Chapter - 12

Parasitoses in brackishwater aquaculture

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“Without pride, man becomes a parasite – and there are already too many parasites”

– Carla H. Krueger

Outline

Concepts

The rapid increase in aquaculture activities raises the quantum of disease incidences globally.

The diseases caused by infectious agents are considered to be most significant as many of them do not cause acute disease but lead to chronic disease and cause persistent economic loss to aquaculture.

Parasites are one of the major infectious disease agents that cause economic loss to aquaculture. Hence it is necessary to understand the major parasitic prevalence, their identification, and prevention and control measures.

Keywords

Acanthocephalan, Amoebozoa, Annelids, Apicomplexa, Aquaculture, Argulids, Arthropods, Caligids, Cestodes, Ciliates, Crab, Cymothoids, Digeneans, Dinoflagellates, Ergasilids, Finfish, Flagellates, Gill flukes, Gregarina, Haplosporida, Helminthiases, Lamproglena, Lernaea, Lernanthropids, Linguatula, Metazoa, Microsporidia, Milkfish, Monogeneans, Myxozoa, Nematodes, Pearlspot, Protozoa, Seabass, Shrimp, Skin flukes, Trematodes

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1. Introduction

Aquaculture represents one of the fastest growing component of the food sectors in the world. In recent times, it has progressed leaps and bounds from the traditional freshwater aquacuture to the brackishwater and marine realms. The brackishwater aquaculture assumes importance as it effectively utilizes the non-potable water which cannot be even used for irrigation of common agriculture crops. There is tremendous scope for the expansion of brackishwater aquaculture world-wide as most of the brackishwater resources under-utilized. Owing to its economic value, shrimp culture dominates the brackishwater aquaculture sector. However, finfish aquaculture is an ancient practice globally and has been continuing significantly to augment the livelihood support of a large population. In addition, the ornamental fish culture, a multi-billion-dollar industry, with very few wild collected marine and brackishwater species, also offers scope for effective utilization of brackishwater resources. Brackishwater ornamental species are hardy to withstand the wide range of water parameters as they naturally thrive in extreme climates. The ultimate goal should be in achieving sustainable brackishwater aquaculture for food with diversification of species, different farming systems, popularisation of the ornamental fish industry, and employment generation. Disease poses the biggest challenge for this growth. Fish and shellfish encounter diseases from all bio-aggressors such as viruses, bacteria, parasites and fungi leading to production and economic losses to the aquaculture industry. With the rapid increase of production, the sector experienced parallel increase in quantum of diseases. In last two decades many diseases and their causative agents have been identified, which necessitate search for innovative means of control. History has shown that diseases and parasites play a significantly detrimental role in aquaculture. In fact, disease-outbreaks have formed one of the major barriers against expanding the industry. Even though brackishwater aquaculture has been making rapid strides, it depends to a large extend on wild fry or fingerlings and natural water sources.

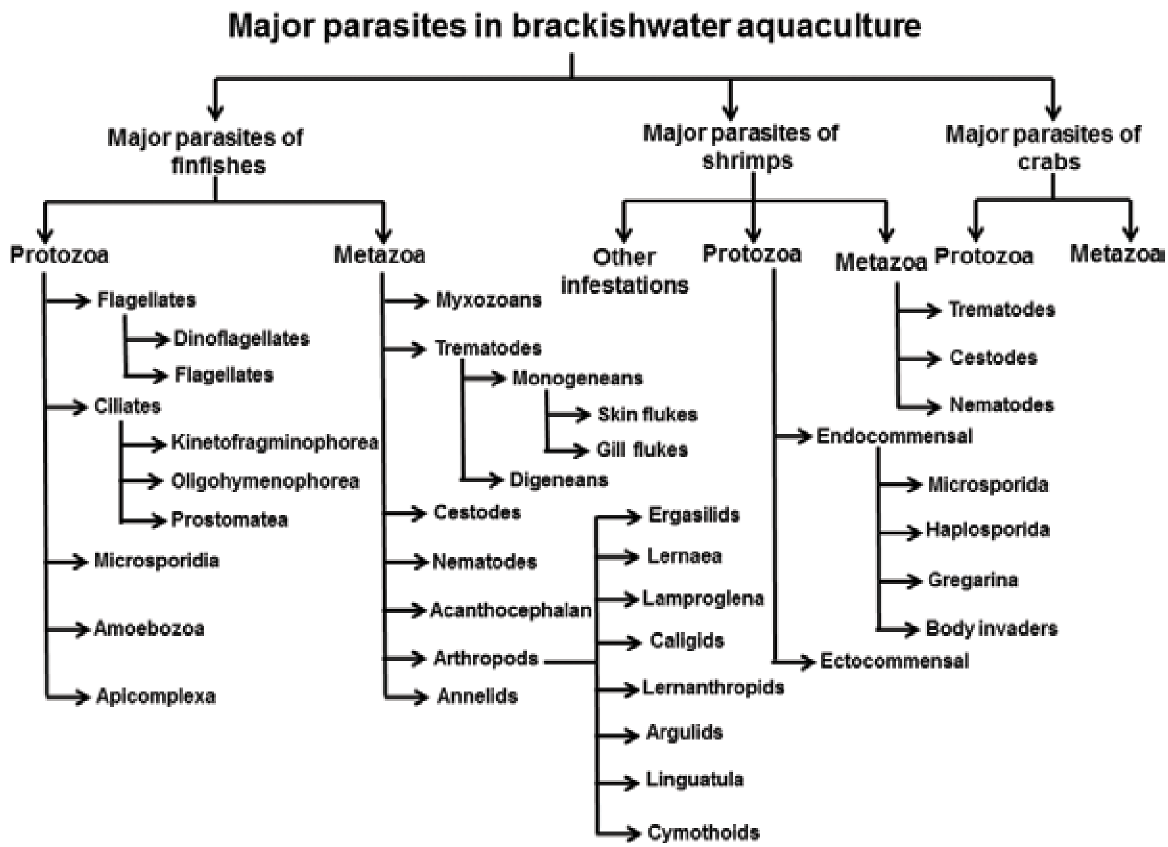
There are many commercially important brackishwater fish and shrimp species with aquaculture significance. Among which, Asian seabass (*Lates calcarifer*), Milk fish (*Chanos chanos*), Grey mullet (*Mugil cephalus*), Pearlspot (*Etroplus suratensis*), Spotted scat (*Scatophagus argus*), Mangrove red snapper (*Lutjanus argentimaculatus*), Silver moony (*Monodactylus argenteus*), Tiger shrimp (*Penaeus monodon*), Pacific white shrimp (*Litopenaeus vannamei* or *Penaeus vannamei*), Indian white shrimp (*Fenneropenaeus indicus* or *Penaeus indicus*), Banana shrimp (*Fenneropenaeus merguiensis*), and Mud crabs (*Scylla serrata* and *Scylla olivacea*) are considered as most potential species of fishes, shrimps and crabs.

This chapter describes the major diseases attributed to parasites of finfishes and shellfishes and their diagnosis, treatment and control. Considering the vast expanse of parasitic world, it is not possible to illustrate each and every species or group in this single chapter, but this chapter does provide a listing of the most parasites identified and reported from brackishwater aquaculture species with some information. This chapter would be a review and also deals with the zoonotic significance of the parasites. Only important references are mentioned considering the spare limitations. The major groups of parasites in brackishwater fishes, shrimps and/or crabs are (i) protozoans and (ii) metazoans. Based on the location / site of attachment on the host fishes, shrimps and/or crabs it can be also categorised as ecto- or endoparasites (Table 1).

Table 1. Worldwide important parasitic infestations of fishes, shrimps and crabs.

Parasitic infestations	Reference(s)
Fish	
Protozoa	
Flagellates (Mastigophora)	
Dinoflagellates (Phytomastigophora)	Paperna, 1980; Paperna and Overstreet, 1981; Ferraz et al., 1998; Cobb et al., 1998; Coats, 1999; Cecchini et al., 2001; Montgomery-Brock et al., 2001; Roberts, 2001; Saraiva et al., 2011; Eiras, 2016; Nozzi et al., 2016; Ramesh Kumar et al., 2016
Flagellates (Zoomastigophora)	Paperna and Overstreet, 1981; Ferguson, 1989; Diamant, 1990; Cruz and Eiras, 1997; Tojo and Santamarina, 1998; Urawa et al., 1998; Roberts, 2001; Woo, 2001; Eiras, 2016
Ciliates (Ciliophora)	
Kinetofragminophorea	Noga, 2000; Eiras, 2016
Oligohymenophorea	Cheung et al., 1980; Paperna and Overstreet, 1981; Ferguson, 1989; Dragesco et al., 1995; Munday et al., 1997; Noga, 2000; Iglesias et al., 2001; Roberts, 2001
Prostomatea	Paperna and Overstreet, 1981; Diggles and Lester, 1996; Noga, 2000; Rigos et al., 2001; Wright and Colorni, 2002
Microsporidia	Paperna and Overstreet, 1981; Estévez et al., 1992; Mathieu-Daude et al., 1992; Abela et al., 1996; Athanassopoulou, 1998; Faye et al., 1998; Speare et al., 1998; Shaw and Kent, 1999
Amoebozoa	Candreviotis 1977; Voelker et al., 1977; Visvesvara et al., 1993; Dykova et al., 1995; Riestra-Castaneda et al., 1997; Leiro et al., 1998; Zilberg et al., 1999; Dykova et al., 2000; Findlay et al., 2000; Douglas-Helders et al., 2001; Munday et al., 2001; Parsons et al., 2001; Powell et al., 2001; Tan et al., 2002; Young et al., 2007; Bustos et al., 2011; Palikova et al., 2011; Constenla et al., 2014
Apicomplexa	Paperna and Overstreet, 1981; Sitjà-Bobadilla et al., 1996; Steinhagen et al., 1998; Alvarez-Pellitero and Sitjà-Bobadilla, 2002; Logana,et al., 2012; Eiras, 2016
Metazoa / Helminthiases	
Myxozoans	Paperna and Overstreet, 1981; Paperna, 1982; Duhamel et al., 1986; Sitjà-Bobadilla and Alvarez-Pellitero, 1992; Alvarez-Pellitero and Sitjà-Bobadilla, 1993; Diamant, 1998; Branson et al., 1999; Canning et al., 1999; Moran et al., 1999; Kent et al., 2001; Padrós et al., 2001; Andree et al., 2002; Palenzuela et al., 2002; Wagner, 2002; Mladineo, 2003; Székely et al., 2009; Eiras, 2016
Trematodes (flukes)	
Monogeneans	
Skin flukes	McHugh et al., 2000; Cable and Harris, 2002; Sterud et al., 2002; Eiras, 2016; Lestari et al., 2018
Gill flukes	González-Lanza et al., 1991; Cecchini et al., 1998; Eiras, 2016; Muller et al., 2016
Digeneans	Buchmann et al., 1997; Midtlyng et al., 1999; Padrós et al., 2001; Eiras, 2016
Cestodes (tape worms)	Rahkonen and Valtonen, 1998; Dick and Choudhury, 1999; Sonune, 2014; Eiras, 2016

Parasitic infestations	Reference(s)
Fish	
Nematodes (round worms)	Deardorff and Overstreet, 1980; Moravec et al., 2011; Sethi et al., 2013; Moravec and Diggles, 2015; Eiras, 2016
Acanthocephalan	Paperna and Overstreet, 1981; Jithendran and Kannappan, 2010; Sanil et al., 2011; Kaur et al., 2017; Verma and Saxena, 2018
Arthropods	
Ergasilids	Paperna and Overstreet, 1981; Johnson et al., 2004; Alaş et al., 2015; Eiras, 2016; Misganaw and Getu, 2016
Lernaea	Daskalov et al., 1999; Piasecki et al., 2004; Hossain et al., 2013; Alaş et al., 2015; Eiras, 2016; Misganaw and Getu, 2016; CIBA, 2019a & 2019b; Ananda Raja et al., 2020; Ananda Raja et al., 2022
Lamproglena	Piasecki, 1993; Tsotetsi et al., 2005; Alaş et al., 2015; Batool et al., 2018
Caligids	Torrisen et al., 2013; Skaala et al., 2014; Alaş et al., 2015; Eiras, 2016; CIBA, 2019a & 2019b; Ananda Raja et al., 2020; Ananda Raja et al., 2022
Lernanthropids	Izawa et al., 2014 &, 2018; Alaş et al., 2015; Eiras, 2016; Raja et al., 2018; CIBA, 2019a & 2019b; Boxshall et al., 2020; Ananda Raja et al., 2022
Argulids	Alaş et al., 2015; Eiras, 2016; Misganaw and Getu, 2016; CIBA, 2019a & 2019b; Ananda Raja et al., 2022
Linguatula	Barnes, 1982; Sudan et al., 2018
Cymothoids	Paperna and Overstreet, 1981; Sethi, 2012; Sethi et al., 2013; Aneesh and Kappalli, 2020
Annelids	CIBA, 2019a & 2019b
Shrimps	
Protozoa	
Endocommensal / Invasive protozoa	
Microsporida	Vedavyasa Rao and Soni, 1988; Brock and LeaMaster, 1992; Johnson, 1995; Stentiford et al., 2007; Tourtip et al., 2009; Ananda Raja et al., 2012; CIBA, 2016; Mukta and Paramveer, 2018
Haplosporida	Vedavyasa Rao and Soni, 1988; Brock and LeaMaster, 1992; Johnson, 1995
Gregarina	Vedavyasa Rao and Soni, 1988; Brock and LeaMaster, 1992; Johnson, 1995; Fajer-Avila et al., 2005; Ananda Raja et al., 2012
Body invaders	Brock and LeaMaster, 1992; Johnson, 1995
Ectocommensal protozoa	Vedavyasa Rao and Soni, 1988; Brock and LeaMaster, 1992; Johnson, 1995; Ananda Raja et al., 2012
Metazoa / Helminthiases	
Trematodes (flukes)	Vedavyasa Rao and Soni, 1988; Johnson, 1995; Ananda Raja et al., 2012
Cestodes (tape worms)	Vedavyasa Rao and Soni, 1988; Johnson, 1995; Ananda Raja et al., 2012
Nematodes (round worms)	Vedavyasa Rao and Soni, 1988; Johnson, 1995; Ananda Raja et al., 2012
Other infestations	Lalitha Devi, 1982; Vedavyasa Rao and Soni, 1988; Johnson, 1995; Ananda Raja et al., 2012
Crabs	
Protozoa	Shields and Overstreet, 2003; McDermott, 2011
Metazoa	Shields and Overstreet, 2003; Jithendran et al., 2009 & 2010; McDermott, 2011



2. Parasitic diseases of finfish

2.1. Protozoa

The protozoa are single celled microscopic eukaryotic organisms with specialized structures for attachment, movement and food gathering (Roberts, 2001; Eiras, 2016). Protozoans can be either ectoparasites or endoparasites depending on their host species and the predilection site of infection. Ciliates and flagellates cause reactive hyperplasia (increase in the number of cells/cell proliferation and within a tissue) of the epithelium and increased mucus production due to their feeding on the skin layers of the epithelium and increased mucus production. Hyperplasia appears as cloudiness on the skin and leads to hypoxia when gills are affected (Noga, 2000). Other groups of parasitic protozoa are Microspora and Myxosporea. Microspora are intracellular affecting a wide variety of vertebrates and invertebrates while Myxosporea are largely intercellular infecting mainly fish (Noga, 2000). Protozoans cause harm to fish mainly by mechanical damage, secretion of toxic substance, occlusion of the blood vessels, depriving the host of nutrition and rendering the host more susceptible to secondary infections. Some of the most common clinical signs are changes in swimming behaviour due to loss of equilibrium, flushing or scraping, inappetence, discoloration, tissue erosion, excess mucus production, haemorrhage and swollen body or distended eyes. The major protozoan parasites in fish are discussed below.

