

1 **High prevalence of the digenean *Plagiorchis* sp. in the wood mouse *Apodemus***
2 ***sylvaticus* in a periaquatic ecosystem.**

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19 Running Title: High prevalence of *Plagiorchis* sp. in a periaquatic ecosystem.

20

21 SUMMARY

22 The prevalence of the digenean *Plagiorchis* sp. was investigated in a natural wood
23 mouse population (*Apodemus sylvaticus*) in a periaquatic environment. Classical
24 identification was complemented with the use of molecular differentiation to determine
25 prevalence and verify species identity. Use of the complete ITS1-5.8S rDNA-ITS2 and
26 partial 28S rDNA gene sequences have confirmed that the species reported at this
27 location was *Plagiorchis elegans* and not *Plagiorchis muris* as reported previously,
28 illustrating the difficulties in identification of these morphologically similar parasites. *P.*
29 *elegans* is typically a gastrointestinal parasite of avian species but has also been reported
30 from small mammal populations. The occurrence of this digenean in *A. sylvaticus* in the
31 UK is rare however in the area immediately surrounding Malham Tarn, Yorkshire, it had a
32 high prevalence of 23% and a mean worm burden of 26.6 ± 61.5 . The distribution of *P.*
33 *elegans* followed a typically overdispersed pattern and both mouse age and sex were
34 determined to be two main factors to be associated with prevalence. Male mice harboured
35 the majority of worms carrying 688 of 717 recovered during the study and had a higher
36 prevalence of 32.4% in comparison to only 8.7% in the small intestine of female mice. A
37 higher prevalence of 43% was also observed in adult mice compared to 14% for young
38 adults. No infection was observed in juvenile mice. These significant differences are likely
39 to be due to differences in the foraging behaviour between the sexes and age cohorts of
40 wood mice.

41

42 Key words: Plagiorchiidae; *Plagiorchis muris*; *Plagiorchis elegans*; wood mouse;
43 *Apodemus sylvaticus*; DNA; aquatic environment.

44

45 INTRODUCTION

46

47 Wild rodent populations are commonly examined for their helminth assemblages
48 and studies conducted worldwide have revealed a plethora of nematode, cestode and
49 trematode species being harboured by various rodent fauna. In the UK, several commonly
50 identified helminth species have been documented in rodents from different sites. The
51 most frequently reported digeneans of the wood mouse *Apodemus sylvaticus* tending to
52 be *Corrigia vitta* and *Brachylaemus recurvum* typically found infecting the pancreatic ducts
53 and the small intestine of their host respectively (Elton *et al.*, 1931; Lewis, 1968; Lewis and
54 Twigg, 1972; Behnke *et al.*, 1999; Abu-Madi 2000). The parasites of *A. sylvaticus* and
55 other small mammals have been extensively studied in populations located in a
56 periaquatic environment at the Malham Tarn Nature Reserve, North Yorkshire, UK,
57 including the discovery of a new parasite species (Allan *et al* 1999; Hughes *et al* 2006,
58 2008; Rogan *et al* 2007; Hide *et al* 2009; Thomasson *et al* 2011; Boyce *et al* 2012).
59 Specifically, Rogan *et al.*, (2007) reported the occurrence of the intestinal digenean
60 *Plagiorchis muris* in the wood mouse *A. sylvaticus* over a 13-year period with an overall
61 prevalence of 16.9%.

62 The occurrence of *P. muris* within the UK is rare. As far as can be determined, the
63 first report of *P. muris* in the UK occurred in Oxford during a study on the health of a wild
64 mouse population which took place from September 1925 to January 1928 (Elton *et al.*,
65 1931). During this study the digenean *Lepoderma muris* (syn. *P. muris*) was described
66 from the small intestine of the wood mouse *A. sylvaticus* at a very low prevalence of 0.1%.
67 *L. muris* was furthermore reported in the brown rat *Rattus norvegicus* in Cambridgeshire,
68 UK (Baylis, 1939) and later an occurrence of *P. muris* was recorded in Scotland in 1963
69 when the digenean was unexpectedly recovered from the intestine of a Scottish Hill sheep
70 during a parasitological necropsy (Fahmy and Rayski, 1963). These studies, including

71 those of Rogan *et al.*, (2007) were all based on classical parasite identification using
72 morphology and have not benefited from the greater precision available for DNA
73 sequencing analysis.

74 Members of the genus *Plagiorchis* are cosmopolitan and tend to demonstrate low
75 definitive host specificity. Species of this genus have been previously described from the
76 intestines of reptiles and birds in addition to mammals (Janssen and Bock, 1990; Biserkov
77 and Kostadinova, 1998; Ito and Itagaki, 2003). *P. muris* is no exception. This species was
78 originally described from the small intestine of the black rat, *Rattus rattus* and the brown
79 rat, *Rattus norvegicus* by Tanabe in Kyoto, Japan (Tanabe, 1922) and has since been
80 considered to be predominantly a digenean of wild rodents (Elton *et al.*, 1931; Seo *et al.*,
81 1964; Ito and Itagaki, 2003; Chai *et al.*, 2007; Rogan *et al.*, 2007). Definitive host variability
82 for *P. muris* has, however been, frequently recorded including that of the domestic dog,
83 *Canis familiaris* in Japan (Saito *et al.*, 1995), the feral Japanese raccoon, *Procyon lotor*
84 (Yamada, 2000; Sato and Suzuki, 2006) and the Mexican Greater funnel-eared bat;
85 *Natalus mexicanus* (Perez-Ponce de Leon *et al.*, 1996) in addition to several cases of
86 natural avian infection in the USA (McMullen, 1937; Cort and Ameel, 1944; Secord and
87 Canaris, 1993). Typically, *P. elegans* has been considered foremost in the genus
88 *Plagiorchis* for infecting birds (Shimalov, 2002) however this species has also been
89 reported to parasitize several species of rodent, including *A. sylvaticus* (Montgomery and
90 Montgomery, 1990a), the yellow necked mouse, *Apodemus flavicollis* (Hildebrand and
91 Zaleśny, 2009), the striped field mouse, *Apodemus agrarius* (Shimalov, 2002; Hildebrand
92 and Zaleśny, 2009), and the bank vole, *Myodes glareolus* (Hildebrand and Zaleśny, 2009).

93 Little is known about the exact life cycle of *Plagiorchis spp.* that infect these rodents
94 and, in particular, the identity and role of intermediate hosts. The high prevalence reported
95 in this periaquatic site (Rogan *et al.*, 2007) suggests that the presence of water and
96 aquatic organisms might be key factors. The unique nature of this site and the

97 development of molecular tools for detection and identification of these parasites presents
98 an opportunity to dissect the ecology of this parasite and its hosts.

99 In the current study we investigate the rare occurrence of *Plagiorchis muris* at
100 Malham Tarn, Yorkshire, UK (Rogan *et al.*, 2007) by examining prevalence, intensity and
101 seasonality of adult stages collected from rodents trapped at defined woodland sites
102 around this upland lake. We furthermore highlight the difficulty in distinguishing *P. muris*
103 and *P. elegans* in the absence of molecular tools and investigate life cycle indicators within
104 this periaquatic environment.

105

106 MATERIALS AND METHODS

107

108 The study was carried out at Malham Tarn Nature Reserve located in North
109 Yorkshire, Northwest England at an altitude of 375m above sea level and is an area that
110 has previously been investigated for a range of host parasite systems (Kennedy and
111 Burrough, 1978; Allan *et al.*, 1999; Hughes *et al.*, 2006, 2008; Rogan *et al.*, 2007; Behnke
112 *et al.*, 2009; Hide *et al.*, 2009; Thomasson *et al.*, 2011; Boyce *et al.*, 2012).

