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Cumulative experience, age-class, sex and season affect the behavioural responses of European badgers (Meles meles) to handling and sedation

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Abstract

*The restraint and sedation of wild animals has welfare implications, thus animal handling procedures should be well-informed and optimised to adhere to welfare standards. Furthermore, it is important that handling procedures should not cause future trap avoidance. This is of particular pertinence to European badgers (*Meles meles*), subject to extensive cage-trapping, relating to bovine tuberculosis epidemiology. We examined 4,288 capture/recapture events for 856 individual badgers, occurring between May 1999–September 2011, recording initial observed behaviour and reaction provoked by injection, on a scale ranging from still (0) to distressed/aggressive (3). Eighty-seven percent of adults and 76% of cubs were still (0) when approached initially and 75% of adults and 62% of cubs remained still when injected. Cubs exhibited significantly higher behavioural responses than adults, while female adults scored higher provoked scores than males. Importantly, the initial behaviour of an individual dictated its provoked response. Previous experience of capture was associated with lower subsequent behavioural response scores, while naïve badgers were most prone to score highly. Individuals first caught as cubs scored lower initial responses than those first caught as adults. Lower initial responses occurred in spring and summer and higher responses were associated with lice infestation. Behavioural criteria have potential to inform and optimise welfare in badger capture operations. This contributes to techniques allowing simple, non-invasive assessment of how wild animals in general respond to temporary restraint, where the psychological perception acts as the precursor to physiological stress.*

Keywords: *animal welfare, badger, behavioural response, injection, restraint, sedation*

Introduction

Attempting to improve the welfare of wild study animals is in the best tradition of ecology, both in terms of ethics and to minimise the chance that the intervention might otherwise influence the animals' behaviour in a way detrimental to research goals (Bekoff 2002; Powell & Proulx 2003). Understanding cognitive phenomena in animals is also essential to scientifically informed ethical reasoning (Dawkins 2004; Allen & Bekoff 2007). Over the course of evolution, organisms undergo adaptation to specific environmental conditions, which von Uexkül (1957) termed their *Umwelt* (see also Tinbergen 1963; Wiepkema 1987). Animals are in a continuous interactional state with their dynamic *Umwelt* and accommodate changes through combining fixed routines with flexible behaviours. Implicitly, there is no 'normal' baseline for the behaviour of an unhabituated wild animal under unnatural conditions, in close proximity to humans. During typical ecological monitoring protocols, such as trapping, restraint, handling and sedation, *Umwelt* expectancies are not fulfilled and there is a reduced predictability and/or controllability of relevant conditions (Weiss 1972; Wiepkema & Koolhaas 1993; Jensen *et al* 1996). With normal 'fight or flight' responses impeded, the animal is forced to cope with its situation, which can induce abnormal behaviour (Moberg 2000), or impact on the animal's health (Tarlow & Blumstein 2007).

Our objective in this study was to determine tractable, observational cues on the extent to which European badgers (*Meles meles*), subjected to restraint and sedation, exhibited signs of behavioural 'distress', which Wiepkema (1987) defined as either acute responses, such as conflict behaviours (eg redirected, ambivalent and displacement activities), or chronic responses (stereotypies, injurious activities etc). In the specific instance of badgers, these ranged from complete immobility (usually connected to sleeping in holding cages) through frustrated attempts to escape holding cages, to overt aggression (see also Berkowitz 1989) toward personnel handling them. We then used this information to establish the appropriate handler response to each 'state' a badger might be in, to avoid worsening behavioural reactions.

The experience an animal undergoes during routine handling (Taraborelli *et al* 2011) can also influence recapture probability; although many other factors, such as prevailing weather, body condition, season etc, tend to eclipse this signal (Noonan *et al* 2015). Practitioners should thus be cognisant of differences between the responses of naïve animals and those of animals handled previously (Caizergues & Ellison 1998; Marai & Rashwan 2004; Littin & Mellor 2005; Montes *et al* 2011).

Previous studies have investigated the risk of trap-related injuries to confined badgers prior to lethal despatch (Woodroffe *et al* 2005; Murphy *et al* 2009), and physiological measures of trapping-induced stress have been derived from glucocorticoids and corticosteroids (eg Breuner *et al* 1999; Schutz *et al* 2006), as well as leukocyte coping capacity (see McLaren *et al* 2003, 2007). Although these measures are undoubtedly involved in why an animal might undergo a problematic cascade and become progressively more difficult to handle, Lazarus (1966) emphasised that the perception of 'psychological stress' by an individual acts as the precursor to physiological stress (triggering the adreno-corticotropic cascade). He proposed that, in order for any situation to be stressful, it must be appraised as such. Therefore, cognitive processes of appraisal are central in determining whether a situation is potentially threatening, constitutes a harm/loss or a challenge, or is benign (see Aldwin 2007). Consequently, what the researcher or veterinarian sees, and must deal with, is this actual behaviour. The advantages of the simple observational methods we describe here are that they gauge the instantaneous behavioural condition of the animal in a very practicable way, where the practitioner can anticipate negative reactions and adapt their approach instantly, while still implementing the required handling and sampling protocol.

The importance of developing and refining criteria able to recognise specific abnormal and undesirable behavioural responses in wild animal species, held in short-term captivity, are reflected in the guidelines and codes of ethics published by various professional animal research societies; for example, the Universities Federation for Animal Welfare (see Dawkins 2004), the American Society of Mammalogists (ASM/ACUC 1998; Sikes *et al* 2011) and the Association for the Study of Animal Behaviour and the Animal Behavior Society (ASAB/ABS 2000). National laws and standards also stipulate the need to monitor the behaviour of captive animals and adapt care as appropriate. In the United States this is a statute of the Animal Welfare Act and the Endangered Species Act, with similar legislation in Canada (the *Guide to Care and Use of Experimental Animals in Canada*) and in the European Union (EU Directive 2010/63/EU on the protection of animals used in scientific research: see Powell & Proulx 2003).

