Human–Wildlife Interactions 15(1):178–190, Spring 2021 • digitalcommons.usu.edu/hwi

Stress response of stone martens and red foxes in two different live traps

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Abstract: Trapping of terrestrial animals is an important tool for harvest, pest control and research worldwide. To catch animals alive, animal welfare has to be ensured, which is reflected in different agreements on trading and trapping of animals between sovereign nations (Council Regulation [EEC] No 3254/91). The red fox *(Vulpes vulpes)* and stone marten *(Martes foina)* represent important predatory animals. Their influence on protected species as well as their increasing appearance in urban areas demand responsible handling. In our study, we evaluated 2 trap systems used for trapping red foxes and stone martens in accordance with criteria stipulated in the Agreement on International Humane Trapping Standards (AIHTS) and International Organisation for Standardisation 10990 Part 5 – Methods for Testing Restraining Traps (ISO 10990). In total, we captured 20 red foxes in a concrete pipe vault trap and 13 stone martens in a Strack's wooden box trap in Schleswig-Holstein, Germany, and observed their behavior inside each trap. After anesthesia, a clinical examination of each animal was conducted, and blood and hair samples were taken. After euthanasia, radiological examinations of the full body were taken, and necropsies and histopathological investigations were performed. No trap-associated lesions were found. Hormone analysis showed no remarkable signs of stress for the animals, according to state-of-the-art methods. Apart from serum cortisol, the quotient of dehydroepiandrosterone in serum and hair seems to be the most predictive value on stress response of the 2 different species. Video observation of the trapped animals emerged as a valuable tool to estimate animal welfare by behavior. This study complements AIHTS and ISO 10990 criteria with results on behavior and hormone analysis, being an additional benefit when evaluating animal welfare of each trapping system.

Key words: AIHTS, Germany, live trap, live trap certification, *Martes foina*, red fox, stone marten, *Vulpes vulpes*

The trapping of predatory mammals constitutes a well-established wildlife management tool in Germany (Gräber et al. 2019). Live traps are also in use for removal of feral animals, research with wild animals, and pest control. Predatory animals like the red fox (*Vulpes vulpes*) and stone marten (*Martes foina*) often come into conflict with human undertakings, either being a threat for livestock or for protected animals (Sillero-Zubiri et al. 2004). The red fox and stone marten species may cause elevated predation pressure and create a habitat-dependent interspecific dietary niche overlap (Padial et al. 2002) with a wide spectrum of food categories (Soe et al. 2017). The population increase of both species in urban areas in Europe is causing conflicts (Adams 2016, Soulsbury and White 2016) and requires

responsible handling that reflects the awareness of decision makers toward animal welfare. The use of traps in urban areas is one of the few possibilities to handle this problem, underlining the necessity of broad acceptance of the general public. At present, in Germany, all kinds of live traps that do not catch animals unharmed are banned (§19 Federal Hunting Law), although indicators for physical harm are missing in this context. Up to now, internationally agreed standards for humane trapping, such as Agreement on International Humane Trapping Standards (AIHTS), are not statutory in Germany. Further, scientists do criticize the level of AIHTS standards and claim for stronger indicators for animal welfare in this context (Proulx et al. 2020). Therefore, trapping methods used for stone martens and red foxes as a minimum requirement should comply with AIHTS and the International Organisation for Standardisation 10990 Part 5 – Methods for Testing Restraining Traps (ISO 10990; European Union 1998), a standardized protocol for the testing of restraining traps. For these animals, 2 types of traps are frequently used by trappers: concrete pipe vault and Strack's wooden box trap. When dealing with animal welfare in live traps, apart from physical injuries, which are the main components of AIHTS and ISO 10990, evaluation of behavior may be a promising approach to complete the picture. In addition, hormonal findings should also reflect the physiological stress response. We used blood counts (BC) and blood analysis to gain closer insight on physiological alterations that might be due to the capture and found that cortisol concentration of serum and hair might help to get a better judgment of stressed animals. In our study, we evaluated the welfare of animals trapped alive in accordance with the abovementioned methods, providing a basis for further research on animal welfare of live traps.

Study area

Our study was conducted from November 2013 to March 2015 in Schleswig-Holstein, Germany, at 11 sites on the Eiderstedt Peninsula, which is located at an altitude of 0–1 m above sea level. The Eiderstedt Peninsula represents a typical marshland environment and at the time of the study was characterized by a maritime climate with a mean total yearly rainfall of 856 mm and a longstanding yearly mean temperature of 8.5°C. At this site, predatory animals are intensively hunted to prevent negative impacts on protected, ground-nesting birds. The area is inhabited by the red fox and stone marten as well as protected species like the black tern (*Chlidonias niger*), black-tailed godwit (*Limosa limosa*), and lapwing (*Vanellus vanellus*), which results in areas identified as Natura 2000 habitats (according to the European Union Habitats Directive that lists 231 natural habitat types [Annex I] and >1,000 animal and plant species [Annexes II, IV, and V; 92/43/EEC of May 21, 1992]). Our study area also served as a European bird sanctuary.

