

Parasitic Diseases in Aquaculture: Their Biology, Diagnosis and Control – Protista

Biology and Taxonomy

Protistans are a large paraphyletic group of organisms that parasitize most, if not all, animal (and plant) groups. Typical groups found in or on aquatic animals include representatives of the flagellates (e.g. *Amyloodinium*, *Cryptobia*, *Gymnodinium*, *Hexamita*, *Ichthyobodo*, *Piscinoodinium*, *Spironucleus*, *Trypanosoma*, *Trypanoplasma*), amoebae or rhizopodes (e.g. *Vermamoeba* [=Hartmannella], *Paramoeba*, *Thecamoeba*), alveolates (e.g. *Perkinsus*), apicomplexans (e.g. *Aggregata*, *Eimeria*, *Goussia*, *Haemogregarina*, *Margolisiella* [=Pseudoklossia]), cercozoans (e.g. *Bonamia*, *Haplosporidium*, *Marteilia*, *Paramarteilia*) and ciliates (e.g. *Anophryoides*, *Apiosoma*, *Capriniana*, *Chilodonella*, *Cryptocaryon*, *Epistylis*, *Ichthyophthirius*, *Tetrahymena*, *Trichodina*, *Uronema*).

Life-Cycle

Generally, protistans have simple life-cycles, reproducing by binary fission, with transmission between and within hosts through contact. In addition, some protistans utilize vectors and/or intermediate hosts in transmission. This includes leeches in the life-cycle of trypanosomes, crustaceans and oligochaetes in coccidian life-cycles, and fomites/substrate in the life-cycle of *Ichthyophthirius multifiliis*, *Cryptocaryon irritans* and *Amyloodinium ocellatum*.

Significant Pathogens within the Group

The majority of problems associated with protistans in aquaculture are mainly due to their ability to transmit without the need for an intermediate host. In many instances, outbreaks on farms are a result of increased stress in the host animal or decreased water quality and, thus, environmental manipulation to reduce these may help to mitigate the lethal outcome of infection. The marine dinoflagellate *Amyloodinium ocellatum* and the freshwater flagellate *Ichthyobodo necator*, occurring on the external surfaces of their hosts, have been implicated in the loss of farmed, wild and aquarium fish in a number of regions around the world. sease (AGD) which leads to gill damage and death of infected fish. The freshwater counterparts, such as *Acanthamoeba*, *Naegleria*, *Protacanthamoeba*, *Rhogostoma*, *Vannella* and *Vermamoeba*, have been associated with high mortalities in rainbow trout, *Oncorhynchus mykiss*. farmed and wild fish, causing the parasitic white spot disease.

Shellfish diseases of concern include the alveolate *Perkinsus marinus*, the causative agent of Dermo disease which leads to mortalities in bivalve molluscs held at high temperature and relatively low salinities. Another important group of shellfish protistans are the cercozoans which include *Bonamia ostreae* and *Bonamia exitiosa*; both infect the haemocytes of their ostreid hosts, with the antipodean *B. exitiosa* being invasive.

Identification

The taxonomy and identification of protistans are based on the morphology of the various life stages. It is clear, however, that there is a great deal of plasticity in these features and as such, there has been a move towards use of molecular tools to discriminate species and genera within the Protista. Flagellates typically have one or more flagellum, generally a single nucleus and body shape is pleomorphic. Parasite size, the organ it infects in its host and the species of host have all been

used to discriminate species. Amoebae are also pleomorphic and while traditionally described on the basis of size, shape and other characteristics, there is an increasing reliance on molecular tools to help discriminate species. For apicomplexans, the structure and the arrangement of the oocyst and sporocyst are of paramount importance in discriminating species and genera. Ciliates, as the name suggests, possess cilia during one or more of their life stages. The arrangement and position of the cilia are of taxonomic importance. Cercozoans are discriminated on the basis of the number, arrangement and size of cells, as well as spore morphology for spore-forming types.

Diagnostic Methods

As described above, protistans are primarily identified using morphological characteristics and the majority of species have been described on this basis. Techniques utilized include light microscopy methods like phase contrast, bright-field and differential interference contrast microscopy. Stained, fixed and permanent preparations of blood and tissue smears and imprints can be made to assist with diagnosis. For some protistans, such as *Uronema* and *Tetrahymena*, the silver nitrate impregnation method (Foissner, 2014; Klein, 1943) to show the ciliary meridians is fundamental. Histology and stains such as Giemsa have been extensively utilized to localize infections and to describe any associated pathology. Refinements to light microscopy methods include the use of in situ hybridization (ISH), monoclonal and polyclonal antibodies and lectins to facilitate the visualization of protistans in histological sections. Transmission electron microscopy has been used extensively to diagnose protistans as internal cellular structures are important in discriminating species and genera. Finally, molecular tools are paramount and of increasing importance in the description of new species – their application in the identification of protistans cannot be overestimated.