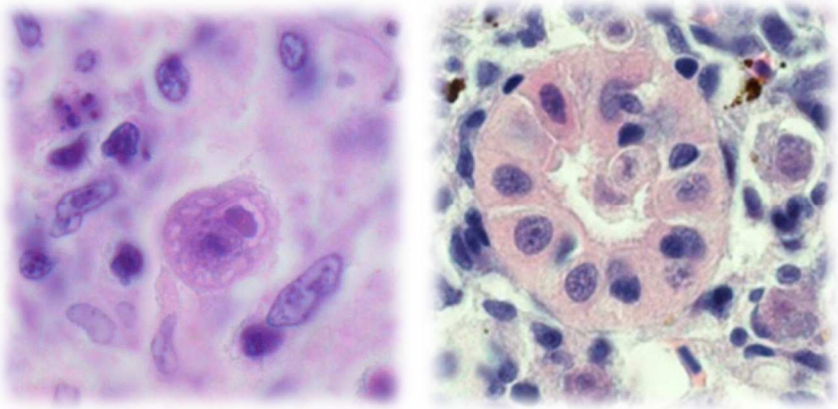
Heike Schmidt-Posthaus, Dr. med vet., Dipl. ECVP, Centre for Fish and Wildlife Health,
Institute of Animal Pathology, Vetsuisse Faculty, Länggassstrasse 122, P.O. Box 8466,
CH-3001 Bern, Tel.: ++41 31 631 24 65, heike.schmidt@vetsuisse.unibe.ch

Investigation for Proliferative Kidney Disease and associated Renal Pathology in Brown Trout from the Rivers Redon and Foron



By Heike Schmidt-Posthaus

Mandate from:

Fédération de Haute-Savoie pour la Pêche et la Protection du Milieu Aquatique
Le Villaret, 2092, Route des Diacquenods,
74370 Saint-Martin-Bellevue,
Tel. 04 50 46 87 55

Funding:

Introduction

Proliferative Kidney Disease (PKD) is a temperature-dependent disease which is considered as an emerging threat to both wild and cultured salmonid fish in Europe (Okamura et al. 2011). The spread and consequences of the disease are suspected to be enhanced by warm water temperatures (Wahli et al. 2008, Bettge et al. 2009, Okamura et al. 2011). The disease is caused by *Tetracapsuloides bryosalmonae* (Myxozoa: Malacosporea) (Hedrick et al. 1993, Canning et al. 2000, Okamura et al. 2001) with bryozoans as invertebrate hosts (Anderson et al. 1999, Longshaw et al. 1999, Okamura et al. 2001) and salmonids as vertebrate hosts (Feist & Bucke 1993, Hedrick et al. 1993). *T. bryosalmonae* infects fish through the skin and gills of the fish (Feist et al. 2001, Longshaw et al. 2002). After invasion, this parasite is distributed systemically with the kidney as the main target organ (Kent & Hedrick 1985). Here, *T. bryosalmonae* multiplies and differentiate into extrasporogonic stages, mainly in the renal interstitium, and then into sporogonic stages in the tubular lumen. In young-of-the-year (YOY), proliferative and granulomatous nephritis and necrotizing vasculitis with thrombus formation are recorded as host reactions to parasite infestation (Hedrick et al. 1993, El-Matbouli & Hoffmann 1994, Bettge et al. 2009). In laboratory studies, surviving rainbow trout can recover with complete regeneration of renal morphology and parasite elimination (Schmidt-Posthaus et al. 2012). However, in field studies kidney regeneration and the parasite elimination in brown trout can be influenced and retarded by additional stress factors, like additional infectious diseases (Schmidt-Posthaus et al., 2013).

The rivers Redon and Foron are two French tributaries of Lake Geneva. In these rivers, decreases in the abundance of young-of-the-year (YOY) brown trout seems to be between 50 and 80% during the summer months of each year (A. Caudron, pers. comm.).

The goal of this investigation was to examine if PKD, which is discussed as a causative factor contributing to brown trout decline in Switzerland, is present in the YOY brown trout in the rivers Redon and Foron. In the following part of the study, we investigated:

(i) if YOY are infected with *T. bryosalmonae*,
(ii) if a possible infection is associated with pathological lesions in the kidney, and
(iii) how a possible infection develops over the summer month.

Material and Methods

Origin of fish

Young-of-the-year brown trout were examined. Fish originated from two different river systems, Redon and Foron. Samples were taken at a weekly basis starting beginning of August, week 31 or 32, until beginning of October, week 40. Always the same location was sampled, near the outlet to the lake. Fish were taken at two consecutive years, 2011 and 2012. At each sampling, 25 brown trout were investigated, resulting in 36 samples or 897 brown trout examined.

Histopathology

Whole fish were fixed in 10% formalin and stored at room temperature. These samples were sent to the Centre for Fish and Wildlife Health for histopathological evaluation. Fish were measured and kidneys were carefully removed, paraffin-embedded and routinely processed for histological examination. 3 µm thick sections were prepared for histopathology and stained with haematoxylin-eosin (H&E). Histopathological lesions were graded as 0 (no), 1 (scattered), 2 (mild), 3 (mild to

moderate), 4 (moderate), 5 (moderate to severe) or 6 (severe). Additionally, lesions were classified as (i) acute, characterized by vasculitis with thrombosis, vascular and interstitial necrosis, hemorrhage and interstitial infiltration with mainly macrophages, (ii) chronic, characterized by macrophage infiltration, interstitial fibrosis and increased tubuloneogenesis, and (iii) chronic active, containing components of acute and chronic inflammation. Infection intensity was measured as density of parasites in the whole histological slide. It was classified as 0 (no parasites), 1 (scattered parasites), 2 (few parasites), 3 (few to moderate numbers of parasites), 4 (moderate numbers of parasites), 5 (moderate to high numbers of parasites) and 6 (high numbers of parasites).

Statistics

PKD prevalence was measured as the sum of animals with *Tetracapsuloides bryosalmonae* in the renal tissue divided by the total number of animals per group. Parasite infection intensity per group was measured as mean of infection intensity values of infected animals (animals with visible parasites in the renal tissue).

