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1 **Identification of Female Sex Pheromone for Monitoring the Barred**
2 **Tooth Striped Moth, *Trichopteryx polycommata*, a Priority**
3 **Conservation Species**

4
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14

15 **Abstract**

16 Pheromone-baited traps can be excellent tools for sensitive detection of insects of
17 conservation concern. Here, identification of the sex pheromone of *Trichopteryx*
18 *polycommata* (Denis & Schiffermüller, 1775), an under-recorded UK priority species, is
19 reported. In analyses of extracts of the pheromone glands of female *T. polycommata* by gas
20 chromatography coupled to electroantennographic recording from the antenna of a male
21 moth, a single active component was detected. This was identified as (Z,Z)-6,9-
22 nonadecadiene (Z,Z6,9-19:H) by comparison of its mass spectrum and retention times with
23 those of the synthetic standard. In a pilot field trial in Kent, UK, *T. polycommata* males were
24 caught in pheromone traps baited with lures loaded with 1mg and 2mg (Z,Z)-6,9-19:H.
25 Optimum lure loading was identified in a further five trials in Kent, Sussex and Lancashire
26 where lures of 0, 0.001, 0.01, 0.1, 1, 2, 5 and 10 mg loadings were tested. Traps baited with 1
27 to 10 mg of ZZ6,9-19:H caught significantly more *T. polycommata* than traps baited with 0
28 mg and 0.001mg. In a pilot survey of *T. polycommata* using pheromone lures around
29 Morecambe Bay, UK, *T. polycommata* males were captured at 122 new sites within the three

30 counties where trials took place, demonstrating the potential of pheromone monitoring to
31 increase knowledge of abundance, distribution and ecology of this elusive species.

32

33 **Key Words** (Z,Z)-6,9-nonadecadiene, electroantennography, insect conservation, lure,
34 detection of endangered species, biodiversity, mapping indicator species, live-catching
35 pheromone traps.

36

37 **Introduction**

38 Biodiversity loss is a global crisis (Brooks et al. 2012; Jenkins 2003) which continues despite
39 international agreements to promote conservation (Larigauderie et al. 2012; Santamaría and
40 Méndez 2012; Waldron et al. 2013). The rate of loss of invertebrate populations exceeds that
41 of vertebrates and vascular plants, possibly by several orders of magnitude (Conrad et al.
42 2006; Dunn 2005; Samways 2007; Thomas et al. 2004). Decline of insect populations is of
43 particular concern, as they are a vital component of ecosystems. Insects provide stability in
44 ecosystems, recycle nutrients and transfer energy between trophic levels. They also supply
45 many ecosystem services essential to humanity, particularly pollination and biological pest
46 control, as well as being of inherent cultural value (Fonseca 2009; Kellert 1993; Kim 1993;
47 Littlewood et al. 2012). However, efforts to conserve insect populations are complicated by a
48 lack of knowledge and data on threatened species. This is due in part to a lack of tools which
49 are suitably sensitive to detect and monitor limited populations of small organisms (Cardoso
50 et al. 2011),

51 Recently, tools employing pheromones have been developed for monitoring insects of
52 conservation value, including *Elater ferrugineus* (Linnaeus, 1758), *Osmoderma eremita*
53 (Scopoli, 1763) (Larsson and Svensson 2009; Larsson et al. 2003), and the luna moth *Actias*
54 *luna* (L.) (Millar et al. 2016). Historically, pheromone traps have been employed as sensitive,
55 species-specific tools for monitoring pest species in and around crops. The same strategy
56 could equally be applied to detection and monitoring of threatened insect populations
57 (Andersson et al. 2014; Larsson & Svensson 2009; Musa et al. 2013). Pheromone monitoring
58 has been shown to be more sensitive and cost-effective than traditional sampling methods and
59 could potentially contribute to solving some of the problems experienced in insect
60 conservation (Andersson et al. 2014; Larsson & Svensson 2009; Musa et al. 2013; Svensson
61 et al. 2009). However, such an approach requires that an attractive pheromone is produced by

62 the target species, and that it can be chemically characterized and produced in sufficient
63 quantities for field use.

64 The aim of this study was to develop and test a pheromone-based system for detecting
65 and monitoring populations of *Trichopteryx polycommata* (Denis & Schiffermüller 1775)
66 (Lepidoptera; Geometridae). Formerly widespread across the UK, this ‘UK Priority Species’
67 (JNCC 2007) is now only found in a few locations in Kent, Sussex, North Hampshire,
68 Wiltshire, Lancashire and South Cumbria, with limited records from Dorset, Herefordshire,
69 Norfolk and Scotland (Wigglesworth et al. 2018). The larvae feed on wild privet (*Ligustrum*
70 *vulgare*) (Linnaeus, 1758) and ash (*Fraxinus excelsior*) (Linnaeus, 1758) (Wigglesworth et
71 al. 2018), and have also been known to feed on species of *Lonicera* (Choi 2007).
72 *Trichopteryx polycommata* is therefore a bioindicator for presence of *L. vulgare*, an important
73 food plant for many species, and could be used to assess the potential impact of ash dieback
74 on insect communities. *Trichopteryx polycommata* also supports a host-specific parasitoid
75 wasp *Earinus transversus* Lyle (Hymenoptera: Braconidae: Agathidinae) found only in the
76 UK, and rediscovered after 100 years in 2005 (Shaw 2010).

