Passage through a hydropower plant affects the physiological and health status of Atlantic salmon smolts

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How the passage through a hydropower plant affects the physiological and health status of Atlantic salmon smolts?

--Manuscript Draft--

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	Damien Sonny
	Syaghalirwa N.M. Mandiki
	Patrick Kestemont
Abstract:	Atlantic salmon is an anadromous species migrating from upper-reach nursery areas in rivers to the oceanic feeding areas at smolt stage and inversely at adult stage requiring unimpeded migration routes. However, dams associated with hydroelectric power plants (HPP) disrupt river connectivity and affect fish movement and survival. The objective of the current study was to evaluate the short and mid-term physiological and immune response of Atlantic salmon smolts after passing through Andenne HPP (Meuse River, Belgium). Several parameters were studied after an in situ deliberate passage including direct mortality and external damages, stress and immune biomarkers as plasma cortisol and glucose levels, complement and peroxidase activities, and immune and oxidative stress related gene expression 24 h, 72 h and 120 h after passage. Survival rate was lower and external damages were more important in fish that confronted the HPP compared to the control ones. Moreover, the passage through the turbine affected plasma glucose levels, complement and peroxidase activities and the expression of some immune genes such as lysg , igm and mpo in a timely manner suggesting that this passage can lead to a great energy expenditure and a disruption of innate immunity. Our observations can partially explain the delayed mortality observed in many studies leading to a poor success of restocking programs. HPPs not only have a direct impact in terms of mortalities and injuries but also an indirect one in terms of physiological and immune changes that can compromise Atlantic salmon smolts ability to escape successfully to the ocean.
Suggested Reviewers:	Pierre Sagnes Agence Francaise pour la Biodiversite pierre.sagnes@afbiodiversite.fr Pierre Sagnes works on river connectivity disruption and its impact on the biodiversity in France
	Johan Coeck Research Institute for Nature and Forest johan.coeck@inbo.be Johan Coeck is a well known scientist working on river and fish species management with a focus on the impact of anthropogenic activities on migrating fish
	Harriet Bakker Rijkswaterstaat

	harriet.bakker@rws.nl Harriet Bakker's work focus on downstream migration especially in Atlantic salmon and European eel with a special interst to the fish mirtality due to hydropower stations in Dutch rivers
	José Maria Santos Universidade de Lisboa Instituto Superior de Agronomia jmsantos@isa.ulisboa.pt José Maria Santos actual research interests focus primarily on ecohydraulics, fish migration and passage, river restoration and on freshwater fish ecology.
	Maria Teresa Ferreira School of Agriculture, University of Lisbon terferreira@isa.ulisboa.pt M.T. Ferreira research is on freshwater ecology and management, with special interest on biological monitoring, fish community ecology, fish habitat requirements and riparian ecology. She has been involved in many applied ecological aspects of river management including fish pass design, fish responses to stressors, management of riparian and invasive vegetation and riparian restoration.
	Lee Baumgartner Institute for Land Water & Society lbaumgartner@csu.edu.au Lee Baumgartner's research has been in several broad areas, including fish passage and fish migration, dietary interactions among native fish species, the impact of human disturbance on aquatic ecosystems and, more recently, mitigating hydropower impacts on tropical rivers.
Opposed Reviewers:	
Response to Reviewers:	Imen Ben Ammar Post-doctoral researcher Institute of Life-Earth-Environment (ILEE), Research Unit in Environmental and Evolutionary Biology (URBE), University of Namur, 61 rue de Bruxelles, 5000 Namur, Belgium Mail: imen.benammar@yahoo.fr Alternative mail: imen.benammar@unamur.be To editorial office of Comparative Biochemistry and Physiology – Part A: Molecular and Integrative Physiology Namur, June 8th, 2020 Dear editor, Please find enclosed the correct manuscript entitled “How the passage through a hydropower plant affects the physiological and health status of Atlantic salmon smolts?” by Ben Ammar et al. with all the corrections made as required by the reviewers. We are very grateful for all the comments that improved the quality of the paper. The corrections in the paper were written in red to highlight them. The answer to the reviewer’s comments in the following pages are written in purple. Thank you very much for considering those revisions. Best regards, Imen Ben Ammar

Reviewer #1: GENERAL COMMENTS
The present study addressed the physiological and immune response of Atlantic salmon smolts upon passing to an experimental Kaplan hydropower turbine. I found the manuscript well-written and structured and easy to read. Further, the manuscript focuses on the issue of delayed mortality which is key to many fish passage and restocking programs. Therefore, the findings of this study can be useful to other contexts and potentially increase attention from different readers. Below is a list of specific comments that readers can use to improve their manuscript.

SPECIFIC COMMENTS
Highlights OK

Line 17 - HPP between parentheses.
Line 17: Correction was made.

Line 20 - Provide river and HPP names, country also.
Line 20: the required information was added “Andenne HPP (Meuse River, Belgium)”

Good abstract, well-structured and written with a sound conclusion and implications to a broader context. Just some minor edits (i.e. outline your study area) to consider.

Line 37 - I think you could provide some more actual references.
Lines 38-39: The references King and O’Hanley, 2016 and McKay et al., 2017 were added.

Line 89 - How many fish? Total length (Mean +- SD?)? This is needed.
Line 94: The required information was added (N=1400, mean length = 5.5 ± 0.4 cm).

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We understand the critic of the reviewer because in the beginning of our project we planned to use wild Atlantic salmon smolts not only because of their swimming behavior but also because they may be carrier of pathogens and, then, may present a kind of vulnerability to the potential impact of stress on their immune status. However, it was impossible for us to obtain a sufficient number of wild Atlantic salmon in the smolt stage to carry on the experiment.
In 2018, we conducted with the Laboratory of Fish Demography and Hydroecology of University of Liège many assessments on a downstream migration trap (Méry, Ourthe, Belgium). The Ourthe river is where the main part of the salmon restocking programs are conducted by the Public service of Wallonia (PSW) in collaboration with the Universities of Liège and Namur. During those assessments, we obtained Atlantic salmon smolts that were in a very bad condition showing saprolegniasis, also known as cotton wool disease and infestation with leech. We sampled those fish in an attempt to measure the same parameters to those measured in our current work (data in progress). Moreover, the quite high water temperatures observed in April 2018 shortened the downstream migration period and decreased the quality of Atlantic salmon smolts.
With regards to the difficulty to conduct our experiment in situ as it needs not only the energy producer collaboration but also good water flow conditions to set the hydropower plant (HPP) at its maximal intake, we chose to use reared individuals as we were confident concerning their availability in terms of size, quantity and quality. Furthermore, the majority of migrating smolts found in the Meuse river come from the restocking programs of PSW and all the restocked salmon fry and parr come from CoSMos hatchery that rear the Loire-Allier strain. We assumed that based on those information, using reared fish coming from the same strain and the same hatchery than the restocked wild ones can help us provide cues about the impact of the passage through the HPP on the restocked wild salmon. We also have some concerns about the extrapolation of this current work conclusions to the wild populations, but we think

based on our observation that he situation may be worse than what we expect based on our findings due to the other threats faced by wild salmon smolts.

Line 91-94 - How many fish per tank? Size? This is needed! Same on line 94. Did you control for ammonia and nitrates? This is very important and can be a cause of fish mortality or unnatural behaviour. Did you place any form of cover (e.g. rocks, boulders, etc.) in the tanks to reduce the stress of the fish? How did you deal with this?
Lines 97-100: Information concerning the number of fish per tank, their size, their rearing condition and water quality during the pre-smolt rearing period were provided as asked by the reviewer.

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We negotiated with the Public Service of Wallonia to release only the healthy and undamaged Atlantic salmon smolts in the Meuse river as they are from the same strain than the restocked ones. All the fish that presented injuries, even minor, were euthanized.

Line 248-250 - Found this sentence quite unclear, in what is n and what is %. could you please re-write clearer?
Lines 263-265: Clarifications were added to this part.

Line 261-262 - Provide teste name and statistic. Same for the p values throughout this section.
Lines 275-280: All the data were analyzed by ANOVA after the linear model (model = lm (Y ~ treatment*sampling time) with Y: dependent variable) was validated as described in the statistical analysis part (paragraph 2.6). Providing the test name every time will lengthen the result part. For p-value, we provided one value (e.g. p = 0.026 in line 267) if we are assessing the p-value of one factor or comparing between 2 means and a maximal threshold (e.g. $p<0.05$) if we are comparing between different means and having different p-values.

Line 317 - I could not find evidence of this in the Results.
Line 320-321 - Same comment as above.
Lines 340-343: More clarification and reference was added in this discussion part to explain the authors conclusion and more details were provided to the results (paragraph 3.1) in lines 265-268.

Line 342-343 - "simulating the passage over the spillways". But on line 123 you say they were released toward the turbine intake. Could you clarify?
Lines 368-371: In the control group, fish are experiencing the passage through the

wetted flexible tube and the turbulences generated from the water flow at the exit of the turbine without the passage through the turbine itself. Those conditions were considered similar to what the fish face when they pass over the spillway with stress due to the turbulence and to the water head. We added, in the control group, in the sentence to clarify that we are speaking about this group and not about the group injected in the turbine intake.

Figures and Tables OK.

Reviewer #2: This paper presents some physiological and immune responses of Atlantic salmon smolts after their passage through a Kaplan type turbine. The results presented are very interesting and useful, as they are currently lacking in the bibliography and could help to better understand certain indirect impacts of human activities on the survival (or fitness) of migratory fish species.
I propose below minor revisions to improve the manuscript before it could be accepted for publication.
General comments:

1) It is stated line 98 that fish were initially acclimated from 16 to 12°C. Please clarify how fish were acclimated in the 1m3 round tanks (line106), as I understood that the natural water temperature (i.e. the water temperature in these tanks?) was about 8°C.
Lines 116-120: Clarification was added concerning the acclimation of the fish. When we arrived on site, the water temperature in the transport tank was about 11°C. We added progressively Meuse river water into the aerated transport tank allowing a temperature drop of less than 1°C per hour. During this acclimation, temperature and fish behavior were monitored in order to ensure the welfare of fish.

2) It is stated line 109 that the bulb turbine tested has four adjustable blades. Can you clarify what was the orientation of the blades during the tests, as it is known in such tests that fish injury or mortality can be linked to the degree of blade closure? (and see general comment #4).
We agree with the reviewer that the degree of blade closure is one aggravating factor for the impact of the passage through the turbine and that having adjustable blades is supposed to improve fish survival. In our study we worked in maximum intake condition which will lead to a lesser mortality due to the minimal blade closure. However, this scenario is related to the common functioning of hydroelectric power producers as explained in comment 4. The required information was added in lines 139-141.

3) Line 120: it is stated that "180 fish from each experimental tank were caught". Did these 180 specimens correspond to all of the fish from each tank or 60 fish from each of the 3 tanks? More generally, the number of fish used in each treatment must be specified line 123. This is still not clear in the results section, where the percentages of survival or recovered fish (e.g. line 248) is not sufficient to have a clear information about the number of fish used.
Lines 133-136: 180 fish from each tank as specified in line 130 were caught to go either into the turbine or inti the net. Every injection (into turbine or net) required the total number of fish in one tank (we have three) tank). We cannot split the 180 fish from three tanks as the capture of fish can stress the fish. So every time, we totally emptied a tank to recover the required number of fish (180) and use them. The total number of fish used for the whole experiment is 540 (3x180). We specified in line 136 the number of fish used for each modality. The number of fish used were also added in the results part in lines 263-264.

4) Lines 126-127: I am not sure that the survival rate presents the lowest values when the turbine is at its maximum intake capacity. In this case, the blade opening of Kaplan type turbines is the largest, which reduces the risk for the fish to be struck by the moving parts of the turbine and, subsequently, the probability for fish injuries. For example, Schoeneman et al. (1961) showed that mortality was higher for smolts when blades were more closed. Please explain better (and see the general comment #2).
Schoeneman D.E., Pressey R.T. & Junge CO., 1961. Mortalities of downstream migrant salmon at McNary dam. Trans. Am. Fish. Soc, 90, 58-72.
Lines 137-141: Actually, we worked with the worst scenario of the maximum intake by injecting fish at the border of blades at their maximal velocity leading to higher risk of strikes even with the blades at their minimal closure. We chose this scenario because it

	is the closest to the reality as hydroelectric power producers always operate a first turbine up to its maximum capacity before putting a second turbine into operation and so on. This information was added to the paper. 5) Lines 132-133: Why were the fish from the first group not weighed and measured (e.g. for a comparison with fish from the other groups)? Lines 147-148: Actually we did weight, measure and examine them (photography taken also) to determine the cause of the death. This information was added to the corresponding part. 6) Line 133-134: How weighing and measuring the fish could help to determine the injuries severity? Please clarify. Moreover, can you present or, at least, say that the fish weights were comparable between treatments, as it may be important to interpret some biochemistry results? Lines 147-149: we specified that fish were examined to determine the type of injury and photographed to further investigate the injuries severity. The fish weight and size were comparable from the beginning of the experiment in order to have a homogenous population for all the group. We already specified the mean total length of the whole population in line 114. We also added the mean weight of fish on Line 114-115 and fish weight did not show significant difference between the groups. 7) Line 140-141: Were the personal data cited here obtained in comparable conditions (i.e. by injecting fish in a quite large turbine)? Also, please explain briefly where the fish can be if they escaped the turbine and were not in the net. Lines 155-158: It would be a bit lengthy to put all those explanations related to the LIFE4FISH project and to the Profish Technology method in the current paper. Profish technology commonly use this method of deliberate passage into the turbine for regulatory incidence studies required by the Public Service of Wallonia. In those incidence studies, they always inject two batch of anesthetized fish one into the turbine and the second in the net. The results coming from many studies showed that anesthesia did not allow fish to show escapement behavior. In fact, as fish bodies are motionless, they were only driven by the water flow. The recovery rate for those anesthetized fish is always 100%. It was considered as a kind of validation of the method itself. With regards to this knowledge, Profish Technology and we assumed that the non-recovered fish are those that successfully escaped the turbine. Some telemetric studies done by Profish technology showed fish that can go in and come back out to the upstream area of the turbine. In a previous experiment in 2018 coupled with a regulatory incidence study, we injected salmon smolts into the turbine and recovered less than 50% of them. However, in the following injection into the turbine with rainbow trout, we recovered some of our Atlantic salmon from the previous injection. Some of those salmon smolts were unharmed. 8) Line 142: To calculate the external damage rate (%), I guess that the "number of damaged fish" can only be obtained from the fish that were collected in the net. Therefore, the "number of surviving fish" used in the same formula has also to be obtained from the fish collected in the net. I mean that the fish that were not collected in the net and that were considered as alive in the previous formula, should not be considered here. Therefore, the "number of surviving fish" should be different between formula of "survival rate" and formula of "external damage rate". Right? If so, please explain. Line 159: Indeed, it is as the reviewer understood. We cannot make assumption about the damages sustained by the non-recovered fish. We added in the formula Number of recovered and surviving fish instead of only number of surviving fish. 9) Line 146-148: It is usual that farmed fish present an "initial" scale loss, before experiment. As this initial situation can influence the results, was it quantified and was it comparable for the different treatments? Before the experiment, the reared fish were selected to form a batch of homogenous population in terms of length, weight and condition and randomly allocated into the three different tanks. 10) Line 246: A 6% loss of fish in the control group seems important. I understand that, in the HPP group, some individuals can remain between the turbine and the net, but in

the control group, where can be the missing fish?
Lines 263-264: As we worked with the maximum intake, we had some water turbulences. In situ, even with the current system, there was some gap between the metallic frame and the turbine output. As we put the wetted tube in this area to make control fish experience the same water flow than the HPP fish, this gap can allow some fish to escape from the net. Based on their field experience, Profish technology can have a recovery rate from 95 to 100% of fish in control group when the all the conditions are quite good. Here, the existence of this gap made the recovery rate lesser than what was expected even for the turbine group. Also, sometimes, Atlantic salmon smolts swim against the water flow and remain in the area between the turbine and the net. As we cannot keep the fish too long in the net because of the debris that can harm them, we generally recover the net 5 to maximum 10 minutes after the injection itself. So if the salmon smolts kept swimming in this area, we cannot recover them.

11) Line 250: what do you mean by "hematoma ≤ 10%"? Did you consider a percentage of the total body surface area?
Lines 267-268: We made photography, as explained in lines 148-149, to estimate the total body surface (both sides) and the total hematoma and descaling surface in order to have percentages of the area with hematoma or scale loss.
12) Line 315: It is stated that mortality due to Kaplan turbines can range between 5 and 46%, which is true. First, it should be stated that these rates generally correspond to "immediate" mortality. Second, it should be explained that, in situations comparable to that of the present study (fish length, head drop, number of blades and turbine diameter, which is not given here but should be about 2 or 3 metres I guess), mortality rates for smolts are generally expected to be closer to 5% than to 46%. Finally, it could be interesting to present this hypothesis in the introduction of the paper, and to state in the discussion section that the present results i) validated this hypothesis and ii) provided other informations that can partially explain delayed mortality.
Lines 53-57: the total mortality rates observed in different studies of the impact of Kaplan turbines were added to the introduction.
Lines 334-340: We added the required statements as suggested by the reviewer.

13) Lines 436-437. I am not sure that the results from the control group can directly be assimilated to fish passage over spillways, because both situations are very different (initial transfer of the fish, their passage through a tube…). Therefore, I would be less conclusive on the wording, saying that speed and water height during the passage over the spillways, in association with potential protruding structures, may also lead to frictions and shocks, that can be harmful and/or stressful, but I would not compare directly this situation with the control group, especially with the aim to infer biochemistry parameters.
Lines 462-464: We corrected as suggested by the reviewer in order to be more nuanced in our conclusions.

Specific comments:
1) Line 21 (summary): this work is not a passage "simulation", but a real passage of fish through a turbine.
Line 21: Simulation was changed by deliberate.

2) Line 109: change "have" to "has".
Line 121: Have was changed to has.

3) Line 125: I was not able to see the video by using the link (video not available). Maybe the link is not valid (or I had a problem with my current "low quality" Web connection…).
The link was removed from the current paper but the video is still available. However, we removed the link as suggested by the reviewer one because it is possible that the video will be removed in the next 5 or 10 years, and it is also possible that it is not available worldwide. We also think that the Figure 1 with its detailed caption and the text will allow readers to fully understand the experiment.

4) Line 134: it seems there is a word missing "the second and latter groups were…"?
Line 150: correction was made to improve the phrasing.

	5) Line 142: please change "surving" to "surviving" in the formula. Line 159: Correction was made. 6) Line 309: "were intermediate in both groups…" should be changed into "were lower in both groups…". Line 327: Correction was made. 7) Line 335: Delete comma after "Bernard et al.". Line 361: Comma deleted. 8) Lines 568-571: Please swap the two references, to present the older one first. Lines 600-603: References swapped. 9) Line 716 (Fig.2 caption): please delete "in" before "changes". Moreover, here and in Figs 5 and 6 captions, I would not write "changes". The figures do not present changes but the visualization of some parameters from different treatments. You can use the term "changes" during the interpretation of these results (in the results and discussion sections). Line 755, 772-773 and 776-778: Corrections were made according to reviewer comment. 10) Line 720 (Fig. 2 caption): there are no "lower case letters" in Fig.2. Line 759: Correction was made. 11) Fig. 5-D: the ordinate axis should refer to "galk 2" (and not "galk") to be consistent with the text. Fig 5-D corrected.

Imen Ben Ammar
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University of Namur, 61 rue de Bruxelles, 5000 Namur, Belgium
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Alternative mail: imen.benammar@unamur.be

To editorial office of Comparative Biochemistry and Physiology – Part A:

Molecular and Integrative Physiology

Namur, March 23rd, 2020

Dear editor,

Please find enclosed the present manuscript entitled "How the passage through a hydropower plant affects the physiological and health status of Atlantic salmon smolts?" by Ben Ammar et *al*. that we would like to submit for publication in Comparative Biochemistry and Physiology – Part A: Molecular and Integrative Physiology as a research paper. We think that the provided data are useful to understand the impact of the passage through the turbine on the physiological and health status of surviving and undamaged Atlantic smolts during their downstream migration. It is already known that the passage through the turbine affect fish survival and their external damages. However, there is no information about the physiological status and the immune defence capacity of the surviving and unharmed fish. Moreover, several studies using telemetric methods observed delayed mortality that occurred after the passage through the turbine. By investigating the mid-term impact, this work was able to provide clues explaining this observed delayed mortality that leads to a poor success of the actual restocking programs.

Thank you very much for considering this submission and we will be happy to answer any criticisms or suggestions from the referees.

Best regards,

Imen Ben Ammar

Imen Ben Ammar
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Namur, June 8th, 2020

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Lines 263-265: Clarifications were added to this part.

Line 261-262 - Provide teste name and statistic. Same for the p values throughout this section.
Lines 275-280: All the data were analyzed by ANOVA after the linear model (model = lm (Y ~ treatment*sampling time) with Y: dependent variable) was validated as described in the statistical analysis part (paragraph 2.6). Providing the test name every time will lengthen the result part. For p-value, we provided one value (e.g. p = 0.026 in line 267) if we are assessing the p-value of one factor or comparing between 2 means and a maximal threshold (e.g. $p<0.05$) if we are comparing between different means and having different p-values.

Line 317 - I could not find evidence of this in the Results.
Line 320-321 - Same comment as above.
Lines 340-343: More clarification and reference was added in this discussion part to explain the authors conclusion and more details were provided to the results (paragraph 3.1) in lines 265-268.

Line 342-343 - "simulating the passage over the spillways". But on line 123 you say they were released toward the turbine intake. Could you clarify?
Lines 368-371: In the control group, fish are experiencing the passage through the wetted flexible tube and the turbulences generated from the water flow at the exit of the turbine without the passage through the turbine itself. Those conditions were considered similar to what the fish face when they pass over the spillway with stress due to the turbulence and to the water head. We added, in the control group, in the sentence to clarify that we are speaking about this group and not about the group injected in the turbine intake.

Figures and Tables OK.

