nature portfolio

Peer Review File

Dams threaten salmonids by triggering temperature-dependent proliferative kidney disease

Corresponding Author: Professor Anti Vasemägi

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Decision Letter:

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Dear Professor Vasemägi,

Your manuscript entitled "Dams threaten salmonids by triggering temperature-dependent disease" has now been seen by 3 referees, whose comments are appended below. You will see from their comments below that while they find your work of considerable interest, some important points are raised. We are interested in the possibility of publishing your study in Communications Biology, but would like to consider your response to these concerns in the form of a revised manuscript before we make a final decision on publication.

We therefore invite you to revise and resubmit your manuscript, taking into account the points raised. In particular, please note that the following revisions would be necessary for us to contact our referees again:

1 – Please address the points 106, 107, 142 and 167 from reviewer #1.

2 – Please address the concerns from reviewer #2 about the measurement of Tb loads and the response variable.

Additional comment from the editor I: Please note that reviewer #2 has provided additional feedback in the attached pdf file.

Additional comment from the editor II: Please note that it is not mandatory to include additional data about bryozoans (intermediate host) as suggested by reviewer #3 (if these data have not been collected already).

We are committed to providing a fair and constructive peer-review process. Do not hesitate to contact us if you wish to discuss the revision in more detail or if there are specific requests from the reviewers that you believe are technically impossible or unlikely to yield a meaningful outcome.

At the same time, we ask that you ensure your manuscript complies with our editorial policies. Specifically:

For all graphs depicting a single point value (e.g., mean) with error bars, you must add individual data points or convert the graph to a boxplot or dot-plot to show data distribution.

It's mandatory to provide access to the numerical source data for graphs and charts either through a repository or by providing the data in a Supplementary Data file (in excel format).

All blots/gels must be accompanied by size markers in every figure panel. Uncropped and unedited blot/gel images must be included as Supplementary Figure(s) in the Supplementary Information pdf.

Please ensure that you have complied with the data deposition policies at the Nature Portfolio, please see here.

Please ensure that you have complied with our policies on research involving animals and humans, see here

Please follow the ARRIVE guidelines for reporting animal experiments. Please fully complete an ARRIVE checklist including both the essential and recommended set of items (adding information to the manuscript where needed) and upload this with your revised manuscript.

Please also see our revision checklist for guidance on formatting the manuscript and complying with our policies. A comprehensive guide to our formatting requirements for final submissions is also available for your reference here.

Please use the following link to submit your revised manuscript, point-by-point response to the referees' comments (which should be in a separate document to the cover letter) and any additional files:

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When submitting the revised version of your manuscript, please pay close attention to our Digital Image Integrity Guidelines.

We would like to receive your revision within 4 weeks, but appreciate that every situation is unique. We look forward to receiving your revised manuscript when it is ready, and will not enforce a hard deadline on this revision.

Please do not hesitate to contact me if you have any questions or would like to discuss these revisions further. We look forward to seeing the revised manuscript and thank you for the opportunity to review your work.

Best regards,

Johannes Stortz, PhD
Senior Editor
Communications Biology
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on behalf of

Eoin O'Gorman, PhD
Editorial Board Member
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orcid.org/0000-0003-4507-5690

Reviewers' comments:

Reviewer #1 (Remarks to the Author):

In this important manuscript, the authors convincingly show how dams in rivers may exacerbate a deadly salmonid kidney disease. This is likely due to a combination of the exposure of stagnant water in the reservoir to solar radiation increasing the temperature more than 2 °C and possibly favourable conditions in the reservoir for bryozoan growth, which are the primary hosts for the parasite causing the kidney disease. The data combine a large study of 14 rivers with dams showing an increase in parasite prevalence, kidney disease, and anaemia from upstream to downstream of the dam. Apart of bringing awareness of the negative effects of damming for sensitive trout populations, the data also show the negative impact on valuable natural trout populations of a temperature rise that must be expected in the near future when global warming is not brought to a halt. The paper thus has both scientific value and interest for the broader public and informs decisions about river management and discussions about future sustainability.

The paper is well written and analysed and I have only minor comments. Mainly I feel that the results section could be optimized, by using more consistency in annotations, and by clarifying the statistical results. Also, please clarify the N-values a bit better (number of rivers or number of sample sites?). These points I have left ordered by line number:

95: Please be consistent with annotations: studied cases, dams, reservoirs, rivers are all the same so only use one not to confuse the reader.

97: Consistency: similar for location, which is used in the statistical analysis for upstream vs downstream. 98 area should be

location. Do not use location as annotation for something else later! I think the whole problem is the use of location and locations where with locations you mean the river stretch in which you sampled. That should be double the number of rivers. in the methods you mention sites: line 312. Anyway, this is an annotation problem you should solve and then keep using it consistently!

100: missing U value and N-value for Wilcoxon test

101: change "no difference" to "no significant difference"

104: the beta is positive but the CI values are negative. I guess you should make them positive.

105: change to: Sampled brown trout did not differ significantly in body size ... (N = 14 but here N = 13, explain why)

106: delete ", neither in total length"?

106: rather than giving ANODEV as information here about the test I would find it more helpful to give LMM as information (or GLMM). The ANODEV of car is a method to get to a statistical evaluation of that test, in this case an ANOVA F-test would be an appropriate solution for the linear approach (but the Wald test you use does fine as well).

106: not so important for such a low chisquare, still what is df for this test?

107: As a query for body condition you regressed log(mass) on log(total length) as given in 391. This apparently gives a beta difference for the effect of location of -0.022 (LMM is the test and could be handled as the other test before to keep the annotations similar). You write that body condition is slightly lower? This is however difficult to envision from the beta you give in your model. What exactly means the -0.022? if this means that the relationship between log(w) and log(tl) changes than you have a change in the allometric relationship w = a * tl ^ b, where b is commonly around a value of 3. That would be a less steep relationship in the upstream fish? Anyway it gets a bit confusing and I wonder why not use Fulton's condition factor? Then you could show how much that factor has changed from upstream to downstream.

112: Here you present the data of two analysis in which the second one is on a subset of the data only including rivers in which the Tetracapsuloides was detected. This takes some effort for the reader to understand. Better would be to show only one of the two, and I find the second one more relevant. The other can remain in the supplement. Then first write that xx of xx rivers were found positive for Tetracapsuloides briosalmonae and then do the rest of the analysis only with this subset.

112-113: the use of locations is confusing here. In the model, you use river (equivalent for dam (with a total N of 14)) as random factor. So should this not be N = 13 and N = 9 rivers? In this respect note that you write "Hence, river level variability has a dominant influence on model predictions" in 115!

113: Tb-present is a complex annotation. If you first give how many rivers were tested positive you can introduce the annotation. Is that 9 of 14 rivers / dams?

118: delete "simple" and write that you used mean values per river / location

119: How do N = 18 locations result in df = 12 in a paired t-test? A df of 12 indicates you had 13 paired values which includes all the rivers (14) -1.

124: Not clear what the beta would tell me at this point. In case searching for additional information you could consider effect size. Furter report which test you used LMM or GLMM and the model p-values etc.

132: Here as for condition you regressed the values. That makes it complex to read the results. Why not test the K/B ratio? Further Kidney hyperplasia is a condition where K/B ratio is high. Consider rewriting this part.

137: would you still call it kidney hyperplasia here? Better refer to K/B ratio.

137: You mean that the fish in downstream locations of Tb absent rivers have lower K/B ratio than fish in ... Or K/B ratio was lower... etc.

142: What is the definition you use of low parasite loads. Better write descriptive and give the numbers and ranges you found. Similar for 147 Hematocrit

167: This is the first time you mention a focus on "brown trout recruitment areas" and as is raises confusion about what you mean with this or how it could affect your measurements. This is further only mentioned in the method section. Best would be to add it to the end of the introduction and give some table where we can see how far up and downstream of the dam the data were collected. Moreover, in 232 you correctly mention that waters may naturally warm up in summer while flowing downstream. However, I assume you do not want this to downgrade your central argumentation that it is the damming that increases the water temperature. Now you mention it please argue how this may not explain the extent of increase in temperature to keep your central argumentation intact.

188: delete "(class Phylactolaemata)"

229: There is some new paper on cool water habitat choice in brown trout in relation to PKD, maybe cite that...

261: The 1C figure does not show: Left: Distribution of dam heights in relation to Tb occurrence. Why are there 16 dams (coloured blocks) in this figure? Also not clear what is the function of the right panel of 1C.

285: and figure 1B, is it possible to calculate a relationship between the distance between the two measure points and temperature change with reservoir size as random variable? How does this value stand in relation to the estimated temperature increase due to the reservoir itself?

400: The square-root transformation should not be between brackets.

700: In the figure you write renal hyperplasia (also in methods line 328) but in the main text kidney hyperplasia. Keep things consistent...

Reviewer #2 (Remarks to the Author):

Dear Authors

It was a pleasure to read you MS. It is an interesting and very well-written MS. It tackles a novel and pressing question based on a solid and cleaver design. I don't have much major comments; all is clear both the data and analyses are strong. My comments are directly embedded into the attached pdf file. Most of these comments can be considered as "minors" but two: (i) the way Tb load is measured (qPCR from kidney DNA) is fine but I would like have a bit more information about whether it is problematic ot not to not correct for trout DNA when estimating TB load, and (ii) this response variable might be better modeled using quasipoisson (or negative-binomial) error terms. The the comments in the file for details and more explanation.

Very noce job, congratulations

Best wishes

Reviewer #3 (Remarks to the Author):

Comments to authors

This study presents a set of very clear results associating dams to infection risk and virulence of Tetracapsuloides bryosalmonae. Association with the PKD disease and temperature is well known but authors are the first to point out that small reservoirs warm up the water and this alone may lead to increase in disease prevalence and virulence. This result is not surprising for those who work in the field but as the data are very convincing this is a very important result for management and restoration of watersheds. It is an especially relevant feedback to the ongoing discussion of the importance of small hydropower plants for sustainable energy production in Europe. Here the message is clear, small hydropower will come with potential disease problems in salmonid fish. It would be very important to have these data in the discussion of watershed management and restoration.

I have largely only positive comments on the actual study design, data analysis and interpretation. The design is a solid pairwise upstream-downstream design with sufficient replication. The key result seems large and robust. There is no reason to doubt that these results would not be more general across the PKD / trout range. Methods to detect the parasite, measure the effects of parasite on fish and record the necessary environmental parameters are state of the art and reliable. Statistical analysis is appropriate, but also the effect sizes and the key response seem very clear and robust. Writing is clear and figures are already standalone giving a clear overview of the results.

I find this study valuable and well-conducted.

I have one comment that authors might want to consider. This comment is about direct and indirect effects of temperature increase due to dams. Authors would have the opportunity to deepen the conceptualization of the study by discussing the direct and indirect effects more clearly. I am sure they actually think like that, but it would be good to spell it out.

I try to explain what I mean. Readers who know the system will be wondering if the temperature increase benefits the bryozoans that are the intermediate host of the myxozoan parasites. Authors devote a paragraph in the discussion to the possible increase in bryozoan population due to changes in the flow regime and temperature. I think it would be useful to bring the thematic of multiple host life cycle already earlier, even mentioning that in the abstract. One could explain that with multiple host life cycles processes in the intermediate host (indirect temperature effects, for example) may be important. Why do I think this would be important? This would allow authors to explain how direct and indirect effects of increasing temperature operate in this system. PKD is an example of a parasite which has higher virulence in stressed host (trout), and here authors measure virulence. Temperature will increase the stress levels of fish. PKD is also an example of a parasite where parasite risk depends on density of infected bryozoans in the environment. Bryozoans are filter feeders that thrive in

rivers with pools of slowly flowing water, solid (built) structures and low water level variation. Bryozoans probably like impoundments like authors write. If there is something missing in the study it is the assessment of the density of infected and uninfected bryozoans upstream, in reservoirs and downstream. It would be a great addition to the study, but probably authors did not sample bryozoans. I might be asking a bit too much, but it would be great to be able to dissect / discuss the direct and indirect effects of the temperature. Direct effects would be the increase in disease prevalence and virulence due to stressed fish (temperature dependent susceptibility and virulence). Indirect effects would be the epidemiological consequences of more favorable bryozoan habitats (increase in parasite spore numbers and infection risk). This distinction might have importance for management decisions. Authors could also write the introduction and motivation of the study by explaining the expectations with respect to direct and indirect effects of temperature, showing that the problematic with abiotic stressors comes with indirect effects through ecological change in the environment.

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Version 1:

Decision Letter:

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Dear Professor Vasemägi,

Your manuscript entitled "Dams threaten salmonids by triggering temperature-dependent disease" has now been seen again by our referees, whose comments appear below. In light of their advice I am delighted to say that we are happy, in principle, to publish a suitably revised version in Communications Biology.

We therefore invite you to edit your manuscript to comply with our format requirements and to maximise the accessibility and therefore the impact of your work. Please note that addressing the remaining points from reviewer #2 is not mandatory.

* Please see the attached document for editorial requests for the final version (.docx file). Please ensure a completed version of this file is uploaded as a Related Manuscript with your final submission.

* Please review our final submission file checklist to ensure all necessary files are present with your final submission and to avoid delays in accepting your manuscript. For your reference, a style and formatting guide is available here and includes all of our style requirements.

It is important that you pay careful attention to the requests in these documents to avoid a delay in formal acceptance of the article.

