Impacts of water temperature and other environmental parameters on proliferative kidney disease in wild brown trout (*Salmo trutta*) populations

Inaugural dissertation
of the Faculty of Science,
University of Bern

presented by

Aurélie Amandine Rubin

from Reichenbach im Kandertal

Supervisor of the doctoral thesis:
Professor Dr. Helmut Segner

Centre for Fish and Wildlife Health

Copyright Notice

Impacts of water temperature and other environmental parameters on proliferative kidney disease in wild brown trout (*Salmo trutta*) populations

Inaugural dissertation
of the Faculty of Science,
University of Bern

presented by

Aurélie Amandine Rubin

from Reichenbach im Kandertal

Supervisor of the doctoral thesis :
Professor Dr. Helmut Segner
Centre for Fish and Wildlife Health

Accepted by the Faculty of Science.

Bern, 4th September 2023

The Dean
Prof. Dr. Marco Herwegh

To my grandfather

“Look deep into nature, and then you will understand everything better.”

Albert Einstein

Acknowledgements

Firstly, I would like to specially thank my supervisor from the University of Bern, Helmut Segner for his guidance and his always relevant remarks and corrections of manuscripts, as well as Thomas Wahli for his help during the whole study and for his tips for the identification of parasites on histological slides during these hours spent on the microscope.

My special thanks go to Nicole Strepparava and Christyn Bailey for their help on the field, on the lab and for data analysis.

I also thank Frédéric Hofmann and the fish ranger from the General Directorate of the Environment of the Canton of Vaud for their help during fish samplings. Many thanks also to Daniel Hefti from the Federal Office for the Environment who believed in this project and to the Federal Food Safety and Veterinary Office which founded a large part of this work through the pilot program “Adaptation to climate change”.

Special thanks to Amandine Bussard and David Bippus, my office partners, for all these past and future great moments and funny discussions on the office, on the field or on the lab. Many thanks also to all the team of the Maison de la Rivière.

I wish also to massively thank my family for its unconditional support throughout my life which has brought me to get where I am today. In particular, my grandmother Olga and my grandfather Jean-Paul, my sisters Adeline and Delphine, my mother Marianne for her kindness and her overall support no matter what happens, and my father Jean-François for having shared his passion for rivers and their inhabitants with me, and for giving me the chance to work with him.

And finally, I would like to thank my husband Christian who shares my life and is always at my side.

Abstract

At the global scale, biodiversity is massively threatened. Pollution, deforestation, habitat loss are only some of the dangers hovering on terrestrial and aquatic biodiversity. Among it, freshwater ecosystems are at severe risk. Habitat quality, food supply, overfishing, water quality and temperature are some of the multiple drivers that impact the biodiversity of lakes and rivers. In addition, infectious diseases are also a major threat for aquatic organisms. One key challenge for salmonids is the emergence of Proliferative Kidney Disease (PKD), caused by the myxozoan parasite *Tetracapsuloides bryosalmonae*. This infection provokes a significant fish mortality, either in the wild or in fish farms. In Switzerland, this infection plays a major role in the decline of wild brown trout (*Salmo trutta*) populations.

Water temperature is considered as the main driver for PKD manifestation. Prevalence, disease symptoms and related mortality are enhanced at high temperature, especially when values reach or surpass 15°C. Up to now, this statement mainly derives from laboratory experiments, using constant water temperature and achieved with rainbow trout (*Oncorhynchus mykiss*) as the vertebrate host. However, conclusions from these studies may not be applied to the field, where brown trout populations must face fluctuating temperature and a multitude of other potential stressors. In this thesis, we thus focused on the effects of PKD on wild brown trout populations under varying water temperature and possible combining impacts of aggravating factors in the field.

In Chapter 2, we investigated the optimal sampling period for a reliable assessment of PKD in the field. Indeed, if samplings are performed too early in the season (when the infection may not be sufficiently developed or not have taken place yet at this moment) or too late (when fish have already died or the parasites have been excreted), the fish status may be wrongly assessed. We sampled young-of-the-year (YOY) brown trout over a three-years period and assessed the fish status by histology. We showed that the optimal period for the detection of *T. bryosalmonae*-infected fish was when a mean of 1500 degree days (dd) or 30 days with a daily mean temperature ≥15°C (ndays15) was reached. Based on long-term temperature values, this time-point could thus be employed in particular streams independent of altitude, location or weather characteristics. This threshold is therefore a useful tool for field researchers and should thus be taken into consideration when planning PKD sampling campaigns.

In Chapter 3, we explored the implication of varying water temperature and potential other environmental parameters on PKD infection. We sampled YOY in 45 stations through the canton of Vaud and recorded long-term water temperature values, water quality data and ecomorphology indicator at each site. PKD prevalence and infection intensity were significantly correlated with the mean water temperature of June, ndays15 and presence of a wastewater treatment plant (WWTP). This chapter hence demonstrates that water temperature influences PKD manifestation in the field and suggests that additional environmental stressors, such as water quality, may be considered in the *T. bryosalmonae*-brown trout system.

In Chapter 4, we focused on the influence of water quality on PKD manifestation. We sampled and tagged fish in two thermal similar stations, upstream and downstream of a WWTP. PKD prevalence and parasite intensity were significantly higher in the downstream site receiving the effluent compared to the upstream station. The apparent survival rate was also reduced in the downstream site. Our results thus show that even a minor alteration of water quality is sufficient to induce consequences on PKD. This factor should thus be considered alongside water temperature as a driver for PKD manifestation.

In conclusion, investigations in wild brown trout populations under varying water temperature regime and possible cumulative influences of environmental parameters need thus to be undertaken in the context of aquatic fieldwork. These outcomes are of primary importance for a better understanding of PKD impacts on fish population dynamics, which could lead to applied protective actions to maintain brown trout populations and to limit their extinctions, particularly in the context of climate change.

Table of contents

1 General Introduction

1.1 The Brown trout

Brown trout *Salmo trutta* belongs to the Salmonid family. Its native European geographical distribution is comprised between Iceland and northern Scandinavia in the North and the Atlas Mountains in North Africa in the South. Due to its high interest of recreational fishing, massive campaigns of stocking were performed in Europe, but also in other continents where brown trout is a non-native fish and now often considered as an invasive species (Elliott, 1994). Today, brown trout is present in all five continents (Figure 1).

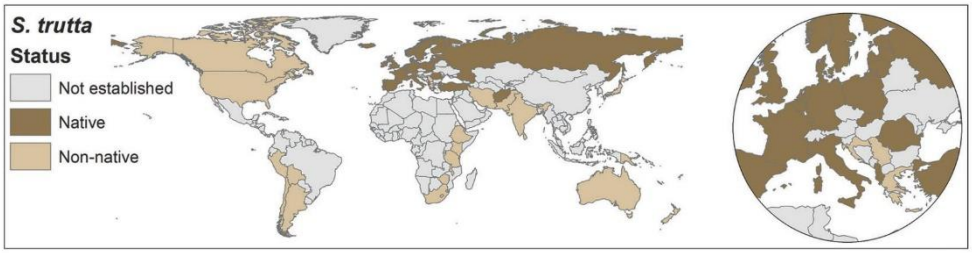


Figure 1 : Worldwide distribution of brown trout. Native distribution (brown) and introduction areas (light brown) are shown (Muhlfeld et al., 2019)

In Switzerland, five native trout species are observed: adriatic trout *Salmo cenerinus* (Po and Adige basins), danubian trout *Salmo labrax* (upper part of Danube basin), marble trout *Salmo marmoratus* (Po and Adige basins), zebra trout *Salmo rhodanensis* (Doubs basin) and brown trout *Salmo trutta* (Rhine and Rhone basins) (Figure 2) (Zaugg, 2018). Among them, brown trout has the widest distribution.

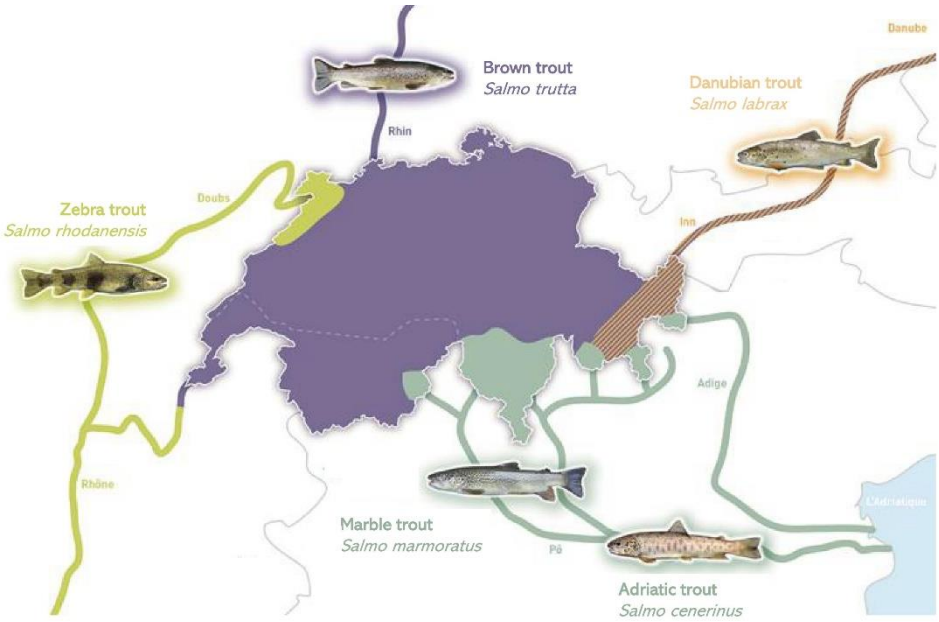


Figure 2 : Swiss distribution of the five native trout species (FIBER, 2014)

Spawning season takes generally place from October to January. All brown trout breed in rivers in areas with clean gravel and running water (Elliott, 1994). The female digs a depression called redd and leaves her eggs on it while one or several males release their sperm for fecundation. On the contrary to some Pacific salmon species experiencing a massive mortality after spawning, brown trout can reproduce several years during their life. Egg to fry development duration in the gravel depends on the environmental thermal conditions. From fecundation to the eyed stage, 220 degree days are needed, hatching takes place at 440 degree days, and emergence occurs when 840 degree days are reached (Elliott, 1984). After hatching, small alevins feed from their yolk. When emergence takes place, macroinvertebrates represent the main part of their diet. Parr stay in their natal stream in the first year. At this moment of their life, juveniles have to make a choice: stay in their natal river for their whole life or migrate downstream to a lake and come back in the river where they were born (homing phenomenon) for spawning (Elliott, 1994). In the first case, trout are termed as "resident form". Individuals are characterized by an orange-brown coloring covered with red dots surrounded by a white or black border (Figure 3 left). Their size rarely exceeds 60 cm in large and food rich streams. In the second case, trout are considered as migratory individuals. As food resources are greater in lakes compared to rivers, growth rate is more important resulting in large individuals colored with silver sides covered by black dots (Figure 3 right).

Figure 3 : Resident (left) and migratory form (right) of brown trout *Salmo trutta* © Christian Genton

Cold water temperatures (4-19.5°C) and oxygen concentration (>5 mg/l) are the main factors for trout typical river habitats (Mills, 1971; Elliott, 1994). In addition, good water quality, diversified morphology and a fine grain size are also necessary (Baglinière & Maisse, 1991). Trout are carnivorous, with macroinvertebrates, shellfish and mollusks as part of their diet. Adult fish are also considered as ichthyophagous.

Fish are poikilothermic animals. Water temperature impacts therefore fish through different ways, as on physiological functions, body temperature and behavior (Elliott, 1994). Thus, growth, period of migration, spawning time and fry emergence are some parameters of fish life influenced by temperature (Jonsson & Jonsson, 2009). Alevins are more vulnerable than adults to temperature fluctuations, while egg life stage is the most sensitive to rising temperatures (Elliott & Elliott, 2010). All fish species tolerate a specific thermal niche with upper and lower limit of temperature characteristics. Brown trout shows preferences to cold water temperature (4-19.5°C). The upper incipient lethal temperature is reached at 24.7°C and the ultimate lethal temperature at 29.7°C (Elliott, 1994). Lower and upper limits for growth are reached at respectively 2.9-3.6°C and 18.7-19.5°C, while growth optimum occurs at 13.1-13.9°C (Elliott & Hurley, 2001).

Since the beginning of the 1980s, a massive decline of brown trout catches of up to 50% has been recorded in Switzerland (Burkhardt-Holm et al., 2002; Burkhardt-Holm et al., 2005; Borsuk et al., 2006; Hari et al., 2006; Burkhardt-Holm & Scheurer, 2007; Burkhardt-Holm, 2008). The reasons for this considerable drop were investigated by a nationwide project called "Fischnetz" (2007) (Burkhardt-Holm et al., 2002). A combination of 12 hypotheses were defined to explain this diminution (e.g.

reproductive failure, pollutions, inadequate fish management, increase of water temperature, etc.). One key factor which was suspected to play an important role is an emerging temperature-dependant infective disease of salmonids, proliferative kidney disease (PKD) (Wahli et al., 2002; Burkhardt-Holm et al., 2005; Burkhardt-Holm & Scheurer, 2007; Wahli et al., 2007; Zimmerli et al., 2007).

1.2 Proliferative kidney disease

1.2.1 The distribution in Switzerland

In Switzerland, this infection is considered as an epizooty to monitor. PKD fighting is described as the eighth of the 10 points program of the Swiss Confederation for improving rivers state and fish populations (Fischnetz, 2007). Fighting the disease is thus a crucial element for maintaining trout populations for the Federal Office of the Environment. The infection was first recorded in Switzerland in 1979 (Wahli et al., 2002). Between 2000 and 2001, a country-wide study revealed that infected fish were present in 56 out of 139 (40.3%) sampled sites (Wahli et al., 2002). In 2004, a complementary monitoring was performed and showed that PKD-positive animals were found in 62 of the 111 (55.9%) study sites (Wahli et al., 2007) (Figure 4). The majority of infected fish were observed in rivers from the Swiss midlands, at an altitude below 800 m (Wahli et al., 2008). The disease is therefore widely distributed over Switzerland.

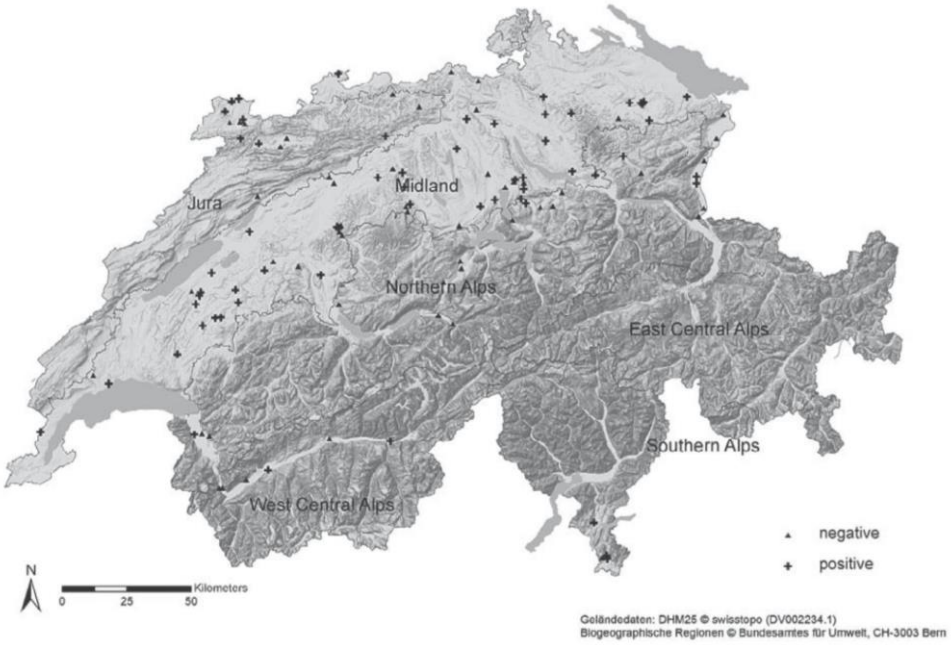


Figure 4 : Distribution of proliferative kidney disease in Switzerland in 2004 (Wahli et al., 2007)

1.2.2 The distribution of PKD in Europe and worldwide

The disease has not been reported only in wild brown trout populations of Switzerland but in a number of other European countries as well, e.g. in England (Peeler et al., 2008), Denmark (Skovgaard & Buchmann, 2012), Estonia (Dash & Vasemägi, 2014; Lauringson et al., 2021), Slovenia (Jenčič et al., 2014), Finland (Vasemägi et al., 2017), Austria (Lewisch & El-Matbouli, 2018; Waldner et al., 2020), Germany (Arndt et al., 2019), Czech Republic (Pojezdal et al., 2020; Seidlova et al., 2021) Iceland (Svavarsdottir et al., 2021) and Norway (Lauringson et al., 2022), as well as in other salmonid species such as Grayling *Thymallus thymallus* and Rainbow trout *Oncorhynchus mykiss* in Switzerland (Wahli et al., 2002), Marble trout *Salmo marmoratus* in Italy (Beraldo et al., 2006), Atlantic salmon *Salmo salar* in Norway and Iceland (Sterud et al., 2007; Mo et al., 2011; Mo & Jørgensen, 2016; Svavarsdottir et al., 2021), Arctic char *Salvelinus alpinus* in Iceland (Kristmundsson et al., 2010; Svavarsdottir et al., 2021) and European whitefish *Coregonus sp.* in Switzerland and Norway (Naldoni et al., 2019; Oredalen et al., 2021). In North America, Cutthroat trout *Oncorhynchus clarkii* (MacConnell & Peterson, 1992), Chinook salmon *Oncorhynchus tshawytscha*, Coho salmon *Onchorhynchus kisutch* (Hedrick et al., 1993), Pink salmon *Oncorhynchus gorbuscha* (Braden et al., 2010), Chum salmon *Oncorhynchus keta*, Sockeye salmon *Oncorhynchus nerka* (Gorgoglione et al., 2020) and Mountain whitefish *Prosopium williamsoni* (Hutchins et al., 2021) are also susceptible to the disease. The vast majority of infected salmonids were observed in riverine systems or shallow lakes. However, *T. bryosalmonae* positive fish were observed also in deep lakes in Norway (Oredalen et al., 2021) and Switzerland (Naldoni et al., 2019).

Fish from other families might also be concerned, as northern Pike *Esox Lucius* (Bucke et al., 1991; Grabner & El-Matbouli, 2008). However, studies are contradictory since no signs of PKD were observed in pike in Grabner and El-Matbouli's (2008) study. More recently, *T. bryosalmonae* was also detected for the first time in the cyprinid fish species European chub *Squalius cephalus*, European bullhead *Cottus gobio* and Minnow *Phoxinus phoxinus* (Lewisch & El-Matbouli, 2018).

1.2.3 *Tetracapsuloides bryosalmonae* life cycle

PKD is caused by the myxozoan parasite *Tetracapsuloides bryosalmonae* (Canning et al., 1999; Canning et al., 2002). Its genus name is linked to its typical cell structure (spores with four polar capsules). Its species name corresponds to the two hosts (bryozoan and salmonids) of the life cycle. Freshwater bryozoans, mainly *Fredericella sultana*, are considered as the invertebrate host and salmonid fish as the vertebrate host (Anderson et al., 1999; Longshaw et al., 2002; Morris & Adams, 2006; Okamura et al., 2001) (Figure 5). Horizontal transmission from fish to fish or from bryozoans to bryozoans has never been demonstrated (Ferguson & Ball, 1979; D'Silva et al., 1984; Tops et al., 2004; Grabner & El-Matbouli, 2008; Fontes et al., 2017). Covert or overt infection stages can appear in bryozoans. During a covert infection, the parasite is associated with the body wall of the bryozoan as a non-virulent single cell (Morris & Adams, 2006). This stage can occur throughout the year (Fontes et al., 2017). Then, overt infection can appear if the necessary conditions are met, as high temperature or sufficient food resources as example (Tops et al., 2009; Hartikainen & Okamura, 2012). During this stage, multicellular sacs of *T. bryosalmonae* spores develop in bryozoan body cavity and are finally released into the water (McGurk et al., 2006). This stage takes place mainly in late spring and autumn (Tops et al., 2006; 2009). If *T. bryosalmonae* spores released by bryozoans get into contact with a susceptible salmonid, they infect the fish through skin and gills (Feist et al., 2001; Longshaw et al., 2002; Grabner & El-Matbouli, 2010). One single spore has been shown to be sufficient to induce the infection (McGurk et al., 2006). Thus, a small colony of infected bryozoans is sufficient to infect a lot of salmonids. Moreover, repeated exposure to *T. bryosalmonae* spores impacts significantly the dynamics of PKD (Strepparava et al., 2020). Transported via the vascular system, *T. bryosalmonae* parasites reach the kidney, which is considered the main target organ for PKD (Kent & Hedrick, 1986; Feist et al., 2001). In the interstitial tissue of the kidney, the extrasporogonic stage multiplies which can induce, depending on the environmental conditions and the fish susceptibility, an inflammatory response resulting mainly in

proliferative and granulomatous nephritis, interstitial and vascular lesions and thrombus formation (Hedrick et al., 1993; Bettge et al., 2009a; Okamura et al., 2011; Schmidt-Posthaus et al., 2015). The parasites then translocate into the tubuli differentiating into the sporogonic stage, where it produces spores which are released via the urine into the environment and can (re)infect bryozoans, thus completing the parasite life cycle (Longshaw et al., 2002; Hedrick et al., 2004; Morris & Adams, 2006; Abd-Elfattah et al., 2014; Soliman et al., 2018). Spores released by brown trout and brook trout *Salvelinus fontinalis* have been shown to be infective for bryozoans, on the contrary to spores excreted by rainbow trout and grayling (Morris & Adams, 2006; Grabner & El-Matbouli, 2008; Abd-Elfattah et al., 2014). After spore excretion, some fish do not totally clear the infection and become asymptomatic carriers (Soliman et al., 2018). *T. bryosalmonae*-positive fish can remain infective for bryozoans for several years and are therefore a reservoir for the infection of the next growing bryozoan colonies in spring as well as for parasite dissemination in PKD-free zones (Abd-Elfattah et al., 2014; Soliman et al., 2018). However, a complete restoration of renal morphology and parasite elimination during winter is also possible depending on concurrent infection and temperature (Schmidt-Posthaus et al., 2013).

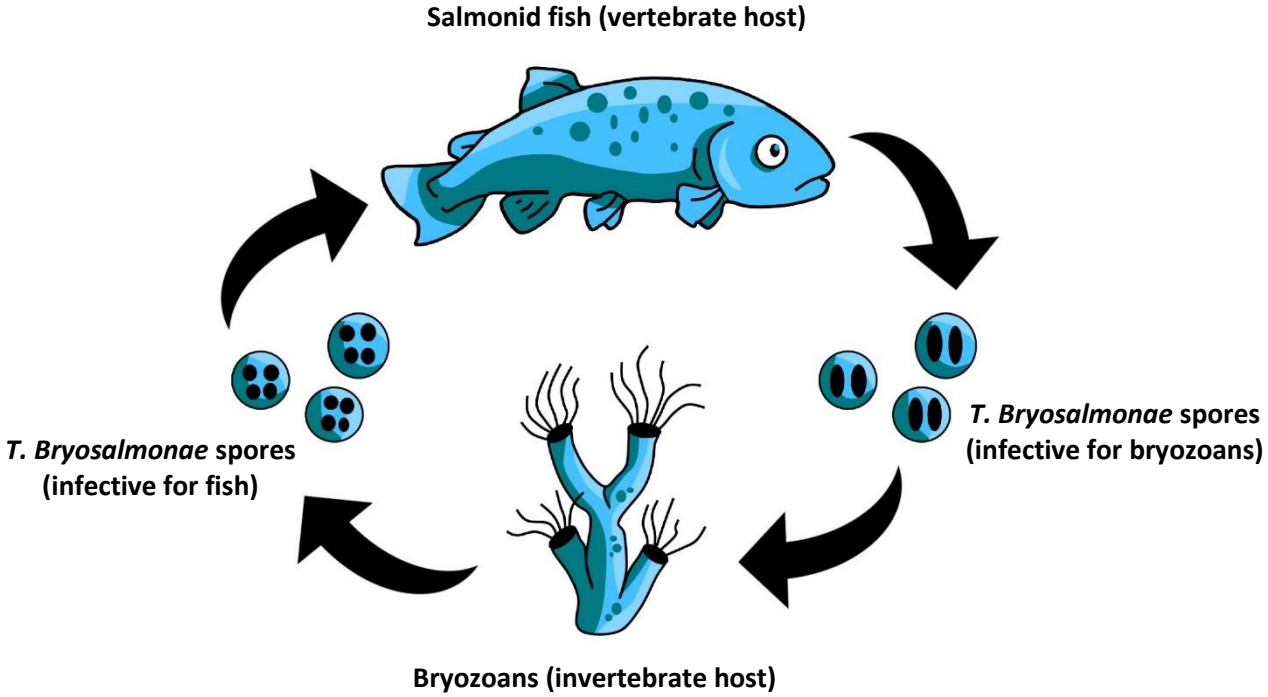


Figure 5 : *T. bryosalmonae* life cycle through invertebrate and vertebrate hosts

Infected fish can show external macroscopic symptoms, as melanism, exophthalmos, swollen abdomen and pale and anaemic gills (Hedrick et al., 1993). Internally, the major clinical sign is a hypertrophic kidney (Figure 6) caused by an inflammatory response of the hematopoietic tissue due to the parasite presence (Clifton-Hadley et al., 1987; Bettge et al., 2009a; Okamura et al., 2011; Schmidt-Posthaus et al., 2015). This kidney impairment will induce anaemia, which results as example in a decrease of oxygen transport in fish blood or in a negative impact on the fish metabolic rate (Bruneaux et al., 2017).

Young-of-the-year fish are particularly susceptible to the infection, since they are in contact with the parasite for the first time (Wahli et al., 2002; Okamura et al., 2011; Feist & Longshaw, 2006; Bruneaux et al., 2017). The parasite proliferation is faster in this life stage compared to older fish, as well as the clinical signs severity (Schmidt-Posthaus et al., 2013; Waldner et al., 2020; Bailey et al., 2021).

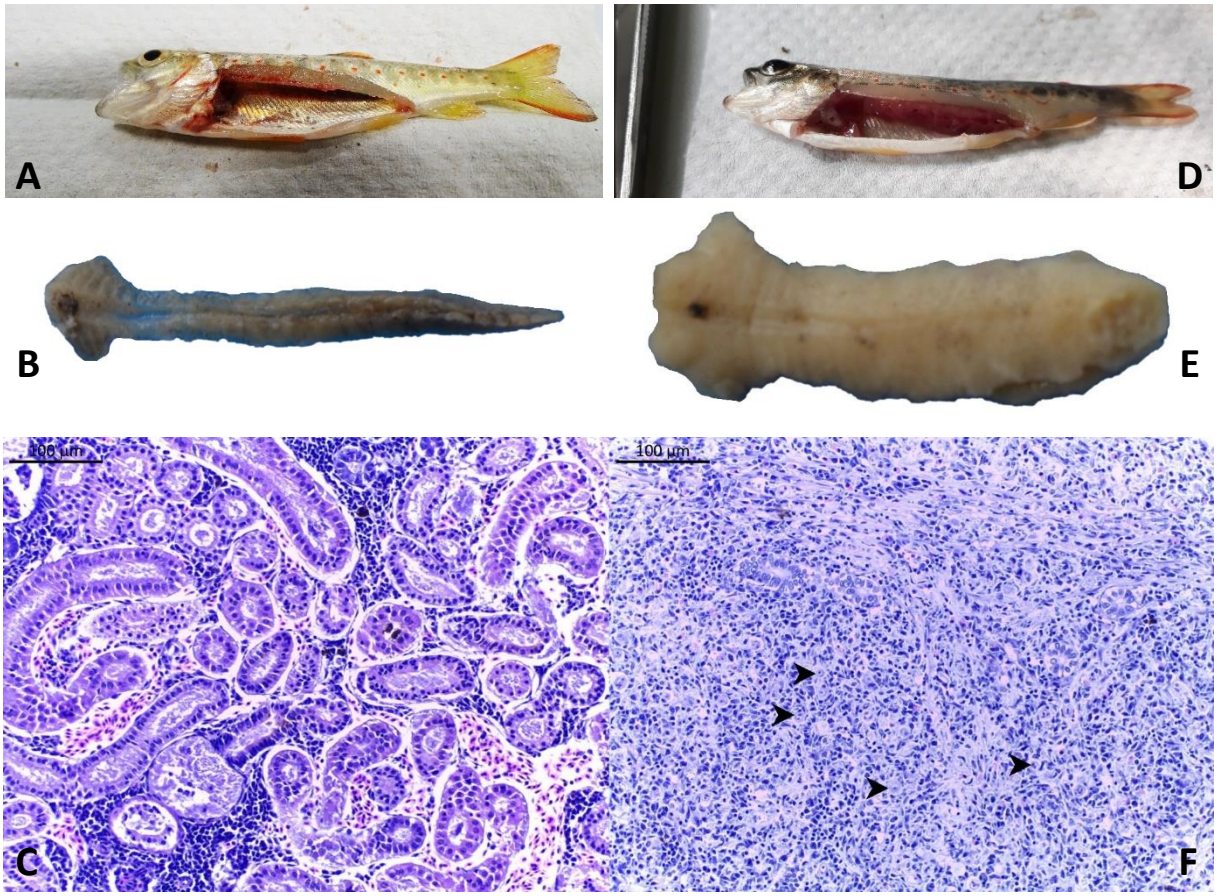


Figure 6 : A) Young-of-the-year brown trout not infected by PKD, B) Macroscopic normal kidney, C) Histological picture of normal kidney, D) *T. bryosalmonae*-infected Young-of-the-year, E) Macroscopic enlarged kidney due to PKD, F) Histological picture of *T. bryosalmonae* infected kidney, showing intensive proliferation. Some *T. bryosalmonae* parasites are shown (arrows).

1.2.4 PKD impact on fish mortality

Even if the infection is widespread in Europe and North America, the PKD-related mortality in wild salmonids populations is not fully understood (Okamura et al., 2011). The true proportion of fish mortality due to PKD infection is complex to elucidate since different confounding factors might be effective (Hutchins et al., 2018; Ros et al., 2022). The disease can lead to heavy losses in fish populations, as observed in Atlantic salmon in Norway, where the infection was responsible for an estimated decrease of salmon parr density of 85% (Sterud et al., 2007). In the Yellowstone River, 295 km of river stretches were closed for several weeks to fishing activities due to a massive PKD-related fish death (Hutchins et al., 2021). Brown trout populations of the Wutach (Germany) decrease by 51.9% over the last 20 years, most likely due to the development of *T. bryosalmonae* (Ros et al., 2021). In Switzerland, a cage-experiment showed that the PKD related-mortality of exposed brown trout reached 15% (Schmidt-Posthaus et al., 2015). More generally, the disease is suspected to contribute to a mortality of approximately >25% in Swiss wild brown trout populations (Borsuk et al., 2006). If co-infection occurs, the mortality can reach up to 100% (Hedrick et al., 1993). However, disease related mortality in wild salmonids populations might be severely underestimated, particularly due to fish stocking or predation of dead fish by other animals (Okamura et al., 2011). Moreover, the mortality needs to be sufficient to be noticed in a stream. In a Czech fish farm, PKD related cumulative mortality of rainbow trout reached 30% (Palikova et al., 2017). However, in intensive fish farm, where high densities occur, the infection can lead to a stock mortality of up to 95%, which results in huge economic losses (Hedrick et al., 1993; Okamura et al., 2001; Feist & Longshaw, 2006).

Nevertheless, some fish can survive the disease and, depending on conditions, can restore a normal kidney structure, acquire resistance to reinfection or develop chronic lesions (Schmidt-Posthaus et al., 2012; Palikova et al., 2017; Bailey et al., 2021).

1.2.5 Pathways for PKD spreading

Several pathways for the spread of pathogens appear in rivers. Among them, animal migration is considered as a main factor (Altizer et al., 2011; Schmidt-Posthaus et al., 2021). Pathogen colonization through animal migration has been demonstrated for mammals, birds, fish and insects (Altizer et al., 2011). As a long-distance migratory fish, particularly during spawning season, salmonids may contribute to disease dispersion. In Switzerland, important campaigns of obstacles removal are in progress in the context of the restoration of river in order to allow fish migration (Federal fisheries act, 1991). However, removal of migration barriers might also be considered as a mean for pathogen spreading through movement of infected-fish, particularly to upper river sites currently disease free (Carraro et al., 2018; Schmidt-Posthaus et al., 2021). Nevertheless, during a five-years monitoring of brown trout migration and PKD distribution after barrier removal, no *T. bryosalmonae*-infected young-of-the-year (YOY) were detected in the upstream area, although adults coming from downstream PKD positive zones reached the sector during spawning season (Schmidt-Posthaus et al., 2021). In addition, spore transmission by other fish or bird species should also be considered (Abd-Elfattah et al., 2017; Schmidt-Posthaus et al., 2020). Even if spores ingested by brown trout and released via the fish faeces have been shown to be broken and thus do not represent a potential pathway for PKD spreading, it seems not to be the case for spores ingested by common carp *Cyprinus carpio* (Abd-Elfattah et al. 2017). *T. bryosalmonae*-infected statoblasts were able to survive after the passage through the fish gut, suggesting that some fish species could be a vector for the spread of the parasite. In this context, the potential impact of bird spreading was also tested by Schmidt-Posthaus and colleagues (2020). Fish-eating birds, goosander *Mergus merganser* were fed with *T. bryosalmonae* infected brown trout. Some parasite DNA was detected in faeces, but no viable spores were observed. Infected fish ingestion by goosander is hence not considered as a potential vector for PKD transmission. However, transport of viable bryozoan spores was also noted in waterbird feathers and faeces (Reynolds & Cumming, 2015). Ship ballast tanks are as well noticed as a potential transport vector of freshwater bryozoans (Kipp et al., 2010). In addition, fish raised in fish farm where parasites are present could also spread the infection in disease free areas during stocking purposes. Finally, infected statoblasts released by bryozoans in stream permits a downstream transport via waterflow to several tens of kilometres, thus possibly inducing PKD infection to various salmonids population as long as bryozoans are present (Carraro et al., 2018).

1.2.6 Influence of water temperature on the disease

Different environmental factors are suspected to play a role on the development of diseases. Among them, water temperature is considered as a main driver for host-pathogen systems. Indeed, it can promote the transmission and the virulence of the pathogen, the host susceptibility, the season of parasite transmission and thus the distribution and the proliferation of diseases, which can lead to population decrease or extinctions (Marcogliese, 2001, 2008; Karvonen et al., 2010; Marcos-Lopez et al., 2010; Johnson & Paull, 2011; Carraro et al., 2016; Bailey et al., 2017a, 2018a; Strepparava et al., 2018). As example, the severity, the frequency and the distribution of some salmonid diseases, as furunculosis and enteric red mouth are expected to increase due to global warming (Marcos-López, 2010). Since PKD is suspected to be temperature-dependent, the same situation is presumed to occur in the near future (Hari et al., 2006; Tops et al., 2006, 2009; Okamura et al., 2011; Carraro et al., 2016), which could thus have a huge impact on native brown trout populations.

Water temperature is suggested as a key parameter modulating the disease (Foott & Hedrick, 1987; Kinkelin & Loriot, 2001; Gay et al., 2001; El-Matbouli & Hoffmann, 2002; Schager et al., 2007; Sterud

et al., 2007; Bettge et al., 2009a and b; Okamura et al., 2011). Water temperature could affect this host-parasite system through different ways: it can influence the vertebrate host, the invertebrate host, or the parasite itself. In fish, this parameter modulates the strategy of the fish immune response (Bailey et al., 2017), the rate of spore production and transmission (Strepparava et al., 2018), as well as the kinetic of *T. bryosalmonae* proliferation in the kidney and the lesion severity in the renal tissue (Bettge et al., 2009a and b). In bryozoans, elevated temperature also induces speeds up and prolongation of the production of infective spores (Tops et al., 2006; 2009) and enhances bryozoan growth (Tops et al., 2009).

T. bryosalmonae infection of salmonids has found to take place at temperatures ≥9°C (Hedrick et al., 1993; Gay et al., 2001). Clinical signs and disease-related mortality are enhanced when water temperature reaches or surpasses ≥15°C (Bettge et al., 2009a and b; Okamura et al., 2011; Schmidt-Posthaus et al., 2012; Bailey et al., 2017; Bruneaux et al., 2017; Strepparava et al., 2018; Bailey et al., 2018). Waldner et al. (2021) observed that brown trout kept at 16°C showed significantly less clinical signs and morbidity than trout kept at 19°C and 22°C. However, they found only few differences between fish raised at 19°C and 22°C.

Nevertheless, these studies pointing that water temperature is a key driver of PKD dynamics and severity mainly result from laboratory experiments, performed with constant temperatures. In the wild, the thermal regime fluctuates widely, often surpassing 20°C in summer for typical Swiss midlands streams (Data from the General Directorate of the Environment, 2022). Thus, since a minor change in water temperature is suggested to modify the PKD dynamic in a lab setting (e.g. Bailey et al., 2017; Strepparava et al., 2018; Waldner et al., 2021), statements observed in laboratory experiments might not be directly applied to native salmonids populations in the wild.

Moreover, PKD laboratory investigations are also largely performed with rainbow trout as model species (e.g. Kinkelin & Loriot, 2001; Gay et al., 2001; Okamura et al., 2001; Longshaw et al., 2002; Bettge et al., 2009a and b; Grabner & El-Matbouli, 2010; Bailey et al., 2017a and b, 2018a). However, rainbow trout and brown trout do not react in the same way to *T. bryosalmonae* infection and differences in susceptibility appear between these salmonid species (Bailey et al., 2017). As example, Schmidt-Posthaus et al. (2001) showed that the mortality rate of brown trout was higher than that of rainbow trout for animals kept in tanks supplied with *T. bryosalmonae*-positive river water. Kumar et al. (2013) observed that the parasite burden in brown trout kidneys was superior to the parasite load measured in rainbow trout kidneys. Moreover, these authors observed a successful transmission of the parasite from brown trout to the bryozoan *Fredericella sultana*, while infected rainbow trout were not able to transmit the infection to bryozoans. Bailey et al. (2017) showed that the prevalence of the disease and the increase of parasite intensity in brown trout were higher compared to rainbow trout. Several differences in the development of PKD appears therefore between rainbow trout and brown trout. Statements derived from laboratory experiments using rainbow trout as model species are thus not directly applicable for wild brown trout populations.

Nevertheless, some studies focused on PKD in wild brown trout populations (e.g. Feist et al., 2002; Skovgaard & Buchmann, 2012; Dash & Vasemägi, 2014; Lewisch & El-Matbouli, 2018; Waldner et al., 2020). Wahli et al. (2008) used altitude as a proxi for water temperature, but no correlation between infection intensity and altitude was found, in contrast to a study of Ros et al. (2021), in which PKD prevalence was negatively correlated to sites elevation. Moreover, due to this temperature dependency, disease symptoms and mortality are not constant through the year (Wahli et al. 2002; Sterud et al. 2007; Ros et al. 2021). Indeed, they are most pronounced during summer and early autumn, following thus a seasonal occurrence of the infection. However, none of these studies used long-term water temperature data or environmental information in order to explain the disease development in a field setting. Up to now, a deepened analysis of long-term temperature values and presence of *T. bryosalmonae* infected brown trout in the wild is missing.

1.2.7 Influence of water quality and other environmental stressors on PKD manifestation

Besides temperature, other environmental stressors may also affect freshwater fish disease dynamics, such as co-infection, pH, species introduction, stocking density, habitat quality, water levels and ecomorphology (Marcogliese, 2001; Jacobson et al., 2003; Graham et al., 2007; Marcogliese, 2008; Peeler & Feist, 2011; Johnson & Sumpter, 2014). Among them, pollution is one important factor influencing fish-parasite systems (Blanar et al., 2009; Vidal-Martinez et al., 2010).

In Switzerland, during the 1950's, lakes and rivers water was highly polluted by untreated urban, industrial and artisanal wastewater (FOEN, 2017). In some places, swimming and bathing were even forbidden due to sanitary reasons. Swiss people asked therefore the government to take actions for an improvement of water quality. The first federal law for water protection against pollution was thus born in 1955. Since that time, numerous sewage pipelines and wastewater treatment plants (WWTPs) were built in the country. In 1965, 14% of the Swiss habitants were connected to a WWTP. Today, this number reaches almost 99%. These installations, together with the reduction and prohibition of problematic substances, permit to eliminate a large number of pollutants and thus reduce greatly water pollution (Binderheim-Bankay et al., 2000). However, even if the majority of WWTP effluent flowing through rivers respects the Swiss legislation (Ordinance on water protection, 1998), some microorganisms, nutrient or micropollutants, such as pharmaceuticals, chemicals, phytosanitary products or hormones, can still reach the stream despite water treatment (Glassmeyer et al., 2008). To date, the major sources of water pollution come from WWTP untreated elements and from agriculture (Binderheim-Bankay et al., 2000).

Up to now, only few studies focused on the role of pollution on fish health and more specifically on the *T. bryosalmonae* – salmonid system (e.g. Schmidt et al., 1999; Schmidt-Posthaus et al., 2001; El-Matbouli & Hoffmann, 2002). Schmidt et al. (1999) observed some minor lesions in internal organs from rainbow and brown trout kept in tanks supplied with an input of WWTP effluent water, compared to fish raised in tap water. However, the authors concluded that these alterations do not permit to assess a direct damaging effect of water quality on fish health. In another experiment, Schmidt-Posthaus et al. (2001) showed that the mortality rate of fish exposed to river water containing WWTP effluent was increased in contrast to animals held in tap water.

Water quality is suspected to influence the PKD prevalence and intensity by three distinct ways: directly by decreasing the fish health, indirectly through the parasite by modifying its virulence or by promoting bryozoan proliferation. Indeed, these animals are filter feeding organisms and thus flourish in areas with an increased concentration of nutrients (El-Matbouli & Hoffmann, 2002; Hartikainen et al., 2009), as it is the case just downstream of WWTPs. El-Matbouli & Hoffmann (2002) observed that the PKD prevalence in wild brown trout sampled upstream a WWTP was significantly lower compared to that in fish sampled downstream the facility. However, only few fish were infected during the whole study. This experiment gives thus a first look of the potential influence of organic pollution on the PKD dynamics but need to be further addressed.

Therefore, both temperature and water quality seem to contribute to the infection prevalence, disease severity and fish mortality. Thus, multiple factors are suspected to play a role on the infection and need to be taken into account to better understand the disease development and their impacts on fish population in the future.

1.3 Climate change

1.3.1 Climate change in Switzerland

Freshwater ecosystems are among the most threatened on earth. Indeed, these habitats cover only 1% of our planet but host more than 30% of all vertebrate species (Dudgeon et al., 2006; Strayer & Dudgeon, 2010). Since 1970, these ecosystems have undergone a reduction of 30% (Dixon et al., 2016). Nowadays, freshwater biodiversity is declining at a frightening rate, higher than terrestrial or marine species (Dudgeon et al., 2006). Some of the main causes of this process are flow alteration, water pollution, overexploitation, alien species invasion and habitat deterioration (Dudgeon et al., 2006). Actually, an even bigger threat, climate change, is at work, which will have enormous repercussions on countless processes of freshwater ecosystems. Changes in geographic distribution, introduction of invasive species, modification in the seasonal activities and emerging diseases are some illustrations of the consequences of climate change that freshwater species are facing (Caissie, 2006; Heino et al., 2009; Strayer & Dudgeon, 2010; Johnson & Paull, 2011; Comte et al. 2013).

