OPEN ACCESS

Haya: The Saudi Journal of Life Sciences
Abbreviated Key Title: Haya Saudi J Life Sci
ISSN 2415-623X (Print) | ISSN 2415-6221 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Review Article

Ichthyophthiriasis (Ich / White Spot Disease) in Aquaculture Systems: Biology, Epidemiology, Pathogenesis, Economic Impacts and Control Strategies

Usman Naveed[1]*, Rimsha Latif[1], Rashid Hussain Dahar[2], Nasim Yasin[1], Zareena Khadim[1], Gumza Mushtaq[1], Mahroz Fatima Zaidi[3], Alishba Fatima[1], Muhammad Usama[1]

[1]Department of Zoology, Wildlife and Fisheries, University of Agriculture Faisalabad 38040, Pakistan
[2]Department of Fisheries Resources, Ocean University of China Qingdao P.R China
[3]Department of Zoology, Government College University, Lahore 54000, Pakistan

DOI: https://doi.org/10.36348/sjls.2026.v11i07.002 | **Received:** 12.05.2026 | **Accepted:** 30.06.2026 | **Published:** 03.07.2026

***Corresponding author:** Usman Naveed (ranausmannaveed26@gmail.com, 2022ag8958@uaf.edu.pk)
Department of Zoology, Wildlife and Fisheries, University of Agriculture Faisalabad 38040, Pakistan

Abstract

One of the most important parasitic diseases in freshwater fish farming due to its clinical and economic importance, ichthyophthiriasis results from I. multifiliis (a ciliate obligate parasite) infection, also known as "white spot disease" or simply "Ich." This literature review is aimed at compiling the existing knowledge about the taxonomical position, biological features, and three-stage life cycle (consisting of the theront, trophont, and tomont) of the parasite under the influence of various environmental parameters such as temperature, salinity, and pH. In addition, the epidemiological features of the infection (host specificity, susceptibility factors, transmission methods, and seasonal prevalence) as well as host-parasite relationships (mechanisms of parasite entry, i-antigen-induced immune evasion, and host response) will be considered. Clinical features and histopathological changes for various cultured species are reviewed along with the diagnosis, which includes both traditional methods as well as molecular diagnostics like qPCR and ddPCR. It is emphasized that the disease creates significant financial loss, costing more than USD 1 billion annually on a worldwide basis. The effect of the disease is significant in aquacultures from Europe, China, and North America. Prevention and management of this disease include chemical treatment, salt treatment, biological control, immunostimulants, vaccine, and biosecurity. There is no method available which gives total protection. Latest innovations in the fields of genomics, transcriptomics, novel treatment methods and DNA vaccines are also mentioned. The conclusion made by the authors is that sustainable management of I. multifiliis calls for an integration of various measures such as decreased use of chemicals, increased biosecurity, biological control, genetic resistance and precision aquaculture, with vaccine efficacy, drug resistance and climate change adaptation highlighted as key research areas.

Keywords: Ichthyophthirius multifiliis, White spot disease, Freshwater aquaculture, Fish parasitology, Host-parasite interaction, i-antigens, Disease control strategies, Vaccine development, Aquaculture biosecurity.

1. INTRODUCTION

Aquaculture constitutes the fast-growing part of food production globally and provides the protein sources necessary for the growing population (FAO,2024). At the same time, intensified aquacultures have made it possible for diseases to be encouraged and parasitic diseases to constitute the most difficult category of all. Ichthyophthiriasis is a parasitic infection caused by the obligate parasite parasitic ciliate named Ichthyophthirius multifiliis (I. multifiliis) (Fouquet, 1876), commonly referred to as "white spot disease" or "Ich". It is among the most economically and medically catastrophic diseases affecting all freshwater cultured fish species.

The geographical spread of I. multifiliis covers all continents that engage in freshwater aquaculture, with reported parasitization in over 100 fish species belonging to different taxonomic categories (Lom & Dykova, 1992; Qasim et al., 2025). This parasite exhibits an incredible ability to reproduce exponentially, in addition to its lack of host specificity and natural protection at various life cycle stages, making traditional management strategies inefficient in most cases (Buchmann *et al.,* 2001). In intensive aquaculture settings, an uncontrolled outbreak may cause mortality of up to 80%, while losses in global economic terms reach over USD 1 billion per year (Peng *et al.,* 2025; Shinn *et al.,* 2015).

