1	Comparison of damage to live vs. euthanized Atlantic salmon Salmo salar smolts from				
2	passage through an Archimedean screw turbine				
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15	Scale loss to S. salar smolts in screw turbines				

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16 Abstract:

This study assessed the usefulness of passing euthanized Atlantic salmon Salmo salar smolts 17 through an Archimedean screw turbine to test for external damage, as compared with live, 18 19 actively swimming smolts. Scale loss was the only observed effect. Severe scale loss was 5.9 times more prevalent in euthanized turbine-passed fish (45%) than the live fish (7.6%). 20 Additionally, distinctive patterns of scale loss, consistent with grinding between the turbine 21 22 helices and housing trough, were observed in 35% of euthanized turbine-passed smolts. This distinctive pattern of scale loss was not seen in live turbine-passed smolts, nor in control 23 groups (live and euthanized smolts released downstream of the turbine). We do not advise the 24 use of euthanized fish to estimate damage rates and severity caused by passage through screw 25 turbines since it is likely that the altered behaviour of dead fish in turbine flows generates 26 27 biased injury outcomes.

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29 Keywords: behaviour, hydropower, impact assessment, migration, run-of-river, smolt

Worldwide, incentives to increase renewable energy production have resulted in the 30 emergence of innovative hydropower turbine technologies designed to exploit very low head 31 hydropower potential (Paish, 2002; Bozhinova et al., 2013). The Archimedean screw turbine 32 (AST) has been increasingly favoured for the installation of new hydropower facilities at 33 existing low-head historic barriers in Europe (Bracken & Lucas, 2013). There is a need to 34 assess the potential impacts of such emerging technologies on aquatic biota, particularly on 35 migrating fish. Passage through conventional hydropower turbine infrastructure can result in 36 high fish mortality as a result of injury caused by mechanical damage, rapid changes in water 37 38 velocity and pressure, and high shear stresses (Coutant & Whitney, 2000; Turnpenny et al., 2000, Larinier & Travade, 2002). ASTs operate at low rotational speeds (up to 30 RPM), 39 with no rapid or extreme changes in water pressure and velocity, or high shear stress. Once a 40 41 fish has passed the leading edges of the helical turbine blades, it is contained within a partially water-filled compartment between the screw helices until it is released at the outflow 42 (Kibel, 2007). Nevertheless, several mechanisms for damage to fish by ASTs have been 43 identified, namely: impact by the leading edges of the turbine, grinding between moving and 44 stationary turbine parts, and abrasion (Bracken & Lucas, 2013). 45

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47 Mortality of radio tagged hatchery-reared Atlantic salmon *Salmo salar* L. 1758 smolts 48 passing through an AST has been estimated as under 10% (Havn *et al.*, 2017). Other studies 49 have reported low rates and severity of sub-lethal damage by ASTs to multiple species, life 50 stages and sizes. Kibel (2007) reported under 10% scale loss, by body area, in 4.4% of AST-51 passed wild *S. salar* smolts (1.4% greater than in net-retention controls using hatchery reared 52 brown trout *Salmo trutta* L. 1758). In the same study 3-4% of hatchery reared *S. trutta* lost 53 less than 10% of their scales, and the remainder none (similar to the rate of damage in

controls). Kibel & Coe (2008) found no damage to S. trutta and S. salar kelts, but one case 54 (0.64% prevalence) of a pinched tail of a European eel Anguilla anguilla (L. 1758). In a 55 further study Kibel et al. (2009) observed no damage to a range of species. Brackley et al. 56 (2016) found 2.5% prevalence of 5-30% descaling, beyond a control prevalence of 5%. 57 Bracken & Lucas (2013) found a damage rate of 1.5% for larval and juvenile lampreys 58 *Lampetra sp.* These reports suggest low risk to live fishes from AST passage. However it has 59 not yet been investigated whether similar conclusions could be reached for these turbines 60 using passively drifting fish models (e.g. euthanized fish) - a replacement that would be 61 62 preferred both ethically and for logistical convenience.