Prevalence (PKD prevalence and prevalence of fish showing renal pathology) is shown as sum of positive animals per group divided through the total number of examined fish per group. Each group was defined as one sampling point per week and river location. Each group consisted of 25 animals. Parasite infection intensity and severity of pathological lesions were calculated by mean values of all affected animals per sampling site and date.

Differences in degree of renal pathology between fish originating from the river Redon or Foron, respectively, were tested for significance using a two way ANOVA test with a p-value of ≤ 0.05 significance level (NCSS 2001, Hintze 2006).

Results and Discussion

River Redon

In 2011, PKD prevalence per sampling was about 60% at the beginning of the study and remained at this level for the first 6 weeks. Thereafter values decreased to 20% until the end of the study (Fig. 1). In 2012, prevalence was 84% at the beginning of the study (first week of August) and increased to 100% in the third week. Afterwards it decreased to a similar level as 2011, i.e. to 16% (Fig. 1).

In 2011, prevalence of animals showing pathological renal lesions remained at about 50% until the end of the study (Fig. 1). The discrepancy between PKD prevalence and prevalence of animals showing pathological lesions, especially in the last three weeks of the study was due to animals still showing chronic lesions in the kidney, while parasites were already eliminated (Table 1, for reference see Fig. 8).

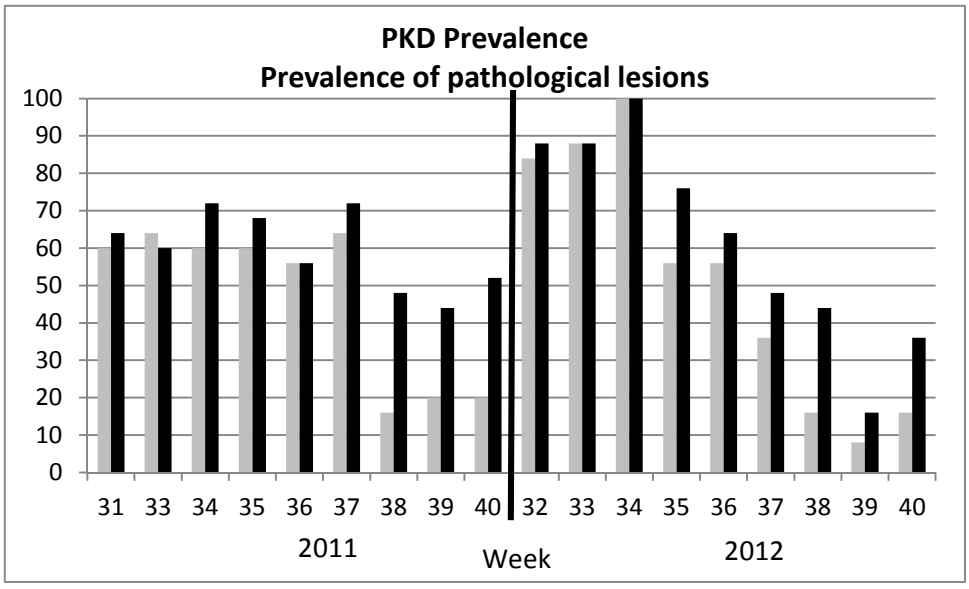


Fig. 1: Redon, prevalence of fish infected with *T. bryosalmonae* (grey bars) and prevalence of fish showing pathological renal lesions (black bars) (%).

In the river Redon, in 2011, mean values of parasite infection intensity per group fluctuated between 1.3 and 3.4 (Fig. 2). However, there was no clear development visible over the whole study period. Despite decreasing prevalences, parasite infection intensity in infected fish remained similar (Fig. 2). The same tendency was visible regarding severity of renal lesions (Fig. 2). Infected fish showed mild to moderate pathology in the kidney over the whole study period. However, the quality of lesions changed by time, in the first two weeks all infected animals showed acute lesions, like vasculitis with thrombus formation, necrosis and hemorrhage in the renal interstitium and macrophage infiltration in the interstitium (granulomatous interstitial nephritis) (for reference see Fig. 7). In the following weeks, quality of lesions changed towards chronic changes, like granulomatous nephritis, interstitial fibrosis and tubuloneogenesis (newly formed tubules) (for reference see Fig. 8). Fish are able to show renal regeneration by repopulation of injured nephrons and, unlike mammals, they are additionally able to produce nephrons de novo. Renal regeneration by nephron neogenesis has been reported in catfish, rainbow trout, tomcod, zebrafish, tilapia, aglomerular toadfish and medaka (Reimschuessel et al. 1990, Reimschüssel et al. 1996, Reimschüssel 2001, Salice et al., 2001, Watanabe et al. 2009, Diep et al. 2011). These nephrons arise from basophilic cell clusters in the interstitium and progress through the normal stages of nephron differentiation. Beginning of September, chronic active changes were visible (for reference see Fig. 9), indicating an ongoing infection during recovery process. In these animals, both acute changes with vasculitis, thrombus formation, interstitial necrosis and hemorrhage and chronic changes, like fibrosis and increased tubuloneogenesis, were present. Additionally, in around 10% of investigated animals acute changes were visible until the end of the study period (Table 1). These results indicate ongoing infections with *T. bryosalmonae* until the beginning of October.

Only one animal in the first week of September showed an additional infection with fungal hyphi associated with a necrotising and purulent process in the kidney.

A similar development of parasite infection intensity and severity of renal pathology was visible in 2012 (Fig. 2). However, in 2012, parasite infection intensity and pathology decreased in parallel to the decrease in PKD prevalence, indicating a beginning of elimination of parasites and a start of recovery process. This was also reflected by the lesions seen in the kidney. In the last two weeks of the study, no chronic active changes were present anymore and only a small percentage of fish showed still acute renal lesions (8%) (Table 1).

