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Effects of Different Preservatives During Ecological Monitoring of Myxozoan Parasite *Tetracapsuloides bryosalmonae* Causing Proliferative Kidney Disease (PKD) in Salmonids

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ABSTRACT

Effective sample preservation is essential in large-scale population monitoring, particularly for molecular genetic analyses of pathogens, and for measuring disease symptoms in hosts. In such monitoring cases, disease symptoms can indicate poor habitat health, as they often coincide with elevated temperatures and suboptimal environmental conditions. This study examines the effect of two preservatives; 95% ethanol and 99% isopropanol on the assessment of proliferative kidney disease (PKD) in the renal tissue of young-of-the-year brown trout (*Salmo trutta*). Specifically, we studied the effect of preservatives on the physical measurement of a primary symptom of PKD, renal hyperplasia. Furthermore, we evaluated the effect of preservatives on the molecular detection and quantification of the causative PKD agent myxozoan parasite *Tetracapsuloides bryosalmonae*. Our results indicate that isopropanol-preserved samples exhibit greater renal tissue shrinkage, with the most pronounced differences observed in smaller fish when compared to ethanol-preserved samples. This difference in shrinkage is great enough to disguise symptomatic fish when observing renal hyperplasia with mixed storage mediums. However, both preservatives were found to be suitable for DNA extraction of sufficient quality for detection and quantification of the parasite using qPCR with no statistically significant differences in DNA yield or parasite load due to the type of preservative. We found that while ethanol is preferable for ease of dissection, isopropanol is a suitable alternative for PKD monitoring in wild fish, especially where access to ethanol may be limited. Understanding the difference in tissue shrinkage caused by the two preservatives can enable compensatory adjustment and maintain higher standards of data accuracy when assessing the severity of *Tetracapsuloides bryosalmonae* infection.

1 | Introduction

In large-scale monitoring programmes involving the sampling and preservation of biological material, multiple actors are often involved to cover large geographic areas within an appropriate period. Therefore, it is important to adopt a common methodology, or when deviation occurs, that methods are validated to harmonise the data for effective analyses.

Salmonids are culturally and economically important, and their populations are therefore systematically monitored in many countries [e.g., within ICES (https://www.ices.dk/) and NASCO

(https://nasco.int/)]. Furthermore, salmonids are often selected as umbrella indicator species within national riverine environmental monitoring, to assess biological effects of water acidification or general ecological status (e.g., Eklöv et al. 1999; van Treeck et al. 2020; Näslund and Strömberg 2023). Several salmonid species and populations are regionally endangered (IUCN 2024) and negative population trends have been observed in several areas in recent years (e.g., Gallagher et al. 2022; Donadi et al. 2023). However, despite substantial interest in salmonid population status and general health, regular large-scale monitoring of salmonid populations for parasites and pathogens is rare, except for Atlantic salmon monitoring for the monogenean *Gyrodactylus salaris* (e.g., Hansen et al. 2024; Degerman et al. 2012).

When collecting samples during large-scale monitoring, ethanol is often the preferred preservative for samples destined for molecular and genetic analyses due to its rapid penetration of cellular membranes (King and Porter 2004). However, pure ethanol solutions (only containing ethanol and water) can be problematic from several perspectives, especially when sampling is conducted by private contractors. For instance, there are countries where high-percentage pure ethanol cannot be purchased without a licence, since ingestion can cause intoxication with psychoactive effects or be subject to misuse. An alternative preservative for storing biological samples is isopropanol (e.g., Rake 1972; Carmon et al. 2014; Basnet et al. 2017), which is commonly used for household and commercial purposes (Slaughter et al. 2014) and is generally not restricted to the same extent as ethanol (Carmon et al. 2014). Furthermore, isopropanol cannot be consumed for intoxicative purposes since the consumer will likely suffer from poisoning (Jammalamadaka and Raissi 2010; Slaughter et al. 2014).

Here, we compare the effects of preserving whole fish (young-of-the-year brown trout *Salmo trutta*), in either of two commonly used preservatives in Sweden, i.e. 95% ethanol or 99% isopropanol, on both the molecular genetic assessment of the presence of the malacosporean parasite *Tetracapsuloides bryosalmonae* and the associated PKD (Canning et al. 1999). The parasite and, in particular, its associated disease are of high interest within ecological risk assessments of salmonid population integrity (e.g., Skovgaard and Buchmann 2012; Dash and Vasemägi 2014; Rubin et al. 2019). PKD is temperature dependent (Strepparava et al. 2018; Lauringson et al. 2022) and can cause substantial mortality in salmonid young of the year (Sterud et al. 2007; Arndt et al. 2019). Clinical symptoms of PKD include anaemia, abnormal swimming behaviour and abdominal swelling whereby the kidney is the most affected organ (Clifton-Hadley et al. 1984). The magnitude of renal hyperplasia is correlated with disease severity and estimated thermal tolerance (Bruneaux et al. 2017), Hence, knowledge of the presence of *T. bryosalmonae* and quantifying renal hyperplasia within populations is highly valuable when assessing salmonid population trends within environmental- and fisheries management and research. This is particularly relevant in locations affected by increasing summer water temperatures caused by climate change or sun-heated epilimnion spillover at small dams (Zaidel et al. 2021), or by excessive nutrient loads (Ros et al. 2021). Therefore, the presence of sick fish in national monitoring can indicate deteriorated or unfavourable environmental conditions.

In this study, young-of-the-year brown trout were collected within Sweden. The monitoring was conducted on a national scale (Figure 1a), in parallel to the yearly fish monitoring programme, and included a large number of electrofishing contractors as well as research staff (21 unique collectors). While some contractors have access to laboratory-grade ethanol for preservation, this is not always the case (or it may be associated with unwanted paperwork). Thus, isopropanol was used as an alternative preservative by approximately half of the contractors in the PKD monitoring. During the dissection of sampled trout, we noticed substantial differences between samples stored in the two preservatives. Specifically, the samples preserved in isopropanol were harder and tissues appeared more dried out; as a consequence, the bodies were sometimes misshaped (Figure 1b). To ascertain comparability of our results when using different preservatives, we assessed differences between samples, with respect to (1) cross-sectional measurements of kidneys for assessment of one of the primary PKD symptoms, renal hyperplasia and (2) successful molecular detection of *T. bryosalmonae* using end-point PCR and quantification using quantitative PCR (qPCR).

