REVIEW

Scuticociliatosis caused by *Philasterides dicentrarchi*

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ABSTRACT: The ciliate *Philasterides dicentrarchi* has been previously identified as a new agent of scuticociliatosis in marine fish. The parasite can cause high mortalities in fish reared on farms or kept in aquariums. *P. dicentrarchi* is usually a free-living protozoan but can become an opportunistic histophagous parasite causing rapid lethal systemic infections in cultured fish. This review provides information about the morphology and biology of the scuticociliate *P. dicentrarchi*, as well as information about the pathological and immunological reactions of the host in response to the infection with the parasite. The epidemiology and the control strategies of the disease are also reviewed.

KEY WORDS: *Philasterides dicentrarchi* • Scuticociliatosis • Aquaculture

1. INTRODUCTION

Scuticociliatosis is a severe disease of marine fish worldwide caused by about 20 species of ciliates belonging to the subclass Scuticociliatia (Small 1967). This group of ciliates is charaterised by the presence of a scutica, which is a transient organelle appearing at a late stage of ontogenesis during stomatogenesis (Lynn 2008). Scuticociliates are free-living marine protozoans that are widely found in world oceans and feed on bacteria and microalgae (Porter et al. 1985). However, under certain circumstances, some scuticociliate species can become opportunistic histophagous endoparasites of other marine organisms, including teleost and elasmobranch fish and crustaceans (Harikrishnan et al. 2010a). Fish infected with these ciliates show various symptoms and pathologies, ranging from weakness, listlessness, anorexia, loss of scales, dermal haemorrhagic and necrotic ulcers, necrotised gill tissue, skin darkening, muscular dystrophy, necrosis of internal organs, hypochromic anaemia, accumulation of ascitic fluid, to systemic infection (Harikrishnan et al. 2010a). In the final stage of the infection, encephalitis associated with the softening and liquefaction of the brain tissue occurs, leading to the death of the affected fish (Harikrishnan et al. 2010a).

A scuticociliate that particularly causes high mortalities in farmed fish is *Philasterides dicentrarchi*. Following a 1993 report of a sudden increase in mortality of sea bass (*Dicentrarchus labrax*) reared in the French Mediterranean lagoon of Thau, this species was described as a new aetiological agent of scuticociliatosis in 1995 (Dragesco et al. 1995). It was recorded that dead fish were heavily infected with a histophagous ciliate, and that this type of infection had never been seen before in fish reared in the lagoon or any other fish farm. In subsequent years, *P. dicentrarchi* was identified as the causative agent of several outbreaks of scuticociliatosis around the world. This parasite has been and still is responsible for significant economic losses to the fish aquaculture industry (Iglesias et al. 2001; Paramá et al. 2003; Jin et al. 2010; Harikrishnan et al. 2012a; Lama et al. 2018; Jalenques et al. 2021). Under normal circumstances, *P. dicentrarchi* is a free-living microaerophilic ciliate of the benthic zone. However, when the ciliate encounters a potential host, it can adopt a parasitic lifestyle. Although previously it was thought that *P. dicentrarchi* is identical with the scuticociliate

species *Miamiensis avidus* (Jung et al. 2007), more recent research confirmed that *P. dicentrarchi* and *M. avidus* are indeed different species (de Felipe et al. 2017).

This review summarises what is currently known about the biology, pathology, immunology, epidemiology, and treatment options of *P. dicentrarchi* infection in fish.

2. MORPHOLOGICAL AND ULTRASTRUCTURAL FEATURES OF *P. DICENTRARCHI*

The morphometric characteristics of fixed *P. dicentrarchi* trophozoites are summarised in Table 1. Compared to other scuticociliates, *P. dicentrarchi* is relative small (Dragesco et al. 1995; Iglesias et al. 2001; de Felipe et al. 2017). The cell body of the ciliate is pear-shaped with a pointed anterior and a rounded posterior end (Fig. 1A). At the posterior end, a caudal cilium emerges from a small cupule (Dragesco et al. 1995; Iglesias et al. 2001; de Felipe et al. 2017). The somatic cilia are about half the length of the caudal cilium, whereby the posterior cilia are longer than the anterior cilia (Dragesco et al. 1995; Iglesias et al. 2001; de Felipe et al. 2017). The cilia have the typical axoneme structure of motile cilia consisting of nine outer microtubule doublets and two central microtubule singlets (Paramá et al. 2006). The somatic ciliature consists of 10-15 kineties, each having 28 to 34 kinetosomes (Fig. 1B) (Dragesco et al. 1995; de Felipe et al. 2017). A non-ciliated meridian is situated between the first and last kineties, starting from the scutica and merging with the first kinety at the posterior end (Fig. 1B) (Iglesias et al. 2001). An anal pore (cytoproct) is posteriorly situated on this meridian (Fig. 1B) (Dragesco et al. 1995; Iglesias et al. 2001). The pore of the single contractile vacuole is located at the posterior end of the second kinety (Fig. 1B) (Dragesco et al. 1995; de Felipe et al. 2017). The oral apparatus is situated in the anterior third to the anterior half of the cell body (Iglesias et al. 2001; De Felipe et al. 2017). The oral cavity contains two paroral membranes (PM_1 and PM_2) and three oral polykineties (OPK_1, OPK_2, and OPK_3) (Fig. 1B) (Dragesco et al. 1995; Iglesias et al. 2001; de Felipe et al. 2017). PM_1 extends from the beginning of OPK_2 to the beginning of OPK_3, whereas PM_2 extends from the beginning of OPK_3 to the end of the buccal

cavity (Dragesco et al. 1995; Iglesias et al. 2001; de Felipe *et al.* 2017). OPK_1 is elongated and consists of two longitudinal rows of a few ciliated kinetosomes (Dragesco et al. 1995; Iglesias et al. 2001; de Felipe et al. 2017). OPK_2 is trapezoidal and has three to four rows of several ciliated kinetosomes (Dragesco et al. 1995; Iglesias et al. 2001; de Felipe et al. 2017). OPK_3 is smaller, and its ciliated kinetosomes are arranged in a crescent-shaped way (Dragesco et al. 1995; Iglesias et al. 2001; de Felipe et al. 2017). The cytostome is localised posterior of OPK_3 and extends alongside PM_2 (Fig. 1B) (Dragesco et al. 1995; Iglesias et al. 2001). The inconspicuous scutica is located posteriorly to the oral apparatus, is Y-shaped, and consists of two to eight non-ciliated kinetosomes (Fig. 1B) (Dragesco et al. 1995; Iglesias et al. 2001).

The cell envelope of *P. dicentrarchi* is composed of three membranes; the outer plasma membrane, the outer alveolar membrane, and the inner alveolar membrane (Paramá et al. 2006). Between the outer and inner alveolar membranes, spaces of variable size occur, the so-called alveolar sacs (Paramá et al. 2006). Directly beneath the alveolar membrane, the subpellicular microtubules are located as part of the ciliate's cytoskeleton (Paramá et al. 2006).

The ciliate has two types of extrusomes, spindle-shaped (fusiform) and spherical ones (Dragesco et al. 1995; Paramá et al. 2006). The fusiform extrusomes are about 1.6-2.0 μm long, are orientated perpendicular to the plasma membrane, and have a compact content (Dragesco et al. 1995; Paramá et al. 2006). The spherical extrusomes are 1.0-1.1 μm in diameter, are positioned below the cell surface, and are in direct contact with the plasma membrane. They are surrounded by a membrane, and their content is amorphous (Dragesco et al. 1995; Paramá et al. 2006). The biological function of extrusomes seems to be the generation of a mucoid capsule through the release of matrix glycoproteins (Folgueira et al. 2019a). The capsule protects the ciliate from attack by the host immune system (Folgueira et al. 2019a).

