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Evaluation of external fish injury caused by hydropower plants based on a novel field-based protocol

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Abstract

Knowledge on the extent and mechanisms of fish damage caused by hydropower facilities is important for their ecological improvement. Herein, a novel field-based fish injury assessment protocol is proposed that includes vitality and four general health criteria, as well as nine lethal and sub-lethal injury types across 18 body parts. The protocol was validated using 3,087 specimens from four species of hatchery-reared fish, as well as 2,262 specimens from 32 species of wild fish. The protocol allowed a detailed and systematic evaluation of different fish injury types in the field. Injuries related to handling and to contact with different parts of the hydropower structure could be distinguished applying multivariate statistics. This approach allows quantification and comparison of fish injuries across sites, and can help to identify the technologies and operational procedures that minimise damage to fish. It may also be useful to assess fish health in other contexts including aquaculture.

KEYWORDS

animal welfare, fish conservation, fish mortality, fish passage, hydropower monitoring

1 | INTRODUCTION

Hydropower technology is considered a clean and renewable energy source of increasing worldwide importance (Zarfl, Lumsdon, Berlekamp, Tydecks & Tockner, 2015). Hydropower is generated by converting the kinetic and potential energy from falling water into rotating shaft power, which can be used to drive an electricity generator (Paish, 2002). Unfortunately, downstream moving fish often enter the hydropower structure where they are exposed to extreme risks of harm (Williams, Armstrong, Katopodis, Larinier & Travade, 2012). Various physical mechanisms can result in various forms of fish damage, including collisions with the machinery (Killgore, Maynord, Chan & Morgan, 2001), bar screens or cleaning devices (Adam & Brujis, 2006; Nettles & Gloss, 1987; Skalski, Mathur & Heisey, 2002), shear stress near the turbine blades, in the draft tube and in the tailrace (Čada, Garrison & Fisher, 2007), barotrauma caused by pressure changes (Brown, Pflugrath, et al., 2012; Brown, Carlson, et al.,2012), cavitation forces within the runner case, turbulences and fluid shear within the suction hose as well as in the tailrace (Abernethy, Amidan & Čada, 2001). Resulting injuries include scale loss, fin damage, haemorrhages, bruises, skin wounds, amputations of body parts or internal injuries, such as swim bladder rupture and emboli (Dedual, 2007; Ebel, 2013; Schneider, Hübner & Korte, 2012). As movement and migration are obligatory elements in the life cycle of many fishes (Lucas, Baras, Thom, Duncan & Slavík, 2001), the injuries and mortalities resulting from passage through turbines are still a major drawback of hydroelectric energy (Hogan, Čada & Amaral, 2014).

The severity of the impact of power plant passage is dependent on technical characteristics such as the rotation speed of the turbine, turbine diameter, number of turbine blades, blade angle and the drop height, which determine the degree of pressure changes, shear stress, cavitation and the collision risk for fish (Ferguson, Ploskey, Leonardsson, Zabel & Lundqvist, 2008; Skalski et al., 2002). Conventional power plants are often equipped with Pelton, Kaplan or Francis turbines, which are known for very high fish mortality due to their high rotation speed, pressure changes and shear forces (Hogan et al., 2014).

Currently, there is increased effort towards development of more fish-friendly hydropower installations, including fish protection screens in combination with bypass systems at conventional turbines, as well as innovative turbine types. Innovative turbine types such as the very-low-head turbine or Archimedes screw turbines are supposed to be more “fish-friendly” than conventional turbines (Hogan et al., 2014), but scientific evidence on lethal and sub-lethal effects of these power plants on fish is still scarce. Single mechanisms causing damage to the fish body, such as pressure changes, have been intensively studied in the laboratory and in mathematical models (e.g. Brown, Pflugrath, et al., 2012; Ferguson et al., 2008; Neitzel et al., 2004; Stephenson et al., 2010). Whilst such studies allow stricter control over dependent variables or stressors, they do not allow a comprehensive assessment of the combined effects from different stressors under real-world conditions. However, field evaluations are more time consuming and resource intensive, and their effectiveness thus needs to be justified. Previous studies on fish damage under field conditions have either only assessed mortality (e.g. Brown, Carlson, et al., 2012; Calles, Karlsson, Vezza, Comoglio & Tielman, 2013; Carlson et al., 2012; Keefer et al., 2013), determined four to five severity categories of fish injury without discriminating injury types or body parts (e.g. Holzner, 1999; Lagarrigue & Frey, 2010; Schneider et al., 2012), qualitatively described some injury patterns (Bochert & Lill, 2004), or specifically focused on single injury types such as barotrauma (Brown, Pflugrath, et al., 2012; Colotelo et al., 2012) or hemorrhaging (Colotelo, Cooke & Smokorowski, 2009). Consequently, there are to date no generally applicable standards for a classification of various injury types and intensities at different body parts, which would be necessary to assess the “fish-friendliness” of conventional and novel hydropower techniques. Also, detecting small differences in survival between turbine operations remains a great challenge (Ferguson, Absolon, Carlson & Sandford, 2006). Different design features and operation conditions of hydropower plants can result in different impacts (e.g. rapid decompression, fluid shear, collision), which are related to different injury types. Thus, a standard method enabling a detailed evaluation of injury patterns after power plant passage, taking into account different body parts and injury types, would help to determine the most injurious design features and improve fish-friendly turbine design and operational management. Because fish-catching techniques (e.g. net-based, electrofishing), as well as natural factors (e.g. predation by birds, mammals and larger fish), can also result in injury patterns, it is necessary to distinguish these injuries from hydropower-derived damage. Consequently, evaluation methods should be highly sensitive and allow discrimination of different causes of injury. This is important because legal regulations on water management, as formulated in the European Water Framework Directive (European Parliament, 2000), necessitate predictions on fish population-level effects of hydropower use that are derived from the survival probability of individuals. The monitoring of fish damage at hydropower plants is therefore currently increasingly enforced by authorities in many member states. This requires a standardised and detailed assessment of fish injury patterns.

Herein, a universally applicable protocol for a systematic field evaluation of external fish injuries is proposed. To validate the methodology, three main questions were investigated using an experiment comprising wild (natural downstream movement of fish) and hatchery-reared fish (standardised damage assessment compared with predamage) at a hydropower plant with Kaplan turbines: (1) Can the method distinguish the effect of turbine passage, fish handling effects as well as catch-related effects? (2) Which injuries or combinations are most important in relation to turbine passage? (3) How and to what extent do injuries vary among different fish species and origin (hatchery-reared versus wild)?

2 | MATERIAL AND METHODS

All data used were produced within an animal experiment in accordance with national laws and regulations (animal care permit number 50.2-1-54-2532-31-2015). All protocols and methods used were evaluated for appropriate animal care and use by the ethics commission of the Bavarian government. Adequate measures minimising pain or discomfort were taken following European guidelines (European Parliament, 2010) and national standards for the use of aquatic animals for experimental purpose (Adam, Schürmann & Schwevers, 2013).

2.1 | Study site

The study was carried out at a hydropower plant at the River Regnitz in Baiersdorf-Wellerstadt, Bavaria, Germany (N 49.6706, E 11.0424). The River Regnitz has a mean annual discharge of 34.8 m^3/s. During the investigation, the average discharge was 27.0 ± 1.0 m^3/s and ranged between 25.1 and 29.8 m^3/s. The power plant is placed in a headrace channel and is equipped with two identical horizontal Kaplan turbines with four turbine blades (diameter—2 m, drive—150 rpm, power—15–320 kW) as well as an inclined (27°) vertical fish protection screen with a bar spacing of 15 mm. The drop height between headrace and tailrace is 2.3 m. During the investigation, the turbines were run at 240 ± 4 kW each. All experiments were conducted within 2 weeks in September 2015.

2.2 | Experimental design

The dataset included four species of hatchery-reared fish (European eel, *Anguilla anguilla* L.—total length: 200-640 mm; common nase, *Chondrostoma nasus* L.: 40-120 mm; brown trout, *Salmo trutta* L.: 80-160 mm and European perch, *Perca fluviatilis* L.: 70-150 mm), which were tested for hydropower-related injuries in a standardised experiment in which specimens were deliberately released at different parts of the facility. Additionally, the naturally downstream moving fish and the upstream fish community at the power plant were considered (Figure 1, Table 1). The four test species were selected according to morphological characteristics and their ecological relevance in Bavarian streams (*A. anguilla*: long-distance migratory species,

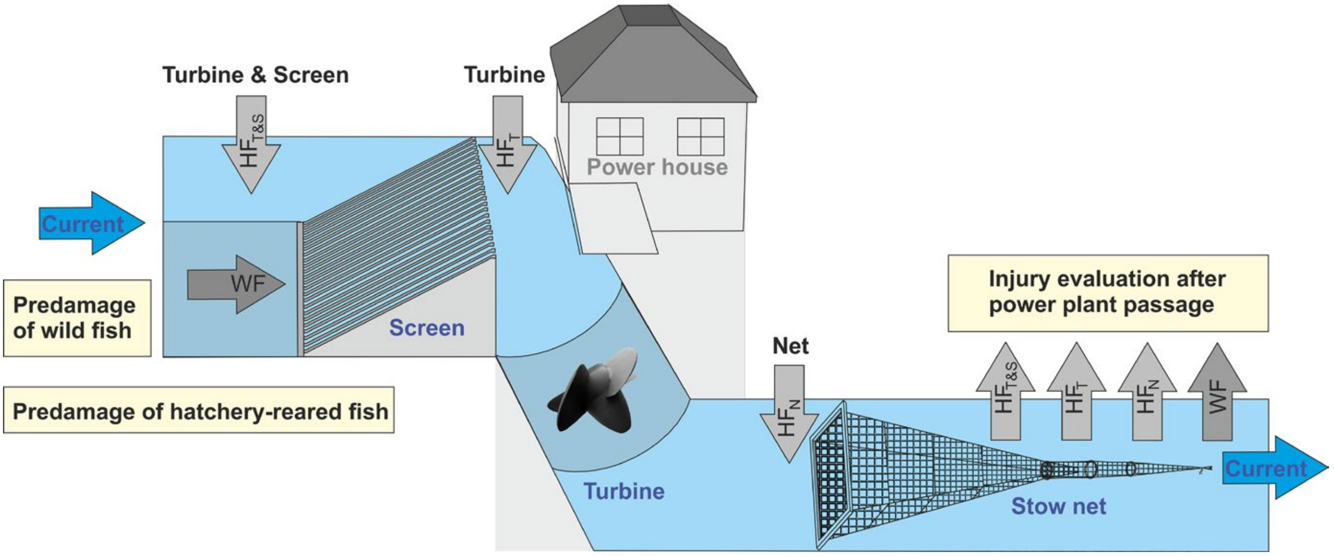


FIGURE 1 Schematic of the experimental design and the hydropower plant comprising assessment endpoints (yellow boxes), and fish release and catching points (grey arrows) differentiating wild (dark grey, WF) and hatchery-reared (light grey, HF) fish. $\mathrm{HF}_{\mathrm{T\&S}}$ = release point upstream of fish protection screen, HF_{T} = release point at turbine inlet, HF_{N} = release point at the entrance of the stow net; blue arrows = flow direction, Screen = angled vertical fish protection screen, Turbine = horizontal Kaplan turbine, Stow net = fish recovery unit. Treatments are indicated in bold black letters: Net = hatchery-reared fish released directly at the entrance of the stow net, Turbine = hatchery-reared fish released at the turbine inlet, Turbine & Screen = hatchery-reared fish released upstream from fish protection screen. Predamage in wild fish was assessed following electrofishing in the headrace, the assessment of predamage in hatchery-reared fish was based on a representative sample of fish without treatment. [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Numbers of hatchery-reared fish and wild fish used in the different treatments of the experiment