2 | Material and Methods

2.1 | Sample Collection

In total, 884 young-of-the-year brown trout were collected using wading electrofishing in conjunction with the Swedish national river monitoring programs (general environmental monitoring, monitoring of liming and monitoring of salmonid populations) in 2022. The mean collection date was August 30 (SD: +/− 16.5 days) ranging from July 18 to October 4. After electrofishing, the fish were euthanized by an overdose of anaesthetic (according to Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes). The type of anaesthetic varied among sampling crews (e.g., MS 222 or benzocaine), fish were measured to the nearest millimetre (maximal total length, caudal fins folded), and tagged with a streamer tag for individual identification. The dead and tagged fish were stored in either 95% ethanol ($n = 445$) or 99% isopropanol ($n = 439$); maximally 50 g fish per 0.5 L preservative fluid (≈ 10% volume). The choice of preservative was decided by the sampling crews based on accessibility; hence, all fish from a given supplier were generally preserved in only one of the preservatives. However, suppliers and storage types overlapped spatially, reducing the concern of any location-level effect on the size of the fish sampled.

Before any handling, samples were stored in preservative fluid for a minimum of 138 days [mean: 202 days (identical for both preservation methods); SD:33, 36 days; range: 138–265, 138–269 days for ethanol and isopropanol, respectively], to ensure full fixation since the majority of shrinkage should occur by 90 days post-fixation (Fox 1996).

2.2 | Dissection and Quantitative Assessment of Renal Hyperplasia

To investigate one of the most characteristic PKD symptoms, renal hyperplasia (excessive proliferation of the kidney tissue), a standardised sagittal cross-section of the fish body was made

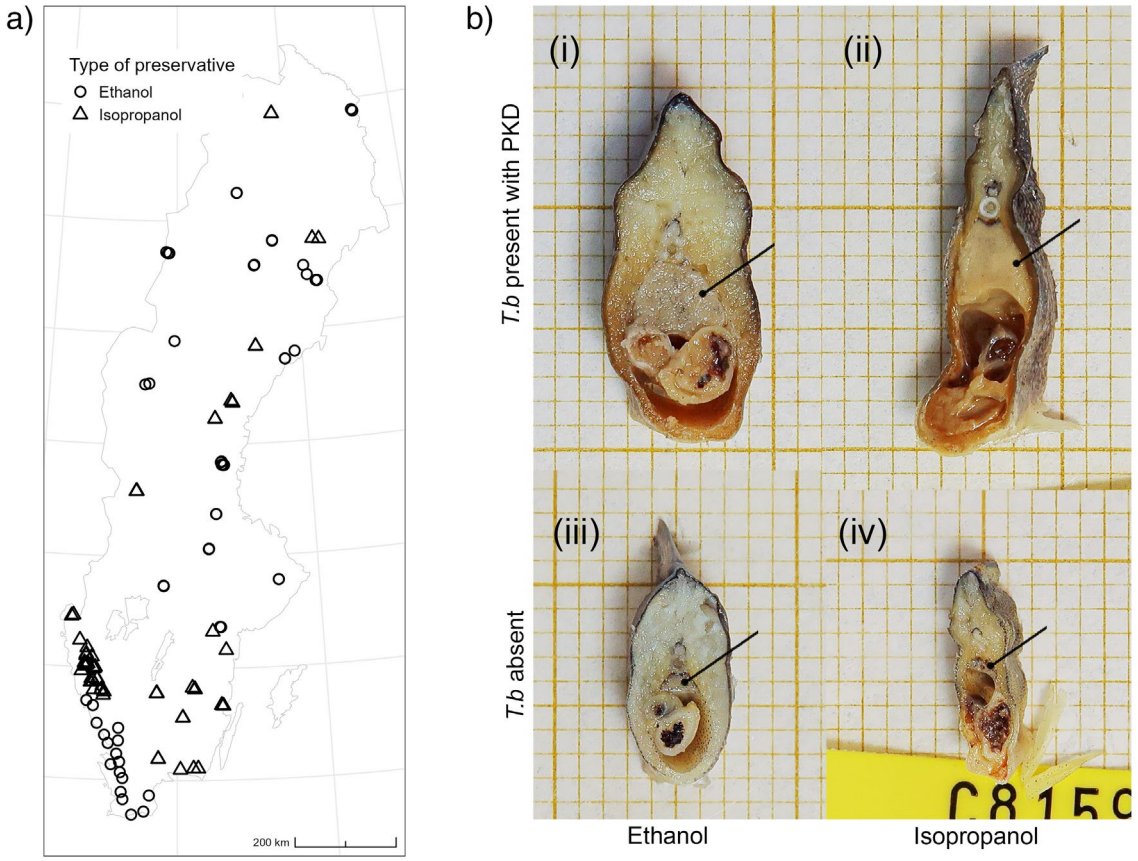


FIGURE 1 | (a) Map of the sampling locations and preservative type, there were 60 locations where ethanol was used and 63 locations for isopropanol. (b) Pictures of sagittal cross-sections of juvenile brown trout, for quantitative assessment of renal hyperplasia. Depicted specimens are: (i, ii) infected with *Tetracapsuloides bryosalmonae* (*T.b*) and displaying PKD symptoms (renal hyperplasia); (iii, iv) uninfected; (i, iii) stored in 95% ethanol; (ii, iv) stored in 99% isopropanol. Fish sizes (maximal total length): (i) 73 mm, (ii) 70 mm, (iii) 48 mm, (iv) 48 mm. Annotation lines indicate kidney tissue. Photos are taken against a 1 mm grid.