The spherical macronucleus and its closely associated micronucleus are located near the middle of the cell body. The macronucleus contains several nucleoli that are peripherally located. The nucleoplasm of the macronucleus is interspersed with chromatin granules. The micronucleus contains compact chromatin and is located towards one of the macronucleus' pores (Dragesco et al. 1995; Iglesias et al. 2001; Paramá et al. 2006; de Felipe et al. 2017).

Mitochondria are located peripheral, directly beneath the cell surface between kinetosomes (Paramá et al. 2006). In close proximity to mitochondria, rough endoplasmatic reticulum and dictyosomes can be found (Paramá et al. 2006).

3. LIFE CYCLE OF *P. DICENTRARCHI*

As most ciliates, *P. dicentrarchi* divides by binary fission along a transverse plane at a right angle to the long axis, which is also known as homothetogenic fission. The initial stage of the cell division in *P. dicentrarchi* is the stomatogenesis which starts with the multiplication of the kinetosomes of the scutica (Dragesco et al. 1995). Next, the PM_2 duplicates giving rise to two primordia. One of the primordia remains the PM_2 of the anterior daughter (proter) cell, while the kinetosomes of the other primordium multiply, producing two second-generation primordia. One of the second-generation primordia becomes the PM_2 of the posterior daughter (opisthe) cell, while the other grows larger, moves to the middle of the cell body, and gives rise to OPK_1 and OPK_2 of the future opisthe cell. At the same time, opisthe's OPK_3 is produced from congregated kinetosomes of the scutica. At the base of both proter's and opisthe's PM_2, kinetosomes are multiplying and differentiating into characteristic scutico-hooks that will form the individual scutica of the two daughter cells. The other oral apparatus structures of the proter cell do not undergo any transformation. During the process of stomatogenesis, the macronucleus and the micronucleus have also duplicated and moved in the proter and opisthe part of the dividing cell (Dragesco et al. 1995).

Sexual reproduction (conjugation) has been observed for *P. dicentrarchi* under starving and high cell density *in vitro* culture conditions (Alvarez-Pellitero et al. 2004). However, conjugation was not detected for the parasite on fish farms, presumably because the specific conditions for this process do usually not arise in marine aquacultures (Budiño et al. 2011b). Besides, conjugation in ciliates seems to be generally a rare and erratic event in the natural environment (Lucchesi & Santangelo 2004).

Three morphological feeding stages can be differentiated in scuticociliates; bacteriovorus microstome, predatory macrostome, and non-feeding, fast-swimming tomite forms. In the presence of nutrients, *P. dicentrarchi* produces only microstome forms (de Felipe et al. 2017). Under nutrient deficiency conditions, the ciliate occurs almost exclusively as tomites which are between 17-24 μm long and 11-16 μm wide (de Felipe et al. 2017). The lack of macrostome forms clearly distinguishes *P. dicentrarchi* from the previously identically considered species *M. avidus*, which produces predatory macrostome stages under both nutrient-rich and nutrient-poor conditions (de Felipe et al. 2017). Cyst formation by *P. dicentrarchi* has not been observed during *in vitro* cultivation or during host infection (Iglesias et al. 2001).

The route by which *P. dicentrarchi* infects fish remains to be established. However, experimental infection studies with turbot and olive flounder indicate that the natural route of entry is probably through lesions in the skin and/or gills (Paramá et al. 2003; Jin et al. 2009). This suggestion is supported by the observation that *P. dicentrarchi* is chemoattracted by blood and blood components of turbot, one of the main hosts of the ciliate (Paramá et al. 2004a). Another infection route may be through the cornea and/or periorbital skin, while infection through the nasal passages shown for other fish-pathogenic ciliates has not been observed for *P. dicentrarchi* (Iglesias et al. 2001; Paramá et al. 2003). In addition, infection via the oral route is unlikely as the ciliate does not survive the acid environment of the host stomach, and experimental oral infections have been unsuccessful (Dragesco et al. 1995; Paramá et al. 2003). The presence of *P. dicentrarchi* on gill lamellae and in the gill epithelium of naturally infected fish (Iglesias et al. 2001) supports the suggestion that the ciliate may enter the host via the branchial route. The increased incidence of exophthalmia, together with abundant ciliates in the periorbital area in fish infected with *P. dicentrarchi* (Paramá et al. 2003) suggests that the parasite may also enter the host via lesions of the cornea. Ocular abrasions can be caused in farmed fish by exposure to water supersaturated with oxygen (Sterud et al. 2000; Speare 2010). Once infected fish have died, ciliates are probably released from the cadavers into the water and will infect new fish (Iglesias et al. 2001). Cadavers may also serve as a reservoir and food source, and thus may be important for sustained infestations of water bodies with *P. dicentrarchi*.

Based on the information described above, a presumptive life cycle of *P. dicentrarchi* is shown in Fig. 2.

4. PHYSIOLOGICAL ADAPTATIONS OF *P. DICENTRARCHI*

P. dicentrarchi has developed adaptations that help the ciliate to survive the microaerophilic environment at the sea bottom. The same adaptations also enable the ciliate to live a parasitic lifestyle.

4.1. Alternative oxidase

P. dicentrarchi has the ability to reduce oxygen via two pathways; by the classical electron transport chain and by an alternative oxidase (*Pdi*AOX). Evidence for this came from experiments showing that the mitochondrial respiration of digitonin-permeabilised *P. dicentrarchi* trophozoites can be partially inhibited by the specific cytochrome c oxidase inhibitor potassium cyanide (KCN) as well as by the alternative oxidase specific inhibitor salicylhydroxamic acid (SHAM) (Mallo et al. 2013). In the presence of both KCN and SHAM, mitochondrial respiration was shown to be completely inhibited (Mallo et al. 2013). In addition, it was also shown that SHAM inhibits the growth of the ciliate both under normoxic and hypoxic conditions (Mallo et al. 2013). While respiration via the electron transport chain is increased during exponential growth, respiration via the alternative oxidase is stimulated during the stationary phase (Mallo et al. 2013). *Pdi*AOX has been shown to be a protein of 305 amino acids with an apparent molecular weight of 42 kDa for the native glycoprotein (Mallo et al. 2013; Folgueira et al. 2020). The enzyme is localised beneath the outer mitochondrial membrane (Folgueira et al. 2020). Phylogenetic analysis revealed that *Pdi*AOX belongs to the alternative oxidase family and is closely related to the alternative oxidases of other scuticociliates (Folgueira et al. 2020). *Pdi*AOX is induced under hypoxic conditions and by

inhibitors of the electron transport chain and alternative oxidase (Mallo et al. 2013; Folgueira et al. 2020). In addition, the expression of *Pdi*AOX is significantly increased during host infection (Folgueira et al. 2020). The expression profile of *Pdi*AOX led to the suggestion that the physiological roles of the oxidase are to support respiration under hypoxic conditions and to protect the ciliate against oxidative stress produced by the host during infection (Folgueira et al. 2020).

4.2. Superoxide dismutases

For the neutralisation of superoxide radicals ($O_2^{\bullet-}$), a reactive oxygen species generated by UV radiation in water and by the host immune system during infection, *P. dicentrarchi* expressed three different types of superoxide dismutase (SOD) enzymes; cupper/zinc-SOD (CSOD), manganese-SOD (MSCO), and iron-SOD (FSOD) (Folgueira et al. 2019b). This is unusual as eukaryotic cells usually have either CSODs and MSODs or only FSODs. Moreover, *P. dicentrarchi* expresses three CSOD isoenzymes (*Pdi*CSOD1-3) of apparent molecular weights ranging between 34-44 kDa for the native proteins (Folgueira et al. 2019b). All three *Pdi*CSODs have been found to be localised in the cytosol and in alveolar sacs, while *Pdi*CSOD2 is also secreted (Folgueira et al. 2019b). The native MSCD and FSOD of *P. dicentrarchi* (*Psi*MSOD and *Psi*FSOD) have apparent molecular weights of 50 kDa and 60 kDa, respectively (Folgueira et al. 2019b). Whereas *Psi*MSOD is localised beneath the outer mitochondrial membrane, *Pdi*FSAO is secreted (Folgueira et al. 2019b). SOD activity is increased in *P. dicentrarchi* trophozoites when the ciliates are exposed to UV radiation and to chemical-generated $O_2^{\bullet-}$ (Folgueira et al. 2019b). The regulable SOD activity and the abundance of different types of SOD enzymes indicate that *P. dicentrarchi* is very well adapted to protect itself from the toxic action of $O_2^{\bullet-}$ generated in the marine environment and by the host during its different life cycle phases.