77 Species-specific monitoring and a need to gain a better understanding of population
78 distribution, status and ecology are crucial to conservation of *T. polycommata* (JNCC 2010).
79 The moths are infrequently caught in light traps (Wigglesworth et al. 2018), and the best
80 current technique for detection is to search for adults resting on *L. vulgare* after dark
81 (Wigglesworth et al. 2018). Here, we collected and identified a putative sex pheromone
82 produced by *T. polycommata*, and confirmed that it elicits a physiological response through
83 electroantennography. Lures releasing the pheromone were formulated and tested through
84 pilot studies to determine whether they attract *T. polycommata* moths, presented alone and in
85 combination with funnel traps. In a second experiment, we examined the effect of amount of
86 pheromone loaded into lures on number of moths caught. Finally, we conducted a
87 preliminary field survey with pheromone-baited traps, to assess their usefulness in detecting
88 populations of *T. polycommata*.

89

90 **Methods and Materials**

91 **Insect Sourcing and Sample Collection** Three adult female and four male *T. polycommata*
92 moths were collected by torch light and netting from Seaford Head Nature Reserve, Sussex
93 (50.756995N, 0.13722479E) on 23 March 2016. The moths were kept in a refrigerator at 5

94 °C in individual plastic containers (17.8 × 11.5 × 4.4 cm). Pieces of damp cotton wool were
95 placed in each container to maintain humidity and provide a drinking source. The next day at
96 1000 h the females were removed from the refrigerator and placed in a dark, controlled-
97 temperature room at 10 °C to mimic the conditions under which the moths are found to be
98 most active. At 1230 h gland extracts were taken from two live females who had been
99 observed calling for approximately 90 min. During calling, females swayed their abdomen
100 back and forth while the pulsing pheromone gland was exposed, at which point it was excised
101 with a pair of microscissors. Excised glands were placed immediately into individual glass
102 vials (1.1mL 12mm x 32mm; Fisher Scientific, Leicestershire, UK) each containing 10 µl of
103 hexane (HPLC Plus; Sigma-Aldrich). After 10 min the hexane was removed using a pipette
104 and retained in a separate vial. A second wash was performed with another 10 µl of hexane
105 and stored separately. Glands were retained individually in vials containing 10µl of hexane.
106 All samples were placed in the freezer at -20 °C until use.

107

108 **Analyses by Gas Chromatography linked to Electroantennographic Detection (GC-EAD)**

109 Male *T. polycommata* moths used for GC-EAD were kept in a refrigerator at 5 °C in
110 individual plastic containers (0.8 × 11.5 × 4.4 cm) containing damp cotton wool. Individuals
111 were removed from the refrigerator 2 h before use to allow them to acclimatize to room
112 temperature. Insects were then anesthetized using carbon dioxide, and the head removed
113 under a dissecting microscope with a razor blade. A borosilicate glass capillary electrode (ID
114 0.86mm, Warner Instruments, Hamden, CT06514), pulled to a fine tip and filled with 0.1M
115 KCl containing 1% polyvinylpyrrolidone as electrolyte, was inserted into the back of the
116 head. The electrode and head were then mounted onto a silver wire held within an electrode
117 holder connected to the earth probe of a portable EAG amplifier (INR-2, Syntech, formerly
118 Hilversum, The Netherlands, now Kirchzarten, Germany). A similar electrode mounted onto
119 the x10 recording preamplifier was then brought into contact with the distal tip of the
120 antenna.

121 Samples were presented to antennal preparations via a gas chromatograph (HP6890,
122 Agilent Technologies, Stockport, Cheshire, UK) fitted with DB-WAX and DB1 fused silica
123 capillary columns (30 m x 0.32 mm i.d. x 0.25 µ film thickness; Supelco, Gillingham, Dorset,
124 UK). The eluents from the columns were combined with a glass Y-piece into a length (10
125 cm) of deactivated fused silica capillary and then split 50:50 using a glass Y-piece to equal
126 lengths of deactivated fused silica tubing leading to the flame ionization detector (FID) and

127 via a heated (250°C) transfer line into silanized glass tube (4 mm i.d.) delivering a continuous
128 flow of air (200 ml/min) over the antennal preparation. Gland extracts (1 µl) were injected at
129 220°C in splitless mode onto the DB-WAX column, with the oven temperature held for 2 min
130 at 50 °C before increasing at 20 °C min⁻¹ or 10 °C min⁻¹ to 250 °C and held for 5 min. Carrier
131 gas was helium at continuous flow of 2.4 ml/min. The EAG signal was digitized by
132 connecting the amplifier as a GC detector and this and the simultaneous FID signal were
133 captured and analyzed using EZchrom Elite (Version 3.3.1, Agilent Technologies). Antennal
134 preparations were only moved under the air flow outlet once the solvent peak had eluted.
135 Two of the four males survived so two EAD runs of the first wash of the gland extract from
136 the same female moth were carried out using an antenna from each male in turn. Standard *n*-
137 alkanes (C8 to C24) were run under the same conditions to calculate retention indices.