Reviewer #2: This paper presents some physiological and immune responses of Atlantic salmon smolts after their passage through a Kaplan type turbine. The results presented are very interesting and useful, as they are currently lacking in the bibliography and could help to better understand certain indirect impacts of human activities on the survival (or fitness) of migratory fish species.
I propose below minor revisions to improve the manuscript before it could be accepted for publication.
General comments:

1) It is stated line 98 that fish were initially acclimated from 16 to 12°C. Please clarify how fish were acclimated in the 1m3 round tanks (line106), as I understood that the natural water temperature (i.e. the water temperature in these tanks?) was about 8°C.
Lines 116-120: Clarification was added concerning the acclimation of the fish. When we arrived on site, the water temperature in the transport tank was about 11°C. We added progressively Meuse river water into the aerated transport tank allowing a temperature drop of less than 1°C per hour. During this acclimation, temperature and fish behavior were monitored in order to ensure the welfare of fish.

2) It is stated line 109 that the bulb turbine tested has four adjustable blades. Can you clarify what was the orientation of the blades during the tests, as it is known in such tests that fish injury or mortality can be linked to the degree of blade closure? (and see general comment #4).
We agree with the reviewer that the degree of blade closure is one aggravating factor for the impact of the passage through the turbine and that having adjustable blades is supposed to improve fish survival. In our study we worked in maximum intake condition which will lead to a lesser mortality due to the

minimal blade closure. However, this scenario is related to the common functioning of hydroelectric power producers as explained in comment 4. The required information was added in lines 139-141.

3) Line 120: it is stated that "180 fish from each experimental tank were caught". Did these 180 specimens correspond to all of the fish from each tank or 60 fish from each of the 3 tanks? More generally, the number of fish used in each treatment must be specified line 123. This is still not clear in the results section, where the percentages of survival or recovered fish (e.g. line 248) is not sufficient to have a clear information about the number of fish used.
Lines 133-136: 180 fish from each tank as specified in line 130 were caught to go either into the turbine or inti the net. Every injection (into turbine or net) required the total number of fish in one tank (we have three) tank). We cannot split the 180 fish from three tanks as the capture of fish can stress the fish. So every time, we totally emptied a tank to recover the required number of fish (180) and use them. The total number of fish used for the whole experiment is 540 (3x180). We specified in line 136 the number of fish used for each modality. The number of fish used were also added in the results part in lines 263-264.

4) Lines 126-127: I am not sure that the survival rate presents the lowest values when the turbine is at its maximum intake capacity. In this case, the blade opening of Kaplan type turbines is the largest, which reduces the risk for the fish to be struck by the moving parts of the turbine and, subsequently, the probability for fish injuries. For example, Schoeneman et al. (1961) showed that mortality was higher for smolts when blades were more closed. Please explain better (and see the general comment #2). Schoeneman D.E., Pressey R.T. & Junge CO., 1961. Mortalities of downstream migrant salmon at McNary dam. Trans. Am. Fish. Soc, 90, 58-72.
Lines 137-141: Actually, we worked with the worst scenario of the maximum intake by injecting fish at the border of blades at their maximal velocity leading to higher risk of strikes even with the blades at their minimal closure. We chose this scenario because it is the closest to the reality as hydroelectric power producers always operate a first turbine up to its maximum capacity before putting a second turbine into operation and so on. This information was added to the paper.

5) Lines 132-133: Why were the fish from the first group not weighed and measured (e.g. for a comparison with fish from the other groups)?
Lines 147-148: Actually we did weight, measure and examine them (photography taken also) to determine the cause of the death. This information was added to the corresponding part.

6) Line 133-134: How weighing and measuring the fish could help to determine the injuries severity? Please clarify. Moreover, can you present or, at least, say that the fish weights were comparable between treatments, as it may be important to interpret some biochemistry results?
Lines 147-149: we specified that fish were examined to determine the type of injury and photographed to further investigate the injuries severity. The fish weight and size were comparable from the beginning of the experiment in order to have a homogenous population for all the group. We already specified the mean total length of the whole population in line 114. We also added the mean weight of fish on Line 114-115 and fish weight did not show significant difference between the groups.

7) Line 140-141: Were the personal data cited here obtained in comparable conditions (i.e. by injecting fish in a quite large turbine)? Also, please explain briefly where the fish can be if they escaped the turbine and were not in the net.
Lines 155-158: It would be a bit lengthy to put all those explanations related to the LIFE4FISH project and to the Profish Technology method in the current paper. Profish technology commonly use this

method of deliberate passage into the turbine for regulatory incidence studies required by the Public Service of Wallonia. In those incidence studies, they always inject two batch of anesthetized fish one into the turbine and the second in the net. The results coming from many studies showed that anesthesia did not allow fish to show escapement behavior. In fact, as fish bodies are motionless, they were only driven by the water flow. The recovery rate for those anesthetized fish is always 100%. It was considered as a kind of validation of the method itself. With regards to this knowledge, Profish Technology and we assumed that the non-recovered fish are those that successfully escaped the turbine. Some telemetric studies done by Profish technology showed fish that can go in and come back out to the upstream area of the turbine. In a previous experiment in 2018 coupled with a regulatory incidence study, we injected salmon smolts into the turbine and recovered less than 50% of them. However, in the following injection into the turbine with rainbow trout, we recovered some of our Atlantic salmon from the previous injection. Some of those salmon smolts were unharmed.

8) Line 142: To calculate the external damage rate (%), I guess that the "number of damaged fish" can only be obtained from the fish that were collected in the net. Therefore, the "number of surviving fish" used in the same formula has also to be obtained from the fish collected in the net. I mean that the fish that were not collected in the net and that were considered as alive in the previous formula, should not be considered here. Therefore, the "number of surviving fish" should be different between formula of "survival rate" and formula of "external damage rate". Right? If so, please explain.
Line 159: Indeed, it is as the reviewer understood. We cannot make assumption about the damages sustained by the non-recovered fish. We added in the formula Number of recovered and surviving fish instead of only number of surviving fish.

9) Line 146-148: It is usual that farmed fish present an "initial" scale loss, before experiment. As this initial situation can influence the results, was it quantified and was it comparable for the different treatments?
Before the experiment, the reared fish were selected to form a batch of homogenous population in terms of length, weight and condition and randomly allocated into the three different tanks.

10) Line 246: A 6% loss of fish in the control group seems important. I understand that, in the HPP group, some individuals can remain between the turbine and the net, but in the control group, where can be the missing fish?
Lines 263-264: As we worked with the maximum intake, we had some water turbulences. *In situ*, even with the current system, there was some gap between the metallic frame and the turbine output. As we put the wetted tube in this area to make control fish experience the same water flow than the HPP fish, this gap can allow some fish to escape from the net. Based on their field experience, Profish technology can have a recovery rate from 95 to 100% of fish in control group when the all the conditions are quite good. Here, the existence of this gap made the recovery rate lesser than what was expected even for the turbine group. Also, sometimes, Atlantic salmon smolts swim against the water flow and remain in the area between the turbine and the net. As we cannot keep the fish too long in the net because of the debris that can harm them, we generally recover the net 5 to maximum 10 minutes after the injection itself. So if the salmon smolts kept swimming in this area, we cannot recover them.

11) Line 250: what do you mean by "hematoma ≤ 10%"? Did you consider a percentage of the total body surface area?
Lines 267-268: We made photography, as explained in lines 148-149, to estimate the total body surface (both sides) and the total hematoma and descaling surface in order to have percentages of the area with hematoma or scale loss.

12) Line 315: It is stated that mortality due to Kaplan turbines can range between 5 and 46%, which is true. First, it should be stated that these rates generally correspond to "immediate" mortality. Second, it should be explained that, in situations comparable to that of the present study (fish length, head drop, number of blades and turbine diameter, which is not given here but should be about 2 or 3 metres I guess), mortality rates for smolts are generally expected to be closer to 5% than to 46%. Finally, it could be interesting to present this hypothesis in the introduction of the paper, and to state in the discussion section that the present results i) validated this hypothesis and ii) provided other informations that can partially explain delayed mortality.
Lines 53-57: the total mortality rates observed in different studies of the impact of Kaplan turbines were added to the introduction.
Lines 334-340: We added the required statements as suggested by the reviewer.

13) Lines 436-437. I am not sure that the results from the control group can directly be assimilated to fish passage over spillways, because both situations are very different (initial transfer of the fish, their passage through a tube…). Therefore, I would be less conclusive on the wording, saying that speed and water height during the passage over the spillways, in association with potential protruding structures, may also lead to frictions and shocks, that can be harmful and/or stressful, but I would not compare directly this situation with the control group, especially with the aim to infer biochemistry parameters.
Lines 462-464: We corrected as suggested by the reviewer in order to be more nuanced in our conclusions.

Specific comments:
1) Line 21 (summary): this work is not a passage "simulation", but a real passage of fish through a turbine.
Line 21: Simulation was changed by deliberate.

2) Line 109: change "have" to "has".
Line 121: Have was changed to has.

3) Line 125: I was not able to see the video by using the link (video not available). Maybe the link is not valid (or I had a problem with my current "low quality" Web connection…).
The link was removed from the current paper but the video is still available. However, we removed the link as suggested by the reviewer one because it is possible that the video will be removed in the next 5 or 10 years, and it is also possible that it is not available worldwide. We also think that the Figure 1 with its detailed caption and the text will allow readers to fully understand the experiment.

4) Line 134: it seems there is a word missing "the second and latter groups were…"?
Line 150: correction was made to improve the phrasing.

5) Line 142: please change "surving" to "surviving" in the formula.
Line 159: Correction was made.

6) Line 309: "were intermediate in both groups…" should be changed into "were lower in both groups…".
Line 327: Correction was made.

7) Line 335: Delete comma after "Bernard et al.".
Line 361: Comma deleted.

8) Lines 568-571: Please swap the two references, to present the older one first.
Lines 600-603: References swapped.

9) Line 716 (Fig.2 caption): please delete "in" before "changes". Moreover, here and in Figs 5 and 6 captions, I would not write "changes". The figures do not present changes but the visualization of some parameters from different treatments. You can use the term "changes" during the interpretation of these results (in the results and discussion sections).
Line 755, 772-773 and 776-778: Corrections were made according to reviewer comment.

10) Line 720 (Fig. 2 caption): there are no "lower case letters" in Fig.2.
Line 759: Correction was made.

11) Fig. 5-D: the ordinate axis should refer to "galk 2" (and not "galk") to be consistent with the text.
Fig 5-D corrected.

How the passage through a hydropower plant affects the physiological and health status of Atlantic salmon smolts?

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Abstract

Atlantic salmon is an anadromous species migrating from upper-reach nursery areas in rivers to the oceanic feeding areas at smolt stage and inversely at adult stage requiring unimpeded migration routes. However, dams associated with hydroelectric power plants (HPP) disrupt river connectivity and affect fish movement and survival. The objective of the current study was to evaluate the short and mid-term physiological and immune response of Atlantic salmon smolts after passing through Andenne HPP (Meuse River, Belgium). Several parameters were studied after an *in situ* deliberate passage including direct mortality and external damages, stress and immune biomarkers as plasma cortisol and glucose levels, complement and peroxidase activities, and immune and oxidative stress related gene expression 24 h, 72 h and 120 h after passage. Survival rate was lower and external damages were more important in fish that confronted the HPP compared to the control ones. Moreover, the passage through the turbine affected plasma glucose levels, complement and peroxidase activities and the expression of some immune genes such as *lysg*, *igm* and *mpo* in a timely manner suggesting that this passage can lead to a great energy expenditure and a disruption of innate immunity. Our observations can partially explain the delayed mortality observed in many studies leading to a poor success of restocking programs. HPPs not only have a direct impact in terms of mortalities and injuries but also an indirect one in terms of physiological and immune changes that can compromise Atlantic salmon smolts ability to escape successfully to the ocean.

Keywords: Hydropower plant, Atlantic salmon smolts, downstream migration, physiological and health status

1. Introduction

Anthropogenic activities as dams, navigation weirs and hydropower stations have led to the reduction of hydrological connectivity (King and O'Hanley, 2016; Larinier, 2001; McKay et al., 2017; Pringle, 2003). These activities have well documented effects such as the delay or the total prevention of fish migratory movements, fish stranding, and mortalities directly and/or indirectly linked to the passages through hydropower plants (HPP) and over the spillways (Freeman et al., 2003; Katopodis and Williams, 2012; Larinier and Travade, 2002; Nagrodski et al., 2012; Renardy et al., 2019). During their passage through the turbines, fish are subjected to various forms of stress that can cause high mortality as strike from parts of the HPP, sudden speed and pressure changes, shear, and cavitation (Coutant and Whitney, 2000; Larinier and Travade, 2002; Mathur et al., 2000; Rivinoja, 2005). Numerous studies were conducted on different types of turbines but only focused on determining the direct (*e.g.* mortality from HPP blade strikes) and indirect (*e.g.* delayed mortality due to minor injuries) fish mortality and damage rates, mainly using telemetric methods or the simulation of the passage through the turbine (Brackley et al., 2018; Ferguson et al., 2006; Havn et al., 2017; Kibel and Coe, 2007; Larinier and Travade, 2002). The better survival rates are higher than 90% in "environmentally friendly" turbines, but it can be lower than 60% in other common used turbine designs (Bickford and Skalski, 2000; Havn et al., 2017; Thorstad et al., 2012). In Kaplan turbines, for example, total mortality rate (combining both direct and delayed) can vary from below 5% to 46 depending on the characteristics of the turbine and fish species and size (Bickford and Skalski, 2000; Čada et al., 2006; Coutant and Whitney, 2011; Larinier, 2008; Larinier and Travade, 2002; Thorstad et al., 2012). However, there is no information about the physiological and health condition of surviving and unharmed fish.

Atlantic salmon (*Salmo salar* Linnaeus, 1758) is an anadromous species that migrates between spawning and nursery habitats in rivers, and feeding and growth areas in the ocean (Thorstad et al., 2011). This species has experienced severe reductions and even the extinction of some strains in Europe and North-America due to the disruption of river connectivity and the limited access to functional habitats (Forseth et al., 2017; Freeman et al., 2003; Nehlsen et al., 1991; Parrish et al., 1998). To prevent population depletion and support commercial and recreational fisheries, many restoration and/or compensatory salmon hatchery-rearing programmes have been established in Europe and North America (Jonsson and Jonsson, 2011). However, the success of such programmes is mitigated and depend on many factors including the quality, size and density of the fish, and time and place of the stocking (Jonsson and Jonsson, 2011; Persson et al., 2019). The decrease of water flow due to the HPP intake

can dramatically decrease the carrying capacity for Atlantic salmon smolts in save passage forcing them to pass through the turbine and compromising the success of the releases (Brevé et al., 2014; Jonsson and Jonsson, 2011; Persson et al., 2019).

In many river systems such as in the Meuse River, Atlantic salmon smolts are confronted to many hydropower plants during their long travel to the sea, and the cumulative impact of these obstacles could constitute, as suggested by some authors, a persistent physiological stress that could impair the immune defence capacity (Thorstad et al., 2017, 2012). Moreover, smolts must complete their migration in a very narrow migration window and face physiological changes during the smoltification process (McCormick et al., 1998; Thorstad et al., 2012). The delay in downstream migration can represent a serious threat for the population maintenance (Mathers et al., 2002; Nyqvist et al., 2017). A disruption in the physiological status can lead to a great energy expenditure that can compromise further migration while a disruption in the immune status can increase fish vulnerability to pathogens and increase the delayed mortality. However, to our knowledge, no information is available about the physiological status and immune defence capacity of Atlantic salmon surviving after the HPP passage and the impact on their migration ability is still largely unknown.

The aim of this study was to assess how the passage through the turbine can affect the survival, the physiological and immune status of Atlantic salmon smolts by various key studying stress and immune biomarkers. We hypothesized that the passage through the turbine can lead to an elevated allostatic charge and affect directly or indirectly the immune system and thereby the overall physiological and health status of fish.

2. Materials and Methods

2.1. Animals and rearing conditions

Atlantic salmon parr (N=1400, mean length = 5.5 ± 0.4 cm) were transferred from CoSMos hatchery (Conservatoire du Saumon Mosan, Erezée, Belgium) to the facilities of the University of Namur in Belgium and were reared until the pre-smolt stage. During the parr stage, fish (about 300 per tank) were reared at 16°C in sub squared tanks of 100 L partially covered by PVC plates and fed at 3% of their weight with Nutra XP 0.5 (Skretting, Canada) and Coppens starts premium (1 mm, Alltech Coppens, Netherland). When fish size reached 8-9 cm, they were transferred into two 1m³ sub-squared tanks (500 per tank) partially covered with PVC plates and totally covered by nets, reared at 16°C and fed at 3% of their weight with Ultra 2 mm (Alltech Coppens, Netherland) (AquaTech, Austria) and Supreme 21 (3 mmAlltech Coppens, The Netherlands) using a belt feeder. During the whole rearing process,

104 temperature, pH and dissolved oxygen were checked every day using a multiparameter
105 measuring device (MultiLine® Multi 3510, WTW, WVR). Water analysis (ammonia, nitrite,
106 and nitrate) was done twice a week, and concentrations did not exceed 0.02, 0.1, and 2 mg/L,
107 respectively. Since Meuse water temperature was about 8°C at the time of Atlantic salmon
108 transfer, water temperature was progressively decreased in the rearing tanks during 10 days
109 from 16 to 12°C in order to prepare the fish for natural conditions.
110 All experiments were carried out in accordance with the International Guiding Principles for
111 Biomedical Research Involving Animals (EU Directive 2010/63/EU for animal experiments).
112
113 **2.2. Experimental protocol and sampling procedures**
114 A total of 540 Atlantic salmons (age: 1 year, mean total length = 140.01 ± 10.16 mm, mean
115 weight 25.5 ± 5.2 g) were transported to the Andenne hydropower plant (Anton Roadway
116 114-144, 5300 Andenne, Belgium, 50°29'30.3"N 5°04'11.9"E). During their transfer, fish
117 were acclimated to the temperature and water quality changes during 4h by progressively
118 adding the Meuse river water into the aerated transport tank. Then fish were allowed to
119 recover into three 1m³ round tanks covered by nets (180 fish per tank) for four days before the
120 experiment. This site was chosen because it was recently equipped with a bulb turbine – a
121 variant of Kaplan-type turbine with a horizontal axis – that has four adjustable blades, a
122 rotational speed of 176.47 rpm and a head of 5.35 m (EDF Luminus, 2015). This model often
123 used on Atlantic salmon river (Thorstad et al., 2012) was meant to improve hydropower
124 production efficiency and enable a broad operating range. As this turbine can function even
125 with a low flow, the probability that the turbine will be in operation during Atlantic salmon
126 downstream migration is relatively high compared to other models which cannot operate
127 under these conditions. Moreover, two hydropower plants (Lixhe and Andenne) are equipped
128 with bulb turbines in the Meuse River, which is our project area (LIFE4FISH). On the 4th of
129 April 2019 (J0), the simulation of fish passage through the turbine was conducted according
130 to Profish Technology (https://www.profish-technology.be/) method commonly used to study
131 the incidence of the hydropower plant *in situ* (Brackley et al., 2018; Kibel and Coe, 2007).
132 The deliberate passage through the turbines is a validated method in Germany, Austria and
133 Switzerland (Schmalz et al., 2015). A total of 180 fish from each experimental tank were
134 caught, transported quickly in a 100 L square tank and gently released from a bucket of water
135 through a wetted flexible plastic pipe (20 cm of diameter) with its exit directly into the turbine
136 intake itself (HPP group, N=2x180) or directly in the net for control group (N=180, Figure 1).
137 During the simulation of the passage, the bulb turbine was set at its maximum intake capacity

(166 m^3/s) coupled with injection at the border of blades which represents the scenario that lead to the lowest survival rate in high water flow conditions. In those conditions, the blades are opened at their maximum improving fish survival. This scenario is the closest to the real operating conditions. After the passage, fish were recovered using a 50 meters' length net fixed on a metallic frame handled by a crane.

Then, fish were sorted into three groups immediately after their recovery:

- Group 1: dead fish + heavily injured ones,
- Group 2: surviving fish with non-life threating external injuries
- Group 3: surviving fish without any external injuries.

Fish from the first group were weighed (g), measured (mm), and examined in order to determine the causes of death. Fish from the second group were weighed, measured, examined and photographed in order to determine the injuries severity. The second and latter groups were put back in the tanks in maximum two hours while the heavily injured fish were euthanized using MS222 (240 mg/L).

The recovery, survival and external damage rates were calculated after retrieving the net as follows:

- $Recovery\ rate\ (\%) = \frac{Number\ of\ recovered\ fish \times 100}{Number\ of\ injected\ fish}$
- $Survival\ rate\ (\%) = \frac{Number\ of\ surviving\ fish \times 100}{Number\ of\ injected\ fish}$, as previous personal data of the same experiment in another site showed 100% of recovery rate after injection of anesthetized fish, assumption was made that the non-recovered fish succeeded in escaping the turbine and were considered alive.
- $External\ damage\ rate\ (\%) = \frac{Number\ of\ surviving\ and\ damaged\ fish \times 100}{Number\ of\ recovered\ and\ surviving\ fish}$

The severity of external damages was assessed post hoc from the photographs taken during the experiment according to Brackley et al., (2018). The damages were considered non-life threatening if fish displayed normal swimming behaviour in the two hours after the recovery and if the fish survived until the end of the monitoring period (120 h post injection). Scale loss were classified following the distribution across the fish's body: 0 – 1% negligible scale loss, 2 – 4% low scale loss, 5 – 9% moderate scale loss, 10 – 30% severe scale loss.

A total of 10 fish were sampled from control and HPP groups for blood (after anaesthesia with MS222, 120 mg/L) and brain, liver and spleen (after euthanasia with overdose of MS222, 240 mg/L) 24 h after injection (24 h pi), 72 h after injection (72 h pi) and 120 h after injection (120 h pi) in order to investigate the response of fish in the short and mid-term.

2.3. Stress indicators

Cortisol was assayed in duplicate using a cortisol ELISA kit (KAPDB270, Diasource, Belgium) following the manufacturer's instructions. The assay dynamic range was between 0 and 600 ng ml^{-1}. The intra-assay coefficient of variation and the analytical sensitivity were respectively 5.8%, and 4 ng ml^{-1}.

Plasma glucose, assayed in triplicate, was determined based on a glucose oxidase/peroxidase method described by Trinder (1969). Briefly, 20 µl of samples and standards were deproteinized using perchloric acid (0.33M) and 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). After an incubation of 15 min at 38°C, the absorbance was measured at 436 nm using the 96-well plate reader (FLUOstar® Omega, BMG LABTECH, Germany).