A large number of badgers are caught (and re-captured) in the UK and the Republic of Ireland each year connected to the management of bovine tuberculosis (bTB, caused by *Mycobacterium bovis*), where various culling strategies undertaken by the UK Government have relied on cage-trapping and shooting (see Tuyttens *et al* 1999); with stopped-restraints (snares) used in the Republic of Ireland (Murphy *et al* 2009). A further development is badger vaccination against bTB, which requires that a specified proportion of any regional populations can be caught and treated, where again ensuring good welfare standards for these animals is important (Lesselier *et al* 2011).

No matter whether processed *in situ* at their sett (den) or transferred to a central facility, the capability of being able to recognise instantly the likelihood that a badger will become difficult or aggressive to approach and sedate has benefits for animal care and operator safety.

Furthermore, there is substantial public opposition to badger trapping and culling (Grant 2009). If trapping for culling and research must be used for wildlife management, it is crucial to address public sentiments on welfare (Schmidt & Bruner 1981; Proulx & Barrett 1991). Even if trapped for euthanasia, badgers should be treated humanely prior to dispatch, because it is also easier to shoot a placid target. A recent report (DEFRA 2014) on the latest culling strategy — shooting free-ranging badgers — concluded that it was 'extremely likely' that 7.4 to 22.8% of badgers that were shot were still alive after five minutes, due to shot misplacement. Consequently, trapping to euthanise may be a more humane and efficient alternative (Smith & Cheeseman 2007) ensuring that targeted animals are killed effectively, and not just wounded, and causing less behavioural change among those animals not targeted, where disease expression and transmission is enhanced by stress (McLaren *et al* 2007), potentially exacerbating the spread of infection to cattle (Riordan *et al* 2011).

In addition to application in these applied control measures, and to the humane treatment of badgers in capture-mark-recapture-based fundamental badger ecology studies (eg Rogers *et al* 1997; Macdonald *et al* 2009; Byrne *et al* 2012), badgers are also frequently injured in road-traffic accidents (Macdonald *et al* 2010), necessitating their capture, restraint and, often, veterinary treatment (Cousquer 2005). Consequently, a wide range of practitioners under a variety of scenarios stand to benefit from being able to recognise if a restrained badger is exhibiting fearful, distressed or aggressive behaviours, facilitating improved conservation practice (Macdonald 2001; Teixeira *et al* 2007).

Working with a 24-year dataset for a high-density badger population, the objectives of this study were three-fold:

- To test if badgers exhibit specific behavioural responses to temporary (i) restraint and (ii) sedation and to analyse if these responses are linked;
- To explore the interactive — and potentially additive — effects of key parameters known to interact with badgers' basic behavioural state (*sensu* Broom 1991; Dawkins 2004). For example, (i) weather (being uncomfortably cold, or hot, or wet will likely predispose the badger to discomfort — see Macdonald *et al* 2010; Noonan *et al* 2014); (ii) parasites (being irritated and itchy is also likely to predispose the badger to discomfort — see Cox *et al* 1999; Johnson *et al* 2004; Newman *et al* 2004; Sin *et al* 2014); (iii) less-experienced juveniles often tend to respond differently than do more experienced adults — connected to

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learning and sensitive developmental periods (eg Bornstein 1989; Fell *et al* 2006); and (iv) males and females might respond differently, especially in terms of aggression, based on hormonal conditions (Yamaguchi *et al* 2006).

• To investigate whether previous experience of trapping alters future trappability and linked to this, if behavioural responses to restraint and sedation change with cumulative experience of the regime.

Even though this final objective is only applicable to studies undertaking repeat trapping, it nevertheless does yield information pertinent to the potential difficulties or advantages of commencing work on a naïve population, relative to those more studied ones detailed in the literature.

From these simple, non-invasive observations we then formulate best-practice recommendations for restrained badgers, subject to sedation (see Schutz *et al* 2006), attempting to reduce the risk that they might escalate injurious or aggressive behaviours, but without adding to procedural demands by applying physiological measures (von Borell 2000; Dawkins 2004).

Materials and methods

Study population and trapping regime

The Wytham Woods badger population (Oxfordshire, UK: GPS 51.774ºN, 1.322ºW) has been studied continuously since 1987, using a systematic trapping regime, with the aim of monitoring the life histories of as many members of the population as thoroughly as possible through seasonal trapping sessions. To 2011, this totalled 1,037 individually tattooed badgers, recorded over 9,145 trapping events, with a mean of 8.5 capture-recapture events per individual, yielding a mean (± SEM) annual population density estimate of *circa* 36.37 (± 2.25) badgers per km^2 (Macdonald *et al* 2009) over the 6 km^2 foraging range. The trapping regime has, as a minimum, involved sessions in spring (May/June — first opportunity to catch annual cub cohort), summer (late July-early Sept), and autumn (Oct/Nov), with winter (Jan) trapping in key years, to diagnose pregnancy using ultrasound (eg Dugdale *et al* 2003). This was supplemented by occasional *ad hoc* trappings, focused on specific experiments, such as the progression of parasitological disease (eg Newman *et al* 2001); developing repellents (eg Baker *et al* 2005) and telemetry studies (eg Woodroffe *et al* 1995; Dyo *et al* 2012).

Badgers were trapped in steel mesh cage traps (850 × 370 × 380 mm; length × width × height) baited (but not pre-baited; Macdonald & Newman 2002) with approximatelt 150 g peanuts, sited at all of the active setts (including outliers) associated with each social-group in the population. More traps were set at each site than the anticipated number of badgers present (from previous trapping history), providing saturation trapping.

All trapping and handling protocols were subject to instituitional ethical review and were performed under Natural England Licence (currently 20104655) and UK Home Office 'Animals (Scientific Procedures) Act, 1986' Licence (currently PPL 30/2835); all personnel handling badgers were qualified Personal Individual Licence (PIL) holders.

As we made refinements continuously over the total study period to streamline the protocol, we limited these total data to a subset from 1999–2011, for which the handling regime and welfare criteria were recorded consistently. During this interval, the trapping regime consistently involved three successive days of effort at each site trapped, dividing the woods into quarters — thus, the full seasonal session spanned 12 days. Traps were set between 1500 and 1800h, and badgers were collected between 0600 and 0830h the following morning. During handling, each badger was marked clearly with a temporary livestock spray, in order to identify recaptures and prevent re-sampling an individual within a session, which were released immediately at the morning round of trap checking.

