

1 Does size matter? A test of size-specific mortality
2 in Atlantic salmon *Salmo salar* smolts tagged with
3 acoustic transmitters

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5 **Final version accepted for publication in *Journal of Fish Biology***

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16 Running headline: SURVIVAL OF SMOLTS BY ACOUSTIC TAGGING

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20 **Abstract**

21

22 Mortality rates of wild Atlantic salmon *Salmo salar* smolts implanted with acoustic
23 transmitters were assessed to determine if mortality was size dependent. The routinely
24 accepted, but widely debated, “2% transmitter mass: body mass” rule in biotelemetry was
25 tested by extending the transmitter burden up to 12.7% of body mass in small (mean fork
26 length 138.3 mm, range 115 – 168 mm) downstream migrating *S. salar* smolts. Over the
27 short timescale of emigration (range 11.9 – 44.5 days) through the lower river and estuary,
28 mortality was not related to *S. salar* size, no relationship was found between mortality
29 probability and transmitter mass: body mass or transmitter length: fork length ratios. This
30 study provides further evidence that smolt migration studies can deviate from the “2% rule”
31 of thumb, to more appropriate study-specific measures, which enables the use of fishes
32 representative of the body size in natural populations without undue effects.

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35 Key Words: Biotelemetry, Migration, Transmitter effects, Tag burden

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Introduction

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43 Recent technological advances have dramatically improved our ability to track fishes in the
44 wild (Cooke *et al.*, 2013; Thorstad *et al.*, 2013). Fuelled by the need to understand the
45 movements of diadromous fishes, particularly salmon smolts, during their estuarine and early
46 marine migration, acoustic transmitters have been miniaturised, thus opening up new and
47 exciting aspects of fisheries research. Previously limited to larger species or older life stages,
48 acoustic telemetry now has the potential to track small fishes through freshwater, estuarine
49 and marine environments for considerable periods of time (Thorstad *et al.*, 2013). Like all
50 battery-powered electronic transmitters, one significant remaining constraint of this
51 technology, for fishes, is the transmitter size relative to that of the fish, which currently
52 precludes use of the technique on small species and very early life stages.

53

54 In fishes, the “2% rule” (Winter, 1996) has been accepted frequently as a ‘rule of thumb’ for
55 maximum tag mass to body mass ratios (tag burden), despite criticism in recent years (Jepsen
56 *et al.*, 2005). Empirical studies have shown negative effects on fishes when tag burden is
57 greater than this and have been used to support this position (McCleave & Stred, 1975; Ross
58 & McCormick, 1981; Marty & Summerfelt, 1986; Adams *et al.*, 1998; Lefrançois *et al.*,
59 2001; Sutton & Benson, 2003).

60

61 More recently, the boundaries of telemetry transmitter burden impacts on small fishes have
62 been explored, stimulated in part by the study of Brown *et al.* (1999) showing no effect on
63 swimming performance of surgically implanted acoustic transmitters (7 x 12 mm, 0.6 g in air)

64 up to 12% of body mass in juvenile hatchery rainbow trout, *Oncorhynchus mykiss* (Walbaum,
65 1792) (mean L_F 88.9, mean mass 7.4 g). Studies on Pacific salmon (*Oncorhynchus* spp.) from
66 hatcheries have attempted to determine a maximum tag burden for surgically intracoelomic
67 implanted transmitters. Species, tag size, survival rate and other measures of performance,
68 however, have varied between studies. For example Zale *et al.* (2005) reported a small
69 decrease in swimming performance with transmitter mass (mass 1-5 g in air, volume 0.5-1.5
70 cm^3) of up to 4% body mass in cutthroat trout, *Oncorhynchus clarkii lewisi* (Richardson,
71 1837) (mean L_T 240 mm, mean mass 132.8 g). Yearling Chinook salmon, *Oncorhynchus*
72 *tshawytscha*, (Walbaum, 1792) (mean L_F 166 mm and mass 50.5 g) exhibited 80 - 100%
73 survival rates with a combined intracoelomic implantation of an acoustic transmitter (7 x 20.5
74 mm, 1.8 g in air) and passive integrated transponder (PIT) tag (2.15×12.0 mm, 0.1 g in air) up
75 to 5.6% of their body mass (Ammann *et al.*, 2013). However, growth and survival impacts in
76 *O.tshawytscha* (L_F 80 – 109 mm, mass 6.8 – 16.3 g) surgically implanted with an acoustic
77 transmitter (mean mass 0.64 g in air; 0.28 ml volume) and a PIT tag (mass 0.10 g in air, 0.04
78 ml volume) were evident at transmitter burdens greater than 6.7% (Brown *et al.*, 2010).

