

Rainbow trout, moss animals and disease: the inside story

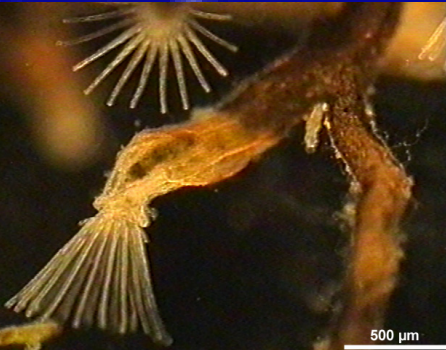


Figure 1: Bryozoan zooids of *Fredericella sultana*



Figure 3: Bryozoan culture system

Bryozoans are kept within cooled water sleeves in the blue tanks, while algae are grown in the brightly lit lower 2 tanks.

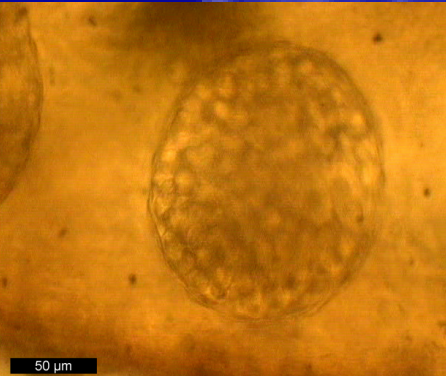


Figure 4: Immature spore sac of *T. bryosalmonae* within *F. sultana*

Introduction

Although the cultivation of aquatic animals and plants dates back at least 2500 years, aquaculture is currently the fastest growing animal based food-producing sector¹. Intensification of production in modern fish farming heightens the impact of disease outbreaks in high value systems. Despite being highly damaging to farmed salmonids throughout Europe and North America while costing the UK trout industry over £2.5 M annually², there are currently no licensed control measures against proliferative kidney disease (PKD). This research project aims to give insight into the relationships between the parasitic causative agent and its vertebrate and invertebrate hosts while moving towards development of successful control measures.

Bryozoa: collection and cultivation

The phylum Bryozoa comprises microscopic sessile colonial invertebrate coelomates known colloquially as moss animals³ (Fig. 1). In 1999, freshwater bryozoans were identified as the elusive alternate hosts of the parasite causing PKD, which was subsequently named *Tetracapsuloides bryosalmonae*⁴. As there has been little published literature regarding the laboratory maintenance of bryozoans, a preliminary aim of this project was to optimise culture conditions. Over 50 species of protozoa and algae were presented to bryozoan colonies as monocultures and subsequent ingestion and digestion were observed (Fig. 2). From these data, several species including *Cryptomonas ovata*, *Euplores viridis*, *Haematococcus lacustris*, *Pediastrum boryanum* and *Synechococcus leopoliensis* were found to be highly nutritious and were included in the formation of a core bryozoan diet. Limitations within existing culture systems were mitigated by the development of a novel automated design that linked an algal tank continuously infused with growth medium to a shaded cooled bryozoan aquarium (Fig. 3).

Developmental stages of *T. bryosalmonae* within bryozoans

Bryozoan colonies were collected from the inlet waters of fish farms within southern England known to be affected by PKD. Following transportation to the laboratory, they were maintained within the laboratory culture system and examined regularly by inverted microscopy. Extended laboratory culture and the resultant development of translucent outer membranes (as opposed to the encrusted opaque cuticles coating freshly collected specimens) allowed the stages of parasitic growth to be discerned and reported for the first time⁵. Sequential parasitic developmental stages were observed from the earliest signs of tiny particles (7 µm in diameter) up to the formation of spherical spore sacs measuring 350 µm across (Fig. 4). Upon maturity, these sacs ruptured in the coelomic cavity, releasing spores which were subsequently voided to the surrounding water. It was found that consecutive waves of parasitism followed, implying the potential long term release of spores from infected wild bryozoans.



Figure 5: A rainbow trout mortality due to PKD.

The swollen abdomen seen in the top image can be attributed to enlargement of the kidney and spleen with pooling of ascitic fluid.

References

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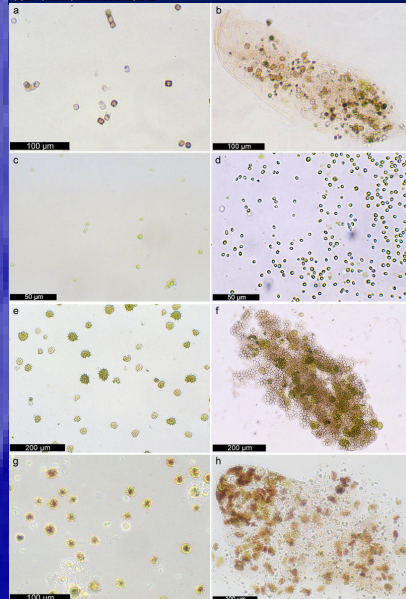


Figure 2: Algae before and after bryozoan ingestion (a & b) *Stephanodiscus* sp., (c & d) *Chlorella vulgaris*, (e & f) *Pediastrum boryanum*, (g & h) *Haematococcus lacustris*

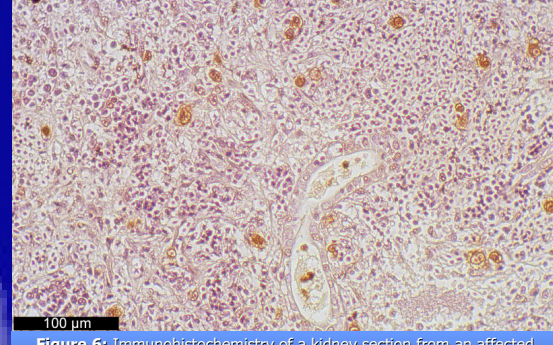


Figure 6: Immunohistochemistry of a kidney section from an affected rainbow trout. Parasitic stages are stained brown.