113 Rodent trapping and examination was conducted according to the methods
114 described by Boyce *et al.*, (2012). In total 117 wood mice (*Apodemus sylvaticus*) and 63
115 voles (54 bank voles *Myodes glareolus* and 9 field voles *Microtus agrestis*) were trapped
116 from four sampling sites around Malham Tarn: Tarn Woods, Tarn Fen, Spiggot Hill and Ha
117 Mire Plantation between January 2010 and October 2011 (**Figure 1**). A permit was
118 granted by the National Trust to allow sampling within the boundaries of the reserve.
119 Plantations within these boundaries are represented by Tarn Woods, Ha Mire Plantation
120 and Spiggot Hill. Ownership boundaries form part of the reserve perimeter restricting
121 sampling in part. Tarn Fen is an area of raised bog covered by deciduous woodland
122 located within the reserve boundaries. As water is considered to be an important aspect in

123 the life cycle of *Plagiorchis* these four trapping sites were selected in order to cover a vast
124 area of woodland throughout the reserve, which is located in close proximity to each of the
125 tarn's borders.

126 Morphological examination of the digenean specimens recovered from the small
127 intestine of *A. sylvaticus* was carried out according to the methods described by Boyce *et*
128 *al.*, (2012). The majority of the specimens were fixed in 70% ethanol suitable for molecular
129 analysis. DNA was extracted from 12 individual worms collected from different host
130 specimens of *A. sylvaticus* when feasible using a phenol: chloroform method modified from
131 Thomasson *et al.*, (2011) which encompassed halving the amount of reagent at each
132 stage of the protocol. DNA was extracted from three individual worms isolated from three
133 different wood mice trapped from each of the four sampling sites. Only one wood mouse
134 however was found to be infected from Spiggot Hill therefore three individual worms from
135 the same host specimen needed to be used in this instance.

136 The Internal transcribed spacer (ITS), including the ITS1, 5.8S, ITS2 and flanking
137 regions of the 3' end of the 18S and 5' end of the 28S were amplified using the forward
138 universal primer BR (5'GTAGGTGAACCTGCGGA^{3'}) and reverse digenean specific primer
139 dig11 (5'GTGATATGCTTAAGTTCAGC^{3'}) according to Tkach *et al.*, (2000a). The partial
140 28S rDNA gene region was amplified using the forward digenean specific primer dig12
141 (5'AAGCATATCACTAAGCGG^{3'}) and the reverse universal primer Lo
142 (5'GCTATCCTGAGRGAACTTCG^{3'}) according to Tkach *et al.*, (2000b).

143 Each 50µl PCR reaction contained 5µl 10X DreamTaq buffer including 2mM MgCl₂
144 (Fermentas, Life Sciences), 0.05µmol dNTPS (100mM, Bioline), 2.5µM forward primer,
145 2.5µM reverse primer, 5U DreamTaq DNA polymerase and 2µl DNA template. All PCR
146 reactions were performed using a Robocycler 96 PCR machine (Stratagene, CA) and
147 visualised on a 1% (w/v) Tris-acetate-EDTA (TAE) agarose gel stained with gel red using a
148 G: Box gel imaging system (Syngene, UK). The amplification profile consisted of 1 cycle at

149 94°C for 10 minutes, followed by 35 cycles of 1 minute at 94°C, 1 minute at 54°C and 1
150 minute at 72°C and one final cycle at 72°C for 10 minutes. The target bands were excised
151 from the gel using a UV transilluminator and purified using a PCR purification kit
152 (Geneflow) according to the manufacturer's instructions. Samples were commercially
153 sequenced in both directions (Source Bioscience, Nottingham, UK). The 12 DNA
154 sequences for the ITS gene were primarily aligned using the multiple sequence alignment
155 program ClustalW (www.genome.jp/tools/clustalw/) to check for sequence homology
156 between specimens from each of the four sampling sites. This procedure was repeated
157 with the 28S rDNA data. In both instances FinchTV trace viewer (Geospiza, Seattle, WA)
158 was utilised in order to verify any regions of ambiguity. The two DNA sequences generated
159 for the ITS and 28S rDNA from Malham Tarn were compared with those held in the
160 National Center for Biotechnology Information (NCBI) database using the BLAST program
161 (www.blast.ncbi.nlm.nih.gov/Blast.cgi).

162 Differences in prevalence observed between sampling sites and seasons were
163 investigated using Chi-squared test for heterogeneity. Host sex and host age were
164 statistically analysed using 2 x 2 contingency tables using Fisher's exact test
165 (<http://www.graphpad.com/quickcalcs/ConfInterval2.cfm>). For determination of age,
166 rodents were split into three age cohorts according to Behnke *et al.*, (1999). Associations
167 between prevalence and host length (cm), weight (g) and rainfall data (mm) were analysed
168 using Spearman's rank of correlation. Monthly rainfall data was provided by Malham Tarn
169 Field Centre. Prevalence calculated during each season over a two-year period was
170 analysed in relation to the previous three months rainfall (mm) adapted from Rogan *et al.*,
171 (2007).

172 In an attempt to identify life cycle indicators of *Plagiorchis* sp. at Malham Tarn,
173 molluscan species were also collected quarterly between January 2010 and October 2011
174 in order to examine for intramolluscan stages. Several water bodies located within close

175 proximity to the rodent trapping sites including the tarn margin were selected for analysis.
176 Snails were collected using a D-frame aquatic dip net and kick sample technique and were
177 also hand-picked from the stems of vegetation and underlying surfaces of rocks. Snails
178 were speciated according to Macan and Cooper (1960) and housed in the laboratory in 4
179 litre glass covered tanks containing pond water. Snails were maintained at 4°C and fed
180 washed lettuce *ad libitum* according to Voutilainen *et al.*, (2009). For the examination of
181 *Plagiorchis* sp. intramolluscan larval stages, a snail crushing method was employed
182 according to Caron *et al.*, (2008). Binomial confidence intervals on parasite prevalences
183 were calculated ($P = 0.05$, two-tailed test), based on standard methods
184 (<http://statpages.org/confint.html>).

185

186 RESULTS

187 Five helminth species were recorded from the sampled wood mouse population;
188 *Heligmosomoides polygyrus*, *Syphacea oblevata*, *Capillaria murissylvatici*, *Brachylaemus*
189 *recurvum* and *Plagiorchis* sp. with species richness varying from 0 to 5 in individual
190 animals. *Plagiorchis* sp. was not detected from any of the vole species despite careful
191 observations.

192 In total, 717 *Plagiorchis* worms were successfully recovered from the small intestine
193 of 27 of 117 examined wood mice between January 2010 and October 2011. An overall
194 prevalence rate of 23% was determined from all four sampling locations: Tarn Woods,
195 Tarn Fen, Spiggot Hill and Ha Mire Plantation indicating this digenean species to be
196 dispersed throughout the Malham Tarn area. Worm burden ranged from 0 to 275 with a
197 mean of 26.6 ± 61.5 (717/27) appearing to be typically overdispersed in distribution
198 (Variance to mean ratio (VMR): $\sigma^2/\mu = 158.89$) with 84.6% of wood mice being infected
199 with zero or just a single worm in comparison to just 1.7% harbouring the majority of
200 parasites (>100 worms). A goodness of fit indicated a good agreement with the negative

201 binomial distribution ($G_{\text{calculated}} = 21.17$, $df = a - 3$ ($12 - 3$) is 21.67 , $p = 0.01$). *Plagiorchis*
202 sp. was identified following the microscopical analysis of 10 fixed and unstained
203 specimens.