If you have any questions or concerns about any of our requests, please do not hesitate to contact me.

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required third party permissions have been obtained, and to provide billing information in order to pay the article-processing charge (APC).

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We hope to hear from you within two weeks. If you expect the process to take longer than one month, please let us know.

Congratulations on an excellent paper!

Best regards,

Johannes Stortz, PhD
Senior Editor
Communications Biology
orcid.org/0000-0002-5928-1850

Eoin O'Gorman, PhD
Editorial Board Member
Communications Biology
orcid.org/0000-0003-4507-5690

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REVIEWERS' COMMENTS:

Reviewer #1 (Remarks to the Author):

All my queries have been satisfactorily answered by the authors. The result is an excellent manuscript that deals with important data on the impact of dams on diseases in a warming world. I have no further comments.

Reviewer #2 (Remarks to the Author):

Dear Authors

Thanks for the corrections you've made on the MS. It seems you have replied to all the comments made by the reviewers rather adequately. I still think that quasipoisson/negbim models are better than Gaussian models when we are to work with count data, but given the strong effects reported by the authors, I don't think choosing one or the other would change the conclusions. Nonetheless, the authors could have ran (just as an information for the reviewers) a negbin or quasipoisson GLMM; this is super straightforward (glmmPQL for quasipoisson, glmmTMB for negbin) and permit reifonrce further the conclusions.

Apart from that I'm fine with the MS as it is.

Reviewer #3 (Remarks to the Author):

Thank you for considering my suggestions and incorporating a bit more discussion of indirect and direct effects of temperature in the discussion. I think the manuscript is very nice and response of the authors sufficient and considerate.

We would like to thank editors and the reviewers for the time and effort spent evaluating our manuscript entitled “Dams threaten salmonids by triggering temperature-dependent disease” (COMMSBIO-25-5900-T) by Magnus Lauringson, Joacim Näslund, Lilian Pukk, Siim Kahar, Riho Gross and Anti Vasemägi. We appreciate the constructive feedback and have carefully revised the manuscript in response to the comments provided. Below, we address each point raised by the editors and reviewers and explain the corresponding changes made.

	Reviewer #1 (Remarks to the Author): In this important manuscript, the authors convincingly show how dams in rivers may exacerbate a deadly salmonid kidney disease. This is likely due to a combination of the exposure of stagnant water in the reservoir to solar radiation increasing the temperature more than 2 °C and possibly favourable conditions in the reservoir for bryozoan growth, which are the primary hosts for the parasite causing the kidney disease. The data combine a large study of 14 rivers with dams showing an increase in parasite prevalence, kidney disease, and anaemia from upstream to downstream of the dam. Apart of bringing awareness of the negative effects of damming for sensitive trout populations, the data also show the negative impact on valuable natural trout populations of a temperature rise that must be expected in the near future when global warming is not brought to a halt. The paper thus has both scientific value and interest for the broader public and informs decisions about river management and discussions about future sustainability. The paper is well written and analysed and I have only minor comments. Mainly I feel that the results section could be optimized, by using more consistency in annotations, and by clarifying the statistical results. Also, please clarify the N-values a bit better (number of rivers or number of sample sites?). These points I have left ordered by line number:	We thank the reviewer for the thorough review and positive feedback. We have now clarified the use on N throughout the manuscript.
1.	95: Please be consistent with annotations: studied cases, dams, reservoirs, rivers are all the same so only use one not to confuse the reader.	Terminology for sampling units has been harmonized. We now use “location(s)” to describe the downstream and upstream sampling sites and “rivers” as a general unit based on analysis of several rivers including their respective sampling locations.
2.	97: Consistency: similar for location, which is used in the statistical analysis for upstream vs downstream. 98 area should be location. Do not use location as annotation for something else later! I think the whole problem is the use	Terminology for sampling units has been harmonized. We now use “location” to describe a single sampling site, in plural both up- and downstream sites and “river(s)” as a general unit based on analysis of both locations.

	of location and locations where with locations you mean the river stretch in which you sampled. That should be double the number of rivers. in the methods you mention sites: line 312. Anyway, this is an annotation problem you should solve and then keep using it consistently!	
3.	100: missing U value and N-value for Wilcoxon test.	We have now added missing df, U and N values for Wilcoxon tests.
4.	101: change "no difference" to "no significant difference".	Corrected to “no significant difference”, line 118 - 119.
5.	104: the beta is positive but the CI values are negative. I guess you should make them positive.	Corrected.
6.	105: change to: Sampled brown trout did not differ significantly in body size ... (N = 14 but here N = 13, explain why).	The variation in sample size is clarified under Materials & Methods section “Sampling of fish”, as we did not catch any YOY trout downstream of one dam (R. Ahja, Saesaare dam) in two consecutive years. We have clarified this also under the current section (lines 123 to 125).
7.	106: rather than giving ANODEV as information here about the test I would find it more helpful to give LMM as information (or GLMM). The ANODEV of car is a method to get to a statistical evaluation of that test, in this case an ANOVA F-test would be an appropriate solution for the linear approach (but the Wald test you use does fine as well).	We have changed the reporting to “(linear mixed model, factor: location; $\chi 2 = 0.03$, $P = 0.870$; Table S2, N = 442 individuals)”. We changed all the similar reporting of statistical results in the same manner.
8.	106: not so important for such a low chisquare, still what is df for this test?	The df for the chisquare tests are 1 (two groups compared; 2-1 = 1). We did not think this was meaningful to report, and we have not added it at this stage (we can add it if deemed necessary). Maybe a brief explanation about why we present chi-square tests at some points will clarify things a bit. The chisquare test is presented when the parameter estimates from the LMM/GLMM models are deemed superfluous or obfuscating. If there is no significant difference, we just present the chisquare test from the ANODEV table (as for body size); when we do not believe strongly in the accuracy of the parameter estimates (as for prevalence; explained in the manuscript, see below) we also only report the chisquare significance. Model summary tables with parameter estimates are, however, always presented in the supplement, so they can be independently evaluated.
9.	107: As a query for body condition you regressed log(mass) on log(total length) as given in 391. This apparently gives a beta difference for the effect of location of -0.022	Using the simple cube law (“Fulton’s K”; although Fulton never proposed K; see Nash et al. 2006: The Origin of Fulton’s Condition Factor—Setting the Record Straight) would likely work OK for

	(LMM is the test and could be handled as the other test before to keep the annotations similar). You write that body condition is slightly lower? This is however difficult to envision from the beta you give in your model. What exactly means the -0.022? if this means that the relationship between log(w) and log(tl) changes than you have a change in the allometric relationship w = a * tl ^ b, where b is commonly around a value of 3. That would be a less steep relationship in the upstream fish? Anyway it gets a bit confusing and I wonder why not use Fulton's condition factor? Then you could show how much that factor has changed from upstream to downstream.	trout, which typically has a b only slightly above 3. However, we prefer to use the more accurate evaluation model, were both a and b are empirically derived for the fish included in the study. Our reported beta-value is the parameter estimate for the difference in a (how much a in upstream fish differ from downstream fish) in the allometric relationship. I.e. it evaluates the intercept a, not the scaling exponent b (our model used the same b for all populations, there is no included term for an interaction between length and location). So it is interpreted as a being 0.22 lower at any given length (i.e. mass is ca. 2.2 % lower in the upstream sections, given that $\exp(-0.022) \approx 0.978$). We have added the estimated percent difference to the text to make it clearer; the rest of the model details as well as the visual illustration of the model are available in the supplement (Table S3 and Fig. S2). The model structure is described in the methods, but it becomes a bit unintuitively presented since the journal format puts the methods at the end. We have noted that we use LMM; LMM is strictly speaking the model, the test of parameter estimates (beta) is a t-test in the lmer-package (the t-value is presented; it is quite rare to see the t-test being explicitly referred to when presenting LMM results, so we have not added this information [similar to not explicitly referring to F-tests when presenting ANOVA results, the presence of a F-value is generally considered enough]).
10.	112: Here you present the data of two analysis in which the second one is on a subset of the data only including rivers in which the Tetracapsuloides was detected. This takes some effort for the reader to understand. Better would be to show only one of the two, and I find the second one more relevant. The other can remain in the supplement. Then first write that xx of xx rivers were found positive for Tetracapsuloides briosalmonae and then do the rest of the analysis only with this subset.	We agree, the first analysis has now been removed from the text, it is provided in the Table S5.
11.	112-113: the use of locations is confusing here. In the model, you use river (equivalent for dam (with a total N of 14)) as random factor. So should this not be N = 13 and N = 9 rivers? In this respect note that you write "Hence, river level variability has a dominant influence on model predictions" in 115!	This probably becomes a bit confusing due to the journal format, where methods are presented at the end of the paper. We have 9 pairs of locations (total N = 18, from N = 9 rivers, in this presented model where we only look rivers with apparent Tb presence [analysing differences in rivers where there is no apparent Tb-infections makes little sense; the difference when there is no Tb at all is

		0 by logic and including such cases obfuscates the results]). The note about river variability refers to the apparent problem for the model to predict good mean effects (it says prevalence is predicted to be above 80 % in upstream locations and 100% for downstream locations, which is far above the median for the pooled data;).
12.	113: Tb-present is a complex annotation. If you first give how many rivers were tested positive you can introduce the annotation. Is that 9 of 14 rivers / dams?	We now added „N = 18 locations (9 pairs/rivers)“.
13.	118: delete "simple" and write that you used mean values per river / location	Done.
14.	119: How do N = 18 locations result in df = 12 in a paired t-test? A df of 12 indicates you had 13 paired values which includes all the rivers (14) -1.	Good point, we have now clarified the use of 13 rivers within the results section as described before.
15.	124: Not clear what the beta would tell me at this point. In case searching for additional information you could consider effect size. Furter report which test you used LMM or GLMM and the model p-values etc.	The beta value (parameter estimate) basically represents the effect size; this is the reason why it is included in the text. The beta-value is the estimated average difference between up- and downstream sites, on the sqrt scale. The estimated effect is also presented graphically. LMM would the type of model used (added), the test of the parameter estimate is the t-test (t-value is presented, which should provide the necessary information?) and the full model summary table (including also the intercept p-value from the model) is presented in the referred to Table S6 (and Table S7 for the second model evaluated).
16.	132: Here as for condition you regressed the values. That makes it complex to read the results. Why not test the K/B ratio? Further Kidney hyperplasia is a condition where K/B ratio is high. Consider rewriting this part.	In general it is considered better to not analyse ratios when there is a viable alternative to instead analyse the numerator as dependent on the denominator – that avoids issues of e.g. disregarding how both values contribute to the outcome, the often problematic distributions that ratios have, and the difficulty of understand what a unit of change is at the ratio scale. Ratios are a bit easier to visualise, and many previous papers have analysed the ratios, so we opted to visualise results based on the K/B ratio. Presenting the results in terms of K at a given B should give the equivalent information as the K/B-ratio, with the additional information about how thick the kidney actually is at a given dorsal muscular thickness. The initial sentence of the paragraph is rewritten to clarify that kidney hyperplasia is indicated by a high K/B-ratio.
17.	137: would you still call it kidney hyperplasia here? Better refer to K/B ratio.	Changed accordingly.

18.	137: You mean that the fish in downstream locations of Tb absent rivers have lower K/B ratio than fish in ... Or K/B ratio was lower... etc.	Changed accordingly.
19.	142: What is the definition you use of low parasite loads. Better write descriptive and give the numbers and ranges you found. Similar for 147 Hematocrit.	We have now added further information on the descriptive statistics of parasite load and haematocrit and also adjusted the text, lines 167 to 172.
20.	167: This is the first time you mention a focus on "brown trout recruitment areas" and as is raises confusion about what you mean with this or how it could affect your measurements. This is further only mentioned in the method section. Best would be to add it to the end of the introduction and give some table where we can see how far up and downstream of the dam the data were collected. Moreover, in 232 you correctly mention that waters may naturally warm up in summer while flowing downstream. However, I assume you do not want this to downgrade your central argumentation that it is the damming that increases the water temperature. Now you mention it please argue how this may not explain the extent of increase in temperature to keep your central argumentation intact.	As suggested, we have now added information to the Introduction (lines 98 - 102) regarding the selection of brown trout recruitment areas as close to the dams as possible. We now emphasize that both of these naturally occurring processes may be present, making proximity to the dams crucial for estimation of dam effects on PKD. Since sampling distances from the dams are already presented in Fig. 1B, upstream-downstream averages in text (lines 324 - 325), and distances from the dam for individual sites in Table S1, we did opt not to repeat this information in additional table. We have also added a sentence that described that the measured temperature increase of 2.64 °C over the short river stretch (average distance between sampling points 5.5 km) exceeds what we expect natural processes. **Added sentence (Lines 98 to 102):** *We focused on brown trout recruitment areas located as close to the dams as possible, in order to minimise the influence of natural environmental factors, such as downstream warming or coldwater inputs from groundwater and tributaries, that could confound the assessment of dam and reservoir effects on PKD.* **Added sentence (Lines 262 - 264)**: *However, the measured water temperature increase (2.64 °C) over the short distances involved (average 5.5 km between sampling points) exceeds what would be expected from natural processes.*
21.	188: delete "(class Phylactolaemata)"	Done.
22.	229: There is some new paper on cool water habitat choice in brown trout in relation to PKD, maybe cite that...	Good point, we have added the reference (ref. no. 48, Oexle et al. 2025) to the line 261.
23.	261: The 1C figure does not show: Left: Distribution of dam heights in relation to Tb occurrence. Why are there 16 dams (coloured blocks) in this figure? Also not clear what is the function of the right panel of 1C.	Well spotted. In two rivers there were two consecutive dams, this is described under lines 334 to 336 ”In two rivers (R. Mustoja and R. Loobu), two consecutive dams were present (distance between dams 0.65 and 3 km, respectively). Therefore, heights of 16 dams are presented in Fig. 1C and Table S1.” We now have described this also in the figure caption. The right