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64 The deliberate passage of fish through turbines has been a widely-used technique for assessing turbine impacts. The use of euthanized fish for this purpose may be a useful initial 65 test for identifying the frequency, severity and character of possible damage to passively 66 drifting fish. However, recent evidence (Vowles et al., 2014) suggests that where low water 67 velocities and turbine rotational speeds are utilized, fish behaviour, as well as size and shape, 68 69 may become relatively more important as a determinant for potential injury or mortality, as 70 compared with high-velocity situations in conventional hydropower turbines. In this study, 71 euthanized S. salar smolts were used to assess the potential for damage to passively drifting 72 fish by an AST. The results are compared with those from tests with live fish in order to determine the utility of such passively drifting models for the assessment of damage to fish 73 by ASTs. 74

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The experiments were carried out at Craigpot hydropower scheme (57.26°N, 2.63°W) on the
River Don, Aberdeenshire, Scotland. The scheme uses a four-bladed, 5.4 m length, 2.9 m

diameter AST (www.landustrie.nl) and head of 2.2 m to generate up to 60 kW at its full capacity of 4 m<sup>3</sup>s<sup>-1</sup>. The screw is mounted in a steel trough set at 22° to horizontal, through which the water flows, driving the screw. The upstream-leading edges of the turbine blades are fitted with rubber bumpers with 35 mm of compression to mitigate the physical impact of blade strike to fish, as recommended by the U.K. regulatory authorities (SEPA, 2015; Environment Agency, 2016). The maximum gap between the screw blades and trough is 5 mm.

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The experiments were carried out under UK Home Office Licence (project licence number 86 PPL 40/3425) and complied with the UK Animals (Scientific Procedures) Act 1986. 87 88 Euthanasia was carried out using an overdose of benzocaine, followed by pithing. Hatchery origin S. salar smolts (www.howietounfishery.co.uk) were used in order to attain predictably 89 sufficient sample sizes during the planned period for the experiments, and to avoid interfering 90 with wild migrating smolts. A lethal endpoint was necessary for all experimental smolts 91 because live hatchery reared smolts could not be released or kept after the experiment. S. 92 salar smolts, were transported to Craigpot on 8 April 2014 and carefully transferred to a 2  $m^2$ 93 94 holding tank, which was supplied with fresh water from an immersion pump in the river. 95 Smolts were exposed to ambient river temperatures and experienced natural photoperiod during the experiments. 96

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98 Damage to smolts was assessed by comparing their external condition before and after the 99 experimental treatment. For both live and euthanized smolts, two experimental groups were 100 used: 1) a turbine treatment group which was released above the turbine and recaptured 101 below it; and 2) a control group which was released below the turbine and recaptured as a control for possible change to fish condition resulting from recapture and handling. Each 102 batch comprised treatment and control groups released simultaneously but distinguishable by 103 104 Visible Implant Elastomer marking (VIE, www.nmt.us) or adipose clip. Live smolts (n = 153, mean fork length (FL)  $\pm$  SD = 180.9  $\pm$  9.2, range = 161-202 mm) were released in batches of 105 14-28 fish between 10 and 21 April 2014. Euthanized smolts (n = 30, mean fork length (FL) 106  $\pm$  SD = 179.8  $\pm$  8.3, range = 163-196 mm) were released on a single occasion on 20 April 107 2014. Turbine speed was set at 26 RPM (maximum operating speed) during the releases. 108 109 Experimental release groups and recaptures are summarized in Table I.

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111 Prior to release, live smolts were lightly sedated (benzocaine, 50 ppm), marked with a batchand treatment-specific VIE and or adipose clip mark and processed. While under anaesthesia, 112 each fish was visually assessed for damage and photographed for post-trial assessment of 113 scale loss. Fork length (mm) and mass (g) were measured, and the fish placed on wetted 114 laminated graph paper and photographed 12 times in order to gain a variety of shading 115 conditions and angles for later assessment of scale coverage. These photographs included a 116 view of each flank as well as dorsal and ventral aspects. Fish data were cross-referenced with 117 118 the assessment photographs. Time from anaesthetic induction to the end of processing averaged 154 s, during which the fish remained wetted. For the euthanized release group, 119 marking and processing were carried out exactly as for the live group, immediately after 120 euthanization, and before release. Damage to the head resulting from pithing or other sources 121 122 was not included in the post-trial damage assessments. Live fish were allowed to recover in a 123 tank supplied with fresh river water for at least 30 minutes and checked to ensure that recovery was complete (normal swimming, good balance, no signs of distress – this was the
case for all live experimental fish) prior to release.

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Treatment fish were gently released from a bucket of water through a wetted plastic pipe with its exit directly into the turbine intake basin, 2 m downstream of the trash rack and 4.5 m upstream from the turbine mouth. In order to prevent live fish from escaping upstream, a fence of 10 mm smooth plastic mesh was fitted across the trash rack and remained in place for the duration of the experimental period (10 April to 21 April). Control fish were released simultaneously with, and in the same way as the treatment fish, but 2 m downstream of the turbine.