79

80 For many salmonids, seaward-migrating smolts are relatively small, so tag burden issues are
81 particularly acute in these studies. In coho salmon, *Oncorhynchus kisutch*, (Walbaum, 1792)
82 smolts L_F 95 – 130 mm, a maximum transmitter size to body size of 17% L_F and 7% by mass
83 showed no adverse effects on survival, growth or physiology using transmitters of 6 x 19
84 mm, and mass of 0.9 g in air (Chittenden *et al.*, 2009). Small *O. mykiss* pre-smolts (L_F 110 –
85 170 mm, mass 16.8 – 53.3 g) have been shown to survive intracoelomic implantation with
86 acoustic transmitters 8 mm diameter, 24 mm long, mass 1.4 g (with a 12 mm PIT tag
87 embedded in the body of the tag) (Welch *et al.*, 2007), however greatest survival rate in that
88 study was with *O. mykiss* larger than 140 mm L_F .

89

90 Although there is a paucity of studies that have directly examined the effects of tag burden
91 specifically on Atlantic salmon, *Salmo salar* L. 1758 smolts in the wild, there is good reason
92 for concern that tag size effects may introduce unwanted biases to smolt movement and
93 mortality studies. Many tracking studies on *S. salar* smolts have been conducted on *S. salar*
94 which have been reared in hatcheries and are typically larger than wild *S. salar*. For study of
95 stocked smolts, this is acceptable, but their use as a surrogate for wild *S. salar* is a poor
96 choice. Hatchery fishes, express different physiological, behavioural and ecological traits to
97 those of wild smolts (*e.g.* Jonsson *et al.* 1991). Physical condition along with physiological
98 status also differs between wild and hatchery fishes due to their exposure to different
99 selection regimes, thus migration preparedness and survival is likely to differ significantly
100 between hatchery origin and wild smolts (McCormick *et al.*, 1998). Fishes reared in hatchery
101 conditions lack exposure to predators and this may result in increased mortality for hatchery
102 origin individuals when released to the wild. Thorstad *et al.* (2012a), for example, reported
103 low survival (12%) for hatchery reared smolts released to the wild, potentially due to reduced
104 freshwater migratory behaviour.

105

106 Also, resulting from tag burden concerns, in most salmon smolt acoustic telemetry studies
107 using widely available 7 x 20 mm sized transmitters, and where wild fishes are used, often
108 only the largest individuals are selected for tagging (*e.g.* Lefèvre *et al.* 2012). Since the size
109 of fishes is thought to play a significant role in survival, bias in initial selection may falsely
110 represent true behaviour and/or mortality (Gingerich *et al.*, 2012; Deng *et al.*, 2015). There is
111 a pressing need for smolt migration studies which focus on wild rather than hatchery reared
112 fish and access the full size range of the natural migrating smolt populations. One route to

113 enabling this, is to better evaluate the effects that exceeding the ‘2% rule’ may have on wild
114 migrating smolts implanted with acoustic transmitters, particularly under natural conditions.
115 The effect of tag burden, beyond 2% of body mass, on mortality is tested here with wild *S.*
116 *salar* smolts implanted with acoustic transmitters.