Methods

The traps were placed on game trails by local trappers, avoiding places in direct sunlight, being partly covered by local flora. The concrete pipe vault consisted of 5 pieces of concrete pipe strung together, each measuring 1 m long with a diameter of 25 cm each. In our study, we used concrete pipe vault traps to trap red foxes. The pipes were arranged in a row placed on concrete pavers, the first and the last pipe remaining empty. The second, third, and fourth pipes were placed on metallic devices; the third pipe, at the center of the trap, contained the rocker, which triggers the trap. Once inside the trap, the metallic trapdoors of pipes 2 and 3 immediately closed behind the animal after the rocker was released. The resulting holding chamber measured 3 m in length (Figure 1). The trapped animal was carefully guided to an external catch box by means of activating a slider in the trap.

The Strack's wooden box trap is made of wood measuring 1.5 m long, 0.73 m high, and 0.38 m wide, with a 29 x 29-cm entrance. In our study, we used Strack's wooden box traps to trap stone martens. In its center, a wooden rocker serves as a trigger to release the metallic trapdoors, which immediately close when activated (Figure 2).

Both traps were checked every 12 hours, in the morning and in the evening, resulting in varying times of entrapping. Trapped animals were weighed inside the catch box to ensure an adequate anesthesia via intramuscular injection of medetomidine (red fox: 0.2 mg/kg; stone marten: 0.1 mg/kg; Cepetor® CP-Pharma,

Figure 1. Concrete pipe vault set up in the Eiderstedt Peninsula, Schleswig-Holstein, Germany (*photo courtesy of the Institute for Terrestrial and Aquatic Wildlife Research*).

Figure 2. Strack's wooden box trap, set up in the Eiderstedt Peninsula, Schleswig-Holstein, Germany (*photo courtesy of the Institute for Terrestrial and Aquatic Wildlife Research*).

Burgdorf, Germany) and ketamine (red fox: 10 mg/kg; stone marten: 10 mg/kg; Ketamin®, CP-Pharma, Burgdorf, Germany). After anesthesia, the sex of the animal was determined, and a clinical examination was conducted. We aged red foxes according to dentition (Brömel and Zettl 1974) and the age of stone martens by weight and physical examination of the animal because information on dentition was not available. For hormonal testing, strands of hair measuring approximately the diameter of a pencil were taken. For blood analysis, we extracted blood by intracardial puncture. Subsequently, we euthanized the animals using embutramide, mebezonium iodide, and tetracaine hydrochloride (T-61®, MSD Animal Health, Unterschleissheim, Germany) intracardially (1.5 ml/kg). Animal trapping, handling and euthanasia were performed in accordance with the German Law of Animal Welfare, and all experiments were approved by the official agency for animal experiments in Schleswig-Holstein, Ministry of Energy, Agriculture, the Environment and Rural Areas (MELUR) under the permit V242-7224.121–19.

We conducted necropsies and collected samples for pathohistological examinations as described by Lempp et al. (2017). The focus of our study was on the occurrence of injuries that are regarded as indicators of poor welfare of trapped animals in accordance with ISO 10990 Annex C trauma scales and the indicators established by AIHTS: fractures, joint luxation proximal to the carpus or tarsus, severance of a tendon or ligament, major periosteal abrasion, severe external hemorrhage or hemorrhage into an internal cavity, major skeletal muscle degeneration, limb ischemia, fracture of a permanent tooth exposing pulp cavity, ocular damage including corneal laceration, spinal cord injury, severe internal organ damage, myocardial degeneration, amputation, or death. We performed radiological examinations of the carcasses diagnosing bone injuries with the system Unimat from Vogedes, Herdege, Germany, and the images were digitized by Kodak Orex CR System. In 1 trap of each kind, a video recording system monitoring the behavior of trapped animals was installed. Video recording started immediately after trapping the animal and lasted approximately 60 minutes. Behavioral indicators recognized as indicators of poor welfare in trapped wild animals by AITHS are: self-directed biting leading to severe injury (self-mutilation) or excessive immobility and unresponsiveness. Trapping complies with AIHTS standards if 80% of at least 20 animals show none of the above-mentioned indicators.

We conducted complete BC with ScilVet ABC™ Animal Blood Counter (Scil Animal Care Company GmbH, Viernheim, Germany). We performed blood chemistry with VET Scan 2 (Scil Animal Care Company GmbH, Viernheim, Germany) and included alanine aminotransferase (ALAT), alkaline phosphatase (ALP), total bilirubin (T-BIL), creatinine, blood urea nitrogen (BUN), inorganic phosphate (Pi), glucose, amylase, serum total protein (PROT), albumin (ALB), globulines, electrolytes potassium (K), natrium (Na), chloride (Cl), and calcium (Ca).

For some of the measured parameters, reference values were available from other studies or related animal species.