4.3. Proton-translocating pyrophosphatases

Since *P. dicentrarchi* is both a free-living marine organism and an endoparasite, the ciliate needs to be able to tolerate environments of different salinity. In order to maintain the intracellular pH homeostasis, *P. dicentrarchi* possesses acidic organelles that contain two H^+-translocating pyrophosphatases; *Pdi*VP1 and *Pdi*VP2 (Mallo et al. 2014, 2016a,b). As *Pdi*VP1 and *Pdi*VP2 are proteins of 764 and 810 amino acids but have apparent molecular weights of 158 kDa and 178 kDa, respectively, both enzymes occur as dimers under native conditions (Folgueira et al. 2021). Sequence analysis revealed that *Pdi*VP1 and *Pdi*VP2 bear close resemblance to the type I vascular pyrophosphatases from plants (Mallo et al. 2014, 2016a). Both enzymes are localised in the membranes of alveolar sacs and intracellular vacuoles (Mallo et al. 2014, 2016a; Folgueira et al. 2021). The addition of pyrophosphate (PP_i) to digitonin-permeabilised *P. dicentrarchi* trophozoites has been shown to lead to an influx of H^+-ions into the acidic organelles (Mallo et al. 2016b). This PP_i-driven H^+-translocation is inhibited by ATP and Ca^{2+} (Mallo et al. 2016b). In addition, treatment of *P. dicentrarchi* trophozoites with ATP or Ca^{2+} has been found to result in the downregulation of *Pdi*VP1/2 expression (Mallo et al. 2016b). NaCl, the main salt in seawater, also inhibits the PP_i-driven intracellular acidification (Mallo et al. 2016b). Based on these findings, it was concluded that the regulation of the pH of intracellular acidic organelles is important for *P. dicentrarchi* to survive the salt stress the ciliate is experiencing when shuttling between different habitats (Mallo et al. 2016b).

4.4. Proteases

The major proteolytic activity present in cell extracts of *P. dicentrarchi* has been identified to be from cysteine proteases (Paramá et al. 2004b, 2007). One of the cysteine proteases has been cloned and characterised as a cathepsin L enzyme (Shin et al. 2014). Proteolytic activity has also been reported in excretion/secretion products of the ciliate, indicating that mature proteases may be released into the environment (Paramá et al. 2004b; Piazzon et al. 2011a).

For example, *P. dicentrarchi* proteases have been demonstrated in serum and ascites fluids of experimentally infected fish (Piazzon et al. 2011a). It has been suggested that released parasite proteases may have roles in host tissue evasion, degradation of nutrients, modulation of host immune response by inducing apoptosis in host leucocytes, and circumventing host immune response by inactivation of antibodies and complement factors (Paramá et al. 2004b, 2007a,b; Piazzon et al. 2011a).

5. PATHOLOGICAL EFFECTS OF *P. DICENTRARCHI*

5.1. External symptoms

The first non-clinical signs of fish infected with *P. dicentrarchi* are abnormal swimming behaviour, lethargy, and anorexia (Iglesias et al. 2001; Rossteuscher et al. 2008; Stidworthy et al. 2014; de Felipe et al. 2017). Another early symptom is that infected fish show darkening of the skin with haemorrhagic ulcers (Iglesias et al. 2001; Ramos *et al.* 2007; Harikrishnan et al. 2010b; Jin et al. 2009; Stidworthy et al. 2014; de Felipe et al. 2017). The gills of affected fish may be congested with mucus, and their eyes may protrude from the eye socket (exophthalmia) (de Felipe et al. 2017). In addition, fish diseased with *P. dicentrarchi* may show abdominal distension as a result of an accumulation of ascitic fluid in the body cavity (Iglesias et al. 2001; Ramos et al. 2007; de Felipe et al. 2017).

5.2. Clinical signs and pathology

During the course of the disease, many affected fish develop systemic infection (Iglesias et al. 2001; Paramá et al. 2003; Harikrishnan et al. 2012a; de Felipe et al. 2017). Once this stage is reached, *P. dicentrarchi* ciliates can be found everywhere in the body of infected fish (Iglesias et al. 2001; Harikrishnan et al. 2010b; Moustafa et al. 2010). Quite often, the blood of

infected fish appears pale red and less viscous and contains numerous ciliates feeding on red blood cells (Dragesco et al. 1995; Jin et al. 2009; Harikrishnan et al. 2012a). In addition, red blood cells of diseased fish are often distorted and smaller (reduced mean cell volume), indicative of microcytic anaemia (Dragesco et al. 1995). Likewise, in the ascitic fluid that many infected fish accumulate in their peritoneal cavities, a large number of ciliates can be usually found (Iglesias et al. 2001). In many cases, ciliates can be detected on the epithelial surface and subepithelial tissue of the gills of affected fish (Iglesias et al. 2001; Rossteuscher et al. 2008). Similarly, smears of skin ulcers would also reveal numerous ciliates (Iglesias et al. 2001). In the brain, ciliates are usually present within the capillary blood vessels and in the meninges (Iglesias et al. 2001; Jin et al. 2009; Stidworthy et al. 2014).

Deep epidermal ulcers that can spread into the underlying muscular tissue are commonly seen in many infected fish (Ramos et al. 2007; Rossteuscher et al. 2008; Moustafa et al. 2010; Harikrishnan et al. 2012a; Jalenques et al. 2021). Oedematous swelling of gill filaments has been observed in several diseased fish (Rossteuscher et al. 2008; Moustafa et al. 2010). Another feature seen in some affected fish is the enlargement of spleen and liver (Stidworthy et al. 2014; Jalenques et al. 2021). Haemorrhages have been recorded on organs, particular on liver, kidney, and pancreas, and on muscles in various diseased fish species (Iglesias et al. 2001; Ramos et al. 2007; Rossteuscher et al. 2008; Jin et al. 2009; Harikrishnan et al. 2012a; Di Cicco et al. 2013; Stidworthy et al. 2014; de Felipe et al. 2017; Jalenques et al. 2021). In the stomach and intestine, the infection can cause oedematous inflammation and in severe cases to a complete loss of the mucosal epithelium of the stomach (Jin et al. 2009; Moustafa et al. 2010; Di Cicco et al. 2013; Stidworthy et al. 2014). Some affected fish may show oedema and congestion of the brain (Iglesias et al. 2001; Stidworthy et al. 2014; Jalenques et al. 2021).