117

118 **Materials and Methods**

119

120 The Foyle catchment (4450 km², 54° 736' N; 007° 083' W) is situated on the border between
121 Northern Ireland (U.K.) and the Republic of Ireland (Fig. 1). Two main tributaries of the
122 catchment are the rivers Finn and Mourne, both of which have significant migrations of *S.*
123 *salar* smolts. The average size of these smolts is relatively small at around 135 mm L_F and 26
124 g (Loughs Agency, 2009). These two rivers form the River Foyle at their confluence, which
125 is a transitional/estuarine water under tidal influences. Salinity levels range from 0.14 at the
126 confluence of the rivers Mourne and Finn (River Foyle) to 22 at Culmore point (Fig. 1). This
127 section of river (confluence to Culmore point) will be referred to as the estuarine section. At
128 Culmore point, the Foyle discharges into a large sea lough, Lough Foyle. Lough Foyle is a
129 shallow embayment, covering approximately 186 km², 20% of which is intertidal mudflats.
130 At its mouth, the lough narrows to a 1 km wide channel before discharging into the Atlantic
131 Ocean. Salinity in the sea lough ranges from 22 at Culmore point to 35 at its mouth and
132 represents the early marine phase of migration for migrating smolts (Fig.1).

133

134 Wild *S. salar* smolts were tagged over a 2 year period (2013 to 2014). Individuals were
135 captured by electrofishing (backpack) in the upper tributaries of the Mourne and Finn in 2013

136 and by rod and line only in the Mourne in 2014. *S. salar* were implanted with acoustic
137 transmitters and released close to their capture site (Fig. 1) following a short period of
138 recovery (approximately 30 minutes) post capture. *S. salar* were anaesthetised with clove oil
139 (0.5 mg l^{-1}); their mass (g) and fork length (L_F , mm) were recorded prior to being placed on a
140 v-shaped surgical sponge saturated with river water. The gills were aspirated with 100%
141 river water throughout the procedure. An incision (11-13 mm) was made along the abdominal
142 wall, anterior to the pelvic girdle. A coded acoustic transmitter (either, Model LP-7.3, 7.3mm
143 diameter, 18mm length, 1.9g mass in air, Thelma Biotel AS, www.thelmabiotel.com or
144 Model V7-2x, 7 mm diameter, 18 mm length, 1.9 g mass in air, Vemco Ltd, ,
145 www.vemco.com) was inserted into the intracoelomic cavity. The incision was closed with
146 two independent sterile sutures (6-0 ETHILON, Ethicon Ltd, <http://www.ethicon.com/>) with
147 a surgeons knot. On completion of the procedure, *S. salar* were placed into a keep-box which
148 was positioned in an area of gentle flow in the river overnight; *S. salar* were released in their
149 tagging groups the following day. No mortality occurred before release. Work was
150 undertaken in accordance with UK Home Office licencing.

151

152 An acoustic receiver array was established to monitor tagged *S. salar* smolts. In this study,
153 specific automatic listening stations [ALS (Vemco VR2W)] from within a larger array were
154 utilised to determine the survival of migrating *S. salar*. Receivers were deployed in March
155 and recovered in the July of each year. Transmitter life was expected to extend into mid-July,
156 receivers were recovered after this point, thus it is assumed all migrating *S. salar* would have
157 been detected within the deployment period of receivers. Fish were deemed to have initiated
158 migration upon detection at ALS M1 or F1 (Fig. 1). Detection on ALS L1 indicated
159 successful freshwater and estuarine migration by tagged *S. salar* and are referred to as
160 successful migrants. Detection at ALS, L2 and/or L3 identified *S. salar* migrating through the

161 sea lough into the Atlantic Ocean. It is assumed any *S. salar* not detected at the consecutive
162 ALS (L1 or L2 and L3) was a mortality (unsuccessful migrant) within that specific stage (Fig.
163 1). De-smoltification has not been previously reported for this population.