204 The adult worm (**Figure 2**) measured 1.66 to 2.93mm (mean 2.64mm) in length by
205 0.46 to 0.76mm (mean 0.58mm) in width at the widest part (across the region of the most
206 anterior testis). The tegument possesses minute spines covering the entire surface. The
207 ventral surface appears to be obscured by a vast array of vitelline glands that fail to
208 maintain confluency within the anterior region. The oral sucker is roughly spherical in
209 shape and measures 200 to 300 μm in length (mean 256 μm) by 200 to 300 μm (mean
210 247 μm) in width. The oral sucker lies anterior to the pharynx from which the intestinal
211 bifurcation occurs. The intestine appears short and indistinct and thereafter extends into
212 two very long blindly ending caeca that are often difficult to observe, commonly being
213 masked by a copious mass of vitelline glands, but reaching the near posterior extremity of
214 the body. The ventral gland is smaller in size than that of the oral sucker measuring 130 to
215 200 μm (mean 163 μm) in length by 130 to 200 μm (mean 163 μm) in width. These
216 measurements indicate an oral to ventral sucker ratio of 1.52: 1 (width ratio) – 1.57: 1
217 (length ratio). Two oval shaped testes are situated posterior to a single ovary. The testes
218 are obliquely positioned with the anterior testis situated slightly right of the median line and
219 the most posterior testis to the left. The ovary lies just posterior to the ventral sucker,
220 separated by the cirrus sac, which curves in a posterior direction along the left hand side
221 of ventral sucker. The metraterm can be visualised to curve posteriorly along the right
222 hand side of the ventral gland adjoining the anterior region of the uterus when not
223 obscured by the vitelline glands. The uterus extends to the posterior extremity of the body
224 presenting a characteristic s-shape that reaches from the region of the ovary and
225 continues intertesticularly towards a posterior vitellarian commissure. The vitelline glands
226 continue along both lateral sides, from the far extremity of the hind body and into the

227 forebody surpassing the ventral sucker and creating confluency often up to the posterior
228 border of the pharynx. The morphological features described in this section hold a
229 sufficient similarity for both *P. muris* and *P. elegans* therefore the use of morphology alone
230 could be considered ambiguous and the use of molecular differentiation be regarded an
231 important means to complement species classification in this case.

232 DNA was successfully extracted and amplified from 12 individual *Plagiorchis* worms
233 recovered from the small intestine of 10 *A. sylvaticus* mice trapped from the four different
234 sites. Amplification of the internal transcribed spacers (ITS) including the ITS1, 5.8S and
235 ITS2 generated a sequence of 1213bp (GenBank accession: JX522536). The 12 ITS
236 sequences generated from all four sampling sites were 100% identical. The ITS sequence
237 generated from Malham Tarn was compared against five DNA sequences generated from
238 *Plagiorchis* adults that were held in the NCBI database: *P. maculosus* (AF316152)
239 collected from the Chaffinch, *Fringilla coelebs* (Snyder and Tkach, 2001), *P. elegans*
240 (AF151952) collected from The Red-Backed Shrike, *Lanius collurio*, *P. koreanus*
241 (AF151944) collected from Kuhl's pipistrelle, *Pipistrellus kuhli*, the common noctule,
242 *Nyctalus noctula* and Daubenton's bat *Myotis daubentoni*, *P. vespertilionis* (AF151949)
243 from *M. daubentoni* and *P. muelleri* (AF151947) from the serotine bat *Eptesicus serotinus*
244 all obtained within the Ukraine (Tkach *et al.*, 2000a). The ITS sequence from Malham Tarn
245 shared a 100% sequence homology with that of *P. elegans* with only one omission of
246 adenosine at site 571. This omission was observed in all 12 generated DNA sequences.
247 The Malham Tarn sequence shared only 94% sequence homology with *P. maculosus*,
248 89% with *P. koreanus*, 91% with *P. vespertilionis*, and 90% with *P. muelleri*.

249 Amplification of the 28S rDNA gene generated a partial sequence of 1263bp
250 (GenBank accession: JX522535). The 12 28S rDNA sequences generated from all four
251 sampling sites were also 100% identical. This sequence was also compared against five
252 available DNA sequences from the NCBI database: *P. elegans* (AF151911) (Tkach *et al.*,

253 1999), *P. muris* (AF096222) obtained from the intestine of a rat in the Republic of Korea
254 (Lee *et al.*, 2004), *P. muelleri* (AF184250) (Tkach *et al.*, 2001), *P. koreanus* (AF151930)
255 and *P. vespertilionis* (AF151931) collected from *N. noctula* in the Sumy region of the
256 Ukraine and *M. daubentoni* in the vicinity of Kiev, Ukraine (Tkach *et al.*, 2000b). The partial
257 28S rDNA sequence available for *P. muris* (AF096222) was only 304bp in length (Lee *et*
258 *al.*, 2004). All other available 28S sequences for *Plagiorchis* species including the
259 sequence generated from Malham Tarn were therefore trimmed and aligned with this
260 sequence for *P. muris*. The 28S sequence generated for specimens collected from
261 Malham Tarn again shared a 100% sequence homology with that of *P. elegans*, 98% with
262 *P. muelleri*, *P. koreanus* and *P. vespertilionis* and only a 95% match with *P. muris* (**Figure**
263 **3**). This data in combination with the 100% sequence homology match demonstrated by
264 the ITS region, questions the identity of *P. muris* at Malham Tarn and infers that the
265 species present at this location is in fact that of *P. elegans*. For the remainder of this
266 paper, references to *Plagiorchis* sp. in this study should be read as *P. elegans*. The
267 prevalence of *P. elegans* at this location was examined.

268 Prevalence was analysed in relation to both extrinsic and intrinsic factors. During
269 the study a comprehensive data set was established which recorded trapping location,
270 date, host sex, host weight and host length. All prevalence data, 95% confidence limits and
271 mean intensities have been summarised in **Table 1**.

272 To determine whether there was an association with prevalence and a particular
273 trapping site, the rate of prevalence was examined between the four sampling sites using
274 chi squared test for heterogeneity. The greatest prevalence was observed at Ha Mire
275 Plantation in which 37.03% (n = 27) of sampled wood mice carried a mean intensity of
276 19 ± 24.2 worms (187/10). The prevalence at Tarn Woods which is the original sampling site
277 reported by Rogan *et al.*, (2007) was less with only 23.07% (n = 52) of wood mice being
278 infected despite a slightly higher mean intensity of 21 ± 50.33 (246/12). At Tarn Fen, only

279 four of the 21 examined wood mice were infected with *P. elegans* giving a prevalence of
280 19.05%. A very low worm burden of just 2 ± 2.5 (9/4) was also observed at this site. Only a
281 single wood mouse was infected with *P. elegans* at Spiggot Hill providing the lowest
282 prevalence of the study at 5.88% (n = 17). This mouse however harboured 275 worms, the
283 highest number recorded during the study. No significant heterogeneity was found
284 between the prevalence of *P. elegans* and any of the four sites ($X^2 = 3.79$, $p = 0.05$, $v = 3$).

285 As studies of *Plagiorchis* infection in natural rodent populations have demonstrated
286 seasonal variation in prevalence and intensity, we examined this hypothesis at Malham
287 Tarn. Mean prevalence and mean intensity was calculated each season over a two year
288 period. Both mean prevalence and mean intensity were zero during the winter (January)
289 sampling periods (n = 8). Only one mouse was infected during the spring (April) giving a
290 prevalence of 12.5% (n = 8). This mouse however harboured 179 worms. Peak prevalence
291 occurred during the summer (July) when 27.3% (n = 22) of mice carried a mean intensity
292 of 3 ± 2.94 worms (20/6). Prevalence thereafter decreased slightly to 25.3% (n = 79) during
293 the autumn (September/October) however mean intensity increased considerably to
294 26 ± 61.46 worms (518/20) for this period. Despite observable differences in prevalence no
295 significant heterogeneity was found between the prevalence of *P. elegans* and season (X^2
296 = 3.38, $p = 0.05$, $v = 3$). A Spearman's rank of correlation also indicated a very weak but
297 significant correlation ($r = 0.095$, $P = <0.05$) between the prevalence of *P. elegans* and
298 rainfall for the three months preceding each sampling session (**Table 2**).

299 In total, 71 male mice and 46 female mice were examined. Prevalence in male mice
300 was greater at 32.4% in comparison to only 8.7% for female mice. Male mice furthermore
301 harboured the majority of worms carrying 688 of 717 recovered with a mean intensity of
302 30 ± 66.09 (688/23) against only 7 ± 12.5 (29/4) for female mice. A highly significant
303 difference between *P. elegans* infection and *A. sylvaticus* sex was identified ($p = 0.003$).

