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Impact of downstream passage through hydropower plants on the physiological and health status of a critically endangered species: The European eel *Anguilla anguilla*

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Abstract

Hydropower plants (HPPs) are a source of “green” energy but also a threat to migrating fish such as the European eel (*Anguilla anguilla*) owing to the disruption of river connectivity and the obstruction of downstream migration. The impact of HPP are well-documented in terms of fish survival and damages but there is no available information concerning the condition of surviving and unharmed fish. The aim of this study is to assess the impact of the passage through HPP on the survival, the physiological and health status of adult eels. Two trials were carried with variants of the Kaplan turbine - one of the most common types in Europe. After a deliberate passage through the turbines, we studied direct mortality, external and internal damages, stress and immune biomarkers such as plasma cortisol and glucose levels, alternative complement (ACH50), lysozyme and peroxidase activities, and total immunoglobulin (Ig) content. Our results showed a lower survival and a higher external and internal damages rates in the HPP groups. Glucose levels, ACH50, lysozyme and peroxidase activities and TIgc were also affected by the passage depending on HPP characteristics. Those
findings suggest a greater energy expenditure and a disruption in innate immunity due to this passage. HPPs can not only have an impact in terms of direct mortality and injuries but also affect the physiological and health condition of the surviving eels. This impact may explain the delayed mortality observed in telemetric studies and the passage through many HPPs may compromise the ability of adult eels to migrate successfully to the ocean.

**Keywords:** Hydropower plant, adult European eel, downstream migration, physiological and health status
1. Introduction

The European eel *Anguilla anguilla* L. is a panmictic species distributed across the majority of the coastal countries in Europe and North-Africa in the geographic area between Mauritania (30°N) and the Barents sea (72°N) and spanning the entire Mediterranean basin (International Council for the Exploration of the Sea Conseil ICES, 2018). Most adults of this long-lived semelparous species migrate downstream from the growing areas in freshwater to the Sargasso Sea to reproduce (Bruijs and Durif, 2009). Since the 1980s, the recruitment of European eels has strongly declined throughout its geographical distribution (Dekker, 2004, 2003; Stone, 2003). The species was deemed to be in critical danger of extinction from 2008 (Jacoby and Gollock, 2014). Multiple factors are suggested to explain this decline including diseases and anthropogenic factors such as pollution, fisheries, habitat loss and migration barriers (Dekker, 2004).

During their downstream migration, silver eels have to pass through many obstacles including flood-control dams, flood gates, weirs, hydropower plants (HPPs), sluices, and pumping stations. Those obstacles, abundant in many European rivers and inland waters, impede the free movement of eels and access to suitable habitats during their growth phase and prevent or delay silver eel downstream migration (Bruijs and Durif, 2009).

With the proliferation of hydroelectricity as “a green energy source”, hydropower plants (HPPs) are widely presents in many European riverine systems. Such barriers present problems through the disruption of the river ecological connectivity, the obstruction of downstream migration and risk for the survival of silver eels (Bruijs and Durif, 2009; Fullerton et al., 2010; Pringle, 2003). The impact of silver eel passage through HPPs was the main concern of several studies assessing the direct mortality by studying the deliberate passage through the turbine (Dainys et al., 2018; Pedersen et al., 2012) or the total mortality (including direct and delayed mortalities) thanks to telemetric surveys of silver eels during
their migration through different barriers including HPPs (Bernaś et al., 2017; Calles et al., 2013; Dębowski et al., 2016; Økland et al., 2019; Trancart et al., 2018). Eel mortality at different HPP areas varied from < 5% to > 70% depending on many factors including turbine’s type, the number of blades, the velocity, the water head height and fish size (Bernaś et al., 2017; Calles et al., 2013; Dainys et al., 2018; Dębowski et al., 2016; Økland et al., 2019; Pedersen et al., 2012; Trancart et al., 2018). Recent reviews and reports suggest a mortality of 10 – 20% in large Kaplan low-head turbines (Larinier, 2008), 20 – 38% in other Kaplan large turbines (Bruijs and Durif, 2009; Klopries and Schüttrumpf, 2020), an average mortality of 41% (International Council for the Exploration of the Sea ICES, 2019) and 50% – 100% in small turbines e.g. CINK turbines (Dainys et al., 2018; Larinier, 2008). The variation in mortality among sites is dependent upon characteristics such as turbine type, size, and velocity eel size and behaviour (Bruijs and Durif, 2009; International Council for the Exploration of the Sea ICES, 2019). During their passage through the turbine, eels face various types of stress such as strike and collision with parts of the HPP (mechanical trauma), sudden variation in speed or pressure (barotrauma), shear and cavitation stress (International Council for the Exploration of the Sea ICES, 2019). The type of injury and the aggravating factors of each form of stress have been compiled by Pracheil et al. (2016) (Figure 1).

Moreover, silver eels are confronted by many HPPs during their downstream migration. Larinier (2008) suggested that the cumulative mortality rate will increase drastically from 2 – 5% to 19 – 40% by passage through 10 HPPs and 33 – 74% by passage through 20 HPPs and may can therefore threaten the entire fish populations.