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A fence (welded metal, covered with 10 mm plastic mesh) was installed below the turbine, along the outlet channel's bed, at an angle of 45 degrees to the direction of flow (plan view) to guide fish into a funnel net with a mesh box at its end. This recapture system remained in place for the duration of the study. Not all live fish arrived in the recapture system naturally and instead held station in the turbine outflow basin. These fish were carefully corralled into the recapture box or captured *in situ* using a section of seine net.

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Recaptured live fish were euthanized before the body condition assessment process was repeated as for prior to release. The recaptured euthanized group were processed equivalently. Care was taken to ensure that handling was kept to a minimum and was consistent across all fish. Scale-loss was assessed *post-hoc* from the photographs taken during fish processing. Photographs were scored blind and in random order. In carrying out this

147	assessment the scorer did not know if a photograph was that of a treatment, control, live or
148	euthanized fish, nor whether the photograph was taken before or after exposure to either
149	treatment. A score from one to four was assigned to each side of each of fish according to the
150	following grading system, and by comparison with reference diagrams (Supplementary Fig.
151	S1) designed to be typical of the grade and aid scoring, though considerable variation in
152	patterns of scale loss distribution occurred:
153	Grade 1: 0-1%; negligible scale loss, scattered and isolated scale loss across the fish's
154	body;
155	Grade 2: 2-4%; low scale loss, scattered across the body but with multiple groups of
156	scale loss several scales high and wide;
157	Grade 3: 5-9%; moderate scale loss, mostly small patches scattered across the body
158	but with at least one larger patch, the height and width of which approximates the
159	width of the wrist of the tail; and
160	Grade 4: 10-30%; extensive scale loss comprising multiple patches, with at least one
161	patch with both dimensions exceeding the width of the wrist of the tail.
162	This grading system was arrived at with prior knowledge of the range and variety of scale
163	loss extent and patterning, the clarity of the photographs and the presence of glare and
164	shading on the fish surface making more precise measurement of scale loss difficult.

Pictures of recaptured fish were matched with those taken of the same individual before release: first by narrowing the number of fish using the batch VIE code or adipose clip mark, then using length and mass data to filter individuals of similar size, and then matching

individuals using distinctive markings. In the first instance spots on the gill cover and 169 distinctive fin shapes (deformed dorsal and pectoral fins were common in these hatchery 170 origin smolts) were used to match individuals. Where these identifiers were not adequate, 171 patterns of pre-existing scale loss and fin damage were also used. It is recognized that scale 172 patterns may have changed as a result of the trials but where matches were made, the patterns 173 used were corroborated with at least two other identifiers on separate areas of the fish. In 174 practice this proved an effective method of identification. Five recaptured fish (two live 175 treatment, and three live control) could not be matched to photographs of released fish, and 176 were excluded from the analysis. 177

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Each side of each fish was scored independently, but the condition, and any change in 179 condition of the two sides of a fish, are not likely to be independent. Hence, in order to carry 180 181 out analyses per fish (rather than per side) the data were summarised to give a single outcome for each fish as follows. Incidences of severe scale loss were defined as those where either 182 side of the fish changed in score by more than one scoring category between release and 183 recapture. Incidences of less severe scale loss, defined as a change by a single category, were 184 more likely to arise from scoring errors for smolts whose condition appeared near the limits 185 186 of a grade. Visual categorization methods of the type used are inevitably prone to a small amount of human error. Therefore the analyses reported here are confined to the more 187 reliable outcome of severe scale loss. The distribution and change of scale coverage grades 188 before release and after recapture, for each fish side, are provided in Supplementary Table 189 190 S1. Association between frequency of severe scale loss and treatment group was tested using 191 Fisher's exact test, both within and between the live and euthanized groups.

193 Scale-loss was the only visible sign of experimentally induced change in any of the treatment/control groups. Prevalence of severe scale loss was significantly greater (by a 194 factor of 5.9) in the euthanized turbine treatment group (45%, 9/20 smolts), than in the live 195 turbine treatment group (7.6%, 6/79 smolts) (score change of two or greater in Figure 1, 196 Fisher's exact test, P < 0.001). There was no significant association between severe scale loss 197 and turbine treatment or control groups, within the live group (severe scale loss in 7/69 198 treatment, and 3/56 control, Fisher's exact test, P > 0.1) or the euthanized group (9/20 199 treatment, and 1/10 control, Fisher's exact test, P > 0.1), although for the euthanized group, 200 201 this is likely due to the small sample size. A substantial portion (35%, 7/20 smolts) of the euthanized treatment group exhibited a consistent and distinctive pattern of scale loss which 202 comprised a curved longitudinal stripe along the flank (Figure 2, and Supplementary Figures 203 204 S12, S16, S19, S20, S22, S24 and S26). This pattern of scale loss was not seen in the live fish, nor in the euthanized control fish. Association between the distinctive scale-loss stripe 205 and treatment or control groups within the euthanized group was not significant (distinctive 206 stripe pattern seen in 7/20 treatment, and 0/10 control, Fisher's exact test, P = 0.06), but again 207 208 this is likely due to the small sample of euthanized smolts. Assessment photographs for all 209 smolts with severe scale loss are provided in the supplementary material (Figures S2-S39).