Citation: Usman Naveed, Rimsha Latif, Rashid Hussain Dahar, Nasim Yasin, Zareena Khadim, Gumza Mushtaq, Mahroz Fatima Zaidi, Alishba Fatima, Muhammad Usama (2026). *Ichthyophthiriasis* (Ich / White Spot Disease) in Aquaculture Systems: Biology, Epidemiology, Pathogenesis, Economic Impacts and Control Strategies. *Haya Saudi J Life Sci, 11*(7): 343-354.

In the past, malachite green was the chief chemotherapy that was used in the treatment of I. Multifiliis; nevertheless, because of its mutagenic nature and persistence in the fish tissues, its usage has been banned in the European Union, North America, and many other parts of the world (Sudova *et al.,* 2007). This ban has triggered a lot of research on alternative therapeutic methods such as formalin, hydrogen peroxide, peracetic acid, and salt therapies, although each one of them has some limitations.

Modern scientific work has paid much attention to immunological methods of disease prophylaxis involving DNA vaccines with i-antigens of I. multifiliis (Clark *et al.,* 1995; Xu *et al.,* 2020). However, despite the significant outcomes obtained through these studies in relation to the stimulation of immune response, there is no sufficient level of protection, which makes it necessary to develop these vaccines further (von Gersdorff Jorgensen *et al.,* 2012; Ye *et al.,* 2024).

In this paper, an extensive analysis of the current state of knowledge about I. multifiliis in aquaculture systems will be carried out, including taxonomic classification and biological characteristics of parasites, life cycle of parasites, epidemiology, host-parasite relationship, diagnostic procedures, economic consequences, and control options. In addition, this study highlights important research needs and priorities for future work.

2. Taxonomy and Biology of Ichthyophthirius multifiliis

The I. multifiliis parasite is classified under the phylum Ciliophora, class Oligohymenophorea, order Hymenostomatida and family Ichthyophthiriidae (Corliss, 1979). The taxonomic classification and biological characteristics of this significant aquaculture pathogen are summarized in Table 1.

Table 1: Taxonomic classification and biological characteristics of I. multifiliis.

Characteristic	Description
Kingdom	Protista
Phylum	Ciliophora
Class	Oligohymenophorea
Order	Hymenostomatida
Family	Ichthyophthiriidae
Genus	Ichthyophthirius
Species	I. multifiliis (Fouquet, 1876)
Common names	White spot disease, Ich, Ichthyophthiriasis
Trophont size	0.05–1.0 mm diameter
Theront size	30–50 µm
Macronucleus	Horseshoe-shaped, C-form
Host range	Virtually all freshwater teleosts (>100 species documented)
Distribution	Global, all freshwater aquaculture regions
I-antigens	GPI-anchored surface proteins, 40–60 kDa; 5 serotypes (A–E) identified

The largest known parasitic protozoan to infect fish, I. multifiliis reaches diameters of 0.5 to 1.0 mm at its mature stage, making it visible to naked eye as small white spots that appear on the body of infected fish (Francis-Floyd *et al.,* 2016). This parasite is completely covered by cilia for locomotion and has a characteristic horseshoe-shaped macronucleus, which is an important diagnostic feature of the parasite (Yanong, 2023).

Surface features of I. multifiliis include i-antigens, which are composed of a number of GPI-anchored protein molecules having a molecular weight of 40-60 kDa (Clark *et al.,* 2001). Surface proteins are regarded as primary antigenic sites for an immunological response by the host, and studies on these surface proteins as potential vaccine candidates have been performed extensively due to their ability to elicit the production of protective antibodies (Clark *et al.,* 1995; Lin *et al.,* 2002). Up to date, at least five distinct serotypes of i-antigens (A-E) have been identified in parasites (Dickerson *et al.,* 1993; Wang & Dickerson, 2002).

Ultrastructural studies have shown that the parasite has developed advanced mechanisms to facilitate parasitism, such as specialized cytostomes used to ingest cells, contractile vacuoles to regulate osmoregulation, and complex cortical arrangements linked with cilial coordination (Chapman & Kern, 1983). The cellular structure of the parasite is indicative of adaptation to its environment in terms of host penetration and rapid asexual reproduction.