The impact of the passage through HPP has been fairly well studied in terms of mortality and injury types. However, to our knowledge, there is no available information about the physiological stress and immune status of surviving and unharmed fish. Our previous work on
another migrating fish species, Atlantic salmon *Salmo salar* smolts, showed an impact in terms of physiological and immune changes that may affect their ability to migrate to the sea (Ben Ammar et al., 2020). During their migration, silver eels cope with various pathogens, chemical pollutant presence in their tissues and digestive tract regression (Durif et al., 2009). Considering the aforementioned stressors, we hypothesised that, as in Atlantic salmon smolts, a disruption in silver eels’ physiological status and health condition during the passage through HPPs could lead to a great energy expenditure, an increased vulnerability of individuals to pathogens, an increased delayed mortality, and, therefore, compromise the success rate of the downstream migration.

The aim of this study was to study the impact of the passage through hydropower plant on the survival, the physiological and health status of adult eels by measuring various stress and immune biomarkers. Two trials were carried out in order to test the impact of two variants of the Kaplan type turbine – the most encountered type in Europe and in the Meuse River basin (Bruijs and Durif, 2009) – under comparable hydrographic conditions. We hypothesise that the passage of eels through turbines may lead to stress affecting the physiological condition and the immune system. Moreover, the impact of this passage can be aggravated by the characteristic of the turbine itself. This study can provide information to better understand the delayed mortality that occurs after the passage through the turbines.

### 2. Materials and Methods

#### 2.1. Animals and study sites

For the first experiment, 70 adult eels (mean total length 720 ± 31.7 mm) were purchased from Royal Danish fish (Hanstholm, Denmark) due to the unavailability of wild silver eels. Eels were acclimated into two 1-m³ round tanks (35 eels per tank) at Grands-Malades (GM) site (Luminus Grands Malades, 5100 Namur, Belgium, 50°27'57.2"N 4°53'54.8"E) on March
The tanks were completely covered by nets and continuously supplemented with water from the Meuse river at a flow rate of 70 L/min (Pump Tallas D-DWP 1000, DAB pumps, Qingao). The study took place on March 28, 2018. This site is equipped with straflo turbine – a lowhead variant of Kaplan-type turbine with a horizontal axis and the generator outside of the water channel (Davies, 1988), four blades, a rotational speed of 132 rpm and a head of 3.8 m (EDF Luminus, 2015). This turbine model is one of the commonly used in European rivers (Bruijs and Durif, 2009) and is also present in two other sites of the Belgian Meuse River Andenne and Lixhe currently being evaluated by our Life European project (EC-Life project - LIFE4FISH).

For the second experiment, 120 adult eels with the same class size (mean total length 682.4 ± 50.4 mm) as in the first experiment were purchased from Nijvis eel farm (Bergeijk, Netherlands). Eels were acclimated into three covered 1-m³ round tanks (40 eels per tank) continuously alimented with water from the Meuse river at Andenne (And) site (Luminus Andenne, Anton Roadway 114-144, 5300 Andenne, Belgium, 50°29'30.3"N 5°04'11.9"E) on April 15, 2019. The experimentation itself took place on April 17, 2019. This site was recently equipped with two bulb turbine – another variant of Kaplan-type turbine with a horizontal axis (Davies, 1988), four blades, a rotational speed of 176.47 rpm and a head of 5.35 m (EDF Luminus, 2015). This model is often used on large rivers (Thorstad et al., 2012) due to its improved hydropower production efficiency and large broad operating range. Moreover, several other hydropower sites in the Meuse River are also equipped with the bulb turbine model.

2.2. Experimental protocol and sampling procedures

On day 0, the simulation of the eel passage through the turbine was conducted according to Profish Technology method as described in Ben Ammar et al. (2020). This method is a
validated method in many European countries (Schmalz et al., 2015) commonly used to assess the impact of fish passage through the turbine in situ (Brackley et al., 2018; Kibel and Coe, 2007). Eels were caught (Figure 2-A), transported in a 100-L square tank and gently released from a bucket of water under continuous water flow through a wetted plastic pipe (Figure 2-B, 1) 20 cm in diameter. The pipe exited either into the turbine intake itself (HPP group, Figure 2-C, 2) or into the net for control group (Figure 2-D). For each experiment, we performed two injections into the turbine intake and one injection into the net in order to decrease the risk linked to the injection through the turbine failure. For GM site, two groups of 20 eels were injected through the turbine and one group of 22 eels were injected directly into the net. For Andenne site, control group consisted of 36 eels and the two HPP groups consisted of 37 eels each. During the deliberate passage through the turbine, both the straflo and bulb turbines were set at their maximum intake capacity (170 and 166 m³/s, respectively) coupled with injection at the border of blades which is considered as the scenario leading to the lowest survival rate in high water flow conditions. This scenario is also the closest to the real operating conditions. Then, fish were recovered using a 50 meters’ length net fixed on a 2000 kg metallic frame handled by a crane (Figure 2-E, 3).

Immediately after the recovery, fish were sorted into three groups:

- **Group 1**: dead and heavily injured fish that were examined to determine the causes of death. The heavily injured fish were euthanized using MS222 (240 mg/L).

- **Group 2**: surviving fish with non-life-threatening external injuries that were weighed and measured and examined in order to determine the injuries nature and severity.

