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20

21 **Abstract**

22 Data collected from wildlife telemetry studies relies on tagging and attachment having
23 minimal impacts on behaviour. Though a widespread technique, relatively few studies
24 evaluate the impacts of differing tagging methods on both welfare and behaviour.
25 Here we use tracking data, collected for other aims, to investigate the impact of inter-
26 peritoneal surgical implantation of acoustic transmitters on the health and behaviour
27 of common bream, *Abramis brama* (L.). In five separate capture events, the behaviour
28 in terms of distances moved and linear range of newly tagged fish (n=61) were
29 compared to previously tagged fish (n=55) present in the same river at the same time.
30 In the first 5 days post-tagging, newly tagged fish moved significantly further than
31 previously tagged fish. Despite this difference, the linear ranges moved by the two
32 groups of fish were equivalent. During 6-10 days post tagging there was no significant
33 differences between the two groups. Thus, the tagging procedure had short term, but
34 not long term behavioural impacts. In addition, a number of tagged fish were
35 recaptured between 51 and 461 days post-surgery. Recaptured fish appeared to have
36 clean, well-healed incisions and exhibited 'normal' behaviour in that they were caught
37 alongside a large number of conspecifics. Three recaptured tagged fish were
38 translocated ~35 km downstream, to ascertain how translocation would affect their
39 behaviour. The translocated fish had a greater linear range than control fish, with all
40 three fish returning to the site of capture within 6 to 24 days, suggesting that common
41 bream can exhibit site fidelity.

42

43 Key words: aquatic telemetry; surgical implantation; effects of tagging; translocation;
44 *Abramis brama* (L.).

45 **1. Introduction**

46 The development of electronic tags has been one of the most important advances in
47 the study of freshwater fish behaviour and ecology (Lucas & Baras 2001; Cooke *et al.*
48 2013). Tag implantation into the peritoneal cavity is commonly used in long-term
49 tracking studies of fishes (Lucas & Batley 1996) and regularly used for cyprinid
50 species (*e.g.* Lucas & Batley 1996; Huber & Kirchhofer 1998; Lyons & Lucas 2002;
51 Winter & Fredrich 2003; Fredrich *et al.* 2003; Kuliskova *et al.* 2009; Gardner *et al.*
52 2013). Such telemetry studies commonly rely on three assumptions: 1) fish condition
53 and mortality are not altered by the tagging procedure or transmitter presence; 2)
54 transmitters are retained for the duration of the observation period; and 3) tagged
55 individuals accurately represent the population being observed (*i.e.* they behave
56 normally; Smith *et al.* 1998; Ramstad & Woody 2003; Neely & Steffensen 2010).
57 Taylor *et al.* (2011) suggests the best approach to evaluate potential tagging effects is
58 to use multiple endpoints that evaluate lethal and sub-lethal effects (*e.g.* healing,
59 swimming activity, and performance; Cooke *et al.* 2011) and that field validations are
60 necessary to ensure that data are relevant to field scenarios (Cooke *et al.* 2011).

61 Assessment of the effects of surgical implantation of transmitters on the behaviour
62 of tagged fish has previously been carried out by observations of fish in captivity prior
63 to release. In addition, examination of recaptured fish (post-release) can determine the
64 degree of healing and the effects of tag implantation on fish condition (*e.g.* weight).
65 However, there is a paucity of data on the effects of tag implantation on fish
66 behaviour in the wild. Unusual long-distance movements have been observed during
67 the first few days of release following transmitter implantation in largemouth bass
68 *Micropterus salmoides* (L.) (Mesing & Wicker 1986) and dace *Leuciscus leuciscus*
69 (L.) (Clough & Beaumont 1998), but without a suitable control population in the

70 watercourse, it is difficult to ascribe this behaviour as a direct effect of the capture,
71 handling and surgical procedure.

72 Here, we present the results of a study in which the immediate post-surgery spatio-
73 temporal behaviour of common bream *Abramis brama* (L.) was compared to that of
74 other common bream that had been tagged between 55 and 378 days previously. The
75 study had other primary objectives (see Gardner *et al.* 2013 & 2015)., However, it
76 also presented the opportunity to analyse the short-term effects of capture, handling
77 and tagging on the behaviour of the bream via a comparison of the movements of the
78 two groups of bream. In recapturing a sample of tagged individuals, incision wound
79 healing could also be assessed. In addition, a small group of recaptured fish were
80 translocated downstream and their behaviour compared to those that were not
81 recaptured nor translocated. A detailed description of the tagging protocol is reported
82 in response to calls for greater scrutiny of the methods used in order to compare,
83 replicate and interpret the growing literature on fish telemetry studies (Thiem *et al.*
84 2011).

85

86 **2. Material and methods**

87 *2.1 Study area*

88 The study area was a continuous open reach of approximately 40 km of the non-tidal
89 lower River Witham and associated tributaries in Lincolnshire, UK, see Gardner *et al.*
90 (2013 & 2015) for further details.

91

92 *2.2 Sampling procedures*

93 In total, eighty-three adult common bream were caught by either rod and line, seine
94 netting or electrofishing from the River Witham, and tagged in seven batches between

95 November 2006 and February 2009 (see summary in Table 1). Fish were retained
96 overnight; on one occasion in net cages placed in the river (the site was secure), or
97 more commonly in holding tanks on shore between capture and tagging. After
98 surgery, all fish were released at the site of capture.

99 Coded acoustic transmitters of two types: Vemco (Nova Scotia, Canada) V9-2L
100 (cylindrical with dimensions of 29 mm by 9 mm diameter, weight in air of 4.7 g,
101 weight in water 2.9 g and with operational life of 80-330 days) and V13-1L
102 (cylindrical with dimensions of 36 mm by 13 mm diameter, weight in air of 11.0 g,
103 weight in water 6.0 g and with operational life of 526-621 days) were implanted into
104 the body cavity. The tag weight in air would represent 0.16-0.57% of the fish's weight
105 out of water.