Sedation, handling protocols and behavioural observations

Badgers were transferred to smaller holding cages *in situ*, and then usually transported back to a central processing facility (barn) using an ATV or cargo vehicle, driven slowly — a journey usually < 15 min (Montes *et al* 2004). Back at the processing facility, holding cages were placed on racks, off the floor, to allow any urine or faeces to fall away. Badgers were then allowed to rest for at least 15 min in the handling facility (Montes *et al* 2004). Holding cages were covered with a blanket prior to procedures commencing, which reduced behaviours such as rolling over in the cage, clawing at the mesh, snapping at handlers etc. This badger population is relatively closed, and during this study interval no bTB was recorded in soil sampled at the study site (in contrast to bTB study sites, see Courteney *et al* 2006) or from the many hundreds of deer killed and inspected for woodland management on the Wytham Estate each year (Wytham Management Committee, personal communication 2015). Close co-confinement and covering might not be appropriate for studies where bTB is known, or suspected, due to enhanced risks of disease transmission — although processing badgers *in situ* at setts does not obviate the need to manage behavioural responses to restraint and sedation carefully.

The procedures room was kept as quiet as practicable, given the need for the personnel present (usually 3–6) to communicate about procedures (but not extraneous matters); all other noise was limited — specifically avoiding any clanking or banging while moving equipment, or any operating sounds from equipment (eg centrifuges). Although badgers rely extensively on olfaction (Buesching & Macdonald 2001) we have not observed any specific reactions to laboratory odours, and every effort was made to keep strong chemicals away from animals during processing.

Badgers were then sedated one after the other, using an intra-muscular injection, usually of ketamine hydrochloride 100 mg ml^{-1} at a dose of 0.2 ml kg^{-1} (McLaren *et al* 2005). If deeper anaesthesia was required, for example, to examine naturally occurring wounds (de Leeuw *et al* 2004), we used ketamine + medetomidine + butorphenol combinations; although ketamine has been the sedative of choice. Thus, typically a 2-ml bolus (for a 10-kg adult) was injected using a 23 G × 1" (0.6 × 25 mm) needle attached to a 2-ml syringe. After sedation, any previously un-caught badger was given a unique identifying tattoo in the inguinal region (Macdonald & Newman 2002).

For each badger, ancillary information on the operating temperature in the procedures room (unheated barn; numeric data), and prevailing rainfall conditions during preceding (trap) night (dry/rained during the trap night/raining during processing), was noted by the anaesthetist, to investigate whether these variables influence the pre-procedural behaviour exhibited by individuals.

The behaviour of each badger was recorded at two stages in the procedure, initially upon lifting the blanket (ie the behavioural state of the individual the anaesthetist would need to interact with to inject) and then the behavioural response of that individual to the provocation of initial needle prick. In particular, we were interested to see if provoked responses were in line with initial behaviour, for example, did aggressive animals become even more aggressive.

We assessed both these 'initial' and 'provoked' behavioural conditions on a four-point scale (0–3 categorical assessment), using the following subjective criteria and definitions to characterise the individual's behaviour.

Initial behaviour (presentation of animal upon lifting cover)

0 = Still: no movement, usually lying down and often sleeping;

1 = Active: alert, usually standing, observant of the anaesthetist's movements, but without responding;

2 = Agitated: moving around in the cage, turning away from the anaesthetist, sometimes shivering or with subdued growling — seeking means of escape; and

3 = Distressed: rolling over in the cage, clawing and snapping at the cage mesh — but without any targeted attack.

Provoked behaviour (in response to injection procedure)

0 = Still: no movement, potentially remaining asleep;

1 = Reactive: aware of injection — either watching the injection site or edging away from the needle;

2 = Agitated: moving around cage, actively resisting the anaesthetist's attempts to get the needle into the individual's rump, possibly with subdued growling — but not attempting to attack anaesthetist; and

3 = Aggressive: vigorous movement within cage — often clawing at mesh, highly averse to needle, orienting toward the anaesthetist, often snarling and snapping — targeting attack against anaesthetist from within cage.

For each badger injected we recorded: age-class (adult or cub), sex (male or female), weight (to nearest 0.1 kg), body condition (score — 5-point scale: emaciated = 1; corpulent = 5), reproductive status (female vulva condition; male testes descent and shape), wounds (location, extent and freshness) and ectoparasite burden. Flea (*Paraceras melis*), burden — was assessed from numbers found by searching through the entire fur per 20 s (described in Cox *et al* 1999); lice (*Trichodectes melis*) and ticks (*Ixodes* spp) were counted within a 4 × 4 cm square frame, placed in the umbilical-illiac region of the abdomen; the presence/absence of harvest mites (*Trombicula autumnalis*) around the face was also recorded. Samples collected during the handling protocol typically included blood (jugular venepuncture), subcaudal gland secretion (via spatula), and more recently anal gland secretion (via palpation). A proportion of animals was also administered enemas (warm soapy water) to induce defaecation (for endoparasite studies). Radio-collars were fitted on some animals. Attempting to be careful but expedient, this handling took approximately 5–10 min, after which individuals were returned to their holding cages and moved to a quiet recovery area in an adjacent room, placed on racks, and covered. Recovery from sedation was monitored to ensure all badgers regained their righting reflex after about 20 min and that no individuals struck themselves repeatedly against the holding cage as they regained consciousness. Respiratory distress was never observed. After approximately 3 h badgers were fully conscious, aware of their surroundings and mobile and transported back to their sites of capture for release.