2.1.1. Flagellates (mastigophora)

Aquatic parasites in this subphylum are separated in to two classes such as Phytomastigophora and Zoomastigophora.

a. Dinoflagellates (Phytomastigophora)

Phytomastigophora contains chloroplasts in their cytoplasm and the dinoflagellate parasites such as *Amyloodinium* spp., *Piscinoodinium* spp. and *Hematodinium* spp. are common under this group (Roberts, 2001). *Amyloodinium* spp. and *Piscinoodinium* spp. cause necrotic dermatitis to their host. Amyloodiniosis also known as velvet disease, is caused by a dinoflagellate, *Amyloodinium ocellatum* (Fig. 1). It is one of the common microscopic ectoparasites with flagella for movement, affecting the gills and skin of many fishes (Coats, 1999; Eiras, 2016). Other dinoflagellate (*Piscinoodinium* spp.) parasitizes many freshwater fish species. The lifecycle of *A. ocellatum* is direct in three stages and can be completed in five to seven days when temperature and salinity are between 23-27 °C and 30-35 parts per thousand (ppt), respectively.

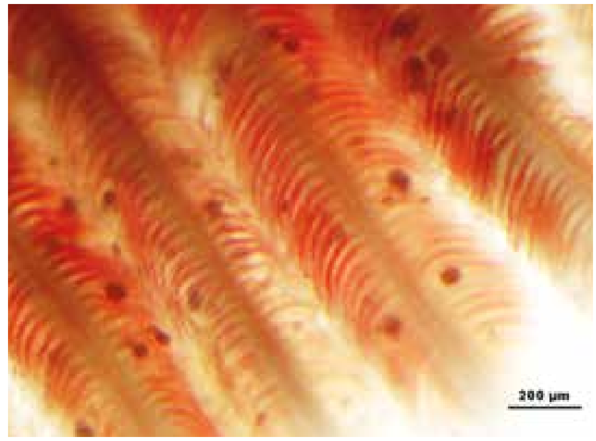
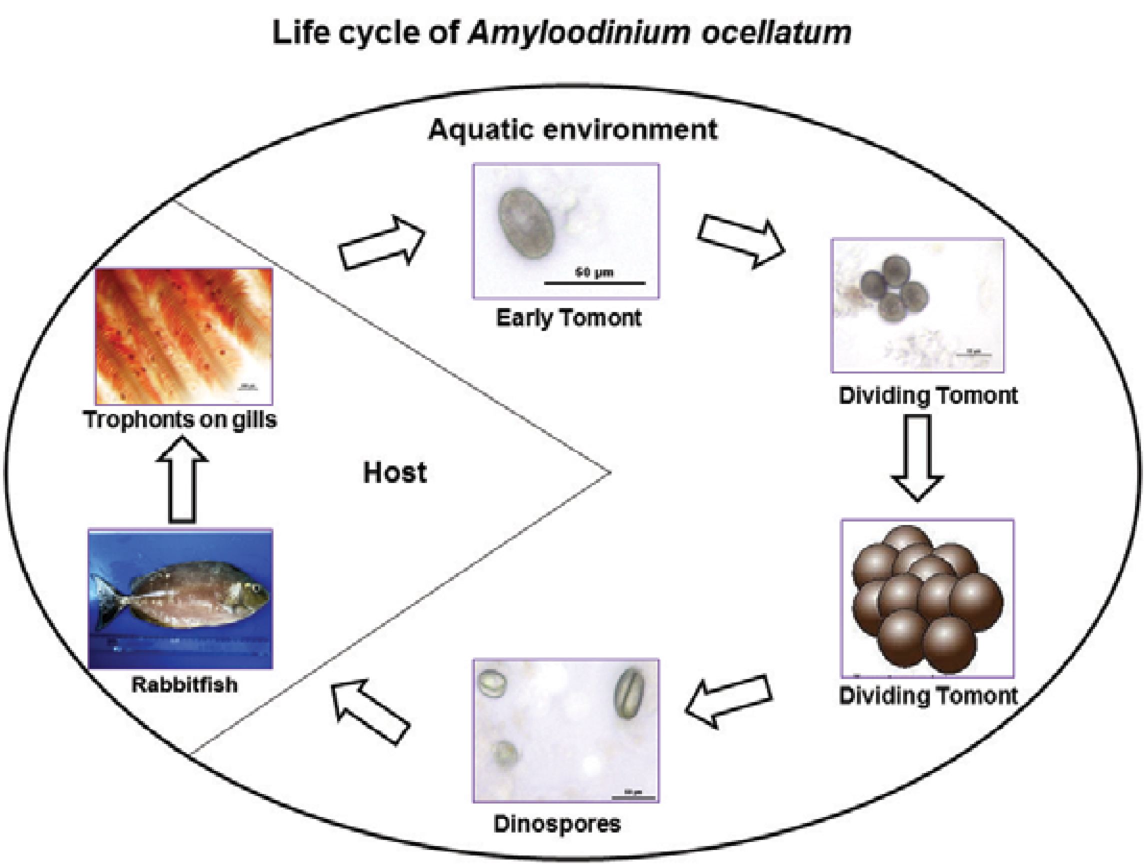


Fig. 1. Dinoflagellate, *Amyloodinium ocellatum* infested in the gills of Rabbitfish, *Siganus javus* maintained at ICAR-CIBA aquarium, India. Wet mount. Scale bar : 200 µm.

The parasitic stage is represented by the sessile trophont with pear-shaped protist enclosed in a



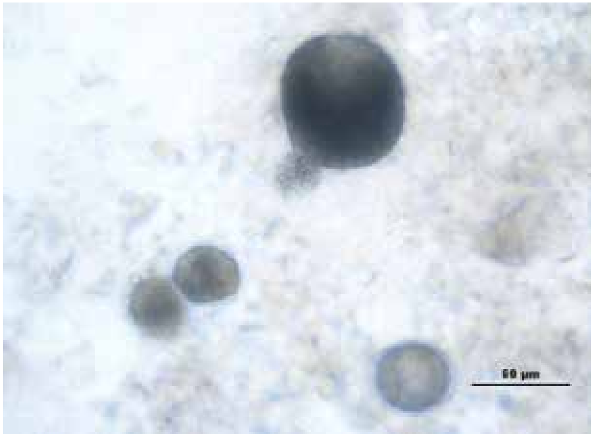


Fig. 2. The parasitic stage, sessile trophont with pear-shaped protist enclosed in a cellulose wall with rhizoids (tentacle like processes) for anchoring to the host epithelia (predominantly gill or skin), collected from Rabbitfish, *Siganus javus*. Wet mount. Scale bar : 50 µm.

Fig. 3. Tomont, the reproductive stage, of dinoflagellate, *Amyloodinium ocellatum* infested in the gills of Rabbitfish, *Siganus javus.* It is round and encapsulated in a thick cellulose wall. Wet mount. Scale bar : 50 µm.

cellulose wall with rhizoids [tentacle like processes] (Fig. 2) that enable it to strictly anchor to host epithelia (predominantly gill or skin). Trophonts can also be found on eyes, fins and in all oropharyngeal cavities in severe infections. Trophonts measuring 27×23 µm while mature ones can reach up to 130×60 µm and more, cause physical injuries to cells due to their continuous and constant movements. They feed directly from the host cells using the stomopode by releasing digestive enzymes. Two to six days after feeding, the trophonts detach from the host and encyst on inert substrates (pond/tank bottom or sea bed) transforming into the tomont, the reproductive stage (Fig. 3). In this stage, the protozoan is round and encapsulated in a thick cellulose wall, which confers an exceptional resistance to unfavourable conditions and to therapeutic treatments. This protozoan reproduces asexually and in two to four days, single tomont releases 256 new dinospores (infective stage). In this stage, the armoured (cellulose wall) protist is capable of actively swimming and with two flagella, one longitudinal and the other transverse. After adhesion to a new host, the dinospore transforms into a trophont within 5 to 20 minutes (Paperna, 1980; Roberts, 2001).

High level of organic matter in water and higher stocking density of fish are considered as predisposing factors. Clinical signs consist of velvet appearance, anorexia, scratching, darkening of the body surface and gathering on the surface or near the source of aeration. Histopathological lesions include gill and skin inflammation, haemorrhages, hyperplasia and necrosis (Paperna, 1980; Ferraz et al., 1998; Saraiva et al., 2011; Ramesh Kumar et al., 2016; Nozzi et al., 2016). Severe infections at high temperatures lead to mass mortalities, both in mariculture and marine aquaria. Diagnosis is mainly based on microscopic observations of fresh samples and histopathological examinations, and ELISA tests are also available (Cobb et al., 1998; Cecchini et al., 2001). There is no possible prevention and control measures developed so far. Freshwater (2-4 minutes) or bath treatment with 200 parts per million (ppm) formalin for 1 h or copper sulphate (0.75 mgL^{-1}, 12-14 days) baths with aeration and daily water replenishment have been suggested to control the trophonts or dinospores, respectively. Hydrogen perroxide is used as suitable treatment in pacific threadfin (*Polydactylus sexfilis*) (Montgomery-Brock et al., 2001). The development of specific antibodies against this parasite has been demonstrated in the sera of infected fish (Cobb et al., 1998), denotes the protection for re-infection.

b. Flagellates (zoomastigophora)

They do not contain chloroplasts in their cytoplasm. Three orders namely Kinetoplastida, Retortamonodida and Diplomonadida are usually reported as fish parasites (Roberts, 2001; Eiras, 2016). Parasites of order Kinetoplastida have one or two flagella as in *Cryptobia* spp. Cryptobiasis in marine fish species is caused by ectoparasitic flagellates such as *Cryptobia branchialis* and *C. eilatica* with a direct life cycle (Diamant, 1990). Few species like *Cryptobia iubilans* parasitize internal organs in aquaria cichlid fish. It causes gastric dilation, submucosal granuloma, gastric perforation, peritonitis and full thickness necrosis of the body wall musculature (Ferguson, 1989). Ectozoic species principally infects gills causing hyperplasia and epithelial destruction leading in sequelae to respiratory impairment. Clinical signs such as anorexia, skin darkness, and persistent cummulative mortality upto 10% after several weeks of infection are commonly observed in Mediterranean seabass and seabream (Ferguson, 1989).

Parasites of order Retortamonodida possess two to four flagella, one turned posteriorly such as genus *Ichthyobodo*. Costiasis or ichthyobodiasis is the infection caused by ectoparasitic flagellate, *Ichthyobodo* spp. (also known as *Costia* spp.) which causes a disease in both freshwater and marine fish and affects the gills and skin (Tojo and Santamarina, 1998; Urawa et al., 1998). Costiasis is mostly observed in larval and juvenile stages. Affected fish show clinical signs such as lethargy, emaciation and flashing behaviour. Clinical lesions such as grey-whitish pellicle on skin, severe epidermal erosion, increased mucus production, ulcers, ulcerative dermatitis, haemorrhages, gill hyperplasia, lamellar fusion (clubbing), oedema, and mortality are observed (Urawa et al., 1998).

Parasites of order Diplomonadida have one to four flagella and two-fold rotational or bilateral symmetry such as *Hexamita* spp. and *Spironucleus* spp. *Hexamita* spp. are endoparasitic flagellates of the intestine and gall bladder in freshwater fish, mainly salmonids but also cyprinids, mullets (Paperna and Overstreet, 1981; Eiras, 2016) and ornamental fish. Hexamitiasis is observed typically in weak fish frequently as a secondary infection. Affected fish can show nervous behaviour, and internally the intestine may appear pale. Mortalities can occur in fry and ornamental fish (Tojo and Santamarina, 1998). *Spironucleus* spp. and *Hexamita* spp. are the cause of

Fig. 4. Flower horn fish infected with flagellate, *Hexamita* spp. showing hole-in-the-head (Hexamitiosis), collected from aquarium at Kolathur, India.

hole-in-the-head disease in aquarium cichlids (Fig. 4). This parasites are associated with large erosions in the cranial cartilages, may ulcerate, resulting often in bilaterally symmetrical lesions (Ferguson, 1989).

Trypanoplasma spp. and *Trypanosoma* spp. are the endoparasitic flagellates of bloodstream and tissues, with indirect life cycles having leeches as the main vectors. The best known is *Trypanoplasma salmositica* (frequently referred as *Cryptobia salmositica*) producing cryptobiasis with severe impact in salmonid cultures and *Trypanosoma mugicola* in *Mugil Cephalus* (Paperna and Overstreet, 1981). Clinical signs consist of exophthalmia, splenomegaly, hepatomegaly, abdominal distension with ascites, anaemia and anorexia. Mortality is dependent on fish stocks and species, but mortality rates may be high in juveniles. Other pathogenic species, *Trypanoplasma borreli,* parasitizes mainly cyprinids in Europe and North America. The genus *Trypanosoma* infects numerous species of both freshwater and marine fish. Some freshwater species are pathogenic for cyprinids (Cruz and Eiras, 1997; Woo, 2001).