106

107 *2.3 Tag implantation protocol*

108 The procedure described was regulated and licensed in the UK by the Home Office
109 under the Animals (Scientific Procedures) Act 1986, and was performed under project
110 licence number PPL 80/2016. The surgery itself took place within the shelter of a
111 mobile laboratory under conditions that were as aseptic as possible in-the-field.

112 A specially constructed operating table was used to secure and restrain the fish
113 in an upside-down position with the incision site clearly accessible. The mobile
114 laboratory and operating table were washed and disinfected before any procedures
115 were started. Surgical equipment was sterilised in 97 % ethanol and then rinsed with
116 sterile water or saline solution. Prior to the procedure the activated tag was tested in
117 water with a hydrophone. Tags were then sterilised with a 25% Dettol™ solution,
118 rinsed with sterile water or saline solution and stored in a sterile swab. A 60 litre
119 anaesthetic bath (Tricaine methanesulphonate MS-222; 50 mg/L; see Carter *et al.*

120 2011), and a similar sized recovery tank, both with aeration were clearly labelled. The
121 anaesthetic bath was tested with a single fish (which was not used again for
122 subsequent anaesthesia and tagging) until anaesthesia was reached (indicated by loss
123 of the righting reflex and a slowed operculum rate, which did not stop). The test fish
124 was allowed to fully recover before any fish to be tagged were anaesthetised.

125 All surgeries were conducted by a single surgeon (CG). The surgeon and assistant
126 thoroughly cleaned their hands with an alcohol-based hand wash and maintained as
127 aseptic a procedure as was possible under field conditions. The surgeon wore sterile
128 surgical gloves, changing them between fish, or after coming into contact with
129 anything away from the surgical area. Fish were firstly weighed, measured, their sex
130 determined, primarily by the presence of tubercles and also by vent & body
131 morphology (Kennedy & Fitzmaurice 1968), and a scale sampled from the mid-flank
132 above the lateral line, for subsequent age determination. The fish was then placed into
133 the aerated anaesthetic bath. When anaesthesia was reached the fish was removed
134 from the bath, inverted and secured in the operating table between wet smooth foam
135 padding with Velcro™ straps and transferred to the mobile laboratory. During the
136 surgery an assistant monitored the operculum movement throughout. If a problem
137 with the fish's health or well-being was encountered during the procedure then the
138 team had the option to administer an overdose of anaesthetic in-line with Schedule 1
139 of the Animals (Scientific Procedures) Act 1986.

140 The incision site was on the ventral surface of the fish, anterior of the pelvic fins
141 and associated muscle blocks. Using tweezers, a line of 4 to 6 scales were removed
142 from the incision site and another scale removed midway and perpendicular to the
143 incision site to allow suture entry. The site was then swabbed with an iodine based
144 antiseptic (Betadine™). An incision was made with a sterile scalpel and was kept to

145 the minimum length required, approximately 20-30 mm. The incision was begun just
146 in front of the pelvic muscle blocks, where the body wall thins, and extended towards
147 the pectoral fins. The incision was made slowly by dragging the scalpel lightly. An
148 assistant used sterile tweezers to hold the incision open while it was being cut
149 allowing the surgeon to see when the incision breached the body cavity and thus
150 avoided making an incision that was too deep, potentially damaging vulnerable
151 internal organs. A sterilised tag was inserted into the incision using a sterile, gloved
152 hand and/or sterile tweezers. The incision was closed with a single suture (Ethicon
153 PDS*II Polydioxanone violet monofilament absorbable W9125; Ethicon, Piscataway,
154 NJ, U.S.A.). One scale had already been removed to allow suture entry and another 1-
155 2 scales were removed with tweezers to achieve suture exit. The suture was secured
156 with a surgeon's knot and excess suture material trimmed with sterile scissors, 5-10
157 mm from the knot.

158 The site was swabbed again and G7 wound sealer (Lincolnshire Fish Health, UK)
159 was applied and allowed to at least partly dry for a few seconds. The site was then
160 liberally covered with Orabase™ (Squibb & Sons, Uxbridge, UK) protective paste to
161 provide a temporary barrier and G7 wound sealer reapplied on top of the Orabase™,
162 to prevent the Orabase™ barrier dissolving too quickly when fish were returned to the
163 water. The whole procedure took approximately three to four minutes.

164 Following surgery, the fish was removed from the operating table and immediately
165 placed in the aerated recovery tank where it was supported by hand in an upright
166 position. An antibiotic injection of 1 ml Baytril at 2.5% directly behind the dorsal fin
167 was given while fish were recovering, although antibiotic use post-surgery has been
168 questioned by Mulcahy (2011). The injection was made on the same line as the erect
169 last dorsal ray between the two main muscle blocks. This site reduces possible scale

170 damage and reduces post-injection leakage of antibiotic. Once each fish was deemed
171 recovered, which took no more than 5 minutes, it was removed to a separate retention
172 net in the river for further observation. Fish were retained in this way for an hour after
173 the last fish was tagged, to ensure they had regained balance and were actively
174 swimming, then collectively released as a group.

175

176 *2.4 Recapture and translocation of tagged fish*

177 After release, the movements of the common bream were monitored in the lower
178 River Witham, Lincolnshire between Short Ferry (53°13'38"N; 0°21'23"W) and
179 Boston (52°58'53"N; 0°1'46"W). Tracking results were obtained from up to twenty-
180 six fixed (marginal, maintained at approximately mid-water depth, a metre below the
181 surface) VR2 and VR2W acoustic receivers (Vemco, Nova Scotia, Canada) which
182 were positioned ~2-3 km apart, as described by Gardner *et al.* (2013).