		panel is visualizing the Tb life-cycle and scheme of the effect of temperature on parasite prevalence, load and PKD. We think this visualization is informative to understand the main focus of this study.
24.	285: and figure 1B, is it possible to calculate a relationship between the distance between the two measure points and temperature change with reservoir size as random variable? How does this value stand in relation to the estimated temperature increase due to the reservoir itself?	We explored the reviewer's suggestion by testing whether temperature change between upstream and downstream sites was related to the distance between measurement points, including reservoir size as a covariate. Several linear models were evaluated, with and without an interaction term. None of the models yielded statistically robust effects. The closest to significance was the model including the interaction term, where distance showed a weak trend ($p = 0.058$), but this effect disappeared when the interaction was removed, and no stable patterns emerged across models. In practical terms, the weak trend would correspond to a few degrees difference over very long distances (e.g. ~2 °C over 10 km), but given the lack of robustness and sensitivity to model structure, this result is most likely spurious. Overall, we find no consistent or statistically supported relationship between temperature change and distance between sampling sites, nor between temperature change and reservoir size.
25.	400: The square-root transformation should not be between brackets.	The brackets have been removed.
26.	700: In the figure you write renal hyperplasia (also in methods line 328) but in the main text kidney hyperplasia. Keep things consistent...	Well noted, we harmonized the disease trait to renal hyperplasia.
	Reviewer #2 (Remarks to the Author): Dear Authors It was a pleasure to read you MS. It is an interesting and very well-written MS. It tackles a novel and pressing question based on a solid and cleaver design. I don't have much major comments; all is clear both the data and analyses are strong. My comments are directly embedded into the attached pdf file. Most of these comments can be considered as "minors" but two: (i) the way Tb load is measured (qPCR from kidney DNA) is fine but I would like have a bit more information about whether it is problematic ot not to not correct for trout DNA when estimating TB load, and (ii) this response variable might be better modeled using quasipoisson (or negative-binomial) error terms. The the comments in the file for details and more explanation.	We thank the reviewer for the kind words and thorough review. We hereby address the main concern of the reviewer: (i): This is a well-justified consideration, as many studies indeed normalize Tb DNA to trout DNA using qPCR. At the same time, many published works are using total genomic DNA extracted from kidney tissue and measure total genomic DNA spectrophotometrically prior to qPCR quantification of Tb (e.g., Bettge et al., 2009; Hutchins et al., 2018; Lauringson et al. 2025). To reduce variation associated with spectrophotometric DNA quantification, the samples were diluted to 20 ng μL^{-1}, and DNA concentration was subsequently re-measured and adjusted if the estimated values deviated by more than 2 ng μL^{-1} from the target concentration. Therefore, we are confident that the DNA concentrations were well equalized

	Very noce job, congratulations Best wishes	before qPCR and we added this detail to M&M. Given that the genome size of brown trout (~2.4 Gb) is orders of magnitude larger than genome size of Tb (~18-20 Mb, unpublished genome assembly by Hanna Hartikainen, University of Nottingham) and other typical myxozoan parasites (~20–200 Mb; Chang et al., 2015), and the kidney sample used for DNA isolation consists of mostly trout cells (based on histology studies on Tb), we are confident that total genomic DNA predominantly consists of trout DNA and provides a suitable reference for estimating Tb load via qPCR. **Added sentence (Lines 379 - 381):** To reduce variation associated with spectrophotometric DNA quantification, DNA dilutions were subsequently re-measured and adjusted if the estimated values deviated by more than 2 ng μL^{-1} from the target concentration. (ii): Answered under point 35.
27.	57: Agreed. Perhaps this paper can be cited as an exception on the impact of weirs on parasite communities: https://pubmed.ncbi.nlm.nih.gov/17647018/	We thank the reviewer for bringing this paper up, we have now added it to the manuscript.
28.	Line 60: Please, indicate explicitely that this is a positive side of dams as this can avoid infection in upstream areas	We have now emphasized that this can be a positive effect on aquatic disease dynamics (line 73 - 74).
29.	63: I would be more integrative as dam restoration does not only include removal. Fishpasses, restoration of an ecologically-suitable flow might also be alternative to removal. I would therefore replace "removal" per "dam ecological restoration" or something like that	Authors response: We have now added ”dam removal and restoration of floodplais” (line 76 - 77).
30.	113: If I well understand, there is a huge amount of variability (in prevalence) that is explained by the river identity, and this statistically overestimates the "true" effect of the location (upstream vs. downstram of the dams). Did I interpret it correctly ? If yes (oe even if no) I would advice you to explain this more explicitly, by avoiding precise statistical terms	The reviewers interpretation is not really correct. There is large variability in terms of several rivers aggregating at 100% and 0% prevalence. In proportional data, these values are extreme and here it leads to high (inflated) estimates (expected values) of prevalence (i.e. the 100% values influence the estimation upwards in the modelling procedure) – in contrast, the effect (difference between up- and downstream locations) appears to be underestimated because both up- and downstream estimates appear inflated. We have made an attempt to clarify without too much statistical terminology

		(although it seems impossible to avoid it completely; this is quite technical…)
31.	129: Why is the df different from the t-test presented for the prevalence? This is not logical, it should be the same as the number of replicate is the same	Well noted by the reviewer. It appears that we initially included test results from all rivers (N = 13), rather than restricting the analysis to Tb-positive rivers (N = 9) for the prevalence comparison. We have now corrected the test results on prevalence accordingly.
32.	167: Could you briefly provide some information regarding the distance of the upstream areas to the closest dam upstream? Said in other words; to which extend the selected upstream areas are impacted (or not) by other dams? We expect these upstream areas to be relatively far away from other dams. Having this information would reinforce their "control" status.	Authors response: We thank the reviewer for pointing this out. We added this discussion now to the sub-section of Limitations and Implications of Discussion. In eight of the studied rivers, there are no dams located upstream of the highest sampling location. In six remaining rivers, additional upstream dams were present at different distances from our studied sites. The average distance between the studied upstream location and the nearest upstream dam was 6.1 km. Thus, we cannot rule out that these upstream dams influenced the collected temperature and disease data. Nevertheless, our paired design makes our results robust. Given the well-supported cumulative impacts of multiple reservoirs on water temperature and aquatic ecosystems (e.g. https://doi.org/10.1007/s13201-023-01902-9), we expect that multiple dams may similarly have cumulative effects on disease dynamics. **Added text (lines 286 – 292):** *Due to the high abundance of dams in Estonia, our analyses included rivers with (N = 6) and without (N = 8) additional upstream dams located upstream of our highest sampling sites. The average distance between the studied upstream location and the nearest upstream dam was 6.1 km. Therefore, it is possible that these upstream dams affected the measured variables. However, the paired design ensures the robustness of our results. Given the well-supported cumulative impacts of multiple reservoirs on water temperature and aquatic ecosystems (60), we expect that multiple dams may have similar cumulative effects on disease dynamics.*
33.	175: Did you observe some differences in the density of YOY downstream and upstream dams? Could you discuss this a bit?	This is very interesting point but also rather speculative as robust estimation of juvenile salmonid abundance require repeated electrofishing and analyses of sufficient areas and number of locations (eg. https://doi.org/10.1577/03-044). Furthermore, in relation to PKD, it would be perhaps more

		informative to estimate change in juvenile abundance during the first summer but at the same time both mortality and emigration is also influenced by density dependence. We chose not to directly measure YOY density during this study because of time and workload limitations. We also decided not to delve into speculations on mortality in this work although we observed for example a total lack of YOY in two consecutive years below Saesaare dam while still catching older trout specimens. Interestingly, the upstream reservoir of Saesaare dam is the largest among our dataset (48.5 ha). On the other hand, some of the studied downstream sea-trout locations with reasonably high juvenile densities do exhibit severe PKD symptoms. In our previous work (Ahmad et al. 2020), we detected a significant negative correlation of YOY brown 37.trout abundance and summer temperature in R Altja below the dam (Fig 1c, this river was not included to current study because of lack of brown trout above the dam). However, in order to better characterize and separate the effects of PKD and temperature, analyses of long-term electrofishing datasets in combination to annual temperature variation, Tb and PKD data is probably needed.
34.	193: OK, I agree but I think here you need to be more explicit on what should be done next to clearly demonstrate this. For instance, quantifying the abundance of bryozoans above and below dams might be done to confirm your hypothesis. Providing clear guidelines would be more interesting.	We have now emphasized future study directions in relation bryozoans. **Added text (lines 218 – 224):** Future studies integrating eDNA data with hydrological and thermal profiles could help identify specific habitat features that facilitate bryozoan colonization. Longitudinal studies across seasons and dam types would further clarify how dam-induced habitat changes influence bryozoan abundances, parasite transmission dynamics and disease risk in salmonid populations. These effects may be indirect, via expanded bryozoan habitats that raise parasite spore abundance and infection risk, or direct, through higher disease prevalence and virulence driven by temperature-induced stress in fish.
35.	400: You may consider using GLMM with a quasipoisson (or negative binomial) distribution of error terms, which is often more adequate that a transformation of the raw data as parasite load is generally distributed as count data. You just need to change the decimal numbers to their closest integers	There is indeed an slight issue with the sqrt-transformation, which leads to poor predictability if the model results are applied on comparisons of populations where the load of the parasite is lower – if the parasite load of trout in a river is below ca. 2200 in the downstream section, the load in the upstream section would be predicted

		to be negative on the untransformed scale. Still, we do not think that quasi-Poisson modelling is appropriate; it does not look like our data represent counts that follow a Poisson process, the mean is very high and in such a case a Poisson-distribution should be near-Gaussian, which is not the case (we have a leptokurtic shape). Number of Tb parasites in a fish body might be more determined by an encounter rate function coupled with an infection probability for each parasite, and they may not behave as count data (this is a bit speculative). If we are not mistaken, the quasi-Poisson would scale the SE to the overdispersion, but at high means the assumption about near-Gaussian distribution would still be there (quasi-Poisson only inflate the variance globally, it has no impact on the assumptions of overall distribution shape). Negative binomial is possibly a better option, but applying a proper theta-parameter to describe the skew is tricky without a large amount of data to really find the appropriate underlying data distribution; appropriate theta is central to the neg.bin. modelling. We tested to log-transform the data [log10(x+1)], and then run a linear model on these data. The statistical significance is qualitatively the same ($p << 0.00001$) but the mean estimates are lower and confidence intervals are much wider for the downstream group, and confidence intervals for the upstream group are much narrower, as compared to the model on sqrt-transformed data. Overall, the sqrt-model has mean estimates closer to the overall medians and the confidence intervals look more reasonable. Perhaps there are more complex data distributions to apply, but we have here opted for keeping our original model as it seems to represent the overall pattern in terms of differences fairly well.
36.	**Reviewer #3 (Remarks to the Author):** Comments to authors This study presents a set of very clear results associating dams to infection risk and virulence of Tetracapsuloides bryosalmonae. Association with the PKD disease and temperature is well known but authors are the first to point out that small reservoirs warm up the water and this alone may lead to increase in disease prevalence and virulence. This result is not surprising for those who work in the field but as the data are very convincing this is a very	We thank the reviewer for the thoughtful feedback and for situating our study within a broader ecological and applied context.