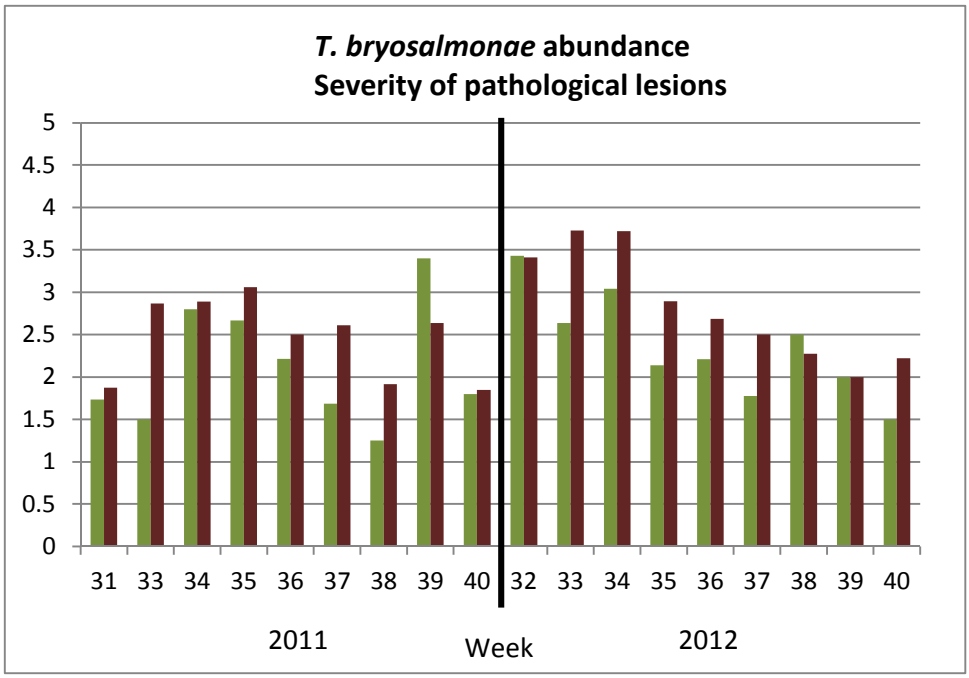


Fig.2: Redon, infection intensity of T. bryosalmonae parasites in the renal tissue (green bars), infection intensity of parasites was classified as 1 (scattered parasites), 2 (few parasites), 3 (few to moderate numbers of parasites), 4 (moderate numbers of parasites), 5 (moderate to high numbers of parasites) and 6 (high numbers of parasites). Shown are mean values of all infected animals per group. Severity of pathological lesions (red bars), histopathological lesions were graded as 0 (no), 1 (scattered), 2 (mild), 3 (mild to moderate), 4 (moderate), 5 (moderate to severe) or 6 (severe). Shown are mean values of all affected animals per group.

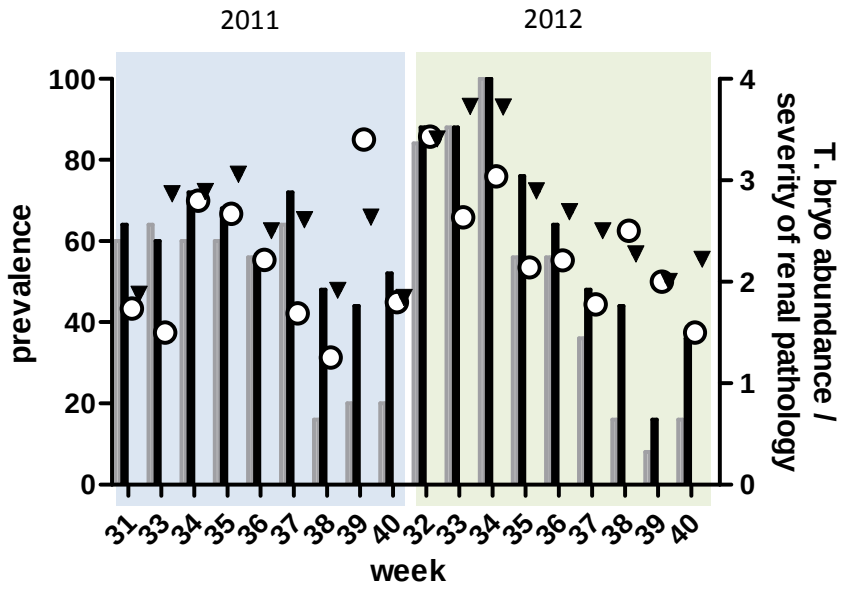


Fig. 3: Redon. Comparison of all parameters. Prevalence of PKD infections (white bars), prevalence of animals showing renal pathological lesions (black bars), parasite infection intensity (white circles) and severity of renal pathology (black triangles); results of 2011 are marked in light blue, results of 2012 are marked in light green.

Table 1: Redon, Prevalence of fish infected with *T. bryosalmonae*, parasite infection intensity (0 = no parasites, 1 = scattered parasites, 2 = few parasites, 3 = few to moderate numbers of parasites, 4 = moderate numbers of parasites, 5 = moderate to high numbers of parasites, 6 = high numbers of parasites) and severity of associated pathology (0 = no, 1 =scattered, 2 =mild, 3 = mild to moderate, 4 =moderate, 5 = moderate to severe, 6 = severe lesions); Prevalence of fish showing acute, chronic or chronic active lesions.

River	Year	Month	Week No.	T. bryo prevalence	T. bryo infection intensity	Patho-logy	Prevalence acute changes (%)	Prevalence chronic changes (%)	Prevalence chronic active changes (%)
Redon	2011	Aug	31	60	1.7	1.8	60		
			33	64	1.5	2.9	64		
			34	60	2.8	2.9	52	8	
			35	60	2.7	3.1	40	20	
		Sep	36	56	2.2	2.5	44	4	8
			37	64	1.7	2.6	40	12	12
			38	16	1.3	1.9	4	32	8
			39	20	3.4	2.6	12	44	
		Oct	40	20	1.8	1.8	8	36	4
	2012	Aug	32	84	3.4	3.4	84		
			33	88	2.6	3.7	48	1	36
			34	100	3.0	3.7	100		
		Sep	35	56	2.1	2.8	48	24	
			36	56	2.2	2.7	24	16	24
			37	36	1.8	2.5	16	16	16
			38	16	2.5	2.3	8	32	8
		Oct	39	8	2	2	8	8	
			40	16	1.5	2.2	8	24	

River Foron

In the river Foron, PKD prevalence was higher in 2011 compared to 2012, reaching 100% in the third week of the study. In the fifth week of 2011, PKD prevalence began decreasing, however at the end of the study in 2011 it was still at 54% (Fig. 4). In 2012, the highest prevalence value was already reached in the second week of the study period with 84%. Afterwards it decreased to 32% by the beginning of October (Fig. 4).