Dataset	Fish species	All treatments	Upstream fish	Predamage	Net	Turbine	Turbine & Screen
Hatchery-reared fish	All species	3087	n.a.	210	631	951	1295
	A. anguilla	544	n.a.	60	188	183	113
	S. trutta	713	n.a.	74	131	251	257
	C. nasus	820	n.a.	33	146	224	417
	P. fluviatilis	1010	n.a.	43	166	293	508
Wild fish	All species	2262	397	n.a.	n.a.	n.a.	1865
	A. alburnus	454	48	n.a.	n.a.	n.a.	406
	A. bipunctatus	358	36	n.a.	n.a.	n.a.	322
	P. parva	285	15	n.a.	n.a.	n.a.	270
	R. rutilus	233	50	n.a.	n.a.	n.a.	183
	L. leuciscus	207	56	n.a.	n.a.	n.a.	151
	S. trutta	140	11	n.a.	n.a.	n.a.	129
	S. lucioperca	107	3	n.a.	n.a.	n.a.	104
	C. nasus	85	0	n.a.	n.a.	n.a.	85
	P. semilunaris	56	8	n.a.	n.a.	n.a.	48
	Other species	337	170	n.a.	n.a.	n.a.	167

All species = sum for all species within each category, other species = sum of all individuals from wild species caught in numbers less than 30, n.a. = not available. Upstream fish = wild fish caught by electrofishing in the headrace of the power plant, Predamage = hatchery-reared fish, Net = hatchery-reared fish released directly at the entrance of the stow net, Turbine = hatchery-reared fish released at the turbine inlet, Turbine & Screen = hatchery-reared fish released upstream from fish protection screen or wild fish caught in the stow net at the turbine outlet. *A. anguilla* = European eel, *Anguilla anguilla* L.; *S. trutta* = brown trout, *Salmo trutta* L., *C. nasus* = common nase, *Chondrostoma nasus* L., *P. fluviatilis* = European perch, *Perca fluviatilis* L.; *A. alburnus* = bleak, *Alburnus alburnus* L.; *A. bipunctatus* = Schneider, *Alburnoides bipunctatus* (Bloch); *P. parva* = topmouth gudgeon, *Pseudorasbora parva* (Temminck & Schlegel); *R. rutilus* = roach, *Rutilus rutilus* L., *L. leuciscus* = common dace, *Leuciscus leuciscus* L.; *S. lucioperca* = pikeperch, *Sander lucioperca* L.; *P. semilunaris* = Western tubenose goby, *Proterorhinus semilunaris.* (Heckel, 1837) elongated body shape, tough skin, very small cycloid scales; *C. nasus*: typical laterally flattened cyprinid with cycloid scales, *S. trutta*: typical salmonid species with torpedo-shaped body form and cycloid scales; *P. fluviatilis*: typical percid species with ctenoid scales). Hatchery-reared fish were used to quantify hydropower-related fish damage under consideration of predamage from aquaculture and transportation, effects from catching them with the net, as well as potential effects of the fish protection screen. For this purpose, four different treatments of hatchery-reared fish were used (Turbine & Screen, Turbine, Net, Predamage; Figure 1) to allow an assignment of specific injury types to specific hydropower structures. For the treatment turbine & screen, fish were introduced in the headrace directly upstream of the fish protection screen. Fish from this treatment were affected by the screen, the turbine and the catching procedure. For the treatment turbine, fish were released in the turbine inlet downstream of the screen. Fish from this treatment were only affected by the turbine and the catching procedure, excluding effects from the screen. For the treatment net, fish were released at the entrance of the stow net. This treatment served as internal control for catch-related effects. For treatment predamage, individuals from each species were investigated for their injuries without any further treatment as a control, with potential damage arising from aquaculture, transportation and other handling effects (e.g. touching and dip-netting from the fish tanks). All fish were carefully released at the different parts of the power plant from a water-filled bucket at the water surface. Fish from each release point were individually marked in accordance with the animal care permit. After power plant passage, fish were caught at the turbine outlets in the tailrace with four knotless stow nets of decreasing mesh size and narrowing diameter (two nets per turbine outlet, length: 22.8 m, mesh sizes: 30 mm, 20 mm, 15 mm, 10 mm and 8 mm; Figure 1), applying 1- to 2-h emptying intervals during the day and night. Naturally downstream moving wild fish in the catch, which could be distinguished from introduced hatchery-reared fish due to their distinct morphological characteristics (colour, body shape, fin shape, size), were also evaluated for injuries. In addition to assessing fish after turbine passage and the predamage of hatchery-reared fish, electrofishing (11 kW, EL 65 II, Grassl, Schönau, Germany; 1 anode, 1 dipnet) was carried out in the headrace to assess the condition of upstream naturally occurring fish before passing the power plant. This additional control was primarily used to compare the injuries of these fish with the caught wild fish after turbine passage. However, it has to be noted that dead fish and fish with strongly reduced vitality cannot be caught using electrofishing, yet they may still be part of the "natural downstream movement" caught in the nets. Data from the natural fish community were used to validate whether standardised experiments with hatchery fish representing different morphological fish types can be used as surrogates for wild fish to determine hydropower effects (question 3).

2.3 | Fish injury assessment protocol

To establish a standardised protocol for recording fish injury in the field, 3,087 specimens from the four species of hatchery-reared fish as well as 2,262 specimens from 32 species of the wild fish population (Table 1) were examined for all externally visible injuries. The injury types examined were identified using a literature search. Available international and national literature on fish damage at hydropower plants was reviewed using the search terms "fish injury," "fish damage," "fish mortality" and "fish health" in ISI Web of Science, Google Scholar and the database of the Bavarian Environmental Authority. The injury categories used are shown in Table 2, with photographs in Figure 2. Intensity of the injuries was distinguished into four categories: no damage=0, minor damage=1, medium damage=3 and severe damage=5. For recording the intensities of the different injuries, the fish body was subdivided into distinct anatomical sections (Fig. S1) : dorsal, ventral, anterior body part (without head, left and right side respectively), posterior body part (left and right side), head, opercula, and fins (dorsal, caudal, anal, left and right ventral, left and right pectoral fins). The 86 possible combinations of body parts with injury types were summarised and the intensity of each injury at each body part recorded (Fig. S2). The assignment of the intensity levels of each injury type follows a detailed score sheet (Table S1).

In addition to the recording of the specific injuries, five general criteria of fish health were documented, including vitality, nutritional status, respiratory movements, fungal infections and parasite infections (Table 2). These criteria provide information on an individual's susceptibility and regeneration potential (Belding, 1929; Tierney & Farrell, 2004). For estimation of fish condition, another scoring system including a fifth category was used according to animal use and care guidelines (0, 1, 2, 3, 4). This scoring system allowed discrimination between dead fish that suffered from severe stress (vitality 0) from severely stressed fish, which still had minor vital functions (e.g. movement of opercula, vitality 1; see Table S2) and that had to be euthanised (using a tenfold overdose of MS 222) for ethical reasons according to national animal use and care guidelines (Adam et al., 2013). As both categories 0 and 1 comprise non-surviving fish, they were pooled as one category in the data analysis, resulting in four categories (0, 1, 3, 5) in the scoring system, with 5 representing severe loss of vitality and 0 representing full vitality.

2.4 | Fish handling and injury assessment procedure

Hatchery-reared fish were directly delivered to the study site from the fish hatcheries one day prior to the experiments. All fish were carefully adapted to the water chemistry and temperature of the River Regnitz and transferred into rectangular fish tanks (300 × 70 × 70 cm, Aquacultur Fischtechnik GmbH, Nienburg, Germany). Fish densities in the tanks were adapted to species-specific according to the national guidelines for keeping fish in animal experiments (Adam et al., 2013). To mimic similar water conditions as those that fish would normally experience after turbine passage, fish tanks were permanently supplied with fresh river water from the tailrace by a submersible pump. Temperature (mean = 14.7 ± 0.2°C), dissolved oxygen (mean = 9.8 ± 0.5 mg/L), pH (mean = 8.4 ± 0.2), turbidity (mean = 4.9 ± 1.7 NTU) and electric conductivity (mean=818 ± 230 μS/cm at 25°C) were controlled

TABLE 2 Description of injury types and general health criteria

Description	
Injury types	
Spine deflection	Externally visible deformation of the spinal column, such as cracks or S-curves.
Amputations	Partly or complete detachment of body parts (e.g. opercula, fins, eyes) or complete transection of the body. Low intensity amputation includes loss of muscle tissue, resulting in an opened body cavity.
Haemorrhages	Externally visible effusions of blood at different parts of the body, including eyes or fins.
Bruises	Pressure marks on the fish body leading to a visible deformation of the natural body shape.
Emboli	Externally visible gas bubbles underneath the skin, e.g. in eyes and fins.
Dermal lesions	Injuries of the skin reaching from small abrasions of epidermis to deeper wounds with injury of the muscle tissue.
Tears in fins	Injury of the skin between fin rays. Tears can range from small cuts between fin rays to completely fissured fins.
Scale loss	Missing scales at naturally scaled body parts.
Pigment anomalies	Change in normal pigmentation of the epidermis, often visible as dark stripes or darkened area of body surface. Brightened areas are also possible.
General health condition criteria	
Vitality	Describes the acute general condition and swimming performance of an individual. It is used to discriminate dead and alive fish, as well as fish with reduced vitality following animal care guidelines.
Respiratory movements	Combined action of mouth and gill cover (Belding, 1929). Water is taken in by opening the mouth. The mouth is then closed, and the water is forced through the gills, causing a visible outward extension of the gills.
Nutritional status	Body condition as a result of feeding and nutrients in the body that permit metabolic integrity.
Fungal infections	Fungal infections usually occur when the fish is in a weakened state. Symptoms include light grey, cottony growths on the skin, fins, gills and eyes.
Parasitic infections	Externally visible parasites (e.g. lichens, ciliates, arthropods) or other visible indicators of parasites (e.g. white or black dots) on the skin, fins, gills and eyes.

in the fish tanks and in the river every three hours using a handheld multimeter (Multi 3420, WTW, Weilheim, Germany). For the treatments Net, Turbine and Turbine & Screen, fish were transferred into a 40-L bucket and carefully released at the water surface from a boat or using ropes fixed at the bucket. To recover the fish after power plant passage, the end of the net was opened from a boat and the content of the trap was filled into a large bucket with fresh river water and oxygen supply. For fish injury assessment, fish were placed individually alive and without being euthanised into a transparent plastic box filled with fresh river water (30 × 17 × 10 cm, ROTHO clear boxes, ROTHO Kunststoff AG, Würenlingen, Switzerland). The water level in the box was chosen so the fish was fully covered with water and all fins were unfolded. A ruler was fixed at the bottom of the box to measure total length to the nearest mm. Vitality, respiratory movements, nutritional status, fungal infections and parasitic infections were evaluated first, followed by presence and intensity of the different injury types at different body parts. Injury intensity was visually estimated by one person, whilst a second person recorded all information in the protocol. After evaluation, fish were collected in 80-L buckets with freshwater and oxygen supply and were afterwards released into the river downstream of the power plant. All investigators (scientists or trained fisheries technicians) previously went through a detailed training exercise on the use of the protocol (Fig. S2) using the score sheet (Tables S1 & S2) as well as the examples in Figure 2. Fish evaluation was done in the same way for all species and treatments. One senior scientist coordinating the experiments was permanently present to assure quality control.