Behavioural responses to handling: effects of age-class, sex, parasite burden and prevailing weather

Given that initial and provoked behavioural responses were ranked ordinal values, and that badger recapture events were repeated measures per individual, we used Cumulative Linear Mixed Models (CLMM), fitted using the CLMM2() function of the 'ordinal' package in R, to analyse: (i) whether initial and provoked responses were affected by the recapture history of individuals; along with (ii) the effect of our set of explanatory parameters, ie, age-class (cub/adult), sex, season, parasite type/burden, weather (temperature and rainfall on the trapping night). The CLMM was fitted as:

$\text{logit}\,(P[Y \le j]) = \theta_j - \beta_1 \times \text{Var}_1 - \beta_2 \times \text{Var}_2 - \ldots \beta_n \times \text{Var}_n - \text{u}$ (random effect)

Where, θ_j represents a threshold coefficient, β_1, β_2, ..., β_n represent the coefficients of the explanatory variables Var_1, Var_2,...Var_n, u indicates a random effect and *j* index signifies ordinal levels. Badger identity was included as a random effect (Christensen 2015). Wald-*Z* and likelihood ratio test statistics are provided (the normal output statistic approximated by the CLMM procedure).

Given the potential for a single badger to be co-infected with four different ectoparasites, we used a separate data sub-set for each species, from the total of 4,288 behavioural response records (badger fleas = 3,389 records; lice = 2,949; ticks = 702; harvest mites = 194: parasite counts ranged from 0 to 100), to examine whether these influenced behavioural responses to handling and sedation. Continuous predictors (eg, temperature and parasite burden/count number) were standardised by subtracting the seasonal mean from each value and dividing by the standard deviation (see Ramaswami & Sukumar 2013).

Table 1 Summary of percentages of badgers displaying different initial and provoked behaviour responses categories by Age-class and Sex.

Categories (n = sampling records)	Initial				Provoked			
	0	1	2	3	0	1	2	3
Adult (n = 3,449)	87.0	11.0	1.6	0.4	75.1	16.6	5.16	3.1
Adult male (n = 1,464)	87.3	10.7	1.6	0.4	77.0	15.9	4.78	2.4
Adult female (n = 1,985)	86.9	11.1	1.7	0.4	73.8	17.2	5.44	3.6
Cub (n = 839)	75.9	18.2	5.4	0.5	61.7	24.4	10.13	3.7
Cub male (n = 400)	76.8	18.8	4.0	0.5	64.2	24.5	8.75	2.5
Cub female (n = 439)	75.2	17.8	6.6	0.5	59.5	24.4	11.39	4.8

Table 2 Summary of Cumulative Linear Mixed Models: compositions, comparisons and best model variable selection performed using likelihood ratio tests (badger identity was included as a random effect).

Model comparison	Variables and step-wise backward removal procedure	AIC	df	LR	*P*-value
1	Response variable: Initial behaviour Explanatory variables: Age, sex, season, rain, temperature and age × sex	4,231.509			
1 vs 2	Removal of term: age × sex	4,229.510	1	0.001	0.973
2 vs 3	Removal of term: rain	4,226.473	2	0.962	0.618
3 vs 4	Removal of term: sex	4,225.001	1	0.528	0.467
4 vs 5	Removal of term: temperature	4,223.004	1	0.003	0.957
5 vs 6	Removal of term: season	4,228.191	3	11.187	0.011*
5 vs 7	Removal of term: age	4,267.749	1	46.745	< 0.001***
1	Response variable: Provoked behaviour Explanatory variables: Initial status, age, sex, rain, season, temperature and age × sex	6,230.947			
1 vs 2	Removal of term: age × sex	6,229.025	1	0.078	0.780
2 vs 3	Removal of term: rain	6,226.740	2	1.716	0.424
3 vs 4	Removal of term: season	6,223.040	3	2.299	0.513
4 vs 5	Removal of term: temperature	6,221.202	1	0.162	0.687
5 vs 6	Removal of term: sex	6,223.992	1	4.790	0.029*
5 vs 7	Removal of term: age	6,241.465	1	22.263	< 0.001***
5 vs 8	Removal of term: initial status	6,823.354	3	608.152	< 0.001***

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$. × Indicates interaction between variables.

Linked initial and provoked responses and individual behavioural typologies

It would help inform the animal handler, if behaviour patterns could be identified that would be likely to result in a more difficult delivery of injection, and if certain individuals could be singled out from previous records that required special consideration. We therefore applied a CLMM framework to test whether the provoked response to injection procedure was affected by an individual's initial response score, and whether individuals could be characterised as conforming to any consistent response types on each capture, treating the initial response score (0–3) as an ordinal categorical variable.

The effect of first exposure to restraint and handling on subsequent behaviour

To investigate whether first experience of the trapping protocol, and in particular initial exposure as a cub during this sensitive developmental period (Knudsen 2004), influenced subsequent initial behavioural responses (assuming the provoked response was largely the product of the initial response), we divided previous capture history into three categorical typologies: 1) first captured as an adult; 2) first captured as a cub (and not subsequently as an adult — implying mortality, or emigration from the population); and 3) first captured as a cub and then recaptured as an

Table 3 Final Cumulative Linear Mixed Model outputs for initial and provoked behavioural responses.

Model	Response variables	Explanatory variables		Estimate	SEM	Wald z	Pr(>|z|)	N
Model I	Initial	Age	Age:Cub	0.770	0.110	7.001	< 0.001***	4,288
		Season	Season:Spring	0.323	0.126	2.570	0.010*	4,288
			Season:Summer	0.391	0.125	3.129	0.002**	4,288
			Season:Winter	0.131	0.227	0.579	0.563	4,288
	Provoked	Initial	Initial1	2.063	0.102	20.302	< 0.001***	4,288
			Initial2	3.510	0.220	15.953	< 0.001***	4,288
			Initial3	3.964	0.604	6.563	< 0.001***	4,288
		Age	Age:Cub	0.455	0.096	4.742	< 0.001***	4,288
		Sex	Sex:Male	–0.220	0.100	–2.191	0.028*	4,288
Model II	Initial	Parasite	Flea	0.072	0.053	1.369	0.171	3,389
		Parasite	Lice	0.107	0.049	2.187	0.029*	2,949
		Parasite	Tick	–0.206	0.475	–0.434	0.664	702
		Parasite	Trombiculids†	–1.812	0.819	–2.211	0.027*	194
	Provoked	Parasite	Flea	–0.055	0.044	–1.264	0.206	3,389
		Parasite	Lice	0.065	0.044	1.485	0.138	2,949
		Parasite	Tick	–0.083	0.116	–0.716	0.474	702
		Parasite	Trombiculids†	–0.081	0.182	–0.447	0.655	194
Model III	Initial	Experience: Category 3		0.330	0.138	2.389	0.017*	3,449
	Provoked	Experience: Category 3		–0.053	0.131	–0.408	0.684	3,449

Model I — effects of age, sex, season and initial response on provoked responses; Model II — effects of parasite burden; and Model III — effect of first experience of capture (Category 1: captured as adult only; Category 3: captured as a cub and as an adult subsequently (where we compare only between subsequent adult responses). N indicates number of observations per model.
*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$. † Indicates CLMMs were ill-defined as Hessian value over 10,000.

adult at least once (where we used only subsequent adult responses in further analyses and disregarded cub responses).