Fish severely infected with *P. dicentrarchi* show diverse histopathological changes in many organs and tissue. Epidermal ulcers are often associated with oedema and liquefactive necrosis of the underlying dermis and muscular tissue (Ramos et al. 2007; Rossteuscher et al. 2008; Moustafa et al. 2010; Harikrishnan et al. 2012a; Jalenques et al. 2021). In addition, inflammation with a large number of lymphocytes and macrophages that extends from the superficial dermis into the subjacent musculature is commonly observed (Ramos et al. 2007;

316 Rossteuscher et al. 2008; Jin et al. 2009; Jalenques et al. 2021). The epidermis adjacent to
317 ulcers can be hyperplastic (Rossteuscher et al. 2008). Similarly, the gill epithelium of affected
318 fish can appear hyperplastic and hypertrophic, accompanied by intra- and subepithelial
319 haemorrhages (Fig. 3A) (Iglesias et al. 2001; Ramos et al. 2007; Rossteuscher et al. 2008;
320 Moustafa et al. 2010). Skeletal muscles are also often affected showing necrotising myositis
321 and extensive myolysis with ciliates and lymphocyte infiltration in and between muscle
322 bundles (Fig. 3B) (Iglesias et al. 2001; Ramos et al. 2007; Rossteuscher et al. 2008;
323 Harikrishnan et al. 2010b, 2012a; Moustafa et al. 2010). Necrotising inflammation of liver,
324 kidney, pancreas and spleen have been recorded in many fish with severe scuticociliatosis
325 (Iglesias et al. 2001; Ramos et al. 2007; Rossteuscher et al. 2008; Jin et al. 2009; Harikrishnan
326 et al. 2012a; Di Cicco et al. 2013; Stidworthy et al. 2014; de Felipe et al. 2017; Jalenques et al.
327 2021). In addition, necrosis of the intestinal epithelium has been found in a number of diseased
328 fish (Jin et al. 2009; Moustafa et al. 2010; Di Cicco et al. 2013; Stidworthy et al. 2014). The
329 infection of the brain is typically associated with necrotising meningoencephalitis (Stidworthy
330 et al. 2014; Jalenques et al. 2021). Characteristically, oedema, increased vascularisation,
331 inflammation with lymphocyte and monocyte, and necrotising vasculitis can be usually seen in
332 infected meninges (Fig. 3C) (Iglesias et al. 2001; Stidworthy et al. 2014; Jalenques et al. 2021).
333 Subsequently, the infected brain is softening and liquefying in connection with vacuolation and
334 necrosis of the grey matter (Jin et al. 2009).

335 The mortality rate of fish infected with *P. dicentrarchi* is usually high and can reach 100%.
336 Studies with experimentally infected fish provided information on how quickly fish can die
337 from a *P. dicentrarchi* infection. Depending on the route of infection and dosages of ciliates
338 inoculated, fish can die within 4-30 days post-infection (Paramá et al. 2003; Jin et al. 2009;
339 Harikrishnan et al. 2012a).

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342 **6. HOST IMMUNE DEFENCE AGAINST *P. DICENTRARCHI* INFECTIONS**

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Most of what is known about the defence of fish against *P. dicentrarchi* infection comes from studies in turbot. Findings thus far indicate that in turbot the humoral immunity, in particular the humoral innate immunity, is critical for the defence against *P. dicentrarchi*. The cellular immune response seems to play only a minor role, if any. For example, inoculation of turbot with live or killed ciliates led to a moderate increase in subsequent *in vitro* phagocytic activity and reactive oxygen production of isolated leucocytes, but only in the presence of serum from infected turbot (Leiro et al. 2004a). This observation was corroborated by *in vitro* experiments showing that a combination of fresh serum and specific antibodies obtained from infected turbot kill the ciliate, but this killing activity was not increased by the addition of live turbot leucocytes (Piazzon et al. 2011b). As specific antibodies alone and heat-inactivated serum in the presence of specific antibodies exhibited no killing activity against *P. dicentrarchi*, it was concluded that the defence against the ciliate in turbot is mainly through the antibody-mediated classical complement pathway (Leiro et al. 2008; Piazzon et al. 2011b). That fish can produce specific antibodies against *P. dicentrarchi* was previously demonstrated in turbot that survived infection with this ciliate (Iglesias et al. 2003a). These antibodies recognise ciliary antigens and induce rapid shedding of these surface proteins, which are then replaced with different antigenic polypeptides (Iglesias et al. 2003a). It has been shown that immunisation of turbot with ciliate lysate in the presence of Freund's complete adjuvant resulted in the production of agglutinating antibodies that provided some protection against challenge infection with the parasite (Iglesias et al. 2003a). These results indicate that ciliary surface antigens play an important role in the defence in turbot against *P. dicentrarchi*.

Other components of the humoral innate immune system have been shown to contribute also to the defence against *P. dicentrarchi* infection in turbot. For instance, the fish coagulation system seems to play an important role in immobilising and killing the scuticociliate. Injection of the parasite in the intraperitoneal space of turbot led to the formation of clots encapsulating the ciliate (Blanco-Abad et al. 2018). *In vitro* studies confirmed that live parasites and cellular components of the parasite are triggers for the clotting (Blanco-Abad et al. 2018). Another humoral innate defence element used by turbot to fight infections is the antimicrobial peptide NK-lysin. Although infection with *P. dicentrarchi* seems not to affect the expression of NK-

lysin mRNA, it changes the protein level of the peptide (Lama et al. 2018). NK-lysin has been shown to kill the ciliate *in vitro* and *in vivo* by disrupting the plasma membrane of the parasite (Lama et al. 2018). A further innate defence molecule that has been shown to exhibit toxicity against *P. dicentrarchi* is the lily-type lectin *Sm*LTL (Huang et al. 2016). The lectin is present in the skin mucus of turbot and is expressed in response to the infection with the ciliate (Huang et al. 2016).

It has also been shown that infection with *P. dicentrarchi* induces a potent acute inflammatory response in turbot and that many inflammatory and defence/immune-related genes are upregulated in immune-relevant tissue and peritoneal cells (Pardo et al. 2008, 2012; Valle et al. 2020). These findings clearly indicate that turbot reacts to an infection with the scuticociliate *P. dicentrarchi* by activating defence mechanisms. In addition, the infection with *P. dicentrarchi* seems to cause stress in turbot as their blood cortisol level increases significantly (Rodríguez-Quiroga et al. 2017). However, cortisol suppresses the immune system and thus may counteract any immune defence activated by the ciliate, making it easier for the parasite to establish an infection in turbot.

7. OUTBREAKS OF SCUTICOCILIATOSIS CAUSED BY *P. DICENTRARCHI*

Since the first report of an outbreak of scuticociliatosis in reared fish due to *P. dicentrarchi* in 1993, further outbreaks of the disease causing high mortality in fish farms have been recorded around the world (Table 2). Clusters of outbreaks of *P. dicentrarchi* scuticociliatosis have been observed in fish farms at two geographical locations: at the Atlantic region of the Iberian Peninsula (Iglesias et al. 2001; Alvarez-Pellitero et al. 2004; Ramos et al. 2007; Budiño et al. 2011a) and at the South Korean Jeju Island (Jin et al. 2009, 2010; Harikrishnan et al. 2010b,c, 2012a). Fish farms rearing flatfish have been primarily affected by outbreaks of *P. dicentrarchi* scuticociliatosis. In some cases, the outbreaks were preceded by periods of increased water temperature (Iglesias et al. 2001; Ramos et al. 2007; de Felipe et al. 2017) which may have enhanced the propagation or pathogenicity of the parasite. In addition,

outbreaks of scuticociliatosis by *P. dicentrarchi* occur more commonly in the summer months when the water temperature is generally higher (Iglesias et al. 2001; Ramos et al. 2007). In fact, a water temperature of 18-23°C has been shown to be optimal for the proliferation of *P. dicentrarchi* (Iglesias et al. 2003b). In addition, the increased water temperature may have affected the fitness of the farmed fish so that they were more susceptible to the infection. As water temperatures will further increase due to climate change, more outbreaks of scuticociliatosis by *P. dicentrarchi* can be expected in the future.

Outbreaks of *P. dicentrarchi* scuticociliatosis have also been reported in fish held captive in public aquariums (Table 2). In most cases, the source of the parasite could not be determined. However, there is the possibility that the parasite may have been introduced via imported wild-caught fish added to aquariums. In particular, newly purchased fish can turn out to be the animals that are mainly affected by the disease (Rossteuscher et al. 2008).

It has also been shown that different variants of *P. dicentrarchi* can occur during scuticociliatosis outbreaks on individual fish farms (Budiño et al. 2011a,b). For instance, one study found that from ten *P. dicentrarchi* isolates obtained during an outbreak on a turbot farm, four different morphotypes could be distinguished (Budiño et al. 2011b). Serological analysis revealed that the four morphotypes could be grouped into three different serotypes belonging to three genotypes (Budiño et al. 2011b). The existence of different serological variants of *P. dicentrarchi* during outbreaks is probably the reason why vaccines often fail to protect against the infection (see below).

