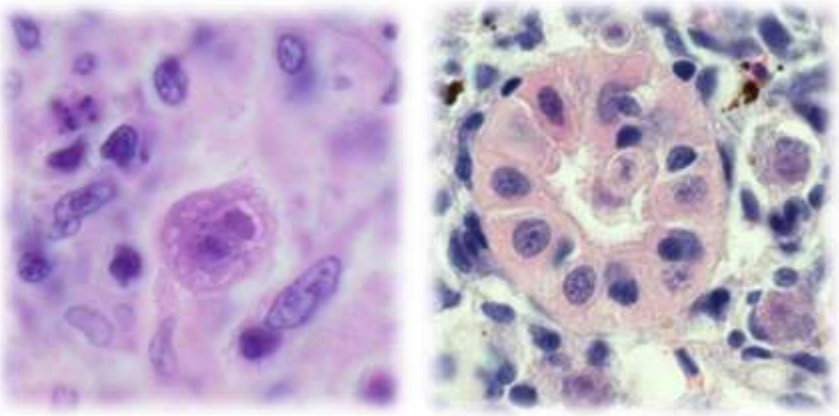
Heike Schmidt-Posthaus, Dr. med vet., Dipl. ECVP, Centre for Fish and Wildlife Health,
Institute of Animal Pathology, Vetsuisse Faculty, Länggassstrasse 122, P.O. Box 8466,
CH-3001 Bern, Tel.: ++41 31 631 24 65, heike.schmidt@vetsuisse.unibe.ch

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



By Heike Schmidt-Posthaus

Arnaud Caudron

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

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Introduction

Proliferative Kidney Disease (PKD) is a temperature-dependent disease which is considered as an emerging threat of both, wild and cultured, salmonid fish in Europe (Okamura et al. 2011). The spread and the consequences of the disease are suspected to be enhanced by water temperature (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 skin and gills (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 from extrasporogonic stages, mainly in the renal interstitium, to 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 reaction to parasite infestation (Hedrick et al. 1993, El-Matbouli & Hoffmann 1994, Bettge et al. 2009). In laboratory studies, we previously showed that surviving rainbow trout can recover with complete regeneration of renal morphology and parasite elimination (Schmidt-Posthaus et al. 2012). However, in field studies using brown trout we could show that these kidney regeneration and the parasite elimination 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 the Lake of Geneva. In these rivers decrease in population size of young-of-the-year (YOY) 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 Proliferative Kidney Disease could be a possible cause for an increased mortality in YOY in the rivers Redon and Foron. In the following part of the study, we wanted to investigate

(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. 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 by Guillaume Bini.

Histopathology

According to river and sampling date, 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 kidney was prepared. Formalin-fixed kidney samples were 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. Abundance 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).

Statistics

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 abundance and severity of pathological lesions were calculated by mean values of all affected animals.

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

Results and Discussion

River Redon

In 2011, PKD prevalence was about 60% at the beginning of the study and remained at this level for the first 6 weeks. Afterwards it decreased down 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, 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).

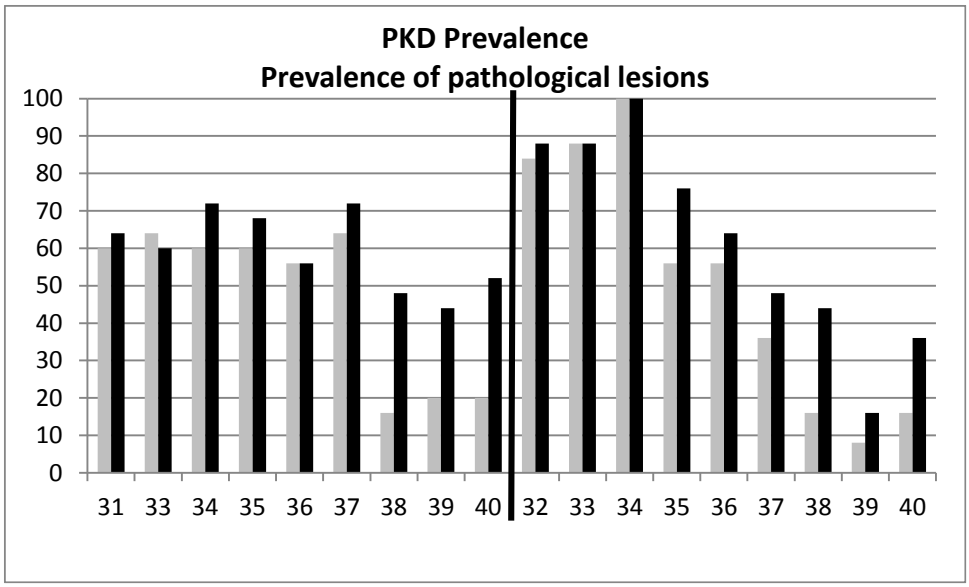

Fig. 1: Redon, prevalence of fish infected with *T. bryosalmonae* (grey bars) and prevalence of fish showing pathological renal lesions (black bars) (%).