183 Fish were recaptured both intentionally, for translocation and unintentionally,
184 during fishing operations to capture new fish for tagging. When new fish were
185 required for tagging, mobile tracking with a VR100 mobile receiver (Vemco, Nova
186 Scotia, Canada) was undertaken to find areas where tagged fish were present and
187 fishing for fresh fish was concentrated in these areas. Common bream are a shoaling
188 species (Backiel & Zawiska 1968) and thus tagged fish were likely to be associated
189 with untagged fish. Recaptured tagged fish were isolated and identified with the
190 VR100 mobile receiver in separate bank side tanks and identification confirmed by
191 the presence of surgery incisions. The incision site was inspected and photographed to
192 determine the extent of post-surgical healing. All fish were returned to the river alive.

193 On 16 March 2010 three fish (tag numbers: 12255, 12257& 12266) were caught by
194 wrap-around seine netting (35 m by 3 m pull down and 50 m by 3 m wrap; Coles *et*

195 *al.* 1985) in the Sincil Dyke, close to Short Ferry (53°12'49"N; 0°20'50"W) when
196 three separate single haul nettings covered a length of drain of ~1 km. Fish were
197 placed in large plastic bags (60 cm by 100 cm) containing approximately 20 L of
198 water, bags were sealed at the top and the air space filled with oxygen. These fish
199 were then translocated ~35 km downstream by road and released as a group so their
200 spatio-temporal behaviour could be compared with 'control' (not captured nor
201 translocated) tagged fish that were present in the river.

202 The definition of the 'control' group used throughout the study warrants some
203 comment; previously tagged fish were used as a 'control' group to compare with
204 newly captured, tagged / translocated and released fish. A more appropriate control
205 would be previously tagged fish recaptured but released at the same site and their
206 behaviour compared with recently tagged (or translocated) and released fish.
207 However, in this case it would not be clear if the difference in behaviour between the
208 two groups was due to recapture itself (which is stressful) rather than the capture,
209 handling and surgery / tag insertion (or translocation) which collectively is a distinct
210 but also stressful event. Recapture of previously tagged fish was very difficult in such
211 a large waterbody, thus it was considered that previously tagged fish still at liberty
212 would act as a reasonable proxy for a control group.

213

214 *2.5 Data handling and statistical analysis*

215 Data were downloaded to a laptop using VR2PC and VUE software packages
216 (Vemco, Nova Scotia, Canada). Allocation of a km value (measured using ArcMap
217 v9.1 Geographic Information System, ESRI Ltd, Redlands, CA, USA) upstream of the
218 tidal limits at Boston for each receiver allowed the movements of individual fish to be
219 quantified.

220 There were five tagging events when ‘new’ fish were tagged while previously
221 tagged fish were also being tracked in the river, allowing the behavioural effects of
222 capture, handling and tagging to be compared between these two groups; newly
223 tagged and previously tagged (details of each tagging event are presented in Table 1).
224 The effects of the single translocation event of three fish were also compared.

225 Two methods of quantifying the spatio-temporal behaviour of the tracked fish were
226 used. The cumulative distance moved between the receivers visited by an individual
227 fish gave the total distance moved (TDM) in km. The longitudinal distance between
228 the most upstream and downstream detections (Young 1999; Ovidio *et al.* 2000) gave
229 the linear range (LR) in km, with positive values indicating ranges upstream and
230 negative values indicating ranges downstream.

231 We analysed the TDM and the LR by newly tagged and previously tagged fish
232 over two periods; the first 5 days (120 h) following release and the second 5 days
233 (120.1 – 240 h) after release. Fish that were not detected during any period were
234 omitted from that part of the analysis. The movement data did not fit a normal
235 distribution, possibly because of the discrete intervals of transmitters in the river.
236 Data instead fitted an overdispersed Poisson distribution. We analysed movement data
237 using a Poisson GLMM with type (newly tagged/previously tagged) as a fixed factor
238 and fish ID and tagging event as random factors. To account for overdispersion, we
239 fitted an individual-level random effect. We then tested whether fish moved upstream
240 or downstream using a binomial GLMM with the same fitted fixed and random
241 effects. Using just newly tagged fish, we tested whether capture method impacted
242 movements. Again, we used Poisson and binomial GLMMs with method
243 (electrofishing, seine netting, rod and line) as a fixed factor and tagging event as a
244 random effect. We fitted an individual level random effect to account for

245 overdispersion. All models were conducted using the *glmer* function from the lme4
246 package (Bates *et al.* 2014) in R version 3.03 (R core team 2014).

247 For the translocation analysis, Mann-Whitney U tests were used to analyse
248 differences in TDM and LR between the two groups during the consecutive five day
249 periods following translocation, as data were not normally distributed, these analyses
250 were performed with Minitab® v15.1.1 (Minitab Inc., PA, USA).

251

252 **3. Results**

253 All eighty-three common bream made a full recovery post-surgery and swam away
254 strongly on release. Another fish implanted was euthanised under Schedule 1 of the
255 Animals Scientific Procedures Act (1986) after it failed to fully recover from
256 anaesthesia; although a post-mortem revealed no surgical complications. Individual
257 fish were tracked from 40-629 days (mean $266.0 \pm \text{SD } 146.7$; see Gardner *et al.*
258 2013). Subsequent analysis of tracking data showed no evidence of tag expulsion and
259 survival rates of 100% were experienced for fish that stayed within the study area in
260 the short term, with all fish released showing upstream movements (indicative of an
261 alive fish) for at least two months, usually substantially more, post-surgery.

262

263 *3.1 Recaptured fish*

264 In total, eight (~10%) of the eighty-three tagged common bream were recaptured
265 during the study. A single haul seine netting on 30 November 2007 resulted in 990
266 adult common bream with masses between ~2-3 kg. The netting recaptured four
267 tagged bream, three 51 days post-surgery and one 275 days post-surgery. One fish
268 was recaptured 76 days post-surgery by electro-fishing in the Sincil Dyke on 24
269 February 2009. One fish 384 days post-surgery and two fish 461 days post-surgery

270 were recaptured during nettings on 16 March 2010, which resulted in 1,270 adult
271 common bream between ~1-3 kg.