- **Group 3**: surviving fish without any visible injuries.

The fish from groups 2 and 3 were then returned to their corresponding tanks.

The recovery, survival and external damage rates were calculated after retrieving the net as follows:
- **Recovery rate (%)** = \( \frac{\text{Number of recovered fish} \times 100}{\text{Number of injected fish}} \)

- **Survival rate (%)** = \( \frac{\text{Number of surviving fish} \times 100}{\text{Number of injected fish}} \). Previous data from the same experiment in another site showed 100% recovery rate after injection of anesthetized fish. Thus, assumption was made that the non-recovered fish succeeded in escaping the turbine and were considered alive.

- **External damage rate (%)** = \( \frac{\text{Number of surviving and damaged fish} \times 100}{\text{Number of recovered and surviving fish}} \)

The severity of external damage was assessed post hoc from the photographs taken during the experiment based on Girard and Elie (2007) injury classification (Annex1) fully described in De Oliveira et al. (2018). The damages were considered non-life-threatening if the fish survived and displayed normal swimming behaviour in the two hours after the recovery. Bruises and scraping were considered minor if the size is less than 10% of fish body per side. Haemorrhaged eye was considered minor if less than 50% while operculum tear was considered minor if less than 5%. Cuts, lacerations, operculum folded under or torn off, bulged eyes and deformed pupils were all considered major injuries.

After anaesthesia with MS222 (120 mg/L), 10 fish were sampled from control and HPP groups for blood at two times: 24 h post-injection (24 h pi) and 144 h post-injection (144 h pi) in order to investigate the response of fish in the short and mid-term. At 144 h pi, X-rays were performed on 12 eels from each group and analysed by a veterinarian to assess the internal injuries (Figure 3). Internal injuries were classified from A and B for minor injuries such as reduction of less than 5 inter-vertebrate spaces (slight internal damages) to C and D such as multiple injuries with a fracture (severe internal damages).

Blood was sampled using heparinised syringes for plasma and non-heparinised syringes for serum and stored on ice. After the sampling, blood was allowed to clot at 4 °C prior to centrifugation (5000 g, 5 min) and aliquoted serum was then stored at -80 °C until analysis.
For plasma, blood was directly centrifuged and stored as above. All experiments were carried out in accordance with the International Guiding Principles for Biomedical Research Involving Animals (EU Directive 2010/63/EU for animal experiments).

### 2.3. Stress indicators

Cortisol was assayed in plasma samples in duplicate using a competitive cortisol ELISA kit (KAPDB270, Diasource, Belgium). Plasma samples were diluted two times when needed. The assay dynamic range was between 0 and 600 ng/mL and the intra-assay coefficient of variation and the analytical sensitivity were 5.8 %, and 4 ng/mL respectively.

Plasma glucose was determined calorimetrically based on a glucose oxidase/peroxidase method described by Trinder (1969) and was performed in triplicate in plasma samples. After a deproteinization step using perchloric acid (0.33 M), the samples and standards were centrifuged 10 min at 850 g (Centrifuge 5424, Eppendorf, Belgium). In flat-bottomed 96-well plate, 10 µL of each sample and standard were mixed with a glucose oxidase/peroxidase reactional solution (glucose oxidase type X-S, peroxidase type 1, ABTS, phosphate buffer 0.1 M, pH 7.5) and incubated during 15 min at 38 °C. Then, the absorbance was measured at 436 nm using the 96-well plate reader (FLUOstar® Omega, BMG LABTECH, Germany).

### 2.4. Humoral immune parameters

The plasma alternative complement pathway (ACH50) procedure was performed in duplicate in plasma samples to measure the haemolytic activity using rabbit red blood cells (RRBC) as targets (Cornet et al., 2018). A serial dilution from 1/50 to 1/25600 into veronal buffer (IDVert, France) was performed in a round-bottomed 96-well plate. Then, 10 µL of RRBC (Biomerieux) suspension (3 % in veronal buffer) was added to each well and the plate incubated at 25 °C for 120 min at 300 rpm using the orbital shaker (KS 4000 ic control, IKA®).
Werke GmbH & Co. KG, Germany). The total haemolysis was obtained by mixing 10 µl of RRBC lysed with bi-distilled water and the spontaneous haemolysis was obtained by adding veronal buffer to 10 µl of RRBC (total volume = 70 µL). After the incubation, the turbidity (inversely proportional to the haemolysis) was measured using the 96-well plate reader (FLUOstar® Omega, BMG LABTECH, Germany) at 650 nm. The ACH50 value is the reciprocal of the plasma dilution which induces the haemolysis of 50 % of the rabbit red blood cells.

The lysozyme activity protocol was adapted from Douxfils et al., (2012). In flat-bottom 96-well plates, samples were assayed in triplicate by mixing 17 µL of serum with 120 µL of lyophilized *Micrococcus lysodeikticus* (Sigma) suspension at 0.6 g/L in phosphate buffer (Na$_2$HPO$_4$, 0.05 M, pH 6.2). A negative control (phosphate buffer) and a positive control (*M. lysodeikticus*) were also assayed in triplicate in the same plate (total volume = 137 µL). The absorbance (OD) at 450 nm was monitored between 0 min and 15 min (linearity range) using the 96 well-plate reader. Lysozyme activity (units) represents the amount of enzyme decreasing the turbidity by 0.001 OD per min. Data are expressed in U/mL.