High performance liquid chromatography method was adapted from Baekelandt et al. (2019) in order to assess the serotonergic and dopaminergic activities expressed as hydroxyl-indole-acetic acid (5-HIAA)/serotonin (5-HT) and 3,4-dihydroxyphenylacetic acid (DOPAC)/dopamine (DA) ratios respectively in the whole fish brain. Brains were weighed and homogenized during 2 min at 8°C using a Bullet Blender Storm 24 (Next Advance) in tubes containing 2 mL/g of tissue absolute methanol (≥ 99.8%, HiPerSolv CHROMANORM, VWR, Belgium) and 0.5 mm zirconium oxide beads (Dutscher). Homogenates were then centrifuged (21 000g, 15 min, 4°C), supernatants were transferred to new tubes and centrifuged a second time before being filtered through 0.5 µm filters (Phenomenex). An aliquot (35 µL) of the filtrate was injected into the HPLC system. The procedure was carried out on ice. HPLC analysis was carried out using GP50 gradient pump (Dionex) equipped with an autosampler FAMOS (LC packings). The filtered homogenates were applied individually on a 2.6 µm particle size (150 × 4.6 mm, ID) C18 analytical Kinetex column (kept at 25°C) at 1 mL/min of mobile phase (65 mmol/L NaH_2PO_4, 1.63 mmol/L octane sulfonic acid, 0.1 mmol/L EDTA-Na^2, and 13% absolute methanol, pH = 2.79 adjusted with orthophosphoric acid). Neurohormones were monitored using a DC amperometry detector (Dionex) with Glassy Carbon Working Electrode (0.700 V, Ag/AgCl-P/N 061677). Chromeleon™ software 6.8 (Dionex) was used for data acquisition and processing. Standard solutions were serially diluted (from 250 nmol/L to 7.8 nmol/L) in absolute methanol from purified hormones (Sigma-Aldrich) and were treated similarly to samples. Concentrations of the compounds

were calculated by interpolation of their respective standard curves. The intra- and inter-assay coefficients of variation for tested hormones were under 5.9% and 7.4% respectively.

2.4. Humoral immune parameters

The plasma alternative complement pathway (ACH50) procedure measure the haemolytic activity in plasma samples using rabbit red blood cells (RRBC) as targets (Cornet et al., 2018). A serial dilution from 1/20 to 1/480 into veronal buffer (IDVert, France) was performed in duplicate for each plasma sample in a round-bottomed 96-well plate. The total haemolysis was obtained by mixing 10 µl of RRBC (3%) lysed with bi-distilled water and the spontaneous haemolysis was obtained by adding veronal buffer to 10 µl of RRBC. After the incubation, the turbidity 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 total peroxidase activity in plasma was assessed according to Quade and Roth (1997). The samples and negative control (water) were assayed in triplicate. In flat-bottomed 96-well plate, 7 µl of plasma was diluted in 68 µl of Hank's buffer (HBSS) without Ca^{2+} or Mg^{2+}. As substrate, 25 µl of reactional solution (20 mM 3,3′,5,5′-tetramethylbenzidine hydrochloride and 5 mM H_2O_2) was added. The reaction was stopped after 2 min by adding 50 µl of 4M sulphuric acid and the absorbance was measured at 450 nm. One unit (U) of peroxidase activity was defined as the amount producing an absorbance change of 1 OD.

2.5. Gene expression

Gene expression procedure was conducted following Cornet et al., (2018). For each sampling time and in each group, total RNA was extracted from the organs (liver and spleen) using Tri Reagent solution (Ambion, Thermofisher Scientific) according to the manufacturer's instructions. The pellet was dried and re-suspended in 50 and 100 µL of RNase-free water for spleen and liver respectively. Total RNA concentration was determined by NanoDrop-2000 spectrophotometer (Thermo Scientific). Genomic DNA was digested for 15 min at 37 °C with 1U of rDNAse I (Thermofischer Scientific) and total RNA was quantified again by NanoDrop-2000 spectrophotometer. Then, 1 µg of total RNA was reverse-transcribed using RevertAid RT kit (Thermofischer Scientific) according to the manufacturer's instructions. The cDNA was used to test the expression of 29 genes using Real-time quantitative polymerase chain reaction qPCR. Two housekeeping genes (β-actin and elongation factor 1α, ef1α) were tested and b-actin was chosen as the reference gene. Only 15 target genes were kept after an amplification test using different dilutions of cDNA. The list of specific primers used is given in Table 1. Real-time qPCR was carried out with iTaq universal SYBR green

supermix (Bio-Rad Laboratories) using a 1:100 dilution of the cDNA. Primers for target and reference genes were used at 100 nM. The thermal conditions were 3 min at 95 °C, followed by 40 cycles at 95°C for 10 s and 60 °C for 30 s, and melting curves were analysed to verify the absence of multiple amplicons. All reactions were performed using QuantStudio5 device (Applied Biosystem) and the relative gene expression was calculated using the standard curve method. Values for each sample were expressed as normalized relative expression (NRE), calculated with the formula $NRE =$

$$Relative\ quantity\ Target\ gene / Relative\ quantity\ Reference\ gene$$

2.6. Statistical analyses

Statistical analyses were performed using the free software R version 3.6.2 (R Core team, 2019). Homogeneity of variances was previously tested for all the dependent variables using Levene test (leveneTest, package "car", Fox et al., 2014). Data were then analysed by a linear mixed model (lm, package "lme4", Bates et al., 2014) with the treatment and the sampling time as fixed effects: *model = lm (Y ~ treatment*sampling time*) with *Y*: dependent variable. 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). 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, or Box-Cox transformed. When the model was validated, an ANOVA table for various statistical models 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 and external damages

The recovery rate was lesser in HPP group (71.1% of the injected fish N = 2x180) than in control one (94%, N = 180). All the recovered fish from control group were alive and unharmed while HPP group showed a survival of 96.4% of the injected fish. The main cause of death was body part loss or crushing (9 fish out of 13) or laceration (4 fish out of 13). In the surviving recovered fish (N = 256), 8 Atlantic salmons showed moderate scale loss ranging from 5 to 10% and 10 showed moderate scale loss combined with hematoma ≤ 10%.

3.2. Stress response

Plasma cortisol levels did not differ between HPP group and control one and remained stable through the time (Figure 2-A). At 120 h pi, cortisol mean values showed a little tendency to diverge between the two groups with 63.9 ± 33.1 ng/mL in HPP group and 41.7 ± 40.7 ng/mL in control group.

The interaction between the treatment and the sampling time affected significantly plasma glucose levels ($p = 0.026$, Figure 2-B). The lowest level was observed in HPP fish sampled at 120 h pi (0.31 ± 0.06 mg/mL) while the highest was observed in HPP fish sampled at 24 h pi (0.62 ± 0.22 mg/mL). Glucose values decreased over the time in HPP fish between 24 h and 120 h pi ($p < 0.05$) while they remained stable until 72 h pi and decreased in control group only between 72 h and 120 h pi ($p < 0.001$).

The content of brain neurohormones (Figure 3) did not show any significant difference except for DOPAC that varied significantly depending on the interaction between the treatment and the sampling time ($p = 0.001$). DOPAC content in brain decreased significantly between 72 h and 120 h pi in HPP group only ($p = 0.034$, 15.7 ± 4.1 ng/g of wet weight and 11.5 ± 3.8 ng/g of wet weight, respectively, Figure 3-D). Serotonergic and dopaminergic ratios in whole brain did not vary significantly in the whole experiment (Figure 3-E and F).

3.3. Humoral immune parameters

Plasma peroxidase levels varied significantly depending on the interaction between the treatment and the sampling time ($p = 0.014$, Figure 4-A). The levels remained stable in all groups through the time until 72 h pi, but values were significantly different between HPP and control group at 120 h pi ($p = 0.008$). Control fish (217.7 ± 148.1 U/mL) showed a significantly lower level compared to HPP groups at 120 h pi (279.3 ± 97.2 U/mL) and HPP group at 72 h pi (241.4 ± 70 U/mL).

The interaction between the treatment and the sampling time significantly influenced the ACH50 levels ($p < 0.001$, Figure 4-B). Twenty-four hours after injection, control fish showed a significantly higher ACH50 level (92.2 ± 60.7) than HPP ones (44.6 ± 48.7, $p = 0.027$). Fish sampled at 72 h and 120 h pi did not show any significant difference between HPP and control groups. However, ACH50 levels decreased over the time, in control groups, between 24 h and 72 h pi ($p = 0.016$) and in HPP group between 72 h and 120 h pi ($p = 0.023$).

3.4. Gene expression

3.4.1. Stress and metabolic related genes in liver

Stress related *hsp70* gene expression did not vary significantly during the experiment (Figure 5-A) while *gr1* (stress response, carbohydrate metabolism and hormone regulation) varied depending on the sampling time with an increase at 120 h pi (Figure 5-B, $p < 0.001$). All the carbohydrate metabolism gene expressions (*gr1, apoa1, galk2, calm1* and *cd36*) varied significantly depending on the sampling time ($p<0.05$, Figure 5-B, C, D, E, F and G) while *fads6* (lipid metabolism) did not show any significant variation during the experiment. The values were similar between 24 h and 72 h pi and increased at 120 h pi for *apoa1* and *galk2* ($p < 0.05$). For *calm1*, the relative gene expression levels increased significantly between 72 h pi and 120 h pi ($p = 0.005$) and were intermediate at 24 h pi, while they increased for *cd36* between 24 h and 120 pi ($p = 0.043$).

For the hormone regulating genes (*gr1, ghr1, igf1* and *igf2)*, the expression varied throughout the time (Figure 5-B, H, I and J). In all groups, *gr1, ghr1* and *igf2* expression levels increased at 120 h pi ($p < 0.05$). *Igf1* relative expression levels and increased at 72 h pi remaining stable until the end of the experiment ($p < 0.005$).

3.4.2. Immune related genes in spleen

The expression of immune genes *lysg* and *igm* varied significantly depending on the interaction between sampling time and treatment ($p = 0.023$ and 0.035 respectively, Figure 6-A and B), while the relative expression of c3 did not show any significant variation during the experiment (Figure 6-C). In HPP group, *lysg* levels was lower at 24 h and increased at 72 h pi ($p < 0.001$, Figure 6-A). Changes in *igm* relative expression occurred only in HPP group with an increase over the time ($p < 0.01$) while the levels remained quite similar in control group. Relative expression levels of *mpo* varied according to the interaction between the sampling time and the treatment ($p = 0.012$, Figure 6-D). At 72 h pi, those levels were higher in HPP group compared to control group and were lower in both groups for the other sampling times ($p = 0.007$). Relative gene expression levels of *cox2* were stable and increased in all groups at 120 h after injection ($p < 0.05$, Figure 6-E).

4. Discussion

4.1. Survival rate and external damages

The survival rate in the HPP fish was consistent with previous findings for Kaplan turbine (mortality from below 5% to 46%, Bickford and Skalski, 2000; Čada et al., 2006; Coutant and Whitney, 2011; Larinier, 2008; Larinier and Travade, 2002; Thorstad et al., 2012). Our findings are close to the direct mortality estimation of 5% found in similar studies (Coutant and Whitney, 2000; Ferguson et al., 2006; Larinier and Travade, 2002; Mathur et al., 2000). It allows us to consider that a part of the higher total mortality observed in telemetric studies

may be due to other factors including potential changes in the animal condition, exhaustion or disorientation due to the passage through the turbine (Ferguson et al., 2006; Havn et al., 2020). The main causes of mortality were body parts loss and crushing and the main external damages observed were descaling and haemorrhage. Those types of injuries are directly related to strikes from part of the HPP and other mechanical wounding and descaling can also be caused by shear and turbulence (Pracheil et al., 2016). As Atlantic salmons are physostomes, they can resist quite well sudden changes in pressure due to their quick regulation of the pressure in the swim bladder through the air canal and the mouth (Larinier and Travade, 2002). This explains the absence of mortality due to the rupture of the swim bladder caused by sudden pressure variations. Moreover, the mortality rate varies between fish species and depends also on fish size with mortality rate in adult eels estimated to be 4 to 5 times higher than in juvenile salmonids (Larinier and Travade, 2002). The recorded damages were mainly scale losses combined or not with hematoma. Those damages are widely encountered in fish after the passage through the turbine (Brackley et al., 2018; Havn et al., 2017; Kibel and Coe, 2007). As large scale loss may reduce the osmoregulatory ability of fish leading to a delayed mortality in the ocean, it is important to record and monitor those non-life threatening damages after the passage through the turbine (Thorstad et al., 2012; Zydlewski et al., 2010).

4.2. Changes in stress status, metabolism and hormonal regulation

Changes in physiological stress status after the passage through the hydropower turbine were evaluated by various reliable stress parameters, including circulating cortisol and glucose, brain neurotransmitters and liver *gr1* and *hsp70* genes expressions. Plasma cortisol levels measured in HPP groups did not vary from the control ones over the time. Those levels are close to those observed by Bernard et al. (2018) in the Loire-Allier strain – the same strain as the one used in this study – at the beginning of the smoltification process when the water temperature is about 7 – 9°C. In the same time, the observed levels were about five times higher than those observed in non-stressed smolts by Carey and McCormick (1998). Plasma cortisol levels in stressed Atlantic salmon smolts can rise sharply and decline to their initial values in 8 h after an acute stress (Carey and McCormick, 1998). This leads to conclude that fish sampled at 24 h post-injection were already in a recovery process from the turbine-induced stress. Moreover, for the control group, the passage in the wetted flexible plastic tube simulating the passage over the spillways seems to have also induced a stress in the fish regarding the fact that no significant differences were found between the control and the HPP group. As Atlantic salmon smolts have a high interrenal responsiveness during the

smoltification process (Carey and McCormick, 1998), it seems possible that the stress due to the handling and the passage through the tube were already enough to trigger an increase in cortisol levels potentially overshadowing the effects of the passage through the turbine itself. Changes in glucose levels are considered as part of a secondary metabolic response to stress as the release of cortisol from the interrenal is involved in maintaining hyperglycaemia through protein catabolism and gluconeogenesis to prevent exhaustion (Soengas et al., 1992; Specker, 1982; Van Der Boon et al., 1991). Catecholamines are involved in the primary metabolic response to stress and can cause an initial rise in plasma glucose by glycogenolysis while cortisol mediates sustained plasma glucose levels (Fabbri and Moon, 2016; Faught et al., 2016). The decrease of plasma glucose levels was more abrupt and occurred earlier in HPP group than in the control group. The plasma glucose levels remained stable until 72 h post injection in control group before decreasing while those levels decreased sharply from the first day post-injection in HPP group. The glucose levels were quite similar at 120 h post-injection. It seems that the passage through the turbine led to a more rapid consumption of plasma glucose and therefore a faster exhaustion. As cortisol levels were quite similar in both groups, it seems that the plasma glucose levels were sustained in the same pattern under cortisol mediation preventing hypoglycaemia and exhaustion (Soengas et al., 1992; Specker, 1982; Van Der Boon et al., 1991).

Glucocorticoid receptor (*gr1*) mRNA levels increased in both groups between 72 h and 120 h post injection when glucose levels were the lowest. Sathiyaa and Vijayan, (2003) demonstrated an upregulation of *gr1* mRNA abundance induced by cortisol in trout hepatocytes and that this higher content in mRNA corresponded to a lower protein expression. In liver, applying cortisol treatment mimicking physiologically elevated plasma concentration led to the increase in *gr1* mRNA levels and a downregulation of gr1 protein content (Vijayan et al., 2003). Cortisol is known to sustain higher glucose production during stress (Faught et al., 2016) and this response seems to be due to hepatic gluconeogenesis mediated by glucocorticoids (Mommsen et al., 1999; Vijayan et al., 1997, 1996, 1994). The higher content in *gr1* mRNA was already found concomitant with a higher content in a glucocorticoid-responsive gene mRNA coding for a key gluconeogenic enzyme the phosphoenolpyruvate carboxykinase (Vijayan et al., 2003). This regulation seems to have partially participated in maintaining plasma glucose levels to face the increased demand relating to the allostatic charge.

The increase in relative expression of galactokinase2 (*galk2*) gene occurred in both groups 120 h after injection when plasma glucose levels dropped. The gene *galk2* is involved in

Leloir pathway converting α-D-galactose into galactose 1-phosphate. Leloir pathway leads to the production of the metabolically useful glucose 1-phosphate from β-D-galactose (Holden et al., 2003). This pathway occurs in the liver and is involved in maintaining glucose levels in blood when necessary. Using one of the minor carbohydrate pathways instead of using body reserves in glycogen and lipids may be one compensatory mechanism to the glucose consumption that occurred during the experiment.

Apolipoprotein A1 mRNA content increased at 120 h after injection in both groups. This protein is involved in reverse cholesterol transport from peripheral tissues to liver before redistributing or removing it. Stressors exposure can induce the expression of *apoa1* (Lu et al., 2012; Simmons et al., 2017; Skolness et al., 2012). As plasma cortisol levels were relatively higher than the levels observed in non-stressed smolts (Carey and McCormick, 1998), they may have induced the expression of *apoa1*. It has been reported that the level of some apolipoprotein isoforms such as apoE, apoA1/A2 increased participating to a more efficient lipid transport to target tissues to sustain the increased energetic demand during confinement stress or bacterial infection in Eurasian perch *Perca fluviatilis* or common carp *Cyprinus carpio* (Concha et al., 2003; Douxfils et al., 2012). Moreover, Concha et al., (2004) reported antimicrobial acivity of apoA1/A2 in common carp and a synergism between apoA1 synthetic peptid and lysozyme suggesting the important role of this multifunctional protein in the innate defence in fish.

Growth hormone GH and Insulin-like growth factors (IGFs) are central to the smolting process (McCormick et al., 2013). Their plasma levels increase at the early stages of this process (February for IGF1 and March for GH, McCormick et al., 2013). The increase of mRNA levels of those proteins in all groups in a timely manner seems to be related to the progression of the smoltification process. Those results suggest that the potential stress due to the experiment did not negatively affect the ability of fish to undergo the smoltification process.

4.3. Disruption in immune response and oxidative stress defence

Plasma complement and peroxidase activities were affected by the passage through the turbine. Complement activity was lower in HPP group at 24 h after injection compared to control group and decreased in all groups afterwards while peroxidase activity was higher in HPP group at 120 h after injection. A transient increase in mRNA content due to the passage in the turbine occurred for lysozyme G (*lysg*) and eosinophil peroxidase (*mpo*) between 24 h and 72 h post injection while immunoglobulin M (*igm*) increased over the time for HPP fish. Complement 3 (*c3*) mRNA did not show any difference while Cytochrome C oxidase subunit

II (*cox2*) mRNA content increased only on 120 h ai in both groups. During the smoltification process, fish may experience a massive immune suppression (Johansson et al., 2016) with decrease in plasma lysozyme and IgM levels (Melingen et al., 1995; Muona and Soivio, 1992). However, the passage through the turbine induced a transient increase in some immune parameters and oxidative stress defence in this study. This increase may be due to a transient immunostimulation due to the stress (Bonga, 1997; Nardocci et al., 2014; Tort, 2011). In fact, acute stress over a short time duration such as the passage through a turbine may activate some immune functions such as enhancing the innate response and leukocyte mobilization (Nardocci et al., 2014; Tort, 2011). However, the cumulative impact of this kind of stress have to be considered. Chronic stress affects negatively the immune system and the energetic-metabolic machinery and leads to an increasing pathogen susceptibility (Nardocci et al., 2014; Tort, 2011).

Cell antioxidant defences protect the cells against reactive oxygen species (ROS) damages (Di Giulio and Meyer, 2008). Those defences include glutathione peroxidases, catalase, transferases, superoxide dismutase, xanthine oxidase and glucose 6-phosphate dehydrogenase (Slaninova et al., 2009). Eosinophil peroxidase (*mpo*) mRNA content and plasma peroxidase levels increase in HPP group may suggest that the passage through the turbine can induce ROS production and lead to damages to cell structure and DNA, lipid peroxidation and protein oxidation (Das and White, 2002; Lawson et al., 2018).

5. Conclusions

It was unexpected that plasma cortisol levels were not affected by the passage through the turbine. However, fish handling seems to be stressful for all groups and led to a general increase of cortisol in fish regardless of their treatment. Eventually, the speed and the water height in association with protruding structures during the passage over the spillways may lead to strikes and shocks and therefore being quite harmful and/or stressful to fish.

The passage through the turbine disrupted lightly carbohydrate metabolism and glucose production and consumption. It seems that the stress and the energy expenditure due to the confrontation with the turbine increased the glucose demand and caused a faster drop in plasma glucose levels in HPP group.

However, the passage through the turbine enhanced innate immune response and oxidative stress defence mechanisms. This immunostimulation seems to be positive but it is well known that a more chronic stress will lead to immune system depression. The cumulative impact of the passage through many turbines need to be investigated as it can represent a chronic stress affecting negatively the immune system and increasing the susceptibility to pathogens.

This work provided some clues explaining the delayed mortality – observed in many studies – that leads to a poor success of restocking programs. Turbines not only have a direct impact in terms of mortalities and injuries but also an indirect one in terms of fish behaviour and physiological and immune changes that can compromise the ability of Atlantic salmon smolts to escape successfully to the ocean.