We recorded 3,755 responses (category 1: 1,859; category 2: 304; and category 3: 1,590).

The effect of cumulative experience of restraint and handling over an individual's trapping history

We examined whether initial and provoked behavioural scores changed with the accumulating recapture experience of individuals, once adult — either to become more or less sensitised to disturbed behaviours, using general linear regression models, applying function lm() in R.

Statistical analysis

We first assessed the effect of explanatory variables separately, using general linear models, and then analysed the contribution each made to model significance using likelihood ratio tests, fitted using the ANOVA()' function of the'ordinal' package in R. A P-value ≥ 0.05 indicated that the model performed equally well with and without the variable. We then produced three global models, subjected to CLMM analysis: Model I (to test effects of extrinsic and intrinsic variables); Model II (effect of parasite burden); and Model III (effect of badger recapture history). A stepwise backward removal procedure was then applied to simplify Model I.

The Hessian number derived from the CLMM output allowed us to test the precision of the model; values within 10,000 of the given model can be considered as well defined (Christensen 2013) and were accepted. From these well-defined models we were able to produce a cumulative probability curve of ranked behavioural responses.

Results

Behavioural responses to handling: effects of age-class, sex, parasite burden and prevailing weather

In the majority, badgers yielded low behavioural reaction scores across the 4,288 responses to handling and injection. Our initial assessment scored 87% of adults and 76% of cubs as remaining still (0) prior to the sedation procedure. Similarly, 75% of adults and 62% of cubs were assessed as remaining still (0) in response to injection (Table 1). Adult badgers tended to exhibit generally lower mean (± SEM) response scores overall than did cubs (Initial:

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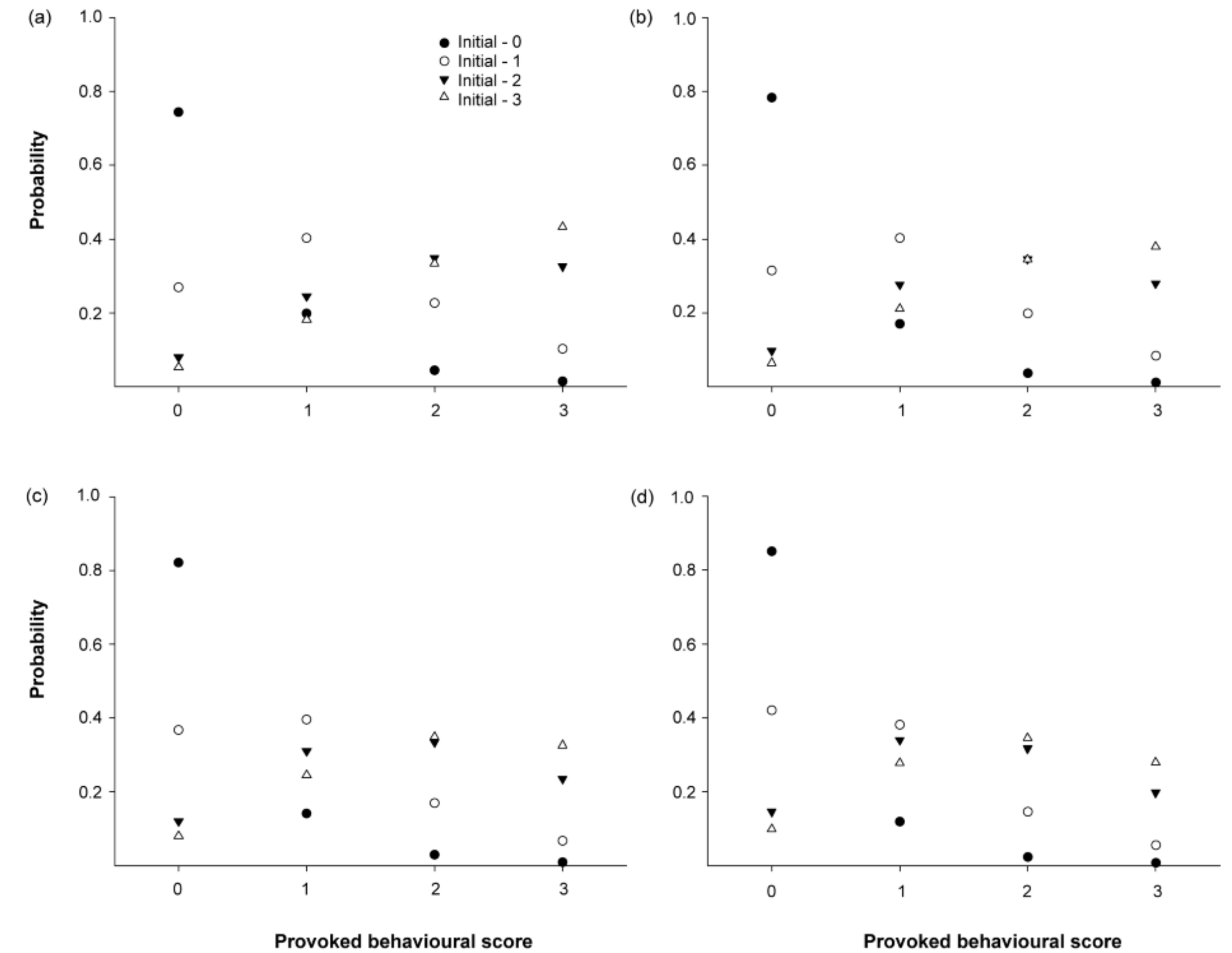
Figure 1



Cumulative probability of inducing provoked score category (x-axis value: 0 = still, 1 = active, 2 = agitated and 3 = aggressive) in relation to levels of initial behaviour for (a) age = cub; sex = female, (b) age = cub; sex = male, (c) age = adult; sex = female and d) age = adult; sex = male.