The total peroxidase activity in plasma was assessed according to Quade and Roth, (1997) as fully described in Ben Ammar et al. (2020). The samples and negative control (water) were assayed in triplicate. In flat-bottomed 96-well plate, the plasma was mixed with 25 µL of reactional solution (20mM 3,3′,5,5′-tetramethylbenzidine hydrochloride and 5mM H$_2$O$_2$) and the reaction was stopped after 2 min by adding 50 µL of 2M sulphuric acid. Then, the absorbance was measured at 450 nm and one unit (U) of peroxidase activity was defined as the amount producing an absorbance change of 1 OD.

The measurement method of total immunoglobulin (Total Ig) concentration in serum was adapted from Milla et al. (2010). Serum was mixed with an equal volume of 12 % of polyethylene glycol 10000 kDA (PEG, Sigma), vortexed, and incubated under constant
shaking for 2h at room temperature. After centrifugation at 1000 g for 10 min, the supernatant was collected and assayed using Bradford method for its protein concentration. As immunoglobulins are precipitated under PEG action, the total Ig concentration was calculated by subtracting this value from the total protein concentration in the serum before precipitation.

2.5. Statistical analyses

Data were analysed using the free software R version 3.6.2 (R Core team, 2019). For all the dependent variables, homogeneity of variances was tested using Levene test (leveneTest, package “car”, Fox et al., 2014). Data were analysed using a linear mixed model (lm, package “lme4”, Bates et al., 2014) with the treatment and the sampling time as fixed effects: \( \text{model} = \text{lm} \ (Y \sim \text{treatment*sampling time}) \) with \( Y \): dependent variable such as cortisol and glucose levels. Outliers were assessed using Cook's distances test (cooks.distance, package “stats”, R Core team, 2019) and Bonferroni outlier test (outlierTest, package “car”, Fox et al., 2014) to detect anomalies that differ greatly from the majority of data. For the model validation, residuals were tested for homogeneity and normality using residuals vs fitted values and sample vs theoretical quantiles (Q-Q) plots, respectively (plotresid, package “RVAideMemoire”, Hervé, 2015). If necessary, data were log-transformed. When the model was validated, an ANOVA table was performed to calculate F-tests (ANOVA, package “car”, Fox et al., 2014) followed by estimated marginal means comparisons as a post hoc test (emmeans, package “emmeans”, Lenth et al., 2019). The level of significance used in all tests was \( p < 0.05 \).

3. Results

3.1. Fish recovery, survival, external and internal damages
The recovery rates in control groups were 90 % and 100 % in the Grands-Malades (GM) and Andenne experiments, respectively. More losses were observed in fish injected through the turbines, with a recovery rate of 93 and 68 % in GM and Andenne, respectively (Figure 4). In both experiments, the survival rate was higher (100 % of the recovered fish) in control groups compared to HPP groups. The survival rate was higher in the HPP group during the GM experiment (92 %) compared to the Andenne experiment (80 %). The external damages were more frequent in the GM experiment (31 %).

The main cause of mortality was body severance (11 out of 16 at Andenne and 2 out of 3 at GM) followed by body part’s crushing (3 out of 16 at Andenne and 1 out of 3 at GM) and large lacerations (3 out of 16 at Andenne). For two dead eels at the Andenne site, large hematomas (more than the third of the body), associated or not to laceration, were observed. Further necropsy showed a fractured vertebral column in one and internal haemorrhage in the second one.

The main external injury types observed in both sites were bruising and hematoma – often in association (7 out of 10 eels at GM and 3 out of 6 eels at Andenne). Lacerations – associated or not with hematoma – also occurred in eels (3 out of 6 at Andenne and 2 out of 10 at GM).

In the GM experiment, both control and HPP groups showed 25 % of severe internal damages including fracture, deviation and reduction of several intervertebral spaces (Figure 5-A). Those damages were generally associated to a bone sclerosis (observed in 7 fish out of 14) and/or vertebrae compaction (observed in 7 fish out of 14). The number of fishes that did not show any internal damages was slightly higher in the control group (67 %) than in the HPP group (58 %).

In the Andenne experiment, no internal damages were observed in the control group, while only 57 % of eels did not show any internal damages in the HPP group (Figure 5-B). In the latest group, 26 % of eels displayed severe internal damages including fracture (observed in 3
fish out of 10) associated to bone sclerosis (6 fish out of 10) and vertebrae compaction (5 fish out of 10).

3.2. Stress response

In both experiments, plasma cortisol levels only varied over time (p<0.01, Figure 6-A, B) and decreased significantly at 144 h post-injection (pi) from 177.9 ± 60.4 ng/mL to 68.5 ± 22.7 ng/mL at GM and from 125.1 ± 76.8 ng/mL to 78.2 ± 70.4 ng/mL at Andenne. Those levels showed greater variability in eels confronted to the passage through the turbine in Andenne (CV varied from 61.4 at 24 h pi to 89.9 % at 144 h pi in Andenne and were about 34 % for both sampling times for the GM site).