During the 21st century, the global mean temperature has increased by 0.99°C (IPCC, 2021). In Switzerland, a warming trend of 1.8°C is in progress, far more than the world average (FOEN, 2012). Three scenarios were developed by the Intergovernmental Panel on Climate Change (IPCC) in order to predict the possible temperature increase in the future depending on the greenhouse gas concentration:

- Representative Concentration Pathway (RCP) 2.6 corresponds to the most optimistic scenario with a massive decrease of greenhouse gases in the near future,
- RCP4.5 (mean scenario) comprises a stabilisation of greenhouse gases,
- RCP8.5 corresponds to the *Business as usual* scenario, with an increase of greenhouse gas concentration.

The models predict that by the end of this century, the global temperature is suspected to increase between 1.8°C (RCP2.6) and 4.4°C (RCP8.5) compared to 1995-2014 (IPCC, 2021).

In Switzerland, the estimated mean increase of temperature should be comprised between +0.6 and +1.9°C for the RCP2.6 and between +3.3°C and +5.4°C for the RCP8.5 scenario (Fischer et al., 2022). The temperature deviations compared to the standard period (1981-2010) will be more intense in summer (Figure 7) and in the Alps area (Fischer et al., 2022).

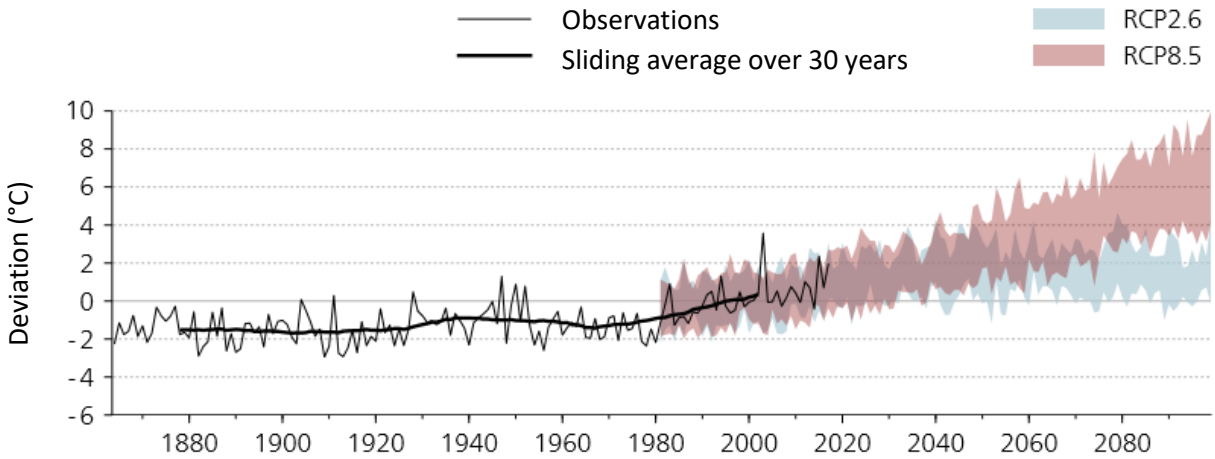


Figure 7 : Swiss summer air temperature deviation from the period 1981-2010 (Fischer et al., 2022)

The increase of air temperature will also have an influence on other phenomenons. Indeed, as air and water temperature are linked (Stefan & Preud'homme, 1993; Mohseni & Stefan, 1999; Caissie, 2006), global warming also influences the temperature of rivers and lakes. In 52 Swiss catchments, water temperature rises of 0.37°C per decade are calculated (Michel et al., 2020). Moreover, these authors

observed a higher frequency and intensity of the number of days per year when temperature reaches 15°C. Michel and colleagues (2022) predicted that the median annual water temperature increase by the end of the century will be comprised between 0.9°C for the RCP2.6 to 3.3°C for the RCP8.5.

Precipitation will also be affected by global warming. Indeed, in Switzerland, models predict a reduction of precipitations during summer (CH2011, 2011). However, intensity and frequency of extreme events will tend to increase. Precipitations will also be more often constituted of rain rather than snow. Thus, the frequency of extreme flood events is suspected to increase, mostly during the wintertime (FOEN 2012). This phenomenon implicates a new threat for infrastructures, agriculture and urban areas, as well as for aquatic wildlife (FOEN, 2020).

1.3.2 Climate change impact on brown trout population

As a temperature-sensitive fish, brown trout populations are thus deeply endangered by rising temperature due to climate change. With increasing water temperature, suitable thermal habitats for brown trout are suspected to dramatically shrink in the future, leading to massive modifications of its worldwide and regional distribution, as well as potential population extinctions as shown in several studies (e.g. Hari et al., 2006; Lassalle & Rochard, 2009; Wenger et al., 2011; Almodóvar et al., 2012; Filipe et al., 2013; Santiago et al., 2016; Carraro et al., 2018; Borgwardt et al., 2020; Santiago et al., 2020; Ros et al., 2021). In western United States, a reduction of 48% of suitable habitat is predicted, mainly driven by rising temperature and winter high flows frequency (Wenger et al., 2011). Forecasts showed that all suitable habitat of the southern European distribution range of brown trout will disappear (Lassalle & Rochard, 2009). Based on models using annual mean temperature and annual precipitation as main factors, Filipe et al. (2013) identified a massive decline of up to 64% in the 2080s of brown trout suitable habitat in the Elbe, Ebro and Danube basins due to global warming. In the Spanish Aragòn River basin, climate models predicted a huge increase of 93% of unsuitable thermal habitat for the period 1993-2004 compared to 1975-1986 (Almodóvar et al., 2012). A decline of 12% of suitable habitat per decade is suspected as well. Also in Spain, a decline of 11% and even 56% of habitat is predicted on the two studied streams (Santiago et al., 2016). The situation is even more dramatic for intragravel stages (egg and fry) (Santiago et al., 2020). In Switzerland, a massive reduction of sustainable habitat is assumed to happen below 600m, while some new favourable areas could be gained above 600m, especially at altitudes between 1000 to 1500m (Hari et al., 2006). Even if some differences in methodological aspects appear between them, all the cited studies conclude that a dramatic decline of suitable thermal habitat is in progress, leading to potential numerous brown trout population extinctions. In addition, PKD remains a significant additional threat for salmonids in predicted suitable thermal habitats. Carraro et al. (2018) observed that a water temperature increase of 4°C results in 25% of PKD related fish loss. Based on Austrian streams, Borgwardt et al. (2020) showed that the actual thermal conditions for the outbreaks of PKD were not reached in 72.6% of rivers, against only 37.7% of stream considering the RCP 8.5 scenario in 2050. Ros et al. (2021) observed that predicted suitable habitats for *T. bryosalmonae* in a German stream are not affected by global change, in contrast to brown trout favourable areas. In this study, forecasts estimate a serious decline of 40.9%-72.3% of brown trout populations in 50 years.

However, global warming will also massively impact other environmental processes, in addition to rising temperatures. Indeed, climate change is predicted to alter precipitation pattern as well (Trenberth, 2011; Döll & Schmied, 2012; IPCC, 2021), thus possibly impacting fish populations dynamics. As example, forecasts predict a reduction of summer waterflow rate leading to a modification of the carrying capacity of streams and hence fish density (Muñoz-Mas et al., 2016; Papadaki et al., 2016; Santiago et al., 2020). Parry et al. (2018) showed that flow reduction, particularly during spawning season, negatively impacts juvenile survival of Atlantic salmon by limiting access to spawning areas. On the contrary, huge waterflow at the end of winter and spring can move the substrate of the riverbed which contains eggs, implying thus death of the fry (Cattanéo et al., 2002; Warren et al., 2009). On the other hand, extreme drought events during summer will lead to dramatic

low water level in some part of rivers, as during the summer 2022, which could provoke disappearance of fish populations. Moreover, fish community could be modified, with warm-water species becoming dominant in downstream part of rivers (Daufresne & Boët, 2007). Cyprinids zone will be extended, in contrast to the salmonids zone that will move upstream and thus be reduced. As a consequence, an upstream shift to upper and cooler areas might appear (Hari et al., 2006; Daufresne & Boët, 2007; Comte et al., 2013, 2015; Isaak et al., 2016). Unfortunately, these upstream potential suitable habitats are still often unreachable by fish migration due to physical barriers. As a consequence of climate change, brown trout populations are therefore at serious risk in the future.

Proactive management is thus of crucial importance. Actions must be undertaken now for freshwater ecosystems protection and limitation of cold-water species extinctions. As example, massive campaigns should be performed by managers for obstacles suppression and free connection to upstream thermal refuges or cooler tributaries (Hari et al., 2006; Elliott & Elliott, 2010; Ros et al., 2021). Riparian vegetation is considered as a significant factor lowering water temperature by shading the stream and thus might be promoted (Garner et al., 2017; Trimmel et al., 2018; Wondzell et al., 2019; Borgwardt et al., 2020). Renaturation measures are as well a trigger point (Roni, 2019). Monitoring of environmental changes, protection and enhancement of riparian vegetation, renaturation measures, as well as water sampling management are of primary concern (Palmer et al., 2009). A better understanding of the impacts of climate change on brown trout populations is therefore important to propose protective actions for a limitation of populations extinction. In this context, the influence of environmental stressors on PKD manifestation is of primary concern.

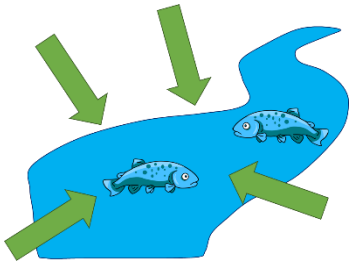
1.4 Thesis aims and objectives

PKD is temperature-dependent, since elevated water temperature seems to exacerbate the infection which leads to acute renal pathology and mortality rate. To date, the influence of water temperature on the disease largely derives from laboratory investigations. Indeed, field surveys investigating PKD with long-term water temperature remain scarce. Moreover, laboratory experiments investigating *T. bryosalmonae*-trout system are often performed with rainbow trout as model species for the vertebrate host. However, the sensitivity to infectious agent, the parasite intensity and the impact of water temperature differ between rainbow trout and brown trout. In addition, despite the fact that water temperature plays a major role for PKD development, the disease is modulated by multiple stressors, as water quality for example. These factors possibly induce cumulative effect on the response of the fish to the parasite. Nevertheless, their accumulative role remains up to now little known.

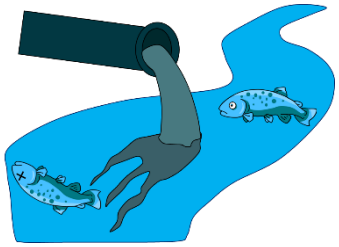
Hence, conclusions from laboratory experiments applying constant temperature, performed under controlled conditions and executed with rainbow trout may thus not be in equivalence with the condition of native brown trout individuals dealing with fluctuating water temperature and multiple stressors. Further studies need thus to be performed in the context of aquatic fieldwork. A better understanding of PKD effects on wild fish population dynamics is crucial. Then, applied protective actions should be conducted for the protection of brown trout populations, especially in the context of climate change.

The goal of this thesis was therefore to focus on the manifestation of PKD in wild brown trout under fluctuating temperature regimes and multiple stressors. Three objectives were evaluated:

- **When is the optimal sampling period for reliable field assessment of PKD?**
  (Chapter 2)



- **What are the combined implications of environmental stressors on PKD prevalence and intensity?**
  (Chapter 3)



- **What is the specific influence of water quality on PKD susceptibility and mortality?**
  (Chapter 4)

2. Reliable field assessment of proliferative kidney disease in wild brown trout, *Salmo trutta*, populations: When is the optimal sampling period?

In the context of aquatic fieldwork, the development of robust sampling approaches for the reliable identification of *T. bryosalmonae*-infected fish in native fish populations is of primary importance. For this purpose, the selection of the diagnosis method, the sample size and the sampling period need to be carefully considered.

The manifestation of PKD in wild population and its related mortality seem to vary through the year. Sample period should therefore well be planned for a reliable determination of the presence/absence of PKD-positive fish. Indeed, if fish are sampled too early in the season, the disease could not have been declared or might not be enough developed to be detected by the selected diagnosis method. Sites may thus be falsely classified as PKD-negative. On the contrary, if individuals are caught too late, lot of fish might already have died due to the disease-induced mortality, or the parasites might have been eliminated by the host immune system. An underestimation of the PKD fish status might thus appear. Hence, the selection of the appropriate sampling time-point is a crucial element for PKD investigations in the wild.

Indications seem to stipulate that this seasonal PKD variation along the year could mainly be driven by water temperature chronicle, but this question needs to be further elucidated. If a correlation between water temperature and PKD status is highlighted, then the water temperature conditions required for PKD detection by the diagnosis method could be noticed and thus the appropriate sampling period could be planned.

The aim of the study was:

- To identify when PKD sampling operations should be planned based on water temperature data for having the highest chance for the histological detection of *T. bryosalmonae* presence/absence in wild fish populations from small streams.

For this purpose, two streams of the canton of Vaud (Switzerland) were selected. In each river, two stations were sampled, with differing temperature chronical between sites. Young-of-the-year brown trout were caught over three consecutive years. The PKD status of each fish was histologically assessed. The percentage of *T. bryosalmonae*-infected fish and the PKD intensity were evaluated. Water temperature was recorded every 15 minutes in the 4 stations during the whole study period. This factor was characterized as the degree days and the number of days with a daily mean temperature ≥15°C. Long-term temperature measurements and infection values were associated for the determination of the optimal sampling time-point for reliable field PKD investigations. The identified time window based on temperature data could therefore be applied to other streams independently of altitude, weather conditions or location.

3. Keeping an Eye on Wild Brown Trout (*Salmo trutta*) Populations: Correlation Between Temperature, Environmental Parameters, and Proliferative Kidney Disease

Water temperature is considered as a major factor modulating PKD infection. However, this conclusion mainly derived from laboratory experiments performed with rainbow trout as model species. Therefore, studies investigating the impact of *in situ* water temperature on wild brown trout populations remain scarce. Moreover, besides temperature, PKD modulation could be affected by other environmental parameters, but these combined impacts were rarely examined.

For this research we linked long-term water temperature measurements and combined effect of aggravating environmental factors with PKD prevalence and severity in a field context.

The aims of the study were:

- To identify the correlation between seasonal variation of water temperature and PKD data (prevalence and degree of infection) on wild brown trout population,
- To highlight if other environmental factors, such as water quality and ecomorphology, play a role on the manifestation of PKD, and thus have cumulative effects,
- To quantitatively predict the combined implications of these environmental parameters on the infection using a statistical model.

For this purpose, 45 stations located through the canton of Vaud streams (Switzerland) were analysed. In each station, 25 young-of-the-year brown trout were sampled during the period when the highest chance to detect the presence of *T. bryosalmonae*-infected fish by means of histology was reached (based on results from Chapter 2). One kidney slide per fish was analysed by means of histology. Long-term water temperature measurements, water quality data (macroinvertebrate community index and presence/absence of a wastewater treatment plant effluent) as well as river ecomorphology were recorded. Environmental data were then correlated with PKD prevalence and degree of infection. The collected data were integrated in a statistical model predicting the variation of PKD infection due to the increase of water temperature and additional cumulative factors. These conclusions could then serve as a basis for identifying and predicting the present and future potential disease hot spots.

4. Do fish get wasted? Assessing the influence of effluents on parasitic infection of wild fish

Water temperature is believed to be a main driver modulating PKD infection, and thus impacting wild brown trout population dynamics. However, multiple other stressors may influence aquatic ecosystems, as water quality. For the *T. bryosalmonae* – brown trout system, pollution may impact infection through the resistance of the host, the replication rate of pathogens, the distribution and abundance of pathogens hosts and the transmission of infectious stages. Only very few studies focused on the influence of a decrease in water quality on PKD manifestation. Up to know, the effect of pollution in this host-parasite system remains little known.

In this study we compared the manifestation of PKD between two close stations situated upstream and downstream a wastewater treatment plant. This decrease in water quality was suspected to induce a more severe susceptibility to the disease for fish inhabiting the effluent site compared to upstream individuals.

The aim of this study was:

- To highlight the potential aggravating role of altered water quality on PKD infection.

We selected two adjacent stations in close proximity (400m apart). No significant differences in terms of water temperature and ecomorphology appear between the two sites, except the presence of a wastewater treatment plant effluent inducing a subtle decrease of water quality. Young-of-the-year brown trout were monthly sampled in the upstream and downstream stations between June and October. Kidneys were removed and PKD status was assessed in terms of infection prevalence, parasite intensity and host health. The apparent survival rate and mortality were also evaluated through capture-tagging-recapture method. The water quality upstream and downstream the wastewater treatment plant was assessed through an evaluation of the macrozoobenthos community. This study could thus highlight if a subtle decrease of water quality may impact *T. bryosalmonae* – brown trout system and should also be considered as a driver of PKD manifestation.

1.5 References

Abd-Elfattah, A., G. Kumar, H. Soliman, and M. El-Matbouli. 2014. Persistence of *Tetracapsuloides bryosalmonae* (Myxozoa) in chronically infected brown trout Salmo trutta. Diseases of Aquatic Organisms 111:41-49.

Abd-Elfattah, A., M. El-Matbouli, and G. Kumar. 2017. Structural integrity and viability of *Fredericella sultana* statoblasts infected with *Tetracapsuloides bryosalmonae* (Myxozoa) under diverse treatment conditions. Veterinary Research 48:19.

Almodóvar, A., G. G. Nicola, D. Ayllón, and B. Elvira. 2012. Global warming threatens the persistence of Mediterranean brown trout. Global Change Biology 18:1549-1560.

Altizer, S., R. Bartel, and B. Han. 2011. Animal Migration and Infectious Disease Risk. Science 331:296-302.

Anderson, C., E. Canning, and B. Okamura. 1999. Molecular data implicate bryozoans as hosts for PKX (Phylum Myxozoa) and identify a clade of bryozoan parasites within the Myxozoa. Parasitology 119:555-561.

Arndt, D., R. Fux, A. Blutke, J. Schwaiger, M. El-Matbouli, G. Sutter, and M. C. Langenmayer. 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.

Baglinière, J.-L., and G. Maisse. 1991. La truite. Biologie et écologie. Editions Quae, Versailles.

Bailey, C., H. Schmidt-Posthaus, H. Segner, T. Wahli, and N. Strepparava. 2018. Are brown trout *Salmo trutta fario* and rainbow trout *Oncorhynchus mykiss* two of a kind? A comparative study of salmonids to temperature-influenced *Tetracapsuloides bryosalmonae* infection. Journal of Fish Diseases 41:191-198.

Bailey, C., H. Segner, A. Casanova-Nakayama, and T. Wahli. 2017a. Who needs the hotspot? The effect of temperature on the fish host immune response to *Tetracapsuloides bryosalmonae* the causative agent of proliferative kidney disease. Fish & shellfish immunology 63:424-437.

Bailey, C., H. Segner, and T. Wahli. 2017b. What goes around comes around: an investigation of resistance to proliferative kidney disease in rainbow trout *Oncorhynchus mykiss* (Walbaum) following experimental re-exposure. Journal of Fish Diseases 40:1599-1612.

Bailey, C., N. Strepparava, A. Ros, T. Wahli, H. Schmidt-Posthaus, H. Segner, and C. Tafalla. 2021. It's a hard knock life for some: Heterogeneity in infection life history of salmonids influences parasite disease outcomes. Journal of Animal Ecology 90: 2573-2593.

Beraldo, P., D. Berton, R. Giavenni, and M. Galeotti. 2006. First report on proliferative kidney disease (PKD) in marble trout (*Salmo trutta marmoratus*, Cuvier 1817). Bulletin-European Association of Fish Pathologists 26:143-150.

Bettge, K., T. Wahli, H. Segner, and H. Schmidt-Posthaus. 2009a. Proliferative kidney disease in rainbow trout: time-and temperature-related renal pathology and parasite distribution. Diseases of Aquatic Organisms 83:67-76.

Bettge, K., H. Segner, R. Burki, H. Schmidt-Posthaus, and T. Wahli. 2009b. Proliferative kidney disease (PKD) of rainbow trout: temperature-and time-related changes of *Tetracapsuloides bryosalmonae* DNA in the kidney. Parasitology 136:615-625.

Binderheim-Bankay, E., Jakob, A., and P. Liechti. 2000. NADUF Messresultate 1977-1998. Nationales Programm für die analytische Daueruntersuchung der schweizerischen Fliessgewässer (in German). Schriftenreihe Umwelt: Vol. 319. BUWAL, BWG, Eawag, Bern.

Blanar, C. A., K. R. Munkittrick, J. Houlahan, D. L. MacLatchy, and D. J. Marcogliese. 2009. Pollution and parasitism in aquatic animals: a meta-analysis of effect size. Aquatic Toxicology 93:18-28.

Borgwardt, F., G. Unfer, S. Auer, K. Waldner, M. El-Matbouli, and T. Bechter. 2020. Direct and indirect climate change impacts on brown trout in central Europe: how thermal regimes reinforce

physiological stress and support the emergence of diseases. Frontiers in Environmental Science 8:59.

Borsuk, M. E., P. Reichert, A. Peter, E. Schager, and P. Burkhardt-Holm. 2006. Assessing the decline of brown trout (*Salmo trutta*) in Swiss rivers using a Bayesian probability network. Ecological Modelling 192:224-244.

Braden, L., G. Prosperi-Porta, E. Kim, and S. Jones. 2010. *Tetracapsuloides bryosalmonae* in spawning pink salmon, *Oncorhynchus gorbuscha* (Walbaum), in the Quinsam River, British Columbia, Canada. Journal of Fish Diseases 33:617-621.

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.

Burkhardt-Holm, P. 2008. Decline of brown trout (*Salmo trutta*) in Switzerland–How to assess potential causes in a multi-factorial cause–effect relationship. Marine Environmental Research 66:181-182.

Burkhardt-Holm, P., A. Peter, and H. Segner. 2002. Decline of fish catch in Switzerland. Aquatic sciences 64:36-54.

Burkhardt-Holm, P., and K. Scheurer. 2007. Application of the weight-of-evidence approach to assess the decline of brown trout (*Salmo trutta*) in Swiss rivers. Aquatic sciences 69:51-70.

Burkhardt-Holm, P., W. Giger, H. Guttinger, U. Ochsenbein, A. Peter, K. Scheurer, H. Segner, E. Staub, and M. J.-F. Suter. 2005. Where have all the fish gone? ACS Publications.

Caissie, D. 2006. The thermal regime of rivers: a review. Freshwater Biology 51:1389-1406.

Canning, E. U., S. Tops, A. Curry, T. S. Wood, and B. Okamura. 2002. Ecology, development and pathogenicity of *Buddenbrockia plumatellae* Schröder, 1910 (Myxozoa, Malacosporea)(syn. *Tetracapsula bryozoides*) and establishment of Tetracapsuloides n. gen. for *Tetracapsula bryosalmonae*. Journal of Eukaryotic Microbiology 49:280-295.

Canning, E., A. Curry, S. 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.

Carraro, L., E. Bertuzzo, L. Mari, I. Fontes, H. Hartikainen, N. Strepparava, H. Schmidt-Posthaus, T. Wahli, J. Jokela, and M. Gatto. 2017. Integrated field, laboratory, and theoretical study of PKD spread in a Swiss prealpine river. Proceedings of the National Academy of Sciences 114:11992-11997.

Carraro, L., L. Mari, M. Gatto, A. Rinaldo, and E. Bertuzzo. 2018. Spread of proliferative kidney disease in fish along stream networks: A spatial metacommunity framework. Freshwater Biology 63:114-127.

Cattanéo, F., N. Lamouroux, P. Breil, and H. Capra. 2002. The influence of hydrological and biotic processes on brown trout (*Salmo trutta*) population dynamics. Canadian Journal of Fisheries and Aquatic Sciences 59:12-22.

CH2011 (2011), Swiss Climate Change Scenarios CH2011, published by C2SM, MeteoSwiss, ETH, NCCR Climate, and OcCC, Zurich, Switzerland.

Clifton-Hadley, R., D. Bucke, and R. Richards. 1987. A study of the sequential clinical and pathological changes during proliferative kidney disease in rainbow trout, *Salmo gairdneri* Richardson. Journal of Fish Diseases 10:335-352.

Comte, L., and G. Grenouillet. 2015. Distribution shifts of freshwater fish under a variable climate: comparing climatic, bioclimatic and biotic velocities. Diversity and Distributions 21:1014-1026.

Comte, L., L. Buisson, M. Daufresne, and G. Grenouillet. 2013. Climate-induced changes in the distribution of freshwater fish: observed and predicted trends. Freshwater Biology 58:625-639.

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.

Daufresne, M., and P. Boet. 2007. Climate change impacts on structure and diversity of fish communities in rivers. Global Change Biology 13:2467-2478.

Dixon, M., J. Loh, N. Davidson, C. Beltrame, R. Freeman, and M. Walpole. 2016. Tracking global change in ecosystem area: The Wetland Extent Trends index. Biological Conservation 193:27-35.

Döll, P., and H. M. Schmied. 2012. How is the impact of climate change on river flow regimes related to the impact on mean annual runoff? A global-scale analysis. Environmental Research Letters 7:014037.

D'silva, J., M. Mulcahy, and P. De Kinkelin. 1984. Experimental transmission of proliferative kidney disease in rainbow trout, *Salmo gairdneri* Richardson. Journal of Fish Diseases 7:235-239.

Dudgeon, D., A. H. Arthington, M. O. Gessner, Z.-I. Kawabata, D. J. Knowler, C. Lévêque, R. J. Naiman, A.-H. Prieur-Richard, D. Soto, and M. L. Stiassny. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biological reviews 81:163-182.

Elliott, J. 1984. Numerical changes and population regulation in young migratory trout *Salmo trutta* in a Lake District stream, 1966-83. The Journal of Animal Ecology:327-350.

Elliott, J., and J. Elliott. 2010. Temperature requirements of Atlantic salmon *Salmo salar*, brown trout *Salmo trutta* and Arctic charr *Salvelinus alpinus*: predicting the effects of climate change. Journal of fish biology 77:1793-1817.

Elliott, J., and M. A. Hurley. 2001. Modelling growth of brown trout, *Salmo trutta*, in terms of weight and energy units. Freshwater Biology 46:679-692.

El-Matbouli, M., and R. Hoffmann. 2002. Influence of water quality on the outbreak of proliferative kidney disease–field studies and exposure experiments. Journal of Fish Diseases 25:459-467.

Federal fisheries act n°923.0 of 21st June 1991

Federal Office for the Environment. 2012. Effects of climate change on water resources and watercourses. Synthesis report on the « Climate Change and Hydrology in Switzerland » (CCHydro) project. Federal Office for the Environment, Bern.

Federal Office for the Environment. 2017. Wastewater treatment in Switzerland, a great success. https://www.bafu.admin.ch/bafu/fr/home/themes/eaux/dossiers/internationaler-tag-des-wassers-2017.html

Federal Office for the Environment. 2020. Adaptation to climate change in Switzerland, 2020-2025 Action plan (in French). Bern.

Feist SW, Longshaw M. Phylum myxozoa. In: Woo PTK, editor. Fish Diseases and Disorders. Protozoan and Metazoan Infections, Vol. 1, 2nd ed. Oxfordshire: CAB International (2006). p. 230–96.

Feist, S. W., M. Longshaw, E. U. Canning, and B. Okamura. 2001. Induction of proliferative kidney disease (PKD) in rainbow trout *Oncorhynchus mykiss* via the bryozoan *Fredericella sultana* infected with *Tetracapsula bryosalmonae*. Diseases of Aquatic Organisms 45:61-68.

Feist, S., E. Peeler, R. Gardiner, E. Smith, and M. Longshaw. 2002. Proliferative kidney disease and renal myxosporidiosis in juvenile salmonids from rivers in England and Wales. Journal of Fish Diseases 25:451-458.

Ferguson, H., and H. Ball. 1979. Epidemiological aspects of proliferative kidney disease amongst rainbow trout *Salmo gairdneri* Richardson in Northern Ireland. Journal of Fish Diseases 2:219-225.

FIBER. 2014. Trout in Switzerland, diversity, biology and breeding (in French).

Filipe, A. F., D. Markovic, F. Pletterbauer, C. Tisseuil, A. De Wever, S. Schmutz, N. Bonada, and J. Freyhof. 2013. Forecasting fish distribution along stream networks: brown trout (*Salmo trutta*) in Europe. Diversity and Distributions 19:1059-1071.

Fischer, A., K. Strassmann, M. Croci-Maspoli, A. Hama, R. Knutti, S. Kotlarski, C. Schär, C. S. Poberaj, N. Ban, and M. Bavay. 2022. Climate Scenarios for Switzerland CH2018–Approach and Implications. Climate services 26:100288.

Fischnetz 2007 Fischnetz. 2007. Final Report of Project Fischnetz (in French). Bern: Eawag Dübendorf and BUWAL.

Fontes, I., H. Hartikainen, N. G. Taylor, and B. Okamura. 2017. Conditional persistence and tolerance characterize endoparasite–colonial host interactions. Parasitology 144:1052-1063.

Foott, J., and R. Hedrick. 1987. Seasonal occurrence of the infectious stage of proliferative kidney disease (PKD) and resistance of rainbow trout, *Salmo gairdneri* Richardson, to reinfection. Journal of fish biology 30:477-483.

Garner, G., I. A. Malcolm, J. P. Sadler, and D. M. Hannah. 2017. The role of riparian vegetation density, channel orientation and water velocity in determining river temperature dynamics. Journal of hydrology 553:471-485.

Gay, M., B. Okamura, and P. De Kinkelin. 2001. Evidence that infectious stages of *Tetracapsula bryosalmonae* for rainbow trout *Oncorhynchus mykiss* are present throughout the year. Diseases of Aquatic Organisms 46:31-40.

Glassmeyer, S. T., D. W. Koplin, E. T. Furlong, and M. Focazio. 2008. Environmental presence and persistence of pharmaceuticals: an overview. CRC Press, Boca Raton.

Gorgoglione, B., C. Bailey, and J. A. Ferguson. 2020. Proliferative kidney disease in Alaskan salmonids with evidence that pathogenic myxozoans may be emerging north. International Journal for Parasitology 50:797-807.

Grabner, D. S., and M. El-Matbouli. 2008. Transmission of *Tetracapsuloides bryosalmonae* (Myxozoa: Malacosporea) to *Fredericella sultana* (Bryozoa: Phylactolaemata) by various fish species. Diseases of Aquatic Organisms 79:133-139.

Grabner, D., and M. El-Matbouli. 2010. *Tetracapsuloides bryosalmonae* (Myxozoa: Malacosporea) portal of entry into the fish host. Diseases of Aquatic Organisms 90:197-206.

Graham, D., C. Staples, C. Wilson, H. Jewhurst, K. Cherry, A. Gordon, and H. Rowley. 2007. Biophysical properties of salmonid alphaviruses: influence of temperature and pH on virus survival. Journal of Fish Diseases 30:533-543.

Hari, R. E., D. M. Livingstone, R. Siber, P. BURKHARDT-HOLM, and H. Guettinger. 2006. Consequences of climatic change for water temperature and brown trout populations in Alpine rivers and streams. Global Change Biology 12:10-26.

Hartikainen, H., and B. Okamura. 2012. Castrating parasites and colonial hosts. Parasitology 139:547-556.

Hedrick, R., D. Baxa, P. De Kinkelin, and B. Okamura. 2004. Malacosporean-like spores in urine of rainbow trout react with antibody and DNA probes to *Tetracapsuloides bryosalmonae*. Parasitology research 92:81-88.

Hedrick, R., E. MacConnell, and P. De Kinkelin. 1993. Proliferative kidney disease of salmonid fish. Annual review of Fish diseases 3:277-290.

Heino, J., R. Virkkala, and H. Toivonen. 2009. Climate change and freshwater biodiversity: detected patterns, future trends and adaptations in northern regions. Biological reviews 84:39-54.

Hutchins, P. R., A. J. Sepulveda, H. Hartikainen, K. D. Staigmiller, S. T. Opitz, R. M. Yamamoto, A. Huttinger, R. J. Cordes, T. Weiss, and L. R. Hopper. 2021. Exploration of the 2016 Yellowstone River fish kill and proliferative kidney disease in wild fish populations. Ecosphere 12:e03436.

Hutchins, P. R., A. J. Sepulveda, R. M. Martin, and L. R. Hopper. 2018. A probe-based quantitative PCR assay for detecting *Tetracapsuloides bryosalmonae* in fish tissue and environmental DNA water samples. Conservation Genetics Resources 10:317-319.

IPCC. 2021. Summary for Policymakers. In: Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Masson-Delmotte, V., P. Zhai, A. Pirani, S.L. Connors, C. Péan, S. Berger, N.

Caud, Y. Chen, L. Goldfarb, M.I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J.B.R. Matthews, T.K. Maycock, T. Waterfield, O. Yelekçi, R. Yu, and B. Zhou (eds.)]. In Press.

Isaak, D. J., M. K. Young, C. H. Luce, S. W. Hostetler, S. J. Wenger, E. E. Peterson, J. M. Ver Hoef, M. C. Groce, D. L. Horan, and D. E. Nagel. 2016. Slow climate velocities of mountain streams portend their role as refugia for cold-water biodiversity. Proceedings of the National Academy of Sciences 113:4374-4379.

Jacobson, K. C., M. R. Arkoosh, A. N. Kagley, E. R. Clemons, T. K. Collier, and E. Casillas. 2003. Cumulative effects of natural and anthropogenic stress on immune function and disease resistance in juvenile Chinook salmon. Journal of Aquatic Animal Health 15:1-12.

Jenčič, V., U. Zajc, D. Kušar, M. Ocepek, and M. Pate. 2014. A survey on *Tetracapsuloides bryosalmonae* infections in Slovene fresh waters. Journal of Fish Diseases 37:711-717.

Johnson, A. C., and J. P. Sumpter. 2014. Putting pharmaceuticals into the wider context of challenges to fish populations in rivers. Philosophical Transactions of the Royal Society B: Biological Sciences 369:20130581.

Johnson, P. T., and S. H. Paull. 2011. The ecology and emergence of diseases in fresh waters. Freshwater Biology 56:638-657.

Jonsson, B., and N. Jonsson. 2009. A review of the likely effects of climate change on anadromous Atlantic salmon *Salmo salar* and brown trout *Salmo trutta*, with particular reference to water temperature and flow. Journal of fish biology 75:2381-2447.

Karvonen, A., P. Rintamäki, J. Jokela, and E. T. Valtonen. 2010. Increasing water temperature and disease risks in aquatic systems: climate change increases the risk of some, but not all, diseases. International Journal for Parasitology 40:1483-1488.

Kent, M. L., and R. Hedrick. 1986. Development of the PKX myxosporean in rainbow trout *Salmo gairdneri*. Diseases of Aquatic Organisms 1:169-182.

Kinkelin, P. d., and B. Loriot. 2001. A water temperature regime which prevents the occurrence of proliferative kidney disease (PKD) in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Journal of Fish Diseases 24:489-493.

Kipp, R., S. Bailey, H. J. MacIsaac, and A. Ricciardi. 2010. Transoceanic ships as vectors for nonindigenous freshwater bryozoans. Diversity and Distributions 16:77-83.

Kristmundsson, Á., T. Antonsson, and F. Árnason. 2010. First record of proliferative kidney disease in Iceland. Bulletin of the European association of fish pathologists 30:35-40.

Kumar, G., A. Abd-Elfattah, M. Saleh, and M. El-Matbouli. 2013. Fate of *Tetracapsuloides bryosalmonae* (Myxozoa) after infection of brown trout *Salmo trutta* and rainbow trout *Oncorhynchus mykiss*. Diseases of Aquatic Organisms 107:9-18.

Lassalle, G., and E. Rochard. 2009. Impact of twenty-first century climate change on diadromous fish spread over Europe, North Africa and the Middle East. Global Change Biology 15:1072-1089.

Lauringson, M., M. Y. Ozerov, M.-E. Lopez, V. Wennevik, E. Niemelä, T. Y. Vorontsova, and A. Vasemägi. 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.

Lauringson, M., I. Nousiainen, S. Kahar, O. Burimski, R. Gross, T. Kaart, and A. Vasemägi. 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.

Lewisch, E., and M. El-Matbouli. 2018. A fish health survey of selected Austrian rivers: implications for stocking practices and a wide distribution of *Tetracapsuloides bryosalmonae*. Wien Tierarztl Monatsschr 105:81-91.

Longshaw, M., R. M. Le Deuff, A. Harris, and S. Feist. 2002. Development of proliferative kidney disease in rainbow trout, *Oncorhynchus mykiss* (Walbaum), following short-term exposure to Tetracapsula bryosalmonae infected bryozoans. Journal of Fish Diseases 25:443-449.

Macconnell, E., and J. E. Peterson. 1992. Proliferative kidney disease in feral cutthroat trout from a remote Montana reservoir: a first case. Journal of Aquatic Animal Health 4:182-187.

Marcogliese, D. 2008. The impact of climate change on the parasites and infectious diseases of aquatic animals. Rev Sci Tech 27:467-484.

Marcogliese, D. J. 2001. Implications of climate change for parasitism of animals in the aquatic environment. Canadian Journal of Zoology 79:1331-1352.

Marcos-López, M., P. Gale, B. Oidtmann, and E. Peeler. 2010. Assessing the impact of climate change on disease emergence in freshwater fish in the United Kingdom. Transboundary and emerging diseases 57:293-304.

McGurk, C., D. J. Morris, N. A. Auchinachie, and A. Adams. 2006. Development of *Tetracapsuloides bryosalmonae* (Myxozoa: Malacosporea) in bryozoan hosts (as examined by light microscopy) and quantitation of infective dose to rainbow trout (*Oncorhynchus mykiss*). Veterinary parasitology 135:249-257.

Mills, D. 1971. Salmon and trout: a resource, its ecology, conservation and management. Oliver and Boyd, Edinburgh.

Mo, T., and A. Jørgensen. 2017. A survey of the distribution of the PKD-parasite *Tetracapsuloides bryosalmonae* (Cnidaria: Myxozoa: Malacosporea) in salmonids in Norwegian rivers–additional information gleaned from formerly collected fish. Journal of Fish Diseases 40:621-627.

Mo, T., I. Kaada, A. Jøranlid, and T. Poppe. 2011. Occurrence of *Tetracapsuloides bryosalmonae* in the kidney of smolts of Atlantic salmon (*Salmo salar*) and sea trout (*S. trutta*). Bulletin of the European association of fish pathologists 31:151-155.

Morris, D. J., and A. Adams. 2006. Transmission of *Tetracapsuloides bryosalmonae* (Myxozoa: Malacosporea), the causative organism of salmonid proliferative kidney disease, to the freshwater bryozoan *Fredericella sultana*. Parasitology 133:701-709.

Muhlfeld, C. C., D. C. Dauwalter, V. S. D'Angelo, A. Ferguson, J. J. Giersch, D. Impson, I. Koizumi, R. Kovach, P. McGinnity, and J. Schöffmann. 2019. Global status of trout and char: Conservation challenges in the twenty-first century. Trout and char of the world:717-760.

Muñoz-Mas, R., A. Lopez-Nicolas, F. Martinez-Capel, and M. Pulido-Velazquez. 2016. Shifts in the suitable habitat available for brown trout (*Salmo trutta* L.) under short-term climate change scenarios. Science of the Total Environment 544:686-700.

Naldoni, J., E. A. Adriano, A. Hartigan, C. Sayer, and B. Okamura. 2019. Malacosporean myxozoans exploit a diversity of fish hosts. Parasitology 146:968-978.

Okamura, B., H. Hartikainen, H. Schmidt-Posthaus, and T. Wahli. 2011. Life cycle complexity, environmental change and the emerging status of salmonid proliferative kidney disease. Freshwater Biology 56:735-753.

Ordinance n°814.201 on water protection of 28th October 1998

Oredalen, T. J., M. Sæbø, and T. A. Mo. 2021. Patterns of *Tetracapsuloides bryosalmonae* infection of three salmonid species in large, deep Norwegian lakes. Journal of Fish Diseases.

Palikova, M., I. Papezikova, Z. Markova, S. Navratil, J. Mares, L. Mares, L. Vojtek, P. Hyrsl, E. Jelinkova, and H. Schmidt-Posthaus. 2017. Proliferative kidney disease in rainbow trout (*Oncorhynchus mykiss*) under intensive breeding conditions: Pathogenesis and haematological and immune parameters. Veterinary parasitology 238:5-16.

Palmer, M. A., D. P. Lettenmaier, N. L. Poff, S. L. Postel, B. Richter, and R. Warner. 2009. Climate change and river ecosystems: protection and adaptation options. Environmental management 44:1053-1068.

Papadaki, C., K. Soulis, R. Muñoz-Mas, F. Martinez-Capel, S. Zogaris, L. Ntoanidis, and E. Dimitriou. 2016. Potential impacts of climate change on flow regime and fish habitat in mountain rivers of the south-western Balkans. Science of the Total Environment 540:418-428.

Parry, E. S., S. D. Gregory, R. B. Lauridsen, and S. W. Griffiths. 2018. The effects of flow on Atlantic salmon (*Salmo salar*) redd distribution in a UK chalk stream between 1980 and 2015. Ecology of Freshwater Fish 27:128-137.

Peeler, E. J., and S. W. Feist. 2011. Human intervention in freshwater ecosystems drives disease emergence. Freshwater Biology 56:705-716.

Peeler, E., S. Feist, M. Longshaw, M. Thrush, and S. St-Hilaire. 2008. An assessment of the variation in the prevalence of renal myxosporidiosis and hepatitis in wild brown trout, *Salmo trutta* L., within and between rivers in South-West England. Journal of Fish Diseases 31:719-728.

Pojezdal, Ľ., M. Adamek, E. Syrová, D. Steinhagen, H. Minářová, I. Papežíková, V. Seidlová, S. Reschová, and M. Palíková. 2020. Health surveillance of wild brown trout (*Salmo trutta fario*) in the Czech Republic revealed a coexistence of proliferative kidney disease and Piscine Orthoreovirus-3 infection. Pathogens 9:604.

Reynolds, C., and G. S. Cumming. 2015. The role of waterbirds in the dispersal of freshwater cladocera and bryozoa in southern Africa. African Zoology 50:307-311.

Roni, P. 2019. Does river restoration increase fish abundance and survival or concentrate fish? The effects of project scale, location, and fish life history. Fisheries 44:7-19.

Ros, A., H. Schmidt-Posthaus, and A. Brinker. 2022. Mitigating human impacts including climate change on proliferative kidney disease in salmonids of running waters. Journal of Fish Diseases 45:497-521.

Ros, A., J. Baer, T. Basen, C. Chucholl, E. Schneider, R. Teschner, and A. Brinker. 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.

Santiago, J. M., C. Alonso, D. Garcia de Jalon, J. Solana-Gutiérrez, and R. Muñoz-Mas. 2020. Effects of climate change on the life stages of stream-dwelling brown trout (*Salmo trutta* Linnaeus, 1758) at the rear edge of their native distribution range. Ecohydrology 13:e2241.