2.1.2. Ciliates (Ciliophora)

Ciliates are microscopic external parasites with cilia for movement in at least one stage of the life cycle. They always have two types of nucleus such as micronucleus and macronucleus. Most of these parasites are classified in to three classes such as

Kinetofragminophorea, Oligohymenophorea and Prostomatea (Eiras, 2016).

a. Kinetofragminophorea

Members of class Kinetofragminophorea belonging to the genus *Chilodonella*, free living organism, have oral ciliature slightly distinct from body ciliature. Only two species *C. piscicola* and *C. hexasticha* are pathogenic for freshwater and marine fish (Noga, 2000). *Chilodonella* spp. attaches on skin and gills, especially in ornamental fish (Koi, goldfish). *Chilodonella* spp. feeds directly on epithelium by penetrating the host cells with its cytostome and sucking their contents. On the skin they may virtually cover the body surface. After infection, fish secrete excessive mucus, with acute to subacute dermatitis, rotting or fraying of fins, hyperplasia, degeneration, necrosis and respiratory distress (Noga, 2000). Chilodonellosis is a serious ectoparasitic infestation causing heavy losses in aquaria and in farming systems.

b. Oligohymenophorea

Class Oligohymenophorea has well-defined oral apparatus and oral ciliature is distinct from somatic ciliature such as *Ichthyophthirius multifilis*, *Trichodina* spp. *Tetrahymena* spp., *Epistylis* spp., (Fig. 5) *Vorticella* spp. and *Scuticociliatida* (*Uronema* spp., *Phylasterides* spp. and *Miamiensis* spp.). *I. multifilis* parasitizes on many species of freshwater fish and cause Ich or white spot disease. This parasite is large in size (about 0.1-1.0 mm) contains a horseshoe-shaped macronucleus. Infected fish have the presence of small white spots on the skin or gills. *I. multifilis* cause acute to sub-acute dermatitis with hyperplasia, they are present within epidermis (Ferguson, 1989). Fish trichodinids include mainly *Trichodina* spp., *Trichodinella* spp., *Tripartiella* spp. *Paratrichodina*, *Hemitrichodina* and *Vauchomia* spp. from freshwater and marine source (Paperna and Overstreet, 1981). *Trichodina* spp. is considered important. It has a circular body with 100 µm diameter and cilia around the perimeter. It infects mainly gills, body surface and fins. High level of organic matter in water or poor

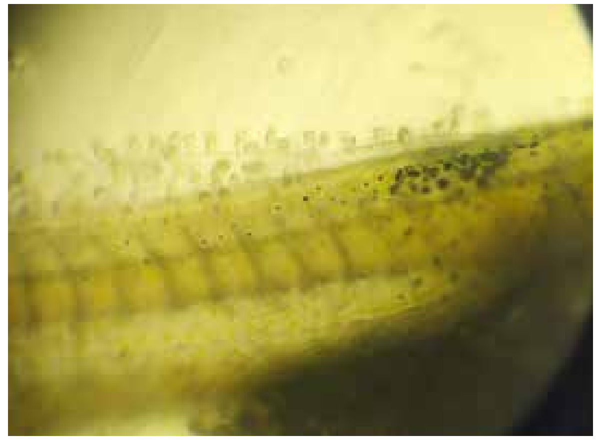


Fig. 5. *Epistylis* spp. in larvae of Pearlspot, *Etroplus suratensis*. Wet mount.

water exchange serves as predisposing factors. The parasites are especially harmful to fingerlings by interfering with the respiration. Clinical signs and lesions of Trichodiniosis / Trichodiniasis include excess mucus production, respiratory distress by clogging of gills by mucus, flashing, debility, pale gills, hyperplasia, irritation on body hence rubs body against objects, subacute dermatitis, necrosis of the epidermis, grey-blue turbid layer on the skin and high mortality. The fin may become badly frayed in heavily infected fish, accompanied by sluggishness and loss of appetite. In incidental case, some species of *Trichodina* may infect the urinary bladder, oviducts or gastrointestinal tract. In this case, unilateral aplasia of a ureter or chronic inflammation may result in cystic dilation of that portion proximal to the obstruction (Ferguson, 1989; Noga, 2000; Roberts, 2001). *Tetrahymena* spp. is the parasite of freshwater fish, cause erosion of the cranium in Atlantic salmon. *Tetrahymena pyriformis* is usually reported parasite of guppy, capable of disseminated infections with dermal ulceration. In some cases, this parasite may invade various internal organs like kidney or brain. The peritrichous ciliate, *Epistylis* spp. and *Scyphidia* spp. are widely prevalent on freshwater fish such as cichlid and cyprinid. They may infect skin, fins, oral cavity and gills. *Epistylis* spp. is the most common and pathogenic type of sessile, colonial ectocommensal ciliate. Host response for this infection occurs by haemorrhagic lesions and necrotic dermatitis with ulceration. *Vorticella* spp. is found on the thoracic appendages and the cercopods of fairy shrimps. In many previous reports, *Vorticella* spp. is reported as a free living organism, however in some cases these free-living ciliophorans become facultative parasites under adverse environmental conditions causing a "grey mat" on the epithelial surface of completely debilitated and moribund animals. Several species of the genera *Uronema*, *Phylasterides* and *Miamiensis* have been recorded under *Scuticociliatida* as facultative parasites of different marine and brackishwater fish species (Cheung et al., 1980; Dragesco et al., 1995; Iglesias et al., 2001). Clinical signs of scuticociliatosis depend on the site of attachment of the parasite. External signs include skin lesions such as ulcers and pigmentation changes, but this histophagous parasite frequently invades the body muscle and the internal organs, causing severe degeneration of internal organs. When this parasite colonises in the nervous system, it can lead to erratic swimming, loss of equilibrium and/or lethargy (Munday et al., 1997). The disease cause severe infections and outbreaks in some cultured fish and mortalities can reach 100% in affected stocks.

c. Prostomatea

Cryptocaryon irritans is the member of class Prostomatea, which cause marine white spot disease. It is the counterpart of the freshwater *I. multifilis* which causes the "White Spot Diseases" or "Ich". *C. irritans* is a pear shaped (0.3-0.5 mm in size) external parasite found on the external body surface of fish. *C. irritans*, parasite of gills and skin, is the causative agent of cryptocaryosis in many important commercial and ornamental marine and brackishwater fish species (Paperna and Overstreet, 1981; Noga, 2000; Rigos et al., 2001; Wright and Colorni, 2002). The surface of invaded fish reveals white pustules or numerous minute and greyish vesicles which are nests of ciliates burrowing under the epidermis. They feed on the host's cells underneath the epithelium and cause heavy irritation resulting in excessive production of mucus, acute to subacute dermatitis, hyperplasia white spots over the body surface, ulcers, wound, secondary bacterial infection, rubbing the body against submerged objects, and finally completely destroying the fine respiratory platelets of the gill filaments leading to respiratory distress and mass mortality in untreated cases. Gill histopathology consists of inflammation, haemorrhages, hyperplasia and lamellar destruction. High stocking density and low water temperature serve as predisposing factor for infection (Diggles and Lester, 1996).

2.1.3. Microsporidia

Microsporidians are intracellular internal parasites. Microsporea are represented in fish by different genera, mainly *Enterocytozoon*, *Glugea*, *Loma*, *Pleistophora* and *Tetramicra* (Mathieu-Daude et al., 1992; Abela et al., 1996; Athanassopoulou, 1998; Shaw and Kent, 1999). In freshwater fish, *Pleistophora* and *Loma* are relatively frequent. Among cultured marine fish, there have been several reports of *Plesitophora senegalensis* in gilthead seabream, whereas *Glugea* spp. and *Tetramicra brevifillum* have been found in turbot. It forms spore and resembles myxozoans. The oval shaped spores have average size of 6 µm. These are small single-celled spores containing a single sporoplasm which extrudes through an everted polar filament into a host cell. Many of these develop within a pansporoblast, and the visible cyst comprises massive numbers of spores. Some species are capable of devastating natural stocks of fish and shellfish and can inflict heavy losses to cultured species, given the right conditions. Pansporoblasts in *Crenimugil crenilabis* in mullet always occurred in association with the myxosporidan *Kudoa* spp. in the intestinal wall of the host (Paperna and Overstreet, 1981). Poor water quality and poor nutrition are the predisposing factors. No visible clinical signs of microsporidiosis are observed. Cysts are observed in various internal organs like intestinal wall, ovary, fat tissue etc. These cysts are brown or black in colour and are of various size and shape called xenoma (Speare et al., 1998). Pathological concern of microsporidiosis in fish is dependent on location and intensity of infection (Estévez et al., 1992; Faye et al., 1998).

2.1.4. Amoebozoa

Amoebozoas are ranked as a phylum within either the kingdom Protista or Protozoa. Protista consists of heterotrophic and autotrophic organisms whereas most protozoa are heterotrophs which are obligatorily parasitize fish species. Hence, they are dealt under protozoa in this chapter. Although different species of amoebas have been associated to amebiasis in fish, *Neoparamoeba pemaquidensis* is identified as specific ethology amoebic gill disease (AGD) in *Salmo salar*, *Scophthalmus maximus*, *Sparus aurata* and *Dicentrarchus labrax* (Dykova et al., 1995; Leiro et al., 1998; Zilberg et al., 1999; Munday et al., 2001; Tan et al., 2002). Amoebas may appear in small numbers trapped in the gills without causing any damage, but in heavy infections, the parasites elicit epithelial hyperplasia, metaplasia, resulting in complete fusion of secondary lamellae and subsequent gill dysfunction (Young et al., 2007; Bustos et al., 2011). Amoebozoa, *Endolimax piscium* can also cause systemic granulomatous disease as reported in cultured sole, *Solea senegalensis* (Constenla et al., 2014). Amoebic infections involving systemic granulomatous inflammatory lesions and abscesses can affect different animal and human organs, especially the liver and the brain as reported in goldfish, *Carassius auratus* and in tench, *Tinca tinca* (Voelker et al., 1977; Candreviotis 1977; Visvesvara et al., 1993; Riestra-Castaneda et al., 1997; Palikova et al., 2012). Fish become lethargic and emaciated. Diagnosis is mainly based on microscopic examination and histopathology (Zilberg et al., 1999; Dykova et al., 2000), immunodiagnosis and species specific PCR are also available (Douglas-Helders et al., 2001; Constenla et al., 2014). The most widely recommended treatment is the use of freshwater baths, though it is not fully effective in killing amoebae. Hydrogen peroxide and levamisole have also been assayed with variable results (Findlay et al., 2000; Parsons et al., 2001; Powell et al., 2001).

2.1.5. Apicomplexa

Many species of coccidia are endoparasitic protozoans belonging to Apicomplexa (currently known as Apicomplexa, it includes most taxa previously regarded as Sporozoa) and are known from freshwater, brackishwater and marine fish species, but their pathological significance for aquaculture is very variable. The genera *Eimeria, Goussia* and *Cryptosporidium* are more frequently reported from cultured fish (Logana,et al., 2012; Eiras, 2016). In freshwater fish, *G. carpelli* and *E. anguillae* are reported in cyprinids and eels, respectively (Steinhagen et al., 1998). In marine fish, *G. sparis* and *E. sparis* have been reported from *Sparus aurata*, *E. dicentrarchi* and *E. bouixi* from *Dicentrarchus labrax* (Sitjà-Bobadilla et al., 1996), and *Eimeria* spp. from *Mugil cephalus* (Paperna and Overstreet, 1981). *Cryptosporidium* spp. infects seabream, seabass, turbot, and aquarium fish, affecting mainly larvae and juveniles resulting in poor condition. *C. molnari* is more frequent in seabream than in seabass (Alvarez-Pellitero and Sitjà-Bobadilla, 2002). The most frequent hosts are marine fish, mainly *S. aurata* and *D. labrax*, followed by *Scophthalmus maximus* and *Diplodus puntazzo*. Freshwater fish hosts include only cyprinids and some exotic fish. Cryptosporidiosis of seabass and seabream is caused by *C. molnari*. Haemogregarines such as *Haemogregarina mugili* Carini and *Haemogregarina bigemina* Laveran and Mesnil infect the red blood cells and white blood cells of mullets, respectively. A life cycle of a piscine species of *Haemogregarina* has not been completed, but transmission was suggested to be conducted by leeches (Paperna and Overstreet, 1981; Logana et al., 2012).

2.2. Metazoa / helminthiases

Metazoans parasites of fish include mainly Myxozoans, helminths of different classes, arthropods dominated by parasitic crustaceans and some annelids such as leeches. Helminthiases have not been observed as serious problem in finfish culture. This is probably due to their complex life cycle and inability to complete their life cycle in the culture systems. Few helminths found in finfish include trematodes or flatworms, cestodes or tape worms and nematodes or round worms. Among helminths, trematodes are of significance in cultured fish though nematodes and cestodes rarely cause massive infections.