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Bibliography

Baekelandt, S., Mandiki, S.N.M., Schmitz, M., Kestemont, P., 2019. Influence of the light spectrum on the daily rhythms of stress and humoral innate immune markers in pikeperch Sander lucioperca. Aquaculture 499, 358–363. https://doi.org/10.1016/j.aquaculture.2018.09.046

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R.H.B., Singmann, H., Dai, B., Grothendieck, G., Maech, Maechler, M., Bolker, B., Walker, S., Christensen, R.H.B., Singmann, H., Dai, B., Grothendieck, G., 2014. Package lme4. R Found. Stat. Comput. Vienna 4, 14–24. https://doi.org/10.1111/j.2041-210x.2012.00251.x

Bernard, B., Sobandi, K.C., Darras, V., Rollin, X., Mandiki, S.N.M., Kestemont, P., 2018. Influence of strain origin on osmoregulatory and endocrine parameters of two non-native strains of Atlantic salmon (Salmo salar L.). Gen. Comp. Endocrinol. 258, 205–212. https://doi.org/10.1016/j.ygcen.2018.01.004

Bickford, S.A., Skalski, J.R., 2000. Reanalysis and Interpretation of 25 Years of Snake–Columbia River Juvenile Salmonid Survival Studies. North Am. J. Fish. Manag. 20, 53–68. https://doi.org/10.1577/1548-8675(2000)020<0053:raioyo>2.0.co;2

Bonga, S.E.., 1997. The Stress Response in Fish. Physiol. Rev. 77, 591–625. https://doi.org/10.1152/physrev.1997.77.3.591

Brackley, R., Lucas, M.C., Thomas, R., Adams, C.E., Bean, C.W., 2018. Comparison of damage to live v. euthanized Atlantic salmon Salmo salar smolts from passage through an Archimedean screw turbine. J. Fish Biol. 92, 1635–1644. https://doi.org/10.1111/jfb.13596

Brevé, N., Vis, H., Spierts, I., de Laak, G., Moquette, F., Breukelaar, A., 2014. Exorbitant mortality of hatchery-reared Atlantic salmon smolts Salmo salar L., in the Meuse river system in the Netherlands. J. Coast. Conserv. 18, 97–109. https://doi.org/10.1007/s11852-013-0237-4

Čada, G., Loar, J., Garrison, L., Fisher, R., Neitzel, D., 2006. Efforts to reduce mortality to hydroelectric turbine-passed fish: Locating and quantifying damaging shear stresses. Environ. Manage. 37, 898–906. https://doi.org/10.1007/s00267-005-0061-1

Carey, J.B., McCormick, S.D., 1998. Atlantic salmon smolts are more responsive to an acute handling and confinement stress than parr. Aquaculture 168, 237–253. https://doi.org/10.1016/S0044-8486(98)00352-4

Concha, M.I., Molina, S., Oyarzún, C., Villanueva, J., Amthauer, R., 2003. Local expression of apolipoprotein A-I gene and a possible role for HDL in primary defence in the carp skin. Fish Shellfish Immunol. 14, 259–273. https://doi.org/10.1006/fsim.2002.0435

Concha, M.I., Smith, V.J., Castro, K., Bastías, A., Romero, A., Amthauer, R.J., 2004. Apolipoproteins A-I and A-II are potentially important effectors of innate immunity in the teleost fish Cyprinus carpio. Eur. J. Biochem. 271, 2984–2990. https://doi.org/10.1111/j.1432-1033.2004.04228.x

Cornet, V., Ouaach, A., Mandiki, S.N.M., Flamion, E., Ferain, A., Van Larebeke, M., Lemaire, B., Reyes López, F.E., Tort, L., Larondelle, Y., Kestemont, P., 2018. Environmentally-realistic concentration of cadmium combined with polyunsaturated fatty acids enriched diets modulated non-specific immunity in rainbow trout. Aquat.

528 Toxicol. 196, 104–116. https://doi.org/10.1016/j.aquatox.2018.01.012

529 Coutant, C.C., Whitney, R.R., 2000. Transactions of the American Fisheries Society Fish
530 Behavior in Relation to Passage through Hydropower Turbines : A Review Fish
531 Behavior in Relation to Passage through Hydropower Turbines : A Review. Trans. Am.
532 Fish. Soc. 8487, 37–41. https://doi.org/10.1577/1548-8659(2000)129<0351

533 Das, K.C., White, C.W., 2002. Redox systems of the cell: possible links and implications.
534 Proc. Natl. Acad. Sci. 99, 9617–9618.

535 Di Giulio, R.T., Meyer, J.N., 2008. Reactive oxygen species and oxidative stress, in: Di
536 Guilio, R.T., Hinton, D.E. (Eds.), The Toxicology of Fishes. CRC Press, Boca Raton,
537 FL, USA, pp. 273–324. https://doi.org/10.1201/9780203647295

538 Douxfils, J., Deprez, M., Mandiki, S.N.M., Milla, S., Henrotte, E., Mathieu, C., Silvestre, F.,
539 Vandecan, M., Rougeot, C., Mélard, C., Dieu, M., Raes, M., Kestemont, P., 2012.
540 Physiological and proteomic responses to single and repeated hypoxia in juvenile
541 Eurasian perch under domestication - Clues to physiological acclimation and humoral
542 immune modulations. Fish Shellfish Immunol. 33, 1112–1122.
543 https://doi.org/10.1016/j.fsi.2012.08.013

544 EDF Luminus, 2015. Andenne et Lixhe, les compactes [WWW Document]. URL
545 https://www.luminus.be/fr/corporate/activites/energie-hydroelectrique/andenne/

546 Fabbri, E., Moon, T.W., 2016. Adrenergic signaling in teleost fish liver, a challenging path.
547 Comp. Biochem. Physiol. Part - B Biochem. Mol. Biol. 199, 74–86.
548 https://doi.org/10.1016/j.cbpb.2015.10.002

549 Faught, E., Aluru, N., Vijayan, M.M., 2016. The Molecular Stress Response, Fish Physiology.
550 Elsevier Inc. https://doi.org/10.1016/B978-0-12-802728-8.00004-7

551 Ferguson, J.W., Absolon, R.F., Carlson, T.J., Sandford, B.P., 2006. Evidence of Delayed
552 Mortality on Juvenile Pacific Salmon Passing through Turbines at Columbia River
553 Dams. Trans. Am. Fish. Soc. 135, 139–150. https://doi.org/10.1577/t05-080.1

554 Forseth, T., Barlaup, B.T., Finstad, B., Fiske, P., Gjøsæter, H., Falkegård, M., Hindar, A., Mo,
555 T.A., Rikardsen, A.H., Thorstad, E.B., Vøllestad, L.A., Wennevik, V., 2017. The major
556 threats to Atlantic salmon in Norway. ICES J. Mar. Sci. 74, 1496–1513.
557 https://doi.org/10.1093/icesjms/fsx020

558 Fox, J., Weisberg, S., Adler, D., Bates, D., Baud-, G., Ellison, S., Firth, D., Friendly, M., Gor-
559 , G., Graves, S., Heiberger, R., Laboissiere, R., Mon-, G., Murdoch, D., Nilsson, H.,
560 Ogle, D., Rip-, B., 2014. Package 'car' (Version 2.1-3), Second. ed, URL https://r-
561 forge.r-project.org/projects/car/, http://CRAN.R-project.org/package=car,
562 http://socserv.socsci.mcmaster.ca/jfox/Books/Companion/index.html. SAGE
563 Publications, Inc.

564 Freeman, M.C., Pringle, C.M., Greathouse, E.A., Freeman, B.J., 2003. Ecosystem-Level
565 Consequences of Migratory Faunal Depletion Caused by Dams. Am. Fish. Soc. Symp.
566 35, 255–266.

567 Havn, T.B., Sæther, S.A., Thorstad, E.B., Teichert, M.A.K., Heermann, L., Diserud, O.H.,
568 Borcherding, J., Tambets, M., Økland, F., 2017. Downstream migration of Atlantic
569 salmon smolts past a low head hydropower station equippped with Archimedes screw
570 and Francis turbines. Ecol. Eng. 105, 262–275.

https://doi.org/10.1016/j.ecoleng.2017.04.043

Havn, T.B., Thorstad, E.B., Borcherding, J., Heermann, L., Teichert, M.A.K., Ingendahl, D., Tambets, M., Sæther, S.A., Økland, F., 2020. Impacts of a weir and power station on downstream migrating Atlantic salmon smolts in a German river. River Res. Appl. 1–13. https://doi.org/10.1002/rra.3590

Hervé, M., 2015. R package 'RVAideMemoire': Diverse Basic Statistical and Graphical Functions. Version: 0.9-45-2.

Holden, H.M., Rayment, I., Thoden, J.B., 2003. Structure and Function of Enzymes of the Leloir Pathway for Galactose Metabolism. J. Biol. Chem. 278, 43885–43888. https://doi.org/10.1074/jbc.R300025200

Johansson, L.H., Timmerhaus, G., Afanasyev, S., Jørgensen, S.M., Krasnov, A., 2016. Smoltification and seawater transfer of Atlantic salmon (Salmo salar L.) is associated with systemic repression of the immune transcriptome. Fish Shellfish Immunol. 58, 33–41. https://doi.org/10.1016/j.fsi.2016.09.026

Jonsson, B., Jonsson, N., 2011. Ecology of Atlantic Salmon and Brown Trout: Habitats as Template for Life Histories, FISH & FISHERIES SERIES 33. Science, Springer 2011, Business Media B.V. https://doi.org/DOI 10.1007/978-94-007-1189-1_11

Katopodis, C., Williams, J.G., 2012. The development of fish passage research in a historical context. Ecol. Eng. 48, 8–18. https://doi.org/10.1016/j.ecoleng.2011.07.004

Kibel, P., Coe, T., 2007. Fish monitoring and live trials. Archimedes screw turbine, River Dart. Phase I Report: Live fish trials, smolts, leading edge assessment, disorientation study, outflow monitoring. Fishtek. Moretonhampstead, Devon.

Kiilerich, P., Kristiansen, K., Madsen, S.S., 2007. Hormone receptors in gills of smolting Atlantic salmon, Salmo salar: Expression of growth hormone, prolactin, mineralocorticoid and glucocorticoid receptors and 11β-hydroxysteroid dehydrogenase type 2. Gen. Comp. Endocrinol. 152, 295–303. https://doi.org/10.1016/j.ygcen.2006.12.018

King, S., O'Hanley, J.R., 2016. Optimal Fish Passage Barrier Removal-Revisited. River Res. Appl. 32, 418–428. https://doi.org/10.1002/rra.2859

Larinier, M., 2001. Environmental issues, dams and fish migration. FAO Fish. Tech. Pap. 419, 45–89.

Larinier, M., 2008. Fish passage experience at small-scale hydro-electric power plants in France. Hydrobiologia 609, 97–108. https://doi.org/10.1007/s10750-008-9398-9

Larinier, M., Travade, F., 2002. Downstream migration: problems and facilities. Bull. Fr. Pech. Piscic. 364 suppl., 181–207.

Lawson, M., Jomova, K., Poprac, P., Kuca, K., Musílek, K., Valko, M., 2018. Free radicals and antioxidants in human disease. Nutr. Antioxid. Ther. Treat. Perspect. 39, 283–305. https://doi.org/10.1007/978-3-319-67625-8_12

Lenth, R., Singmann, H., Love, J., Buerkner, P., Herve, M., 2019. Package "emmeans": Estimated marginal means, aka least-squares means. Compr. R Arch. Netw 1–67.

Lu, X.J., Chen, J., Huang, Z.A., Zhuang, L., Peng, L.Z., Shi, Y.H., 2012. Influence of acute cadmium exposure on the liver proteome of a teleost fish, ayu (Plecoglossus altivelis).

613 Mol. Biol. Rep. 39, 2851–2859. https://doi.org/10.1007/s11033-011-1044-3

614 Mathers, R.G., De Carlos, M., Crowley, K., Ó Teangana, D., 2002. A review of the potential
615 effect of Irish hydroelectric installations on Atlantic salmon (Salmo salar L.) populations,
616 with particular reference to the River Erne. Biol. Environ. 102, 69–79.
617 https://doi.org/10.3318/BIOE.2002.102.2.69

618 Mathur, D., Heisey, P.G., Skalski, J.R., Kenney, D.R., 2000. Salmonid Smolt Survival
619 Relative To Turbine Efficiency and Entrainment Depth in Hydroelectric Power
620 Generation. J. Am. Water Resour. Assoc. 36, 737–747.
621 https://doi.org/https://doi.org/10.1111/j.1752-1688.2000.tb04302.x

622 McCormick, S.D., Hansen, L.P., Quinn, T.P., Saunders, R.L., 1998. Movement, migration,
623 and smolting of Atlantic salmon (Salmo salar). Can. J. Fish. Aquat. Sci. 55, 77–92.
624 https://doi.org/10.1139/cjfas-55-S1-77

625 McCormick, S.D., Sheehan, T.F., Björnsson, B.T., Lipsky, C., Kocik, J.F., Regish, A.M.,
626 O'Dea, M.F., 2013. Physiological and endocrine changes in Atlantic salmon smolts
627 during hatchery rearing, downstream migration, and ocean entry. Can. J. Fish. Aquat.
628 Sci. 70, 105–118. https://doi.org/10.1139/cjfas-2012-0151

629 McKay, S.K., Cooper, A.R., Diebel, M.W., Elkins, D., Oldford, G., Roghair, C., Wieferich,
630 D., 2017. Informing Watershed Connectivity Barrier Prioritization Decisions: A
631 Synthesis. River Res. Appl. 33, 847–862. https://doi.org/10.1002/rra.3021

632 Melingen, G.O., Stefansson, S.O., Berg, A., Wergeland, H.I., 1995. Changes in serum protein
633 and IgM concentration duringsmolting and early post-smolt period in vaccinated and
634 unvaccinated Atlantic salmon (Salmo salar L.). Fish Shellfish Immunol. 5, 211–221.

635 Metzger, D.C., Luckenbach, J.A., Dickey, J.T., Beckman, B.R., 2013. Development of a
636 multiplex gene expression assay for components of the endocrine growth axis in coho
637 salmon. Gen. Comp. Endocrinol. 189, 134–140.
638 https://doi.org/10.1016/j.ygcen.2013.04.022

639 Mommsen, T.P., Vijayan, M.M., Moon, T.W., 1999. Cortisol in teleosts: dynamics,
640 mechanisms of action, and metabolic regulation. Rev. Fish Biol. Fish. 9, 211–268.

641 Muona, M., Soivio, A., 1992. Changes in plasma lysozyme and blood leucocyte levels of
642 hatchery-reared Atlantic salmon (Salmo salar L.) and sea trout (Salmo trutta L.) during
643 parr-smolt transformation. Aquaculture 106, 75–87.

644 Myrnes, B., Seppola, M., Johansen, A., Øverbø, K., Callewaert, L., Vanderkelen, L.,
645 Michiels, C.W., Nilsen, I.W., 2013. Enzyme characterisation and gene expression
646 profiling of Atlantic salmon chicken- and goose-type lysozymes. Dev. Comp. Immunol.
647 40, 11–19. https://doi.org/10.1016/j.dci.2013.01.010

648 Nagrodski, A., Raby, G.D., Hasler, C.T., Taylor, M.K., Cooke, S.J., 2012. Fish stranding in
649 freshwater systems: Sources, consequences, and mitigation. J. Environ. Manage. 103,
650 133–141. https://doi.org/10.1016/j.jenvman.2012.03.007

651 Nardocci, G., Navarro, C., Cortés, P.P., Imarai, M., Montoya, M., Valenzuela, B., Jara, P.,
652 Acuña-Castillo, C., Fernández, R., 2014. Neuroendocrine mechanisms for immune
653 system regulation during stress in fish. Fish Shellfish Immunol. 40, 531–538.
654 https://doi.org/10.1016/j.fsi.2014.08.001

655 Nehlsen, W., Williams, J.E., Lichatowich, J.A., 1991. Pacific Salmon at the Crossroads:

656 Stocks at Risk from California, Oregon, Idaho, and Washington. Fisheries 16, 4–21.
657 https://doi.org/10.1577/1548-8446(1991)016<0004:psatcs>2.0.co;2

658 Nyqvist, D., Greenberg, L.A., Goerig, E., Calles, O., Bergman, E., Ardren, W.R., Castro-
659 Santos, T., 2017. Migratory delay leads to reduced passage success of Atlantic salmon
660 smolts at a hydroelectric dam. Ecol. Freshw. Fish 26, 707–718.
661 https://doi.org/10.1111/eff.12318

662 Olsvik, P.A., Vikeså, V., Lie, K.K., Hevrøy, E.M., 2013. Transcriptional responses to
663 temperature and low oxygen stress in Atlantic salmon studied with next-generation
664 sequencing technology. BMC Genomics 14. https://doi.org/10.1186/1471-2164-14-817

665 Parrish, D.L., Behnke, R.J., Gephard, S.R., Mccormick, S.D., Reeves, G.H., 1998. Why aren't
666 there more Atlantic salmon (Salmo salar)? Can. J. Fish. Aquat. Sci. 55 Suppl.1, 281–287.
667 https://doi.org/10.1139/cjfas-55-S1-281

668 Persson, L., Kagervall, A., Leonardsson, K., Royan, M., Alanärä, A., 2019. The effect of
669 physiological and environmental conditions on smolt migration in Atlantic salmon
670 Salmo salar. Ecol. Freshw. Fish 28, 190–199. https://doi.org/10.1111/eff.12442

671 Pracheil, B.M., DeRolph, C.R., Schramm, M.P., Bevelhimer, M.S., 2016. A fish-eye view of
672 riverine hydropower systems: the current understanding of the biological response to
673 turbine passage. Rev. Fish Biol. Fish. 26, 153–167. https://doi.org/10.1007/s11160-015-
674 9416-8

675 Pringle, C.M., 2003. What is hydrologic connectivity and why is it ecologically important?
676 Hydrol. Process. 17, 2685–2689. https://doi.org/10.1002/hyp.5145

677 Quade, M.J., Roth, J.A., 1997. A rapid, direct assay to measure degranulation of bovine
678 neutrophil primary granules. Vet. Immunol. Immunopathol. 58, 239–248.
679 https://doi.org/10.1016/S0165-2427(97)00048-2

680 R Core team, 2019. R: A Language and Environment for Statistical Computing. Version
681 3.6.2.

682 Renardy, S., Benitez, J.P., Tauzin, A., Dierckx, A., Nzau Matondo, B., Ovidio, M., 2019.
683 How and where to pass? Atlantic salmon smolt's behaviour at a hydropower station
684 offering multiple migration routes. Hydrobiologia. https://doi.org/10.1007/s10750-019-
685 04108-w

686 Rivinoja, P., 2005. Migration Problems of Atlantic Salmon (Salmo salar L.) in Flow
687 Regulated Rivers.

688 Sathiyaa, R., Vijayan, M.M., 2003. Autoregulation of glucocorticoid receptor by cortisol in
689 rainbow trout hepatocytes. Am J Physiol Cell Physiol 284, 1508–1515.
690 https://doi.org/10.1152/ajpcell.00448.2002.-We

691 Schmalz, W., Wagner, F., Sonny, D., 2015. Arbeitshilfe zur standörtlichen Evaluierung des
692 Fischschutzes und Fischabstieges, Fischschutz und Fischabstieg.

693 Simmons, D.B.D., Miller, J., Clarence, S., McCallum, E.S., Balshine, S., Chandramouli, B.,
694 Cosgrove, J., Sherry, J.P., 2017. Altered expression of metabolites and proteins in wild
695 and caged fish exposed to wastewater effluents in situ. Sci. Rep. 7, 1–14.
696 https://doi.org/10.1038/s41598-017-12473-6

697 Skolness, S.Y., Durhan, E.J., Jensen, K.M., Kahl, M.D., Makynen, E.A., Villeneuve, D.L.,

698 Ankley, G.T., 2012. Effects of gemfibrozil on lipid metabolism, steroidogenesis, and
699 reproduction in the fathead minnow (Pimephales promelas). Environ. Toxicol. Chem. 31,
700 2615–2624. https://doi.org/10.1002/etc.1989

701 Slaninova, A., Smutna, M., Modra, H., Svobodova, Z., 2009. A review: Oxidative stress in
702 fish induceed by pesticides. Neuroendocr. Lett. 30, 2–12. https://doi.org/NEL300709R01
703 [pii]

704 Soengas, J.L., Rey, P., Rozas, G., Andrés, M.D., Aldegunde, M., 1992. Effects of cortisol and
705 thyroid hormone treatment on the glycogen metabolism of selected tissues of
706 domesticated rainbow trout, Oncorhynchus mykiss. Aquaculture 101, 317–328.
707 https://doi.org/10.1016/0044-8486(92)90034-I

708 Specker, J.L., 1982. Interrenal function and smoltification. Aquaculture 28, 59–66.
709 https://doi.org/10.1016/0044-8486(82)90008-4

710 Thorstad, E.B., Havn, T.B., Sæther, S.A., Heermann, L., Teichert, M.A.K., Diserud, O.H.,
711 Tambets, M., Borcherding, J., Økland, F., 2017. Survival and behaviour of Atlantic
712 salmon smolts passing a run-of-river hydropower facility with a movable bulb turbine.
713 Fish. Manag. Ecol. 24, 199–207. https://doi.org/10.1111/fme.12216

714 Thorstad, E.B., Whoriskey, F., Rikardsen, A.H., Aarestrup, K., 2011. Aquatic nomads: the life
715 and migrations of the Atlantic salmon, in: Atlantic Salmon Ecology. Wiley-Blackwell:
716 Singapore, pp. 1–32. https://doi.org/10.1002/9781444327755.ch1

717 Thorstad, E.B., Whoriskey, F., Uglem, I., Moore, A., Rikardsen, A.H., Finstad, B., 2012. A
718 critical life stage of the Atlantic salmon Salmo salar: Behaviour and survival during the
719 smolt and initial post-smolt migration. J. Fish Biol. 81, 500–542.
720 https://doi.org/10.1111/j.1095-8649.2012.03370.x

721 Tipsmark, C.K., Madsen, S.S., 2009. Distinct hormonal regulation of Na+,K+-atpase genes in
722 the gill of Atlantic salmon (Salmo salar L.). J. Endocrinol. 203, 301–310.
723 https://doi.org/10.1677/JOE-09-0281

724 Tort, L., 2011. Stress and immune modulation in fish. Dev. Comp. Immunol. 35, 1366–1375.
725 https://doi.org/10.1016/j.dci.2011.07.002

726 Trinder, P., 1969. Determination of glucose in blood using glucose oxidase with an alternative
727 oxygen acceptor. Ann. Clin. Biochem. 6, 24–27.

728 Van Der Boon, J., Van Den Thillart, G.E.E.J.M., Addink, A.D.F., 1991. The effects of
729 cortisol administration on intermediary metabolism in teleost fish. Comp. Biochem.
730 Physiol. -- Part A Physiol. 100, 47–53. https://doi.org/10.1016/0300-9629(91)90182-C

731 Vijayan, M.M., Mommsen, T.P., Glémet, H.C., Moon, T.W., 1996. Metabolic effects of
732 cortisol treatment in a marine teleost, the sea raven. J. Exp. Biol. 199, 1509–1514.

733 Vijayan, M.M., Pereira, C., Grau, E.G., Iwama, G.K., 1997. Metabolic responses associated
734 with confinement stress in tilapia: the role of cortisol. Comp. Biochem. Physiol. Part C
735 Pharmacol. Toxicol. Endocrinol. 116, 89–95.