	important result for management and restoration of watersheds. It is an especially relevant feedback to the ongoing discussion of the importance of small hydropower plants for sustainable energy production in Europe. Here the message is clear, small hydropower will come with potential disease problems in salmonid fish. It would be very important to have these data in the discussion of watershed management and restoration. I have largely only positive comments on the actual study design, data analysis and interpretation. The design is a solid pairwise upstream-downstream design with sufficient replication. The key result seems large and robust. There is no reason to doubt that these results would not be more general across the PKD / trout range. Methods to detect the parasite, measure the effects of parasite on fish and record the necessary environmental parameters are state of the art and reliable. Statistical analysis is appropriate, but also the effect sizes and the key response seem very clear and robust. Writing is clear and figures are already standalone giving a clear overview of the results. I find this study valuable and well-conducted.	
37.	I have one comment that authors might want to consider. This comment is about direct and indirect effects of temperature increase due to dams. Authors would have the opportunity to deepen the conceptualization of the study by discussing the direct and indirect effects more clearly. I am sure they actually think like that, but it would be good to spell it out. I try to explain what I mean. Readers who know the system will be wondering if the temperature increase benefits the bryozoans that are the intermediate host of the myxozoan parasites. Authors devote a paragraph in the discussion to the possible increase in bryozoan population due to changes in the flow regime and temperature. I think it would be useful to bring the thematic of multiple host life cycle already earlier, even mentioning that in the abstract. One could explain that with multiple host life cycles processes in the intermediate host (indirect temperature effects, for example) may be important. Why do I think this would be important? This would allow authors to explain how direct and indirect effects of	We fully agree that distinguishing between direct and indirect temperature effects, particularly in the context of multiple-host life cycles, offers valuable perspectives and has important implications for management. However, since we did not quantify bryozoan abundances or their fine-scale spatial distribution, we are not in a position to rigorously assess the relative contributions of direct and indirect effects in our study. For this reason, and to keep the focus of the current work aligned with our main research objectives, we have not reframed the introduction or abstract around this theme. That said, following the reviewer's comment, we have expanded the discussion of future research directions to explicitly acknowledge the importance of this distinction. Specifically, we now emphasize the need to better understand dam-induced disease processes through both direct effects (e.g. increased disease prevalence and virulence due to temperature-induced stress in fish) and indirect effects (e.g. enhanced bryozoan habitat suitability and associated increases in parasite spore abundance and

	increasing temperature operate in this system. PKD is an example of a parasite which has higher virulence in stressed host (trout), and here authors measure virulence. Temperature will increase the stress levels of fish. PKD is also an example of a parasite where parasite risk depends on density of infected bryozoans in the environment. Bryozoans are filter feeders that thrive in rivers with pools of slowly flowing water, solid (built) structures and low water level variation. Bryozoans probably like impoundments like authors write. If there is something missing in the study it is the assessment of the density of infected and uninfected bryozoans upstream, in reservoirs and downstream. It would be a great addition to the study, but probably authors did not sample bryozoans. I might be asking a bit too much, but it would be great to be able to dissect / discuss the direct and indirect effects of the temperature. Direct effects would be the increase in disease prevalence and virulence due to stressed fish (temperature dependent susceptibility and virulence). Indirect effects would be the epidemiological consequences of more favorable bryozoan habitats (increase in parasite spore numbers and infection risk). This distinction might have importance for management decisions. Authors could also write the introduction and motivation of the study by explaining the expectations with respect to direct and indirect effects of temperature, showing that the problematic with abiotic stressors comes with indirect effects through ecological change in the environment.	infection risk). We believe this addition provides a forward-looking perspective while maintaining the primary focus of the present study. **Added text (lines 218 – 224):** Future studies integrating eDNA data with hydrological and thermal profiles could help identify specific habitat features that facilitate bryozoan colonization. Longitudinal studies across seasons and dam types would further clarify how dam-induced habitat changes influence bryozoan abundances, parasite transmission dynamics and disease risk in salmonid populations. These effects may be indirect, via expanded bryozoan habitats that raise parasite spore abundance and infection risk, or direct, through higher disease prevalence and virulence driven by temperature-induced stress in fish.

Title: Dams threaten salmonids by triggering temperature-dependent disease

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Classification: Biological Sciences, Environmental Sciences

Keywords: Climate change, fish disease, emerging disease, parasites, habitat degradation

Abstract

Dams are ubiquitous in river systems, providing essential services such as drinking water, electricity and irrigation to human society. At the same time, dams significantly impact ecosystems by disrupting flow, and altering natural water temperature regimes. Here, we describe a novel, unappreciated threat posed by dams and reservoirs to one of the world's most popular game fish, brown trout (*Salmo trutta*). We show that small river impoundments elevate downstream water temperature in summer, which increase the prevalence and abundance of malacosporean parasite *Tetracapsuloides bryosalmonae* triggering proliferative kidney disease (PKD), an emerging disorder in salmonids across North America and Europe. Our study highlights the role of reservoirs in creating parasite and disease hotspots, while providing limited evidence that dams act as barriers to *T. bryosalmonae* spread. As global temperatures continue to rise, reservoirs are likely to cause unsustainable effects on downstream riverine fishes through temperature-induced diseases, with particularly severe consequences for cold-water salmonids. This makes downstream areas from reservoirs valuable sentinel sites for monitoring climate impacts on riverine ecosystems. Ultimately, the assessment of dams requires a more holistic approach, where the disease risks are included in the decision-making process balancing human needs with the health of aquatic ecosystems.

Main Text

Introduction

Building dams to manipulate running water is as old as human civilization. Recent estimates suggest that millions of dams have been constructed worldwide (1) and their continued construction progresses at a high pace to provide renewable energy, water security and flood management, particularly in emerging economies (2, 3). However, these man-made barriers impede free flow of water and their associated impoundments have significant effects on water physicochemistry, biodiversity, and ecological connectivity, with far-reaching negative consequences for environmental sustainability (4, 5). Increased attention to environmental recovery has rendered removal of barriers, in particular obsolete ones, an important part of current river restoration activities, often with special emphasis on ecological connectivity and movement of aquatic organisms within river networks (6, 7).

In addition to habitat fragmentation, climate change is increasingly threatening river ecosystems by altering hydrological patterns and thermal regimes (8). Dams exacerbate these impacts by disrupting the natural flow of water, nutrients and sediment, which can lead to eutrophication, algal blooms, oxygen depletion and release of greenhouse gases (9). Their impact on downstream thermal conditions varies depending on river hydrology, reservoir characteristics, and, most critically, the depth from which water is released – either from the surface or the hypolimnion (10). Small, surface-release dams, which constitute the majority of dams globally, can elevate summer water temperatures by several degrees, mirroring short-term projections of climate warming (11, 12).

While many negative environmental consequences of barriers in natural lotic ecosystems are widely recognised, we currently have very limited knowledge on how dams alter epidemiology of temperature-dependent diseases in aquatic wildlife as global temperatures rise (13). Given that the climate acts as an important driver of spatial and seasonal patterns of infections (14), reservoirs that accumulate heat during the hot summer months (11, 12) can drive disease outbreaks. Alternatively, dams may function as barriers for upstream pathogen spread (15). Despite their significant

ecological implications, the role of pathogens and diseases remains largely overlooked by decision-makers and environmental managers when setting priorities for dam removal.

In this study, we focus on an emerging temperature-dependent disease in salmonid fish (Proliferative Kidney Disease; PKD), caused by the malacosporean *Tetracapsuloides bryosalmonae* (*Tb*). This microparasite is widely distributed in North America and Europe, and PKD has been implicated in the decline of salmonid populations in many countries (16). The parasite's lifecycle alternates between colonial bryozoans as its primary host and salmonids as the vertebrate vector. Parasite development in bryozoans is positively correlated to temperature and nutrient content of water (17). Furthermore, severity and fatality of PKD in salmonids is strongly temperature-dependent (18, 19). Resulting disease symptoms, such as enlargement of the kidney (granulomatous nephritis with vascular necrosis) and anemia, leads to high mortality when the water temperature exceeds 15-17 °C over extended period of time (18, 20). Given that parasite lifecycle, host resilience, and disease outcome are all temperature-dependent, the impact of PKD on salmonid populations is expected to exacerbate with global warming (21). However, there is a current gap in understanding the potential role of dams and associated reservoirs in triggering PKD, and more broadly, in contributing to the rise of temperature-dependent diseases in aquatic wildlife.

In this study, we investigated the impact of man-made dams on parasite abundance and disease severity by examining juvenile brown trout (*Salmo trutta*) from up- and downstream of small reservoirs and artificial impoundments. To assess the role of dams as catalysts of PKD or alternatively, as migration barriers for parasite spread, we measured *Tb* infection prevalence and abundance (parasite load), together with main disease symptoms (renal hyperplasia and anemia). Our findings provide compelling evidence that reservoirs function as catalysts for temperature-driven disease in brown trout, while providing limited evidence that dams act as barriers to *Tb* spread. Notably, lotic environments downstream of small reservoirs emerge as parasite and disease hotspots, rendering salmonids particularly vulnerable to rising temperatures.

Results

Water temperature and body size

Compared to upstream river sections, the water temperature was consistently higher downstream of the reservoirs in all studied cases (N = 14 dams/reservoirs, Fig. 1, A and D, Table S1). On average, water temperature was 2.64 °C higher (95% CI: 1.69 to 3.58 °C, range: 0.24 to 5.73 °C) downstream of the dams, as compared to the upstream location (Wilcoxon signed-rank test $P <$ 0.001; Fig. 1D). Similarly, downstream areas exhibited water temperature over 15 °C (clinical PKD threshold) for longer time period compared to upstream locations (average no. of days >15 °C, up- vs. downstream: 33.21 vs 51.3, Wilcoxon signed-rank test $P < 0.001$; Fig 1E, Table S1). There was no difference in diurnal water temperature fluctuation between upstream and downstream locations (up- vs. downstream: 2.05 °C vs. 1.96 °C, Wilcoxon signed-rank test $P = 0.552$, Fig. S1, Table S1). The extent of downstream warming was positively associated with reservoir size, though this did not reach statistical significance (β: 1.3576, 95% CI: -0.514 to -2.592, $P = 0.074$, LM; Fig. 1F). Body size did not differ significantly between locations upstream and downstream of dams ($N = 13$ dams), neither in total length (ANODEV (location): $\chi^2 = 0.03$, $P = 0.870$; Table S2, $N = 442$

individuals). Body mass at a given length (body condition), however, was on average slightly lower in the upstream locations ($\beta_{upstream}$: -0.022 ± 0. 01 SE, t = -2.83, P = 0.005; Table S3, Fig. S2).

Infection prevalence and parasite load

Infection prevalence was significantly lower upstream of dams compared to downstream locations (ANODEV$_{\text{all data}}$ (location): χ^2 = 42.3, P < 0.001, N = 26 locations, Table S4; ANODEV$_{Tb\text{-present rivers}}$ (location): χ^2 = 42.1, P < 0.001, N = 18 locations, Table S5). A closer look at the GLMM for *Tb*-present rivers show that SD = 5.72 for the random effect, compared to the fixed effect ($\beta_{upstream}$ = -4.97), indicating substantial variability in baseline log-odds of the response variable. Hence, river-level variability has a dominant influence on model predictions, where expected values become inflated (Fig. 2A) such that the model overestimates the mean tendency when compensating for high variability. Using a simple pairwise *t*-test to compare infection prevalence upstream and downstream locations in *Tb* present rivers, the differences are still significant (t = 2.40, df = 12, P = 0.034, N = 18 locations).

Parasite load, quantified as copies of *Tb* fragments per qPCR reaction was generally higher downstream of the dams (Fig. 2B), with effects detected using both the full data set ($\beta_{upstream}$: -47.2 ± 5.1 SE, t = -9.30, P < 0.001, N = 444 individuals, Table S6) and the reduced set excluding apparently *Tb*-absent rivers ($\beta_{upstream}$: -65.4 ± 6,8 SE, t = -9.60, P < 0.001, N = 315 individuals, Table S7). The maximum parasite loads for each river are presented in Fig. S3 and the average values (± standard error) for each river are presented in Fig. S4. Using a simple pairwise *t*-test to compare parasite load upstream and downstream locations in *Tb* present rivers, the differences are still significant (t = 2.1, df = 8, P = 0.034, N = 18 locations).

Kidney hyperplasia and anemia

Kidney hyperplasia, measured as K/B ratio and analysed as K at a given B, was more severe in the downstream locations ($\beta_{downstream}$: 0.50 ± 0.04 SE, t = 11.8, P < 0.001, N = 443 individuals, Table S8). From the follow-up model (Table S9), the latter result was verified in the *Tb* present rivers [estimated difference at average body length (upstream – downstream): -0.64 mm, t = -13.1, P < 0.001], but not in *Tb* absent rivers (estimated difference: -0.14 mm, t = -1.70, P = 0.323). Downstream locations in *Tb* absent rivers had lower levels of kidney hyperplasia than downstream locations in *Tb* present rivers (estimated difference: -0.78 mm, t = -3.22, P = 0.032) and the same was the case in the upstream locations (estimated difference: -0.92 mm, t = -3.82, P = 0.012) (Fig. 2, C and D, Fig. S4).

The K/B-ratio started to increase already at low parasite loads and the average K/B-ratio plateaued at parasite loads above approximately 8,000 Tb copies/reaction (N = 213 individuals, Fig. 3A). After plateauing, there was a substantial variation in K/B-ratio, but no values were found to be lower than the average for fish in *Tb* absent rivers.

Hematocrit was found to be generally lower at the highest parasite loads (N = 209 individuals, Fig. 3B). Hematocrit showed a clear negative relationship with K/B-ratio in *Tb* present rivers, which clearly deviates from the uninfected individuals (N = 217 individuals, Fig. 3C). Severe anemia (hematocrit: < 0.25) was observed among individuals with enlarged kidneys (N = 27 individuals, K/B-ratio: > 0.2).

Discussion

The majority of rivers in Europe and North America are fragmented by numerous dams and weirs, which disrupt flow, habitat connectivity, and natural water temperature regime (1, 7). Our study reveals that surface-release dams amplify temperature-dependent disease with warmer lotic environments downstream of small reservoirs serving as parasite and disease hotspots. This adds further pressure on cold-water salmonid populations, which are increasingly threatened by the effects of climate change across their global range (22, 23).

Reservoirs warm up water

We observed increased water temperature downstream of all studied dams, reflecting a widespread effect. Notably, the average water temperatures were up to 5.73 °C warmer than upstream locations (mean 2.64 °C). These values are comparable to reported summertime warming caused by small surface-release dams elsewhere (12). However, unlike earlier studies, we did not measure temperature directly below the dam or upstream of the reservoir. Instead, we focused on brown trout recruitment areas to obtain water temperature data relevant for the disease progression. Consequently, the combined warming effect of the reservoir and associated disease risk may be even more pronounced immediately downstream of the dam.

