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² Tick hazard in the South Downs National Park (UK):

species, distribution, key locations for future interventions, site density, habitats

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22 Abstract [500/500]

Background. The South Downs National Park (SDNP) is the UK's most visited National Park, and a foci of tick-borne Lyme disease. A range of human pathogens have been detected in UK ticks and related hosts, and the first presumed autochthonous cases of tick-borne encephalitis and babesiosis were recorded in 2019–20. SDNP's key objectives include conserving wildlife and encouraging enjoyment of the countryside, so interventions are needed that reduce hazard without negatively affecting ecosystem health. To be successful these require knowledge of site hazards, and we aimed to provide this to enable action.

Methods. British Deer Society volunteers submitted ticks removed from deer. Key potential intervention sites were selected and ticks collected by drag-sampling six 50 m² transects per site, in most cases twice yearly for two years. Ticks were identified in-lab (sex, life stage, species), hazard measured as tick presence, Density of Ticks (all life stages, DOT), and Density of Nymphs (DON). Sites and habitat types were analysed for association with hazard. Distribution across SDNP was mapped in a Geographic Information System (GIS), by combining and comparing our fieldwork results with records from five other data sources (recent and historic).

37 **Results.** 87 *Ixodes ricinus* (all but one adults, 82%F) were removed from 14 deer (*Dama dama*

n=10; *Capreolus capreolus* n=3; 1 not recorded; tick burden, 1–35) at 12 locations (commonly

39 woodland). Five potential key intervention sites were identified and drag-sampled 2015–16,

40 collecting 623 ticks (238 on-transects): 53.8% nymphs, 42.5% larvae, 3.7% adults (13M, 10F).

41 Ticks were present on-transects at all sites drag-sampled (*I.ricinus* at three, *Haemaphysalis*

42 *punctata* at two). The Mens (TM, the quietist site for human visitors) had the highest DOT at

43 $30/300 \text{ m}^2$ (DON= $30/300 \text{ m}^2$), followed by Queen Elizabeth Country Park (QECP, the busiest) at

44 $22/300 \text{ m}^2 (12/300 \text{ m}^2)$, Cowdray Estate (CE) at $8/300 \text{ m}^2 (6/300 \text{ m}^2)$, and Seven Sisters Country 23 March 2021 – j.middleton@bsms.ac.uk Page 2 of 47

45	Park (SSCP) at 1/300 m ² (1/300 m ²). Ditchling Beacon Nature Reserve (DBNR) was sampled
46	2016 only (one adult H.punctata collected). Woodland had significantly higher hazard than
47	grazed downland, but ticks were present at all downland sites drag-sampled. GIS mapping
48	showed <i>I.ricinus</i> identified in 33/37 of SDNPs 10 km ² grid squares, <i>Ixodes hexagonus</i> 10/37,
49	H.punctata 7/37, Dermacentor reticulatus 1/37.

50 **Conclusions.** Mapping shows tick hazard is broadly distributed across SDNP. *Ixodes ricinus* was 51 most common, though the seeming range expansion of *H.punctata* is concerning, particularly as 52 it seems to thrive better on grazed downland than *I.ricinus*. Site specific recommendations 53 include: management of small high hazard plots with heavy visitor numbers (QECP); signage on 54 post-visit precautions (all sites); repellent impregnated clothing for deerstalkers (CE); flock trials 55 to control *H.punctata* (SSCP, DBNR). Further research at TM, which has high tick density, may 56 contribute to knowledge on ecological dynamics underlying infection density, and the potential 57 use of predator re-introduction/protection as a public health intervention. Ecological research on 58 *H.punctata* would aid control. The SDNP Authority is ideally placed to link and champion site-59 based and regional policies to reduce hazard, whilst avoiding or reducing conflict between public 60 health and ecosystem health.

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61 INTRODUCTION

The South Downs National Park (SDNP) covers 1,627 km² of the south-east of the British Isles, 62 across Hampshire, West Sussex, and East Sussex. It encompasses two bioregions, the 140 km 63 64 long chalk ridge of the South Downs, and the wooded lowland Weald. Though much of the Park 65 is subject to industrial agriculture, substantive fragments of rare and species rich semi-natural chalk grassland can be found on its windy hills, whilst some of its woodlands are truly ancient 66 67 (>1000 y) and harbor biodiverse ecological communities (Crane & Williams, 2013). It is the most 68 visited national park in the UK, with an estimated 39 million visitor days per year (NPUK, 2014), 69 c.120,000 people live and/or work within its borders, two million live within 5 km (SDNPA, 2020). A substantial part of the national park is private land with limited or no public access 70 71 (Bangs, 2008). However, the area is crisscrossed by 3218 km of public rights-of-way (TTC, 72 2018), and there is sizable local authority owned country parks and myriad nature reserves. Some 73 stretches of downland are legally classed as 'Access Land' (SDS, 2021) and some landowners 74 also allow permissive paths. The South Downs Way is one of 15 UK national trails and is very 75 popular with walkers, cyclists, and horse-riders. Over one year 61,191 people were counted passing one point of the trail (ESCC, 2016), locations closer to carparks can be far busier still 76 77 (HCC, 2020).

Ticks (Ixodida) are second only to mosquitoes globally as vectors of human pathogens (Lawrie *et al.*, 2004). Twenty species of tick are native to Great Britain (Jameson & Medlock, 2011), 26 to northwestern Europe as a whole (Hillyard, 1996). Most are relatively host specific and primarily nidicolous (i.e. living in or near shelters used by their hosts), and therefore of minimal risk to humans (Gray, Estrada-Pena & Vial, 2014). (Throughout this article we use 'tick hazard' to refer to tick species that parasitise humans, and 'tick risk' as tick hazard x chance of human exposure).

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84	In contrast to nidicolous species, some ticks feed on diverse host communities, climbing
85	undergrowth or litter and attaching to passing potential vertebrate hosts, including humans. In
86	three regions in England and Wales, patients consulted General Practitioners about tick-bites at a
87	rate of 54–204 per 100 000 inhabitants in 2011, 72.5% of respondents in Cumbria had removed
88	ticks from patients 2011–13 (101/100 000 population) (Gillingham et al., 2020). This is only a
89	partial glimpse of the full extent of bites; an estimated $\frac{1}{3}-\frac{2}{3}$ of those fed upon remain unaware
90	(Hofhuis et al., 2015), particularly if bitten by smaller instars, and even if noticed many don't
91	seek medical advice. For example, a 2007 population survey in the Netherlands found a tick bite
92	incidence of 7198/100 000, $c.1.1$ million bites were reported. This equates to approximately
93	fifteen times the number of tick-bite related general practice consultations (Hofhuis et al., 2015).
94	Lyme disease is the primary human tick-borne disease of concern in the UK. Cairns et al. (2019)
95	used general practice data to estimate a 1-year Lyme disease incidence of 12/100 000 (cautious
96	interpretation is warranted, 59% of these clinical diagnoses lacked documented laboratory
97	confirmation). The causative pathogen of Lyme disease, Borrelia burgdorferi s.l., was only
98	identified in 1983 (Sood, O'Connell & Weber, 2011), and over the last decade other human
99	pathogens have been detected in ticks and related hosts in the British Isles, including: spotted
100	fever group rickettsia (Tijsse-Klasen et al., 2011; 2013), Borrelia miyamotoi (Hansford et al.,
101	2015), tick-borne encephalitis virus (Holding, Dowall, Hewson, 2020), and Babesia venatorum
102	(Gray et al., 2019; Weir et al., 2020). In 2019–20 the first presumed autochthonous human cases
103	of tick-borne encephalitis and babesiosis were recorded in the UK (PHE, 2020a). Some of these
104	recently detected health threats may result from emerging foci of imported pathogens. However,
105	it is also possible that in addition to Lyme disease, there may be considerable levels of
106	undiagnosed tick-borne infections affecting persons in the British Isles.

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107	Public Health England have mapped UK tick distributions at 10 km ² resolution by combining
108	historical records (Pietzsch et al., 2005) with samples sent by the public, who in most cases found
109	them attached to themselves or their pets (Jameson & Medlock, 2011). It should be noted that UK
110	general practice records of arthropod bites do not identify by species (Newitt et al., 2016).
111	Hospital Episode Statistics (HES) have been used to map Lyme disease distribution across
112	England (Cooper et al., 2017). However, HES uses residential postcodes of patients, not where
113	they were bitten. Thus, whilst HES is valuable to understanding disease burden given that UK
114	tick-borne infections are very often linked to recreational exposure (Dobson, Taylor & Randolph,
115	2011), the use of this to map differing geographic tick hazard is limited. This is especially true in
116	places such as the SDNP, with high numbers of regional, national, and international visitors.
117	Knowledge on tick density, the most reliable metric of site tick hazard (Ostfeld, 2011), is
118	therefore restricted to the relatively small number of places in Britain actively field-sampled.
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130	researchers (Randolph & Dobson, 2012). Sheep grazing supports vector populations in some UK
131	grass uplands, and though not host competent for Borrelia burgdorferi s.l., sheep can support
132	transmission cycles via tick co-feeding (Ogden, Nuttall & Randolph, 1997), and also host
133	Babesia venatorum (Gray et al., 2019). However, compared to wildlife, the role of livestock in
134	propagation of tick-borne diseases of human concern is under-researched (Stanek et al., 2012).
135	Increased wildlife populations have been implicated elsewhere in rising incidence of tick-borne
136	disease (e.g. Crimean-Congo hemorrhagic fever in Turkey (Randolph, 2009a); tick-borne
137	encephalitis in East Europe (Randolph, 2009b)) setting up a potential conflict between
138	biodiversity and human health. Given UK National Parks aim to enhance wildlife and encourage
139	public enjoyment of the countryside (NPUK, 2017), such conflict would be problematic for the
140	South Downs National Park Authority (SDNPA) and the local governments from which most of
141	its members are drawn. However, its joint remit, bioregional framing, and coalition of
142	stakeholder members makes it the ideal body to link and champion site-based and regional
143	policies to reduce hazard, whilst avoiding or reducing conflict between public health and
144	ecosystem health.