272 Recaptured fish appeared to be behaving naturally, in that they were associated
273 with numbers of untagged fish, sometimes in very high densities. Physically they
274 exhibited clean healing and tissue regeneration (Figure 1), although in one case there
275 was some haemorrhaging around the incision site. There was no evidence of the tag
276 expulsion process described by Jepsen *et al.* (2008). All recaptured fish were
277 visually inspected and appeared to be in no worse a physical condition than when they
278 were tagged. All three fish recaptured 51 day post-surgery and the single fish
279 recaptured 76 days post-surgery still had sutures present. All four fish recaptured 275-
280 461 days post-surgery displayed clean healing and suture absorption, with a small scar
281 being the only evidence of the surgical procedure. This indicates that the sutures were
282 absorbed in line with the manufacturers' specifications, which state that absorption is
283 minimal until about the 90 days post-surgery.

284

285 *3.2 Effects of capture, handling and tagging procedure*

286 There was a lot of variability between results from individual fish in some cases,
287 hence the relatively large standard errors (Figures 2 & 3). In the first 5 days (120 h)
288 following release the newly tagged fish moved significantly further than fish
289 previously tagged and already in the river (Poisson GLMM: Estimate = 0.67 ± 0.21 , Z
290 = 3.12, $P = 0.002$; Figure 2), but the linear range they covered did not differ
291 significantly (Poisson GLMM: Estimate = 0.30 ± 0.22 , $Z = 1.38$, $P = 0.169$; Figure 3).
292 There was no significant difference in the proportion of fish moving upstream or
293 downstream (Binomial GLMM: Estimate = 0.17 ± 0.48 , $Z = 0.36$, $P = 0.721$; total $n =$
294 87, control upstream $n = 20$, control downstream $n = 11$, newly tagged upstream $n =$

295 40, newly tagged downstream $n = 16$), although when fish that did not register either
296 an upstream or downstream movement (*i.e.* linear distance = zero) were included in
297 the analysis, newly tagged fish tended to move upstream more often (Binomial
298 GLMM: Estimate = 1.11 ± 0.41 , $Z = 2.76$, $P = 0.006$; total $n = 116$, control upstream
299 $n = 20$, control downstream $n = 11$, control non-movers $n = 24$, newly tagged
300 upstream $n = 40$, newly tagged downstream $n = 16$, newly tagged non-movers $n = 5$).
301 By contrast, newly tagged fish did not move significantly further than previously
302 tagged fish already in the river at 6-10 days (120.1 – 240 h) post-release (Poisson
303 GLMM: Estimate = 0.17 ± 0.43 , $Z = 0.40$, $P = 0.689$; Figure 2), nor did the linear
304 range they moved differ significantly (Poisson GLMM: Estimate = 0.16 ± 0.36 , $Z =$
305 0.434 , $P = 0.665$; Figure 3). There was no significant difference in the proportion of
306 fish moving upstream or downstream (Binomial GLMM: Estimate = -0.52 ± 0.62 , $Z =$
307 -0.84 , $P = 0.401$; total $n = 55$, control upstream $n = 20$, control downstream $n = 9$,
308 newly tagged upstream $n = 15$, newly tagged downstream $n = 11$). This result did not
309 change when fish that did not register either an upstream or downstream movement
310 were included in the analysis (Binomial GLMM: Estimate = -0.36 ± 0.48 , $Z = -0.80$,
311 $P = 0.421$; total $n = 105$, control upstream $n = 20$, control downstream $n = 9$, control
312 non-movers $n = 29$, newly tagged upstream $n = 15$, newly tagged downstream $n = 11$,
313 newly tagged non-movers $n = 21$).

314 Method of capture had a significant effect on total distance moved in the first 5
315 days (120 h) following release by the newly tagged fish (Table 2). Fish caught by
316 seine netting ($P = 0.039$) moved significantly further than those caught by
317 electrofishing, whilst there was a marginally non-significant tendency for rod caught
318 fish to move further ($P = 0.069$). Similarly method of capture had a significant effect
319 on the linear range (Table 2), with rod caught fish ($P = 0.001$) having larger linear

320 ranges than those caught by electrofishing, and a non-significant tendency for seine
321 net caught fish to have larger linear ranges ($P = 0.155$). Method of capture did not
322 impact the direction fish moved within the first 5 days post-release (Table 2).

323 By contrast, method of capture had no significant effect on the total distance
324 moved 6-10 days post-release (Table 3), though fish caught by seine net had a
325 marginally non-significant tendency to move less ($P = 0.073$). Similarly, method of
326 capture had no significant effect on the linear ranges of newly tagged fish (Table 3),
327 or the direction fish moved (Table 3).

328

329 *3.3 Effects of translocation*

330 Following translocation all three fish returned to the Sincil Dyke 6-24 days after being
331 released ~35 km downstream (Figure 4). Individual fish showed considerable
332 variability in their behaviour but there was no significant difference in mean TDM
333 between the translocated and control fish (Figure 5). Mann-Whitney U tests revealed
334 no significant effect on TDM in any five day period following translocation, Table 4.
335 However, the translocated fish ranged over longer distances upstream as they returned
336 to the Sincil Dyke, while the control fish slowly ranged downstream (Figure 6).
337 Mann-Whitney U tests revealed significant differences in LR in every concurrent five
338 day period (Table 4).

339

340 **4. Discussion**

341 *4.1 Recaptured fish*

342 Whenever surgery is involved fish will be subjected to disturbance and post-surgical
343 healing rates vary according to species, age, the size of the incision and associated
344 trauma, and water temperature (Lucas & Baras 2001). Although the survival of tagged

345 fish is often not the best measure of the impact of the surgical procedure on fish
346 (Jepsen *et al.* 2008), it remains the simplest to measure without invasive or destructive
347 techniques. Although the sample size here was relatively small and may not be
348 random or representative, all recaptured fish in this study were alive and showed
349 advanced healing and no suture loss after 51 days with water temperatures between 4-
350 14 °C. The four fish recaptured 275-461 days post tagging showed complete external
351 recovery, with the incision site hard to identify and no signs of sutures, being
352 absorbed in line with the manufacturer's specifications. Retention of sutures until
353 healing is advanced is preferable to premature loss which may increase tag loss and
354 mortality risk (Jepsen *et al.* 2002).