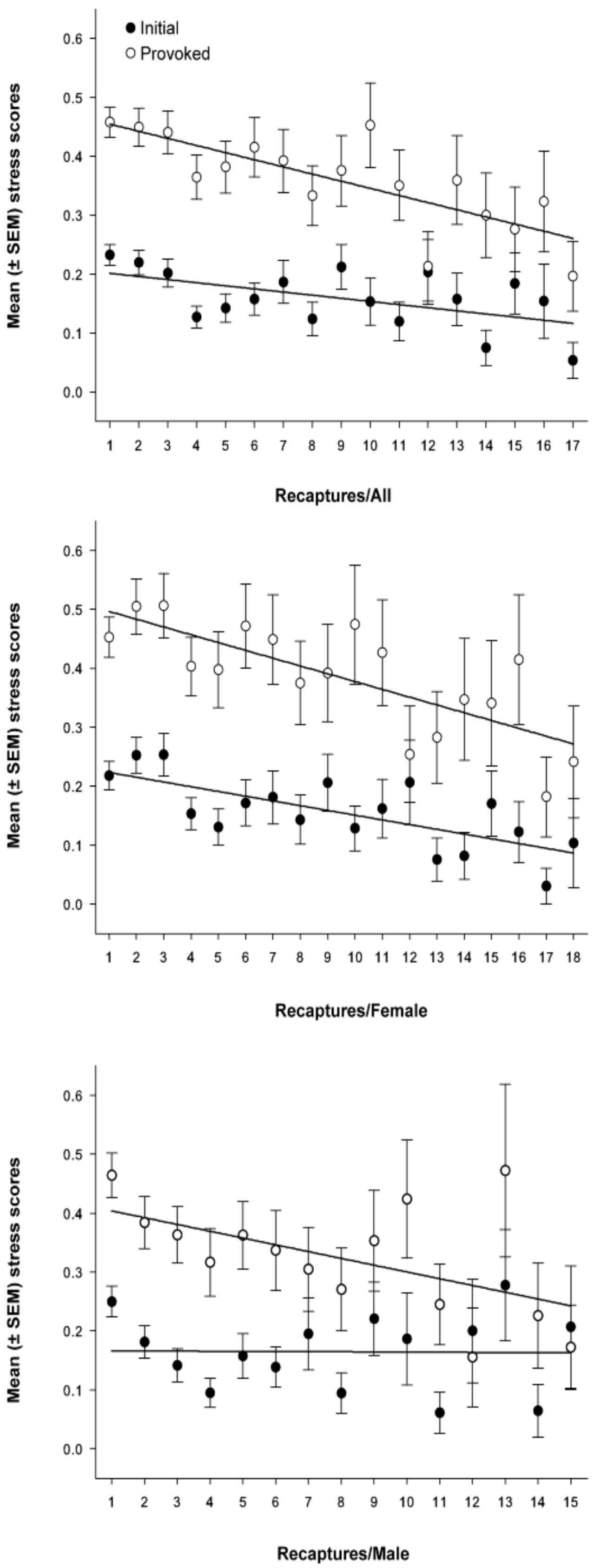
adult = 0.153 [± 0.007]; Initial: cub = 0.304 [± 0.020]; Provoked: adult = 0.362 [± 0.012]; Provoked: cub = 0.558 [± 0.028]). This effect of age-class was significant for initial responses (Likelihood ratio test/LR = 46.745; $P < 0.001$), while provoked responses differed significantly with both age-class (LR = 22.263; $P < 0.001$) and with sex (LR = 4.790; $P = 0.029$); males (Provoked: male = 0.362 [± 0.016]) scored significantly lower than females (Provoked: female = 0.429 [± 0.016]). No significant interactive effect of age and sex on badger initial or provoked behaviour was apparent (Table 2).

From our CLMMs, as would be expected, extrinsic variables foremost affected initial responses, where provoked responses were most strongly influenced by the initial response of the individual (Table 3). Badgers exhibited significantly higher initial response scores in spring and summer than autumn or winter (LR = 11.187; $P = 0.011$; Table 2). Weather effects were nested within this season effect, such that examining the influence of temperature and rainfall as explanatory variables separately with general linear models (using the 'CLMM2' function fitted 'ordinal' package in R) indicated that higher initial response scores were associated with warmer conditions (Full dataset: $P = 0.0061$), but not with rainfall ($P = 0.239$); restricting these data to initial response scores of 1 and 2 only (n = 2,165) gave $P = 0.079$ for temperature and $P = 0.078$ for rainfall. The independent contributions these weather variables made, however, were not influential in the context of overall model significance, as shown by likelihood ratio tests to compare the global model with and without these specific explanatory variables (using 'ANOVA' function fitted in the 'ordinal' package).

After standardising parasite counts, CLMMs indicated that badgers with more lice exhibited higher initial scores (positive coefficient estimate = 0.1068 and $P = 0.029$; Table 3). By contrast, infestation with harvest mites was associated significantly with initial response 0 (still) (Estimate = –1.8115 and $P = 0.027$), although these CLMM model simulations for harvest mites were ill-defined, with a high Hession value (> 10,000). We observed no interactive effect of flea and tick numbers on initial or provoked responses.

Neither the climatic variables, nor parasite type or burden affected badger-provoked responses significantly ($P > 0.05$; Table 3).

Figure 2



General linear regression analyses showing the mean (± SEM) number of recaptures for relationships between initial/provoked response score.