2.5 | Validation of visual injury estimation

As visual estimation of injury intensity may be biased, accuracy was validated by comparing visual assessment in the field with digital evaluation. Scale loss was chosen as a model injury type because it extends over larger areas of the fish body than other injuries such as tears/rips in the fins or haemorrhages and thus can be digitally determined with high accuracy. Two cycloid-scaled species from the natural fish community with a high variety and different patterns of scale loss were chosen: bleak, *Alburnus alburnus* L. (Cyprinidae) and *S. trutta* (Salmonidae).

Digital analyses were performed based on photographs taken from dead fish using a Canon EOS 70D camera on a repro stand with a grey background board and constant settings of remote release and lighting (F11, ISO 100, exposure time 0"6 to 1, Stabilizer off, Auto Focus, focal distance 35 mm). Digital analysis of scale loss was performed in Adobe® Photoshop® CS6 (Adobe Systems Software Ireland Limited, Dublin, Ireland). As a first step, the body was edged in PS CS 6 using the *Quick Selection Tool*. When edging front parts of the fish, the pectoral fin was excluded, because it was impossible to determine scale loss beneath. In accordance with the field-based visual estimation, the fish was divided into the individually analysed four body regions: left and right, front and back (Fig. S1). Scaled areas were marked using the *Magnetic Lasso Tool* in PS CS 6, and the proportion of scale loss was calculated using the number of pixels of scaled area versus potentially scaled area. Using the same pictures as for digital analyses, proportions of areas without scales were visually estimated by three people who had been calibrated before and by two people who had not been calibrated.

2.6 | Statistical analyses

Injury incidence (% fish affected), the average injury intensity of all fish and of all injured fish were calculated per treatment and injury type (pooled for both body halves). Injury intensities of all fish, all injured fish and injury incidence were compared between treatments across all injury types and body parts using univariate statistics in R (version 3.1.2, R Team 2014).

A multivariate approach was used to test differences in fish injury patterns between treatments, as it allows simultaneous inclusion of all injuries at each part of the body (Table S3). For all multivariate analyses, raw data on fish injury intensity were transformed into a resemblance matrix containing similarity values for each comparison of samples (fish individuals) or variables (injuries). An additional resemblance matrix based on injury presence/absence was calculated to test for the importance of quantitative information on injury intensity. Bray-Curtis Coefficient (Bray & Curtis, 1957) was chosen as the similarity measure because its values are not affected by inclusion or exclusion of injuries, which are jointly absent from two compared samples. As it is unlikely that all injuries included in the protocol occur in each fish, this is an important prerequisite for the calculation of similarities from the fish injury data. If variables among samples happened to be entirely zero, a zero-adjusted Bray-Curtis coefficient, including a virtual dummy variable being one for all objects, was used as suggested by Clarke, Somerfield and Chapman (2006). Nonmetric multidimensional scaling (NMDS) based on Bray-Curtis similarities between individuals was used to visualise differences in fish injury patterns between treatments and hatchery-reared test fish species (Table S3).

To test for significant differences between multivariate injury patterns of different treatments, body halves as well as fish types and species, one-way and two-way PERMANOVAs (PERMutational ANalysis Of VAriance, Anderson, Gorley & Clarke, 2008) were applied on Bray-Curtis Similarities (Table S3). PERMANOVA was chosen because it achieves a partitioning of multivariate variability in complex experimental designs (Anderson et al., 2008), which was particularly relevant for the dataset comprising different species and treatments. Pseudo-*F* ratios were used as a measure of strength of evidence in PERMANOVA as they are dependent on the denominator degrees of freedom, *P*-values and the similarity between groups (average from pairwise comparisons between samples) (Anderson et al., 2008).

Underlying patterns of injury types causing the differences identified via PERMANOVA were examined using one-way SIMilarity PERcentages analysis (SIMPER, Clarke & Warwick, 2014). SIMPER tested differences in constantly occurring injury types to be responsible for between-group dissimilarities. To analyse whether there are common patterns of jointly occurring injuries, the complete dataset of hatchery-reared fish was used for hierarchical clustering with group-average linking based on pairwise Bray-Curtis similarities between variables (injuries) using the routine CLUSTER in PRIMER v7 (Clarke & Warwick, 2014). A dendrogram was generated to identify any grouping or co-occurrence of the most frequent injuries and combined with a table giving the incidence, average intensity across all individuals and average intensity across injured individuals for the different treatments. Similarity profiles (SIMPROF, Clarke & Warwick, 2014) were used to test for significant groupings in the multivariate data.

Visually estimated values for scale loss from the field were tested against expected values gained from digital analysis using Chi-square test in R.

3 | RESULTS

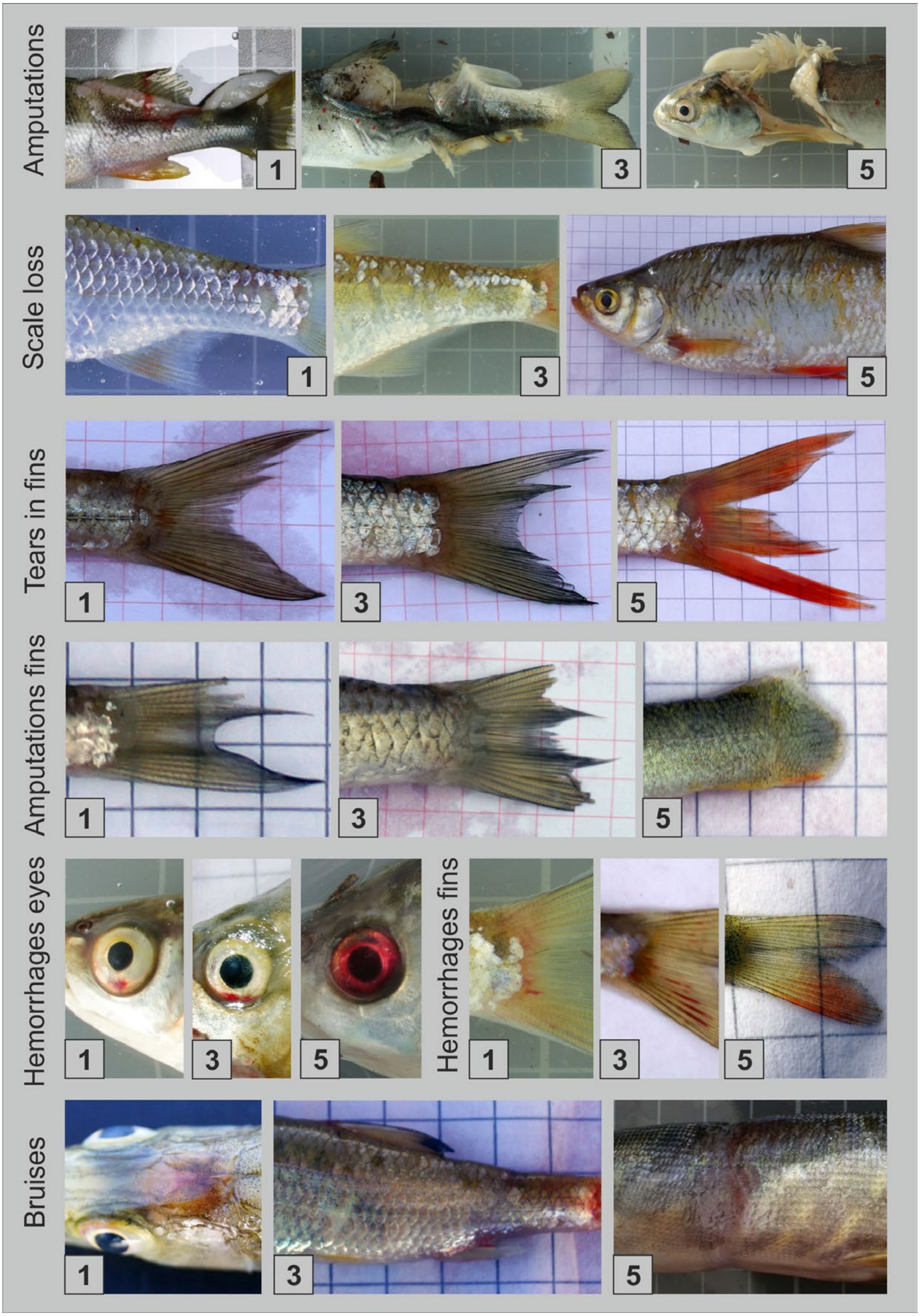
3.1 | General prevalence of fish injuries

Over all fish tested, tears in the fins (63% of all fish) and scale loss (60%) were the most frequently observed injury types, followed by haemorrhages (44%) dermal lesions (43%), partial amputations of fins (31%), pigment anomalies (24%) and bruises (11%, Figure 3). Emboli in the eyes (7%) and amputations of body parts (2%) occurred less frequently (Figure 3). Emboli in fins and spinal deflection were the least frequent injury types, only occurring in less than 1% of all fish (Figure 3).

A pattern of four main clusters of injuries was found using a similarity threshold of 20% (Figure 3, cophenetic correlation=0.92, $P < .05$). The first cluster contained three severe injury types (amputations of anterior and posterior body parts, spine deflection) that exclusively occurred after power plant passage at rather low incidence, but typically with high intensity in affected individuals. There was no significant sub-structure within the first cluster according to SIMPROF tests (Figure 3). The second cluster comprised injury types of medium severity that were detected in increased intensity in fish after power plant passage, such as haemorrhages, dermal lesions and emboli in the eyes (Figure 3). According to SIMPROF tests ($\pi = 5.99$, $P \leq .001$), there was significant sub-structure in the second cluster, separating different injury types as well as body regions, i.e. head, eyes, opercula, fins and main body. The third group of injuries included the least severe and most frequent injuries across all treatments (scale loss, tears in fins, pigment anomalies). Similar to the second cluster, there was also significant structure in the third cluster, separating injury types for the same body regions (Figure 3). The fourth cluster comprised a mixture of injuries with different intensities that generally occurred at very low intensities or with very low incidence, with no significant sub-structure (e.g. amputation of head, pigment anomalies in fins and opercula, amputations of eyes, emboli in fins, Figure 3).