138

139 **Analyses by Gas Chromatography coupled to Mass Spectrometry (GC-MS)** Pheromone
140 gland extracts were analyzed by gas chromatography-mass spectrometry (GC-MS) on a CP-
141 3800 GC coupled directly to a Saturn 2200 MS (Varian, now Agilent Technologies) in
142 electron impact mode. The GC was equipped with a polar DB-Wax column and non-polar
143 VF5 column (Agilent; 30m × 0.25mm i.d. × 0.25µ film thickness) connected to the transfer
144 line via a Quick-Switch Valve. The GC was programmed at 40 °C for 2 min, then increased
145 by 10 °C min⁻¹ to 240 °C and held for 5 min. Injections were made in splitless mode at 220
146 °C and the transfer line temperature was 250 °C. Carrier gas was helium at a constant flow of
147 1 ml/min. Gas chromatography retention times were converted to retention indices by
148 comparison with the retention times of *n*-alkanes as above.

149

150 **Field Trials** (*Z,Z*)-6,9-Nonadecadiene (ZZ6,9-19:H; ≥ 98% pure by GC-MS analysis on the
151 polar GC column) was obtained from Pherobank (Wijk bij Duurstede, The Netherlands).
152 Lures were prepared by loading the required amount in hexane solution (100 µl) onto rubber
153 septa (13 mm diameter, Sigma Aldrich, Gillingham, Dorset, UK). Once the hexane had
154 evaporated, lures were wrapped in aluminum foil and placed in the freezer until required.

155 For a preliminary field trial, lures were loaded with 1 mg or 2 mg ZZ6,9-19:H. In the
156 field, lures were attached to garden canes approximately 1 m above ground level, the height
157 at which *T. polycommata* rest on privet hedges. The lures were tested in this way at the
158 following 17 key British locations known to contain *T. polycommata* populations in Sussex

159 (50.827194N, 0.455016W), Inverness (57.425511N, 4.499708W), Norfolk (52.402082N,
160 0.754747E; 52.509296N, 0.627334E; 52.466495N, 0.769076E; 52.478686N, 0.786043E;
161 52.488902N, 0.614333E; 52.568607N, 0.596493E; 52.451878N, 0.942289E; 52.402082N,
162 0.754747E; 52.468944N, 0.772028E); Argyll and Bute (56.558925N, 5.253752W;
163 56.558678N, 5.254284W); Yorkshire (54.082715N, 2.022893W; 54.084423N, 2.021212W;
164 54.137356N, 2.036545W and 54.083614N, 2.022893W) between 28 March and 11 May
165 2017. Searches for *T. polycommata* by torchlight are typically carried out between 1900 h and
166 2130 h when the moths can be seen resting on *L. vulgare*. Participants given lures to test
167 without traps trialed the lures during this time period. Lures were tested for 15-20 min before
168 being moved to a new location at least 50 m away.

169 In a parallel trial, two economy funnel traps (Oecos Ltd, Kimpton, UK; height 22 cm,
170 diam. 13 cm), one baited with the 1 mg lure the other baited with the 2 mg lure, were placed
171 at St Margaret's Bay, Kent (51.141445N, 1.372182E). The traps were hung from a large *L.*
172 *vulgare* bush approximately 1 m above the ground and approximately 5 m apart from each
173 other on 21 March 2017. The traps were checked daily between 22-28 March 2017 and any
174 moths caught were released within a 5 m radius of the trap. This inevitably meant that there
175 would be some recaptures of individual males. Ideally recaptured moths would have been
176 identified by mark recapture methods, but we were not able to mark captured moths due to
177 restrictions on handling this rare species. Recapture rates in moth pheromones tend to be
178 fairly low after a few days of recapture (Oleander et al. 2018), so the overall effect can likely
179 be considered negligible.

180 To investigate the effect of lure loading on catches, lures were loaded with 0.001 mg,
181 0.01 mg, 0.1 mg, 1 mg, 2 mg, 5 mg and 10 mg of ZZ6,9-19H. Control lures were made by
182 adding 100 µl of hexane to the rubber septum. The field trials were carried out at five
183 locations: St Margaret's Bay, Kent (51.141445N, 1.372182E); Seaford Head, Sussex
184 (50.756995N, 0.137225E); Warton Crag, Lancashire (54.148923N, 2.783226W); Roudsea,
185 Lancashire (54.234726N, 3.026337W); Challan Hall, Lancashire (54.188482N, 2.808341W
186 and 54.195082N, 2.802492W); and Sizergh, Lancashire (54.285892N, 2.788926W). Trials
187 took place between 29 March – 4 April 2017 in Kent; 30 March and 13 April 2017 in Sussex
188 and between 13-25 April 2017 in Lancashire. At each location, eight economy funnel traps
189 (Oecos Ltd) K were baited with the lures. The traps were positioned 2 m apart and were
190 placed in the order of lowest loading to highest. In Kent the traps were arranged in two
191 parallel lines, each line containing four traps positioned 2 m apart and the lines were also 2 m

192 apart. In Sussex and Lancashire, on each site the traps were arranged in an approximate semi-
193 circle and were 2 m apart. Traps were placed in this close proximity due to the limited size of
194 the locations, which are narrow open pathways through a woodland. The traps were checked
195 daily and any moths caught were identified and released within a 5 m radius of the traps.
196 Traps were moved round one position daily to reduce any positional effect. As a
197 precautionary measure, the traps were removed every two or three days for two days to
198 ensure the local population of moths would have the opportunity to mate.