at the rostral base of the dorsal fin as in Bruneaux et al. (2017). Each cross-section was photographed perpendicularly from above, against a millimetre paper, using a digital camera mounted on a copy stand (Canon PowerShot G7 X Mk II, Canon; Tokyo, Japan). The photos were captured in RAW file type and post-processed in Adobe Photoshop Lightroom Classic (Version 13.0.1, Adobe Systems; Mountain View, CA, USA) with white balance and exposure adjustments to compensate for the fluctuations in the colour temperature of the on-camera flash. From the digital photographs, the cross-sectional area of the kidney for each individual was measured using ImageJ software (Schneider et al. 2012). Previous studies have assessed renal hyperplasia by a kidney-to-body thickness ratio, based on one-dimensional measures of fresh fish (e.g., Bruneaux et al. 2017; Debes et al. 2017; Lauringson et al. 2021). Here, this ratio was considered unsuitable because isopropanol samples tended to be misshapen, in comparison to ethanol samples, possibly distorting the shape of the kidney (Figure 1) whereas total area is hypothesized to be better conserved despite distortion.

2.3 | DNA Isolation

During dissection, a piece of the kidney from the sagittal cross-section of each sample, regardless of the initial preservative, was stored in 95% ethanol for molecular detection of *T. bryosalmonae*. Stored kidney fragments were standardised to ca. 2 mm in diameter.

DNA was extracted using QIAamp 96 DNA QIAcube HT Kit (Qiagen GmbH; Hilden, Germany), with a QIAcubeHT robot (Qiagen GmbH) for nucleic acid purification automation following the manufacturer's protocol. The volume of the elution buffer (Buffer AE, Qiagen GmbH) used in the final step was 100 μL for all extractions. DNA quality was measured using a NanoPhotometer N60 (Implen; Munich, Germany) and subsequently diluted with the same elution buffer to 20 ng μL^{-1}.

2.4 | End-Point Multiplex PCR

A subset of samples ($n = 161$) was selected across all batches of DNA extraction such that there was at least one sample per location for end-point multiplex PCR and equal sampling effort per batch. The multiplex PCR assay developed by Dash and Vasemägi (2014) provided a check for DNA integrity by utilising primers amplifying parasite and host DNA of different base pair lengths; PKX3F, PKX4R (Kent et al. 1998) 298 bp, PKD-realF, PKD-realR, 166 bp (Grabner and El-Matbouli 2009) to target the 18 s rRNA gene of *T.*

bryosalmonae, and a salmonid-specific fragment ca 500 bp (Vasemägi et al. 2010) for control. The use of both PKD-realF and PKX4R primers in the multiplex reaction also amplifies a 756 bp fragment of the *T. bryosalmonae* 18 s rRNA gene (Dash and Vasemägi 2014). The PCR protocol was followed as per Lauringson et al. (2022) with the alteration of an increase to 35 PCR cycles and the inclusion of *T. bryosalmonae* and salmonid-positive controls. Lastly, the final product after PCR amplification was inspected using ethidium bromide stained 2% agarose gel electrophoresis.

2.5 | Real-Time qPCR Parasite Detection

Since multiplex PCR is not a quantitative method, we applied real-time qPCR to quantify the parasite load and increase the sensitivity of detecting *T. bryosalmonae*. The assay consisted of a 10 μL reaction comprised of 4.94 μL dH_2O, 2 μL 5× HOT FIREPol Probe qPCR Mix Plus (Solis BioDyne; Tartu, Estonia) and 0.02 μL of forward and reverse primers (PKX18s1266f-1426r, 91 bp, Hutchins et al. 2018), probe (Taqman double quenched PKX18s_1399probe, Hutchins et al. 2018) (100 pmol μL^{-1} concentration) and 3 μL of DNA template (20 ng μL^{-1} concentration). qPCR was conducted on a CFX384 Touch Real-Time PCR Detection System (Bio-Rad Laboratories Inc.; Hercules, CA, USA) with analyses performed in CFX Maestro 2.3 software (Bio-Rad Laboratories Inc.). A standard hot-start two-step qPCR protocol was used with 12 min at 95°C, then 40 cycles of 15 s of denaturation at 95°C, 60 s of annealing at 60°C and a plate read.

The 884 samples that required screening necessitated 9 qPCR plate runs, therefore inter-assay variation was assessed using a synthetic PKX target (91 bp) in a dilution series with 5 steps of a tenfold dilution starting at 1.81×10^8 copies. Subsequently, the slope, intercept and R^2 of the standard curve fit to the threshold values of the dilutions were compared between plates. For robust quantification of *T. bryosalmonae*, the samples were analysed with three technical replicates. The limit of detection (LoD) and limit of quantification (LoQ) of the assay for a single reaction was determined as 18.6 and 19 copies, respectively, as calculated by the method of Klymus et al. (2020). When considering all three replicates the effective LoD was 2.8 copies of the target DNA.

2.6 | Data Analyses

Out of 884 samples, 19 individuals (2.1%; 3 from ethanol and 16 from isopropanol) were excluded due to missing or erroneous body length data. Due to shrinkage and curved body fixation, body lengths were not remeasured from preserved specimens. To investigate storage effects on the kidney cross-sectional area and avoid potentially confounding effects due to infection, only confirmed *T.b*-negative samples were used ('*T.b*' = *T. bryosalmonae*). Confirmation of parasite infection was based on qPCR results (ethanol: $n = 291$; isopropanol: $n = 193$). The analysis was based on linear regression, with a general linear model where the square root of the cross-sectional area was the dependent variable ($\sqrt{A}$), and maximum total body length (L) (range median and mean) and preservative (P; fixed factor, two levels: ethanol and isopropanol) were independent variables:

$$\sqrt{A} \sim L + P$$

The cross-sectional area was square root transformed to improve normality and homoscedasticity (the cross-sectional area is a two-dimensional measure being related to a body length, a one-dimensional measure).

Specimens being *T.b*-positive were later added in an analysis of all samples, with isopropanol samples being recalculated to the predicted ethanol-storage size based on parameter estimates from the regression model above.