Santiago, J. M., D. Garcia de Jalon, C. Alonso, J. Solana, J. Ribalaygua, J. Pórtoles, and R. Monjo. 2016. Brown trout thermal niche and climate change: Expected changes in the distribution of cold-water fish in central Spain. Ecohydrology 9:514-528.

Schager, E., A. Peter, and P. Burkhardt-Holm. 2007. Status of young-of-the-year brown trout (*Salmo trutta fario*) in Swiss streams: factors influencing YOY trout recruitment. Aquatic sciences 69:41-50.

Schmidt, H., D. Bernet, T. Wahli, W. Meier, and P. Burkhardt-Holm. 1999. Active biomonitoring with brown trout and rainbow trout in diluted sewage plant effluents. Journal of fish biology 54:585-596.

Schmidt-Posthaus, H., D. Bernet, T. Wahli, and P. Burkhardt-Holm. 2001. Morphological organ alterations and infectious diseases in brown trout *Salmo trutta* and rainbow trout *Oncorhynchus mykiss* exposed to polluted river water. Diseases of Aquatic Organisms 44:161-170.

Schmidt-Posthaus, H., E. Schneider, N. Schölzel, R. Hirschi, M. Stelzer, and A. Peter. 2021. The role of migration barriers for dispersion of Proliferative Kidney Disease—Balance between disease emergence and habitat connectivity. PloS one 16:e0247482.

Schmidt-Posthaus, H., K. Bettge, U. Forster, H. Segner, and T. Wahli. 2012. Kidney pathology and parasite intensity in rainbow trout *Oncorhynchus mykiss* surviving proliferative kidney disease: time course and influence of temperature. Diseases of Aquatic Organisms 97:207-218.

Schmidt-Posthaus, H., P. Mattmann, R. Hirschi, and E. Schneider. 2020. On the potential role of *Mergus merganser* as transport hosts for *Tetracapsuloides bryosalmonae*. Transboundary and emerging diseases 67:3056-3060.

Schmidt-Posthaus, H., P. Steiner, B. Müller, and A. Casanova-Nakayama. 2013. Complex interaction between proliferative kidney disease, water temperature and concurrent nematode infection in brown trout. Diseases of Aquatic Organisms 104:23-34.

Schmidt-Posthaus, H., R. Hirschi, and E. Schneider. 2015. Proliferative kidney disease in brown trout: infection level, pathology and mortality under field conditions. Diseases of Aquatic Organisms 114:139-146.

Seidlova, V., E. Syrova, H. Minarova, J. Zukal, V. Balaz, M. Nemcova, I. Papezikova, J. Pikula, H. Schmidt-Posthaus, and J. Mares. 2021. Comparison of diagnostic methods for *Tetracapsuloides bryosalmonae* detection in salmonid fish. Journal of Fish Diseases 44:1147-1153.

Skovgaard, A., and K. Buchmann. 2012. *Tetracapsuloides bryosalmonae* and PKD in juvenile wild salmonids in Denmark. Diseases of Aquatic Organisms 101:33-42.

Soliman, H., G. Kumar, and M. El-Matbouli. 2018. *Tetracapsuloides bryosalmonae* persists in brown trout Salmo trutta for five years post exposure. Diseases of Aquatic Organisms 127:151-156.

Sterud, E., T. Forseth, O. Ugedal, T. T. Poppe, A. Jørgensen, T. Bruheim, H.-P. Fjeldstad, and T. A. Mo. 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.

Strayer, D. L., and D. Dudgeon. 2010. Freshwater biodiversity conservation: recent progress and future challenges. Journal of the North American Benthological Society 29:344-358.

Strepparava, N., A. Ros, H. Hartikainen, H. Schmidt-Posthaus, T. Wahli, H. Segner, and C. Bailey. 2020. Effects of parasite concentrations on infection dynamics and proliferative kidney disease pathogenesis in brown trout (*Salmo trutta*). Transboundary and emerging diseases 67:2642-2652.

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.

Svavarsdóttir, F. R., M. A. Freeman, Þ. Antonsson, F. Ámason, and Á. Kristmundsson. 2021. The presence of sporogonic stages of *Tetracapsuloides bryosalmonae* in Icelandic salmonids detected using in situ hybridisation. Folia Parasitologica 68:1-8.

Tops, S., D. Baxa, T. McDowell, R. Hedrick, and B. Okamura. 2004. Evaluation of malacosporean life cycles through transmission studies. Diseases of Aquatic Organisms 60:109-121.

Tops, S., H.-L. Hartikainen, and B. Okamura. 2009. The effects of infection by *Tetracapsuloides bryosalmonae* (Myxozoa) and temperature on *Fredericella sultana* (Bryozoa). International Journal for Parasitology 39:1003-1010.

Tops, S., W. Lockwood, and B. Okamura. 2006. Temperature-driven proliferation of *Tetracapsuloides bryosalmonae* in bryozoan hosts portends salmonid declines. Diseases of Aquatic Organisms 70:227-236.

Trenberth, K. E. 2011. Changes in precipitation with climate change. Climate Research 47:123-138.

Trimmel, H., P. Weihs, D. Leidinger, H. Formayer, G. Kalny, and A. Melcher. 2018. Can riparian vegetation shade mitigate the expected rise in stream temperatures due to climate change during heat waves in a human-impacted pre-alpine river? Hydrology and earth system sciences 22:437-461.

Vasemägi, A., I. Nousiainen, A. Saura, J.-P. Vähä, J. Valjus, and A. Huusko. 2017. First record of proliferative kidney disease agent *Tetracapsuloides bryosalmonae* in wild brown trout and European grayling in Finland. Diseases of Aquatic Organisms 125:73-78.

Vidal-Martinez, V. M., D. Pech, B. Sures, S. T. Purucker, and R. Poulin. 2010. Can parasites really reveal environmental impact? Trends in parasitology 26:44-51.

Wahli, T., D. Bernet, H. Segner, and H. Schmidt-Posthaus. 2008. Role of altitude and water temperature as regulating factors for the geographical distribution of Tetracapsuloides bryosalmonae infected fishes in Switzerland. Journal of fish biology 73:2184-2197.

Wahli, T., D. Bernet, P. A. Steiner, and H. Schmidt-Posthaus. 2007. Geographic distribution of *Tetracapsuloides bryosalmonae* infected fish in Swiss rivers: an update. Aquatic sciences 69:3-10.

Wahli, T., R. Knuesel, D. Bernet, H. Segner, D. Pugovkin, P. Burkhardt-Holm, M. Escher, and H. Schmidt-Posthaus. 2002. Proliferative kidney disease in Switzerland: current state of knowledge. Journal of Fish Diseases 25:491-500.

Waldner, K., M. Borkovec, F. Borgwardt, G. Unfer, and M. El-Matbouli. 2021. Effect of water temperature on the morbidity of *Tetracapsuloides bryosalmonae* (Myxozoa) to brown trout (*Salmo trutta*) under laboratory conditions. Journal of Fish Diseases.

Waldner, K., T. Bechter, S. Auer, F. Borgwardt, M. El-Matbouli, and G. Unfer. 2020. A brown trout (*Salmo trutta*) population faces devastating consequences due to proliferative kidney disease and temperature increase: A case study from Austria. Ecology of Freshwater Fish 29:465-476.

Warren, D. R., A. G. Ernst, and B. P. Baldigo. 2009. Influence of spring floods on year-class strength of fall-and spring-spawning salmonids in Catskill mountain streams. Transactions of the American Fisheries Society 138:200-210.

Wenger, S. J., D. J. Isaak, C. H. Luce, H. M. Neville, K. D. Fausch, J. B. Dunham, D. C. Dauwalter, M. K. Young, M. M. Elsner, and B. E. Rieman. 2011. Flow regime, temperature, and biotic interactions drive differential declines of trout species under climate change. Proceedings of the National Academy of Sciences 108:14175-14180.

Wondzell, S. M., M. Diabat, and R. Haggerty. 2019. What matters most: are future stream temperatures more sensitive to changing air temperatures, discharge, or riparian vegetation? Journal of the American Water Resources Association 55:116-132.

Zaugg, B. 2018. Pisces. Atlas. Fauna Helvetica 7. Info fauna CSCF, Neuchâtel.

Zimmerli, S., D. Bernet, P. Burkhardt-Holm, H. Schmidt-Posthaus, P. Vonlanthen, T. Wahli, and H. Segner. 2007. Assessment of fish health status in four Swiss rivers showing a decline of brown trout catches. Aquatic sciences 69:11-25.

2 Reliable field assessment of proliferative kidney disease in wild brown trout, *Salmo trutta*, populations: When is the optimal sampling period?

Aurélie Rubin, Christyn Bailey, Nicole Strepparava, Thomas Wahli, Helmut Segner, Jean-François Rubin

Paper published in *Pathogens, Volume 11, June 2022, Article 681*.

pathogens

MDPI

Article

Reliable Field Assessment of Proliferative Kidney Disease in Wild Brown Trout, *Salmo trutta*, Populations: When Is the Optimal Sampling Period?

Aurélie Rubin [1,2,3,*], Christyn Bailey [1,4], Nicole Strepparava [1], Thomas Wahli [1], Helmut Segner [1] and Jean-François Rubin [2,3]

[1] Centre for Fish and Wildlife Health, University of Bern, 3012 Bern, Switzerland; christyn.bailey@outlook.com (C.B.); nicole.strepparava@bluewin.ch (N.S.); thomas.wahli@vetsuisse.unibe.ch (T.W.); helmut.segner@vetsuisse.unibe.ch (H.S.)
[2] La Maison de la Rivière, 1131 Tolochenaz, Switzerland; jf.rubin@maisondelariviere.ch
[3] Land, Nature, Environment Institute, University of Applied Sciences and Arts Western Switzerland, 1202 Geneva, Switzerland
[4] Fish Immunology and Pathology Laboratory, Animal Health Research Centre (CISA-INIA), 28130 Madrid, Spain
* Correspondence: aurelie.rubin@maisondelariviere.ch; Tel.: +41-215-462-060

Abstract: Proliferative kidney disease (PKD), caused by the myxozoan parasite *Tetracapsuloides bryosalmonae*, is suspected to contribute to the decline of wild brown trout *Salmo trutta* populations. Different factors need to be taken into consideration for PKD outbreaks. Among them, water temperature appears as a main driver of the disease. To understand the epidemiology and impact of the disease on wild fish populations, reliable sampling approaches to detect the presence of *T. bryosalmonae*-infected fish are needed. This study aimed to characterize the seasonal variation of the prevalence of *T. bryosalmonae*-infected fish in brown trout populations in two small streams with differing temperature regimes between upstream and downstream sites. As water temperature is known to influence PKD manifestation in brown trout, we hypothesized that the number of *T. bryosalmonae*-positive fish, as well as their seasonal distribution, will vary between upper and downstream parts of the two streams. Since, in field studies, results can strongly vary across years, we extended the study over a 3-year-period. The number of infected fish and the intensity of infection were assessed by histology. The results confirmed the hypothesis of pronounced temporal- and site-related differences in the percentage of PKD-positive fish and the intensity of the infection. Comparison of water temperatures (total degree days as well as the number of days with a daily mean temperature ≥15 °C) with PKD data indicated that temperature was the driving factor for the temporal development and the intensity of the infection. A mean of 1500 degree days or 30 days with a daily mean temperature ≥15 °C was required before the infection could be detected histologically. From our findings, recommendations are derived for a water temperature-driven sampling strategy campaigns that enables the detection of PKD infection and prevalence in wild brown trout populations.

Keywords: proliferative kidney disease; *Salmo trutta*; *Tetracapsuloides bryosalmonae*; sampling time point; water temperature; degree days

Citation: Rubin, A.; Bailey, C.; Strepparava, N.; Wahli, T.; Segner, H.; Rubin, J.-F. Reliable Field Assessment of Proliferative Kidney Disease in Wild Brown Trout, *Salmo trutta*, Populations: When Is the Optimal Sampling Period? *Pathogens* **2022**, *11*, 681. https://doi.org/10.3390/pathogens11060681

Academic Editor: Pantelis Katharios

Received: 10 May 2022
Accepted: 10 June 2022
Published: 14 June 2022

1. Introduction

Brown trout (*Salmo trutta*, L.) populations are declining in Switzerland [1–4]. A combination of different factors might explain this diminution (e.g., reproductive failure, pollutions, inadequate fish management, increase of water temperature, genetic admixture caused by illegal translocations, etc.) [1–5]. Among them, proliferative kidney disease (PKD) is also involved in this decrease [6–8]. The infection is caused by the myxozoan

parasite *Tetracapsuloides bryosalmonae* [9]. The parasite possesses a heteroxenus life cycle, with salmonid fish species as the vertebrate host and freshwater bryozoans as the invertebrate host [10–12]. In bryozoans, covert and overt infection stages can occur. During a covert infection, the parasite is under its non-virulent single cell form, associated with the body wall of the bryozoan [11]. This stage can be found throughout the year [13]. If the necessary conditions are met (food resources and temperature), overt infection can occur [14]. Multicellular sacs of *T. bryosalmonae* spores develop in bryozoans and are then undulatingly released into the water, with major peaks in spring and summer [11,12,15]. If the parasite come into contact with a susceptible salmonid (e.g., brown trout; marble trout, *Salmo marmoratus*; grayling, *Thymallus thymallus*; or rainbow trout *Oncorhynchus mykiss*), it infects the fish through skin and gills [16–18]. In the fish host, the parasite targets the kidney and can induce pronounced renal pathology, and mortalities. The disease severity is strongly influenced by environmental conditions, in particular, temperature [19–22]. The fact that water temperature affects the development and intensity of PKD in salmonids has been shown for rainbow trout, e.g., [19,20,23–28], and brown trout, e.g., [8,27,29–32]. Laboratory studies using constant temperature regimes provided evidence that water temperatures $\geq$15 °C are associated with a strong increase in disease severity and disease-related mortalities, e.g., [17,19,20,27,33,34]. Indeed, on this host–parasite system, water temperature could affect the fish, the bryozoans, or the parasite itself. Temperature impacts the kinetic of *T. bryosalmonae* proliferation in the kidney, the lesions severity and their distribution in the renal tissue [19,20], and the strategy of the fish immune response [26], as well as the rate of spore production and transmission [33]. In the invertebrate host, temperature also induces, speeds up, and prolongs the production of infective spores and enhances bryozoan growth [12].

The question is how such laboratory findings can be transferred to the field situation, which is characterized by daily and seasonal temperature fluctuations. Moreover, under field conditions, additional stressors, such as limited food supply or poor water quality, may influence the response of the fish to the parasite [27,31,35].

Given the fact that PKD can impact wild salmonid stocks, the availability of robust sampling approaches to reliably assess the PKD status of fish populations is essential. Successful field diagnosis depends mainly on three parameters: (1) appropriate sample size that avoids or minimizes false negatives, (2) reliable detection methods, and (3) sampling time point. In this study, we focused on the last factor. Indeed, the time point for field sampling campaigns should be well planned. Given the temperature dependence of PKD manifestation, we hypothesize that early or late in the year, when field water temperatures are low, only a few individuals may be infected and/or display clear signs of infection, and, therefore, field sites where PKD is present may be falsely classified to be PKD-negative. On the other hand, when water temperatures are high, i.e., during the summer season, infected fish may have already been eliminated from the population due to PKD-induced mortality, which again would result in an underestimation of the true PKD presence in the rivers. Therefore, the present study aimed to follow the manifestation of PKD in wild brown trout populations concerning the seasonal variations in water temperature. The expected outcome of this study is to identify the suitable period for reliable PKD diagnosis which is based on the actual water temperature regime at a field site. To this end, we investigated two streams that host PKD-positive brown trout populations (Rubin and Wahli, unpublished data), and which show different temperature regimes along the river course. The water temperatures were continuously recorded utilizing a data logger. Young-of-the-year (YOY) brown trout were selected for sampling, as this life stage appears to be most susceptible to the parasite [16,29,36,37]. A sampling period of 3 consecutive years was selected as this is necessary to account for the inter-annual variability [38]. For PKD diagnosis, histology was employed, as the technique is suited for practical field sampling, and it enables to estimation of both disease prevalence and infection intensity.

2. Results

The results of the histology-based detection of *T. bryosalmonae*-infected fish (expressed as the percentage of *T. bryosalmonae*-infected fish and the infection intensity per site and time point) and water temperature values (expressed as the degree days (dd) and the number of days with a daily mean temperature ≥15 °C (ndays15)) are summarized in Table 1.

Table 1. Percentage of *T. bryosalmonae*-infected fish based on histological examination and temperature results for the Boiron de Morges (BM1 and BM2) and the Venoge (V1 and V2). Upstream sites = BM1 and V1, downstream sites = BM2 and V2. Grey-shaded boxes correspond to sites in which *T. bryosalmonae*-infected fish were detected based on histological examination.

Station	Year	Sampling Date	Number of Sampled Fish	% of *T. bryosalmonae*-Infected Fish	Infection Severity	Degree Days	Number of Days with a Daily Mean Temperature ≥ 15 °C
BM1	1	5 June	25	0	0.0	737	0
		8 July	25	0	0.0	1144	1
		22 August	25	0	0.0	1814	18
		12 September	25	0	0.0	2099	20
		8 November	31	0	0.0	2777	20
	2	7 July	2	0	0.0	1391	11
		8 September	25	0	0.0	2316	35
	3	14 July	4	0	0.0	1499	15
		31 August	14	7 *	1.0 *	2271 *	49 *
BM2	1	5 June	25	0	0.0	749	0
		8 July	25	0	0.0	1188	5
		22 August	25	68	3.9	1931	49
		12 September	25	68	3.2	2240	57
		8 November	26	31	1.5	2950	57
	2	7 July	18	0	0.0	1417	28
		8 September	25	88	4.6	2444	88
	3	14 July	25	68	3.3	1627	34
		31 August	25	88	3.5	2488	82
V1	1	19 June	25	0	0.0	834	0
		12 July	25	0	0.0	1047	0
		20 August	25	0	0.0	1465	0
		2 October	25	0	0.0	1892	0
		29 November	26	0	0.0	2339	0
	2	1 September	26	0	0.0	1730	0
V2	1	19 June	25	0	0.0	1056	3
		12 July	26	0	0.0	1397	13
		20 August	25	40	2.9	2064	50
		2 October	25	44	1.8	2661	63
		29 November	25	44	1.3	3278	63
	2	1 September	24	38	2.8	2366	59

* Only one infected fish (infection intensity = 1.0) was found for the first time in BM1. Therefore, it was categorized as a migratory animal coming from an infected downstream zone and was not considered in the discussion.

In the first study year, no PKD-positive fish were found in the upstream sites of the Boiron (BM1) and the Venoge (V1). This was true for all five sampling dates from June to November. For the downstream sites of the Boiron (BM2) and the Venoge (V2), infected fish were not observed in June and July. PKD-positive fish were detected for the first time in August, with 68% positive fish at BM2 and 40% at V2. The infection intensity had a mean value of 3.9 at BM2 and of 2.9 at V2. In September, the values were comparable to August, with 68% *T. bryosalmonae*-infected fish and an infection intensity of 3.2 at BM2. At the V2 site, the next sampling after August was conducted in October, yielding 44% positive fish and an infection intensity of 1.8. In November, 31% of sampled fish were assessed as *T. bryosalmonae*-positive for BM2 and 44% for V2. While the percentage of positive fish did not decline from August to November, a decrease was evident in the infection intensity, suggesting a declining number of parasites per fish.

The results of the second study year were comparable to those of year 1. Again, no *T. bryosalmonae*-infected fishes were detected at the upstream sites, BM1 and V1. In the downstream sites, positive fish were not detected in July, but from August onwards. The percentage of infected fish reached 88% in September for BM2 and 38% for V2. The corresponding intensity values were 4.6 in BM2 and 2.8 in V2.

In the third sampling year, at BM2, a high percentage of positive fishes (68%), with a high infection intensity (3.3), were already detected in July, whereas V2 was still PKD-

negative in July. In August, the populations at both downstream sites contained infected fishes. Moreover, in August, for the first time, a *T. bryosalmonae*-positive fish was detected at the upstream BM1-site. However, since this was the only isolated observation of a PKD-positive fish at this site during the whole study period, we assume that this was a migratory fish coming from a downstream location.

The samplings showed a clear seasonal pattern of the PKD-infected fish percentage, which raises the question of the potential driving factors. One such parameter might be the water temperature. To examine the possible relation between water temperature and PKD, we continuously monitored water temperatures at the study sites and expressed the data as (a) degree-days (dd), i.e., the sum of daily temperature values, and (b) the number of days with a mean water temperature ≥15 °C (ndays15). The cut-off value of ≥15 °C was selected since we know from previous studies that PKD-induced pathologies and mortalities are strongly intensified at temperatures above 15 °C e.g., [19,20,34].

During the three study years, the water temperature regime showed a distinct seasonal pattern (Table 1). In the two streams, the dd values were higher at the downstream sites compared to the upstream sites. Differences between sites and streams were also evident for the number of days with mean water temperatures of ≥15 °C. At BM1, water temperature reached mean values of ≥15 °C at 20–49 days, whereas at BM2 this was the case for 57–88 days. For instance, in the first study year, brown trout at BM1 experienced 20 days of mean water temperatures of ≥15 °C (maximum of 9 consecutive days with mean water temperature ≥15 °C), but brown trout at the downstream site BM2 were exposed to such temperatures for 57 days (maximum of 25 consecutive days with mean water temperature ≥15 °C). In the third study year, the summer was very hot and dry, resulting in low water levels and particularly high-water temperatures (71 consecutive days with mean water temperature ≥15 °C obtained at BM2). The Venoge generally had fewer days with elevated water temperature. At the V1 upstream site, no single day with a mean water temperature of ≥15 °C was recorded. At the downstream site V2, the respective values were 63 and 59 ndays15 for years 1 and 2.

The relationship between PKD values (percentage of *T. bryosalmonae*-infected fish and infection intensity) and temperature data (dd and ndays15) for the two streams was assessed using Pearson's correlation. A positive linear correlation was found between the percentage of *T. bryosalmonae*-infected fish and dd ($r = 0.476$). For ndays15, the correlation was even stronger, with a Pearson's coefficient value of 0.857. Furthermore, infection intensity and dd were positively correlated, although the coefficient was relatively low ($r = 0.245$). On the contrary, the correlation was stronger with ndays15 ($r = 0.556$). In addition, when combining data from all sites, Student's *t*-test showed that the dd and ndays15 mean was significantly higher for *T. bryosalmonae* positive fish (respectively 2328 dd and 62.6 ndays15) than for PKD-free trout (respectively 1762 dd and 19.3 ndays15) (Figure 1), suggesting an influence of water temperature on the infection.

When combining the data of all three years in BM2 and V2, sites were assessed as PKD-negative with maximum temperature values of, respectively, 1417 dd or 28 ndays15 at BM2, and 1397 dd or 13 ndays15 at V2. When these values were surpassed, PKD-positive fish could be detected by histological diagnosis (Figure 2).

This suggests that the threshold for histology-based detection of PKD in wild brown trout populations appears to be between 1400 and 1600 dd or 28 to 34 ndays15. Hence, 1500 dd and 30 ndays15 were assessed as a mean.

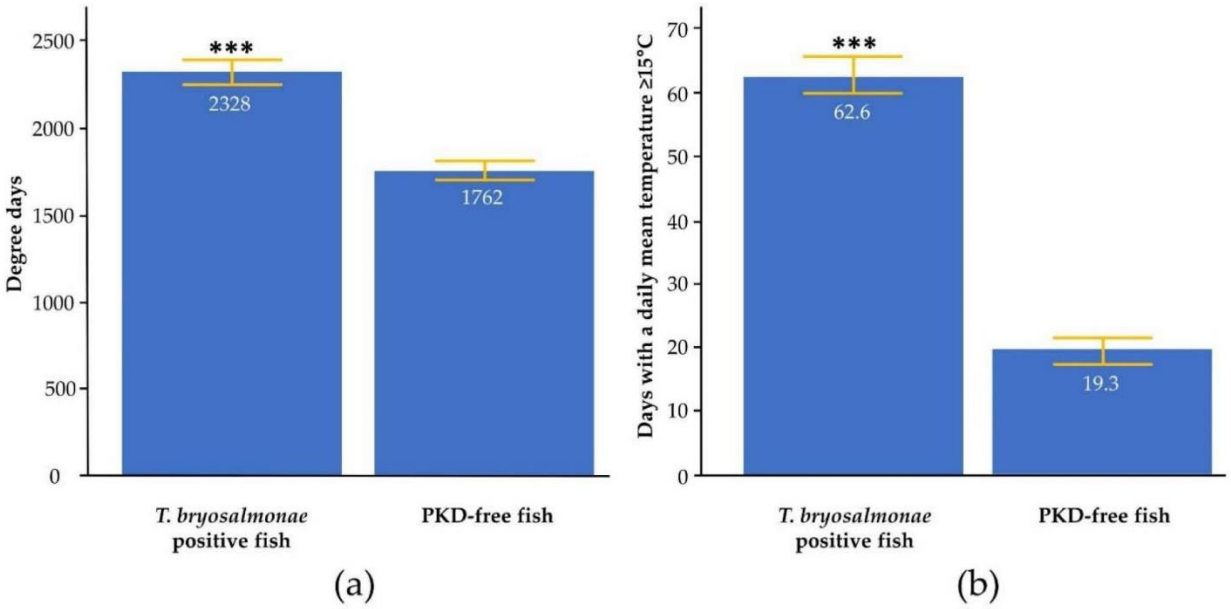


Figure 1. Mean degree days (**a**) and the number of days with a daily mean temperature ≥15 °C (**b**) between *T. bryosalmonae*-infected and PKD-free fish from all sites. White scores indicate means results, yellow lines indicate the standard error, and asterisks indicate levels of significance (*t*-test), *** $p < 0.001$.

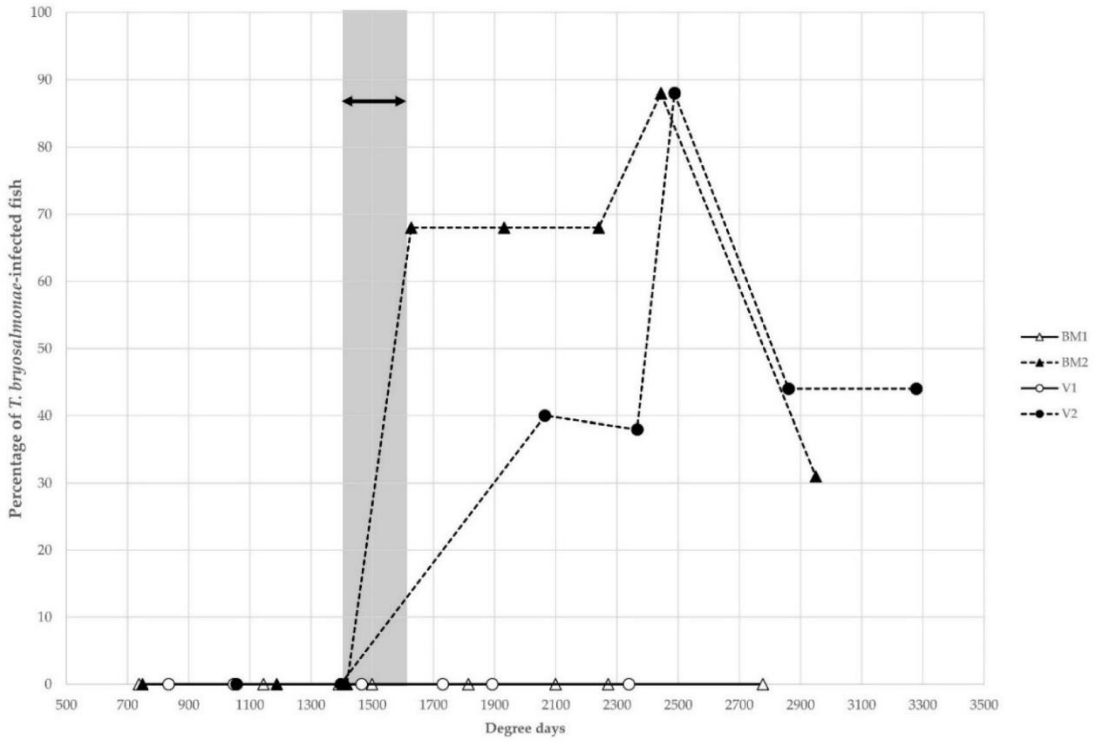


Figure 2. Long-term water temperature measurements expressed as degree days linked with the percentage of *T. bryosalmonae*-infected fish values in the Boiron de Morges (BM) and the Venoge (V) (BM1 and V1 = upstream sites; BM2 and V2 = downstream sites). The grey zone corresponds to the critical degree days threshold between PKD-free and *T. bryosalmonae*-infected fish.

3. Discussion

When planning effective fieldwork campaigns, several questions need to be addressed; in particular, the number of samples [8], choice of diagnostic methods [19,20,37], and

selection of sampling periods [28–31]. In this study, we investigated to what extent the sampling season influences the detectability of the presence of PKD infections in wild brown trout populations by means of histology. The higher the percentage of infected fish is in a population, the higher is the likelihood to detect the infection, particularly when only small sample sizes are possible [8]. Wahli et al. [8] showed that a sample of 20 fish is adequate for the detection of PKD when the prevalence in the examined population is equal or higher than 10%. To accurately measure prevalence below 10%, substantially higher sample sizes would be needed. However, if it comes to small streams carrying relatively small brown trout populations, such high sample sizes cannot be justified from ecological and ethical reasons. Therefore, we decided that the accuracy that is achievable with a sample size of 25 fish is sufficient for the purpose of our study.

Typical methods for PKD diagnosis comprise histology and qPCR [19,20,37]. Differences between the microscopic technique (histology) and the molecular technique (qPCR) were investigated in previous papers [19,20,37]. In these studies, qPCR was shown to detect parasite material for a longer period in the kidneys of PKD infected rainbow trout in contrast to microscopic technique. When using histology as a diagnostic method, the likelihood to detect parasites on a tissue section increases with the intensity of the infection. However, in fish with very low infection levels and few parasites present in the kidney, histological examination may have missed the parasites, particularly, as only one slide was analyzed. In contrast, qPCR methodology relying on whole tissue extraction, is less dependent on parasite quantity and on heterogeneous distribution of the parasites within the target organ. Nevertheless, use of histology provides some advantages, as an easier field procedure for the fixation of sampled fish. Moreover, this method is the only one able to assess the severity of the pathological response and the state of the disease, as presence of fibrous tissue revealing begin of recovery and was thus used here.

Since the water temperature regime is the main driver of PKD manifestation in brown trout, we hypothesized that the number of infected fish in a population, as well as the infection intensity, will co-vary with the seasonal variation of water temperature at a given field study site. The results of our study are in agreement with this hypothesis.

Studies conducted in the laboratory with constant temperatures showed that *T. bryosalmonae* infection in brown trout has a pattern of temporal variation. For example, in the study of Strepparava et al. [33], a sharp increase in parasites' number was detected after exposure, followed by a plateau phase, and, finally, a slow decrease of parasites was observed. In lab studies, however, initial exposure to parasites is synchronous and further infection is not possible as the parasite is not transmitted from fish to fish [13,18,39]. In the wild the situation is different: diurnal and seasonal water temperature regimes are fluctuating, long time exposure to new parasites could appear, co-stressors might be present, etc. Despite evidence that this seasonal distribution of PKD cases may depend on temperature [8,21,29–32,40], it has not yet been fully elucidated whether this course of PKD in the field is directly linked to the course of the water temperature over the year.

Our results showed that water temperature is the main factor for PKD infection. Differing temperature regimes appear between upstream and downstream sites in the two rivers, as well as water quality as observed in Rubin et al. [31]. PKD-positive fish were detected in the two downstream sites from the Boiron and the Venoge, as regularly previously observed (Rubin and Wahli, unpublished data). A positive correlation between PKD values (percentage of *T. bryosalmonae*-infected fish and infection intensity) and temperature data (dd and ndays15) was observed. In early summer, no parasites were detected in the evaluated fish, while the highest percentage of *T. bryosalmonae*-infected fish were detected in the period from late August to the beginning of September for years 1 and 2. Thus, samplings taken too early in the season, (e.g., BM2 05 June and 07 August samplings of year 1) could miss the infection. At these dates, either infection could not have been taken place yet or not have developed enough to be detected by histology. However, later in the season, when 1500 dd were surpassed, *T. bryosalmonae*-infected fish were detected. Thus, sites might be wrongly classified as PKD negatives if the sampling period is not adequately

chosen. On the contrary, too late in the season, as in the November samples, a large number of animals might have died because of the infection, or parasites might have already been excreted or eliminated by the fish immune system [11,19–21,41], resulting in low parasite number. In addition, the correct timepoint for fish sampling is not linked to a month, but rather to the course of water temperature, as it happens in the July BM2 sampling of year 3. Indeed, PKD-positive fish were already observed at this date, as a result of the extraordinary temperature conditions in this particular year (1627 dd). Hence, a very hot summer may lead to a shift in the period for PKD sampling. With rising temperature due to global warming [42], this phenomenon may become more and more frequent.

In the wild, parasite infection and PKD-related mortalities appear throughout the year and seem to be more noticeable in summer and the beginning of autumn [30,31], which corresponds to our observations. Wahli et al. [29] identified PKD-positive fish between June to November, with major peaks in August and September. Dash and Vasemägi [43] detected the highest prevalence in August. Palikova et al. [28] found that the highest parasite loads were observed in September in rainbow trout raised on fish farms, but under natural water temperature conditions. These authors also observed that only sporadically single *T. bryosalmonae* parasites were present in fish kidneys sampled in November and December, indicating a significant decline of the parasite presence during the winter months, which corresponds to our observations. In other studies, PKD-positive fish were observed in streams where the water temperature exceeded 15 °C for 31 days [40], 39 days [22], and, respectively, 48, 83, and 80 days along the three investigation years [44]. Thus, these studies support our results, even if we obtained a smaller ndays15 (mean of 30 days) for the detection of *T. bryosalmonae* positive fish. The Swiss Fischnetz project [45] predicted 14 to 28 days at 15 °C for PKD infection. In a previous YOY sampling campaign encompassing 45 sites, Rubin et al. [31] observed that PKD prevalence ranging from 4 to 100% were always found in sites with a minimum of 1900 dd.

4. Materials and Methods

4.1. Fish Sampling

Sampling campaigns were carried out from 2013 to 2015 (2013 = year 1; 2014 = year 2; 2015 = year 3). Fieldwork was performed at the Boiron de Morges (BM) and the Venoge (V), two streams of the Canton of Vaud (Switzerland) (Figure 3). Two sites were selected in BM (upstream BM1 46.49812° N 6.43836° E, downstream BM2 46.49567° N 6.47410° E), and two sites in V (upstream V1 46.62722° N 6.42770° E, downstream V2 46.55494° N 6.53233° E). Five electrofishing campaigns were carried out between June and November of year 1. BM sites were also sampled in July and September of year 2 and in July and August of year 3. V sites were tested in September of year 2 as well. Whenever possible, 25 fish were caught. These individuals were selected based on their total length in order to correspond to the average size class for young of the year brown trout from typical Swiss midland streams (<100 mm) (Rubin, unpublished data).

4.2. Histological Analysis

After capture, YOY were euthanized with MS222® (3-aminobenzoic acid ethyl ester, 300 mg l-1, Argent Chemical Laboratories, Redmont, WA, USA), fixed, and stored in containers containing 4% buffered formalin. Kidneys were removed from the carcasses in the laboratory, embedded in paraffin, and cut following routine histological methods. Histological slides were stained with haematoxylin and eosin. One section of a full-length kidney per animal was analyzed for the presence of *T. bryosalmonae*, determined through its typical cell structure (spores with four polar capsules). The infection intensity (estimation of the numbers of observed parasites) was assessed for each kidney sample with a microscope (Olympus BX41). For this purpose, a scoring system from 0 (no parasite) to 6 (at least 10 parasites per high power field with 400× magnification) was used (Figure 4) following Bettge et al. [19] and Schmidt-Posthaus et al. [22]. A fish was classified as *T. bryosalmonae*-infected if at least one parasite was detected in the analyzed kidney section. The infection

intensity per site was obtained as the addition of infection scores divided by the number of PKD-positive fish. The percentage of *T. bryosalmonae*-infected fish was determined as the number of infected individuals divided by the total number of sampled fish at a particular site and time-point as described in Bush et al. [46].

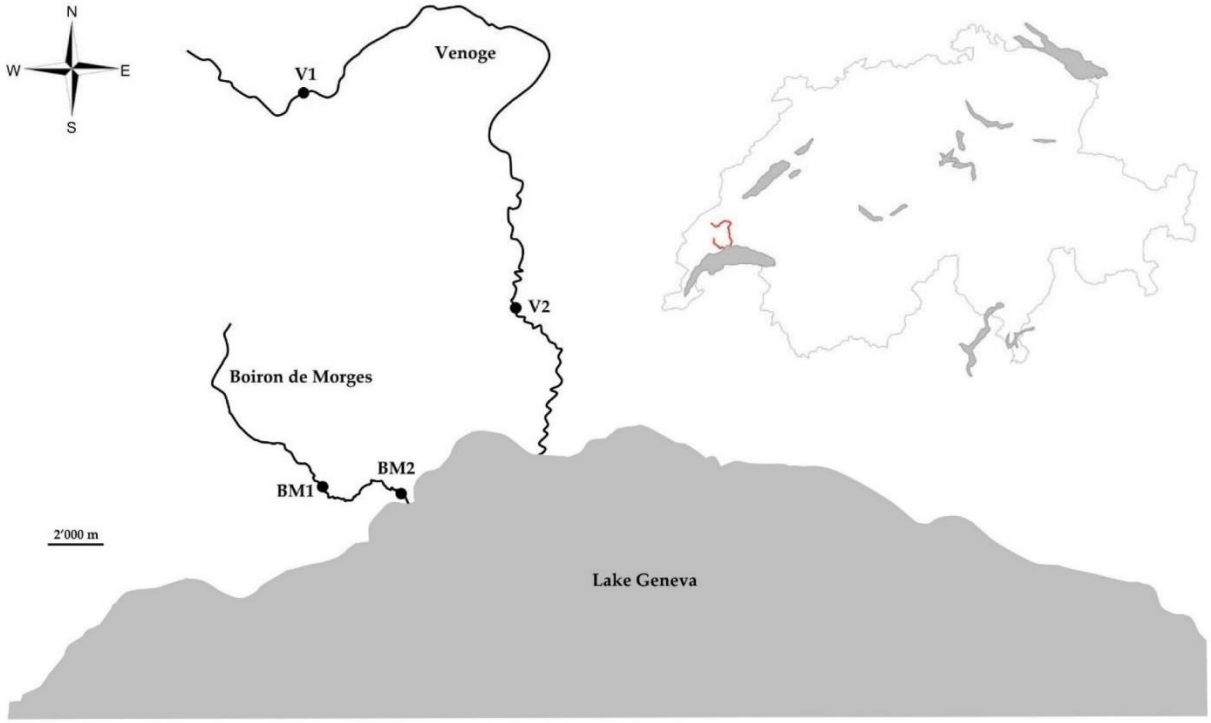


Figure 3. Location of study sites in the Boiron de Morges (BM1 and BM2) and the Venoge (V1 and V2). The two rivers are shown on the map of Switzerland (top right).

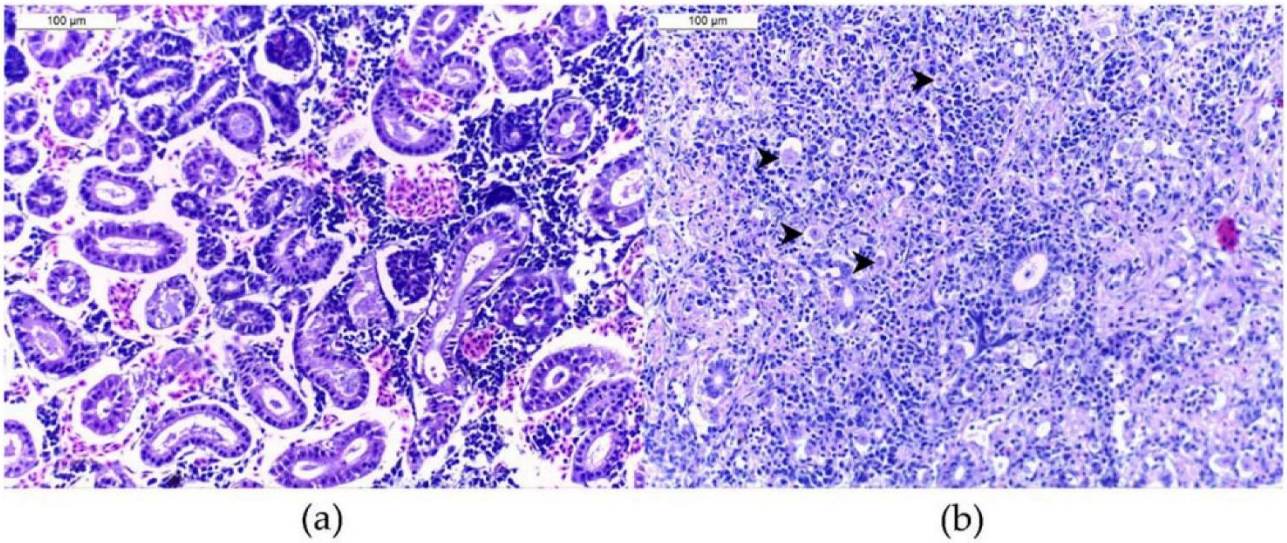


Figure 4. Histological assessment of brown trout *Salmo trutta* posterior kidney H&E-stained slides (**a**) without *T. bryosalmonae* parasite (infection score of 0), (**b**) with a severe infection (score of 6). Four parasites are pointed.

4.3. Water Temperature

Water temperature was recorded at all sites every 15 min with loggers (HOBO® Water Temp Pro v2 Data Logger, Onset, Cape Cod, MA, USA). Temperature data started on the 1st of March, as from this date on fry hatching is beginning in the Boiron (Rubin, pers. obs.).

The degree days (dd, sum of the daily mean temperature values from 1st of March to the date of fish capture) and the number of days with a daily mean temperature ≥ 15 °C (ndays15) from the 1st of March to the date of the catch were calculated from the logger data.

4.4. Statistical Analysis

A total of 697 fish were sampled and used for statistical analysis, which was performed applying the software RStudio (version 2021.09.1, RStudio, Inc, Boston, MA, USA). The correlation between PKD data (percentage of *T. bryosalmonae*-infected fish and infection intensity) and temperature values (dd and ndays15) was assessed with a univariate analysis using Pearson's coefficient. The significant differences ($p < 0.05$) in the dd and ndays15 means between all *T. bryosalmonae*-infected fish and parasite-free trout were tested with Student's *t*-test.

