Comparison of damage to live *vs.* euthanized Atlantic salmon *Salmo salar* smolts from passage through an Archimedean screw turbine

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Scale loss to *S. salar* smolts in screw turbines

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This study assessed the usefulness of passing euthanized Atlantic salmon *Salmo salar* smolts through an Archimedean screw turbine to test for external damage, as compared with live, 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%). Additionally, distinctive patterns of scale loss, consistent with grinding between the turbine 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 groups (live and euthanized smolts released downstream of the turbine). We do not advise the use of euthanized fish to estimate damage rates and severity caused by passage through screw turbines since it is likely that the altered behaviour of dead fish in turbine flows generates biased injury outcomes.

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

Worldwide, incentives to increase renewable energy production have resulted in the emergence of innovative hydropower turbine technologies designed to exploit very low head hydropower potential (Paish, 2002; Bozhinova *et al*., 2013). The Archimedean screw turbine (AST) has been increasingly favoured for the installation of new hydropower facilities at existing low-head historic barriers in Europe (Bracken & Lucas, 2013). There is a need to assess the potential impacts of such emerging technologies on aquatic biota, particularly on migrating fish. Passage through conventional hydropower turbine infrastructure can result in high fish mortality as a result of injury caused by mechanical damage, rapid changes in water 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), with no rapid or extreme changes in water pressure and velocity, or high shear stress. Once a 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 (Kibel, 2007). Nevertheless, several mechanisms for damage to fish by ASTs have been identified, namely: impact by the leading edges of the turbine, grinding between moving and stationary turbine parts, and abrasion (Bracken & Lucas, 2013).

Mortality of radio tagged hatchery-reared Atlantic salmon *Salmo salar* L. 1758 smolts passing through an AST has been estimated as under 10% (Havn *et al.*, 2017). Other studies have reported low rates and severity of sub-lethal damage by ASTs to multiple species, life stages and sizes. Kibel (2007) reported under 10% scale loss, by body area, in 4.4% of AST-passed wild *S. salar* smolts (1.4% greater than in net-retention controls using hatchery reared brown trout *Salmo trutta* L. 1758). In the same study 3-4% of hatchery reared *S. trutta* lost 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 (0.64% prevalence) of a pinched tail of a European eel *Anguilla anguilla* (L. 1758). In a further study Kibel *et al*. (2009) observed no damage to a range of species. Brackley *et al*. (2016) found 2.5% prevalence of 5-30% descaling, beyond a control prevalence of 5%. Bracken & Lucas (2013) found a damage rate of 1.5% for larval and juvenile lampreys *Lampetra sp*. These reports suggest low risk to live fishes from AST passage. However it has not yet been investigated whether similar conclusions could be reached for these turbines using passively drifting fish models (e.g. euthanized fish) - a replacement that would be preferred both ethically and for logistical convenience.

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 test for identifying the frequency, severity and character of possible damage to passively drifting fish. However, recent evidence (Vowles *et al.*, 2014) suggests that where low water velocities and turbine rotational speeds are utilized, fish behaviour, as well as size and shape, may become relatively more important as a determinant for potential injury or mortality, as compared with high-velocity situations in conventional hydropower turbines. In this study, euthanized *S. salar* smolts were used to assess the potential for damage to passively drifting 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 by ASTs.

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^3s^{-1}. 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.

The experiments were carried out under UK Home Office Licence (project licence number PPL 40/3425) and complied with the UK Animals (Scientific Procedures) Act 1986. 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 sufficient sample sizes during the planned period for the experiments, and to avoid interfering with wild migrating smolts. A lethal endpoint was necessary for all experimental smolts because live hatchery reared smolts could not be released or kept after the experiment. *S. salar* smolts, were transported to Craigpot on 8 April 2014 and carefully transferred to a 2 m^2 holding tank, which was supplied with fresh water from an immersion pump in the river. Smolts were exposed to ambient river temperatures and experienced natural photoperiod during the experiments.

Damage to smolts was assessed by comparing their external condition before and after the experimental treatment. For both live and euthanized smolts, two experimental groups were used: 1) a turbine treatment group which was released above the turbine and recaptured 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 batch comprised treatment and control groups released simultaneously but distinguishable by Visible Implant Elastomer marking (VIE, www.nmt.us) or adipose clip. Live smolts (*n* = 153, mean fork length (FL) ± SD = 180.9 ± 9.2, range = 161-202 mm) were released in batches of 14-28 fish between 10 and 21 April 2014. Euthanized smolts (*n* = 30, mean fork length (FL) ± SD = 179.8 ± 8.3, range = 163-196 mm) were released on a single occasion on 20 April 2014. Turbine speed was set at 26 RPM (maximum operating speed) during the releases. Experimental release groups and recaptures are summarized in Table I.