3 | Results

3.1 | DNA Extraction and Molecular Detection of the Parasite

A comparison of the DNA extractions revealed no significant difference in DNA concentration between the two storage mediums (Wilcoxon rank-sum test, $W = 27{,}488$, $p = 0.4867$). The mean DNA concentrations yielded for ethanol and isopropanol were 240 (SD: 129) ng μL^{-1} and 238 (SD: 157) ng μL^{-1} respectively. Of the 365 samples in which the parasite DNA was detected, the presence of the parasite DNA in the kidney tissue did not contribute to a significant difference in the concentration of the extracted DNA. Of the ethanol-stored samples, those without parasite detection ($n = 295$) yielded a mean DNA concentration of 237 (SD: 140) ng μL^{-1} and in isopropanol ($n = 222$), the yield was 244 (SD: 179) ng μL^{-1}. When the parasite was detected mean yields were 248 (SD: 104) and 227 (SD: 131) ng μL^{-1} for ethanol ($n = 150$) and isopropanol ($n = 215$), respectively.

Inspection of end-point PCR using agarose gels revealed seven samples where the 500 bp salmonid control fragment did not amplify (Figure 2a). Six of these samples were stored in isopropanol and one of these six samples retained amplification for the shorter parasite 166 bp DNA fragment. Similarly, the single ethanol stored sample where the salmonid control DNA target did not amplify also featured amplification of the parasite, suggesting that some degradation of the DNA had occurred.

The threshold values of amplification of the 91 bp parasite fragment based on the qPCR revealed no significant difference between storage media (Wilcoxon rank-sum test, $W = 15{,}628$, $p = 0.8009$). Mean C_t values for ethanol stored samples were 27.7 (SD: 4.1) cycles and 27.5 (SD: 3.5) cycles for isopropanol (Figure 2b) implying that parasite quantification was unaffected by the choice of preservative.

3.2 | Sagittal Kidney Cross-Sections

We found a significant effect of the preservation medium on the cross-sectional area of the kidney (Figure 3a,b), where fish stored in isopropanol had on average smaller kidneys than kidneys from fish stored in ethanol. Considering the square-root

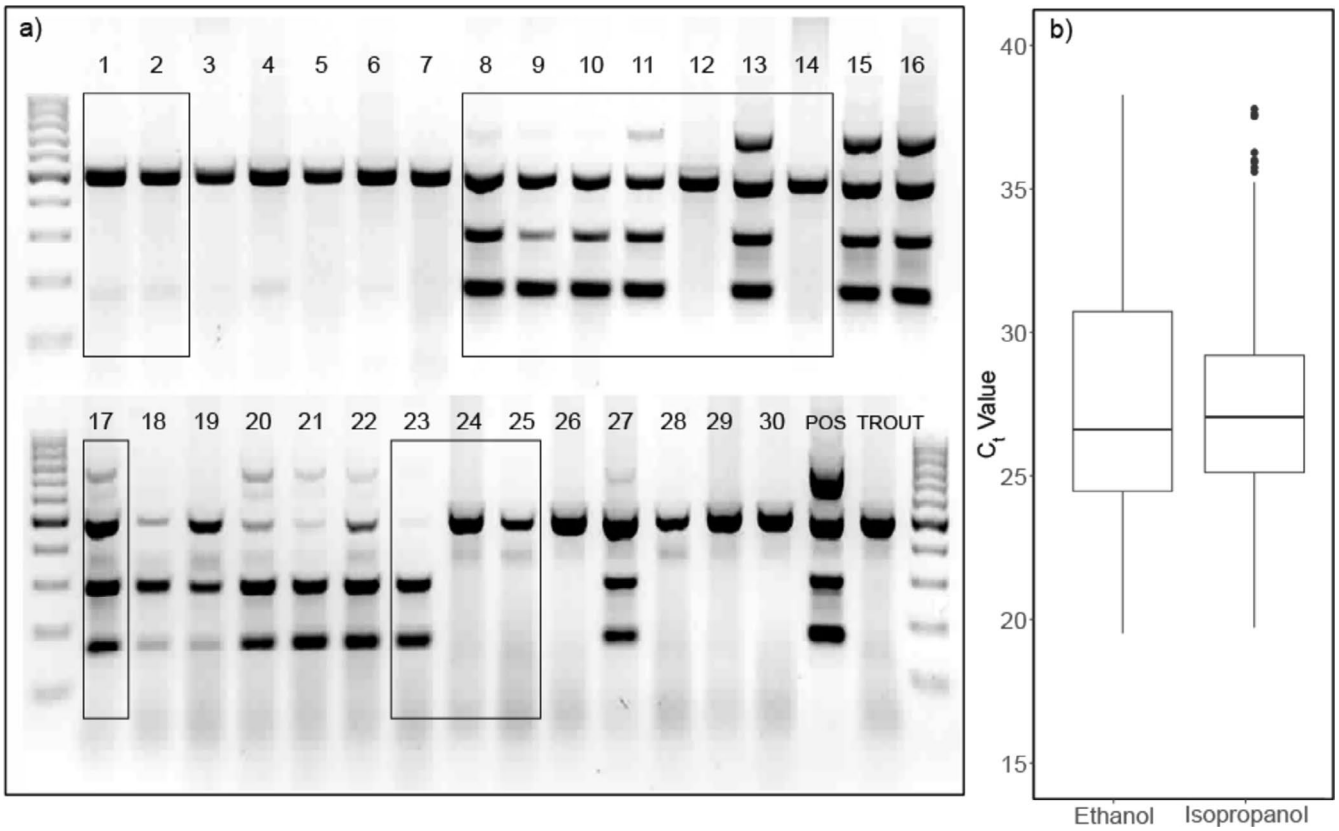


FIGURE 2 | (a) 2% agarose gel electrophoresis of end-point multiplex PCR product. Samples that were preserved in isopropanol are shown within rectangles. The ladder ranges from 100 to 1000 bp with the 166 bp PKD-realF/PKD-realR, 298 bp PKX3F/PKX4R and 756 bp PKD-realF/PKX4R *T. bryosalmonae* amplicons in addition to the 500 bp salmonid target. POS—*T. bryosalmonae* positive control, TROUT—salmonid positive control. Sample #23 provides an example of where the salmonid amplicon was not present due to DNA degradation yet the shorter *T. bryosalmonae* amplicons are still present. (b) Effect of preservative on parasite detection using qPCR, mean C_t value of three technical replicates displayed on the y-axis. Less cycles indicate a higher starting quantity of parasite DNA in the fish kidney tissue. Line, box, whiskers and dots represent the median, first to third quartile, variability as 1.5 times interquartile range and outliers respectively.