2.2.1. Myxozoans

Myxozoans (Class Myxosporea; Phylum Myxozoa) are microscopic internal fish parasites. They are characterised by a spore with one to several valves, one or more infective sporoplasms and one to several polar capsules with a coiled polar filament inside (Kent et al., 2001; Mladineo, 2003). In freshwater fish the most significant myxosporidiosis are whirling disease (*Myxobolus cerebralis* or *Myxosoma cerebralis*) (Andree et al., 2002; Wagner, 2002), proliferative kidney disease or PKD (*Tetracapsuloides bryosalmonae, Tetracapsula bryosalmonae* or *T. renicola*) (Canning et al., 1999), sphaerosporosis (*Spaherospora renicola*) and ceratomyxosis (*Ceratomyxa shasta*). Myxosporea reported from cultured marine fish include species of the genera *Ceratomyxa* in gall bladder (Alvarez-Pellitero and Sitjà-Bobadilla, 1993), *Enteromyxum* in intestinal tract causing severe chronic enteritis (extreme thinness; "knife-fish") (Paperna and Overstreet, 1981; Diamant, 1998; Branson et al., 1999; Padrós et al., 2001; Palenzuela et al., 2002; Eiras, 2016), *Kudoa* in muscle (white plasmodial soft textured cysts; soft flesh condition) (Paperna, 1982; Moran et al., 1999), *Sphaerospora* in the connective tissue of gall bladder and intestine (*Sphaerospora dicentrarchi*) and in testes (*Sphaerospora testicularis*) (Sitjà-Bobadilla and Alvarez-Pellitero, 1992), *Lepthoteca polysporoplasma* in kidney, *Sinuolinea* in urinary bladder, *Myxobolus parvus* in gills of *Mugil cephalus*, *Myxosoma branchialis* (Markewitsch) in the branchial region of *M. cephalus*, *M. inteslinalis* in the intestinal epithelium of *Mugil waigensis*, and *Myxosoma cephalus* in the meninges, gill arches and filaments, buccal cavity, jawbone, crop, oesophagus, intestine, liver and mesentery of *M. cephalus* (Paperna and Overstreet, 1981). *Myxobolus omari* and *M. leptobarbi* are found in the muscles of *Pangasianodon hypophthalmus* and *Leptobarbus hoevenii*, respectively (Székely et al., 2009). The parasites either infect tissue of various organs or live freely in cavities like gall bladder, abdominal cavity, etc (Alvarez-Pellitero and Sitjà-Bobadilla, 1993). Poor water quality, high stocking density, feeding with infected trash fish and lack of quarantine measures facilitate infection. Common examples of myxozoans are *Myxobolus, Myxidium, Kudoa, Ceratomyxa* etc. Clinical signs are not apparently visible. However, white or black cysts may be seen on body surface, gills, fins and internal organs. The parasites invade all major organs and forms cysts or freely floating mass called pansporoblast. They destroy gills and all major target organs of the fish (Paperna and Overstreet, 1981).

Henneguya spp. is a flagellated myxosporidan parasite found attached mainly to the gills (Fig. 6). Plasmodia and spores of *Thelohanellus zahrahae* and *Henneguya daoudi* are detected in the gills of *Barbonymus gonionotus* and *Trichogaster trichopterus*, respectively (Székely et al., 2009). In heavy infestations, it may be found in the skin also. Diseased fish exhibits chronic mortalities with clinical sign of anaemic gills. The major lesions are enlarged bulbus arteriosus and internal haemorrhages in the pericardial cavity. Irregular-shaped plasmodia developed in the bulbus arteriosus which releases mature spores accumulating in the lumen. Massive influx of spores into the gills causes local occlusion and congestion of gill capillaries resulting in proliferative and granulomatous branchitis, lamellar hypertrophy, degeneration of the gill epithelium, and degenerative cardiomyopathy. It is commonly called as "Hamburger Gill Disease" or "proliferative gill disease" (Duhamel et al., 1986).

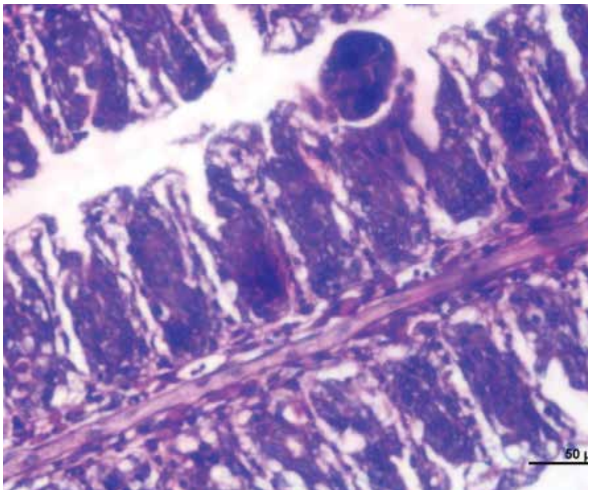


Fig. 6. Spores of *Henneguya* spp. in gills of Pearlspot, *Etroplus suratensis,* collected from Pazhaverkadu, India. H&E. Scale bar: 50 μm.

2.2.2. Trematodes (flukes)

Trematodes constitute mainly external parasites with adhesive structures for attachment to the host. They are of two types: Monogeneans and Digeneans. Monogenetic trematodes can be observed throughout the year. Temperature apparently plays an important role in determining outbreaks of certain Monogenean parasites.

a. Monogeneans

Monogeneans are mostly ectoparasites with specialized posterior attachment organs. It needs single host for completing its life cycle and multiply very fast in confined water bodies. Monogeneans are usually very host specific, though in certain culture conditions some species can be found in unusual hosts. Monogenea include two main groups, *Monopisthocotylea* (with a simple adhesive disc) and *Polyopisthocotylea* (with a complex adhesive disc including clumps and hooks). The most significant species infecting cultured fish among *Monopisthocotylea* are *Gyrodactylus* spp., *Dactylogyrus* spp., *Diplectanum* spp. and *Furnestinia* spp. (Paladini et al., 2014; Eiras, 2016; Muller et al., 2016). *Polyopisthocotylea* includes several species of pathological concern for fish cultures, most of them belonging to the family *Microcotylidae* (Faisal and Iman, 1990; Sanz, 1992; Khidr et al., 2012) and *Heteraxinidae* (Grau et al., 2003). There are four families such as Gyrodactylidae (viviparous), Dactylogyridae (oviparous), Ancyrocephalidae (oviparous) and Capsalidae (oviparous), and parasites of these families are commonly called gyrodactylids, dactylogyrids, ancyrocephalids, and capsalids, respectively. Distinguishing characteristics include the presence or absence of eye spots, and the number of pairs of anchors (hamuli), transverse bars, and marginal hooks on their haptors. These features can only be seen with a compound microscope. Monogenosis is caused by skin and gill flukes which can cause different degrees of damage in parasitized fish (Sterud, 2002).

i) Skin flukes

Skin flukes are 2-6 mm long and the most common flukes are *Gyrodactylus* spp., *Benedinea* spp., *Neobenedenia* spp. and *Pseudorhabdosynochus* spp. (Eiras, 2016; Lestari et al., 2018). Gyrodactylosis are mainly typical from freshwater fish (*G. salaris*), however, it has also been reported in marine fish (McHugh et al., 2000; Cable and Harris, 2002; Sterud et al., 2002). Gyrodactylids have a pair of anchors with both dorsal and ventral bars and 16 marginal hooks, and do not have eye spots. Attachment to the fish is made with the marginal hooks; the anchors are used as a spring-like device to assist attachment with the marginal hooks. An embryo with its pair of anchors may frequently be seen inside an adult gyrodactylid. The infection sets in when there is high stocking density, poor water exchange and polyculture. The parasites mainly affect body surface, fins, eyes, and sometimes gills. Mass mortalities may appear in moderate or heavy infections, mainly in juvenile fish, increasing with water temperature. Clinical signs include lethargy, anoxia, loss of appetite, scratching, excessive mucus production, corneal opacity, blindness and ulcers or haemorrhages with secondary bacterial infection.

ii) Gill flukes

These common ectoparasites in fish are *Diplectanum* spp., *Dactylogyrus* spp. and Ancyrocephalids. The best known species of the genus *Diplectanum* are *D. aequans* and *D. laubieri*. Their dispersion is very wide in the Mediterranean and Atlantic areas, mostly coinciding with the distribution of seabass. *D. aequans* is considered more pathogenic, mainly for juveniles and broodstocks (González-Lanza et al., 1991; Cecchini et al., 1998; Eiras, 2016). Dactylogyrids primarily found in freshwater fish, have two pairs of eye spots and occasionally have a vestigial pair of anchors in addition to a pair of anchors and one transverse bar. They usually have 1214 marginal hooks, but these may be absent in some species. Dactylogyrids are oviparous (egg-layers), and eggs are occasionally seen in association with gill tissue during microscopic examination. The best known species of the family *Dactylogyridae* are

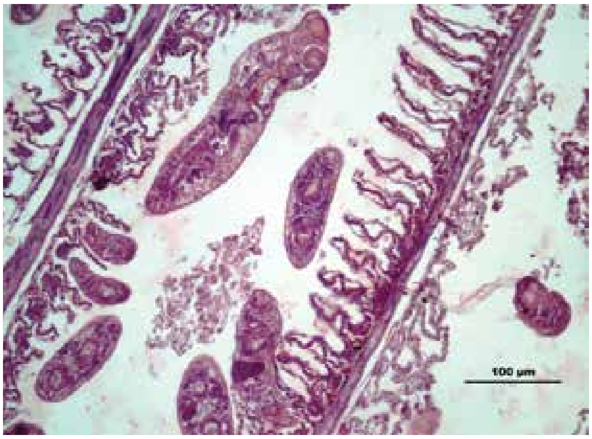


Fig. 7. *Dactylogyridae, Mymarothecium* spp., the haptor penetrating into the basement membrane and connective tissue of the lamellae in the gills of Grey mullet, *Mugil cephalus,* collected from Muttukadu Lagoon, India . H&E. Scale bar : 100 µm.

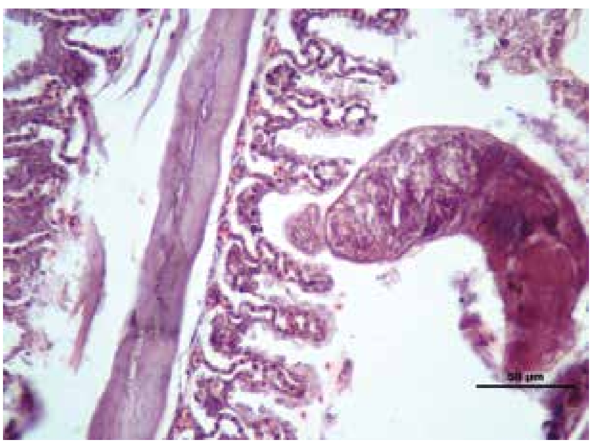


Fig. 8. *Dactylogyridae*, *Anacanthorus* spp., the haptor penetrating into the basement membrane and connective tissue of the lamellae in the gills of Grey mullet, *Mugil cephalus,* collected from Muttukadu Lagoon, India. H&E. Scale bar : 50 µm.

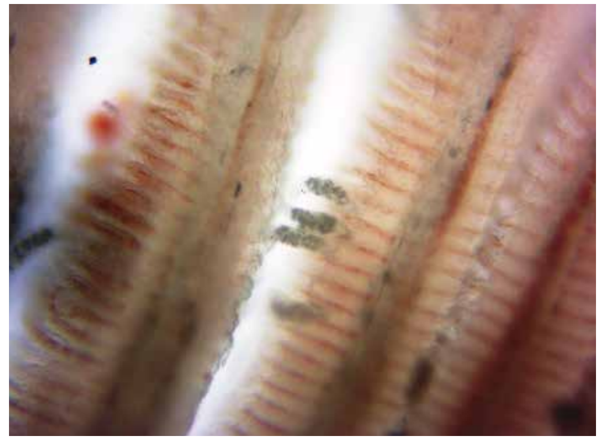


Fig. 9. Ancyrocephalids penetrating into the basement membrane and connective tissue of the lamellae in the gills of Pearlspot, *Etroplus suratensis,* collected from the aquarium of ICAR-CIBA, Chennai, India. Wet mount. Scale bar : 200 µm.

Fig. 10. Ancyrocephalid collected from the the basement membrane and connective tissue of the lamellae in the gills of Pearlspot, *Etroplus suratensis,* collected from the aquarium of ICAR-CIBA, Chennai, India. Wet mount. Scale bar : 100 µm.

Anacanthorus spp. (Fig. 7) and *Mymarothecium* spp. (Fig. 8) [Muller et al., 2016]. Ancyrocephalids are closely related to the Dactylogyrids. They are usually found on gills of freshwater, marine and brackishwater fish. However, some genera (*Neodiplectanotrema* and *Paradiplectanotrema*) are reported in the oesophagus of marine fish and one genus, *Enterogyrus*, lives in the stomach cichlid species. Ancyrocephalids (Fig. 9 & 10) have two pairs of eye spots, and two pairs of anchors. Each pair of anchors has a transverse bar. Marginal hooks usually number 1214, but may be absent in some species. Some species produce an adhesive substance to assist in attachment to the fish host. In microcotylosis besides the gill dysfunction, increased damage due to hematophagous condition of the microcotylids, causing anaemia and poor fish condition. The important species of the microcotylids are *Sparicotyle chrysophry* in Sparidae and *Serranicotyle labracis* in seabass. High density and poor sanitation serve as predisposing factors. Mass mortality with respiratory problems is observed in severely affected fish. Other clinical signs are pale gills, low consumption of feed, erratic swimming behavior and mucus production on gills. Gill histopathological lesions include focal hyperplasia, lamellar fusion, haemorrhages and inflammatory infiltration.

b. Digeneans

Digeneans are generally hermaphroditic platyhelminths that typically have a selectively absorptive tegument, a blindly ending alimentary tract, and two suckers. They are mostly intestinal parasites and involves more than one host for completing their life cycle. Digenetic trematodes such as *Lecithochirium* spp. and *Pseudometadena celebesensis* are found in the intestine especially in wild fish. The metacercarial phase of *Diplostomum* spp., digeneans parasitizes the eye of numerous fish species, though it can be occasionally found in other organs, including the brain (Eiras, 2016). Clinical signs consist of cloudiness of eye lens leading to crystalline opacity, blindness, dark body coloration, emaciation and mortality (Buchmann et al., 1997; Midtlyng et al., 1999). Members of the family Sanguinicolidae are parasites of the circulatory system of freshwater and marine fish causing sanguinicolosis. Adult parasites are located in the heart or kidney. Eggs can accumulate in blood vessels, and are quite frequent in the gills, producing vascular obstruction. Haemorrhages can also occur at miracidial eclosion. Moderate inflammatory response can be produced and the lesions are not extremely severe in moderate infections (Padrós et al., 2001). These are of variable size based on the species. No treatment method is suggested, although anthelmintic drugs are used. Digeneans complete their life cycle in a molluscan host, therefore, elimination of molluscs from the culture facility should stop the transmission cycle of the parasite.