3. Life Cycle and Environmental Influences

I. multifiliis has a direct life cycle which does not involve any intermediate host. This includes three functional phases of the parasite, namely, the infective theront, the parasitic trophont, and the reproductive tomont (Ewing & Kocan, 1992; Noe & Dickerson, 1995). Knowledge of this life cycle is necessary to design control programs since each phase offers different targets and problems for treatment

3.1 Life Cycle Stages

The parasitic trophont constitutes the feeding stage of the I. multifiliis parasite and accounts for the symptoms observed in white spot disease. Following infection of the host epithelial tissues with the theronts, the trophonts become established between the epithelial layers of the skin, fins, and gills of the fish, and here they proceed to ingest host cells, leukocytes, and tissue fluids (Ventura & Paperna, 1985). Being protected by the host epithelium and mucus, the trophonts become immune to the effects of chemicals in this stage. During this period which lasts from 7 to 10 days at optimum temperatures, the trophonts enlarge from 50 µm to more than 1 mm in size and become visible to the naked eyes as white spots (Meyer, 1969).

After their maturation process, trophonts leave the host as protomonts and eventually develop into tomonts which then encyst themselves on the surface of the substrate in the aquatic habitat. Inside the gelatinous cyst, tomonts undergo a series of binary fission resulting in the formation of 50 to 2,000 tomites depending on the temperature and the size of tomont (Aihua & Buchmann, 2001; Meyer, 1969).

Tomites metamorphose to theronts, which become pear-shaped free-swimming parasites with a size of 30-50 µm after emerging from the cyst. Theronts have a non-ciliated anterior part that contains a perforatorium, which is useful for penetrating through the tissues of the host (MacLennan, 1935). The theront stage is very brief because theronts live for about 96 hours at 3-4°C but only 24 hours at 25°C (Maharsi, 1979; Suzuki, 1935).

Table 2: Life cycle stages, duration, and environmental conditions affecting development of I. multifiliis

Stage	**Characteristics**	**Duration at 25°C**	**Susceptibility to Treatment**
Trophont	Parasitic feeding stage on fish skin, fins, gills; 0.05–1.0 mm; feeds on host epithelial cells and leukocytes	7–10 days	Protected by host epithelium, not susceptible
Protomont	Transitional stage after exiting host; free-swimming in water column	2–24 hours	Moderately susceptible
Tomont	Encysted reproductive stage attached to substrates; undergoes binary fission producing 50–2,000 tomites	18–24 hours (division); 2–7 days total	Protected by gelatinous cyst, low susceptibility
Tomite	Daughter cells within tomont cyst; differentiate into theronts	Variable	Protected within cyst
Theront	Free-swimming infective stage; 30–50 µm; seeks fish host	Up to 24 hours at 25°C; 40 hours at 20°C	Highly susceptible—primary target for chemical control

3.2 Environmental Influences

Temperature of water plays the most important role in the development of I. multifiliis, influencing the time required for the completion of life cycles. The life cycle takes 6-9 days in an optimum temperature range of 24-25°C, but it is completed in more than five weeks when the temperature is 10°C and in several months in the temperature range of 3-4°C (Meyer, 1969; Wagner, 1960; Qasim et al., 2026). Temperature sensitivity poses some problems in the treatment of diseases, as changes in treatment protocols have to be made as per the temperature of water.

Another environmental factor affecting the parasites' viability and infectivity is salinity. Investigations conducted by Aihua and Buchmann (2001) revealed that high salinity (above 5 ppt) inhibits the formation of tomites at 11.6°C. The recent studies performed by Tange et al. (2020) shed light on the tolerance levels of the pH, showing that theronts generated at pH 8 survived under the pH range of 4-10 for an hour, whereas tomonts were capable of releasing theronts at pH levels of 5-10. The size of theronts increased with increasing pH.

There are other environmental elements such as the hardness of water, the ions in it, the amount of dissolved oxygen, and the quantity of organic matter that affect the spread of disease. Cysts of tomonts are able to stick easily to any organic matter. This is because organic matter acts as sources of infection in aquaculture systems (Francis-Floyd *et al.,* 2016).

4. Epidemiology and Disease Transmission

I. multifiliis epidemiology is defined through the interplay between the parasite's biology, host vulnerability, environmental conditions, and aquaculture management. Knowledge about its transmission dynamics is crucial to formulate effective prevention and control measures.

4.1 Host Range and Susceptibility

I. multifiliis is characterized by its highly low host specificity where infections have been reported to occur in all species of freshwater teleost fish (Noga, 1996). Even though it is said that all freshwater fish are susceptible, interspecific and intraspecific variations in disease severity have been found. The rainbow trout (Oncorhynchus mykiss), channel catfish (Ictalurus punctatus) and common carp (Cyprinus carpio) are the fish species that have a high degree of susceptibility and suffer mortalities in excess of 80–90% when no measures are taken (Peng *et al.,* 2025; Matthews, 2005). However, some cyprinid species such as zebra fish (Danio rerio) have better natural resistance and can clear their infections through strong immune response.

