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Assessing the impact of live-capture, confinement, and translocation on stress and fate in eastern gray squirrels

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Live-capture and translocation are methods to deal with nuisance eastern gray squirrels in North America, but it is unknown how these methods affect squirrel physiology or survival. In this study we validated an enzyme immunoassay (EIA) to measure fecal cortisol metabolites (FCMs) in gray squirrels; assessed their stress response relative to the positioning of the live trap (sun, shade, or control); and assessed the impact of translocation on their long-term stress, movement patterns, and survival using FCM levels, body mass changes, and radiotelemetry. We found that a 5α-pregnane-3β,11β,21-triol-20-one EIA reliably detected acute stress in gray squirrel feces 12–24 h after the stressor; live traps positioned in the sun resulted in higher peak FCM levels compared with traps positioned in the shade; translocated squirrels experienced a 10% mortality rate, compared with no mortality in the controls, although overall fates were the same; translocated squirrels initially explored more and dispersed farther than controls, but after 2 weeks made similar movements; and after controlling for the effect of season, translocation did not affect long-term FCM levels or body mass; this conclusion must be tempered by the low number of recaptures. Our study demonstrates the utility of the FCM assay; that gray squirrels are extremely sensitive to capture, handling, and confinement; and that live-capture must be done in a way that minimizes exposure to additive environmental stressors.

Key words: cortisol, ecophysiology, glucocorticoids, gray squirrel, invasive species, noninvasive technique, relocation, *Sciurus carolinensis*, urban habitat

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Live-capture and translocation are commonly used management tools for mediating conflicts between wildlife and humans, bolstering or reintroducing natural populations, or a combination of these goals (Fischer and Lindenmayer 2000; Teixeira et al. 2007; Dickens et al. 2010). Here, we define translocation as the deliberate movement of free-living animals from one part of their range to another. The benchmarks of successful translocation are the establishment of animals into new habitat with limited mortality or health issues, and limited dispersal (Dickens et al. 2010). Stress is sometimes cited as the cause of translocation failure, but evidence for this explanation is wanting. The acute stress of the initial capture and translocation is rarely distinguished from the chronic stress of being moved into an existing population of conspecifics or to habitat vacant of all conspecifics (Teixeira et al. 2007; Aguilar-Cucurachi et al. 2010; Dickens et al. 2010). Much may depend on the sensitivity of the species, the duration of stressors, and the cumulative effects of stressors (Dickens et al. 2010).

Central to the stress response is the hypothalamic–pituitary–adrenal axis permitting the maintenance of homeostasis throughout unpredictable and predictable challenges (Sapolsky 2002; Reeder and Kramer 2005; Boonstra 2013). Glucocorticoids are the main hormones secreted by the hypothalamic–pituitary–adrenal axis. Because glucocorticoids can be secreted in response to anthropogenic and natural perturbations (Sheriff et al. 2011) and emotions such as fear (Rodrigues et al. 2009; Clinchy et al. 2011), they are of particular interest in the study of stress and translocation. There are several ways to measure glucocorticoid levels, with blood sampling being a common but invasive method. Measurement of fecal cortisol–corticosterone metabolites (FCMs) has emerged as a viable, noninvasive alternative with some benefits, and has provided

new avenues for stress research (Palme et al. 2005; Touma and Palme 2005; Sheriff et al. 2011).

Eastern gray squirrels (*Sciurus carolinensis*; hereafter, gray squirrel; approximately 600 g) are common small mammals native to eastern North America, but are among the most invasive alien species in the world (Lowe et al. 2000). Gray squirrels were introduced into western North America and parts of Europe (e.g., British Isles and Italy), where they flourished and displaced native Eurasian red squirrels (*Sciurus vulgaris*) and caused timber damage (Bertolino and Genovesi 2003; Tattoni et al. 2006; Bertolino 2008). In North America, gray squirrels commonly associate with humans near or in buildings and may be considered a nuisance, so they are commonly livetrapped for removal. When thus trapped, they are often moved elsewhere to "eliminate" the problem. This is in contrast to how these animals are dealt with in United Kingdom, where any captured gray squirrel cannot be released, even at the site of capture (Wildlife and Countryside Act, 1981 section 14[1]).

In this study, we had 4 goals: to validate an enzyme immunoassay (EIA) to measure the species-specific FCMs excreted by eastern gray squirrels; to use FCMs to evaluate the short-term stress experienced due to trap-positioning treatments; to use FCMs to evaluate the long-term stress experienced due to translocation; and to assess the fate and movement patterns of translocated gray squirrels.

Materials and Methods

Species background and biology.—Gray squirrels are nonterritorial, but have a dominance hierarchy determined primarily by sex and age, with males and older individuals dominating over females and young animals (Pack et al. 1967). Gray squirrels also defend core areas in the fall season when juvenile dispersal is greatest. In their preferred habitat of deciduous forest, their diet consists mainly of nuts, berries, fungi, and seeds, but they also may prey upon small animals such as insects and bird nestlings, as well as on bird eggs.

General trapping and handling protocols.—Gray squirrels were live-captured (model 102 or 103 live trap; Tomahawk Live Trap Co., Tomahawk, Wisconsin) in the morning (between 0700 h and 1100 h) using peanut butter or whole roasted peanuts as bait. We inspected traps hourly, and upon finding a captured squirrel, we immediately collected a fecal sample from it. The maximum duration from initial capture to the collection of the sample was about 1 h, and thus the effect of capture stress on the baseline sample was eliminated. To ensure we collected uncontaminated samples, we attached a fine mesh screen to the base of each trap to allow urine to drain, but to also keep fecal samples separate from soil and debris. Each squirrel was weighed (± 10 g; Pesola spring scale; Pesola, Baar, Switzerland) at every capture inside a cloth bag, and then its reproductive condition was determined (for males, testes scrotal or abdominal; for females, lactating or not, based on mammae color and condition). Lactating females were immediately released. We ear-tagged all squirrels with Monel tags (fingerling fish tags; National Band and Tag Co., Newport, Kentucky). We also attached a unique combination of colored wire to the Monel tags (allowing us to identify them with binoculars) for the translocation experiment.