5. Conclusions

With the anticipated increase in water temperature as a result of ongoing global warming, far-reaching, long-lasting, and, in many cases, dramatic consequences for aquatic ecosystems are to be expected. In particular, PKD of salmonids could become an even greater issue for the survival of many wild brown trout populations. In this light, one of the key challenges facing researchers is to develop a suite of tools for detecting and assessing the impacts of climate change on infectious diseases in complex ecosystems. Therefore, research as presented here is essential to provide a baseline for field studies for a better understanding of the impact of PKD on our wild brown trout populations. For this purpose, long-term temperature parameters, such as the degree days and the number of days with a daily mean temperature $\geq$15 °C, are a useful tool to compare the influence of temperature across field sites and determine the optimal sampling period for field investigations. This study aimed to follow the temporal variation of PKD manifestation in wild brown trout populations from two rivers with different temperature regimes in order to identify the time window which is most suitable for the robust determination of the disease prevalence. Our findings indicate that water temperature is the main driver for the temporal manifestation of the infection, which, hence, has important implications for the practical design of field investigations. Careful consideration must be given to the choice of the sampling period when aiming to identify the PKD presence in a native brown trout population. Too early, the infection could not have been declared or could have been kept under the histology detection limit. Too late, infected fish could have died due to the infection or parasites could have already been excreted. Average values of ~1500 dd or 30 ndays15 are necessary for having the highest probability to detect histologically *T. bryosalmonae*-infected fish in wild brown trout populations. This threshold should, therefore, be carefully considered when planning sampling campaigns for PKD assessment in a particular stream and could be applied independent of location, level above sea, and weather conditions.

Author Contributions: Conceptualization, A.R., T.W. and J.-F.R.; methodology, A.R., T.W. and J.-F.R.; validation, A.R., C.B., N.S., T.W., H.S. and J.-F.R.; formal analysis, A.R. and N.S.; investigation, A.R. and N.S.; resources, A.R., N.S. and J.-F.R.; data curation, A.R.; writing—original draft preparation, A.R., C.B., N.S., T.W., H.S. and J.-F.R.; writing—review and editing, A.R., C.B., N.S., T.W., H.S. and J.-F.R.; visualization, A.R.; supervision, T.W., H.S. and J.-F.R.; project administration, J.-F.R.; funding acquisition, J.-F.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research was partly funded by the SWISS federal food safety and veterinary office through the pilot program "Adaptation to climate change", partly by the SWISS national science foundation Sinergia grant number CRSII3_147649_1, and partly by the general directorate of the environment of the canton of Vaud. CB was funded by SNF Post Doc Mobility Fellowship number P400PB_183824.

Institutional Review Board Statement: The animal study protocol was approved by the Institutional Review Board of the general directorate for agriculture; viticulture and veterinary affairs of the canton of Vaud (protocol code 2871 and date of approval 7 July 2014).

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors would like to thank the team of the Maison de la Rivière and the fish rangers of the Canton of Vaud for their help in the field.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Burkhardt-Holm, P.; Peter, A.; Segner, H. Decline of fish catch in Switzerland. *Aquat. Sci.* **2002**, *64*, 36–54. [CrossRef]
2. Burkhardt-Holm, P.; Giger, W.; Güttinger, H.; Ochsenbein, U.; Peter, A.; Scheurer, K.; Segner, H.; Staub, E.; Suter, M.J.-F. Where have all the fish gone? The reasons why fish catches in Swiss rivers are declining. *Environ. Sci. Technol.* **2005**, *39*, 441A–447A. [CrossRef] [PubMed]
3. Zimmerli, S.; Bernet, D.; Burkhardt-Holm, P.; Schmidt-Posthaus, H.; Vonlanthen, P.; Wahli, T.; Segner, H. Assessment of fish health status in four Swiss rivers showing a decline of brown trout catches. *Aquat. Sci.* **2007**, *69*, 11–25. [CrossRef]
4. Burkhardt-Holm, P. Decline of brown trout (*Salmo trutta*) in Switzerland—How to assess potential causes in a multi-factorial cause-effect relationship. *Mar. Environ. Res.* **2008**, *66*, 181–182. [CrossRef] [PubMed]
5. Giantsis, I.A.; Sapounidis, A.; Koutrakis, E.; Apostolidis, A.P. Assessment of Stocking Activities on the Native Brown Trout Populations from Nestos River (Southern Balkans) Inferred by mtDNA RFLP and Sequencing Analyses. *Appl. Sci.* **2021**, *11*, 9034. [CrossRef]
6. Borsuk, M.E.; Reichert, P.; Peter, A.; Schager, E.; Burkhardt-Holm, P. Assessing the decline of brown trout (*Salmo trutta*) in Swiss rivers using a Bayesian probability network. *Ecol. Model.* **2006**, *192*, 224–244. [CrossRef]
7. Schager, E.; Peter, A.; Burkhardt-Holm, P. Status of young-of-the-year brown trout (*Salmo trutta fario*) in Swiss streams: Factors influencing YOY trout recruitment. *Aquat. Sci.* **2007**, *69*, 41–50. [CrossRef]
8. Wahli, T.; Bernet, D.; Steiner, P.A.; Schmidt-Posthaus, H. Geographic distribution of *Tetracapsuloides bryosalmonae* infected fish in Swiss rivers: An update. *Aquat. Sci.* **2007**, *69*, 3–10. [CrossRef]
9. Canning, E.; Curry, A.; Feist, S.; Longshaw, M.; Okamura, B. *Tetracapsula bryosalmonae* N.SP. for PKX organism, the cause of PKD in salmonid fish. *Bull. Eur. Assoc. Fish Pathol.* **1999**, *19*, 203–206.
10. Anderson, C.L.; Canning, E.U.; Okamura, B. Molecular data implicate bryozoans as hosts for PKX (phylum Myxozoa) and identify a clade of bryozoan parasites within the Myxozoa. *Parasitology* **1999**, *119*, 555–561. [CrossRef]
11. Morris, D.J.; Adams, A. Transmission of *Tetracapsuloides bryosalmonae* (Myxozoa: Malacosporea), the causative organism of salmonid proliferative kidney disease, to the freshwater bryozoan *Fredericella sultana*. *Parasitology* **2006**, *133*, 701–709. [CrossRef] [PubMed]
12. Tops, S.; Hartikainen, H.L.; Okamura, B. The effect of infection by *Tetracapsuloides bryosalmonae* (Myxozoa) and temperature on *Fredericella sultana* (Bryozoa). *Int. J. Parasitol.* **2009**, *39*, 1003–1010. [CrossRef] [PubMed]
13. Fontes, I.; Hartikainen, H.; Taylor, N.G.H.; Okamura, B. Conditional persistence and tolerance characterize endoparasite-colonial host interactions. *Parasitology* **2017**, *144*, 1052–1063. [CrossRef]
14. Hartikainen, H.L.; Okamura, B. Castrating parasites and colonial hosts. *Parasitology* **2012**, *139*, 547–556. [CrossRef] [PubMed]
15. McGurk, C.; Morris, D.J.; Auchinachie, N.A.; Adams, A. Development of *Tetracapsuloides bryosalmonae* (Myxozoa: Malacosporea) in bryozoan hosts (as examined by light microscopy) and quantitation of infective dose to rainbow trout (*Oncorhynchus mykiss*). *Vet. Parasitol.* **2006**, *135*, 249–257. [CrossRef]
16. Feist, S.W.; Longshaw, M. Phylum Myxozoa. In *Fish Diseases and Disorders. Protozoan and Metazoan Infections*, 2nd ed.; Woo, P.T.K., Ed.; CAB International: Wallington, UK, 2006; Volume 1, pp. 230–296.
17. Longshaw, M.; Le Deuff, R.M.; Harris, A.F.; Feist, S.W. Development of proliferative kidney disease in rainbow trout, *Oncorhynchus mykiss* (Walbaum), following short-term exposure to *Tetracapsula bryosalmonae* infected bryozoans. *J. Fish Dis.* **2002**, *25*, 443–449. [CrossRef]
18. Grabner, D.; El-Matbouli, M. Transmission of *Tetracapsuloides bryosalmonae* (Myxozoa: Malacosporea) to *Fredericella sultana* (Bryozoa: Phylactolaemata) by various fish species. *Dis. Aquat. Org.* **2008**, *79*, 133–139. [CrossRef]
19. Bettge, K.; Wahli, T.; Segner, H.; Schmidt-Posthaus, H. Proliferative kidney disease in rainbow trout: Time- and temperature-related renal pathology and parasite distribution. *Dis. Aquat. Org.* **2009**, *83*, 67–76. [CrossRef]
20. Bettge, K.; Segner, H.; Burki, R.; Schmidt-Posthaus, H.; Wahli, T. Proliferative kidney disease (PKD) of rainbow trout: Temperature- and time-related changes of *Tetracapsuloides bryosalmonae* DNA in the kidney. *Parasitology* **2009**, *136*, 615–625. [CrossRef]
21. Okamura, B.; Hartikainen, H.L.; Schmidt-Posthaus, H.; Wahli, T. Life cycle complexity, environmental change and the emerging status of salmonid proliferative kidney disease. *Freshw. Biol.* **2011**, *56*, 735–753. [CrossRef]
22. Schmidt-Posthaus, H.; Hirschi, R.; Schneider, E. Proliferative kidney disease in brown trout: Infection level, pathology and mortality under field conditions. *Dis. Aquat. Org.* **2015**, *114*, 139–146. [CrossRef] [PubMed]
23. Foott, J.S.; Hedrick, R.P. Seasonal occurrence of the infectious stage of proliferative kidney disease (PKD) and resistance of rainbow trout, *Salmo gairdneri* Richardson, to reinfection. *J. Fish Biol.* **1987**, *30*, 477–483. [CrossRef]

24. De Kinkelin, P.; Loriot, B. A water temperature regime which prevents the occurrence of proliferative kidney disease (PKD) in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *J. Fish Dis.* **2001**, *24*, 489–493. [CrossRef]
25. Gay, M.; Okamura, B.; De Kinkelin, P. Evidence that infectious stages of *Tetracapsula bryosalmonae* for rainbow trout *Oncorhynchus mykiss* are present throughout the year. *Dis. Aquat. Org.* **2001**, *46*, 31–40. [CrossRef] [PubMed]
26. Bailey, C.; Segner, H.; Casanova-Nakayama, A.; Wahli, T. Who needs the hotspot? The effect of temperature on the fish host immune response to *Tetracapsuloides bryosalmonae* the causative agent of proliferative kidney disease. *Fish Shellfish Immunol.* **2017**, *63*, 424–437. [CrossRef]
27. Bailey, C.; Schmidt-Posthaus, H.; Segner, H.; Wahli, T.; Strepparava, N. Are brown trout *Salmo trutta fario* and rainbow trout *Oncorhynchus mykiss* two of a kind? A comparative study of salmonids to temperature-influenced *Tetracapsuloides bryosalmonae* infection. *J. Fish Dis.* **2018**, *41*, 191–198. [CrossRef]
28. Palikova, M.; Papezikova, I.; Markova, Z.; Navratil, S.; Mares, J.; Mares, L.; Vojtek, L.; Hyrsl, P.; Jelinkova, E.; Schmidt-Posthaus, H. Proliferative kidney disease in rainbow trout (*Oncorhynchus mykiss*) under intensive breeding conditions: Pathogenesis and haematological and immune parameters. *Vet. Parasitol.* **2017**, *238*, 5–16. [CrossRef]
29. Wahli, T.; Knuesel, R.; Bernet, D.; Segner, H.; Pugovkin, D.; Burkhardt-Holm, P.; Escher, M.; Schmidt-Posthaus, H. Proliferative kidney disease in Switzerland: Current state of knowledge. *J. Fish Dis.* **2002**, *25*, 491–500. [CrossRef]
30. Bailey, C.; Rubin, A.; Strepparava, N.; Segner, H.; Rubin, J.-F.; Wahli, T. Do fish get wasted? Assessing the influence of effluents on parasitic infection of wild fish. *PeerJ* **2018**, *6*, e5956. [CrossRef]
31. Rubin, A.; de Coulon, P.; Bailey, C.; Segner, H.; Wahli, T.; Rubin, J.-F. Keeping an eye on wild brown trout (*Salmo trutta*) populations: Correlation between temperature, environmental parameters, and proliferative kidney disease. *Front. Vet. Sci.* **2019**, *6*, 281. [CrossRef]
32. Borgwardt, F.; Unfer, G.; Auer, S.; Waldner, K.; El-Matbouli, M.; Bechter, T. Direct and Indirect Climate Change Impacts on Brown Trout in Central Europe: How Thermal Regimes Reinforce Physiological Stress and Support the Emergence of Diseases. *Front. Environ. Sci.* **2020**, *8*, 59. [CrossRef]
33. Strepparava, N.; Segner, H.; Ros, A.; Hartikainen, H.; Schmidt-Posthaus, H.; Wahli, T. Temperature-related parasite infection dynamics: The case of proliferative kidney disease of brown trout. *Parasitology* **2018**, *145*, 281–291. [CrossRef] [PubMed]
34. Waldner, K.; Borkovec, M.; Borgwardt, F.; Unfer, G.; El-Matbouli, M. Effect of water temperature on the morbidity of *Tetracapsuloides bryosalmonae* (Myxozoa) to brown trout (*Salmo trutta*) under laboratory conditions. *J. Fish Dis.* **2021**, *44*, 1005–1013. [CrossRef] [PubMed]
35. Rehberger, K.; Wernicke von Siebenthal, E.; Bailey, C.; Bregy, P.; Fasel, M.; Herzog, E.L.; Neumann, S.; Schmidt-Posthaus, H.; Segner, H. Long-term exposure to low 17α-ethinylestradiol (EE2) concentrations disrupts both the reproductive and the immune system of juvenile rainbow trout, *Oncorhynchus mykiss*. *Environ. Int.* **2020**, *142*, 105836. [CrossRef]
36. Bruneaux, M.; Visse, M.; Gross, R.; Pukk, L.; Saks, L.; Vasemägi, A. Parasite infection and decreased thermal tolerance: Impact of proliferative kidney disease on a wild salmonid fish in the context of climate change. *Funct. Ecol.* **2017**, *31*, 216–226. [CrossRef]
37. Schmidt-Posthaus, H.; Bettge, K.; Forster, U.; Segner, H.; Wahli, T. Kidney pathology and parasite intensity in rainbow trout *Oncorhynchus mykiss* surviving proliferative kidney disease: Time course and influence of temperature. *Dis. Aquat. Org.* **2012**, *97*, 207–218. [CrossRef]
38. Chapman, J.M.; Kelly, L.A.; Teffer, A.K.; Miller, K.M.; Cooke, S.J. Disease ecology of wild fish: Opportunities and challenges for linking infection metrics with behaviour, condition, and survival. *Can. J. Fish Aquat. Sci.* **2021**, *78*, 995–1007. [CrossRef]
39. Tops, S.; Baxa, D.V.; McDowell, T.S.; Hedrick, R.P.; Okamura, B. Evaluation of malacosporean life cycles through transmission studies. *Dis. Aquat. Org.* **2004**, *60*, 109–121. [CrossRef]
40. 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. [CrossRef]
41. Soliman, H.; Kumar, G.; El-Matbouli, M. *Tetracapsuloides bryosalmonae* persists in brown trout *Salmo trutta* for five years post exposure. *Dis. Aquat. Org.* **2018**, *127*, 151–156. [CrossRef] [PubMed]
42. IPCC. *Climate Change 2021: Synthesis Report. Working Group I Contribution to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*; IPCC: Geneva, Switzerland, 2021; 40p.
43. Dash, M.; Vasemägi, A. Proliferative kidney disease (PKD) agent *Tetracapsuloides bryosalmonae* in brown trout populations in Estonia. *Dis. Aquat. Org.* **2014**, *109*, 139–148. [CrossRef] [PubMed]
44. Schmidt-Posthaus, H.; Schneider, E.; Schölzel, N.; Hirschi, R.; Stelzer, M.; Peter, A. The role of migration barriers for dispersion of Proliferative Kidney Disease—Balance between disease emergence and habitat connectivity. *PLoS ONE* **2021**, *16*, e0247482. [CrossRef] [PubMed]
45. Fischnetz. *Final Report of Project Fischnetz*; Eawag: Dübendorf, Switzerland; BUWAL: Bern, Switzerland, 2004; 188p.
46. Bush, A.O.; Lafferty, K.D.; Lotz, J.M.; Shostak, A.W. Parasitology meets ecology on its own terms: Margolis et al. revisited. *J. Parasitol.* **1997**, *83*, 575–583. [CrossRef] [PubMed]

3 Keeping an eye on wild brown trout (*Salmo trutta*) populations: Correlation between temperature, environmental parameters, and proliferative kidney disease

Aurélie Rubin, Pauline de Coulon, Christyn Bailey, Helmut Segner, Thomas Wahli and Jean-François Rubin

Paper published in *Frontiers in Veterinary Science, Volume 6, August 2019, Article 281*.

frontiers in Veterinary Science

ORIGINAL RESEARCH
published: 22 August 2019
doi: 10.3389/fvets.2019.00281

Keeping an Eye on Wild Brown Trout (*Salmo trutta*) Populations: Correlation Between Temperature, Environmental Parameters, and Proliferative Kidney Disease

Aurélie Rubin[1,2,3]*, *Pauline de Coulon*[4], *Christyn Bailey*[1,5], *Helmut Segner*[1], *Thomas Wahli*[1] and *Jean-François Rubin*[2,3]

[1] Department of Infectious Diseases and Pathobiology, Centre for Fish and Wildlife Health, University of Bern, Bern, Switzerland, [2] La Maison de la Rivière, Tolochenaz, Switzerland, [3] Land, Nature, Environment Institute, University of Applied Sciences and Arts Western Switzerland, Geneva, Switzerland, [4] Department of Ecology and Evolution, Faculty of Biology and Medicine, University of Lausanne, Lausanne, Switzerland, [5] Fish Immunology and Pathology Laboratory, Animal Health Research Center (CISA-INIA), Madrid, Spain

OPEN ACCESS

Edited by:
Robert James Ossiboff,
University of Florida, United States

Reviewed by:
Franz Lahnsteiner,
Bundesamt für Wasserwirtschaft, Austria
Bartolomeo Gorgoglione,
Michigan State University, United States

***Correspondence:**
Aurélie Rubin
aurelie.rubin@students.unibe.ch

Specialty section:
This article was submitted to Zoological Medicine, a section of the journal Frontiers in Veterinary Science

Received: 03 May 2019
Accepted: 07 August 2019
Published: 22 August 2019

Citation:
Rubin A, de Coulon P, Bailey C, Segner H, Wahli T and Rubin J-F (2019) Keeping an Eye on Wild Brown Trout (Salmo trutta) Populations: Correlation Between Temperature, Environmental Parameters, and Proliferative Kidney Disease. Front. Vet. Sci. 6:281. doi: 10.3389/fvets.2019.00281

Proliferative kidney disease (PKD) is an emerging disease of salmonids caused by the myxozoan parasite *Tetracapsuloides bryosalmonae*, which plays a major role in the decrease of wild brown trout (*Salmo trutta*) populations in Switzerland. Strong evidence demonstrated that water temperature modulates parasite infection. However, less knowledge exists on how seasonal water temperature fluctuations influence PKD manifestation under field conditions, how further environmental factors such as water quality may modulate the disease, and whether these factors coalesce with temperatures role possibly giving rise to cumulative effects on PKD. The aims of this study were to (1) determine the correlation between seasonal course of water temperature and PKD prevalence and intensity in wild brown trout populations, (2) assess if other factors such as water quality or ecomorphology correlate with the infection, and (3) quantitatively predict the implication of these factors on PKD prevalence with a statistical model. Young-of-the-year brown trout were sampled in 45 sites through the Canton of Vaud (Switzerland). For each site, longitudinal time series of water temperature, water quality (macroinvertebrate community index, presence of wastewater treatment plant effluent) and ecomorphological data were collected and correlated with PKD prevalence and intensity. 251 *T. bryosalmonae*-infected trout of 1,118 were found (overall prevalence 22.5%) at 19 of 45 study sites (42.2%). Relation between PKD infection and seasonal water temperature underlined that the mean water temperature for June and the number of days with mean temperature $\geq$15°C were the most significantly correlated parameters with parasite prevalence and intensity. The presence of a wastewater treatment plant effluent was significantly correlated with the prevalence and infection intensity. In contrast, macroinvertebrate diversity and river ecomorphology were shown to have little impact on disease parameters. Linear and logistic regressions highlighted quantitatively the prediction of PKD prevalence depending on environmental parameters at a given site and

its possible increase due to rising temperatures. The model developed within this study could serve as a useful tool for identifying and predicting disease hot spots. These results support the importance of temperature for PKD in salmonids and provides evidence for a modulating influence of additional environmental stress factors.

Keywords: proliferative kidney disease, *Salmo trutta*, *Tetracapsuloides bryosalmonae*, water temperature, water quality, ecomorphology, wild fish population, aquatic fieldwork

INTRODUCTION

Beginning in the 1980s, the catch of brown trout *Salmo trutta* in Switzerland experienced a massive decline of up to 50% (1–4). This process is still ongoing. Hence, the wild salmonid populations are considered to be threatened (5–7). Investigations within a nationwide project suggested the decrease was due to multifactorial drivers (2). Among the parameters involved, a parasitic disease of salmonids, proliferative kidney disease (PKD) was considered to play a major role (2, 3, 8–10).

The causative agent of PKD is the myxozoan parasite *Tetracapsuloides bryosalmonae* (11, 12). The life cycle of the parasite comprises salmonid fish species as vertebrate hosts and bryozoans, mainly *Fredericella sultana* (Blumenbach), as invertebrate hosts (13–16). Spores, which have developed in bryozoans, are released into the water and upon contact with a suitable vertebrate host, infect the fish through skin and gills (14, 17). Via the circulatory system, the parasites eventually reach the target organs, mainly the kidney. The infection may cause renal swelling (18–20). In the kidney, the parasite normally develops sporogonic stages in the tubuli, from where spores are released via the urine and can infect bryozoans again (15, 21, 22). Depending on host susceptibility and seasonal conditions, extrasporogonic stages might instead proliferate in the interstitial tissue causing PKD, which may lead to high mortality rates (18, 19, 23). Juvenile fish getting in contact with the parasite for the first time appear to be particularly susceptible to the infection and to PKD pathogenesis (8, 19, 24, 25).

Several freshwater fish diseases are suggested to be sensitive to the rising temperatures associated with climate change (26–30). Also for PKD, water temperature is a key parameter for the disease prevalence and intensity for Swiss wild brown trout populations (8–10, 31), wild Atlantic salmon *Salmo salar* L. in Norway (32), and brown trout (33) and rainbow trout *Oncorhynchus mykiss* (Walbaum) from laboratory experiments (34–37) as examples. Renal pathology and trout mortalities are enhanced at water temperatures of ≥15°C (19, 23, 25, 38, 39). Consequently, PKD-associated mortalities and disease symptoms show a seasonal occurrence and appear to be most pronounced during summer and early autumn (8, 32, 34, 40). Temperature could act directly or indirectly on this host-parasite system. Indeed, high temperature could promote spore production by bryozoans (41), accelerate parasite proliferation in fish (18, 23, 36), modulate the host immune response (37) and modify the parasite transmission opportunities (33).

Abbreviations: PKD, Proliferative kidney disease; IBCH, Swiss biological index; WWTP, Wastewater treatment plant; N days ≥ x, Number of days with a daily mean temperature ≥ x°C.

In addition, freshwater fish diseases can be influenced by a multitude of stressors besides temperature (27, 42, 43), such as habitat quality, water levels, pH, and water quality (26, 44, 45). This last factor might indeed have an influence on PKD prevalence, intensity and mortality as previously investigated (31, 46, 47).

To date, data on the influence of temperature on *T. bryosalmonae* infection of fish is largely derived from laboratory experiments using constant temperature(s). In the wild, the influence of long-term water temperature measurements on the development of the disease has rarely been investigated. Besides temperature, other environmental factors have been individually revealed to influence *T. bryosalmonae* infection, but seldom in a combined way. In addition, rainbow trout is often used as a model species for laboratory investigations of fish disease, including to reproduce PKD in controlled conditions [e.g., (14, 17, 18, 23, 34–37)]. Though, rainbow trout and brown trout react differently to *T. bryosalmonae* infection, in terms of sensitivity to infectious agent and environmental stress (46), intensity of *T. bryosalmonae* infection (48), or temperature-dependant modulation of the parasite (49) for example. Therefore, some of the conclusions based on experiments with rainbow trout might not correspond to the situation of wild brown trout populations in their natural habitat with fluctuating temperatures. Thus, the influence of the course of water temperature over time, with its seasonal, local and diurnal variations, on PKD infection in wild brown trout populations need to be investigated. Moreover, the possible cumulative effects of other environmental factors and their predicted consequences on the disease should also be taken under consideration. Field investigations are therefore of crucial importance for a better understanding of the brown trout—*T. bryosalmonae* host parasite system and possible future implications by the global warming on population dynamics.

The aims of the present study were to (1) determine the correlation between seasonal course of water temperature and PKD prevalence and intensity in wild brown trout populations, (2) identify if additional environmental parameters, such as water quality or ecomorphology, might have cumulative effects on PKD infection, and (3) quantitatively predict the combined consequences of these environmental parameters on the disease prevalence.

MATERIALS AND METHODS

Study Sites

Forty five stations located over 18 rivers of the Canton of Vaud in Switzerland were analyzed (**Figure 1**). For each station, data on *T. bryosalmonae* infection status of fish (prevalence and

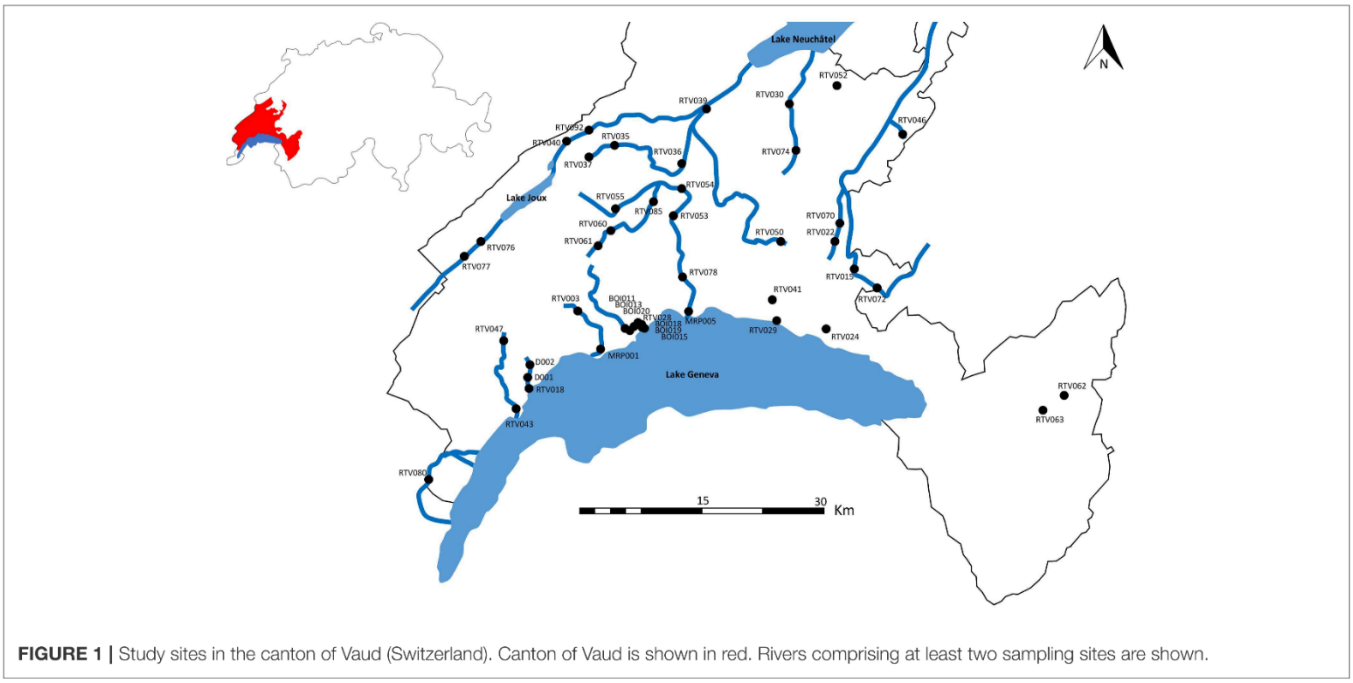


FIGURE 1 | Study sites in the canton of Vaud (Switzerland). Canton of Vaud is shown in red. Rivers comprising at least two sampling sites are shown.

intensity), water temperature, biological water quality assessed through macroinvertebrate sensitivity, presence/absence of a wastewater treatment plant (WWTP) and ecomorphology were collected (**Table 1**).

Temperature Data

Water temperature was measured with temperature loggers (HOBO® Water Temp Pro v2 Data Logger, Onset, Cape Cod Massachusetts, USA) recording the water temperature every 15 min. Before placing the loggers into the water and upon sampling, they were compared to a reference thermometer to determine possible drifts which could then be considered for the temperature values. Temperature measurements were selected from the 1st of March 2014, which was considered as the fry emergence date for streams of the Midlands (Rubin, pers. obs.), until the 31st of August 2014 since fish were sampled at the beginning of September. For two sites (RTV063 and RTV022), water temperatures were extrapolated with upstream or downstream loggers. No temperature data were available for RTV092.

Six types of temperature values are described in the study: (1) the monthly mean temperature from March to August, (2) the mean temperature from the "spring" period (March–May), the mean temperature from the "summer" period (June–August) and the total mean during the whole period, (3) the maximum temperature (absolute maximum and daily mean maximum), (4) the number of days with a daily mean temperature $\geq$ 13°C to $\geq$ 19°C, and (5) the degree days, calculated as the sum of the daily mean temperature values from the 1st of March to the 31st of August 2014.

Additional Environmental Factors

Macroinvertebrate Analysis

A good indicator for the assessment of water quality and ecological quality is the macroinvertebrate community, depending on the observed taxa and their sensitivity to pollutants (50–56). The standardized method "IBCH" (Swiss biological index) developed by the Swiss Federal Office for the Environment (53) is used to determine the biological water quality and ecological status through assessment of the macroinvertebrate community. This method, also applied in Bailey et al. (31), was performed at all 45 study sites except the station RTV41, where water was loaded with a huge amount of sediment due to construction works preventing sample collection. For an assessment of the biological water quality during the life period of sampled fish (March to September 2014), macrozoobenthos samplings were performed between the 12th of March and 22nd of April 2015, following the favorable sampling periods in function of the altitude, defined by the method (53). 35 macroinvertebrates samplings were performed by our team and eight stations were analyzed by collaborators of the department of water protection from the General Directorate of the Environment from the canton of Vaud.

This standardized method (53) consisted of eight macroinvertebrate samplings with a normalized net in function of the substratum and waterflow. After capture, all material was fixed in a container filled with 85% ethanol. In the laboratory, the material was sorted and determined using a binocular loupe. The determination of each individual was performed until family taxonomic level, using the reference book of Tachet et al. (50). Abundance of all taxa was recorded. Finally, the IBCH index, a score between 0 (very low water quality) and 20 (excellent), was calculated based on the diversity of observed taxa and their sensitivity to water quality. Based

TABLE 1 | Study sites, PKD status, temperature, and environmental data.

	PKD data				Environmental data			Temperature data				
Site index	**Number of infected fish**	**PKD prevalence (%)**	**Infection degree**	**Alteration degree**	**IBCH note**	**Upstream WWTP**	**Ecomorphology**	**Mean June [°C]**	**Mean Summer (June-July-August) [°C]**	**Days with daily mean ≥ 14°C**	**Days with daily mean ≥ 15°C**	**Days with daily mean ≥ 16°C**
BOI011	0	0	0.0	0.0	13	Yes	1	13.9	14.5	62	35	4
BOI013	0	0	0.0	0.0	9	Yes	1	14.7	15.1	72	54	24
BOI020	0	0	0.0	0.0	9	No	1	15.0	16.2	85	82	56
RTV028	3	13	3.7	3.7	14	Yes	1	15.3	15.6	83	65	38
BOI018	17	68	3.8	3.5	12	Yes	1	15.0	15.8	84	81	41
BOI019	22	88	4.6	4.4	7	Yes	1	15.1	15.9	85	80	47
BOI015	22	88	4.6	4.4	12	Yes	1	15.7	16.2	86	83	60
D002	0	0	0.0	0.0	8	No	4	15.2	16.3	86	83	68
D001	0	0	0.0	0.0	7	No	1	12.6	13.3	11	1	0
RTV018	0	0	0.0	0.0	6	No	1	11.8	11.3	4	0	0
RTV063	0	0	0.0	0.0	13	No	1	8.5	8.6	0	0	0
RTV062	0	0	0.0	0.0	13	No	1	9.3	9.3	0	0	0
RTV003	0	0	0.0	0.0	14	Yes	2	10.2	7.3	0	0	0
MRP001	0	0	0.0	1.7	17	Yes	1	10.6	9.4	0	0	0
RTV072	1	4	3.0	3.0	17	Yes	1	15.3	15.0	70	50	20
RTV015	1	4	3.0	2.0	17	Yes	1	15.3	15.0	68	50	20
RTV022	8	31	4.4	4.4	12	No	1	14.1	14.4	63	31	10
RTV070	6	24	3.7	3.8	12	No	1	14.4	14.6	67	40	12
RTV046	0	0	0.0	0.0	11	No	1	13.3	14.1	55	19	1
RTV074	24	96	3.0	2.6	11	Yes	1	15.0	15.1	70	55	26
RTV030	12	48	1.8	2.0	15	Yes	1	15.9	15.9	79	67	51
RTV037	0	0	0.0	0.0	15	No	1	11.1	9.7	0	0	0
RTV035	0	0	0.0	0.0	13	Yes	1	13.4	12.4	16	5	0
RTV036	0	0	0.0	0.0	13	Yes	3	14.2	13.5	41	14	1
RTV077	10	38	3.3	2.7	17	No	1	17.5	16.3	79	64	49
RTV076	19	70	3.0	3.1	16	No	2	15.3	13.8	38	30	15
RTV040	0	0	0.0	0.0	14	No	1	10.0	9.7	0	0	0
RTV092	0	0	0.0	0.0	15	No	2	NA	NA	NA	NA	NA
RTV039	1	4	3.0	2.0	8	Yes	4	15.0	13.7	45	31	7
RTV055	0	0	0.0	0.0	14	Yes	3	11.8	10.3	0	0	0
RTV054	8	32	2.3	2.3	14	Yes	2	14.4	13.2	33	12	2
RTV053	23	92	3.7	3.7	13	Yes	4	16.5	15.0	62	48	33
RTV078	9	38	2.8	3.2	8	Yes	1	16.3	15.5	84	59	35
MRP005	18	72	3.9	3.9	9	Yes	2	18.0	16.7	90	74	59
RTV061	0	0	0.0	0.0	11	Yes	1	13.6	12.7	14	6	1

(Continued)

TABLE 1 | Continued

	PKD data				Environmental data			Temperature data			
Site index	Number of infected fish	PKD prevalence (%)	Infection degree	Alteration degree	IBCH note	Upstream WWTP	Ecomorphology	Mean June [°C]	Mean Summer (June-July-August) [°C]	Days with daily mean ≥ 14°C	Days with daily mean ≥ 15°C	Days with daily mean ≥ 16°C
RTV060	21	81	4.0	4.2	15	Yes	1	14.5	13.2	39	13	6
RTV085	26	100	3.7	3.8	15	Yes	1	14.0	13.8	47	23	3
RTV024	0	0	0.0	0.0	10	No	1	15.0	15.1	82	50	17
RTV029	0	0	0.0	0.0	8	No	1	15.3	15.2	71	53	28
RTV041	0	0	0.0	0.0	NA	No	2	14.7	14.7	67	44	20
RTV043	0	0	0.0	0.0	16	No	1	13.6	12.5	17	2	0
RTV047	0	0	0.0	0.0	13	No	1	13.2	13.5	33	2	0
RTV050	0	0	0.0	0.0	15	No	1	13.0	13.1	25	3	0
RTV052	0	0	0.0	0.0	10	No	1	12.3	13.3	22	3	0
RTV080	0	0	0.0	0.0	10	No	1	7.8	7.7	0	0	0

NA, no data for this site.

on this index, a water quality class was assigned to each station, with the scale: very good water quality = index 17–20, good = 13–16, medium = 9–12, poor = 5–8, bad = 0–4. The macrozoobenthos samplings were performed relating to ethical approvals (permission delivered by the Service of Hunting, Fishing and Surveillance of the Biodiversity and Landscape Division from the General Directorate of the canton of Vaud).

Wastewater Treatment Plant

The presence/absence of a wastewater treatment plant effluent upstream of the study site was also determined using topographic maps.

Ecomorphology

Ecomorphology is also a potential stressor affecting fish health (57–59) and fish density in streams (60–62). The ecomorphology was determined using the standardized method "Ecomorphology" from the Swiss Federal Office for the Environment (63). This method aims to determine the structural diversity of the stream, its connectivity and interactions with the surrounding area. A class was attributed to each of 45 river sections depending on the state of the riverbed, riverbank and shore, following the scale 1 = natural, 2 = little affected, 3 = very affected, 4 = artificial, 5 = underground pipe. These data were obtained from the geodatabase from the Federal Office of Topography (Swisstopo) (64).

Fish Analysis

Twenty five young-of-the-year (YOY) brown trout were sampled by electrofishing at each site, resulting in a total number of 1,118 fish. The sampling campaign took place over a period of 10 days, between the 1st and 11th of September 2014. This period was chosen based on previous PKD studies in Swiss streams (8–10) and PKD analysis in the river Boiron de Morges (Rubin, unpublished data), showing that the peak of *T. bryosalmonae* infection in brown trout occurs in late August, beginning of September. After this period, typical PKD kidney alterations could still be visible but parasites might already have been cleared out by the immune system of the fish or have left the fish, causing therefore difficulties for the diagnosis (10).

After electrofishing, juvenile brown trout (total body length 40–118 mm, mean of 76 mm ± standard deviation of 13 mm) were euthanized with an overdose of MS222 (3-aminobenzoic acid ethyl ester, 300 mg l-1, Argent Chemical Laboratories). The fish body cavity was opened, and whole animals were fixed in 4% buffered formalin until later kidney removal in the laboratory. Whole fixed kidneys were embedded in paraffin. Organs were cut using routine histological methods and stained with haematoxylin and eosin (H&E). One section of the full length of the kidney was prepared per fish. Thus, one slide per fish was examined for the presence of *T. bryosalmonae*. The degree of infection (infection intensity corresponding to an estimation of the numbers of observed parasites) and the alteration degree (tissue proliferation due to pathological alterations) were recorded for each sample using a microscope. For each slide a common score system from 0 (no parasite/no

alteration) to 6 (at least 10 parasites per high power field with 400x magnification/severe alteration) was applied as in Bettge et al. (18) and Schmidt-Posthaus et al. (39). If at least one parasite was observed, the slide was considered as histologically positive. The infection/alteration degree per station were then calculated as the mean of all infection/alteration scores from a site. The prevalence of PKD (%) was calculated as the percentage of *T. bryosalmonae* infected individuals within all sampled animals at a particular site.

Histology was chosen for PKD diagnosis instead of Polymerase Chain Reaction (PCR) due to time, material and crew resources in the field. This method also permits to assess the severity of pathological response and the state of the disease. However, histological examination may have missed the parasites for fish with very low infection levels. Thus, immunohistochemistry was performed on three randomly selected fish per station where no *T. bryosalmonae* parasites were observed on any H&E stained slide, in order to corroborate negative results. Therefore, immunohistochemistry was performed on 78 samples using an anti-*T. bryosalmonae* (PKX) monoclonal antibody (AquaMab-P01, Aquatic Diagnostic Ltd., Stirling, Scotland) applying the method described by Bettge et al. (18).

Fish samplings were performed relating to ethical approvals (permission number VD2871 delivered by the Service of Consumption and Veterinary business of the canton of Vaud).

Statistical Analysis

The sample size used for the statistical analysis, contains 1,068 samples. Data of sites RTV041 and RTV092 were excluded because of the absence of, respectively, IBCH index and temperature data.

The software RStudio (version 1.1.463) was used to perform the statistical analysis. An univariate analysis was conducted using Pearson's coefficient to investigate the correlation between the prevalence of *T. bryosalmonae* infected fish, infection degree, alteration degree and temperature data, as well as potentially aggravating environmental parameters. Student's *t*-test was also applied to test the significant differences ($p < 0.05$) in the means of temperature variables and other environmental factors between *T. bryosalmonae* positive fish and PKD healthy trout from all sampling sites.

Multivariate analysis was applied using a linear probability model (LPM) and a logistic model (Logit) that quantified the implication of the different parameters on the PKD infection. The following equation was applied for the linear regression:

$$PKD_i = \beta_0 + \beta_1 * Temp_j + \beta_2 * IBCH_j + \beta_3 * WWTP_j + \beta_4 * Ecomorphology_j + \epsilon_i$$

Where:

PKDi is a binary variable taking the value of 1 if the trout *i* is infected with *T. bryosalmonae*, and 0 otherwise,
Temp is an indicator of temperature,
IBCH is the value of the IBCH index,
WWTP is a binary variable with a value of 1 if a WWTP is present upstream the study site, and 0 otherwise,
Ecomorphology is a categorial variable (from 1 for a natural section to 4 for a very anthropized sector),
ϵ is the error term or the residual variation that the model cannot explain,
Index *i* represents variable at the trout level and index *j* represents variable at the site level.

The average marginal effect was applied for the Logit model, in order to get comparable results and not "log odd ratio" data. The conditional expectation was also applied to predict the variation of PKD prevalence due to temperature, while controlling the other environmental factors.

RESULTS

Water Temperature

A selection of the temperature results is given in **Table 1** (all temperature data are given in **Supplementary Material**). Highest mean temperature during the study period (March to August 2014), and during the summer (June to August) was observed

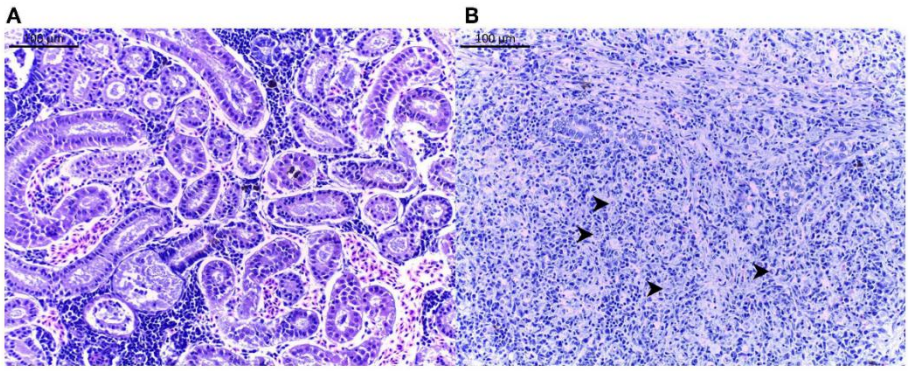


FIGURE 2 | Histological pictures of brown trout *Salmo trutta* posterior kidney. **(A)** Histological assessment of an infection score of 0 (no parasite). **(B)** Histological assessment of an infection score of 6 (severe infection). Some parasites *Tetracapsuloides bryosalmonae* are shown (arrows). Scale bar = 100 µm. Pictures are taken from H&E stained slides.

TABLE 2 | Pearson's correlation between *T. bryosalmonae* infected brown trout prevalence, infection degree, alteration degree, and variables.

