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Article

A Physical and Behavioral Barrier for Enhancing Fish Downstream Migration at Hydropower Dams: The Flexible FishProtector

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Abstract: Fish protection at hydropower plants is important for the sustainability of hosting ecosystems and the acceptance of hydropower. On their way downstream, fish are exposed to hydropower plants and various related negative effects, ranging from a delay in downstream movement to being injured or killed by a turbine. Understanding the behavior of fish in close proximity to protection devices is essential in order to establish efficient fish protection facilities. In this study, physical (horizontal steel cables) and behavioral barriers (electric field) for fish protection were developed (Flexible FishProtector) and their effectiveness was investigated. The behavior of brown trout (*Salmo trutta fario*), rainbow trout (*Oncorhynchus mykiss*), grayling (*Thymallus thymallus*) and chub (*Squalius cephalus*) at the Flexible FishProtector was analyzed using video evaluation. The experimental setup was a non-scaled section model of a runoff river power plant. The used electric field induced a flight reaction at a corresponding distance to the Flexible FishProtector that significantly increased the protection rate. Furthermore, an increase in guiding efficiency was achieved with the use of a physical as well as a physical and behavioral barrier, supporting safe downstream migration with the narrower cable clearance (30 mm versus 60 mm).

Keywords: ethohydraulic experiments; video analysis; hybrid barrier; fish behavior; fish guiding; potamodromous species; hydropower; fish protection

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1. Introduction

Fish migrate in rivers both up- and downstream to fulfill specific needs corresponding to their life stage, the time of the year, and environmental stressors [1–3]. Depending on the species, migration routes can vary from a few meters up to thousands of kilometers [4]. For fish species that migrate long distances, fragmented rivers can have serious consequences for the population status of fish, eventually resulting in extinction [5–9]. While upstream migration corridors are widely being restored using nature-based and technical fishway solutions [10], the passage efficiency can vary greatly [11]. Downstream migrating fish are still confronted with river fragmentation caused by hydropower plants. Turbine inlets are mostly screened with trash racks [12,13]. Generally, these trash racks are designed for turbine and human protection. Trash racks in many cases cannot sufficiently prevent fish from entering the turbine passage [2]. Both turbine passages and the trash racks themselves can cause injuries and occasionally fatalities [2,14]. Even if fish are not harmed, downstream migration can be fully blocked or at least delayed, which may seriously influence effected populations [15,16]. Hence, fish protection measures for downstream migration at hydropower plants have to be effective for various species and sizes. Besides efficiently protecting fish from injuries, guidance is required to a safe downstream migration corridor without any appreciable delay [15–19].

To minimize negative impacts on fish and increase the acceptance of hydropower as a renewable energy source, the Flexible FishProtector (University of Innsbruck, HyFish GmbH, Innsbruck, Austria) was invented as a hybrid fish protection and guiding system [20–22]. There are different types of FishProtectors in terms of spatial arrangement, electrodes used (steel cables or bars), and cleaning methods used. The hybrid barrier Flexible FishProtector consists of horizontally tensioned steel cables (physical barrier) that are simultaneously used as electrodes and surrounded by a moderate pulsating electric field (behavioral barrier) [22–24]. The pulsed electric field is created using the Neptun fish guidance and deterrence system (Procom System S.A., Wrocław, Poland) [25]. Due to the combination of physical barriers and graduated electric fields with moderate voltage and expansion, relatively wide cable clearances (in the experiments, 30 mm and 60 mm) are applicable [24]. The intensity and pulsation of deployed electric fields can be modified. As discussed in Tutzer et al. [24], the use of the correct proportion of electric field size and intensity to trigger an avoidance reaction while preventing immobilization or narcosis for all species and life stages is crucial [26–29]. Improper electric fields have the potential to cause severe injuries [30].

The Flexible FishProtector, as a hybrid barrier, has to be combined with a bypass system at its downstream end [1,2]. The hydraulic conditions and geometry of the entrance section are crucial for an efficient bypass [13]. The implementation of the Flexible FishProtector, together with an efficient bypass system, can fulfill the EU Council Directive 2000/60/EEC (Water Framework Directive), which mandates the limitation of adverse effects on fish caused by the anthropogenic use of rivers [31,32].

As presented in Tutzer et al. [24], the hybrid barrier exhibits great protective potential for all investigated fish species and lengths in all performed ethohydraulic experiments; such experiments lie in the interdisciplinary intersection of ethology and hydraulics. The authors obtained mean protection rates higher than 97% for all their applied hybrid setups. PIT-tag technology was used to determine fish protection rates for various species and lengths in previous studies [24,33]. However, these results do not provide detailed information concerning fish behavior at the hybrid barrier, e.g., the guiding behavior induced along the Flexible FishProtector or the flight reactions caused by the electric fields. Therefore, in this study simultaneously recorded video data were used to fill the information gap regarding fish behavior.

The objective of the present work is to investigate the behavior of fish in close proximity to the Flexible FishProtector using video evaluation. A key point of this study is to determine factors such as the size of the electric field, cable clearance, and exposition angle as well as corresponding fish behavior in order to increase the efficacy with regard to the protection and guiding rates of the Flexible FishProtector. Two hypotheses related to Tutzer et al. [24] were tested: (1) the electric field induces flight reactions and therefore increases fish protection, and (2) the narrower cable clearance enhances guiding activities, leading to a safe downstream migration corridor.

2. Materials and Methods

2.1. Fish Species

For the experiments, wild specimens of the four potamodromous species, brown trout (*Salmo trutta fario*), rainbow trout (*Oncorhynchus mykiss*), grayling (*Thymallus thymallus*), and chub (*Squalius cephalus*), were used. All four species are typical for the grayling zone (hyporhithral) and barbel zone (epipotamal) in Europe. Experimental fish originated from wild fish stocks and were taken from surrounding river sections immediately before the start of the test period. The water temperature (8–13 °C) and flow conditions (mean velocity 0.43 m/s) during the experiments were within the typical range for the analyzed species and life-stages [34–37]. Brown trout and rainbow trout were considered as one species (trout) in this context due to their similar behavior in terms of swimming performance and movement patterns. For every experiment, 15 individuals of each species were randomly chosen, and all experiments were performed as mixed experiments. Hence, in every single experiment, 45 fish were planned to be utilized. Due to a lack of grayling, the first 12 experiments were conducted without grayling and the last 19 experiments with less than 15 graylings (one experiment with 14 graylings, 15 experiments with nine graylings, and three experiments with seven graylings). Consequently, the first 12 experiments were carried out with 30 individuals (chub and trout only), whereas in the last 19 experiments, on average 39 individuals were used. For every individual, a minimum rest period of seven days was ensured between the two experiments.

The number of individuals per species with the mean number of experiments per specimen and mean fish length with the corresponding standard deviation are shown in Table 1.

Table 1. Fish species—trout (*Salmo trutta fario* and *Oncorhynchus mykiss*), chub (*Squalius cephalus*), and grayling (*Thymallus thymallus*)—used in the evaluated ethohydraulic experiments with the total number of available individuals, mean frequency of use per specimen across the 77 experiments, and mean and standard deviation (SD) of fish length.

Species	Number of Individuals	Mean Frequency of Use per Specimen	Length Mean ± SD (mm)
Trout	179	6.5	187 ± 38
Chub	226	5.1	171 ± 38
Grayling	73	11.6	257 ± 16

In the experiments, a natural downstream migration of utilized fish species was not expected. The reason why fish started exploring or swimming around in the experimental area could be their curiosity, their motivation to swim, or the lack of space in the adaption area.

2.2. Experimental Setup and Video Observation

The ethohydraulic experiments (from here on called experiments) were conducted at the research facility "Hydromorphology and Temperature Experimental Channel—HyTEC" in Lunz am See, Austria, from August to November 2017 [38]. The experimental plant consisted of two outdoor channels, which were fed with nutrient-poor lake water. The inflow to both channels was regulated independently. One channel was used for the experimental setup, whereas the second channel was used as a water reservoir and for holding fish. To guarantee the a priori fixed discharge of 650 L/s for all experiments, 250 L/s were constantly diverted from lake Lunz. A pump (KSB, Type KRTK 300-400/218UG-S, KSB GmbH, Vienna, Austria) in the upstream section of the second channel (connected to the experimental channel at the downstream end) circulated the remaining 400 L/s. The water flowed over a weir into the experimental channel.

The experimental area began with the adaption area approx. 20 m downstream of the inflow weir. Due to the width (3.0 m) of the experimental area and the adjusted water depth of 0.5 m, the mean flow velocity was 0.43 m/s. Velocity measurements were provided in Haug [39]. The water depth was adjusted using two weirs situated downstream of the experimental area. The experimental area was delineated with a fine mesh upstream and downstream. Fish could swim only within these limits during the experiments. At the upstream end, the grid to the adaption area was open during the conducted experiments. The distance from the upper limit to the beginning of the Flexible FishProtector was 4.0 m for both exposition angles (Figure 1). The experimental area was covered with a tarp to minimize external influences, e.g., weather effects, shading, and birds. The channel substrate consisted of fine gravel.