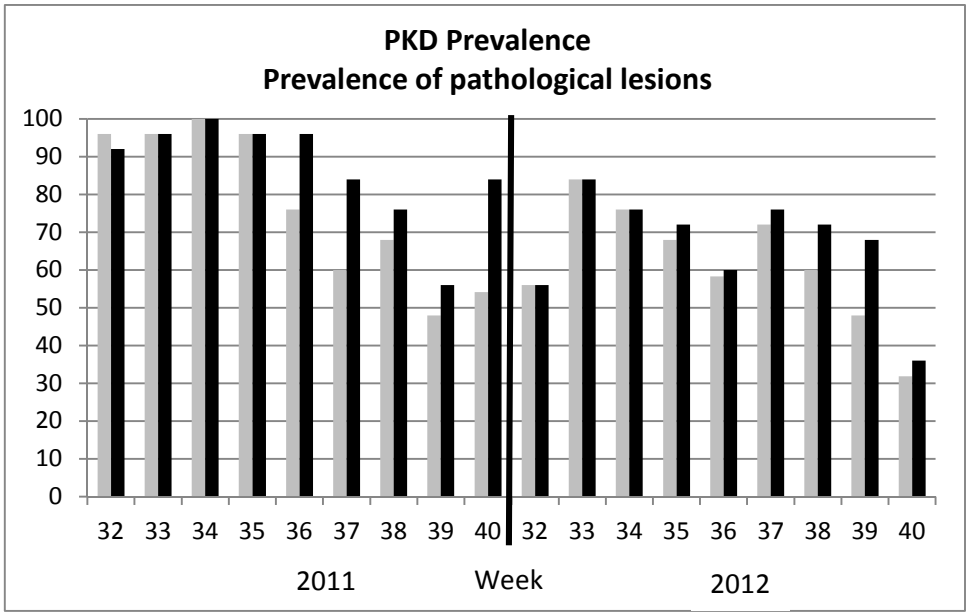


Fig. 4: Foron, prevalence of fish infected with *T. bryosalmonae* (grey bars) and prevalence of fish showing pathological renal lesions (black bars)

In the river Foron, mean values of parasite infection intensity per group and pathology severity developed in parallel to the PKD prevalence (Fig. 5). However, compared to the river Redon, parasite infection intensity and pathology severity were higher (Fig. 11). In 2011 and 2012, at the end of the study, fish showed still moderate pathological lesions (Fig. 5). Compared to the river Redon, pathological lesions were more severe over the whole study period and remained high until the end of the study (Fig. 11).

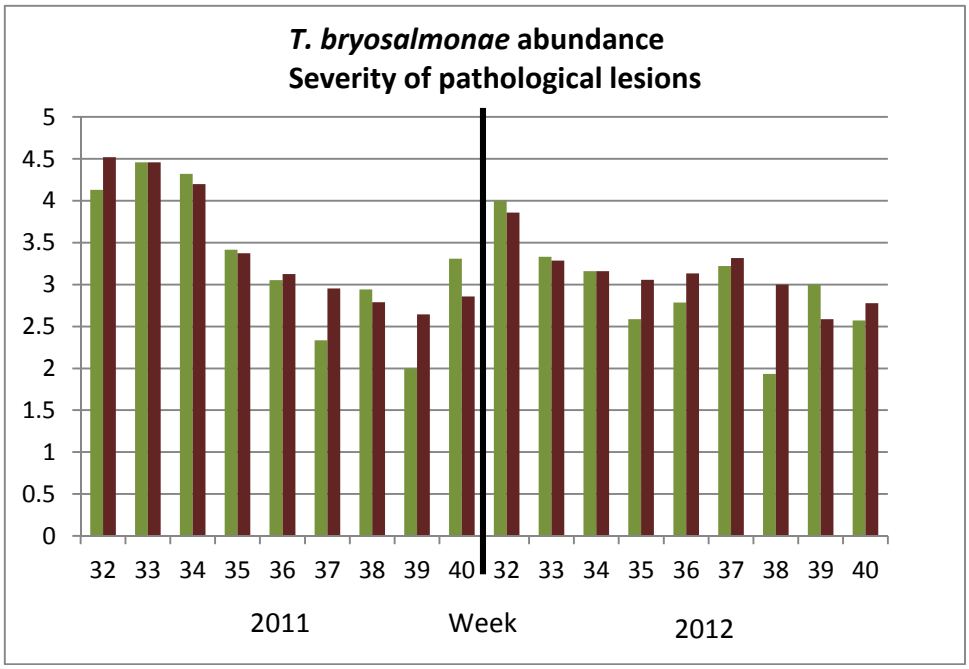


Fig.5: Foron, infection intensity of *T. bryosalmonae* parasites in the renal tissue (green bars), infection intensity of parasites was classified as 0 (no parasites), 1 (scattered parasites), 2 (few parasites), 3 (few to moderate numbers of parasites), 4 (moderate numbers of parasites), 5 (moderate to high numbers of parasites) and 6 (high numbers of parasites). Severity of pathological lesions (red bars), histopathological lesions were graded as 0 (no), 1 (scattered), 2 (mild), 3 (mild to moderate), 4 (moderate), 5 (moderate to severe) or 6 (severe).