In the river Redon, in 2011, parasite abundance 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 abundance 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). In the following weeks, quality of lesions changed towards chronic changes, like granulomatous nephritis, interstitial fibrosis and tubuloneogenesis (newly formed tubules). 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, 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, around 10% of investigated animals remained to show acute changes 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 abundance and severity of renal pathology was visible in 2012 (Fig. 2). However, in 2012, parasite abundance 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 and only a small percentage of fish showed still acute renal lesions (8%) (Table 1).

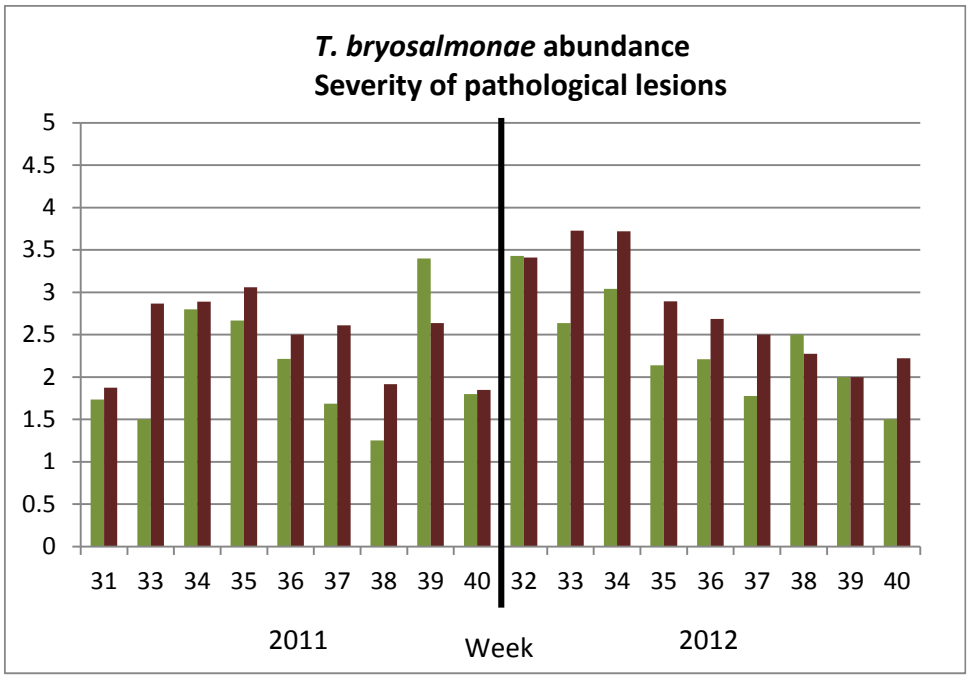

Fig.2: Redon, abundance of T. bryosalmonae parasites in the renal tissue (green bars), abundance of parasites was classified as 1 (scattered parasites), 2 (few parasites), 3 (few to moderate numbers of parasites), 4 (moderate numbers of parasites), 5 (moderate to high numbers of parasites) and 6 (high 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.