Variables	PKD prevalence	Infection degree	Alteration degree
Mean temperature of march [°C]	0.069	0.091	0.098
Mean temperature of April [°C]	0.209	0.209	0.199
Mean temperature of May [°C]	0.311	0.296	0.286
Mean temperature of June [°C]	0.406	0.366	0.351
Mean temperature of July [°C]	0.304	0.289	0.267
Mean temperature of August [°C]	0.307	0.297	0.276
Mean temperature of spring (March–May) [°C]	0.213	0.216	0.211
Mean temperature of summer (June–August) [°C]	0.346	0.325	0.304
Total mean temperature [°C]	0.306	0.294	0.279
Maximum temperature [°C]	0.146	0.114	0.106
Maximum daily mean temperature [°C]	0.315	0.307	0.301
Days with daily mean temperature ≥ 13°C	0.315	0.298	0.282
Days with daily mean temperature ≥ 14°C	0.358	0.343	0.322
Days with daily mean temperature ≥ 15°C	0.373	0.363	0.337
Days with daily mean temperature ≥ 16°C	0.360	0.350	0.323
Days with daily mean temperature ≥ 17°C	0.308	0.288	0.266
Days with daily mean temperature ≥ 18°C	0.224	0.202	0.187
Days with daily mean temperature ≥ 19°C	0.150	0.138	0.116
Degree days	0.307	0.295	0.277
IBCH note	0.044	0.002	−0.001
WWTP	0.321	0.303	0.295
Ecomorphology	0.041	0.026	0.042

at the outlet of the river Venoge (MRP005) with a value of, respectively, 13.8 and 16.7°C. Mean temperature of June reached <10°C in three stations, was comprised between 10 and 15°C in 28 cases, and surpassed 15°C in 13 stations. The warmest daily mean temperature (20.6°C) was observed in the Orbe (RTV077) and the Venoge (MRP005). Some stations never reached a daily mean temperature of ≥15°C, while a maximum of 83 days was measured in the Boiron (BOI015) and the Dullive (D002). The degree days varied from 1,090 (RTV063) to 2,539 (MRP005).

Additional Environmental Factors

IBCH scores were calculated for each site (**Table 1**) based on the sensitivity and abundance of macroinvertebrate taxa. The lowest obtained IBCH score was 6 (RTV018) and the highest IBCH score obtained was 17 (MRP001, RTV015, RTV072, and RTV077). Four stations corresponded to the water quality class "very good," 20 belonged to the class "good," 13 sites were classified as "medium" and seven were in the class "poor." Thus, all water quality classes were represented in the samples, except the poorest class.

A WWTP was present upstream at 24 of the study sites (53.3%) (**Table 1**). Among them, 15 stations were assessed as PKD-positive (62.5%), with prevalence ranging from 4 to 100% (mean of 52 ± 34%) of *T. bryosalmonae* infected fish.

The ecomorphology class indicates the condition of the riverbed. Thirty four stations belonged to the class 1, six were in the class 2, two belonged to class 3 and three were considered as anthropized (class 4) (**Table 1**). No site belonged to the class 5.

PKD Manifestation

Stocking of fry or juvenile brown trout for sustaining natural populations was performed since several years in 16 study sites (stocking performed at a maximum of 2 km upstream the study site). Thus, for a comparison of wild fish between all sites, no stocking took place before our fish sampling in 2014, in agreement with the fish inspectorship of the canton of Vaud. All YOY caught here originate therefore from natural spawning. Among the 45 sampled sites, 19 stations (42.2%) were assessed as *T. bryosalmonae*-positive (**Table 1**). The disease was present in the rivers Boiron de Morges, Broye, Flon de Carrouge, Mentue, Orbe, Venoge, and Veyron. Presence of *T. bryosalmonae* was observed in 251 brown trout from the 1,118 sampled animals (22.5%). The observed prevalence ranged from 0 to 100%. Among the 19 PKD-positive sites, the highest site-specific infection degree (score of 4.64) was found in the Boiron (BOI019), while the lowest (score of 1.84) was seen in the Mentue (RTV030) (**Figure 2**). However, even if PKD prevalence was low (≤10%), infected fish showing high infection degree (≥3.0) were found. When considering the degree of alteration, mean values between 1.7 to 4.4 at sites with infected fish were found.

Correlation of Environmental Variables and PKD

Pearson's correlation coefficient between *T. bryosalmonae* prevalence/infection degree/alteration degree and temperature parameters/other environmental variables is given in **Table 2**. A positive linear relation appeared between infected fish/infection degree/alteration degree and every temperature variable but remained rather low ($r < 0.5$). The strongest correlation was obtained with the mean temperature of June ($r = 0.406$ with prevalence, 0.366 with infection degree, 0.351 with alteration degree), followed by the number of days with a daily mean temperature ≥15°C (N days ≥ 15) ($r = 0.373$ with prevalence, 0.363 with infection degree, 0.337 with alteration degree). No correlation was found between PKD data and IBCH index ($r = 0.044$ with prevalence, 0.002 with infection degree, −0.001 with alteration degree) and ecomorphology ($r = 0.041$ with prevalence, 0.026 with infection degree, 0.042 with alteration degree), in contrast to the presence of an upstream WWTP ($r = 0.321$ with prevalence, 0.303 with infection degree, 0.295 with alteration degree). Site-specific infection degree and

prevalence of infected fish were positively correlated ($r = 0.808$), as well as alteration degree and prevalence of infected fish ($r = 0.750$). Infection degree and alteration degree were strongly correlated ($r = 0.952$).

Average Differences

Significant differences in the means of temperature parameters between sites with infected and not infected fish were observed. Mean temperature of June was significantly higher (mean of 15.4°C) for sites with infected fish than for sites with trout without parasites (mean of 13.4°C) (**Figure 3A**). The same applied for the N days ≥ 15 (**Figure 3B**).

The presence of an upstream WWTP significantly positively influenced the PKD prevalence (**Figure 4A**). On the contrary, no significant difference appeared between sites with infected and sites with parasite-free fish for IBCH score (**Figure 4B**) and ecomorphology (**Figure 4C**).

Quantification of the Predicted Effect of Temperature and Additional Environmental Factors on PKD Prevalence

Two models, a linear model (LPM) and a logistic model (Logit), were compared to quantify the predicted variation of PKD prevalence due to an increase of one unit of the temperature variables and other environmental factors. These analyses were performed by separating the temperature variables in two categories (temperature means and N days ≥ x°C), which permit an estimate of the best temperature indicator.

Table 3a (LPM results) and **Table 3b** (Logit results) show the estimated effect of an increase of 1°C of the different means of temperatures on the prevalence of infected fish. All mean temperature parameters were significantly associated with infection prevalence ($p < 0.001$). For example, the predictions showed that a one-degree increment elevation in the mean temperature of June induces an increase of 6.9% of the prevalence of infected fish for the LPM [**Table 3a**, Model (1)] and an increase of 9.9% for the Logit [**Table 3b**, Model (1)].

The temperature parameter that explained at best the variance of the prevalence of infected fish (R2) was obtained with the model using the mean temperature of June [**Table 3a**, Model (1), R2 = 0.211] followed by the model taking into account the mean temperature of summer [Model (4), R2 = 0.196] for the LPM. The same findings appeared for the Logit [**Table 3b**, Model (1), Pseudo R2 = 0.373; Model (4), Pseudo R2 = 0.339]. **Table 4a** (LPM) and **Table 4b** (Logit) give the quantification of the average influence of the N days ≥ x°C and other environmental factors on the prevalence of the disease. Every temperature variable was significant ($p < 0.01$). For the LPM model, the N days ≥ 16 appeared to be the best explanatory temperature variable (R2 = 0.208), followed by the N days ≥ 15 (R2 = 0.203). The N days ≥ 14 (Pseudo R2 = 0.313) and then the N days ≥ 15 (Pseudo R2 = 0.309) had the higher Pseudo R2 for the Logit models. Therefore, all models showed that an increase in water temperature induced an increment in the predicted prevalence of infected fish. For the mean temperature category, the mean temperature of June and the mean temperature of the summer period explained at best the variance of the infection prevalence, both LPM and Logit combined. For the number of days with a daily mean temperature ≥ x°C, the LPM and Logit did not result in the same findings for the best explanatory variable for the variance of PKD prevalence (respectively, N days ≥ 16 and N days ≥ 14) but were coincident for the second explanatory variable (N days ≥ 15). Variance of each parameter was better explained with the Logit model than with the LPM model. However, R2 and Pseudo R2 remained low for every model.

Concerning the additional environmental parameters, the IBCH index was significantly associated with PKD prevalence ($p < 0.05$) only in Logit models (four out of five models dealing with means of temperature and in five of the eight models of the number of days with a daily mean ≥ x°C). An increase of one unit in the IBCH score induced a predicted increase smaller than 2.1% in the PKD prevalence. The presence of an upstream WWTP was significantly linked to the disease ($p < 0.01$) in all models. The presence of such a facility increased the predicted PKD prevalence between 18 and 30.8% at a given site. On the contrary, ecomorphology was never significantly linked to PKD prevalence ($p < 0.05$). Therefore, presence of an upstream WWTP seemed to play a role in the predicted PKD prevalence, while IBCH score and ecomorphology had less influence.

Since both LPM and Logit models predicted that the mean temperature of June and the N days ≥ 15 explained well the variance of the infection prevalence, these two parameters were chosen for the following graphs. The LPM and Logit predicted PKD prevalence depending on the mean temperature of June, with study sites projection, are shown (**Figures 5A,B**). Infected fish were detected in stations with a mean temperature in June of 14°C at least. LPM predicted negative prevalence for temperature below 10°C, which highlighted limits of the model. On the contrary, the prediction issued from the Logit better corresponds to the observations. The predicted prevalence started to be positive from a mean temperature of June at 13°C and then increased. This model predicted a marked threshold effect from which infected fish may be found in the wild. The predictions of LPM and Logit models in function of the N days ≥ 15 are shown (**Figures 6A,B**). Infected fish were observed at sites with a mean temperature of ≥15°C for at least 12 days. The difference between the LPM and Logit model was weaker compared to the prediction of the mean temperature of June.

DISCUSSION

We performed a large-scale field investigation with longitudinal temperature measurements to demonstrate that temperature does indeed influence PKD prevalence and intensity in the wild. The possible combined influence of additional environmental parameters upon disease dynamics was also tested. Moreover, we developed a statistical model to highlight the predicted increase of PKD prevalence with rising water temperatures and/or additional environmental factors. Our analysis confirmed a positive relationship existing between water temperature and PKD prevalence and intensity in wild trout. The mean temperature of June and the N days ≥ 15 were the two parameters

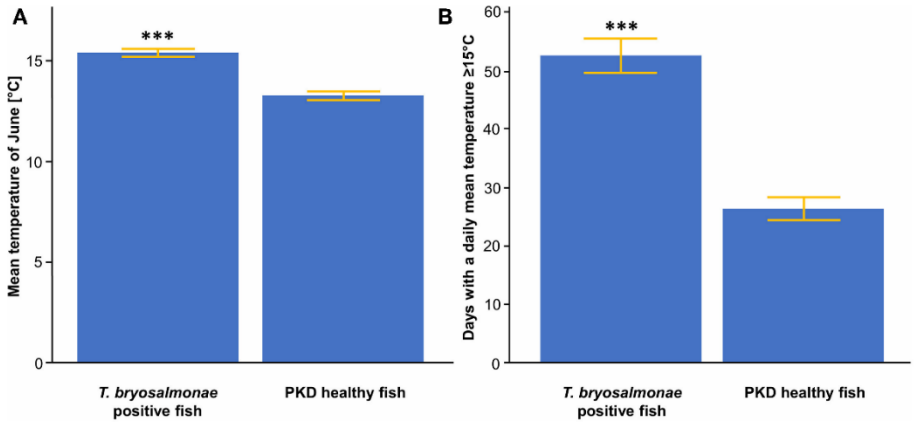


FIGURE 3 | (A) Mean temperature of June between *T. bryosalmonae* infected and PKD healthy fish. **(B)** Number of days with a daily mean temperature ≥ 15°C between *T. bryosalmonae* infected and PKD healthy fish. Yellow lines indicate standard error, asterisks indicate levels of significance (*t*-Test), ***$p < 0.001$.

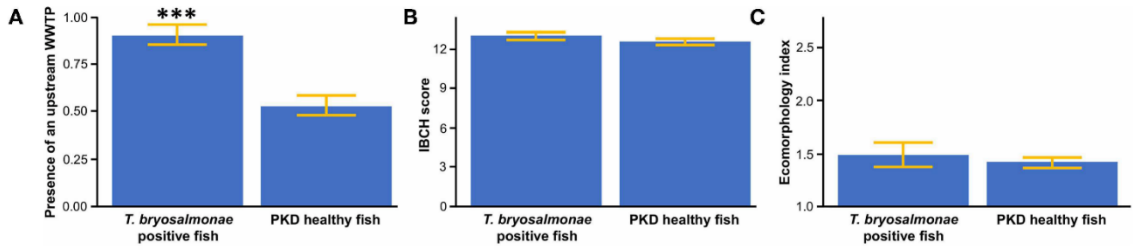


FIGURE 4 | (A) Mean presence of an upstream wastewater treatment plant (WWTP) between *T. bryosalmonae* infected and PKD healthy fish. **(B)** Mean biological score (IBCH) between *T. bryosalmonae* infected and PKD healthy fish. **(C)** Mean ecomorphology index between *T. bryosalmonae* infected and PKD healthy fish. Yellow lines indicate standard error, asterisks indicate levels of significance (*t*-test), ***$p < 0.001$.

that were most strongly associated with PKD prevalence. The mean temperature of the summer period (June to August) was also a strong indicator for the prevalence of infected fish. Depending on the models, the macroinvertebrate index might sometimes have a positive correlation with the disease prevalence. The presence of an upstream WWTP showed significant aggravating influence on the infection prevalence, while the ecomorphology had less importance. Logistic model had a better explanatory power than linear model. However, the variance of the models remained low, suggesting that other parameters than the factors tested here might also have an influence on the disease. Thus, our findings (1) increased the knowledge of the implication of water temperature in PKD prevalence and intensity by investigating the impact of long-term temperature data, with seasonal, diurnal and site-specific changes, on PKD infection, in a large number of field sites within a specific region, (2) determined other disease modulating factors, such as water quality, and (3) quantitatively predicted the further impact of environmental parameters on the given prevalence.

PKD Manifestation

In our study, PKD infected fish were generally detected in the downstream part of the rivers, while stations close to the source were free of the disease, as observed in the Boiron (upstream sites BOI011 and BOI013 were PKD free, while infected fish were found from the site RTV028 and downstream), the Venoge (no infected fish were observed in the site RTV055 but *T. bryosalmonae* positive fish appeared from station RTV054 and downstream) and the Veyron (upstream site RTV061 was free of the disease while parasites were found in downstream stations RTV060 and RTV085). Based on a study of 287 sampling sites throughout Switzerland, Wahli et al. (38) observed that the disease was present at an altitude lower than 800 m in most cases. Feist et al. (65) observed PKD prevalence ranging from 0 to 43% in England, which corresponds also to the findings of Peeler et al. (66) (PKD prevalence ranging from 2.5 to 36%). In our study, PKD prevalence ranged from 0 to 100% at a scale level of one canton, as observed by Wahli et al. (38) at a scale of a country. Therefore, PKD prevalence is also highly spatially variable, even within the same region.

Relationship Between Temperature and PKD Infection

The influence of water temperature on PKD infection has been investigated mostly in laboratory with rainbow trout as model species, but studies comprising *in situ* water temperature data sets over seasons and their impact on wild brown trout populations

Table 3a | Linear predicted increase/decrease of *T. bryosalmonae* infected fish prevalence due to an increase of 1 unit in the mean temperature variables and additional environmental parameters.

	Model 1	Model 2	Model 3	Model 4	Model 5
Mean temperature June	0.069*** (0.005)				
Mean temperature July		0.048*** (0.004)			
Mean temperature August			0.050*** (0.004)		
Mean temperature Summer				0.057*** (0.004)	
Total mean temperature					0.063*** (0.005)
IBCH (note)	0.006 (0.004)	0.013** (0.005)	0.014** (0.005)	0.012** (0.004)	0.013** (0.005)
WWTP	0.181*** (0.027)	0.230*** (0.026)	0.235*** (0.026)	0.216*** (0.026)	0.212*** (0.027)
Ecomorphology	−0.030* (0.014)	0.001 (0.015)	0.002 (0.015)	−0.006 (0.015)	−0.014 (0.015)
N	1,068	1,068	1,068	1,068	1,068
R2	0.211	0.181	0.188	0.196	0.169

Robust standard error in parentheses.
**p < 0.10, **p < 0.05, ***p < 0.01.*

Table 3b | Logistic predicted increase/decrease of *T. bryosalmonae* infected fish prevalence due to an increase of 1 unit in the mean temperature variables and additional environmental parameters.

	Model 1	Model 2	Model 3	Model 4	Model 5
Mean temperature June	0.099*** (0.007)				
Mean temperature July		0.065*** (0.006)			
Mean temperature August			0.069*** (0.006)		
Mean temperature Summer				0.084*** (0.007)	
Total mean temperature					0.100*** (0.010)
IBCH (note)	0.006 (0.004)	0.016*** (0.004)	0.019*** (0.004)	0.016*** (0.004)	0.020*** (0.004)
WWTP	0.190*** (0.025)	0.225*** (0.024)	0.235*** (0.024)	0.213*** (0.084)	0.206*** (0.250)
Ecomorphology	−0.021* (0.012)	0.021* (0.012)	0.026* (0.012)	0.015 (0.012)	0.001 (0.012)
N	1,068	1,068	1,068	1,068	1,068
Pseudo R2	0.373	0.299	0.315	0.339	0.296

Robust standard error in parentheses.
**p < 0.10, **p < 0.05, ***p < 0.01.*

are scarce. Wahli et al. (38) correlated PKD prevalence with altitude (as a substitution for temperature) in a wide variety of sites spread all over Switzerland, but they found no relationship between these two elements. On the contrary, in this study, a relationship between longitudinal temperature data and PKD prevalence and intensity was assessed within a field setting. *T. bryosalmonae* infection might appear when temperature reaches 9°C (36). Clinical signs and mortality are enhanced when the water temperature is >15°C (19, 23, 25, 39). Bettge et al. (18) showed that renal pathology of rainbow trout and parasite numbers were more intense at elevated temperature. Immune response strategy chosen by rainbow trout is also dependant of water temperature (37). Moreover, high temperature results in an accelerated rate for parasite release of brown trout, modifying therefore the parasite transmission period (33).

Our results do not support the findings of Gay et al. (36) since infected fish were observed in sites with warmer temperature than 9°C, even if trout were also sampled in colder streams. However, Gay et al. used rainbow trout as model species and experiments were performed in the laboratory. We also observed infected fish in sites with a minimum of 12 days with a daily mean of ≥15°C, which is slightly < the 14 to 28 days at 15°C predicted by Fischnetz (67) for PKD development in the wild. Lewisch et al. (68) found a significant increased number of *T. bryosalmonae* infected brown trout in Austria at sampling sites comprising a minimum of 115 days with at least 1 hourly maximum water temperature measurement exceeding 15°C. However, no long-term water temperature measurements at the precise sampling sites were used for these two studies, which might explain the differences with our results.

Based on statistical analysis, we observed that, when comparing the LPM and Logit, the Logit seemed to better fit to the data. This model should therefore be favored for further investigations. We found that the explanatory power of the variance from the mean temperature of summer (Pseudo R2 = 0.373) was also very close to the mean temperature of June results (Pseudo R2 = 0.339). Therefore, for a more global approach, the mean temperature of the summer period might also be a good indicator, instead of a precise month. Indeed, monthly temporal variations are more likely to depend in a particular

Table 4a | Linear predicted increase/decrease of *T. bryosalmonae* infected fish prevalence due to an increase of 1 unit in the in the number of days with a daily mean ≥ x°C variables and additional environmental parameters.

	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6	Model 7	Model 8
Days with daily mean ≥ 13°C	0.004*** (0.000)							
Days with daily mean ≥ 14°C		0.004*** (0.000)						
Days with daily mean ≥ 15°C			0.005*** (0.000)					
Days with daily mean ≥ 16°C				0.007*** (0.001)				
Days with daily mean ≥ 17°C					0.012*** (0.001)			
Days with daily mean ≥ 18°C						0.016*** (0.003)		
Days with daily mean ≥ 19°C							0.035*** (0.007)	
Degree days								0.004*** (0.000)
IBCH (note)	0.012* (0.005)	0.012** (0.004)	0.014** (0.004)	0.012** (0.004)	0.004 (0.004)	−0.001 (0.004)	−0.004 (0.004)	0.015** (0.005)
WWTP	0.222*** (0.026)	0.200*** (0.026)	0.191*** (0.027)	0.221*** (0.026)	0.265*** (0.026)	0.272*** (0.026)	0.290*** (0.026)	0.209*** (0.026)
Ecomorphology	−0.002 (0.015)	−0.002 (0.015)	−0.009 (0.015)	−0.024 (0.015)	−0.028 (0.015)	−0.02 (0.015)	−0.016 (0.016)	−0.018 (0.015)
N	1,068	1,068	1,068	1,068	1,068	1,068	1,068	1,068
R2	0.18	0.194	0.203	0.208	0.191	0.148	0.133	0.171

Robust standard error in parentheses.
**p < 0.10, **p < 0.05, ***p < 0.01.*

Table 4b | Logistic predicted increase/decrease of *T. bryosalmonae* infected fish prevalence due to an increase of 1 unit in the in the number of days with a daily mean ≥ x°C variables and additional environmental parameters.

	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6	Model 7	Model 8
Days with daily mean ≥ 13°C	0.005*** (0.000)							
Days with daily mean ≥ 14°C		0.005*** (0.000)						
Days with daily mean ≥ 15°C			0.005*** (0.000)					
Days with daily mean ≥ 16°C				0.006*** (0.001)				
Days with daily mean ≥ 17°C					0.010*** (0.001)			
Days with daily mean ≥ 18°C						0.013*** (0.002)		
Days with daily mean ≥ 19°C							0.031*** (0.005)	
Degree days								0.005*** (0.001)
IBCH (note)	0.015** (0.004)	0.017*** (0.004)	0.017*** (0.004)	0.014*** (0.004)	0.005 (0.004)	0.001 (0.004)	−0.002 (0.004)	0.021*** (0.004)
WWTP	0.225*** (0.024)	0.203*** (0.024)	0.201*** (0.025)	0.234*** (0.025)	0.282*** (0.026)	0.285*** (0.026)	0.308*** (0.027)	0.204*** (0.028)
Ecomorphology	0.017 (0.012)	0.022* (0.012)	0.015 (0.012)	−0.003 (0.012)	−0.013 (0.013)	−0.019 (0.014)	−0.015 (0.014)	−0.001 (0.001)
N	1,068	1,068	1,068	1,068	1,068	1,068	1,068	1,068
Pseudo R2	0.299	0.313	0.309	0.304	0.277	0.214	0.201	0.298

Robust standard error in parentheses.
**p < 0.10, **p < 0.05, ***p < 0.01.*

year than the mean of the whole summer season. Taking into account the summer mean temperature is therefore a possibility to reduce the influence of a specific month, in particular for year to year comparisons. Given the water temperature regime of a stream is known, our results permit to assess if the thermal conditions are reached for the development of the infection or to estimate the predicted increase of PKD prevalence following rising water temperature due to global warming. Solutions to prevent this increase of water temperature might therefore be proposed to counteract the possible spread of the disease. Our model estimated that an increase of one degree in the mean temperature of summer might result in an increase of 5.7% in infection prevalence. Therefore, with rising temperature in Switzerland (69–71) due to the ongoing global warming (72), the PKD infection prevalence might intensify in already infected zones possibly leading to the parasite colonizing new areas, thus, extending the geographic range of *T. bryosalmonae* (16, 19, 29). This phenomenon is also relevant for other emerging diseases sensitive to temperature (28, 30), such as furunculosis caused by the bacterium *Aeromonas salmonicida* (73) or enteric redmouth disease due to *Yersinia ruckeri* (74).

The Relationship Between Additional Environmental Factors and PKD Infection Prevalence

Studies have shown that low water quality can induce a decrease in freshwater biodiversity, such as macroinvertebrate community (56, 75, 76) or fish diversity (77, 78). In this study, we investigated

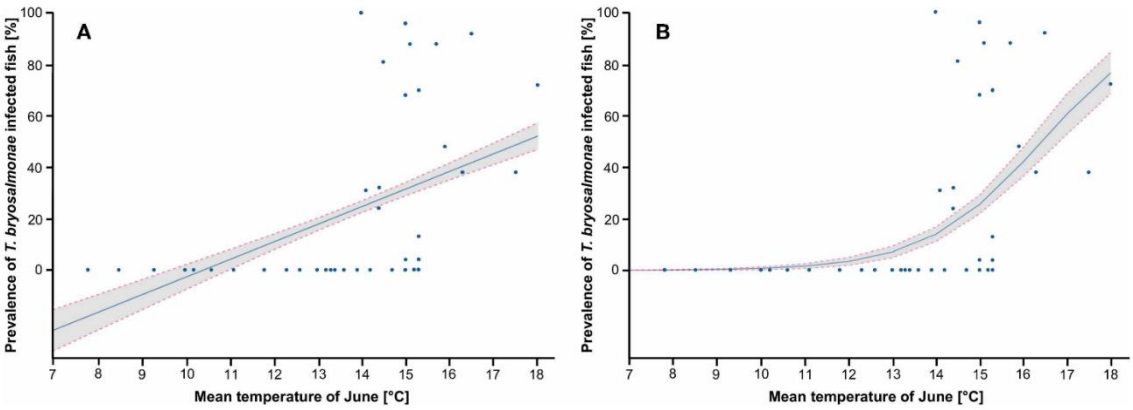


FIGURE 5 | Prediction of the prevalence of *T. bryosalmonae* infected fish depending on the mean temperature of June. **(A)** Prediction of the linear probability model (LPM), R2 is 0.211. **(B)** Prediction of the logistic model (Logit), Pseudo R2 is 0.373. Blue points correspond to study sites, blue line corresponds to the prediction, gray zone corresponds to the 95% confidence intervals.

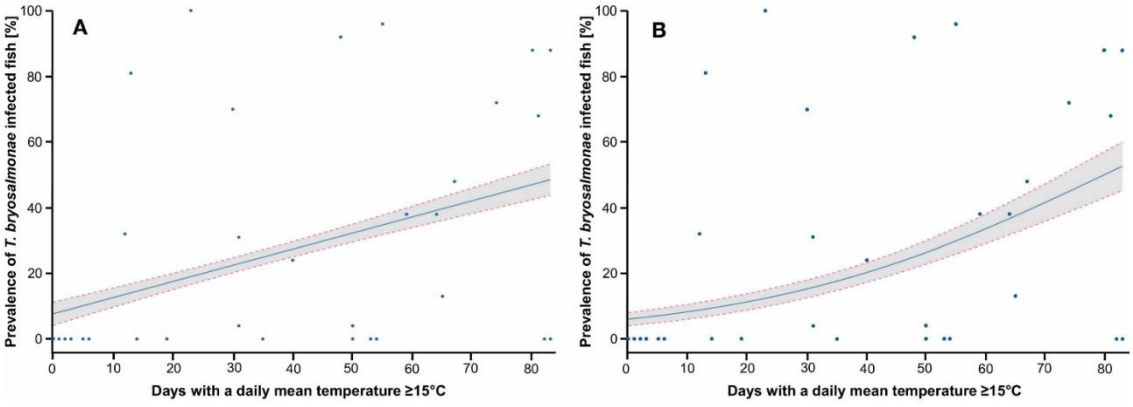


FIGURE 6 | Prediction of the prevalence of *T. bryosalmonae* infected fish depending on the number of days with a daily mean temperature ≥ 15°C. **(A)** Prediction of the linear probability model (LPM), R2 is 0.203. **(B)** Prediction of the logistic model (Logit), Pseudo R2 is 0.309. Blue points correspond to study sites, blue line corresponds to the prediction, gray zone corresponds to the 95% confidence intervals.

the combined effect of different environmental factors and their relationship with PKD disease dynamics. Our analyses revealed that PKD prevalence was not strongly influenced by the state of the macroinvertebrate community measured by means of the Swiss biological index "IBCH," which assessed water quality depending on the presence or absence of taxon sensitive to pollutants, as stoneflies for example. The presence of macroinvertebrate community is also driven by other factors, such as hydrology and habitat of a stream and does not focus only on water pollutants. The benthic fauna might therefore not be an ideal predictor for the disease presence.

We also analyzed the presence of an upstream WWTP as in indicator for water quality. Schmidt et al. (79) explored the effect of a sewage plant effluent on rainbow and brown trout health. When comparing trout kept in tap water and fish raised in water with an input of water coming from the WWTP, they found some alterations in internal organs but nothing that highlighted a real impact of low water quality on the decrease of brown trout populations. Bernet et al. (80) observed a decrease in brown trout health due to wastewater effluent. El-Matbouli and Hoffman (47) investigated the influence of water quality on *T. bryosalmonae*-rainbow and brown trout system. A significant difference in the PKD prevalence from sampled trout upstream and downstream of a WWTP effluent was observed. Significant differences in PKD prevalence and parasite intensity also appeared between two environmentally similar sites distant of 400 m, upstream and downstream of a WWTP in Switzerland (31). Our results corroborate these findings, since the presence of a WWTP appeared to be a significant aggravating parameter. Indeed, PKD prevalence increased by 20% if there was a WWTP present. However, the present study as well as the abovementioned referenced reports do not allow to assess if the decrease in water quality influences the disease prevalence either by directly affecting the fish health making them more vulnerable to the parasite infection, or influenced indirectly *T. bryosalmonae* infection by favoring the presence of

bryozoans, since polluted water seems to favor the proliferation of filter feeding bryozoans (19, 36) which, in turn, increases the number of parasites in water. For instance, Hartikainen et al. (81) showed that an increase in nutrient concentration promotes bryozoan biomass and growth rates of *Fredericella sultana*, the most common bryozoan hosting *T. bryosalmonae*. Moreover, the concentration of statoblast was significantly promoted by phosphorus concentrations. A possibility for discriminating these two hypotheses would be to test the direct impact of water quality on fish health by performing a controlled experiment with infected trout raised in good and low water quality. Therefore, even if water quality has been considerably improved by sewage treatment in Switzerland (60) and the majority of WWTP effluent respect the Swiss legislation (82), we found that WWTP effluents play a role in the infection and might thus be considered when studying PKD in the wild. However, the variable used here was "presence" or "absence" of a WWTP, therefore more detailed investigations of this factor should be conducted, such as taking into account the dilution ratio of the effluent in the stream, the distance between the WWTP and the study site or the inhabitant equivalent of WWTP, which might reveal further information on the influence of WWTP effluents.

We observed that ecomorphology was not a discriminant factor for PKD prevalence. Thus, this parameter does not seem to act directly on the infection. However, this factor might characterize the habitat complexity of the stream, which could impact the trout density (61–63). Moreover, a stream with canalized riverbanks without trees could lead to higher water temperature. With rising temperature due to global change, this situation could become even worse. Therefore, especially in the downstream part of anthropized rivers, some habitats are susceptible to turn thermally unsuitable for brown trout, which could lead to a population decrease (6), as suspected in the southern periphery of brown trout distribution range (83–85). Thus, restoration measures should be taken in rivers, as planting trees along open streams, which will thus reduce the temperature increase by making shadow or remove physical barriers to restore the migration to upstream thermal refuges.

CONCLUSION

Our results highlight the combined influence of water temperature, water quality and ecomorphology on PKD prevalence and intensity in wild brown trout populations. Water temperature appeared to be the major factor for PKD prevalence. When the necessary water temperature conditions are reached, the presence of an upstream WWTP might also play a role. These findings are crucial, especially in the context of global warming. Indeed, an increase in summer mean temperature and temperature variability might have consequences in the *T. bryosalmonae*-brown trout system, together with the potential geographical expansion of PKD. Therefore, knowing the significant parameters influencing the infection will allow to identify and to predict the potential disease hot spots in the future. Solutions to prevent this temperature increase and actions against the further spread of the disease might be proposed in terms of river management, mainly in downstream parts of river where water temperatures are higher and water quality is reduced.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the Swiss Confederation. The protocol of macrozoobenthos sampling was approved by the Service of Hunting, Fishing, and Surveillance of the Biodiversity and Landscape Division from the General Directorate of the canton of Vaud, and the protocol of fish sampling was approved by the Service of Consumption and Veterinary business of the canton of Vaud (permission number VD2871).

AUTHOR CONTRIBUTIONS

AR, J-FR, and TW conceived the experiment. PdC, AR, and J-FR performed the sampling. PdC and AR carried out the laboratory analysis. CB, PdC, AR, J-FR, HS, and TW analyzed the data. AR wrote the draft of the paper. All authors commented on the manuscript.

FUNDING

This study was partly funded by the General Directorate of the Environment from the canton of Vaud (DGE) and partly by the Federal Food Safety and Veterinary Office (FSVO) through the pilot program Adaptation to climate change. CB was funded by SNF Post Doc Mobility Fellowship number P400PB_183824.

ACKNOWLEDGMENTS

The authors wish to thank the department of Water Protection from the General Directorate of the Environment from the canton of Vaud (DGE-DIREV-PRE) for providing the IBCH index from eight stations and the fish-rangers for their help in the fish sampling. We also thank Quentin Gallea for his instruction in the statistical analysis.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2019.00281/full#supplementary-material

REFERENCES

1. Burkhardt-Holm P. Peter A, Segner H. Decline of fish catch in Switzerland. *Aquatic Sci.* (2002) 64:36–54. doi: 10.1007/s00027-002-8053-1
2. Burkhardt-Holm P, Giger W, Güttinger H, Peter A, Scheurer K, Suter MJF, et al. Where have all the fish gone? The reasons why fish catches in Swiss rivers are declining. *Environ Sci Technol.* (2005) 39:441–7. doi: 10.1021/es053375z
3. Burkhardt-Holm P, Scheurer K. Application of the weight-of-evidence approach to assess the decline of brown trout (*Salmo trutta*) in Swiss rivers. *Aquatic Sci.* (2007) 69:51–70. doi: 10.1007/s00027-006-0841-6
4. Burkhardt-Holm P. Decline of brown trout (*Salmo trutta*) in Switzerland - How to assess potential causes in a multi-factorial cause-effect relationship. *Mar Environ Res.* (2008) 66:181–2. doi: 10.1016/j.marenvres.2008.02.055
5. Borsuk ME, Reichert P, Peter A, Schager E, Burkhardt-Holm P. Assessing the decline of brown trout (*Salmo trutta*) in Swiss rivers using a Bayesian probability network. *Ecol Model.* (2006) 192:224–44. doi: 10.1016/j.ecolmodel.2005.07.006
6. Hari RE, Livingstone DM, Siber R, Burkhardt-holm P, Güttinger H. Consequences of climatic change for water temperature and brown trout populations in Alpine rivers and streams. *Glob Change Biol.* (2006) 12:10–26. doi: 10.1111/j.1365-2486.2005.001051.x
7. Zimmerli S, Bernet D, Burkhardt-Holm P, Schmidt-Posthaus H, Vonlanthen P, Wahli T, et al. Assessment of fish health status in for Swiss rivers showing a decline of brown trout catches. *Aquatic Sci.* (2007) 69:11–25. doi: 10.1007/s00027-006-0844-3
8. Wahli T, Knuesel R, Bernet D, Segner H, Pugovkin D, Burkhardt-Holm P, et al. Proliferative kidney disease in Switzerland: current state of knowledge. *J Fish Dis.* (2002) 25:491–500. doi: 10.1046/j.1365-2761.2002.00401.x
9. Schager E, Peter A, Burkhardt-Holm P. Status of young-of-the-year brown trout (*Salmo trutta fario*) in Swiss streams: factors influencing YOY trout recruitment. *Aquatic Sci.* (2007) 69:41–50. doi: 10.1007/s00027-007-0845-x
10. Wahli T, Bernet D, Steiner PA, Schmidt-Posthaus H. Geographic distribution of *Tetracapsuloides bryosalmonae* infected fish in Swiss rivers: an update. *Aquatic Sci.* (2007) 69:3–10. doi: 10.1007/s00027-006-0843-4
11. Canning E, Curry A, Feist S, Longshaw M, Okamura B. *Tetracapsula bryosalmonae* N. SP. for PKX organism, the cause of PKD in salmonid fish. *Bull Eur Assoc Fish Pathol.* (1999) 19:203–6.
12. Canning E, Tops S, Curry A, Wood T, Okamura B. Ecology, development and pathogenicity of *Buddenbrockia plumatellae* Schröder, 1910 (*Myxozoa,* Malacosporea) (syn. *Tetracapsula bryozoides*) and establishment of *Tetracapsuloides* n. gen. for *Tetracapsula bryosalmonae*. *J Eucaryotic Microbiol.* (2002) 49:280–95. doi: 10.1111/j.1550-7408.2002.tb00371.x
13. Anderson CL, Canning EU, Okamura B. Molecular data implicate bryozoans as hosts for PKX (phylum Myxozoa) and identify a clade of bryozoan parasites within the *Myxozoa*. *Parasitol.* (1999) 119:555–61. doi: 10.1017/S003118209900520X
14. Longshaw M, Le Deuff RM, Harris AF, Feist SW. Development of proliferative kidney disease in rainbow trout, *Oncorhynchus mykiss* (Walbaum), following short-term exposure to *Tetracapsula bryosalmononae* infected bryozoans. *J Fish Dis.* (2002) 25:443–9. doi: 10.1046/j.1365-2761.2002.00353.x
15. Morris DJ, Adams A. Transmission of *Tetracapsuloides bryosalmonae* (*Myxozoa*: Malacosporea), the causative organism of salmonid proliferative kidney disease, to the freshwater bryozoan *Fredericella sultana*. *Parasitology.* (2006) 133:701–9. doi: 10.1017/S003118200600093X
16. Tops S, Hartikainen H, Okamura B. The effect of infection by *Tetracapsuloides bryosalmonae* (*Myxozoa*) and temperature on *Fredericella sultana* (Bryozoa). *Int J Parasitol.* (2009) 39:1003–10. doi: 10.1016/j.ijpara.2009.01.007
17. Grabner DS, El-Matbouli M. *Tetracapsuloides bryosalmonae* (*Myxozoa*: Malacosporea) portal of entry into the fish host. *Dis Aquatic Organ.* (2010) 90:197–206. doi: 10.3354/dao02236
18. Bettge K, Wahli T, Segner H, Schmidt-Posthaus H. Proliferative kidney disease in rainbow trout: time- and temperature-related renal pathology and parasite distribution. *Dis. Aquatic Organ.* (2009) 83:67–76. doi: 10.3354/dao01989
19. Okamura B, Hartikainen HL, Schmidt-Posthaus H, Wahli T. Life cycle complexity, environmental change and the emerging status of salmonid proliferative kidney disease. *Freshw Biol.* (2011) 56:735–53. doi: 10.1111/j.1365-2427.2010.02465.x
20. Schmidt-Posthaus H, Hirschi R, Schneider E. Proliferative kidney disease in brown trout: infection level, pathology and mortality under field conditions. *Dis Aquatic Organ.* (2015) 114:139–46. doi: 10.3354/dao02855
21. Abd-Elfattah A, Kumar G, Soliman H, El-Matbouli M. Persistence of *Tetracapsuloides bryosalmonae* (*Myxozoa*) in chronically infected brown trout *Salmo trutta*. *Dis Aquatic Organ.* (2014) 111:41–9. doi: 10.3354/dao02768
22. Soliman H, Kumar G, El-Matbouli M. *Tetracapsuloides bryosalmonae* persists in brown trout *Salmo trutta* for five years post exposure. *Dis Aquatic Organ.* (2018) 127:151–6. doi: 10.3354/dao03200
23. Bettge K, Segner H, Burki R, Schmidt-Posthaus H, Wahli T. Proliferative kidney disease (PKD) of rainbow trout: temperature- and time-related changes of *Tetracapsuloides bryosalmonae* DNA in the kidney. *Parasitology.* (2009) 136:615–25. doi: 10.1017/S0031182009005800
24. Feist SW, Longshaw M. Phylum *myxozoa*. In: Woo PTK, editor. *Fish Diseases and Disorders. Protozoan and Metazoan Infections*, Vol. 1, 2nd ed. Oxfordshire: CAB International (2006). p. 230–96.
25. Bruneaux M, Visse M, Gross R, Pukk L, Saks L, Vasmägi A. Parasite infection and decrease thermal tolerance: impact of proliferative kidney disease on a wild salmonid fish in the context of climate change. *Funct Ecol.* (2017) 31:216–26. doi: 10.1111/1365-2435.12701
26. Marcogliese D. Implications of climate change for parasitism of animals in the aquatic environment. *Can J Zool.* (2001) 79:1331–52. doi: 10.1139/cjz-79-8-1331
27. Marcos-López M, Gale P, Oidtmann B, Peeler E. Assessing the impact of climate change on disease emergence in freshwater fish in the United Kingdom. *Transbound Emerg Dis.* (2010) 57:293–304. doi: 10.1111/j.1865-1682.2010.01150.x
28. Adlard R, Miller T, Smit N. The butterfly effect: parasite diversity, environment, and emerging disease in aquatic wildlife. *Trends Parasitol.*(2015) 31:160–6. doi: 10.1016/j.pt.2014.11.001
29. Carraro L, Bertuzzo E, Mari L, Fontes I, Hartikainen H, Strepparava N, et al. Integrated field, laboratory, and theoretical study of PKD spread in a Swiss prealpine river. *PNAS.* (2017) 114:11992–7. doi: 10.1073/pnas.1713691114
30. Debes PV, Gross R, Vasemägi A. Quantitative genetic variation in, and environmental effects on, pathogen resistance and temperature-dependent disease severity in a wild trout. *Am Natural.* (2017) 190:244–65. doi: 10.1086/692536
31. Bailey C, Rubin A, Strepparava N, Segner H, Rubin J-F, Wahli T. Do fish get wasted? Assessing the influence of effluents on parasitic infection of wild fish. *Peer J.* (2018) 6:e5956. doi: 10.7717/peerj.5956
32. Sterud E, Forseth T, Ugedal O, Poppe TT, Jørgensen A, Bruheim T, et al. Severe mortality in wild Atlantic salmon *Salmo salar* due to proliferative kidney disease (PKD) caused by *Tetracapsuloides bryosalmonae* (*Myxozoa*). *Dis Aquatic Organ.* (2007) 77:191–8. doi: 10.3354/dao01846
33. Strepparava N, Segner H, Ros A, Hartikainen H, Schmidt-Posthaus H, Wahli T. Temperature-related parasite infection dynamics: the case of proliferative kidney disease of brown trout. *Parasitology.* (2017) 145:281–91. doi: 10.1017/S0031182017001482
34. Foott JS, Hedrick RP. Seasonal occurrence of the infectious stage of proliferative kidney disease (PKD) and resistance of rainbow trout, *Salmo gairdneri* Richardson, to reinfection. *J Fish Biol.* (1987) 30:477–83. doi: 10.1111/j.1095-8649.1987.tb05771.x
35. De Kinkelin P, Loriot B. A water temperature regime which prevents the occurrence of proliferative kidney disease (PKD) in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *J Fish Dis.* (2001) 24:489–93. doi: 10.1046/j.1365-2761.2001.00312.x
36. Gay M, Okamura B, De Kinkelin P. Evidence that infectious stages of *Tetracapsula bryosalmonae* for rainbow trout *Oncorhynchus mykiss* are present throughout the year. *Dis Aquatic Organ.* (2001) 46:31–40. doi: 10.3354/dao046031
37. Bailey C, Segner H, Casanova-Nakayama A, Wahli T. Who needs the hotspot? The effect of temperature on the fish host immune response to *Tetracapsuloides bryosalmonae* the causative agent of proliferative kidney disease. *Fish Shellfish Immunol.* (2017) 63:424–37. doi: 10.1016/j.fsi.2017.02.039
38. Wahli T, Bernet D, Segner H, Schmidt-Posthaus H. Role of altitude and water temperature as regulating factors for the geographical distribution