Age is one of the main determinants of susceptibility; it has been shown that the young fish become more susceptible because of the weak immune system (Hines & Spira, 1974). The scale-less fish and the ones that produce little mucus are more susceptible. Environmental factors like crowding, poor water quality, nutrition deficiency, and handling can suppress the immune system of the hosts (Abdel-Hafez *et al.,* 2014).

4.2 Transmission Pathways

Transmission is mainly carried out through direct contact between the infected and uninfected fish with theronts being the only infectious stage. In highly intensive fish rearing systems, the high fish populations help to speed up the spread of parasites, thus allowing for epizootic infection outbreaks to arise within days (Dickerson, 2006). The movement of infected fish, contaminated equipment, or water from the infected system provides the main route of transmission of I. multifiliis.

The parasites that manage to infect the fish could act as asymptomatic carriers by carrying sub-clinical levels of parasites, which can be used to infect naive populations (Yanong, 2023). The implication is that visually healthy fish can infect naive populations, hence the need for stringent biosecurity practices. There have been no reports of vertical transmission, and the parasite cannot survive for long without fish hosts.

4.3 Outbreak Patterns

Epizootics of I. multifiliis are usually associated with changes in seasonal temperatures and stress in fish culture practices. In temperate climates, such epizootics happen in spring and autumn, as the water temperatures help the parasites reproduce and fish immune system becomes compromised under heat stress (Leao *et al.,* 2015). Tropical and subtropical aquaculture systems face an ever-present danger of disease occurrence all year round, but the highest incidence is connected with rainy seasons or rapid changes in temperatures (Corrêa *et al.,* 2019).

5. Host-Parasite Interactions and Pathogenesis

The relationship between the parasite I. multifiliis and their fish hosts comprises numerous molecular, cellular, and histological factors that affect disease outcomes. New knowledge obtained using techniques such as transcriptomics and immunology has greatly enhanced our understanding of these relationships.

5.1 Parasite Invasion Mechanisms

The invasion of the host epithelium by theronts includes mechanical penetration and enzymatic degradation of barriers. The presence of a perforatorium at the anterior region of theronts helps in the initial penetration of the host, while proteolytic enzymes like cysteine proteases (ICP), such as cathepsin L, help in degrading the host extracellular matrix, thereby allowing the parasite to penetrate and colonize (Zhao *et al.,* 2009). Once the parasite penetrates the host, the trophonts move into the basal epithelium of the host and make feeding cavities.

The i-antigens found on the surface of theronts and trophonts are involved in immune system evasion. They are GPI-anchored antigens, and their antigenic variation makes it possible for the parasite to avoid the already existing response from the immune system of the host (Clark *et al.,* 1992). Additionally, there is a regulation in the expression of these antigens in different developmental stages of the parasite.

5.2 Host Immune Responses

The immune response of fish to I. multifiliis is multi-faceted with the participation of innate and adaptive responses. Early immune response is particularly significant for the development of the disease because of studies that compared transcriptomes of fish species that were resistant and susceptible to the infection (Noethiger *et al.,* 2025). The zebrafish, which is naturally resistant, exhibits early transcriptional response that peaks 24 hours after infection through up-regulation of chemokines (Cxcl8a, Cxcl11, Cxcl13), cytokines, and pathogen recognition receptor signal transduction pathways.

The antibody-mediated immune system, especially the production of mucosal IgT and systemic IgM responses against i-antigens, represents the most important means of protection (Clark *et al.,* 1995). The fish that survive infection acquire good, serotype-specific immunity to challenge infections, and hence help in vaccine research work. Passive immunoexperiments conducted using monoclonal antibodies to i-antigens ensure full protection in naïve fish (Clark *et al.,* 1995; Wang *et al.,* 2002).

5.3 Pathogenesis Pathway

The I. multifiliis pathogenesis is associated with tissue damage caused by the parasites' feeding activities, as well as inflammation reactions from the host organisms, and complications. Trophont invasion affects the integrity of the epithelial tissues and leads to an impaired osmotic balance and electrolyte disturbances. Infection of the gills proves especially lethal, because the lamellae fusion, hyperplasia, and excessive mucus secretion impede gas exchange, thus causing lethal hypoxia at even moderate parasitism levels (Ventura & Paperna, 1985). Moreover, the exit of the adult trophonts causes other tissue damages that allow bacteria and fungi to enter the body.