164

165 Extensive range tests were undertaken throughout the array, and specifically at ALS L2 and
166 L3 (Fig.1) to ensure detection coverage at this location was adequate to determine
167 escapement success. To test for acoustic breaches at L2 and L3, an acoustic transmitter
168 (Model LP-7.3, 139 dB re 1 μ Pa power, Thelma Biotel AS, Trondheim, Norway 2013) was
169 suspended at 3 m depth and trolled (~1500 m x 4; ebbing and flooding tide) by a drifting boat
170 (engine off). Tests identified an acoustic range of 450 m ensuring an overlap in detection
171 ranges of ALS L2 and L3. Transmitter failure rate reported by manufacturers is low (<2%);
172 for Thelma transmitters of the same model used here, Gauld *et al.* (2013) reported control
173 transmitter failure rates of 0% within field test environments. Thus relevant precautionary
174 steps were taken to maximise detection efficiency within the study and enable the
175 determination of transmitter fate.

176

177 The hypothesis that tag burden affects survival in *S. salar* smolts was tested by examining
178 the influence of four characteristics (F_L , *S. salar* mass, transmitter length to F_L ratio and
179 transmitter mass to body mass ratio) on mortality. Tests were conducted on all tagged (AT)
180 *S. salar* to investigate outright mortality, along with a subset of these which initiated
181 migration (ST) to investigate the effect of tag burden during migration. ST *S. salar* were
182 analysed separately as a subset of AT as they were deemed to have initiated migration and
183 thus may be exposed to delayed mortality post tag implantation. *S. salar* were grouped
184 depending on their survival outcome, Welch's two sample *t*-tests were used to compare

185 between each group (survive vs. mortality) for each variable. All analysis was conducted
186 using R statistical computing package (R Development Core Team, 2014).

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189

Results

190

191 Sixty eight wild *S.salar* smolts were implanted with acoustic transmitters (39 in 2013 and 29
192 in 2014) over a 2 year period. *S. salar* fork length (L_F) ranged from 115 to 168 mm and mass
193 from 15 to 44 g (Table I). A lower proportion of *S. salar* (41%) was detected within the array
194 in 2014 compared to 85% in 2013. There was no difference in L_F or transmitter mass to body
195 mass ratio between fish tagged in the Mourne 2014 detected within the array and those not
196 detected (L_F , t -test, $t = -0.8$, $df = 23.3$, $P = >0.05$. transmitter mass: body mass, t -test, $t = 1.3$,
197 $df = 27.0$, $P = >0.05$). Similarly there was no difference between *S. salar* detected in the array
198 and those not in 2013 in the Mourne (L_F , t -test, $t = -1.4$, $df = 2.9$, $P = >0.05$. transmitter mass:
199 body mass, t -test, $t = 1.2$, $df = 2.6$, $P = >0.05$) or between all *S. salar* in the study (L_F , t -test, t
200 $= -0.9$, $df = 35.7$, $p = >0.05$. transmitter mass: body mass, t -test, $t = 0.9$, $df = 36.6$, $P = >0.05$).
201 All *S. salar* were detected in the array from the river Finn in 2013. The exact fate of
202 undetected *S. salar* cannot be directly determined.

203

204 Across the size range of *S. salar* tagged in this study (L_F 115 – 168 mm, mass 15 – 44 g),
205 (Table I) there was no evidence to support the hypothesis that tag burden had any effect on
206 survival. t -tests between all measured parameters of *S. salar* size and transmitter size to *S.*
207 *salar* size ratios showed no significant difference between successful [*S. salar* detected at L1

208 (Fig. 1)] and unsuccessful migrants (Table I). This holds true for all tagged *S. salar* (AT, $n =$
209 68) as well as a subset of these *S. salar* (ST, $n = 41$) which were deemed to have initiated
210 migration.

211

212 Indeed, the smallest tagged *S. salar* within the study ($L_F = 115$ mm, mass = 15 g) successfully
213 migrated through fresh water and the estuary. Of the 10 smallest fish within the study (mean
214 $L_F = 120.1 \pm 3$ mm, mean mass = 18.5 ± 3 g) six were successful migrants, entering the sea
215 lough. Similarly, of the 10 largest fish within the study (mean $L_F = 160.5 \pm 5.8$ mm, mean
216 mass = 38.0 ± 5.0 g) six were also successful migrants reaching the sea lough. The two fish
217 with highest transmitter mass to body mass ratios (both 12.7%) also survived. Mean time \pm
218 S.D. from release to escapement into Atlantic Ocean (last detection within the array for
219 successful migrants) was 24.9 ± 8.8 days (range 11.9 – 44.5 days).