Again, the quality of lesions changed by time, for the first three weeks in 2011 and 1012, all infected animals showed acute lesions, like vasculitis with thrombus formation, necrosis and hemorrhage in the renal interstitium and macrophage infiltration in the interstitium (granulomatous nephritis) (Table 2, Fig. 7). In the following weeks, quality of lesions changed towards chronic changes, like granulomatous nephritis, interstitial fibrosis and tubuloneogenesis (Fig. 8). In animals showing chronic renal lesions, *T. bryosalmonae* parasites were also visible in the tubular lumen, indicating a migration of the parasites from the renal interstitium towards the tubular lumen. Starting in the fourth week of the study, chronic active changes were already visible together with acute and chronic changes in the same group (Table 2, Fig. 9). Additionally, the percentage of animals showing chronic active changes was higher compared to animals from the river Redon at the same sampling times (Table 1, 2). This finding indicates a higher percentage of ongoing infections during the recovery process. Additionally, 28% in 2011 and 23% of investigated animals in 2012 still showed acute renal lesions at the end of the sampling period, beginning of October (Table 2). Therefore, the recovery process characterized by shifting of renal lesions towards chronic changes and migration of parasites from the renal interstitium towards the tubular lumen, seems to be retarded in the river Foron in comparison to the river Redon.

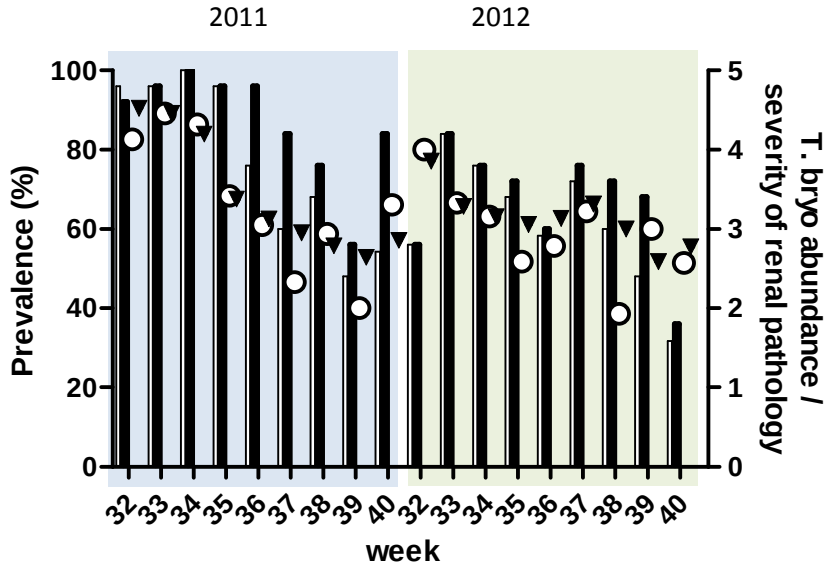


Fig.6: Foron. Prevalence of PKD infections (white bars), prevalence of animals showing renal pathological lesions (black bars), parasite infection intensity (white circles) and severity of renal pathology (black triangles); results of 2011 are marked in light blue and results of 2012 are marked in light green.

Starting end of September in 2011 and end of August in 2012, additional infections with a fugal hyphi, most probable *Exophiala* sp., were diagnosed (Table 2, Fig. 9b). This as a further indication that affected fish were more susceptible to secondary infections and possibly immunosuppressed. In earlier studies, the recovery process following a PKD infection and the elimination of the parasites was retarded in brown trout infested with additional infective agents (Schmidt-Posthaus et al., 2013). The fish immune response against myxozoan infections generally consists of cellular components, like macrophages, lymphocytes or granulocytes, and of humoral components, like lysozyme, peroxidases, complement or specific antibodies (Sitjà-Bobadilla 2008). It is possible that this immune response is influenced and possibly retarded by concurrent infections. A synergistic effect of two concurrent infections is also described for e.g. tilapia and catfish concurrently infected with *Myxobolus tilapiae* and *Flavobacterium columnare* (Eissa et al. 2010). In mammals, co-infection with different parasites can polarize the T cell response in susceptible hosts (Zeidner et al. 2000). The exact mechanisms for this polarization are still unknown.

Table 2: Foron, Prevalence of fish infected with *T. bryosalmonae*, parasite infection intensity (0 = no parasites, 1 = scattered parasites, 2 = few parasites, 3 = few to moderate numbers of parasites, 4 = moderate numbers of parasites, 5 = moderate to high numbers of parasites, 6 = high numbers of parasites) and severity of associated pathology (0 = no, 1 =scattered, 2 =mild, 3 = mild to moderate, 4 =moderate, 5 = moderate to severe, 6 = severe lesions); Prevalence of fish showing acute, chronic or chronic active lesions; Prevalence of fish showing additional infections with fungal hyphi.

River	Year	Month	Week No.	T. bryo prevalence	T. bryo infection intensity	Pathology	Prevalence acute changes (%)	Prevalence chronic changes (%)	Prevalence chronic active changes (%)	Additional infection with fungi (%)
Foron	2011	Aug	32	96	4.1	4.5	96			
			33	96	4.6	4.6	96			
			34	100	4.3	4.2	92		8	
		Sep	35	96	3.4	3.4	40	16	40	
			36	76	3.1	3.1	20	40	36	
			37	60	2.3	3.0	24	28	28	
			38	68	2.9	2.8	28	28	20	
		Oct	39	48	2.0	2.6	28	16	12	4
			40	54	3.3	2.9	28	32	12	4
	2012	Aug	32	56	4.0	3.9	56			
			33	84	3.3	3.3	84			
			34	76	3.2	3.2	76			
		Sep	35	68	2.6	3.1	44	8	16	4
			36	58	2.8	3.1	24	8	28	4
			37	72	3.2	3.3	60	8	8	
			38	60	1.9	3.0	28	16	24	8
		Oct	39	48	3.0	2.6	28	12	20	4
			40	32	2.6	2.8	23	9	5	