3.2 | Determination of turbine effects

A significant effect differentiation of power plant passage (treatments Turbine and Turbine & Screen) from handling effects was


Amputations
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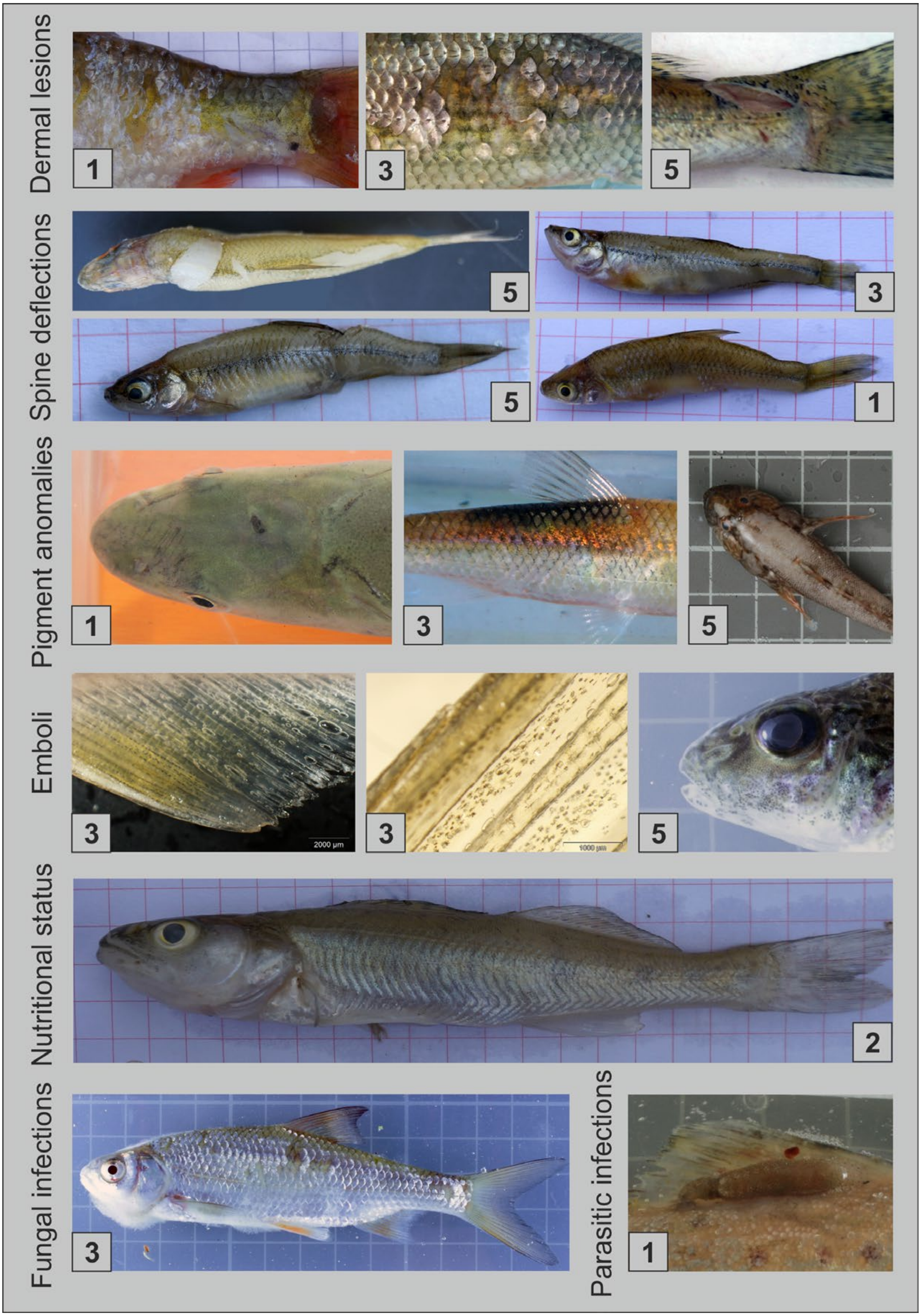
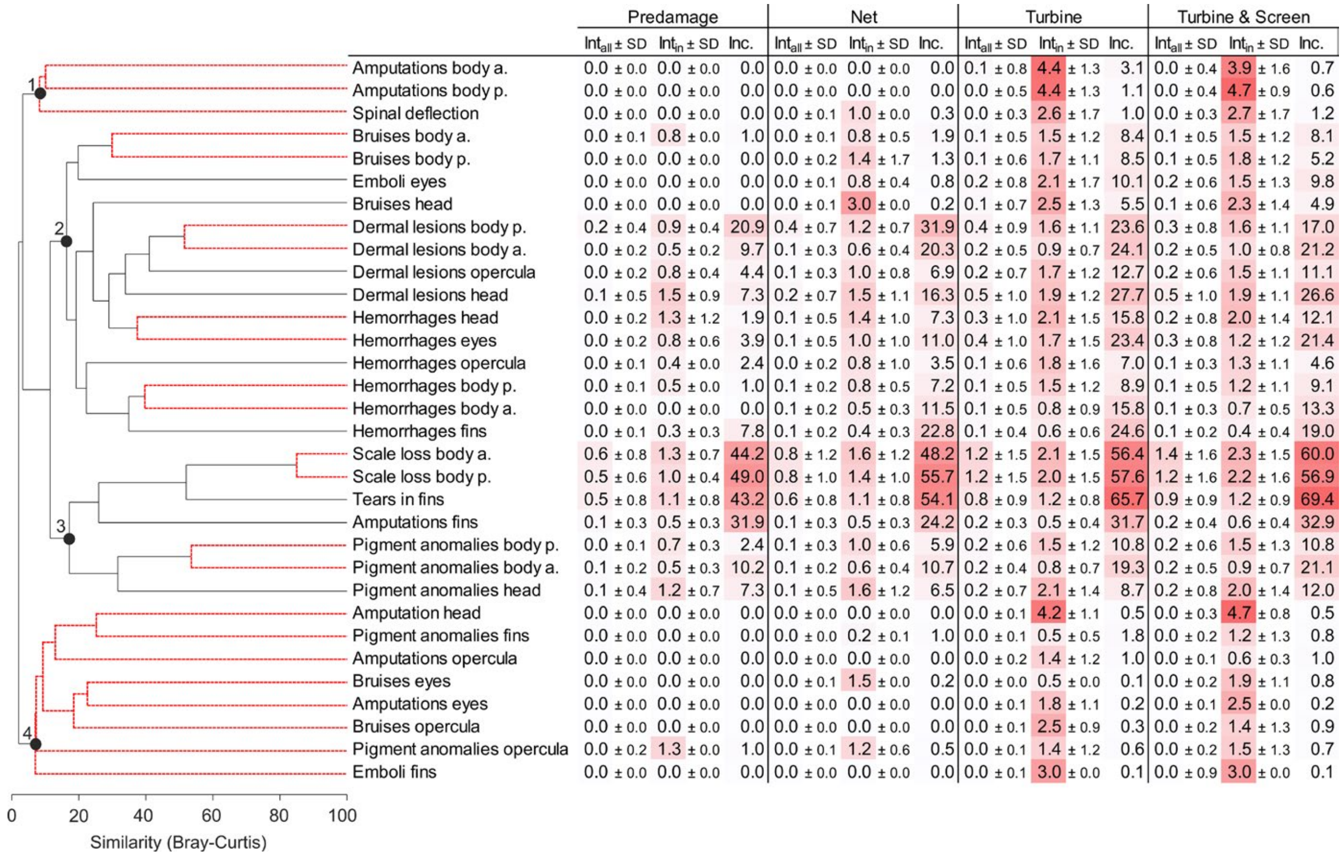


FIGURE 2 Examples of fish injuries. Typical examples of fish damage patterns as described in Tables 2 & Table S1, and summarised in the fish injury protocol in Fig. S2. The boxed numbers in the respective images indicate the severity of injury in the categories 0, 1, 3 and 5 or the evaluation of general criteria such as nutritional state, fungal infection or individual parasite infestation with the severity from 0 to 4 . [Colour figure can be viewed at wileyonlinelibrary.com]