2.2.3. Cestodes (tape worms)

These platyhelminthes may parasitize fish in larval or adult stages, sometimes causing diseases in cultured fish with variable economic impact. The life cycle involves at least one intermediate host. Most species causing disease in fish of economic importance fall within four orders: Caryophyllidea (*Caryophyllaeus* and *Khawia*), Pseudophyllidea (*Bothriocephalus, Diphillobothrium, Ligula* [ligulosis] and *Triaenophorus*), Proteocephalidea (*Proteocephalus*) and Tetraphyllidea (*Acanthobothrium*). *Bothriocephalus* spp., *Eubothrium* spp. and *Triaenophorus* spp. of order Pseudophyllidea causing bothriocephalosis, eubothriosis and trienophorosis in freshwater and marine fish, respectively (Dick and Choudhury, 1999; Eiras, 2016). *Triaenophorus* spp. can cause severe pathology in some fish. Fish are usually the final hosts, but some small fish may act as reservoirs of certain species. Larval stages (plerocercoids) of *Diphyllobothrium* spp. parasitize the muscle and visceral organs of different freshwater fish. Mammals are the final hosts of these tapeworms, which can also infect humans. Thus, diphyllobothriasis in fish must be controlled due to its zoonotic character (Rahkonen and Valtonen, 1998). Plerocercoids of *Ligula* spp. parasitize the visceral cavity of different freshwater fish. Fish can act as definitive and intermediate hosts of different *Proteocephalus* spp. Cestodes in fish usually do not cause mortality, though poor condition is frequently observed, mainly in heavy infections. Adult cestodes of the genera *Caryophyllaeus* and *Khawia* parasitize the digestive tract of cyprinids and salmonids, producing different degrees of damage and economic impact in the cultures. In heavy infections, abdominal swelling and poor condition can be observed, mainly in small fish. After necropsy, cestodes can be seen with the naked eye in the intestine. *Acanthobothrium* spp. are found as encysted "grubs" in the skin and muscle of fish, which can be a common human parasite. This parasite has a micro crustacean as first intermediate host and marine fish are required as second intermediate host (Sonune, 2014).

2.2.4. Nematodes (round worms)

Nematodes or roundworms are large, intestinal parasites with un-segmented body and mostly 1-2 cm length. Nematode of the genus *Cucullanus* is found more commonly in the gut of larger fish than in that of young fish. Ascaridoid *Contracaecum robustum* Chandler 1935 or *Contracaecum multipapillatum* is the nematode with potential public health hazard. Third-stage larvae (L3's) of various *Contracaecum* spp. have been reported from marine fish. C. *robustum* larvae encapsulated in the liver, kidneys, and mesentery of mullets [*Mugil cephalus,* M. *curema,* and *Liza ramada* and others] (Deardorff and Overstreet, 1980). Related ascaridoid nematodes, *Anisakis* spp. otherwise called as herring worms (Fig. 11-16) similarly found in mullets cause human anisakiasis. Anisakiasis is reported in many countries

Fig. 11. Grey mullet, *Mugil cephalus* harbouring anisakid larvae (L3) encysted in the posterior kidney, collected from Kovalam coast, India.

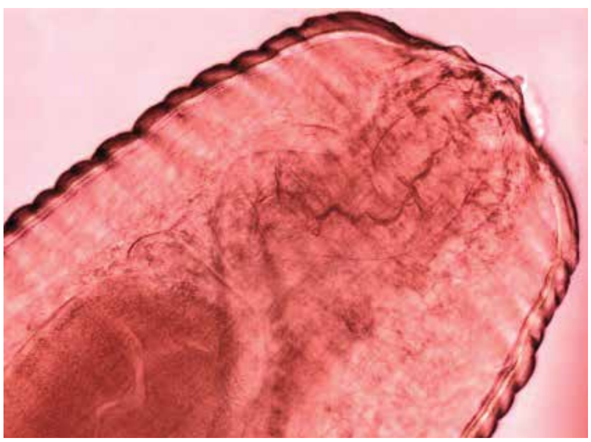


Fig. 12. Female cephalic end of the third stage anisakid larvae (L3) from Grey mullet, *Mugil cephalus*, collected from Kovalam coast, India.

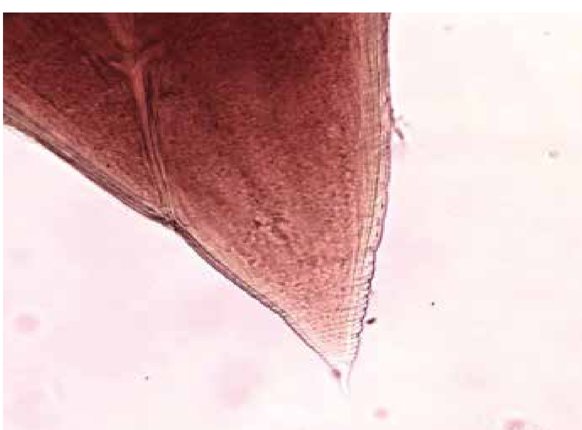


Fig. 13. Male caudal end of the third stage anisakid larvae (L3) from Grey mullet, *Mugil cephalus*, collected from Kovalam coast, India.

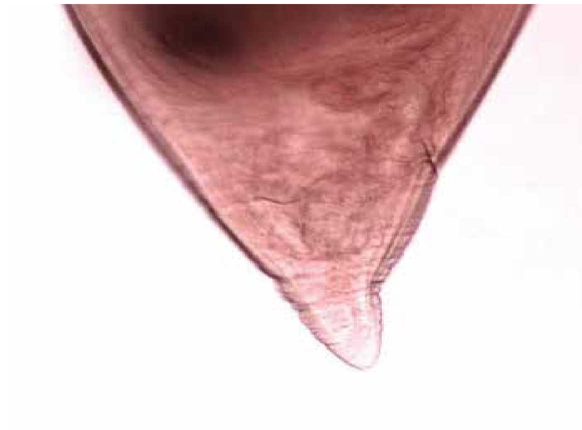


Fig. 14. Female caudal end of the third stage anisakid larvae (L3) from Grey mullet, *Mugil cephalus*, collected from Kovalam coast, India.

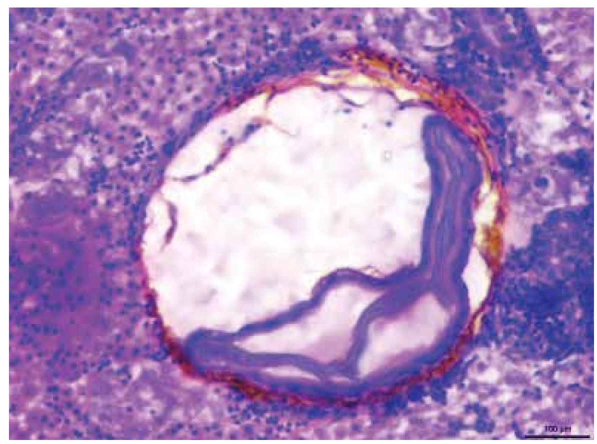


Fig. 15. Anisakid larvae cyst in the posterior kidney with mononuclear cells infiltration [MNC] in Grey mullet, *Mugil cephalus*. H & E. Scale bar : 100 µm.

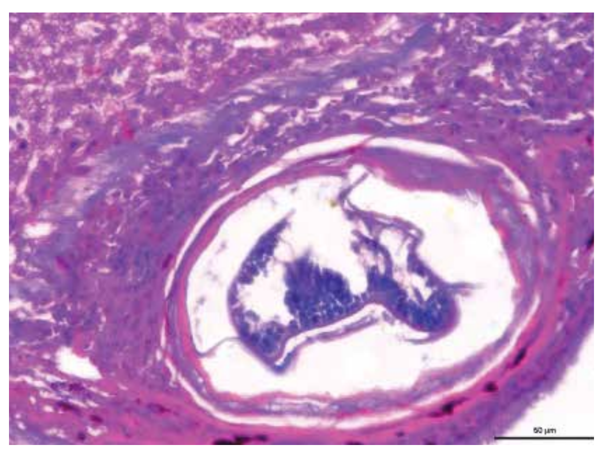


Fig. 16. Anisakid larvae cyst in the Glisson's capsule with mononuclear cells infiltration [MNC] in Grey mullet, *Mugil cephalus*. H & E. Scale bar : 50 µm.

as zoonotically significant parasitic disease causing serious health hazard in humans. Infection is prevented by maintaining hygienic conditions and avoid eating raw and/or under cooked fish. *Philometra barnesi* and *P. lateolabracis* (Philometridae) are described from the skin, muscle and ovary of the marine teleost fish causing reproductive failures (Moravec et al., 2011; Sethi et al., 2013; Moravec and Diggles, 2015; Eiras, 2016).

2.2.5. Acanthocephalan

Acanthocephalid worms with their fearsome-looking proboscis and their rows of hooks have been observed as serious pathogens of fish. Several acanthocephalans, also known as 'thorny-headed' or 'spiny-headed' worms infect the intestines of many fish species (Paperna and Overstreet, 1981). Members of this phylum superficially appear like nematodes, but closer examination of these reveals a protrusible spiny proboscis and no intestine. They have simple life-cycle. Once the embryo within a diatom-like shell reaches a certain point of development, it can be released from the female worm and continues development only when eaten by a specific arthropod. The acanthor stage metamorphoses soon to become a juvenile which is infective to the fish. Occasionally, paratenic hosts (reservoir host) are involved in the life cycles. Mostly reported Acanthocephalan are *Neoechinorhynchus bangoni* Tripathi (in *Mugil tade*), *N. agilis*, *N. chitkaensis* Podder (in *M. cephalus*), *N. elongatus* Tripathi (in *M. subviridis* and *M. dussumieri*), *N. karachiensis* Bilqees (in *M. spegieleri*), *N. agite* (Rudolphi) (in *Mugil* spp.), *N. coiliae* (in *Liza carinata*), *Gracitisentis mugilus* Gupta & Lata (in *Mugil* spp.), *Dispiron mugili* Bilqees (in *M. buchanani*), *Floridosentis mugilis* (Machado Filho) and *F. elongatus* [in *Mugil* spp.], *Paulisentis* spp. (in *Mugil* spp.), *Tenuiproboscis* spp. (in *Lutjanus argentimaculatus*) and *Tenuiproboscis keralensis* (in *Scatophagus argus*) [Jithendran and Kannappan, 2010; Sanil et al., 2011; Kaur et al., 2017; Verma and Saxena, 2018].

2.2.6. Arthropods

There are several hundreds of parasites considered to be of economic importance in brackishwater aquaculture under phylum Arthropoda and sub-phylum Crustacea. They are of Class or Sub-class such as Copepoda (Order: Cyclopoida and Siphonostomatoida), Branchiura (Order: Arguloida), Pentastomida (Order: Porocephalida) and Malacostraca (Order: Isopoda). Order Cyclopoida is further divided at genus level in to *Ergasilus* (Family: *Ergasilidae*), *Lernaea* and *Lamproglena* (Family: *Lernaeidae*). Order Siphonostomatoida is divided at genus level in to *Caligus* (Family: *Caligidae*) and *Lernanthropsis* (Family: *Lernanthropidae*). Order Arguloida is identified at genus level as *Argulus* (Family: *Argulidae*). Similarly, Order Porocephalida is identified at genus level as *Linguatula* (Family: *Linguatulidae*) while the Order Isopoda is recognized at genus level as *Cymothoa* (Family: *Cymothoidae*) [Johnson et al., 2004; Jithendran et al., 2008; Yatabe et al., 2011; Sahoo et al., 2013; Alaş et al., 2015; Eiras, 2016; Misganaw and Getu, 2016; CIBA, 2019a & 2019b; Ananda Raja et al., 2020].

a. Ergasilids

Ergasilids commonly known as 'gill maggot', are small and barely visible to the naked eye, clinging to the gill filaments with their characteristic second antennae. Few genus paraergasilus are found attached other than gills. The cephalothorax constitutes half or more of the body length. The parasite body part is segmented with thorax (except the first segment, fused with the head) and the abdomen is distinct. In Ergasilidae only the female is parasitic, and is found on the gills of fish. Males are free-living and there is a prolonged, free living larval development which includes three to six stages of nauplii and four to six stages of copepodites (lasting from 10 days to over a month). These free-living stages feed on nanoplankton (Johnson et al., 2004; Alaş et al., 2015; Eiras, 2016; Misganaw and Getu, 2016). They infest a wide range of fresh-water to marine hosts. Ergasilids attached to gill filaments produce digestive secretions which partially dissolve tissue, allowing easier ingestion leading to the destruction of respiratory epithelium and responsive hyperplasia. Erosion can extend beyond the epithelial lining, resulting in obstructed branchial blood vessels (Paperna and Overstreet, 1981).

Fig. 17. Cage culture of Asian Seabass, *Lates calcarifer* encountered with Anchor worm, *Lerneae cyprinacea,* infestation in Puducherry, India.

b. Lernaea

Lernaea spp. (Copepoda, Lernaeidae) is an important freshwater fish parasite which has a worldwide geographical range. The parasite was introduced to many countries by translocation of cyprinids (Piasecki et al., 2004; Alaş et al., 2015; Eiras, 2016). Its economic importance in fish farming is increasing due to the numerous epizootics occurring among the most important farmed fish species. The rod-shaped, unsegmented, or partly segmented parasitic stage lernaeid female is anchored, with the aid of the first thoracic segment, a specialized holdfast organ (anchors), to the host skin or buccal mucosa. The anchor consists of two ventral processes and two branched dorsal processes. All thoracic segments enlarge greatly, but the swimming legs do not, so that the legs become difficult to observe. Mature

Fig. 18. Asian Seabass, *Lates calcarifer* fry infested with anchor worm, *Lernaea cyprinacea* in Puducherry, India.

females have two eggs sacs immediately posterior to the pregenital prominence. Copepodites of

Fig. 19. Asian Seabass, *Lates calcarifer* fry infested with Anchor worm, *Lernaea cyprinacea* in Puducherry, India.