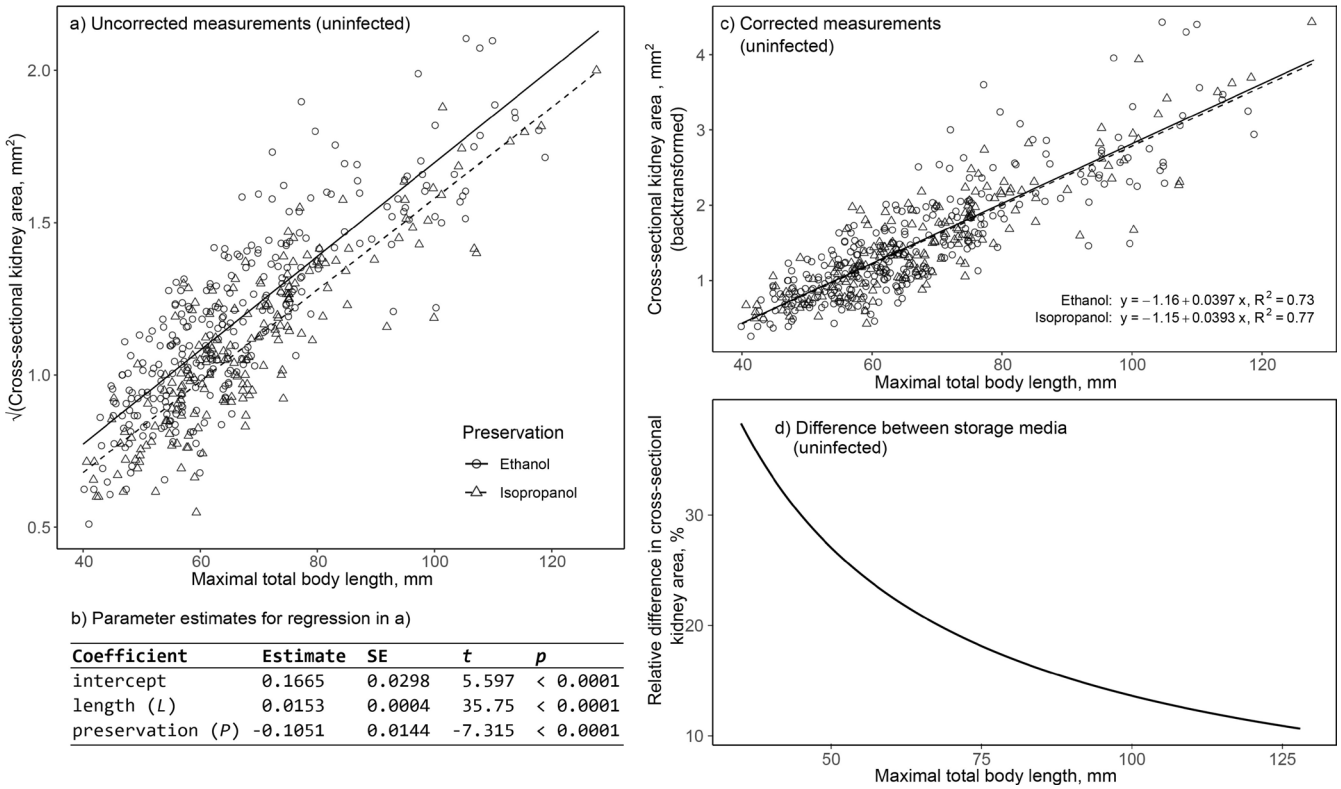
transformed scale, isopropanol samples required adjustment by a value of +0.1051 (Figure 3b) to match the ethanol samples. (Figure 3c,d; note that back transformed values are plotted in the graphs).

4 | Discussion

Our study shows that using different preservatives results in significant differences in the estimation of *T. bryosalmonae*-induced renal hyperplasia in brown trout. We found that 99% isopropanol leads to more shrinkage of the kidney as compared to 95% ethanol and this effect becomes more pronounced as the fish size decreases. For example, when considering the 1st, 2nd and 3rd quartiles at 56 mm, 64 mm and 75 mm respectively, the difference between storage media is, approximately, as large as 24% for the 1st quartile, 21% for the 2nd and small as 18% for the 3rd. For a fish greater than 200 mm in length the difference is approaching 6%, however, this assumes the relationship between fish length and kidney cross-sectional area holds beyond the juvenile year (Figure 3d). Generally, as fish length increases, the relative size difference between the two storage mediums becomes less pronounced. The magnitude of these differences is sufficient to disguise symptomatic fish stored in isopropanol among asymptomatic, yet parasite infected fish stored in ethanol.

It should be noted that the alcohol concentration in the solutions differed slightly (i.e., 95% vs. 99%), which limits the validity of the results to these specific concentrations. Studies on whole-body shrinkage in fish larvae (*Diaphus* spp.) showed that 70% isopropanol caused more shrinkage than 90% ethanol (Moku et al. 2004). Studies on wet mass loss in worms show the same effect using 70% solutions for both alcohols (Howmiller 1972). Hence, while the alcohol concentration could play a role in the shrinkage (dehydration) factor (Smith and Walker 2003), storage in isopropanol is likely to cause more shrinkage than storage in ethanol in general. Storage duration can affect shrinkage rates in small fish; however, the majority of distortion occurs early on in the fixation process and can plateau by 90 days of storage (Moku et al. 2004; Fox 1996). Nevertheless, the main take-home message is that there is a clear need to estimate and correct estimates obtained from different preservation fluids when quantifying renal hyperplasia for PKD monitoring.