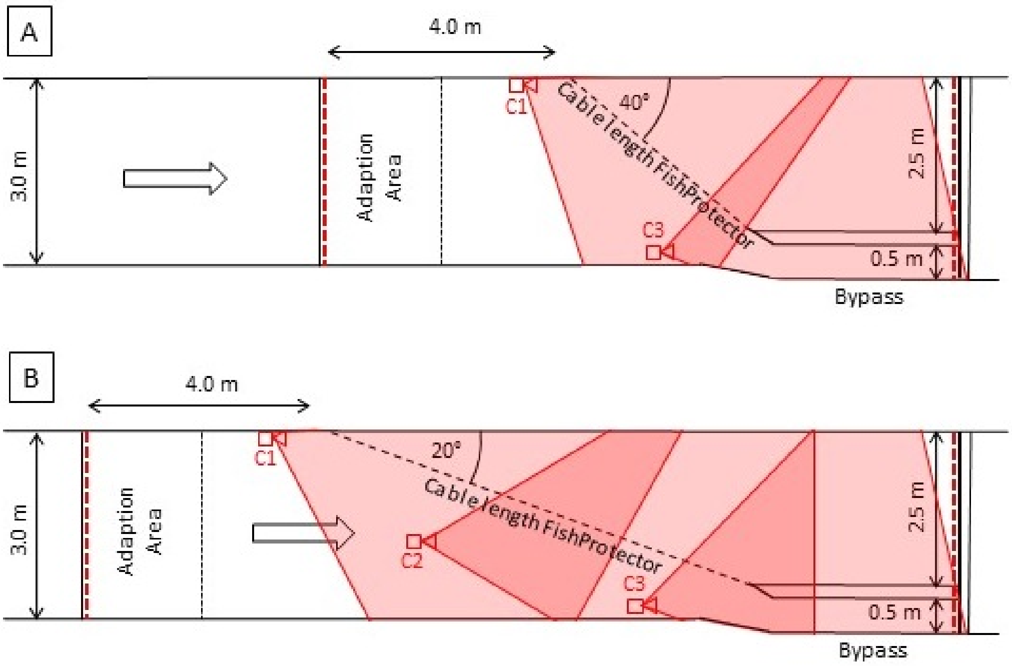

Figure 1. Design of the 3.0 m-wide experimental area with the two investigated exposition angles, 40° (**A**) or 20° (**B**), with two or three cameras (depending on the cable length of the Flexible FishProtector C1 and C3 or C1–C3) with the covered areas of the cameras shaded in red. Red dashed lines show the limits of the experimental area, the black dotted line (grid) shows the limit of the adaption area, and the grid was pulled after the adaption time of 0.5 h.

The experimental setup was primarily designed to evaluate the fish protection potential of the Flexible FishProtector as a hybrid barrier. In order to find the most applicable parameters for this purpose, the exposition angle as well as the cable clearance and electric field were varied, resulting in 12 different setups within the framework of the experiments (Table 2). The video-based analysis enabled an understanding of the protection principle and a further understanding of general behavioral patterns in close proximity to the hybrid barrier. All experiments were repeated at least five times. Every independent experiment consisted of two phases. Fish were stocked in the adaption area to adapt to the flow velocity. After an adaption time of 30 min, the fine meshed grid separating the adaption and experimental area (Figure 1, black dotted line) was lifted carefully by pulling a cable; thus, the experiment, with a duration of one hour, began.

Table 2. The number (#) of independent experiments used for the statistical analysis dependent on the investigated setups: cable clearance (30 mm, 60 mm), electric field (none, small field, large field), and exposition angle (20°, 40°).

Cable Clearance	Electric Field	# Experiments 20°	# Experiments 40°
30 mm	None	6	7
	Small Field	5	9
	Large Field	7	6
60 mm	None	6	6
	Small Field	6	6
	Large Field	6	7

The hybrid barrier was arranged in the 3.0 m-wide experimental channel. Due to the exposition angle of 20° (40°) the cable length of the Flexible FishProtector was 7.3 m (3.9 m) (Figure 1). The 0.5 m-wide bypass was located at the downstream end of the Flexible FishProtector as a natural extension. The total width of the section with the bypass remained 3.0 m (2.5 m experimental channel + 0.5 m bypass) [24]. The steel cables, tensioned within a frame, had a diameter of 8.0 mm and cable clearances of 30 mm and 60 mm, respectively. In addition to their function as a physical barrier, they were also used as electrodes, inducing a graduated electric field in the water surrounding the physical structure. The small electric field expanded approx. 10 cm upstream of the structure and the large field approx. 20 cm [24]. The indicated extensions of 10 cm and 20 cm considered a threshold value of 60 V/m for fish deterrence [29]. The intensity of the graduated and pulsed electric field increased towards the electrodes. The control unit Neptun (Procom System S.A.) [25] supplied the Flexible FishProtector with a pulsed direct current of 80 volts in this instance. Further details of the electrification are provided in Tutzer et al. [24]. Numerical modelling results and measurements of the size and intensity of the electric fields, which were conducted in the laboratory and during field work, are shown in Haug [39] and Knoll [40]. Control experiments without an electric field were performed for each setup. For the video analysis, up to three underwater cameras (GoPro Hero 3, GoPro Inc., San Mateo, CA, USA) were used. The number of cameras depended on the exposition angle and, thus, on the length of the Flexible FishProtector. One camera (C1 Figure 1A) or two underwater cameras (C1 and C2 Figure 1B) were installed along the Flexible FishProtector. In each case, one camera was installed at the downstream end of the Flexible FishProtector, filming the downstream section of the Flexible FishProtector and the entrance to the bypass (C3 in Figure 1A,B). The cameras used to film all the experiments in the downstream direction covered the whole experimental area in such a way that an action-based evaluation of fish behavior in close proximity to the Flexible FishProtector was possible (Figure 1). Experiments were performed exclusively during the daytime to guarantee suitable lighting conditions for analysis. Due to the setup limit, e.g., the distance to a camera and clarity of the water, the fish species could not be determined in every case, but their behavior could be observed appropriately.

2.3. Protection Principle of the Flexible FishProtector and Data Evaluation

Possible behavioral patterns and sequences of fish in close range to the FishProtector are described below and illustrated in detail in Figure 2. The following numbers in brackets correspond to the numbers in Figure 2. When a fish moved downstream (1), the physical barrier was perceived first (2) and the fish took a positive rheotactic swimming position (3), followed by a further careful approach closer to the barrier (4). Entering the electric field (5) induced a flight reaction (6). Due to the previously taken positive rheotactic swimming position, fish showed a flight reaction basically in an upstream direction, enlarging the distance to the Flexible FishProtector. This flight reaction could be repeated several times (7). Due to the arrangement of the Flexible FishProtector, fish were guided (parallel or showing a wavy path) along the Flexible FishProtector in both directions. As the fish moved further downstream along the barrier, they were eventually directed into the bypass system (8).

For video data evaluation, the same 77 out of 92 experiments as those presented by Tutzer et al. [24] were used. Experiments influenced by external factors, e.g., stormy or windy conditions that could lead to irregular effects on the behavioral patterns of experimental fish—were excluded (15 experiments).

During the experiments, fish were kept within the experimental area so that every fish could trigger several actions. In order to guarantee a consistent standardized evaluation of all experiments, actions in close range to the Flexible FishProtector were counted as an independent action if they belonged to one of five categories. These five categories were defined after a first review of the video data: (1) fish guiding (fish were guided in a parallel or wavy line along the Flexible FishProtector in an up- or downstream direction), (2) flight reaction (fish showed a flight reaction induced by the electric field; flight reactions could, by definition, only occur with hybrid setups), (3) FishProtector passage (fish passed through the physical or hybrid barrier), (4) FishProtector passage from downstream to upstream, and (5) bypass use (fish entered the bypass system). The fourth category (FishProtector passage from downstream to upstream) was not statistically investigated due to its sparse occurrence for hybrid setups. On average, only one such activity for two experiments for this category was recorded.