Gray squirrels for the validation study were live-captured in the Highland Creek valley (43°78′N, 79°19′W) in Toronto, Ontario, Canada, in June 2010 (permit 11057300), and for the translocation study in Hamilton, Ontario, Canada, in July–November 2010 (permit 1057276) under permits from the Ontario Ministry of Natural Resources. The University of Toronto Animal Care Committee approved the use of live gray squirrels for the validation and trap-positioning experiments (protocol 20008265), and the Ontario Ministry of Natural Resources Animal Care Committee approved the use of gray squirrels for the translocation experiment (10–214). All protocols followed guidelines set by the American Society of Mammalogists for research on live animals (Sikes et al. 2011).

Validation: housing and sample collection times.—We housed 10 male and 4 female gray squirrels at the wildlife research facility at University of Toronto's Scarborough campus. The animals were held under simulated local ambient environmental conditions (20°C; 16L:8D; lights on at 0600 h) in stainless steel cages (91.5 × 61 × 46 cm), each containing a nest box lined with cotton. The squirrels were fed ad libitum with apple, black oil sunflower seeds, peanut butter, and water.

We collected a fecal sample when the squirrels were 1st captured, and then all squirrels were habituated to laboratory conditions for 6–10 days in captivity, during which time feces were collected every 4 h. This was followed by 2 days of the adrenocorticotropic hormone (ACTH) challenge experiment (12–15 June) to assess the adrenal cortisol release response and its signature in FCMs. This was followed by 3 days of the radiometabolism experiment (16–19 June) and by 2 days of the trap-positioning sun and shade treatments (20–21 June). Finally, the trap-positioning control treatment was performed (27–29 June), after which all animals were released at their site of capture.

The radiometabolism cages had stainless steel slatted floors that urine and feces fell freely through into a pan below. The pans were covered with fine metal mesh, which permitted urine to fall through, but retained feces. We collected fecal samples from all squirrels every 4 h (at 0600 h, 1000 h, 1400 h, 1800 h, and 2200 h) each day while in captivity using forceps. The only exception to this sampling protocol occurred during the radiometabolism experiment (described in a later section), when we collected both feces and urine every 2 h for 2 days (at 0600 h, 0800 h, 1000 h, 1200 h, 1400 h, 1600 h, 1800 h, 2000 h, and 2200 h), followed by every 4 h for a 3rd day. We collected the urine by lining the pans with 46 × 57-cm filter paper (Fisher Scientific, Toronto, Ontario, Canada), and then removing the paper with urine spots.

Validation: ACTH injections.—At 0600 h, 6 male and 4 female squirrels were restrained and injected with a 4-IU/kg intramuscular injection of ACTH in 250 μl of saline. The

injection procedure lasted about 5 min per squirrel. We monitored squirrels for 48 h post–ACTH injection.

Validation: radiometabolism.—At 0600 h, 4 male and 4 female squirrels were given an intramuscular injection (in the thigh) with a 250-μl solution containing 23 μCi (851 kBq) of hydrocortisone–[1,2,6,7^{3}H] (78.4 μCi/mMol, 95.2% volume–volume saline, 4.8% volume–volume ethanol; Perkin-Elmer, Waltham, Massachusetts). We monitored squirrels for 72 h post–ACTH injection.

Trap-positioning experiments.—For these experiments, we used the same housing conditions and the same 10 male squirrels listed above. Squirrels were confined inside live traps (model 103; Tomahawk Live Trap Co.) during 3 trap-positioning treatments. For the 1st treatment (hereafter called the sun treatment), we positioned 5 male squirrels in the sun for 1 h beginning at 0900 h. For the 2nd treatment (hereafter called the shade treatments), we positioned 5 male squirrels in the shade for 1 h beginning at 0900 h. The air temperature near the soil surface (10 cm above the soil) at 0900 h was 24°C in the sun and 22°C in the shade, and at 1000 h was 29°C in the sun and 24°C in the shade. The 3rd treatment was a control (hereafter called the control treatment), wherein we positioned the 9 male squirrels inside traps in their housing cages for an hour beginning at 0900 h. We began the sun and shade treatments on the same day, and we used the same animals for the control treatment on a different day. Following the trap-positioning procedures, we returned the squirrels to their cages at the research facility.

Translocation: study area.—We undertook the translocation study in the city of Hamilton, Ontario, Canada (43°15′0″N, 79°50′0″W; population approximately 505,000). The study area consisted of a source area (where we initially trapped all animals and released them as controls), and a sink area (where we released the translocated animals). The source area was a residential neighborhood having paved roads and moderate traffic speed ($\leq$ 50 km/h). Although we did not measure the density of gray squirrels in the source area, squirrels were very abundant. The sink area was a deciduous forest (43°12′53″N, 79°48′40″W) about 5 km from the source. There were 2 release sites, both of which were in habitat suitable for gray squirrels. The release sites were 480 m apart, and no more than 2 squirrels were released at the same site on any given day. The forest and area surrounding the release sites were approximately 425 ha in size (they included Albion Falls Park, Rosedale Park, Kings Forest Golf Course, Upper King's Forest, Lower King's Forest, Mohawk Sports Park, and the Glendale Golf and Country Club). Deciduous forest (*Quercus* sp., *Acer* sp., and *Juglans nigra*) was predominant. Other squirrel species were present at the release site in addition to gray squirrels, including southern flying squirrels (*Glaucomys volans*), eastern chipmunks (*Tamias striatus*), and red squirrels (*Tamiasciurus hudsonicus*). Predators at the release site included domestic dogs (*Canis lupus familiaris*), coyotes (*Canis latrans*), great horned owls (*Bubo virginianus*), broad-winged hawks (*Buteo platypterus*), and northern goshawks (*Accipiter gentilis*).

Translocation: attachment of transmitters and release.—After collecting a fecal sample, we attached necklace-style radiotransmitters (model PD-2C weighing 4.3 g; Holohil Systems Ltd., Carp, Ontario, Canada) to 41 squirrels. We randomly assigned squirrels into either the control group (13 males and 8 females) or the translocated group (10 males and 10 females). The controls were released at the site of capture and translocated squirrels were moved by vehicle inside covered live traps to the sink area.