199 For statistical analysis, at each of the six sites at which lures were tested, the total
200 number of *T. polycommata* captured by each lure loading was divided by number of nights of
201 trapping to give mean catch night⁻¹. The resultant means were transformed to log (n+1) and
202 entered as the dependent variable into a linear model with site (six level factor) and lure
203 loading (eight level factor) as independent variables. Significance of terms within the model
204 were assessed by *F* tests, with Tukey's test ($P < 0.05$) of estimated marginal means used to
205 identify significant differences between lure loadings, controlling for effect of site. Estimated
206 marginal means (and 95% confidence intervals) of catch night⁻¹ for each lure loading were
207 back-transformed onto the original scale for presentation. All data analysis was performed in
208 R (R Core Team, 2018, Lenth 2019)

209

210 ***T. polycommata* Survey** A field survey was carried out in order to establish the potential for
211 increasing detection of *T. polycommata* moths using a pheromone-based method of sampling.
212 Pheromone traps baited with 2 mg lures were placed overnight at 168 locations at 102 sites in
213 Morecambe Bay between 11 April – 1 May 2017. *T. polycommata* had previously been
214 recorded at 26 of the 168 locations. One economy funnel trap (Oecos Ltd, UK) was used per
215 location. Each trap was hung on a tree or bush approximately 1 m above the ground. The
216 traps were set in position by 17:00 h and checked by 11:00 h the following day. Any moths
217 caught were identified and released into suitable vegetation on site. Maps of distribution were
218 produced using ArcMap 10.2.2 (ESRI (Environmental Systems Resource Institute) 2014).

219

220 **Results**

221 **Pheromone Identification** A single, reproducible response was observed in GC-EAD
222 analyses of ovipositor extracts of female *T. polycommata* run using a polar GC column. The

223 antenna of a male moth responded to a putative major sex pheromone component (Fig. 1) at
224 Retention Index (RI) 1964. The amount present was up to 150 ng per ovipositor. In GC-MS
225 analyses this major peak had RI 1950 on the polar column and 1869 on the non-polar column.
226 The mass spectrum (Fig. 2) showed a probable molecular ion at m/z 264 and base peak at m/z
227 67. The data were consistent with those for a straight-chain, 19-carbon hydrocarbon with two
228 non-conjugated double bonds, most likely in the 6,9-positions as the 3,6- configuration would
229 have been expected to give a strong ion at m/z 79 (e.g. Yamamoto et al. 2008). Synthetic
230 (*Z,Z*)-6,9-nonadecadiene (ZZ6,9-19:H) was subsequently obtained and had identical mass
231 spectrum (Fig. 2) and RI's to the natural compound, although insects were not available by
232 then to test the EAG response to the synthetic compound.

233

234 **Field Trials** In the first trial with volunteers and lures suspended on canes, *T. polycommata*
235 males were observed to be attracted to the 1 mg and 2 mg ZZ6,9-19:H lures at 50.827194N,
236 0.455016W and 54.082715N, 2.022893W but not at any of the other sites. In the parallel live
237 trapping trial, one male *T. polycommata* was caught in the trap baited with the 1 mg lure
238 while 16 males were caught in the trap baited with the 2 mg lure. No other species of moths
239 were caught in these traps.

240 In the trial to compare catches with different lure loadings, controlling for a
241 significant effect of trapping location ($F_{5,35} = 8.7$, $P < 0.001$), an overall significant effect of
242 pheromone loading was found on mean catch night⁻¹ ($F_{7,35} = 11.1$, $P < 0.001$, Fig. 3). Traps
243 baited with 1 to 10 mg of ZZ6,9-19:H caught significantly more *T. polycommata* than traps
244 baited with 0 mg and 0.001mg. Traps baited with 10 mg ZZ6,9-19:H also caught significantly
245 more *T. polycommata* than traps baited with 0.01 mg.

246 No other species were attracted to the lures in Kent and Sussex, but in Lancashire *T.*
247 *carpinata* (Borkhausen, 1794) and *Chloroclystis v-ata* (Haworth, 1809) (Lepidoptera:
248 Geometridae) were caught in the pheromone baited traps, although in much lower numbers
249 than *T. polycommata* (67 and 1 respectively, compared to 514 *T. polycommata*).

250 It was observed on 31 March 2017 at Seaford Head that at 2130 h no *T. polycommata*
251 had been caught in the pheromone traps, but by 1000 h the following morning 61 had been
252 caught. During the Lancashire field trials, it was observed that activity at the pheromone traps
253 began at 0045 h and lasted for approximately 45 min.

254

255 **Survey of *T. polycommata*.** The pilot study increased the number of records of *T.*
256 *polycommata* from 107 to 881 in the region and the number of known *T. polycommata* sites
257 from 48 to 88. Fig. 4 shows the known distribution and abundance of *T. polycommata* in the
258 Morecambe Bay area before 2017 and after the pilot pheromone study.