145 Aims

146 Our overall project (*Tick-borne hazards in the SDNP and the potential for Planetary Health*

based interventions) includes (1) mapping and fieldwork to better understand tick hazard across
the SDNP, including crucially at key potential locations for future interventions, (2) a systematic
review of proposed interventions to reduce site hazard of the most common tick-borne disease in
Britain, Lyme disease, with a focus on those actions not expected to negatively affect ecosystem
health. Here we report on our mapping and fieldwork, information on our systematic review can
be found in Middleton, Cooper & Rott (2016).

153 Study objectives:

- identify and describe potential key locations for future interventions;
- map distribution of tick hazard across the SDNP;
- determine tick hazard (species and density) at potential intervention sites; and
- analyse habitat associations with tick hazard in the SDNP.

158 MATERIALS AND METHODS

159 Sites selection for drag-sampling and potential future interventions

160 Five sites were selected: three prospectively, and two responsively after submission of ticks 161 obtained by deerstalkers from sentinel deer. The three prospectively chosen sites were located 162 one in each of the SDNP's three counties. We took this approach so as to sample from along the 163 National Park's length, and because one of our project's primary audiences is county authorities 164 which manage countryside sites within the SDNP with high numbers of recreational visitors (e.g. 165 UK accredited country parks as defined by NE & DEFRA (2014)). These authorities are key to 166 implementing potential interventions to reduce tick-borne disease risks in the SDNP as they elect 167 governing members to SDNPA (responsible for strategic action across SDNP), and directly 168 manage downland and woodland sites with high visitor numbers where interventions could be 169 trialed. Of the three counties within SDNP's borders, two county councils manage such sites in 170 the National Park: Hampshire County Council (SDNP's western section), and East Sussex 171 County Council (SDNP's eastern section). West Sussex County Council (SDNP's central section) 172 does not perform this function within the National Park. The SDNP's ranger service was 173 consulted about which Hampshire County Council and East Sussex County Council sites had the 174 highest visitor numbers (subsequently confirmed by councils themselves). Given West Sussex 175 County Council do not manage an appropriate site for sampling, a third site was chosen at

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SDNP's center which represented a sizeable wealden woodland owned by a key Park stakeholder(Sussex Wildlife Trust).

178 Tick collection from Deer

- 179 Deerstalkers were recruited through the British Deer Society (bds.org.uk) newsletter and website,
- 180 sent kits, and asked to collect ticks from deer culled for reasons unrelated to this project.
- 181 Participants were instructed to inspect the whole animal, collect every visible tick, place them in
- 182 pre-coded 1ml cryovials (pre-filled with 0.5ml 70% ethanol) and return by post (safety measures,
- 183 Supplementary Material p. 2). On receipt cryovals were deposited in a laboratory fridge (approx.
- 184 5 °C), and after identification transferred to a freezer (approx. -20 °C). Deerstalkers recorded:
- habitat type; deer species; six-figure grid reference (using 'OS Locate' (Ordnance Survey,
- 186 London, 2014)); body sites ticks found at; and whether ticks were attached or not.

187 Tick collection by drag-sampling

188 Sites were sampled April to November inclusive. To collect questing ticks, sampling was not 189 carried out when air temperature was <7 °C 50 cm above the ground or when vegetation was wet 190 from recent rain/dew, as per James et al. (2013). Four sites were sampled in both 2015 and 2016, 191 with an additional site sampled in 2016. At each, six 1m x 50m transects were sampled as per 192 Dobson, Taylor & Randolph (2011). The first two transects chosen were those suspected to have 193 the highest potential exposure of humans to ticks (e.g. vegetation alongside a footpath). Where 194 sites included grassland and woodland, one chosen transect was selected from each. All others 195 were selected using dice and a random number table. To reduce spurious conclusions from single 196 sampling, each transect was planned to be sampled twice yearly, for two years. However, at one 197 private site used for game shooting, it was not possible to visit twice in year-2 due to a 198 requirement to be accompanied by a deerstalker with restricted availability. To improve chances

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199	of picking up disease signals in planned follow-up research (usually only a minority of ticks at
200	any site are infected (Vollmer et al., 2011)), extras were acquired by drag-sampling between
201	transects, and at follow up visits where possible. Tick sampling techniques differ in efficacy and
202	are affected by habitat/vegetation type (Dantas-Torres et al., 2013). To reduce bias ticks were
203	collected simultaneously along transects using woollen blanket, flags, and chaps (Fig. 1). Wools
204	were examined after each transect, ticks placed individually in 70% ethanol filled micro-
205	centrifuge tubes, deposited same day in a laboratory freezer (approx20°C).
206	At each transect, at each sampling, photos were taken along with field notes including: ticks
207	collected; date/time; weather; habitat; visitors observed; dominant vegetation; vegetation height;
208	main litter constituents; relative humidity and temperature (both at 50cm and in litter, measured
209	with a Fisher Scientific Traceable Hygrometer). Locations were recorded (10-figure OS grid
210	references, bearings) using 'OS Locate' and 'OS Mapfinder' (Ordnance Survey, London, 2014)
211	on a Samsung Galaxy Note II phone. (Researcher safety and inter-site contamination control,
212	Supplementary Material p. 2.)

213 Tick identification

214 Identification was conducted in-lab with a hand lens (Hilkinson Ruper x20 15mm achromatic), 215 and where necessary a dissecting microscope (Leica EZ4). A species key was used (Hillyard, 216 1996), and identification aided by reference to Baker (1999) and Beati, Needham & Klompen 217 (2016). Species and life stage was recorded, adult ticks sexed. Larvae were not fully keyed as 218 clearing for slide mounting would have reduced the sample pool available for future pathogen 219 detection. However, each larva was inspected for characters which identified them to genus, and 220 indicated likely species. If nymphs/adults of more than one species were identified at any site, 221 10% of larvae from that site (to a maximum of 50) would have been slide mounted and keyed. A 23 March 2021 - j.middleton@bsms.ac.uk Page 10 of 47

222	limitation of many similar studies has been not enabling retrospective evaluation of species
223	identification (Estrada-Pena et al., 2013). To corroborate identification, voucher specimens
224	(including all life stages/sexes) are stored at approx20 °C at the University of Brighton, to be
225	deposited into the Natural History Museum acarology collection on publication.

226 Mapping distribution of tick hazard

227 Using ArcMap 10.7 (ESRI, Redlands USA) sites where ticks had been submitted by deerstalkers or drag-sampled by JM were mapped, indicated by points at 100 m² resolution. In addition, to 228 map recorded presence of tick hazard at 10 km² resolution by species, data from the following 229 230 sources were compared and combined as layers: (1) the most recent published Public Health 231 England/Health Protection Agency tick maps for England and Wales (Cull et al., 2018; PHE, 232 2016; HPA, 2013a, b, c, d), (2) National Biodiversity Network Atlas (NBN, 2020) (which 233 includes historic data 1890 onwards, and into which Public Health England now submits tick 234 records (PHE, 2020b)), (3) a single site drag-sample in 2014 by Layzell et al. (2018), (4) point 235 locations of ticks submitted from culled deer or collected by drag-sampling in this project 2015-236 16, (5) point locations drag-sampled for Haemaphysalis punctata by Public Health England and 237 the Animal and Plant Health Agency, primarily 2015-18 (Medlock et al., 2018), (6) records from 238 pan-species surveying at Sussex Wildlife Trust reserves, mainly 2016-17 (previously unpublished 239 data). Digital basemaps were obtained from OS OpenData (2020) and Natural England (2020). 240 (Layer generation detailed in Supplementary Material, p. 3.)