To assess a possible stress reaction, we measured serum cortisol with chemiluminescence immunoassay (Immulite©/Immulite 1000 Cortisol Kit; LKCO1, Siemens Healthcare Diagnostics GmbH, Eschborn, Germany) and the concentrations of serum dehydroepiandrosterone (DHEA), with Radioimmunassay (DHEA RIA Kit, DSL8900, Immunotech, Beckman Coulter, Brea, California, USA). We additionally determined cortisol and DHEA in the hair samples of both species. Hair samples were decontaminated using methanol/H2O (v:v; 35:65), and subsequently 100 mg of powdered hair was extracted using methanol. After purification via solid-phase extraction, samples were admitted to LC–MS (liquid chromatography–mass spectrometry) technology at the Institute of Doping Analysis and Sports Biochemistry, Kreischa, Germany. Due to the slow growth of hair, this analysis represented a possibility of retrospective evaluation of potential stress hormone activity within the last weeks or months depending on coat change and hair growth (Sheriff et al. 2011) and in this case served to check on species-specific differences of the obtained values. The quotient between stress parameters measured in the serum and those measured in the hair allowed us to evaluate whether the species had reacted abnormally to the stress of being caught compared to its species-dependent difference in general stress physiology. For statistical analysis of stress hormone effects, we performed a *t*-test using R version 3.6.1.3.

Results

General findings

We captured 20 red foxes and 13 stone martens. Of the 20 red foxes, 2 were juveniles (deciduous teeth) and 18 were adults (permanent teeth), with a sex distribution of 8 males and 12 females with an average body weight of 5.6 kg (3.5–9.0 kg). The 13 stone martens were comprised of 3 juveniles and 10 adults, split into 6 male and 7 female animals with an average body weight of 1.4 kg (1.2–1.9 kg). All stone martens and 18 red foxes were in good general and nutritional condition. Only 1 juvenile red fox displayed a reduced general condition in terms of low body weight and fur neatness, and 1 adult red fox presented a bad general condition in combination with emaciation and matted fur. No non-target species entered the traps during the study.

Pathological and radiological findings

Radiological examination revealed cases of chronic disease in 2 stone martens (arthrosis, lumbar stenosis) and in 6 red foxes (spondylosis, hip dysplasia, mended rib fracture, joint arthrosis) that were not related to trapping. Pathological findings with special emphasis on infectious and zoonotic agents were presented by Lempp et al. (2017) and are not part of this paper. Necropsies did not reveal any of the above-mentioned criteria, concerning neither AIHTS nor ISO 10990. The animals caught in our study showed several individual underlying health problems of differing severity, as described in Lempp et al. (2017) without further consequences for wildlife or population issues.

Blood analysis

Blood counts. Blood counts revealed a lower number of erythrocytes in 3 red foxes, whereas 11 red foxes showed a higher number of erythrocytes and 7 red foxes lay outside the observed range of hemoglobin. One red fox showed an increase in white blood cells, which was in line with findings of multiple inflammations in this animal. Thrombocyte counts of 4 red foxes showed high platelet numbers (Table 1). For the stone martens, hematocrit, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) were decreased compared to values of ferrets (*Mustela putorius furo*), resulting in an increase of mean corpuscular hemoglobin concentration (MCHC; Table 2).

Blood chemistry. Low calcium values were discovered in 2 stone martens; these values were compared to ranges in calcium for ferrets (Gabrisch 2010). Four blood samples taken from red foxes displayed mild hypernatremia, and 8 red fox samples were classified as hyperpotassic. Total protein increased in 2 red foxes and 6 stone martens compared to the mentioned ranges (Tables 3 and 4). A wider range of the lactate levels in red foxes was found, ranging from 0.9–10.8 mmol/L in comparison with the narrower range of 0.7–4.2 mmol/L in stone martens. Liver-specific enzymes were typically wider ranging in red foxes than in previous studies (Table 3),

Table 1. Blood count for red foxes (*Vulpes vulpes*). Columns for min (minimum) and max (maximum) are for this study; ranges are also reported from Couper (2016) and Brash (2003). MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; RDW = red cell distribution width. Data collected from the Eiderstedt Peninsula, Schleswig-Holstein, Germany, November 2013 to March 2015.

Parameter	Min	Max	*n*	Couper (2016)	*n*	Brash (2003)
Leukocytes*10^9/L	3.2	15.2	20	2.5–11.2	29	3.7–7.9
Erythrocytes*10^9/L	5.5	14.1	20	6.6–92.8	23	8.6–10.6
Hemoglobin (g/dL)	8.5	20.0	20	11.0–18.9	25	13.4–17.8
Hematocrit (%)	24.0	58.2	20	23–59	31	36–55
MCV (fL)	37.0	47.0	20	5.3–55.7	23	34–49
MCH (pg/cell)	12.6	15.4	20	1.7–55.7	21	14–18
MCHC (g/dL)	32.0	37.8	20	29.4–45.2	25	33–37
RDW	13.7	15.8	20			
Thrombocytes 10^9/L	581.0	1,498.0	5	240–660	9	
Granulocytes %	39.4	92.8	19			
Granulocytes*10^9/L	2.4	13.4	19			
Lymphocytes %	5.1	52.2	19			
Lymphocytes*10^9/L	0.2	3.1	19	0.16–4.29	27	0.9–2.8
Monocytes %	2.1	8.4	19			
Monocytes*10^9/L	0.0	0.5	19	0.00–0.62	23	0.04–0.3

Table 2. Blood count for stone martens (*Martes foina*). Columns for min (minimum) and max (maximum) are for this study; ranges are also reported from Gabrisch (2010) for ferrets (*Mustela putorius furo*). MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; RDW = red cell distribution width. Data collected from the Eiderstedt Peninsula, Schleswig-Holstein, Germany, November 2013 to March 2015.

