

Comparative pathology of rosette agent (*Sphaerothecum destruens*) in sunbleak (*Leucaspius delineatus*)

Introduction

Sphaerothecum destruens is a mesomycetozoa parasite initially recorded from North American salmonids, particularly cage-reared Chinook salmon (*Oncorhynchus tshawytscha*) (Arkush *et al.*, 1998). The pathogen has recently been discovered for the first time in a non-native cyprinid host, the sunbleak (*Leucaspius delineatus*), in the UK. It had been postulated that declines in sunbleak populations across Europe may have been caused by transmission of a rosette-like agent (recently shown to be *S. destruens*, based on 18s rDNA sequence data) from another non-native cyprinid, the topmouth gudgeon (*Pseudorasbora parva*) (Gozlan *et al.*, 2005). However, *S. destruens* has never been observed in topmouth gudgeon tissues and the hypothesis that this species acts as a healthy carrier of *S. destruens* causing emaciation and decline in sunbleak remains to be confirmed. The aims of the current investigation were to establish whether sunbleak populations in the UK harbour *S. destruens* in the absence of topmouth gudgeon, to describe the histopathology of infections in sunbleak and to confirm the identity of the agent using ultrastructural features and *in situ* hybridisation (ISH). Additionally, a comparison with infections in salmonids and related organisms in different fish species were undertaken.

Materials and methods

Sunbleak were sampled from a site in southern England, which had previously been used for cohabitation experiments with topmouth gudgeon (n=100). Bullheads (*Cottus gobio*) were obtained from the river Allen in southern England and dab (*Limanda limanda*) were sampled from the North Sea. Fish or selected tissues were fixed whole in neutral buffered formalin and processed using standard histological techniques. Sections were cut at 5µm thickness and stained with haematoxylin and eosin and with Gram's stain. Selected specimens of sunbleak were used for ISH in order to localise *S. destruens* cells in organs and tissues. Archive specimens of *S. destruens* infections in North American Atlantic salmon (*Salmo salar*) and *Dermocystidium* sp. in *S. salar* from Scotland were examined for comparative purposes.

Sphaerothecum destruens in sunbleak

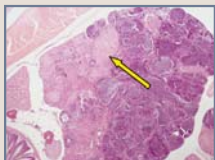


Figure 1: Low power view showing multiple granuloma in the testis.

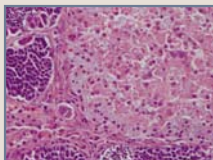


Figure 2: Granuloma in the testis with numerous eosinophilic stages of *S. destruens*.



Figure 3: High power view of kidney with numerous intra- and extracellular rosettes of *S. destruens*.

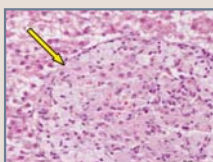


Figure 4: Well circumscribed hepatic granuloma containing numerous *S. destruens* stages.



Sunbleak (*Leucaspius delineatus*)

Results

The infection was confirmed histologically in 5 fish with a further 7 fish showing pathological changes without cells of *S. destruens* being observed. Affected fish did not show any gross signs of disease and were not emaciated. The infection was systemic with the kidney and testis particularly showing intense inflammation. Granulomatous pathology was rarely seen. Low levels of infection could be discerned using Gram stain, which clearly demonstrated Gram +ve granules in the cytoplasm of *S. destruens* and ISH. These findings are consistent with the disseminated form of the disease in salmonids. Comparison with infections of *Dermocystidium* sp. in Atlantic salmon and *D. salmonis* in bullheads (*Cottus gobio*) showed clear differences in the appearance of the agents and their pathological response. Infections with a similar agent in the marine flatfish, dab (*L. limanda*) were seen in liver and spleen and appeared to be primarily associated with macrophage aggregates and produced a granulomatous host response. Ultrastructure of the agent is similar to that of *S. destruens* with differences in the structure of the cell wall.

Related infections

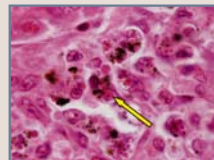


Figure 5: *S. destruens* in the kidney of North American *S. salar*.



Figure 6: *Dermocystidium* sp. infecting renal haematopoietic tissue of *S. salar*.



Figure 7: *D. salmonis* forming cysts in the dermis of bullhead (*C. gobio*).

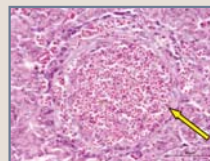
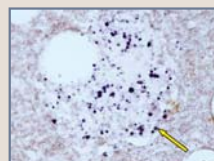
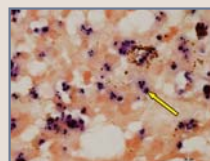


Figure 8: Hepatic granuloma containing numerous *Sphaerothecum*-like stages in the dab (*L. limanda*).

ISH and Gram staining



Figure's 9 & 10: Specific labeling of *S. destruens* in sunbleak liver by ISH and selective staining of *S. destruens* using Gram's stain (Figure 10).



Ultrastructure

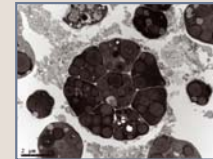
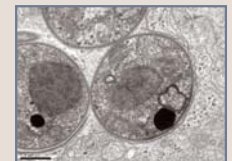
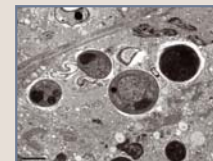


Figure 11: 'Rosette' of *S. destruens* from sunbleak cultured in EPC cells.



Figure 12: *S. destruens* cells from North American salmonids cultured in EPC cells.



Figure's 13 & 14: Sunbleak *S. destruens* stages in renal tissue.

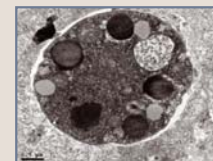


Figure 15: North American isolate of *S. destruens* cultured in CHSE cells.

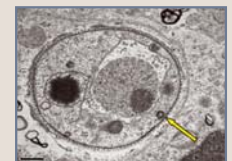


Figure 16: *Sphaerothecum*-like organism in the liver of dab. Note the surface projections on the cell wall.

Conclusions

- Rosette agent *S. destruens* is prevalent in an established population of sunbleak in southern England in the absence of topmouth gudgeon.
- The pathology of the disease in sunbleak was not associated with emaciation.
- The disease in sunbleak showed a similar pattern to the disseminated form of the disease in salmonids.
- Histologically, the disease is easily distinguished from infections with *Dermocystidium* spp.
- The agent in dab may be a new but related species.

Acknowledgements

This work was funded by Defra contract F1166. We thank Dr's Ron Hedrick and David Bruno for providing histological samples of *S. destruens* in North American *S. salar* and *Dermocystidium* sp. infections in *Salmo salar* from Scotland.

References

- Arkush, K.D., Frasca, S. (Jr) and Hedrick R.P. (1998) Pathology associated with the Rosette agent, a systemic protist infecting salmonid fishes. *J. Aquat. Anim. Health.* 10:1-11
 Gozlan, R.E., St-Hilaire, S., Feist, S.W., Martin, P. and Kent, M.L. (2005) Biodiversity: disease threat to European fish. *Nature.* 2005 435(7045):1046.