8. EVALUATION OF STRATEGIES FOR CONTROLLING *P. DICENTRARCHI* INFECTIONS

To date, there are no approved therapies or vaccines available to fight infections caused by the scuticociliate *P. dicentrarchi* in fish. In search of agents able to kill *P. dicentrarchi*, numerous anti-infective drugs, chemical agents, and natural compounds have been tested for cidal activity against the ciliate. In addition, immunostimulants and dietary supplements have

been investigated for their effectiveness to boost the immune response in fish against the parasite. Furthermore, various antigens have been evaluated for their ability to protect fish against infection with *P. dicentrarchi*. Another strategy to control *P. dicentrarchi* infections is the selective breeding of fish for resistance traits to scuticociliatosis.

8.1. Anti-infective drugs

In vitro screening studies of antibacterial and antiparasitic medications identified a few compounds exhibiting cidal activity against *P. dicentrarchi* (Iglesias et al. 2002; Paramá et al. 2004c). Of the antibiotics tested, only furaltadone, norfloxacin, and doxycycline were found to display toxic activity against the ciliate with minimum lethal concentrations (MLC) of 25 mg l^{-1}, 50 mg l^{-1}, and 50 mg l^{-1}, respectively (Iglesias et al. 2002; Paramá et al. 2004c). Among antiparasitic drugs, the following nine agents were discovered to be able to kill the scuticociliate at the indicated MLC values: albendazole (100 mg l^{-1}), carnidazole (100 mg l^{-1}), pyrimethamine (100 mg l^{-1}), quinacrine hydrochloride (100 mg l^{-1}), quinine sulphate (100 mg l^{-1}), toltrazuril (6.2 mg l^{-1}), bithionol sulfoxide (3.1 mg l^{-1}), oxyclozanide (0.8 mg l^{-1}), and niclosamide (0.8 mg l^{-1}) (Iglesias et al. 2002). Only oxyclozanide and niclosamide (Fig. 4), two anthelmintic medications, exhibited promising toxic activity against *P. dicentrarchi* with MLC values below 1 mg l^{-1} and thus may be good candidates for the control of scuticociliatosis caused by this protozoan parasite.

The nitroimidazole metronidazole, an antiprotozoal veterinary drug, has been shown to clear *P. dicentrarchi* infections in seahorses kept in aquaculture (Di Cicco et al. 2013). The treatment consisted of a 10 days bath at a dosage of 50 mg l^{-1}. The fish survived the treatment and showed improved physical condition and feeding behaviour (Di Cicco et al. 2013). No ciliates were detected in two seahorses, which were euthanised for post-mortem examination (Di Cicco et al. 2013). According to the results of this preliminary study, metronidazole seems to be an effective treatment of seahorses infected with *P. dicentrarchi*.

Although indomethacin is mainly known as a nonsteroidal anti-inflammatory drug, this agent has been shown to inhibit the growth of *P. dicentrarchi in vitro* (Paramá et al. 2007c). However, the inhibitory effect on the growth of the ciliate was only moderate; at a concentration of 100 μM, the proliferation of the protozoan was reduced by around 50%. As no cidal activity was observed for indomethacin, the agent seems not to be an option for the treatment of *P. dicentrarchi* infections.

8.2. Chemical agents

The disinfectants formalin and hydrogen peroxide were among the first chemicals to be tested for anti-proliferative activity against *P. dicentrarchi*. However, both chemicals were not very effective in killing the protozoan *in vitro*. Only after exposure to 400 mg l^{-1} formalin for 180 min and 300 mg l^{-1} hydrogen peroxide for 150 min, respectively, all ciliates were killed (Harikrishnan et al. 2010c; Jin et al. 2010). For both formalin and hydrogen peroxide, the effective concentrations for killing *P. dicentrarchi* are above the recommended dosages of 250 mg l^{-1} and 50-100 mg l^{-1}, respectively, for short bath treatments of fish up to 1 h (Leal et al. 2018; Yanong 2018). Hence, both chemicals seem not to be suitable as therapeutic reagents to treat *P. dicentrarchi* infection of fish in aquacultures.

An interesting alternative to the traditional formalin bathing of fish for controlling parasites are beta-cyclodextrin containing chitosan microspheres crosslinked with glutaraldehyde (Paramá et al. 2005). In particular, chitosan microspheres with low crosslinks (0.15% glutaraldehyde) and low beta-cyclodextrin content (0.1%) seem to exhibit high toxicity against *P. dicentrarchi in vitro* at low microsphere concentration (10 μg ml^{-1}) (Paramá et al. 2005).

In two other studies, about 100 newly synthesised compounds (simple piperazines, simple pyrimidines, isoxazolpyrimidines, pyridothienopyrimidines, naphthyridines, pyridothienodiazines, and pyridothienotriazines) were tested for *in vitro* cidal activity against *P. dicentrarchi* (Quintela et al. 2003; Paramá et al. 2004c). The majority of the compounds were inactive; only 28 compounds exhibited killing activity against the ciliate with MLC values

of ≤100 mg l^{-1} and only one compound (12k; 8-cyano-7-(4-methylpiperidino)-4-piperazinopyrido[3',2':4,5]thieno[3,2-d]-1,2,3-triazine; Fig. 4) with an MLC value of below 1 mg l^{-1} (Quintela et al. 2003; Paramá et al. 2004c). As the toxicity of compound 12k for fish is not known, it remains unclear whether this pyridothienotriazine can be developed into a therapeutic drug for the treatment of *P. dicentrarchi* infections.

8.3. Natural compounds

The phytochemical polyphenols, resveratrol, mangiferin, (–)-epigallocatechin-3-gallate, and curcumin, have been investigated for *in vitro* activity against *P. dicentrarchi*. All four compounds were shown to display only moderate growth inhibitory activity against the ciliate (Leiro et al. 2004b; Mallo et al. 2017). At 500 μM, resveratrol, mangiferin, and (–)-epigallocatechin-3-gallate inhibited the proliferation of the ciliate by 96%, 93%, and 56%, respectively (Leiro et al. 2004b). Curcumin was found to exhibit stronger antiprotozoal activity; at 100 μM, the compound inhibited the growth of the ciliate almost completely (Mallo et al. 2017). However, the limited water solubility of resveratrol and curcumin, the only compounds with reasonable activity against the ciliate, makes them unsuitable for their use in controlling scuticociliatosis caused by *P. dicentrarchi*.

In a recent study, 26 compounds of natural origin were tested for their *in vitro* toxicity to *P. dicentrarchi*. The highest anti-proliferating activity was observed for 2',4'-dihydroxychalcone, plumbagin (a naphthoquinone), and tomatine (a glycoalkaloid), all exhibiting 48 h 50% growth inhibition values (GI_{50}) of <10 μM (Sueiro et al. 2022). However, only 2',4'-dihydroxychalcone was found to display low *in vitro* cytotoxicity towards cultured fish cells (Epithelioma Papulosum Cyprini (EPC) cells). At 7.5 μM, the 24 h GI_{50} value for *P. dicentrarchi*, only about 10% of EPC cells were killed (Sueiro et al. 2022). In the same study, the bacterial biosurfactant from the *Pseudomonas* strain H6 (PS) was also investigated for *in vitro* activity against *P. dicentrarchi* (Sueiro et al. 2022). When *P. dicentrarchi* cells were exposed to the lipopeptide for 3 h, the 50% lethal concentration of PS was determined to be