241 Analysis

As well as site vector presence/absence, for sites drag-sampled both years tick hazard was

assessed as (1) questing Density of Ticks, all life stages (DOT), and (2) questing Density of

Nymphs (DON). These were calculated as means of totals of four site samplings: six 1m x 50m
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245	transects, sampled twice yearly for two years. To determine significance of difference of tick (all
246	life stages) and nymph counts between the four sites sampled in both years, Kruskal-Wallis Tests
247	with follow-on Dunn's Tests were carried out on counts from all individual transect samplings
248	(i.e. 90 50m x 1m drag-samplings). A further Kruskal-Wallis Test with follow-on Dunn's Test
249	examined habitat types determining tick hazard. Statistical analysis was carried out in Mintab17
250	(Minitab Inc, State College, Pennsylvania). Drag-sampling results from the additional site
251	sampled in 2016 are reported as presence/absence, and in distribution mapping, but were not
252	included otherwise in analysis. (Justification of analysis, Supplementary Material p.3.)
253	Data availability
254	All relevant data are included in this article or Supplementary Material (machine readable data
255	deposited at https://sussex.figshare.com/bsms). On publication all novel tick records will be
256	uploaded to NBN Atlas.
257	
258	RESULTS

Figure 2 maps the locations where ticks were submitted from by deerstalkers, and the five
potential future intervention sites drag-sampled across the SDNP by the first author. The tick
species collected at each site and the locations of nearby towns are also given.

262 Sites selected for drag-sampling and potential future interventions

- 263 *Prospectively selected*
- East Sussex County Council's site with the highest annual visits (est. 350,000 (ESCC & SDCB,
- 265 2004)), was the 280 ha Seven Sisters Country Park (sevensisters.org.uk) in the SDNP's eastern
- section. Its visitors centre had 52,124 visitors Jan–Dec 2019 (ESCC, 2020), and only a minority

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267	of trips to Seven Sisters Country Park are expected to include a visit to the centre. Unlike the
268	other sites it attracts a large number of international tourists, its white cliffs having featured in
269	major films and as a default Microsoft Windows wallpaper (BBC, 2017; Tsang, 2018; Baddeley,
270	2020). Seven Sisters Country Park is easily reachable by day-visitors from Eastbourne (8 km
271	away; est. 2019 pop. 114,809 (ONS, 2020)) and Brighton and Hove (24 km away; est. 2019 pop.
272	244,917 (ONS, 2020)). It is composed of chalk grassland, saltmarsh, shingle seashore, woodland,
273	and a meandering river. Conservation designations include Site of Special Scientific Interest
274	(SSSI), Area of Outstanding Natural Beauty (AONB), Heritage Coast, and Marine Conservation
275	Area. Transects (Fig. 3A–F) consisted of sheep grazed chalk downland and woodland, primarily
276	beech (Fagus sylvatica) and sycamore (Acer pseudoplatanus) (Supplementary Material Table
277	S1). Hampshire County Council's site with the highest annual visits (est. 327,000 (Speller et al.,
278	2010)) was the 564 ha Queen Elizabeth Country Park
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278 279 280 281 282	2010)) was the 564 ha Queen Elizabeth Country Park (hants.gov.uk/thingstodo/countryparks/qecp) in the SDNP's western section. In March 2019– April 20 Queen Elizabeth Country Park's number plate recognition system recorded 202,559 vehicle entries (HCC, 2020). It is 19 km from Portsmouth (est. 2019 pop. 229,851 (ONS, 2020)), popular with walkers, mountain bikers and picnickers, and hosts outdoor events such as
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278 279 280 281 282 283 284 285	2010)) was the 564 ha Queen Elizabeth Country Park (hants.gov.uk/thingstodo/countryparks/qecp) in the SDNP's western section. In March 2019– April 20 Queen Elizabeth Country Park's number plate recognition system recorded 202,559 vehicle entries (HCC, 2020). It is 19 km from Portsmouth (est. 2019 pop. 229,851 (ONS, 2020)), popular with walkers, mountain bikers and picnickers, and hosts outdoor events such as marathons. It consists of downland and wooded hills, designations include: SSSI, National Nature Reserve, Special Area for Conservation, Scheduled Ancient Monuments. All transects (<i>Fig. 3G–L</i>) were in woodland (beech, conifer, or hazel (<i>Corylus avellana</i>)). Some had sparse

The third site selected prospectively was woodland at The Mens in West Sussex, a 166 ha naturereserve owned by Sussex Wildlife Trust in the central section of the SDNP

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290	(sussexwildlifetrust.org.uk/visit/the-mens). There are less major conurbations close to this section
291	of the SDNP compared to its eastern and western parts. The nearest mid-sized town is Horsham
292	(18 km away; 2011 pop. 49,000 (Horsham District Council, 2016)). The Mens has a small
293	carpark and a network of paths, but is otherwise largely unmanaged wealden forest with
294	relatively few visitors. The site is especially rich in plants, saproxylic invertebrates, and fungi
295	(c.600 species). The sampled transects (<i>Fig.</i> $4A-F$) followed footpath borders tufted with grass,
296	and cut across ground with sparse undergrowth under high canopies of predominantly beech, and
297	sections with dense waist-high brambles (Supplementary Material, Table S3).
298	Responsively selected
299	The 6677 ha Cowdray Estate (cowdray.co.uk) is owned by Viscount Cowdray in the SDNP's
300	central section in West Sussex, 9 km from Chichester (2011 pop. 26,795 (ONS, 2015)). It is a
301	large private landholding with commercial deerstalking and mostly consists of forestry,
302	downland, arable, and dairy/livestock farming. Cowdray Estate has visitor attractions (golf
303	course, holiday cottages, conference/wedding venue, farm shop and café), and is crossed by well-
304	used public paths. Transects (Fig. $4G-L$) sampled conifer plantation and sheep-grazed downland
305	(Supplementary Material, Table S4). The final site (sampled 2016 only) was the 24 ha Ditching
306	Beacon Nature Reserve in East Sussex (sussexwildlifetrust.org.uk/visit/ditchling-beacon),
307	managed by Sussex Wildlife Trust in the SDNP's eastern section. Ditching Beacon Nature
308	Reserve is 4 km from Brighton and consists of downland plateau and steep scarp slopes of chalk
309	grassland and woods. The plateau is next to a busy National Trust carpark and is popular with
310	walkers, mountain bikers, and picnickers. Parts of Ditching Beacon Nature Reserve are under
311	conservation grazing with sheep/cattle. The escarpment is an SSSI harboring flower rich chalk
312	grassland, rare orchids, and butterflies. Transects ran through grazed downland, some bordering

- 313 hawthorn (*Crataegus monogyna*) and ash (*Fraxinus excelsior*) scrub, and along verges of
- 314 footpaths leading from carparks (Supplementary Material, *Table S5*).
- 315 Extent of tick hazard across the SDNP
- 316 Distribution and species

317 Ticks collected by drag-sampling or submitted by deerstalkers confirmed presence across much 318 of SDNP (Fig. 2). Ticks were present in both of its characteristic habitats: sheep grazed 319 downland, and the wealden woods. Ticks were found at all sites drag-sampled (though not on all 320 transects, Figs. 3 and 4; Supplementary Material, Tables S1–4). All ticks submitted from deer 321 were Ixodes ricinus (Fig. 5A), also the only species collected at three of the four sites drag-322 sampled in both 2015 and 2016 (Queen Elizabeth Country Park, The Mens, Cowdray Estate). 323 The nationally rare *H.punctata* (*Fig. 5B*) was the sole tick collected from the remaining site 324 sampled in both years (Seven Sisters Country Park), and was also found at Ditchling Beacon 325 Nature Reserve in 2016.

Figure 6 maps distribution of tick records at 10 km² resolution. The first report for *I.ricinus* is 326 327 from 1964, 33/37 of the Parks grid squares have had at least one record (often multiple) in the 328 last 15 years (Fig. 6A). In contrast, Ixodes hexagonus has been recorded far less, most squares 329 where presence has been recorded represent historic records only (*Fig. 6B*). The earliest 330 *H.punctata* report is from 1920, but all related grid squares have had recorded presence in the last 331 decade, mostly in its known foci in the far east of the Park (Fig. 6C). Locations included from 332 recent drag-sampling by Public Health England and Animal and Plant Health Agency suggests it 333 has spread westwards somewhat, and this observation by Medlock et al. (2018) is confirmed by 334 drag-sampling in our study at Ditchling Beacon Nature Reserve which extends its known range 335 further still, as does a previously unpublished isolated recording by Sussex Wildlife Trust 44 km

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337	Wildlife Trust at one of its West Sussex reserves in 2004 (Fig. 6D). To our knowledge
338	D. reticulatus has not otherwise been recorded in the SDNP or its constituent counties; this record
339	was not previously included in National Biodiversity Network Atlas or Public Health
340	England/Health Protection Agency published mapping. There are a few records of Ixodes
341	frontalis and Ixodes trianguliceps. These have not been mapped as they are highly host type
342	specific and not routinely hazardous for humans (Mysterud et al., 2015; Drehmann et al., 2019).
343	Ticks collected from Deer
344	Eighty-seven ticks were submitted (Table 1) obtained from 14 deer at 12 locations (Fig. 2). All
345	bar one were adult ticks, and 82% were females. The majority of males were attached in mating;
346	the majority of females were engorged (73%, n=71, two not recorded by collectors), as was the
347	sole nymph. The commonest habitat ticks were collected off deer at was 'wood' (59%, n=87).
348	Most of the remaining were from deer shot at mixed edge-habitats involving wood (28%, n=87),
349	e.g. 'wood-heath'. The majority of hosts were fallow deer (Dama dama) (10, 71%), followed by
350	roe (Capreolus capreolus) (3, 21%) (n=14, one host species not recorded by collector). Tick
351	burden was 1–35 per deer. The most common attachment sites were abdomen and sternum, and
352	posterior and frontal axillae (Fig. 7).