At the GM site, plasma glucose levels varied significantly depending on the sampling time (p<0.001) and treatment (p<0.05, Figure 6-C, D). In both groups, glucose levels were higher (p<0.001) 24 h pi (0.51 ± 0.14 mg/mL) than 144 h pi (0.35 ± 0.15 mg/mL). Moreover, they were always higher (p=0.032) in the HPP group (0.49 ± 0.19 mg/mL) compared to the control group (0.37 ± 0.11 mg/mL). In Andenne, plasma glucose levels varied depending on the interaction between the treatment and the sampling time (p<0.01). At 24 h pi, both groups showed similar values (1.18 ± 0.33 mg/mL and 1.06 ± 0.29 mg/mL for control and HPP group respectively). Glucose levels decreased significantly over the time in both groups (p<0.001) with lower (p=0.038) glucose levels at 144 h pi in control group (0.22 ± 0.12 mg/mL) compared to the HPP group (0.54 ± 0.25 mg/mL). Glucose levels at 24 h pi were twice as high in Andenne (1.15 ± 0.37 mg/mL) than in GM (0.51 ± 0.14 mg/mL).

3.3. Humoral immune parameters

At the GM site, plasma ACH50 levels varied significantly depending on the sampling time (p<0.001, Figure 7-A) while in Andenne, no significant variation was observed (Figure 7-B).
At GM, ACH50 values were higher at 24 h pi (4276.2 ± 710.2) compared to 144 h pi (3360.1 ± 619.7) in both groups.

Serum lysozyme activity varied depending on the interaction between the sampling time and the treatment in both sites (p<0.05). At GM (Figure 7-C), lysozyme activity decreased only in HPP group between 24 h (1498.7 ± 331.3 U/mL) and 144 h pi (901.7 ± 255.6 U/mL, p<0.001) and remained stable in the control group (1266.7 ± 203 and 1159.1 ± 352.2 U/mL for 24h and 144h pi respectively). At Andenne (Figure 7-D), the highest lysozyme activity was observed in the control group at 24 h pi (1747.1 ± 244.1 U/mL, p<0.001) while the other showed quite similar values (between 804.7 ± 327.5 U/mL for control group at 144 h pi and 1027.1 ± 448.1 U/mL for HPP group at 144h pi).

Values for plasma peroxidase activity did not significantly differ between treatments or over time at GM (Figure 8-A). However, they varied significantly depending on the interaction between the sampling time and the treatment at Andenne (p<0.05, Figure 8-B). The control group at 24 h pi showed the highest peroxidase activity (168.5 ± 79.9 U/mL) while the other values were quite similar (from 119.1 ± 16.6 U/mL for control group at 144 h pi to 137.8 ± 62.8 U/mL for HPP group at 144 h pi).

Serum total immunoglobulin content (total Ig) varied depending on the interaction between the sampling time and the treatment at GM (p<0.001, Figure 8-C). Total Ig values increased from 2.2 ± 0.7 mg/mL to 5.9 ± 1.4 mg/mL throughout the time in control group (p<0.001) while Ig content remained stable in HPP group (3.6 – 3.8 mg/mL). Therefore, at 24 h pi, control group displayed lower values compared to HPP one (3.8 ± 1 mg/mL) while it showed a higher value at 144 h pi (3.6 ± 0.9 mg/mL). At Andenne, total Ig content varied only depending on the treatment (p<0.05, Figure 8-D) with higher value in HPP group (6.6 ± 1.4 mg/mL) compared to control group at both sampling times (5.6 ± 1.6 mg/mL).
4. Discussion

4.1. Survival rate, external and internal damages

In both sites, the survival rate of the eels after the passage through the turbine was above 80 %
and 100 % for the control group (Figure 4). The survival rate in HPP group was consistent
with previous data for large Kaplan turbines (Bruijs and Durif, 2009; Dainys et al., 2018;
Larinier, 2008; Økland et al., 2019; Pracheil et al., 2016; Trancart et al., 2018). However, the
results of this study showed that the effects on survival can vary according to variants of this
type of Kaplan turbine. Indeed, the survival rate at Grands-Malades Straflo turbine was higher
(92 %) than for the bulb turbine (80 %) at the Andenne site mainly due to the water head. In
fact, Larinier (2008) suggested a lesser impact (between 10 and 20 % of mortality) on survival
in large low-head turbines compared to the other turbines from the same category (between 20
and 38 %). In their review, Pracheil et al. (2016) suggested that turbine type was one
important aggravating factor for mortality and injury caused by mechanical and shear stress as
well as barotrauma. The main injuries inducing mortality were body severance and body part
flushing in both sites. Those type of injuries are mainly due to strikes with mobile and non-
mobile parts of the HPP (Pracheil et al., 2016). Moreover, fish size is considered as an
aggravating factor because it increases the risk for the individuals to be subjected to blade
strike or other mechanical wounding (Coutant and Whitney, 2000; Pracheil et al., 2016).
Because eel size was comparable in the two trials of the current study, the lower survival
observed at Andenne compared to GM would be mainly related to the HPP characteristics.