517 7.8 μg ml^{-1} (Sueiro et al. 2022). Under the same incubation conditions, only 14.9% of EPC
518 cells were killed (Sueiro et al. 2022). Interestingly, PS has been previously shown to exhibit
519 similar anti-proliferating activity against another fish pathogenic ciliate, *Ichthyophthirius*
520 *multifilis* (Al-Jubury et al. 2018). Whether 2',4'-dihydroxychalcone and PS can be developed
521 into treatments to control *P. dicentrarchi* infections in fish remains to be shown.
522
523
524 **8.4. Immunostimulants and dietary supplements**
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526 Synthetic oligodeoxynucleotides containing unmethylated cytosine-phosphate-guanine
527 motifs (CpG-ONDs) are recognised as a pathogen-associated molecular pattern (PAMP) or
528 danger signal by the innate immune system of vertebrates and can induce potent non-specific
529 immune activation (Klinman 2004). When fish were injected with CpG-ONDs, it was found
530 that their serum displayed significantly higher lytic activity against *P. dicentrarchi in vitro*
531 compared to serum from untreated fish (Lee & Kim 2009). In addition, fish treated with CpG-
532 ONDs had a higher relative survival rate than control fish receiving the vehicle alone (Lee &
533 Kim 2009). The latter result suggests that CpG-ONDs may be useful in the stimulation of the
534 innate immune response to reduce the mortality in farmed fish due to scuticociliatosis caused
535 by *P. dicentrarchi*.
536 Natural products and extracts have been documented to possess immunostimulatory
537 properties in animals. For this reason, diets supplemented with natural products and extracts
538 have been evaluated in order to determine whether these feed additives can boost the immune
539 response in fish to increase the resistance against *P. dicentrarchi*. Initially, a mixed herb-
540 enriched diet containing extracts prepared from leaves of pomegranate (*Punica granatum*),
541 Dalmatian chrysanthemum (*Chrysanthemum cinerariaefolium*), and mastic-leaved prickly-ash
542 (*Zanthoxylum schinifolium*) was tested. The herbal-enriched diet at 50 mg kg^{-1} was shown to
543 enhance positively the innate immune response in fish and to reduce the cumulative mortality
544 against infection with *P. dicentrarchi* compared to a diet without the herbal extracts
545 (Harikrishnan et al. 2010d). Similar results were also obtained with fish feeds enriched with

546 pure extracts of mistletoe (*Viscum album*; 1-2%), castor aralia (*Kalopanax pictus*; 0.1%), or
547 pomegranate (*Punica granatum*; 1%) (Harikrishnan et al. 2011b,c, 2012c). Furthermore,
548 isolated natural compounds were shown to produce comparable effects. For instance, a diet
549 enriched with 1% chitin or chitosan stimulated the innate immune response in fish with
550 concomitant reduction of cumulative mortality against infection with the ciliate (Harikrishnan
551 et al. 2012c). Together, these results indicate that a diet enriched with natural extracts can
552 improve the innate immune response of fish and provide partial resistance against *P.*
553 *dicentrarchi* infection.
554
555
556 **8.5. Vaccines**
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558 It has been shown that fish inoculated with lysate of *P. dicentrarchi* together with Freund's
559 complete adjuvant or with formalin-fixed ciliates conferred some protection (73.7% and
560 31.6%, respectively) against challenge infection with the parasite (Iglesias et al. 2003a). It was
561 suggested that antibodies produced against the surface immobilisation antigen (i-antigen) were
562 responsible for the partial protection (Iglesias et al. 2003a). However, as there are various types
563 of i-antigens, it was questioned whether this surface antigen is the best target for vaccine
564 development to protect fish against infection with *P. dicentrarchi* (Lee & Kim 2008). In
565 another study, a membrane fraction was used as antigen, which was encapsulated and
566 covalently linked to a polymeric microsphere formulation composed of chitosan and poly-
567 methyl vinyl ether-co-maleic anhydride (PMVE/MA) (León-Rodríguez et al. 2012). The
568 relative percentage survival of fish vaccinated with the antigen-microsphere formulation was
569 68%, while that of fish immunised with the antigen together with Freund's complete adjuvant
570 was 58% (León-Rodríguez et al. 2012). Moreover, the antigen-microsphere formulation was
571 shown to enhance the innate immune response in fish (León-Rodríguez et al. 2013). These
572 results indicate that microsphere-based vaccines are more effective than traditional oil-based
573 vaccines.

One problem in the development of vaccines to protect fish against infection with *P. dicentrarchi* is the existence of different strains of the parasite (Iglesias et al. 2003a; Lee & Kim 2008). Most antigen candidates tested so far provide immunity only against the strain used for the preparation of the vaccine (Iglesias et al. 2003a; Lee & Kim 2008; León-Rodríguez et al. 2012). These autologous vaccines do not generally afford protection against other strains of the parasite. The development of a protective anti-*P. dicentrarchi* vaccine is further hampered by the ability of the scuticociliate to use antigen shedding and antigen variation strategies in order to evade the immune response of the host (Iglesias et al. 2003a; Lee & Kim 2008). Therefore, other invariant and constitutively expressed antigens need to be identified if universal protective vaccines are to be developed as immunoprophylaxis against *P. dicentrarchi*.

8.6. Selective breeding

In order to undertake successful breeding programmes to produce broodstock fish that are more resilient to scuticociliatosis caused by *P. dicentrarchi*, the quantitative trait loci (QTLs) affecting resistance and survival time need to be known. The identification of QTLs-bearing linkage groups has been previously achieved by a genome scan for such QTLs after injection of *P. dicentrarchi* in four turbot families using a homogeneously distributed microsatellite panel and applying two different statistical methods (Rodríguez-Ramilo et al. 2013). In total, eight suggestive and three significant QTLs were detected for resistance, and eleven suggestive and three significant QTLs were detected for survival time (Rodríguez-Ramilo et al. 2013). In another study, the association of the genetic basis of resistance and survival time was disentangled (Saura et al. 2019). It was found that genetic variation exists for resistance and survival time and also for the composite trait resilience (resistance + survival time) (Saura et al. 2019). However, as a very high genetic correlation (0.90) was detected between resistance and resilience, it seems that these two traits are mainly the same (Saura et al. 2019). On the other hand, no significant genetic correlation was observed between resistance and survival

time (Saura et al. 2019). The findings of this study are essential information for future breeding programmes as they indicate that selection on resilience will lead to improvement of both resistance and survival time.

9. CONCLUSION

Scuticociliatosis caused by *P. dicentrarchi* has been recognised as an emerging problem in the aquaculture industry worldwide. The disease has been responsible for significant economic losses to the aquaculture sector. The infection affects mainly flatfish reared at fish farms but also various other fish species kept in public aquariums. Infected fish develop quickly severe pathologies leading to the death of the animals in most instances. Humoral innate immunity and antibody-mediated classical complement pathway appear to be the important defence mechanisms of fish against *P. dicentrarchi* infection.

There are no satisfactory therapies for the treatment of fish infected with *P. dicentrarchi*. At present, fish farmers use a variety of different chemicals to manage outbreaks of *P. dicentrarchi* scuticociliatosis. Generally, this involves a short-term bath of affected fish in the presence of the chemicals. However, no systematic study has been carried out to determine the efficacy of bath treatment under field conditions. In addition, some of the more widely used chemicals for bath treatment (e.g. formalin) have been banned in food fish production as they pose a risk to human health. Although few compounds have been identified to be able to kill the ciliate *in vitro* with high efficiency, no agent has been developed yet that is effective in treating systematic *P. dicentrarchi* infections of fish. The best way, however, to prevent outbreaks of *P. dicentrarchi* scuticociliatosis is probably species-appropriate farming to reduce stress and injuries of the fish as healthy animals most likely will not get infected with this opportunistic protozoan parasite.