355 Efforts to recapture tagged fish often resulted in the capture of large numbers of
356 untagged conspecifics, indicating tight shoaling behaviour (as described by Backiel &
357 Zawiska 1968; Phillips & Rix 1985; Borcharding *et al.* 2002.). The observation of
358 tagged fish with other untagged conspecifics (*e.g.* Clough & Ladle 1997; Clough &
359 Beaumont 1998; Jepsen & Berg 2002), and of tagged fish engaged in migration (*e.g.*
360 Baras 1997) and spawning (*e.g.* Lucas & Batley 1996) have been interpreted to
361 indicate 'normal' behaviour by the tagged fish. However, few studies have quantified
362 the effects of tagging on behaviour (see review by Bridger & Booth 2003).

363

364 *4.2 Effects of capture, handling and tagging procedure*

365 The tagging procedure was designed with the highest regard for fish welfare and
366 animal ethics, aseptic techniques, incision dressing and antibiotics were employed as
367 a 'belt and braces' approach to safeguard against post-operative infection, despite
368 recent evidence that such measures do not increase post-operative survival (see Jepsen
369 *et al.* 2013). This approach was adopted following advice from the regulator. This

370 subject has been cause for recent debate and some now consider it important to
371 discourage researchers from taking unnecessary precautions unless there are specific
372 (documented) problems with infections (see Jepsen *et al.* 2013, 2014a, 2014b).
373 However, the use of aseptic techniques has also been championed (see Mulcahy 2013
374 & 2014) as standard best practice.

375 In this study the capture, handling and tagging procedure appeared to impact
376 behaviour for the first 5 days post-tagging, detectable at the resolution of the tracking
377 undertaken. These differences had disappeared at 6–10 days post-surgery. Similarly
378 temporary effects of tagging have been observed elsewhere. Robertson *et al.* (2003)
379 recorded negative effects on growth up to day 36 of a 45 day experiment assessing the
380 impacts of tagging on wild Atlantic salmon *Salmo salar* (L.) parr implanted with
381 dummy transmitters and observed in flow-through aquaria.

382 It is important to know if tagging disrupts behaviour in order to validate the data
383 collected and conclusions drawn. For example, in a study of dace in the River Frome,
384 UK, 88% of tagged fish moved upstream immediately after release, with some
385 making large excursions on the day of release and three fish moving so far that they
386 were lost outside of the study area (Clough & Beaumont 1998). In response to such
387 reports, some studies have either not recorded data from the period immediately after
388 tagging (*e.g.* one week by Allouche *et al.* 1999) or excluded it from analyses to
389 mitigate against the effects of tagging and handling on fish behaviour (Winter 1996).

390 Mesing & Wickler (1986) report unusual long-distance movements in largemouth
391 bass during the first days of release after transmitter implantation. By contrast, Lyons
392 & Lucas (2002) observed no large movements (> 100m) of tagged common bream in
393 the River Trent, UK during the first hour after release. It is possible the effect of
394 surgery on behaviour is taxon specific and may reflect differences in ecology, such as

395 the likelihood of predation. For instance, dace in the river Frome are common prey of
396 pike (Masters *et al.* 2003). Alternatively, differences between studies may reflect
397 differences in the spatial resolution of the tracking devices used, such that small
398 changes in behaviour may not be picked up by static receivers positioned several km
399 apart.

400 Capture by seine net and rod and line had the most effect on TDM and LR after 0-5
401 and 5-10 days, it might be that electrofishing is less disruptive than these other two
402 methods. Disruption of an entire school of shoaling fish may elicit this response, with
403 released fish trying to relocate their school.

404

405 *4.4 Effects of translocation*

406 Translocation did not significantly affect the activity levels of the fish, as determined
407 by the mean total distance moved (although this may reflect the low statistical power
408 associated with the movement of just 3 fish). However, linear ranges were
409 significantly greater in the translocated fish as they appeared to display site fidelity,
410 moving upstream towards the original capture site.

411 How fish navigate in complex habitats is still unclear, although it is likely to
412 involve several mechanisms (see Hasler & Wisby 1958; Malinin 1970; Carlson &
413 Haight 1972; Mesing & Wicker 1986; Hert 1992; Baras 1997, Odling-Smee &
414 Braithwaite 2003). Fourteen radio tagged brown trout (*Salmo trutta* L.) were
415 displaced over 0.8 to 3.6 km upstream and downstream in the river Eden, Scotland.
416 Twelve of these fish subsequently returned to the areas from which they were taken,
417 seemingly to follow specific orientation cues (*e.g.* olfactory) rather than searching at
418 random (Armstrong & Herbert 1997).

419 Here data lend support for the notion that fish are capable of relocating by directed
420 movements using specific orientation cues, rather than by accident or a random search
421 pattern. Prior to translocation all fish were relatively sedentary in the Sincil Dyke, and
422 had been for some time. Following translocation, once fish had returned 'home', they
423 tended to remain in relatively localized areas of the channel. The preceding tracking
424 data of the fish allows some assessment of each fish's 'familiar area' (the zone
425 through which the fish could remember having moved; Bovet 1992). Interestingly the
426 fish that took the longest to return 'home' had prior knowledge of the lower river
427 where it was translocated. Therefore, this fish could be using olfactory beacons (the
428 presence or absence of familiar odours emanating from the familiar area; Halvorsen &
429 Stabell 1990), or any of a range of other potential cues, such as visual and olfactory
430 landmarks, or areas of distinct water flow patterns (Armstrong & Herbert 1997). By
431 contrast, the other two translocated fish had not experienced the lower river in the
432 preceding ~15 months and may have no experience of its visual and olfactory
433 landmarks to use as navigational aids, and were the quickest to return 'home'.