	Predamage			Net			Turbine			Turbine & Screen		
	Int_{all} ± SD	Int_{in} ± SD	Inc.	Int_{all} ± SD	Int_{in} ± SD	Inc.	Int_{all} ± SD	Int_{in} ± SD	Inc.	Int_{all} ± SD	Int_{in} ± SD	Inc.
Amputations body a.	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0 ± 0.0	0.0 ± 0.0	0.0	0.1 ± 0.8	4.4 ± 1.3	3.1	0.0 ± 0.4	3.9 ± 1.6	0.7
Amputations body p.	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0 ± 0.5	4.4 ± 1.3	1.1	0.0 ± 0.4	4.7 ± 0.9	0.6
Spinal deflection	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0 ± 0.1	1.0 ± 0.0	0.3	0.0 ± 0.3	2.6 ± 1.7	1.0	0.0 ± 0.3	2.7 ± 1.7	1.2
Bruises body a.	0.0 ± 0.1	0.8 ± 0.0	1.0	0.0 ± 0.1	0.8 ± 0.5	1.9	0.1 ± 0.5	1.5 ± 1.2	8.4	0.1 ± 0.5	1.5 ± 1.2	8.1
Bruises body p.	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0 ± 0.2	1.4 ± 1.7	1.3	0.1 ± 0.6	1.7 ± 1.1	8.5	0.1 ± 0.5	1.8 ± 1.2	5.2
Emboli eyes	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0 ± 0.1	0.8 ± 0.4	0.8	0.2 ± 0.8	2.1 ± 1.7	10.1	0.2 ± 0.6	1.5 ± 1.3	9.8
Bruises head	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0 ± 0.1	3.0 ± 0.0	0.2	0.1 ± 0.7	2.5 ± 1.3	5.5	0.1 ± 0.6	2.3 ± 1.4	4.9
Dermal lesions body p.	0.2 ± 0.4	0.9 ± 0.4	20.9	0.4 ± 0.7	1.2 ± 0.7	31.9	0.4 ± 0.9	1.6 ± 1.1	23.6	0.3 ± 0.8	1.6 ± 1.1	17.0
Dermal lesions body a.	0.0 ± 0.2	0.5 ± 0.2	9.7	0.1 ± 0.3	0.6 ± 0.4	20.3	0.2 ± 0.5	0.9 ± 0.7	24.1	0.2 ± 0.5	1.0 ± 0.8	21.2
Dermal lesions opercula	0.0 ± 0.2	0.8 ± 0.4	4.4	0.1 ± 0.3	1.0 ± 0.8	6.9	0.2 ± 0.7	1.7 ± 1.2	12.7	0.2 ± 0.6	1.5 ± 1.1	11.1
Dermal lesions head	0.1 ± 0.5	1.5 ± 0.9	7.3	0.2 ± 0.7	1.5 ± 1.1	16.3	0.5 ± 1.0	1.9 ± 1.2	27.7	0.5 ± 1.0	1.9 ± 1.1	26.6
Hemorrhages head	0.0 ± 0.2	1.3 ± 1.2	1.9	0.1 ± 0.5	1.4 ± 1.0	7.3	0.3 ± 1.0	2.1 ± 1.5	15.8	0.2 ± 0.8	2.0 ± 1.4	12.1
Hemorrhages eyes	0.0 ± 0.2	0.8 ± 0.6	3.9	0.1 ± 0.5	1.0 ± 1.0	11.0	0.4 ± 1.0	1.7 ± 1.5	23.4	0.3 ± 0.8	1.2 ± 1.2	21.4
Hemorrhages opercula	0.0 ± 0.1	0.4 ± 0.0	2.4	0.0 ± 0.2	0.8 ± 1.0	3.5	0.1 ± 0.6	1.8 ± 1.6	7.0	0.1 ± 0.3	1.3 ± 1.1	4.6
Hemorrhages body p.	0.0 ± 0.1	0.5 ± 0.0	1.0	0.1 ± 0.2	0.8 ± 0.5	7.2	0.1 ± 0.5	1.5 ± 1.2	8.9	0.1 ± 0.5	1.2 ± 1.1	9.1
Hemorrhages body a.	0.0 ± 0.0	0.0 ± 0.0	0.0	0.1 ± 0.2	0.5 ± 0.3	11.5	0.1 ± 0.5	0.8 ± 0.9	15.8	0.1 ± 0.3	0.7 ± 0.5	13.3
Hemorrhages fins	0.0 ± 0.1	0.3 ± 0.3	7.8	0.1 ± 0.2	0.4 ± 0.3	22.8	0.1 ± 0.4	0.6 ± 0.6	24.6	0.1 ± 0.2	0.4 ± 0.4	19.0
Scale loss body a.	0.6 ± 0.8	1.3 ± 0.7	44.2	0.8 ± 1.2	1.6 ± 1.2	48.2	1.2 ± 1.5	2.1 ± 1.5	56.4	1.4 ± 1.6	2.3 ± 1.5	60.0
Scale loss body p.	0.5 ± 0.6	1.0 ± 0.4	49.0	0.8 ± 1.0	1.4 ± 1.0	55.7	1.2 ± 1.5	2.0 ± 1.5	57.6	1.2 ± 1.6	2.2 ± 1.6	56.9
Tears in fins	0.5 ± 0.8	1.1 ± 0.8	43.2	0.6 ± 0.8	1.1 ± 0.8	54.1	0.8 ± 0.9	1.2 ± 0.8	65.7	0.9 ± 0.9	1.2 ± 0.9	69.4
Amputations fins	0.1 ± 0.3	0.5 ± 0.3	31.9	0.1 ± 0.3	0.5 ± 0.3	24.2	0.2 ± 0.3	0.5 ± 0.4	31.7	0.2 ± 0.4	0.6 ± 0.4	32.9
Pigment anomalies body p.	0.0 ± 0.1	0.7 ± 0.3	2.4	0.1 ± 0.3	1.0 ± 0.6	5.9	0.2 ± 0.6	1.5 ± 1.2	10.8	0.2 ± 0.6	1.5 ± 1.3	10.8
Pigment anomalies body a.	0.1 ± 0.2	0.5 ± 0.3	10.2	0.1 ± 0.2	0.6 ± 0.4	10.7	0.2 ± 0.4	0.8 ± 0.7	19.3	0.2 ± 0.5	0.9 ± 0.7	21.1
Pigment anomalies head	0.1 ± 0.4	1.2 ± 0.7	7.3	0.1 ± 0.5	1.6 ± 1.2	6.5	0.2 ± 0.7	2.1 ± 1.4	8.7	0.2 ± 0.8	2.0 ± 1.4	12.0
Amputation head	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0 ± 0.1	4.2 ± 1.1	0.5	0.0 ± 0.3	4.7 ± 0.8	0.5
Pigment anomalies fins	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0 ± 0.0	0.2 ± 0.1	1.0	0.0 ± 0.1	0.5 ± 0.5	1.8	0.0 ± 0.2	1.2 ± 1.3	0.8
Amputations opercula	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0 ± 0.2	1.4 ± 1.2	1.0	0.0 ± 0.1	0.6 ± 0.3	1.0
Bruises eyes	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0 ± 0.1	1.5 ± 0.0	0.2	0.0 ± 0.0	0.5 ± 0.0	0.1	0.0 ± 0.2	1.9 ± 1.1	0.8
Amputations eyes	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0 ± 0.1	1.8 ± 1.1	0.2	0.0 ± 0.1	2.5 ± 0.0	0.2
Bruises opercula	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0 ± 0.1	2.5 ± 0.9	0.3	0.0 ± 0.2	1.4 ± 1.3	0.9
Pigment anomalies opercula	0.0 ± 0.2	1.3 ± 0.0	1.0	0.0 ± 0.1	1.2 ± 0.6	0.5	0.0 ± 0.1	1.4 ± 1.2	0.6	0.0 ± 0.2	1.5 ± 1.3	0.7
Emboli fins	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0 ± 0.1	3.0 ± 0.0	0.1	0.0 ± 0.9	3.0 ± 0.0	0.1

FIGURE 3 Dendrogram for hierarchical clustering (using group-average linking) of injury types by body part, based on Bray-Curtis Similarities (cophenetic correlation = 0.92, $P \le .05$). Continuous black lines indicate significant tree structure which is supported by SIMPROF tests, red dashed lines indicate no significant tree structure. Black circles with number 1-4 indicate four main significant injury clusters using a Bray-Curtis Similarity threshold of 20%. The table next to the dendrogram gives Int_{all} = arithmetic mean of injury intensity across all individuals, Int_{in} = arithmetic mean of injury intensity across injured individuals and Inc. = incidence in %/percentage of affected individuals for each of the treatments Predamage = hatchery-reared fish (n = 210), Net = hatchery-reared fish released directly at the entrance of the stow net (n = 631), Turbine = hatchery-reared fish released at the turbine inlet (n = 951), Turbine & Screen = hatchery-reared fish released upstream from fish protection screen (n = 1295); SD = standard deviation. The shading of the table in different intensities of red indicates increasing injury intensity/incidence with increasing intensity of red. The shading is graded separately for intensity and incidence due to the different numerical scale of both measures. [Colour figure can be viewed at wileyonlinelibrary.com]

detected by PERMANOVA for all hatchery-reared fish species used in the standardised experiment. However, average similarity values between treatments ranging from 25 (power plant passage in *A. anguilla*) to 52 (power plant passage in *S. trutta*) indicated a pronounced overlap between treatments (Table 3, Figure 4). Injury patterns generally were highly species-specific and overall injuries differed between tested species, with most pronounced effects of turbine passage detected for *A. anguilla* (Table 3, Figures 4 and 5). The treatments turbine and turbine & screen only differed significantly for *S. trutta* and *A. anguilla* (Table 3). A significant catch-related effect was detected in all species, being most pronounced in *C. nasus*. As indicated by the average similarity between treatments, the strength of evidence was lower for catch-related effects than for hydropower-related effects in *A. anguilla* and in *P. fluviatilis*, whilst both effects were of similar strength for *C. nasus* and *S. trutta* (Table 3).

Injury intensity and incidence was generally higher in fish after power plant passage (treatments turbine and turbine & screen) than in reference treatments (predamage and net, Kruskal–Wallis test: injury intensity all fish Chi-square = 175.86, *df* = 3, $P \le .001$; injury intensity injured fish Chi-square = 224.39, $P \le .001$; incidence Chi-square = 13.61, $P \le .01$; Figure 3). For instance, incidence of emboli in the eyes increased 12-fold, incidence of bruises increased up to six-fold and haemorrhages increased up to four-fold in intensity after turbine passage (Figure 3). A reduction of vitality, scale loss, tears in the fins, dermal lesions and haemorrhages most strongly contributed to the differences between the treatments net and turbine, as well as between the treatments net and turbine & screen (SIMPER; Figure 5). For pooled data over all species, screen passage (treatment turbine & screen) was mainly characterised by a higher intensity of scale loss than exclusive turbine passage (treatment turbine). For *A. anguilla*, dermal lesions also increased after screen passage. Catch-related damage (net) was mainly distinguished from predamage due to reduced vitality as well as enhanced scale loss, dermal lesions and tears in the fins. Considering single species, specific

TABLE 3 Results from PERMANOVA comparisons of injury patterns between treatments over all four tested species of hatchery-reared fish and for each species separately, based on two different levels of detail: injury intensities 0,1,3,5 for each injury (= Intensity) and injury presence/absence (1= presence, 0=absence)

Level of detail	Comparisons of treatments	All species AvS	t	P	df	*Anguilla anguilla* AvS	t	P	df	*Salmo trutta* AvS	t	P	df	*Chondrostoma nasus* AvS	t	P	df	*Perca fluviatilis* AvS	t	P	df
Intensity	Predamage, Net	30	2.8	***	831	42	3.0	***	244	50	1.9	*	201	41	3.4	***	175	55	1.9	**	205
	Net, Turbine	25	5.3	***	1567	25	8.2	***	365	49	2.3	***	378	41	3.2	***	363	40	2.9	***	455
	Net, Turbine & Screen	26	5.6	***	1916	25	9.0	***	297	52	1.8	*	384	40	3.4	***	559	40	2.6	***	670
	Turbine, Turbine & Screen	25	2.0	**	2231	34	2.3	***	290	48	2.4	**	327	42	1.2		634	36	1.1		797
Presence/absence	Predamage, Net	37	2.8	***	831	47	3.2	***	244	59	2.0	*	201	55	3.8	***	175	60	2.1	***	205
	Net, Turbine	34	4.5	***	1567	39	7.6	***	365	58	1.8	*	378	53	2.8	***	363	48	2.9	***	455
	Net, Turbine & Screen	34	5.1	***	1916	40	8.8	***	297	62	1.5		384	53	3.1	***	559	48	2.6	***	670
	Turbine, Turbine & Screen	35	2.2	**	2231	51	2.7	***	290	57	2.6	**	327	53	1.7	**	634	46	1.1		797

Treatments: Predamage = hatchery-reared fish, Net = hatchery-reared fish released directly at the entrance of the stow net, Turbine = hatchery-reared fish released at the turbine inlet, Turbine & Screen = hatchery-reared fish released upstream from fish protection screen. AvS = Average Bray-Curtis Similarity between treatments (averaged for each pair of samples), *P* = level of significance.

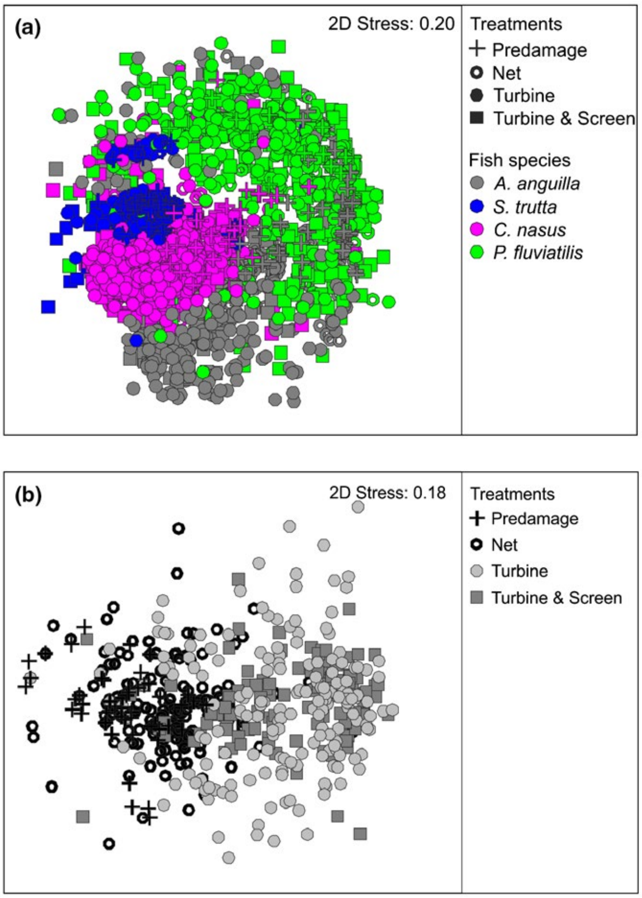


FIGURE 4 Nonmetric multidimensional scaling (NMDS) of fish injuries (a) in all four hatchery-reared fish species tested in the standardised experiment and (b) exemplarily for hatchery-reared *Anguilla anguilla*. Analyses are based on pairwise Bray-Curtis Similarities between each pair of fish calculated from injury intensities and vitality. Each symbol in the NMDS plots indicates one specimen. The different colours in plot (a) indicate the different test species, different symbols indicate the different treatments Predamage = hatchery-reared fish without further treatment (a: n = 210, b: n = 60), Net = hatchery-reared fish directly released at the entrance of the stow net (a: n = 631, b: n = 188), Turbine = hatchery-reared fish released at the turbine inlet (a: n = 951, b: n = 183), Turbine & Screen = hatchery-reared fish released upstream of the fish protection screen (a: n = 1295, b: n = 113). [Colour figure can be viewed at wileyonlinelibrary.com]

injuries contributed to the differences between treatments. Whilst *A. anguilla* was more affected by dermal lesions, haemorrhages and emboli than other species, *P. fluviatilis* was the only species in which scale loss played a minor role for differentiation between treatments (Figure 5).