304 The data was furthermore analysed to determine whether there was an age-
305 dependent prevalence occurring at this location. *P. elegans* was recorded in a total of 19
306 out of 44 adult wood mice (43.2%) which was greater than that observed for young adult
307 mice (14.3%, n = 56). No juvenile mice were infected during the present study (n = 17). A
308 similar pattern was observed for mean intensity with adult wood mice harbouring the vast
309 majority of infection carrying 677 of 717 worms recovered. Adult males (n = 34) however
310 carried 650 of these worms in comparison to just 27 worms carried by adult female mice (n
311 = 10). Mean worm burden in the adult group was 36 ± 71.72 (677/19) in comparison to only
312 5 ± 7.86 (40/8) for young adults and zero for juvenile mice. No significant difference was
313 observed between the juvenile and young adult age categories ($p = 0.185$) however the
314 difference between adult wood mice and young adults ($p = 0.002$) and adult and juvenile
315 ($p = 0.001$) age cohorts was found to be highly statistically significant. Wood mice ranged
316 in weight from 4.0 to 29g and length from 4.5 to 9.8cm. A Spearman's rank of correlation
317 using these data against prevalence gave a very strong correlation in both cases ($r =$
318 0.929 $P = <0.05$ and $r = 0.955$, $P = <0.05$ respectively) indicating an age-dependent
319 prevalence to be evident at this location.

320 In an attempt to identify life cycle indicators of *P. elegans* at Malham Tarn, a total of
321 2021 snails consisting of 11 species were examined for intramolluscan stages by crushing
322 (**Table 3**). No larval stages of *P. elegans* were found in any of the snails despite careful
323 observation and the frequent detection of other trematode larvae such as *Notocotylus*.
324 Binomial confidence intervals were calculated ($P = 0.05$, two-tailed test) to establish
325 whether zero prevalence was a significant result using these samples. The results
326 however indicate that none of the examined snail species can be currently ruled out as a
327 potential intermediate host for *P. elegans* with zero prevalence not being significant given
328 the small sample sizes. Despite this for snail species where sample sizes were high, very
329 low prevalences (of less than 1%) are likely. Further investigation is required.

330

331 DISCUSSION

332

333 In the present study we employed the use of molecular differentiation to investigate
334 further the occurrence of *Plagiorchis muris* at Malham Tarn. Results indicate that the
335 currently identified specimens of *P. muris* at this location are *Plagiorchis elegans*. As far as
336 we can determine, our previous report (Rogan *et al.*, 2007) was the fourth known report of
337 *P. muris* in British wildlife (Elton *et al.*, 1931; Baylis 1939; Fahmy and Rayski, 1963)
338 although the conclusion from this study indicates that this trematode was, in fact, *P.*
339 *elegans*, and also raises the question as to whether other previous studies have correctly
340 identified the species. Elton *et al.*, (1931) reported only a prevalence and Baylis, (1939)
341 simply listed an occurrence of the digenean. Neither author described the morphology of
342 the parasite involved nor was DNA sequencing an aspect of biological surveys at that time.
343 The third report by Fahmy and Rayski (1963) was a short report which provided no written
344 account, but rather incorporated a diagram as a means of description. Unfortunately, the
345 diagram contained insufficient detail to clarify its status as *P. muris* from that of *P. elegans*.

346 Previously, Fahmy (1954) described a new species of *Plagiorchis*, *P. lutrae* from the
347 otter (*Lutra lutra*) in Scotland. This new species closely resembled the description of *P.*
348 *muris* but was differentiated on the basis of size. The measurements of this new species
349 did however coincide with those provided in the original description of *P. muris* described
350 by Tanabe in Kyoto, Japan in 1922; despite such *P. lutrae* was compared with *P. muris*
351 described by McMullen (1937) from Douglas Lake in Michigan State, USA which was
352 much larger in size.

353 The description of *P. muris* from various locations appears ambiguous. The majority
354 of reports describing *P. muris* from outside of Southeast Asia indicate both a greater range
355 in length and oral to ventral sucker ratio. The description of *P. muris* by McMullen (1937)

356 was based upon adult digeneans recovered from a range of experimental hosts in addition
357 to a range of naturally infected avian fauna. The average length and oral to ventral sucker
358 ratio of these specimens was however beyond the maximum dimensions reported for
359 Southeast Asian *P. muris* (Tanabe, 1922; Seo *et al.*, 1964).

360 Interestingly, the measurements provided for *P. muris* by McMullen (1937), Rogan
361 *et al.*, (2007) and during the current study overlap with the description generated for *P.*
362 *elegans* from a PhD study conducted by Gorman at Leeds University in 1980.
363 Experimental evidence provided by Gorman (1980) did however identify intraspecific
364 variation within a pure strain of *P. elegans*. The study identified various manifestations in
365 several anatomical structures, not only between different definitive host species but
366 furthermore within the same definitive host including differences in the extent of the
367 vitellaria and aperture shape of both the oral and ventral suckers (Gorman, 1980).
368 Confluency of the vitelline glands have been used on several occasions as a means of
369 differentiation for *Plagiorchis* species (Fahmy, 1954; Tkach *et al.*, 2000a) however as
370 pointed out by Blankespoor (1974) and Gorman (1980) use of the glands for diagnosis
371 may not be appropriate due to the intraspecific variation observed in this feature. For
372 instance the vitelline glands of *P. muris* have been reported to extend to either the
373 posterior border of the pharynx (Tanabe, 1922; Hong *et al.*, 1996) or the level of the oral
374 sucker (Fahmy and Rayski, 1963; Seo *et al.*, 1964; Hong *et al.*, 1998). Hong *et al.*, (1996)
375 nonetheless described *P. muris* from a human case of plagiorchiasis using the positioning
376 of the vitelline glands to morphologically differentiate *P. muris* from both *P. vespertilionis*
377 and *P. koreanus*. There was however no mention of *P. elegans* in this report despite this
378 species appearing to display the most morphological similarity to *P. muris* in the
379 distribution of the vitellaria. As far as can be determined there have currently been no
380 reported cases of *P. elegans* infection in either Korea or Japan where *P. muris* appears to
381 be considered the typical dominant *Plagiorchis* species found in rodents.

382 Currently, there appears to be ambiguity in the criteria used to morphologically
383 differentiate *P. muris* and *P. elegans*. Fortunately, the use of DNA sequencing could be
384 employed in the current study to confirm the identity of the Malham Tarn specimens. The
385 use of the internal transcribed spacer regions and the 28S rDNA gene indicate the
386 specimens recovered from Malham Tarn to be *P. elegans*. Based on these results and
387 taking into consideration the unreliability of morphological differentiation for the two
388 species in question (Blankespoor, 1974; Gorman, 1980; Hong *et al.*, 1998), it could be
389 speculated that other reports describing *P. muris* based purely on morphology have also
390 misidentified the species involved. For example, in his report McMullen (1937) commented
391 that the cercariae used for experimental infection possessed seven or eight pairs of
392 penetration glands on either side of the stylet which is a combination typical of *P. elegans*
393 (Faltýnková *et al.*, 2007) as opposed to the four pairs originally described for *P. muris* by
394 Tanabe (1922). This morphological description provided for *P. muris* by McMullen (1937)
395 has since been a basis for morphological comparison made by some European authors
396 (Fahmy, 1954; Rogan *et al.*, 2007).

397 Despite the questionable identity over *Plagiorchis* at Malham Tarn, the occurrence
398 of this digenean at this location within the UK is nonetheless considered rare, in particular
399 with a consistent prevalence recorded since 1993 (Rogan *et al.*, 2007). Furthermore, the
400 overall prevalence rate of 23% recorded during this study appears to be much greater than
401 that reported in the literature. Other UK reports involving either *P. muris* or *P. elegans*
402 have encompassed very low prevalence rates of 0.1% and 0.05% respectively (Elton *et*
403 *al.*, 1931; Montgomery and Montgomery, 1990a). A further two reports of *P. muris* in the
404 wood mouse *A. sylvaticus* in Ireland have also indicated very low prevalence rates of 1%
405 or less (Langley and Fairley, 1982; O'Sullivan *et al.*, 1984). Further afield, Ito and Itagaki,
406 (2003) reported a prevalence of just 1.7% in the large Japanese field mouse *Apodemus*
407 *speciosus* in Japan and Chai *et al.*, (2007) recorded an overall prevalence of 5.3% in the

408 striped field mouse *Apodemus agrarius* in Korea. *P. elegans* does however appear to be
409 the species reported most often from small mammals within Europe. Hildebrand and
410 Zaleśny (2009) reported a prevalence of 1.3% in the bank vole *Myodes glareolus* trapped
411 in Poland. A single specimen of *P. elegans* recovered from *M. glareolus* in Pallasjärvi,
412 Finland, gave a prevalence of just 0.5% (Tenora *et al.*, 1983) and a slightly higher
413 prevalence rate of 3.1% was reported by Shimalov (2002) from *A. agrarius* in Belarus.