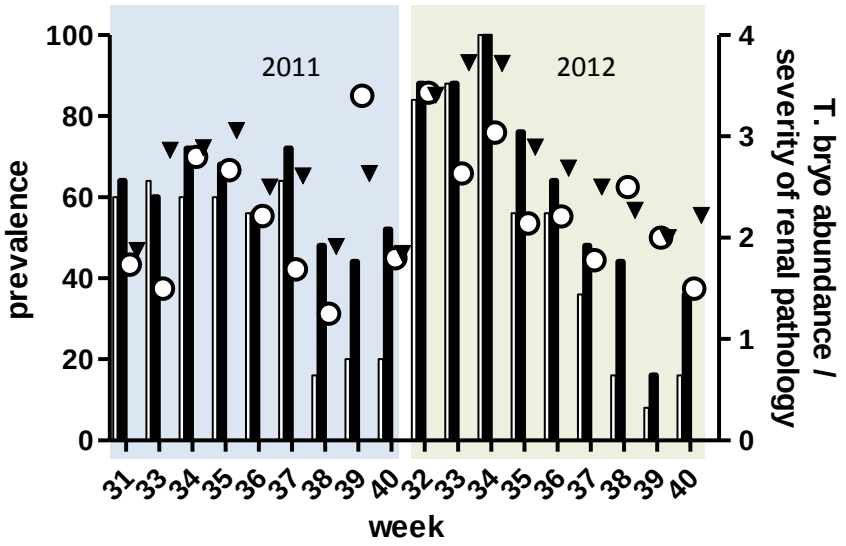

Fig. 3: Redon. Comparison of all parameters. Prevalence of PKD infections (white bars), prevalence of renal pathological lesions (black bars), parasite abundance (white circles) and severity of renal pathology (black triangles); results of 2011 are marked in light blue, results of 2012 are marked in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

Table 1: Redon, Prevalence of fish infected with *T. bryosalmonae*, parasite abundance (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 abundance	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		
			35	56	2.1	2.8	48	24	
		Sep	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
			39	8	2	2	8	8	
		Oct	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, PKD prevalence was decreasing, however at the end of the study in 2011 it was still at 54% (Fig. 4). In 2012, highest prevalence value was already reached in the second week of the study period with 84%. Afterwards it decreased to 32% beginning of October (Fig. 4).

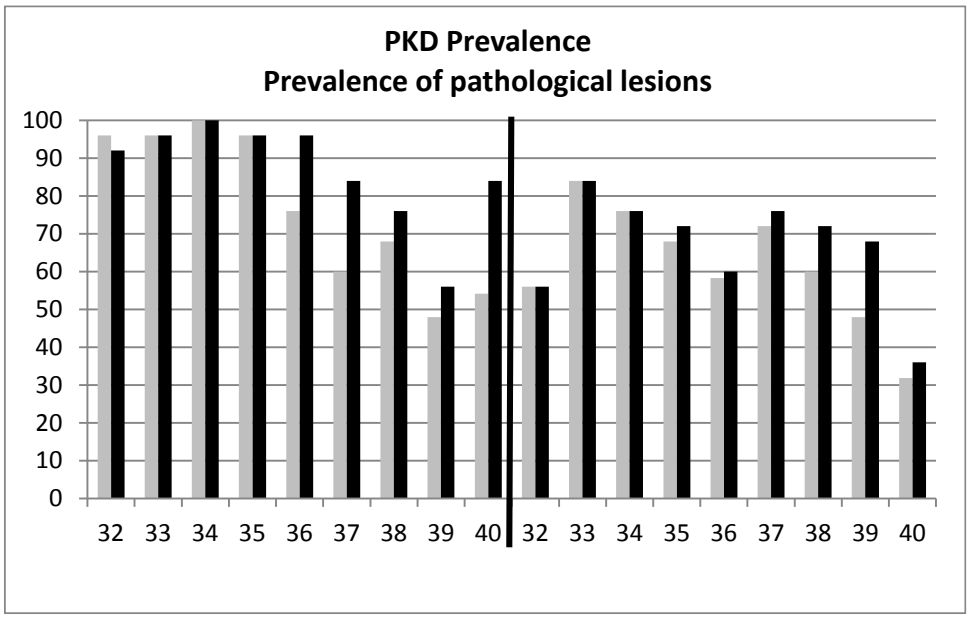

Fig. 4: Foron, prevalence of fish infected with *T. bryosalmonae* (grey bars) and prevalence of fish showing pathological renal lesions (black bars)

In the river Foron, parasite abundance and pathology severity developed in parallel to the PKD prevalence (Fig. 5). However, compared to the river Redon, parasite abundance and pathology severity were higher (Fig. 7). 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 advanced over the whole study period and remained to be high until the end of the study (Fig. 8).