Amputations of body parts, spinal deflection and emboli in the fins were not detected by SIMPER to contribute to differences between treatments in any species. Emboli in the fins, amputations of body parts (including head, opercula and eyes), bruises of opercula and spine deflections were exclusively detected in fish from treatments turbine and turbine & screen, but with much lower incidence than the less severe injury types detected by SIMPER (Figure 3). However,

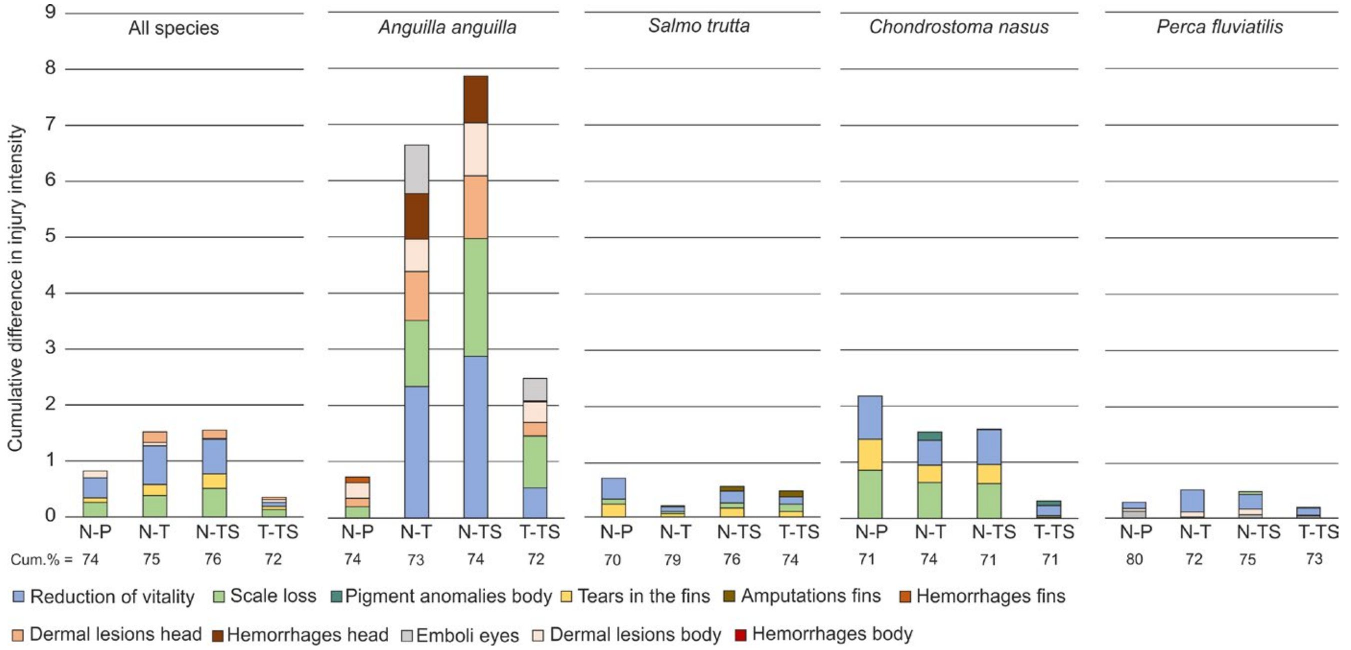


FIGURE 5 Absolute differences in injury intensity between pairs of treatments (plotted cumulatively) for injuries with a contribution to between-group dissimilarity larger than 5% according to similarity percentage analysis (SIMPER). The size of the bar parts indicates the delta in intensity values for the respective injury type and treatment comparison. Cum.% = cumulative contribution to between-group dissimilarity according to SIMPER. Contribution to between-group dissimilarity of single injury types ranged between 5% and 44%, Diss/SD ranging between 0.36 and 1.37. Average Bray-Curtis Similarities between treatments are given in Table 3. P = Predamage (hatchery-reared fish without further treatment), N = Net (hatchery-reared fish directly released at the entrance of the stow net), T = Turbine (hatchery-reared fish released at the turbine inlet), TS = Turbine & Screen (hatchery-reared fish released upstream of the fish protection screen). For the number of replicates (n) within each species and treatment see Table 1. [Colour figure can be viewed at wileyonlinelibrary.com]

amputations of body parts showed the highest average injury intensity of affected individuals compared with all other injury types (Figure 3). Considering exclusively injury presence and absence, the effect size, as measured by the average similarity between treatments, was generally lower than the consideration of injury intensities (Table 3). However, results of PERMANOVA were still significant except for the comparison of treatments net and turbine & screen for *S. trutta*, which was no longer significant, and an additional significant differentiation of turbine and turbine & screen for *C. nasus*. There was no significant difference within any treatment when comparing injuries on the left and right body half (PERMANOVA: *df* = 4, den. *df* = 6124, Pseudo-*F* = 1.4, *P* = .1).

3.3 | Applicability of surrogates for wild migrating fish

The comparison of caught wild fish that had passed the hydropower plant with the reference fish caught upstream of the power plant also identified significant effects of power plant passage on injury patterns (PERMANOVA: *df* = 1, den. *df* = 2258, Pseudo-*F* = 447.2, *P* ≤ .001). Generally, injury patterns of wild fish strongly differed between fish types after turbine passage, with most pronounced differences between species with ctenoid and cycloid scales (PERMANOVA: *df* = 1, den. *df* = 2071, Pseudo-*F* = 206.1, *P* ≤ .001). Within wild fish from the family Cyprinidae (cycloid scales), injury patterns of species with similar body shape differed significantly from each other after turbine passage (small-sized, laterally flattened species: *C. nasus*, *A. alburnus*, roach, *Rutilus rutilus* (L.), Schneider, *Alburnoides bipunctatus* (Bloch), dace, *Leuciscus leuciscus* (L.), topmouth gudgeon, *Pseudorasbora parva* (Temminck & Schlegel), PERMANOVA: *df* = 5, den. *df* = 1411, Pseudo-*F* = 17.5, *P* ≤ .001). However, the average similarity between species ranged between 43 and 56, indicating a much higher level of overlap compared with species that have ctenoid vs cycloid scales (average similarity=25). Wild and hatchery-reared individuals from the same species significantly differed in their injury patterns after power plant passage (PERMANOVA: *df* = 3, den. *df* = 1507, Pseudo-*F* = 17.9, *P* ≤ .001, average similarity ranging from 32 to 40). According to SIMPER analysis, differences between hatchery-reared and wild fish were mainly attributed to a higher intensity of scale loss in wild fish (average intensity wild fish = 1.94, hatchery-reared fish = 1.18, Contrib. % = 29.81, Diss/SD = 1.19), a pronouncedly stronger reduction of vitality in wild fish (average intensity wild fish = 2.20, hatchery-reared fish = 0.8, Contrib. % = 27.08, Diss/SD = 0.97) and slightly less tears in the fins of wild fish (average intensity wild fish = 0.74, hatchery-reared fish = 0.85, Contrib. % = 17.16, Diss/SD = 0.89).

3.3 | Validation of visual estimation

The comparison between visual estimation of scale loss and digital determination did not result in significant differences (Chi-square test: *df* = 47, X-squared = 22.72, *P* = .99). Scale loss category 1 was always correctly assigned. Non-significant false visual estimations of category 3 and 5 occurred in both species, with a tendency of scale loss being visually underestimated. Non-significant underestimation most frequently occurred when digital analysis suggested a scale loss of category 3 (data not shown).

4 | DISCUSSION

Previous field evaluations on hydropower-induced fish damage were often species-specific and focused on survival without considering sub-lethal endpoints. The novelty of the present study is that it takes into account lethal and nonlethal injuries across multiple species. With nine different injury types and 18 fish body parts assessed, the new protocol presented herein covers a large diversity of injury patterns that were all detected in fish after turbine passage at the investigated hydropower field site. Recovery times from sub-lethal injuries can be significant and delayed mortality accounted for a high percentage of total mortality in other studies (e.g. 46%-70% of total mortality in Ferguson et al., 2006; 36% of total mortality in Dedual, 2007), which justifies that the injury types included in the protocol proposed herein are of high biological relevance. For instance, rainbow trout with moderate to severe scale losses died within 96 hr after turbine passage (Dedual, 2007). Even injuries of low severity that can usually heal in fish within a timespan of days to weeks, such as scale loss (Bereiter-Hahn & Zylberberg, 1993; Vieira et al., 2011), tears in the fins (Azevedo, Grotek, Jacinto & Weidinger, 2011), dermal lesions (Anderson & Roberts, 1975; Roubal & Bullock, 1988) and pigment anomalies, may cause delayed mortality. This can result from severe loss of protection from infections and mechanical threats (Dastjerdi & Barthelat, 2015; Vernerey & Barthelat, 2010) or reduced swimming performance and therefore enhance predation risk (Dastjerdi & Barthelat, 2015; Noble et al., 2012).

Different parts of the hydropower machinery can cause distinctive damage patterns across the fish body (Pracheil, DeRolph, Schramm & Bevelhimer, 2016). Broad-scale estimates of survival, as provided by previous field studies, were found to be of limited value for understanding sub-lethal effects from turbine passage and improving turbine design, because they only provide generalised information (Ferguson et al., 2006). By contrast, the comprehensive assessment of multiple fish injury patterns across various body parts, as proposed herein, allows distinctive injury types to be linked to specific construction details of the hydropower plant. For instance, the increased intensity of scale loss and dermal lesions of eel that passed the bar screen in this study indicates that this feature of the power plant, originally intended to protect fish, may cause additional injury, particularly to medium-sized fish that barely fit through the bar spacing and have intense contact with the bars of the screen whilst passing. Such information can be of high relevance for deducing species-specific risk potentials of different hydropower plant types as well as scaling to population-level effects. Additionally, the identification of power plant-type specific injury potential is of particular importance in context of the high diversity of techniques used and hydropower developments in different countries (Hogan et al., 2014).