353 Ticks collected by drag-sampling

Drag-sampling four sites in both 2015 and 2016 collected 622 ticks (*Table 2*). Of these, 237 were along transects and are included in calculations of vector densities and analysed statistically. 385 extras were stockpiled to aid future pathogen detection. Ticks were present at all four sites and the additional site sampled twice in 2016 (1 adult only). Of ticks collected by drag-sampling at all sites (n=623), most were nymphs (53.8%), followed by larvae (42.5%), and a small number of

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359	adults (3.7%, 13 males, 10 females (Supplementary Material, Tables S1-4). 93.3% (222) of ticks
360	gathered along transects (n=238) had attached to woollen blankets, 6.7% (16) to woollen chaps,
361	and none were attached to flags (Table 2). Figure 8 shows a breakdown of tick data from
362	transects by collection month, and by tick life stage (all sites for y1 and y2, n=238).
363	Tick hazard at potential sites for interventions
364	Site-by-site
365	Seven Sisters Country Park: Figures 3A-F show the number of ticks obtained at each transect, at
366	each sampling (Supplementary Material, Table S1). 78 H.punctata ticks were collected, four
367	along transects, 74 off-transect, representing all life stages, attached to both blanket (76) and
368	chaps (2) (Table 2). On-transect ticks were nymphs (2) and adults (2). All ticks were collected on
369	downland, none having been collected on the wooded transects (Supplementary Material, Table
370	S1). Tick hazard ranged 0–2 ticks per 50 m ² individual sampling, site DON and DOT were both 1
371	per 300 m ² (<i>Table 3</i>). There were hundreds of visitors observed every day. The area which

provided most off-transect ticks is in the background of the photo of transect 3 (*Fig.* 3C), hosting

a school picnic.

374 Queen Elizabeth Country Park: *Figures* 3G–L show the number of ticks obtained at each

transect, at each sampling (Supplementary Material, *Table S2*). 183 *I.ricinus* ticks were collected,

376 89 on-transect, 94 off-transect, from all life stages, attached to both blanket (175) and chaps (8)

377 (*Table 2*). On-transect ticks were larvae (37), nymphs (47), and adults (5). Ticks were found on

- 378 five of the six transects, all of which were wooded or on paths verging woodland (Supplementary
- Material, *Table S2*), range 0–25 per 50 m² sampling, DON=12 per 300 m², DOT=22 per 300 m²
- 380 (*Table 3*). A very large number of visitors were present during all site visits, and though some

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381	transects were especially busy (e.g. Fig. 2G, running from the visitor centre) people were
382	observed during sampling at all transects, even far from carparks.
383	The Mens: Figures 4A–F show the number of ticks obtained at each transect, at each sampling
384	(Supplementary Material, Table S3). 330 I.ricinus ticks were collected, 121 on-transect, 209 off-
385	transect, representing all life stages, attached to both blanket (296) and chaps (4) (Table 2). On-
386	transect ticks were larvae (35), nymphs (82) and adults (4). Ticks were obtained from all six
387	transects (Supplementary Material, <i>Table S3</i>), range 0–21 per 50 m ² sampling, DON=21 per 300
388	m ² , DOT=30 per 300 m ² (<i>Table 3</i>). Few visitors were met during sampling visits and, unlike any
389	of the other sites, sometimes none were encountered.
390	Cowdray Estate: Figures 4G-L show the number of ticks obtained at each transect, at each
391	sampling (Supplementary Material, Table S4). 31 I.ricinus ticks were collected, 23 along
392	transects, eight off-transect, from all life stages, attached to both blanket (29) and chaps (1)
393	(Table 2). On-transect ticks were nymphs (22) and adult (1). Ticks were collected on five of six
394	transects, on both woodland and downland (Supplementary Material, Table S4), range 0-7 per 50
395	m ² sampling, DON=6 per 300 m ² , DOT=8 per 300 m ² (<i>Table 3</i>). Public footpaths along transects

(Fig.4H and L) were in use by walkers during all visits. One of two downland transects on which

a tick was found ran along the South Downs Way.

Ditchling Beacon Nature Reserve: One single *H.punctata* tick was collected on-transect on semigrazed downland adjacent to scrub woodland, none on the other five transects at either set of
samplings in 2016. The adult male tick had attached to the blanket (*Table 2*). Densities were not

401 calculated due to 1-year only sampling (Supplementary Material, p. 3). Every day during

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- 402 sampling large numbers of visitors were observed crossing the high plateau, few on the lower403 scarp slope where the tick was collected.
- 404 *Comparisons between sites*
- 405 Tick hazard was detected at all four sites surveyed in both 2015 and 2016, but levels (range per
- 406 sampling, DON, DOT) differed between them, as outlined above and shown in *Table 3*. Kruskal-
- 407 Wallis Tests showed average ranks for (i) nymphs, and (ii) ticks (all life stages) differed
- 408 significantly (<0.05) for at least one of the four sites (nymphs: H=31.59 (DF 3, n=90), p=0.000
- 409 (adjusted for ties); ticks (all life stages): H=31.93 (DF 3, n=90), p=0.000 (adjusted for ties)).
- 410 Post-hoc Dunn's Tests were used to carry out pairwise site comparisons (magnitudes and
- 411 directions of differences, Supplementary Material Fig. S1). The Mens had significantly more
- 412 nymphs than Cowdray Estate (Z=2.128, p=0.0000), Seven Sisters Country Park (p=0.0005), and
- 413 Queen Elizabeth Country Park (p=0.0121). Seven Sisters Country Park had significantly less than
- 414 Queen Elizabeth Country Park (p=0.0029). For ticks (all life stages) The Mens had significantly
- 415 more ticks than Cowdray Estate (Z=2.128, p=0.0013) and Seven Sisters Country Park (p=0.000)
- 416 which in turn had significantly less ticks than Queen Elizabeth Country Park (p=0.006).
- 417 However, unlike for nymphs, The Mens did not have significantly more ticks (all life stages) than
- 418 Queen Elizabeth Country Park.

419 Habitat associations with tick hazard in the SDNP

420 Of the four sites drag-sampled in both years, sites with transects entirely in woodland (Queen

- 421 Elizabeth Country Park, The Mens) had the highest tick hazards (*Tables 2 and 3*; Supplementary
- 422 Material, *Tables S2* and *S3*). However, tick hazard was present at all sites, including on the
- 423 grazed downland sections of the two sites (Seven Sisters Country Park and Cowdray Estate)
- 424 which had transects in downland and woodland (Supplementary Material, *Tables S1* and *S4*). The

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425	tick hazard present was not universal in wooded sections of sites; no ticks were found in the
426	forested part of Seven Sisters Country Park (Supplementary Material, Table S1). A Kruskal-
427	Wallis Test performed on multiyear means of ticks collected (all life stages) on each habitat
428	coded transect showed average ranks differed significantly (<0.05) for at least one of the three
429	coded habitat types (H=6.39 (DF 2, n=24), p=0.041 (adjusted for ties)). A post-hoc Dunn's Test
430	was used to carry out pairwise habitat type comparisons (magnitudes and directions of
431	differences, Supplementary Material Fig. S2). There was not a statistically significant difference
432	in the number of ticks (all life stages) between deciduous woodland and conifer
433	woodland/planting. However, both these habitats had significantly more ticks than downland
434	(Z=1.834, p=0.0226, and p=0.0390 respectively).

435 **DISCUSSION**

436 *Ixodes ricinus* or *H.punctata* ticks were recorded at all sites drag-sampled, on some transects in

437 high numbers (*Fig. 3* and 4). The extent of tick hazard differed between sites, and was

438 significantly higher in woodland compared to grazed downland. Although it must be noted that

439 tick hazard was still present at all downland sites. The mapping presented in this paper indicates

that tick hazard is widely distributed across the SDNP, as confirmed by the deerstalker

submission of ticks from sites across the National Park.