414 The reasons for the occurrence of *P. elegans* at such a high prevalence at Malham
415 Tarn are unclear. Malham Tarn is a 'Site of Special Scientific Interest' (SSSI) boasting a
416 vast array of plant and animal species. The surface area of the tarn is approximately 150
417 acres with an average depth of 2.4m and a maximum depth of 4.4m in various regions
418 (Woof and Jackson, 1988). Similarly to this study, previous studies have observed the
419 occurrence of *Plagiorchis* in regions of close proximity to significant water bodies (Cort and
420 Olivier, 1943; Cort and Ameel, 1944; Bock, 1984; Hong *et al.*, 1999; Hildebrand and
421 Zaleśny, 2009). Being the only upland marl lake of its kind in Britain (Rogan *et al.*, 2007), it
422 could be speculated that Malham Tarn itself may play an important role by providing
423 important breeding sites for intermediate host species. Molluscs of the genus *Lymnaea* are
424 the dominant snails acting as the first intermediate host for *Plagiorchis* species worldwide
425 (Tanabe, 1922; Velasquez, 1964; Bock, 1984; Manga-Gonzalez *et al.*, 1994; Zakikhani
426 and Rau, 1999; Väyrynen *et al.*, 2000; Faltýnková *et al.*, 2007). Four species of *Lymnaea*
427 have been recorded at Malham Tarn including *L. stagnalis*, *L. peregra*, *L. palustris* and *L.*
428 *truncatula* (Norris, 2003) although currently none of these hosts have been positively
429 implicated in the life cycle of *P. elegans* at this location. Negative infections, in all of the
430 1603 *Lymnaea* specimens examined, suggest that these are unlikely to be intermediate
431 hosts, but this is difficult to absolutely rule out. Insects act as the second intermediate host
432 and dragonflies are the most commonly reported insects found to be naturally infected
433 (Hong *et al.*, 1999). Malham Tarn is home to several species of dragonfly (Shorrocks and

434 Sutton, 2010) however further study is required in order to identify first and second
435 intermediate host species involved in transmission at this location.

436 In the present study, *P. elegans* was recorded from all four trapping sites and no
437 association between prevalence and rainfall amount was identified suggesting that
438 temporary water bodies may not be an important determinant for transmission and that
439 rather infection is related to the presence of intermediate host species that breed within the
440 tarn body itself. Each trapping site however is separated from the tarn by a narrow shingle
441 beach and earth ridge, terrain that wood mice are unlikely to cross. A crude morphological
442 examination of the stomach contents of *A. sylvaticus* (n = 117) at Malham Tarn
443 demonstrated the presence of adult insect remains suggesting the main source of infection
444 for *A. sylvaticus* to be adult insects infected with metacercariae that may migrate into the
445 home range of the wood mouse following emergence from the tarn body. Other studies
446 have also indicated the diet of *A. sylvaticus* to include various adult insects (Montgomery
447 and Montgomery, 1990b; Khammes and Aulagnier, 2007).

448 The distribution of *P. elegans* at Malham Tarn demonstrated a typical pattern of
449 over-dispersion with rodent age and sex being the two main factors associated with
450 prevalence. Khammes and Aulagnier (2007) used three age categories to examine the
451 differential diet of *A. sylvaticus*; juvenile, sub-adults and adults and indicated arthropod
452 remains to be more abundant in the stomach contents of adult mice. In the present study,
453 the prevalence of *P. elegans* was significantly greater in adult mice than younger age
454 cohorts. This is likely due to differences in exposure to infective stage parasites through
455 differences in diet as a result of adult mice demonstrating greater foraging behaviour
456 (Lewis, 1968; Lewis and Twigg, 1972).

457 Several studies have indicated a change in the feeding habits of *A. sylvaticus* from
458 a granivorous diet to one that consumes animal material during periods when seeds are
459 scarce during the spring and early summer months (Lewis, 1987; Montgomery and

460 Montgomery, 1990b). A study by Montgomery and Montgomery (1990b) in County Down,
461 Northern Ireland, compared the stomach contents of *A. sylvaticus* at two locations. In both
462 sample sets animal material was seen to rapidly decline after September. The typical
463 consumption of insect material by *A. sylvaticus* between spring and autumn coincides with
464 the detectable prevalence period of *P. elegans* in the present study. Other studies have
465 furthermore identified seasonal patterns of infection in natural rodent populations (Chai *et*
466 *al.*, 2007). Seasonality in prevalence is likely due to the developmental cycle of the
467 intermediate host species involved in transmission. Hong *et al.*, (1998) demonstrated that
468 up to 96% of *Plagiorchis* worms were expelled from the intestine of albino laboratory rats
469 within 28 days post infection indicating the likelihood of finding adult digeneans from the
470 previous season to be low.

471 Male mice carried both a statistically higher prevalence and a higher worm burden
472 than their female counterparts. It is likely that the differences observed between male and
473 female and adult and younger adult mice are due to variation in behaviour between the
474 various groups. It has been well documented that the home range of male rodents is much
475 greater than that for female rodents (Langley and Fairley, 1982; Wolton, 1985;
476 Attuquayefio *et al.*, 1986), particularly during the breeding season between April and
477 October (Buesching *et al.*, 2007) during which male wood mice have been observed to
478 increase their home range by as much as five times (Corp *et al.*, 1997). Male adult wood
479 mice are also much more arboreal than their younger counterparts as well as female wood
480 mice (Buesching, 2007). Arboreality in wood mice is considered to be due to their
481 insectivorous nature, with insects and other small invertebrates often inhabiting the tree
482 canopy. Climbing as a means to acquire food may however be energetically expensive
483 (Buesching *et al.*, 2007) and female wood mice that have a greater dependency on
484 resources have been observed establishing mutually exclusive breeding territories
485 (Flowerdew, 1993) and as such are less likely to become infected due to a reduced

486 exposure to metacercarial infected insects than adult males that appear more prone to
487 wanderlust.

488 This study sheds light on the detailed parasite-multihost interactions within this
489 complex periaquatic ecosystem but demonstrates the difficulties associated with
490 understanding these interactions. A key issue is clearly the need for accurate identification
491 of all stages of the parasite, an issue that molecular tools can significantly enhance in
492 particular for species such as *P. muris* and *P. elegans* that are difficult to distinguish by
493 classical morphology alone. The greatest challenges lie in linking the life cycle stages
494 together and to link transmission dynamics to the ecology of the parasites and hosts within
495 the ecosystem. Further studies are required to fully understand these complex
496 interactions.

497

498 ACKNOWLEDGEMENTS

499

500 This project was funded by the University of Salford PhD Studentship scheme and the
501 Nuffield Foundation. We would also like to thank the National Trust for granting the licence
502 that enabled this work to be conducted in addition to all staff at Malham Tarn FSC field
503 centre, in particular the current warden Mike Cawthorn and Kate Martin for support and
504 assistance. We would also like to thank Dr Belgees Boufana for technical advice and
505 Jaroslav Bajnok for assistance during the summer sampling in addition to the many
506 undergraduate and postgraduate students who contributed to the fieldwork during the
507 autumn sampling period.

508

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510 REFERENCES

511

512 **Abu-Madi, M. A., Behnke, J. M., Lewis, J. W. and Gilbert, F. S.** (2000). Seasonal and
513 site specific variation in the component community structure of intestinal helminths in
514 *Apodemus sylvaticus* from three contrasting habitats in south-east England. *Journal of*
515 *Helminthology* **74**, 7–15.