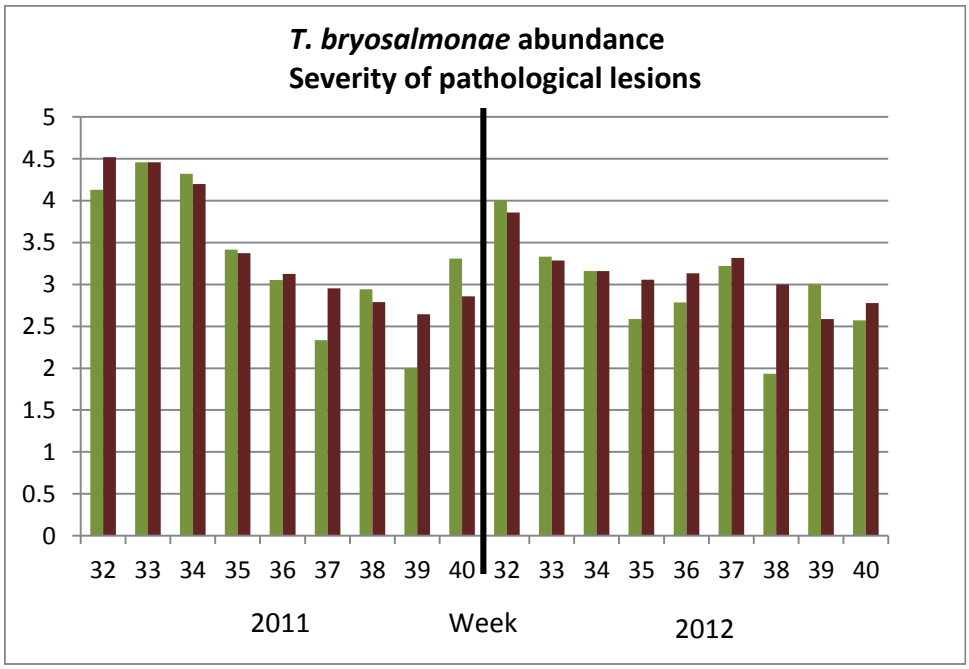

Fig.5: Foron, abundance of *T. bryosalmonae* parasites in the renal tissue (green bars), abundance of parasites was classified as 0 (no parasites), 1 (scattered parasites), 2 (few parasites), 3 (few to moderate numbers of parasites), 4 (moderate numbers of parasites), 5 (moderate to high numbers of parasites) 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). In the following weeks, quality of lesions changed towards chronic changes, like granulomatous nephritis, interstitial fibrosis and tubuloneogenesis. 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). Additionally, 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 circumstance indicates a higher percentage of ongoing infections during 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, 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.

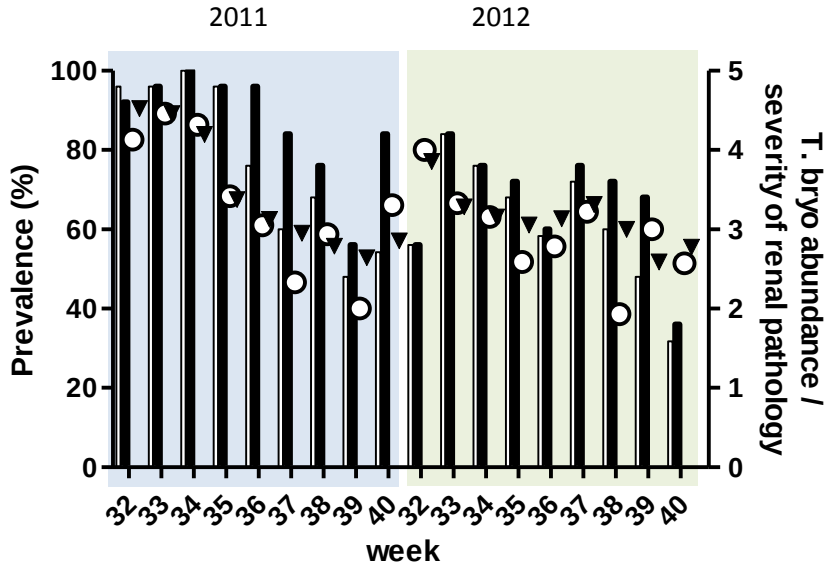

Fig.6: Foron. Prevalence of PKD infections (white bars), prevalence of renal pathological lesions (black bars), parasite abundance (white circles) and severity of renal pathology (black triangles); results of 2011 are marked in light blue and results of 2012 are marked in light green.