Fig. 20. Female Anchor worm, *Lernaea cyprinacea* collected from Asian Seabass, *Lates calcarifer* fry in Puducherry, India.

lernaeaids attached to gills are readily differentiated from Ergasilid parasitic females in lacking the hook (Spine) type terminal segment of the second antenna (Misganaw and Getu, 2016). *Lernaea* spp. is often isolated from the external surface of eye, lips, gills, nostrils, fins, operculum and body [Fig. 17-20] (Ananda Raja et al., 2022). *Lernaea* spp. which feeds on the blood of the host, causing mechanical injuries and erosions leading to increased susceptibility of fish to super-infections, anaemia and death (Daskalov et al., 1999; Misganaw and Getu, 2016; CIBA, 2019a & 2019b; Ananda Raja et al., 2020). Clinical signs of the infection are haemorrhage, ulceration, frequent rubbing or "flashing", inflammation on the body of fish, tiny white green or red worms in wounds, respiratory distress, general lethargy, sluggish movement, retarded growth, anaemia, epithelia hypertrophy, restlessness and finally death. Infection by a single or 2-3 female is very damaging or even deadly to young or small fish. Major injury is caused by loss of blood and by secondary infection with bacteria, fungi and other organisms (Hossain et al., 2013).

c. Lamproglena

Many species from the genus *Lamproglena* Von Nordmann, 1832 (Copepoda, Lernaeidae) are reported both from freshwater and marine fish worldwide. These are reported to cause severe histopathological changes in the gills, loss of weight gain and mass mortality if untreated (Piasecki, 1993; Tsotetsi et al., 2005; Alaş et al., 2015; Batool et al., 2018). .

d. Caligids

Caligoid copepods are capable of damaging fish by scraping the fish's gill filaments or integument with their mandibles. Most common of these are members of the family Caligidae. Like ergasilids, several caligids have a wide geographic range and pose taxonomic problems. There are hundreds of *Caligus* spp. widely found in marine and brackishwater fish (Fig. 21-28). *Lepeophtheirus* commonly called as 'sea lice' parasitizes different freshwater and marine fish (Torrisen et al., 2013; Skaala et al., 2014; Alaş et al., 2015; Eiras, 2016; CIBA, 2019a & 2019b; Ananda Raja et al., 2020; Ananda Raja et al., 2022). The general life cycle has been worked out. After a short free-living larval stage, a chalimus stage attaches, usually to a gill filament, where it progresses through three or four moults to the adult stage. Most adults infest the buccal cavity or integument, whereas larvae attach, first by the second antennae and then by a chitinous secretion called as frontal filament, to the gills or elsewhere on the body. Affected fish had sluggish behaviour, shows anorexia, occasional rubbing, inflamed wounds, ulcers. The fish show small white-grey spots on the skin, excess mucous production, emaciation, gill damage leading to respiration problems and become weak due to heavy infestation. Gills and skin have erosions and secondary bacterial infection leading to high mortality, if not treated.

Fig. 21. Female crustacean ecto-parasite, *Caligus minimus* collected from Asian seabass, *Lates calcarifer* in Andhra Pradesh, India. Scale bar : 1000 µm.

Fig. 22. Male crustacean ecto-parasite, *Caligus minimus* collected from Asian seabass, *Lates calcarifer* in Andhra Pradesh, India. Scale bar : 500 µm.

Fig. 23. Female crustacean ecto-parasite, *Caligus epidemicus* collected from Asian seabass, *Lates calcarifer* in Tamil Nadu, India. Scale bar : 500 µm.

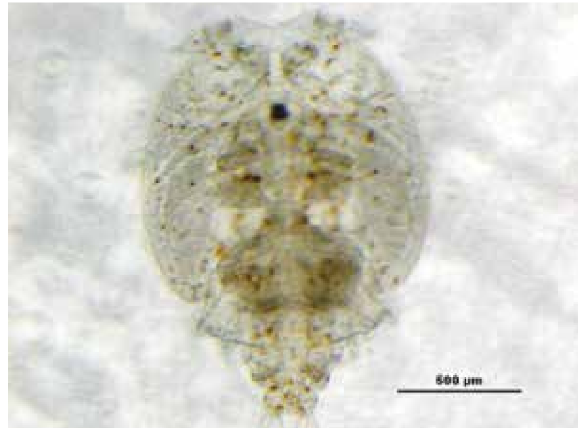


Fig. 24. Male crustacean ecto-parasite, *Caligus epidemicus* collected from Asian seabass, *Lates calcarifer* in Andhra Pradesh, India. Scale bar : 500 µm.

Fig. 25. Female crustacean ecto-parasite, *Caligus rogercresseyi* collected from Pearlspot, *Etroplus suratensis* in Tamil Nadu, India. Scale bar : 1000 µm.

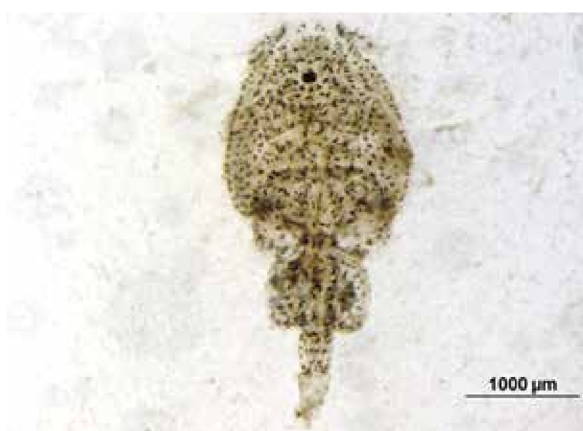


Fig. 26. Male crustacean ecto-parasite, *Caligus rogercresseyi* collected from Pearlspot, *Etroplus suratensis* in Tamil Nadu, India. Scale bar : 1000 µm.

Fig. 27. Female crustacean ecto-parasite, *Caligus rotundigenitalis* collected from Pearlspot, *Etroplus suratensis* in Tamil Nadu, India. Scale bar : 1000 µm.

Fig. 28. Male crustacean ecto-parasite, *Caligus rotundigenitalis* collected from Pearlspot, *Etroplus suratensis* in Tamil Nadu, India. Scale bar : 500 µm.

Fig. 29. Female crustacean ecto-parasite, *Lernanthropsis mugilii* collected from mullet, *Mugil cephalus* in Kerala, India. Scale bar : 5000 µm.

Fig. 30. Male crustacean ecto-parasite, *Lernanthropsis mugilii* collected from mullet, *Mugil cephalus* in Kerala, India. Scale bar: 1000 µm.

e. Lernanthropids

Family *Lernanthropidae* under Copepoda consists of more than 150 species of gill parasites and some species cause high mortalities (Izawa et al., 2014 &, 2018; Alaş et al., 2015; Eiras, 2016; Raja et al., 2018; CIBA, 2019a & 2019b; Boxshall et al., 2020; Ananda Raja et al., 2022). *Lernanthropsis* spp. and *Lernanthropus* spp. are important copepods causing anaemia by attaching to the gills especially in cage cultured fish. As compared to other copepods infecting gills, Lernanthropids are larger in size and can be seen with the naked eye (Fig. 29-30). It causes mass mortality (40%) over a period of one week with clinical signs such as dullness, depression, inappetence, erratic swimming, gasping for air, lethargy, loss of equilibrium and anaemia. Post-mortem gross lesions such as cutaneous haemorrhages, pallor gill with excessive mucus production, anaemic and blanched abdominal viscera are also prominently observed.

f. Argulids

The parasites of the genus *Argulus,* commonly called 'fish-lice' infesting both freshwater and marine fish species (Fig. 31-34). Severe infestations can lead to anaemia and death. Body of *Argulus* comprises a head of five limb-bearing segments and a trunk, divided into a thoracic region carrying four pairs

Fig. 31. Female crustacean ecto-parasite, *Argulus siamensis* collected from rogu, *Labeo rohita* in Andhra Pradesh, India. Scale bar : 1000 µm.

Fig. 33. Female crustacean ecto-parasite, *Argulus quadristriatus* collected from Asian seabass, *Lates calcarifer* in Tamil Nadu, India. Scale bar : 1000 µm.

Fig. 32. Male crustacean ecto-parasite, *Argulus siamensis* collected from rogu, *Labeo rohita* in Andhra Pradesh, India. Scale bar : 1000 µm.

Fig. 34. Male crustacean ecto-parasite, *Argulus quadristriatus* collected from Asian seabass, *Lates calcarifer* in Tamil Nadu, India. Scale bar : 1000 µm.

of strong swimming legs, and a short abdomen, comprise of a single bi-lobed unit (caudal fin). Dorso-ventrally flattened and covered dorsally by a rounded or horseshoe shaped carapace. Ventrally positioned head appendages, the cup-like sucker of the second maxilla, are developed for attachment. Argulids are active swimmers. Argulus is parasitic throughout life, but leave the host to moult or to lay eggs, and during this process will also change hosts. Both males and females may remain free living for as long as 15 days. The sexes are separate and in most brachyurans, males transfer sperm directly to the females using a variety of modified structures on the third and fourth thoracic legs. Mating takes place while they are free-swimming. A mature female will lay eggs in rows on any hard, submerged surface. Eggs hatch into free-swimming larvae. These larvae function as a dispersal phase and moult into the second stage. The first larval stage lasts about six days and moults occur at intervals until maturity. Branchiurans are parasitic from the second larval stage onwards but appear to leave the host and then find a new host of intervals throughout development. In order to feed, they protrude an organ referred to as a pre-oral sting, or stylet, through a tubular mouth and pierce the host. Rapid thrusting of this organ accompanied with the release of toxic secretions can result in severe pathological changes in the host. Rather than carrying eggs in egg-sacs as do copepods, female argulids leave their host and lay eggs in mucus strips on rocks, weeds, submerged objects and the sides of aquaria. Confined fish are easily infested by the larval stage. Argulids use no intermediate hosts. Brachyurans damage the fish directly by extracting blood and vital tissue fluids from the host with their modified mouth parts. The mode of feeding involves secretion and injection of relatively large quantities of digestive fluids, which are toxic to the fish. The sting of one fish lice can kill a small fish. Feeding sites become haemorrhagic ulcerated and provide access to secondary infections by other pathogens. Mucus is secreted when skin, fin and gills become infected. Persistent irritation caused in heavy infections may affect fish appetite with resulting in anorexia, and cessation of growth. The infected fish scratch itself against rocks or repeatedly jump out of the water in an effort to dislodge the parasites and erratic swimming is also commonly observed (Alaş et al., 2015; Eiras, 2016; Misganaw and Getu, 2016; CIBA, 2019a & 2019b; Ananda Raja et al., 2020; Ananda Raja et al., 2022).

g. Linguatula

Tongue worm, *Linguatula* spp. is classified in the subclass Pentastomida under the order Porocephalida. It lives in the upper respiratory tract of reptiles, birds, and mammals, where they lay eggs. It is gonochoric (unisexual, having two sexes) and employ internal fertilisation. The eggs are either coughed out by the host or leave the host body through the digestive system. When the eggs are ingested by an intermediate host such as fish or small herbivorous mammals (Barnes, 1982), the larva hatches in the intermediate host and breaks through the wall of the intestine. It then forms a cyst in the intermediate host's body. The larva is initially rounded in form, with four or six short legs, but moults several times to achieve the adult form. *Linguatula* spp. enters the main host when the main host has eaten the infected intermediate host. The parasite then crawls into the respiratory tract, the predilection site from the oesophagus. *L. serrata* is the most common species distributed worldwide and is of zoonotic significance (Barnes, 1982; Sudan et al., 2018).

h. Cymothoids

Isopods parasitize both fresh-water and marine fish. Most parasitic species infecting fish differ only slightly from free-living ones. They have prehensile anterior appendages, modified mouthparts and a somewhat altered alimentary tract. These are serious threats to the host's health, especially to fish reared in cages in their natural habitat. Cymothoids comprise about half the species of isopods infesting fish. Cymothoids attract attention because some are protandrous hermaphrodites. After the initial molt from juvenile into a male, the isopod will eventually become a female. Mature female Cymothoids usually attach to the fish and feeds on the host (Fig. 35-37). After copulation the female matures and

Fig. 35. Cymothoid found attached to the mouth of Pearlspot, *Etroplus suratensis,* collected from Pazhaverkadu, India.

Fig. 36. Dorsal view of Cymothoid collected from Pearlspot, *Etroplus suratensis,* collected from Pazhaverkadu, India. Scale bar: 2000 µm.

Fig. 37. Ventral view of Cymothoid collected from Pearlspot, *Etroplus suratensis,* collected from Pazhaverkadu, India. Scale bar : 2000 µm.

produces egg sacs while the male dies. They cause enormous lesions on the body, destroy most of the gill filaments, transmit viral diseases and/or irritate the tissue allowing a virus to enter (Paperna and Overstreet, 1981; Sethi, 2012; Sethi et al., 2013; Aneesh and Kappalli, 2020). Clinical signs such as inappetence, anaemia, stunted growth and sudden mortality in 2–3 days in heavily infested young fish are observed.

2.2.7. Annelids

Leeches are segmented parasitic or predatory worms that belong to the phylum Annelida and comprise the subclass Hirudinea. Leeches act as micropredators

Fig. 38. Leech, *Zeylanicobdella* spp. collected from Asian seabass, *Lates calcarifer,* cage culture in Maharashtra, India. Scale bar : 2000 µm.

rather than parasites, but some leeches exhibit considerable host-specificity. Excessive blood-loss probably occurs only with intense and prolonged infestations. Small hosts, of course, could be more easily harmed and secondary infections can follow any infestation. They probably act as vectors for the blood protozoan parasites. The most common genera are *Myzobdella* and *Zeylanicobdella* (Fig. 38) [CIBA, 2019a & 2019b].

3. Parasitic diseases of shrimp

Apart from virus, bacteria and fungus, parasites are responsible for causing significant economic loss in shrimp aquaculture in slow attrition manner. Parasitic infestations cause continuous deterioration of the health status of shrimp by weakening, disorienting, reducing their ability to survive variations in the physical environment, blinding, altering behaviour and in ways that render shrimp more vulnerable to cannibalism and/or predation. Knowledge on shrimp parasitic infestations is still primitive and rudimentary in the field of isolation, identification, treatment, prevention and control.