434 In conclusion, this study detected short term impacts of capture, handling and
435 tagging procedure on the behaviour of bream with newly tagged fish moving greater
436 distances in the first five days post-operation, these differences had become non-
437 significant during the 6-10 day period. Recaptured fish had clean healing incisions
438 and appeared to be exhibiting 'normal' behaviour in that they were part of large
439 shoals of common bream. Translocated fish appeared to exhibit site fidelity, moving
440 quickly back to the site of capture.

441

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593

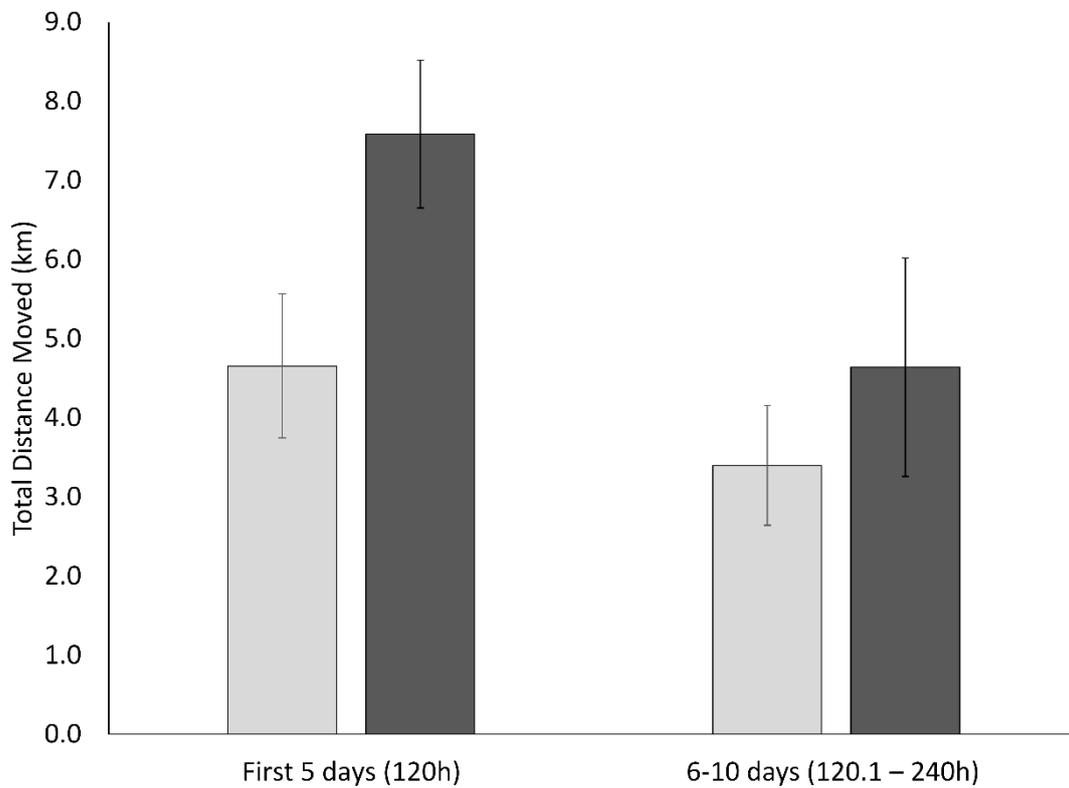
594 **Figure Captions**



595

596 Figure 1. Incision site of fish 6073 - 51 days post tagging. Note the very clean

597 advanced healing and incision closure, suture still present.

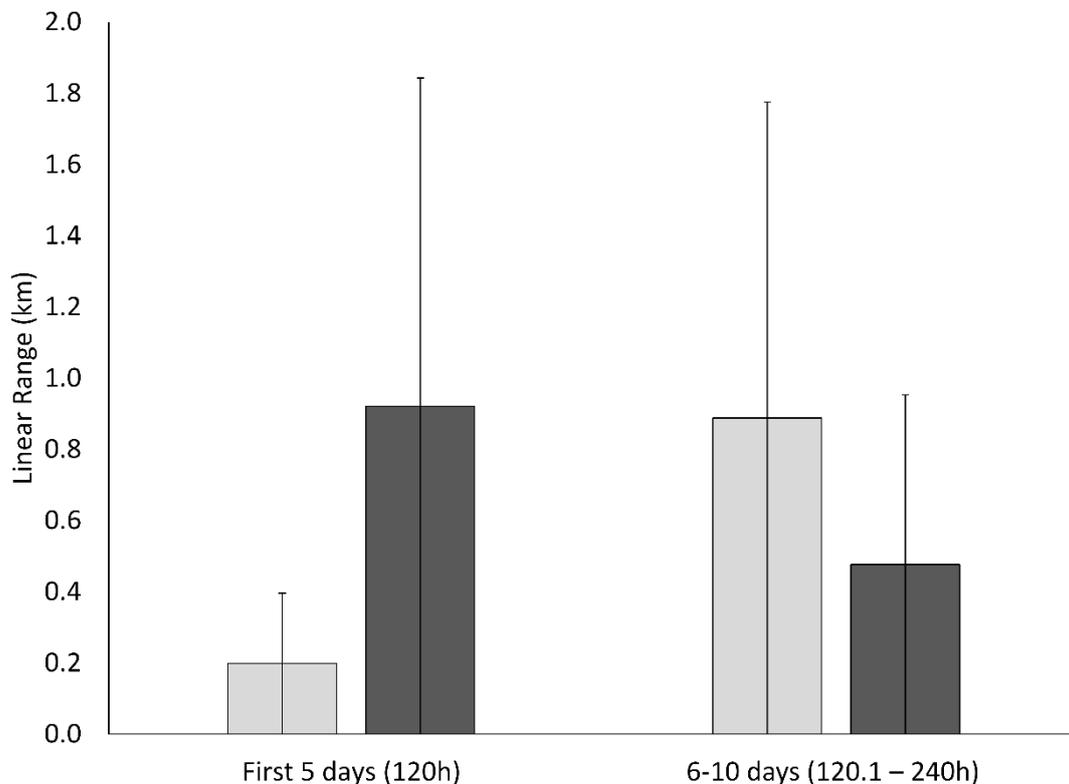


598
599

600 Figure 2. The mean total distance moved TDM (\pm SE) from five different tagging

601 events over two concurrent five day periods (0-5 days and 6-10 days) post-surgery of