We located each animal using radiotelemetry between 1200 and 2000 h every day for the 1st week after release (including the day of release), and then on every other day for the 2nd, 3rd, and 4th week after release. If the target animal was accessible, we recorded its position directly with a global positioning system unit (eTrex Venture HC; Garmin Inc., Olathe, Kansas). If the animal was inaccessible, we triangulated its position with a minimum of 3 global positioning system locations and 3 bearings using Locate III (version 3.33; Pacer Computing, Tatamagouche, Nova Scotia, Canada).

At the end of the telemetry period, we attempted to recapture the squirrels that remained in the study by setting traps where they were observed. We collected a fecal sample from recaptured animals, and reweighed them. We recaptured 10 squirrels (the other 31 were not recaptured due to mortality, lost radiotransmitter signals, or detached radiotransmitters).

Laboratory methods: fecal sample storage and preparation.—We lyophilized (Labconco Corp., Kansas City, Missouri) the frozen fecal samples overnight, pulverized each separately with a mortar and pestle, and weighed 65 $\pm$ 5 mg of the resulting powder into 2-ml conical-bottomed microvials. We vortexed each sample in 1 ml of 80% (volume–volume) methanol for 30 min, and then centrifuged the samples for 15 min at 800 $\times$ *g*. A portion of the supernatant was diluted 1:10 in assay buffer and stored frozen at –20°C.

Laboratory methods: EIAs.—We analyzed samples from the physiological validation (ACTH) experiment using 2 different EIAs, and then used the best-performing EIA to analyze all other samples, including the reverse-phase high-performance liquid chromatography fractions (Touma and Palme 2005). The 5α-pregnane-3β,11β,21-triol-20-one EIA detects metabolites with a 5α-3β,11β-diol structure and was 1st described for use with laboratory mice (for details of the EIA, including cross-reactions of the antibody, see Touma et al. [2003]). This EIA has been successfully used for European rabbits (*Oryctolagus cuniculus*, Monclús et al. 2006), laboratory rats (Lepschy et al. 2007, 2010), Columbian ground squirrels (*Urocitellus columbianus*, Bosson et al. 2009), North American red squirrels (Dantzer et al. 2010), and eastern chipmunks (Montiglio et al. 2012). The 11-oxoetiocholanolone EIA detects metabolites with a 5β-3α-ol-11-one structure and has been used for guinea pigs (*Cavia porcellus*, Keckeis et al. 2012), Syrian hamsters (*Mesocricetus auratus*, Chelini et al. 2010), snowshoe hares (*Lepus americanus*, Sheriff et al. 2009), mountain hares (*Lepus timidus*, Rehnus et al. 2009), and arctic ground squirrels (*Urocitellus parryii*, Sheriff et al. 2012). High- and low-concentration quality-control pools were run in