259 Discussion

260 Prior to this study, the recommended way of surveying for *T. polycommata* was to search *L.*
261 *vulgare* bushes after dark by torchlight looking for adults resting on the twigs. The results
262 presented here demonstrate that pheromone-baited traps could provide a more practical and
263 sensitive method of detection. (Z,Z)-6,9-nonadecadiene (ZZ6,9-19:H) was identified as a
264 component of the female sex pheromone of this species, which attracts male moths in the
265 field. This is the first pheromone component to be identified in the genus *Trichopteryx* which
266 contains 11 other species. Given the small numbers of individuals available for this study and
267 the somewhat artificial conditions used prior to gland extraction, the possibility of there being
268 additional components in the complete pheromone cannot be excluded. However, only a
269 single reproducible EAG response was recorded from males in GC-EAD analyses of
270 pheromone gland extracts.

271 (Z,Z)-6,9-Nonadecadiene has been identified as a sex pheromone or attractant in one
272 member of the Arctiidae family and 14 members of the Geometridae family in the
273 subfamilies Alsophilinae, Ennominae and Larentiinae (Pherobase, 2017). Of these species the
274 following occur in the UK: *Alcis repandata* (Linnaeus, 1758), *Bupalus piniaria* (Linnaeus,
275 1758), *Campaea margaritata* (Linnaeus, 1761), *Ecliptopera silaceata* (Dennis &
276 Schiffermuller, 1775), *Operophtera fagata* (Scharfenberg, 1805), *Epirrhoe alternata* (Muller,
277 1764) and *Epirrhoe tristata* (Linnaeus, 1758) (Bogenschuetz et al. 1985; Chittamuru 2000;
278 Francke et al. 1998; Millar et al. 1992; Subchev et al. 1986; Szocs et al. 2004; Wong et al.
279 1985). None of these species was caught in the traps baited with ZZ6,9-19:H in our studies,
280 probably due, at least in part, to differences in flight seasons and distributions. *Trichopteryx*
281 *polycommata* flies from March to early May while *A. repandata* flies in June and July,
282 *B. piniarius* flies in May and June, *C. margaritata* flies from June to September, *E. silaceata*
283 flies from May to September, *O. fagata* flies from October to December, *E. alternata* flies
284 from May to September and *E. tristata* flies from May to July (Kimber 2018).

285 In Kent and Sussex, only *T. polycommata* were caught in the pheromone traps.
286 However, in Lancashire adults of *T. carpinata*, the only other species in the *Trichopteryx*
287 genus found in the UK, were also caught. Despite being more common than *T. polycommata*,
288 *T. carpinata* were trapped in lower numbers. This suggests that Z,Z6,9-19:H may not be the
289 complete pheromone blend for *T. carpinata*, and additional pheromone components may play
290 a role in maintaining reproductive isolation from *T. polycommata*. Moths of the two species

291 have different markings and can be distinguished and identified by eye. Thus, cross-attraction
292 does not present a problem for monitoring *T. polycommata* using pheromone lures, and
293 indeed, traps baited with this compound can potentially be used to monitor both species to
294 some extent.

295 The limited data acquired so far on the timing of response of *T. polycommata* males to
296 the pheromone indicate that this is much later than the times when surveys have previously
297 been carried out. Searches for *T. polycommata* by torchlight are typically carried out between
298 1900 h and 2130 h when the moths can be seen resting on *L. vulgare*. However, it is probable
299 that male moths are responding to the pheromone after this time, which may explain why few
300 moths were observed flying to the lures in the initial tests with volunteers. Consequently, in
301 order to use the lures effectively they must be deployed overnight in pheromone traps. For
302 successful trapping programs, optimum trap height for this species still needs to be
303 established, as height and trap design can have significant influence on number caught
304 (Yonce et al. 1976). If a pheromone trap is not available or appropriate, observations in this
305 study suggest that the lures should be used after midnight. Further investigation is needed to
306 identify when the males are most responsive to the pheromone and therefore the optimum
307 time to use the lures.

308 The lures loaded with 10 mg ZZ6,9-19:H attracted the highest numbers of *T.*
309 *polycommata*, but not significantly more than those attracted to lures containing 1, 2 and 5
310 mg. We therefore recommend the lower loadings for monitoring this species. Using 2 mg
311 pheromone lures to survey for *T. polycommata* increased the number of records in
312 Morecambe Bay, Lancashire from 107 to 881 and the number of sites where *T. polycommata*
313 has been recorded from 48 to 88.

314 Using pheromone traps requires less survey effort than searching by torchlight, so a
315 greater number of sites can be surveyed and therefore knowledge of distribution, status and
316 ecology of the species can be improved (Burman et al. 2016; Giangregorio 2015; Zauli et al.
317 2014). This has been demonstrated with *Synanthedon vespiformis*, and saproxylic beetles
318 *Osmoderma eremita* and *Elater ferrugineus* (Burman et al. 2016; Giangregorio 2015; Zauli et
319 al. 2014). Improved knowledge of insect distribution can be used to help inform management
320 practices and to predict the effects of factors such as habitat fragmentation (Giangregorio
321 2015; Zauli et al. 2014). The Biodiversity Action Plan (BAP) for *T. polycommata* identifies a
322 need to encourage survey work to gain a better understanding of the moth's distribution and
323 this pilot study clearly shows that pheromone monitoring achieved this. The pheromone lures

324 enable low-effort species-specific monitoring to be carried out by volunteers and
325 conservation organizations. Such activities will support other BAP actions, including
326 understanding the ecology of *T. polycommata*, and better managing the sites where it is
327 found.