After the passage through the turbine, 25 % of the injected eels at the GM site showed
external damages while only 8 % displayed external injuries at the Andenne site (Figure 5). In
both sites, no external damages were recorded in the control group. The main types of injuries
observed at both sites were bruising associated with hematoma, mainly caused by strike with
parts of the HPP (Pracheil et al., 2016). The difference in the external damages rate between
the two sites can be related to the fact that we observed more life-threatening injuries such as tail severance and holes in fish head at Andenne leading to death in the two hours after eel recovery. This increased the direct mortality rate and decreased the external damages rate in comparison to GM. Differences in survival rates between the two sites can be explained by the difference in water head between the sites. In fact, the water head in Andenne is higher (5.35 m) than in Grands-Malades (3.8 m) site. In the literature, water head is considered as an aggravating factor which can lead to more severe injuries (Larinier, 2008; Larinier and Travade, 1998).

Surprisingly, at the GM site, internal damages rate for severe injuries was quite similar between the control and HPP group while half of the eel showed slight internal damages in control group compared to HPP group. A great prevalence of bone sclerosis or osteosclerosis – defined as “the increase of compact bone in the medullary region in place of spongy bone and/or the filling in the medullary cavity resulting in an increased density” (Gray et al., 2007) – was observed in both groups. This skeletal deficiency may increase the risk of fracture during handling or shock. Skeletal deficiencies have been described in many cultured fish species and can be due to nutritional imbalances, water temperature, heavy metal contamination or hydrodynamic conditions (Afonso et al., 2000; Bengtsson, 1979; Berntssen et al., 2003; Brown et al., 2010; Divanach et al., 1997; Roberts and Rodger, 2012; Sassi et al., 2010). The presence of osteosclerosis in both control and HPP groups at GM can explain the severe internal damages rate in both groups while the slight internal damages rates represented only the half in control group compared to HPP one. This can also explain why the control group results differ between the two sites. In fact, no internal damages were observed in the control group at the Andenne site while the HPP group displayed internal damage rates quite similar to those recorded in the HPP group of the GM site.
4.2. Changes in stress status

Physiological stress status is usually evaluated by assaying plasma cortisol and glucose levels (Bonga, 1997; Faught et al., 2016; Mommsen et al., 1999; Sopinka et al., 2016). Changes in circulating glucose levels are triggered by the production and release of cortisol from the interrenal gland to help animals survive and recover from the physiological challenges (Bonga, 1997; Sopinka et al., 2016). This secondary metabolic response to stress is involved in maintaining sustainable glucose levels in order to prevent hypoglycaemia and exhaustion (Soengas et al., 1992; Sopinka et al., 2016; Specker, 1982; Van Der Boon et al., 1991). The passage through the turbine triggered an initial rise in plasma glucose levels followed by a decrease during the recovery period (between 24 h and 144 h pi, Figure 6-C and 6-D). At GM, the HPP group showed higher glucose levels at both sampling times suggesting a rapid mobilization of mechanisms such as protein catabolism and gluconeogenesis to sustain plasma glucose levels. This can be linked to the higher cortisol levels observed at the GM compared to Andenne site at 24 h post-injection (pi). In the latter site, glucose levels were high and similar in both groups at 24 h but decreased following two different slopes at 144 h pi with a sharper decrease in the control group. The decrease of glucose levels at both sites at 144 h pi closely followed the observed decrease in cortisol levels. In fact, cortisol clearance seems to have triggered a decrease in glucose levels. It is important to observe that even with cortisol clearance and drop, glucose levels remained higher in HPP for both sites at 144 h pi compared to the control group, suggesting the maintenance of mechanisms preventing hypoglycaemia and exhaustion and providing a better understanding of the stress status of fish. In fact, some critics were raised concerning the usefulness of cortisol as stress indicator because its plasma levels can drop to resting levels even if the fish may still be responding to the stressor (Mommsen et al., 1999). Moreover, glucose levels were two-fold higher at 24 h pi in the Andenne experiment compared to the GM experiment. This also can be explained by
the impact of a higher water head on the fish condition. As the Andenne site has a higher water head, the eels may have faced a more intense stress leading to higher levels of glucose. This may highlight the importance of the stress encountered when eels passed through the turbine and the importance of turbine characteristics on the severity of this stress.

Plasma cortisol levels measured in both control and HPP groups were quite similar. However, in both sites, plasma cortisol levels decreased from 125-178 ng/mL at 24 h pi to 68-78 ng/mL at 144 h pi (Figure 6-A and 6-B). Cortisol levels observed at 24 h pi are relatively close to those found in stressed eels while the levels observed at 144 h pi are only slightly higher than those found in non-stressed eels (Teles et al., 2004; Van Ginneken, 2006). As previously observed in Atlantic salmon *Salmo salar*, it seems that eel handling, their passage through the wetted flexible tube and their recovery with the net were already enough to trigger an increase in circulating cortisol levels (Ben Amar et al., 2020). This increase of cortisol levels found in stressed eels due to the experiment itself (Teles et al., 2004) seems to potentially overshadow the impact of the passage through the turbine. It is also important to note that the absence of clearly elevated cortisol levels does not always mean the absence of stressors (Bonga, 1997). After 144 h, cortisol levels decreased and eels displayed values close to those found in non-stressed eels (Teles et al., 2004; Van Ginneken et al., 2002). It is well known that circulating glucocorticoid levels respond rapidly to acute stress and those changes can provide useful information on the impact of specific *stimuli*. Moreover, the return to normal levels can start one hour after the stimulus as the clearance is non-stressor dependent (Bonga, 1997; Mommsen et al., 1999; Sopinka et al., 2016).