of *Tetracapsuloides bryosalmonae* infected fishes in Switzerland. *J Fish Biol.* (2008) 73:2184–97. doi: 10.1111/j.1095-8649.2008.02054.x
39. Schmidt-Posthaus H, Bettge K, Forster U, Segner H, Wahli T. Kidney pathology and parasite intensity in rainbow trout *Oncorhynchus mykiss* surviving proliferative kidney disease: time course and influence of temperature. *Dis Aquatic Organ.* (2012) 97:207–18. doi: 10.3354/dao02417
40. Dash M, Vasemägi A. Proliferative kidney disease (PKD) agent *Tetracapsuloides bryosalmonae* in brown trout populations in Estonia. *Dis Aquatic Organ.* (2014) 109:139–48. doi: 10.3354/dao02731
41. Tops S, Lockwood W, Okamura B. Temperature-driven proliferation of *Tetracapsuloides bryosalmonae* in bryozoan hosts portends salmonid declines. *Dis Aquatic Organ.* (2006) 70:227–36. doi: 10.3354/dao070227
42. Jacobson KC, Arkoosh MR, Kagley AN, Clemons ER, Collier TK, Casillas E. Cumulative effects of natural and anthropogenic stress on immune function and disease resistance in juvenile Chinook salmon. *J Aquatic Anim Health.* (2003) 15:1–12. doi: 10.1577/1548-8667(2003)015<0001:CEONAA>2.0.CO;2
43. Thrush M, Murray A, Brun E, Wallace S, Peeler E. The application of risk and disease modelling to emerging freshwater diseases in wild aquatic animals. *Freshw Biol.* (2011) 56:658–75. doi: 10.1111/j.1365-2427.2010.02549.x
44. Graham DA, Staples C, Wilson CJ, Jewhurst H, Cherry K, Gordon A, et al. Biophysical properties of salmonid alphaviruses: influence of temperature and pH on virus survival. *J Fish Dis.* (2007) 30:533–43. doi: 10.1111/j.1365-2761.2007.00811.x
45. Marcogliese D. The impact of climate change on the parasites and infectious diseases of aquatic animals. *OIE Rev Sci Tech.* (2008) 27:467–84. doi: 10.20506/rst.27.2.1820
46. Schmidt-Posthaus H, Bernet D, Wahli T, Burkhardt-Holm, P. Morphological organ alterations and infectious diseases in brown trout *Salmo trutta* and rainbow trout *Oncorhynchus mykiss* exposed to polluted river water. *Dis Aquatic Organ.* (2001) 44:161–70. doi: 10.3354/dao044161
47. El-Matbouli M, Hoffmann RW. Influence of water quality on the outbreak of proliferative kidney disease - field studies and exposure experiments. *J Fish Dis.* (2002) 25:459–67. doi: 10.1046/j.1365-2761.2002.00393.x
48. Kumar G, Abd-Elfattah A, Saleh M, El-Matbouli M. Fate of *Tetracapsuloides bryosalmonae* (*Myxozoa*) after infection of brown trout *Salmo trutta* and rainbow trout *Oncorhynchus mykiss*. *Dis Aquatic Organ.* (2013) 107:9–18. doi: 10.3354/dao02665
49. Bailey C, Schmidt-Posthaus H, Segner H, Wahli T, Strepparava N. Are brown trout *Salmo trutta fario* and rainbow trout *Oncorhynchus mykiss* two of a kind? A comparative study of salmonids to temperature-influenced *Tetracapsuloides bryosalmonae* infection. *J Fish Dis.* (2018) 41:191–8. doi: 10.1111/jfd.12694
50. Tachet H, Richoux P, Bournaud M, Usseglio Polatera P. *Invertébrés D'eau Douce, Systématique, Biologie, Écologie*. Paris: CNRS&Editions (2000). p. 588.
51. Liess M, Von Der Ohe P. Analyzing effects of pesticides on invertebrate communities in stream. *Environ Toxicol Chem.* (2005) 24:954–65. doi: 10.1897/03-652.1
52. Schriever C, Hansler Ball M, Holmes C, Maund S, Liess M. Agricultural intensity and landscape structure: influences on the macroinvertebrate assemblages of small streams in northern Germany. *Environ Toxicol Chem.* (2007) 26:346–57. doi: 10.1897/05-629R.1
53. Federal Office for the Environment (FOEN). *Méthodes D'analyse et D'appréciation des cours d'eau, Macrozoobenthos—Niveau R (Région) (in French)*. Bern. (2010). Available online at: https://www.bafu.admin.ch/bafu/fr/home/themes/eaux/publications/publications-eaux/methodes-d-analyse-et-d-appreciation-des-cours-d-eau-en-suisse-macrozoobenthos.html
54. Marcel R, Bouchez A, Rimet F. Influence of herbicide contamination on diversity and ecological guilds of river diatoms. *Cryptogam Algol.* (2013) 34:169–83. doi: 10.7872/crya.v34.iss2.2013.169
55. Malaj E, Von der Ohea P, Groted M, Kühnee R, Mondyf C, Usseglio-Polaterag P, et al. Organic chemicals jeopardize the health of freshwater ecosystems on the continental scale. *PNAS.* (2014) 111:9549–54. doi: 10.1073/pnas.1321082111
56. Kattwinkel M, Reichert P, Rüegg J, Liess M, Schuwirth N. Modelling macroinvertebrate community dynamics in stream mesocosms contaminated with a pesticide. *Environ Sci Technol.* (2016) 50:3165–73. doi: 10.1021/acs.est.5b04068
57. Näslund J, Rosengren M, Del Villar D, Gansel L, Norrgård J, Persson L, et al. Hatchery tank enrichment affects cortisol levels and shelter-seeking in Atlantic salmon (*Salmo salar*). *Can J Fish Aquatic Sci.* (2013) 70:585–90. doi: 10.1139/cjfas-2012-0302
58. Rosengren M, Kvingedal E, Näslund J, Johnsson J, Sundell K. Born to be wild: effects of rearing density and environmental enrichment on stress, welfare, and smolt migration in hatchery-reared Atlantic salmon. *J Can Sci Halieutiq Aquatiq.* (2017) 74:396–405. doi: 10.1139/cjfas-2015-0515
59. Cogliati K, Herron C, Noakes D, Schreck C. Reduced stress response in juvenile Chinook Salmon reared with structure. *Aquaculture.* (2019) 504:96–101. doi: 10.1016/j.aquaculture.2019.01.056
60. Federal Office for the Environment (FOEN). *NADUF–Messresultate 1977–1998. Schriftenreihe Umwelt Nr.319 (in German)*. Bern: Federal Office for the Environment (FOEN); Swiss Federal Institute of Aquatic Science and Technology (EAWAG) (2000).
61. Horan D, Kershner J, Hawkins C, Crowl T. Effects of Habitat area and complexity on colorado river cutthroat trout density in uinta mountain streams. *Trans Am Fish Soc.* (2000) 129:1250–63. doi: 10.1577/1548-8659(2000)129<1250:EOHAAC>2.0.CO;2
62. Ayllón D, Almodóvara A, Nicolab G, Parraa I, Elvira B. Modelling carrying capacity dynamics for the conservation and management of territorial salmonids. *Fish Res.* (2012) 134–6:95–103. doi: 10.1016/j.fishres.2012.08.004
63. Federal Office for the Environment (FOEN). *Ecomorphologie des cours D'eau Suisses (in French)*. Bern (2009). Available online at: https://www.bafu.admin.ch/bafu/fr/home/themes/eaux/publications/publications-eaux/ecomorphologie-cours-eau-suisses.html
64. Federal Office of Topography (Swisstopo). *Ecomorphology Map of Switzerland*. Available online at: https://map.geo.admin.ch/ (accessed September 27, 2018).
65. Feist SW, Peeler E, Gardiner R, Smith E, Longshaw M. Proliferative kidney disease and renal myxosporidiosis in juvenile salmonids from rivers in England and Wales. *J Fish Dis.* (2002) 25:451–8. doi: 10.1046/j.1365-2761.2002.00361.x
66. Peeler EJ, Feist SW, Thrush MA, St-Hilaire S. An assessment of the variation in the prevalence of renal myxosporidiosis and hepatitis in wild brown trout, *Salmo trutta* L., within and between rivers in South-West England. *J Fish Dis.* (2008) 31:719–28. doi: 10.1111/j.1365-2761.2008.00942.x
67. Fischnetz. *Final Report of Project Fischnetz (in French)*. Bern: Eawag Dübendorf and BUWAL (2004).
68. Lewisch E, Unfer G, Pinter K, Bechter T, El-Matbouli M. Distribution and prevalence of *T. bryosalmonae* in Austria: a first survey of trout from rivers with a shrinking population. *J Fish Dis.* (2018) 41:1549–57. doi: 10.1111/jfd.12863
69. Federal Office of Meteorology and Climatology (MeteoSwiss). *Climate change in Switzerland*. Available online at: https://www.meteoswiss.admin.ch/home/climate/climate-change-in-switzerland.html (accessed June 6, 2019).
70. Beniston M. *Changements Climatiques et Impacts*. Lausanne: Presses Polytechniques et Universitaires Romandes (2012). p. 247.
71. Rebetez M. *La Suisse se Réchauf*fe. Lausanne: Presses Polytechniques et Universitaires Romandes (2014). p. 144.
72. IPCC. Summary for policymakers. In: *Global Warming of 1.5°C. An IPCC Special Report on the Impacts of Global Warming of 1.5°C Above Pre-Industrial Levels and Related Global Greenhouse Gas Emission Pathways, in the Context of Strengthening the Global Response to the Threat of Climate Change, Sustainable Development, and Efforts to Eradicate Poverty*. Geneva: World Meteorological Organization (2018). p. 32.
73. Nordmo R, Ramstad A. Variables affecting the challenge pressure of *Aeromonas salmonicidae* and *Vibrio salmonicida* in Atlantic salmon (*Salmo salar* L.). *Aquaculture.* (1999) 171:1–12. doi: 10.1016/S0044-8486(98)00426-8
74. Tobback E, Decostere A, Hermans K, Haesebrouck F, Chiers K. *Yersinia ruckeri* infections in salmonid fish. *J Fish Dis.* (2007) 30:257–68. doi: 10.1111/j.1365-2761.2007.00816.x
75. Duan X, Wang Z, Xu M. Effects of fluvial processes and human activities on stream macro-invertebrates. *Int J Sediment Res.* (2011) 26:416–30. doi: 10.1016/S1001-6279(12)60002-X
76. Chiu M, Hunt L, Resh V. Response of macroinvertebrate communities to temporal dynamics of pesticide mixtures: a case study from the Sacramento River watershed, California. *Environ Pollut.* (2016) 219:89–98. doi: 10.1016/j.envpol.2016.09.048

77. Shetty A, Venkateshwarlu M, Muralidharan M. Effect of water quality on the composition of fish communities in three coastal rivers of Karnataka, India. *Int J Aquatic Biol.* (2015) 3:42–51. doi: 10.22034/ijab.v3i1.46
78. Guo Q, Liu X, Ao X, Qin J, Wu X, Ouyang S. Fish diversity in the middle and lower reaches of the Ganjiang River of China: threats and conservation. *PLoS ONE.* (2018) 13:e0205116. doi: 10.1371/journal.pone.0205116
79. Schmidt H, Bernet D, Wahli T, Meier W, Burkhardt-Holm, P. Active biomonitoring with brown trout and rainbow trout in diluted sewage plant effluents. *J Fish Biol.* (1999) 54:585–96. doi: 10.1111/j.1095-8649.1999.tb00637.x
80. Bernet D, Schmidt H, Bernet D, Wahli T, Burkhardt-Holm P. Effects of treated waste water on infectious agents in brown trout. *Fish Ecol.* (2001) 12:1–16.
81. Hartikainen H, Johnes P, Moncrieff C, Okamura B. Bryozoan populations reflect nutrient enrichment and productivity gradients in rivers. *Freshw Biol.* (2009) 54:2320–34. doi: 10.1111/j.1365-2427.2009.02262.x
82. *Ordinance on Water Protection (in French).* (1998). Available online at: https://www.admin.ch/opc/fr/classified-compilation/19983281/201401010000/814.201.pdf
83. Jonsson B, Jonsson N. A review of the likely effects of climate change on anadromous Atlantic salmon *Salmo salar* and brown trout *Salmo trutta*, with particular reference to water temperature and flow. *J Fish Biol.* (2009) 75:2381–447. doi: 10.1111/j.1095-8649.2009.02380.x
84. Lassalle G, Rochard E. Impact of twenty-first century climate change on diadromous fish spread over Europe, North Africa and the Middle East. *Glob Change Biol.* (2009) 15:1072–89. doi: 10.1111/j.1365-2486.2008.01794.x
85. Almodóvar A, Nicola GG, Ayllón D, Elvira B. Global warming threatens the persistence of Mediterranean brown trout. *Glob Change Biol.* (2012) 18:1549–60. doi: 10.1111/j.1365-2486.2011.02608.x

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary Material:

Table S1: Data of temperature parameters related to sites.

Study sites data					Water temperature data													
Watershed	Site index	X coordinate	Y coordinate	Altitude [m]	Mean March [°C]	Mean April [°C]	Mean May [°C]	Mean July [°C]	Mean August [°C]	Mean spring (March-April-May) [°C]	Total mean [°C]	Maximum [°C]	Daily mean max [°C]	Days with daily mean ≥13°C	Days with daily mean ≥17°C	Days with daily mean ≥18°C	Days with daily mean ≥19°C	Degree days
Boiron	BOI011	522977	150418	439	7.6	9.9	10.9	14.8	14.7	9.5	12.0	18.0	16.6	84	0	0	0	2203
Boiron	BOI013	524491	149751	407	7.5	10.1	11.2	15.4	15.1	9.6	12.3	18.4	17.1	86	3	0	0	2270
Boiron	BOI020	524896	150320	397	9.0	11.4	12.5	16.7	16.8	11.0	13.6	18.5	18.3	102	27	2	0	2498
Boiron	RTV028	525286	150255	390	7.7	10.5	11.5	15.9	15.6	9.9	12.7	18.9	17.7	92	11	0	0	2346
Boiron	BOI018	525647	149944	383	7.2	10.1	11.4	16.0	16.3	9.6	12.7	17.7	17.7	86	5	0	0	2331
Boiron	BOI019	526021	149843	378	6.8	9.8	11.2	16.2	16.4	9.3	12.6	18.2	18.1	88	13	1	0	2318
Boiron	BOI015	526310	149480	373	8.2	11.2	12.2	16.4	16.5	10.5	13.4	18.0	17.9	101	19	0	0	2460
Dullive	D002	511788	143720	410	9.3	11.3	12.5	16.7	17.0	11.0	13.7	24.4	18.0	103	21	0	0	2518
Dullive	D001	511758	142863	390	8.8	10.3	11.0	13.5	13.6	10.0	11.6	18.9	15.2	65	0	0	0	2140
Dullive	RTV018	512072	142386	377	5.4	9.2	7.9	11.4	10.8	7.5	9.4	33.7	14.4	12	0	0	0	1733
Hongrin	RTV063	574280	139824	1389	1.3	3.3	5.2	8.6	8.6	3.3	5.9	13.7	10.4	0	0	0	0	1090
Torneresse	RTV062	577548	141318	1135	3.7	5.3	6.7	9.4	9.4	5.2	7.3	13.3	10.8	0	0	0	0	1340
Aubonne	RTV003	517221	152486	562	5.0	5.3	5.9	6.0	5.8	5.4	6.4	15.3	12.7	0	0	0	0	1553
Aubonne	MRP001	520670	147128	387	7.1	7.5	8.0	8.7	8.9	7.5	8.4	14.8	12.4	0	0	0	0	1169
Broye	RTV072	554527	155044	652	5.3	8.6	10.6	15.0	14.8	8.2	11.6	19.9	18.0	86	6	1	0	2131
Broye	RTV015	553384	155852	625	5.3	8.7	10.6	15.0	14.7	8.2	11.6	20.2	18.1	86	5	2	0	2131
Broye	RTV022	549098	159212	724	5.6	9.2	10.3	14.7	14.3	8.4	11.4	18.1	16.6	78	0	0	0	2091
Broye	RTV070	549941	162756	668	5.9	9.5	10.6	14.9	14.6	8.7	11.7	18.4	16.8	85	0	0	0	2146
Broye	RTV046	557627	173811	656	6.4	9.2	10.4	14.6	14.5	8.6	11.4	17.6	16.0	80	0	0	0	2092
Mentue	RTV074	544810	171780	562	5.6	9.4	10.8	15.4	14.9	8.6	11.8	19.8	17.7	86	6	0	0	2176
Mentue	RTV030	543934	177253	481	6.0	9.9	11.4	16.2	15.5	9.1	12.5	20.6	13.7	94	21	4	0	2300
Nozon	RTV037	519488	170861	941	4.1	6.2	7.1	9.6	8.6	5.8	7.8	18.7	18.5	6	0	0	0	1428
Nozon	RTV035	522292	172236	870	5.7	8.4	9.4	12.4	11.4	7.8	10.1	19.1	16.0	38	0	0	0	1857
Nozon	RTV036	530983	170027	448	7.4	9.9	10.6	13.7	12.7	9.3	11.4	17.7	16.0	60	0	0	0	2101
Orbe	RTV077	503859	158761	1028	4.1	8.7	11.1	15.7	15.6	8.0	12.1	26.7	20.6	94	37	21	11	2227
Orbe	RTV076	506113	160586	1014	4.5	7.1	9.2	12.9	13.3	6.9	10.4	21.4	18.1	53	9	2	0	1908
Orbe	RTV040	516672	172906	756	6.4	7.0	7.7	9.2	10.0	7.1	8.4	12.6	12.0	0	0	0	0	1544
Orbe	RTV092	517745	173550	750	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Orbe	RTV039	533272	176747	434	6.9	9.3	10.5	12.9	13.3	8.9	11.3	19.1	16.9	60	0	0	0	2080
Venoge	RTV055	522317	164378	639	8.2	8.4	8.8	9.8	9.4	8.5	9.4	15.5	13.3	1	0	0	0	1730
Venoge	RTV054	530241	166846	452	7.9	9.6	10.6	12.8	12.3	9.3	11.2	18.0	16.3	48	0	0	0	2069
Venoge	RTV053	529473	163453	432	7.9	9.8	11.4	14.5	13.9	9.7	12.3	23.9	18.7	81	14	4	0	2269
Venoge	RTV078	530615	156165	392	7.2	10.4	12.5	15.2	15.1	10.0	12.8	19.3	18.4	97	10	3	0	2351
Venoge	MRP005	531176	151787	375	8.3	11.3	13.0	16.5	15.7	10.8	13.8	21.3	20.6	107	44	30	8	2539
Veyron	RTV061	520403	159776	663	6.5	8.8	9.9	12.2	12.4	8.4	10.6	16.8	17.7	39	0	0	0	1943
Veyron	RTV060	521930	161657	633	6.3	8.4	10.2	12.5	12.5	8.3	10.7	19.9	16.0	52	3	0	0	1976
Veyron	RTV085	526720	165520	527	6.5	9.1	10.8	13.7	13.7	8.8	11.3	17.2	16.4	69	0	0	0	2076
Forestay	RTV024	548620	149614	603	7.3	10.1	11.2	15.3	14.9	9.5	12.3	19.3	17.4	86	2	0	0	2258
Lutrive	RTV029	542912	151613	507	6.9	9.9	10.9	15.3	14.9	9.2	12.2	23.7	18.4	88	12	1	0	2245
Paudèze	RTV041	541884	153119	608	5.4	8.8	10.1	15.1	14.4	8.1	11.4	20.6	17.3	79	3	0	0	2101
Promenthouse	RTV043	510031	140063	393	7.6	9.1	10.1	11.9	12.0	8.9	10.7	17.5	15.1	32	0	0	0	1971
Serine	RTV047	508635	148310	727	6.5	8.9	10.0	13.8	13.6	8.5	11.0	17.2	15.2	71	0	0	0	2023
Talent	RTV050	541978	160294	760	4.5	7.8	9.3	13.4	12.9	7.2	10.2	17.2	15.5	54	0	0	0	1869
Vaux	RTV052	549314	179487	655	6.3	8.7	9.8	13.6	13.9	8.3	10.8	17.7	15.4	58	0	0	0	1983
Versoix	RTV080	499369	131241	464	4.0	5.1	5.8	7.5	7.8	5.0	6.4	11.0	9.3	0	0	0	0	1169

NA = no data for this site

4 Do fish get wasted? Assessing the influence of effluents on parasitic infection of wild fish

Christyn Bailey, Aurélie Rubin, Nicole Strepparava, Helmut Segner, Jean-François Rubin and Thomas Wahli

Paper published in *PeerJ, 6, e5956, November 2018.*

Do fish get wasted? Assessing the influence of effluents on parasitic infection of wild fish

Christyn Bailey[1], Aurélie Rubin[1,2], Nicole Strepparava[1], Helmut Segner[1], Jean-François Rubin[2,3] and Thomas Wahli[1]

[1] Department of Infectious Diseases and Pathobiology, Vetsuisse Faculty, Centre for Fish and Wildlife Health, University of Bern, Bern, Switzerland
[2] University of Applied Sciences, Hepia, Geneva, Switzerland
[3] Maison de la Rivière, Tolochenaz, Switzerland

ABSTRACT

Many ecosystems are influenced simultaneously by multiple stressors. One important environmental stressor is aquatic pollution via wastewater treatment plant (WWTP) effluents. WWTP effluents may contribute to eutrophication or contain anthropogenic contaminants that directly and/or indirectly influence aquatic wildlife. Both eutrophication and exposure to anthropogenic contaminants may affect the dynamics of fish-parasite systems. With this in mind, we studied the impact of WWTP effluents on infection of brown trout by the parasite *Tetracapsuloides bryosalmonae*, the causative agent of proliferative kidney disease (PKD). PKD is associated with the long-term decline of wild brown trout (*Salmo trutta*) populations in Switzerland. We investigated PKD infection of brown trout at two adjacent sites (≈400 m apart) of a Swiss river. The sites are similar in terms of ecology except that one site receives WWTP effluents. We evaluated the hypothesis that fish inhabiting the effluent site will show greater susceptibility to PKD in terms of prevalence and disease outcome. We assessed susceptibility by (i) infection prevalence, (ii) parasite intensity, (iii) host health in terms of pathology, and (iv) estimated apparent survival rate. At different time points during the study, significant differences between sites concerning all measured parameters were found, thus providing evidence of the influence of effluents on parasitic infection of fish in our study system. However, from these findings we cannot determine if the effluent has a direct influence on the fish host via altering its ability to manage the parasite, or indirectly on the parasite or the invertebrate host via increasing bryozoa (the invertebrate host) reproduction. On a final note, the WWTP adhered to all national guidelines and the effluent only resulted in a minor water quality reduction assessed via standardized methods in this study. Thus, we provide evidence that even a subtle decrease in water quality, resulting in small-scale pollution can have consequences for wildlife.

Submitted 7 June 2018
Accepted 17 October 2018
Published 13 November 2018

Corresponding author
Christyn Bailey,
christyn.bailey@vetsuisse.unibe.ch

Academic editor
Esteban María Ángeles

Additional Information and Declarations can be found on page 16

DOI **10.7717/peerj.5956**

Subjects Aquaculture, Fisheries and Fish Science, Parasitology, Ecotoxicology, Freshwater Biology

Keywords Wild fish, Aquatic pollution, Wastewater, Multiple stressors, Host-parasite interactions, PKD, *Tetracapsuloides bryosalmonae*

INTRODUCTION

Proliferative kidney disease (PKD) has been strongly associated with the long-term decline of wild brown trout (*Salmo trutta*) populations in Switzerland (*Burkhardt-Holm, 2002*; *Wahli et al., 2002*, *2007*). PKD is caused by the myxozoan parasite *Tetracapsuloides bryosalmonae*. *T. bryosalmonae* has a two-host life cycle, comprising of salmonid fish (the vertebrate host) and freshwater bryozoa (the invertebrate host). Infective *T. bryosalmonae* spores are released from the bryozoans into the water. On encounter of a suitable fish host, spores infect the fish through the gills and/or skin and are then transported by the circulatory system to the target organs, primarily the kidney (*Clifton-Hadley, Bucke & Richards, 1987*; *Hedrick, Monge & De Kinkelin, 1992*). In the kidney, *T. bryosalmonae* penetrates the interstitial tissue, multiplies and differentiates from extrasporogonic to sporogenic stages (*Longshaw et al., 2002*). During PKD infection, a huge renal swelling and mortality may occur in severely diseased fish (*Kent & Hedrick, 1985*; *Hedrick, MacConnell & De Kinkelin, 1993*).

In Switzerland, elevated water temperatures have been suggested to exacerbate PKD infections and drive disease related mortality of wild brown trout (*Wahli et al., 2002*, *2007*). Moreover, several lab and field studies have demonstrated the influence of temperature on host-parasite dynamics in the salmonid—*T. bryosalmonae* system (*Bettge et al., 2009a*, *2009b*; *Bailey et al., 2017*, *2018*; *Strepparava et al., 2017*). However, given the strong interference of human activities with the diversity and functioning of freshwater ecosystems (*Søndergaard & Jeppesen, 2007*; *Vörösmarty et al., 2010*; *Dodds, Perkin & Gerken, 2013*), PKD dynamics may not only be driven by temperature change, but by multiple stressors. In fact, a number of laboratory and field studies have already demonstrated the influence of multiple stressors upon aquatic wildlife (*Johnson et al., 2007*; *Rohr et al., 2008*; *Acevedo-Whitehouse & Duffus, 2009*; *Buck et al., 2012*; *Segner, Schmitt-Jansen & Sabater, 2014*) and, more specifically, for diseases of freshwater fish (*Schisler, Bergersen & Walker, 2000*; *Jacobson et al., 2003*; *Peeler & Feist, 2011*; *Johnson & Sumpter, 2014*). The cumulative impact of multiple stressors may ensue in nonlinear effects and ecological surprises (*Segner, Schmitt-Jansen & Sabater, 2014*).

One important environmental stressor in freshwater ecosystems is pollution. Both eutrophication and exposure to anthropogenic contaminants have been shown to affect the manifestation and dynamics of infectious diseases of various aquatic species (*Johnson et al., 2007*; *Rohr et al., 2008*; *Peeler & Feist, 2011*) and are able to influence fish-parasite systems (*Poulin, 1992*; *Blanar et al., 2009*; *Vidal-Martínez et al., 2010*). Gross aquatic pollution has been reduced in recent decades through the construction of wastewater treatment plants (WWTPs). Although depending on the quality of the WWTPs, a mix of micropollutants, microorganisms, or nutrients can make their way into the waterbody and decrease water quality (*Daughton & Ternes, 1999*; *Heberer, 2002*; *Costanzo, Murby & Bates, 2005*; *Stackelberg et al., 2007*; *Glassmeyer et al., 2008*). This is particularly pronounced when the dilution factor of the wastewater in the receiving freshwater system is low, as is often the case in small streams or during the dry season.

Aquatic pollution may contribute to infectious disease processes in following ways: (1) directly influencing the resistance of the host, through adverse effects on multiple

physiological functions, including the immune system, (2) enhancing the replication rate of pathogens, (3) indirectly influencing the abundance and distribution of pathogens hosts and vectors and/or (4) influencing the transmission of infectious stages. For instance, micropollutants including pharmaceuticals such as diclofenac, hormonally active compounds or chemicals activating the aryl hydrocarbon receptor possess the potential to compromise the immunocompetence of fish, and thereby increase their susceptibility to pathogens (*Arkoosh et al., 2010*; *Casanova-Nakayama et al., 2011*; *Arkoosh et al., 2015*; *Rehberger et al., 2017*). Or, sewage-derived organic enrichment of sediments, resulting in bottom-up effects via increasing populations of invertebrate hosts, leading to greater parasite species richness and subsequently increased presence of parasitic infection in fish can occur (*Marcogliese & Cone, 2001*; *Krueger et al., 2006*; *McKenzie & Townsend, 2007*). Either way, directly and/or indirectly the presence of aquatic pollution and its potential influence on host-parasite interactions is a massive concern for wildlife and one that clearly requires much more attention.

Considering, both the evidence for the impact of PKD on wild salmonids and the influence pollution may have upon aquatic diseases, thus far only one study has attempted to explore the influence of a decrease in water quality on the salmonid—*T. bryosalmonae* host-parasite system. *El-Matbouli & Hoffmann (2002)* studied the effect of WWTP effluents on the prevalence of PKD infection in farmed rainbow trout (*Oncorhynchus mykiss*) and wild brown trout. They observed that infection prevalence decreased in both farmed and wild fish populations after the effluents were removed. While their study provided some initial indications that PKD may be influenced by pollution it was limited by the overall low prevalence of PKD in wild fish (*El-Matbouli & Hoffmann, 2002*). Taking this into account, and considering that over the last decade, greater knowledge has been generated concerning the relationship between fish disease and pollution it is undoubtedly time to readdress the PKD-pollution question and broaden our horizons relating to this potential interaction. Such a study can complement earlier findings and elucidate insight into the combined effects of a chemical and natural stressor and its effects upon wildlife.

With this in mind, an ideal study site in Switzerland is provided at the Boiron de Morges. Here two stations in close proximity (~400 m), the Amont step and the Aval step in the river Boiron exist. These stations are almost identical in terms of ecology and both PKD positive sites, inhabited by bryozoans, brown trout, and parasites and experience virtually matching water temperatures throughout the year. Furthermore, river structure is the same. However, the caveat is that the decrease in biological water quality from the Amont to the Aval step triggered by WWTP effluents may be too subtle to have a clear-cut impact on PKD.

The goal of this study was to explore the implications of altered water quality for parasitic infection of wild fish. More specifically, we tested the hypothesis that brown trout inhabiting the stretch downstream to the WWTP effluent (Aval step) will show greater susceptibility to PKD infection than trout at the upstream site. We investigated susceptibility by (i) infection prevalence, (ii) parasite intensity, and (iii) host health: in terms of disease driven pathology. We predicted that PKD infection

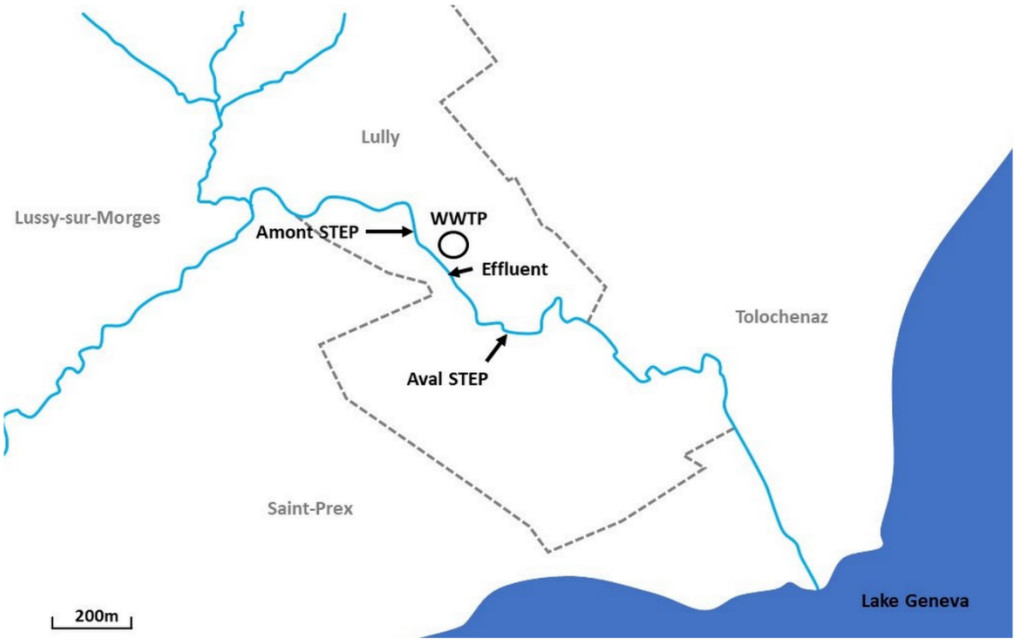


Figure 1 Location of sampling sites along the Boiron in the canton of Vaud, Switzerland. Two sites, a short distance apart (~400 m) were sampled: the Amont step (46°29′56.171″N 6°28′01.204″E) and the Aval step (46°29′47.961″N 6°28′13.301″E). Also included is the location of the wastewater treatment plant (WWTP) (46°29′52.513″N 6°28′05.500″E) and the effluent (just below the highway).
Full-size DOI: 10.7717/peerj.5956/fig-1

prevalence, parasite intensity and the impact on host health in terms of pathology would be increased in fish at the Aval step. In addition, we evaluated (iv) estimated apparent survival rate and mortality of fish at each site. We expected that at the Aval step there would be a decreased apparent survival rate as an outcome of the increase in PKD susceptibility, leading to greater mortality in comparison to the Amont step. For these purposes, the field campaign consisted of monthly samplings at both sites from June to October 2017. Infection prevalence and parasite intensity were measured via concentration of parasite DNA copies in the fish kidney. Pathological changes in the kidneys of infected fish were examined by histopathology. Furthermore, we used the capture-mark-recapture method to estimate apparent fish survival and mortality at each site.

MATERIALS AND METHODS

Study sites

Samplings were performed at the Boiron de Morges in the canton of Vaud, Switzerland. The Boiron (14 km long) is a stream that flows into Lake Geneva. Two sites, at a short distance apart (~400 m) were sampled: the Amont step (46°29′56.171″N 6°28′01.204″E) and the Aval step (46°29′47.961″N 6°28′13.301″E) (Fig. 1). No tributary reaches the stream between these two sites, except the arrival of the WWTP effluent (46°29′52.513″N 6°28′05.500″E) upstream from the Aval step (also Fig. 1).

While there is no barrier preventing migration between the Amont and Aval, based on almost 20 years of field investigations performed at the Boiron; the majority of young-of-the-year (YOY) brown trout do not migrate between the sites until they reach juvenile or adult stages (J-F. Rubin, 2018, personal communication). In our study, we only investigated YOY fish. YOY fish are defined as age 0 fish, that is, those fish born within the year of sampling. Moreover, based on electrofishing and a fish tagging investigation described in detail within this study, only three out of 69 YOY fish tagged at the Amont step were detected downstream at the Aval step, whereas only one out of 99 YOY fish tagged at the Aval step was found upstream at the Amont step. Therefore, we exclude migration between the sites as a confounding variable. This means we consider only fish sampled at the Aval step to be influenced by the WWTP.

WWTP effluent

The Aval step receives effluent from the WWTP of Lully–Lussy, which treats sewage from 1,412 inhabitants, with no industries or hospitals in the area, the majority of the waste water treated at the WWTP originates from households (*Direction générale de l'environnement, 2017*). The WWTP uses a combined fluidized bed reactor and activated sludge system. The WWTP is equipped for nitrification (removal of nitrogen) and a reed bed sludge treatment system.

During period of low-water level, the "Q347" of the river Boiron (defined as the flow rate which, averaged over 10 years, is reached or exceeded on an average of 347 days per year and which is not substantially affected by damming, withdrawal, or supply of water, that is, 95% of the time) at the level of the WWTP is 42 l/s. The dilution ratio between wastewater and river water is 1:14 (1 volume of wastewater per 14 volume of river water). This indicates the effluent represents 7% (1/14 = 7%) of the water flow.

At the WWTP outlet, the concentration of the 5 day biochemical oxygen demand, which represents the biodegradable organic matter, reaches a concentration of 4.0 mg O_2/l. The output for the chemical oxygen demand that quantifies the oxidizable materials has a value of 32 mg O_2/l. The organic carbon intensity reaches a concentration of 8.0 mg C/l. Finally, a concentration of 1.1 mg N/NH4 is measured at the WWTP outlet (*Direction générale de l'environnement, 2017*). All these values correctly adhere to the national standards enacted by the Federal Office for the Environment (FOEN). In this manner, nationally as well as internationally the effluent is not deemed an illegal or in no circumstances major source of pollution (*Direction générale de l'environnement, 2017*). However, a mixture of micropollutants, microorganisms, excessive nutrients and/or metals in low concentrations may remain untreated or poorly treated within the WWTP and make their way into the waterbody and thus decrease water quality.

Water quality assessment

To determine the difference in water quality, a standardized method according to the guidelines of FOEN was used. This technique uses the IBCH index (Indice biologique suisse - The standardized biological index adapted to Switzerland) which indicates the ecological status of the site taking into account water quality, habitat morphology, and

hydrology via, the evaluation of macrozoobenthos (*Federal Office for the Environment (FOEN), 2011*). Depending on the sensitivity to water quality of the observed taxon, a score between 0 (very poor ecological quality) and 20 (excellent ecological status) is defined for each investigated site. Samplings were performed in March 2015. The General Directorate of the Environment from the canton of Vaud (DGE) collected and determined the material from the Amont step station while we performed the sampling and analysis of the Aval step station. Macrozoobenthos material was sampled according to field study approvals from the DGE.

Water temperature

Water temperature is an essential mechanism modulating PKD-induced clinical signs and mortality (*Bettge et al., 2009a*, *2009b*; *Bailey et al., 2017*, 2018; *Strepparava et al., 2017*). Importantly, if water temperature is very much identical at both sites, then any difference in PKD infection dynamics is not driven by water temperature but by another factor. To confirm that the water temperature was comparable at both sampling sites throughout the sampling year, it was examined with temperature loggers (HOBO® Water Temp Pro v2 Data Logger, Onset, Cape Cod, MA, USA) recording data every 15 min over a period for the year. In addition, we also compared the amount of days with water temperatures $\geq$15 °C, as this critical temperature threshold is linked with elevations in PKD related mortality (*Ferguson, 1981*; *Clifton-Hadley, Richards & Bucke, 1986*).

Fish sampling

No restocking is done at either site, therefore all fish sampled in the study are wild. Monthly samplings from June to October 2017, were performed at each site. At each sampling 25 YOY brown trout per site were captured by electrofishing over a section of 100 m long (N = 125 experiment total per site). YOY fish sampled here would have hatched from eggs spawned in March, being around 3-months-old at the first sampling (Rubin, 2018, personal communication). Therefore, these fish would have had a fully competent immune system (*Magnadóttir et al., 2005*). After capture, fish were euthanized using 3-aminobenzoic acid ethyl ester (MS 222®; Argent Chemical Laboratories, Redmond, WA, USA). Total length and total weight were recorded. The kidney was carefully removed and cut in two halves longitudinally: one part was weighed (kidney weight, KW) and stored in one ml RNAlater (Qiagen, Basel, Switzerland) for downstream DNA extraction, while the other half was put in formalin to be prepared for histology. Fish and ICBH samplings were carried out relating to ethical approvals (Service of Consumption and Veterinary business of the canton of Vaud, permission number VD3253).

Fish tagging

In July at both sites, additional YOY fish were captured and tagged with a passive integrated transponder-tag before release. The tags allowed us to track fish movements using a portable antenna and investigate apparent survival rates and fish mortality at each site. A total of 69 YOY fish from the Amont step and 99 YOY fish from the Aval step were captured and tagged. After capture, fish were anesthetized (MS 222) and a small

cut with a scalpel was performed above the pelvic fins. The tag was injected in the abdominal cavity and its unique number was obtained with a reader.

In October, tagged fish were recaptured to determine the apparent survival rate, which is calculated as the number of tagged recaptured fish divided by the total amount of tagged fish. At the same sampling before returning the recaptured fish to the sampling sites, we used the portable antenna to detect the remaining tags. All detected tags found on the river floor were considered as dead fish and thus the percent mortality was calculated from these samples. Individual fish not recaptured or detected were deemed to have emigrated. It could be assumed that these individuals may have dispersed and died, although this is impossible to confirm. Thus, for the sake of the study, these fish were defined as emigrated. In addition, through monitoring movement of tagged fish allowed us to investigate if migration was occurring between the sampling sites.

Determination of infection prevalence and parasite intensity

Proliferative kidney disease infection prevalence and parasite intensity were assessed by qPCR. Approximately 25 mg of tissue was used for DNA extraction. DNA extractions were carried out using the Blood and Tissue DNA extraction kit (QIAGEN, Basel, Switzerland) following manufacturer's guidelines. Then DNA was eluted with 100 µl EB buffer, provided in the kit and stored at −20 °C, until qPCR was carried out. qPCR was performed with primers and probe according to *Strepparava et al. (2017)* for this model, using an Applied Biosystem analyser (Applied Biosystems, Rotkreuz, Switzerland). The qPCR was carried out in a final volume of 20 µl containing 1× TaqMan universal Master Mix (Applied Biosystems, Switzerland), 0.5 µM of each primer (PKDtaqf1: 5′-GCGAGA TTTGTTGCATTTAAAAAG-3′ and PKDtaqr1: 5′-GCACATGCAGTGTCCAATCG-3′), 0.2 µM of the probe PKD (5′-CAAAATTGTGGAACCGTCCGACTACGA-3′) labelled with FAM-TAMRA, 1× of IC DNA (TaqMan Univ. MMix w Exog IntPostC; Applied Biosystems, Switzerland), and two µl of template DNA. Standard curves were generated for each qPCR cycle using plasmids containing the amplified fragment. For all plate 5 logs of plasmid dilution standards were amplified (from 10^6–10^2 gene copies number). To concur reliability of the qPCR, the coefficient of the standard regression had to be in the range −3.6 to −3.0 as per the manufacturer's instructions (Applied Biosystems, Switzerland), the coefficient of variation of quantification within each standard and sample in duplicate had not to surpass 25% and the non-target control (water) had to show no amplification (*Mackay, 2004*; *Joly & Bruneau, 2006*; *Yun et al., 2006*; *Hellemans et al., 2007*). If reliability criteria were not met, the qPCR was repeated. Percent prevalence was calculated per each time point, while parasite intensity (DNA copy number) was taken for each individual and standardized to KW.

Histological assessment

Following routine processing and paraffin embedding, kidney sections of 3–5 µm thickness were prepared on SuperFrost® Plus positively charged glass slides. The slides were stained with hematoxylin and eosin for histological examination. Histopathological alterations throughout the kidney sections were assessed, these included, tissue

proliferation, infection degree (presence and distribution of parasites), and presence of fibrous tissue. All parameters were scored individually 0–6. The degree of tissue proliferation in terms of proliferation/pathological alterations was scored as: 0 (none), 1 (scattered), 2 (mild), 3 (mild to moderate), 4 (moderate), 5 (moderate to severe), or 6 (severe), modified from (*Schmidt-Posthaus et al., 2012*). Concerning infection degree: 0 indicated no presence of parasites, whereby a 6 specified a very high number of parasites per view. Relating to the presence of fibrous tissue: fibrous tissue is an indicator of the tissue regeneration process which is stimulated to recover/regenerate organ structure and function. Its presence has been observed in salmonids recovering from PKD infection (*Schmidt-Posthaus et al., 2012*, *2017*). Therefore, a strong score in this category from one sample site in comparison to the other sample site may indicate a fish in recovery stage. Presence of fibrous tissue was thus also scored from 0–6 using the same index as tissue proliferation (*Schmidt-Posthaus et al., 2012*). This scoring system was used for statistical investigation between the two sites. One slide was evaluated per fish.