Previous studies have shown that typical PKD symptoms, such as renal hyperplasia and anemia, emerge when the water temperature exceeds 15 °C for extended periods of time (19, 24). We found that reservoirs significantly increase the duration of time with mean water temperature over 15 °C (on average with 18.1 days), which likely contributes to disease progression and increased mortality. Moreover, even very small reservoirs (1-3 ha) caused a rise in downstream water temperatures by several °C. This highlights that, under low flow conditions, small impoundments can substantially warm up the water, with potentially severe consequences on downstream biodiversity (25, 26).

Dams give rise to parasite hotspots

We observed consistently higher infection prevalence and parasite load downstream of the dams. This is in line with both experimental- and field studies that demonstrate how increased water temperature results in higher *Tb* infection prevalence and parasite load in salmonids (27, 28). The temperature-driven proliferation of *Tb* within bryozoans and salmonids further enhances infectious *Tb* spore abundance in the environment (17, 19). Reservoirs also likely promote the growth, abundance and diversity of bryozoans, as in natural lakes (29). Primary production is enhanced by increased water temperature, solar exposure and longer water residence times (30), leading to a boost in food availability (31) for the primary host of *Tb,* bryozoans (class *Phylactolaemata*) (32). Hence, reservoirs and associated downstream river stretches likely become strongholds for bryozoans and *Tb*, as hinted by recent environmental DNA and niche modelling analyses (33). The latter work illustrates the importance of water temperature, agricultural land use, sediment fineness and cumulative height of upstream dams in explaining *Tb* occurrence and abundance in brown trout. Therefore, our findings, together with existing ecological and parasitological knowledge (33, 34. 35), suggest that dams generate parasite hotspots for *Tb* downstream of reservoirs by creating favourable habitat for bryozoans, the parasite's primary host.

Dams as triggers of disease

Below the dams, brown trout exhibited higher levels of kidney hyperplasia compared to their upstream counterparts. We also observed severe anemia, but only among individuals suffering from severe renal swelling. Elevated water temperature accelerates *Tb* proliferation in fish (20, 28) and modulates the host's immune response (36). At elevated temperatures, the fish immune system

becomes overactive, leading to uncontrolled proliferation of interstitial kidney tissue, i.e., renal hyperplasia (36). Acute PKD also reduces the concentration of red blood cells, limiting oxygen transportation capacity of the circulatory system and thereby reducing both aerobic scope and thermal tolerance of fish (37). Thus, in addition to thermal stress, PKD-affected fish face a complex set of challenges, enduring reduced oxygen transport capacity, diminished aerobic scope, and compromised thermal tolerance (37), which further challenges their ability to withstand elevated water temperatures.

Additionally, variable flow rates and increased evaporation from reservoirs affect fish through limiting habitat use (38). During low-flow periods, salmonids often migrate to deeper pools and ponds (39), which increases their risk of predation (40). Reduced flow rates also elevate stress, increasing the risk of infection, as fish congregate in small areas leading to higher transmission rates (14, 41). Therefore, given widespread temperature- and density-dependence of many diseases (42, 43), reservoirs and dams that create migration barriers and thermal stress are likely to function as disease hotspots for other pathogens beyond PKD.

Dams as potential barriers for parasite spread

In two studied rivers, we detected Tb in trout downstream of dams, while no parasites were found upstream. Therefore, it is possible that these dams function as barriers for upstream parasite spread. Interestingly, in one of these rivers, the dam separating upstream and downstream location was removed in 2015 and functioning fish passage was built to allow anadromous trout to reach upstream spawning grounds. Seven years after construction of the fish passage, we did not observe *Tb* among juvenile trout upstream of the dam. This resembles a case in southern Germany where *Tb* spread has been not observed in five years after connectivity restoration (44). Thus, while questions remain about the long-term risks of introducing parasites and invasive species to currently pathogen-free areas, restoring connectivity and natural lotic sections of the river is expected to provide substantial ecological benefits (6, 45, 46). These include cooling down water, reduced algal growth, and improved access to cold-water refugia, ultimately benefiting a wide range of species. Furthermore, migration serves as a life-history strategy for salmonid fish to evade unfavorable parasite-rich environments, potentially leading to the evolution of pathogens with reduced virulence (47). River temperatures also naturally rise from headwaters to downstream reaches due to cumulative effects of solar radiation, tributary inflows and decreasing elevation (48). Therefore, while there is a potential risk of *Tb* spread to new upstream sections after dam removal, the environmental conditions will likely become less suitable for clinical PKD progression, and ecological benefits may outweigh the associated risks. Equally important is restoring habitats in other heavily modified river sections to ensure connectivity, improved ecological functions and climate change resilience (49). From the perspective of temperature-driven diseases such as PKD, another important measure is enhanching riverbank shading in artificially modified stretches that lack riparian vegetation, as this could significantly improve the temperature regime (50).

Limitations and implications

Although this study covers a single country, it provides important insights with potential implications beyond its borders. In Europe alone, the mean density of dam-like structures is estimated at 0.74 per river kilometre (1). Along with habitat fragmentation, this is affecting temperature- and hydrological regimes of rivers. *Tb* infects a wide range of salmonid fishes (e.g., Atlantic and Pacific salmon, whitefish, char, grayling and trout) and is widely distributed in Europe and North America, with approximately half of the investigated salmonid populations being infected (33, 51, 52, 53, 54). Recent outbreaks of PKD in North America (54), coupled with an increase in disease prevalence in Europe, underscore PKD as an emerging climate-change-associated threat to salmonids (16, 56, 57). Given that our analyses were based on surviving wild

fish, the most severe disease cases were likely missed due to mortality, leading to an underrepresentation of the true impact of PKD (58).

Considering the ongoing threat of further fragmentation due to planned dam constructions (59, 60), our results are highly relevant for future efforts in hydropower development, dam removal, connectivity restoration, and riverscape rehabilitation. This urgency is further underscored by the rapid degradation of freshwater ecosystems (4) and the accelerating pace of global warming, which together highlight the need to better understand the interactive effects of disease and human-induced disturbances in aquatic systems (42, 61).

Materials and Methods

Experimental design

To investigate the impact of small dams on parasite infection dynamics and host pathology in wild brown trout, we employed a paired upstream - downstream sampling design. This approach allowed us to directly compare environmental and biological parameters across dam-influenced and non-impacted sites while minimizing confounding spatial effects. We selected 14 river systems in Estonia and identified the nearest suitable upstream and downstream habitats for spawning and young-of-the-year (YOY) brown trout. These paired sites were used to assess infection prevalence, parasite load and disease symptoms associated with *Tb*, the causative agent of PKD. Summer water temperature profiles were recorded using high-resolution loggers (Onset HOBO MX; HOBO Data Loggers, Bourne, MA, USA). Biological sampling and molecular diagnostics were then employed to link dam-induced thermal changes with parasite load and disease symptoms.

Site selection and water temperature measurements

To estimate the effect of dams on infection prevalence, parasite load and PKD symptoms (renal hyperplasia, anemia), we sampled wild YOY brown trout from the closest spawning and recruitment areas immediately downstream and upstream of the dams to minimize the potential spatial effects (Fig 1, A and B). A total of 28 locations from fourteen rivers containing man-made dams in Estonia were included to the analyses (Fig. 1A, Table S1). All studied dams and associated reservoirs were small (average dam height: 2.7 m, range: 0.95 - 7.65 m; average reservoir size: 8.1 ha, range: 0.5 - 48.5 ha) (Fig. 1C and F, Table S1). Eight of the studied dams have a functioning fish passage. Average study site distance from the dam was 0.9 km downstream and 4.6 km upstream of the dam (Fig 1B, Table S1). Brown trout from five studied rivers were known to be infected with *Tb* (62), whereas for the other nine rivers no prior parasite information was available. To measure daily water temperature, temperature data loggers (Onset HOBO MX) were placed in each site at the end of May in 2022 (26 sites, 2 loggers per site) and at the end of June 2023 (2 sites). Water temperature data was measured every six hours (06:00, 12:00, 18:00, and 00:00). For all studied locations the mean summer water temperature was calculated for the period of June 29 to August 22 (55 days; longer time series available for 13 rivers). Based on maximum and minimum daily temperatures, we calculated diurnal variation for the same period of time (Table S1, Fig. S1). Dam height and reservoir area data was obtained from the Estonian Environmental Portal public database (https://register.keskkonnaportaal.ee/). In two rivers (R. Mustoja and R. Loobu), two consecutive dams were present (distance between dams 0.65 and 3 km, respectively). Therefore, heights of 16 dams are presented in Fig. 1C and Table S1. The area of the two consecutive reservoirs were summed when estimating the correlation between reservoir area and temperature change downstream of the dam (Fig. 1F, separate areas presented in Table S1). Sampling of brown trout in those two rivers was carried out upstream of the upper dam and downstream of the lower dam. For comparative purpose, we extracted temperature data using https://plotdigitizer.com/app from a

study which presents downstream mean water temperature change (August) for 35 small dams in Massachusetts, USA (Fig. 1F) (11).

Sampling of fish

Standard electrofishing of YOY brown trout was conducted over 12 day period in Aug-Sept 2022 (14 reservoirs, 28 sites, permit no. 10-1/22/42-2 for 2022 and permit no. 10-1/23/50-2 for 2023, issued by Ministry of Regional Affairs and Agriculture of Estonia). In September 2023, we sampled one additional river (R. Loobu) and repeated electrofishing in R. Ahja where YOY brown trout were not detected downstream of the dam in 2022. However, similar to 2022, YOY trout were not detected in 2023 although several older trout specimens with detected *Tb* infection were caught in both years. Therefore, R. Ahja was not included to parasitological analyses, and 26 sites located upstream and downstream of 13 rivers were analysed further. Previous studies have shown that *Tb* prevalence in infected salmonid populations is typically > 0.2 (50, 51, 63). Therefore, we aimed to collect tissue samples from ca 20 YOY brown trout per location to minimize the number of lethally sampled fish, while maintaining sufficient power (95%) to distinguish non-infected and infected populations when true prevalence is > 0.2 (64). Caught YOY brown trout were killed with an overdose of benzocaine (> 250 mg L^{-1}, Caesar & Loretz GmbH, Hilden, Germany).

Fork length and total mass (mg) of each fish was measured with a precision scale (Radwag WLC 2/A2/C/2; RADWAG, Radom, Poland). Blood samples were collected from the caudal artery using heparinized microcapillary tubes (0.5–0.6 mm in diameter; Paul Marienfeld GmbH & Co.KG, Lauda-Königshofen, Germany) and centrifuged immediately after sampling at 12,250 g for five minutes (QBC Capillary Centrifuge; Drucker Diagnostics, Port Matilda, PA, USA). Blood plasma and packed red blood cell estimates were carried out using a standard ruler (to closest 0.5 mm) (65). One, two or three microcapillaries were collected per fish from 427, 222 and 6 individuals, respectively, and mean hematocrit values per fish was calculated when multiple estimates were available. In order to quantify renal hyperplasia, a sagittal cross section of each fish was produced by cutting the body between the front tip and the end of the dorsal fin (37). This section was photographed against 1 mm grid (Fig. 1J) with a digital camera for the measurement of the kidney-to-body thickness ratio (K/B-ratio) as a quantitative estimate of kidney hyperplasia using ImageJ software (37, 66). The cross section was stored in 96% ethanol (5 mL screw-cap centrifuge tubes) for DNA extraction from the kidney tissue for quantification of *T. bryosalmonae (Tb)*.

DNA extraction and molecular quantification of the parasite

Total genomic DNA from kidney tissues of the 446 YOY brown trout were extracted using QIAamp 96 DNA QIAcube HT kit and the QIAcube® HT Instrument for automated nucleic acid purification (QIAGEN, Hilden, Germany). DNA concentrations were measured with NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA) and diluted to 20 ng μL^{-1}. The quantification of *Tb* was performed in a set of three replicates for each kidney extraction using real-time quantitative PCR (qPCR) based on Taqman probe on a LightCycler 480 (Roche, Basel, Switzerland). The assay employed *Tb* specific primers and TaqMan hydrolysis probe to amplify a 90-bp 18S rDNA sequence of the parasite (66). Each 10 µL amplification reaction consisted of 4.4 µL of nuclease-free water, 2 µL of 5x HOT FIREPol Multiplex qPCR Mix (NO-ROX; Solis BioDyne OÜ, Tartu, Estonia), 0.2 µL of 200 nM forward and reverse primers, 0.2 µL of 200 nM probe, and 3 µL of genomic kidney DNA (20 ng μL^{-1}). qPCR was conducted using the following thermal cycling conditions: an initial denaturation step at 95°C for 10 minutes, followed by 45 cycles of 95°C for 15 seconds and 60°C for 60 seconds. All qPCR plates were manually prepared and run by the same individual.

A ten-fold serial dilution of pooled *Tb*-positive kidney DNA, with total genomic DNA concentrations ranging from 40 to 0.004 ng μL^{-1} (five concentrations), was prepared and included on each plate in six technical replicates per concentration as a standard dilution series. Negative PCR controls were included on each plate in six replicates (36 total negative PCR controls). None of the negative PCR controls showed positive amplification signal. Additionally, one negative DNA extraction control was included on each qPCR plate (six plates total), with three technical replicates per plate. None of the negative DNA extraction controls showed positive amplification.