220

221 Mortality within the sea lough was high, only seven individuals were detected at L2 and L3
222 of the initial 41 detected entering the Lough. A two sample *t*-test between *S. salar* which
223 were successful in migrating to L2/3 and those successful in reaching L1 but not L2/3 (Fig.1)
224 showed no difference in transmitter mass to body mass ratio (*t*-test, $t = 0.1$, $df = 10$, $P = 0.9$).

225

226

Discussion

227

228 The range of sizes (Table I) of *S. salar* used in this study include some of the smallest *S.*
229 *salar* smolts used in electronic tagging studies, providing a unique opportunity to determine

230 the effect of tagging on short term (up to 44 days) survival rates and migration patterns of
231 these fish. Mortality of small, wild *S. salar* smolts implanted with acoustic transmitters, was
232 not associated with tag burden, for transmitters 7 x 20 mm in size and 1.9 g mass in air.
233 Survival of the smallest *S. salar* in the study to the sea lough, with a transmitter mass to body
234 mass ratio of 12.7% and 115 mm L_F along with another *S. salar* of the same tag burden,
235 12.7% (L_F 123 mm), demonstrate the ability of small *S. salar* to successfully cope with
236 relatively large acoustic transmitters. This is supported by the high survival rate (60%) to the
237 sea lough of the 10 smallest *S. salar* within the study, equivalent to that of the largest 10
238 (60%). Despite only small numbers of *S. salar* being detected exiting the sea lough, no size
239 difference in mortalities was present. No tagged *S. salar* were recorded on an ALS which had
240 not been recorded previously at an upstream ALS. Combined with no acoustic breaching
241 during range tests and high transmitter reliability, it is assumed the telemetry array design
242 was adequate to determine migration success. High mortality within the lough (83%) was
243 probably due to predation, although mortality by other means (*e.g.* osmoregulatory
244 incompetence) cannot be ruled out. High estuarine predation is commonly reported in smolt
245 migration studies (Hvidsten & Møkkelgjerd, 1987; Serrano *et al.*, 2009; Hedger *et al.*, 2011;
246 Thorstad *et al.*, 2012*b*). Reduced numbers of *S. salar* were detected within the array in 2014
247 despite this not being related to size. No mortalities occurred during the tagging process.
248 This difference might be due, in part, to the change in capture method between the 2 years
249 but the exact fate of these individuals could not be determined. Indeed the need for further
250 investigation on the effects of capture and handling in fishes telemetry studies has recently
251 been highlighted (Jepsen *et al.*, 2015).

252

253 Body size is a limiting factor in acoustic tagging studies, and although the effects of tagging
254 on Pacific salmonids (*Oncorhynchus* spp) are relatively well studied (Jepsen *et al.*, 2005),

255 extrapolation of data across even closely related species should be done with caution (Ebner
256 *et al.*, 2009). The findings of the study presented here do not define tag size or a limit to tag
257 mass ratios, however they do specifically demonstrate the potential to successfully implant
258 small wild *S. salar* smolts with acoustic transmitters at a size much smaller than previously
259 reported. Lacroix *et al.* (2004) recommend a transmitter mass of 8% body mass and a
260 transmitter length of 16% or less of L_F for juvenile *S. salar* following a laboratory
261 experiment. Several studies utilising *S. salar* smolts for tagging have not identified any
262 abnormal mortality rates despite using transmitter mass: body mass ratios above 2%. Urke *et*
263 *al.* (2013) although not specifically reporting on the effect of tag size, indicate high survival
264 rates to sea for wild smolts (775 survival, mean L_F 127 mm, mean mass 16.5 g) implanted
265 with acoustic transmitters (7.3 mm diameter, 1.2 g in water) and hatchery *S. salar* (85%
266 survival, mean L_F 157 mm, mean mass 40.8 g) with transmitter mass to body mass ratios
267 equating to approximately 7%. In addition Thorstad *et al.* (2007) indicated no effect of
268 transmitter to body mass ratio (mean = 6%) on survival of wild *S. salar* post smolts (mean L_T
269 152 mm, mean mass 25 g) implanted with acoustic transmitters (7 x 19 mm 1.9 g in air).
270 Lefèvre *et al.* (2012) utilised transmitter mass (9 x 20 mm, 2.9 g in air) to body mass ratios of
271 up to 14% (mean 12%) with wild *S. salar* smolts and post smolts (>131 mm L_F and >20 g)
272 with no reported effect on mortality.