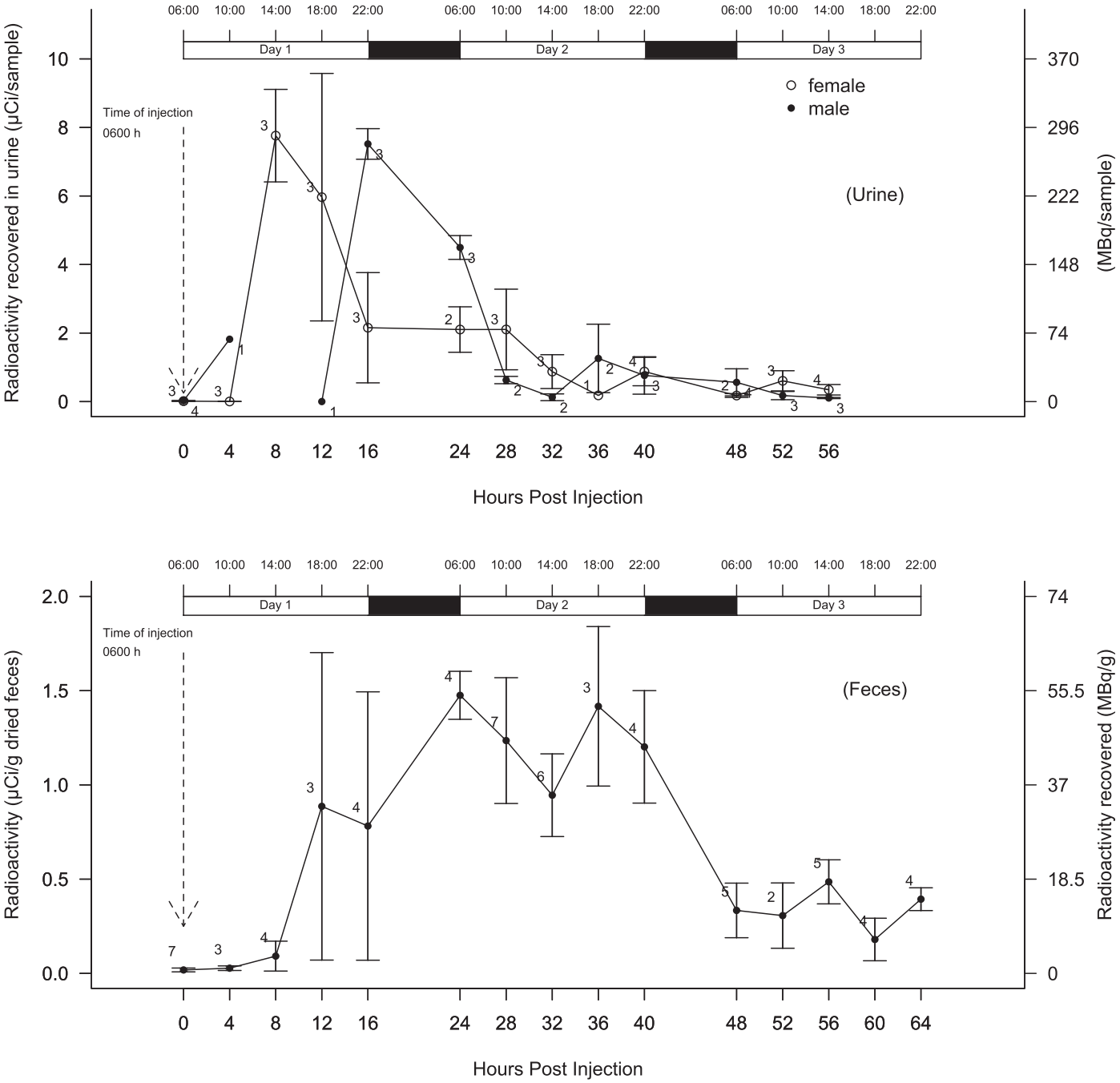

Fig. 1.—^{3}H-cortisol metabolites ($\bar{X} \pm SE$) recovered from the urine (upper panel: 6 males and 4 females) and feces (lower panel: males and females combined) from eastern gray squirrels (*Sciurus carolinensis*) following intramuscular injection of 23 µCi (851 MBq) of ^{3}H-cortisol at 0600 h. Sample sizes at each time are shown above and to the left of each point (females) or below and to the right of each point (males). These varied because of variation in defecation frequency.

quadruplicate on each microtiter plate. The intra- and interassay coefficients of variation were 8.1% and 9.6%, and 17.1% and 15.7%, respectively.

Lab methods: measurement of radioactivity.—For feces, we measured radioactivity in 100 µl of the methanolic suspension in 3 ml of liquid scintillation fluid (Biosafe II; Research Products International Corp., Mount Prospect, Illinois) using a reader with quench correction (Packard Tri-Carb 2900TR; Packard, Boston, Massachusetts). For the urine, we shredded the filter paper with dried urine into 0.5-cm-wide strips, and then vortexed the strips with 10 ml of distilled H_2O in 20-ml tubes (water was found completely effective at recovering urinary radioactivity). We measured the radioactivity in 100 µl of the resulting aqueous solution in 3 ml of scintillation fluid.

TABLE 1.—Percent recovery and time lag to peak recovery of ^{3}H ($\bar{X} \pm SE$). Recovery from the urine and feces is shown for eastern gray squirrels (*Sciurus carolinensis*) injected at 0600 h with ^{3}H-cortisol. Males and females are compared with a *t*-test.

	Males ($n = 4$)	Females ($n = 3$[a])	t	P	Combined ($n = 7$)
^{3}H recovered (%)					
Urine	51.5 ± 5.2	71.2 ± 6.0	2.5	0.05	—
Feces	11.1 ± 2.0	7.1 ± 2.3	−1.3	NS[b]	9.4 ± 1.6
Lag time to peak recovery (h)[c]					
Urine	16.5 ± 2.0	8.0 ± 2.4	−2.7	0.04	—
Feces	25.0 ± 8.0	36.7 ± 9.3	1.0	NS	30.0 ± 6.0

[a] One female was excluded from the analysis because it did not defecate for 60 h.

[b] NS = not significant ($P < 0.05$).

[c] Peak recovery was measured as μCi/g from the feces, and μCi/sample from the urine.

Laboratory methods: reverse-phase high-performance liquid chromatography.—Fecal suspensions with the highest ^{3}H concentrations from 4 males and 2 females were pooled by sex, and separated into 100 fractions using reverse-phase high-performance liquid chromatography. The radioactivity and immunoreactivity (using a 5α-pregnane-3β,11β,21-triol-20-one EIA) was measured in each fraction. Further details of this method can be found in Lepschy et al. (2007) and Touma et al. (2003).

Data analysis.—All data in the text, tables, and graphs are presented as mean ± 1 *SE* unless noted otherwise. We calculated 2 distance measures for each squirrel in the translocation study: a dispersal distance equaling the maximum Euclidian distance between the release site and any other position the squirrel was observed and an exploration rate (m/day) equaling the Euclidian distance between day-to-day observations of the same squirrel. Both distance measures were nonnormal in distribution and could not be transformed, and thus we used Kruskal–Wallis tests to analyze the distance measures. FCM concentrations followed a log-normal distribution, and thus they were $\log_{10}$ transformed prior to analysis. We used a 2×2 chi-square test to analyze the fate frequencies. We used a chi-square goodness of fit to analyze the distribution of fecal samples among the sample times and a Kruskal–Wallis test to analyze whether there was an effect of handling stress on gut motility and fecal sample frequency the following day (when squirrels were handed in captivity).

Linear mixed-model analyses were used to analyze longitudinal data from the validation and trap-positioning experiments. For this, we used R (version 2.12.1, R Foundation for Statistical Computing, Vienna, Austria) and the NLME package (version 3.1–97, Pinheiro et al. 2010). Linear mixed models are ideal for longitudinal data with missing values because they handle unbalanced designs well and can effectively deal with multiple observations from the same individual (Bolker et al. 2009). In all linear mixed models, individual animal was used as a random effect. We used linear mixed models to examine changes in FCM levels throughout the habituation phase of the validation (fixed effect: days postcapture); the effect of ACTH on FCMs (fixed effects: time + sex + time*sex); the effect of trap positioning (sun, shade, and control treatments separately) to determine whether there was a time effect on FCM levels using days postcapture as the fixed effect; and the effect of trap positioning on peak FCM levels using treatment (sun, shade, or control) as the fixed effect.

RESULTS

Validation experiment: distribution of fecal sample frequencies.—Gray squirrels, unlike other rodent species with which we have worked in captivity, may not defecate regularly throughout the day (probably because of the stress of capture and confinement). Consequently, the numbers of fecal samples collected at the different sample times were not randomly distributed ($\chi^2_4 = 53.2$, $P < 0.0001$). The majority of samples (32%) were collected at 0600 h (when the lights were turned on), followed by the 1000 h, 1400 h, 1800 h, and 2200 h sampling periods yielding 20%, 17%, 16%, and 15% of the samples, respectively. Although we did not sample overnight, we did check the cages once at 0200 h and there were no samples. We handled the squirrels 4 times after the habituation phase (once for the radiometabolism experiment, once for the ACTH experiment, and twice for the trap-positioning experiment). Handling affected how many squirrel produced feces at 0600h ($\chi^2_1 = 5.5$, $P = 0.02$; before handling: 91.3% ± 5.4%, $n = 4$ versus after handling: 54.0% ± 7.8%, $n = 4$). Thus, the stress of handling may inhibit gut motility and increase the retention of feces.