736 Vijayan, M.M., Raptis, S., Sathiyaa, R., 2003. Cortisol treatment affects glucocorticoid
737 receptor and glucocorticoid-responsive genes in the liver of rainbow trout. Gen. Comp.
738 Endocrinol. 132, 256–263. https://doi.org/10.1016/S0016-6480(03)00092-3

739 Vijayan, M.M., Reddy, P.K., Leatherland, J.F., Moon, T.W., 1994. The effects of cortisol on

740 hepatocyte metabolism in rainbow trout: a study using the steroid analogue RU486. Gen.
741 Comp. Endocrinol. 96, 75–84.

742 Zydlewski, J., Zydlewski, G., Danner, G.R., 2010. Descaling Injury Impairs the
743 Osmoregulatory Ability of Atlantic Salmon Smolts Entering Seawater. Trans. Am. Fish.
744 Soc. 139, 129–136. https://doi.org/10.1577/t09-054.1

745

746 Table 1: Primers sequences used for analyses of selected genes expression in *Salmo salar*

	Gene	Accession	Primers (5'-3')	Protein	Function	Reference
Liver	*hsp70*	BG933934	CCCCTGTCCCTGGGTATTG CACCAGGCTGGTTGTCTGAGT	Heat shock protein 70	Stress response	Olsvik et al., 2013
	gr1	AF209873	ACGACGATGGAGCCGAAC ATGGCTTTGAGCAGGGATAG	Glucocorticoid receptor	Stress response, carbohydrate metabolism and hormone regulation	Kiilerich et al., 2007
	galk2	BT045062.1	GGTTATGCTGTGCTCCCAAT TCATCCCAGACAGAGGAACC	Galactokinase 2	Carbohydrate metabolism	Bernard et al., submitted
	apoa1	NM_001123663.1	TGGTCCTCGCACTAACCATC GCAGTCAACTTCACCTGAGCTA	Apolipoprotein A-I		
	calm1	NM_001139713	CGACAAGGATGGTAACGGCT GTTGACAGTGAGTGTGTTGC	Calmodulin		
	cd36	NM_001124511	GGATGAACTCCCTGCAT TGAGGCCAAAGTACTCGTCGA	Cluster of differentiation 36		
	fads6	NM_001123575	TACCCAGTGGGCAAAGAGAC CAACGGCTTCAGAAACTTCC	Delta-6 fatty acyl desaturase	Lipid metabolism	Bernard et al., submitted
	igf1	NM_001123623	GATGTCTTCAAGAGTGCGATGTG CGCCGAAGTCAGGGTTAGG	Insulin-like growth factor 1	Hormone regulation	Metzger et al., 2013
	igf2	NM_001123647	TGCCCACACTCAAACAGG CTTCCTCTGCCACACCTCA	Insulin-like growth factor 2		Bernard et al., submitted
	ghr1	AY462105	TCCCAACATGCAGCTGTAGA TGTGGCACCTTGAAGAACAG	Growth hormone receptor 1		Tipsmark and Madsen, 2009
Spleen	*igm*	Y12457.1	TGAGGAGAACTGTGGGCTACACT TGTTAATGACCACTGAATGTGCAT	Immunoglobuline M	Immune response	Cornet et al., personal data
	lysg	AM493682	GGCTGGGGTAGTGTCAATC TGACCTTGCTGCCATGAACA	Lysozyme G		Myrnes et al., 2013
	c3	BI468074	GTGACAGGTGGAGAGCAGA CCAGGCCAATATCCTCCCA	Complement C3		

Spleen	*mpo*	XM_014128513.1	GAGAGGTGCCTTGCTTCATAG ATCTTGCGAGCCTCCTGATA	Eosinophil peroxidase	Immune response and Oxidative stress defence	Cornet et al., personal data
	cox2		CTCTAAATCGTTTGGACTGTCCT AGGTGTGGGTCATTAATTTCGTC	Cytochrome C oxidase subunit II	Oxidative stress defence	Cornet et al., personal data
	β-actin	**BG933897**	ACTGGGACGACATGGAGAAG GGGGTGTTGAAGGTCTCAAA	Beta-actin	Reference gene	Cornet et al., personal data

747

Figure captions

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

Figure 2: Plasma cortisol (2-A) levels of control (white) and HPP (red) groups and changes in plasma glucose levels (2-B) of control and HPP groups depending on the interaction between sampling time and treatment. 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 ($p<0.05$).

Figure 3: Content of brain serotonin (3-A), dopamine (3-B), their metabolites 5HIAA (3-C) and DOPAC (3-D) and the serotonergic (3-E) and dopaminergic (3-F) ratios related to sampling time and treatment 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 ($p<0.05$).

Figure 4: Changes in peroxidase activity (4-A) and ACH50 levels (4-B) depending on the interaction between sampling time and treatment 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 ($p<0.05$).

Figure 5: Relative genes expression in liver in function of sampling time in control (white) and HPP (red) groups. The horizontal line in the boxplot represents the median. Triangles represent the mean. Lower case letters indicate significant differences among the sampling times ($p<0.05$).

Figure 6: Relative genes expression in spleen in function of sampling time (6-D) or of the interaction between sampling time and treatment (6-A, B, and E) 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 ($p<0.05$).

Highlights :

- Fish passage through turbine not only affect survival but physiological condition too
- This passage disrupted carbohydrate metabolism increasing glucose demand
- It also enhanced innate immune response and oxidative stress defence mechanisms
- The cumulative impact can represent a chronic stress and need further investigation
- This impact may be a clue to explain the delayed mortality in migrating fish

Graphical Abstract

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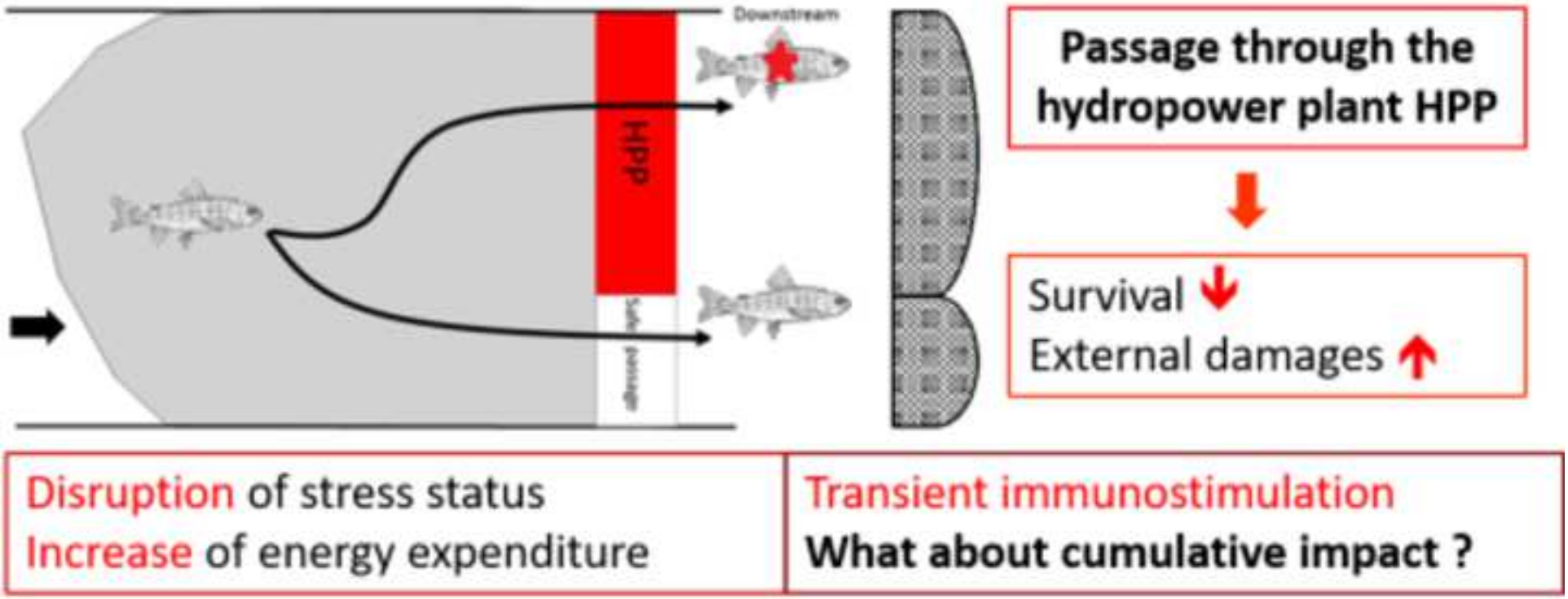

How the passage through a hydropower plant affects the physiological and health status of Atlantic salmon smolts?

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Abstract

Atlantic salmon is an anadromous species migrating from upper-reach nursery areas in rivers to the oceanic feeding areas at smolt stage and inversely at adult stage requiring unimpeded migration routes. However, dams associated with hydroelectric power plants (HPP) disrupt river connectivity and affect fish movement and survival. The objective of the current study was to evaluate the short and mid-term physiological and immune response of Atlantic salmon smolts after passing through Andenne HPP (Meuse River, Belgium). Several parameters were studied after an *in situ* deliberate passage including direct mortality and external damages, stress and immune biomarkers as plasma cortisol and glucose levels, complement and peroxidase activities, and immune and oxidative stress related gene expression 24 h, 72 h and 120 h after passage. Survival rate was lower and external damages were more important in fish that confronted the HPP compared to the control ones. Moreover, the passage through the turbine affected plasma glucose levels, complement and peroxidase activities and the expression of some immune genes such as *lysg*, *igm* and *mpo* in a timely manner suggesting that this passage can lead to a great energy expenditure and a disruption of innate immunity. Our observations can partially explain the delayed mortality observed in many studies leading to a poor success of restocking programs. HPPs not only have a direct impact in terms of mortalities and injuries but also an indirect one in terms of physiological and immune changes that can compromise Atlantic salmon smolts ability to escape successfully to the ocean.

Keywords: Hydropower plant, Atlantic salmon smolts, downstream migration, physiological and health status

1. Introduction

Anthropogenic activities as dams, navigation weirs and hydropower stations have led to the reduction of hydrological connectivity (King and O'Hanley, 2016; Larinier, 2001; McKay et al., 2017; Pringle, 2003). These activities have well documented effects such as the delay or the total prevention of fish migratory movements, fish stranding, and mortalities directly and/or indirectly linked to the passages through hydropower plants (HPP) and over the spillways (Freeman et al., 2003; Katopodis and Williams, 2012; Larinier and Travade, 2002; Nagrodski et al., 2012; Renardy et al., 2019). During their passage through the turbines, fish are subjected to various forms of stress that can cause high mortality as strike from parts of the HPP, sudden speed and pressure changes, shear, and cavitation (Coutant and Whitney, 2000; Larinier and Travade, 2002; Mathur et al., 2000; Rivinoja, 2005). Numerous studies were conducted on different types of turbines but only focused on determining the direct (*e.g.* mortality from HPP blade strikes) and indirect (*e.g.* delayed mortality due to minor injuries) fish mortality and damage rates, mainly using telemetric methods or the simulation of the passage through the turbine (Brackley et al., 2018; Ferguson et al., 2006; Havn et al., 2017; Kibel and Coe, 2007; Larinier and Travade, 2002). The better survival rates are higher than 90% in "environmentally friendly" turbines, but it can be lower than 60% in other common used turbine designs (Bickford and Skalski, 2000; Havn et al., 2017; Thorstad et al., 2012). In Kaplan turbines, for example, total mortality rate (combining both direct and delayed) can vary from below 5% to 46 depending on the characteristics of the turbine and fish species and size (Bickford and Skalski, 2000; Čada et al., 2006; Coutant and Whitney, 2011; Larinier, 2008; Larinier and Travade, 2002; Thorstad et al., 2012). However, there is no information about the physiological and health condition of surviving and unharmed fish.

Atlantic salmon (*Salmo salar* Linnaeus, 1758) is an anadromous species that migrates between spawning and nursery habitats in rivers, and feeding and growth areas in the ocean (Thorstad et al., 2011). This species has experienced severe reductions and even the extinction of some strains in Europe and North-America due to the disruption of river connectivity and the limited access to functional habitats (Forseth et al., 2017; Freeman et al., 2003; Nehlsen et al., 1991; Parrish et al., 1998). To prevent population depletion and support commercial and recreational fisheries, many restoration and/or compensatory salmon hatchery-rearing programmes have been established in Europe and North America (Jonsson and Jonsson, 2011). However, the success of such programmes is mitigated and depend on many factors including the quality, size and density of the fish, and time and place of the stocking (Jonsson and Jonsson, 2011; Persson et al., 2019). The decrease of water flow due to the HPP intake

can dramatically decrease the carrying capacity for Atlantic salmon smolts in save passage forcing them to pass through the turbine and compromising the success of the releases (Brevé et al., 2014; Jonsson and Jonsson, 2011; Persson et al., 2019).

In many river systems such as in the Meuse River, Atlantic salmon smolts are confronted to many hydropower plants during their long travel to the sea, and the cumulative impact of these obstacles could constitute, as suggested by some authors, a persistent physiological stress that could impair the immune defence capacity (Thorstad et al., 2017, 2012). Moreover, smolts must complete their migration in a very narrow migration window and face physiological changes during the smoltification process (McCormick et al., 1998; Thorstad et al., 2012). The delay in downstream migration can represent a serious threat for the population maintenance (Mathers et al., 2002; Nyqvist et al., 2017). A disruption in the physiological status can lead to a great energy expenditure that can compromise further migration while a disruption in the immune status can increase fish vulnerability to pathogens and increase the delayed mortality. However, to our knowledge, no information is available about the physiological status and immune defence capacity of Atlantic salmon surviving after the HPP passage and the impact on their migration ability is still largely unknown.

The aim of this study was to assess how the passage through the turbine can affect the survival, the physiological and immune status of Atlantic salmon smolts by various key studying stress and immune biomarkers. We hypothesized that the passage through the turbine can lead to an elevated allostatic charge and affect directly or indirectly the immune system and thereby the overall physiological and health status of fish.

2. Materials and Methods

2.1. Animals and rearing conditions

Atlantic salmon parr (N=1400, mean length = 5.5 ± 0.4 cm) were transferred from CoSMos hatchery (Conservatoire du Saumon Mosan, Erezée, Belgium) to the facilities of the University of Namur in Belgium and were reared until the pre-smolt stage. During the parr stage, fish (about 300 per tank) were reared at 16°C in sub squared tanks of 100 L partially covered by PVC plates and fed at 3% of their weight with Nutra XP 0.5 (Skretting, Canada) and Coppens starts premium (1 mm, Alltech Coppens, Netherland). When fish size reached 8-9 cm, they were transferred into two 1m³ sub-squared tanks (500 per tank) partially covered with PVC plates and totally covered by nets, reared at 16°C and fed at 3% of their weight with Ultra 2 mm (Alltech Coppens, Netherland) (AquaTech, Austria) and Supreme 21 (3 mmAlltech Coppens, The Netherlands) using a belt feeder. During the whole rearing process,

temperature, pH and dissolved oxygen were checked every day using a multiparameter measuring device (MultiLine® Multi 3510, WTW, WVR). Water analysis (ammonia, nitrite, and nitrate) was done twice a week, and concentrations did not exceed 0.02, 0.1, and 2 mg/L, respectively. Since Meuse water temperature was about 8°C at the time of Atlantic salmon transfer, water temperature was progressively decreased in the rearing tanks during 10 days from 16 to 12°C in order to prepare the fish for natural conditions.

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).

2.2. Experimental protocol and sampling procedures

A total of 540 Atlantic salmons (age: 1 year, mean total length = 140.01 ± 10.16 mm, mean weight 25.5 ± 5.2 g) were transported to the Andenne hydropower plant (Anton Roadway 114-144, 5300 Andenne, Belgium, 50°29'30.3"N 5°04'11.9"E). During their transfer, fish were acclimated to the temperature and water quality changes during 4h by progressively adding the Meuse river water into the aerated transport tank. Then fish were allowed to recover into three 1m³ round tanks covered by nets (180 fish per tank) for four days before the experiment. This site was chosen because it was recently equipped with a bulb turbine – a variant of Kaplan-type turbine with a horizontal axis – that has four adjustable blades, a rotational speed of 176.47 rpm and a head of 5.35 m (EDF Luminus, 2015). This model often used on Atlantic salmon river (Thorstad et al., 2012) was meant to improve hydropower production efficiency and enable a broad operating range. As this turbine can function even with a low flow, the probability that the turbine will be in operation during Atlantic salmon downstream migration is relatively high compared to other models which cannot operate under these conditions. Moreover, two hydropower plants (Lixhe and Andenne) are equipped with bulb turbines in the Meuse River, which is our project area (LIFE4FISH). On the 4th of April 2019 (J0), the simulation of fish passage through the turbine was conducted according to Profish Technology (https://www.profish-technology.be/) method commonly used to study the incidence of the hydropower plant *in situ* (Brackley et al., 2018; Kibel and Coe, 2007). The deliberate passage through the turbines is a validated method in Germany, Austria and Switzerland (Schmalz et al., 2015). A total of 180 fish from each experimental tank were caught, transported quickly in a 100 L square tank and gently released from a bucket of water through a wetted flexible plastic pipe (20 cm of diameter) with its exit directly into the turbine intake itself (HPP group, N=2x180) or directly in the net for control group (N=180, Figure 1). During the simulation of the passage, the bulb turbine was set at its maximum intake capacity

(166 m^3/s) coupled with injection at the border of blades which represents the scenario that lead to the lowest survival rate in high water flow conditions. In those conditions, the blades are opened at their maximum improving fish survival. This scenario is the closest to the real operating conditions. After the passage, fish were recovered using a 50 meters' length net fixed on a metallic frame handled by a crane.

Then, fish were sorted into three groups immediately after their recovery:

- Group 1: dead fish + heavily injured ones,
- Group 2: surviving fish with non-life threating external injuries
- Group 3: surviving fish without any external injuries.

Fish from the first group were weighed (g), measured (mm), and examined in order to determine the causes of death. Fish from the second group were weighed, measured, examined and photographed in order to determine the injuries severity. The second and latter groups were put back in the tanks in maximum two hours while the heavily injured fish were euthanized using MS222 (240 mg/L).

The recovery, survival and external damage rates were calculated after retrieving the net as follows:

- $Recovery\ rate\ (\%) = \frac{Number\ of\ recovered\ fish \times 100}{Number\ of\ injected\ fish}$
- $Survival\ rate\ (\%) = \frac{Number\ of\ surviving\ fish \times 100}{Number\ of\ injected\ fish}$, as previous personal data of the same experiment in another site showed 100% of recovery rate after injection of anesthetized fish, assumption was made that the non-recovered fish succeeded in escaping the turbine and were considered alive.
- $External\ damage\ rate\ (\%) = \frac{Number\ of\ surviving\ and\ damaged\ fish \times 100}{Number\ of\ recovered\ and\ surviving\ fish}$

The severity of external damages was assessed post hoc from the photographs taken during the experiment according to Brackley et al., (2018). The damages were considered non-life threatening if fish displayed normal swimming behaviour in the two hours after the recovery and if the fish survived until the end of the monitoring period (120 h post injection). Scale loss were classified following the distribution across the fish's body: 0 – 1% negligible scale loss, 2 – 4% low scale loss, 5 – 9% moderate scale loss, 10 – 30% severe scale loss.

A total of 10 fish were sampled from control and HPP groups for blood (after anaesthesia with MS222, 120 mg/L) and brain, liver and spleen (after euthanasia with overdose of MS222, 240 mg/L) 24 h after injection (24 h pi), 72 h after injection (72 h pi) and 120 h after injection (120 h pi) in order to investigate the response of fish in the short and mid-term.

2.3. Stress indicators

Cortisol was assayed in duplicate using a cortisol ELISA kit (KAPDB270, Diasource, Belgium) following the manufacturer's instructions. The assay dynamic range was between 0 and 600 ng ml^{-1}. The intra-assay coefficient of variation and the analytical sensitivity were respectively 5.8%, and 4 ng ml^{-1}.

Plasma glucose, assayed in triplicate, was determined based on a glucose oxidase/peroxidase method described by Trinder (1969). Briefly, 20 µl of samples and standards were deproteinized using perchloric acid (0.33M) and 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). After an incubation of 15 min at 38°C, the absorbance was measured at 436 nm using the 96-well plate reader (FLUOstar® Omega, BMG LABTECH, Germany).

High performance liquid chromatography method was adapted from Baekelandt et al. (2019) in order to assess the serotonergic and dopaminergic activities expressed as hydroxyl-indole-acetic acid (5-HIAA)/serotonin (5-HT) and 3,4-dihydroxyphenylacetic acid (DOPAC)/dopamine (DA) ratios respectively in the whole fish brain. Brains were weighed and homogenized during 2 min at 8°C using a Bullet Blender Storm 24 (Next Advance) in tubes containing 2 mL/g of tissue absolute methanol (≥ 99.8%, HiPerSolv CHROMANORM, VWR, Belgium) and 0.5 mm zirconium oxide beads (Dutscher). Homogenates were then centrifuged (21 000g, 15 min, 4°C), supernatants were transferred to new tubes and centrifuged a second time before being filtered through 0.5 µm filters (Phenomenex). An aliquot (35 µL) of the filtrate was injected into the HPLC system. The procedure was carried out on ice. HPLC analysis was carried out using GP50 gradient pump (Dionex) equipped with an autosampler FAMOS (LC packings). The filtered homogenates were applied individually on a 2.6 µm particle size (150 × 4.6 mm, ID) C18 analytical Kinetex column (kept at 25°C) at 1 mL/min of mobile phase (65 mmol/L NaH_2PO_4, 1.63 mmol/L octane sulfonic acid, 0.1 mmol/L EDTA-Na^2, and 13% absolute methanol, pH = 2.79 adjusted with orthophosphoric acid). Neurohormones were monitored using a DC amperometry detector (Dionex) with Glassy Carbon Working Electrode (0.700 V, Ag/AgCl-P/N 061677). Chromeleon™ software 6.8 (Dionex) was used for data acquisition and processing. Standard solutions were serially diluted (from 250 nmol/L to 7.8 nmol/L) in absolute methanol from purified hormones (Sigma-Aldrich) and were treated similarly to samples. Concentrations of the compounds

were calculated by interpolation of their respective standard curves. The intra- and inter-assay coefficients of variation for tested hormones were under 5.9% and 7.4% respectively.