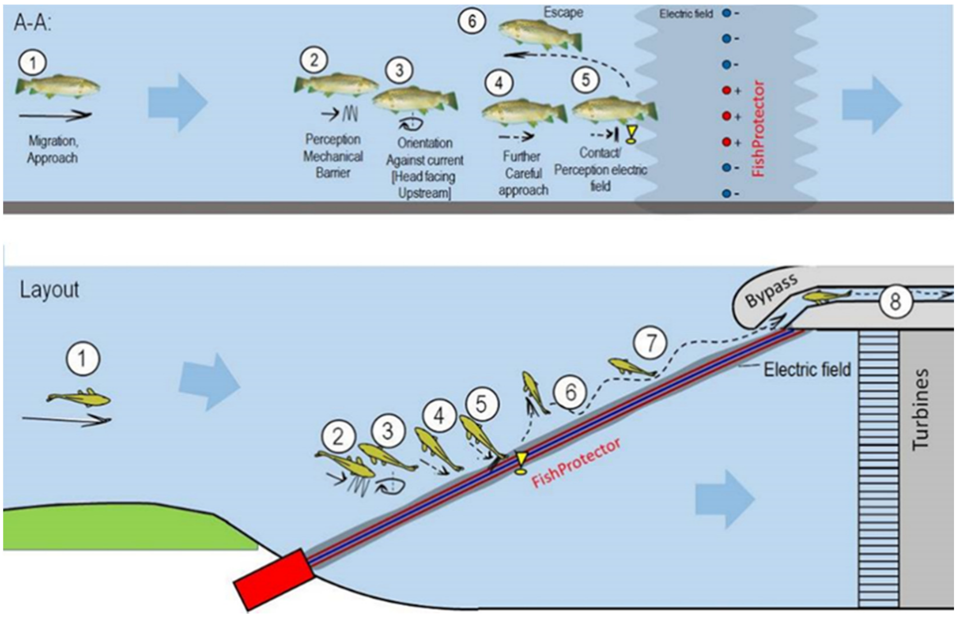

Figure 2. Fish protection principle at the hybrid barrier Flexible FishProtector: interaction of the physical barrier and the behavioral barrier. Numbers from 1 to 8 indicate the sequence of fish behavior when fish approach the Flexible FishProtector. (1) Downstream migration, (2) perception of physical barrier, (3) rheotactic swimming position, (4) approaching FishProtector, (5) entering electric field, (6) flight reaction, (7) guiding, and (8) bypass use. Protection principle shown in A-A as section and underneath as layout. Source: Aufleger, 2019 [41].

For the statistical analysis, four categories (fish guiding, flight reaction, FishProtector passage, bypass use) were investigated (Figure 3). The counts in each category were divided by the number of fish in the experiment and used as response parameter for the further analyses.

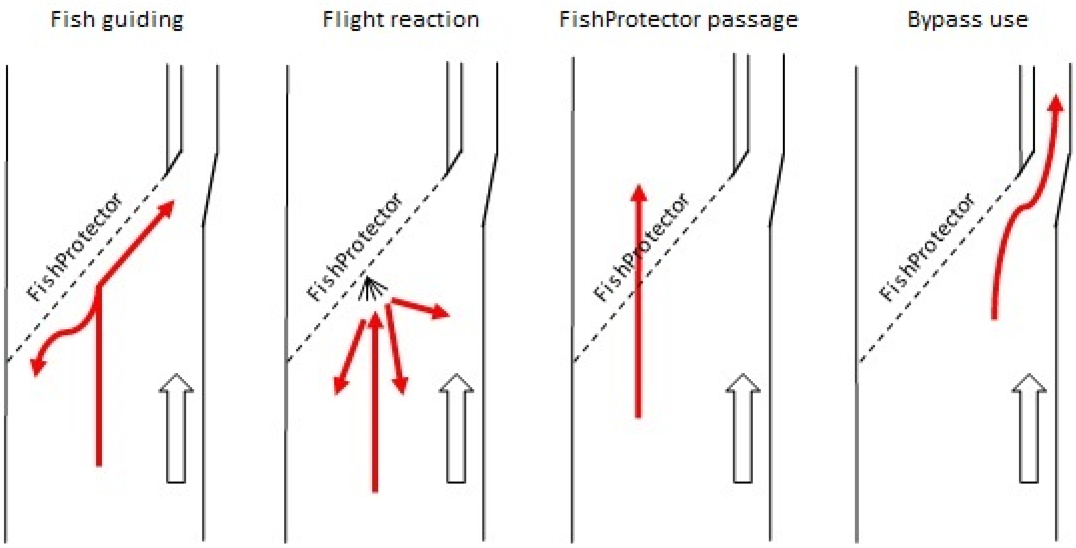

Figure 3. The four behavioral categories that were used in the (statistical) analysis are visualized in the sketch of the experimental layout: fish guiding, flight reaction, FishProtector passage, and bypass use

from left to right. The fish movement is shown in red, while the white arrow denotes flow direction. The physical and behavioral barrier are indicated by the dashed line.

These chosen categories were strongly linked to the Flexible FishProtector, actions were induced by physical or hybrid barriers. The subjectivity of the evaluators played a minor role due to the clear and distinct movements of fish within the four categories.

2.4. Statistical Analysis

The quantities of interest (y) were the four fish behaviors divided by the number of fish per experiment, i.e., guiding per fish, flight reaction per fish, FishProtector passage per fish, and bypass use per fish. As y was skewed to the right and heteroskedastic, a generalized linear model with a gamma distribution and the natural log link was used for investigating the parameters $\left(\left(x^1, \ldots, x^p\right),\ p \ldots \text{number of parameters}\right)$ with respect to their statistical significance. To guarantee that y had no zero values, few zero values were replaced by a small value c, with $c \ll$ minimum of y. Note, however, that the findings were independent of c. The model equation used for the expected behavior of each fish conditional on the parameters $(E(y_i|x_i))$ is:

$$\log(E(y_i \middle| x_i)) = x_i^T \beta, \tag{1}$$

where y_i denotes the specific behavior of each fish in the experiment i $(i = 1, \ldots, n)$ and is gamma-distributed, x_i^T is a row vector summarizing the values of the parameters as well as possible interaction terms, and β is the vector of the regression coefficients. The estimated coefficients and their standard errors were obtained using maximum likelihood estimation. Residuals and outlier diagnostics were carried out to check the model's appropriateness. For all fish behaviors, Equation (1) was carried out separately, and for each model appropriateness a pseudo R^2 was provided, where pseudo R^2 is the squared correlation between y and its fitted values. The gamma regression output provided the estimates, their standard errors, the values of the test statistic and p-values.

Decision trees were used for the visualization of the effects of the parameters. The algorithm worked by splitting the dataset recursively. At each step, the split was performed based on the parameter that results in the smallest Akaike information criterion (AIC) [42] of the corresponding gamma regression. The subsets that arose from a split were further split until the difference in the AIC was less than 2, i.e., no substantial support for a further split [43]. The decision tree showed the importance of the parameters from top to bottom.

A statistical significance level of 5% was used, statistical results in the test provided an estimate and corresponding p-value in brackets. All statistical analyses were carried out in R (Version 4.0.3) [44] and visualizations were obtained using the ggplot2 package [45].

3. Results

3.1. Observational Results

When a fish moved downstream, the physical barrier was perceived first and the fish took a positive rheotactic swimming position. This was the first fish reaction observed at a distance of approximately 0.5 to 1.0 m to the Flexible FishProtector. This behavior was observed for all species, for both single individuals and fish schools. Even the absence or presence of an electric field caused no difference in this behavior. Thus, fish turned around in a positive rheotactic swimming position before they approached the barrier and entered the electric field in the case of a hybrid system. This behavior did not change depending on the exposition angle (20°/40°) or cable clearance (30 mm/60 mm) within the experiments. Due to the slight exposition angle to the approach flow (<45°), fish were guided along the Flexible FishProtector in up- and downstream directions, showing straight or wavy swimming patterns. This was observed for both electrified and non-electrified setups and for all species used. The electric field kept fish a certain distance from the

Flexible FishProtector depending on the size of the electric field; at this distance, guiding was mainly observed.

Fish were guided to the entrance of the bypass along the Flexible FishProtector (common behavior in experiments) or swam along the orographic right side in the experiment to finally arrive at the entrance section of the bypass. Swarm behavior was observed as well as behaviors of single individuals. In the case of a school advancing to the entrance section of the bypass system, every possible behavior configuration was observed (school swam away, one fish of the school entered the bypass, one fish of the school entered the bypass and some followed, almost the whole school entered the bypass system, and the whole school entered the bypass system). Once fish were at the entrance to the bypass system, they could enter the bypass, rest there, or return upstream. The duration of a fish staying at the entrance section could be from seconds to minutes. Fish entering the bypass system showed no general pattern regarding behavior or the time they stood in the bypass system. Every possible configuration of behavior, e.g., fish swimming into the bypass system and staying a long time, fish swimming in and out, fish entering the bypass system several times, or fish entering the bypass system only once, was observed.

Entering the graduated electric field evoked a flight reaction. Due to the previously taken positive rheotactic swimming position, fish showed a flight reaction basically in an upstream direction, enlarging the distance to the Flexible FishProtector by 10 to 50 cm. The closer the fish were to the Flexible FishProtector (high intense electric field) the more flight reactions resulting in rather sharp turnings or abrupt changes in swimming direction were observed. In case a school got close to the Flexible FishProtector and entered the electric field, the fish swimming closest to the barrier entered the electric field first and subsequently performed the flight reaction. This fish could influence the school, which then also showed a flight reaction (the whole or part of the school) or the movement of the individuals stopped when they encountered the next upstream swimming fish. The distance to the Flexible FishProtector where the flight reaction occurred depended on the size of the electric field. Fish showed the same flight reaction several times while approaching the hybrid barrier. A special flight reaction that appeared rarely (only 20 flight reactions out of a total of 801) was the fish passing through the Flexible FishProtector without leaving the electric field to repass the Flexible FishProtector in an upstream direction immediately afterwards.