Parameter	Min	Max	*n*	Gabrisch (2010)
Leukocytes*10^9/L	3.2	13.6	13	3.0–16.7
Erythrocytes*10^9/L	9.3	13.7	13	7.4–13.0
Hemoglobin (g/dL)	13.4	18.4	13	13.6–21.9
Hematocrit (%)	34.0	48.6	13	40–70
MCV (fL)	34.0	38.0	13	49.6–60.6
MCH (pg/cell)	11.6	13.6	13	16.1–19.3
MCHC (g/dL)	32.8	38.1	13	17.8–20.9
RDW	13.4	16.7	12	
Thrombocytes 10^9/L	1,620.0	1,620.0	1	
Granulocytes %	56.4	96.0	13	
Granulocytes*10^9/L	2.2	13.2	13	
Lymphocytes %	2.7	31.1	13	
Lymphocytes*10^9/L	0.2	1.6	13	0.6–10.5
Monocytes %	1.1	12.5	13	
Monocytes*10^9/L	0.1	1.6	13	0–0.5

Table 3. Serum chemistry analyses for red foxes (*Vulpes vulpes*). Columns for min (minimum) and max (maximum) are for this study; ranges are also reported from Couper (2016) and Brash (2003). ALP = alkaline phosphatase; ALAT = alanine aminotransferase; T-BIL = total bilirubin; BUN = blood urea nitrogen; Pi = inorganic phosphate; Na = natrium; K = potassium; Ca = calcium; PROT = serum total protein; ALB = albumin. Data collected from the Eiderstedt Peninsula, Schleswig-Holstein, Germany, November 2013 to March 2015.

Parameter	Min	Max	*n*	Couper (2016)	*n*	Brash (2003)
ALP (U/L)	15.0	87.0	20	19–249	27	11–157
ALAT (U/L)	43.0	398.0	20	39–607	27	<117
T-BIL (mg/dL)	0.4	1.2	20	0.41–1.52	24	0.41–0.99
Amylase (U/L)	175.0	814.0	20	0–191.1	9	
Glucose (mg/dL)	108.0	263.0	20	67–240	32	87.27–160.54
BUN (mg/dL)	11.0	64.0	20	21–130	32	31.74–79.04
Pi (mg/dL)	3.7	9.4	20	1.6–9.1	27	2.51–6.91
Creatinine (mg/dL)	0.4	1.2	20	0.4–2.19	28	0.69–1.3
Na (mmol/L)	138.0	167.0	20	129–156	23	135–151
K (mmol/L)	3.4	10.1	20	3.5–5.0	23	4.0–4.8
Ca (mg/dL)	6.7	11.2	20	6.8–11.2	31	8.04–10.6
PROT (g/dL)	5.9	8.3	20	3.5–7.6	28	4.7–6.5
ALB (g/dL)	1.7[a]	4.1	20	2.3–4.4	22	2.9–3.7
Globulines (g/dL)	2.1	5.4	20	1.8–4.0	20	2.3–3.5
Lactat (mmol/L)	0.9	10.8	13			

[a]Animal showing chronic diseases in necropsy.

Table 4. Serum chemistry analyses for stone martens (*Martes foina*). Columns for min (minimum) and max (maximum) are for this study; ranges are also reported for other mustelids (ferrets [*Mustela putorius furo*] and pine martens [*Martes martes*]) from Bourne (2016) and Gabrisch (2010). ALP = alkaline phosphatase; ALAT = alanine aminotransferase; T-BIL = total bilirubin; BUN = blood urea nitrogen; Pi = inorganic phosphate; Na = natrium; K = potassium; Ca = calcium; PROT = serum total protein; ALB = albumin. Data collected from the Eiderstedt Peninsula, Schleswig-Holstein, Germany, November 2013 to March 2015.

Parameter	Min	Max	*n*	Bourne (2016)	Gabrisch (2010)
ALP (U/L)	63.0	217.0	13	77±29, 115±35	13.3–141.6
ALAT (U/L)	66.0	158.0	13	1723±120, 222±106	49–242
T-BIL (mg/dL)	0.3	0.4	13		0.0–0.18
Amylase (U/L)	243.0	726.0	13		19.4–61.9
Glucose (mg/dL)	134.0	262.0	13	209±14.4, 310±70.2	54.05–153.15
BUN (mg/dL)	15.0	36.0	13	49.1±24, 72.5±29	28.74–101.2
Pi (mg/dL)	2.7	6.0	13	2.79±0.93, 4.96±0.93	3.1–9.6
Creatinine (mg/dL)	0.2	0.5	13	0.68±0.06, 0.79±0.18	0.26–0.77
Na (mmol/L)	141.0	158.0	13	155±3, 165±3	140.1–169.7
K (mmol/L)	3.8	5.3	13	3.8±0.4, 4±0.2	3.9–5.9
Ca (mg/dL)	6.4	9.5	13	8.8±0.4, 9.2±0.4	8.0–10.4
PROT (g/dL)	6.5	7.8	13	6±0.4, 6.2±1.2	5.47–7.79
ALB (g/dL)	3.8	4.5	13	3±0.4, 3.7±0.2	2.8–4.39
Globulines (g/dL)	2.7	3.9	13	2.3±0.2, 3.1±0.4	
Lactat (mmol/L)	0.7	4.2	10		