To estimate the Limit of Detection (LOD) and Limit of Quantification (LOQ), a synthetic dilution series was created by using an artificially synthesized 90 bp *Tb* 18S gene sequence fragment (67). The series began with a ten-fold serial dilution ranging from 6,022,142 to 60.2 copies μL^{-1} (five concentrations, with 32 technical replicates per concentration). This was followed by an eight-fold dilution, reducing the concentration from 60.2 to 7.5 copies μL^{-1} (32 technical replicates per concentration). Finally, two five-fold dilutions were performed, further decreasing the concentration from 7.5 to 1.5 and then to 0.3 copies μL^{-1} (64 technical replicates per concentration).

Due to the substantial variation in parasite load among infected samples (e.g., up to 1.6×10^6 orders of magnitude) (68), an SD threshold of <1 was used to identify outliers. Regression analysis of the standard dilution series was performed for each plate to determine qPCR plate efficiencies (Table S10). LOD (3.6 copies/reaction) and LOQ (9 copies/reaction) were determined using the synthetic *Tb* 18S rRNA gene dilution series (69, 70). The effective LOD for three replicates was calculated as 1.1 *Tb* target copies/reaction.

Statistical analyses

All analyses were run in R 4.4.2 (R Core Team, Vienna; https://www.r-project.org). For statistical comparisons, we ran linear (LM), or generalized linear mixed models (LMM/GLMM) using the *lme4* (71) and *lmerTest* (72) packages (presented below in *lme4* syntax). Analyses of Deviance (ANODEV) were run through the *car* package (73). Graphs were constructed based on elements created in *ggplot2* (74) and *cowplot* (75) and formatted in Inkscape 1.3.2 (Inkscape Project 2020, https://inkscape.org/). Statistical summary tables from raw data and models are presented in the supplementary materials (Table S2-S10).

The non-parametric Wilcoxon signed-rank test was used to compare water temperature, days with water temperature >15 °C and diurnal water temperature fluctuation up- and downstream of the dams and reservoirs.

Body size differences between upstream and downstream sites were assessed based on *total body length* (tl), using the LMM: tl ~ location + (1|river), where location is a fixed factor (two levels: upstream- and downstream of dam) and river is a random factor [13 levels (river names)]. Body condition between upstream and downstream sites was evaluated as the *relative body mass* (m) given the total length of the individuals; model: $\log_e(m) \sim \log_e(tl)$ + location + (1|river).

Infection prevalence (proportion of infected individuals; pv) was analysed with the GLMM: pv ~ location + (1|river), weights = tot.obs, family = binomial. For weights, we used the total number of individuals (tot.obs) that each location-specific proportion value was based on. Overdispersion was tested through the *DHARMa* package (76) and found non-significant (dispersion = 1.14, $p = 0.30$). The same model was run using a data subset including only rivers where the parasite was detected, given that effects are mainly of interest in rivers with confirmed parasite presence.

Parasite load (pl) was analysed (square-root transformed to accommodate for positive skew) with the LMM: sqrt(pl) ~ location + (1|river); focusing on comparing upstream and downstream sites overall. We also ran the same model using a data subset including only rivers where the parasite was detected.

Renal hyperplasia was analysed as the relative thickness of the kidney to dorsal muscular tissue (Fig. 1J). In models, *kidney thickness* (k) was the dependent variable, height of dorsal musculature (*body thickness*; b) was added as a covariate [LMM: k ~ b + location + (1|river)] representing a proxy of the overall size of the fish; in data plots, we use the calculated K/B-ratio ('K/B-ratio' = k/b). A follow-up model was built utilizing the fact that some rivers (N = 4) were completely parasite-free in the analysed samples (*ad hoc* controls), adding the factor *parasite presence* (par.pres) in interaction with location [model: k ~ b + location × par.pres + (1|river)]. Results from the latter model were analysed using pairwise location × par.pres contrasts (Kenward-Roger method for degrees-of-freedom; Tukey adjustment for multiple comparisons), using the *emmeans* package (77).

Progression of renal hyperplasia (as K/B-ratio) in relation to parasite load was analysed for infected individuals, using a non-linear loess regression (pooling all rivers). The relationship between the variables was assessed graphically based on the 95% confidence band relative to the estimated mean K/B-ratio for uninfected individuals (non-overlapping confidence bands indicating significant difference).

Hematocrit was analysed in relation to parasite load and renal hyperplasia using non-linear loess regressions, as above. Reservoir area and downstream temperature rise was assessed using linear regression.

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Figures and Tables

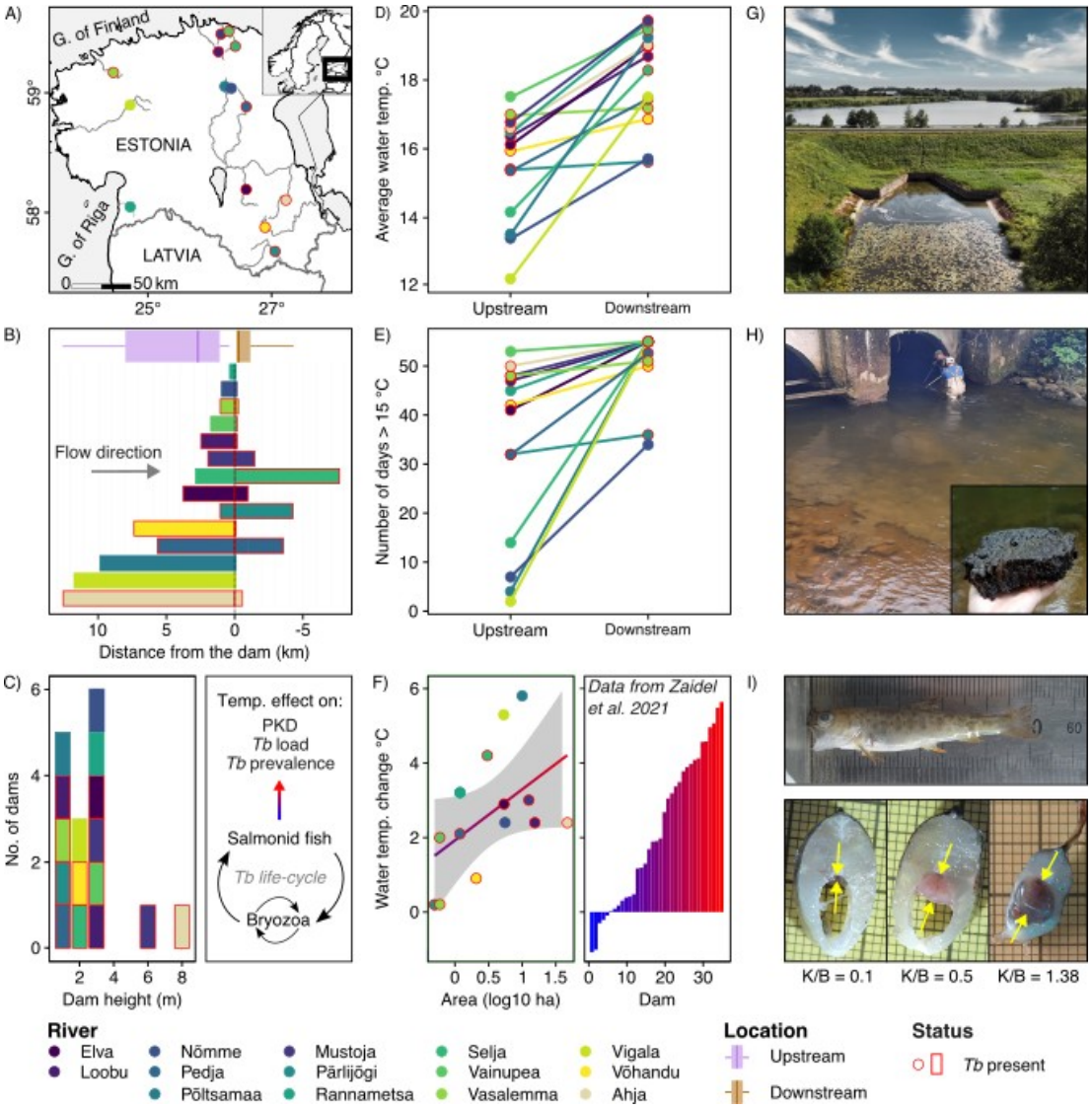


Figure 1. Studied rivers and dams, water temperature and proliferative kidney disease (PKD) in brown trout. A, The study area consisting 14 dams. **B**, Distances of the study sites from the dam, boxplots showing the distributions for up-and downstream sites. **C.** Left: Distribution of dam heights in relation to *Tb* occurrence. Right: *Tb* life-cycle and scheme of the effect of temperature on parasite prevalence, load and PKD. **D**, Mean summer water temperature with lines connecting upstream and downstream locations. **E**, Number of days over 15 °C upstream and downstream of the dam. **F**, Left: Effect of reservoir area (log10 ha) on water temperature change. Right: Temperature change downstream of 35 small dams according to Zaidel et al. (2021). **G**, Aerial photo of small dam and reservoir (10.6 ha) at R. Mustoja. **H**, Photo of a large bryozoan colony covering most of the bottom downstream of the dam at R. Ahja with a close-up image of the bryozoan colony (*Plumatella fungosa*). **I**, Dead wild YOY brown trout found from downstream section of R. Mustoja with extreme renal hyperplasia. **J**, Sagittal cross section of a trout with normal (left), swollen (middle) kidney and extreme renal hyperplasia (right, dead trout from Fig. 1I). The yellow arrows mark the location of the kidney, K/B indicates kidney-to-body thickness ratio.

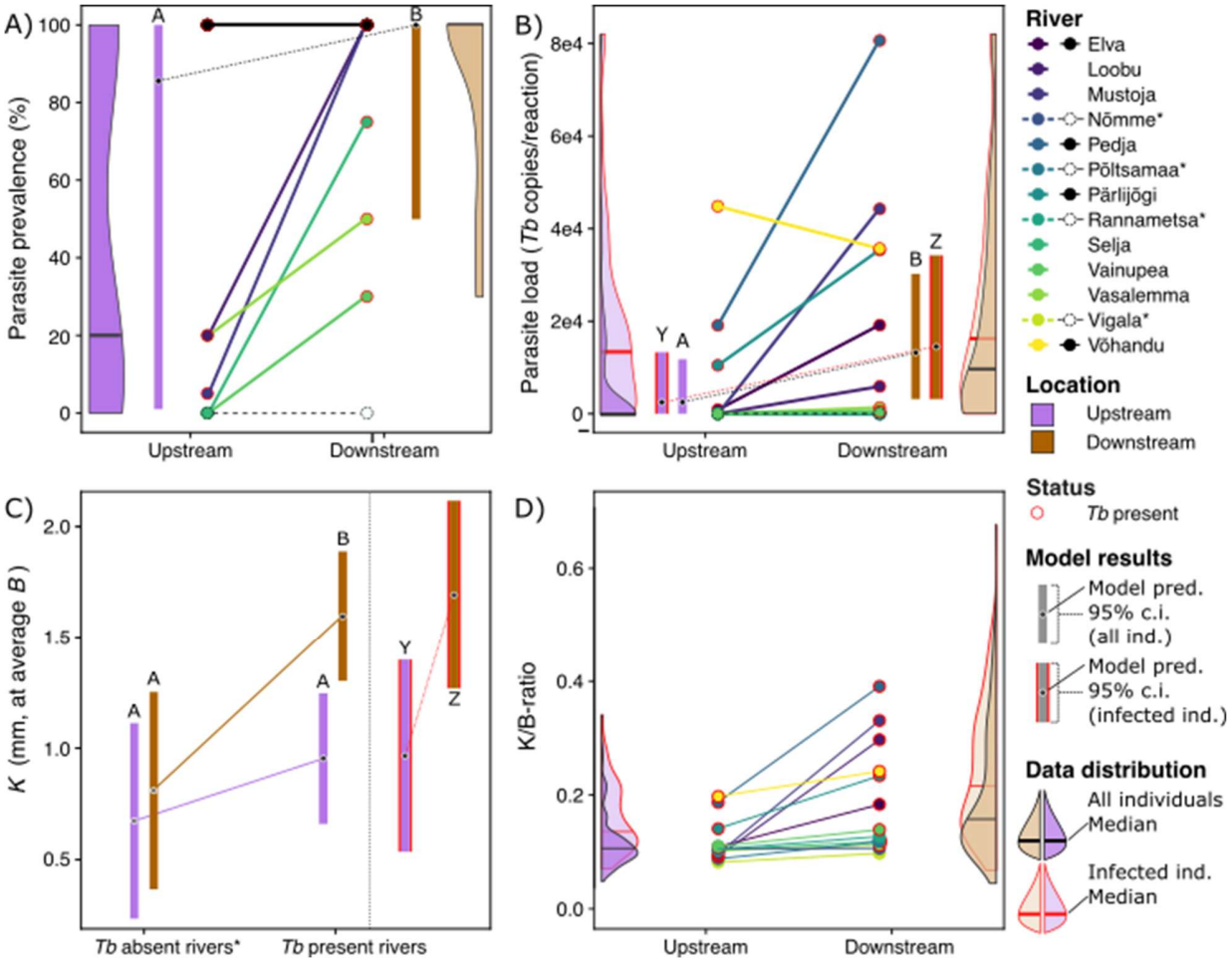


Figure 2. Effect of dams on infection prevalence, parasite load and renal hyperplasia. A, Parasite prevalence (% of individuals infected) up- and downstream of the dams. Note that all rivers with 100 % total prevalence (up- and downstream of dams) are shown with black colour and all rivers with 0 % total prevalence are coloured white (see legend). **B**, Parasite load (copies of *Tb* per reaction) up- and downstream of dams (upper clip at 8.2e4 for graphing purposes; see max values in Fig. S3). **C**, Relative kidney thickness (*K*) in relation to body thickness (dorsal muscular thickness, *B*), evaluated at the average *B*. **D**, Relative kidney thickness illustrated as K/B-ratio (kidney thickness divided by dorsal muscular thickness). For panels **B**, **C**, and **D**, data and results are presented both for all trout individuals and for infected individuals only (see legend for colour scheme explanation of model results and data distribution). Significant differences are marked with different letters above model estimates (A-B for models including all individuals; Y-Z for models including only infected individuals).

