

# Renal myxosporidiosis in wild brown trout (*Salmo trutta*) in south-west England

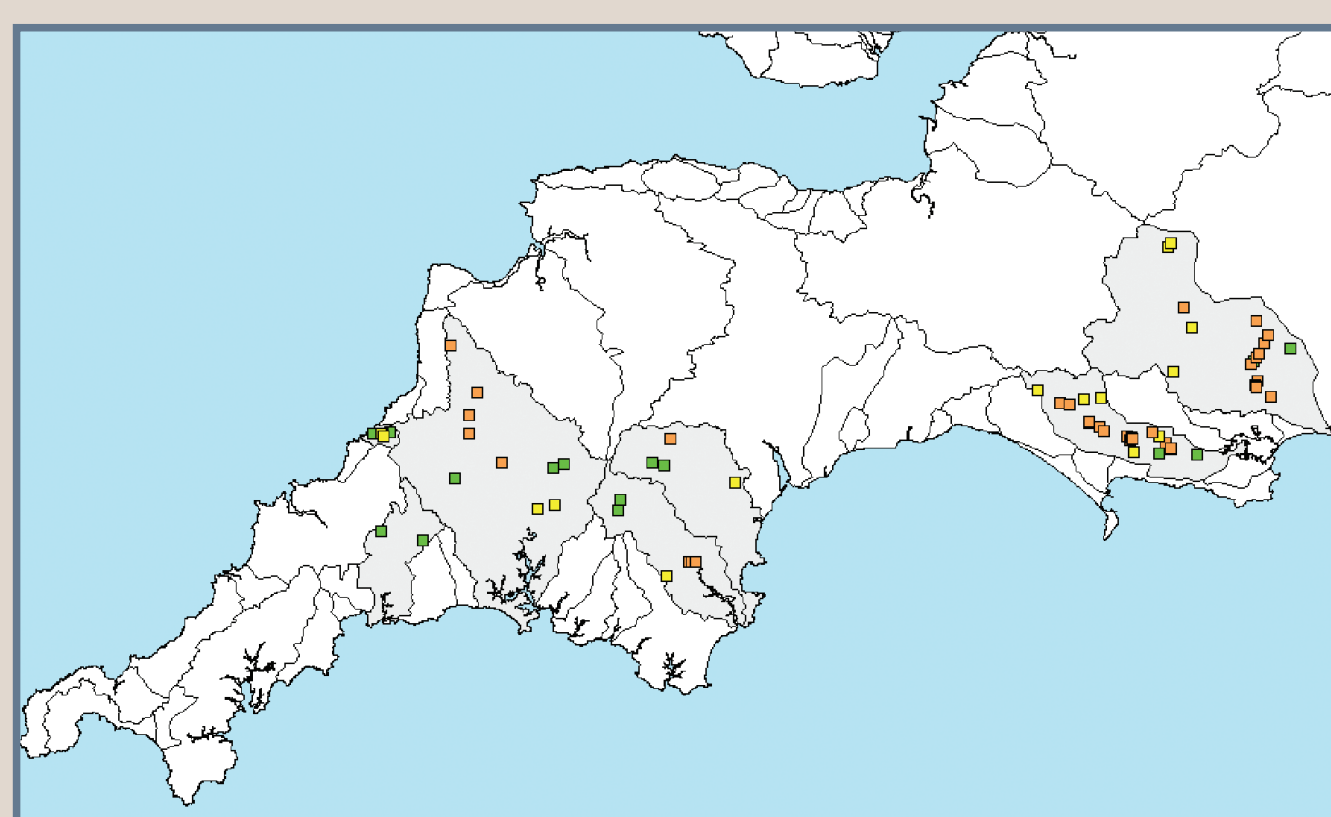
## Introduction

The impact of proliferative kidney disease (PKD) on cultured salmonids in Europe and North America is well recognised, but the prevalence and effect of *T. byosalmonae* and other myxozoan parasite infections on wild populations has not been investigated thoroughly. Published data available do not allow a comparison of within and between river variance. Estimates of these variances are needed to design studies, specifically the increase in standard error (SE) that will arise from cluster compared with random sampling – the design effect (D). Cluster sampling is inevitable when investigating disease in wild fish, i.e. sampling at one or more defined locations on one or more river catchments.

The main objectives of this study were to:

- investigate the prevalence of *Tetracapsuloides byosalmonae* and *Chloromyxum schurovi*, and their association with pathology in juvenile wild brown trout (*Salmo trutta*) in south-west England.
- assess the variation in prevalence within site, between site and river, and calculate the design effect.

## Material and methods



Brown trout were collected by electrofishing from 16 sites on seven river catchments in the south-west of England (Figure 1). A total of 327 fish (0+) were caught (approximately 20 from each site). The visceral cavity was incised and the fish were placed directly in 10% buffered formalin for histological evaluation.