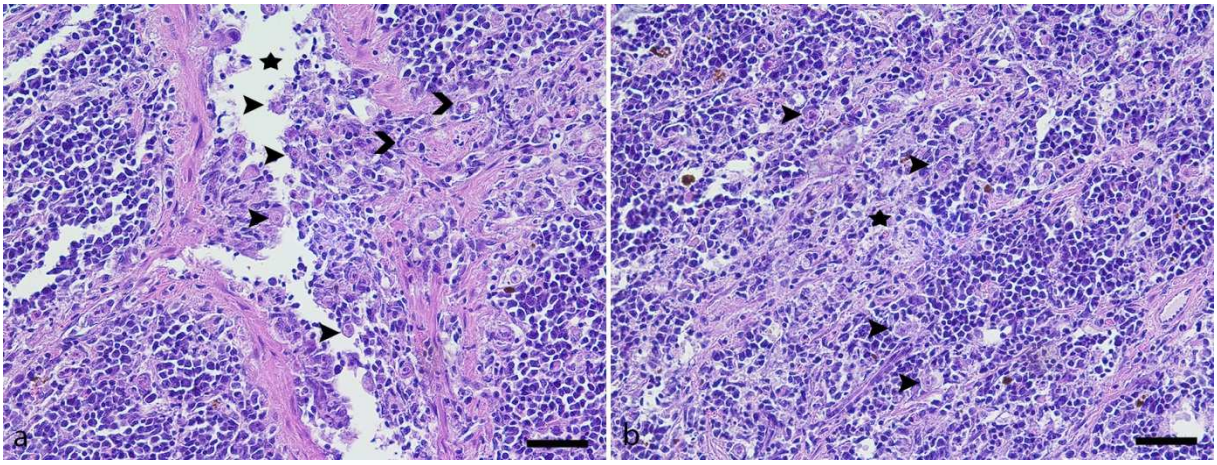


Fig.7: Characteristics of acute histopathological changes, Foron, a. vessel filled with thrombus (star), thrombus composed of fibrin, inflammatory cells and *Tetracapsuloides bryosalmonae* (arrowheads), multifocally parasites migrating trough vessel wall in surrounding interstitial tissue (open arrowheads), vessel wall multifocally degenerated and infiltrated with different inflammatory cells, HE stain, bar = 25µm; b. renal interstitial tissue is expanded by multiple necrotic areas (star) and numerous *T. bryosalmonae* (arrowheads), HE stain, bar = 25µm.

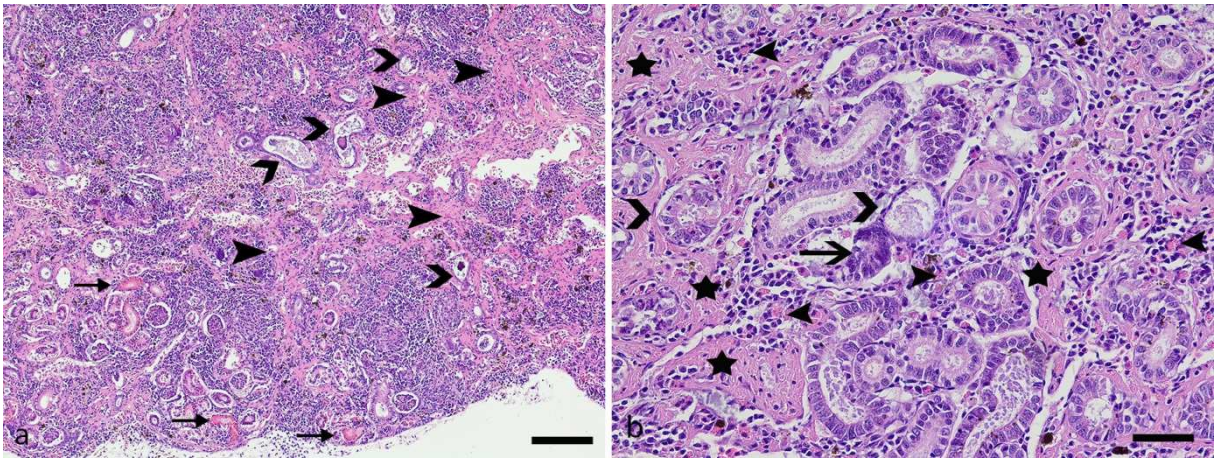


Fig.8: Characteristics of chronic histopathological changes, Foron, a. multifocal interstitium is distended by fibrosis (arrowheads), multiple tubuli show necrosis of tubular epithelial cells (tubulonephrosis) (open arrowheads) or hyaline droplet degeneration (arrows), HE stain, bar = 100µm; b. higher magnification of tubulonephrosis (open arrowheads) and newly formed tubuli (tubuloneogenesis) (arrow), tubuli are surrounded by fibrosis (stars), multifocally there are increased numbers of eosinophilic granular cells (arrowheads), HE stain, bar = 25µm.

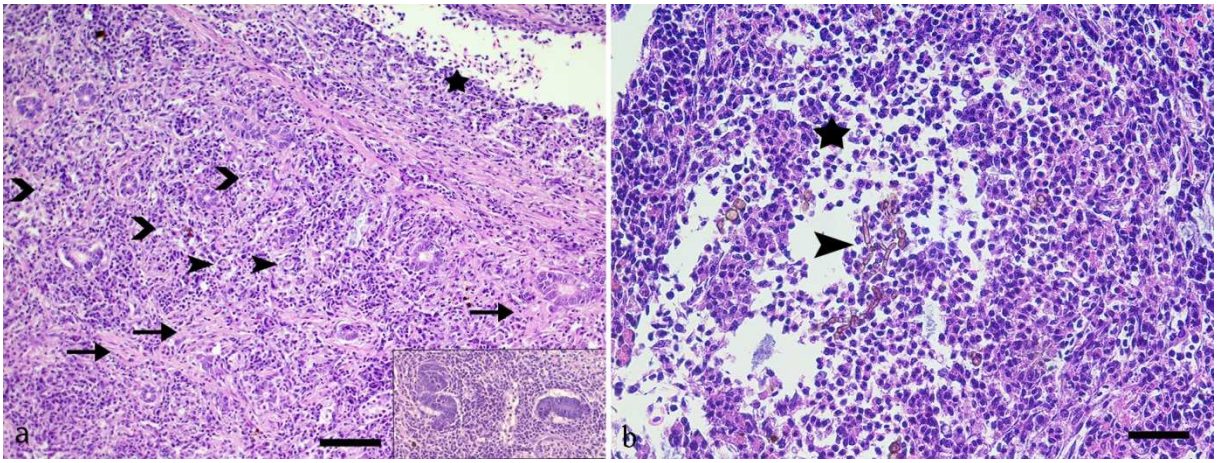


Fig.9: Characteristics of chronic-active histopathological changes, Foron, a. vessel is filled with thrombi (star), parasites are present in interstitial tissue (arrowheads) and are associated with interstitial necrosis (open arrowheads), in parallel interstitial fibrosis is present (arrows) and multifocally there is tubuloneogenesis (insert), HE stain, bar = 50µm; b. occasionally fungal hyphi are present (arrowhead) associated with widespread necrosis (star) and infiltration with macrophages, lymphocytes and neutrophilic granulocytes, HE stain, bar = 25µm.