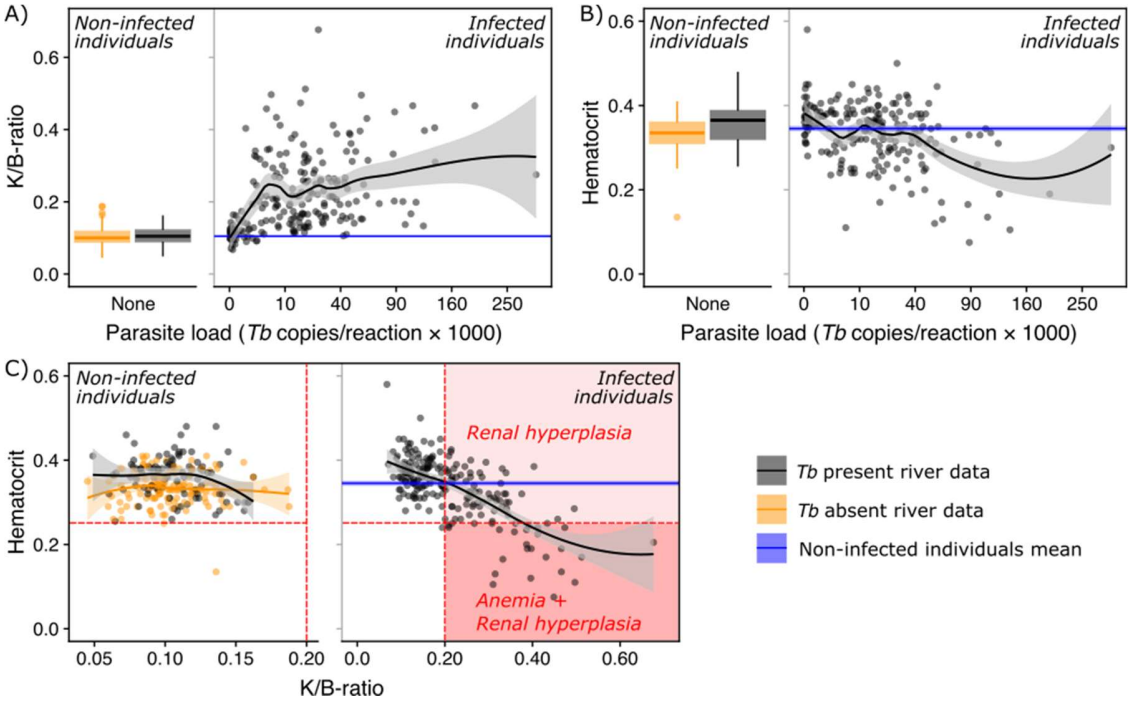
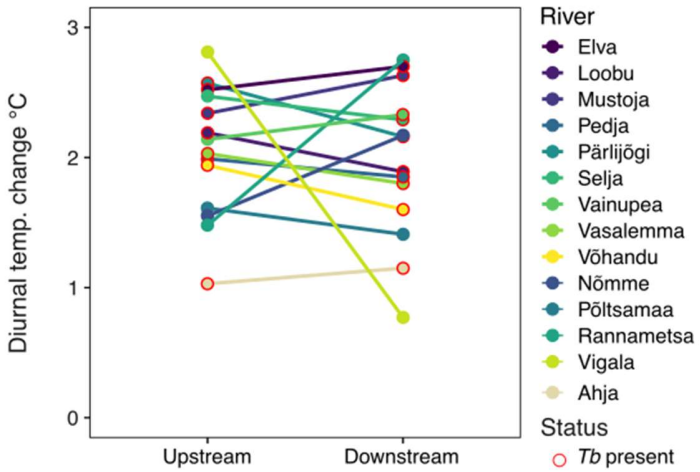


Figure 3. Relationships between parasite load and PKD symptoms. a, Parasite load and renal hyperplasia (measured as K/B-ratio), **b**, Parasite load and hematocrit, and **c**, Renal hyperplasia and hematocrit, presented as loess regressions. Individuals from *Tb* absent rivers and uninfected individuals from *Tb* present rivers are treated as two sets of control data (all parasite loads = 0), presented as a Tukey boxplots to the left of the regressions, with their pooled mean values represented as a blue line (with 95% CI) crossing the regression graph.

706 **Supplementary Materials**
707
708 **Figs. S1 to S4**
709 Supplementary Fig. 1. Diurnal water temperature changes up- vs. downstream of the dams.
710 Supplementary Fig. 2. Residual body mass for downstream and upstream locations.
711 Supplementary Fig. 3. Maximum parasite load (*Tb* copies/reaction) up- and downstream of dams.
712 Supplementary Fig. 4. Disease traits, parasite load and prevalence of individual rivers.
713
714 **Tables S1 to S10**
715 Supplementary Table 1. Sampling site information with dam/reservoir characteristics, parasite
716 prevalence and temperature estimates.
717 Supplementary Table 2. Summary table from linear mixed model for analysis of total length.
718 Supplementary Table 3. Summary table from linear mixed model for analysis of body condition.
719 Supplementary Table 4. Summary table from binomial generalized mixed model (logit-link) for
720 analysis of parasite prevalence (all rivers).
721 Supplementary Table 5. Summary table from binomial generalized mixed model (for analysis of
722 parasite prevalence (only including *Tb* present rivers).
723 Supplementary Table 6. Summary table from linear mixed model for analysis of parasite load (all
724 rivers).
725 Supplementary Table 7. Summary table from linear mixed model for analysis of parasite load (only
726 including *Tb* present rivers).
727 Supplementary Table 8. Summary table from linear mixed model for analysis of renal hyperplasia
728 (all data).
729 Supplementary Table 9. Summary table from linear mixed model for analysis of renal hyperplasia.
730 Model includes factor parasite presence in interaction with location to compare *Tb* present/absent
731 rivers.
732 Supplementary Table 10. Estimated qPCR parameters associated with *Tb* quantification.



733
734 **Fig. S1:** Diurnal water temperature changes up- vs. downstream of the dams.
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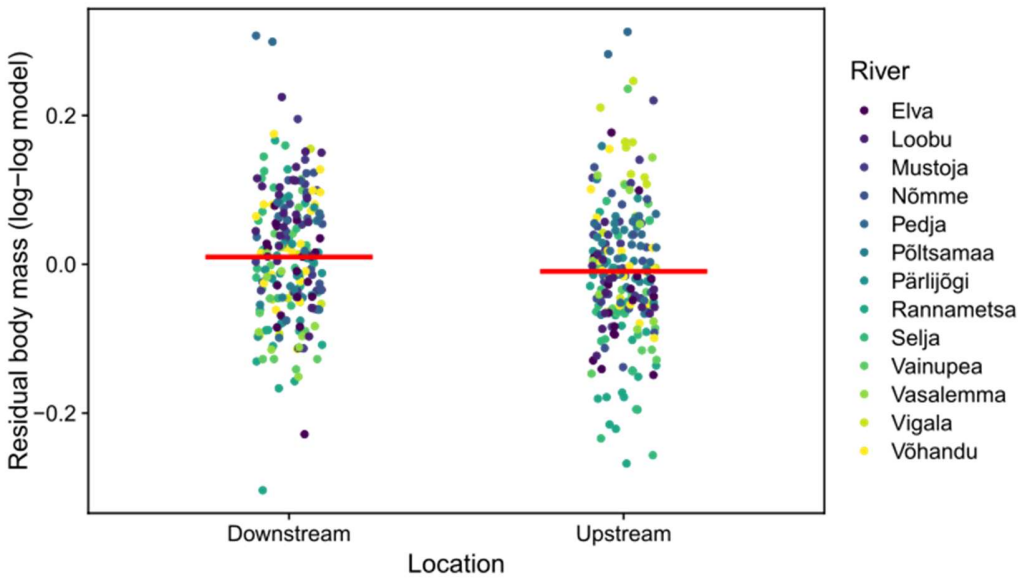

Fig. S2: Residual body mass for downstream and upstream locations. Residuals from model: tm ~ log(tl) + location + (1|river). Red horizontal line shows the overall mean.

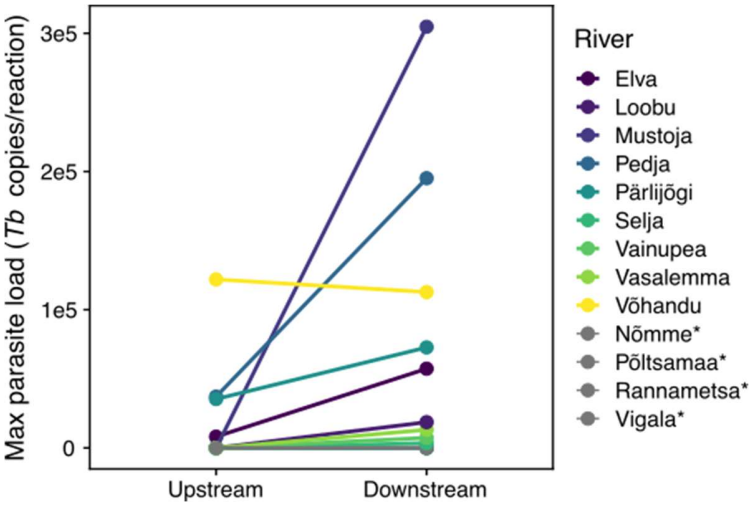

Fig. S3: Maximum parasite load (*Tb* copies/reaction) up- and downstream of dams.

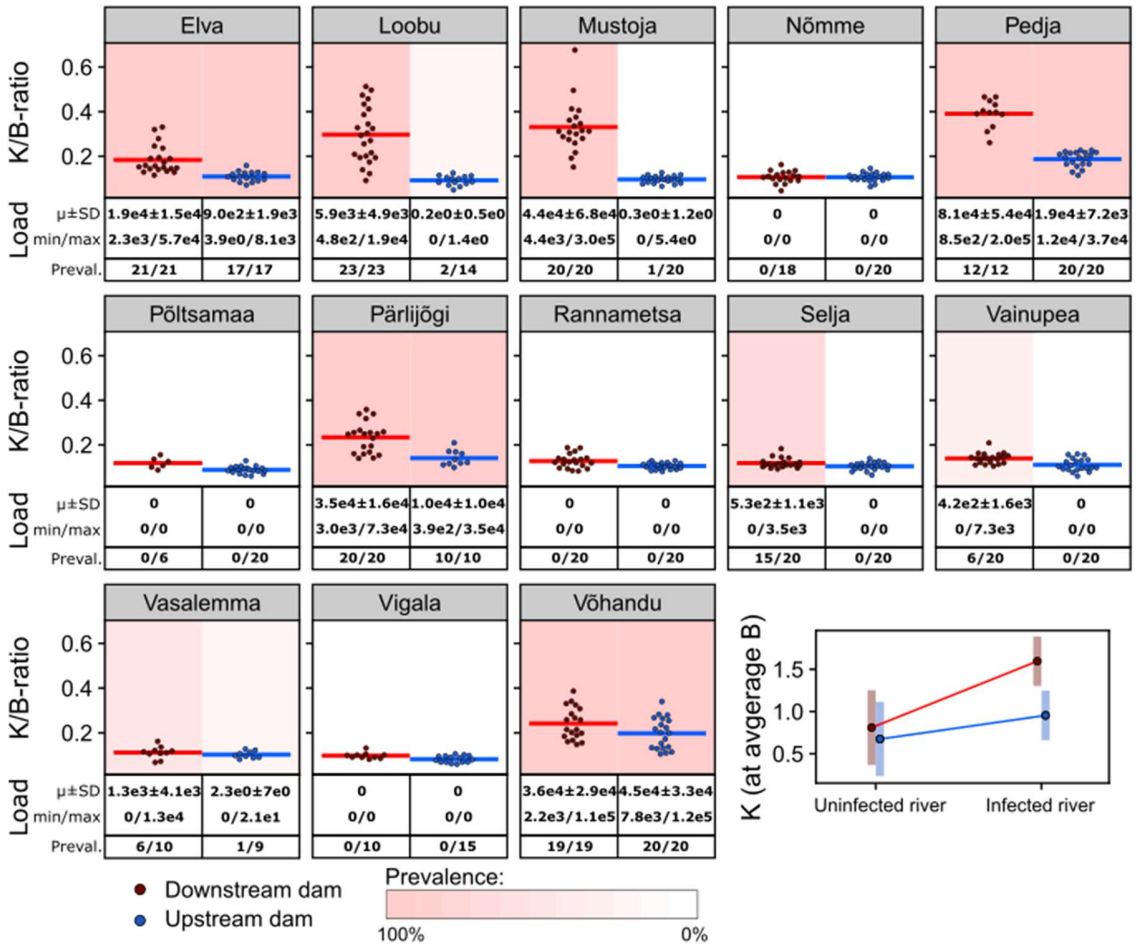

Fig. S4: Summarizing figure of renal hyperplasia (K/B-ratio; kidney thickness divided by dorsal muscle thickness), parasite loads (Load; *Tb* copies/reaction) and prevalence (% infected individuals) in brown trout in Estonian rivers (upstream and downstream of dams). Parasite loads are summarised for each river, in terms of mean and standard deviation (μ ± SD), minimum and maximum values (min/max), and number of individuals with detected loads out of total sample ('Preval.'). For each river location, the prevalence is also indicated by graph background shading. The K/B-ratio is presented for each river (points: individual fish; horizontal bar = mean), as well as in the form of estimated marginal means with 95% confidence intervals (lower right); in the latter graph, the estimates relate to the mean kidney thickness (mm) at the mean body thickness.