Validation: FCM level change due to capture and the habituation phase.—Days postcapture had an effect on FCM levels throughout the habituation phase ($F_{9,285} = 25.2$, $P < 0.0001$). FCM concentrations on the day of capture and the day after capture were similar ($t_{285} = -0.3$, $P = 0.8$). However, by days 2 and 6 after capture FCM concentrations were 67% higher ($t_{285} = 3.9$, $P = 0.001$), and 242% higher ($t_{285} = 9.3$, $P < 0.0001$), respectively, than on the day of capture.

Validation: the route and lag time of ^{3}H-cortisol metabolites.—We recovered 71% of the injected ^{3}H in urine and feces, and found no sex difference ($t_6 = 1.9$, $P = 0.11$). Peak recovery in urine from females occurred at a time when no urine was collected from the males (Fig. 1), which probably caused the statistical difference in lag time among males and females

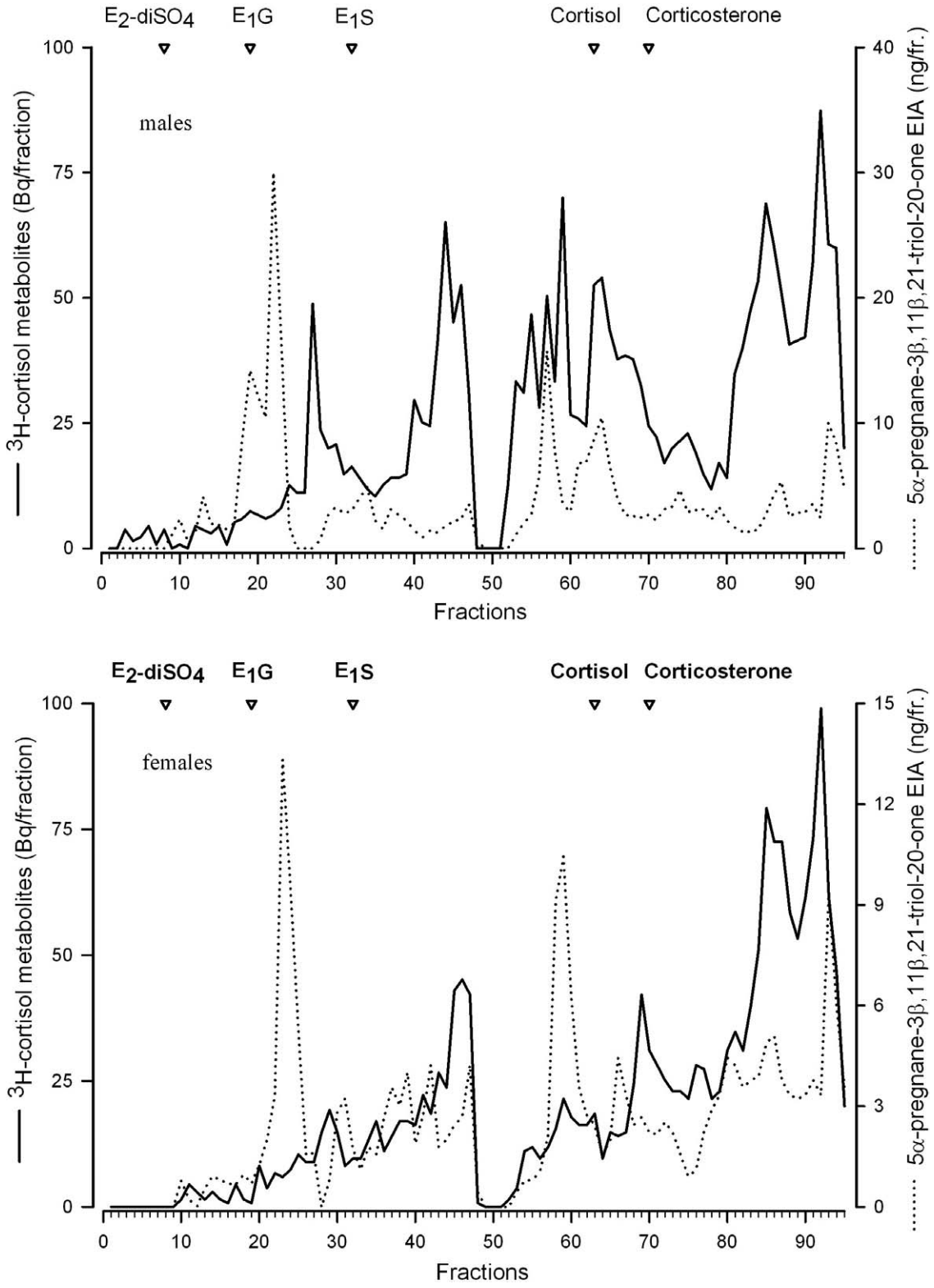

Fig. 2.—Reverse-phase high-performance liquid chromatography immunograms were prepared for eastern gray squirrels (*Sciurus carolinensis*) from fecal suspensions having peak radioactivity from 4 males (pooled) and 2 females (pooled). The radioactivity (solid line) and immunoreactivity with the 5α-pregnane-3β,11β,21-triol-20-one enzyme immunoassay (dotted line) is shown for each fraction. Elution times for standards (estradiol disulfate [E2-diSO4], estrone glucuronide [E1G], estrone sulfate [E1S], cortisol, and corticosterone) are marked with open triangles.