whereas ALP increased in 4 stone martens, probably due to larva migrans as in previous studies (Lempp et al. 2017; Table 4).

Hormonal findings. To assess a possible stress reaction, we measured concentrations of cortisol and the steroid hormone DHEA in the serum and in the hair of the examined foxes and stone martens. No statistically significant differences in stress parameters measured in serum and hair were found between sexes within the species.

Endocrine examinations of 13 stone martens (6 males, 7 females) and 17 red foxes (6 males, 11 females) revealed distinct lower levels of serum cortisol in stone martens than in red foxes (30.4 [21.0–62.1] ng/mL vs. 128.0 [108.0–157.0] ng/mL, Mann-Whitney U = 217.0, $P < 0.001$; Figure 3). Stone martens also showed lower serum DHEA concentrations (0.2 [0.1–0.2] ng/mL) compared to the foxes (0.4 [0.2–0.9] ng/mL, Mann-Whitney U = 186.5, $P = 0.001$; Figure 3). The DHEA concentrations did not correlate directly with the cortisol values, but the calculated cortisol/DHEA quotient resulted in comparable values for the sampled stone martens (230.0 [132.0–477.0]) and the examined foxes (290.0 [173.0–650.0], $P > 0.05$).

The cortisol concentrations of the stone marten hair samples ($n = 9$, 5 males, 3 females) gave similar values (7.9 [6.4–9.3] pg/mg) compared to the foxes ($n = 17$, 6 males, 11 females; 11.8 [6.7–16.9] pg/mg). However, significantly lower DHEA values were found in the hair of the stone martens in contrast to the foxes (1.0 [0.8–1.6] pg/mg vs. 7.5 [3.9–14.5] pg/mg, Mann-Whitney U = 125.0, $P < 0.001$; Figure 3). The DHEA concentrations measured in hair did not correlate directly with the hair cortisol values, but the calculated cortisol/DHEA quotient resulted in significantly higher values for the sampled stone martens (7.5 [3.1–10.3)] and the examined foxes (1.9 [0.4–3.8], Mann-Whitney U = 25.0, $P = 0.013$; Figure 3).

The serum/hair DHEA quotient was comparable between the species (0.04 [0.02–0.23] vs. 0.17 [0.08–0.38] for red foxes and stone martens, respectively; Mann-Whitney U = 32.0, $P = 0.1$), but the serum/hair cortisol quotient was significantly higher in foxes (9.35 [6.33–17.92] vs. 4.77 [3.50–6.15] for red foxes and stone martens, respectively; Mann-Whitney U = 99.0, $P = 0.002$; Figure 4).

We found no statistical difference between the hair samples of stone martens taken after phenological change in fur in November and December ($n = 5$, cortisol 8.3 [4.9–9.3] pg/mg, DHEA 1.2 [0.7–1.2] pg/mg) and the hair samples obtained in January ($n = 3$, cortisol 7.5 [7.2–8.5] pg/mg, DHEA 0.9 [0.8–1.9] pg/mg). There was also no statistical difference between the hair samples of red foxes sampled up until December ($n = 8$, cortisol 13.6 [7.5–18.5] pg/mg, DHEA 5.7 [3.1–8.5] pg/mg) compared with the results of the hair tests sampled during January and February ($n = 9$, 11.0 [6.7–14.6] pg/mg, DHEA 9.6 [6.2–26.0] pg/mg).

Video recordings

As video recordings were only possible for 2 traps, documentation succeeded in recording 1 red fox and 9 stone martens over a period of approximately 60 minutes each. Stone martens stayed calm throughout the recording. Most of the time, the animals rested; 6 slept and did not show signs of distress (Table 5) in accordance with AIHTS. The red fox did not move at first. After 20 minutes of being in the trap, it began to move and explore, even trying to escape for a few seconds. Hereafter, it continued exploring for 30 minutes (Table 6).