Considering the hybrid barrier, the passage movement could be described as, (a) a fast slip through. Individuals passing through the Flexible FishProtector were mostly in a positive rheotactic swimming position, turning around abruptly to pass through without testing the barrier. Other passage movements were, (b) fish swimming out of the bypass system, sharply turning around the pillar, passing through the Flexible FishProtector, or (c) the fish swimming head first through the Flexible FishProtector. In the case of a school getting close to the Flexible FishProtector and entering the electric field, the fish swimming closest to the barrier showed the described flight reaction. If the school eliminated possible flight options, fish could turn around and show a flight reaction through the Flexible FishProtector. Fish coming close to the non-electrified physical barrier approached the physical barrier until they could touch the barrier with their tailfin (thigmotactic behavior), constantly remaining in a positive rheotactic swimming position. This could happen several times. Fish became confident and finally passed through the physical barrier. The movement through the barrier could be described as turning sideways and drifting through the barrier head on. If the first fish passed through the physical barrier, other fish often followed and passed through the physical barrier as well. In the case where only a physical barrier was used, fish also returned upstream through the physical barrier. This happened especially for cable clearances of 60 mm and only rarely for cable clearances of 30 mm.

In the experiments, fish swam as single individuals, in small groups of up to four individuals, or in schools. Schools could be composed of various species. Fish approaching the barrier several times showed the same behavioral patterns. Thereby, it was irrelevant if a fish got close to the hybrid barrier the first time or if the activity rate within an experiment was high or low. Fish behavior was similar within an experiment as well as across all experiments. Regarding the behavior of fish related to the Flexible FishProtector, no signs of short-term learning effects (within an experiment) could be observed, since fish approached the Flexible FishProtector several times. Additionally, no dependence on the time of the single actions within the experiments could be detected. As fish were utilized several times across the experiments and always showed the same behavior when faced with the Flexible FishProtector, no long-term learning effects (over all experiments) or habituation to the Flexible FishProtector were observed.

The counted actions corresponding to the defined four categories obtained by video analysis are provided in Table A1 in Appendix A as well as a descriptive statistics table of the response variables, which are analyzed below (Table A2 in Appendix A). Supplementary video clips showing the described fish behavior (Video S1–Video S6 in Supplementary Materials) are available online at https://www.mdpi.com/article/10.3390/w14030378/s1.

3.2. Fish Guiding

Figure 4 shows the guiding activity per fish dependent on the setups. The lowest guiding activities per fish were observed for the setup 60 mm and 20°.

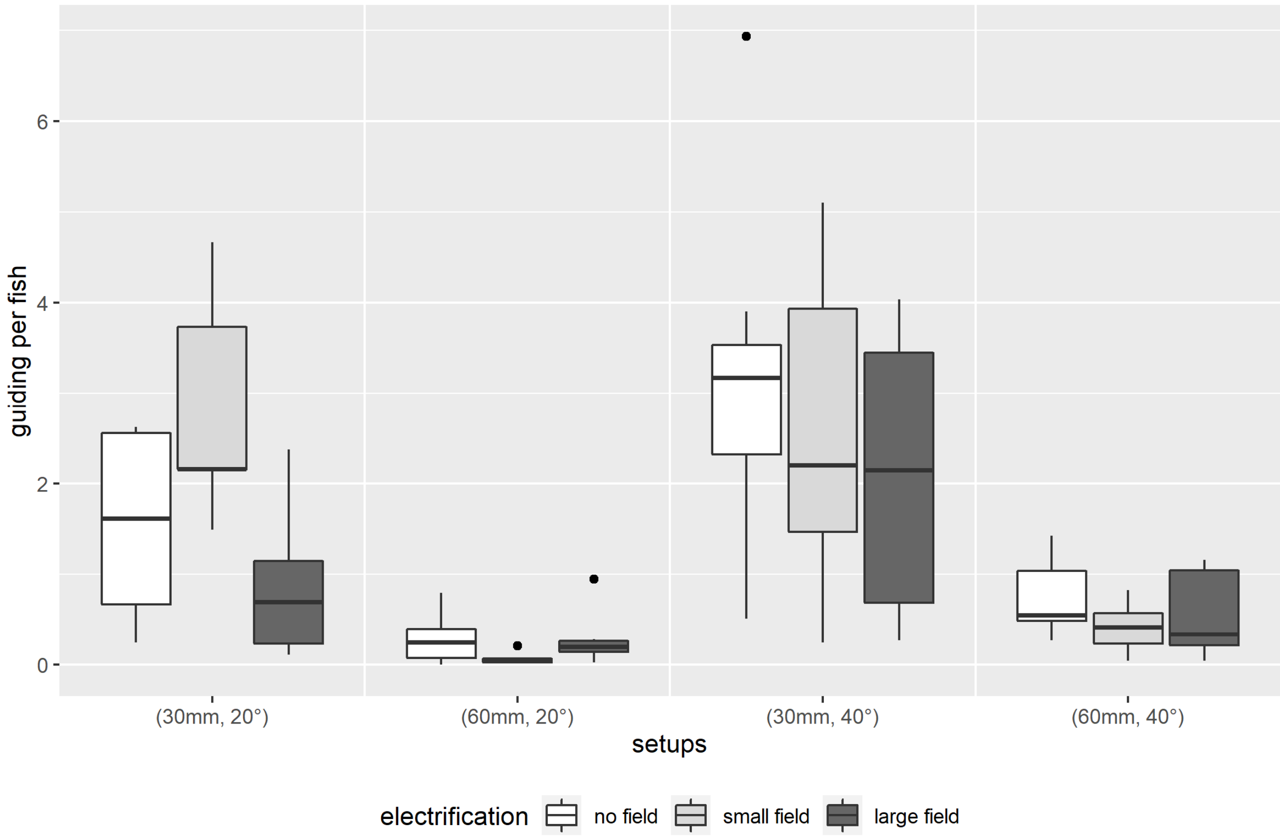

Figure 4. Guiding activities along the Flexible FishProtector per fish, dependent on the setups with the parameter exposition angle (°), cable clearance (mm), and electric field (no electric field (white), small electric field (light gray), large electric field (dark gray)). A single boxplot represents robust statistical measures, such as minimum, 1st quartile, median, 3rd quartile, maximum, as well as extreme values defined as 3rd quartile plus 1.5 times the interquartile range, interquartile range equals 3rd quartile minus 1st quartile.

The guiding efficiency along the Flexible FishProtector was mainly influenced by the cable clearance (Table 3, Figure 4). Without an electric field, the wider cable clearance of 60 mm had a statistically significant lower number of guiding activities compared to the cable clearance of 30 mm (−1.551, p-value < 0.001). With a small electric field, this effect became even larger (−1.049, *p*-value = 0.023). Other than this effect, no statistical evidence of a main effect of the electric fields was found.

Table 3. For guiding along the Flexible FishProtector per fish, a summary of the coefficient estimates of the gamma regression model with interactions (cf. Equation (1)) is provided.

Parameters	Estimate	Std. Error	z Value	*p*-Value	Sig.
(intercept)	0.398	0.244	1.633	0.107	ns
electrification small field	0.180	0.310	0.582	0.562	ns
electrification large field	−0.508	0.315	−1.613	0.111	ns
spacing 60 mm	−1.551	0.321	−4.828	0.001	***
angle 40°	0.780	0.184	4.231	0.001	***
spacing 60 mm: electrification small	−1.049	0.451	−2.327	0.023	*
spacing 60 mm: electrification large	0.387	0.450	0.859	0.393	ns
pseudo R^2 (sample size)	0.447 (*n* = 77)				

Note: Reference group (intercept): angle 20°, cable clearance 30 mm, no electric field. Std. error denotes the standard error of the estimate; z value provides the value of the z-statistic; *p*-value gives the two-sided *p*-value; and sig. denotes significance with the significance codes '***' *p*-value < 0.001, '*' *p*-value < 0.05, and 'ns' denotes not statistically significant.

The exposition angle showed a statistically significant influence on guiding along the Flexible FishProtector (Table 3). The exposition angle of 40° provoked a higher number of guiding activities compared to the exposition angle of 20° (0.780, *p*-value < 0.001).

The decision tree (Figure 5) started to split the sample with cable clearance. If the spacing equaled 60 mm, the left branch was used (spacing = 60 mm *yes* denotes the *left* branch); if the spacing equaled 30 mm the right branch has to be followed (spacing = 60 mm *no* denotes the *right* branch). Following the right branch (cable clearance = 30 mm), neither the exposition angle nor the electrification gave substantial support for a further split in this branch. The leaf with the highest value of mean guiding per fish is colored in the darkest gray, i.e., for guiding, the highest activity per fish was 2.34 if the cable clearance was 30 mm (right branch of Figure 5). Following the left branch, the next parameter was the exposition angle. The experiments were further split into experiments with exposition angles of 20° and 40°, respectively. Following the left branch again, the next parameter was electrification. Experiments were further split into experiments with a small electric field (left branch) and no or a large electric field (right branch). The final leaf indicated 0.10 mean guiding activities per fish; if the cable clearance was 60 mm, the exposition angle was 20° and a small electric field was applied. All other leaves can be interpreted in an analog manner.