Infection of fish: quantitation of the infective dose

It is now well established that exposure of salmonid fish to spores of *T. bryosalmonae* leads to characteristic signs of PKD⁶, including enlargement of the kidney and spleen (Fig. 5). Histological examination of kidney sections reveals a profound immunological response localised around numerous parasitic extrasporogonic stages (Fig. 6). The resulting pathological damage can be sufficient to lead to large scale mortality levels on affected farms during the summer months. It had previously been postulated that low doses of spores might be capable of infecting individual fish⁶, however, the exact infective dose was unknown. Within this project, it was found that when populated with infected colonies, the medium from the bryozoan culture system could contain sufficient spores to lead to development of PKD following experimental exposure to rainbow trout. Additionally, spores were collected by dissection of bryozoans and micromanipulation techniques, allowing fish to be exposed to known numbers. It was found that one spore was capable of infecting a rainbow trout. This led to development of full-blown PKD following multiplication of the parasite by autoinfection. The infective nature of the spores explains the high level of morbidity (up to 100%) reported on endemic farms⁷.

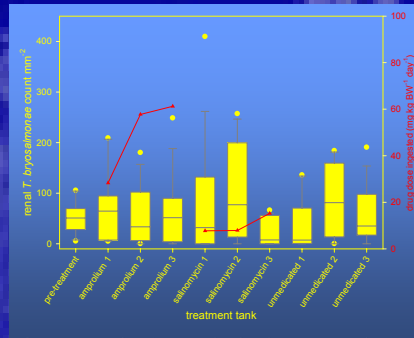


Figure 8: Box plot of parasite burden data from PKD drug trial treating rainbow trout with amprolium or salinomycin

Tetracapsuloides bryosalmonae spores: confocal microscopy and 3D modelling

Mature spores were dissected from infected colonies of the bryozoan *Fredericella sultana* stained using fluorophores and examined by confocal laser scanning microscopy. Staining with the optical brightener Blankophor revealed the presence of four spherical polar capsules within capsular cells which formed a cruciate arrangement, abutting eight structural valve cells (Fig. 7). The non-polar lipophilic dye BODIPY 505/515 revealed internal structures including the germinative sporoplasms that are infective to fish, whereas the nucleic acid stain DAPI confirmed the location of cell nuclei. Sequences of images from each of 18 spores were taken along the optical Z-axis using the confocal optical sectioning facility allowing 3D representations to be processed using Leica Confocal Software. The features obtained from the multiple scans were compiled to allow the formation of rotational 3D computerised models of the structure of spores of *T. bryosalmonae* within bryozoans⁸.

Control of PKD in trout: the farmers' dilemma

The current lack of a licensed drug or vaccine for PKD places heavy reliance upon the success of remedial management practices. However, the success of this approach is tenuous, with severe outbreaks still occurring especially in the face of high summer temperatures and secondary stress factors. Within this project, two oral anti-coccidial agents that are licensed for terrestrial livestock were efficacy tested against PKD in rainbow trout. Whereas amprolium did not mitigate pathological changes, salinomycin was found at higher ingestion doses to lower the parasite burden within trout kidney sections (Fig. 8). However, the high dose required combined with poor palatability of the drug make this candidate less promising for commercial application. Several experimental vaccines have been developed and tested. These have included preparations of refined *T. bryosalmonae* material extracted from infected bryozoan colonies reared within the laboratory culture system. Development of a recombinant sub-unit vaccine is now underway, with a cDNA library of parasite tissue sourced from Arctic charr having recently been established.

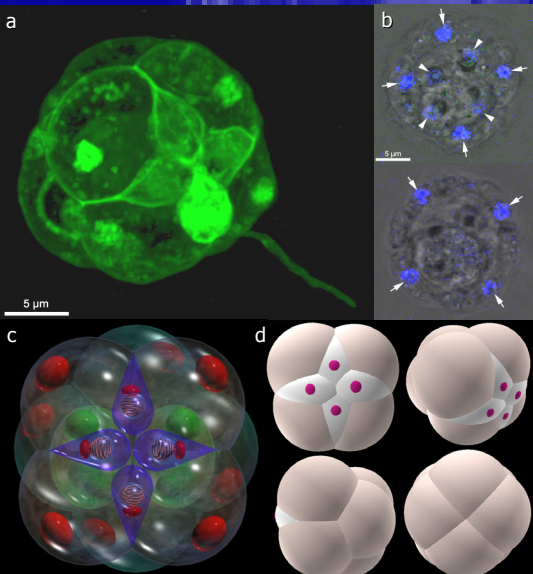


Figure 7: Representations of spores of *T. bryosalmonae*

- (a) 3D confocal laser scanning microscopy (CLSM) reconstruction.
 (b) CLSM sections through a spore displaying nuclei (denoted by arrows).
 (c & d) Computer models of the proposed 3D structure of the spore.



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Conclusions

The development of a robust bryozoan laboratory culture system has allowed observation of the developmental stages of *T. bryosalmonae*. The lack of processing required to image spores by confocal microscopy has allowed the 3D structure of the pathogen to be ascertained. Additionally, the long term maintenance of infection due to continuous waves of development has provided an invaluable resource that can be utilised in simulating natural infection of fish. The highly infective nature of the spores combined with their mass release from bryozoans suggests that widespread control of the disease by invertebrate population reduction may not be feasible. Therefore, concerted efforts continue towards the development of a vaccine that could lessen the effects of this killer disease.