Discussion

The present study intends to define additional criteria that might improve international standards on humane trapping. The concrete pipe vault matched AIHTS standards. The sample size for stone martens did not comply with the expectations of 20 animals stipulated in AIHTS, but for the animals caught, standards were met for animal welfare concerning behavioral and physical indicators of AIHTS and ISO 10990. Further investigations of that kind should be dispensed with, especially because ISO 10990 does not specify an exact number but instead states a "sufficient number of individuals of the target species" if certain conditions are met. With a number of 20 animals being unharmed and therefore meeting the required standards of both ISO 10990 and AIHTS, the concrete pipe vault serves as an appropriate device for trapping red foxes, being in line with several studies on trapping welfare of animals (Muñoz-Igualada et al. 2008, Ziegler et al. 2018). Additionally, in both traps, non-target species were not caught, which is another advantage for management because it may indicate the specificity of the traps to capture specific spe-

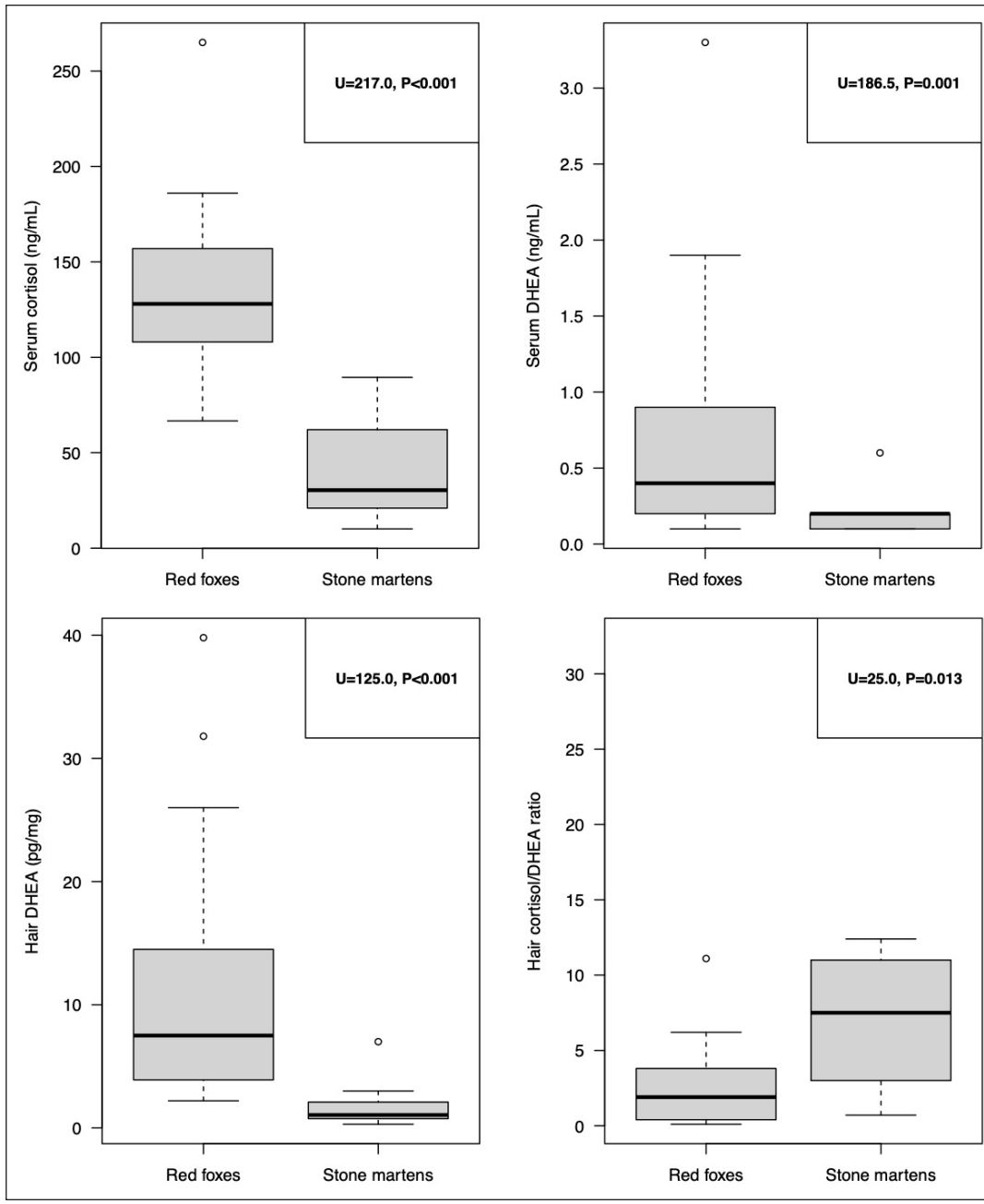

Figure 3. Serum cortisol, serum DHEA, hair DHEA, and hair cortisol/DHEA ratio in red foxes (*Vulpes vulpes*) and stone martens (*Martes foina*), using non-parametric tests considering the small sample size. Data collected from the Eiderstedt Peninsula, Schleswig-Holstein, Germany, November 2013 to March 2015.

cies, which might be proved by camera settings near the traps in future research. In addition to these indicators, we found that hormone analysis may improve evaluation criteria. Stone martens showed lower levels of stress hormones and remained calmer during their stay in the trap than foxes, which was in part shown in video-based observation. Evaluation of stress in wildlife animals is a challenging task. Several studies indicate that the best concept is to combine different methods to have the most effective outcome (Veasey et al. 1996, Iossa et al. 2007, Whitham and Wielebnowski 2013).

