

The relationship between PKD & Bryozoa: an inside story

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Proliferative kidney disease (PKD) is an economically important parasitic condition - costing the trout industry well over £1 million per annum in the UK alone. It primarily affects first season freshwater salmonid fish in areas of Western Europe and North America (Clifton-Hadley, Bucke and Richards 1984; Hedrick, MacConnell and de Kinkelin 1993). The causative agent involved was originally known as PKX, denoting its uncertain taxonomic position in the phylum Myxozoa Grassé (Seagrave, Bucke and Alderman 1980). Subsequent studies discovered that bryozoans (Fig. 1) – known colloquially as “moss animals” - acted as alternate hosts, and the organism was recently named *Tetracapsuloides bryosalmonae* (Anderson, Canning and Okamura 1999; Canning, Tops, Curry, Wood and Okamura 2002).

Seasonal outbreaks of the disease, typically between May and September, have been linked to increased water temperatures permitting severe development of PKD in affected fish (Ferguson and Needham 1978; Foott and Hedrick 1987). The characteristic disease sign is severe renal swelling with granulomatous hyperplasia encompassing interstitial extrasporogonic *T. bryosalmonae* cells (Ferguson and Needham 1978). Disease leads to increased production costs with levels of mortality ranging from below 20% in uncomplicated cases, to 100% in fish suffering from secondary diseases and stressors (Clifton-Hadley, Bucke and Richards 1986; Ferguson and Ball 1979). Fish that have recovered from clinical disease exhibit apparent resistance to future challenge (Ferguson and Ball 1979; Klontz, Rourke and Eckblad 1986).

Various control methods have been developed to counter PKD, with varying levels of success. Husbandry measures - including lowering summer water temperature (using bore-hole water), delaying transfer of naïve stocks to enzootic waters, eliminating

secondary pathogens and reducing feeding rates - have been implemented in attempting to limit economic losses (Bucke, McGregor, Hudson and Scott 1981).



Figure 1: A single zooid of a bryozoan colony

Malachite green, the antibiotic fumagillin DCH and its synthetic analogue TNP-470 have been used therapeutically with some efficacy, but concerns over toxicity to fish, residue levels and environmental issues have prevented wide adoption of these treatments (Hedrick *et al.* 1993). The perceived specific immunity that previously exposed fish demonstrate to *T. bryosalmonae* has suggested that there is potential for the development of a vaccine to combat the condition, although no such product is currently available.

In the current study, laboratory culture techniques have been developed to allow continuous maintenance of the bryozoan hosts of the parasite. Overwintering stages (statoblasts) of Bryozoa were collected from a Scottish loch, germinated and maintained in aquaria. A feeding trial of the Bryozoa was undertaken, gauging the nutritional value of more than 50 species of protozoa and algae. Several species of algae and protozoa were found to be readily ingested and digested by the bryozoans, presumably proving beneficial to their maintenance. No single species of algae or protozoa was identified which in isolation could maintain the bryozoan colonies, so

mixtures of digestible cultures were added to the aquaria. Algal cultures were reared in Jaworski's medium & the bryozoans were kept in Chalkley's medium.



Figure 2: Coiled myxozoan parasite *Buddenbrockia* within *P. repens*

Field trips were made to trout farms enzootic for PKD in the South of England, and wild bryozoan specimens were collected. Culture in the laboratory of the bryozoans revealed myxozoan infection (Fig. 2) of several colonies. Two distinct myxozoan infections were seen: *Buddenbrockia* sp. (Fig. 2) and *T. bryosalmonae* (Fig. 3), the former producing worm-like spore sacs, the latter developing spherical sacs. The ensuing development of these parasites was observed under an inverted microscope. Both still and video images were captured, revealing the movement of the parasitic developmental stages within the coelom of the bryozoan host.

With both myxozoan infections, the first observable sign was the presence of numerous microscopic bodies swirling within the Bryozoa. After one or two days, larger irregularly shaped bodies formed, in the case of *Buddenbrockia* adhered to the internal wall of the host, while with *T. bryosalmonae* they were free of attachment. Subsequently, the observed stages enlarged, *Buddenbrockia* forming elongating oblong forms, while *T. bryosalmonae* infection led to formation of spherical bodies. Over the period of about a week, mature spore sacs were seen to develop. The worm-like spore sacs of *Buddenbrockia* seen within the bryozoan *Plumatella repens* were up

to 2 mm in length, apparently writhing independently of their host. Mature spore sacs of *T. bryosalmonae* within *Fredericella sultana* were of approximate diameter 100 – 200 µm and were seen to rotate and flow in the currents within the coelom of the host Bryozoa. Upon maturation of the sacs, many spores were released into the bryozoan, subsequently being ejected into the surrounding water. The spores of both parasites were approximately 20 µm in diameter, each possessing the four characteristic polar capsules.