273

274 This study adds to the growing evidence challenging rigid application of the '2% rule' in
275 biotelemetry (Brown *et al.*, 1999; Jepsen *et al.*, 2005). Brown *et al.* (1999) for example
276 suggest moving away from the 2% rule towards a new standard with a more scientific basis
277 which takes into account the relative buoyancy of a tag and physical dimensions. They argue
278 that there may be a requirement of a fish to compensate for tag buoyancy by transferring gas
279 into their swim bladder. Hence a more buoyant tag may have less impact upon a fish

280 compared with a denser tag of similar dimensions. Jepsen *et al.* (2005) similarly argue that
281 any tag/fish size relationship should be driven by the study objectives and empirical evidence.
282 In some cases, large tags may be utilised without significant effects on behaviour and
283 physiology, whilst in other circumstances, effects such as reduced growth and swimming
284 ability may result from the use of smaller tags (Jepsen *et al.*, 2005; Thorstad *et al.*, 2013).
285 Nevertheless, several longer-term studies have shown growth impacts on fishes with higher
286 tag burdens (Larsen *et al.*, 2013) and concerns over subtle impacts on behaviour and the need
287 to minimize impacts in handling and tagging continue to drive forward tag miniaturisation
288 processes (McMichael *et al.*, 2010; Deng *et al.*, 2015).

289

290 Telemetry has helped unlock an understanding of fish migration ecology providing essential
291 knowledge to manage and conserve declining anadromous fish populations. The ability to
292 identify migration routes, bottlenecks, sources of mortality and species interactions will
293 enable development of more effective conservation strategies. The study presented here has
294 shown that the 2% tag mass to body mass ratio is not an immutable threshold for tagging
295 studies. If *S. salar* smolt migration studies are to adequately represent wild salmon behaviour
296 there is a requirement to move away from the 2% tag mass to body mass rule of thumb
297 adhered to in the past, and towards tested criteria which are species-specific and suitable to
298 address study outcomes, without compromising the natural behaviour of the individual.

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Acknowledgements

303

304 This work was supported by funding from the European Union's INTERREG IVA
305 Programme (project 2859 'IBIS') managed by the Special EU programmes Body. The
306 authors would like to J.Pollock and his staff at the Loughs Agency for technical assistance.
307 The authors would also like to thank IBIS students & staff who helped with field work and
308 technical assistance.