While this study focuses on brown trout, the generality of the results is likely relevant for soft tissue storage regardless of



b) Parameter estimates for regression in a)

Coefficient	Estimate	SE	t	p
intercept	0.1665	0.0298	5.597	< 0.0001
length (*L*)	0.0153	0.0004	35.75	< 0.0001
preservation (*P*)	-0.1051	0.0144	-7.315	< 0.0001

FIGURE 3 | Illustration of differences between preservation media (95% ethanol and 99% isopropanol) and results of data corrections. (a) Scatterplot visualising all data from *T.b*-negative (uninfected) brown trout and the estimated difference in cross-sectional kidney area between samples stored in ethanol and isopropanol. (b) Summary table for the regression for panel a. For the 'preservation' coefficient, the effect of isopropanol relative to ethanol (intercept) is presented. Model statistics: $F_{2,470} = 653.2$; $p < 0.0001$; $R^2 = 0.734$. Model residuals: Min = −0.472; max = 0.556; median = −0.007. (c) Result of applying the empirical correction factor, visualised using the back-transformed data from *T.b*-negative individuals. (d) Relative difference between ethanol and isopropanol stored samples for a given fish length (ethanol relative to isopropanol). For a calculation of the relative difference, see Appendix A.

focal species (Howmiller 1972; Moku et al. 2004). However, exact correction factors need to be preferably investigated separately for other salmonid species, tissues and types of measures.

DNA extraction followed by end-point PCR and qPCR was successfully performed on the samples stored in both ethanol and isopropanol. However, there was a lack of amplification of the salmonid positive control target in six isopropanol-preserved samples after end-point PCR in comparison to a single sample for ethanol. It was observed that three of these isopropanol samples were received from a single location in a shared container with an insufficient quantity of preservative. A lack of preservative can result in residual water leading to accelerated DNA degradation through the processes of hydrolysis (Lindahl 1993). If DNA degradation occurs, it is usually the shortest fragments that are most resilient as is often observed in the extreme cases of ancient DNA (Dabney et al. 2013). Ultimately, we saw no difference in detection thresholds between the two preservatives when using the short 91 bp fragment of *T. bryosalmonae* as a target for the qPCR assay, confirming the performance of isopropanol as a suitable preservative for molecular detection and quantification of the parasite comparable to that of ethanol. This result was expected, given that isopropanol is used in some protocols for precipitating DNA (e.g., Moore and Dowhan 2002) and previously suggested as an alternative to ethanol to preserve animal specimens and tissues for DNA extraction (e.g., Rake 1972; Basnet et al. 2017). Thus, our molecular results confirm the suitability of isopropanol as an alternative to ethanol, which in some circumstances makes it easier to collect field samples since ethanol is subject to certain restrictions in some legislations.

5 | Conclusions

Overall, we found that the preservation of fish in isopropanol is a suitable alternative to ethanol for the assessment of the prevalence and load of *T. bryosalmonae*, and for the quantification of renal hyperplasia as a characteristic symptom of PKD. However, additional tissue shrinkage requires correction when assessing renal hyperplasia as an indicator of disease severity and samples stored in ethanol are easier to process in terms of dissection procedures. By underlining the differences in the usage of the preservative, while at the same time providing a correction factor, our study enables the flexible choice of preservative medium and facilitates monitoring of *T. bryosalmonae* infection and PKD in wild salmonid populations over wide geographic areas involving researchers, electrofishing contractors, as well as, commercial and sport fishers.

Author Contributions

Duncan Philpott: conceptualization, methodology, validation, formal analysis, investigation, data curation, writing – original draft, visualization. **Joacim Näslund:** conceptualization, methodology, writing – original draft, supervision, funding acquisition. **Serena Donadi:** conceptualization, writing – review and editing, supervision, funding acquisition. **Oksana Burimski:** methodology, resources, data curation. **Magnus Lauringson:** data curation, resources, methodology. **Lilian Pukk:** methodology, data curation, resources. **Anti Vasemägi:** conceptualization, methodology, resources, writing – review and editing, visualization, supervision, project administration, funding acquisition.

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We thank all electrofishing survey crews who agreed to collect samples for our monitoring of PKD in Swedish rivers.

Ethics Statement

The study has been conducted in accordance with ethical approvals, following Swedish law (each sampling event being associated with a specific licence; a prerequisite for receiving permission to do electrofishing in Sweden). Euthanized trout individuals are part of a national-scale age analysis.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in Data: Effects of different preservatives during PKD screen at https://figshare.com/s/1bbf711138a582e80b54, reference number 10.6084/m9.figshare.27316878.

References

Arndt, D., R. Fux, A. Blutke, et al. 2019. "Proliferative Kidney Disease and Proliferative Darkening Syndrome Are Linked With Brown Trout (*Salmo trutta* Fario) Mortalities in the Pre-Alpine Isar River." *Pathogens* 8: 177. https://doi.org/10.3390/pathogens8040177.

Basnet, S., R. B. Narain, and S. T. Kamble. 2017. "Preservation Techniques Affecting DNA Extraction From *Cimex lectularius* (Hemiptera: Cimicidae)." In *Proceedings of the Ninth International Conference on Urban Pests*, 93–97. Pureprint Group.

Bruneaux, M., M. Visse, R. Gross, L. Pukk, L. Saks, and A. Vasemägi. 2017. "Parasite Infection and Decreased Thermal Tolerance: Impact of Proliferative Kidney Disease on a Wild Salmonid Fish in the Context of Climate Change." *Functional Ecology* 31: 216–226. https://doi.org/10.1111/1365-2435.12701.

Canning, E. U., A. Curry, S. W. Feist, M. Longshaw, and B. Okamura. 1999. "*Tetracapsula bryosalmonae* n. Sp. for PKX Organism, the Cause of PKD in Salmonid Fish." *Bulletin of the European Association of Fish Pathologists* 19: 203–206.