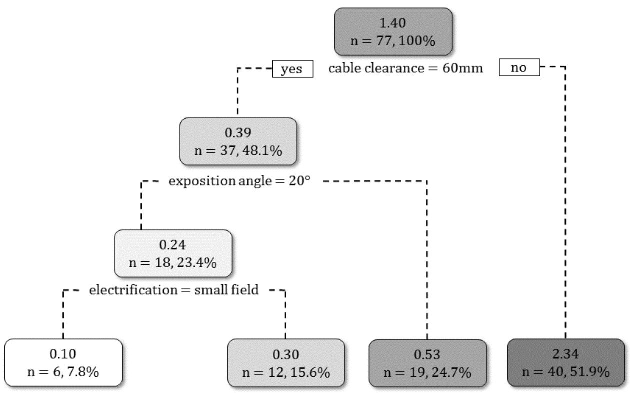

Figure 5. Decision tree for guiding along the Flexible FishProtector per fish showing the importance of the parameters. Following the left branch at a split always means "yes" for the stated parameter value, while following the right branch at a split means "no". In the boxes, the corresponding mean guiding per fish is provided as well as the number of experiments and its percentage of all experiments.

Hence, for guiding along the Flexible FishProtector, the most important parameter was cable clearance. Neither the exposition angle nor the electric field could substantially improve the guiding activities if the cable clearance was 60 mm.

3.3. Flight Reaction

A flight reaction could occur by definition only for hybrid setups due to the electric field; therefore, the number of experiments used in this analysis was 52. The strength of the reaction varied according to the position of the fish relative to the Flexible FishProtector.

Figure 6 visualizes the flight reactions per fish dependent on the setups, which do not seem to have strong influences on this behavior.

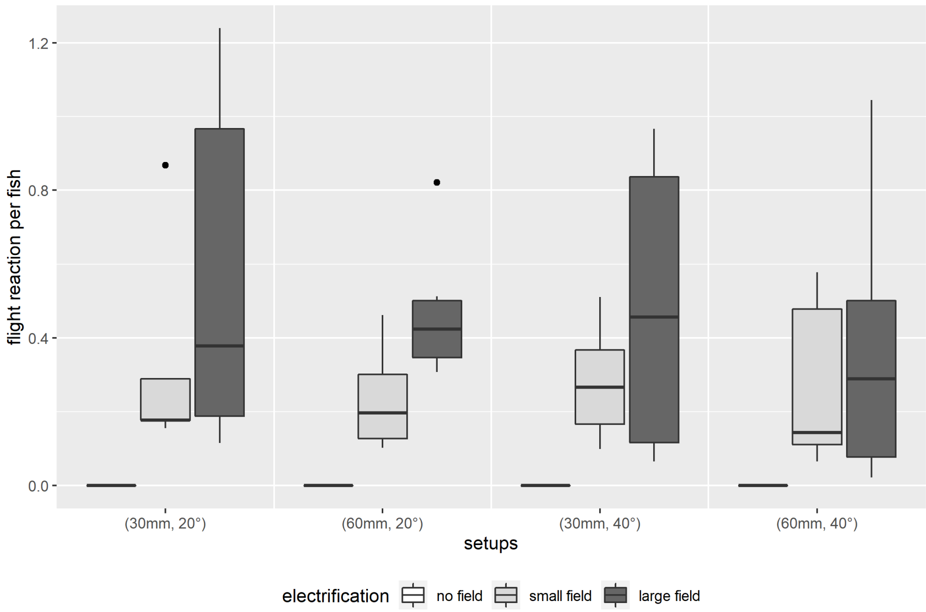

Figure 6. Flight reactions per fish dependent on the setups with the parameter exposition angle (°), cable clearance (mm), and electric field (no electric field (white), small electric field (light gray), large electric field (dark gray)). A single boxplot represents robust statistical measures, such as minimum, 1st quartile, median, 3rd quartile, maximum, as well as extreme values defined as 3rd quartile plus 1.5 times the interquartile range, interquartile range equals 3rd quartile minus 1st quartile.

Only the electric field parameter showed a significant influence on the occurrence of flight reactions (Table 4). The large electric field provoked a higher number of flight reactions compared to the small electric field (0.507, p-value = 0.016). As only one parameter is statistically significant, no decision tree for flight reaction was provided.

Table 4. For the flight reactions per fish, a summary of the coefficient estimates of the gamma regression model (cf. Equation (1)) without interactions, as they were not statistically significant, is provided.

Parameters	Estimate	Std. Error	z Value	p-Value	Sig.
(Intercept)	−1.099	0.209	−5.249	0.001	***
electrification large field	0.507	0.203	2.495	0.016	*
spacing 60 mm	−0.220	0.203	−1.085	0.283	ns
angle 40°	−0.120	0.204	−0.590	0.558	ns
Pseudo R^2 (sample size)	0.138 (n = 52)				

Note: Reference group (intercept): angle 20°, cable clearance 30 mm, small field. Std. error denotes the standard error of the estimate; z value provides the value of the z-statistic, p-value gives the two-sided p-value; and sig. denotes significance with the significance codes '***' p-value < 0.001, '*' p-value < 0.05, and 'ns' denotes not statistically significant.

3.4. Passage Occurrences

Due to the cable clearance (30/60 mm) used, passage through the physical barrier was in principle possible for all species and lengths of fish, but occurred more often for 60 mm if no electric field was used. The strong dependence of fish passage through the physical barrier on cable clearance was no longer visible for fish passing through the physical barrier with an electric field (Figure 7).

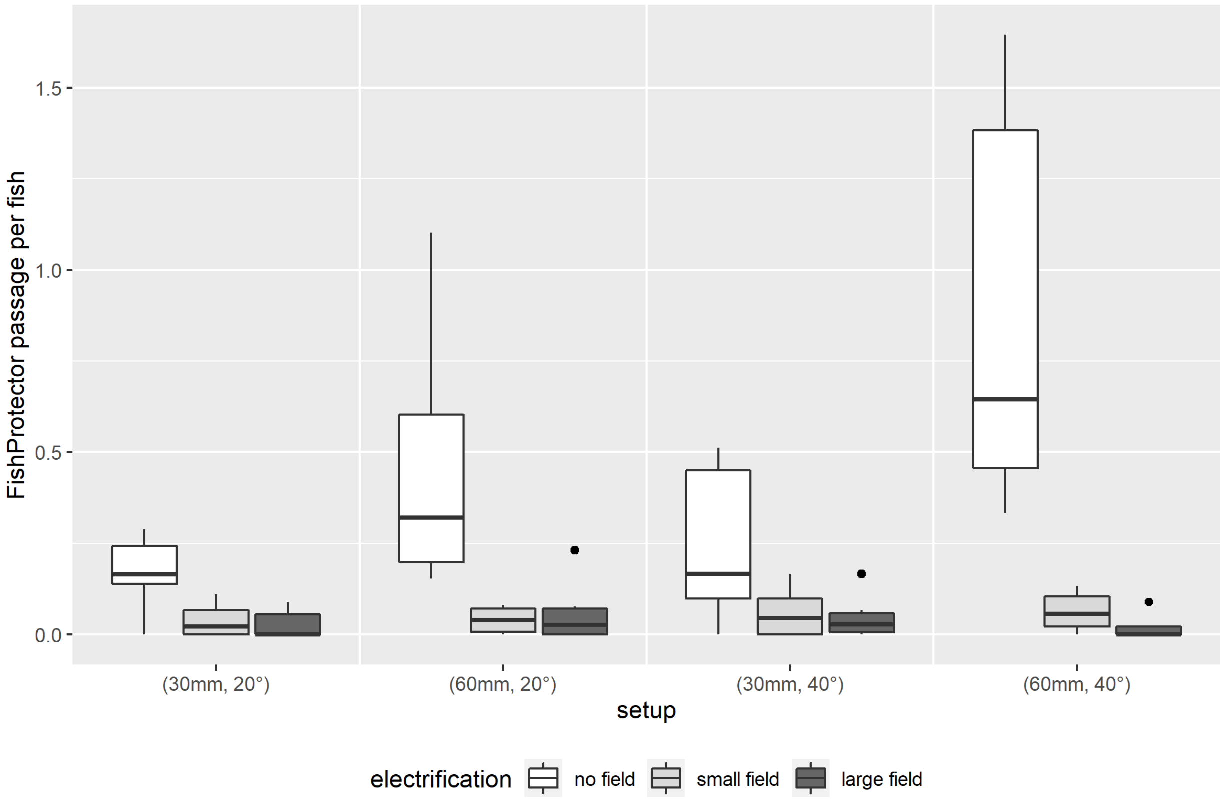

Figure 7. FishProtector passages per fish dependent on the setups with the parameter exposition angle (°), cable clearance (mm), and electric field (no electric field (white), small electric field (light gray), large electric field (dark gray)). A single boxplot represents robust statistical measures, such as minimum, 1st quartile, median, 3rd quartile, maximum, as well as extreme values defined as 3rd quartile plus 1.5 times the interquartile range, interquartile range equals 3rd quartile minus 1st quartile.