Figure 1: Location of rivers catchments sampling sites (green circles) farms (red circles) and restocking sites (yellow circles)

The SE and 95% confidence intervals adjusted for clustering at the site level were calculated using a formula developed by Bennett *et al.*, (1991):

$$SE = \sqrt{\sum \frac{(p_i - p)^2}{c(c-1)}}$$

where  $p_i$  = prevalence at the  $i$ th site,  $p$  = overall prevalence,  $c$  = number of sites. The design effect (D) was calculated using the following formula (Bennett *et al.*, 1991):

$$D = \frac{SE^2_{\text{cluster sample}}}{SE^2_{\text{simple random sample}}}$$

Odds ratios (OR) were calculated for the presence of *T. byosalmonae* or *C. schurovi* and hepatitis and adjusted to account for clustering by river using the Mantel-Haenszel (MH) procedure. A variance component model using MLwiN statistical software 2.01 (Rasbash *et al.*, 2000) was used to partition the variance in the prevalence of *T. byosalmonae*, *C. schurovi* and hepatitis. Binomial hierarchical models with a logit link function were constructed for each outcome where fish (level 3) were modelled within site (level 2) and site within river catchment (level 1).

## Results

- PKD infections were found in 16.5% of fish (Table 1), and in fish from all rivers except river 7 (river level prevalence from 2.5% to 36%), and at 11 of 16 sites (Table 2).
- 51% of fish with PKD had moderate (39%) or severe (12%) nephritis (Figure 2).
- C. schurovi* infections were found in 25% of fish (Table 1) from all rivers except for river 3 (river level prevalence 2.4 to 63%) and at 10 of 16 sites (Table 2).
- Hepatitis was found in 21% of fish (Table 1) (river level prevalence 5% to 45%).
- Almost 72% of fish with *T. byosalmonae* and 3% of fish infected with *C. schurovi* had hepatitis.
- The presence of *T. byosalmonae* was strongly associated with hepatitis (unadjusted OR = 20.2, chi-squared value = 97.4,  $P < 0.001$ ).
- The association between the presence of *C. schurovi* and hepatitis was strongly negative (unadjusted OR = 0.10, chi-squared value = 19.3,  $P < 0.001$ ).
- The design effects were 5.27 and 10.70 for *T. byosalmonae* and *C. schurovi*, respectively (Table 1).
- For PKD 70% of the variance was at the site level, whereas for *C. schurovi* 78% of the variation was found at the level of the river.

Table 1: Prevalences and design effects of renal myxozoan parasites and hepatitis in wild brown trout

Parameter	<i>Tetracapsuloides byosalmonae</i>	<i>Chloromyxum schurovi</i>	Hepatitis
Prevalence	16.5	25.1	20.1
SE	4.7	7.9	5.2
Upper 95% CI	25.8	40.5	31.2
Lower 95% CI	7.3	9.6	10.6
Design effect	5.3	10.8	5.4

Confidence intervals (CI) adjusted to account for clustering by site.

Table 2: Prevalence of renal myxozoan parasites and hepatitis in wild brown trout by sampling site.

River	Site	<i>T. byosalmonae</i>		<i>Chloromyxum schurovi</i>		Hepatitis	
		%	N	%	N	%	N
1	1	27.3	22	0.0	22	42.9	21
1	2	45.0	20	5.0	20	45.0	20
2	3	0.0	20	5.3	19	0.0	19
2	4	5.0	20	55.0	20	10.0	20
3	5	10.0	20	0.0	20	10.0	20
3	6	15.0	20	0.0	20	25.0	20
4	7	45.0	20	5.0	20	55.0	20
4	8	15.0	20	0.0	20	35.0	20
5	9	20.0	20	0.0	20	52.9	17
5	10	60.0	20	0.0	20	35.0	20
5	11	0.0	21	14.3	21	0.0	21
6	12	4.4	23	60.8	23	13.0	23
6	13	19.0	21	61.9	21	19.0	21
7	14	0.0	21	42.8	21	0.0	21
7	15	0.0	20	75.0	20	0.0	20
7	16	0.0	19	73.4	19	0.0	19

N = number of observations



Figure 2: Juvenile brown trout with swollen kidney characteristic of PKD



Figure 3: Sampling fish

## Conclusions

It may be tentatively concluded that:

- site level factors are most important in determining the prevalence of *T. byosalmonae*.
- river level factors are most important in determining the prevalence of *C. schurovi*.
- infection with *T. byosalmonae* causes hepatitis (in addition to nephritis).

Spatially distributed determinants of disease that may explain these results include: poor water quality, habitat type, water flow and alternate hosts, all of which may vary widely between sites in the same river. For *T. byosalmonae*, the alternate host is bryozoans whilst for *C. schurovi*, the alternate stage is *Neoaactinomyxum eiseniellae* from the oligochaete *Eiseniella tetraedra*.

The higher design effect of *C. schurovi*, compared with *T. byosalmonae* indicates a greater degree of infectiousness, although this may be attributable to the abundance and distribution of the alternate host, and not parasite biology.

Future larger scale studies should:

- investigate the association between disease outcomes at sampling sites and spatially distributed explanatory variables (including location of fish farms).
- use the design effects estimates for sample size calculations.
- assess whether myxozoan parasite infections adversely effect brown trout population levels.

## Acknowledgements

This work was supported by Defra funding (F1168, F1165). We thank staff of the Environment Agency for the sample collection and supplying additional information.

## References

- Bennett, S., Woods, T., Liyanage, W.M., Smith, D.L., 1991. A simplified general method for cluster-sample surveys of health in developing countries. *World Health Statistics Quarterly* 44, 98-106.
- Rasbash, J., Browne, W., Goldstein, H., Yang, M., Plewis, I., Healy, M., Woodhouse, G., Draper, D., Langford, I., Lewis, T., 2000. A user's guide to MLwiN.