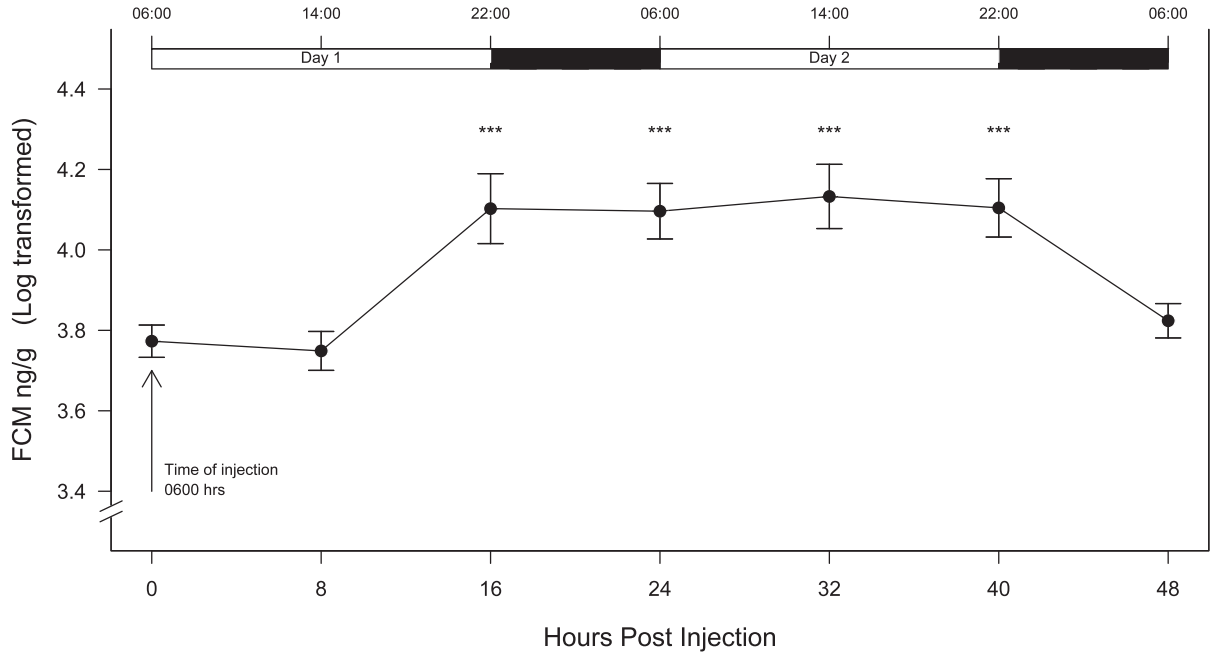

Fig. 3.—Fecal corticosteroid metabolite (FCM) levels ($\bar{X} \pm SE$) measured with a 5α-pregnane-3β,11β,21-triol-20-one enzyme immunoassay from eastern gray squirrels (*Sciurus carolinensis*) following an intramuscular injection of adrenocorticotropic hormone at 0600 h. Males ($n = 6$) and females ($n = 4$) are pooled at each point. Asterisks (***) indicate sampling periods when FCM concentrations were significantly higher ($P < 0.001$) than at the time of injection.

(Table 1). Most (78%) urinary ^{3}H was cleared within 24 h and most (88%) fecal ^{3}H within 48 h. Peak recovery in feces occurred 24–40 h after injection. Sometimes the variation in ^{3}H in the feces was large (e.g., 12 and 16 h postinjection; Fig. 1) but this was not due to lower sample size.

Validation: characterization of ^{3}H-cortisol metabolites by reverse-phase high-performance liquid chromatography.—Because the 5α-pregnane-3β,11β,21-triol-20-one EIA measured a significant increase in immunoreactivity following ACTH (the most important validation step, because it guarantees that plasma cortisol is reflected by FCM), and the 11-oxoetiocholanolone EIA did not (thus disqualifying it), we ran the reverse-phase high-performance liquid chromatography fractions against the 5α-pregnane-3β,11β,21-triol-20-one EIA only. Nonpolar cortisol metabolites dominated the reverse-phase high-performance liquid chromatography immunograms for males and females (Fig. 2), and there were no apparent sex differences. Although we did not run the 5α-pregnane-3β,11β,21-triol-20-one standard, it was found to be slightly less polar than corticosterone (Touma et al. 2003). Thus, one of the smaller ^{3}H peaks eluting around fraction 75 could represent the standard (Fig. 2). Around fraction 50, ^{3}H metabolites were absent in both males and females, and this was mirrored by an absence of binding using the 5α-pregnane-3β,11β,21-triol-20-one EIA (Fig. 2). Immunoreactive peaks detected by the EIA co-eluted with some of the ^{3}H metabolites.

Validation: ACTH.—For the EIA measuring metabolites with 5α-3β,11β-diol structures, we found an effect of time ($F_{6,31} = 12.4$, $P < 0.0001$), not of sex ($F_{1,8} = 0.2$, $P = 0.64$), and no interaction ($F_{6,31} = 2.2$, $P = 0.07$). FCM concentrations at 16 h postinjection were different from those at base ($t_{57} = 4.7$, $P < 0.0001$; Fig. 3). FCM levels returned to baseline levels 48 h later ($t_{57} = 1.4$, $P < 0.18$; Fig. 3). For the 11-oxoetiocholanolone EIA, we found no effect of time ($F_{6,31} = 2.3$, $P = 0.06$), sex ($F_{1,8} = 0.07$, $P = 0.8$), or their interaction ($F_{6,31} = 1.5$, $P = 0.2$). Thus, the 5α-pregnane-3β,11β,21-triol-20-one EIA was significantly better at measuring FCM levels in gray squirrels compared with the 11-oxoetiocholanolone EIA, and thus we used the former in all subsequent assays.