In experiments without an electric field, the fish passages depended mainly on the cable clearance (Table 5). The wider the physical barrier was, the more fish swam through the Flexible FishProtector (1.111, *p*-value < 0.001). However, this influence depended on the presence of the electric field. With a hybrid system, the strong dependence on cable clearance decreased, as the barrier effect was strengthened with the presence of an electric field. For a cable clearance of 30 mm, the small electric field (−1.369, *p*-value < 0.001) as well as the large electric field (−1.601, *p*-value < 0.001) significantly decreased the fish passage compared to the experiments without electric fields. Additionally, the model main effects of a cable clearance of 60 mm, i.e., more occurrences of fish passage than with a cable clearance of 30 mm and no field (1.111, *p*-value < 0.001), were strongly reduced by both electric fields, not yet at a significance level of 5% (small field: −1.081, *p*-value = 0.063; large field: −1.049, *p*-value = 0.071).

Table 5. For passing through the Flexible FishProtector per fish, a summary of the coefficient estimates of the gamma regression model with interactions (cf. Equation (1)) is provided.

Parameters	Estimate	Std. Error	z Value	*p*-Value	Sig.
(Intercept)	−1.648	0.309	−5.330	0.001	***
electrification small field	−1.369	0.393	−3.483	0.001	***
electrification large field	−1.601	0.400	−4.006	0.001	***
spacing 60 mm	1.111	0.408	2.725	0.010	**
angle 40°	0.212	0.234	0.904	0.369	ns
spacing 60 mm: electrification small	−1.081	0.572	−1.889	0.063	.
spacing 60 mm: electrification large	−1.049	0.571	−1.836	0.071	.
Pseudo R^2 (sample size)	0.572 (n = 77)				

Note: Reference group (intercept): angle 20°, cable clearance 30 mm, no electric field. Std. error denotes the standard error of the estimate; z value provides the value of the z-statistic; *p*-value gives the two-sided *p*-value; and sig. denotes significance with significance codes '***' p-value < 0.001, '**' p-value < 0.01, '.' p-value < 0.1, and 'ns' denotes not statistically significant.

For the exposition angle, no statistically significant evidence of an influence on fish passage occurrences was found.

For FishProtector passage, the most important parameter was the electric field (Figure 8). If an electric field was applied, neither the cable clearance nor the exposition angle provided substantial support for a further split. For the non-electrified FishProtector, the cable clearance was the most important parameter. The exposition angle offered no substantial support for a further split in this case.

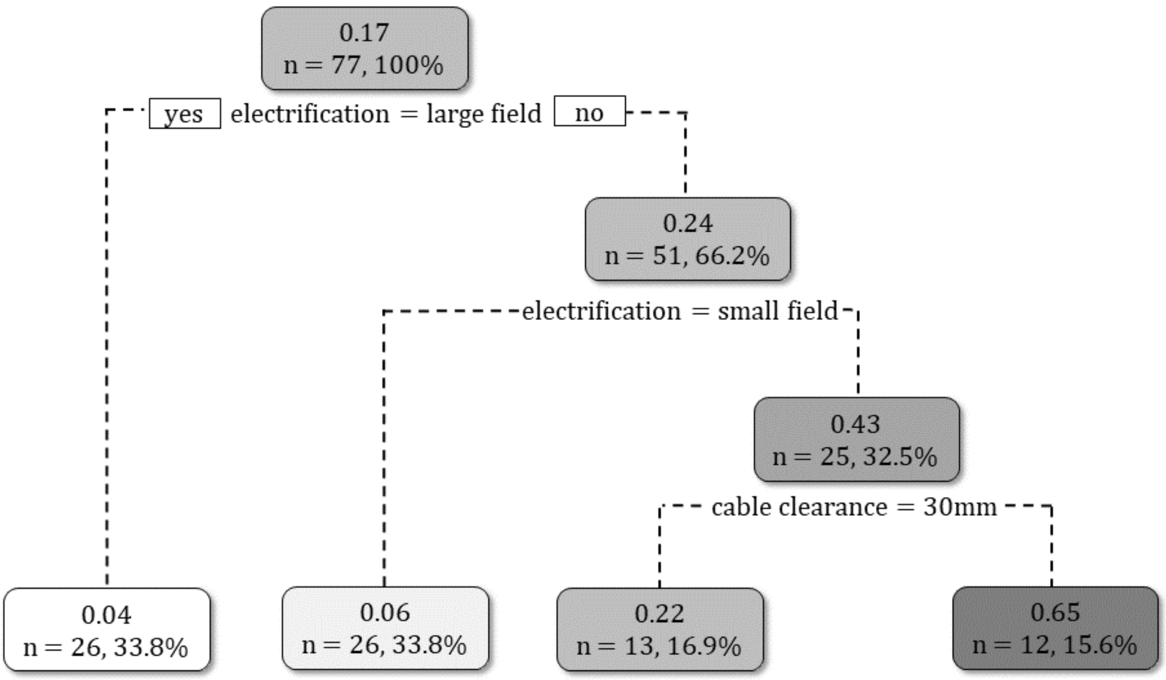

Figure 8. Decision tree for FishProtector passage per fish showing the importance of the parameters. Following the left branch at a split always means "yes" for the stated parameter value, while following the right branch at a split means "no". In the boxes, the mean passages per fish are provided as well as the number of experiments and the percentage of all experiments.

3.5. Bypass Use

Clearly, 30 mm for bypass use was the best value of parameter cable clearance (Figure 9). The exposition angle seems to have a much weaker influence.

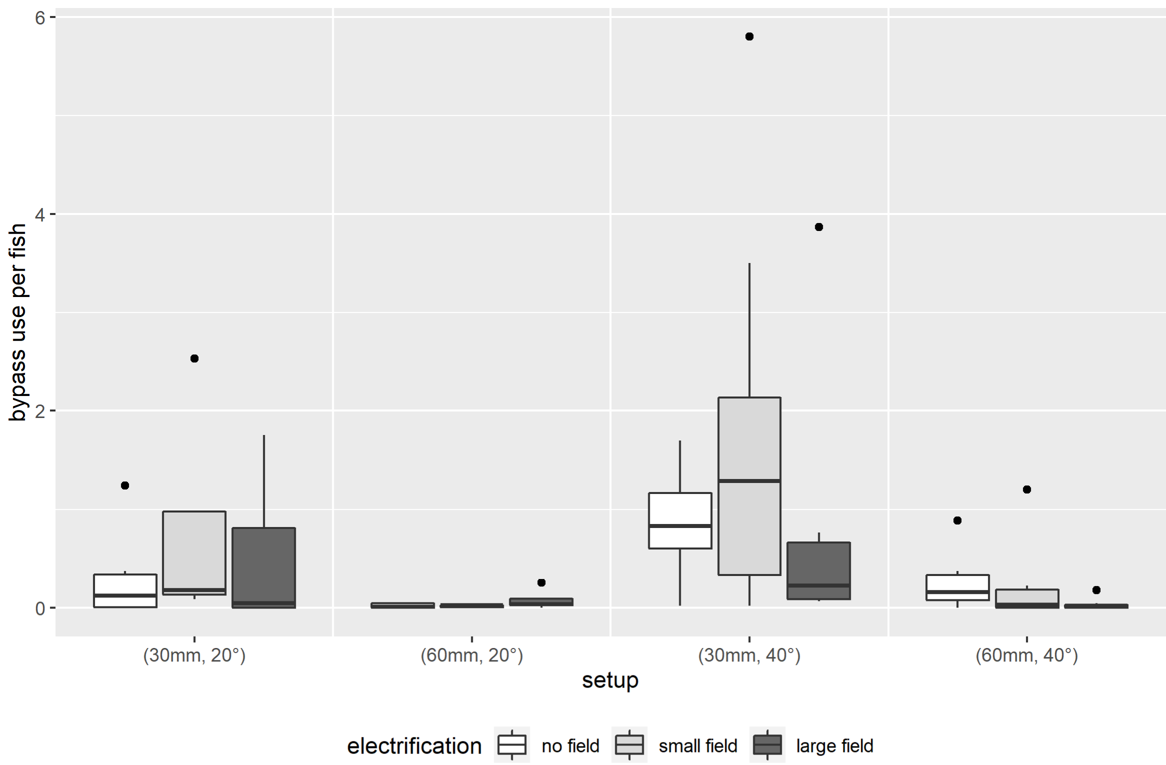

Figure 9. Bypass use activities per fish dependent on the setups with the parameter exposition angle (°), cable clearance (mm), and electric field (no electric field (white), small electric field (light gray), large electric field (dark gray)). A single boxplot represents robust statistical measures, such as minimum, 1st quartile, median, 3rd quartile, maximum, as well as extreme values defined as 3rd quartile plus 1.5 times the interquartile range, interquartile range equals 3rd quartile minus 1st quartile.