To further evaluate the variance in infection dynamics between the sites we examined the relationship between infection variables host health and parasite intensity. In this manner, we used increasing tissue proliferation as a proxy for decreasing host health during infection, as increasing proliferation in the kidney during PKD infection results from an enhanced inflammatory response and associated nephritis, corresponding with host immunopathology and even mortality.

Statistical analysis

The statistical differences between sites were tested for significant differences using a *t*-test. Correlations between host health and parasite intensity were assessed by calculating the Pearson product—moment correlation coefficient (r). All tests were performed using SigmaPlot 12.0 (Systat Software, San Jose, CA, USA) and graphically presented with GraphPad Prism 5 (GraphPad Software, Inc., San Diego, CA, USA) Excel 2010 (Microsoft, Redmond, WA, USA) or SigmaPlot 12.0. Significance was set at $P \leq 0.05$.

RESULTS

Water quality assessment

As an integrative parameter to assess the ecological status at the Amont and Aval sites, we used the IBCH index, which is based on the evaluation of macrozoobenthos. Using this methodology, the Amont step reached a score of 14, and the Aval step a score of 12—on a scale ranging from 0 (very poor ecological quality) to 20 (excellent ecological status). Based on this data, the ecological status at both sites appears to be moderate to good, as indicated from the IBCH scores, and, importantly, there is only a subtle difference between the two sites.

Water temperature assessment

We used data loggers to monitor water temperature at the two study sites. As temperature is a key driver of PKD infection and disease severity it was critical to our study that water temperature was nearly identical at the sites. Importantly water temperatures recorded

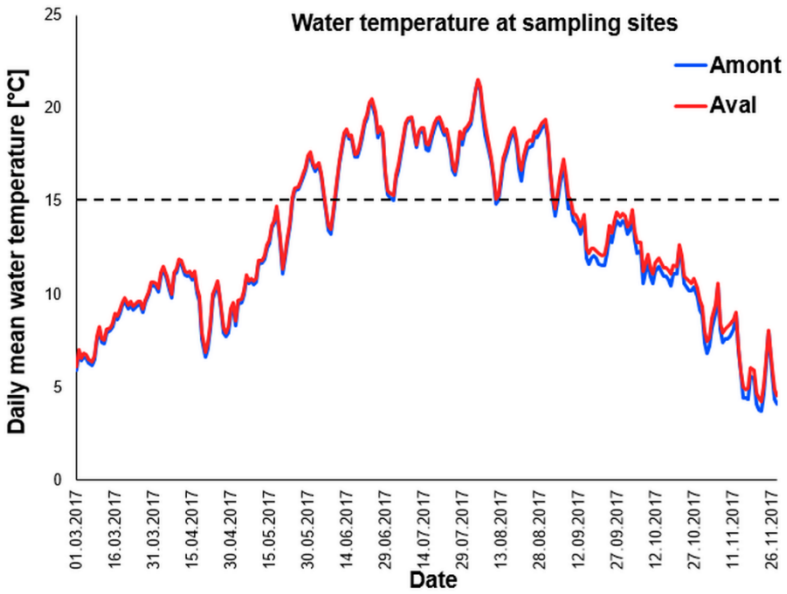


Figure 2 Daily mean temperature of sampling sites. Water temperature curves measured throughout the year at the sampling sites the Amont step (blue line) and the Aval step (red line). Black dotted line indicates 15 °C, the critical water temperature for proliferative kidney disease-related clinical signs and mortality in trout. Full-size DOI: 10.7717/peerj.5956/fig-2

Table 1 Total number of days with daily mean water temperature ≥15 °C at each sampling at Amont and Aval step.

Monthly sampling	Amont	Aval
June	16	17
July	36	37
August	60	61
September	88	90
October	99	104

Note:
15 °C is the critical water temperature for proliferative kidney disease-related clinical signs and mortality in trout.

throughout the sampling campaign and beyond; March until November 2017 followed an almost identical pattern at the two study sites (Fig. 2). Furthermore, when comparing the total number of days with daily mean water temperature ≥15 °C, there was no significant difference between the sites (Table 1).

Infection prevalence and parasite intensity

Over the course of the experiment PKD infection prevalence at the Amont step ranged from 16% to 96%, whereas at the Aval step prevalence ranged from 72% to 100% (Fig. 3A). Overall PKD infection prevalence, consisting of data from all monthly samplings was significantly greater at the Aval step, relative to the Amont step. The parasite intensity of fish sampled at the Aval step was also significantly increased in June and August and overall in a comparison of all infected samples relative to the Amont step (Fig. 3B).

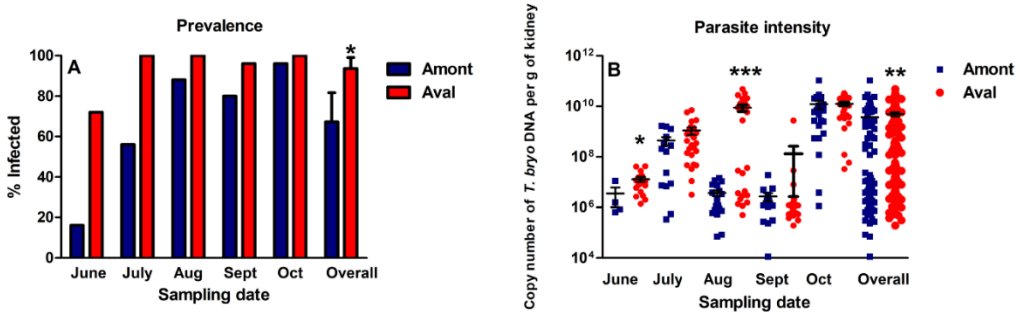


Figure 3 Proliferative kidney disease dynamics observed in infected fish sampled at the Amont and Aval step. (A) Infection prevalence and (B) Parasite intensity (black lines indicate mean ± SE) at sampling point of the Amont (blue bars (A) or blue squares (B)) and the Aval (red bars (A) or red circles (B)) sites. Parasite intensity was determined using copy numbers of parasite DNA as a proxy. Asterisks indicate levels of significance (*t*-Test), $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. $N = 25$ per site, per monthly sampling per site.
Full-size DOI: 10.7717/peerj.5956/fig-3

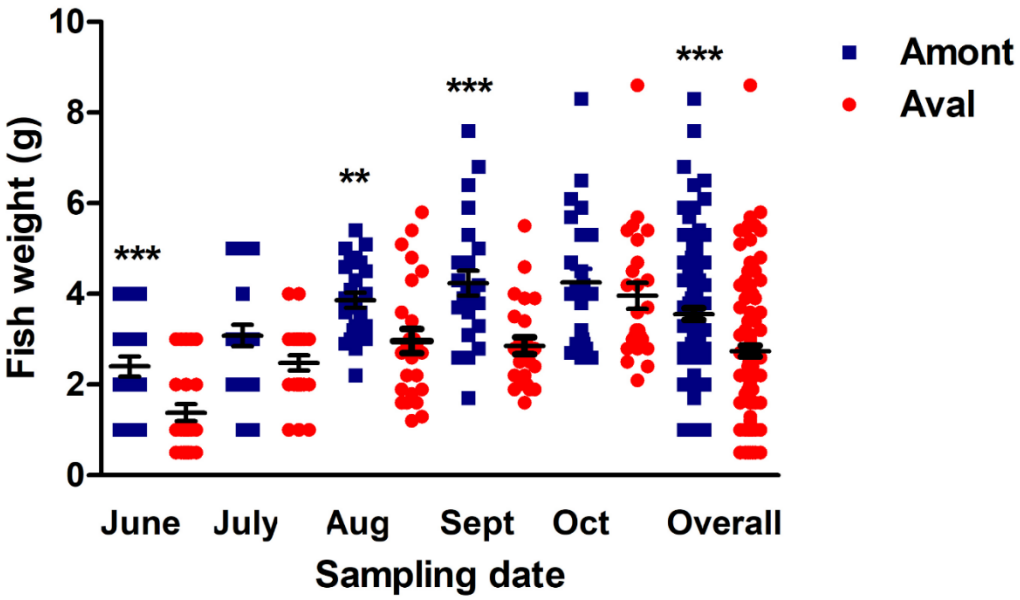


Figure 4 Fish body weight at the Amont and Aval. Amont (blue squares) and Aval (red circles) (black lines indicate mean ± SE) at different sampling points. Asterisks indicate levels of significance (*t*-Test), $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. $N = 25$ per site, per monthly sampling per site.
Full-size DOI: 10.7717/peerj.5956/fig-4

Fish weight

Fish body weight was compared between the sites to investigate if there was an impact of the effluent on the growth of YOY fish at the Amont or Aval step. The mean weight of fish sampled at the Amont step was greater at every sampling. More specifically, at the Amont step there were significant increases in fish weight at the June, August, and September samplings and overall in a comparison of all samples relative to the Aval step (Fig. 4).

Table 2 Histopathological changes of the kidney section observed in infected fish sampled at the Amont and Aval step concerning infection degree, tissue proliferation, and fibrous tissue.

Site	Monthly sampling	Infection degree	Tissue proliferation	% of fish with tissue proliferation	Fibrous tissue	% of fish with fibrous tissue
Amont	July	1.2 ± 0.36	1.33 ± 0.18	33	None	None
	August	1.89 ± 0.24	1.81 ± 0.21	78	1.87 ± 0.35	32
	September	2.35 ± 0.38	2.00 ± 0.28	78	1.91 ± 0.38	69
	October	3.05 ± 0.32	3.00 ± 0.22	82	2.46 ± 0.33	65
	Overall	2.12 ± 0.32	2.03 ± 0.22	68	2.08 ± 0.35	55
Aval	July	1.83 ± 0.24	1.77 ± 0.23	50	None	None
	August	3.33 ± 0.15*	3.04 ± 0.12***	100	2.1 ± 0.31	60
	September	3.16 ± 0.19***	3.26 ± 0.21**	96	2.26 ± 0.34	62
	October	3.4 ± 0.40	3.12 ± 0.25	77	2.35 ± 0.24	91
	Overall	2.93 ± 0.98***	2.79 ± 0.20*	81	2.23 ± 0.29	71

Notes:
No infected fish at either site had presence of fibrous tissue in July sampling. Asterisks indicate levels of significance (*t*-Test).
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

Histological assessment

Here is an overall description and summary of the significant histology results. For a complete outline of the histology scores refer to Table 2. Owing to size of the YOY fish at the June sampling no histology samples were taken. From August parasite induced microscopic alterations of the kidney associated with PKD infection of salmonids were observed in fish at both sites. These included proliferation of the interstitial tissue, granulomatous inflammation, multiple areas of necrosis and haemorrhage, necrotising vasculitis, formation of thrombi, and fibrous tissue.

Concerning the statistical differences observed when examining alterations in the severity of infection; in terms of renal proliferation and degenerative alterations evaluated via tissue proliferation scores, there were significant increases in these scores from fish sampled at the Aval step relative to fish sampled at the Amont step in August and September and in overall in a comparison of all infected samples. In addition, there was a significant increase in the degree of infection, observed in terms of parasite presence per slide at the Aval step relative to the Amont step in August and September and overall in a comparison of all infected samples. By assessing the amount of parasites observed histologically it allows us to correlate the increase in parasite intensity seen by qPCR when comparing the Aval and the Amont providing further evidence of the differences in parasite intensity when comparing the two sites. Concerning the presence of fibrous tissue no significant differences were found between the two sites, at any of the samplings. Images illustrating a zero score and the highest observed in the case of each category of the histological assessment are shown (Fig. 5). The relationship between parasite intensity and host health in terms of tissue proliferation was plotted and compared statistically for each site, although, there was no significant relationship between the variables at either site (Fig. 6).

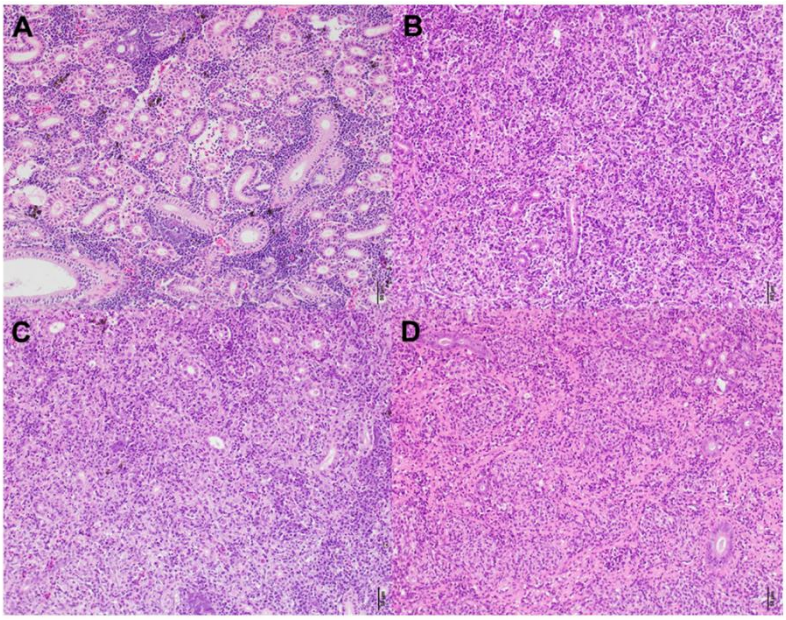


Figure 5 Histologicalimages of the posterior kidney. (A) Demonstrates a zero in all categories according to the histological assessment and (B–D) the highest scores in each category. (A) Is from a fish sampled at the Amont in June. (B) Is from a fish sampled at the Aval in August showing a score of six for infection degree (presence and distribution of parasites). (C) Is also from a fish sampled at the Aval in August showing a score of five (moderate to severe) for tissue proliferation. (D) Is from a fish sampled at the Amont in September showing a score of five for fibrous tissue. Scale bar = 50 µm. All pictures are taken from slides stained with H&E. Full-size DOI: 10.7717/peerj.5956/fig-5

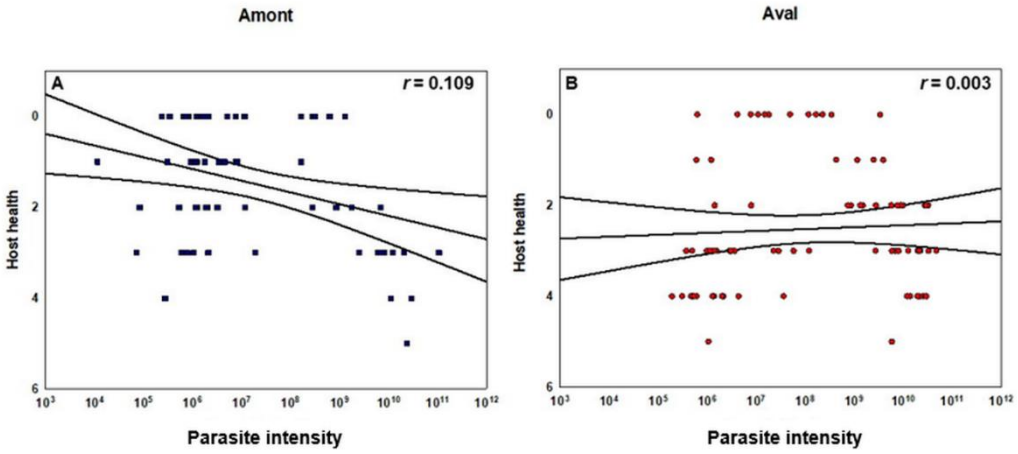


Figure 6 Correlation of copy number of *T. bryosalmonae* DNA per gram of kidney tissue and host health at each sampling site. (A) Amont step (blue squares) and (B) Aval step (red circles). Pearson correlation coefficients *r* as well as confidence intervals are given (black lines). Tissue proliferation score determined via histology and used as a proxy for host health. *Y*-axis is inverted to indicate that an increase in tissue proliferation is associated with a decrease in host health. There were no significant correlations between the variables at either sampling site. For the Amont step $N = 63$ and for the Aval step $N = 82$. Full-size DOI: 10.7717/peerj.5956/fig-6

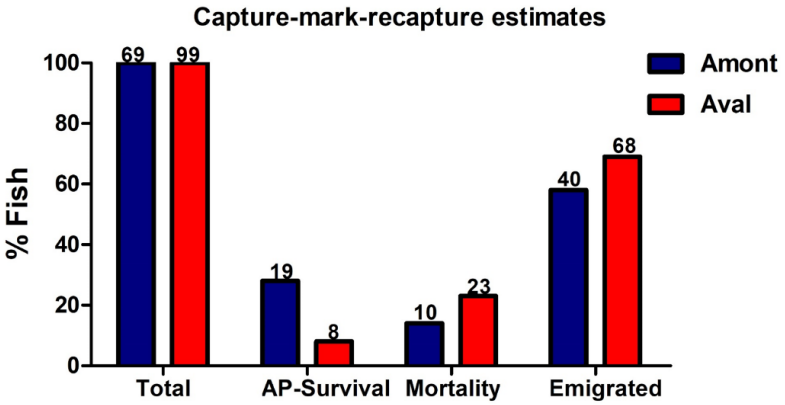


Figure 7 Estimates generated from the capture-mark-recapture method using PIT tags of fish at the Amont and Aval step. Estimations include apparent (AP) survival rate, mortality and possible emigration at Amont step (blue) and Aval step (red). While could be assumed that emigrated fish may have died post dispersal, though this is impossible to confirm, thus, for the sake of the study, these fish were defined as emigrated. *N* is indicated above the respective bar. Full-size DOI: 10.7717/peerj.5956/fig-7

Apparent survival, mortality, emigration, and migration

In July 2017, 69 YOY fish at the Amont step and 99 YOY from the Aval step were captured and tagged before being returned to the waterbody (Fig. 7). In October 2017 during the recapture, 19-tagged fish were recaptured at the Amont step and eight at the Aval step. The estimated apparent survival rate reached 28% at Amont step relative to 8.0% at Aval step, thus indicating a greater apparent survival rate at the Amont step. In addition, mortality calculated from detected tags found lying on the river floor was greater at the Aval step in comparison to the Amont step (23–14%). While the percent of fish deemed to have dispersed (emigration) was also greater at the Aval step (68%) in comparison to the Amont step (40%).

DISCUSSION

Here, we assessed the ecological implications of WWTP effluents on parasitic infection of wild fish. We evaluated the hypothesis that brown trout inhabiting the lower water quality site (Aval step) will show greater susceptibility to PKD. We assessed susceptibility by (i) infection prevalence, (ii) parasite intensity, (iii) host health in terms of pathology, and (iv) estimated apparent survival rate. At different time points during the study, significant differences between sites concerning all measured parameters were found, thus providing evidence of the influence of effluents on parasitic infection of fish in our study system. In addition, fish at the Amont step had increased body weight at all the time points investigated. While fish weight could be influenced by a swollen kidney. However, given the fish in the Amont step were heavier but had less alterations, this underpins a better growth at his particular site.

Taken together our results indicate that the fish inhabiting the Aval step had greater susceptibility to PKD infection. The main goal here was not to find major differences in terms of disease severity or mass mortality events, but to provide information on what impacts a subtle difference in ecological quality may have for this host-parasite system.

Assessment of environmental pollution often focuses on medium to large-scale impacts and the influence of small-scale effluent discharges on the natural environments can be overlooked. The novelty of the present study is that it investigates these small-scale impacts and their role in PKD from the perspective of the fish host.

In our study system, the impact of effluents may have influenced PKD through a direct and/or in-direct process. Relating to the brown trout—*T. bryosalmonae* host-parasite system these processes may occur under the following synopsis: (1) through the fish host via altering its ability to manage the parasite, (2) through the parasite via effects on virulence, transmission, or reproduction, or (3) through the invertebrate host via increasing bryozoa (the invertebrate host) reproduction. Concerning the above synopses, it could be suggested that this is due to: (1) through the fish host via its ability to manage the parasite being lowered. It is widely documented environmental pollution can interfere with host physiology and/or immune processes (*Colborn & Clement, 1992*; *Kavlock et al., 1996*). In this context, a lower water quality induced modulation of the host physiology or immune response may not be dramatic *per se*, but it may lead to immunosuppression and as a result increased susceptibility to infection (*Kimber & Dearman, 2002*). This outcome would offer support to what we have seen in the present study: that the subtle difference in water quality caused via effluents does not lead to an extreme outcome or mass mortality event but increases of susceptibility possibly via an impact on immunocompetence.

On the other hand, it does not mean we can simply speculate that if we had seen a greater deterioration in water quality it would necessarily correlate to a greater negative outcome for the fish host. An example of this is a report evaluating the effects of environmentally relevant polybrominated biphenyl ethers (PBDEs) on Chinook salmon (*O. tshawytscha*) and the impacts associated with susceptibility to infectious bacterial diseases (*Vibrio anguillarum*) (*Arkoosh et al., 2010*). In this study, Arkoosh et al (2010) found that fish fed the 1× PBDE diet showed significantly greater disease-related mortality when challenged with a pathogen than control fish. In contrast, the Chinook salmon fed the 10× PBDE diet were less susceptible to disease than the fish fed the control diet. The 1× PBDE diet was proposed to have a concentration reflecting contaminant levels found in gut contents of wild Chinook salmon (*Arkoosh et al., 2010*). The authors did not know the reason for this result; it could be conceivable that it is due the impact of PBDEs directly on the bacteria, or indirectly through an impact on the host in terms of composition and metabolic activity of the gut microbiota, which acts as the niche for the bacteria. Either way, the study outcome demonstrates that the net impact of multiple stressors may ensue in ecological surprises. In a further example of an unexpected outcome a controlled lab multiple stressor experiment incorporating *T. bryosalmonae*, Burki et al. exposed fish to an E2 (estrogen 17β-estradiol) concentration and *T. bryosalmonae* challenge. Remarkably, fish exposed only to the parasite had greater parasite intensity and increased mortality relative to the fish exposed to both E2 and *T. bryosalmonae* (*Burki et al., 2013*). Therefore, it could be suggested that in the Burki study, E2 may have either benefited the host or hindered the parasite, in contrast to our results in which decreased water quality had a negative effect on the host (indirect

through a pro-parasitic impact or directly upon host homeostatic processes). Though in our study system the WWTP effluents will contain a temporally varying combination of compounds, in comparison to the aforementioned lab study that used a pre-determined concentration of E2, hence a more drastic impact upon the host might be expected.

To stay with the role of pathogen, it might be suggested that the increase of PKD susceptibility at the Aval step maybe due to synopsis: (2) through an influence on the parasite through enhanced virulence. To avoid any conflictions, we define virulence as the host's parasite-induced fitness loss referring to the degree of pathology caused by the pathogen. In this manner, we discuss that the differences we observed in pathology between the Amont and Aval step were due to a difference in the parasites impact on host health. Micropollutants found in effluents may include nutrients (especially carbon, nitrogen, and oxygen), and various metals; which have been shown to act as mediators of the metabolism in pathogenic bacteria and important regulators of their virulence mechanisms (*Somerville & Proctor, 2009*; *Rohmer, Hocquet & Miller, 2011*). Envisaging this, WWTP effluents may support the expression of virulence factors of pathogens (*Alizon et al., 2009*). For instance, it has been shown that increases in nutrient levels can moderate putative virulence factors of the salmonid pathogen *Flavobacterium columnare,* resulting in elevated expression of tissue degrading enzymes chondroitinase and collagenase, which play a role in skin lesions, fin erosion, and gill necrosis (*Penttinen et al., 2016*). Thus, the differences in pathology in our system may have been due to increased parasitic virulence. While *T. bryosalmonae* is able to persistently exploit bryozoan hosts by undergoing host-condition dependent development (cycling between virulent overt and avirulent covert infections), little is known in terms of identifying virulence factors and other adaptations, despite its relevance as a disease of both aquaculture and wild fish. As PKD infection of brown trout is characterized by persistent infections selection for low virulence may occur for this opportunistic parasite (*Okamura, 2016*). Therefore, suggesting this synopsis may be the most speculative. However, as the environment outside the host is unpredictable, such opportunistic pathogens as *T. bryosalmonae* have to adapt rapidly to the changing conditions, for example, water temperature (*Guijarro et al., 2015*), or even WWTP effluents, which may alter the parasite characteristics and also its impact upon the host. In the case of PKD, and its association between an increase in disease severity and mortality with elevated water temperatures, clearly *T. bryosalmonae* as an opportunistic pathogen is influenced by changes in the environment. However, if micropollutants or nutrients in the water may have a similar influence on the parasite clearly requires greater elucidation.

Finally, we consider that the differences in susceptibility we have seen are due to an influence of effluents on: (3) through the invertebrate host, via increasing bryozoan reproduction. A bryozoa survey along the Boiron de Morges performed in 2014 by H. Hartikainen and J-F. Rubin (2018, personal communication) described a greater abundance of bryozoa at the Aval step in comparison the Amont step. Furthermore, *Hartikainen et al. (2009)* utilizing a combination of both lab and field studies demonstrated that greater nutrient levels promote both parasite and bryozoa growth resulting in a greater number of spores available for infection of fish. Therefore, the increased nutrient intake due to the WWTP

effluent at the Aval step may have promoted growth of the bryozoans and led to higher parasite densities for fish infection, similar to the results of *Hartikainen et al. (2009)*.

Concerning other myxozoan diseases, there are several examples in which the relationship between eutrophic-like nutrients enriched environments and the intermediate hosts (in PKD the bryozoa) which suggest that the parasite biomass is also influenced by bottom-up effects (*Marcogliese & Cone, 2001*; *Krueger et al., 2006*; *McKenzie & Townsend, 2007*). Granting this, while all these studies clearly identify a link between an increase of intermediate host populations and greater presence of parasites via pollution enrichment and diseased fish, they do not investigate the impact on fish health, making it difficult to understand how infection severity of the fish host may be moderated by nutrients in these myxozoan diseases.

CONCLUSION

While from our results we can indicate that there is a negative impact for the fish, we cannot clearly disentangle the exact synopsis (1, 2, or 3) or combination thereof in which the host-pathogen interaction is modulated. This would require dedicated lab experiments encompassing each player in the PKD life cycle to be conclusive. Moreover, our field study only investigated two sites and only through including an increased number of replicates would have allowed us to confirm with greater certainty if pollution was the true causation for the differences reported here. This is a common issue that impacts environmental studies when attempting to link a particular factor with disease without suitable replicates. However, we do provide critical baseline information for the development of future studies focusing on the impact of a subtle decrease in water quality and point to the importance of studying pollution on a small scale.

In closing, our results indicate that environmental pollution should be considered alongside temperature as a driver of PKD infection of wild populations of brown trout even if it occurs on a small-scale. In this context, while the pathogen may be the ultimate factor in diseases related mortality there are still clearly other factors that may drive mortality and to comprehensibly understand these effects we need to integrate multiple stressors studies if we are to attempt to disentangle the current and growing threats biodiversity faces.

ACKNOWLEDGEMENTS

The authors wish to thank The General Directorate of the Environment from the canton of Vaud (DGE) for providing the IBCH index score from the Amont step station.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

This research was supported by the Federal Office for the Environment of the Swiss Confederation and by the Canton de Vaud (general survey of Boiron). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosure

The following grant information was disclosed by the authors:
The Federal Office for the Environment of the Swiss Confederation.

Competing Interests

The authors declare that they have no competing interests.

Author Contributions

- Christyn Bailey performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Aurélie Rubin conceived and designed the experiments, performed the experiments, contributed reagents/materials/analysis tools, approved the final draft.
- Nicole Strepparava performed the experiments, analyzed the data, approved the final draft.
- Helmut Segner analyzed the data, authored or reviewed drafts of the paper, approved the final draft.
- Jean-François Rubin conceived and designed the experiments, performed the experiments, contributed reagents/materials/analysis tools, approved the final draft.
- Thomas Wahli analyzed the data, authored or reviewed drafts of the paper, approved the final draft.

Animal Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

Fish and ICBH samplings were carried out relating to ethical approvals (Service of Consumption and Veterinary business of the canton of Vaud, permission number VD3253).

This is indicted in the methods section of the manuscript.

Field Study Permissions

The following information was supplied relating to field study approvals (i.e., approving body and any reference numbers):

Samplings were carried out relating to ethical approvals (Service of Consumption and Veterinary business of the canton of Vaud, permission number VD3253).

Data Availability

The following information was supplied regarding data availability:

All other raw data is included within the article and in Supplementary File 1.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.5956#supplemental-information.

REFERENCES

Acevedo-Whitehouse K, Duffus AL. 2009. Effects of environmental change on wildlife health. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* **364**(**1534**):3429–3438 DOI 10.1098/rstb.2009.0128.

Alizon S, Hurford A, Mideo N, Van Baalen M. 2009. Virulence evolution and the trade-off hypothesis: history, current state of affairs and the future. *Journal of Evolutionary Biology* **22**(**2**):245–259 DOI 10.1111/j.1420-9101.2008.01658.x.

Arkoosh MR, Boylen D, Dietrich J, Anulacion BF, Bravo CF, Johnson LL, Loge FJ, Collier TK. 2010. Disease susceptibility of salmon exposed to polybrominated diphenyl ethers (PBDEs). *Aquatic Toxicology* **98**(**1**):51–59 DOI 10.1016/j.aquatox.2010.01.013.

Arkoosh MR, Van Gaest AL, Strickland SA, Hutchinson GP, Krupkin AB, Dietrich JP. 2015. Dietary exposure to individual polybrominated diphenyl ether congeners BDE-47 and BDE-99 alters innate immunity and disease susceptibility in juvenile Chinook salmon. *Environmental science and technology* **49**(**11**):6974–6981 DOI 10.1021/acs.est.5b01076.

Bailey C, Segner H, Casanova-Nakayama A, Wahli T. 2017. Who needs the hotspot? The effect of temperature on the fish host immune response to *Tetracapsuloides bryosalmonae* the causative agent of proliferative kidney disease. *Fish & Shellfish Immunology* **63**:424–437 DOI 10.1016/j.fsi.2017.02.039.

Bailey C, Schmidt-Posthaus H, Segner H, Wahli T, Strepparava N. 2018. Are brown trout *Salmo trutta* fario and rainbow trout *Oncorhynchus mykiss* two of a kind? A comparative study of salmonids to temperature-influenced *Tetracapsuloides bryosalmonae* infection. *Journal of Fish Diseases* **2**:191–198 DOI 10.1111/jfd.12694.

Bettge K, Wahli T, Segner H, Schmidt-Posthaus H. 2009a. Proliferative kidney disease in rainbow trout: time- and temperature-related renal pathology and parasite distribution. *Diseases of Aquatic Organisms* **83**:67–76 DOI 10.3354/dao01989.

Bettge K, Segner H, Burki R, Schmidt-Posthaus H, Wahli T. 2009b. Proliferative kidney disease (PKD) of rainbow trout: temperature- and time-related changes of *Tetracapsuloides bryosalmonae* DNA in kidney. *Journal of Parasitology* **136**(**6**):615–625 DOI 10.1017/S0031182009005800.

Blanar CA, Munkittrick KR, Houlahan J, MacLatchy DL, Marcogliese DJ. 2009. Pollution and parasitism in aquatic animals: a meta-analysis of effect size. *Aquatic Toxicology* **93**(**1**):18–28 DOI 10.1016/j.aquatox.2009.03.002.

Buck JC, Scheessele EA, Relyea RA, Blaustein AR. 2012. The effects of multiple stressors on wetland communities: pesticides, pathogens and competing amphibians. *Freshwater Biology* **57**(**1**):61–73 DOI 10.1111/j.1365-2427.2011.02695.x.

Burkhardt-Holm P. 2002. Proliferative kidney disease: why is it of interest for the Swiss project 'Fishnet'? *Journal of Fish Diseases* **25**(**8**):441–442 DOI 10.1046/j.1365-2761.2002.00399.x.

Burki R, Krasnov A, Bettge K, Rexroad IIICE, Afanasyev S, Antikainen M, Burkhardt-Holm P, Wahli T, Segner H. 2013. Molecular crosstalk between a chemical and a biological stressor and consequences on disease manifestation in rainbow trout. *Aquatic Toxicology* **127**:2–8 DOI 10.1016/j.aquatox.2012.02.026.

Casanova-Nakayama A, Wenger M, Burki R, Eppler E, Krasnov A, Segner H. 2011. Endocrine disrupting compounds: can they target the immune system of fish? *Marine Pollution Bulletin* **63**(**5–12**):412–416 DOI 10.1016/j.marpolbul.2011.05.007.

Clifton-Hadley RS, Bucke D, Richards RH. 1987. A study of the sequential clinical and pathological changes during proliferative kidney disease in rainbow trout, *Salmo gairdneri* Richardson. *Journal of Fish Diseases* **10**(**5**):335–352 DOI 10.1111/j.1365-2761.1987.tb01081.x.

Clifton-Hadley R, Richards R, Bucke D. 1986. Proliferative kidney disease (PKD) in rainbow trout *Salmo gairdneri*: further observations on the effects of water temperature. *Aquaculture* **55**(**3**):165–171 DOI 10.1016/0044-8486(86)90112-2.

Colborn T, Clement C. 1992. *Chemically-Induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection*. Vol. 21. Princeton: Princeton Scientific Publishing.

Costanzo SD, Murby J, Bates J. 2005. Ecosystem response to antibiotics entering the aquatic environment. *Marine Pollution Bulletin* **51**(**1–4**):218–223 DOI 10.1016/j.marpolbul.2004.10.038.

Daughton CG, Ternes TA. 1999. Pharmaceuticals and personal care products in the environment: agents of subtle change? *Environmental Health Perspectives* **107**(**suppl 6**):907–938 DOI 10.1289/ehp.99107s6907.

Direction génrale de l'environnement (DGE). 2017. Direction de l'environnement industriel, urbain et rural. Bilans 2017 de l'épuration vaudoise, Lausanne. Available at *https://www.vd.ch/fileadmin/user_upload/themes/environnement/eau/fichiers_pdf/DIREV_PRE/Bilans_de_l_%C3%A9puration_2017.pdf* (accessed 23 February 2018).

Dodds WK, Perkin JS, Gerken JE. 2013. Human impact on freshwater ecosystem services: a global perspective. *Environmental Science & Technology* **47**(**16**):9061–9068 DOI 10.1021/es4021052.

El-Matbouli M, Hoffmann R. 2002. Influence of water quality on the outbreak of proliferative kidney disease-field studies and exposure experiments. *Journal of Fish Diseases* **25**(**8**):459–467 DOI 10.1046/j.1365-2761.2002.00393.x.

Federal Office for the Environment (FOEN). 2011. Méthodes d'analyse et d'appréciation des cours d'eau, Macrozoobenthos—niveau R (région). Available at *https://www.bafu.admin.ch/bafu/fr/home/themes/eaux/publications/publications-eaux/methodes-analyse-appreciation-cours-eau-vue.html* (accsessed 15 September 2017).

Ferguson HW. 1981. The effects of water temperature on the development of proliferative kidney disease in rainbow trout, *Salmo gairdneri* Richardson. *Journal of Fish Diseases* **4**(**2**):175–177 DOI 10.1111/j.1365-2761.1981.tb01122.x.

Glassmeyer ST, Koplin DW, Furlong ET, Focazio M. 2008. Environmental presence and persistence of pharmaceuticals: an overview. In: Aga D, ed. *Fate of the Pharmaceuticals in the Environment and in Water Treatment Systems*. Boca Raton: CRC/Taylor and Francis Press, 3–51.

Guijarro JA, Cascales D, García-Torrico AI, García-Domínguez M, Méndez J. 2015. Temperature-dependent expression of virulence genes in fish-pathogenic bacteria. *Frontiers in Microbiology* **6**:700 DOI 10.3389/fmicb.2015.00700.

Hartikainen H, Johnes P, Moncrieff C, Okamura B. 2009. Bryozoan populations reflect nutrient enrichment and productivity gradients in rivers. *Freshwater Biology* **54**(**11**):2320–2334 DOI 10.1111/j.1365-2427.2009.02262.x.

Heberer T. 2002. Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. *Toxicology Letters* **131**(**1–2**):5–17 DOI 10.1016/S0378-4274(02)00041-3.

Hedrick RP, MacConnell E, De Kinkelin P . 1993. Proliferative kidney disease of salmonid fish. *Annual Review of Fish Diseases* **3**:277–290 DOI 10.1016/0959-8030(93)90039-E.

Hedrick RP, Monge D, De Kinkelin P . 1992. Transmission of PKX, the causative agent of proliferative kidney disease (PKD), to rainbow trout *Oncorhynchus miykiss* following filtration of water and sediments. *Diseases of Aquatic Organisms* **14**:237–240 DOI 10.3354/dao014237.

Hellemans J, Mortier G, De Paepe A, Speleman F, Vandesompele J. 2007. qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. *Genome Biology* **8**(**2**):R19 DOI 10.1186/gb-2007-8-2-r19.

Jacobson KC, Arkoosh MR, Kagley AN, Clemons ER, Collier TK, Casillas E. 2003. Cumulative effects of natural and anthropogenic stress on immune function and disease resistance in juvenile Chinook salmon. *Journal of Aquatic Animal Health* **15**:1–12 DOI 10.1577/1548-8667(2003)015<0001:CEONAA>2.0.CO;2.

Johnson PT, Chase JM, Dosch KL, Hartson RB, Gross JA, Larson DJ, Sutherland DR, Carpenter SR. 2007. Aquatic eutrophication promotes pathogenic infection in amphibians. *Proceedings of the National Academy of Sciences of the United States of America* **104**(**40**):15781–15786 DOI 10.1073/pnas.0707763104.

Johnson AC, Sumpter JP. 2014. Putting pharmaceuticals into the wider context of challenges to fish populations in rivers. *Philosophical Transactions of the Royal Society B: Biological Sciences* **369**(**1656**):20130581 DOI 10.1098/rstb.2013.0581.

Joly S, Bruneau A. 2006. Incorporating allelic variation for reconstructing the evolutionary history of organisms from multiple genes: an example from Rosa in North America. *Systematic Biology* **55**(**4**):623–636 DOI 10.1080/10635150600863109.

Kavlock RJ, Daston GP, DeRosa C, Fenner-Crisp P, Gray LE, Kaattari S, Lucier G, Luster M, Mac MJ, Maczka C, Miller R, Moore J, Rolland R, Scott G, Sheehan DM, Sinks T, Tilson HA. 1996. Research needs for the risk assessment of health and environmental effects of endocrine disruptors: a report of the US EPA-sponsored workshop. *Environmental Health Perspectives* **104**:715 DOI 10.2307/3432708.

Kent LM, Hedrick RP. 1985. PKX, the causative agent of proliferative kidney disease (PKD) in Pacific salmonid fishes and its affinities with the Myxozoa. *Journal of Protozoology* **32**(**2**):254–259 DOI 10.1111/j.1550-7408.1985.tb03047.x.

Kimber I, Dearman RJ. 2002. Chemical respiratory allergy: role of IgE antibody and relevance of route of exposure. *Toxicology* **181**:311–315 DOI 10.1016/S0300-483X(02)00299-8.

Krueger RC, Kerans B, Vincent ER, Rasmussen C. 2006. Risk of *Myxobolus cerebralis* infection to rainbow trout in the Madison River. *Ecological Applications* **16**(**2**):770–783 DOI 10.1890/1051-0761(2006)016[0770:ROMCIT]2.0.CO;2.

Longshaw M, Le Deuff R-M, Harris AF, Feist SW. 2002. Development of proliferative kidney disease in rainbow trout, *Oncorhynchus mykiss* (Walbaum), following short-term exposure to *Tetracapsula bryosalmonae* infected bryozoans. *Journal of Fish Diseases* **25**(**8**):443–449 DOI 10.1046/j.1365-2761.2002.00353.x.

Mackay IM. 2004. Real-time PCR in the microbiology laboratory. *Clinical Microbiology and Infection* **10**(**3**):190–212 DOI 10.1111/j.1198-743X.2004.00722.x.

Magnadóttir B, Lange S, Gudmundsdottir S, Bøgwald J, Dalmo RA. 2005. Ontogeny of humoral immune parameters in fish. *Fish & Shellfish Immunology* **19**(**5**):429–439 DOI 10.1016/j.fsi.2005.03.010.

Marcogliese DJ, Cone DK. 2001. Myxozoan communities parasitizing *Notropis hudsonius* (Cyprinidae) at selected localities on the St. Lawrence River, Quebec: possible effects of urban effluents. *Journal of Parasitology* **87**(**5**):951–956 DOI 10.1645/0022-3395(2001)087[0951:MCPNHC]2.0.CO;2.

McKenzie VJ, Townsend AR. 2007. Parasitic and infectious disease responses to changing global nutrient cycles. *EcoHealth* **4**(**4**):384–396 DOI 10.1007/s10393-007-0131-3.

Okamura B. 2016. Hidden infections and changing environments. *Integrative and Comparative Biology* **56**(**4**):620–629 DOI 10.1093/icb/icw008.

Peeler EJ, Feist SW. 2011. Human intervention in freshwater ecosystems drives disease emergence. *Freshwater Biology* **56(4)**:705–716 DOI 10.1111/j.1365-2427.2011.02572.x.

Penttinen R, Kinnula H, Lipponen A, Bamford JK, Sundberg L-R. 2016. High nutrient concentration can induce virulence factor expression and cause higher virulence in an environmentally transmitted pathogen. *Microbial Ecology* **72(4)**:955–964 DOI 10.1007/s00248-016-0781-1.

Poulin R. 1992. Toxic pollution and parasitism in freshwater fish. *Parasitology Today* **8(2)**:58–61 DOI 10.1016/0169-4758(92)90090-O.

Rehberger K, Werner I, Hitzfeld B, Segner H, Baumann L. 2017. 20 Years of fish immunotoxicology—what we know and where we are. *Critical Reviews in Toxicology* **47(6)**:516–542 DOI 10.1080/10408444.2017.1288024.

Rohmer L, Hocquet D, Miller SI. 2011. Are pathogenic bacteria just looking for food? Metabolism and microbial pathogenesis. *Trends in Microbiology* **19(7)**:341–348 DOI 10.1016/j.tim.2011.04.003.

Rohr JR, Schotthoefer AM, Raffel TR, Carrick HJ, Halstead N, Hoverman JT, Johnson CM, Johnson LB, Lieske C, Piwoni MD, Schoff PK, Beasley VR. 2008. Agrochemicals increase trematode infections in a declining amphibian species. *Nature* **455(7217)**:1235–1239 DOI 10.1038/nature07281.

Schisler GJ, Bergersen EP, Walker PG. 2000. Effects of multiple stressors on morbidity and mortality of fingerling rainbow trout infected with Myxobolus cerebralis. *Transactions of the American Fisheries Society* **129(3)**:859–865 DOI 10.1577/1548-8659(2000)129<0859:EOMSOM>2.3.CO;2.

Schmidt-Posthaus H, Bettge K, Forster U, Segner H, Wahli T. 2012. Kidney pathology and parasite intensity in rainbow trout *Oncorhynchus mykiss* surviving proliferative kidney disease: time course and influence of temperature. *Diseases of Aquatic Organisms* **97(3)**:207–218 DOI 10.3354/dao02417.

Schmidt-Posthaus H, Ros A, Hirschi R, Schneider E. 2017. Comparative study of proliferative kidney disease in grayling *Thymallus thymallus* and brown trout *Salmo trutta* fario: an exposure experiment. *Diseases of Aquatic Organisms* **123(3)**:193–203 DOI 10.3354/dao03102.

Segner H, Schmitt-Jansen M, Sabater S. 2014. Assessing the impact of multiple stressors on aquatic biota: the receptor's side matters. *Environmental Science & Technology* **48(14)**:7690–7696 DOI 10.1021/es405082t.