4.1 | Discrimination of hydropower-specific injury patterns

For a detailed evaluation of fish injury, it is essential to catch fish below hydropower structures. Both the catch with nets and the handling afterwards can cause stress to the fish resulting in injuries such as scale loss, tears in the fins or dermal lesions. According to the results of this study, similar injury patterns can be caused by hydropower structures. Consequently, it is crucial to distinguish between handling controls and treatment groups to avoid an over-estimation of fish damage caused by the hydropower facility. Frequently occurring injuries, such as scale loss, tears in the fins, dermal lesions, haemorrhages or bruises could exclusively be disaggregated from handling effects due to higher intensity. For instance, the pronounced increase of haemorrhages (four-fold intensity) and bruises (six-fold occurrence) after turbine passage indicates that the quantification of injury intensity is important for the discriminatory power between turbine passage and handling effects. This is also supported by the higher average similarity between treatments in the PERMANOVA analyses of injury presence/absence. In addition to frequently occurring injuries, the consideration of rare but biologically important injuries such as amputations of the head or other body parts are also highly relevant, because these injuries usually immediately result in severe stress or mortality and exclusively occurred after turbine passage in this and other studies (Colotelo et al., 2012; Deng et al., 2005; Neitzel et al., 2004).

High similarity in injury patterns was found between body sides as well as anterior and posterior body parts in this study (PERMANOVA analysis and SIMPROF tests). However, there is a high diversity of hydropower techniques and operation modes used worldwide and the different designs of fish protection screens, rotation direction, speed or blade distance of the turbine potentially cause body-part-specific injuries (Deng et al., 2005; Schneider et al., 2012). Thus, it cannot be recommended to disclaim the differentiation of body parts.

4.2 | Suitability of morphological fish types as surrogates for single species

Fish diversity in rivers can be very high (Poff et al., 2001), which is also confirmed by the >30 species present in this study at one single site. Field studies into hydropower-induced injury of the whole fish community can thus be time-consuming and resource intensive. This raises the question if it is possible to improve the effectiveness of such studies by analysing surrogates for single species or morphological fish types. This is also of high relevance in all animal experiments in the context of the principle of “reduction,” “replacement” and “refinement” in animal use and care (Russel & Burch, 1959; European Parliament, 2010). The results of this study, with lower dissimilarity between injury patterns of different morphological fish types compared with different species from the same type, indicate that testing representatives for different types can help reduce effort in field studies. In the context of selecting representative fish species, it is important to consider several morphological characteristics that may influence susceptibility to turbine effects, including scale type, body shape, body size and swim bladder morphology (Čada & Richmond, 2011; Čada & Schweizer, 2012). Consequently, fish families comprising morphologically differing species, such as cyprinids, which strongly differ in body shape (e.g. laterally flattened, dorsoventrally flattened, high-backed), need to be represented by several species.

4.3 | Suitability of hatchery-reared fish as surrogates for wild fish

The finding that wild specimens suffered from much stronger impacts on vitality and higher intensity of scale loss after turbine passage compared with hatchery-reared fish indicates that many of them may have been already damaged before turbine passage, e.g. due to predator encounters or the cumulative effects of other hydropower plants upstream (Schneider et al., 2012). As evident from the dataset on the wild fish caught above the studied hydropower plant, different types of injuries and intensities are already present in these fish. Consequently, predamage may strongly affect the evaluation of turbine effects, especially if only assessing injuries in fish from natural downstream movement. Such a bias is problematic because it cannot be corrected sufficiently by assessing the natural fish community of the headrace (e.g. via electrofishing catches). Moreover, catch-related injuries are difficult to account for in investigations of wild fish as they cannot be determined beforehand. Sample size, as well as sufficient representation of morphological fish types may also be an issue if the species inventory of the study river is restricted and natural downstream movement is low. Therefore, it cannot be recommended to investigate wild fish exclusively for the determination of turbine-related injury, especially if a comparison of facilities from different river systems is intended. However, hatchery-reared fish also often have distinct injuries related to aquaculture conditions and transportation, typically comprising injuries of the fins. This predamage needs to be accounted for by including control treatments in standardised experiments. A major advantage of using hatchery-reared fish is that these can be released at specific parts of the power plant, e.g. at the screen or at the turbine inlet. However, it may be difficult to find representative species for each morphological type that are available from aquaculture in sufficient numbers, especially in areas of high species richness. Even in central Europe where this study was carried out, some rare or highly endangered species were not available from aquaculture (e.g. lampreys or bullhead, *Cottus gobio* L.). Furthermore, there may be behavioural differences between hatchery-reared and wild fish (Berejikian, 1995; Fleming & Gross, 1992), particularly when it comes to the choice of the corridor and the timing of downstream passage. Thus, to relate turbine effects detected in standardised experiments using hatchery-reared fish to real field conditions of the study site (e.g. set of species, percentage of individuals using the turbine corridor, timing of downstream movement, abiotic conditions during downstream movement), it is inevitable to combine standardised experiments with a seasonal assessment of natural downstream movements of the wild fish populations.

4.4 | Statistical power and replication

Statistical power is determined by the effect size and replication (Cohen, 1992). Replication is particularly important for assessments of hydropower-induced fish injury, because small effect sizes can be highly relevant, e.g. when validating novel hydropower techniques proposed as fish-friendly solutions. For instance, sub-lethal injuries such as increase in scale loss intensity often occur with low effect size but can be highly relevant for individual survival and population-level effects. Moreover, a weak immediate response to turbine effects of some species (e.g. *S. trutta* in this study), pronounced catch-related effects (e.g. *C. nasus* in this study) and high variability within the data can minimise effect detectability.

To distinguish treatments despite the pronounced overlap evident from NMDS, a large number of fish individuals have to be investigated. This is undesirable from an animal care principle and it can be very time consuming. Because time is often a limiting factor in field monitoring studies, an effective procedure is needed to ensure a high number of replicates. In this study, visual estimation of injuries in the field following the protocol proposed took on average 2 min per fish for assessing all body parts and injury types, and thus was much more effective than digital determination of single injury types which took on average 6.5 min for the exclusive determination scale loss on only one body part. Because loss of precision due to visual estimation was non-significant and can be minimised by adequate staff training, the fish injury assessment procedure proposed herein proved to be an ideal tool to assess a high number of fish replicates in the field.

Based on the observed catch numbers of 113 to 508 individuals per species in treatment groups (turbine, turbine & screen) and 33 to 188 individuals in control groups (predamage, net), PERMANOVA was powerful enough to distinguish turbine and handling effects. Beyond detecting significant differences between treatments, a powerful tool for identifying treatment-specific injury patterns is needed. In this study, SIMPER analysis proofed to be an appropriate tool to identify frequently occurring injury types, which increase in intensity after turbine passage. However, more severe injuries with lower incidence but with potential consequences for survival, such as emboli in the eyes, bruises and amputations of body parts, are under-represented in SIMPER results. This is related to the mathematical routine of SIMPER, which is designed to detect variables that occur with high persistence and therefore contribute to within-group similarity or between-group dissimilarity (Clarke & Warwick, 2014). As low injury incidence does not imply low biological relevance, such injuries should additionally be addressed using univariate and descriptive statistics.

Generally, intensive training of fish injury evaluators is needed to minimise variability in the data and ensure high accuracy. This can be done by providing detailed descriptions and pictures of all injuries and intensity categories (such as provided in Figure 2, Table 2 and Tables S1 and S2). In this study, a joint evaluation of example fish in regular training was additionally performed and a quality control was established, which cross-checked the visual estimations of the different observers in the field.

4.5 | Prospects for future studies

As fish injury patterns are not exclusively externally visible, but also comprise internal injuries, an additional method should be established for X-ray and necropsy assessments (e.g. Brown et al., 2013) which cannot easily be carried out in the field for high sample numbers. Due to the potentially high diversity of internal injuries (Ebel, 2013), a standardised laboratory-based protocol should be developed analogously to the field-based protocol for external injuries proposed herein. To fill current knowledge gaps on relations between physical mechanisms in the turbine and sub-lethal injuries, future studies on fish injury should ideally compare different hydropower facilities (e.g. including innovative solutions), turbine operation modes (e.g. blade adjustments) and designs (e.g. conventional and novel techniques) under consideration of biological and physical data (e.g. assessed using sensor fish, Carlson & Ducan, 2003). Data collected according to the fish injury assessment protocol can also be used to assess fish vitality and health condition in aquaculture, fish transportation or in the context of predation-related injuries.

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REFERENCES

Abernethy, C. S., Amidan, B. G., & Čada, G. F.. (2001). *Laboratory studies of the effects of pressure and dissolved gas supersaturation on turbine-passed fish*. Richland, WA: Pacific Northwest National Laboratory. PNNL-13470.

Adam, B., & Brujis, M.. (2006). Allgemeine Anforderungen an Fischschutz- und Fischabstiegsanlagen/Untersuchungen im Maas-System. DWA-Themen: Durchgängigkeit von Gewässern für die aquatische Fauna. Internationales DWA-Symposium zur Wasserwirtschaft 3. – 7. April 2006. Deutsche Gesellschaft für Wasserwirtschaft, Abwasser und Abfall e.V., Hennef.

Adam, B., Schürmann, M., & Schwevers, U. (2013). *Zum Umgang mit aquatischen Organismen – Versuchstierkundliche Grundlagen*, Wiesbaden, Germany: Springer Spektrum.

Anderson, M. J., Gorley, R. N., & Clarke, K. R. (2008). *PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods*, 1st ed.. Plymouth, UK: PRIMER-E Ltd.

Anderson, C. D., & Roberts, R. J. (1975). A comparison of the effects of temperature on wound healing in a tropical and a temperate teleost. *Journal of Fish Biology*, *7*(2), 173–182.

Azevedo, A. S., Grotek, B., Jacinto, A., & Weidinger, G. (2011). The regenerative capacity of the zebrafish caudal fin is not affected by repeated amputations. *PLoS ONE*, *6*(7), e22820.

Belding, D. L. (1929). The respiratory movements of fish as an indicator of a toxic environment. *Transactions of the American Fisheries Society*, *59*(1), 238–245.

Bereiter-Hahn, J., & Zylberberg, L. (1993). Regeneration of teleost fish scale. *Comparative Biochemistry and Physiology Part A: Physiology*, *105*(4), 625–641.

Berejikian, B. A. (1995). The effects of hatchery and wild ancestry and experience on the relative ability of steelhead trout fry (*Oncorhynchus mykiss*) to avoid a benthic predator. *Canadian Journal of Fisheries and Aquatic Sciences*, *52*(11), 2476–2482.

Bochert, R., & Lill, D. (2004). Untersuchung von möglichen Fischschäden im Wirkungsbereich von Kleinwasserkraftwerken in Mecklenburg-Vorpommern. *Fischerei & Fischmarkt in Mecklenburg-Vorpommern*, *4*, 8–16.

Bray, J. R., & Curtis, J. T. (1957). An ordination of upland forest communities of southern Wisconsin. *Ecological Monographs*, *27*(4), 325–349.

Brown, R. S., Carlson, T. J., Gingerich, A. J., Stephenson, J. R., Pflugrath, B. D., Welch, A. E., … Townsend, R. L. (2012). Quantifying mortal injury of juvenile Chinook salmon exposed to simulated hydro-turbine passage. *Transactions of the American Fisheries Society*, *141*(1), 147–157.