Carmon, J., J. A. Keele, S. F. Pucherelli, and D. Hosler. 2014. "Effects of Buffer and Isopropanol Alcohol Concentration on Detection of Quagga Mussel (*Dreissena bugensis*) Birefringence and DNA." *Management of Biological Invasions* 5: 151–157. https://doi.org/10.3391/mbi.2014.5.2.08.

Clifton-Hadley, R. S., D. Bucke, and R. H. Richards. 1984. "Proliferative Kidney Disease of Salmonid Fish: A Review." *Journal of Fish Diseases* 7: 363–377. https://doi.org/10.1111/j.1365-2761.1984.tb01201.x.

Dabney, J., M. Meyer, and S. Pääbo. 2013. "Ancient DNA Damage." *Cold Spring Harbor Perspectives in Biology* 5: a012567. https://doi.org/10.1101/cshperspect.a012567.

Dash, M., and A. Vasemägi. 2014. "Proliferative Kidney Disease (PKD) Agent Tetracapsuloides Bryosalmonae in Brown Trout Populations in Estonia." *Diseases of Aquatic Organisms* 109: 139–148. https://doi.org/10.3354/dao02731.

Debes, P. V., R. Gross, and A. Vasemägi. 2017. "Quantitative Genetic Variation in, and Environmental Effects on, Pathogen Resistance and Temperature-Dependent Disease Severity in a Wild Trout." *American Naturalist* 190: 244–265. https://doi.org/10.1086/692536.

Degerman, E., E. Petersson, P.-E. Jacobsen, L. Karlsson, E. Lettevall, and F. Nordwall. 2012. "Laxparasiten *Gyrodactylus salaris* i Västkustens Laxåar. *Aqua reports* 2012:8, 68 p. Uppsala: Department of Aquatic Resources, Swedish University of Agricultural Sciences." https://res.slu.se/id/publ/42124.

Donadi, S., J. Näslund, L. Sandin, B. Sers, A. Vasemägi, and E. Degerman. 2023. "Contrasting Long-Term Trends in Juvenile Abundance of a Widespread Cold-Water Salmonid Along a Latitudinal Gradient: Effects of Climate, Stream Size and Migration Strategy." *Ecography* 2023: e06522. https://doi.org/10.1111/ecog.06522.

Eklöv, A. G., L. A. Greenberg, C. Brönmark, P. Larsson, and O. Berglund. 1999. "Influence of Water Quality, Habitat and Species Richness on Brown Trout Populations." *Journal of Fish Biology* 54: 33–43. https://doi.org/10.1111/j.1095-8649.1999.tb00610.x.

Fox, C. J. 1996. "Length Changes in Herring (*Clupea harengus*) Larvae: Effects of Capture and Storage in Formaldehyde and Alcohol." *Journal of Plankton Research* 18, no. 4: 483–493. https://doi.org/10.1093/plankt/18.4.483.

Gallagher, B. K., S. Geargeoura, and D. J. Fraser. 2022. "Effects of Climate on Salmonid Productivity: A Global Meta-Analysis Across Freshwater Ecosystems." *Global Change Biology* 28: 7250–7269. https://doi.org/10.1111/gcb.16446.

Grabner, D. S., and M. El-Matbouli. 2009. "Comparison of the Susceptibility of Brown Trout (*Salmo Trutta*) and Four Rainbow Trout (*Oncorhynchus Mykiss*) Strains to the Myxozoan Tetracapsuloides Bryosalmonae, the Causative Agent of Proliferative Kidney Disease (PKD)." *Veterinary Parasitology* 165: 200–206. https://doi.org/10.1016/j.vetpar.2009.07.028.

Hansen, H., G. J. Fornes, S. N. Mohammad, A. N. Fiske, M. M. Amundsen, and H. I. Welde. 2024. "The Surveillance Programme for Gyrodactylus Salaris in Atlantic Salmon and Rainbow Trout in Norway 2023." In *Surveillance Program Report 16*. Norwegian Veterinary Institute.

Howmiller, R. P. 1972. "Effects of Preservatives on Weights of Some Common Macrobenthic Invertebrates." *Transactions of the American Fisheries Society* 101: 743–746. https://doi.org/10.1577/1548-8659(1972)101<743:EOPOWO>2.0.CO;2.

Hutchins, P. R., A. J. Sepulveda, R. M. Martin, and L. R. Hopper. 2018. "Improved Conventional PCR Assay for Detecting Tetracapsuloides Bryosalmonae DNA in Fish Tissues." *Journal of Aquatic Animal Health* 30: 164–170. https://doi.org/10.1002/aah.10020.

IUCN. 2024. "The IUCN Red List of Threatened Species." IUCN: Gland, Switzerland. https://www.iucnredlist.org/.

Jammalamadaka, D., and S. Raissi. 2010. "Ethylene Glycol, Methanol and Isopropyl Alcohol Intoxication." *American Journal of the Medical Sciences* 339: 276–281. https://doi.org/10.1097/MAJ.0b013e3181c94601.

Kent, M. L., J. Khattra, D. M. L. Hervio, and R. H. Devlin. 1998. "Ribosomal DNA Sequence Analysis of Isolates of the PKX Myxosporean and Their Relationship to Members of the Genus Sphaerospora." *Journal of Aquatic Animal Health* 10: 12–21. https://doi.org/10.1577/1548-8667(1998)010<0012:RDSAOI>2.0.CO;2.

King, J. R., and S. D. Porter. 2004. "Recommendations on the Use of Alcohols for Preservation of Ant Specimens (Hymenoptera, Formicidae)." *Insectes Sociaux* 51: 197–202. https://doi.org/10.1007/s00040-003-0709-x.

Klymus, K. E., C. M. Merkes, M. J. Allison, et al. 2020. "Reporting the Limits of Detection and Quantification for Environmental DNA Assays." *Environmental DNA* 2: 271–282. https://doi.org/10.1002/edn3.29.

Lauringson, M., I. Nousiainen, S. Kahar, et al. 2021. "Climate Change-Driven Disease in Sympatric Hosts: Temporal Dynamics of Parasite Burden and Proliferative Kidney Disease in Wild Brown Trout and Atlantic Salmon." *Journal of Fish Diseases* 44: 689–699. https://doi.org/10.1111/jfd.13330.