Setups with a cable clearance of 30 mm showed significantly higher bypass use compared to setups with a cable clearance of 60 mm (−2.087, p-value < 0.001, Table 6). The exposition angle of 40° provoked a higher value of bypass use compared to the exposition angle of 20° (1.053, $p = 0.002$, Table 6). No evidence was found of a statistically significant impact of the electric field on the bypass use (Table 6).

Table 6. For swimming into the bypass per fish, a summary of the coefficient estimates of the gamma regression model (cf. Equation (1)) without interactions, as these were not statistically significant, is provided.

Parameters	Estimate	Std. Error	z Value	p-Value	Sig.
(Intercept)	−0.913	0.380	−2.402	0.019	*
electrification small field	0.351	0.411	0.854	0.396	ns
electrification large field	−0.033	0.411	−0.080	0.936	ns
spacing 60 mm	−2.087	0.335	−6.227	0.001	***
angle 40°	1.053	0.336	3.132	0.002	**
Pseudo R^2 (sample size)	0.287 ($n = 77$)				

Note: Reference group (intercept): angle 20°, cable clearance 30 mm, no electric field. Std. error denotes the standard error of the estimate; z value provides the value of the z-statistic; p-value gives the two-sided p-value; and sig. denotes significance with the significance codes '***' p-value < 0.001, '**' p-value < 0.01, '*' p-value < 0.05, and 'ns' denotes not statistically significant.

For bypass use, the most important parameter was the cable clearance (Figure 10). The use of a cable clearance of 30 mm was most beneficial for bypass use (mean bypass use per fish = 0.93). All other parameters given a cable clearance of 60 mm could not increase the mean usage of the bypass substantially.

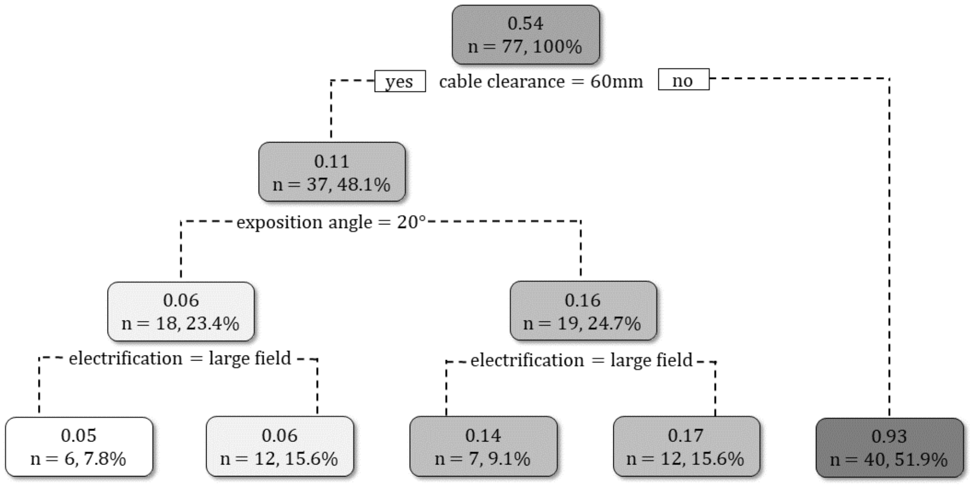

Figure 10. Decision tree for bypass use per fish showing the importance of the parameters. Following the left branch at a split always means "yes" for the stated parameter value, while following the right branch at a split means "no". In the boxes, the mean bypass use for per fish is provided as well as the number of experiments and the percentage of all experiments.

4. Discussion and Conclusions

Fish behavioral patterns in close range to a hybrid (physical and electric) barrier were investigated. Four species, brown trout (*S. trutta*), rainbow trout (*O. mykiss*), grayling (*T. thymallus*), and chub (*S. cephalus*), were used and their behavior was evaluated by video data analysis. Fish behavior was categorized into fish guiding, flight reaction, FishProtector passage, and bypass use. Advantageous parameters for each behavioral category were investigated with various setups. The setups were combinations of cable clearance (30 mm versus 60 mm), exposition angle (20° versus 40°), and electric field (no field, small field, or large field).

The simultaneous use of different species within an experiment might have an effect on the behavior of individual fish or species due to rivalry and the lack of habitat diversity in the experimental area. However, such effects on fish behavior were not observed; rather, the size of the experimental area was large enough to keep the fish density at a suitable level during the experiment.

Fish passage is a direct measure of the protection rate. A hybrid system decreases the strong dependence of protection rates on cable clearance, which was evident for physical barriers [1,2,46]. Both cable clearances and applied electric fields showed fish protection rates at a similar high magnitude. The small electric field as well as the large electric field significantly increased the fish protection compared to the one obtained with no electric field.

Fish protection involves more than just keeping fish away from the turbine inlet. It is at least equally important to guide fish to a suitable downstream migration corridor without delay [7,18,19]. Due to the higher bypass rates for setups with cable clearances of 30 mm compared to cable clearances of 60 mm, Tutzer et al. [24] hypothesized that the guiding efficiency along the Flexible FishProtector would be higher if the physical barrier was denser. Guiding was observed in both up- and downstream directions and in straight or wavy paths. Our second hypothesis of an association between guiding and denser barriers was confirmed with video data. The most important parameter for guiding was even cable clearance. Importantly for the hybrid barrier, the electric field did not influence the guiding efficiency negatively for a cable clearance of 30 mm. In addition, the electric field played a minor role in the wider cable clearance settings.

As shown in Tutzer et al. [24] and confirmed in this work, bypass use was not negatively influenced by the electric field. Still, the most important parameter for bypass use was the cable clearance. Setups with cable clearances of 30 mm showed significantly higher bypass use compared to setups with cable clearances of 60 mm.

This study also faced some limitations concerning the generalizability of the findings. Experiments were conducted with a mean flow velocity of 0.43 m/s, based on previous work [46] and recommendations from the literature [2,30,47]. The bypass system in the experimental channel was constructed as a natural extension of the Flexible FishProtector. To avoid the shading effects of the electric field in the entrance section of the bypass system, the entrance section was slightly enlarged. Hence, the mean flow velocity decreased from 0.43 m/s to 0.35 m/s. Specific hydromorphological conditions at the bypass entrance defined, for example, in Ebel [2] could not fully be considered in order to enable proper downstream migration corridors for fish [48,49]. Although this velocity drop was not that strong, the literature suggested increasing the velocity to the bypass entrance, thus probably making the bypass use higher. Therefore, in future experiments, improvements in the conditions at the bypass entrance are recommended in order to avoid negatively influencing bypass use due to the experimental design [2].

The same fish were used repeatedly in the experiments, maintaining a minimum resting period of seven days between the participations in the experiments. Hence, a slight chance of learning effects due to trial and error, which could thus result in reduced fish activity, could exist [50]. We are convinced that these factors were not present in the study because the interaction with the hybrid barrier was not a profound enough experience to necessitate avoidance in future. No fish injuries resulted from the electric fields used in this application. During the experimental time of one hour and across the independent experiments, fish repeatedly approached the Flexible FishProtector and entered the electric field, showing the same flight reaction.

Due to the restricted dimensions of the 3.0 m-wide test flume, the direct transfer of fish behavior to real hydropower plants should be treated with caution. Nevertheless, the results suppose that the observed behavior patterns, which in the end determined the protection rate and the downstream migration success (with the use of an appropriate bypass system assumed), could also be observed at real sites. Further experiments at real sites are strongly recommended and should consider various flow velocities, fish species, and deeper water. Additionally, experiments should be carried out in low light conditions.

The video evaluation was an action-based and categorized evaluation of fish behavior in close range to the Flexible FishProtector. Although the experimental design allowed for detailed analysis, there were still certain limitations, such as species identification due to the large distance between the fish and camera and the level of water turbidity. Additionally, fish length could not be determined by video analyses. However, fish behavior was clearly observable for all experiments and was classified into the four categories. Experiments were performed exclusively during daytime because the video observation with cameras required sufficient lighting conditions. Due to the high number of individuals used in each experiment, it was not possible to gain information on the activity of a single individual. The number of fish that were not actively participating in an experiment could vary systematically across the experiments. However, the number of inactive fish was shown to be stable [24]. A robustness check concerning the varying number of graylings in the experiments, as provided in Tutzer et al. [24], supported the assumption that there was no effect due to the varying number of graylings.