2.4. Humoral immune parameters

The plasma alternative complement pathway (ACH50) procedure measure the haemolytic activity in plasma samples using rabbit red blood cells (RRBC) as targets (Cornet et al., 2018). A serial dilution from 1/20 to 1/480 into veronal buffer (IDVert, France) was performed in duplicate for each plasma sample in a round-bottomed 96-well plate. The total haemolysis was obtained by mixing 10 µl of RRBC (3%) lysed with bi-distilled water and the spontaneous haemolysis was obtained by adding veronal buffer to 10 µl of RRBC. After the incubation, the turbidity 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 total peroxidase activity in plasma was assessed according to Quade and Roth (1997). The samples and negative control (water) were assayed in triplicate. In flat-bottomed 96-well plate, 7 µl of plasma was diluted in 68 µl of Hank's buffer (HBSS) without Ca^{2+} or Mg^{2+}. As substrate, 25 µl of reactional solution (20 mM 3,3′,5,5′-tetramethylbenzidine hydrochloride and 5 mM H_2O_2) was added. The reaction was stopped after 2 min by adding 50 µl of 4M sulphuric acid and the absorbance was measured at 450 nm. One unit (U) of peroxidase activity was defined as the amount producing an absorbance change of 1 OD.

2.5. Gene expression

Gene expression procedure was conducted following Cornet et al., (2018). For each sampling time and in each group, total RNA was extracted from the organs (liver and spleen) using Tri Reagent solution (Ambion, Thermofisher Scientific) according to the manufacturer's instructions. The pellet was dried and re-suspended in 50 and 100 µL of RNase-free water for spleen and liver respectively. Total RNA concentration was determined by NanoDrop-2000 spectrophotometer (Thermo Scientific). Genomic DNA was digested for 15 min at 37 °C with 1U of rDNAse I (Thermofischer Scientific) and total RNA was quantified again by NanoDrop-2000 spectrophotometer. Then, 1 µg of total RNA was reverse-transcribed using RevertAid RT kit (Thermofischer Scientific) according to the manufacturer's instructions. The cDNA was used to test the expression of 29 genes using Real-time quantitative polymerase chain reaction qPCR. Two housekeeping genes (β-actin and elongation factor 1α, ef1α) were tested and b-actin was chosen as the reference gene. Only 15 target genes were kept after an amplification test using different dilutions of cDNA. The list of specific primers used is given in Table 1. Real-time qPCR was carried out with iTaq universal SYBR green

supermix (Bio-Rad Laboratories) using a 1:100 dilution of the cDNA. Primers for target and reference genes were used at 100 nM. The thermal conditions were 3 min at 95 °C, followed by 40 cycles at 95°C for 10 s and 60 °C for 30 s, and melting curves were analysed to verify the absence of multiple amplicons. All reactions were performed using QuantStudio5 device (Applied Biosystem) and the relative gene expression was calculated using the standard curve method. Values for each sample were expressed as normalized relative expression (NRE), calculated with the formula $NRE =$

$$Relative\ quantity\ Target\ gene / Relative\ quantity\ Reference\ gene$$

2.6. Statistical analyses

Statistical analyses were performed using the free software R version 3.6.2 (R Core team, 2019). Homogeneity of variances was previously tested for all the dependent variables using Levene test (leveneTest, package "car", Fox et al., 2014). Data were then analysed by a linear mixed model (lm, package "lme4", Bates et al., 2014) with the treatment and the sampling time as fixed effects: *model = lm (Y ~ treatment*sampling time*) with *Y*: dependent variable. 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). 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, or Box-Cox transformed. When the model was validated, an ANOVA table for various statistical models 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 and external damages

The recovery rate was lesser in HPP group (71.1% of the injected fish N = 2x180) than in control one (94%, N = 180). All the recovered fish from control group were alive and unharmed while HPP group showed a survival of 96.4% of the injected fish. The main cause of death was body part loss or crushing (9 fish out of 13) or laceration (4 fish out of 13). In the surviving recovered fish (N = 256), 8 Atlantic salmons showed moderate scale loss ranging from 5 to 10% and 10 showed moderate scale loss combined with hematoma ≤ 10%.

3.2. Stress response

Plasma cortisol levels did not differ between HPP group and control one and remained stable through the time (Figure 2-A). At 120 h pi, cortisol mean values showed a little tendency to diverge between the two groups with 63.9 ± 33.1 ng/mL in HPP group and 41.7 ± 40.7 ng/mL in control group.

The interaction between the treatment and the sampling time affected significantly plasma glucose levels ($p = 0.026$, Figure 2-B). The lowest level was observed in HPP fish sampled at 120 h pi (0.31 ± 0.06 mg/mL) while the highest was observed in HPP fish sampled at 24 h pi (0.62 ± 0.22 mg/mL). Glucose values decreased over the time in HPP fish between 24 h and 120 h pi ($p < 0.05$) while they remained stable until 72 h pi and decreased in control group only between 72 h and 120 h pi ($p < 0.001$).

The content of brain neurohormones (Figure 3) did not show any significant difference except for DOPAC that varied significantly depending on the interaction between the treatment and the sampling time ($p = 0.001$). DOPAC content in brain decreased significantly between 72 h and 120 h pi in HPP group only ($p = 0.034$, 15.7 ± 4.1 ng/g of wet weight and 11.5 ± 3.8 ng/g of wet weight, respectively, Figure 3-D). Serotonergic and dopaminergic ratios in whole brain did not vary significantly in the whole experiment (Figure 3-E and F).

3.3. Humoral immune parameters

Plasma peroxidase levels varied significantly depending on the interaction between the treatment and the sampling time ($p = 0.014$, Figure 4-A). The levels remained stable in all groups through the time until 72 h pi, but values were significantly different between HPP and control group at 120 h pi ($p = 0.008$). Control fish (217.7 ± 148.1 U/mL) showed a significantly lower level compared to HPP groups at 120 h pi (279.3 ± 97.2 U/mL) and HPP group at 72 h pi (241.4 ± 70 U/mL).

The interaction between the treatment and the sampling time significantly influenced the ACH50 levels ($p < 0.001$, Figure 4-B). Twenty-four hours after injection, control fish showed a significantly higher ACH50 level (92.2 ± 60.7) than HPP ones (44.6 ± 48.7, $p = 0.027$). Fish sampled at 72 h and 120 h pi did not show any significant difference between HPP and control groups. However, ACH50 levels decreased over the time, in control groups, between 24 h and 72 h pi ($p = 0.016$) and in HPP group between 72 h and 120 h pi ($p = 0.023$).

3.4. Gene expression

3.4.1. Stress and metabolic related genes in liver

Stress related *hsp70* gene expression did not vary significantly during the experiment (Figure 5-A) while *gr1* (stress response, carbohydrate metabolism and hormone regulation) varied depending on the sampling time with an increase at 120 h pi (Figure 5-B, $p < 0.001$). All the carbohydrate metabolism gene expressions (*gr1, apoa1, galk2, calm1* and *cd36*) varied significantly depending on the sampling time ($p<0.05$, Figure 5-B, C, D, E, F and G) while *fads6* (lipid metabolism) did not show any significant variation during the experiment. The values were similar between 24 h and 72 h pi and increased at 120 h pi for *apoa1* and *galk2* ($p < 0.05$). For *calm1*, the relative gene expression levels increased significantly between 72 h pi and 120 h pi ($p = 0.005$) and were intermediate at 24 h pi, while they increased for *cd36* between 24 h and 120 pi ($p = 0.043$).

For the hormone regulating genes (*gr1, ghr1, igf1* and *igf2),* the expression varied throughout the time (Figure 5-B, H, I and J). In all groups, *gr1, ghr1* and *igf2* expression levels increased at 120 h pi ($p < 0.05$). *Igf1* relative expression levels and increased at 72 h pi remaining stable until the end of the experiment ($p < 0.005$).

3.4.2. Immune related genes in spleen

The expression of immune genes *lysg* and *igm* varied significantly depending on the interaction between sampling time and treatment ($p = 0.023$ and 0.035 respectively, Figure 6-A and B), while the relative expression of c3 did not show any significant variation during the experiment (Figure 6-C). In HPP group, *lysg* levels was lower at 24 h and increased at 72 h pi ($p < 0.001$, Figure 6-A). Changes in *igm* relative expression occurred only in HPP group with an increase over the time ($p < 0.01$) while the levels remained quite similar in control group. Relative expression levels of *mpo* varied according to the interaction between the sampling time and the treatment ($p = 0.012$, Figure 6-D). At 72 h pi, those levels were higher in HPP group compared to control group and were lower in both groups for the other sampling times ($p = 0.007$). Relative gene expression levels of *cox2* were stable and increased in all groups at 120 h after injection ($p < 0.05$, Figure 6-E).

4. Discussion

4.1. Survival rate and external damages

The survival rate in the HPP fish was consistent with previous findings for Kaplan turbine (mortality from below 5% to 46%, Bickford and Skalski, 2000; Čada et al., 2006; Coutant and Whitney, 2011; Larinier, 2008; Larinier and Travade, 2002; Thorstad et al., 2012). Our findings are close to the direct mortality estimation of 5% found in similar studies (Coutant and Whitney, 2000; Ferguson et al., 2006; Larinier and Travade, 2002; Mathur et al., 2000). It allows us to consider that a part of the higher total mortality observed in telemetric studies

may be due to other factors including potential changes in the animal condition, exhaustion or disorientation due to the passage through the turbine (Ferguson et al., 2006; Havn et al., 2020). The main causes of mortality were body parts loss and crushing and the main external damages observed were descaling and haemorrhage. Those types of injuries are directly related to strikes from part of the HPP and other mechanical wounding and descaling can also be caused by shear and turbulence (Pracheil et al., 2016). As Atlantic salmons are physostomes, they can resist quite well sudden changes in pressure due to their quick regulation of the pressure in the swim bladder through the air canal and the mouth (Larinier and Travade, 2002). This explains the absence of mortality due to the rupture of the swim bladder caused by sudden pressure variations. Moreover, the mortality rate varies between fish species and depends also on fish size with mortality rate in adult eels estimated to be 4 to 5 times higher than in juvenile salmonids (Larinier and Travade, 2002). The recorded damages were mainly scale losses combined or not with hematoma. Those damages are widely encountered in fish after the passage through the turbine (Brackley et al., 2018; Havn et al., 2017; Kibel and Coe, 2007). As large scale loss may reduce the osmoregulatory ability of fish leading to a delayed mortality in the ocean, it is important to record and monitor those non-life threatening damages after the passage through the turbine (Thorstad et al., 2012; Zydlewski et al., 2010).

4.2. Changes in stress status, metabolism and hormonal regulation

Changes in physiological stress status after the passage through the hydropower turbine were evaluated by various reliable stress parameters, including circulating cortisol and glucose, brain neurotransmitters and liver *gr1* and *hsp70* genes expressions. Plasma cortisol levels measured in HPP groups did not vary from the control ones over the time. Those levels are close to those observed by Bernard et al. (2018) in the Loire-Allier strain – the same strain as the one used in this study – at the beginning of the smoltification process when the water temperature is about 7 – 9°C. In the same time, the observed levels were about five times higher than those observed in non-stressed smolts by Carey and McCormick (1998). Plasma cortisol levels in stressed Atlantic salmon smolts can rise sharply and decline to their initial values in 8 h after an acute stress (Carey and McCormick, 1998). This leads to conclude that fish sampled at 24 h post-injection were already in a recovery process from the turbine-induced stress. Moreover, for the control group, the passage in the wetted flexible plastic tube simulating the passage over the spillways seems to have also induced a stress in the fish regarding the fact that no significant differences were found between the control and the HPP group. As Atlantic salmon smolts have a high interrenal responsiveness during the

smoltification process (Carey and McCormick, 1998), it seems possible that the stress due to the handling and the passage through the tube were already enough to trigger an increase in cortisol levels potentially overshadowing the effects of the passage through the turbine itself. Changes in glucose levels are considered as part of a secondary metabolic response to stress as the release of cortisol from the interrenal is involved in maintaining hyperglycaemia through protein catabolism and gluconeogenesis to prevent exhaustion (Soengas et al., 1992; Specker, 1982; Van Der Boon et al., 1991). Catecholamines are involved in the primary metabolic response to stress and can cause an initial rise in plasma glucose by glycogenolysis while cortisol mediates sustained plasma glucose levels (Fabbri and Moon, 2016; Faught et al., 2016). The decrease of plasma glucose levels was more abrupt and occurred earlier in HPP group than in the control group. The plasma glucose levels remained stable until 72 h post injection in control group before decreasing while those levels decreased sharply from the first day post-injection in HPP group. The glucose levels were quite similar at 120 h post-injection. It seems that the passage through the turbine led to a more rapid consumption of plasma glucose and therefore a faster exhaustion. As cortisol levels were quite similar in both groups, it seems that the plasma glucose levels were sustained in the same pattern under cortisol mediation preventing hypoglycaemia and exhaustion (Soengas et al., 1992; Specker, 1982; Van Der Boon et al., 1991).

Glucocorticoid receptor (*gr1*) mRNA levels increased in both groups between 72 h and 120 h post injection when glucose levels were the lowest. Sathiyaa and Vijayan, (2003) demonstrated an upregulation of *gr1* mRNA abundance induced by cortisol in trout hepatocytes and that this higher content in mRNA corresponded to a lower protein expression. In liver, applying cortisol treatment mimicking physiologically elevated plasma concentration led to the increase in *gr1* mRNA levels and a downregulation of gr1 protein content (Vijayan et al., 2003). Cortisol is known to sustain higher glucose production during stress (Faught et al., 2016) and this response seems to be due to hepatic gluconeogenesis mediated by glucocorticoids (Mommsen et al., 1999; Vijayan et al., 1997, 1996, 1994). The higher content in *gr1* mRNA was already found concomitant with a higher content in a glucocorticoid-responsive gene mRNA coding for a key gluconeogenic enzyme the phosphoenolpyruvate carboxykinase (Vijayan et al., 2003). This regulation seems to have partially participated in maintaining plasma glucose levels to face the increased demand relating to the allostatic charge.

The increase in relative expression of galactokinase2 (*galk2*) gene occurred in both groups 120 h after injection when plasma glucose levels dropped. The gene *galk2* is involved in

Leloir pathway converting α-D-galactose into galactose 1-phosphate. Leloir pathway leads to the production of the metabolically useful glucose 1-phosphate from β-D-galactose (Holden et al., 2003). This pathway occurs in the liver and is involved in maintaining glucose levels in blood when necessary. Using one of the minor carbohydrate pathways instead of using body reserves in glycogen and lipids may be one compensatory mechanism to the glucose consumption that occurred during the experiment.

Apolipoprotein A1 mRNA content increased at 120 h after injection in both groups. This protein is involved in reverse cholesterol transport from peripheral tissues to liver before redistributing or removing it. Stressors exposure can induce the expression of *apoa1* (Lu et al., 2012; Simmons et al., 2017; Skolness et al., 2012). As plasma cortisol levels were relatively higher than the levels observed in non-stressed smolts (Carey and McCormick, 1998), they may have induced the expression of *apoa1*. It has been reported that the level of some apolipoprotein isoforms such as apoE, apoA1/A2 increased participating to a more efficient lipid transport to target tissues to sustain the increased energetic demand during confinement stress or bacterial infection in Eurasian perch *Perca fluviatilis* or common carp *Cyprinus carpio* (Concha et al., 2003; Douxfils et al., 2012). Moreover, Concha et al., (2004) reported antimicrobial acivity of apoA1/A2 in common carp and a synergism between apoA1 synthetic peptid and lysozyme suggesting the important role of this multifunctional protein in the innate defence in fish.

Growth hormone GH and Insulin-like growth factors (IGFs) are central to the smolting process (McCormick et al., 2013). Their plasma levels increase at the early stages of this process (February for IGF1 and March for GH, McCormick et al., 2013). The increase of mRNA levels of those proteins in all groups in a timely manner seems to be related to the progression of the smoltification process. Those results suggest that the potential stress due to the experiment did not negatively affect the ability of fish to undergo the smoltification process.

4.3. Disruption in immune response and oxidative stress defence

Plasma complement and peroxidase activities were affected by the passage through the turbine. Complement activity was lower in HPP group at 24 h after injection compared to control group and decreased in all groups afterwards while peroxidase activity was higher in HPP group at 120 h after injection. A transient increase in mRNA content due to the passage in the turbine occurred for lysozyme G (*lysg*) and eosinophil peroxidase (*mpo*) between 24 h and 72 h post injection while immunoglobulin M (*igm*) increased over the time for HPP fish. Complement 3 (*c3*) mRNA did not show any difference while Cytochrome C oxidase subunit

II (*cox2*) mRNA content increased only on 120 h ai in both groups. During the smoltification process, fish may experience a massive immune suppression (Johansson et al., 2016) with decrease in plasma lysozyme and IgM levels (Melingen et al., 1995; Muona and Soivio, 1992). However, the passage through the turbine induced a transient increase in some immune parameters and oxidative stress defence in this study. This increase may be due to a transient immunostimulation due to the stress (Bonga, 1997; Nardocci et al., 2014; Tort, 2011). In fact, acute stress over a short time duration such as the passage through a turbine may activate some immune functions such as enhancing the innate response and leukocyte mobilization (Nardocci et al., 2014; Tort, 2011). However, the cumulative impact of this kind of stress have to be considered. Chronic stress affects negatively the immune system and the energetic-metabolic machinery and leads to an increasing pathogen susceptibility (Nardocci et al., 2014; Tort, 2011).

Cell antioxidant defences protect the cells against reactive oxygen species (ROS) damages (Di Giulio and Meyer, 2008). Those defences include glutathione peroxidases, catalase, transferases, superoxide dismutase, xanthine oxidase and glucose 6-phosphate dehydrogenase (Slaninova et al., 2009). Eosinophil peroxidase (*mpo*) mRNA content and plasma peroxidase levels increase in HPP group may suggest that the passage through the turbine can induce ROS production and lead to damages to cell structure and DNA, lipid peroxidation and protein oxidation (Das and White, 2002; Lawson et al., 2018).

5. Conclusions

It was unexpected that plasma cortisol levels were not affected by the passage through the turbine. However, fish handling seems to be stressful for all groups and led to a general increase of cortisol in fish regardless of their treatment. Eventually, the speed and the water height in association with protruding structures during the passage over the spillways may lead to strikes and shocks and therefore being quite harmful and/or stressful to fish.

The passage through the turbine disrupted lightly carbohydrate metabolism and glucose production and consumption. It seems that the stress and the energy expenditure due to the confrontation with the turbine increased the glucose demand and caused a faster drop in plasma glucose levels in HPP group.

However, the passage through the turbine enhanced innate immune response and oxidative stress defence mechanisms. This immunostimulation seems to be positive but it is well known that a more chronic stress will lead to immune system depression. The cumulative impact of the passage through many turbines need to be investigated as it can represent a chronic stress affecting negatively the immune system and increasing the susceptibility to pathogens.

This work provided some clues explaining the delayed mortality – observed in many studies – that leads to a poor success of restocking programs. Turbines not only have a direct impact in terms of mortalities and injuries but also an indirect one in terms of fish behaviour and physiological and immune changes that can compromise the ability of Atlantic salmon smolts to escape successfully to the ocean.

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Bibliography

Baekelandt, S., Mandiki, S.N.M., Schmitz, M., Kestemont, P., 2019. Influence of the light spectrum on the daily rhythms of stress and humoral innate immune markers in pikeperch Sander lucioperca. Aquaculture 499, 358–363. https://doi.org/10.1016/j.aquaculture.2018.09.046

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R.H.B., Singmann, H., Dai, B., Grothendieck, G., Maech, Maechler, M., Bolker, B., Walker, S., Christensen, R.H.B., Singmann, H., Dai, B., Grothendieck, G., 2014. Package lme4. R Found. Stat. Comput. Vienna 4, 14–24. https://doi.org/10.1111/j.2041-210x.2012.00251.x

Bernard, B., Sobandi, K.C., Darras, V., Rollin, X., Mandiki, S.N.M., Kestemont, P., 2018. Influence of strain origin on osmoregulatory and endocrine parameters of two non-native strains of Atlantic salmon (Salmo salar L.). Gen. Comp. Endocrinol. 258, 205–212. https://doi.org/10.1016/j.ygcen.2018.01.004

Bickford, S.A., Skalski, J.R., 2000. Reanalysis and Interpretation of 25 Years of Snake–Columbia River Juvenile Salmonid Survival Studies. North Am. J. Fish. Manag. 20, 53–68. https://doi.org/10.1577/1548-8675(2000)020<0053:raioyo>2.0.co;2

Bonga, S.E.., 1997. The Stress Response in Fish. Physiol. Rev. 77, 591–625. https://doi.org/10.1152/physrev.1997.77.3.591

Brackley, R., Lucas, M.C., Thomas, R., Adams, C.E., Bean, C.W., 2018. Comparison of damage to live v. euthanized Atlantic salmon Salmo salar smolts from passage through an Archimedean screw turbine. J. Fish Biol. 92, 1635–1644. https://doi.org/10.1111/jfb.13596

Brevé, N., Vis, H., Spierts, I., de Laak, G., Moquette, F., Breukelaar, A., 2014. Exorbitant mortality of hatchery-reared Atlantic salmon smolts Salmo salar L., in the Meuse river system in the Netherlands. J. Coast. Conserv. 18, 97–109. https://doi.org/10.1007/s11852-013-0237-4

Čada, G., Loar, J., Garrison, L., Fisher, R., Neitzel, D., 2006. Efforts to reduce mortality to hydroelectric turbine-passed fish: Locating and quantifying damaging shear stresses. Environ. Manage. 37, 898–906. https://doi.org/10.1007/s00267-005-0061-1

Carey, J.B., McCormick, S.D., 1998. Atlantic salmon smolts are more responsive to an acute handling and confinement stress than parr. Aquaculture 168, 237–253. https://doi.org/10.1016/S0044-8486(98)00352-4

Concha, M.I., Molina, S., Oyarzún, C., Villanueva, J., Amthauer, R., 2003. Local expression of apolipoprotein A-I gene and a possible role for HDL in primary defence in the carp skin. Fish Shellfish Immunol. 14, 259–273. https://doi.org/10.1006/fsim.2002.0435

Concha, M.I., Smith, V.J., Castro, K., Bastías, A., Romero, A., Amthauer, R.J., 2004. Apolipoproteins A-I and A-II are potentially important effectors of innate immunity in the teleost fish Cyprinus carpio. Eur. J. Biochem. 271, 2984–2990. https://doi.org/10.1111/j.1432-1033.2004.04228.x

Cornet, V., Ouaach, A., Mandiki, S.N.M., Flamion, E., Ferain, A., Van Larebeke, M., Lemaire, B., Reyes López, F.E., Tort, L., Larondelle, Y., Kestemont, P., 2018. Environmentally-realistic concentration of cadmium combined with polyunsaturated fatty acids enriched diets modulated non-specific immunity in rainbow trout. Aquat.