4.3. Disruption in immune response

Changes in immune response was evaluated with commonly used immune parameters such as plasma alternative haemolytic complement (ACH50) and peroxidase activities, serum
lysozyme activity and serum total immunoglobulin content. Plasma ACH50 values decreased between 24 h and 144 h pi at GM while no significant differences were found at Andenne (Figure 7-A and 7-B). However, a trend of decrease induced by the treatment was found in the latter site despite the great inter-individual variability observed in the control group (CV range 31 – 34 %) and HPP group at 24 h pi (CV = 30 %). This decrease in ACH50 activity in Andenne may be related to the high stress response observed at 24h. It is well known that acute stress can over a short time lead to the activation of some immune functions enhancing, then, the innate response and leukocyte mobilization (Bonga, 1997; Nardocci et al., 2014; Tort, 2011). This transient immunostimulation due to the stress is mediated by the catecholamines and/or cortisol which are involved in the primary metabolic response to stress (Bonga, 1997; Tort, 2011).

Lysozyme is an important defence molecule in the fish innate system involved in a large range of defence mechanisms such as bacteriolysis, opsonisation, immune response potentiation (Saurabh and Sahoo, 2008). Changes in immune function features such as lysozyme activity are considered part of the secondary responses to stress (Barton, 2002). In our study, lysozyme activity varied in both sites depending on the interaction between the treatment and the time highlighting an effect of the passage through the turbine on this immune biomarker. In GM, a sharp decrease of lysozyme activity was observed only in the HPP group at 144 h while in Andenne, lysozyme activity was lower at 24 h in the HPP group (Figure 7-C and 7-D). Those results suggest a reduction of lysozyme activity due to the stress caused by the passage through the turbine. Strong or chronic stressors such as long-duration handling, acute contamination or abrupt variations of light environment can decrease the lysozyme activity significantly in stressed fish (Caruso et al., 2002; Möck and Peters, 1990). On the other hand, less stressful situation such as short-duration handling can either increase or decrease this activity (Demers and Bayne, 1997; Möck and Peters, 1990). In both sites, the
control group as assessed by the glucose levels faced a less stressful situation than the HPP group resulting in high lysozyme levels. Those levels decreased afterwards in the control group in the Andenne site to reach similar values than the HPP group. The HPP group, however, facing a more stressful condition, experienced a decrease in the lysozyme activity in the GM site at 144 h pi or showed lower values earlier in And site.

Peroxidase is involved in the protection against reactive oxygen species (ROS) damages and is considered as one of the first lines of defence of fish against microorganisms as well as a biomarker of oxidative stress (Bonga, 1997; Di Giulio et al., 1989; Di Giulio and Meyer, 2008; Martínez-Álvarez et al., 2005). Oxidative stress can be related to many biotic and abiotic factors including xenobiotics and environmental stress (Martínez-Álvarez et al., 2005) and can be a cost of a strenuous energy expenditure such as migration (Sopinka et al., 2016).

Peroxidase activity did not show any significant variation in GM but was higher in the control group at 24 h after the injection before decreasing to the same levels than the HPP group in Andenne (Figure 8-A and 8-B). The lower peroxidase activity observed in the HPP group at 24 h after a stressful situation can be due to a rapid immune depression due to the stress caused by the passage through the turbine (Bonga, 1997). In fact, the inhibition of the immune function due to the stress is pretty well documented and considered as part of the secondary and adaptive responses (Barton, 2002; Bonga, 1997).

Stressors can act on fish and affect many functions including antibody production as part of the secondary responses to a stress in fish (Barton, 2002; Tort, 2011). High cortisol levels are generally associated with a reduction of circulating lymphocyte levels and antibody production (Bonga, 1997; Tort, 2011). At 24 h pi, both groups showed high cortisol levels in both sites (Figure 8-C and 8-D). Under those levels, control group at GM site showed its lowest total immunoglobulin content, slightly lower than the HPP group. At 144 h pi, total Ig content increased in the control group but remained at the same level in the HPP group. Those
findings suggest a longer immune suppressive action on antibody production due to the stress in the HPP group as observed in many studies (for review Bonga, 1997; Tort, 2011).

5. Conclusions

The results show that mortality and damages are generally more severe when eels encounter the HPP, especially in the case of high-head turbines. Migration barriers as well as HPP result in a high stress response in terms of cortisol and glucose induction. However, the passage through the turbine can trigger a higher energy consumption and a partial achievement of the recovery process after the challenge. Finally, the stress due to the passage through the turbine and/or the confrontation to a migration barrier seems to have an inhibitory action on some immune innate and specific functions. Those results are in concordance with the previous findings reporting a suppressive action of acute stress response on the fish immune and health status. Those findings may partially explain the delayed mortality observed in several telemetric studies. Moreover, the cumulative impact of the passage through many HPPs may in fine compromise the ability of adult eels to escape successfully to the ocean.