Linked initial and provoked responses and individual behavioural typologies

Provoked scores, although on the mean (± SEM) was low (0.400 [± 0.011]), were significantly higher than initial scores (0.183 [± 0.007]; LR = 608.152; $P < 0.001$; Table 2, Figure 1). These responses were inter-related per individual; badgers exhibited provoked responses (coefficients) in line with their initial response category (Table 3). Where initial scores were 0 there was a high probability that the provoked response of that individual would also be 0 (female cub = 0.74; male cub = 0.78; female adult = 0.82; male adult = 0.85); in turn, initial scores of 3 were significantly associated with a high probability of provoked scores of 3, per individual response (female cub = 0.43; male cub = 0.38; female adult = 0.33; male adult = 0.28; Figure 1).

In terms of individual pre-disposition to an aggressive reaction (score 3), given that high provoked scores were clearly related to high initial scores, we investigated whether specific adults exhibited consistent tendencies to exhibit initial hostility. Only eleven individuals (13 records of 4,288) registered an initial response score of 3. There was, however, no repeated pattern of scoring upon recapture (Table 4), where the more often these individuals were caught the more diluted the extreme response became within an individual's record. The same lack of any consistent pattern in response typology was also evident when examining initial score 2 (agitated).

The effect of first exposure to restraint and handling on subsequent behaviour

Age when first caught influenced behavioural response scores. Limiting analysis to those response scores measured for each individual once they had reached adulthood (that is, excluding cub scores per individual, because implicitly those individuals first caught as adults had no cub scores for comparison), adult initial response scores for individuals first caught as adults (category 1 = 0.136 [± 0.009]) were, on average, around 29% lower (Estimate = 0.330; $P = 0.017$) than for subsequent adult scores for badgers first caught as cubs (Category 3 = 0.174 [± 0.011]; Table 3). While this shows that being a cub for their first experience of trapping had a real effect in sensitising badgers to restraint, this is within the context that, even for badgers first caught as cubs, mean scores were < 6% of a maximal value (3), and so this sensitisation is likely of limited biological significance.

The effect of cumulative experience of restraint and handling over an individual's trapping history

From the complete data set (n = 4,288) the maximum number of times any individual was caught was 32 (over nine years) where, obviously, the potential to be recaptured was a function of the age to which each individual survived. The complete data set (n = 4,288) yielded 32 potential recapture-history groups, where 856 individuals yielded just a single capture data-point (Group 1), 601 individuals yielded a further second data-point (Group 2), and so forth, until a single individual yielding 32 recaptures.

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We limited our analyses to recapture history groups that included at least 50 records for the whole dataset and 25 records per sex class, because some groups in the spectrum had few, or no, representatives. This effectively restricted the maximum recapture-history group to 17 recaptures; a restricted maximum of 15 times for males and 18 times for females. We then calculated the mean and standard error of each group's initial and provoked response scores for further analyses (Figure 2).

Linear regression models indicated that behavioural responses decreased with increasing experience of recapture (General linear model: Initial score = 0.2068–0.0053 × recaptures, $F_{1,15}$ = 6.155, P = 0.025, R^2 = 0.291; Provoked score = 0.4667–0.0121 × recaptures, $F_{1,15}$ = 23.26, P = 0.002, R^2 = 0.608; Figure 2).

We repeated this approach, separating the sexes, and found that for males, recapture history had no effect on initial scores (General linear model: Male initial score = 0.1667–0.0002 × recaptures, $F_{1,13}$ = 0.003, P = 0.953, R^2 = 0); but experience did reduce their provoked responses significantly (General linear model: Male provoked score = 0.4153–0.0115 × recaptures, $F_{1,13}$ = 5.196, P = 0.040, R^2 = 0.286). For females, both initial and provoked scores decreased significantly with greater experience of the protocol (Female initial score = 0.2312–0.0080 × recaptures, $F_{1,16}$ = 16.37, P < 0.001, R^2 = 0.506; Female provoked score = 0.5098–0.0132 × recaptures, $F_{1,16}$ = 21.09, P < 0.005, R^2 = 0.567; Figure 2).

Discussion

With regard to behavioural responses to cage restraint and pre-sedation procedures, we found that more than three-quarters of all badgers were still (initial score 0) when our handling and sedation protocol commenced and the substantial majority remained still (provoked 0) throughout the injection procedure. Consequently, across all records, we assigned a mean initial score of just 0.183 (cub 0.304; adult 0.153) and a provoked score of 0.40 (cub 0.558; adult 0.362) from potential maximal behavioural response scores of 3. Our results exemplify that if the initial behavioural response score is low, there is a high likelihood of achieving a low provoked-response score. In practical terms this meant that when holding cages were first partially uncovered from beneath blankets covering the target badger, it would actually be asleep (not in a stationary but tremulous state, which would be indicative of fear), and the needle could be inserted without the badger reacting. We found no evidence of any consistent individual behavioural typologies prone to exhibit heightened agitation or aggression when injected, other than the population sub-class effects reported. That is, there was no such thing as a consistently 'difficult' badger. No specific 'trigger' for an escalating aggressive response was apparent to us.

That badgers typically remain still for injection if treated calmly and gently makes them relatively easy wild animals to work with. This also reflects the authors' experience when working on other badger populations, and when

Table 4 Frequency of observing various provoked scores on subsequent recaptures for the eleven badgers that had an initial score of 3 (distressed) on first capture.

Number	Initial				Total recaptures
	0	1	2	3	
1	18	3	5	1	27
2	22	1	0	1	24
3	8	1	1	1	11
4	14	2	1	2	19
5	6	3	0	1	10
6	11	1	0	1	13
7	3	5	2	2	12
8	11	5	0	1	17
9	12	5	3	1	21
10	10	2	0	1	13
11	0	0	0	1	1

processing badgers at their setts, without transportation. Anecdotally, but of interest to practitioners working with badgers, or with wildlife generally, we have found that a well-organised and quiet operating environment is key to reducing initial and provoked responses. Loud and unnecessary conversation should be avoided (although this was impossible to quantify in retrospect), along with other noise from extraneous sources, such as metallic clanking, centrifuges, fans etc. Subdued lighting has also proven beneficial. We have not, however, been aware of any response to scent *per se*, other than the handling facility must smell generically strange to a badger.