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Table 1. Morphometric data for fixed *P. dicentrarchi* trophozoites. Shown are the means of means ($\mu_{\bar{x}}$) and the standard deviations of the means of means ($\sigma_{\bar{x}}$) of morphometric characteristics from the following studies: Dragesco et al. (1995), Iglesias et al.(2001), and De Felipe et al. (2017). Measurements are in µm. PM: paroal membrane; OPK: oral polykineties

Character	$\mu_{\bar{x}}$	$\sigma_{\bar{x}}$	Min	Max
Body length	34.8	2.4	32.6	39.5
Body width	19.0	2.1	15.2	21.5
Caudal cilium length	13.0	3.3	10.7	15.8
Somatic cilia length	7.0	2.0	5.6	9.3
Oral apparatus length	15.3	3.1	14.4	18.8
PM_1 length	3.6	0.4	3.1	4.1
PM_2 length	6.3	1.1	5.0	8.1
Distance apex-OPK_1	3.5	0.3	3.0	3.9
OPK_1 length	1.9	0.6	1.2	2.6
OPK_2 length	2.5	0.7	1.6	3.1
OPK_3 length	0.6	0.2	0.4	0.9
Macronucleus diameter	6.3	0.7	5.4	7.0
Micronucleus diameter	1.5	0.4	1.0	2.1

883 Table 2. Documented outbreaks of scuticociliatosis caused by *P. dicentrarchi*

Year of outbreak	Geographic location	Infected fish species	Type of aquaculture	Reference
1993	France, Mediterranean coast	*Dicentrarchus labrax* (sea bass)	Mariculture	Dragesco et al. (1995)
1999/2000	Spain, Galician southwestern coast	*Scophthalmus maximus* (turbot)	Recirculatory flow system	Iglesias et al. {2001)
2000-2006	South Korea, Jeju Island coast	*Paralichthys olivaceus* (olive flounder)	Raceway culture system	Jin *et al.* (2010); Harikrishnan et al. (2010b)
2001	Spain, Atlantic coast	*Scophthalmus maximus* (turbot)	Raceway culture system (seawater)	Alvarez-Pellitero et al. (2004)
2002	France, Cantabric coast	*Scophthalmus maximus* (turbot)	Raceway culture system (well water)	Alvarez-Pellitero et al. (2004)
2002	Portugal, Atlantic coast	*Scophthalmus maximus* (turbot)	Raceway culture system (well water)	Alvarez-Pellitero et al. (2004)
2002	Spain, Cantabric coast	*Scophthalmus maximus* (turbot)	Raceway culture system (seawater)	Alvarez-Pellitero et al. (2004)
2004/2005	Portugal, northern Atlantic coast	*Scophthalmus maximus* (turbot)	Raceway culture system (seawater)	Ramos et al. (2007)
2004/2005	Switzerland, Basle	*Phycodurus eques* (leafy sea dragon); *Phyllopteryx taeniolatus* (weedy sea dragon)	Aquarium	Rossteuscher et al. (2008)
2008	South Korea, Seoul	*Hippocampus kuda* (common seahorse)	Aquarium	Shin et al. (2011)
2009	Turkey, eastern Black Sea coast	*Scophthalmus maximus* (turbot)	Grow-out tank in a research facility	Kayis et al. (2011)
2010	Europe[a]	*Heterodontus japonicus* (Japanese bullhead shark); *Heterodontus portusjacksoni* (Port Jackson shark);	Aquarium	Stidworthy et al. (2014)

		Stegostoma fasciatum (zebra shark)		
2012	Canada, Vancouver	*Hippocampus abdominalis* (Australian pot-belly seahorse)	Aquarium	Di Cicco et al. (2013)
2014	Peru, Ancash coast	*Paralichthys adspersus* (fine flounder)	Mariculture	de Felipe et al. (2017)
2018/2019	Canada, Québec	*Amphistichus rhodoterus* (redtail surfperch); *Anarrhichthys ocellatus* (wolf-eel); *Sebastes miniatus* (vermillion rockfish)	Aquarium	Jalenques et al. (2021)

884 [a] No location details regarding country and city were provided

885

886 Fig. 1

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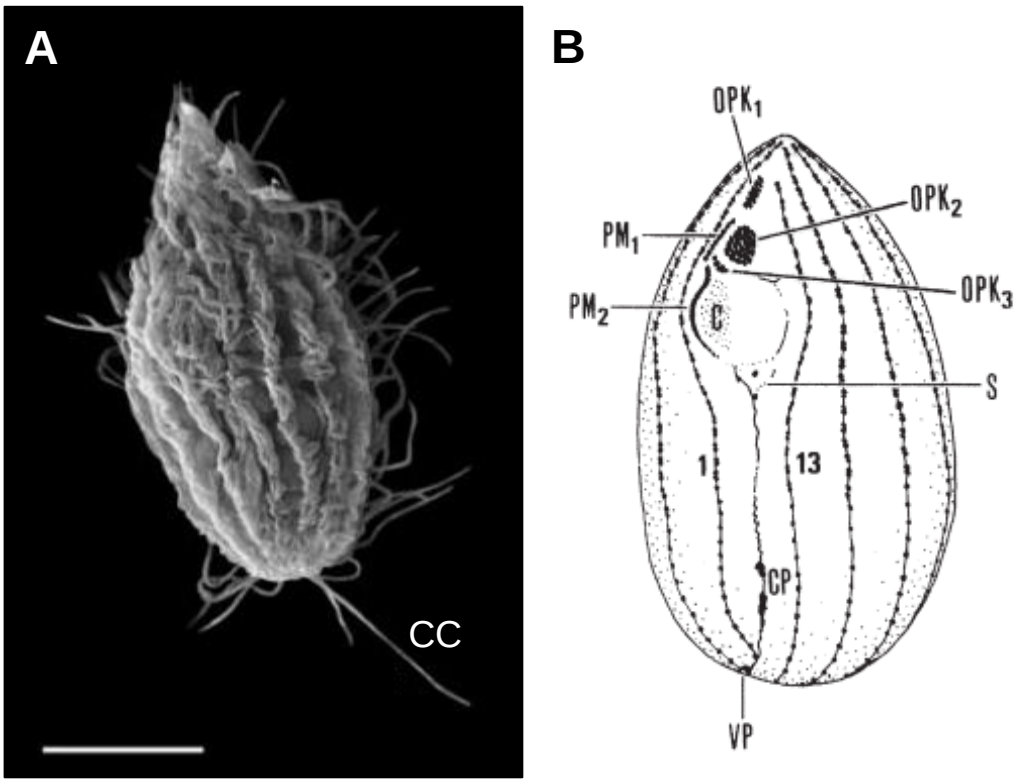

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890 Fig. 1. Morphological characteristics of *Philasterides dicentrarchi*. (A) Scanning electron
891 micrograph of the scuticociliate. CC: caudal cilium; scale bar = 10 μm. (B) Schematic drawing
892 of *P. dicentrarchi*. C: cytostome; CP: cytoproct; OPK_1, OPK_2, and OPM_3: oral polykineties 1,
893 2, and 3; PM_1 and PM_2: paroal membrane 1 and 2; S: scutica; VP: pore of contractile vacuole;
894 1 and 13: first and last kineties of the somatic ciliature. Images were taken from Iglesias et al.
895 (2001)

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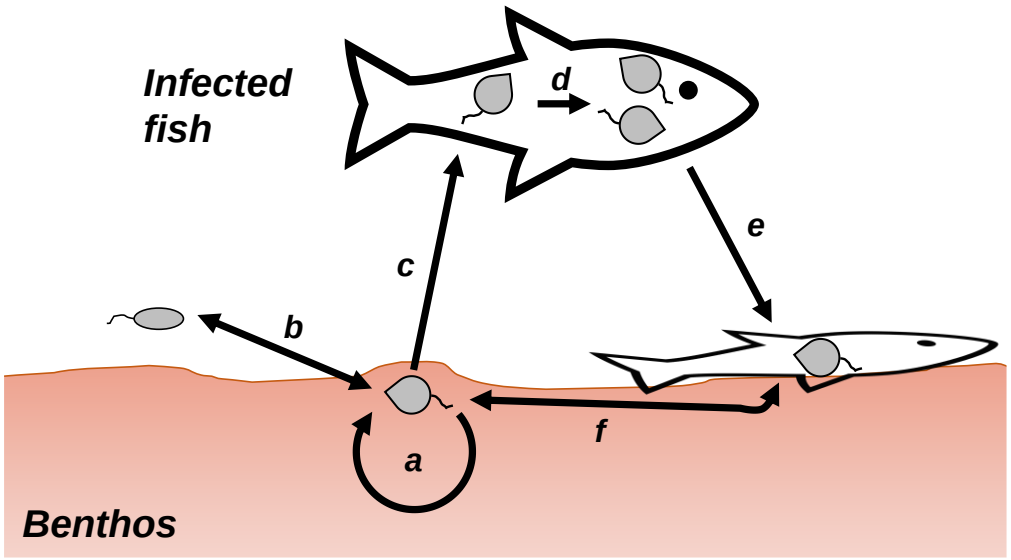