328 In light of this successful pilot study, a nationwide survey of *T. polycommata* using
329 pheromone lures is now being conducted with a number of conservation organizations across
330 the UK. In most European countries, the population trends of *T. polycommata* are unknown
331 or assumed to be stable, except in Belgium where the species is reported to be no longer
332 present (JNCC 2010). International surveys using pheromone lures would contribute to *T.*
333 *polycommata* conservation programs across Europe, and could lead to rediscovery of
334 populations in places where it was previously thought to have become extinct.

335

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341 allowed access to their property in aid of insect conservation research, and Canterbury Christ
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343

344

345 **References**

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477 **Figure Captions**

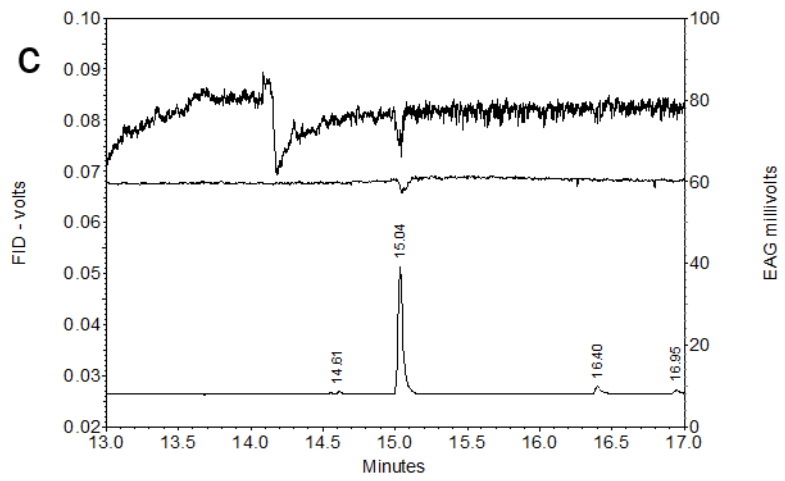
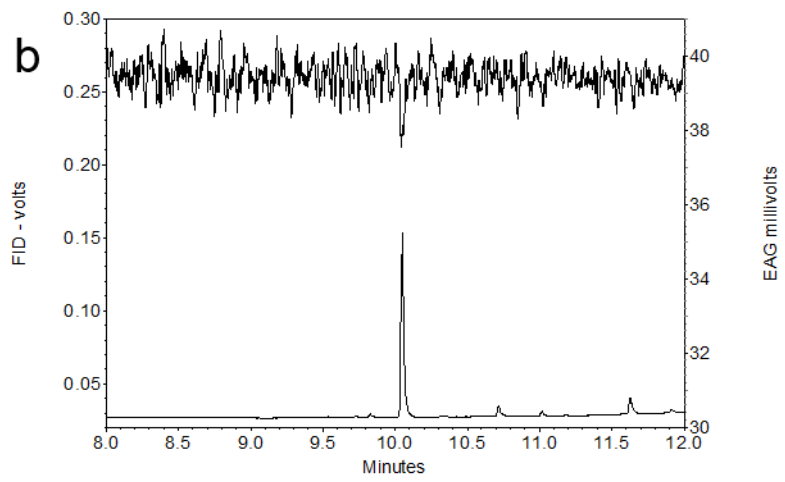
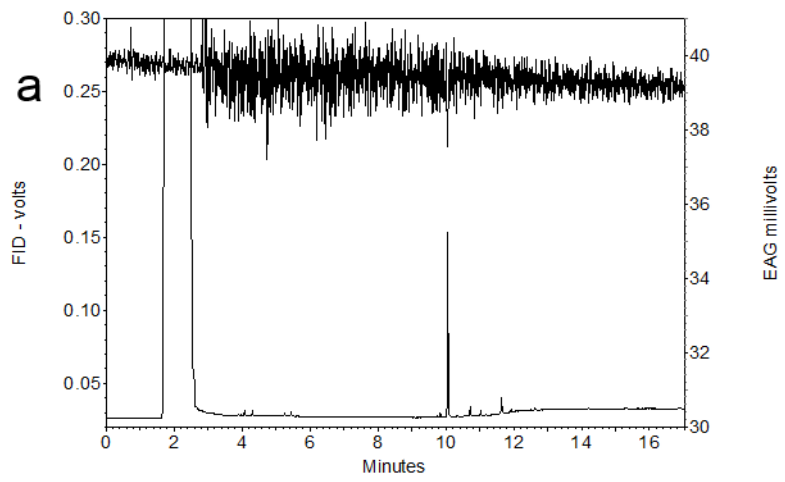
478 **Fig.1** Coupled gas chromatography-electroantennogram analyses of pheromone gland extract
479 from female *Trichopteryx polycommata* with antenna from male moth. Fig. 1(a) shows
480 complete analysis and (b) expanded portion with single EAG response to peak at 10.05 min
481 (run with temperature program of 20 °C min⁻¹); Fig. 1(c) additional runs with the same
482 extract and antenna of second male moth showing response to peak at 15.04 min (run with
483 temperature program of 10 °C min⁻¹). In each, top trace is the EAG response from the male
484 moth antenna; bottom trace is the GC-FID trace.