Prior to release, live smolts were lightly sedated (benzocaine, 50 ppm), marked with a batch- and treatment-specific VIE and or adipose clip mark and processed. While under anaesthesia, each fish was visually assessed for damage and photographed for post-trial assessment of scale loss. Fork length (mm) and mass (g) were measured, and the fish placed on wetted laminated graph paper and photographed 12 times in order to gain a variety of shading conditions and angles for later assessment of scale coverage. These photographs included a view of each flank as well as dorsal and ventral aspects. Fish data were cross-referenced with 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, marking and processing were carried out exactly as for the live group, immediately after euthanization, and before release. Damage to the head resulting from pithing or other sources was not included in the post-trial damage assessments. Live fish were allowed to recover in a 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.

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.

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.

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 assessment the scorer did not know if a photograph was that of a treatment, control, live or euthanized fish, nor whether the photograph was taken before or after exposure to either treatment. A score from one to four was assigned to each side of each of fish according to the following grading system, and by comparison with reference diagrams (Supplementary Fig. S1) designed to be typical of the grade and aid scoring, though considerable variation in patterns of scale loss distribution occurred:

Grade 1: 0-1%; negligible scale loss, scattered and isolated scale loss across the fish's body;

Grade 2: 2-4%; low scale loss, scattered across the body but with multiple groups of scale loss several scales high and wide;

Grade 3: 5-9%; moderate scale loss, mostly small patches scattered across the body but with at least one larger patch, the height and width of which approximates the width of the wrist of the tail; and

Grade 4: 10-30%; extensive scale loss comprising multiple patches, with at least one patch with both dimensions exceeding the width of the wrist of the tail.

This grading system was arrived at with prior knowledge of the range and variety of scale loss extent and patterning, the clarity of the photographs and the presence of glare and 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 distinctive fin shapes (deformed dorsal and pectoral fins were common in these hatchery origin smolts) were used to match individuals. Where these identifiers were not adequate, patterns of pre-existing scale loss and fin damage were also used. It is recognized that scale patterns may have changed as a result of the trials but where matches were made, the patterns used were corroborated with at least two other identifiers on separate areas of the fish. In practice this proved an effective method of identification. Five recaptured fish (two live treatment, and three live control) could not be matched to photographs of released fish, and were excluded from the analysis.

Each side of each fish was scored independently, but the condition, and any change in condition of the two sides of a fish, are not likely to be independent. Hence, in order to carry 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 side of the fish changed in score by more than one scoring category between release and recapture. Incidences of less severe scale loss, defined as a change by a single category, were more likely to arise from scoring errors for smolts whose condition appeared near the limits 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 reliable outcome of severe scale loss. The distribution and change of scale coverage grades before release and after recapture, for each fish side, are provided in Supplementary Table S1. Association between frequency of severe scale loss and treatment group was tested using Fisher's exact test, both within and between the live and euthanized groups.

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 factor of 5.9) in the euthanized turbine treatment group (45%, 9/20 smolts), than in the live turbine treatment group (7.6%, 6/79 smolts) (score change of two or greater in Figure 1, Fisher's exact test, $P < 0.001$). There was no significant association between severe scale loss and turbine treatment or control groups, within the live group (severe scale loss in 7/69 treatment, and 3/56 control, Fisher's exact test, $P > 0.1$) or the euthanized group (9/20 treatment, and 1/10 control, Fisher's exact test, $P > 0.1$), although for the euthanized group, 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 comprised a curved longitudinal stripe along the flank (Figure 2, and Supplementary Figures 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 and treatment or control groups within the euthanized group was not significant (distinctive stripe pattern seen in 7/20 treatment, and 0/10 control, Fisher's exact test, $P = 0.06$), but again this is likely due to the small sample of euthanized smolts. Assessment photographs for all smolts with severe scale loss are provided in the supplementary material (Figures S2-S39).

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, 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 dead treatment fish supports the practical conclusion that passively drifting euthanized fish 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-passed and non-turbine-passed was marginally insignificant ($P = 0.06$), we cannot conceive any mechanism, other than passage through the turbine, likely to produce this pattern. We rather attribute the lack of a significant effect to the limited sample of euthanized smolts. The 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 the findings of some assessments (Kibel, 2007; Kibel & Coe, 2008; Kibel *et al*., 2009, Brackley *et al*., 2016) though impacts may be higher in other studies (Havn *et al*., 2017). Nevertheless the grinding effect observed on euthanized fish identifies a potentially important hazard. Fish with reduced swimming or reaction ability due to low temperature, infection or disorientation may be at higher risk from this damage mechanism. Smaller fish, with weaker swimming ability may also be at more risk of being drawn into the hazardous area.

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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