Comparison between river Redon and river Foron

Comparison between the two rivers, Redon and Foron, showed that both, PKD prevalence and prevalence of fish showing renal pathology, were higher in fish originating from the river Foron in comparison to the Redon (Fig. 10). A few exceptions were visible in the first three weeks of 2012. However, the dynamics of the infection over the study period was comparable between the two examined rivers (Fig. 10).

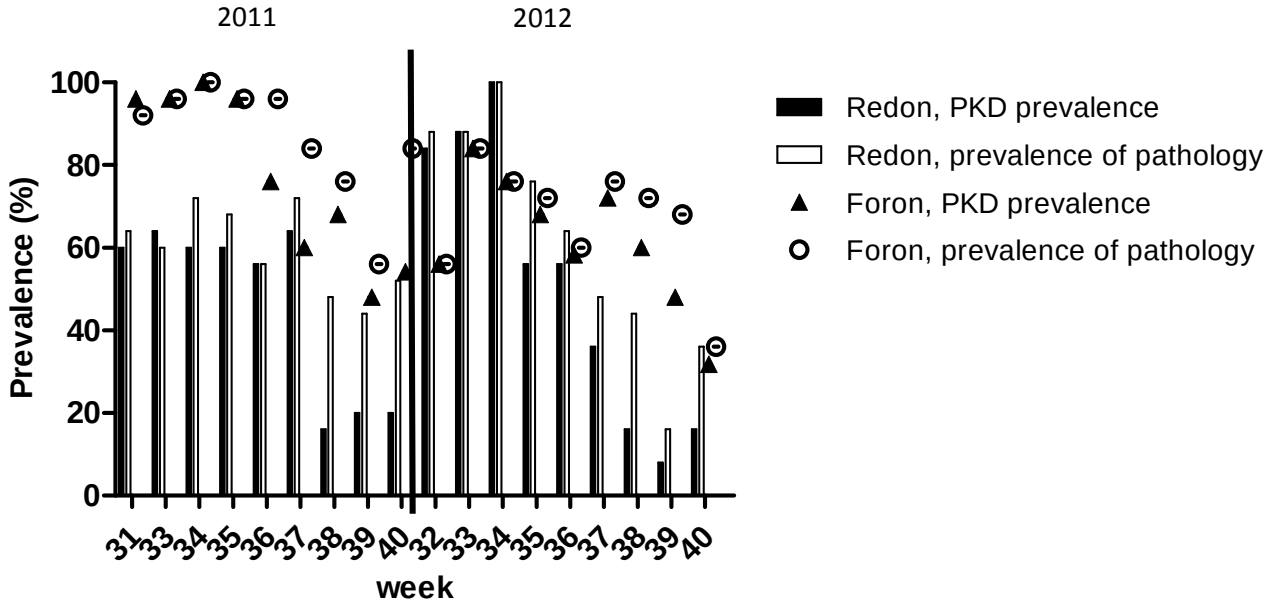


Fig.10: Comparison between Redon and Foron, PKD prevalence in the river Redon (black bars), prevalence of fish showing renal pathology (white bars), PKD prevalence on the river Foron (black triangles), prevalence of fish showing renal pathology (white circles)

Comparison of the degree of renal pathology showed a significant difference between fish from the river Foron and the river Redon, respectively, with brown trout from the river Foron showing significantly higher severity of renal lesions ($p<0.01$) (Fig. 11).

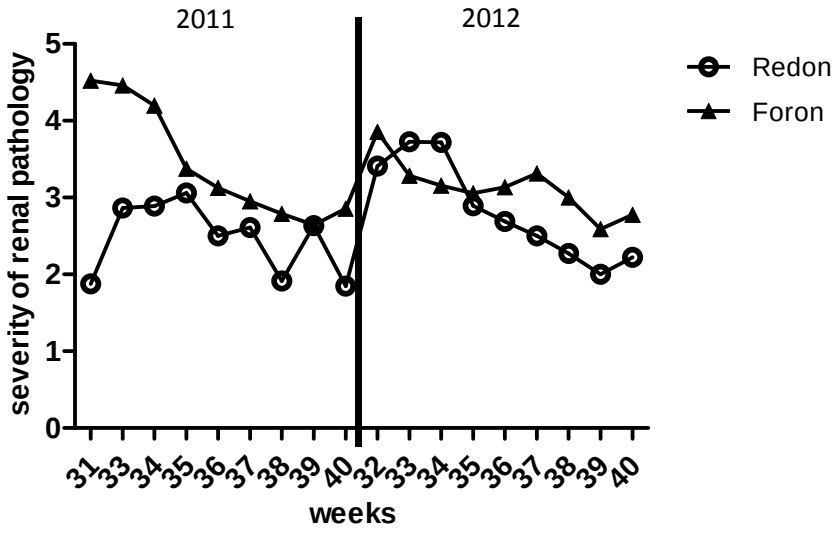


Fig.11: Severity of renal pathology, fish originating from river Redon (white circle), fish originating from river Foron (black triangle); 0 = no, 1 =scattered, 2 =mild, 3 = mild to moderate, 4 =moderate, 5 = moderate to severe, 6 = severe lesions;

In summary, in this study we could show that:

- *(i)* brown trout originating from both rivers, Redon and Foron, are infected with *T. bryosalmoane*,
- *(ii)* that this infection is associated with moderate renal pathology,
- *(iii)* that brown trout from the river Foron showed significantly more severe lesions over the whole study period compared to animals from the river Redon,
- *(iv)* that in the river Foron, in contrast to the river Redon, concurrent infections with fungal hyphi were diagnosed, possibly influencing the recovery process in the kidney and the parasite elimination.