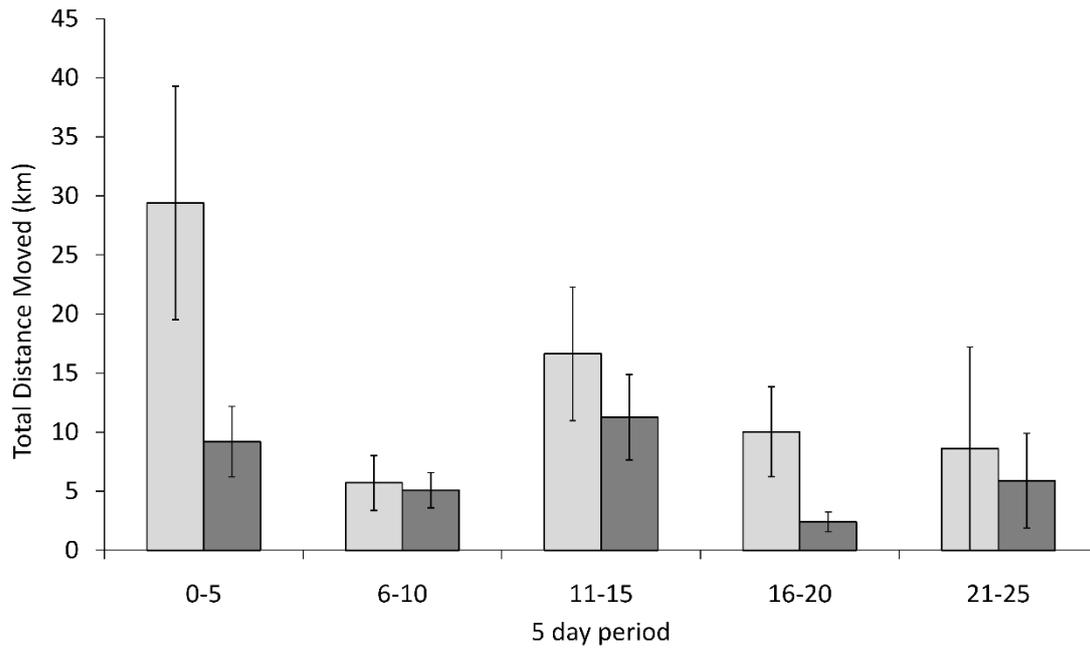
602 newly tagged fish (light grey bars; 0-5 days n= 61; 6-10 days n= 47) and control
 603 previously tagged fish (dark grey bars; 0-5 days n= 55; 6-10 days n= 58).



604
 605 Figure 3. The mean linear range LR (\pm SE) from five different tagging events over
 606 two concurrent five day periods (0-5 days and 6-10 days) post-surgery of newly
 607 tagged fish (light grey bars; 0-5 days n= 61; 6-10 days n= 47) and control previously
 608 tagged fish (dark grey bars; 0-5 days n= 55; 6-10 days n= 58).

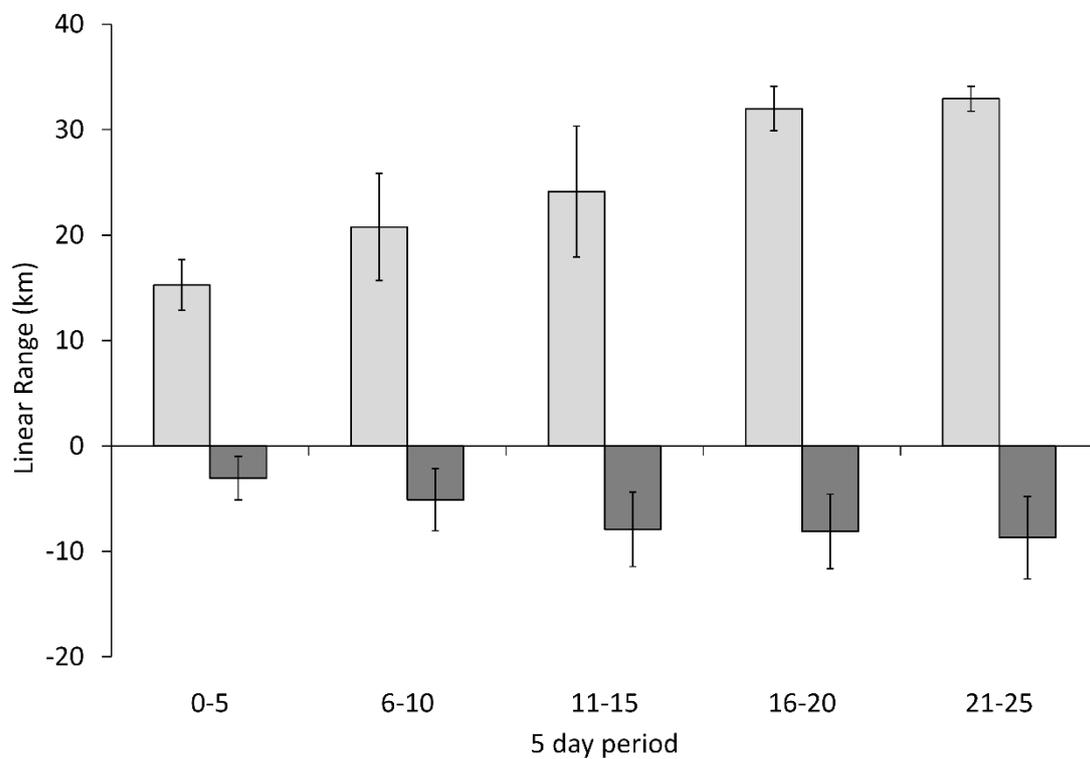
609
 610 Figure 4. Movement patterns for A; tag ID 12255 (male), B; 12257 (female) and C;
 611 ID 12266 (male) showing behaviour before and after translocation. Return journey to
 612 Sincil Dyke shown in red, which took 11, 6 and 24 days after release approx. 35km
 613 downstream respectively.