Brown, R. S., Cook, K. V., Pflugrath, B. D., Rozeboom, L. L., Johnson, R. C., McLellan, J. G., … White, T. A.. (2013). Vulnerability of larval and juvenile white sturgeon to barotrauma: Can they handle the pressure? *Conservation Physiology*, *1*(1), cot019. https://doi.org/10.1093/conphys/cot019

Brown, R. S., Pflugrath, B. D., Colotelo, A. H., Brauner, C. J., Carlson, T. J., Deng, Z. D., & Seaburg, A. G. (2012). Pathways of barotrauma in juvenile salmonids exposed to simulated hydroturbine passage: Boyle's law vs. Henry's law. *Fisheries Research*, *121*, 43–50.

Čada, G. F., Garrison, L., & Fisher, R. (2007). Determining the effect of shear stress on fish mortality during turbine passage. *Hydro Review*, *26*(7), 52–59.

Čada, G. F., & Richmond, M. C. (2011). *Can fish morphological characteristics be used to re-design hydroelectric turbines?* (No. PNNL-SA-81384)Richland, WA: Pacific Northwest National Laboratory (PNNL).

Čada, G. F., & Schweizer, P. E. (2012). *The application of traits-based assessment approaches to estimate the effects of hydroelectric turbine passage on fish populations*. ORNL/TM-2012/110, (pp. 37).Oak Ridge, Tenn: Oak Ridge National Laboratory.

Calles, O., Karlsson, S., Vezza, P., Comoglio, C., & Tielman, J. (2013). Success of a low-sloping rack for improving downstream passage of silver eels at a hydroelectric plant. *Freshwater Biology*, *58*(10), 2168–2179.

Carlson, T. J., Brown, R. S., Stephenson, J. R., Pflugrath, B. D., Colotelo, A. H., Gingerich, A. J., … Townsend, R. L. (2012). The influence of tag presence on the mortality of juvenile chinook salmon exposed to simulated hydroturbine passage: Implications for survival estimates and management of hydroelectric facilities. *North American Journal of Fisheries Management*, *32*, 249–261.

Carlson, T. J., & Ducan, J. P. (2003). *Evolution of the sensor fish device for measuring physical conditions in severe hydraulic environments*, Richland, WA: Pacific Northwest National Laboratory. PNNL-13470

Clarke, K. R., Somerfield, P. J., & Chapman, M. G. (2006). On resemblance measures for ecological studies, including taxonomic dissimilarities and a zero-adjusted Bray-Curtis coefficient for denuded assemblages. *Journal of Experimental Marine Biology and Ecology*, *330*(1), 55–80. https://doi.org/10.1016/j.jembe.2005.12.017

Clarke, K. R., & Warwick, R. M.. (1994, 2001, 2014). Change in marine communities: an approach to statistical analysis and interpretation. PRIMER-E, Plymouth, UK. 1st ed 144 pp; 2nd ed 172 pp; 3rd ed (authors Clarke K. R., Gorley R. N., Somerfield P. J., Warwick R. M.) 260 pp.

Cohen, J. (1992). Statistical power analysis. *Current Directions in Psychological Science*, *1*(3), 98–101.

Colotelo, A. H., Cooke, S. J., & Smokorowski, K. E. (2009). Application of forensic techniques to enhance fish conservation and management: Injury detection using presumptive tests for blood. *Endemic Species Research*, *9*(3), 169–178.

Colotelo, A. H., Pflugrath, B. D., Brown, R. S., Brauner, C. J., Mueller, R. P., Carlson, T. J., … Trumbo, B. A. (2012). The effect of rapid and sustained decompression on barotrauma in juvenile brook lamprey and Pacific lamprey: Implications for passage at hydroelectric facilities. *Fisheries Research*, *129*, 17–20.

Dastjerdi, A. K., & Barthelat, F. (2015). Teleost fish scales amongst the toughest collagenous materials. *Journal of Mechanical Behavior of Biomedical Materials*, *52*, 95–107.

Dedual, M. (2007). Survival of juvenile rainbow trout passing through a Francis turbine. *North American Journal of Fisheries Management*, *27*(1), 181–186.

Deng, Z., Guensch, G. R., McKinstry, C. A., Mueller, R. P., Dauble, D. D., & Richmond, M. C. (2005). Evaluation of fish-injury mechanisms during exposure to turbulent shear flow. *Canadian Journal of Fisheries and Aquatic Sciences*, *62*(7), 1513–1522.

Ebel, G.. (2013). Fischschutz und Fischabstieg an Wasserkraftanlagen – Handbuch Rechen- und Bypasssysteme. Ingenieurbiologische Grundlagen, Modellierung und Prognose, Bemessung und Gestaltung. Büro für Gewässerökologie und Fischereibiologie Dr. Ebel, 1. Auflage, Halle (Saale).

Ferguson, J. W., Absolon, R. F., Carlson, T. J., & Sandford, B. P. (2006). Evidence of delayed mortality on juvenile Pacific Salmon passing through turbines at Columbia River dams. *Transactions of the American Fisheries Society*, *135*(1), 139–150.

Ferguson, J. W., Ploskey, G. R., Leonardsson, K., Zabel, R. W., & Lundqvist, H. (2008). Combining turbine blade-strike and life cycle models to assess mitigation strategies for fish passing dams. *Canadian Journal of Fisheries and Aquatic Sciences*, *65*(8), 1568–1585.

Fleming, I. A., & Gross, M. R. (1992). Reproductive behavior of hatchery and wild coho salmon (*Oncorhynchus kisutch*): Does it differ? *Aquaculture*, *103*(2), 101–121.

Hogan, T. W., Čada, G. F., & Amaral, S. V. (2014). The status of environmentally enhanced hydropower turbines. *Fisheries*, *39*(4), 164–172.

Holzner, M.. (1999). *Untersuchungen zur Vermeidung von Schäden im Kraftwerksbereich, dargestellt am Kraftwerk Dettelbach am Main/Unterfranken* (pp. 224). München: Schriftenreihe LFV Bayern 1.

Keefer, M. L., Taylor, G. A., Garletts, D. F., Helms, C. K., Gauthier, G. A., Pierce, T. M., & Caudill, C. C. (2013). High-head dams affect downstream fish passage timing and survival in the Middle Fork Willamette River. *River Research and Applications*, *29*(4), 483–492.

Killgore, J. K., Maynord, S. T., Chan, M. D., & Morgan, R. P. (2001). Evaluation of propeller-induced mortality on early life stages of selected fish species. *North American Journal of Fisheries Management*, *21*(4), 947–955.

Lagarrigue, T., & Frey, A.. (2010). Test for evaluating the injuries suffered by downstream-migrating eels in their transiting through the new spherical discharge ring VLH turbogenerator unit installed on the Moselle river in Frouard. Report E. CO. GEA for MJ2 Technologies.

Lucas, M. C., Baras, E., Thom, T. J., Duncan, A., & Slavík, O.. (2001). *Migration of freshwater fishes*(47th ed.): Oxford: Blackwell Science.

Neitzel, D. A., Dauble, D. D., Čada, G. F., Richmond, M. C., Guensch, G. R., Mueller, R. P., … Amidan, B. (2004). Survival estimates for juvenile fish subjected to a laboratory-generated shear environment. *Transactions of the American Fisheries Society*, *133*(2), 447–454.

Nettles, D. C., & Gloss, S. P. (1987). Migration of landlocked Atlantic salmon smolts and effectiveness of a fish bypass structure at a small-scale hydroelectric facility. *North American Journal of Fisheries Management*, *7*(4), 562–568.

Noble, C., Jones, H. A. C., Damsgård, B., Flood, M. J., Midling, K. Ø., Roque, A., … Cottee, S. Y. (2012). Injuries and deformities in fish: Their potential impacts upon aquacultural production and welfare. *Fish Physiology and Biochemistry*, *38*, (1)61–83. https://doi.org/10.1007/s10695-011

Paish, O. (2002). Small hydro power: Technology and current status. *Renewable and Sustainable Energy Reviews*, *6*, 537–556.

Parliament, E. (2000). Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for the Community action in the field of water policy. *Official Journal of the European Union*, *327*, 1–73.

Parliament, E. (2010). Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. *Official Journal of the European Union*, *276*, 33–77.

Poff, N. L., Angermeier, P. L., Cooper, S. D., Lake, P. S., Fausch, K. D., Winemiller, K. O., … Rahel, F. J. (2001). Fish diversity in streams and rivers. In F. S. Chapin, O. E. Sala, & E. Huber-Sannwald (Eds.), *Global biodiversity in a changing environment* (pp. 315–349). New York, USA: Springer.

Pracheil, B. M., DeRolph, C. R., Schramm, M. P., & Bevelhimer, M. S. (2016). A fish-eye view of riverine hydropower systems: The current understanding of the biological response to turbine passage. *Reviews in Fish Biology and Fisheries*, *26*(2), 153–167.

Roubal, F. R., & Bullock, A. M. (1988). The mechanism of wound repair in the skin of juvenile Atlantic salmon, *Salmo salar* L., following hydrocortisone implantation. *Journal of Fish Biology*, *32*(4), 545–555.

Russel, W. M. S., & Burch, R. L. (1959). *The principles of human experimental technique*. London, UK: Methuen & Co., Ltd.

Schneider, J., Hübner, D., & Korte, E.. (2012). Funktionskontrolle der Fischaufstiegs- und Fischabstiegshilfen sowie Erfassung der Mortalität bei Turbinendurchgang an der Wasserkraftanlage Kostheim am Main. Endbericht 2012. Bürogemeinschaft für Fisch- & Gewässerökologische Studien, Frankfurt am Main.

Skalski, J. R., Mathur, D., & Heisey, P. G. (2002). Effects of turbine operating efficiency on smolt passage survival. *North American Journal of Fisheries Management*, *22*(4), 1193–1200.

Stephenson, J. R., Gingerich, A. J., Brown, R. S., Pflugrath, B. D., Deng, Z., Carlson, T. J., … Seaburg, A. G. (2010). Assessing barotrauma in neutrally and negatively buoyant juvenile salmonids exposed to simulated hydro-turbine passage using a mobile aquatic barotrauma laboratory. *Fisheries Research*, *106*(3), 271–278.

Tierney, K. B., & Farrell, A. P. (2004). The relationships between fish health, metabolic rate, swimming performance and recovery in return-run sockeye salmon, Oncorhynchus nerka (Walbaum). *Journal of Fish Diseases*, *27*(11), 663–671.

Vernerey, F. J., & Barthelat, F. (2010). On the mechanics of fishscale structures. *International Journal of Solids and Structures*, *47*(17), 2268–2275.

Vieira, F. A., Gregório, S. F., Ferraresso, S., Thorne, M. A., Costa, R., Milan, M., … Power, D. M. (2011). Skin healing and scale regeneration in fed and unfed sea bream, *Sparus auratus*. *BMC Genomics*, *12*(1), 490.

Williams, J. G., Armstrong, G., Katopodis, C., Larinier, M., & Travade, F. (2012). Thinking like a fish: A key ingredient for development of effective fish passage facilities at river obstructions. *River Research and Applications*, *28*, 407–417.

Zarfl, C., Lumsdon, A. E., Berlekamp, J., Tydecks, L., & Tockner, K. (2015). A global boom in hydropower dam construction. *Aquatic Sciences*, *77*(1), 161–170.

SUPPORTING INFORMATION

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