Out-migrant smolts from a river on the west coast of Norway were sampled prior to seawater entry and screened for the occurrence of *Tetracapsuloides bryosalmonae* by histology and real-time PCR. *T. bryosalmonae* was found in more than 50% of the fish by real-time PCR, while no exstrasporogonic stages were found by histology. Heavily melanization in the kidney tissue was found in less than half the examined which could be the result of a past or ongoing subclinical PKD or caused by other infections.

In wild salmonids populations, it is generally stated that salmonid specimens that have survived a PKD outbreak become resistant and will not suffer from PKD again (Ferguson, 1981). However, this statement seems almost exclusively to be based on studies on
farmed rainbow trout *Oncorhynchus mykiss* (Ferguson and Ball, 1979; Foott and Hedrick, 1987), and little is known about resistance to later re-infections of *T. bryosalmonae* and repeated development of PKD among most other salmonid genera. Studies in Norway indicate that parr of Atlantic salmon or brown trout parr that have survived a PKD outbreak, can suffer from PKD again the next year. In the yearly outbreaks of PKD in the river Åelva, Northern Norway (N 65° 4.71’ E 12° 27.35’), most of the yearlings, but also many older parr suffer from PKD (Sterud et al., 2007). Most likely, the older parr suffered from clinical or subclinical PKD the year before without succumbing. Furthermore, in a study in the river Jølstra, *T. bryosalmonae* was found in one and two year old parr in the early spring after ice melting and low water temperatures (< 7 °C) (Eriksson-Kallio and Jørnandl, 2008). This indicates that the fish carried the parasites throughout the winter after being infected in the previous year(s). Thus, a second PKD outbreak in an individual salmonid could either be the result of a new infection or activation of latent infection when the water temperature increases in the summer and autumn. These observations have led us to the following question: Could smolts of Atlantic salmon and sea trout still be infected with *T. bryosalmonae* when they leave the rivers and migrate to sea in the spring? If the kidney is affected, this could be of importance for the smolt survival in the first period at sea. The aim of the study presented in this note was firstly, to examine smolts of Atlantic salmon and sea trout just prior to their migration to the sea for the presence of *T. bryosalmonae* by realtime-PCR in a river where PKD has been diagnosed in younger parr stages, and secondly, to examine the kidney by histology to look for pathological changes in the tissue, associated with extrasporgonic *T. bryosalmonae* stages, that potentially could affect the marine adaptation and survival.

The river Nausta (N 61° 30.40’ E 5° 43.20’) is draining into the fjord Førdefjorden in Sogn and Fjordane county, western Norway. On 10th and 11th May and 1st June 2010, 29 Atlantic salmon smolts and 21 sea trout smolts were sampled by electro-fishing, just prior to their migration to the sea. The fish were killed by a blow to the head, the abdomen opened with a scalpel and a pair of pincers was used to take out kidney samples. From each fish, two tissue samples were taken from the mid-kidney; one piece was transferred to a tube containing 96 % ethanol and the other to a tube containing 10 % buffered formalin. All samples were stored separately. Formalin-fixed kidney samples were cut to approximately 10 x 15 mm pieces, embedded in paraffin wax and processed for routine histology. Sections were cut to 5 µm and stained with hematoxylin and eosin (H&E) (Bancroft and Gamble, 2002). Alcohol-preserved kidney samples were DNA-extracted, diluted and subjected to real-time PCR following the protocol in Grabner and El-Matbouli (2009). The specificity of the primer pair in this realtime-PCR has been tested for several myxozoan species and uninfected rainbow trout kidney tissue, and the test showed no amplification of the fish tissue or the myxozoans except from DNA of *T. bryosalmonae* (Grabner and El-Matbouli, 2009).

Classical findings in fish with clinical PKD include characteristic interstitial PKX-cells surrounded by macrophages and a granulomatous response. No such structures were found in any of the fish. However, the interstitial tissue was heavily melanized in 9 of the 29 Atlantic
salmon smolts and 14 of the 21 sea trout smolts. The explanation for this can only be surmised, but could be the result of a past or ongoing subclinical PKD or caused by another infection. Melanization could be related to age but this is unlikely in this case as the smolts are young fish (2-3 years of age). In 10 out of the 29 Atlantic salmon smolts and 11 out of the 21 sea trout smolts spore-like structures were seen in the kidney tubules. The exact identity of these structures could not be identified with the techniques used. There were no obvious correlation between melanization of the interstitial tissue and the occurrence of spore-like structures in the kidney tubules. By real-time PCR, *T. bryosalmonae* was found in 16 out of 29 Atlantic salmon smolts and in 15 out of 21 sea trout smolts. There were no obvious correlations between the occurrences of spore-like structures in the kidney tubules in the fish positive for *T. bryosalmonae* by real-time PCR. On the other hand, spore-like structures were seen in the kidney tubules of 6 Atlantic salmon and 4 sea trout smolt that were negative for the presence of *T. bryosalmonae*. This could indicate that myxozoans other than *T. bryosalmonae* were involved. However, this can also be explained by the relatively small tissue samples for DNA extraction and histology, and that they were taken from slightly different, although adjacent, parts of the kidney.

Even if *T. bryosalmonae* was demonstrated in 31 of the 50 examined smolts, real-time PCR results give no information about the parasite localization and stage in the fish. In histological sections, no classical observations associated with clinical PKD or characteristic interstitial PKX-cells surrounded by macrophages could be observed. The spore-like structures observed in the kidney tubules could have been *T. bryosalmonae* spores, but could also have been other myxozoan-spores or structures belonging to other parasite taxa, especially in fish negative for *T. bryosalmonae* by real-time PCR. Even if no interstitial extrasporogonic stages were observed in histological sections in real-time PCR positive fish, the presence of such parasite stages can not be excluded as the slides only represent tiny fractions of the kidney. As pointed out by Kent et al. (1995) and Foor et al. (2007), occurrence of *T. bryosalmonae* (formerly PKX) and PKD may impact ocean survival of salmon during their early seaward entry phase because the disease causes osmoregulatory imbalances and anaemia. To the best of our knowledge, this has, so far, not been studied.

It is also relevant to question whether *T. bryosalmonae* still can be present in the kidney when the adult Atlantic salmon or sea trout return to the rivers to spawn, and if so, could the presence of this parasite affect the freshwater adaptation, upstream migration capacity and spawning success? This question remains unanswered. Between 2003 and 2008, Braden et al. (2010) found *T. bryosalmonae* in the kidney of 43 % to 100 % of the examined spawning pink salmon, *Oncorhynchus gorbuscha*, in the Quinsam River, BC, Canada. Examination of histological kidney sections indicated a recent establishment of the parasites due to an ongoing infection pressure towards the adult fish during river upstream migration to the spawning redds. The possibility that the adult fish could have been infected prior to river arrival was not discussed. The conclusion from our study were that more than half of Atlantic salmon and sea trout smolts, ready to leave the River Nausta and migrate to the sea, had a *T. bryosalmonae* infection, but
no extrasporogonic parasite stages could be observed in the kidney tissue.

References


Grabner D and El-Matbouli M (2009). Comparison of the susceptibility of brown trout (Salmo trutta) and four rainbow trout (Oncorhynchus mykiss) strains to the myxozoan Tetracapsuloides bryosalmonae, the causative agent of proliferative kidney disease (PKD). Veterinary Parasitology 165, 200-206.