Despite these limitations, the Flexible FishProtector is a promising solution for future improvement for fish protection at hydropower plants. The Flexible FishProtector technology can be adjusted to various sites and species by varying the cable clearance, exposition angle, and size and intensity of the electric field. Our first hypothesis was confirmed, behavior of fish was influenced by the interaction of both barrier types in such a way that fish protection was significantly improved. This could be explained by the mode of action of the physical barrier in combination with the electric field as a behavioral barrier. Fish approached the Flexible FishProtector cautiously and subsequently showed a flight reaction. In addition, fish were guided along the Flexible FishProtector in up- and downstream directions, which increased the chance of safe downstream migration. The narrower cable clearance enhanced guiding activities (hypothesis two).

5. Ethics

We confirm that the research meets the ethical guidelines and legal requirements under permission (LF1-TVG-54/001) from Lower Austria.

6. Patents

The University of Innsbruck, HyFish GmbH, Innsbruck, has a European patent registration (No. EP2839080B1) with the title "Cable screen for fish protection purposes" licensed.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/w14030378/s1: Video S1: Fish taking a positive rheotactic swimming position; Video S2: Flight reaction; Video S3: Fish guided along the Flexible FishProtector; Video S4: Bypass use; Video S5: Passage physical barrier (no electric field); Video S6: Passage hybrid barrier.

Author Contributions: Conceptualization, R.T., J.W. and B.Z.; methodology, R.T., B.Z., S.F. and J.W.; software, S.R., J.W., J.H. and R.T.; validation, M.A., G.U., B.Z. and J.W.; formal analysis, R.T. and B.Z.; investigation, R.T., J.H., S.F. and B.Z.; resources, M.A., B.B. and G.U.; data curation, R.T., S.R. and J.W.; writing—original draft preparation, R.T.; writing—review and editing, R.T., J.H., S.F., G.U., B.Z., J.W., S.R. and M.A.; visualization, R.T., S.R. and J.W.; supervision, M.A. and B.B.; project administration, M.A., B.Z., R.T. and B.B.; funding acquisition, M.A. and B.B. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: This study was supported by a grant from the Austrian Research Promotion Agency (FFG). In addition, the University of Innsbruck, HyFish GmbH, Innsbruck, has a European patent registration (No. EP2839080B1) with the title "Cable screen for fish protection purposes" licensed. This work has been reviewed and approved by the University of Innsbruck and the University of Natural Resources and Life Sciences, Vienna, in accordance with their policy on objectivity in research.

Appendix A

Table A1. Counted actions out of four defined categories (guiding, flight reaction, FishProtector passage, and bypass use) arranged by the 12 possible setups of evaluated 77 independent experiments (raw data of video evaluation).

Setup (*)	ID	Individuals	Guiding	Flight Reaction	FishProtector Passage	Bypass Use	Sum
40°/30 mm/no_field	V_12	30	95	0	4	25	124
40°/30 mm/no_field	V_13	30	88	0	13	25	126
40°/30 mm/no_field	V_17	30	208	0	14	51	273
40°/30 mm/no_field	V_19	30	117	0	5	37	159
40°/30 mm/no_field	V_20	30	95	0	2	33	130
40°/30 mm/no_field	V_57	45	77	0	23	17	117
40°/30 mm/no_field	V_59	45	23	0	0	1	24
40°/30 mm/small_field	V_14	30	153	11	1	54	219
40°/30 mm/small_field	V_15	30	118	5	3	174	300
40°/30 mm/small_field	V_16	30	113	15	5	105	238
40°/30 mm/small_field	V_18	30	44	8	0	2	54

Table A1. *Cont.*

Setup (*)	ID	Individuals	Guiding	Flight Reaction	FishProtector Passage	Bypass Use	Sum
40°/30 mm/small_field	V_21	30	133	3	3	32	171
40°/30 mm/small_field	V_56	45	34	5	2	58	99
40°/30 mm/small_field	V_58	45	97	23	3	15	138
40°/30 mm/small_field	V_60	45	11	13	0	1	25
40°/30 mm/small_field	V_61	45	99	11	0	96	206
40°/30 mm/large_field	V_10	30	121	27	5	116	269
40°/30 mm/large_field	V_11	30	113	29	0	23	165
40°/30 mm/large_field	V_22	45	112	29	3	4	148
40°/30 mm/large_field	V_23	45	14	3	0	4	21
40°/30 mm/large_field	V_24	45	81	3	1	16	101
40°/30 mm/large_field	V_55	30	8	8	1	2	19
40°/60 mm/no_field	V_27	45	54	0	74	17	145
40°/60 mm/no_field	V_28	45	21	0	19	3	43
40°/60 mm/no_field	V_30	45	64	0	15	0	79
40°/60 mm/no_field	V_34	45	25	0	33	5	63
40°/60 mm/no_field	V_35	45	24	0	72	40	136
40°/60 mm/no_field	V_36	45	12	0	25	9	46
40°/60 mm/small_field	V_25	45	37	26	6	10	79
40°/60 mm/small_field	V_26	45	19	26	5	0	50
40°/60 mm/small_field	V_29	45	18	5	0	0	23
40°/60 mm/small_field	V_37	45	8	3	1	3	15
40°/60 mm/small_field	V_38	45	28	8	4	54	94
40°/60mm/small_field	V_44	45	2	5	1	0	8
40°/60 mm/large_field	V_32	45	52	30	0	1	83
40°/60 mm/large_field	V_40	45	50	47	1	8	106
40°/60 mm/large_field	V_47	45	44	15	1	0	60
40°/60 mm/large_field	V_48	45	6	4	0	1	11
40°/60 mm/large_field	V_49	45	13	3	4	0	20
40°/60 mm/large_field	V_52	45	2	1	0	2	5
40°/60 mm/large_field	V_54	45	15	13	0	0	28
20°/60 mm/no_field	V_84	39	11	0	27	2	40
20°/60 mm/no_field	V_85	39	8	0	13	0	21
20°/60 mm/no_field	V_89	39	31	0	12	0	43
20°/60 mm/no_field	V_91	39	17	0	43	1	61
20°/60 mm/no_field	V_92	39	0	0	6	0	6
20°/60 mm/no_field	V_95	37	1	0	6	2	9
20°/60 mm/small_field	V_81	39	2	8	2	0	12
20°/60 mm/small_field	V_82	39	8	18	3	0	29
20°/60 mm/small_field	V_83	39	3	13	0	1	17
20°/60 mm/small_field	V_94	39	1	4	0	1	6
20°/60 mm/small_field	V_96	37	1	7	3	1	12
20°/60 mm/small_field	V_97	37	1	4	1	1	7
20°/60 mm/large_field	V_80	39	7	32	9	1	49
20°/60 mm/large_field	V_86	39	1	20	2	1	24
20°/60 mm/large_field	V_87	39	8	13	3	2	26
20°/60 mm/large_field	V_88	39	37	15	0	4	56
20°/60 mm/large_field	V_90	39	11	12	0	10	33
20°/60 mm/large_field	V_93	39	5	18	0	0	23
20°/30 mm/no_field	V_64	45	116	0	13	56	185
20°/30 mm/no_field	V_66	45	118	0	7	1	126
20°/30 mm/no_field	V_71	45	112	0	0	10	122
20°/30 mm/no_field	V_74	45	11	0	6	17	34
20°/30 mm/no_field	V_75	46	34	0	8	0	42
20°/30 mm/no_field	V_77	45	29	0	12	0	41
20°/30 mm/small_field	V_62	45	97	8	0	44	149
20°/30 mm/small_field	V_63	45	97	13	3	6	119
20°/30 mm/small_field	V_65	45	210	39	5	114	368
20°/30 mm/small_field	V_72	45	168	7	1	8	184
20°/30 mm/small_field	V_73	45	67	8	0	4	79
20°/30 mm/large_field	V_67	45	107	46	3	0	156
20°/30 mm/large_field	V_68	45	68	41	4	79	192
20°/30 mm/large_field	V_69	45	31	8	0	2	41
20°/30 mm/large_field	V_70	45	5	9	0	0	14
20°/30 mm/large_field	V_76	45	8	17	0	0	25
20°/30 mm/large_field	V_78	46	36	57	2	66	161
20°/30 mm/large_field	V_79	43	12	5	0	8	25

(*) Setup: exposition angle/cable clearance/electric field.

Table A2. Descriptive statistics for the counted activities in each of the four defined categories (guiding, flight reaction, FishProtector passage, and bypass use) divided by the number of fish in the experiment (response parameter) is provided.

Category	Response Parameter	Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
Fish guiding	fish guiding activities per fish	0	0.24	0.69	1.34	2.16	6.93
Flight reaction	flight reactions per fish	0	0.00	0.16	0.25	0.37	1.24
FishProtector passage	FishProtector passages per fish	0	0.00	0.07	0.17	0.17	1.64
Bypass use	bypass use activities per fish	0	0.02	0.07	0.53	0.77	5.80

Note: Min. denotes minimum, 1st Qu. denotes first quartile, 3rd Qu. denotes third quartile, and Max. denotes maximum.

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