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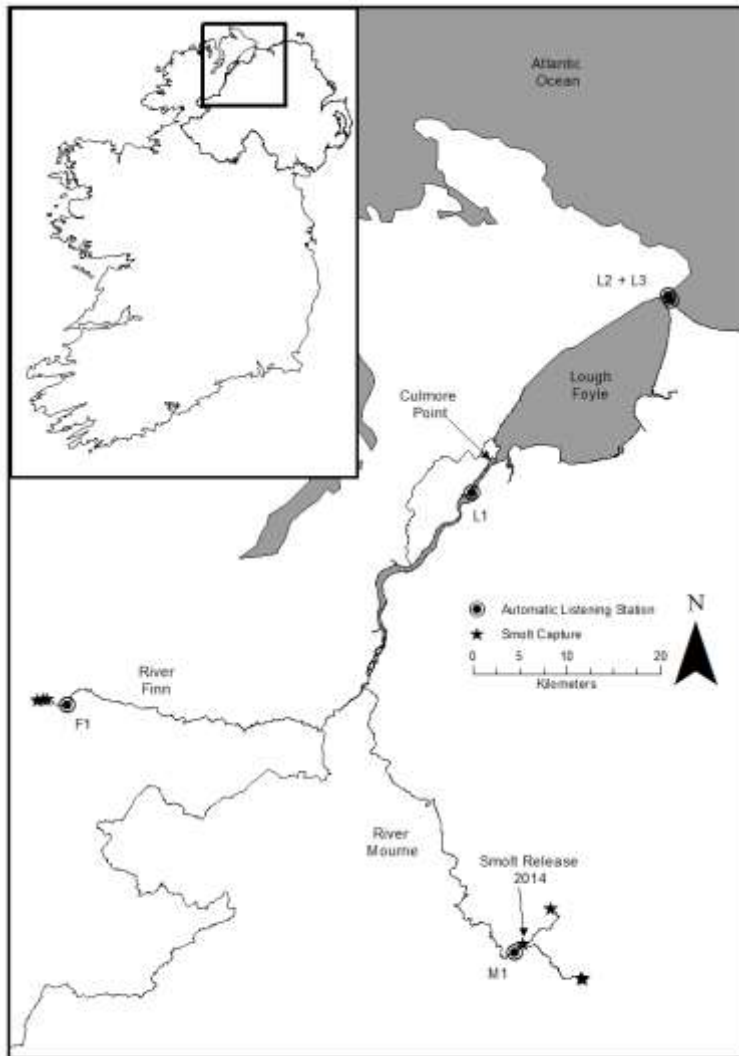
435 Table I: Tests of the differences in a range of *Salmo salar* and tag parameters in smolts that
 436 were successful [detected at ALS L1 (Fig. 1) and unsuccessful in migrating to the sea lough
 437 [not detected at ALS L1 (Fig. 1)], and descriptive statistics for each variable. Tag mass: body
 438 mass (mass %) and tag length: fork length (Length %) ratios are expressed as a percentage.
 439 *Salmo salar* are grouped as all tagged *S. salar* (AT) and a subset of these *S. salar* which were
 440 detected within the acoustic array and deemed to initiate migration (ST)

Group	Test variable	Successful (n) Mean \pm SD	Unsuccessful (n) Mean \pm SD	Range	DF	t-value	P-value
AT	Length (mm)	(41) 138.8 \pm 12.7	(27) 138.3 \pm 13.8	115-168	56.8	-0.2	0.8
AT	Length %	(41) 14.5 \pm 1.3	(27) 14.6 \pm 1.4	11.9- 17.4	57.0	0.3	0.8
AT	Mass (g)	(41) 28.6 \pm 6.5	(27) 28.1 \pm 7.1	15-44	58.4	-0.2	0.8
AT	Mass %	(41) 7.2 \pm 1.9	(27) 7.2 \pm 1.9	4.3-12.7	62.3	0.2	0.9
ST	Length (mm)	(33) 139.1 \pm 12.2	(8) 143.0 \pm 13.5	115-168	9.5	0.8	0.5
ST	Length %	(33) 14.5 \pm 1.3	(8) 14.1 \pm 1.3	11.9- 17.4	10.0	-0.8	0.4
ST	Mass (g)	(33) 28.6 \pm 6.6	(8) 30.65 \pm 7.3	15-44	9.5	0.8	0.5
ST	Mass %	(33) 7.1 \pm 2.0	(8) 6.5 \pm 1.3	4.3-12.7	14.3	-1.1	0.3

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445 FIGURE 1: The Foyle catchment showing location on the border between Northern Ireland
 446 and the Republic of Ireland within the small inset, and the study site location. The large map
 447 outlines the study site, Automatic Listening Station (ALS) locations along with smolt capture
 448 and release points in 2013 and a single point in 2014. The river section between the
 449 confluence of the Mourne and Finn and Culmore point is estuarine.

450

Comment [M1]: I believe the capture points only represent those sites in 2013 and this should be stated in the legend and/or on the figure, that they are capture AND release sites 2013.