Trap-positioning experiment.—One squirrel entered a state of shock and died following the sun treatment, despite being brought inside to recover (hence it was excluded from the analysis). There was an effect of the sun treatment on FCM levels ($F_{6,59} = 2.3$, $P = 0.05$), being 66% higher on the day after the sun treatment (7,762 ± 1,570 ng/g versus 12,882 ± 330 ng/g; $t_{59} = 2.1$, $P = 0.04$). There was no effect of the shade treatment on FCM levels over time ($F_{6,54} = 2.0$, $P = 0.08$), but levels were 38% higher on the following day (6,316 ± 1,124 ng/g versus 8,686 ± 2,326 ng/g), suggesting that there was a smaller effect. Finally, there was an effect of the control treatment on FCM levels ($F_{2,52} = 13.1$, $P < 0.0001$), being 41% higher on the day after the control treatment (5,129 ± 897 ng/g versus 7,244 ± 699 ng/g; $t_{52} = 3.8$, $P < 0.0001$). Thus, simply putting animals into a live trap (control or shade) stresses the squirrels and putting them into the sun stresses them even more. The mean peak FCM level after the sun treatment was higher than after either the shade ($t_4 = 4.79$, $P = 0.009$) or control ($t_4 = 3.91$, $P = 0.02$) treatments; the latter 2 did not differ ($t_4 = -2.11$, $P = 0.1$).

Table 2.—Exploration rate (m/day) relative to the number of days after (post) release for eastern gray squirrels (*Sciurus carolinensis*) that were not moved (control) or moved away from initial capture site (translocated). n = number of squirrels, NS = not significant ($P < 0.05$).

	Control				Translocated					
Days post	*n*	*Median*	*Minimum*	*Maximum*	*n*	*Median*	*Minimum*	*Maximum*	χ^2	*P*
0	21	97	17	549	20	62	8	362	4.9	0.05
1	20	44	0	1,059	19	427	36	1,429	14.0	< 0.001
2	21	65	0	2,169	18	382	96	1,265	16.5	< 0.001
3	21	62	13	390	17	329	2	2,112	11.7	< 0.001
4	21	41	2	1,110	16	123	0	1,728	5.3	0.02
5	19	46	0	2,921	16	53	2	1,467	0.4	NS
6	18	50	0	2,882	17	205	21	1,795	3.9	0.05
7	20	55	0	1,517	17	103	12	1,519	2.2	NS
9	21	79	9	2,588	16	105	0	1,383	0.02	NS
11	17	57	8	182	14	88	0	3,148	1.5	NS
13	19	32	4	211	16	123	32	2,291	11.4	< 0.001
15	18	34	4	218	16	65	5	1,199	2.5	NS
17	18	44	2	300	15	106	0	841	0.6	NS
19	18	61	9	420	14	92	0	1,233	1.0	NS
21	18	40	6	196	13	73	0	3,081	3.4	0.07
23	18	37	0	243	13	47	20	1,212	2.1	NS
25	15	50	0	164	13	36	4	1,077	0.3	NS
27	15	36	0	184	11	69	10	1,632	0.6	NS

Translocation experiment: impact on fates.—At the end of the telemetry study, 29 squirrels remained (18 control versus 11 translocated). One remaining translocated squirrel (a female) homed 5.2 km in 24 days. Of the 12 squirrels that failed to make it to the end of the study, 2 died (0 control versus 2 translocated), the radiotransmitters of 7 were found (3 control versus 4 translocated), and the radiotransmitter signal of 3 disappeared (0 control versus 3 translocated). The fate of translocated and controls did not differ ($\chi^2_1 = 3.3$, $P = 0.07$). However, 3 times the number of translocated squirrels dropped out of the study compared with controls, suggesting a possible biological difference.

Two translocated squirrels died. The 1st was discovered 3 days postrelease after a day of heavy and prolonged rain. It likely died from exposure, exacerbated by the new environment and lack of knowledge of good shelter (its mass was 17% greater than at release). The 2nd was discovered close to the release site 18 days postrelease with its right front foot lodged under its collar, a broken incisor, and a small head wound. The ultimate cause of death may have been that we attached the collar too loosely (however, the foot under the collar might have occurred accidentally during death), and the proximate cause of death was likely from injury and starvation (16% loss in body mass).

Translocation experiment: impact on dispersal distance.—We obtained 304 positions from the translocated squirrels and 369 positions from the control squirrels. Translocated squirrels dispersed farther than controls ($n = 20$, median = 1,558 m, range = 4,994 m versus $n = 21$, median = 202 m, range = 2,428 m, respectively; $\chi^2_1 = 15.7$, $P < 0.001$).

Translocation experiment: impact on exploration rate.—On the day of release (day 0; Table 2), translocated squirrels moved less than controls, but moved more on day 1 than controls (Table 2). Although translocated squirrels continued to make large movements on days 2–4, the median distance decreased daily (Table 2). Translocated squirrels also made larger movements than controls on days 6 and 13, but on all other days there was no difference (Table 2). There was large variation in the distances traveled by the translocated squirrels, even near the end of the telemetry period (day 27; Table 2). Thus, some likely had not yet settled into a core area.

Translocation experiment: impact on FCM and body mass.—The FCM levels were related to Julian day ($F_{1,40} = 18.7$, $P < 0.0001$), but not sex ($F_{1,40} = 0.1$, $P = 0.7$), or their interaction ($F_{1,40} = 1.8$, $P = 0.2$). FCM concentrations were positively related to Julian day ($b = 0.011$, $r = 0.63$, $t = 5.14$, $P < 0.0001$). At 1st capture, the mean body mass of all squirrels was 591.5 ± 6.8 g. Body mass was not related to Julian day ($F_{1,40} = 1.79$, $P = 0.2$), sex ($F_{1,40} = 0.03$, $P = 0.8$), or their interaction ($F_{1,40} = 0.002$, $P = 0.9$).

Recapture proved to be extremely difficult, and we were only able to recapture 6 control and 4 translocated squirrels. Even though we could radiolocate 18 controls and 11 translocated squirrels at the end of the month, we continued to find radiotransmitters that had fallen off. FCM levels in the control group increased by 3,086 ± 2,004 ng/g and in the translocated group by 3,777 ± 2,454 ng/g over the course of the study ($F_{1,9} = 0.13$, $P = 0.7$). Body mass gain in control (+ 16.7 g) and translocated (+ 12.5 g) squirrels did not differ ($F_{1,9} = 0.17$, $P = 0.7$). Thus, we failed to see a long-term effect of translocation on FCM levels and body mass, but sample size was low.