Somerville GA, Proctor RA. 2009. At the crossroads of bacterial metabolism and virulence factor synthesis in Staphylococci. *Microbiology and Molecular Biology Reviews* **73(2)**:233–248 DOI 10.1128/MMBR.00005-09.

Søndergaard M, Jeppesen E. 2007. Anthropogenic impacts on lake and stream ecosystems, and approaches to restoration. *Journal of Applied Ecology* **44(6)**:1089–1094 DOI 10.1111/j.1365-2664.2007.01426.x.

Stackelberg PE, Gibs J, Furlong ET, Meyer MT, Zaugg SD, Lippincott RL. 2007. Efficiency of conventional drinking-water-treatment processes in removal of pharmaceuticals and other organic compounds. *Science of The Total Environment* **377(2–3)**:255–272 DOI 10.1016/j.scitotenv.2007.01.095.

Strepparava N, Segner H, Ros A, Hartikainen H, Schmidt-Posthaus H, Wahli T. 2017. Temperature-related parasite infection dynamics: the case of proliferative kidney disease of brown trout. *Parasitology* **145(3)**:281–291 DOI 10.1017/s0031182017001482.

Vidal-Martínez VM, Pech D, Sures B, Purucker ST, Poulin R. 2010. Can parasites really reveal environmental impact? *Trends in Parasitology* **26**(**1**):44–51 DOI 10.1016/j.pt.2009.11.001.

Vörösmarty CJ, McIntyre PB, Gessner MO, Dudgeon D, Prusevich A, Green P, Glidden S, Bunn SE, Sullivan CA, Liermann CR. 2010. Global threats to human water security and river biodiversity. *Nature* **467**(**7315**):555–561 DOI 10.1038/nature09440.

Wahli T, Bernet D, Steiner PA, Schmidt-Posthaus H. 2007. Geographic distribution of *Tetracapsuloides bryosalmonae* infected fish in Swiss rivers: an update. *Aquatic Sciences-Research Across Boundaries* **69**(**1**):3–10 DOI 10.1007/s00027-006-0843-4.

Wahli T, Knuesel R, Bernet D, Segner H, Pugovkin D, Burkhardt-Holm P, Escher M, Schmidt-Posthaus H. 2002. Proliferative kidney disease in Switzerland: current state of knowledge. *Journal of Fish Diseases* **25**(**8**):491–500 DOI 10.1046/j.1365-2761.2002.00401.x.

Yun JJ, Heisler LE, Hwang II, Wilkins O, Lau SK, Hyrcza M, Jayabalasingham B, Jin J, McLaurin J, Tsao M-S. 2006. Genomic DNA functions as a universal external standard in quantitative real-time PCR. *Nucleic Acids Research* **34**(**12**):e85 DOI 10.1093/nar/gkl400.

Supplementary Material:

Table S1: Histology individual fish scores – July sample results

FV number	ID	Location	HISTO K	Histo K Infection degree	HISTO K Prolif	Histo K Fibro
FV17/15	1	Amont STEP	R17/334.1			
FV17/15	2	Amont STEP	R17/334.2			
FV17/15	3	Amont STEP	R17/334.3			
FV17/15	4	Amont STEP	R17/334.4			
FV17/15	5	Amont STEP	R17/334.5	1	1	
FV17/15	6	Amont STEP	R17/334.6	1		
FV17/15	7	Amont STEP	R17/334.7			
FV17/15	8	Amont STEP	R17/334.8	3	2	
FV17/15	9	Amont STEP	R17/334.9			
FV17/15	10	Amont STEP	R17/334.10	1		
FV17/15	11	Amont STEP	R17/334.11			
FV17/15	12	Amont STEP	R17/334.12	1		
FV17/15	13	Amont STEP	R17/334.13			
FV17/15	14	Amont STEP	R17/334.14	1		
FV17/15	15	Amont STEP	R17/334.15			
FV17/15	16	Amont STEP	R17/334.16	1	1	
FV17/15	17	Amont STEP	R17/334.17	1		
FV17/15	18	Amont STEP	R17/334.18			
FV17/15	19	Amont STEP	R17/334.19			
FV17/15	20	Amont STEP	R17/334.20			
FV17/15	21	Amont STEP	R17/334.21	1		
FV17/15	22	Amont STEP	R17/334.22			
FV17/15	23	Amont STEP	R17/334.23			
FV17/15	24	Amont STEP	R17/334.24			
FV17/15	25	Amont STEP	R17/334.25	1		
FV17/16	1	Aval STEP	R17/335.1			
FV17/16	2	Aval STEP	R17/335.2	2	1	
FV17/16	3	Aval STEP	R17/335.3	1		
FV17/16	4	Aval STEP	R17/335.4	3	2	
FV17/16	5	Aval STEP	R17/335.5			
FV17/16	6	Aval STEP	R17/335.6	4	3	
FV17/16	7	Aval STEP	R17/335.7			
FV17/16	8	Aval STEP	-			
FV17/16	9	Aval STEP	R17/335.8	1		
FV17/16	10	Aval STEP	R17/335.9	3	2	
FV17/16	11	Aval STEP	-	-	-	
FV17/16	12	Aval STEP	R17/335.10	4	2	
FV17/16	13	Aval STEP	R17/335.11	1		
FV17/16	14	Aval STEP	R17/335.12	1		
FV17/16	15	Aval STEP	-	-	-	
FV17/16	16	Aval STEP	R17/335.13	1		
FV17/16	17	Aval STEP	R17/335.14	1		
FV17/16	18	Aval STEP	R17/335.15	1	1	
FV17/16	19	Aval STEP	R17/335.16	2	2	
FV17/16	20	Aval STEP	R17/335.17	2	2	
FV17/16	21	Aval STEP	R17/335.18	1		
FV17/16	22	Aval STEP	R17/335.19			
FV17/16	23	Aval STEP	R17/335.20	2		
FV17/16	24	Aval STEP	R17/335.21	2	1	
FV17/16	25	Aval STEP	R17/335.22	1		

Table S2: Histology individual fish scores – August sample results

FV number	ID	Location	HISTO K	Histo K Infection degree	HISTO K Prolif	Histo K Fibro
FV17/15=FV17/84	1	Amont STEP	R17/624/1	2	1	
FV17/15=FV17/84	2	Amont STEP	R17/624/2	1		
FV17/15=FV17/84	3	Amont STEP	R17/624/3	1	1	3
FV17/15=FV17/84	4	Amont STEP	R17/624/4	3	3	
FV17/15=FV17/84	5	Amont STEP	R17/624/5	3	2	
FV17/15=FV17/84	6	Amont STEP	R17/624/6	3	3	
FV17/15=FV17/84	7	Amont STEP	R17/624/7	1	1	1
FV17/15=FV17/84	8	Amont STEP	R17/624/8	1	1	1
FV17/15=FV17/84	9	Amont STEP	R17/624/9			
FV17/15=FV17/84	10	Amont STEP	R17/624/10	1	1	3
FV17/15=FV17/84	11	Amont STEP	R17/624/11	2	2	1
FV17/15=FV17/84	12	Amont STEP	R17/624/12			
FV17/15=FV17/84	13	Amont STEP	R17/624/13	1	1	
FV17/15=FV17/84	14	Amont STEP	R17/624/14			
FV17/15=FV17/84	15	Amont STEP	R17/624/15	1	1	
FV17/15=FV17/84	16	Amont STEP	R17/624/16			
FV17/15=FV17/84	17	Amont STEP	R17/624/17	1	1	
FV17/15=FV17/84	18	Amont STEP	R17/624/18	2	3	2
FV17/15=FV17/84	19	Amont STEP	R17/624/19	1		
FV17/15=FV17/84	20	Amont STEP	R17/624/20	2	2	3
FV17/15=FV17/84	21	Amont STEP	R17/624/21			
FV17/15=FV17/84	22	Amont STEP	R17/624/22	4	3	1
FV17/15=FV17/84	23	Amont STEP	R17/624/23			
FV17/15=FV17/84	24	Amont STEP	R17/624/24	3	3	
FV17/15=FV17/84	25	Amont STEP	R17/624/25	3		
FV17/16=FV17/85	1	Aval STEP	R17/625/1	4	4	
FV17/16=FV17/85	2	Aval STEP	R17/625/2	3	3	
FV17/16=FV17/85	3	Aval STEP	R17/625/3	5	4	
FV17/16=FV17/85	4	Aval STEP	R17/625/4	4	3	
FV17/16=FV17/85	5	Aval STEP	R17/625/5	3	3	1
FV17/16=FV17/85	6	Aval STEP	R17/625/6	4	3	
FV17/16=FV17/85	7	Aval STEP	R17/625/7	4	4	1
FV17/16=FV17/85	8	Aval STEP	R17/625/8	4	3	
FV17/16=FV17/85	9	Aval STEP	R17/625/9	3	3	1
FV17/16=FV17/85	10	Aval STEP	R17/625/10	4	4	3
FV17/16=FV17/85	11	Aval STEP	R17/625/11	3	3	
FV17/16=FV17/85	12	Aval STEP	R17/625/12	3	3	3
FV17/16=FV17/85	13	Aval STEP	R17/625/13	3	3	3
FV17/16=FV17/85	14	Aval STEP	R17/625/14	4	3	2
FV17/16=FV17/85	15	Aval STEP	R17/625/15	2	2	3
FV17/16=FV17/85	16	Aval STEP	R17/625/16	2	3	3
FV17/16=FV17/85	17	Aval STEP	R17/625/17	3	4	1
FV17/16=FV17/85	18	Aval STEP	R17/625/18	3	2	
FV17/16=FV17/85	19	Aval STEP	R17/625/19	4	2	
FV17/16=FV17/85	20	Aval STEP	R17/625/20			
FV17/16=FV17/85	21	Aval STEP	R17/625/21	3	3	
FV17/16=FV17/85	22	Aval STEP	R17/625/22	3	3	
FV17/16=FV17/85	23	Aval STEP	R17/625/23	3	3	
FV17/16=FV17/85	24	Aval STEP	R17/625/24	4	3	
FV17/16=FV17/85	25	Aval STEP	R17/625/25	2	2	

Table S3: Histology individual fish scores – September sample results

FV number	ID	Location	HISTO K	Histo K Infection degree	HISTO K Prolif	Histo K Fibro
FV17/17=FV17/86	1	Amont STEP	R17/626/1	2	3	2
FV17/17=FV17/86	2	Amont STEP	R17/626/2	5	3	
FV17/17=FV17/86	3	Amont STEP	R17/626/3			
FV17/17=FV17/86	4	Amont STEP	R17/626/4			3
FV17/17=FV17/86	5	Amont STEP	R17/626/5			
FV17/17=FV17/86	6	Amont STEP	R17/626/6	3	2	0
FV17/17=FV17/86	7	Amont STEP	R17/626/7			
FV17/17=FV17/86	8	Amont STEP	R17/626/8	1	1	
FV17/17=FV17/86	9	Amont STEP	R17/626/9	2	2	2
FV17/17=FV17/86	10	Amont STEP	R17/626/10			
FV17/17=FV17/86	11	Amont STEP	R17/626/11	1		3
FV17/17=FV17/86	12	Amont STEP	R17/626/12			
FV17/17=FV17/86	13	Amont STEP	R17/626/13	5	2	2
FV17/17=FV17/86	14	Amont STEP	R17/626/14	3	2	
FV17/17=FV17/86	15	Amont STEP	R17/626/15			
FV17/17=FV17/86	16	Amont STEP	R17/626/16			
FV17/17=FV17/86	17	Amont STEP	R17/626/17	2	1	
FV17/17=FV17/86	18	Amont STEP	R17/626/18	4	4	1
FV17/17=FV17/86	19	Amont STEP	R17/626/19	1		
FV17/17=FV17/86	20	Amont STEP	R17/626/20			2
FV17/17=FV17/86	21	Amont STEP	R17/626/21			
FV17/17=FV17/86	22	Amont STEP	R17/626/22	2	1	2
FV17/17=FV17/86	23	Amont STEP	R17/626/23	1	1	0
FV17/17=FV17/86	24	Amont STEP	R17/626/24			4
FV17/17=FV17/86	25	Amont STEP	R17/626/25	1		
FV17/18=FV17/87	1	Aval STEP	R17/627/1	1		1
FV17/18=FV17/87	2	Aval STEP	R17/627/2	2	1	
FV17/18=FV17/87	3	Aval STEP	R17/627/3	5	3	
FV17/18=FV17/87	4	Aval STEP	R17/627/4	3	3	
FV17/18=FV17/87	5	Aval STEP	R17/627/5	4	5	1
FV17/18=FV17/87	6	Aval STEP	R17/627/6	4	4	4
FV17/18=FV17/87	7	Aval STEP	R17/627/7	4	4	1
FV17/18=FV17/87	8	Aval STEP	R17/627/8	3	3	1
FV17/18=FV17/87	9	Aval STEP	R17/627/9	3	3	1
FV17/18=FV17/87	10	Aval STEP	R17/627/10	2	2	
FV17/18=FV17/87	11	Aval STEP	R17/627/11	2	3	
FV17/18=FV17/87	12	Aval STEP	R17/627/12	2	1	
FV17/18=FV17/87	13	Aval STEP	R17/627/13	4	4	2
FV17/18=FV17/87	14	Aval STEP	R17/627/14	4	4	2
FV17/18=FV17/87	15	Aval STEP	R17/627/15	3	3	
FV17/18=FV17/87	16	Aval STEP	R17/627/16	4	4	4
FV17/18=FV17/87	17	Aval STEP	R17/627/17	4	4	4
FV17/18=FV17/87	18	Aval STEP	R17/627/18	3	3	
FV17/18=FV17/87	19	Aval STEP	R17/627/19	4	2	2
FV17/18=FV17/87	20	Aval STEP	R17/627/20	3	4	4
FV17/18=FV17/87	21	Aval STEP	R17/627/21	3	4	4
FV17/18=FV17/87	22	Aval STEP	R17/627/22			
FV17/18=FV17/87	23	Aval STEP	R17/627/23	2	4	1
FV17/18=FV17/87	24	Aval STEP	R17/627/24	4	4	2
FV17/18=FV17/87	25	Aval STEP	R17/627/25	3	3	

Table S4: Histology individual fish scores – October sample results

FV number	ID	Location	HISTO K	Histo K Infection degree	HISTO K Prolif	Histo K Fibro
FV17/19=FV17/88	1	Amont STEP	R17/628/1	3	3	2
FV17/19=FV17/88	2	Amont STEP	R17/628/2			
FV17/19=FV17/88	3	Amont STEP	R17/628/3	4	3	
FV17/19=FV17/88	4	Amont STEP	R17/628/4	3	2	3
FV17/19=FV17/88	5	Amont STEP	R17/628/5	2	2	5
FV17/19=FV17/88	6	Amont STEP	R17/628/6			
FV17/19=FV17/88	7	Amont STEP	R17/628/7			
FV17/19=FV17/88	8	Amont STEP	R17/628/8	3	3	
FV17/19=FV17/88	9	Amont STEP	R17/628/9			
FV17/19=FV17/88	10	Amont STEP	R17/628/10	2	2	
FV17/19=FV17/88	11	Amont STEP	R17/628/11	3	4	
FV17/19=FV17/88	12	Amont STEP	R17/628/12	3	3	1
FV17/19=FV17/88	13	Amont STEP	R17/628/13	3	3	1
FV17/19=FV17/88	14	Amont STEP	R17/628/14	1		
FV17/19=FV17/88	15	Amont STEP	R17/628/15			2
FV17/19=FV17/88	16	Amont STEP	R17/628/16	6	4	
FV17/19=FV17/88	17	Amont STEP	R17/628/17	1		2
FV17/19=FV17/88	18	Amont STEP	R17/628/18	5	5	
FV17/19=FV17/88	19	Amont STEP	R17/628/19	2		2
FV17/19=FV17/88	20	Amont STEP	R17/628/20			4
FV17/19=FV17/88	21	Amont STEP	R17/628/21	3	2	3
FV17/19=FV17/88	22	Amont STEP	R17/628/22	3	3	1
FV17/19=FV17/88	23	Amont STEP	R17/628/23			3
FV17/19=FV17/88	24	Amont STEP	R17/628/24	5	3	3
FV17/19=FV17/88	25	Amont STEP				
FV17/20=FV17/89	1	Aval STEP	R17/629/1			
FV17/20=FV17/89	2	Aval STEP	R17/629/2			
FV17/20=FV17/89	3	Aval STEP	R17/629/3	3	3	3
FV17/20=FV17/89	4	Aval STEP	R17/629/4	5	3	2
FV17/20=FV17/89	5	Aval STEP	R17/629/5	2	2	3
FV17/20=FV17/89	6	Aval STEP	R17/629/6			
FV17/20=FV17/89	7	Aval STEP	R17/629/7	3	3	3
FV17/20=FV17/89	8	Aval STEP	R17/629/8			2
FV17/20=FV17/89	9	Aval STEP	R17/629/9	5	5	3
FV17/20=FV17/89	10	Aval STEP	R17/629/10			
FV17/20=FV17/89	11	Aval STEP	R17/629/11			
FV17/20=FV17/89	12	Aval STEP	R17/629/12	5	4	1
FV17/20=FV17/89	13	Aval STEP	R17/629/13	2	3	2
FV17/20=FV17/89	14	Aval STEP	R17/629/14		1	1
FV17/20=FV17/89	15	Aval STEP	R17/629/15			4
FV17/20=FV17/89	16	Aval STEP	R17/629/16	1	2	3
FV17/20=FV17/89	17	Aval STEP	R17/629/17	3	3	1
FV17/20=FV17/89	18	Aval STEP	R17/629/18	6	4	2
FV17/20=FV17/89	19	Aval STEP	R17/629/19	5	4	1
FV17/20=FV17/89	20	Aval STEP	R17/629/20			1
FV17/20=FV17/89	21	Aval STEP	R17/629/21	1		5
FV17/20=FV17/89	22	Aval STEP	R17/629/22		2	2
FV17/20=FV17/89	23	Aval STEP	R17/629/23	4	4	2
FV17/20=FV17/89	24	Aval STEP	R17/629/24	3	4	2
FV17/20=FV17/89	25	Aval STEP	R17/629/25	3	3	4

5 General discussion

5.1 General discussion

Water temperature is considered as the main driver for PKD dynamics and severity. However, the majority of studies dealing with PKD are performed in a laboratory setting using constant temperatures. Moreover, rainbow trout is often used as the model species for PKD investigations. Thus, findings from these experiments do not necessarily reflect the situation of the infection in streams, where brown trout populations must deal with fluctuating thermal regimes, as well as potential additional stressors. Studies performed on wild salmonids populations under environmental conditions are hence crucially needed for a better understanding of the infection development and its impacts in the field (Figure 8). Respective findings could then be used to take actions against PKD geographical expansion and for the protection of salmonids populations.

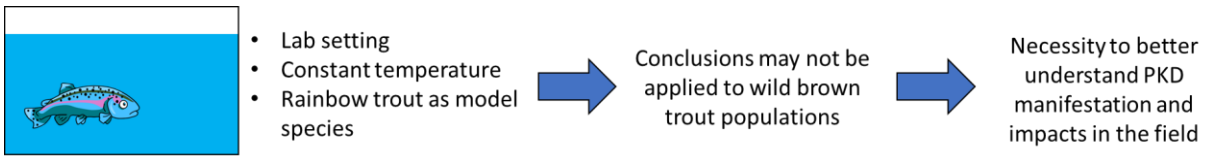


Figure 8 : Simplified drawing of PKD actual gaps, leading to thesis objectives

This thesis aimed therefore to focus on a field setting and with brown trout as model species for the *T. bryosalmonae*-salmonid system. We first determined the suitable period for PKD sampling campaigns based on temperature data (Chapter 2), then highlighted the environmental parameters suspected to influence the disease prevalence and infection intensity (Chapter 3), and finally focused on the impact of water quality as potential aggravating factor for the infection (Chapter 4) (Figure 9).

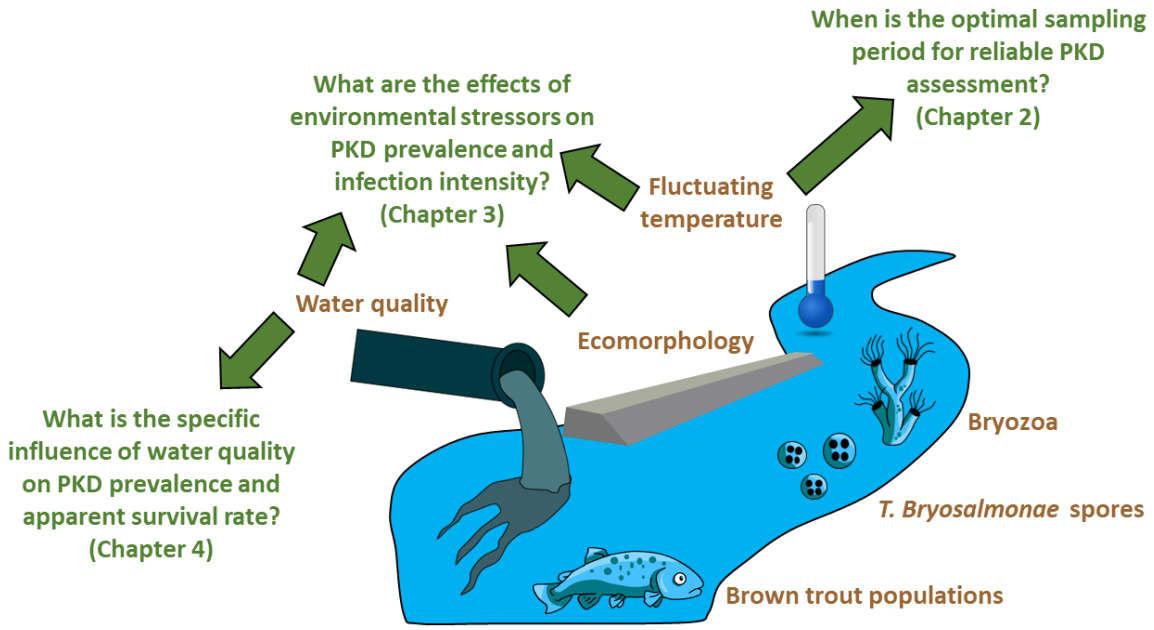


Figure 9 : Simplified drawing of the *T. bryosalmonae*-brown trout system in a field setting (in brown) and thesis objectives (in green).

5.2 Reliable field assessment of proliferative kidney disease in wild brown trout, *Salmo trutta*, populations: When is the optimal sampling period?

PKD can have huge consequences on wild and farmed salmonids stocks. Thus, researchers need to use robust sampling methods for a reliable identification of *T. bryosalmonae*-infected fish in wild brown trout populations. In particular, the choice of the sample size, the diagnosis method and the sampling time-point are of primary concern. In this chapter, the period for having the highest probability for the detection of PKD-positive fish was evaluated.

In the wild, PKD manifestation and its related mortality seem to follow a seasonal fluctuation through the year, with the development of disease symptoms mainly in summer or early autumn (e.g. Foott & Hedrick, 1987; Wahli et al., 2002; Sterud et al., 2007). However, since PKD is temperature-dependant, this period may be reached earlier or later depending on the thermal characteristics of the stream. For a general application, we thus aimed to define the optimal PKD sampling period based on long-term water temperature values and not on season.

Our results showed that a mean of 1500 degree days or 30 days with a daily mean temperature ≥15°C was necessary for a reliable histological detection of the infection. When knowing temperature chronicles of a particular stream, samplings could thus be carefully planned for having the highest probability to detect *T. bryosalmonae*-positive fish and thus assess at best the PKD status of brown trout populations. These values have then been taken into account for the planning of the sampling campaign for Chapter 3.

5.3 Keeping an eye on wild brown trout (*Salmo trutta*) populations: correlation between temperature, environmental parameters and proliferative kidney disease

Water temperature is a key factor for the disease development and severity (e.g. Kinkelin & Loriot, 2001; Longshaw et al., 2002; Bettge et al., 2009a and b). In the wild, some other factors are suspected to affect the interactions between host and parasites, such as water quality (e.g. Schmidt-Posthaus et al., 2001; El-Matbouli & Hoffman, 2002). However, the combined effect of potential other aggravating parameters on the infection has rarely been investigated. The present study thus tried to gain some information about this problematic. We focused on the combined implications of seasonal fluctuating temperature and other stress factors on the infection of wild brown trout populations under field settings. The aims were to 1) observe the correlation between fluctuating water temperature and PKD prevalence and intensity, 2) point out if other environmental aggravating factors influence the infection, and 3) predict the increase of PKD prevalence with water temperature and potential additional parameters through statistical models.

Laboratory experiments have shown that the water temperature threshold for PKD manifestation and mortality appears at 15°C (e.g. Clifton-Hadley et al., 1986; Bettge et al., 2009a and b; Okamura et al., 2011). However, in a field context, with seasonal and diurnal fluctuating thermal regimes, the application of this value seems complex. We thus described six different thermal parameters using long-term temperature measurement data and correlated them with PKD prevalence and severity values. *T. bryosalmonae*-infected fish were observed in 19 sites among the 45 sampled stations (42%), with prevalence ranging from 0 to 100%. The strongest correlation between prevalence values and temperature data was obtained with the mean water temperature of June. The number of days with a daily mean temperature ≥15°C was considered as the second most relevant indicator.

Other environmental factors might also impact the disease dynamics. To this purpose, the possible cumulative effect of water quality (measured via macroinvertebrate analysis and presence/absence of an upstream wastewater treatment plant (WWTP)) and ecomorphology on PKD prevalence and infection intensity was assessed. No correlation was observed between macroinvertebrate data and

infection values. The same statement applied for ecomorphology. In contrast a correlation was noticed between presence of an upstream WWTP and PKD data.

The prediction of PKD prevalence increase following the variation of the highlighted key factors was then determined with statistical models. Our results showed that prevalence was significantly linked to the defined water temperature factors. Thus, an increment of water temperature generated higher predicted prevalence values.

These results provide evidence for a correlation between water temperature and PKD prevalence and severity in brown trout populations and under field conditions. The indication for the implication of additional environmental stress factors, especially the presence of an upstream WWTP, on the disease manifestation was also highlighted. Eutrophication might thus as well be considered as a significant factor for the infection alongside with water temperature. However, this variable is only characterised as “presence” / “absence”. This parameter should thus be further investigated to better understand its implication in the *T. bryosalmonae*-brown trout system. The pathway through which eutrophication influences prevalence (fish health alteration, bryozoan proliferation boost or parasite promotion) could not be highlighted in this study. Further investigations on this issue should hence be conducted for a better understanding of this mechanism. These interrogations have thus been explored in Chapter 4.

5.4 Do fish get wasted? Assessing the influence of effluents on parasitic infection of wild fish

Water temperature has shown to be a key factor for PKD infection (e.g. Kinkelin & Loriot, 2001; Longshaw et al., 2002; Bettge et al., 2009a and b). However, other additional factors might influence disease dynamics (e.g. Marcogliese, 2001; Marcogliese, 2008; Marcos-López et al., 2010). Among them, eutrophication is supposed to play a role on PKD manifestation (Schmidt-Posthaus et al., 2001; El-Matbouli & Hoffman, 2002), as also suggested in Chapter 3. This study aimed therefore to focus on the effect of eutrophication on the PKD infection of wild brown trout populations. We hypothesized that fish living in poor water quality will show greater susceptibility to the disease, and thus lower apparent survival rate.

For this purpose, two stations 400m apart that were similar in terms of temperature regime and ecomorphology, but with differing water quality (upstream and downstream WWTP) were selected. Young of the year brown trout were monthly sampled and tagged between June and October.

Our results confirmed that fish inhabiting the downstream station influenced by the WWTP effluent showed significant higher PKD prevalence (prevalence ranged from 16% to 96%) and infection scores compared to trout residing in the upstream station (scores from 72% to 100%). The apparent survival rate was also lower in the downstream site (28% for the upstream site and 8% for the downstream station).

Thus, even if the studied installation respects the Swiss legislation for WWTP effluents, our results showed that a subtle decrease in water quality is able to strongly influence PKD manifestation. However, we cannot figure out if this factor impacts the *T. bryosalmonae*-brown trout system (1) through the alteration of the fish immune system, (2) via the virulence, transmission and reproduction rate of the parasite, or (3) through the promotion of bryozoan reproduction.

Multiple stressors appear therefore in the ecosystem and may affect PKD dynamics in the wild. Further investigations for a better understanding of these cumulative effects are necessary in order to propose specific measures for the protection of brown trout populations.

5.5 References

Bettge, K., T. Wahli, H. Segner, and H. Schmidt-Posthaus. 2009a. Proliferative kidney disease in rainbow trout: time-and temperature-related renal pathology and parasite distribution. Diseases of Aquatic Organisms 83:67-76.

Bettge, K., H. Segner, R. Burki, H. Schmidt-Posthaus, and T. Wahli. 2009b. Proliferative kidney disease (PKD) of rainbow trout: temperature-and time-related changes of *Tetracapsuloides bryosalmonae* DNA in the kidney. Parasitology 136:615-625.

Clifton-Hadley, R., D. Bucke, and R. Richards. 1987. A study of the sequential clinical and pathological changes during proliferative kidney disease in rainbow trout, *Salmo gairdneri* Richardson. Journal of Fish Diseases 10:335-352.

El-Matbouli, M., and R. Hoffmann. 2002. Influence of water quality on the outbreak of proliferative kidney disease–field studies and exposure experiments. Journal of Fish Diseases 25:459-467.

Foott, J., and R. Hedrick. 1987. Seasonal occurrence of the infectious stage of proliferative kidney disease (PKD) and resistance of rainbow trout, *Salmo gairdneri* Richardson, to reinfection. Journal of fish biology 30:477-483.

Kinkelin, P. d., and B. Loriot. 2001. A water temperature regime which prevents the occurrence of proliferative kidney disease (PKD) in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Journal of Fish Diseases 24:489-493.

Longshaw, M., R. M. Le Deuff, A. Harris, and S. Feist. 2002. Development of proliferative kidney disease in rainbow trout, *Oncorhynchus mykiss* (Walbaum), following short-term exposure to *Tetracapsula bryosalmonae* infected bryozoans. Journal of Fish Diseases 25:443-449.

Marcogliese, D. 2008. The impact of climate change on the parasites and infectious diseases of aquatic animals. Rev Sci Tech 27:467-484.

Marcogliese, D. J. 2001. Implications of climate change for parasitism of animals in the aquatic environment. Canadian Journal of Zoology 79:1331-1352.

Marcos-López, M., P. Gale, B. Oidtmann, and E. Peeler. 2010. Assessing the impact of climate change on disease emergence in freshwater fish in the United Kingdom. Transboundary and emerging diseases 57:293-304.

Okamura, B., H. Hartikainen, H. Schmidt-Posthaus, and T. Wahli. 2011. Life cycle complexity, environmental change and the emerging status of salmonid proliferative kidney disease. Freshwater Biology 56:735-753.

Schmidt-Posthaus, H., D. Bernet, T. Wahli, and P. Burkhardt-Holm. 2001. Morphological organ alterations and infectious diseases in brown trout *Salmo trutta* and rainbow trout *Oncorhynchus mykiss* exposed to polluted river water. Diseases of Aquatic Organisms 44:161-170.

Sterud, E., T. Forseth, O. Ugedal, T. T. Poppe, A. Jørgensen, T. Bruheim, H.-P. Fjeldstad, and T. A. Mo. 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.

Wahli, T., R. Knuesel, D. Bernet, H. Segner, D. Pugovkin, P. Burkhardt-Holm, M. Escher, and H. Schmidt-Posthaus. 2002. Proliferative kidney disease in Switzerland: current state of knowledge. Journal of Fish Diseases 25:491-500.

6 Conclusion and perspectives

6.1 Conclusion

Proliferative kidney disease is an emerging disease in Switzerland, believed to have huge implications either on farmed or on wild salmonids. A better understanding of disease development and impact on fish is of primary concern for the protection of trout populations in present and future times. The present thesis explored PKD manifestation and impacts on wild brown trout populations under *in situ* conditions. More precisely, the suitable time point for PKD sampling (Chapter 2), the implications of environmental parameters on disease prevalence and severity (Chapter 3) and a focus on the involvement of water quality (Chapter 4) have been evaluated (Figure 10 in green).

The main findings of this study are summarized as (Figure 10 in orange):

- The suitable sampling time-point could be assessed based on water temperature data rather than period of the year and could thus be applied to individual streams independently of altitude, location or weather conditions (Chapter 2),
- Water temperature is a main driver for the disease prevalence and infection intensity. However, other additional aggravating environmental parameters, as water quality, should be taken into consideration (Chapter 3).
- Altered water quality could truly be considered as an additional factor affecting PKD prevalence, infection intensity, pathology and apparent survival rate (Chapter 4).

However, even if this study sorted out some issues of the impact of PKD on wild brown trout populations, resulting questions need to be further addressed (Figure 10 in purple). In particular, the pathway through which altered water quality affects the PKD manifestation in fish remains unknown. Three possibilities are plausible:

- Through the vertebrate host via the alteration of its immune system,
- Through the invertebrate host by increasing its reproduction,
- Through the parasite via the modification of its virulence, transmission and reproduction rate.

In addition, potential risk areas for PKD expansion should be highlighted in order to propose measures for the protection of brown trout population.

To answer these questions, laboratory experiments should be performed, studying specifically one of the three possible pathways for the implication of altered water quality on PKD manifestation (Figure 10 in deep blue). Other sampling campaigns upstream and downstream WWTPs could also bring a lot of information.

Moreover, predictions of geographical expansion of the distribution of PKD should be modelled based on the impacts of significant environmental factors (Figure 10 in deep blue). Potential risk areas in the future could thus be highlighted and actions to prevent high PKD development or brown trout populations extinctions should be undertaken.

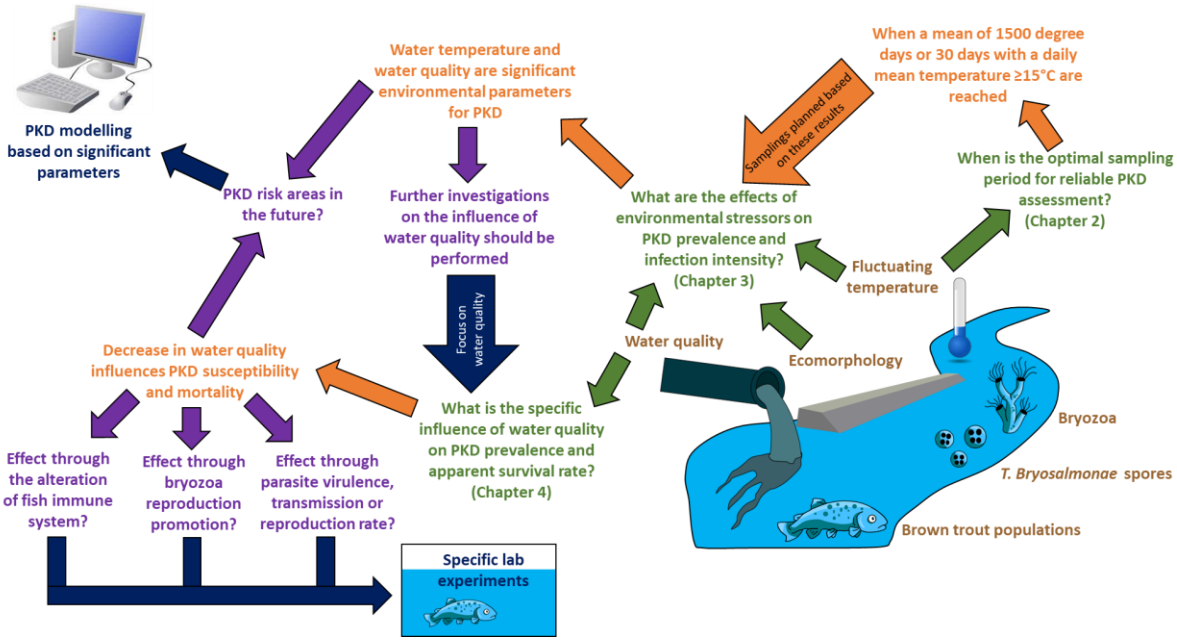


Figure 10 : Simplified drawing of the *T. bryosalmonae*-brown trout system in a field setting (in brown), thesis objectives (in green), general results (in orange), resulting questions (in purple) and further investigations (in deep blue).

6.2 Perspectives

Water temperature data have been used in various modelling studies, in particular for the distribution of present and future suitable thermal habitat for brown trout in Europe (e.g. Lassalle and Rochard, 2009; Almodóvar et al., 2012; Filipe et al., 2013; Muñoz-Mas et al., 2016; Santiago et al., 2020). All these studies coincide on a dramatic reduction in the near or far future of thermal suitable habitats, leading to numerous brown trout population extinctions. However, critical incipient lethal temperature or optimum values were mainly selected as the significant temperature variables. Borgwardt et al. (2020) went one step further. Indeed, since PKD infection is mainly modulated by water temperature, the disease could be considered as an additional threat, potentially lowering even more the remaining thermal favourable habitat assessed by climate models. This study is among the very few experiments modelling the possible emergence of PKD using temperature data (in this case <14 consecutive days over 15°C as the critical thermal parameter for PKD outbreak). They found that, in the past (1971-2000), temperature regime promoting PKD outbreak was not reached in more than 80% of the Austrian studied streams. This value dropped to ~65% in the near future and even to 37% in the most pessimistic model for the far future.

Critical thermal values supporting PKD development may hence be applied in temperature models resulting in a mapping of risk areas for possible PKD expansion. Moreover, potential aggravating factors for the infection, as water quality, may be included as well in PKD modelling. Studies for identification of risk areas should therefore be explored over the next years in order to examine the potential expansion of the parasite geographical range and its impact on wild brown trout population dynamics. One corresponding analysis could be performed on the Boiron de Morges stream since a wide long-term water temperature measurement grid is in place and the distribution of PKD is followed.

In addition, measures have to be initiated by stream managers to protect fish populations from climate change and expansion of PKD infection. Ros and colleagues (2022) explored some actions for fighting PKD impacts. As example, brown trout stocks raised in fish-farm could be released in stream later in the season. This late exposure could produce disease but not mortality. Surviving fish could thus develop immunity and, the next year, they will show less susceptibility to parasite infection. Free

connection to upstream thermal refuges or cooler tributaries will also be of primary importance (Hari et al., 2006; Elliott & Elliott, 2010). Promotion of riparian vegetation shading streams and thus lowering water temperature or limitation of water sampling for agriculture that induces reduction of flow rate may also be proposed (Garner et al., 2017; Trimmel et al., 2018; Wondzell et al., 2019; Borgwardt et al., 2020). Measures for the improvement of water quality and reduction of stream eutrophication will be beneficial as well (Hartikainen et al., 2009; Ros et al., 2022). Awareness campaigns should also be carried out among fishermen for the cleaning of material that was in contact with water to avoid parasite spread due to human movements. Careful oversight should therefore be performed to follow the future disease expansion and its implication in the field. Actions need thus to be undertaken now by stream managers for a better protection of fish biodiversity and limitation of brown trout population extinctions in the future.

6.3 References

Almodóvar, A., G. G. Nicola, D. Ayllón, and B. Elvira. 2012. Global warming threatens the persistence of Mediterranean brown trout. Global Change Biology 18:1549-1560.

Borgwardt, F., G. Unfer, S. Auer, K. Waldner, M. El-Matbouli, and T. Bechter. 2020. Direct and indirect climate change impacts on brown trout in central Europe: how thermal regimes reinforce physiological stress and support the emergence of diseases. Frontiers in Environmental Science 8:59.

Elliott, J., and J. Elliott. 2010. Temperature requirements of Atlantic salmon *Salmo salar*, brown trout *Salmo trutta* and Arctic charr *Salvelinus alpinus*: predicting the effects of climate change. Journal of fish biology 77:1793-1817.

Filipe, A. F., D. Markovic, F. Pletterbauer, C. Tisseuil, A. De Wever, S. Schmutz, N. Bonada, and J. Freyhof. 2013. Forecasting fish distribution along stream networks: brown trout (*Salmo trutta*) in Europe. Diversity and Distributions 19:1059-1071.

Garner, G., I. A. Malcolm, J. P. Sadler, and D. M. Hannah. 2017. The role of riparian vegetation density, channel orientation and water velocity in determining river temperature dynamics. Journal of hydrology 553:471-485.

Hari, R. E., D. M. Livingstone, R. Siber, P. Burkhardt-Holm, and H. Guettinger. 2006. Consequences of climatic change for water temperature and brown trout populations in Alpine rivers and streams. Global Change Biology 12:10-26.

Hartikainen, H., P. Johnes, C. Moncrieff, and B. Okamura. 2009. Bryozoan populations reflect nutrient enrichment and productivity gradients in rivers. Freshwater Biology 54:2320-2334.

Lassalle, G., and E. Rochard. 2009. Impact of twenty-first century climate change on diadromous fish spread over Europe, North Africa and the Middle East. Global Change Biology 15:1072-1089.

Muñoz-Mas, R., A. Lopez-Nicolas, F. Martinez-Capel, and M. Pulido-Velazquez. 2016. Shifts in the suitable habitat available for brown trout (*Salmo trutta* L.) under short-term climate change scenarios. Science of the Total Environment 544:686-700.

Ros, A., H. Schmidt-Posthaus, and A. Brinker. 2022. Mitigating human impacts including climate change on proliferative kidney disease in salmonids of running waters. Journal of Fish Diseases 45:497-521.

Santiago, J. M., C. Alonso, D. Garcia de Jalon, J. Solana-Gutiérrez, and R. Muñoz-Mas. 2020. Effects of climate change on the life stages of stream-dwelling brown trout (*Salmo trutta* Linnaeus, 1758) at the rear edge of their native distribution range. Ecohydrology 13:e2241.

Trimmel, H., P. Weihs, D. Leidinger, H. Formayer, G. Kalny, and A. Melcher. 2018. Can riparian vegetation shade mitigate the expected rise in stream temperatures due to climate change during heat waves in a human-impacted pre-alpine river? Hydrology and earth system sciences 22:437-461.

Wondzell, S. M., M. Diabat, and R. Haggerty. 2019. What matters most: are future stream temperatures more sensitive to changing air temperatures, discharge, or riparian vegetation? Journal of the American Water Resources Association 55:116-132.

Declaration of consent

on the basis of Article 18 of the PromR Phil.-nat. 19

Name/First Name: Rubin Aurélie Amandine

Registration Number: 07-418-825

Study program: PhD in Ecology and Evolution

Bachelor ☐ Master ☐ Dissertation ☑

Title of the thesis: Impacts of water temperature and other environmental parameters on proliferative kidney disease in wild brown trout (Salmo trutta) populations

Supervisor: Prof. Dr. Helmut Segner

I declare herewith that this thesis is my own work and that I have not used any sources other than those stated. I have indicated the adoption of quotations as well as thoughts taken from other authors as such in the thesis. I am aware that the Senate pursuant to Article 36 paragraph 1 litera r of the University Act of September 5th, 1996 and Article 69 of the University Statute of June 7th, 2011 is authorized to revoke the doctoral degree awarded on the basis of this thesis.
For the purposes of evaluation and verification of compliance with the declaration of originality and the regulations governing plagiarism, I hereby grant the University of Bern the right to process my personal data and to perform the acts of use this requires, in particular, to reproduce the written thesis and to store it permanently in a database, and to use said database, or to make said database available, to enable comparison with theses submitted by others.

Montricher, 02.07.2023

Place/Date

Signature