Fig. 2. Proposed life-cycle of *Philasterides dicentrarchi*. The scuticociliate is a microarophilic protozoan living in the benthic zone where it feeds on bacteria. When nutrients are abundant, the ciliate produces only proliferating microstome forms (a). When there is a lack of nutrients, the ciliate produces smaller, non-feeding, fast-swimming tomites (b), possibly in order to reach new feeding grounds. The ciliate can also infect fish and adopt a parasitic lifestyle (c). The protozoan multiplies within fish causing systemic infection (d). Fish infected with *P. dicentrarchi* will eventually die (e). The ciliate are released from the cadavers into the environment but cadavers may also serve as a food scours for the protozoan (f)

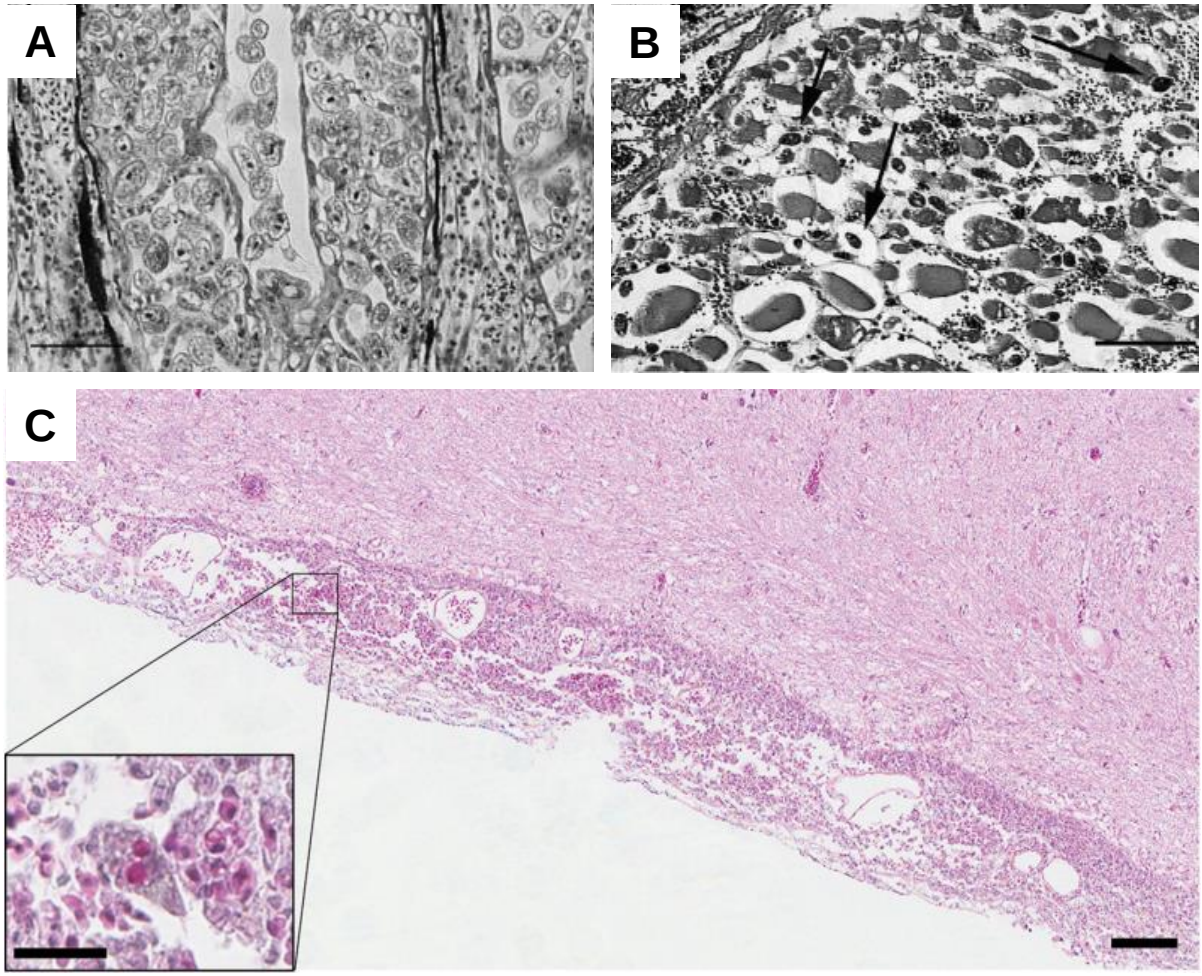
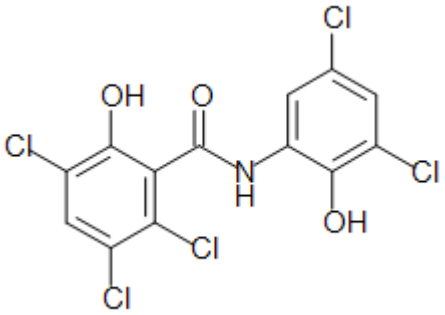
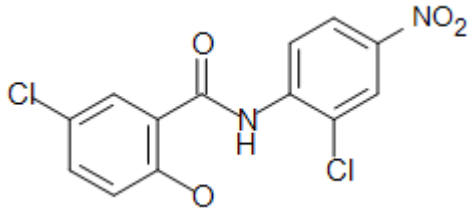


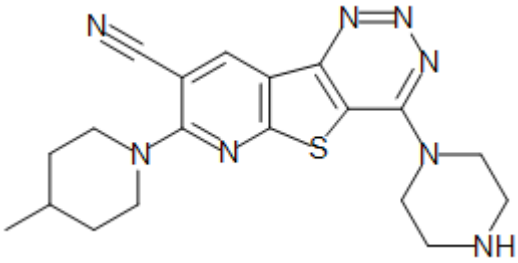
Fig. 3. Histopathological changes associated with *Philasterides dicentrarchi* scuticociliatosis in fish. (A) Hyperplastic and necrotic branchial tissue in turbot infected with *P. dicentrarchi*. Several ciliates are present in primary and secondary lamellae; scale bar = 50 μm. (B) Myolysis of skeletal muscle with haemorrhages in turbot infected with *P. dicentrarchi*. A few ciliates can be seen in the destructed muscle (arrows); scale bar = 100 μm. (C) Meningitis in a wolf-eel infected with *P. dicentrarchi*. The meninges are thickened, haemorrhagic, and infiltrated with macrophages and lymphocytes; scale bar = 100 μm. The enlarged section shows one ciliate within the meninges containing phagocytosed red blood cells; scale bar = 20 μm. Images A and B were taken from Ramos et al. (2007) while image C was taken from Jalenques et al. (2021)



Oxyclozanide



Niclosamide



12k

Fig. 4. Structures of compounds with promising activity against *Philasterides dicentrarchi*. The two related drugs oxyclozanide and niclosamide are approved anthelmintics for the treatment of fascioliasis in domestic ruminants and tapeworm infestation in humans, respectively. The active substance 12k is an experimental compound. All three agents show promising activity against the scuticociliate *P. dicentrarchi* with MLC values below 1 mg l^{-1}