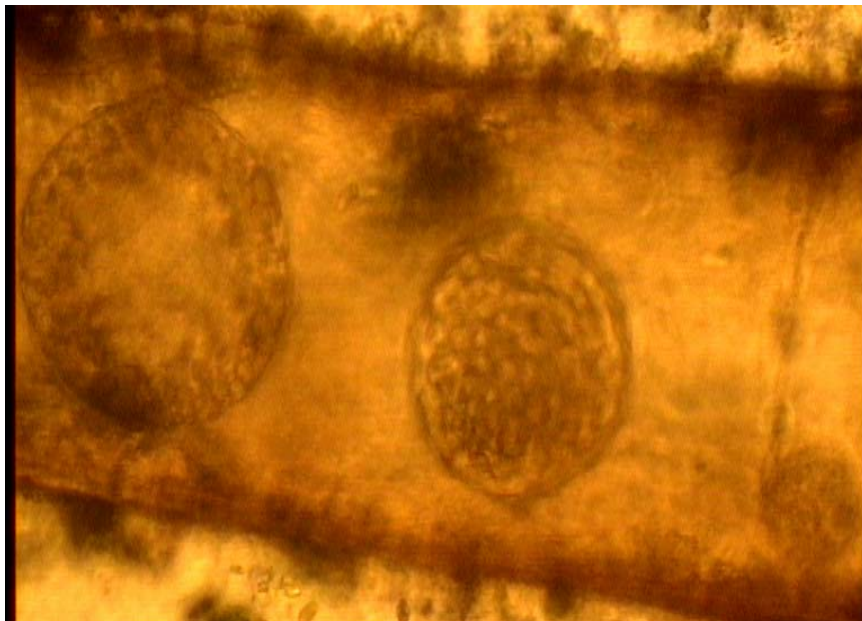


Figure 3: Spore sacs of *T. bryosalmonae* within *F. sultana*

Infected parts of bryozoan colonies were seen to thrive less well than unaffected regions. Waves of infection were seen within *Buddenbrockia*-infected Bryozoa, with a variety of developmental stages seen within one colony simultaneously. Although statoblast formation was noted in infected colonies, it was at a lower level than in comparable uninfected colonies. As statoblasts represent the only overwintering stage of fresh water Bryozoa (except *Fredericella* which can overwinter as colonies), this development could present a means of maintenance of parasites from one season to the next.

PKD was experimentally induced in rainbow trout following exposure to material released from bryozoans infected with *T. bryosalmonae*, but similar exposure to *Buddenbrockia* material did not result in noticeable disease. No evidence of horizontal transmission of myxozoan infection between bryozoan colonies was obtained – despite prolonged cohabitation trials and injection of material between colonies – supporting the theory that the parasites rely on other hosts (such as fish) to complete their life cycles.

The successful laboratory culture of infected bryozoan colonies seems crucial in furthering our understanding of PKD. The potential would be increased of discovering the missing links in the life cycles of these significant malacosporean parasites. The translucent nature of bryozoan colonies cultured in the laboratory system presents a unique opportunity to observe myxozoan parasite development directly within the host. Year-round maintenance of *T. bryosalmonae* could allow controlled infection models to be developed without relying upon seasonally available material. Potentially, increased knowledge of the dynamics and interactions involved could lead toward successful vaccine development against this highly damaging disease.

References

- Anderson C.L., Canning E.U. and Okamura B. (1999) 18S rDNA Sequences indicate that PKX organism parasitizes Bryozoa. *Bulletin of the European Association of Fish Pathologists* **19**, 94-97.
- Bucke D., McGregor D., Hudson E.B. and Scott P. (1981) Control measures fail to stop the spread of PKD. *Fish Farmer* **4**, 25.
- Canning E.U., Tops S., Curry A., Wood T.S. and Okamura B. (2002) Ecology, development and pathogenicity of *Buddenbrockia plumatellae* Schröder, 1910 (Myxozoa, Malacosporea) (syn. *Tetracapsula bryozoides*) and establishment of *Tetracapsuloides* n. gen. for *Tetracapsula bryosalmonae*. *Journal of Eukaryotic Microbiology* **49**, 280-295.
- Clifton-Hadley R.S., Bucke D. and Richards R.H. (1984) Proliferative kidney disease of salmonid fish: a review. *Journal of Fish Diseases* **7**, 363-377.

- Clifton-Hadley R.S., Bucke D. and Richards R.H. (1986) Economic importance of proliferative kidney disease in salmonid fish in England and Wales. *Veterinary Record* **119**, 305-306.
- Ferguson H.W. and Ball H.J. (1979) Epidemiological aspects of proliferative kidney disease amongst rainbow trout *Salmo gairdneri* Richardson in Northern Ireland. *Journal of Fish Diseases* **2**, 219-225.
- Ferguson H.W. and Needham E.A. (1978) Proliferative kidney disease in rainbow trout *Salmo gairdneri* Richardson. *Journal of Fish Diseases* **1**, 91-108.
- Foott J.S. and Hedrick R.P. (1987) Seasonal occurrence of the infectious stage of proliferative kidney disease (PKD) and resistance of rainbow trout, *Salmo gairdneri* Richardson to reinfection. *Journal of Fish Biology* **30**, 477-483.
- Hedrick R.P., MacConnell E. and de Kinkelin P. (1993) Proliferative kidney disease of salmonid fish. *Annual Review of Fish Diseases* **3**, 277-290.
- Klontz G.W., Rourke A.W. and Eckblad W. (1986) The immune response during proliferative kidney disease in rainbow trout: a case history. *Veterinary Immunology and Immunopathology* **12**, 387-393.
- Seagrave C.P., Bucke D. and Alderman D.J. (1980) Ultrastructure of a Haplosporean-like organism: the possible causative agent of proliferative kidney disease in rainbow trout. *Journal of Fish Biology* **16**, 453-459.