516

517 **Allan, J. C., Craig, P. S., Sherington, J., Rogan, M. T., Storey, D. M., Heath, S. and**
518 **Iball, K.** (1999). Helminth parasites of the wild rabbit *Oryctolagus cuniculus* near Malham
519 Tarn, Yorkshire, UK. *Journal of Helminthology* **73**, 289-294.

520

521 **Attuquayefio, D. K., Gorman, M. L. and Wolton, R. J.** (1986). Home range sizes in the
522 Wood mouse *Apodemus sylvaticus*: habitat, sex and seasonal differences. *Journal of*
523 *Zoology* **210** (1), 45 – 53.

524

525 **Baylis, H. A.** (1939). Further records of Parasitic Worms from British Vertebrates. *The*
526 *Annals and Magazine of Natural History, 11th series* **23**, 473–798.

527

528 **Behnke, J. M., Lewis, J. W., Mohd Zain, S. N. and Gilbert, F. S.** (1999). Helminth
529 infections in *Apodemus sylvaticus* in Southern England: interactive effects of host age, sex
530 and year on the prevalence and abundance of infections. *Journal of Helminthology* **73**, 31–
531 44.

532

533 **Behnke, J. M., Eira, C., Rogan, M., Gilbert, F. S., Torres, J., Miquel, J. and Lewis, J.**
534 **W.** (2009). Helminth species richness in wild wood mice, *Apodemus sylvaticus*, is
535 enhanced by the presence of the intestinal nematode *Heligmosomoides polygyrus*.
536 *Parasitology* **136**, 793–804.

537

538 **Biserkov, V. and Kostadinova, A.** (1998). Intestinal helminth communities in the green
539 lizard, *Lacerta viridis*, from Bulgaria. *Journal of Helminthology* **72**, 267 – 271.
540

541 **Blankespoor, H. D.** (1974). Host-induced variation in *Plagiorchis noblei* Park, 1936
542 (Plagiorchiidae: Trematoda). *American Midland Naturalist* **92** (2), 415 – 433.
543

544 **Bock, D.** (1984). The lifecycle of *Plagiorchis* spec. 1, a species of the *Plagiorchis elegans*
545 group (Trematoda, Plagiorchiidae). *Z Parasitenkd* **70**, 359 – 373.
546

547 **Boyce, K., Hide, G., Craig, P. S., Harris, P. D., Reynolds, C., Pickles, A. and Rogan,**
548 **M. T.** (2012). Identification of a new species of digenean *Notocotylus malhamensis* n. sp.
549 (Digenea: Notocotylidae) from the bank vole (*Myodes glareolus*) and the field vole
550 (*Microtus agrestis*). *Parasitology*, DOI: <http://dx.doi.org/10.1017/S0031182012000911>
551

552 **Buesching, C. D., Newman, C., Twell, R. and Macdonald, D. W.** (2007). Reasons for
553 arboreality in wood mice *Apodemus sylvaticus* and bank voles *Myodes glareolus*.
554 *Mammalian Biology* **73**, 318 – 324.
555

556 **Caron, Y., Rondelaud, D. and Losson, B.** (2008). The detection and quantification of a
557 digenean infection in the snail host with special emphasis on *Fasciola* sp. *Parasitology*
558 *Research* **103** (4), 735 – 744.
559

560 **Chai, J. Y., Park, J. H., Guk, S. M., Kim, J. L., Kim, H. J., Kim, W. H., Shin, E. H., Klein,**
561 **T. A., Kim, H. C., Chong, S. T., Song, J. W. and Baek, L. J.** (2007). *Plagiorchis muris*
562 infection in *Apodemus agrarius* from northern Gyeonggi-do (Province) near the
563 demilitarized zone. *Korean Journal of Parasitology* **45** (2), 153 – 156.

564

565 **Corp, N., Gorman, M. L. and Speakman, J. R.** (1997). Ranging behaviour and time
566 budgets of male wood mice *Apodemus sylvaticus* in different habitats and seasons.
567 *Oecologia* **109** (2), 242 – 250.

568

569 **Cort, W. W. and Ameel, D. J.** (1944). Further studies on the development of the sporocyst
570 stages of Plagiorchiid trematodes. *The Journal of Parasitology* **30** (2), 37 – 56.

571

572 **Cort, W. W. and Olivier, L.** (1943). The development of the larval stages of *Plagiorchis*
573 *muris* Tanabe, 1922, in the first intermediate host. *The Journal of Parasitology* **29** (2), 81 –
574 98.

575

576 **Elton, C., Ford, E. B. and Baker, J. R.** (1931). The health and parasites of a Wild Mouse
577 population. *Proceedings of the Zoological Society of London* **101** (3), 657–721.

578

579 **Fahmy, M.** (1954). On Some Helminth Parasites of the Otter, *Lutra lutra*. *Journal of*
580 *Helminthology* **28** (3-4), 189 – 204.

581

582 **Fahmy, M. and Rayski, C.** (1963). Unusual hosts for two trematodes in Scotland. *Journal*
583 *of Helminthology* **37** (4), 287 - 290.

584

585 **Faltýnková, A., Našincová, V. and Kablášková, L.** (2007). Larval trematodes (Digenea)
586 of the great pond snail, *Lymnaea stagnalis* (L), (Gastropoda, Pulmonata) in central
587 Europe: A survey of species and key to their identification. *Parasite* **14** (1), 39 – 51.

588

589 **Flowerdew, J. R.** (1993). *Mice and Voles* (British Natural History Series). Whittet Books
590 Limited: UK.
591

592 **Gorman, A. M.** (1980). *Studies on the biology of Plagiorchis elegans (Rudolphi, 1802),*
593 *(Trematoda: Digenea) in its mammalian and molluscan hosts.* (PhD thesis), University of
594 Leeds, UK. Available internet: <http://www.theses.whiterose.ac.uk/655/> [Accessed:
595 01/06/2012].
596

597 **Hide, G., Morley, E. K., Hughes, J. M., Gerwash, O., Elmahaishi, M. S., Elmahaishi, K.**
598 **H., Thomasson, D., Wright, E. A., Williams, R. H., Murphy, R. G. and Smith, J. E.**
599 (2009). Evidence for high levels of vertical transmission in *Toxoplasma*
600 *gondii*. *Parasitology*, **136**, 1877 - 1886.
601

602 **Hildebrand, J. and Zaleśny, G.** (2009). Host and site specific pattern of occurrence of
603 digenetic trematodes in rodent communities from Lower Silesia, Poland. *Wiad Parazytol*
604 **55** (4), 389 – 393.
605

606 **Hong, S. J., Ahn, J. H. and Woo, H. C.** (1998). *Plagiorchis muris*: recovery, growth and
607 development in albino rats. *Journal of Helminthology* **72**, 215-256.
608

609 **Hong, S. J., Woo, H. C. and Chai, J. Y.** (1996). A Human Case of *Plagiorchis muris*
610 (Tanabe, 1922: Digenea) Infection in the Republic of Korea: Freshwater Fish as a Possible
611 Source of Infection. *Journal of Parasitology* **82** (4), 647 – 649.
612

613 **Hong, S. J., Woo, H. C., Lee, S. U. and Huh, S.** (1999). Infection status of dragonflies
614 with *Plagiorchis muris* metacercariae in Korea. *The Korean Journal of Parasitology* **37** (2),
615 65 – 70.

616

617 **Hughes, J. M., Williams, R. H., Morley, E. K., Cook, D. A. N., Terry, R. S., Murphy, R.**
618 **G., Smith, J. E. and Hide, G.** (2006). The Prevalence of *Neospora caninum* and co-
619 infection with *Toxoplasma gondii* by PCR analysis in naturally occurring mammal
620 populations. *Parasitology* **132**, 29-36.