Discussion

There were 5 major findings from our study: a 5α-pregnane-3β,11β,21-triol-20-one EIA reliably detected acute stress in gray squirrel fecal samples and maximal levels occurred 12–24 h after the stressor; live traps positioned in the sun resulted in higher peak FCM levels than traps positioned in the shade;

there were more casualties and other problems with the translocated squirrels, suggesting a biological difference, but the overall fates of control and translocated squirrels were statistically similar; translocated squirrels explored more and dispersed significantly farther than did controls but after 2 weeks they became similar; and control and translocated squirrels had similar changes in FCM levels and body mass, measured at the beginning and end of the study; however, low recapture number limits the power of this conclusion.

Validation.—In gray squirrels, the percentage of cortisol metabolites in the feces is similar to that in Columbian ground squirrels (6.6%—Bosson et al. 2009) and snowshoe hares (9%—Sheriff et al. 2009), but lower than in North American red squirrels (29.7%—Dantzer et al. 2010) and laboratory rats (74.8%—Lepschy et al. 2007). Gray squirrels have a relatively long lag time in the feces compared with Columbian ground squirrels (7 h—Bosson et al. 2009), North American red squirrels (11 h—Dantzer et al. 2010), and laboratory rats (15 h—Lepschy et al. 2007). Gray squirrels have a relatively long small intestine (approximately 212 cm) compared with related species such as Abert's squirrel (*Sciurus aberti*, 167 cm), fox squirrel (*Sciurus niger*, 144 cm—Murphy and Linhart 1999), and laboratory rats (101–115 cm—Younoszai et al. 1978), which may explain the longer lag time. However, a more likely cause of the lag time is the negative effect that housing and handling stress had on reducing gut motility and defecation frequency. Gray squirrels appear to be much more sensitive to capture and handling than other species we have worked on.

To estimate baseline stress levels using FCM, trap-check frequency should be shorter than the lag for the appearance of FCM in the feces, because trapping and handling can increase plasma glucocorticoids levels (Bosson et al. 2012). A 2nd precaution from our results is that feces should not be collected from the same animal within a 3-day window, because the stress of the 1st capture might influence FCM levels at the 2nd capture. It would be instructive to know if a stressor in nature would result in a similar lag time, or if the lag time was unusually long because the stressor (confinement in the laboratory) was both chronic and outside the animal's normal experience.

The 5α-pregnane-3β,11β,21-triol-20-one EIA proved suited to measure adrenocortical activity in gray squirrels, because it detected an increase following ACTH injection. Interestingly, we found a relatively large proportion of nonpolar ^{3}H-metabolites (some recognized by the EIA) in the feces, which is an unusual result compared with other squirrel species (Bosson et al. 2009; Dantzer et al. 2010; Montiglio et al. 2012; Sheriff et al. 2012). The reverse-phase high-performance liquid chromatography separations help to characterize the excreted cortisol metabolites by indicating what is actually recognized by the EIA. For the application of the method, it is not so important that all the main radioactive peaks are recognized (because a complex mixture of metabolites is present), but it is important that immunoreactivity be related to the radioactive peaks, which was clearly the case.

Trap positioning.—Traps positioned in the sun, but not the shade, resulted in higher peak FCM levels the next day. Within physiological limits, rapid changes in temperature elicit a stress response, but it is usually followed by attenuation to normal values for the new temperature (Dantzer and Mormède 1983). The casualty occurring during the sun treatment was likely from the combined stress of confinement in the live trap followed by the sudden increase in temperature. Gray squirrels rapidly enter shock when confined in traps (Guthrie et al. 1967) due to severe hypoglycemia, leucopenia, suppressed cortisol, and elevated hematocrit. This then leads to a lack of muscular coordination, an inability to maintain equilibrium, convulsions, unconsciousness, and often death (Guthrie et al. 1967; Merson et al. 1978). However, we rarely observe trap mortality in field studies if live traps are placed in shaded areas and are inspected frequently (e.g., every 1–2 h). When trapping nuisance squirrels, we conclude that traps need to be positioned to minimize exposure to temperature extremes.

Translocation.—Extensive exploration is often observed in translocated animals (Dickens et al. 2010). In their 1st weeks after release, the translocated squirrels moved significantly more than control squirrels did. Translocated squirrels also had difficulty homing from a distance of about 5 km, which is similar to other observations of gray squirrel homing ability (Hungerford and Wilder 1941). Gray squirrels often make repeated forays outside their core area (we observed controls foraging up to about 3 km from their core area—Taylor et al. 1971), which might help with homing if they reenter terrain they recognize.

The FCM levels in our study were lower in July and August than in September (when the effects of the breeding were minimized), which may be explained if increased social pressure, especially from juveniles born from the 2nd litter in early summer, caused more competition for nesting and high-quality food resources in the fall. Other reasons could be seasonal shifts in predation risk (Monclús et al. 2009), or the modulation of glucocorticoid-induced behavioral changes (Romero 2002).

We did not detect an effect of translocation on fate. After 1 month, only 10% of the translocated squirrels died, compared with 41% in a comparable study (Adams et al. 2004). Mortality is often the cause of translocation failure, and predation is often the proximate cause (Kenward and Hodder 1998, Calvete and Estrada 2004). The ultimate cause may be lack of familiarity with the environment and competition with resident conspecifics.

We expected to see an effect of translocation on increased FCM levels and reduced body mass, but observed no long-term effects. The translocated squirrels probably experienced acute stress from live-capture and handling, but this was not reflected in FCM levels in recaptured animals 1 month later. Recaptured squirrels were no longer making large exploratory movements, and hence they were likely less stressed than the squirrels making larger exploratory movements. The capacity to adapt to a new environment is likely species dependent (Hartup et al. 2005; Franceschini et al. 2008; Dickens et al. 2009; Vick et al.

2012). We expect that the success of translocation will very much depend on the environment into which the animals are introduced, and on the social structure of the species. If they are introduced into an existing dense population of territorial conspecifics, then the consequences are likely dire. If they are a nonterritorial species or if there are no conspecifics in suitable habitat, then there is a much higher probability of success.

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