528 Toxicol. 196, 104–116. https://doi.org/10.1016/j.aquatox.2018.01.012

529 Coutant, C.C., Whitney, R.R., 2000. Transactions of the American Fisheries Society Fish
530 Behavior in Relation to Passage through Hydropower Turbines : A Review Fish
531 Behavior in Relation to Passage through Hydropower Turbines : A Review. Trans. Am.
532 Fish. Soc. 8487, 37–41. https://doi.org/10.1577/1548-8659(2000)129<0351

533 Das, K.C., White, C.W., 2002. Redox systems of the cell: possible links and implications.
534 Proc. Natl. Acad. Sci. 99, 9617–9618.

535 Di Giulio, R.T., Meyer, J.N., 2008. Reactive oxygen species and oxidative stress, in: Di
536 Guilio, R.T., Hinton, D.E. (Eds.), The Toxicology of Fishes. CRC Press, Boca Raton,
537 FL, USA, pp. 273–324. https://doi.org/10.1201/9780203647295

538 Douxfils, J., Deprez, M., Mandiki, S.N.M., Milla, S., Henrotte, E., Mathieu, C., Silvestre, F.,
539 Vandecan, M., Rougeot, C., Mélard, C., Dieu, M., Raes, M., Kestemont, P., 2012.
540 Physiological and proteomic responses to single and repeated hypoxia in juvenile
541 Eurasian perch under domestication - Clues to physiological acclimation and humoral
542 immune modulations. Fish Shellfish Immunol. 33, 1112–1122.
543 https://doi.org/10.1016/j.fsi.2012.08.013

544 EDF Luminus, 2015. Andenne et Lixhe, les compactes [WWW Document]. URL
545 https://www.luminus.be/fr/corporate/activites/energie-hydroelectrique/andenne/

546 Fabbri, E., Moon, T.W., 2016. Adrenergic signaling in teleost fish liver, a challenging path.
547 Comp. Biochem. Physiol. Part - B Biochem. Mol. Biol. 199, 74–86.
548 https://doi.org/10.1016/j.cbpb.2015.10.002

549 Faught, E., Aluru, N., Vijayan, M.M., 2016. The Molecular Stress Response, Fish Physiology.
550 Elsevier Inc. https://doi.org/10.1016/B978-0-12-802728-8.00004-7

551 Ferguson, J.W., Absolon, R.F., Carlson, T.J., Sandford, B.P., 2006. Evidence of Delayed
552 Mortality on Juvenile Pacific Salmon Passing through Turbines at Columbia River
553 Dams. Trans. Am. Fish. Soc. 135, 139–150. https://doi.org/10.1577/t05-080.1

554 Forseth, T., Barlaup, B.T., Finstad, B., Fiske, P., Gjøsæter, H., Falkegård, M., Hindar, A., Mo,
555 T.A., Rikardsen, A.H., Thorstad, E.B., Vøllestad, L.A., Wennevik, V., 2017. The major
556 threats to Atlantic salmon in Norway. ICES J. Mar. Sci. 74, 1496–1513.
557 https://doi.org/10.1093/icesjms/fsx020

558 Fox, J., Weisberg, S., Adler, D., Bates, D., Baud-, G., Ellison, S., Firth, D., Friendly, M., Gor-
559 , G., Graves, S., Heiberger, R., Laboissiere, R., Mon-, G., Murdoch, D., Nilsson, H.,
560 Ogle, D., Rip-, B., 2014. Package 'car' (Version 2.1-3), Second. ed, URL https://r-
561 forge.r-project.org/projects/car/, http://CRAN.R-project.org/package=car,
562 http://socserv.socsci.mcmaster.ca/jfox/Books/Companion/index.html. SAGE
563 Publications, Inc.

564 Freeman, M.C., Pringle, C.M., Greathouse, E.A., Freeman, B.J., 2003. Ecosystem-Level
565 Consequences of Migratory Faunal Depletion Caused by Dams. Am. Fish. Soc. Symp.
566 35, 255–266.

567 Havn, T.B., Sæther, S.A., Thorstad, E.B., Teichert, M.A.K., Heermann, L., Diserud, O.H.,
568 Borcherding, J., Tambets, M., Økland, F., 2017. Downstream migration of Atlantic
569 salmon smolts past a low head hydropower station equippped with Archimedes screw
570 and Francis turbines. Ecol. Eng. 105, 262–275.

https://doi.org/10.1016/j.ecoleng.2017.04.043

Havn, T.B., Thorstad, E.B., Borcherding, J., Heermann, L., Teichert, M.A.K., Ingendahl, D., Tambets, M., Sæther, S.A., Økland, F., 2020. Impacts of a weir and power station on downstream migrating Atlantic salmon smolts in a German river. River Res. Appl. 1–13. https://doi.org/10.1002/rra.3590

Hervé, M., 2015. R package 'RVAideMemoire': Diverse Basic Statistical and Graphical Functions. Version: 0.9-45-2.

Holden, H.M., Rayment, I., Thoden, J.B., 2003. Structure and Function of Enzymes of the Leloir Pathway for Galactose Metabolism. J. Biol. Chem. 278, 43885–43888. https://doi.org/10.1074/jbc.R300025200

Johansson, L.H., Timmerhaus, G., Afanasyev, S., Jørgensen, S.M., Krasnov, A., 2016. Smoltification and seawater transfer of Atlantic salmon (Salmo salar L.) is associated with systemic repression of the immune transcriptome. Fish Shellfish Immunol. 58, 33–41. https://doi.org/10.1016/j.fsi.2016.09.026

Jonsson, B., Jonsson, N., 2011. Ecology of Atlantic Salmon and Brown Trout: Habitats as Template for Life Histories, FISH & FISHERIES SERIES 33. Science, Springer 2011, Business Media B.V. https://doi.org/DOI 10.1007/978-94-007-1189-1_11

Katopodis, C., Williams, J.G., 2012. The development of fish passage research in a historical context. Ecol. Eng. 48, 8–18. https://doi.org/10.1016/j.ecoleng.2011.07.004

Kibel, P., Coe, T., 2007. Fish monitoring and live trials. Archimedes screw turbine, River Dart. Phase I Report: Live fish trials, smolts, leading edge assessment, disorientation study, outflow monitoring. Fishtek. Moretonhampstead, Devon.

Kiilerich, P., Kristiansen, K., Madsen, S.S., 2007. Hormone receptors in gills of smolting Atlantic salmon, Salmo salar: Expression of growth hormone, prolactin, mineralocorticoid and glucocorticoid receptors and 11β-hydroxysteroid dehydrogenase type 2. Gen. Comp. Endocrinol. 152, 295–303. https://doi.org/10.1016/j.ygcen.2006.12.018

King, S., O'Hanley, J.R., 2016. Optimal Fish Passage Barrier Removal-Revisited. River Res. Appl. 32, 418–428. https://doi.org/10.1002/rra.2859

Larinier, M., 2001. Environmental issues, dams and fish migration. FAO Fish. Tech. Pap. 419, 45–89.

Larinier, M., 2008. Fish passage experience at small-scale hydro-electric power plants in France. Hydrobiologia 609, 97–108. https://doi.org/10.1007/s10750-008-9398-9

Larinier, M., Travade, F., 2002. Downstream migration: problems and facilities. Bull. Fr. Pech. Piscic. 364 suppl., 181–207.

Lawson, M., Jomova, K., Poprac, P., Kuca, K., Musílek, K., Valko, M., 2018. Free radicals and antioxidants in human disease. Nutr. Antioxid. Ther. Treat. Perspect. 39, 283–305. https://doi.org/10.1007/978-3-319-67625-8_12

Lenth, R., Singmann, H., Love, J., Buerkner, P., Herve, M., 2019. Package "emmeans": Estimated marginal means, aka least-squares means. Compr. R Arch. Netw 1–67.

Lu, X.J., Chen, J., Huang, Z.A., Zhuang, L., Peng, L.Z., Shi, Y.H., 2012. Influence of acute cadmium exposure on the liver proteome of a teleost fish, ayu (Plecoglossus altivelis).

613 Mol. Biol. Rep. 39, 2851–2859. https://doi.org/10.1007/s11033-011-1044-3

614 Mathers, R.G., De Carlos, M., Crowley, K., Ó Teangana, D., 2002. A review of the potential
615 effect of Irish hydroelectric installations on Atlantic salmon (Salmo salar L.) populations,
616 with particular reference to the River Erne. Biol. Environ. 102, 69–79.
617 https://doi.org/10.3318/BIOE.2002.102.2.69

618 Mathur, D., Heisey, P.G., Skalski, J.R., Kenney, D.R., 2000. Salmonid Smolt Survival
619 Relative To Turbine Efficiency and Entrainment Depth in Hydroelectric Power
620 Generation. J. Am. Water Resour. Assoc. 36, 737–747.
621 https://doi.org/https://doi.org/10.1111/j.1752-1688.2000.tb04302.x

622 McCormick, S.D., Hansen, L.P., Quinn, T.P., Saunders, R.L., 1998. Movement, migration,
623 and smolting of Atlantic salmon (Salmo salar). Can. J. Fish. Aquat. Sci. 55, 77–92.
624 https://doi.org/10.1139/cjfas-55-S1-77

625 McCormick, S.D., Sheehan, T.F., Björnsson, B.T., Lipsky, C., Kocik, J.F., Regish, A.M.,
626 O'Dea, M.F., 2013. Physiological and endocrine changes in Atlantic salmon smolts
627 during hatchery rearing, downstream migration, and ocean entry. Can. J. Fish. Aquat.
628 Sci. 70, 105–118. https://doi.org/10.1139/cjfas-2012-0151

629 McKay, S.K., Cooper, A.R., Diebel, M.W., Elkins, D., Oldford, G., Roghair, C., Wieferich,
630 D., 2017. Informing Watershed Connectivity Barrier Prioritization Decisions: A
631 Synthesis. River Res. Appl. 33, 847–862. https://doi.org/10.1002/rra.3021

632 Melingen, G.O., Stefansson, S.O., Berg, A., Wergeland, H.I., 1995. Changes in serum protein
633 and IgM concentration duringsmolting and early post-smolt period in vaccinated and
634 unvaccinated Atlantic salmon (Salmo salar L.). Fish Shellfish Immunol. 5, 211–221.

635 Metzger, D.C., Luckenbach, J.A., Dickey, J.T., Beckman, B.R., 2013. Development of a
636 multiplex gene expression assay for components of the endocrine growth axis in coho
637 salmon. Gen. Comp. Endocrinol. 189, 134–140.
638 https://doi.org/10.1016/j.ygcen.2013.04.022

639 Mommsen, T.P., Vijayan, M.M., Moon, T.W., 1999. Cortisol in teleosts: dynamics,
640 mechanisms of action, and metabolic regulation. Rev. Fish Biol. Fish. 9, 211–268.

641 Muona, M., Soivio, A., 1992. Changes in plasma lysozyme and blood leucocyte levels of
642 hatchery-reared Atlantic salmon (Salmo salar L.) and sea trout (Salmo trutta L.) during
643 parr-smolt transformation. Aquaculture 106, 75–87.

644 Myrnes, B., Seppola, M., Johansen, A., Øverbø, K., Callewaert, L., Vanderkelen, L.,
645 Michiels, C.W., Nilsen, I.W., 2013. Enzyme characterisation and gene expression
646 profiling of Atlantic salmon chicken- and goose-type lysozymes. Dev. Comp. Immunol.
647 40, 11–19. https://doi.org/10.1016/j.dci.2013.01.010

648 Nagrodski, A., Raby, G.D., Hasler, C.T., Taylor, M.K., Cooke, S.J., 2012. Fish stranding in
649 freshwater systems: Sources, consequences, and mitigation. J. Environ. Manage. 103,
650 133–141. https://doi.org/10.1016/j.jenvman.2012.03.007

651 Nardocci, G., Navarro, C., Cortés, P.P., Imarai, M., Montoya, M., Valenzuela, B., Jara, P.,
652 Acuña-Castillo, C., Fernández, R., 2014. Neuroendocrine mechanisms for immune
653 system regulation during stress in fish. Fish Shellfish Immunol. 40, 531–538.
654 https://doi.org/10.1016/j.fsi.2014.08.001

655 Nehlsen, W., Williams, J.E., Lichatowich, J.A., 1991. Pacific Salmon at the Crossroads:

656 Stocks at Risk from California, Oregon, Idaho, and Washington. Fisheries 16, 4–21.
657 https://doi.org/10.1577/1548-8446(1991)016<0004:psatcs>2.0.co;2

658 Nyqvist, D., Greenberg, L.A., Goerig, E., Calles, O., Bergman, E., Ardren, W.R., Castro-
659 Santos, T., 2017. Migratory delay leads to reduced passage success of Atlantic salmon
660 smolts at a hydroelectric dam. Ecol. Freshw. Fish 26, 707–718.
661 https://doi.org/10.1111/eff.12318

662 Olsvik, P.A., Vikeså, V., Lie, K.K., Hevrøy, E.M., 2013. Transcriptional responses to
663 temperature and low oxygen stress in Atlantic salmon studied with next-generation
664 sequencing technology. BMC Genomics 14. https://doi.org/10.1186/1471-2164-14-817

665 Parrish, D.L., Behnke, R.J., Gephard, S.R., Mccormick, S.D., Reeves, G.H., 1998. Why aren't
666 there more Atlantic salmon (Salmo salar)? Can. J. Fish. Aquat. Sci. 55 Suppl.1, 281–287.
667 https://doi.org/10.1139/cjfas-55-S1-281

668 Persson, L., Kagervall, A., Leonardsson, K., Royan, M., Alanärä, A., 2019. The effect of
669 physiological and environmental conditions on smolt migration in Atlantic salmon
670 Salmo salar. Ecol. Freshw. Fish 28, 190–199. https://doi.org/10.1111/eff.12442

671 Pracheil, B.M., DeRolph, C.R., Schramm, M.P., Bevelhimer, M.S., 2016. A fish-eye view of
672 riverine hydropower systems: the current understanding of the biological response to
673 turbine passage. Rev. Fish Biol. Fish. 26, 153–167. https://doi.org/10.1007/s11160-015-
674 9416-8

675 Pringle, C.M., 2003. What is hydrologic connectivity and why is it ecologically important?
676 Hydrol. Process. 17, 2685–2689. https://doi.org/10.1002/hyp.5145

677 Quade, M.J., Roth, J.A., 1997. A rapid, direct assay to measure degranulation of bovine
678 neutrophil primary granules. Vet. Immunol. Immunopathol. 58, 239–248.
679 https://doi.org/10.1016/S0165-2427(97)00048-2

680 R Core team, 2019. R: A Language and Environment for Statistical Computing. Version
681 3.6.2.

682 Renardy, S., Benitez, J.P., Tauzin, A., Dierckx, A., Nzau Matondo, B., Ovidio, M., 2019.
683 How and where to pass? Atlantic salmon smolt's behaviour at a hydropower station
684 offering multiple migration routes. Hydrobiologia. https://doi.org/10.1007/s10750-019-
685 04108-w

686 Rivinoja, P., 2005. Migration Problems of Atlantic Salmon (Salmo salar L.) in Flow
687 Regulated Rivers.

688 Sathiyaa, R., Vijayan, M.M., 2003. Autoregulation of glucocorticoid receptor by cortisol in
689 rainbow trout hepatocytes. Am J Physiol Cell Physiol 284, 1508–1515.
690 https://doi.org/10.1152/ajpcell.00448.2002.-We

691 Schmalz, W., Wagner, F., Sonny, D., 2015. Arbeitshilfe zur standörtlichen Evaluierung des
692 Fischschutzes und Fischabstieges, Fischschutz und Fischabstieg.

693 Simmons, D.B.D., Miller, J., Clarence, S., McCallum, E.S., Balshine, S., Chandramouli, B.,
694 Cosgrove, J., Sherry, J.P., 2017. Altered expression of metabolites and proteins in wild
695 and caged fish exposed to wastewater effluents in situ. Sci. Rep. 7, 1–14.
696 https://doi.org/10.1038/s41598-017-12473-6

697 Skolness, S.Y., Durhan, E.J., Jensen, K.M., Kahl, M.D., Makynen, E.A., Villeneuve, D.L.,

698 Ankley, G.T., 2012. Effects of gemfibrozil on lipid metabolism, steroidogenesis, and
699 reproduction in the fathead minnow (Pimephales promelas). Environ. Toxicol. Chem. 31,
700 2615–2624. https://doi.org/10.1002/etc.1989

701 Slaninova, A., Smutna, M., Modra, H., Svobodova, Z., 2009. A review: Oxidative stress in
702 fish induceed by pesticides. Neuroendocr. Lett. 30, 2–12. https://doi.org/NEL300709R01
703 [pii]

704 Soengas, J.L., Rey, P., Rozas, G., Andrés, M.D., Aldegunde, M., 1992. Effects of cortisol and
705 thyroid hormone treatment on the glycogen metabolism of selected tissues of
706 domesticated rainbow trout, Oncorhynchus mykiss. Aquaculture 101, 317–328.
707 https://doi.org/10.1016/0044-8486(92)90034-I

708 Specker, J.L., 1982. Interrenal function and smoltification. Aquaculture 28, 59–66.
709 https://doi.org/10.1016/0044-8486(82)90008-4

710 Thorstad, E.B., Havn, T.B., Sæther, S.A., Heermann, L., Teichert, M.A.K., Diserud, O.H.,
711 Tambets, M., Borcherding, J., Økland, F., 2017. Survival and behaviour of Atlantic
712 salmon smolts passing a run-of-river hydropower facility with a movable bulb turbine.
713 Fish. Manag. Ecol. 24, 199–207. https://doi.org/10.1111/fme.12216

714 Thorstad, E.B., Whoriskey, F., Rikardsen, A.H., Aarestrup, K., 2011. Aquatic nomads: the life
715 and migrations of the Atlantic salmon, in: Atlantic Salmon Ecology. Wiley-Blackwell:
716 Singapore, pp. 1–32. https://doi.org/10.1002/9781444327755.ch1

717 Thorstad, E.B., Whoriskey, F., Uglem, I., Moore, A., Rikardsen, A.H., Finstad, B., 2012. A
718 critical life stage of the Atlantic salmon Salmo salar: Behaviour and survival during the
719 smolt and initial post-smolt migration. J. Fish Biol. 81, 500–542.
720 https://doi.org/10.1111/j.1095-8649.2012.03370.x

721 Tipsmark, C.K., Madsen, S.S., 2009. Distinct hormonal regulation of Na+,K+-atpase genes in
722 the gill of Atlantic salmon (Salmo salar L.). J. Endocrinol. 203, 301–310.
723 https://doi.org/10.1677/JOE-09-0281

724 Tort, L., 2011. Stress and immune modulation in fish. Dev. Comp. Immunol. 35, 1366–1375.
725 https://doi.org/10.1016/j.dci.2011.07.002

726 Trinder, P., 1969. Determination of glucose in blood using glucose oxidase with an alternative
727 oxygen acceptor. Ann. Clin. Biochem. 6, 24–27.

728 Van Der Boon, J., Van Den Thillart, G.E.E.J.M., Addink, A.D.F., 1991. The effects of
729 cortisol administration on intermediary metabolism in teleost fish. Comp. Biochem.
730 Physiol. -- Part A Physiol. 100, 47–53. https://doi.org/10.1016/0300-9629(91)90182-C

731 Vijayan, M.M., Mommsen, T.P., Glémet, H.C., Moon, T.W., 1996. Metabolic effects of
732 cortisol treatment in a marine teleost, the sea raven. J. Exp. Biol. 199, 1509–1514.

733 Vijayan, M.M., Pereira, C., Grau, E.G., Iwama, G.K., 1997. Metabolic responses associated
734 with confinement stress in tilapia: the role of cortisol. Comp. Biochem. Physiol. Part C
735 Pharmacol. Toxicol. Endocrinol. 116, 89–95.

736 Vijayan, M.M., Raptis, S., Sathiyaa, R., 2003. Cortisol treatment affects glucocorticoid
737 receptor and glucocorticoid-responsive genes in the liver of rainbow trout. Gen. Comp.
738 Endocrinol. 132, 256–263. https://doi.org/10.1016/S0016-6480(03)00092-3

739 Vijayan, M.M., Reddy, P.K., Leatherland, J.F., Moon, T.W., 1994. The effects of cortisol on

740 hepatocyte metabolism in rainbow trout: a study using the steroid analogue RU486. Gen.
741 Comp. Endocrinol. 96, 75–84.