442 *Ixodes ricinus (Fig. 5A)* is the tick most often affecting humans and pets in the UK (Jameson &

443 Medlock, 2011; Abdullah et al., 2016; Davies et al., 2017). It is therefore unsurprising it was the

species most frequently recovered in the drag-sampling and deerstalker submissions, and the

445 most spatially reported (*Fig. 6*). The larvae and nymphs of this species feed primarily on rodents

- and small birds, while the adults mainly parasitise larger mammals. Transovarial transmission of
- some pathogens can sometimes cause larvae to hatch as infectious (Hauck *et al.*, 2020). However,
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448 a nymph feeding on a human will also have had opportunity to become infected when feeding as 449 larva, and an adult will have had two blood meals, potentially from very different animals. Thus 450 *Licinus* can act as a vector for the transmission of pathogens to humans from diverse taxa 451 (Hillyard, 1996; Randolph, 2009b; Mannelli et al., 2012). It is the UK's most common Lyme 452 disease vector, followed by *I.hexagonus* (Jameson & Medlock, 2011; Medlock & Leach, 2015), 453 and based upon the data presented here from the drag-sampling and GIS mapping, it is likely to 454 be responsible for the majority of Lyme disease cases contracted in the SDNP. *Ixodes ricinus* has 455 also been reported to be Europe's major tick borne encephalitis vector (Brugger et al., 2017). 456 Tick borne encephalitis virus has been detected in one of the Park's host counties, but to our 457 knowledge no ticks within the SDNP have been tested. Layzell et al. (2018) drag-sampled a 458 single site within the National Park in 2014 (West Dene, West Sussex) and isolated Borellia 459 *miyamotoi* in *I.ricinus* ticks from that site. Like Lyme disease, *Borellia miyamotoi* disease is 460 caused by *Borrelia* species, but signs and symptoms markedly differ so that it is classed 461 separately (Telford et al., 2015). In the USA, a case series of 94 individuals (identified by 462 retrospectively testing stored patient samples) indicated a clinical presentation of chills, 463 headache, generalised/joint pain, thrombocytopenia, and high fever. Of these people, 24% of 464 cases required hospitalisation, and all responded well to antibiotics (Molloy et al., 2015). Borellia 465 *miyamotoi* disease was discovered far more recently than Lyme disease (the first confirmed 466 Western European case was in 2013 (Fonville et al., 2014)). A clear picture of disease burden in 467 humans is, therefore, not yet available. *Borellia miyamotoi* detection in the SDNP, in what we 468 found to be the Park's most well distributed tick vector, adds further weight to the need to conduct interventions. 469

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470	UK countryside workers perceive spatial overlaps between widening deer abundance and
471	I.ricinus (Scharlemann et al., 2008), but wider ecological determinants such as host community
472	compositions affect densities of infected ticks and thus actual disease hazard and determinants
473	vary between site (Kurtenbach et al., 1998; Keesing et al., 2010). For instance, whilst deer likely
474	have roles in most, but not all, UK Lyme disease systems (Ogden et al., 1997; Gilbert et al.,
475	2012) they are non-competent hosts for the pathogen; small mammals/birds are usually required
476	as disease reservoirs (Franke, Hilebrandt & Dorn, 2013). Ixodes ricinus is often associated with
477	forests (Ehrmann et al, 2017), and in our fieldwork its presence and densities were highest in
478	wooded areas. However, we also collected the species on sheep-grazed land (as elsewhere in the
479	UK (Evans, Sheals & Macfarlane, 1968; Ogden et al., 1997; Gilbert et al., 2017)). Management
480	strategies across the SDNP should take this into account, especially where downland is bounded
481	by woods (Gilbert et al., 2017).

482 Haemaphysalis punctata's (Fig. 5B) continued and expanding presence in the SDNP is evident in 483 our tick hazard mapping (Fig. 6C), raising concerns about pathogens it can vector, including 484 B.burgdorferi s.l. (Tälleklint, 1996), tick-borne encephalitis virus (Estrada-Peña & Jongejan, 485 1999), and spotted fever group rickettsiae. UK *H. punctata* testing has so far been negative for 486 B.burgdorferi s.l. (Tijsse-Klasen et al., 2013), to our knowledge un-conducted for tick-borne 487 encephalitis virus (known ranges do not presently overlap), but positive for spotted fever group 488 rickettsiae at some sites outside the Park (Tijsse-Klasen et al., 2013). Spotted fever group 489 rickettsiae are an emerging European disease threat (Lindblom et al., 2013), but one that may 490 have been present yet unidentified for some time (Vitale et al., 2006). For example, Rickettsia 491 *massiliae* was first identified as a human pathogen in 2005 after isolation from a clinical sample 492 collected 20 years prior (Vitale et al., 2006). Spotted fever group rickettsiae related misdiagnoses

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493 and under-reporting still likely continue (Tijsse-Klasen et al., 2013). Haemaphysalis punctata is 494 known to parasitise humans (Hillyard, 1996) and tick submissions to Public Health England by 495 the public show this is happening in the SDNP (Medlock et al., 2018; Phipps, 2019). Sheep and 496 cattle are its main adult hosts, others include horses, hedgehogs, rabbits, birds, goats, deer, and 497 mustelids (Evans, Sheals & Macfarlane, 1968; Hillyard, 1996). Despite flock treatments, sheep 498 infestation at Seven Sisters Country Park (one of the sites we collected it at) has been present for 499 decades (personal communication with site sheep farmer, 2015; Medlock et al., 2018). In 2020, 500 11.5% of a sheep flock in the SDNP near Lewes suffered fatal tick pyaemia, the first such UK 501 outbreak connected to *H.punctata* (Macrelli *et al.*, 2020). Also in 2020, on Brighton's downland 502 outskirts (near the second site we collected *H.punctata*) sheep used for conservation grazing had 503 to be removed on welfare grounds following heavy infestations with the tick (Phipps *et al.*, 504 2020). Given these and related incidents Animal Plant Health Agency and Public Health England 505 are investigating further in the Park and working with farmers.

506 Medlock *et al.* (2018) state that there does not generally seem to be habitat overlap in the UK between *H.punctata* and *I.ricinus*. Though *Fig.6A* and *C* show that in all 10 km² OS grid squares 507 508 where *H.punctata* have been detected *I.ricinus* has also been recorded, this is not in fact in 509 contradiction. On a finer scale of the individual sites JM drag-sampled, tick presence was either 510 *H.punctata* or *I.ricinus*, not both. *I.ricinus* grassland preference in the UK for rough grazing is 511 likely connected to the rapid desiccation it experiences in short grass lacking thick mats of 512 vegetation and litter (Evans, Sheals & Macfarlane, 1968). In contrast, *H.punctata* can likely 513 survive better in short grass, its traditional range includes deserts (Nosek, 1973). Outside the UK 514 *H.punctata* is also found in forest (Borşan *et al.*, 2020). Whilst its established foci in the eastern 515 Downs is relatively unwooded, if allowed to expand its range westward along the downland ridge

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it will increasingly encounter patchworks of grazing, scrub, and woods. The two species site
occupancy and host community may then begin to overlap, with implications for pathogen carry
and thus hazard to humans.

519 Recommendations for key locations for future interventions

520 Queen Elizabeth Country Park: Given its high tick hazard and very high annual visitor numbers 521 this site is the highest priority for interventions. It also lays in Hampshire where tick-borne 522 encephalitis virus has been detected and would be a logistically simple trial setting for action 523 which may be required elsewhere in the county. Basic measures, such as increased frequency of 524 mowing verges (Medlock et al., 2012; Del Fabbro, 2015) and leaf litter removal (Schulze, Jordan 525 & Hung, 1995) would be implausible across the whole Country Park, but could reduce contact at 526 small high risk plots: e.g. edges of marked picnic areas; such as along the path that goes past the 527 visitor centre (*Fig. 3G*). It hosts large outdoor sports events, and elsewhere tick 528 removal/submission from participants has been used to tick-sample (Hall et al., 2017). Site 529 sampling by this method would be inexpensive, and along with increased signage would raise 530 awareness of tick presence and the value of carrying out post-activity tick-checks. These are 531 important as early tick removal reduces transmission, and during-activity recommendations 532 aimed at individuals to minimise exposure are unlikely to be headed (Middleton, Cooper & Rott, 533 2016).

The Mens: The site had the greatest DOT and DON, but fewest visitors. Thus though its DOT
was thirty times Seven Sisters Country Park's (annual visitors, est. >300,000 (ESCC and SDCB,
2004)), the tick risk to public health is far smaller. Nevertheless, signage in the carpark would be
beneficial and the site could become a useful research/trial location. It's impressive beach masts
(the dominant litter constituent of most of its transects) may support high rodent densities, which
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539	can be very host-competent for ticks and pathogens, amplifying disease hazard (Keesing et al.,
540	2009; Ostfeld et al., 2014; Krawcyk et al., 2020). Such a relationship has been observed in
541	northeastern USA where high acorn masts cause subsequent year surges in rodents, followed by
542	elevated Densities of Infected Nymphs (DIN) (Ostfeld et al., 2006). Predator protection and
543	reintroduction have been proposed as ecologically beneficial interventions (Nilsen et al. 2007;
544	Levi et al., 2012). For example, Hofmeester et al. (2017) observed an indirect negative
545	correlation of red fox and stone marten activity with DON and DIN at forest plots across the
546	Netherlands, and called for wider predator appreciation and protection. Tick pathogen testing
547	could establish if the very high tick densities at The Mens are matched by high densities of
548	infection. If so, measurement of vertebrate, especially rodent, tick and pathogen burden would
549	determine if predator re-introduction (i.e. pine martens) and protection (i.e. foxes) should be
550	trialed as health interventions at the site and environs. If successful, this could be extended
551	further through the wooded wealden section of the SDNP of which it is a part. Given The Mens is
552	a relatively large biodiverse reserve for South-East England (Whitbread, 2013), if infection
553	densities are lower than expected based on tick densities, explanatory work could contribute to
554	scientific debate on relationships between biodiversity and health (see: Randolph & Dobson,
555	2012; Levy, 2013; Foley & Piovia-Scott, 2014; Civitello et al., 2015).