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Figures captions

Figure 1: Types of injuries, known and potential aggravating factors among the physical forces that causes injury and mortality during downstream migrating fish passage through turbines from Pracheil et al. (2016)

Figure 2: Eel injection process. Eels were caught (A), transported and injected (B) into a wetted flexible tube (1). Then, the tube leads them in front of the turbine (2) allowing them to pass through it (C) during 10 min (HPP group). Another group (control group) was injected using the same tube directly into the net (3) to mimic a safe passage (D). After each injection, eels were recovered using the net (E) and sorted into three groups depending on their state. Arrows: water flow direction

Figure 3: Different type of internal injuries depending on their severity. From the slightest to the more severe injury, A: reduction of intervertebral (IV) spaces, B: reduction of IV spaces associated to a vertebrate compaction, C: reduction of IV spaces associated to a vertebrate compaction and a bone sclerosis, D: reduction of Iv spaces associated to vertebrate compaction, bone sclerosis and spine deviation leading to a fracture

Figure 4: Recovery, survival and external damages rates after the European eel passage through the turbines at Grands-Malades (A) and Andenne (B) in control and HPP groups

Figure 5: Internal damages rates observed by radiography after the European eel passage through the turbines at Grands-Malades (A) and Andenne (B) in control and HPP groups

Figure 5: Changes in plasma cortisol (A, B) and glucose (C, D) levels in European eels after the passage through the turbines at Grands-Malades (A, C) and Andenne (B, D) in control (white) and HPP (red) groups. The horizontal line in the boxplot represents the median. Triangles represent the mean. Different capital letters indicate significant differences due to the interaction between sampling time and treatment, lower-case letters indicate significant differences among the sampling times, and asterisks indicate differences between groups for one given sampling time (p<0.05)
Figure 6: Changes in plasma ACH50 (A, B) and seric lysozyme (C, D) activities in European eels after the passage through the turbines at Grands-Malades (A, C) and Andenne (B, D) in control (white) and HPP (red) groups. The horizontal line in the boxplot represents the median. Triangles represent the mean. Different capital letters indicate significant differences due to the interaction between sampling time and treatment and lower-case letters indicate significant differences among the sampling times (p<0.05).

Figure 7: Changes in plasma peroxidase activity (A, B) and serum total immunoglobulin content (C, D) in European eels after the passage through the turbines at Grands-Malades (A, C) and Andenne (B, D) in control (white) and HPP (red) groups. The horizontal line in the boxplot represents the median. Triangles represent the mean. Different capital letters indicate significant differences due to the interaction between sampling time and treatment and asterisks indicate differences between groups for one given sampling time (p<0.05).
Highlights

- Hydropower plants are considered as a threat to downstream migrating silver eels
- Eels passage through the turbine affected survival, and caused internal and external damages
- This passage affected energy expenditure and innate immunity
- This impact may explain the delayed mortality observed in migrating eels
- The cumulative impact may compromise the ability of eel to escape to the ocean
Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:
Shear / Turbulence

- Types of injuries:
  - Descaling
  - Loss of epithelium
  - Loss of mucus layers
  - Disorientation
  - Increased predation
  - Elongation
  - Compression
  - Torsion
  - Rotation
  - Deformation

- Factors affecting injury severity:
  - Fish length
  - Fish morphology
  - Fish life history stage

- Factors that might affect injury severity:
  - Turbine type

Cavitation

- Type of injuries:
  - Unknown

- Factors affecting injury severity:
  - Turbine tube aeration

- Factors that might affect injury severity:
  - Very little is known about effects of cavitation on fish because they are difficult to isolate. Although engineers work to minimize cavitation, this is accomplished through building materials that would not necessarily minimize cavitation for fish.

Pressure

- Types of injuries:
  - Stomach eversion
  - Exophthalmia
  - Swim bladder rupture
  - Embolism
  - Haemorrhage

- Factors affecting injury severity:
  - Pressure change ratio
  - Pressure change ratio
  - Swim bladder morphology
  - Acclimation depth

- Factors that might affect injury severity:
  - Fish length
  - Fish life history stage
  - Fish morphology
  - Turbine type

Blade strike / Mechanical wounding

- Type of injuries:
  - Bruising
  - Descaling
  - Laceration
  - Haemorrhage
  - Amputation
  - Decapitation

- Factors affecting injury severity:
  - Fish length
  - Fish behaviour
  - Turbine type
  - Turbine revolution speed
  - Blade configuration
  - Number of blades
  - Blade shape
  - Blade spacing

- Factors that might affect injury severity:
  - Fish morphology

Figure 1
Figure 3

Slight internal injuries

A

B

Severe internal injuries

C

D

Figure 3
Figure 4

A  GM control group

- 100%
- 0%

B  And control group

- 90%
- 10%
- 0%

A  GM HPP group

- 60%
- 25%
- 8%
- 7%

B  And HPP group

- 40%
- 32%
- 20%
- 8%
- Dead

Legend:
- Loss
- Alive without damages
- Alive with external damages
- Dead
Figure 6

(A) Cortisol ng/mL over 24h pi and 144h pi with treatment a and b.

(B) Cortisol ng/mL over 24h pi and 144h pi with treatment a and b.

(C) Glucose mg/mL over 24h pi and 144h pi with treatment a and b.

(D) Glucose mg/mL over 24h pi and 144h pi with treatment A, B, and C.