742 Zydlewski, J., Zydlewski, G., Danner, G.R., 2010. Descaling Injury Impairs the
743 Osmoregulatory Ability of Atlantic Salmon Smolts Entering Seawater. Trans. Am. Fish.
744 Soc. 139, 129–136. https://doi.org/10.1577/t09-054.1

745

746 Table 1: Primers sequences used for analyses of selected genes expression in *Salmo salar*

	Gene	Accession	Primers (5'-3')	Protein	Function	Reference
Liver	*hsp70*	**BG933934**	CCCCTGTCCCTGGGTATTG CACCAGGCTGGTTGTCTGAGT	Heat shock protein 70	Stress response	Olsvik et al., 2013
	gr1	**AF209873**	ACGACGATGGAGCCGAAC ATGGCTTTGAGCAGGGATAG	Glucocorticoid receptor	Stress response, carbohydrate metabolism and hormone regulation	Kiilerich et al., 2007
	galk2	**BT045062.1**	GGTTATGCTGTGCTCCCAAT TCATCCCAGACAGAGGAACC	Galactokinase 2	Carbohydrate metabolism	Bernard et al., submitted
	apoa1	**NM_001123663.1**	TGGTCCTCGCACTAACCATC GCAGTCAACTTCACCTGAGCTA	Apolipoprotein A-I		
	calm1	**NM_001139713**	CGACAAGGATGGTAACGGCT GTTGACAGTGAGTGTGTTGC	Calmodulin		
	cd36	**NM_001124511**	GGATGAACTCCCTGCAT TGAGGCCAAAGTACTCGTCGA	Cluster of differentiation 36		
	fads6	**NM_001123575**	TACCCAGTGGGCAAAGAGAC CAACGGCTTCAGAAACTTCC	Delta-6 fatty acyl desaturase	Lipid metabolism	Bernard et al., submitted
	igf1	**NM_001123623**	GATGTCTTCAAGAGTGCGATGTG CGCCGAAGTCAGGGTTAGG	Insulin-like growth factor 1	Hormone regulation	Metzger et al., 2013
	igf2	**NM_001123647**	TGCCCACACTCAAACAGG CTTCCTCTGCCACACCTCA	Insulin-like growth factor 2		Bernard et al., submitted
	ghr1	**AY462105**	TCCCAACATGCAGCTGTAGA TGTGGCACCTTGAAGAACAG	Growth hormone receptor 1		Tipsmark and Madsen, 2009
Spleen	*igm*	**Y12457.1**	TGAGGAGAACTGTGGGCTACACT TGTTAATGACCACTGAATGTGCAT	Immunoglobuline M	Immune response	Cornet et al., personal data
	lysg	**AM493682**	GGCTGGGGTAGTGTCAATC TGACCTTGCTGCCATGAACA	Lysozyme G		Myrnes et al., 2013
	c3	**BI468074**	GTGACAGGTGGAGAGCAGA CCAGGCCAATATCCTCCCA	Complement C3		

Spleen	*mpo*	**XM_014128513.1**	GAGAGGTGCCTTGCTTCATAG ATCTTGCGAGCCTCCTGATA	Eosinophil peroxidase	Immune response and Oxidative stress defence	Cornet et al., personal data
	cox2		CTCTAAATCGTTTGGACTGTCCT AGGTGTGGGTCATTAATTTCGTC	Cytochrome C oxidase subunit II	Oxidative stress defence	Cornet et al., personal data
	β-actin	**BG933897**	ACTGGGACGACATGGAGAAG GGGGTGTTGAAGGTCTCAAA	Beta-actin	Reference gene	Cornet et al., personal data

747

Figure captions

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

Figure 2: Plasma cortisol (2-A) levels of control (white) and HPP (red) groups and changes in plasma glucose levels (2-B) of control and HPP groups depending on the interaction between sampling time and treatment. 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 ($p<0.05$).

Figure 3: Content of brain serotonin (3-A), dopamine (3-B), their metabolites 5HIAA (3-C) and DOPAC (3-D) and the serotonergic (3-E) and dopaminergic (3-F) ratios related to sampling time and treatment 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 ($p<0.05$).

Figure 4: Changes in peroxidase activity (4-A) and ACH50 levels (4-B) depending on the interaction between sampling time and treatment 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 ($p<0.05$).

Figure 5: Relative genes expression in liver in function of sampling time in control (white) and HPP (red) groups. The horizontal line in the boxplot represents the median. Triangles represent the mean. Lower case letters indicate significant differences among the sampling times ($p<0.05$).

Figure 6: Relative genes expression in spleen in function of sampling time (6-D) or of the interaction between sampling time and treatment (6-A, B, and E) 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 1

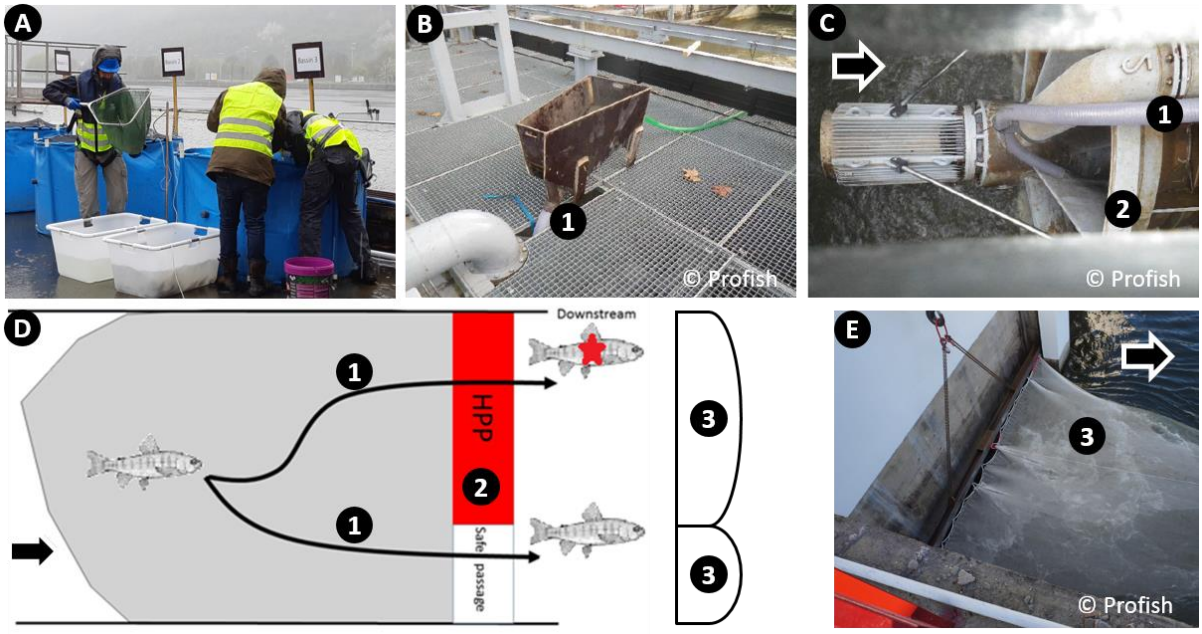

A
B
1
© Profish
C
1
2
© Profish
D
Downstream
1
1
HPP
2
Safe passage
3
3
E
3
© Profish

Figure 2

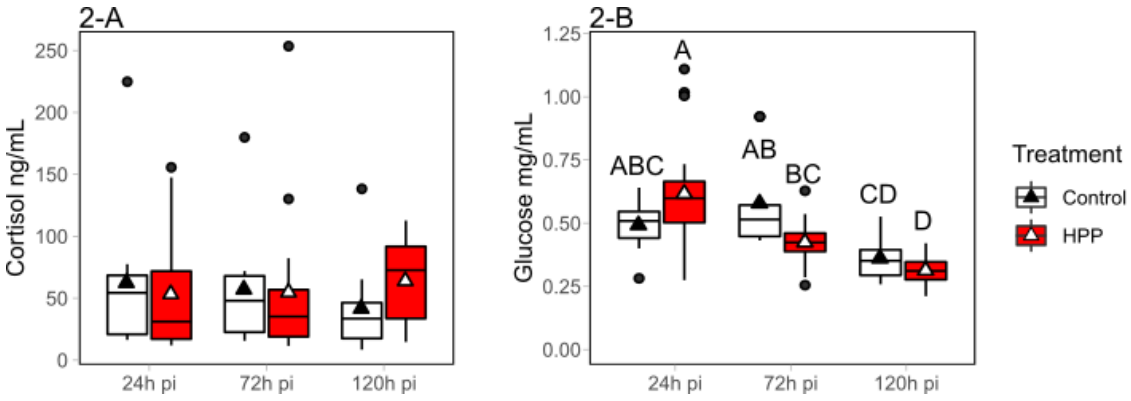

2-A
Cortisol ng/mL
250
200
150
100
50
0
24h pi
72h pi
120h pi
2-B
Glucose mg/mL
1.25
1.00
0.75
0.50
0.25
0.00
A
ABC
AB
BC
CD
D
24h pi
72h pi
120h pi
Treatment
Control
HPP

Figure 3

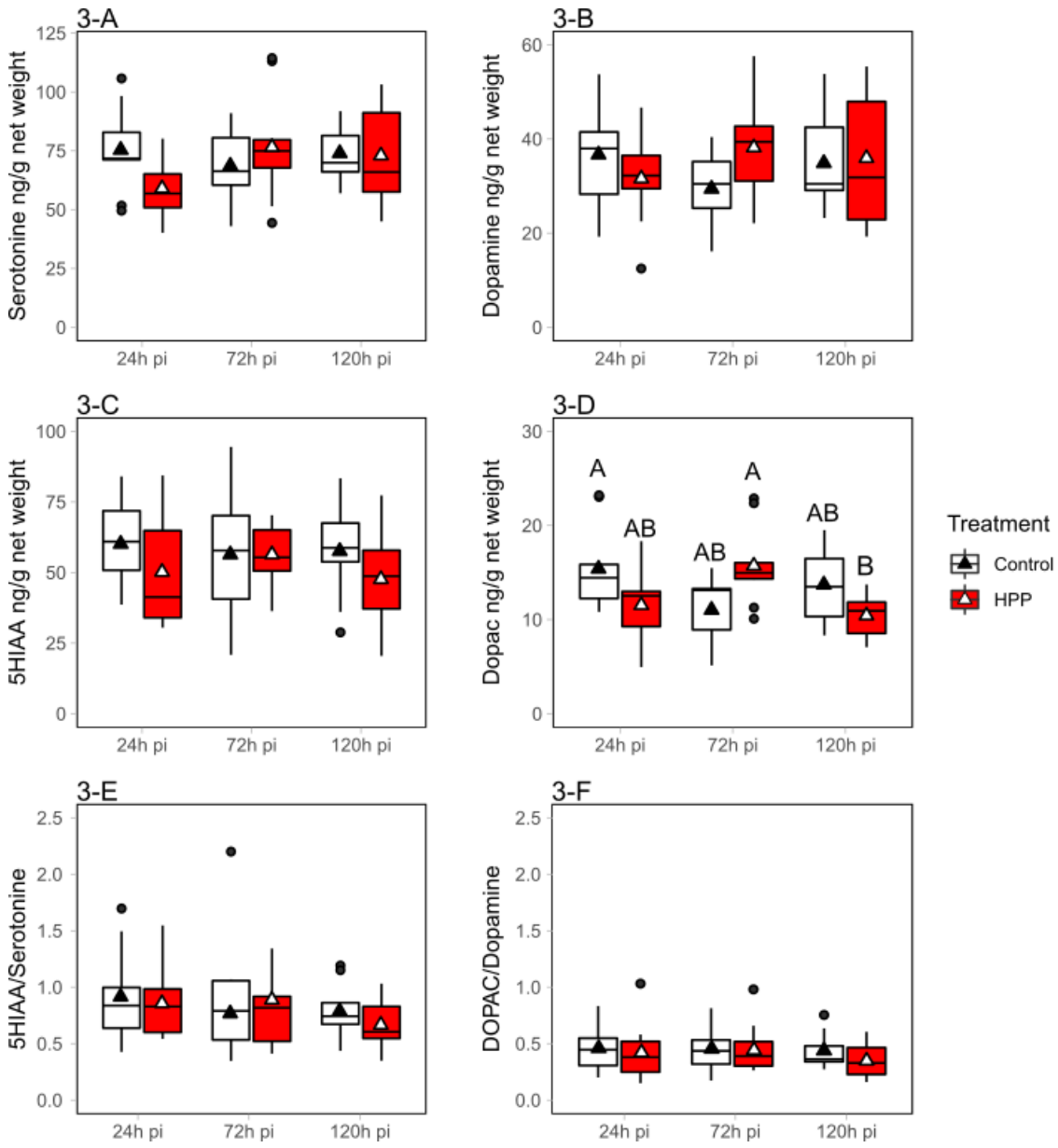

3-A
Serotonine ng/g net weight
125
100
75
50
25
0
24h pi
72h pi
120h pi
3-B
Dopamine ng/g net weight
60
40
20
0
24h pi
72h pi
120h pi
3-C
5HIAA ng/g net weight
100
75
50
25
0
24h pi
72h pi
120h pi
3-D
Dopac ng/g net weight
30
20
10
0
A
AB
A
AB
AB
B
24h pi
72h pi
120h pi
Treatment
Control
HPP
3-E
5HIAA/Serotonine
2.5
2.0
1.5
1.0
0.5
0.0
24h pi
72h pi
120h pi
3-F
DOPAC/Dopamine
2.5
2.0
1.5
1.0
0.5
0.0
24h pi
72h pi
120h pi

Figure 4

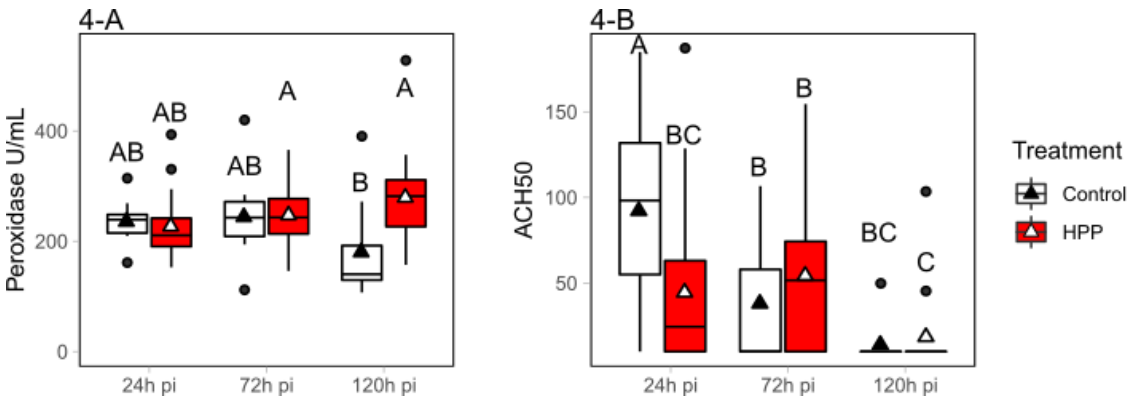

4-A
Peroxidase U/mL
400
200
0
AB
AB
AB
A
B
A
24h pi
72h pi
120h pi
4-B
ACH50
150
100
50
A
BC
B
B
BC
C
24h pi
72h pi
120h pi
Treatment
Control
HPP

Figure 5

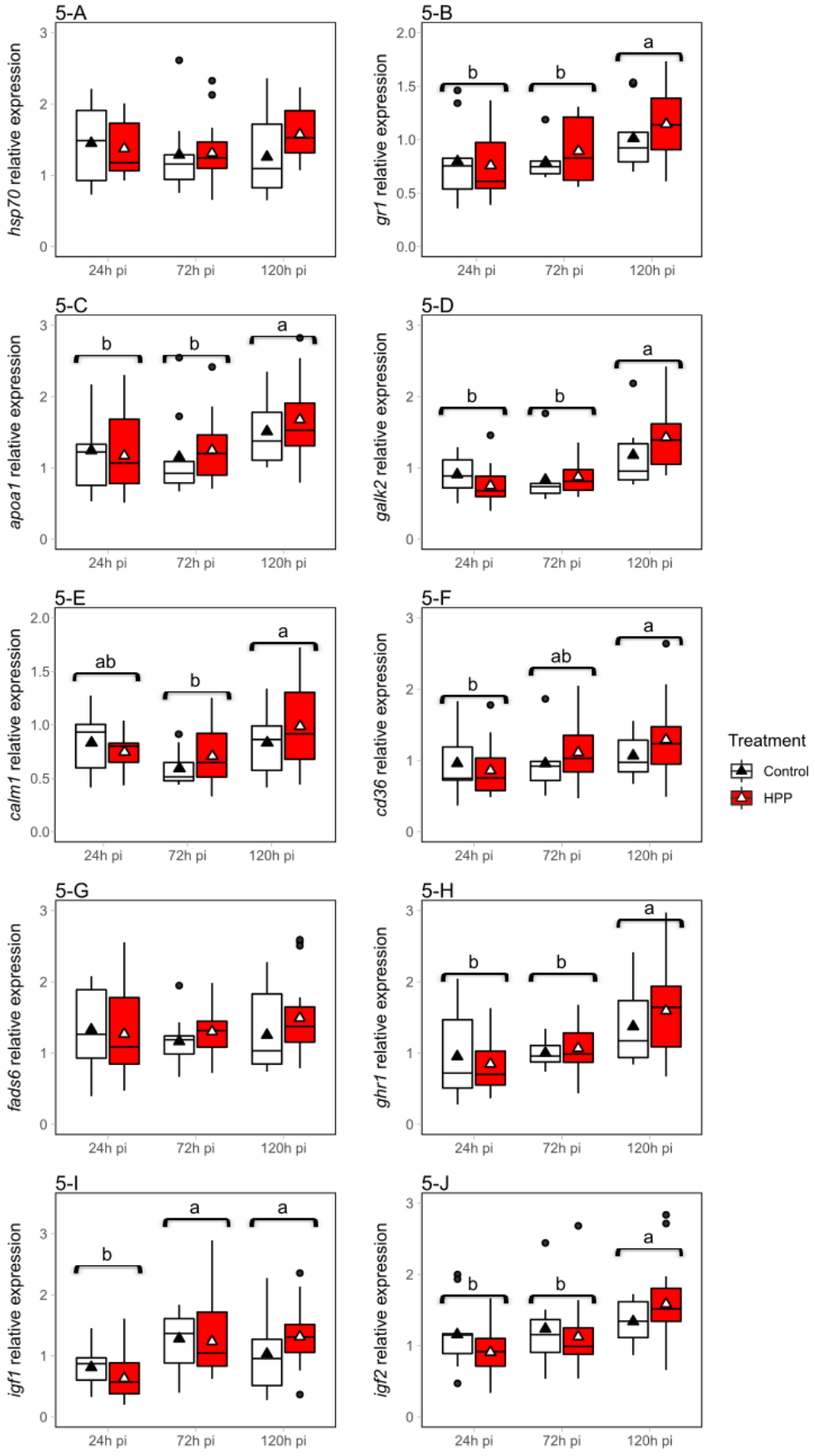
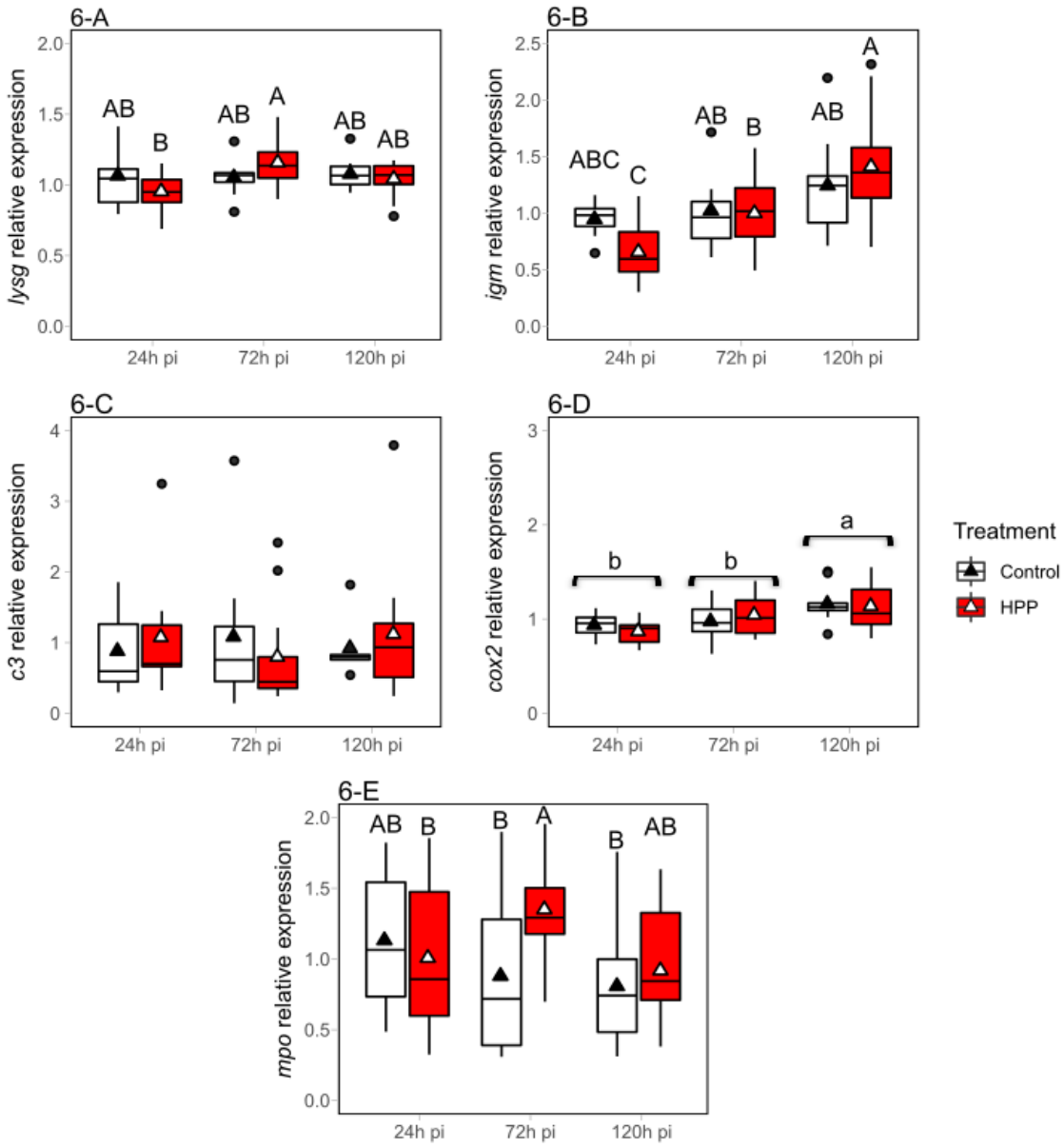

5-A
hsp70 relative expression
5-B
gr1 relative expression
5-C
apoa1 relative expression
5-D
galk2 relative expression
5-E
calm1 relative expression
5-F
cd36 relative expression
5-G
fads6 relative expression
5-H
ghr1 relative expression
5-I
igf1 relative expression
5-J
igf2 relative expression
24h pi
72h pi
120h pi
Treatment
Control
HPP

Figure 6


6-A
lysg relative expression
2.0
1.5
1.0
0.5
0.0
AB
B
AB
A
AB
AB
24h pi
72h pi
120h pi
6-B
igm relative expression
2.5
2.0
1.5
1.0
0.5
0.0
ABC
C
AB
B
AB
A
24h pi
72h pi
120h pi
6-C
c3 relative expression
4
3
2
1
0
24h pi
72h pi
120h pi
6-D
cox2 relative expression
3
2
1
0
b
b
a
24h pi
72h pi
120h pi
Treatment
Control
HPP
6-E
mpo relative expression
2.0
1.5
1.0
0.5
0.0
AB
B
B
A
B
AB
24h pi
72h pi
120h pi

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: