Received: 6 August 2019 | Revised: 15 November 2019 | Accepted: 27 November 2019

DOI: 10.1111/eff.12528

SPECIAL ISSUE ARTICLE

Ecology of FRESHWATER FISH WILEY

A brown trout (*Salmo trutta*) population faces devastating consequences due to proliferative kidney disease and temperature increase: A case study from Austria

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Funding information
Klima- und Energiefonds, Grant/Award Number: B670143; ACRP, Grant/Award Number: KR16AC0K13263.

Abstract

Proliferative kidney disease (PKD) is an emerging parasitic disease that affects wild and farmed salmonid fishes worldwide. Currently, it is widespread in Europe with records in many countries. This study assessed the status of PKD in the river Wulka, a small Austrian river, which was known to host a vital brown trout population despite having high water temperatures. Contrary to the initial hypothesis that the river was free from PKD, we observed an overall PKD prevalence of 92%. Noninfected fish were restricted to the uppermost river section. Twenty-two out of 87 PKD-positive fish of all age classes showed clinical signs, while five individuals exhibited signs of recovering from PKD infection. The severity of clinical signs was significantly dependent on the fish length and age, with smaller or younger individuals being more severely affected. Comparison of data from the earlier fish sampling events with those results from 2017 and 2018, together with the analyses of the pattern of water temperature since 2010, indicates that Wulka's brown trout population might become extinct in the near future.

KEYWORDS

climate change, emerging threat, fish decline, fish parasite, kidney disease, temperature

1 | INTRODUCTION

Brown trout (*Salmo trutta*) is the dominant species of upstream river reaches in Europe. It prefers clean and relatively cool water with high oxygen saturation. Adult brown trout has an optimal temperature range of 4–19°C (Elliott & Elliott, 2010). They may tolerate temperatures of up to a critical incipient lethal temperature of 22–25°C for a short term, depending on the life stage (Elliott & Elliott, 2010). However, factors such as age, acclimatisation time and genetic origin influence the tolerance of water temperature (Beitinger, Bennett, & McCauley, 2000; Carline & Machung, 2001).

Wild brown trout populations are declining in Austria (Lahnsteiner, Haunschmid, & Mansour, 2009) and other European countries such as Switzerland (Hari, Livingstone, Siber, Burkhardt-Holm, & Guttinger, 2006) due to several reasons such as habitat deterioration, overfishing (Borsuk, Reichert, Peter, Schager, & Burkhardt-Holm, 2006), returning predators like the otter (*Lutra lutra*) (Sittenthaler, Bayerl, Unfer, Kuehn, & Parz-Gollner, 2015) and upcoming diseases like the proliferative kidney disease (PKD) (Burkhardt-Holm, 2008). Additionally, climate change is increasingly putting pressure on this sensitive species (Hari et al., 2006). Further, stocking propagated trout to mitigate decreasing populations is a

common practice, which can create further negative implications for autochthonous populations such as genetic homogenisation and competition for habitat and food (Unfer & Pinter, 2018).

Proliferative kidney disease is widespread in wild brown trout populations across Europe and North America (Dash & Vasemägi, 2014; Hutchins, Sepulveda, Martin, & Hopper, 2017; Jencic, Zajc, Kusar, Ocepek, & Pate, 2014; Lewisch, Unfer, Pinter, Bechter, & El-Matbouli, 2018; Peeler, Feist, Longshaw, Thrush, & St-Hilaire, 2008; Skovgaard & Buchmann, 2012). Furthermore, PKD is considered as a major reason for the decline of brown trout in Switzerland (Wahli, Bernet, Steiner, & Schmidt-Posthaus, 2007). The myxozoan parasite *Tetracapsuloides bryosalmonae* belonging to the class Malacosporea (Canning, Curry, Feist, Longshaw, & Okamura, 2000) is known as the causative agent of PKD in salmonid fish (Hedrick, MacConnell, & Kinkelin, 1993). The parasite enters the fish through their gills (Grabner & El-Matbouli, 2010; Morris, Adams, & Richards, 2000) or skin (Longshaw, Le Deuff, Harris, & Feist, 2002), after which its developmental stages migrate through the blood to target the kidney (Holzer, Sommerville, & Wootten, 2006; Kent & Hedrick, 1985). The presence of the proliferating parasite (extrasporogonic stages) in the kidney causes an inflammatory response of the renal tissue (Okamura, Hartikainen, Schmidt-Posthaus, & Wahli, 2011). The external signs of PKD are skin darkening, both sided exophthalmia, ascites and pale gills as a result of haemolytic anaemia (Hedrick et al., 1993; Hoffman & Lommel, 1984).

The parasite needs two hosts to complete its life cycle: a vertebrate (salmonid fish) and an invertebrate host (bryozoa). After infection, brown trout releases viable spores via its urine and thus passes the PKD causing agent *T. bryosalmonae* to the bryozoa (Grabner & El-Matbouli, 2008). The main bryozoan host is *Fredericella sultana* (Hartikainen, Gruhl, & Okamura, 2014). Bryozoan colonies are sessile, filtrating freshwater organisms (Okamura & Hatton-Ellis, 1995).

Proliferative kidney disease is a temperature-dependent disease that causes mortalities at higher water temperatures. Severe effects of PKD have been reported when the water temperature exceeds 15°C (Okamura et al., 2011). Studies performed using rainbow trout under laboratory conditions support these findings, with mortality rates of up to 45.5% at 16°C and 85% at 19°C (Bettge, Segner, Burki, Schmidt-Posthaus, & Wahli, 2009; Bettge, Wahli, Segner, & Schmidt-Posthaus, 2009). Palikova et al. (2017) observed decreasing mortalities in rainbow trout when the water temperature drops below 10°C. In a field trial using brown trout, PKD-associated mortality was not higher than 15% although the water temperature exceeded 15°C for 39 days. Therefore, PKD-associated mortality under natural conditions remained at low levels compared to mortality observed in rainbow trout held at 16°C under laboratory conditions (Bettge, Segner, et al., 2009; Schmidt-Posthaus, Hirschi, & Schneider, 2015).

In Austria, PKD was first reported in 2014 from a fish farm supplied with river water (Gorgoglione, Kotob, Unfer, & El-Matbouli, 2016; Unfer et al., 2015). Further investigation following this

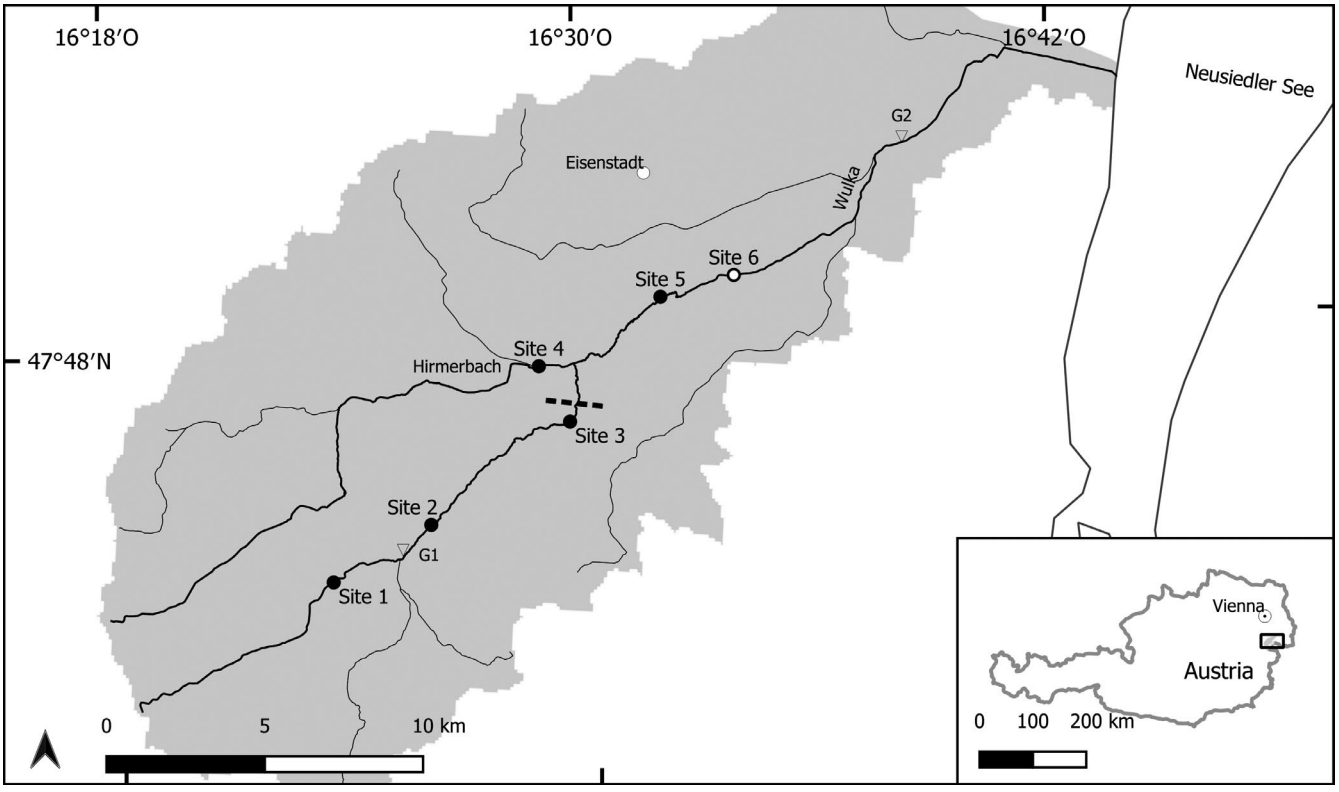

FIGURE 1 (Main) Location of the fish, bryozoan and temperature recording sites (●), bryozoan site ([O]) and the nonpassable barrier (--- dashed line) in the Wulka catchment (grey shaded). G1 and G2 indicate public gauging stations (▾). Water temperatures for Site 1 and Site 2 were recorded in G1. (Right bottom) Location of the case-study river Wulka in Austria

outbreak has confirmed the occurrence of PKD in Austrian rivers (Lewisch et al., 2018). Further evidence on PKD in Austrian rivers is still needed to understand the mechanisms leading to disease outbreaks and develop management strategies for fisheries but also nature conservation purposes. The aim of this study was to investigate and characterise the status of PKD (including the investigation of the brown trout population, bryozoan colonies and their respective infection status) in a lowland river (the Wulka) in East Austria, draining into Lake Neusiedl and thus isolated from other river catchments. The Wulka was originally studied during an ongoing Austrian research project on PKD and its relation to climate change ("ClimateTrout") as a reference river, as it was known to host a vital brown trout population despite marginal temperatures for the occurrence of trout (Unfer, 2013). Therefore, it was initially hypothesised that the river was free from PKD.

2 | MATERIALS AND METHODS

2.1 | Study area

The Wulka is a 38-km-long river, situated in the East of Austria, and is the major tributary of Lake Neusiedl, the largest steppe lake in Central Europe (Figure 1). The Wulka originates from the Rosalian Hills at an elevation of 693 m.a.s.l. and enters the Lake Neusiedel at 111 m.a.s.l. The catchment size at the river mouth is approximately 400 km^2, and the mean discharge is 1.16 m^3/s. The Wulka is characterised by a summer-pluvial regime in the upstream section with the highest discharge during summer, changing to a pluvio-nival regime in the downstream part of the river. The biocoenotic regions range from the Epirhithral in the upper river section to the Epipotamal in the lower section. The tributary "Hirmerbach" is classified as a gudgeon brook (Haunschmid et al., 2006). The Wulka is morphologically highly degraded due to channelisation and river regulation, with the uppermost sections periodically drying up during summer. A nonpassable barrier separates the study sites (Figure 1) (BMLFUW, 2009).

2.2 | Sampling of fish and bryozoa

Brown trout of all age classes were sampled at five sites between June and November of 2017 and 2018 (Figure 1; Table 1): Site 1 (Epirhithral), Site 2 (Metarhithral), Site 3 (Metarhithral), Site 5 (Epipotamal) in the Wulka and Site 4 in the Hirmerbach. Single pass samplings were carried out at low-flow situations using a backpack electrofishing unit (1.5 kW). According to a prior study (Unfer, 2013), Site 5 (mean channel width: 7.5 m) was sampled wading upstream along a 480 m long stretch that was confined by a ruinous weir at the upstream end. All trout caught from Site 5 were kept for further examinations. The other sites (1–4) were fished until a sample size of 10 fish per site (where available) was reached. The sampled lengths for each site were recorded to calculate the catch per unit effort (CPUE), represented as the number of trout caught per 100 m. After the catch, fish were tagged using PIT tags according to their place of origin and transported alive to the laboratory, where all the biometric data were measured.

TABLE 1 Channel width at sampling locations, discharge at sampling dates, total number of caught brown trout and CPUE (Individuals/100 m). Results of kidney scoring of brown trout show the allocation of brown trout in the class depending on the degree of kidney enlargement (0–4) according to Clifton-Hadley et al. (1987); KSI (kidney swelling index); H (healing); numbers of brown trout infected by *T. bryosalmonae* verified by PCR (Pos. BT)

	Width	Discharge	Date	Sampled trout CPUE			KSI 0	KSI 1	KSI 2	KSI 3–4	KSI H	Pos. BT
Site	(m)	(m^3/s)	(dd. mm.yy)	(*n*)	Section length (m)	(Individuals/100 m)	(*n*)	(*n*)	(*n*)	(*n*)	(*n*)	(*n*)
1	1.2	0.1	13.11.18	9	600	2	9	0	0	0	0	1
2	3.0	0.1	26.09.13	128	100	128						
		0.1	30.08.18	10	25	40	6	1	2	0	1	10
		0.1	13.11.18	10	25	40	7	0	0	1	2	10
3	5.0	0.2	09.10.13	19	158	12						
		0.3	23.08.17	10	25	40	10	0	0	0	0	10
		0.2	30.08.18	10	320	3	9	0	0	0	1	10
4	2.0	0.1	23.08.17	10	30	33	8	0	2	0	0	10
		0.1	30.08.18	5	150	3	4	1	0	0	0	5
5	7.5	0.7	15.06.10	148	480	31						
		0.5	01.10.13	40	160	25						
		0.5	25.06.15	117	650	18						
		0.3	14.06.17	16	480	3	5	5	6	0	0	16
		0.3	23.08.17	5	480	1	2	2	0	1	0	5
		0.3	30.08.18	10	480	2	8	0	0	1	1	10

Additionally, we included electrofishing data from 2010, 2013 and 2015 for Site 5 and from 2013 for Site 3. This facilitated the evaluation of the population trends as the sampling methodology was the same across all years.

The presence of bryozoan colonies was checked in Sites 1–6 (Figure 1) and was carried out at the same dates as fish sampling. Stretches of 100 m were waded in a group of four people, and river bed structures suitable for bryozoan growth were examined. The locations of their presence were recorded, and the colonies were stored in 2-ml tubes at −20°C until DNA extraction and subsequent PCR for the detection of *T. bryosalmonae*.

2.3 | Dissection

The sampled fish were euthanised with an overdose of MS 222 (Sigma-Aldrich, Germany) and then subjected to a general examination. The total body weight and length were recorded. Fish were categorised into age classes (0+, 1+, 2+ or older) according to Unfer (2013). Fish smaller than 15 cm were classified as 0+. Age class 1+ comprised fish with lengths between 15 and 25 cm, and fish larger than 25 cm were considered 2+ and older. Mucus smears from skin and gills were subjected to parasitological investigation. Blood was collected by puncturing the caudal vein. Blood smears were studied to check for parasite stages. After opening the body cavity, the parenchymal organs were assessed and the kidney swelling index (KSI) was scored according to Clifton-Hadley, Bucke, and Richards (1987). Pieces of the kidney and spleen were stored in sterile 2-ml tubes at −20°C until DNA isolation.

2.4 | Statistical analyses

To correlate the KSI results with fish lengths, we used contingency table analyses (CTA). First, the observed fish lengths were classified (trichotomised) and the KSI values were summarised in three classes: KSI-0 = no clinical signs, KSI 1 = mild symptoms and KSI 2–4 = substantial symptoms (Table 2). The table was tested globally applying chi-square and likelihood ratio (LQ) tests. The contingency coefficient (Cramér's *V*) was used to indicate the effect size. A bootstrap simulation (k = 1,000) was applied to corroborate the upper and lower limits of Cramér's *V*. The cells were locally tested using residual tests (z) whereby α (0.05) had to be adjusted to α^* (0.00055) and α (0.01) to α^{**} (0.00011) based on the table's nine cells.

2.5 | Molecular genetics

The DNA was extracted from kidney and spleen tissue of all dissected specimen using DNeasy Blood & Tissue Kit (Qiagen); further, we extracted DNA from one bryozoan colony per site using DNeasy®PowerLyzer®PowerSoil® Kit (Qiagen) according to the manufacturer's instructions. Concentration of the extracted DNA was estimated using NanoDrop Spectrophotometer. A nested PCR was applied in 25 µl reaction mixture in two steps: in step 1 primers 5F and 6R (annealing temperature 55°C) (Kent, Khattra, Hervio, & Devlin, 1998) and in step 2 PKD real primers (annealing temperature 61°C) (Grabner & El-Matbouli, 2009) were used. Positive bands corresponding to an amplicon size of 435 bp after the first step and 166 bp after the second step were cut, purified using MinElute®GelExtraction Kit (Qiagen) and sent for sequencing (LGC). The obtained sequences were matched with the available sequences in GenBank using the software BLAST (Basic Local Alignment Search Tool) for detecting the presence of *T. bryosalmonae*.

2.6 | Water temperature

The thermal regime of the Wulka was characterised during the warm period of the year (April–September). For this, the water temperature was continuously logged at hourly intervals from the beginning of April till the 30th of September in 2017 and 2018, using three

TABLE 2 Contingency table results concerning the relation of classified fish length of investigated 0+ brown trout and KSI values (KSI = 0: no clinical signs, KSI = 1: mild symptoms, KSI = 2–4: substantial symptoms). Test statistics: z = (observed − expected)/expected); expected: estimated frequencies supposing total independence of the variables length class and KSI, significance decision: for positive/negative differences $^*p < .0055$, $^{**}p < .0011$ (Bonferroni Adj.). Global chi-square test results (χ^2 = 28.76, LQ = 35.29 df = 4, p = .000; Cramér's V = 0.531). Bootstrap simulation of Cramér's V: lower limit = 0.429, upper limit = 0.645 (CI-95%)

	Length class (mm)	≤85	86–115	≥116
KSI = 0	Observed frequency	2	13	15
	Expected frequency	10.6	10.6	8.8
	Adjusted residuals (z)	−5.1**	1.4	3.9**
	p (z)	.0001	.1615	.0001
KSI = 1	Observed frequency	6	2	0
	Expected frequency	2.8	2.8	2.4
	Adjusted residuals (z)	2.6	−0.7	−2.0
	p (z)	.0093	.4839	.0455
KSI = 2–4	Observed frequency	10	3	0
	Expected frequency	4.6	4.6	3.8
	Adjusted residuals (z)	3.6**	−1.1	−2.7
	p (z)	.0003	.2713	.0069

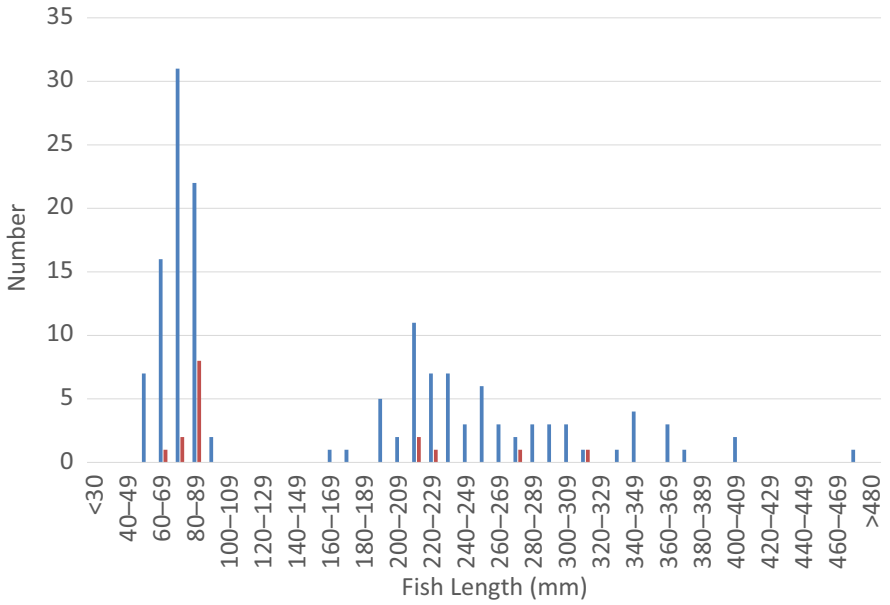

FIGURE 2 Population structure of brown trout from Site 5 in June 2010 (black bars, *n* = 148) and June 2017 (grey bars, *n* = 16)

temperature loggers (iButton-Logger Maxim Integrated DS1922L) having an accuracy of 0.5°C and secured by a waterproof aluminium capsule. This method is proofed by several authors in groundwater and rivers (Johnson et al., 2005; Roznik & Alford, 2012). These water temperature data were supplemented with records from two existing gauging stations (G1, G2) for the period 2000–2018 (Figure 1). Water temperatures for Site 1 and Site 2 were recorded at G1. The temperature loggers were installed in 35–50 cm water depth in sections with flowing water. For this purpose, a steel rod with an eyelet was anchored into the riverbed and the temperature logger was attached to the eyelet. The loggers were programmed using the software Te.M.P. (2016).

Water temperature associated with clinical proliferative kidney disease (PKD) outbreaks, PKD-related mortality and physiological

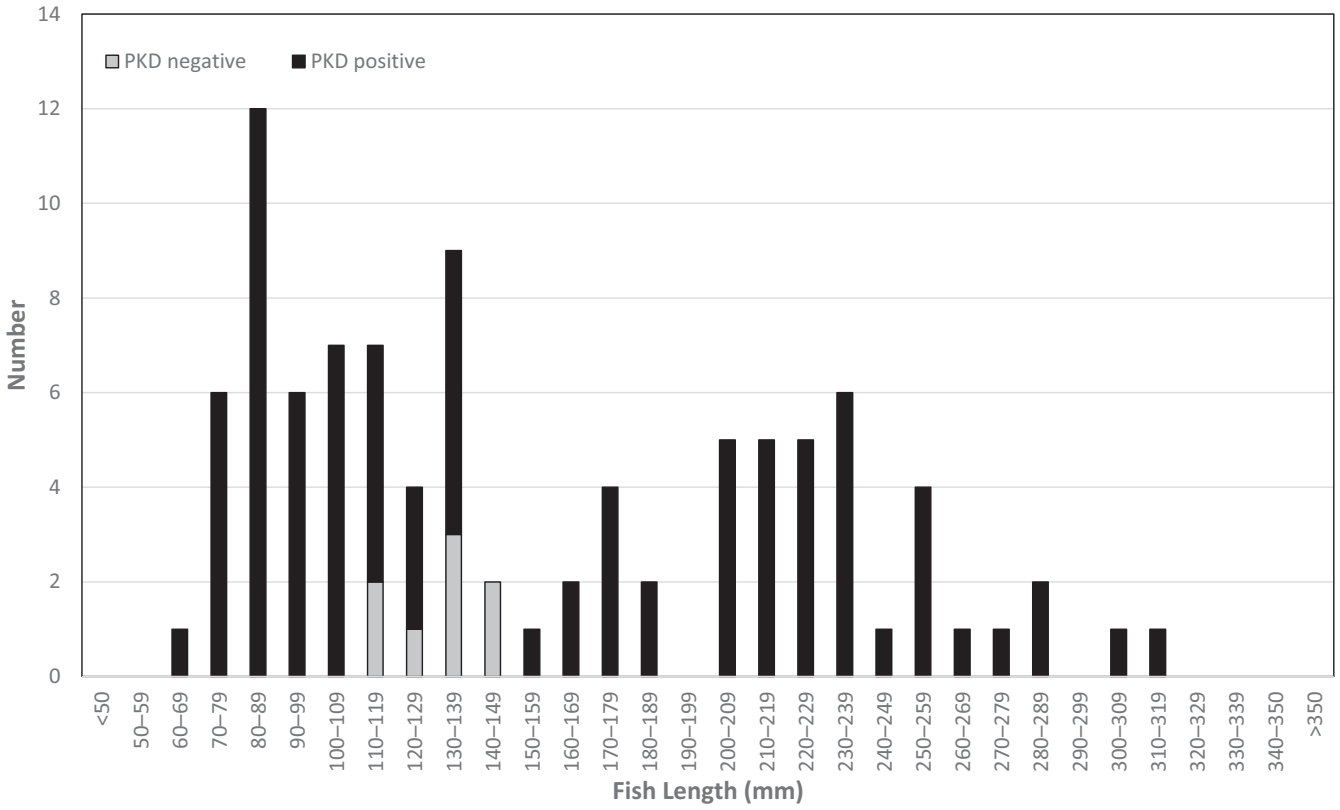

FIGURE 3 Length-frequency plot of brown trout from all sampling sites 2017 and 2018 (*n* = 95); black bars: PKD-positive fish (*n* = 87), grey bars: PKD-negative fish (*n* = 8)

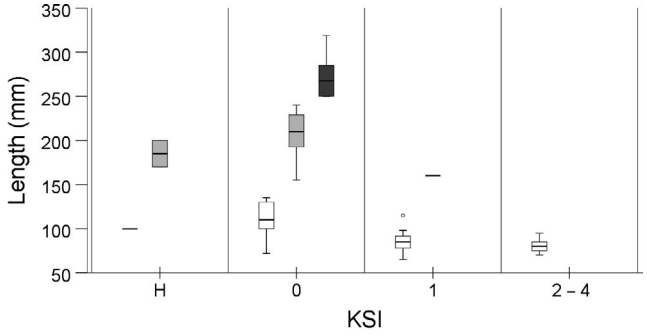

FIGURE 4 Box plot presenting the length distribution of sampled brown trout (n°=°95) according to the KSI (kidney swelling index) (Clifton-Hadley et al., 1987) for different age classes (white boxes: 0+, grey boxes: 1+ and dark grey box: 2+ and older)

stress in brown trout was taken from the literature and used to calculate temperature metrics based on the hourly data. The metrics involve the total number of days (TD) and the total number of consecutive days (ConD) with water temperatures of ≥15°C (Burkhardt-Holm, 2009; Schmidt-Posthaus et al., 2015), ≥18°C (Bettge, Segner, et al., 2009) and ≥19.5°C (Elliott & Elliott, 2010) between 1st of May and 30th of September. Additionally, the date of the first day with a mean temperature of ≥15°C and ≥18°C was extracted from the complete data set.

3 | RESULTS

3.1 | Sampling of fish and bryozoa

In total, 95 brown trout were withdrawn from the Wulka at five locations on four different sampling dates in 2017 and 2018 (Table 1). The detailed information about number of brown trout caught in Sites 1–5, section length and widths and calculated CPUE values is shown in Table 1.

Electrofishing data from 2010, 2013 and 2015 for Site 5 and from 2013 for Site 3 were compared with those from 2017 and 2018. In 2010, 148 brown trout of different age classes and up to a length of 480 mm were caught, whereas, in June 2017, only 16 trout were found at Site 5, all of which were positive for PKD. Figure 2 compares the size structure of the trout population in 2010 and 2017. Additional samplings from 2013 and 2015 (for CPUE values see Table 1) show a slow but steady decline of brown trout between 2010 and 2015, followed by drastically reduced numbers in 2017 and 2018. A further decrease in the population of brown trout was observed during the study period at Sites 3 and 4, where the CPUE decreased by around 90% between 2017 and 2018 (Table 1).

Bryozoan colonies were found at Sites 5 and 6 only. Stereomicroscopic examination revealed one living and 30 empty zooids as well as 2 statoblasts of the species *F. sultana* in the colony from Site 6. The colony originating from Site 5 consisted of 10 living zooids, 150 empty zooids and two statoblasts of *F. sultana*. The PCR results showed that the bryozoan colony from Site 5 was infected with *T. bryosalmonae* whereas the colony from Site 6 was not.

3.2 | General examination of fish—PKD (Proliferative Kidney Disease) status

The mean total length of the sampled trout was 15.8 ± 7.5 cm. The length-frequency plot (Figure 3) showed that the majority of fish (smaller than 15 cm) belonged to the first age class (0+), followed by 1+ (from 15 to 25 cm), while only a few adult fish (larger than 25 cm) were caught. In Site 1, only 0+ specimens were caught (Table S1).

PCR analysis revealed the presence of a DNA amplicon in 87 kidney–spleen tissue samples (92%) (Table 1). Comparison of the obtained sequences with the NCBI database confirmed that the amplified sequence matched 100% with the published *T. bryosalmonae* sequences. Eight brown trout were PKD-negative (8%). All the negative samples originated from the most upstream location (Site 1).

Dissection of brown trout revealed that predominantly fish from Site 5 (15 individuals) had kidney enlargement typical for PKD. Additionally, four fish from Site 2 and three from Site 4 showed

TABLE 3 Overview of temperature parameters derived from hourly records at the five sampling sites in the river Wulka including elevation (S.I.), recording days (RD), total days (TD), consecutive days (ConD) and first day (FirstD) exceeding PKD-related temperature thresholds 15°C, 18°C and 19.5°C

			Fish sampling August				
ID	Year	S.I.	RD	TD ≥ 15°C	TD ≥ 18°C	ConD ≥ 18°C	TD ≥ 19.5°C
Site 1/2	2017	222	115	82	13	5	0
	2018		122	91	14	13	1
Site 3	2017	173	115	100	58	15	32
	2018		122	117	75	43	37
Site 4	2017	170	115	100	71	24	44
	2018		122	120	87	53	54
Site 5	2017	152	115	101	80	66	49
	2018		122	122	87	55	54

clinical signs of PKD. Thus, 22 brown trout from the Wulka had enlarged kidneys (Table 1; Figure 4). Five brown trout (two 1+ and three 0+) showed signs of recovery from PKD symptoms such as spherical nodules and cream-coloured patches (Clifton-Hadley et al., 1987). These recovering fish were found in Sites 2, 3 and 5 in August (n = 3), as well as in Site 2 in November 2018 (n = 2). The detailed KSI scoring is presented in Table 1.

3.3 | Water temperature

The highest water temperatures in both years were observed at Site 5, followed by Site 4 (Table 3). The lowest water temperatures were recorded at Site 1. At Site 4, the mean water temperature exceeded 15°C for the first time on the 7th of May 2017. At Site 3, the 15°C threshold was exceeded 18 days earlier on the 19th of April 2018. Overall, the year 2018 was warmer than 2017 (Figure 5b) at all sites and for all water temperature parameters, except for TD ≥19.5°C and ConD ≥18°C. Regarding the latter, the number at Site 5 was slightly higher in 2017 than in 2018 (Table 3).

Before the first fish sampling at Site 5 in June 2017, water temperature exceeded 15°C on 31 days and 18°C on 12 days out of the 45 recording days (RD) and was associated with kidney swelling in brown trout (Table 1). In contrast, Site 1 being the only site with PKD-free brown trout, water temperature surpassed 18°C for only 13 recording days in 2017 and 14 recording days in 2018. In 2018, kidney alterations were observed in two brown trout at Site 5, after 122 recording days above 15°C and 87 recording days above 18°C, before sampling in August. At Site 3, one brown trout showing signs of recovery from clinical PKD was found after a temperature regime of 117 days ≥15°C and 75 days ≥18°C before sampling.

A full time-series of water temperatures since the year 2000 was available from the gauging station in the lower course of the Wulka (G2). At this station, water temperature exceeded 15°C for 138 days and 18°C for 94 days in 2018, 61 of them being consecutive (Figure 6a,b). During the period from 2000 to 2018, the mean temperature increased by nearly 3°C (Figure 5a). Notable is the early increase in the water temperature right from the end of April in 2018 (Figure 5b).

3.4 | Relation between fish length and PKD symptoms

All fish of the first age class (0+) and their related KSI values were analysed according to proof or falsify a potential correlation between fish length and the severity of kidney enlargement. The derived cross-table (Table 2) includes 51 specimens as three fish were excluded due to signs of recovery from clinical symptoms (Clifton-Hadley, Richards, & Bucke, 1986) (Table S1). Significant correlations were found for 0+ trout with lengths ≤85 mm and ≥116 mm. While the latter show a significantly reduced probability to display kidney enlargement (z = 3.9), small juveniles (≤85 mm) exhibit significantly increased rates of kidney enlargements on the one hand (z = 3.6) and a significantly reduced probability to be free from symptoms (z = −5.1) on the other hand (Table 2).

4 | DISCUSSION

In this study, we investigated the prevalence of *T. bryosalmonae* and associated PKD (proliferative kidney disease) in the brown trout population of the river Wulka. Eighty-seven of the 95 brown trout surveyed during the study period (2017/2018) were PKD-positive. Fish with clinical signs of PKD were mainly caught at the lowermost sampling site (Site 5), where they already displayed kidney enlargement at the first sampling date in June 2017. Uninfected fish were found in the uppermost river section only. Twenty-one out of the total 54 0+ trout exhibited acute symptoms of PKD. Furthermore, three 0+ and two 1+ specimen showed signs of recovery from the disease. The probability of exhibiting clinical signs of PKD among the 0+ age class was significantly dependent on fish length (Table 2). This result confirmed that smaller 0+ fish

Fish sampling June (45 RD)				Entire recording period (166 RD)					
TD ≥ 15°C	TD ≥ 18°C	ConD ≥ 18°C	TD ≥ 19.5°C	TD ≥ 15°C	TD ≥ 18°C	ConD ≥ 18°C	TD ≥ 19.5°C	FirstD ≥ 15°C	FirstD ≥ 18°C
14	0	0	0	95	13	5	0	19.05	10.07.
23	0	0	0	113	14	13	1	03.05	20.06
30	8	6	0	119	65	15	33	13.05.	30.05.
42	22	11	6	142	75	43	37	19.04	03.05
33	10	6	0	126	75	24	43	07.05	30.05
43	30	22	16	144	97	53	54	20.04	04.05
31	12	8	1	140	94	76	57	13.05.	30.05.
45	20	19	5	157	109	55	56	20.04	24.05

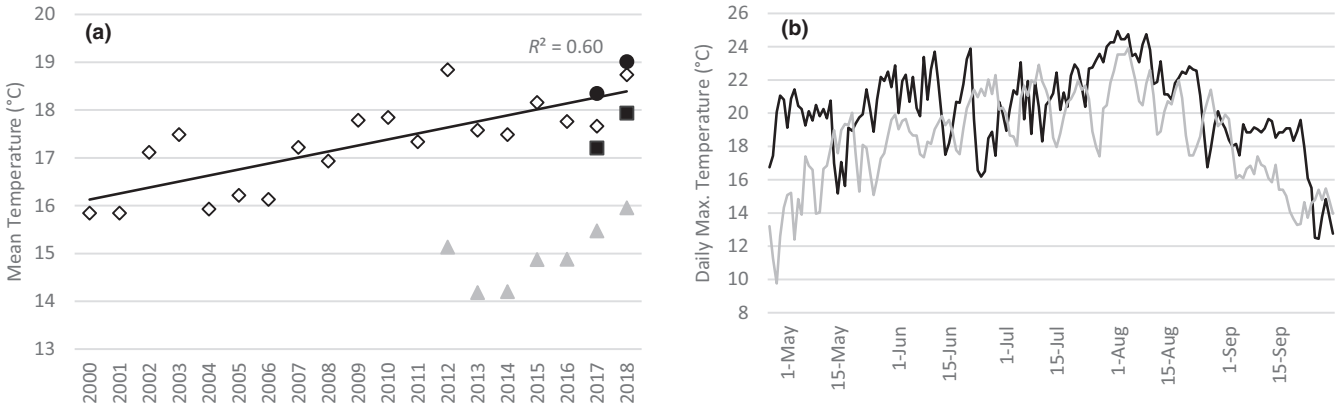

FIGURE 5 (a) Mean temperature (in °C) of the period May to September at G2 (black rhombuses), Site 1/2 (grey triangles), Site 3 (black rectangles) and Site 5 (black circles). The linear regression line for G2 (solid). (b) The daily maximum temperature at Site 3 during the warm season in the years 2017 (grey) and 2018 (black)

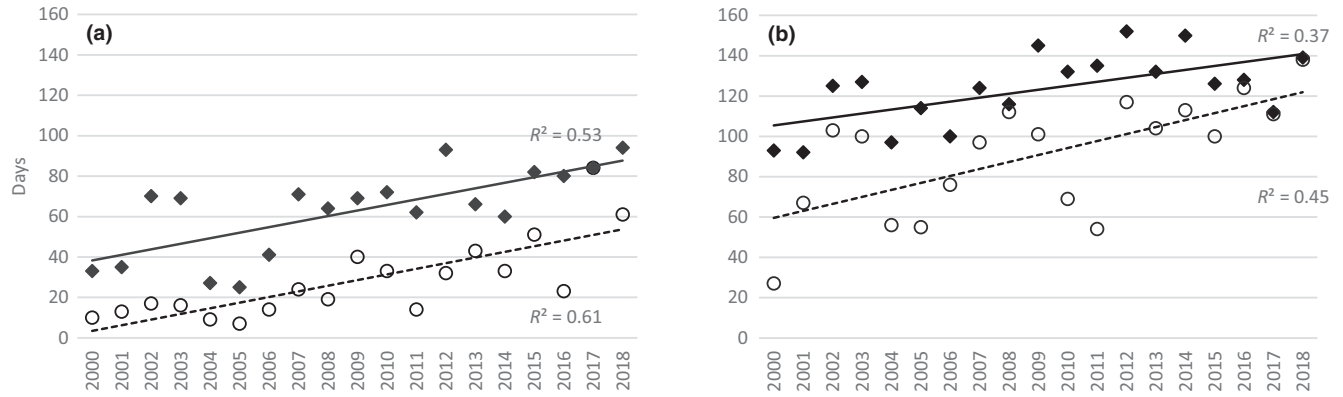

FIGURE 6 Total days (black rhombuses) and consecutive days (black circles) exceeding the temperature thresholds of (a) 18°C and (b) 15°C within 365/366 days of the years 2000–2018 at G1 (see Figure 1). Linear regression lines for total days (solid) and consecutive days (dashed)

are more likely to develop clinical signs of PKD. However, the question of whether the consequences of a *T. bryosalmonae* infection are more pronounced in retarded hatchlings or whether infected fish exhibit reduced growth rates remains open. The novel finding that symptoms of PKD within the group of 0+ specimens are size-dependent enhances the results of Schmidt-Posthaus, Steiner, Müller, and Casanova-Nakayama (2013) who found an increased probability of clinical signs in 0+ fish compared to 1+. In contrast to Wahli et al. (2002) who found acute symptoms also in fish of larger size classes, we registered just one individual (length: 160 mm) of an older age class (1+) showing mild clinical signs of PKD (KSI = 1), while all larger individuals (>15 cm, n = 41) were PKD-positive asymptomatic carriers. This is in line with Bailey, Segner, and Wahli (2017) who confirmed that reinfection does not lead to a new disease outbreak in years following the first infection in rainbow trout (*Oncorhynchus mykiss*).

The higher susceptibility of smaller fish to develop acute symptoms leads us to assume that PKD-related mortality is primarily arising in 0+ brown trout (Carraro et al., 2017). However, the mortality of 0+ brown trout may pass unnoticed as small dead fish can be easily overlooked. High mortalities among the early stages (0+) further alter both, the population structure and abundance, as fewer fish can grow older.

Although PKD is a widespread disease, the actual mortality rates of wild populations of brown trout due to infection with *T. bryosalmonae* are yet not sufficiently elucidated (Okamura et al., 2011; Skovgaard & Buchmann, 2012). In recent studies, PKD-associated mortality in brown trout that were exposed to a river environment in a cage experiment was not higher than 15% (Schmidt-Posthaus et al., 2015; Schmidt-Posthaus, Ros, Hirschi, & Schneider, 2017). In the current field study, mortality rates likewise remain unquantified at the Wulka as we sampled wild fish. Still, the observed population collapse since 2010 (Figure 2), as well as the massive population decline from 2017 to 2018 (Table 1), points to increased mortality rates.

As the hydro-morphological habitat and water quality at the Wulka did not change during this period, water temperature and PKD must be considered to be two major driving factors for this decline. A major reason for the decline at Site 3 within the study period (2017–2018) could be the early increase in the water temperature, right from the end of April in 2018 (Figure 5b). High water temperatures (>18°C) close to the emergence period could lead to

high rates of infection in 0+ fish, resulting in high mortality rates among the early life stages (Carraro et al., 2017; Tops, Lockwood, & Okamura, 2006). Currently, only Site 2 still harbours an adequate number of brown trout (CPUE: 40 individuals/100 m), although the numbers at this site have significantly decreased since 2013. This decline in CPUE clearly demonstrates the decreasing population trend of brown trout in the river Wulka.

The decrease of the brown trout population in the river Wulka was accompanied by a remarkable increase in the water temperatures, showing a clear warming trend. The number of total days when the temperature exceeded 15°C in 2000 was equal to the total number of days when water temperature surpassed 18°C in 2018, clearly underlining a warming trend in the thermal regime (Figure 6a,b).

The water temperature thresholds that trigger clinical signs of PKD (Clifton-Hadley et al., 1986; Palikova et al., 2017) were exceeded at all sites (except Site 1, Table 3) in 2017 and 2018. Except for the uppermost gauging station (G1, between Site 1 and Site 2), the water temperature exceeded 15°C for more than three months at all sites during both recording periods. Moreover, almost all of the sites showed water temperatures higher than 18°C (65–109 TD)—a temperature that has been shown to result in PKD-related mortality rates in rainbow trout (Bettge, Segner, et al., 2009; Bettge, Wahli, et al., 2009). The higher number of TD and ConD over 18°C before the second fish sampling particularly characterises a thermal regime that supports PKD, as indicated by the observed kidney swellings of sampled brown trout. However, only 12 days over 18°C and 31 days over 15°C were sufficient to cause kidney enlargement in the sampled fish during the first sampling in June 2017. This finding was consistent with the results of Schmidt-Posthaus et al. (2015), where 39 days over 15°C led to PKD symptoms. Regarding the data of 2018, the exceeding of critical temperatures early in the year (18°C already in April) could further boost mortalities, as younger fish being exposed to the parasite are more susceptible, exhibiting acute symptoms in the wild (Schmidt-Posthaus et al., 2013). Asymptomatic carrier fish (Carraro et al., 2017) that are still colonising the Wulka can shed spores over several years. Thus, they contribute to maintaining the lifecycle of *T. bryosalmonae* in the river Wulka (Soliman, Kumar, & El-Matbouli, 2017).

Higher water temperatures also enhance the spore production in bryozoan colonies, (Tops et al., 2006) and higher spore densities, therefore, might lead to pronounced clinical signs in PKD-infected fish (Bailey, Schmidt-Posthaus, Segner, Wahli, & Strepparava, 2018). The high abundance of bryozoan colonies at Site 5 could explain the high prevalence of acute PKD symptoms there, as a higher number of bryozoan colonies can produce more spores (Carraro et al., 2017). Another explanation for the numerous diseased 0+ brown trout at Site 5 is that they migrated there from upstream locations due to density-dependent population processes (Elliott, 1994). Diseased fish have an impaired capacity to withstand flow velocity; thus, brown trout might have got passively drifted to lower river reaches (Bruneaux et al., 2017).

As the brown trout is a temperature-sensitive species with an optimum temperature range from 4 to 19°C (Elliott, 1981), thermal regimes play a crucial role for the population dynamics regardless of the occurrence of PKD. Water temperature influences all the biochemical and physiological processes in fish (Beitinger et al., 2000). Stress caused by high water temperatures and leading to an increase in plasma cortisol levels can suppress the adaptive immune system of fish (Le Morvan, Troutaud, & Deschaux, 1998; Pickering & Pottinger, 1989). Therefore, it is plausible that the warm water temperature regime of the river Wulka may also have increased the recent susceptibility regarding infections with PKD. The ability of fish to adapt to environmental pressures such as increasing water temperature and parasites might explain why the Wulka harboured a vital brown trout population despite marginal temperatures. However, the increase in temperature during the past few years may have passed a critical limit, which might lead to the local extinction of brown trout from the Wulka in the near future. This conclusion is underlined by the fact that healthy fish were only found in the uppermost reaches of the Wulka, where the occurrence of trout was restricted to a single pool. The numbers of remaining fish are presumably too low for the recolonisation of downstream sections. Accordingly, the Wulka can be considered to be an early warning system for the other pre-alpine rivers that still have cooler thermal regimes. Thus, climate change will put additional pressure on brown trout populations over larger spatial extents (Filipe et al., 2013).

The complex interplay of different parameters influencing PKD outbreaks cannot be fully untangled on the basis of the existing data set. Hence, more field data quantifying the seasonal developments of the brown trout population (i.e., seasonal infection and mortality rates), the thermal regime and the severity of the disease may help to understand the complex process. However, strategies to control emerging diseases such as PKD need to be developed and considered in the fisheries management of rivers as further and more pronounced interactions with climate change are quite probable in the future.

IP-NUMBERS

Te.M.P. Te.M.P. V 6.0.12 Temperature -Measure - Package. www.blattfisch.at, 2016. Last access: 07.03.2019.

ACKNOWLEDGEMENTS

This work was financed by the Klima- und Energiefonds Österreich under contract number B670143, ACRP—ClimateTrout—KR16AC0K13263. We want to thank DI Christof Giefing for the permission to withdraw fish from the Wulka; DI Georg Fürnweger for providing the fish data from 2015; the water authorities of the province of Burgenland for providing the flow and additional temperature data; Prof. Dr. Dr. Erwin Lautsch for his assistance with the statistical analyses; and Dr. Goklesh Kumar for his help with histology. We also thank four anonymous reviewers for their valuable comments on earlier versions of the manuscript.

AUTHOR'S CONTRIBUTION

B., F.; El-M., M.; and U., G. conceived and designed the investigation. W., K.; B., T.; A., S.; and U., G. performed field and/or laboratory work. W., K.; B., T; B., F.; and U., G. analysed the data.: B., F.; El-M., M.; and U., G. contributed materials, reagents and/or analysis tools. W., K.; B., T; B., F.; U., G.; El-M., M.; and A., S. wrote the paper.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Waldner K, Bechter T, Auer S, Borgwardt F, El-Matbouli M, Unfer G. A brown trout (*Salmo trutta*) population faces devastating consequences due to proliferative kidney disease and temperature increase: A case study from Austria. *Ecol Freshw Fish*. 2020;29:465–476. https://doi.org/10.1111/eff.12528