3.1. Protozoa

3.1.1. Endocommensal / Invasive protozoa

a. Microsporida

Microsporidia are obligate intracellular parasites known to infect a wide range of eukaryotic hosts. Shrimps are infected by ingestion of spores. The spores extrude a filament that penetrates the gut wall and deposits an infective unit which enters nucleus of the gut cells where schizonts are developed. The schizonts divide and develop to form spores which are located in muscles and other organs of the shrimp. The infected shrimp is infective to fish species and vice versa but not to the shrimp itself. The development generally occurs within the cytoplasm of the host-cell via nuclear proliferation, and spore formation (sporogony). There are certain genera known to undergo similar development within the host nucleoplasm also (Stentiford et al., 2007; Tourtip et al., 2009). There are several genera of microsporidia found to infect crustacean hosts such as *Thelohania, Agmasoma* (8 spores / envelope), *Ameson, Nosema, Pleistophora* (>8 spores / envelope),

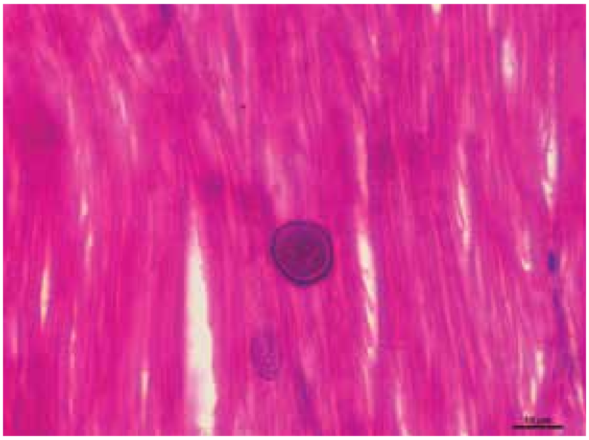


Fig. 39. Microsporidia found in the muscle of Pacific white shrimp, *Litopenaeus vannamei* at Andhra Pradesh,India. H&E. Scale bar : 10 µm.

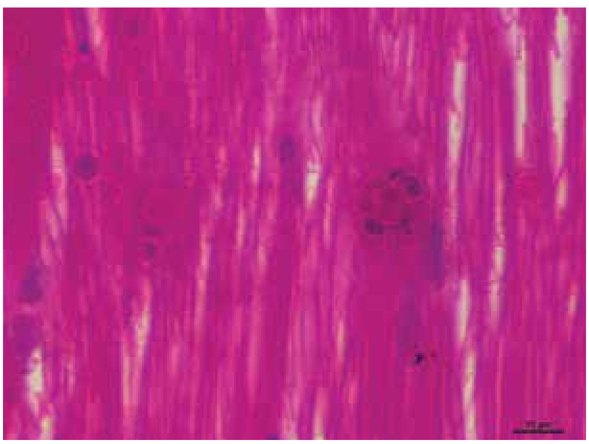


Fig. 40. Microsporidia found in the muscle of Pacific white shrimp, *Litopenaeus vannamei* at Andhra Pradesh,India. H&E. Scale bar : 10 µm.

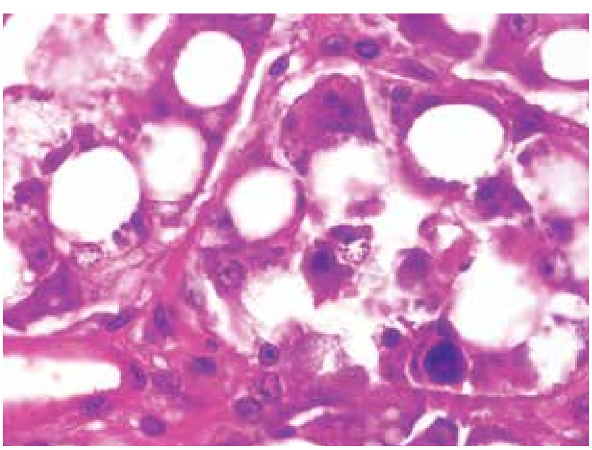


Fig. 41. Microsporidia found in the hepatopancreas of Pacific white shrimp, *Litopenaeus vannamei* at Andhra Pradesh,India. H&E. Scale bar : 10 µm.

Tuzetia, Flabelliforma, Glugoides, Vavraia, Ordospora, Nadelspora, Enterospora and *Enterocytozoon* (Fig. 39-41) [Tourtip et al., 2009]. Microsporidia infects various crustacean tissues for example *Agmasoma penaei* infects muscle and connective tissue, *Tuzetia weidneri* affects muscle, *Enterospora canceri* in hepatopancreas, and *Enterocytozoon hepatopenaei* (EHP) in hepatopancreas (intranuclear). Microsporidia affecting the muscle tissue causes "milk or cotton shrimp disease" or "cotton tail disease" and EHP is affecting the hepatopancreas, which causes "hepatopancreatic microsporidiosis (HPM)". Recently, many studies reported the EHP organism as a microsporidian fungal parasite in shrimp (CIBA, 2016; Mukta and Paramveer, 2018). The affected shrimp show cooked-muscle appearance and the exoskeleton appears bluish black, and white tumor-like swellings may be found on gills and subcuticle. Microsporidia may also localize in shrimp gonads leading to reproductive failure or vertical transmission (Vedavyasa Rao and Soni, 1988; Brock and LeaMaster, 1992; Johnson, 1995; Tourtip et al., 2009; Ananda Raja et al., 2012) granulomation lesions in hepatopancreas.

b. Haplosporida

Haplosporidia is another spore forming protozoan group affecting the digestive glands (hepatopancreas) of shrimp but the incidence is rare among the well maintained shrimp farms (Vedavyasa Rao and Soni, 1988; Brock and LeaMaster, 1992; Johnson, 1995). They are *Bonamia ostereae, Bonamia exitiosa* in *Ostrea* spp. (intracellular) and *Haplosporidium nelsoni* in *Crassostrea* spp. (extracellular). The large multinucleate plasmodia release haplosporosomes or their contents which damage surrounding cells. This leads to a graulomation response by the host (Utari et al., 2012).

c. Gregarina

Gregarina (Protozoa, Apicomplexa) affects the digestive tracts, hepatopancreas and other tissues of shrimp. Worldwide, penaeid shrimps are natural host for a number of gregarine species which are placed into either of three genera, *Nematopsis*, *Cephalolobus* and *Paraophioidina* (Brock and LeaMaster, 1992). Larval stages and cultured shrimps are infected by ingestion of the gregarine spores in the slime of clams or tissues of the polychaete worms. The developed sporozoites attach to the gut wall, grows into trophozoites and form gametocysts. Gametocysts undergo multiple divisions to produce gymnospores which are released outside and are an infective stage for invertebrates such as clams, snails or marine worms. Spores are developed in the intermediate hosts and released in mucous strings which become infective to shrimps. Heavily infected shrimp shows sluggish movement, retarded growth, increased feed conversion ratio (FCR), yellow discoloration of midgut and reduced survival (Vedavyasa Rao and Soni, 1988; Brock and LeaMaster, 1992; Johnson, 1995; Fajer-Avila et al., 2005; Ananda Raja et al., 2012).

d. *Peridiniales*

The genus *Hematodinium*, is one of the most economically significant parasitic dinoflagellate which proliferate internally in the hemolymph and tissues of crustaceans. To date, *Hematodinium* or *Hematodinium*-like infections has been reported in over 40 species of marine crabs, shrimps, lobsters and amphipods (Li et al., 2021). It is highly pathogenic to the blue crab which causes a condition known as milky disease, bitter crab disease (BCD) or pink crab disease (PCD) with 100% mortality. Clinical signs include chalky or yellow discoloration of the hemolymph, lack of clotting ability, decline in hyalinocytes, reduced hemolymph proteins and enzymes, reduced total hemocyte count, loss of transparency of the cuticle in the appendages due to large numbers of parasite life stages within the haemolymph of infected shrimp and leading to mass mortality. The waterborne route via dinospores has been considered to be the main route of disease transmission based on the present understanding of the life cycle of this parasite (Li et al., 2021).

e. Body invaders

There are several protozoa which invade the body and feed on tissues of weakened and diseased shrimps. They are *Parauronema* spp., *Leptomonas* spp. and *Paranophrys* spp. and amoeba (amebiasis) [Brock and LeaMaster, 1992; Johnson, 1995].

3.1.2. Ectocommensal protozoa

They are found on the gill and body surface of the shrimp. They are peritrich protozoans with body cilia (*Zoothamnium* spp., *Epistylis* spp., *Vorticella* spp., *Lagenophrys* spp. and *Apostome* ciliates.) and suctorians without body cilia (*Acineta* spp., and *Ephelota* spp.) [Brock and LeaMaster, 1992]. *Zoothamnium* spp. (Fig. 42) is a frequent inhabitant of the gills of shrimp which are grown in ponds with low dissolved oxygen, leading to suffocation and mass mortality. *Apostome* ciliates interfere with larval moulting in hatchery. Infestations on cultured shrimp are usually a mixture of protozoans, filamentous and non-filamentous bacteria, and algae. Special staining technique like silver impregnation staining is used for identification of Apostome ciliates. They cause "protozoan fouling" and "Fuzzy mat-like appearance" due to ciliate fouling. The affected shrimps show restlessness and difficulty in locomotion and respiration (Vedavyasa Rao and Soni, 1988; Johnson, 1995; Ananda Raja et al., 2012).

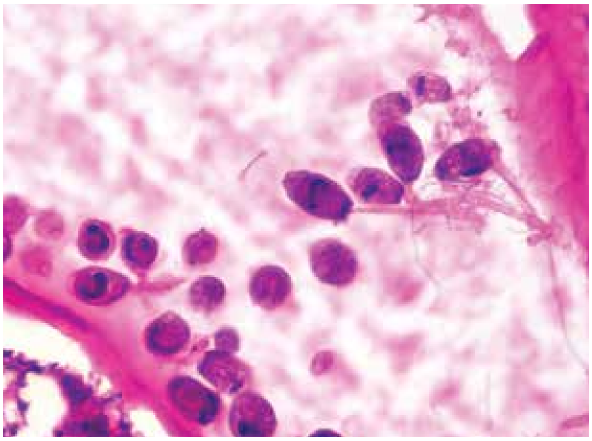


Fig. 42. *Zoothamnium* spp. in gills of Pacific white shrimp, *Litopenaeus vannamei,* collected Andhra Pradesh, India. H&E. Scale bar : 50 µm.

Another condition called black gill (Fig. 43) is reported in Pacific white shrimp, *Penaeus*

(*Litopenaeus*) *vannamei*, but not harmful to humans and are safe to eat (Gambill et al., 2015). Black Gill may not kill shrimp directly but rather compromise individuals, making shrimp increased number of molting events, to remove a damaged gill, more vulnerable to predators and environmental pressures (Gooding et al., 2020). Reports have revealed that black gill in Georgia shrimp is caused by a cryptic parasite, ciliate, a one-celled protozoan animal during the late summer and fall period (Gambill et al., 2015). Sequencing the 18S rRNA gene and electron microscopy have tentatively identified the ciliate as a species closely related to *Hyalophysa chattoni* commonly found in shrimp and other crustaceans. The ciliate invades and kill the gill tissue. The presence of the invading ciliate stimulates an immune response in the shrimp thereby increase the melanin production. The dark protective layer of melanin forms around the ciliate on the gill tissue, possibly attempting to minimize the amount of tissue damage from the ciliate. It is also surprising to note that some shrimp carrying the ciliate develop black gill and some do not. It is possible that the quantity of ciliates infecting a single shrimp may determine if infection is visually displayed as black gill.

Fig. 43. Black gill in Pacific white shrimp, *Litopenaeus vannamei* collected from Andhra Pradesh, India.

3.2. Metazoa / Helminthiases

Metazoan parasites in shrimp are categorized as trematodes, cestodes, and nematodes. Immature forms and adult worms are found in the different parts of the body in shrimps.

3.2.1. Trematodes (flukes)

The cercarial forms of the flukes are infective to the shrimps. The cercaria penetrates the shrimp and encysted in the form of metacercarial forms in tissues which are infective to the first intermediate host (fish). The metacercaria develops in to adult and release eggs. The eggs are hatched out and miracidia released which penetrate second invertebrate host, snail and develops into sporocysts. Cercarias develop inside the sporocysts in second intermediate host and released in to water which become infective stage for shrimp. Eg. *Opecoeloides fimbriatus*, *Microphallidae* spp. and *Echinostomatidae* spp. (Vedavyasa Rao and Soni, 1988; Johnson, 1995; Ananda Raja et al., 2012)

3.2.2. Cestodes (tape worms)

Shrimp ingest copepods or other crustaceans with larval form of tape worm which develop into advanced larval stage in shrimp. The advanced larval stage enters the first intermediate host (sting ray) by ingestion of the infested shrimp and develops in to adult and release eggs. The eggs are eaten by copepods, second intermediate host and develop into larvae which are infective to shrimps. Eg. *Prochristianella penaei*, *Parachristianella* spp., *Renibulbus* spp., Pear shaped worms, and *Cyclophyllidean* group (Vedavyasa Rao and Soni, 1988; Johnson, 1995; Ananda Raja et al., 2012).

3.2.3. Nematodes (round worms)

Round worm larvae also get entry into shrimp through copepods or other small crustaceans. The larvae develop in to an advanced larval stage in shrimp tissues. The advanced larval stage enters the first intermediate host (toad fish) by ingestion of the infested shrimp and develops in to adult worm in the fish gut. Adult worm releases eggs, which are eaten by copepods, second intermediate host and develop in to larvae. The copepods carrying larvae are infective to shrimps. Eg. *Spirocumallanus pereirai*, *Leptolaimus* spp., *Ascaropsis* spp., and *Hysterothylactum reliquens* (Vedavyasa Rao and Soni, 1988; Johnson, 1995; Ananda Raja et al., 2012).

3.3. Other infestations

Single cell plant diatoms (on larval stages), multiple species of algae, barnacle, leeches, colonial hydriod *Obelia bicuspidata*, insects eggs, isopods - *Aega* spp. are occasionally observed among wild and poorly farmed shrimp populations. 'Red rostrum' condition is observed in shrimp grown in ponds with abundant diatom, *Peridinium* spp. Bopyrid isopod parasitic infestation caused by *Epipenaeon* spp. belong to family *Bopyridae.* The parasites lodged in the brachial cavity leads to impaired respiration and reproductive failures (Lalitha Devi, 1982; Vedavyasa Rao and Soni, 1988; Johnson, 1995; Ananda Raja et al., 2012).