621

622 **Hughes, J. M., Thomasson, D. T., Craig, P. S., Georgin, S., Pickles, A. and Hide, G.**
623 (2008). *Neospora caninum*: detection in wild rabbits and coinfection with *Toxoplasma*
624 *gondii* by PCR analysis. *Experimental Parasitology* **120**, 255-260

625

626 **Ito, M. and Itagaki, T.** (2003). Survey on Wild Rodents for Endoparasites in Iwate
627 Prefecture, Japan. *The Journal of Veterinary Medical Science* **65** (10), 1151 – 1153.

628

629 **Janssen, O. and Bock, D.** (1990). Identification and prevalence of two Plagiorchiid
630 trematodes from Hirundinidae in their intermediate and definitive hosts in the surroundings
631 of Ulm, FRG. *Parasitology Research* **76**, 451 – 453.

632

633 **Kennedy, C. R. and Burrough, R. J.** (1978). Parasites of Trout and Perch in Malham
634 Tarn. *Field Studies* **4**, 617 – 629.

635

636 **Khammes, N. and Aulagnier, S.** (2007). Diet of the wood mouse, *Apodemus sylvaticus* in
637 three biotopes of Kabylie of Djurdjura (Algeria). *Folia Zoologica* **56** (3), 246 – 252.

638

639 **Langley, R. and Fairley, J. S.** (1982). Seasonal variations in infestations of parasites in a
640 wood mouse *Apodemus sylvaticus* population in the west of Ireland. *Journal of Zoology*
641 **198**, 249 – 261.

642

643 **Lee, S. U., Huh, S. and Sohn, W. M.** (2004). Molecular phylogenetic location of the
644 *Plagiorchis muris* (Digenea, Plagiorchiidae) based on sequences of partial 28S D1 rDNA
645 and mitochondrial cytochrome C oxidase subunit I. *The Korean Journal of Parasitology* **42**
646 (2), 71 – 75.

647

648 **Lewis, J. W.** (1968). Studies on the helminth parasites of the long tailed field mouse
649 *Apodemus sylvaticus sylvaticus* from Wales. *Journal of Zoology* **154** (3), 287 – 312.

650

651 **Lewis, J. W.** (1987). Helminth parasites of British rodents and insectivores. *Mammal*
652 *Review* **17** (2-3), 81 – 93.

653

654 **Lewis, J. W. and Twigg, G. I.** (1972). Study of internal parasites of small rodents from
655 woodland areas in Surrey. *Journal of Zoology* **166**, 61 – 77.

656

657 **Macan, T. T. and Cooper, R. D.** (1960). *A key to the British Fresh and Brackish water*
658 *Gastropods*. Freshwater Biological Association. Ambleside, UK.

659

660 **Manga-Gonzalez, Y., Gonzalez-Lanza, C. and Kanev, I.** (1994). *Lymnaea truncatula*,
661 intermediate host of some Plagiorchiidae and Notocotylidae species in Leon, NW Spain.
662 *Journal of Helminthology* **68**, 135 – 141.

663

664 **McMullen, D. B.** (1937). The life histories of three trematodes, parasitic in birds and
665 mammals, belonging to the genus *Plagiorchis*. *The Journal of Parasitology* **23** (3), 235 –
666 243.

667

668 **Montgomery, S. S. J. and Montgomery W. I.** (1990a). Structure, stability and species
669 interactions in helminth communities of wood mice, *Apodemus sylvaticus*. *International*
670 *Journal for Parasitology* **20** (2), 225 – 242.

671

672 **Montgomery, S. S. J. and Montgomery, W. I.** (1990b). Intrapopulation variation in the
673 diet of the wood mouse *Apodemus sylvaticus*. *Journal of Zoology* **222**, 641 – 651.

674

675 **Norris, A.** (2003). The Mollusca of Malham. *Proceedings of the Malham Tarn Field*
676 *Centre 2003 conference on Life in a Limestone Landscape*. Available internet:
677 [http://www.fieldstudiescouncil.org/malhamtarn/additional/Research%20Seminarsresearch](http://www.fieldstudiescouncil.org/malhamtarn/additional/Research%20Seminarsresearch%20seminar%202003/Index.aspx)
678 [%20seminar%202003/Index.aspx](http://www.fieldstudiescouncil.org/malhamtarn/additional/Research%20Seminarsresearch%20seminar%202003/Index.aspx) [Accessed: 20/03/2010].

679

680 **O’Sullivan, H. M., Small, C. M. and Fairley, J. S.** (1984). A study of parasite infestations
681 in populations of small rodents (*Apodemus sylvaticus* and *Clethrionomys glareolus*) on
682 Ross Island, Killarney. *Journal of Life Sciences, Royal Dublin Society* **5**, 29 – 42.

683

684 **Perez-Ponce de Leon, G., Leon-Regagnon, V. and Garcia-Vargas, F.** (1996). Helminth
685 parasites of bats from the Neotropical Region of Mexico. *Bat Research News* **37** (1), 3 – 6.

686

687 **Rogan, M. T., Craig, P. S., Hide, G., Heath, S., Pickles, A. and Storey, D. M.** (2007).
688 The occurrence of the trematode *Plagiorchis muris* in the wood mouse *Apodemus*
689 *sylvaticus* in North Yorkshire, UK. *Journal of Helminthology* **81**, 57–62.

690

691 **Saito, T., Morishige, K. and Tongu, Y.** (1995). Parasites of pet dogs and cats in
692 Fukuyama city. *Japanese Journal of Parasitology, Tokyo* **44** (2), 149 – 153 (In Japanese
693 with English summary).

694

695 **Sato, H. and Suzuki, K.** (2006). Gastrointestinal helminths of feral raccoons (*Procyon*
696 *lotor*) in Wakayama Prefecture, Japan. *The Journal of Veterinary and Medical Science* **68**
697 (4), 311 – 318.

698

699 **Secord, M. L. and Canaris, A. G.** (1993). The metazoan parasite community of migrating
700 greater yellowlegs, '*Tringa melanoleuca*' from the Rio Grande Valley, Texas and New
701 Mexico. *Journal of Parasitology* **79** (3), 690 – 694.

702

703 **Seo, B. S., Rim, H. J. and Lee, C. W.** (1964). Studies on the Parasitic Helminths of Korea
704 I. Trematodes of Rodents. *The Korean Journal of Parasitology* **2** (1), 20 – 26.

705

706 **Shimalov, V. V.** (2002). Helminth fauna of the striped field mouse (*Apodemus agrarius*
707 Pallas, 1778) in ecosystems of the Belorussian Polesie transformed as a result of
708 reclamation. *Parasitology Research* **88**, 1009 – 1010.

709

710 **Shorrocks, B. and Sutton, R.** (2010). *Malham Tarn, Wildlife and Weather Report 2010*.
711 Malham Tarn Field Centre: UK.

712

713 **Snyder, S. D. and Tkach, V. V.** (2001). Phylogenetic and biogeographical relationships
714 among some holarctic frog lung flukes (Digenea: Haematoloechidae). *Journal of*
715 *Parasitology* **87** (6), 1433 – 1440.

716

717 **Tanabe, H.** (1922). A contribution to the study of the life cycle of digenetic trematodes. A
718 study of a new species *Lepoderma muris* n. sp. *Okayama Igakkai Zasshi* **385**, 47 – 58. (In
719 Japanese).

720

721 **Tenora, F., Henttonen, H. and Haukisalmi, V.** (1983). On helminths of rodents in
722 Finland. *Annals Zool. Fennici* **20**, 37 – 45.

723

724 **Thomasson, D., Wright, E. A., Hughes, J. M., Dodd, N.S., Cox, A. P., Boyce, K.,**
725 **Gerwash, O., Abushahma, M., Lun, Z. R., Murphy, R.G., Rogan, M. T. and Hide. G.**
726 (2011). Prevalence and Co-infection of *Toxoplasma gondii* and *Neospora caninum* in
727 *Apodemus sylvaticus* in an area relatively free of cats. *Parasitology* **138**, 1117-1123.

728

729 **Tkach, V., Grabda-Kazubska, B., Pawlowski, J. and Swiderski, Z.** (1999). Molecular
730 and morphological evidences for close phylogenetic affinities of the genera *Macrodera*,
731 *Leptophallus*, *Metaleptophallus* and *Paralepoderma* (Digena, Plagiorchiodea). *Acta*
732 *Parasitologica* **44** (3), 170 – 179.