Cowdray Estate and Deerstalkers: With comparably low tick hazard and fewer visitors than most
other sites, the Estate itself does not need to carry out interventions to reduce site tick hazard.
However, action aimed at walkers crossing Cowdray Estate and deerstalkers working in it (and
by implication elsewhere under similar circumstances) is warranted. Publicly/NGO maintained
car parks along the route of the South Downs Way in Cowdray Estate would benefit from tick
related signage (absent during our visits). To our knowledge no study of *B.burgdorferi* s.l.

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562	seropositivity or Lyme disease cases has been conducted for professional UK deerstalkers as has
563	been for more commonly considered occupationally at risk groups such as foresters (e.g. De
564	Keukeleire et al., 2018). However, British Deer Society volunteers at multiple sites stated to the
565	lead author of this paper that during stalking and butchery they regularly encounter ticks, often
566	getting bitten. This statement is plausible given data presented in Table 1 and Fig. 7. On a
567	precautionary principle, professional deerstalkers would thus benefit from provision of
568	permethrin-impregnated clothing which is effective against ticks (Faulde et al., 2015), and
569	targeted tick-related education. They would also be a priority group for future tick-related disease
570	vaccination efforts. A vaccine against European B.burgdorferi s.l. strains is under development
571	(Nayak et al., 2020), and one for tick-borne encephalitis is available but only recommended at
572	present for those doing outdoor activities in a country where tick-borne encephalitis virus is
573	common (NHS, 2021).

574 Seven Sisters Country Park and Ditchling Beacon Nature Reserve: Haemaphysalis punctata's 575 original distribution at Cuckmere Haven and surroundings suggests importation via migratory 576 birds (Tijsse-Klasen et al., 2013), but how it is spreading westward is unclear. This may be 577 facilitated by livestock movements (including conservation grazing (Medlock et al., 2018)), birds 578 (immature tick stages), or pets (Public Health England has received submissions taken off dogs 579 locally (Phipps, 2019)). Public Health England and Animal Plant Health Authority are carrying 580 out targeted surveys to determine its invasion boundary (Fig. 6C) and means of spread, 581 knowledge required for effective region-level intervention. Additional work to understand its 582 ecology and control is needed (Medlock et al., 2018). Acaricide application to sheep on the Lewes site resolved the situation in-year (Phipps et al., 2020). However, as the data from Seven 583 584 Sisters Country Park indicates, livestock treatment alone may be insufficient, potentially because

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585 *H.punctata* instars are supported by non-domestic hosts. Pasture spelling (i.e. temporary flock 586 removal for c.6 months) is a traditional practice to reduce tick numbers (Hillyard, 1996), yet may 587 be unsuccessful in clearing *H.punctata* from grazing land given unfed individuals can survive 588 relatively long periods without blood meals: nymphs, 252 days; adults, 255 (Evans, Sheals & 589 Macfarlane, 1968). Trials at Seven Sisters Country Park, Ditchling Beacon Nature Reserve, and 590 related sites could evaluate approaches for control whilst reducing on-site tick hazards. No tick-591 related notices were seen during sampling or subsequent visits (up to January 2021) at either site. 592 Instructive, but not alarming, signs could be placed in carparks at both, as has been done 593 unobtrusively at another site, Mount Caburn, which is a less visited local site, but where 594 *H.punctata* is also present. Notices should emphasize strongly the need for post visit checks, 595 including vigilance over longer subsequent periods than normally recommended (*H. punctata* 596 nymphs usually feed for one week, but may attach up to 33 days (Evans, Sheals & Macfarlane, 597 1968)).

598 Strengths and weaknesses

599 This work is part of a regional intervention planning exercise, so findings are highly site specific 600 limiting generalisation. However, this study may be useful as a model for intervention planning 601 elsewhere, and the data is available for meta-analyses. One strength was deerstalker involvement, 602 whose submissions enabled responsive site selection and contributed to SDNP wide tick hazard 603 mapping. Another was our use of Density of Ticks, rather than only Density of Nymphs, which 604 starting in the USA with Lyme disease is more common. *H. punctata* presence illustrates its 605 reduced appropriateness for European work. Transovarial transmission of spotted fever group 606 rickettsiae is established, so a hazard metric that excludes larvae (as Density of Nymphs does) 607 will under-count vector density for spotted fever group rickettsiae and other pathogens (e.g.

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608 B.miyamotoi (Hansford et al., 2015)). Similarly, tick-borne encephalitis virus cycles require 609 larvae-nymph co-feeding (PHE, 2019). The idea, based on US data, that transovarial transmission 610 of *B.burgdorferi* s.l. is rare or non-existent (Ostfeld, 2011, p.43) is the main basis on which 611 Density of Nymphs has been used for Lyme disease site hazard assessment. However, van 612 Duijvendijk et al. (2016) showed a Borellia commonly causing Lyme disease in Europe can be 613 transmitted by larvae, and in a UK study by Hall et al. (2017), 0.7% were B.burgdorferi s.l. 614 positive. A study weakness concerns number and months of repeat sampling. Firstly, our first 615 four sites were intended to be drag-sampled twice per year, for two years. However, this was not 616 possible for one site, which was sampled three times only. Secondly, some species lifecycles 617 cause seasonal differences in questing tick numbers/life-stage proportions. However, for 618 logistical reasons the months' individual site samplings took place in varied. This reduces 619 confidence in validity of site comparisons somewhat, though this is partially balanced by in-year 620 and multiple-year repeat samplings. GIS tick hazard mapping successfully brought together all 621 publicly available, and some unpublished, tick records from SDNP. However, to our knowledge 622 vouchers are unavailable for the historic and pan-species records included, leaving some 623 uncertainty regarding correct species identification. In contrast a strength of our fieldwork is our 624 vouchers.

625 **Conclusions**

We set out to map tick hazard distribution across the SDNP, analyse habitat associations, identify and describe potential key locations for future interventions and determine their tick hazard (species and density). Against a background of increased concern about tick-borne pathogens in the UK (*B.burgdorferi* s.l., *B.miyamotoi*, *B.venatorum*, tick-borne encephalitis virus, spotted fever group rickettsiae), our mapping shows tick hazard is broadly distributed across SDNP.

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631 *Ixodes ricinus* was the most common tick found, though the potential range expansion of 632 *H.punctata* from its historic foci at SDNPs far east is concerning, not least as it seems better able 633 to thrive on grazed downland than *I.ricinus*. Our study confirms woodland is the habitat in the 634 SDNP most associated with tick hazard, but ticks (including *I.ricinus*) were collected on 635 downland, and if *H.punctata* is allowed to expand its range westward, this is only likely to 636 increase. Tick hazard does not reflect negatively on land managers, but should be a stimulus for 637 action, especially at those sites with high tick risk: i.e. those with high DOT/DON and high 638 visitor numbers. We identified key potential sites for interventions and based on measured tick-639 density, site description, and visitor levels have provided site specific recommendations for 640 control measures (which should be evaluated in-situ during roll-out) and future research. These 641 include targeted management at small high tick hazard plots with heavy visitor numbers (Queen 642 Elizabeth Country Park), signage to increase awareness of post-visit precautions (all sites), 643 repellent impregnated clothing for deerstalkers (Cowdray Estate), and flock-based experimental 644 trials to control H.punctata (Seven Sisters Country Park, Ditchling Beacon Nature Reserve). 645 Further research at one of the sites with very high tick density (The Mens) may valuably 646 contribute to an understanding of ecological dynamics underlying infection density, and potential 647 use of predator re-introduction and protection as a public health intervention. Ecological research 648 on *H.punctata* would also contribute towards control strategies. Whilst interventions are 649 necessarily site-specific, this does create the danger of implementation becoming fragmented. 650 However, SDNPA is ideally placed to link and champion site-based and regional policies to 651 reduce hazard, whilst avoiding or reducing conflict between public health and ecosystem health.

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673 **Competing interests**

674 None declared.

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675 Ethical approval

676 Not required.

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TABLES 1030

1031 Table 1:

Ticks submitted by deerstalkers. 1032

Deer culled for reasons unrelated to this project. Percentages rounded to whole numbers. Engorged status 1033

1034 covers adult females and nymphs only, as adult males do not engorge. *in addition the single nymph was engorged.