4. Parasitic diseases of crabs

Parasites could be viewed as spanning the gamut of disease in the way of either parasitic, hyperparasitic, commensalistic, mutualistic, or phoretic, but not predatory, relationships. Portunid crabs species of aquaculture importance (flattening of the fifth pair of legs into broad paddles for swimming) such as American blue crab (*Callinectes sapidus*), green shore crab (*Carcinus maenas*), shore crabs (*Hemigrapsus* spp.), mud crabs or Mangrove crabs (*Scylla serrata* and *Scylla olivacea*) and the sand crab (*Portunus pelagicus*) are mostly infected with protozoan and/or metazoan parasites or epicommensals or symbionts (*Vorticella* oysters, polychaetes, hydrozoans, amphipods and turbellarians) [Shields and Overstreet, 2003; Jithendran et al., 2009 & 2010; McDermott, 2011].

4.1. Protozoa

Peritrich ciliates (*Zoothamnium* spp., *Vorticella* spp. and *Epistylis* spp.) and suctorian ciliates (*Acineta* spp. and *Ephelota* spp.) are fouling protozoans in crabs. They infest gills, body and appendages. Peritrichs interfere with locomotion, feeding and moulting of crab larvae, while the suctorians feed mainly on other protozoans and in high numbers may interfere with respiration leading to mass mortality. These parasitic disease outbreaks occur with poor water quality and low dissolved oxygen.

Additionally, the genus *Hematodinium*, is a parasitic dinoflagellate which proliferate internally in the hemolymph and tissues of crustaceans. It is highly pathogenic to the blue crab with 100% mortality. *Hematodinium* spp. is one of the most economically significant diseases of marine decapod Crustacea, which causes a condition known as milky disease, bitter crab disease (BCD) or pink crab disease (PCD) due to the bitter aspirin-like flavor of infected crabs (McDermott, 2011). Diagnosis is done based on the presence of vermiform plasmodium, multinucleate plasmodia, ameboid trophont or vegetative stage, prespore or effete stages with many large vacuoles in the hemolymph. Hemolymph is stained with neutral red, an excellent vital stain for the parasite in fresh hemolymph; because the dye is taken up in the Golgi apparatus of the actively synthesizing parasites while the hemocytes do not generally acquire the stain. Clinical signs include chalky or yellow discoloration of the hemolymph, lack of clotting ability, decline in hyalinocytes, reduced hemolymph proteins and enzymes, reduced total hemocyte count, and leading to mass mortality. Formalin as bath treatment is used effectively in case of the affected crabs (Shields and Overstreet, 2003; McDermott, 2011).

Microspora is a phylum containing strictly intracellular parasitic species that produce small (usually <6 μm) unicellular spores with an imperforate wall. The spores lack mitochondria, but contain a sporoplasm and a hatching apparatus, including an extrusible hollow polar tube that injects the sporoplasm into the host cell. Genera such as *Ameson* (single spore), *Pleistophora* (32 to >100 spores) and *Thelohania* (eight spores) are parasitic to crabs. Spores develop inside a membranous structure termed the sporophorous vesicle (SPV, previously termed pansporoblast). All microsporidians are parasitic, with some infecting vertebrates and others infecting invertebrates; some have a direct life cycle not requiring an intermediate or additional host, but others need another host or life stage. The life cycle for most species is not known. When infected crab tissues or spores are ingested by an uninfected crab, the spore everts its coiled polar tube by means of its lamellar polaroplast and rapidly injects the sporoplasm into an epithelial cell lining the lumen of the mid-gut. The vegetative cell then invades, develops, and multiplies in the hemocytes in the adjacent submucosal connective tissue of the midgut. When the hemocytes reach skeletal muscles, the parasite in these cells undergoes further development in the myofibrils, first forming chains of eight meronts (merogony) that separate into pairs of cells that finally result in isolated mature spores (sporogony). Crabs infected by *A. michaelis* are considered to have "sick crab disease," or "cotton crab disease" with high mortality. The parasite lyses infected muscle and adjacent tissues. The actin and myosin filaments of the host disassemble in the presence of the sporoblasts, and a cell free extract of infected tissue can produce lysis of normal blue crab muscle tissue. Grossly, the infected muscles appear chalky white through joints of the appendages, and the abdomen may appear greyish (Shields and Overstreet, 2003).

Haplosporidia is a small phylum of spore forming protozoans which have a multinucleated naked plasmodial stage in their life cycle. Members contain uninucleated spores without extrudible polar tubules, but they contain mitochondria, characteristic haplosporosomes, and an anterior orifice or operculum. Haplosporidian, *Urosporidium crescens* causes "pepper crabs," "pepper-spot," or "buckshot," in the skeletal muscles, visceral organs, and gills of the blue crab. It does not infect the actual crab tissue, but rather it hyperparasitizes the encysted metacercaria of the digenean *Microphallus basodactylophallus* that infects the crab. When this parasite infects the fluke and undergoes extensive multiplication, its brownish colored spores in the greatly enlarged worm create a black spot readily visible to the naked eye. Affected crabs exhibited opaque hemolymph with uninucleated cells containing perinuclear haplosporosomes and mitochondria; interstitially, multinuclear plasmodial stages of this parasite occupied much of the vascular spaces (Shields and Overstreet, 2003; McDermott, 2011).

Amoeba, *Paramoeba perniciosa* belonging to Rhizopoda causes "Gray crab" disease, a systemic infection in crabs. The disease is named so for the darkly discolored sternum and ventral surfaces of heavily infected and dead crabs (Shields and Overstreet, 2003). The amoeba causes mortalities in high saline waters.

4.2. Metazoa

Trematodes (*Microphallus basodactylophallus*, *Microphallus pygmaeum* and *Levinseniella capitanea*), cestodes (*Prochristianella* spp. in the hepatopancreas of the crab and *Rhynchobothrium* spp. encysted in body cavity), nematodes (ascaridoid *Hysterothylacium reliquens* in hepatopancreatic tubules, *Gammarinema* spp., *Tripylium* spp., *Monhystrium* spp., *Monhystrium* spp., nemertean worm *Carcinonemertes* spp. in gill chambers), annelids (leeches: Hirudinea, *Myzobdella* spp.; branchiobdellid worm: mullet bug, *Cambarincola vitreus* in gills and carapace) and Carripeds (burnacles [Fouling balanid barnacle *Balanus* spp. in the carapace, acorn barnacle *Chelonibia* spp., and gooseneck barnacle *Octolasmis muelleri* in gills, rhizocephalan barnacle *Loxothylacus* spp. in gonads] and *Sacculina* spp.) are reported in different species of crabs (Fig. 44-45). Further studies are required for identification, life cycle and pathogenesis of these parasites in crabs (Shields and Overstreet, 2003; Jithendran et al., 2009 & 2010;

Fig. 44. Stalked barnacle, *Octolasmis* spp. infestation in mud crab, *Scylla serrata,* collected from Andhra Pradesh,India.

Fig. 45. Stalked barnacle, *Octolasmis* spp. collected from mud crab, *Scylla serrata,* collected from Andhra Pradesh, India. Scale bar : 5000 µm.

McDermott, 2011).

5. Diagnosis

The correct diagnosis is obviously a critical step in any disease control program. Major diagnostic methodologies are mentioned below as it would be very exhaustive to elaborate all methodologies under this chapter.

a. Rapid diagnosis based on the anamnesis, clinical signs and post-mortem findings.
b. Wet mount microscopical findings.
c. Isolation and identification of aetiological agents.
d. Bioassay.
e. Histopathology and histochemistry.
f. Electron microscopy.
g. Molecular methods.
h. Serological methods.

6. Prophylactic and therapeutic approach

Application of therapeutic agents directly into the pond water is not an effective and safe method as it requires higher quantities of chemicals and possible toxic effects on target and/or non-target organisms in the pond ecosystem. Toxic accumulation of those compounds and their metabolites in fish tissues and other organisms could pose risk to the safety of consumer and affect the biodiversity leading to ecological imbalance. Hence application of safe and effective chemical agents through feed is commonly practiced in many countries in Europe and South America. But, no such approval is available in India due to lack of scientific data. The following details are provided from the published reports related to fish culture but not as an approved recommendation for use in aquaculture except anti-parasitic drug, emamectin benzoate.

6.1. External parasites

1. Oral in-feed anti-parasitic drug, emamectin benzoate @ 50 µg kg^{-1} body weight day^{-1} for 7-10 consecutive days is recommended via medicated feed, against all crustacean parasites. No recurrence of parasitic infestation for a period of 60 days is reported post medication.
2. Formalin 15–25 ppm as a pond treatment or dip treatment of affected fish with 200-250 ppm for one hour at temperature below 15.6 °C and 100 ppm for one hour at higher temperature or 100 ppm hydrogen peroxide for 30 minutes against all species of ectoparasites. Mild infestations can be controlled by simple freshwater bath for 10-15 minutes.
3. Though use of organophosphates for treatment in aquaculture has legal restrictions, Dichlorvos @ 1 ppm is used as an effective control against external parasites.
4. Ergasilosis can be treated successfully with a combination of 0.5 ppm copper sulphate and 0.2 ppm ferric sulphate for 6 to 9 days.
5. Combination of 25 ppm formalin and 0.1 ppm malachite green dip is found to be effective against cryptocaryon.
6. Malachite green as a dip treatment at a concentration of 1:15000 effectively controls fungal infection on both fish and eggs.
7. Acetic acid at 1:5 concentration for 1–2 minutes and acriflavin at 3–5 ppm to control external parasites.

8. Organo phosphorus compounds, Dylox (Dimethyl phosphonate) at the concentration of 0.25 ppm helps to control anchor worm, crustaceans, gill and body flukes but is not effective against protozoans.
9. Cypermethrin 10% w/v– 10-15 mL/1000 sq. m. water spread area.
10. Costiasis can be treated with formalin 1:4000 or 1:6000 in baths with a good aeration.
11. Formalin baths (150 mL/L) can be effective for treatment of Cryptobiasis.
12. Cryptocaryosis can be treated with copper sulphate bath with 25 ppm and/or formalin bath with 0.5 ppm for 5-7 days with aeration and daily replenishment of water.
13. Short bath treatment with 200 ppm formalin for 30-60 minutes with strong aeration or extended bath treatment with 25 ppm formalin for 1-2 days with good aeration and daily replacement of water is practiced against Trichodiniasis.
14. Flukes are treated by short bath with 100 ppm formalin or freshwater for 10-30 minutes or 150 ppm hydrogen peroxide for 10-30 minutes with strong aeration.

6.2. Internal parasites

1. Oral in-feed anti-parasitic drug, emamectin benzoate @ 50 µg kg^{-1} body weight day^{-1} for 7-10 consecutive days is practiced against some nematodes (Ananda Raja et al., 2020; Ananda Raja et al., 2022).
2. Antihelminthes Di-N-Butyl tin oxide mixed in the food at the rate of 1% and fed at 3% of BW for three days.
3. Control of animal coccidians is based on the use of different coccidiostatics or coccidiocides, but information regarding fish coccidia is very scarce. Furazolidone, amprolium chloride and furanace, among others, have been tried to treat different fish coccidia.
4. Toltrazuril has apparently given better results than fumagillin and amprolium against Microsporidiosis.

7. Prevention and control

No scientifically effective and legally approved treatments are internationally available at present. Hence prevention and control is followed based on breaking of transmission chain, avoiding the presence of intermediate hosts, and minimising or following standard stocking density. Further, a sustainable integrated pest management (IPM) approach should be based on knowledge of the ecology of the parasite along with adoption of several prevention and control methods.

a. Prevention relies on hygienic measures.
b. Dry the pond before starting the culture.
c. Use sieve at water inlet, bleach before stocking to weed out wild shrimp, fish and intermediate hosts.
d. Maintain good water quality throughout the culture.
e. Use disease-free genetic strain of broodstock and develop resistant stocks.
f. Supply of adequate balanced nutrition.
g. Regulate population density, periodical size grading and proper disposal of dead animals.
h. Handle the animals with good care and control entry of other animals.
i. Proper chemical prophylaxis and vaccine development for immunological protection.
j. Regulations to prevent transfer of pathogens from one host population to another, nationally or internationally.
k. Proper destruction and disposal of infected animals.
l. Sanitation and disinfection of hatchery and equipment.

m. Efficient water exchange, good feeding practices (avoiding trash fish as feed) and quarantine measures.

8. Concluding remarks

Many parasitic diseases such as Amyloodiniosis, Costiasis, Hole-in-the-head disease, Trypanoplasmosis, Chilodonellosis, Trichodiniosis, Necrotic dermatitis, Scuticociliatosis, White spot diseases or Ich, Cryptocaryosis, Microsporidiosis, Coccidiosis, Myxosporidiosis or Whirling disease, Proliferative kidney disease or PKD, Hamburger gill disease or Proliferative gill disease, Gyrodactylidosis, Dactylogyridosis, Ancyrocephalidosis, Microcotylosis, Sanguinicolosis, Diphyllobothriasis, Ascariasis, Anisakiasis, Acanthocephalosis, Lernaeosis, Caligosis, Lernanthropidosis, Argulosis, Cymothoidosis, Hirudineasis, Milk or cotton shrimp disease or Cotton tail disease, Hepatopancreatic microsporidiosis (HPM), Zoothamniosis, Hematodiniosis, and Haplosporidiosis are frequently observed among the farmed fishes, shrimps and crabs species, but their presence is not felt probably due to lack of per-acute or acute incidences. But, the chronic parasitic diseases lead to significant economic loss in terms of decrease in productivity and increase in production cost worldwide. Hence, significant attention should be paid towards the parasitic infestations in fish, shrimps and crabs farming worldwide in view of the change in climatic conditions. There is plenty of scope for future research in the major areas of parasitic taxonomy, epidemiology, host-parasite interactions, immunological interventions, diagnostics, therapeutics and prophylactics.

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