Of course, our study circumstances are somewhat unusual. More typically, badgers are trapped for veterinary treatment (Cousquer 2005), relocation (Brown & Cheeseman 1996) or vaccination (Wilkinson *et al* 2004). Where badgers are to be euthanised as part of bTB control measures (eg Woodroffe *et al* 2006), they will not typically have prior experience of capture and ensuring good welfare is still ethically important. Of relevance in this regard is that we found that naïve animals responded with the most agitated or aggressive behaviour. Moreover, on the few occasions badgers exhibited maximal initial response scores (3) they almost always continued to be aggressive (3) toward the anaesthetist when provoked by injection. When individuals are first caught as adults, the tendency for a potentially fierce 15 kg+ carnivore to resist injection needs an experienced practitioner to minimise further provocation. The handling of naïve badgers therefore clearly warrants careful procedural planning and well-trained personnel. We recommend (i) re-covering an agitated badger for 10 min to see if it relaxes, and if not, (ii) having a colleague distract the badger while the anaesthetist makes a quick and confident injection.

Sixty-nine percent of individuals in our study population were first trapped as cubs (Annavi *et al* 2014), and we observed that cubs proved the most challenging age-class to work with, because they are smaller, with more space to move out of reach of the needle, and require a lower dose of sedative (where our protocol is not to sedate animals < 2 kg). Anecdotally, we found that earlier during their development cubs < 2.5 kg tend to respond to captivity by going to sleep in holding cages while awaiting sedation, and were generally compliant — perhaps reflecting a stage of development where they are unaccustomed to 'fighting back' successfully. By contrast, more mature cubs (> 3.5 kg) are often agitated and resistant to injection, where a cub of this size may be able to rebut a fully grown adult (Macdonald *et al* 2002), and thus attempts to fight back against the anaesthetist.

A number of studies report that handling in early life (eg Boissy & Bouissou 1988; Pedersen 1993) can reduce an animal's subsequent behavioural reaction to new situations, rendering it apparently more able to adapt to, or accept, capture events (Pedersen & Jeppesen 1990; Meerlo *et al* 1999). We observed some evidence that juvenile experience of the protocol led to lower adult behavioural response scores, but the small difference in mean response scores that we observed is probably of limited biological significance. Of more relevance, we found that with greater experience of the capture and sampling protocol, individual badgers steadily exhibited lower mean initial and provoked response scores. This amelioration in the extent to which badgers reacted to the protocol with cumulative experience applied to the provoked responses of both sexes, but to initial responses only in females; perhaps reflecting aggressive tendencies that may arise from maternal instinct to be fundamentally defensive, even without explicit provocation.

In addition to sex, age and experiential effects, a range of extrinsic (eg season, weather conditions, parasitic infestations) variables are known to affect badger activity patterns (Noonan *et al* 2014), and so may influence experience of trap restraint preferentially or detrimentally. In our analysis, season was influential on behavioural responses to restraint and sedation (with temperature and rainfall effects nested therein), with higher scores associated with warmer, drier spring and summer conditions. Initial scores were exacerbated by lice infestation, leading us to speculate that conditions likely to cause itching (warmth × lice) while in the trap, prior to morning collection, might lead to badgers being more irritable when processed subsequently. While fleas may cause similar irritation, these can hop off, or be groomed off by captive badgers awaiting sedation (Cox *et al* 1999), potentially explaining the lack of any apparent effect. Harvest mites are highly seasonal, being prevalent only during the autumn when mean stress scores were lower. Based on this effect of season, our recommendation for minimising effects of parasites on agitated and aggressive behaviours during pre-sedation restraint would be to trap in autumn.

Animal welfare implications

Bekoff (2002; p 23) argued that scientists should approach research with the basic principles used in everyday life: "Do no intentional harm, respect all life, treat all individuals with compassion, and step lightly into the lives of other beings, bodies of water, air, and landscapes". To these ends the need to be attentive to animal welfare is widely recognised in the guidelines and codes of ethics published by professional animal research societies and by national laws and standards that stipulate the need to monitor the behaviour of captive animals and adapt care as appropriate (see details in the *Introduction*). Although clinical stress is defined as the physiological disruption to homeostasis (eg Romero *et al* 2009) resulting in elevated corticosteroids, adrenalin or leukocyte activity (eg Miller & O'Callaghan 2002), it would prove not only impractical and expensive to make these types of measures for routine monitoring, but also likely to add to the burden of stress placed upon the animal. This andrenocorticotropic cascade is triggered, however, by psychological stress manifest in behavioural patterns (Lazarus 1966). Consequently, it is important to be able to recognise simple behavioural signs that the subject animal is appraising its situation as challenging (Dawkins 2004).

These types of behavioural criteria are well established for monitoring 'pain, suffering, distress and lasting harm' (to use the terminology of the ASPA 1986) among laboratory animals (eg Wallace *et al* 1990; Manser 1992), for farm animals (Duncan & Dawkins 1983), and even zoo animals (Wielebnowski 2003), and yet there is scant literature applying this approach to the temporary restraint of wild animals (but see Clubb & Mason 2003). Not least, all unhabituated wild animals will find restraint and the proximity of humans challenging, and so the question becomes one of degrees — to recognise how disturbed the animal is, and, vitally, how best to deal with the animal to avoid causing further distress. Not least, ideographic differences in traits associated with an animal's trappability may also be heritable, affecting an individual's life history and fitness (Poissant *et al* 2013), and therefore of evolutionary significance.

The behavioural monitoring criteria for the European badger we present here offer a wide range of researchers and practioners involved in the handling and sedation of this much-trapped species a method with which to recognise the likelihood that an individual will become increasingly more agitated or aggressive during the procedure, so that approaches can be modified accordingly. Crucial information for those involved in research and vaccination of populations where interventions have not taken place previously is that we identify naïve badgers as typically presenting the biggest challenge to the handler, and that behavioural responses decreased with increasing experience of recapture. We emphasise that the thorough training of personnel is vital to ensure the highest possible standards of animal welfare.

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