1035

	n=87	Tick
		(%)
Tick species		
Ixodes ricinus		87 (100)
Tick life stage and sex		
Adult females		71 (82)
Adult males		15 (17)
Nymph		1(1)
Larvae		0 (0)
Female Engorgement (n=71)		
Females engorged*		52 (73)
Females not engorged		19 (27)
Not recorded		2 (3)
Habitat		
Wood		51 (59)
Wood-chalk grassland		15 (17)
Wood-pasture		7 (8)
Pasture		6 (7)
Wood-arable		3 (3)
Wood-heath		3 (3)
Other		2 (2)
Host species		
Fallow deer, Dama dama (n=10))	45 (52)
Roe deer, Capreolus capreolus ((n=3)	38 (44)
Not recorded (host n=1)		4 (5)
Range of ticks per host	·	1-35

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1037 **Table 2:**

1038 Ticks collected by site drag sampling.

1039 Results of individual samplings in Supplementary Material, *Tables S1-4*.

	On-transect					Off-transect			ct	Site Totals	
-	Life stages and sex					Life stages and sex					
	Species	Collected on	Larvae	Nymphs	Adults	Totals	Larvae	Nymphs	Adults	Totals*	
Seven Sisters Country Park Six 50m ² transects, each sampled four times over y1 & 2.	I. ricinus	Blanket		2			65	8	1₽	74	
		Chaps			2♀	4					78
		Flags									
Queen Elizabeth Country Park Six 50m ² transects,	H.punctata	Blanket	37	39	1♀ 4♂		25	65	3♀ 1♂	94	
		Chaps		8		89					183
each sampled four times over y1 & 2.	H.I	Flags				_					
The Mens Six 50m ² transects,	sn	Blanket	35	78	1♀ 3♂		100	104	2♀ 3♂	209	
each sampled four	I.ricinus	Chaps		4		121					330
times over y1 & 2.		Flags				_					
Cowdray Estate	I. ricinus	Blanket		21	18		3	5		8	
Six $50m^2$ transects, each sampled three times over y1 & 2.		Chaps		1		23					31
		Flags									
Ditchling Beacon Nature Reserve	H.punctata	Blanket			1ð				0		
Six $50m^2$ transects, each sampled twice in y2.		Chaps				1					1
		Flags									
		Totals	72	153	13	238	193	182	10	385	62.

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1041 **Table 3:**

1042 Tick hazard and visitors observed during transect drag sampling.

1043 Results of individual samplings in Supplementary Material, *Tables S1-4*.

	Visitor numbers observed	Tick species	Ticks found on transects	Ticks per individual 50 m ² sampling	Site Density of Nymphs (DON)	Site Density of Ticks (DOT)
The Mens	Low to none	I.ricinus	121	0–21	30/300 m ²	30/300 m ²
Queen Elizabeth Country Park	Very high	I.ricinus	89	0–25	12/300 m ²	22/300 m ²
Cowdray Estate	Low	I.ricinus	23	0–7	6/300 m ²	8/300 m ²
Seven Sisters Country Park	Very high	H.punctata	4	0–2	1/300 m ²	1/300 m ²
Ditchling Beacon	Moderate	H.punctata	1	0–1	n/a	n/a

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1045 **FIGURES**

[Placed in Materials and Methods]

- 1047 Figure 1: Site tick-sampling equipment.
- 1048 JM drag-sampling along a path border at The Mens, West Sussex. (A) Woollen blanket. (B)

1049 Woollen chaps. (C) Woollen flags. Design as per Dobson, Taylor & Randolph (2011). Photo:1050 ASR.

1051

1046

[Placed in results]

1052 Figure 2: Tick sample collection sites in the South Downs National Park.

- 1053 Sites where ticks had been submitted by deerstalkers or drag-sampled by JM marked by points at
- 1054 100 m^2 resolution. All ticks collected from deer were *Ixodes ricinus*, which was also the only tick
- species drag-sampled at Queen Elizabeth Country Park, Cowdray Estate, and The Mens.
- 1056 *Haemaphysalis punctata* was the only tick drag-sampled at Seven Sisters Country Park and
- 1057 Ditchling Beacon Nature Reserve. Map contains OS data © Crown Copyright (OS OpenData,
- 1058 2020) and a National Park base layer (unmodified) from Natural England (2020)
- 1059 (https://creativecommons.org/licenses/by-nc-nd/2.0/). Map: JM.

1060 Figure 3: Seven Sisters Country Park, and Queen Elizabeth Country Park.

- 1061 Both sites sampled twice each in 2015 and 2016. Where ticks were present along $50m^2$ transects
- 1062 2-year totals are given (individual samplings in brackets). Photos: JM.

1063 Figure 4: The Mens, and Cowdray Estate.

- 1064 Both sites sampled twice each in 2015 and 2016. Where ticks were present along $50m^2$ transects
- 1065 2-year totals are given (individual samplings in brackets). Photos: JM.

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1066 Figure 5: Tick species collected.

- 1067 Ticks collected during study. (A) *Ixodes ricinus* (drag-sampled at The Mens, West Sussex, 2016).
- 1068 (B) Haemaphysalis punctata (drag-sampled at Ditchling Beacon Nature Reserve, East Sussex,
- 1069 2016). Photos: JM.

1070 Figure 6: Recorded tick hazard in the South Downs National Park.

- 1071 (A) Ixodes ricinus. (B) Ixodes hexagonus. (C) Haemaphysalis punctata. (D) Dermacentor
- 1072 *reticulatus*. Orange OS grid squares indicate the most recent record/s of tick presence are since
- 1073 2005 (inclusive). Yellow squares indicate the most recent record/s found were prior to 2005
- 1074 (latest record date shown). Empty squares represent no records found, but should not be taken as
- 1075 on-the-ground tick absence. Map combines our data of drag-sampling and ticks submitted from
- 1076 culled deer, national maps from the Public Health England/Health Protection Agency tick
- 1077 surveillance scheme (Cull *et al.*, 2018; PHE, 2016; HPA, 2013a, 2013b, 2013c, 2013d), the
- 1078 National Biodiversity Network Atlas (NBN, 2020), Medlock et al. (2018), Layzell et al. (2018),
- 1079 and previously unpublished records from pan-species surveying at Sussex Wildlife Trust
- 1080 reserves. In addition, a case report by Phipps et al. (2020) states there were H.punctata
- 1081 infestations within the confines of Brighton & Hove in 2019. Maps contain OS data © Crown
- 1082 Copyright (OS OpenData, 2020) and a National Park base layer (unmodified) from Natural
- 1083 England (2020) (https://creativecommons.org/licenses/by-nc-nd/2.0/). Maps: JM.

1084 Figure 7: Tick attachment sites on sentinel deer

1085 Ticks collected by British Deer Society members from deer culled for other reasons, attachment

1086 sites for four ticks not recorded. Body zones as per Pacilly *et al.* (2014) *Instructions listed

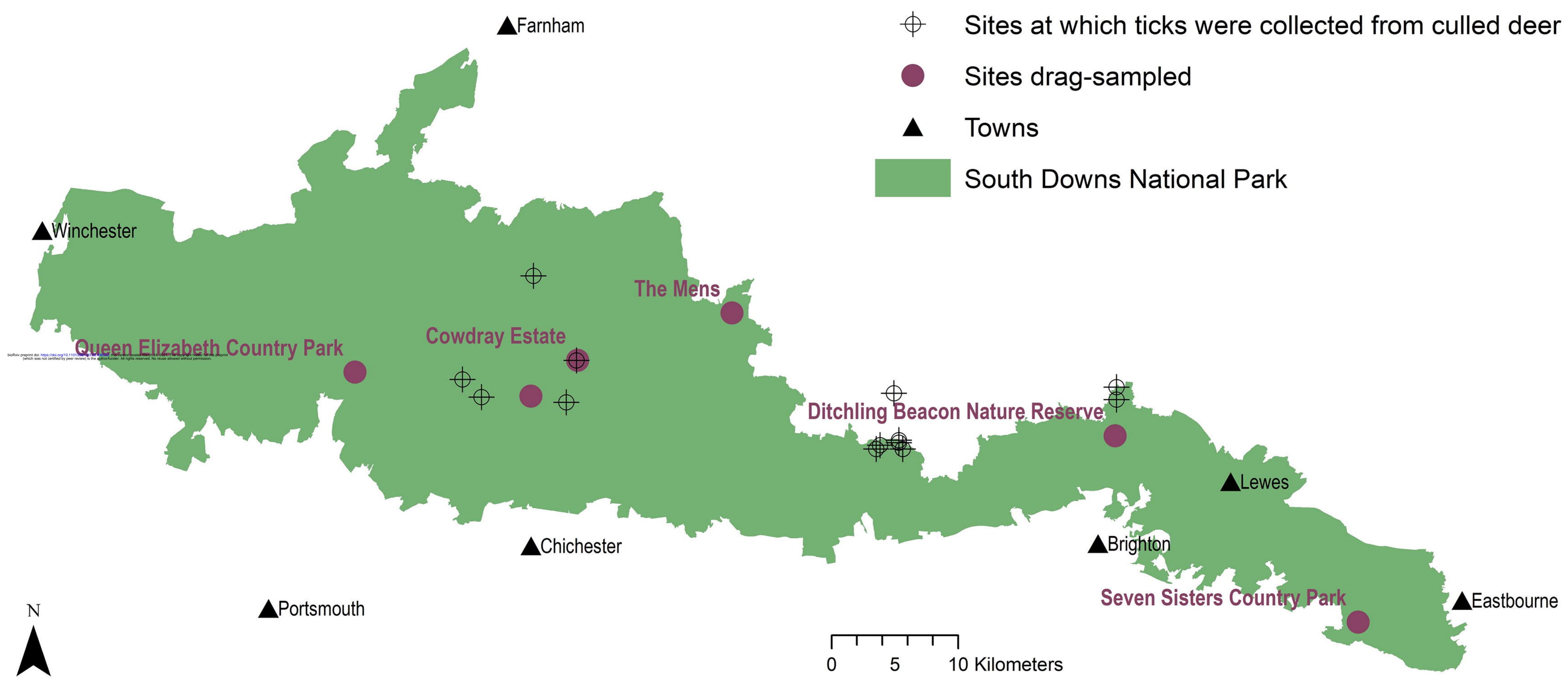
- 1087 abdomen and sternum as separate zones to record, but for 10 ticks this was not done so zones
- 1088 were merged in this figure (reported attachment sites: abdomen, 24; sternum and abdomen, 10;