733

734 **Tkach, V. V., Pawlowski, J. and Sharpilo, V. P.** (2000a). Molecular and morphological
735 differentiation between species of the *Plagiorchis vespertilionis* group (Digenea,
736 Plagiorchiidae) occurring in European bats, with a re-description of *P. vespertilionis*
737 (Müller, 1780). *Systematic Parasitology* **47**, 9-22.

738

739 **Tkach, V., Pawlowski, J. and Mariaux, J.** (2000b). Phylogenetic analysis of the suborder
740 Plagiorchiata (Platyhelminthes, Digenea) based on partial 18S rDNA sequences. *International*
741 *Journal for Parasitology* **30**, 83–93.

742

743 **Tkach, V., Pawlowski, J., Mariaux, J. and Swiderski, Z.** (2001). Molecular phylogeny of
744 the suborder Plagiorchiata and its position in the system of Digenea. In: *Interrelationships*
745 *of the Platyhelminthes* (eds, Littlewood, D. T. J. & Bray, R. A.). Systematics Association
746 Special Publication, Taylor & Francis, London. Pp186-193.

747

748 **Väyrynen, T., Siddall, R., Valtonen, E. T. and Taskinene, J.** (2000). Patterns of
749 trematode parasitism in lymnaeid snails from northern and central Finland. *Annales*
750 *Zoologici Fennici* **37**, 189 – 199.

751

752 **Velasquez, C. C.** (1964). Observations on the life history of *Plagiorchis dilimanensis* sp. n.
753 (Trematoda: Digenea). *The Journal of Parasitology* **50** (4), 557 – 563.

754

755 **Voutilainen, A., Ooik, T. V., Puurtinen, M., Kortet, R., Taskinen, J.** (2009). Relationship
756 between prevalence of trematode parasite *Diplostomum* sp. and population density of its
757 snail host *Lymnaea stagnalis* in lakes and ponds in Finland. *Aquatic ecology* **43**, 351 –
758 357.

759

760 **Wolton, R. J.** (1985). The ranging and nesting behaviour of Wood mice, *Apodemus*
761 *sylvaticus* (Rodentia: Muridae), as revealed by radio-tracking. *Journal of Zoology London*
762 **206**, 203 – 224.

763

764 **Woof, C. and Jackson, E.** (1988). Some aspects of the water chemistry in the area
765 around Malham Tarn, North Yorkshire. *Field Studies* **7**, 159 – 187.

766

767 **Yamada, D.** (2000). Studies on the parasite fauna of raccoon (*Procyon lotor*) naturalised in
768 Hokkaido, Japan. *Japanese Journal of Veterinary Research* **48** (1), 70 – 71.

769

770 **Zakikhani, M, and Rau, M. E.** (1999). *Plagiorchis elegans* (Digenea: Plagiorchiidae)
771 infections in *Stagnicola elodes* (Pulmonata: Lymnaeidae): Host susceptibility, growth,
772 reproduction, mortality and cercarial production. *Journal of Parasitology* **85**, 454 – 463.

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793 **Table 1.** Summarised prevalence and mean intensity data for *P. elegans* in *A. sylvaticus*
 794 from Malham Tarn. * Figure derived from a single host infection.

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	Prevalence (%)	95% Confidence limits		Mean intensity ± S.E.M
		Lower	Upper	
<u>Overall</u>	23.0	16.33	31.54	27 ± 61.5
<u>Location</u>				
Tarn Woods	23.07	13.58	36.28	21 ± 50.33
Tarn Fen	19.05	7.80	40.59	2 ± 2.5
Spiggot Hill	5.88	0.01	28.92	275*
Ha Mire	37.03	21.47	55.84	19 ± 24.2
<u>Season</u>				
Winter	0	0	37.22	0
Spring	12.5	0.11	49.22	179*
Summer	27.3	12.88	48.43	3 ± 2.94
Autumn	25.3	16.96	35.96	26 ± 61.46
<u>Sex</u>				
Male	32.4	22.62	43.98	30 ± 66.09
Female	8.7	2.90	20.86	7 ± 12.5
<u>Age cohort</u>				
Adult	43.2	29.67	57.79	36 ± 71.72
Young adult	14.3	7.16	26.00	5 ± 7.86
Juvenile	0	0	21.63	0

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806 **Table 2.** Correlation of rainfall data and *P. elegans* prevalence (%). Total rainfall over a
 807 three monthly period was analysed, using Spearman's rank of correlation, with parasite
 808 prevalence Rainfall data was supplied by Malham Tarn Field centre. Numbers in bold are
 809 the figures that have been used in the statistical analysis.

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Month	Total monthly rainfall (mm)	Total three months rainfall (mm)	Trapping month/ year	<i>P. elegans</i> prevalence (%)
<u>2009</u>				
October	128.3			
November	378.9			
December	123.6	630.8	January 2010	0
<u>2010</u>				
January	074.4			
February	064.4			
March	098.3	237.1	April 2010	0
April	027.2			
May	021.2			
June	033.5	081.9	July 2010	11
July	151.4			
August	116.4			
September	296.1	559.9	September 2010	35
October	121.4			
November	189.1			
December	041.5	352.0	January 2011	0

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January	150.5			
February	221.4			
March	036.2	408.3	April 2011	20
April	031.4			
May	126.2			
June	094.1	251.7	July 2011	38
July	090.6			
August	170.8			
September	157.7	419.1	October 2011	18
October	NA			

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824 **Table 3.** Prevalence of intramolluscan stages found in snail species. Confidence intervals
 825 (95%, P = 0.05, two tailed test) were calculated using an online Binomial Confidence
 826 Interval calculator (<http://statpages.org/confint.html>).

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Molluscan species	Number examined (n)	Prevalence (%)	95% Confidence interval	
			Lower	Upper
<i>Lymnaea stagnalis</i>	32	0	0.00	10.89
<i>Lymnaea palustris</i>	58	0	0.00	06.16
<i>Lymnaea peregra</i>	1270	0	0.00	00.29
<i>Lymnaea truncatula</i>	243	0	0.00	01.51
<i>Anisus leucostoma</i>	35	0	0.00	10.00
<i>Bithynia tentaculata</i>	10	0	0.00	30.85
<i>Physa fontinalis</i>	8	0	0.00	36.94
<i>Planorbis</i> sp.	7	0	0.00	40.96
<i>Potamopyrgus antipodarum</i>	188	0	0.00	01.94
<i>Sphaerium corneum</i>	33	0	0.00	10.58
<i>Valvata cristata</i>	137	0	0.00	02.66

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835 **Figure 1.** Map of Malham Tarn Nature Reserve and adjacent woodlands. Redrawn from
836 Shorrock and Sutton (2010). **TW:** Tarn Woods; **TF** Tarn Fen; **SP** Spiggot Hill; **HM** Ha Mire
837 Plantation. The dotted line represents the reserve boundary.

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839 **Figure 2.** *Plagiorchis* specimen recovered from the small intestine of *Apodemus sylvaticus*
840 at Malham Tarn. The drawing was made from a photograph taken with a Leica ICC50
841 digital camera attached to a Leica DM500 microscope. Abbreviations: **OS**, oral sucker; **P**,
842 pharynx; **C**, cirrus; **CS**, cirrus sac; **VS**, ventral sucker; **M**, metraterm; **O**, ovary; **T**, testis;
843 **Ca**, caecum; **U**, uterus; **V**, vitellaria; **Vc**, vitellarian commissure. **Scale bar = 500µm.**

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845 **Figure 3.** Comparison of the partial 28S rDNA sequence of *P. muris* collected from
846 Malham Tarn with other *Plagiorchis* species retrieved from the NCBI database: *P. elegans*
847 (AF151911); *P. koreanus* (AF151930); *P. muelleri* (AF184250); *P. vespertilionis*
848 (AF151931); *P. muris* (AF096222). The comparison was made using the GeneDoc
849 alignment tool. Black shading indicates regions of conserved homology. Grey indicates
850 regions of conservation between four or more species.