Starting end of September in 2011 and end of August in 2012, additional infections with a fugal hyphi, most probable *Exophiala* sp., was diagnosed (Table 2). This as an additional indication that affected fish were more susceptible to secondary infections and possible immunosuppressed. In earlier studies we could show that the recovery process following a PKD infection and the elimination of the parasites are retarded in brown trout infested with additional infective agents (Schmidt-Posthaus et al., 2013). The fish immune response against myxozoan infections in general 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 it was shown before, that 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 abundance (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 abundance	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	
			35	96	3.4	3.4	40	16	40	
		Sep	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	
			39	48	2.0	2.6	28	16	12	4
		Oct	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			
			35	68	2.6	3.1	44	8	16	4
		Sep	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
			39	48	3.0	2.6	28	12	20	4
		Oct	40	32	2.6	2.8	23	9	5	

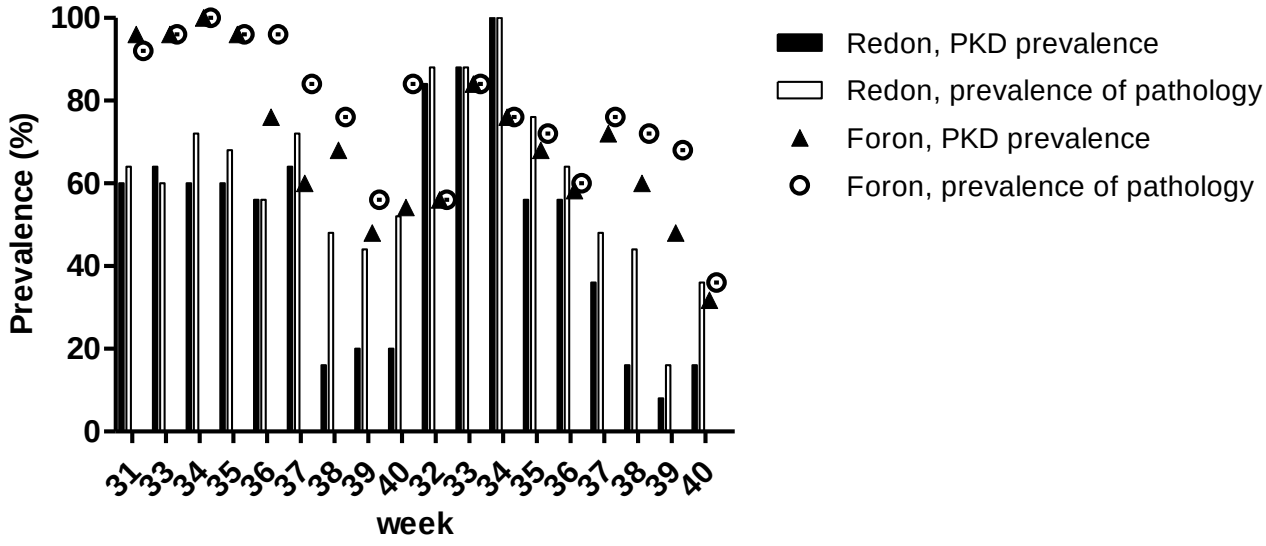

Fig.7: Comparison between Redon and Foron, PKD prevalence in the river Redon (black bars), prevalence of fish showing renal pathology (white bars), PKD prevalence on the river Foron (black triangles), prevalence of fish showing renal pathology (white circles)

Comparison 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. 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.

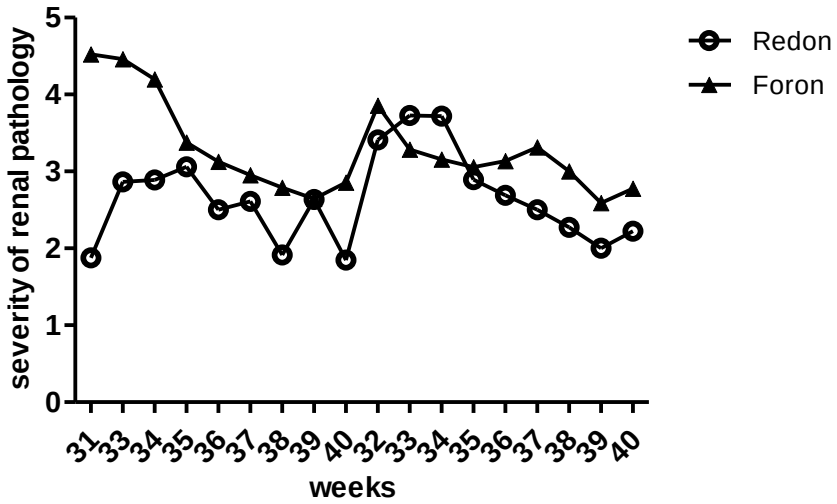

Fig.8: Severity of renal pathology, fish originating from river Redon (white circle), fish originating from river Foron (black triangle); 0 = no, 1 =scattered, 2 =mild, 3 = mild to moderate, 4 =moderate, 5 = moderate to severe, 6 = severe lesions;

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 ($p<0.01$).

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