754 **Table S1:** Sampling site information with dam/reservoir characteristics, parasite prevalence and temperature estimates.
755

River - Dam name	Date (dd.mm.yyyy)	Location from dam	Coordinates (N, E)	Distance from the dam (km)	No. of juveniles sampled	No. of infected	No. of uninfected	*Tb* prev. (95% CI)	Parasite load (*Tb* copies/reaction)	K/B ratio	Hematocrit	Dam height (m)	Reservoir size (ha)	Average water temp. (°C)	No. Of days > 15 °C	Diurnal water temp. difference (°C)
Rannametsa - Laiksaare	22.08.2022	Downstream	58°05'46.5, 24°40'02.7"	0.1	20	0	20	0 (0–0.161)	0	0.127	0.329	3.35	1.2	19.68	55	2.75
Rannametsa – Laiksaare	22.08.2022	Upstream	58°05'40.3, 24°40'23.6"	0.4	20	0	20	0 (0–0.161)	0	0.105	0.336			16.45	45	1.48
Vigala – Kuusiku	23.08.2022	Downstream	58°57'54.3, 24°43'01.4"	0.1	10	0	10	0 (0–0.277)	0	0.098	0.368	2.15	5.4	17.51	55	0.77
Vigala – Kuusiku	23.08.2022	Upstream	58°55'25.7, 24°51'09.0"	11.8	15	0	15	0 (0–0.204)	0	0.082	0.299			12.20	2	2.81
Vainupea – Pajuveski	24.08.2022	Downstream	59°34'02.4, 26°15'28.7"	0.2	20	6	14	0.2 (0.145–052)	1404.7	0.139	0.321	2.65	0.6	19.47	55	2.33
Vainupea – Pajuveski	24.08.2022	Upstream	59°34'02.4, 26°15'28.7"	1.8	20	0	20	0 (0–0.161)	0	0.110	0.341			17.50	53	2.14

Selja - Päide	25.08.2022	Downstream	59°23'27.4", 26°23'30.4"	7.7	20	15	5	0.75 (0.531−0.888)	699.5	0.118	0.379	2.25	3.1	18.29	55	2.29
Selja - Päide	25.08.2022	Upstream	59°22'31.5, 26°17'13.6"	2.9	20	0	20	0 (0−0.204)	0	0.104	0.333			14.14	14	2.47
Mustoja - Vihula II/III	25.08.2022	Downstream	59°33'09.9, 26°10'59.3"	1.5	20	100	0	1 (0.839−1)	44255	0.331	0.29	2.55/6	2.2/10.6	15.72	55	2.63
Mustoja - Vihula II/III	26.08.2022	Upstream	59°31'48.0, 26°10'44.4"	2	20	1	19	0.05 (0−0.236)	5.4	0.096	0.382			13.38	48	2.34
Põltsamaa – Ao	27.08.2022	Downstream	59°00'22.7, 26°12'27.7"	0.1	6	0	6	0 (0−0.39)	0	0.118	0.318	0.95	10.3	19.24	55	1.41
Põltsamaa – Ao	27.08.2022	Upstream	59°03'30.8, 26°10'38.8"	9.9	20	0	20	0 (0−0.161)	0	0.088	0.359			13.50	4	1.61
Nõmme - Nõmmeveski	28.08.2022	Downstream	59°02'32.8, 26°13'29.7"	0.2	18	0	18	0 (0−0.176)	0	0.107	0.35	3.2	5.7	15.72	34	2.17
Nõmme - Nõmmeveski	28.08.2022	Upstream	59°02'42.1, 26°14'20.1"	1	20	0	20	0 (0−0.161)	0	0.106	0.313			13.38	7	1.55
Ahja – Saesaare	29.08.2022	Downstream	58°06'52.2,	0.6	0	N/A	N/A	N/A	N/A	N/A	N/A	7.65	48.5	19.02	55	1.15

Ahja – Saesaare	29.08.2022	Upstream	27°03'05.5" 58°08'38.9, 26°58'28.9"	12.6	15	15	0	1 (0.796−1)	10938.1	0.121	0.34			16.57	50	1.03
Võhandu – Hutita	31.08.2022	Downstream	57°53'00.1, 26°44'32.5"	0.1	19	19	0	1 (0.832−1)	35690	0.242	0.339	2.25	2.1	16.87	50	1.6
Võhandu – Hutita	29.08.2022	Upstream	57°55'32.7, 26°45'05.5"	7.4	20	20	0	1 (0.839−1)	44827.5	0.198	0.297			15.92	42	1.94
Pärlijõgi - Alaveski	31.08.2022	Downstream	57°45'23.6, 26°45'55.6"	4.3	20	20	0	1 (0.839−1)	35368.9	0.234	0.329	1	0.5	15.63	36	2.16
Pärlijõgi - Alaveski	31.08.2022	Upstream	59°02'42.1, 26°14'20.1"	1.1	10	10	0	1 (0.722−1)	10468	0.141	0.407			15.39	32	2.57
Elva - Hellenurme	01.09.2022	Downstream	58°08'33.8, 26°23'51.7"	1	21	21	0	1 (0.845−1)	19150.3	0.183	0.384	2.9	5.5	18.99	55	2.7
Elva - Hellenurme	01.09.2022	Upstream	58°06'54.8, 26°23'17.7"	3.8	17	17	0	1 (0.816−1)	897.9	0.109	0.368			16.10	41	2.52
Pedja – Käruveski	02.09.2022	Downstream	57°45'23.6, 26°45'55.6"	3.6	12	12	0	1 (0.757−1)	80640	0.19	0.391	1	1	17.45	53	1.85

Pedja – Käruveski	02.09.2022	Upstream	58°56'52.8, 26°30'25.4"	5.7	20	20	0	1 (0.839–1)	19100.2	0.187	0.36			15.35	32	1.99
Vasalemma – Töökmani	05.09.2022	Downstream	59°12'25.1, 24°25'06.7"	0.3	10	6	4	0.6 (0.313–0.832)	2224.1	0.112	0.443	1	0.6	17.19	51	1.8
Vasalemma – Töökmani	05.09.2022	Upstream	59°12'10.4, 24°26'10.6"	1.1	10	1	9	0.1 (0–0.04)	21	0.103	0.346			16.97	48	2.03
Loobu - Undla/Kadrina	30.08.2023	Downstream	59°21'00.5, 26°06'31.8"	0.2	23	23	0	1 (0.857–1)	5903.8	0.297	0.295	1/2.65	0.5/15.5	18.70	55	1.89
Loobu - Undla/Kadrina	30.08.2023	Upstream	59°18'46.5, 26°09'53.4"	2.5	15	2	13	0.13 (0–0.038)	1.3	0.092	0.377			16.35	47	2.19

756

Table S2: Summary table from linear mixed model for analysis of total length (mm): tl ~location + (1|river).

Random effects

Groups		Variance	SD
River	(Intercept)	85.9	9.3
Residual		106.9	10.3
N (obs.): 442			
N (River): 13			

Fixed effects

	Estimate	SE	df	*t*	*p*
(Intercept)	74.2	2.7	13.1	27.8	5.17e-13
Location (upstream)	-0.16	1.00	429.4	-0.16	0.870

Table S3: Summary table from linear mixed model for analysis of body condition (relative total mass, g): tm ~ log(tl) + location + (1|river).

Random effects

Groups		Variance	SD
River	(Intercept)	0.002	0.042
Residual		0.007	0.081

N (obs.): 442
N (River): 13

Fixed effects

	Estimate	SE	df	*t*	*p*
(Intercept)	-12.32	0.12	371.2	-106.7	< 2e-16
$\log_e$(total length)	3.18	0.03	388.8	118.9	< 2e-16
Location (upstream)	-0.02	0.01	430.0	-2.83	0.005

Table S4: Summary table from binomial generalized mixed model (logit-link) for analysis of parasite prevalence: PV ~ location + (1|river); PV is the proportion of *Tb*-positive samples and the total sample size for each river was used as weights in the model. Model run on all data.

Random effects

Groups		Variance	SD
River	(Intercept)	79.49	8.92

N (obs.): 26
N (River): 13

Fixed effects

	Estimate	SE	*z*	*p*
(Intercept)	2.01	2.99	0.67	0.502
Location (upstream)	-4.96	0.76	-6.50	< 0.001

Table S5: Summary table from binomial generalized mixed model (logit-link) for analysis of parasite prevalence: PV ~ location + (1|river); PV is the proportion of *Tb*-positive samples and the total sample size for each river was used as weights in the model. Model run on a subset of data, only including *Tb* present rivers.

Random effects

Groups		Variance	SD
River	(Intercept)	32.67	5.72

N (obs.): 18
N (River): 9

Fixed effects

	Estimate	SE	*z*	*p*
(Intercept)	6.55	2.65	2.47	0.013
Location (upstream)	-4.97	0.77	-6.49	< 0.001

Table S6: Summary table from linear mixed model for analysis of parasite load: sqrt(PL) ~ location + (1|river). Model run on all data.

Random effects

Groups		Variance	SD
River	(Intercept)	5190	72.04
Residual		2749	52.43

N (obs.): 444
N (River): 13

Fixed effects

	Estimate	SE	df	*t*	*p*
(Intercept)	82.32	20.31	12.41	4.05	0.002
Location (upstream)	-47.15	5.07	430.60	-9.30	<2e-16

Table S7: Summary table from linear mixed model for analysis of parasite load: pl ~ location + (1|river). Model run on a subset of data, only including *Tb* present rivers.

Random effects

Groups		Variance	SD
River	(Intercept)	5731	75.71
Residual		3571	59.76

N (obs.): 315
N (River): 9

Fixed effects

	Estimate	SE	df	*t*	*p*
(Intercept)	115.03	25.68	8.25	4.48	0.002
Location (upstream)	-65.44	6,82	305.26	-9.60	<2e-16

Table S8: Summary table from linear mixed model for analysis of renal hyperplasia (kidney height, K relative to dorsal musculature height, B): k ~ b + location + (1|river). Model is run on all data without separation of infected and *Tb* absent rivers.

Random effects

Groups		Variance	SD
River	(Intercept)	0.21	0.46
Residual		0.19	0.44

N (obs.): 443
N (River): 13

Fixed effects

	Estimate	SE	df	*t*	*P*
(Intercept)	1.12	0.17	38.8	6.4	1.63e-7
Body height	0.04	0.02	440.0	2.2	0.028
Location (upstream)	-0.50	0.04	429.0	-11.8	<2e-16

Table S9: Summary table from linear mixed model for analysis of renal hyperplasia (kidney height, K relative to dorsal musculature height, B): k ~ b + location × par.pres + (1|river). Model includes factor parasite presence (par.pres) in interaction with location to compare infected and *Tb* present/absent rivers.

Random effects

Groups		Variance	SD
River	(Intercept)	0.15	0.39
Residual		0.18	0.43

N (obs.): 443
N (River): 13

Fixed effects

	Estimate	SE	df	*t*	*p*
(Intercept)	0.38	0.24	22.5	1.59	0.125
Body height	0.06	0.02	435.1	3.65	0.0003
Location (upstream)	-0.14	0.08	430.5	-1.70	0.090
Parasite presence (infected river)	0.79	0.24	11.9	3.21	0.008
Location (upstream) × Par. pres. (infected river)	-0.51	0.09	431.1	-5.34	1.51e-7

Table S10: Estimated qPCR parameters associated with *Tb* quantification. *Calculations were performed with first four ten-fold serial dilution concentrations.

Parameter	
Mean SD for technical replicate Cq values	0.110
Median SD for technical replicate Cq values	0.053
25 percentile for technical replicate Cq values	0.031
75 percentile for technical replicate Cq values	0.113
Plate 1 amplification efficiency %	91.45*
Plate 2 amplification efficiency %	92.16
Plate 3 amplification efficiency %	92.25*
Plate 4 amplification efficiency %	93.20
Plate 5 amplification efficiency %	91.68*
Plate 6 amplification efficiency %	93.60
Plate 1 r^2 of calibration curve	0.999
Plate 2 r^2 of calibration curve	0.993
Plate 3 r^2 of calibration curve	0.999
Plate 4 r^2 of calibration curve	0.997
Plate 5 r^2 of calibration curve	0.999
Plate 6 r^2 of calibration curve	0.998
Synthetic dilution series plate efficiency %	91.78
Synthetic dilution series r^2	0.998