Cortisol is the main mediator of the stress

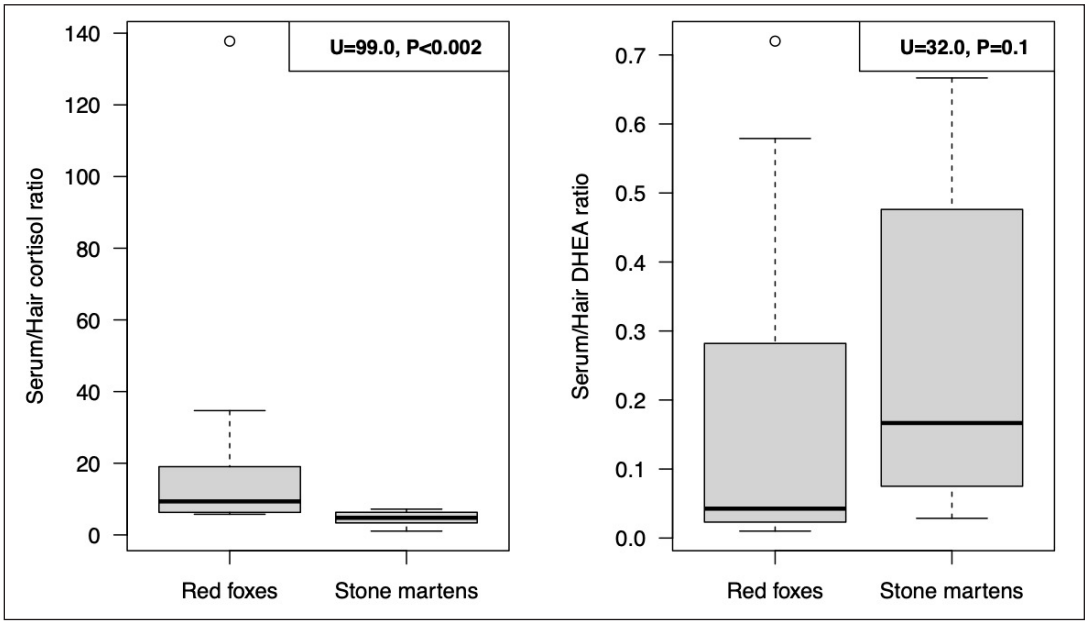

Figure 4. Serum/hair cortisol ratio and serum/hair DHEA ratio suggesting a lower stress response to trapping procedures in stone martens (*Martes foina*) as compared to red foxes (*Vulpes vulpes*). Data collected from the Eiderstedt Peninsula, Schleswig-Holstein, Germany, November 2013 to March 2015.

response, and blood cortisol levels usually increase within a few minutes after a stressful event (Wingfield et al. 1997). However, a single determination of cortisol in the blood primarily reflects the short-term response, also induced by the handling of the animal (Sheriff et al. 2011). Therefore, the level of DHEA in serum and hair samples as well as their quotient are important when assessing stress in mammals (Kaiser et al. 2003, Macbeth et al. 2010, Trevisan et al. 2017, Bergamin et al. 2019).

Levels of cortisol and DHEA were significantly different between the 2 species in serum as well as in hair samples. These results might indicate a species-specific difference in basal values that should be verified in further studies, where seasonal and diurnal variations also should be taken into account (Barja et al. 2007, 2011). The values measured in the serum should correspond to the acute reaction to the trapping, and the interventions and the values measured in the hair correspond to the stress hormone activity of the previous weeks or months. Comparing the quotient of these values between species allows us to ascertain if the species reacted similarly to the trapping procedure. The serum/hair DHEA quotient was comparable between species, but the cortisol quotient was much higher in foxes. This is an indication of the fact that stone martens showed a lower stress response than red foxes under comparable trapping conditions, which again should be clarified in further studies.

A possible influence of the different species-specific times of coat change on the measured hormone concentrations in the hair as well as the associated time differences in the incorporation of cortisol into the hair shaft must be taken into account for the evaluation. Stone martens change fur at the beginning of the winter. In the case of the red foxes, however, both the change of coat in spring and the regrowth of the undercoat in the fall are described in the literature (Maurel et al. 1986). Further investigations based on a higher number of samples will be necessary to rule out interference of the different time of fur change of each species. In our study, we found no statistical difference between the hair samples of the stone marten or red fox relative to the season. This indicates that hair samples can be used for analyzing stress hormones regardless of the time period, which can also be deduced from a study on fur change in minks (*Mustela vison*; Johnston and Rose 1999).

In addition, stone martens showed fewer behavioral symptoms of stress during video observation. Nevertheless, studies on coping strategies confirm that seemingly calm ani-

Table 5. Ethogram for stone martens (*Martes foina*) in a Strack's wooden box trap ($n = 9$). Total time of each video: 60 minutes. Data collected from the Eiderstedt Peninsula, Schleswig-Holstein, Germany, November 2013 to March 2015.

Parameter observed (number of animals)	Description of behavior
Exploring (4)	The animal explores the trap, including sniffing and watching the entrance.
Sniffing	
Watching	
Moving / "walking" (2)	The animal moves back and forth inside the trap.
Walking back and forth	
Trotting back and forth	
Lying down (9)	The animal lies still, even sleeps.
Curled up	
Sleeping	
Grooming (3)	The animal grooms itself while standing or lying down.

