a small animal. The issue of exposure time should be taken into account when developing the *O. nitens* experiment as an internationally harmonized soil toxicity test [13].

In conclusion, 1 mite species cannot represent the enormous diversity of oribatids. Soil protection should take into account the biodiversity of soil invertebrates, which represent also a biodiversity of ecotoxicological responses. At the same time, inclusion of *Oppia* in the collection of model species will confront researchers with a considerable trade-off between the added value of the species and the length of tests needed to obtain useful results.

**Table 3. Estimated uptake rates of cadmium by the mite *Oppia nitens* for each exposure concentration separately, exposed over 7 wk in spiked LUFA 2.2 soil**

| Measured total cadmium in soil (μg/g dry soil) | Slope (μg/g dry soil/wk) | $k_1$ (g dry soil/g dry animal/wk) |
|-----------------------------------------------|--------------------------|-----------------------------------|
| 15.5                                         | 0.330                    | 0.021                             |
| 29.8                                         | 0.538                    | 0.018                             |
| 57.0                                         | 0.954                    | 0.017                             |
| 117                                          | 3.04                     | 0.026                             |
| 258                                          | 8.79                     | 0.034                             |
| 506                                          | 19.5                     | 0.039                             |

*The estimation was based on a linear uptake model, including a background body concentration fixed as 1.1 μg/g dry body weight (mean of control animals). $k_1$ = uptake rate constant.*
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Data availability—All data are available on the data repository site of the department of Animal Ecology, Vrije Universiteit, Amsterdam, The Netherlands, and can be requested by email to: AEWarchive.falw@vu.nl

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