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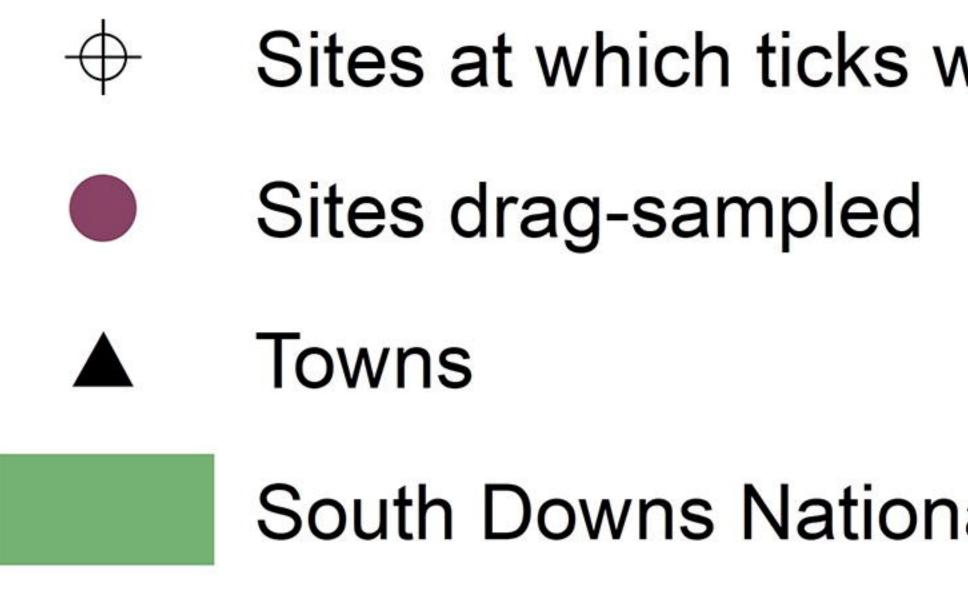
- 1089 sternum, 1). Photo: Johann-Nikolaus Andreae (2008), use and changes made under CC-BY-SA-
- 1090 2.0 which also applies to this figure. Original:
- 1091 https://web.archive.org/web/20200930080151/https://commons.wikimedia.org/wiki/File:Fallow_
- 1092 deer_in_field_%28cropped%29.jpg.
- 1093 Figure 8: Life stages of ticks collected along study transects by month.
- 1094 2015 and 2016: Seven Sisters Country Park, Queen Elizabeth Country Park, The Mens, Cowdray
- 1095 Estate. 2016 only: Ditchling Beacon Nature Reserve. Nymphal and larval proportions in
- sampling months partly reflect *Ixodes ricinus* annual lifecycles, and are not presented to indicate
- 1097 changing *quantity* of hazard, as monthly total differences may be partly explained by which sites,
- 1098 and how many, were visited.





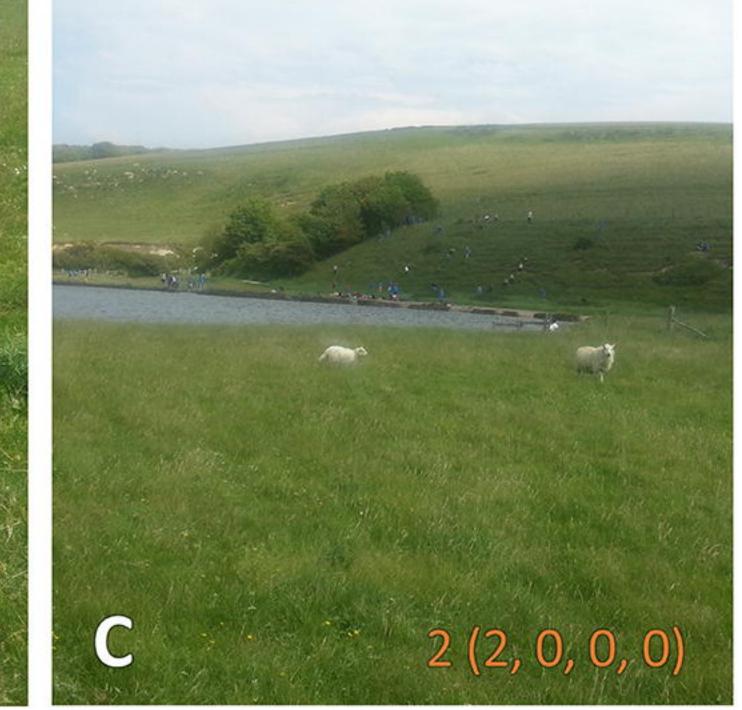








SEVEN SISTERS COUNTRY PARK





B













B

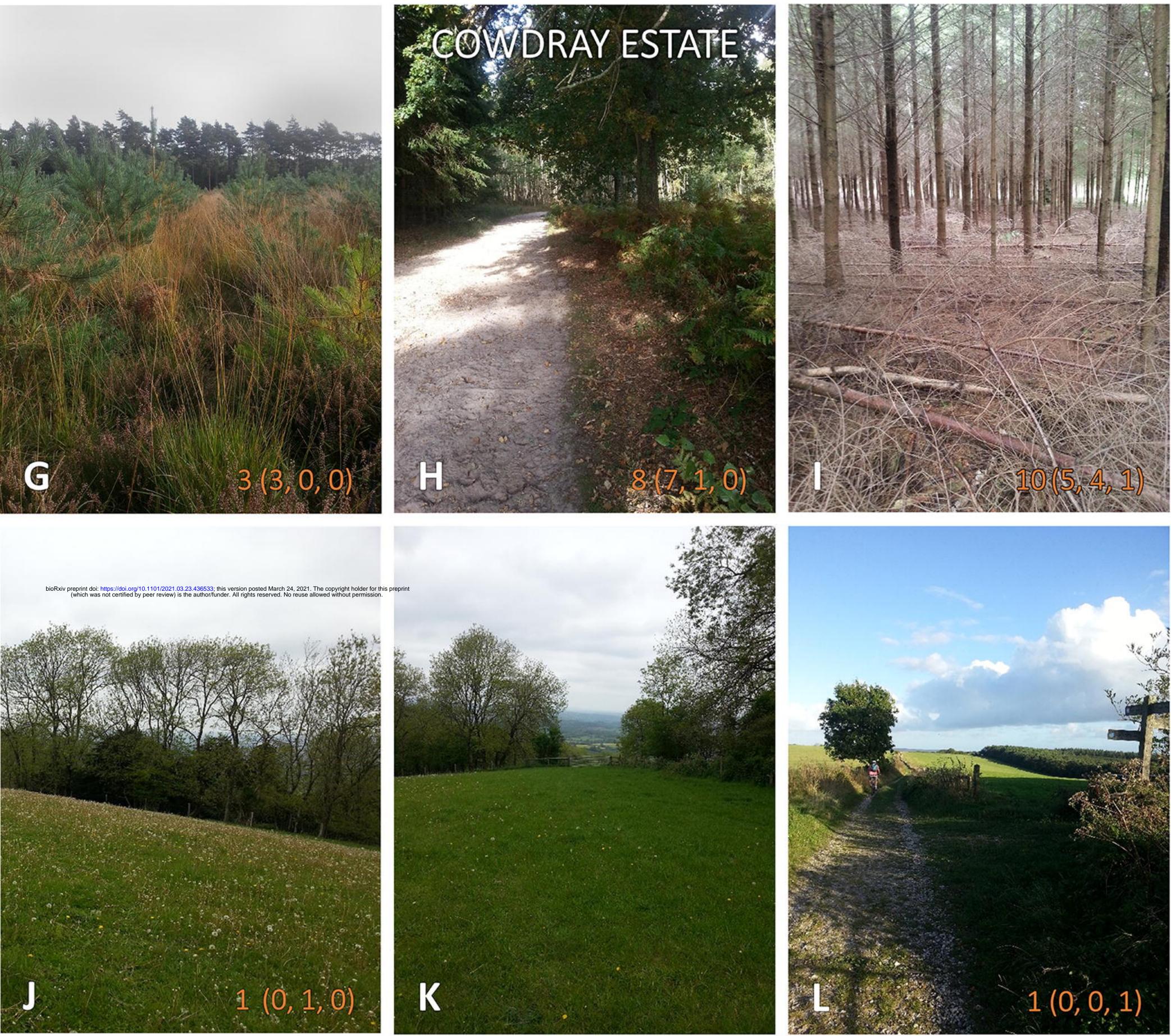














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