485 **Fig.2** Mass spectra of compound in pheromone gland extract from female *Trichopteryx*
486 *polycommata* (upper) and synthetic (Z,Z)-6,9-nonadecadiene (lower)

487 **Fig 3.** Mean catch night⁻¹ (\pm 95% CI) of *T. polycommata* at six sites using traps baited with
488 lures loaded with varying amounts of (Z,Z)-6,9-nonadecadiene. Trap catches were log (n+1)
489 transformed for analysis and back-transformed to the original scale for presentation. Different
490 letters indicate significant differences in mean catches (Tukey's test, $P < 0.05$)

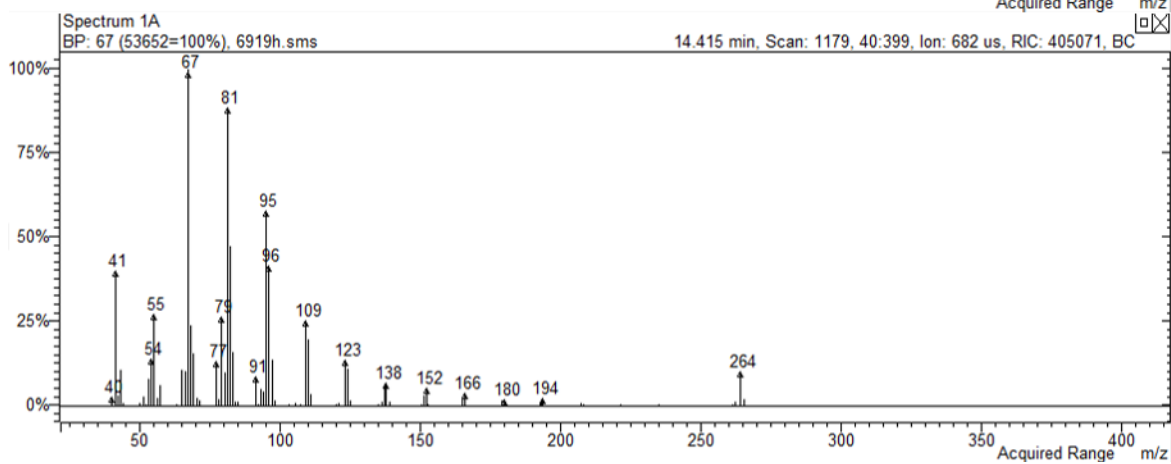
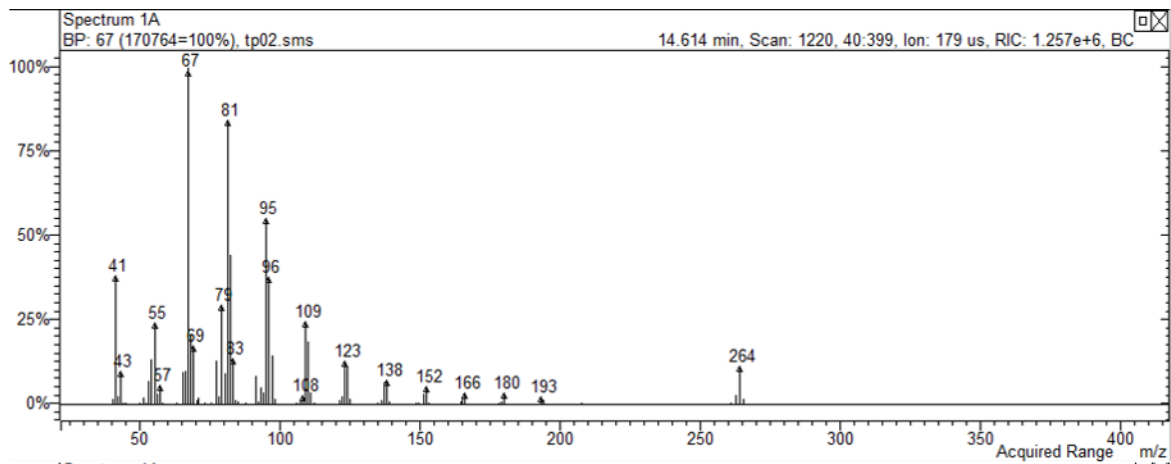
491 **Fig.4** Map of known geographical distribution of *Trichopteryx polycommata* in Morecombe
492 Bay, UK, before and after pheromone survey work in 2017. Circles are proportional to the
493 number of moths caught at any particular location. White circles represent trap catches at
494 locations where the species had already been recorded prior to the 2017 pheromone survey.
495 Black circles represent trap catches at locations where the moth has never been recorded
496 before but was attracted in the pheromone survey.