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The distinctive patterning of descaling observed in seven of the euthanized treatment fish is consistent with that expected from abrasion by the outer edge of the turbine blade, if a fish was to lodge against the gap between the trough and the turbine blade, once within the turbine. It is proposed that the euthanized fish were drawn towards this gap by water flowing from upper to lower turbine compartments under the differential head. This distinctive pattern of damage was not observed in any of the much larger sample of live turbine-passed fish, 217 suggesting that live fish were avoiding contact with these hazard areas in the turbine by active swimming. The significant difference in substantial new scale loss between live and 218 dead treatment fish supports the practical conclusion that passively drifting euthanized fish 219 220 are not appropriate models for assessing potential damage from ASTs. Although within the euthanized group the difference in the prevalence of the scale loss stripe between turbine-221 passed and non-turbine-passed was marginally insignificant (P = 0.06), we cannot conceive 222 any mechanism, other than passage through the turbine, likely to produce this pattern. We 223 rather attribute the lack of a significant effect to the limited sample of euthanized smolts. The 224 225 lack of any significant proportion of the much larger sample of live fish with severe new scale loss is suggestive of no substantive impact to live fish at the AST studied, and supports 226 the findings of some assessments (Kibel, 2007; Kibel & Coe, 2008; Kibel et al., 2009, 227 Brackley et al., 2016) though impacts may be higher in other studies (Havn et al., 2017). 228 Nevertheless the grinding effect observed on euthanized fish identifies a potentially important 229 hazard. Fish with reduced swimming or reaction ability due to low temperature, infection or 230 disorientation may be at higher risk from this damage mechanism. Smaller fish, with weaker 231 swimming ability may also be at more risk of being drawn into the hazardous area. 232

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By contrast to the present study, findings by Vowles *et al.* (2014) suggested an increased likelihood of damage to live salmonids as compared to passively drifting euthanized salmonids when encountering a waterwheel type hydrostatic energy converter. By comparing blade strike models which did and did not incorporate behavioural parameters observed from flume experiments, they found that for rainbow trout *Oncorhynchus mykiss* (Walbaum 1792), the exposure time in the hazardous blade swept region was increased because live fish tended to orientate upstream and maintain swimming whilst approaching the turbine. These opposing directions of effect for salmonids between passive and active models in these two studies highlight the importance of considering each of the potential mechanisms for damage from turbine passage, and identifying the differential effects of these on fish of differing size, morphology and swimming behaviour in order to arrive at a sensible compromise on design and operational constraints to protect the fish species present. These considerations are more widely applicable to emerging novel turbine technologies, both in rivers and those utilizing tidal currents.

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TABLE I. Sample numbers of live and euthanized smolts used to assess damage from passage through an Archimedean screw turbine. Treatment groups were released above the turbine and recovered below after they had passed through it. Control groups were equivalently handled, but were released and recaptured below the turbine. Given are the numbers of smolts released, numbers recaptured, and number of recaptured smolts identified and matched to records of released smolts.

	Live group		Euthanized group	
	Treatment	Control	Treatment	Control
Released	89	64	20	10
Recaptured	81	59	20	10
Recaptures matched	79	56	20	10

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321 FIG 1. Frequencies of changes in condition to live (A, B, upper panels), and euthanized (C,D, lower panels), smolts that passed through an Archimedean screw turbine (A,C, left panels) or 322 were equivalently handled but released and recaptured below the turbine without passing 323 through it (B,D, right panels). Here condition was measured by assigning a score from one to 324 four for scale coverage to each side of each smolt, before and after either turbine-passage or 325 non-turbine-passage. Change in condition was the difference in score from before to after the 326 experiment, for the side of the fish with the greater change. The small number of negative 327 changes are the result of human error during scoring. 328



FIG 2. The distinctive curved longitudinal stripe of scale loss that was observed in euthanized smolts that had passed through the Archimedean screw turbine. This distinctive pattern was not seen in equivalently handled non-turbine-passed smolts, nor in live turbine-passed, and live non-turbine-passed smolts.