Table 6. Ethogram for a red fox (*Vulpes vulpes*) in a concrete pipe vault ($n = 1$). Data collected from the Eiderstedt Peninsula, Schleswig-Holstein, Germany, November 2013 to March 2015.

Parameter observed	Description of behavior
Exploring	The animal explores the trap, including sniffing and watching the entrance, sniffing and biting at the bait.
Sniffing	
Watching	
Retreating	The animal attentively watches and carefully retreats from the entrance.
Attempting to escape	The animal scratches itself, turns, retreating fearfully (big eyes, ears laid back).
Moving / "walking"	The animal moves back and forth inside the trap.
Walking back and forth	
Trotting back and forth	
Lying down	The animal lies still, panting or licking its mouth.

mals suffer from stress (Koolhaas et al. 1999, Coppens et al. 2010). However, the video material clearly shows an animal that was exploring and subsequently sleeping. Because the video material was limited in duration, as was the number of recorded animals, further studies should expand the video recording to cover the whole stay in the trap.

To further clarify the dimension of stress for the trapped animals, we compared the behavioral observations and glucose concentration in both species with cortisol levels. In red foxes with elevated cortisol levels, however, we could not detect elevated glucose levels, which are expected during a stress-induced hyperglycemia. One male stone marten showed an increased glucose and cortisol concentration compared to the other examined stone martens. However, we did not observe supposed stress-related behavior in this animal. As previously mentioned, for a more precise assessment, samples of non-stressed stone martens and red foxes would have to be compared with the obtained data. The ethogram of the stone marten showed no obvious behavioral components of distress, although little is known about distress behavior in this species, even in the ferret (Vinke and Schoemaker 2012). The red fox clearly showed signs of distress compared to ethograms on dog (*Canis lupus familiaris*) behavior (Van den Berg et al. 2003). In both species, the behavior did not reach the dimensions stipulated in AIHTS.

There are different explanations for the observed blood deviations in some animals. The number of erythrocytes and the hematocrit were probably increased due to a reduced plasma volume in connection with dehydration. This might be caused by a lack of fluid absorption prior to trapping. Nevertheless, the time spent in the trap should be as short as possible to ensure animal welfare in this regard. For stone martens, a wider range of hematocrit, MCV and MCH, and calcium values might be possible because reference ranges for Mustelidae species differ (Kollias and Fernandez-Moran 2015). Eight samples of the 20 red foxes showed hyperpotassemia, which in 2 cases could be explained by hemolysis of the blood samples and might also indicate the poor quality of further samples. The increased ALP in 4 stone martens was probably due to larva migrans diagnosed by Lempp et al. (2017). Since there are no references for lactate levels of either the red fox or stone marten, it might be assumed that the range of values is higher in red foxes.

Relating to the interception of foxes entering the trap, in 6 cases, a strong defense reaction of the animal occurred (e.g., turning, averting, biting the box). This might result in tissue damage

and also explains the increased lactate values. It is striking that these values do not correlate with the cortisol levels of the animals, which means that they might not be related to the perceived stress. The deviations caused by the interception procedure are not relevant to the AIHTS but have to be implemented in managerial implications.

Conclusions

Our study focuses on some aspects that might help to improve the standards stipulated in AIHTS and ISO 10990. Additional factors considered in other studies (e.g., heart rate and blood pressure) were omitted, although these might also serve to optimize the quality of animal welfare (Powell and Proulx 2003, Proulx et al. 2012). Because non-target species were not caught, their welfare could not be assessed as was previously done in comparable studies (Shivik et al. 2005, Muñoz-Igualada et al. 2008). Up to now, the evaluation of stress in trapped wild animals in Germany has not been clearly defined or agreed upon by trappers or by scientists or policy. Thorough evaluations of physiological parameters in blood analyses showed alterations that could not be directly allocated with the animals stress. Hormone analysis, especially the cortisol serum/hair quotient, though, did show significant outcomes in both species that might be a promising approach for further research on animal welfare in trapping.

In general, when using traps, the following aspects should be considered: the site should not be exposed to direct sunlight, and removing the animal from the trap should be performed quietly, using a cage in combination with a slide inside the trap to guarantee a quick release into the cage. Time that animals are held in the traps after capturing should be kept as short as possible to reduce stress.

Acknowledgments

Our study was funded by the German Hunting Union. Funding was also received from the European Union's Horizon 2020 research and innovation program COMPARE (grant agreement no. 643476). Special thanks go to H. Behrens who helped to organize the study, to the hunters who trapped our study animals, the Landesjagdverband Schleswig-Holstein, and the University of Kiel for their support in conducting the investigations. This publication was supported by Deutsche Forschungsgemeinschaft and University of Veterinary Medicine Hannover, Foundation within the funding program Open Access Publishing. Comments provided by J. Beck, HWI associate editor, and an anonymous reviewer greatly improved an earlier version of our paper.

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