497



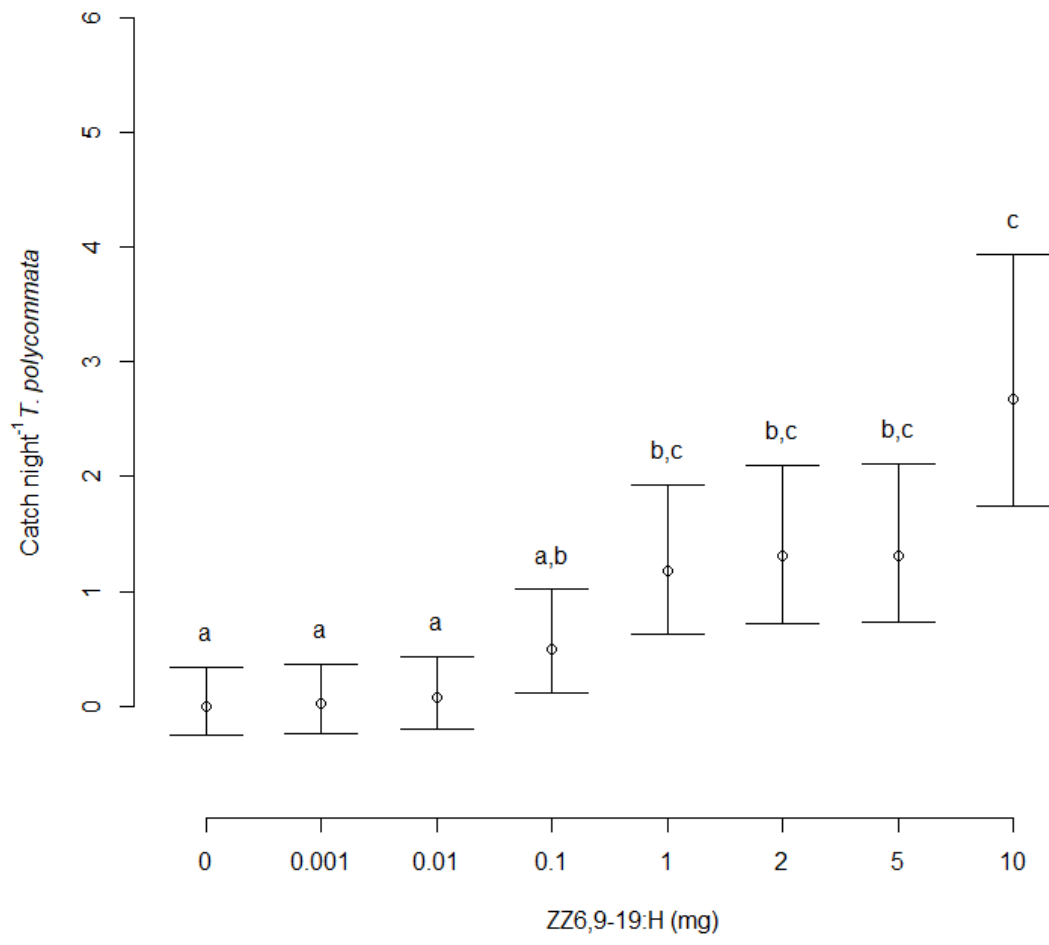
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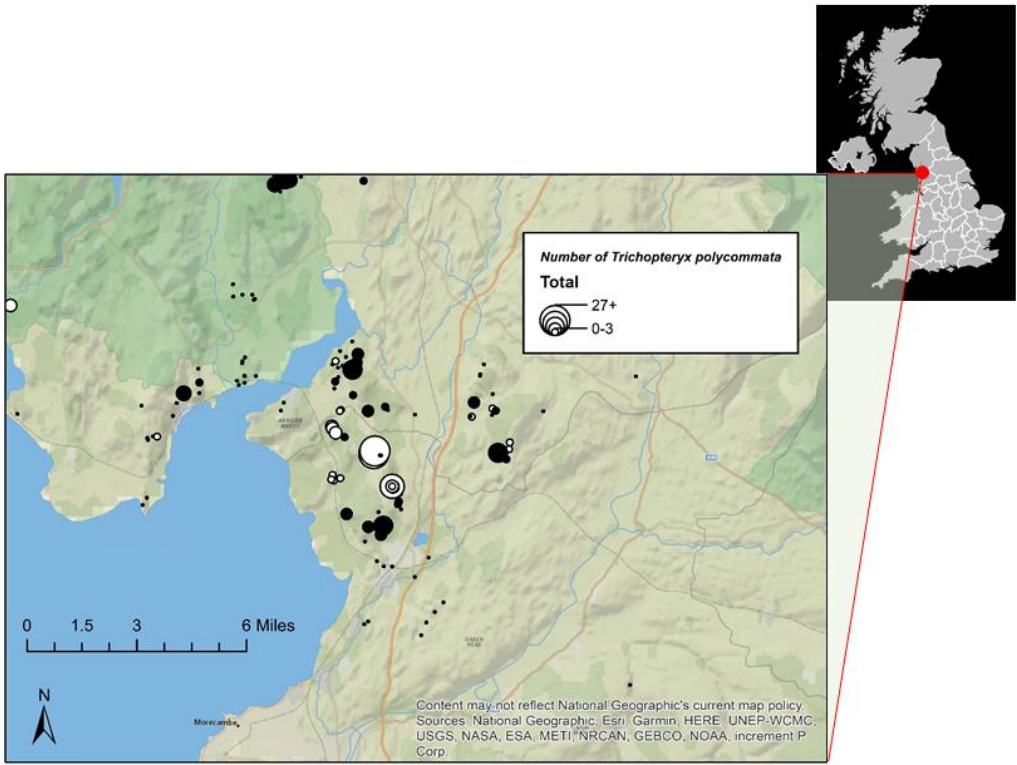


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