Literature

Anderson CL, Canning EU, Okamura B (1999) 18S rDNA sequences indicate that PKX organism parasitizes bryozoa. Bull Eur Ass Fish Pathol 19: 94-97

Bettge K, Wahli, T, Segner H, Schmidt-Posthaus H (2009) Proliferative kidney disease in rainbow trout: time- and temperature-related renal pathology and parasite distribution. Dis Aquat Org 83 (1): 67-76

Canning EU, Curry A, Feist SW, Longshaw M, Okamura B (2000) A new class and order of myxozoans to accommodate parasites of bryozoans with ultrastructural observations on *Tetracapsula bryosalmonae* (PKX Organism). J Eucaryot Microbiol 47: 456-468

Diep CQ, Ma D, Deo RC, Holm TM, Naylor RW, Arora N, Wingert RA, Bollig F, Djordjevic G, Lichman A, Zhu H, Ikenaga T, Ono F, Englert C, Cowan CA, Hukriede NA, Handin RI, Davidson AJ (2011) Identification of adult nephron progenitors capable of kidney regeneration in zebrafish. Nature doi:10.1038/nature09669

Eissa AE, Zaki MM, Aziz AA (2010) Flavobacterium columnare / Myxobolus tilapiae concurrent infection in the earthen pond reared Nile tilapia (*Oreochromis niloticus*) during the early summer. IBC, doi: 10.4051/ ibc.2010.2.2.0005

El-Matbouli M, Hoffman RW (1994) Proliferative kidney disease (PKD) as an important myxosporean infection in salmonid fish. In: Parasitic Diseases of Fish. (Eds A.W. Pike & J.W. Lewis), pp. 3–15. Samara Publishing Limited, Tresaith, Wales.

Feist SW, Bucke D (1993) Proliferative kidney disease in wild salmonids. Fish Res 17: 51-58

Feist SW, Longshaw M, Canning EU, Okamura B (2001) Induction of proliferative kidney disease (PKD) in rainbow trout *Oncorhynchus mykiss* via the bryozoan *Fredericella sultana* infected with *Tetracapsula bryosalmonae*. Dis Aquat Org 45: 61-68

Hedrick RP, Adkinson MA, MacConnell E. (1998) Whirling disease: re-emergence among wild trout. Immunol Rev 166: 365–76

Hedrick RP, MacConnell E, de Kinkelin P (1993) Proliferative kidney disease of salmonid fish. Ann Rev Fish Dis 3: 277-290

Hintze J (2006) NCSS, PASS, and GESS. NCSS. Kaysville, Utah. WWW.NCSS.COM, released: May 19, 2006

Kent ML, Hedrick RP (1985) Development of the PKX myxosporean in rainbow trout *Salmo gairdneri*. Dis Aquat Org 1: 169-182

Longshaw M, Feist SW, Canning EU, Okamura B (1999) First identification of PKX in bryozoans from the United Kingdom – Molecular evidence. Bull Eur Ass Fish Pathol 19: 146-148

Longshaw M, Le Deuff RM, Harris A.F, Feist SW (2002) Development of proliferative kidney disease in rainbow trout, *Oncorhynchus mykiss* (Walbaum), following short-term exposure to *Tetracapsula bryosalmonae* infected bryozoans. J Fish Dis 25: 443-449

Okamura B, Anderson CL, Longshaw M, Feist SW, Canning EU (2001) Patterns of occurrence and 18S rDNA sequence variation of PKX (*Tetracapsula bryosalmonae*), the causative agent of salmonid proliferative kidney disease. J Parasitol 87: 379-385

Okamura B, Hartikainen H, Schmidt-Posthaus H, Wahli T (2011) Life cycle complexity, environmental change and the emerging status of salmonid proliferative kidney disease. Freshwater Biol 56 (4): 735-753. doi:10.1111/j.1365-2427.2010.02465.x.

Reimschuessel R (2001) A fish model of renal regeneration and development. Ilar J 42: 285-291

Reimschuessel R, Chamie SJ, Kinnel M (1996) Evaluation of gentamicin-induced nephrotoxicosis in toadfish. J Am Vet Med Assoc 209: 137-139

Salice CJ, Rokous JS, Kane AS, Reimschuessel R (2001) New nephron development in goldfish (*Carassius auratus*) *k*idneys following repeated gentamicin-induced nephrotoxicosis. Comp Med 51: 56-59

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. Dis Aquat Org 97(3): 207-218

H. Schmidt-Posthaus, P. Steiner, B. Müller, A. Nakayama Casanova (2013). Complex interaction between Proliferative Kidney Disease, water temperature, and concurrent nematode infection in brown trout. Dis Aquat Org 104: 23-34

Sitjà-Bobadilla A (2008) Fish immune response to myxozoan parasites. Parasite 15: 420-425

Wahli T, Bernet D, Segner H, Schmidt-Posthaus H (2008) Role of altitude and water temperature as regulating factors for the geographical distribution of *Tetracapsuloides bryosalmonae* infected fish in Switzerland. J Fish Biol 73: 2184-2197

Watanabe N, Kato M, Suzuki N, Inoue C, Fedorova S, Hashimoto H, Maruyama S, Matsuo S, Wakamatsu Y (2009) Kidney regeneration through nephron neogenesis in medaka. Development, Growth & Differentiation 51: 135-143.

Zeidner NS, Dolan MC, Massung R, Piesman J, Fish D. (2000) Coinfection with *Borrelia burgdorferi* and the agent of human granulocytic ehrlichiosis suppresses IL-2 and IFNγ production and promotes an IL-4 response in C3H/HeJ mice. Parasite Immunol 22 (11): 581-588