Lauringson, M., M. Y. Ozerov, M. E. Lopez, et al. 2022. "Distribution and Prevalence of the Myxozoan Parasite Tetracapsuloides Bryosalmonae in Northernmost Europe: Analysis of Three Salmonid Species." *Diseases of Aquatic Organisms* 151: 37–49. https://doi.org/10.3354/dao03688 Lindahl, T., 1993.

Lindahl, T. 1993. "Instability and Decay of the Primary Structure of DNA." *Nature* 362: 709–715. https://doi.org/10.1038/362709a0.

Moku, M., K. Mori, Y. Watanabe, and J. W. Armbuster. 2004. "Shrinkage in the Body Length of Myctophid Fish (Diaphus Slender-Type Spp.) Larvae With Various Preservatives." *Copeia* 2004, no. 3: 647–651. https://doi.org/10.1643/CI-03-113R.

Moore, D., and D. Dowhan. 2002. "Purification and Concentration of DNA From Aqueous Solutions." *Current Protocols in Molecular Biology* 59: 2–11. https://doi.org/10.1002/0471142727.mb0201as59.

Näslund, J., and H. Strömberg. 2023. "Miljöövervakning–Elprovfiske i Trendvattendrag 2022 [Environmental monitoring–Electrofishing in Trend Rivers 2022]." https://doi.org/10.54612/a.e02jkqa2j1.Aqua Notes 2023:23, 29 p. Uppsala: Department of Aquatic Resources, Swedish University of Agricultural Sciences.

Rake, A. V. 1972. "Isopropanol Preservation of Biological Samples for Subsequent DNA Extraction and Reassociation Studies." *Analytical Biochemistry* 48: 365–368. https://doi.org/10.1016/0003-2697(72)90088-7.

Ros, A., J. Baer, T. Basen, et al. 2021. "Current and Projected Impacts of the Parasite Tetracapsuloides Bryosalmonae (Causative to Proliferative Kidney Disease) on Central European Salmonid Populations Under Predicted Climate Change." *Freshwater Biology* 66: 1182–1199. https://doi.org/10.1111/fwb.13709.

Rubin, A., P. De Coulon, C. Bailey, H. Segner, T. Wahli, and J. F. Rubin. 2019. "Keeping an Eye on Wild Brown Trout (*Salmo trutta*) Populations: Correlation Between Temperature, Environmental Parameters, and Proliferative Kidney Disease." *Frontiers in Veterinary Science* 6: 281. https://doi.org/10.3389/fvets.2019.00281.

Schneider, C. A., W. S. Rasband, and K. W. Eliceiri. 2012. "NIH Image to ImageJ: 25 Years of Image Analysis." *Nature Methods* 9: 671–675. https://doi.org/10.1038/nmeth.2089.

Skovgaard, A., and K. Buchmann. 2012. "Tetracapsuloides Bryosalmonae and PKD in Juvenile Wild Salmonids in Denmark." *Diseases of Aquatic Organisms* 101: 33–42. https://doi.org/10.3354/dao02502.

Slaughter, R. J., R. W. Mason, D. M. G. Beasley, J. A. Vale, and L. J. Schep. 2014. "Isopropanol Poisoning." *Clinical Toxicology* 52: 470–478. https://doi.org/10.3109/15563650.2014.914527.

Smith, B. B., and K. F. Walker. 2003. "Shrinkage of 0+ Carp (*Cyprinus Carpio*) After Preservation in Ethanol." *Marine and Freshwater Research* 54: 113–116. https://doi.org/10.1071/MF02089.

Sterud, E., T. Forseth, O. Ugedal, et al. 2007. "Severe Mortality in Wild Atlantic Salmon *Salmo Salar* due to Proliferative Kidney Disease (PKD) Caused by Tetracapsuloides Bryosalmonae (Myxozoa)." *Diseases of Aquatic Organisms* 77: 191–198. https://doi.org/10.3354/dao01846.

Strepparava, N., H. Segner, A. Ros, H. Hartikainen, H. Schmidt-Posthaus, and T. Wahli. 2018. "Temperature-Related Parasite Infection Dynamics: The Case of Proliferative Kidney Disease of Brown Trout." *Parasitology* 145: 281–291. https://doi.org/10.1017/S0031182017001482.

van Treeck, R., J. van Wichelen, and C. Wolter. 2020. "Fish Species Sensitivity Classification for Environmental Impact Assessment, Conservation and Restoration Planning." *Science of the Total Environment* 708: 135173. https://doi.org/10.1016/j.scitotenv.2019.135173.

Vasemägi, A., R. Gross, D. Palm, T. Paaver, and C. R. Primmer. 2010. "Discovery and Application of Insertion-Deletion (INDEL) Polymorphisms for QTL Mapping of Early Life-History Traits in Atlantic Salmon." *BMC Genomics* 11: 156. https://doi.org/10.1186/1471-2164-11-156.

Zaidel, P. A., A. H. Roy, K. M. Houle, et al. 2021. "Impacts of Small Dams on Stream Temperature." *Ecological Indicators* 120: 106878. https://doi.org/10.1016/j.ecolind.2020.106878.

Appendix A

Calculation of Percentage Difference Between Storage Mediums at a Given Fish Length (l)

$$\text{KidneyArea} = \left(\sqrt{\text{KidneyArea}}\right)^2 = (\text{coefficient} \times \text{l} + \text{intercept} + \text{preservative})^2$$

$$\%\,\text{Difference} = \left(\frac{\text{KidneyArea}_{\text{ethanol}} - \text{KidneyArea}_{\text{isopropanol}}}{\text{KidneyArea}_{\text{isopropanol}}}\right) \times 100$$

$$\%\,\text{Difference} = \left(\left(\frac{0.0153 \times \text{l} + 0.1665}{0.0153 \times \text{l} + 0.1665 - 0.1051}\right)^2 - 1\right) \times 100$$