614



615

616 Figure 5. The mean total distance moved TDM (\pm SE) over five concurrent five day
 617 periods up to twenty five days after release for the translocated group (n=3; light grey
 618 bars) and non-translocated control group (n=7; dark grey bars).



619

620 Figure 6. The mean linear range LR (\pm SE) over five concurrent five day periods up to
 621 twenty five days after release for the translocated group (n=3; light grey bars) and
 622 non-translocated control group (n=7; dark grey bars).

623

624 Table 1 Details of the seven groups of fish that were tagged between 2006 and 2009
 625 and the tagging events used to compare the spatial-temporal behaviour of newly
 626 tagged fish with fish that had been tagged previously, which were present in the same
 627 waterbody and therefore subject to the same environmental variables.

Group No.	Tagging event No. for effects of tagging analysis (ETA)	Date Tagged	No. of new fish	No. of previously tagged fish used in ETA	Tag Type	Capture Method	Mass mean \pm SD [range], (kg)	Fork Length (mean \pm SD [range], mm)	Stated Tag Life (days)	No. days Tracked (days)
1	-	21/11/2006	7	-	V9	Electro fishing	2.46 \pm 0.34 [1.92-2.94]	480.0 \pm 23.4 [440-512]	135-330	108-501
2	1	28/02/2007	7	7	V9	Rod and Line	2.30 \pm 0.14 [2.09-2.49]	485.1 \pm 17.4 [460-510]	80-330	69-495
3	2	10/10/2007	10	6	V9	Rod and Line	2.31 \pm 0.24 [1.92-2.66]	490.5 \pm 18.8 [461-520]	210	208-210
4	3	04/12/2007	19	16	V9	Seine netting	2.40 \pm 0.25 [1.95-2.91]	485.5 \pm 14.7 [458-511]	210	153-210
5	-	01/10/2008	15	-	V13	Electro fishing	2.48 \pm 0.21 [1.98-2.83]	486.3 \pm 9.79 [468-505]	526-621	202-629
6	4	10/12/2008	13	15	V13	Electro fishing	2.22 \pm 0.23 [1.92-2.83]	479.8 \pm 16.3 [454-522]	526-621	132-543
7	5	25/02/2009	12	28	V13	Electro fishing	2.34 \pm 0.22 [2.04-2.72]	491.1 \pm 17.7 [452-518]	526-621	40-534

628

629

630 Table 2 Results of binomial GLMM, using just the newly tagged fish to test whether
 631 capture method impacted movements in the first 5 days (120h).

Model	Parameter	Estimate	Z	P
(a) Total distance moved	Electric fishing	0		
	Rod and line	0.54 \pm 0.30	1.82	0.069
	Seine net	0.59 \pm 0.29	2.06	0.039
(b) Linear range	Electric fishing	0		
	Rod and line	1.08 \pm 0.33	3.28	0.001
	Seine net	0.48 \pm 0.33	1.42	0.155
(c) Direction	Electric fishing	0		
	Rod and line	-0.32 \pm 0.69	-0.47	0.637
	Seine net	0.38 \pm 0.74	0.51	0.613

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638

639 Table 3 Results of binomial GLMM, using just the newly tagged fish to test whether
 640 capture method impacted movements at 6-10 days (120.1 – 240h) post-release.

Model	Parameter	Estimate	Z	P
(a) Total distance moved	Electric fishing	0		
	Rod and line	0.45 ± 0.79	0.57	0.566
	Seine net	-1.79 ± 0.99	-1.79	0.073
(b) Linear range	Electric fishing	0		
	Rod and line	0.68 ± 0.63	1.09	0.278
	Seine net	-1.25 ± 0.82	-1.54	0.125
(c) Direction	Electric fishing	0		
	Rod and line	0.74 ± 1.07	0.69	0.491
	Seine net	-0.12 ± 1.23	-0.10	0.922

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 642

643 Table 4 Mann-Whitney U tests results for comparisons of TDM and LR between fish
 644 captured and translocated ~35 km downstream, and control fish not captured or
 645 translocated. * denotes significance at 0.05.

Concurrent five day period	Total Distance Moved	Linear Range
1 (1-5 days)	U = 25, n ₁ = 3, n ₂ = 7, P = 0.0674	U = 27, n ₁ = 3, n ₂ = 7, P = 0.0227*
2 (6-10 days)	U = 19, n ₁ = 3, n ₂ = 7, P = 0.6475	U = 27, n ₁ = 3, n ₂ = 7, P = 0.0227*
3 (11-15 days)	U = 19, n ₁ = 3, n ₂ = 7, P = 0.6485	U = 27, n ₁ = 3, n ₂ = 7, P = 0.0227*
4 (16-20 days)	U = 24.5, n ₁ = 3, n ₂ = 7, P = 0.0855	U = 27, n ₁ = 3, n ₂ = 7, P = 0.0227*
5 (21-25 days)	U = 16, n ₁ = 3, n ₂ = 7, P = 1.0000	U = 27, n ₁ = 3, n ₂ = 7, P = 0.0227*

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