6. Clinical Signs and Histopathological Changes

Clinical signs of I. multifiliis depend on several factors including intensity of infestation, type of fish infected, site of infection, and environmental factors. Prompt identification of clinical signs of disease is important for timely treatment.

6.1 Clinical Signs

The distinctive feature of I. multifiliis is the presence of small white spots measuring 0.3–1.0 mm on the fish's skin, fins, and sometimes eyes. These white spots indicate individual trophonts located inside the epithelium. Nevertheless, the lack of such spots should not rule out the occurrence of the disease since it is possible that the parasite is localized only in the gills protected by the operculum (Yanong, 2023).

Behavioral changes often precede the development of white spots on the fish body. The fish become restless, show flashing, breathe faster, are lethargic, lose appetite and congregate around areas where the water level of oxygen is higher. With the progression of the disease, the fish become darker in color, clamp their fins, become separated from other fish. With the development of severe infections, erratic swimming, imbalance, and even recumbency are possible (Noga, 1996).

Table 3: Clinical signs and pathological effects of I. multifiliis infection in different fish species.

Fish Species	Clinical Signs	Pathological Effects
Rainbow trout (O. mykiss)	High mortality; severe respiratory distress; flashing; lethargy; white spots on skin/fins	Lamellar fusion; epithelial hyperplasia; marked mucus hypersecretion; severe hypoxia
Channel catfish (I. punctatus)	High mortality; prominent white spots; anorexia; surface breathing; erratic swimming	Extensive gill damage; epithelial necrosis; secondary bacterial infection common
Common carp (C. carpio)	Moderate to high mortality; behavioral changes; visible white spots; respiratory difficulty	Gill hyperplasia; inflammatory infiltration; osmoregulatory dysfunction
Grass carp (C. idella)	Moderate mortality; white spots; flashing; reduced feeding activity	Epidermal hyperplasia; gill tissue damage; secondary infections
Nile tilapia (O. niloticus)	Lower mortality than salmonids; behavioral changes; white spots; respiratory distress	Milder gill pathology; better tolerance; epithelial changes
Goldfish (C. auratus)	Variable mortality; white spots; flashing; lethargy; respiratory changes	Lamellar fusion; cell hyperplasia; hyperemia; inflammatory infiltration; necrosis
Zebrafish (D. rerio)	Minimal mortality in wild-type; behavioral changes; transient white spots	Rapid parasite clearance; effective epithelial repair; minimal pathology

6.2 Histopathological Changes

The tissue changes in the histopathological analysis show distinct changes in tissue that result from the effects of I. multifiliis. In the gills, trophont attachment causes epithelial hyperplasia and hypertrophy, secondary lamellae fusion, goblet cell hyperplasia accompanied by mucus formation, and inflammatory cells. Eosinophilic granulocytes have been reported to increase considerably in the gills during recent studies conducted on infected hosts.

Histopathological changes in the skin include epidermal hyperplasia, spongiosis and focal necrosis at the trophont attachment site. As a consequence of trophont detachment from the lesion, an ulcerative change accompanied by exposure of dermis may occur, making the area more prone to secondary microorganisms.

Recently, studies have shown that infection with I. multifiliis causes a significant change in the microbiota of the hosts. The gill microbiota of the infected fish show lower alpha and beta diversity with an increase in the number of opportunistic pathogens such as Aeromonas and Achromobacter (Zhang *et al.,* 2025).

7. Diagnostic Approaches

Diagnosis of I. multifiliis requires accuracy and timeliness to ensure that proper control measures are put in place. Methods of diagnosis include traditional clinical diagnosis to advanced molecular methods, which have unique strengths and weaknesses.

7.1 Conventional Diagnosis

Diagnosis of I. multifiliis using white spots on skin and fins as a clinical sign is only a presumptive indication. Nevertheless, this diagnostic procedure is not accurate since there are various diseases that could mimic the appearance of I. multifiliis, such as fungal infections, granulomas caused by bacteria, and other parasitic infections like Cryptocaryon irritans (in saltwater fish) (Yanong, 2023). Infections that are only found on the gills could kill without producing any white spots

7.2 Microscopic Diagnosis

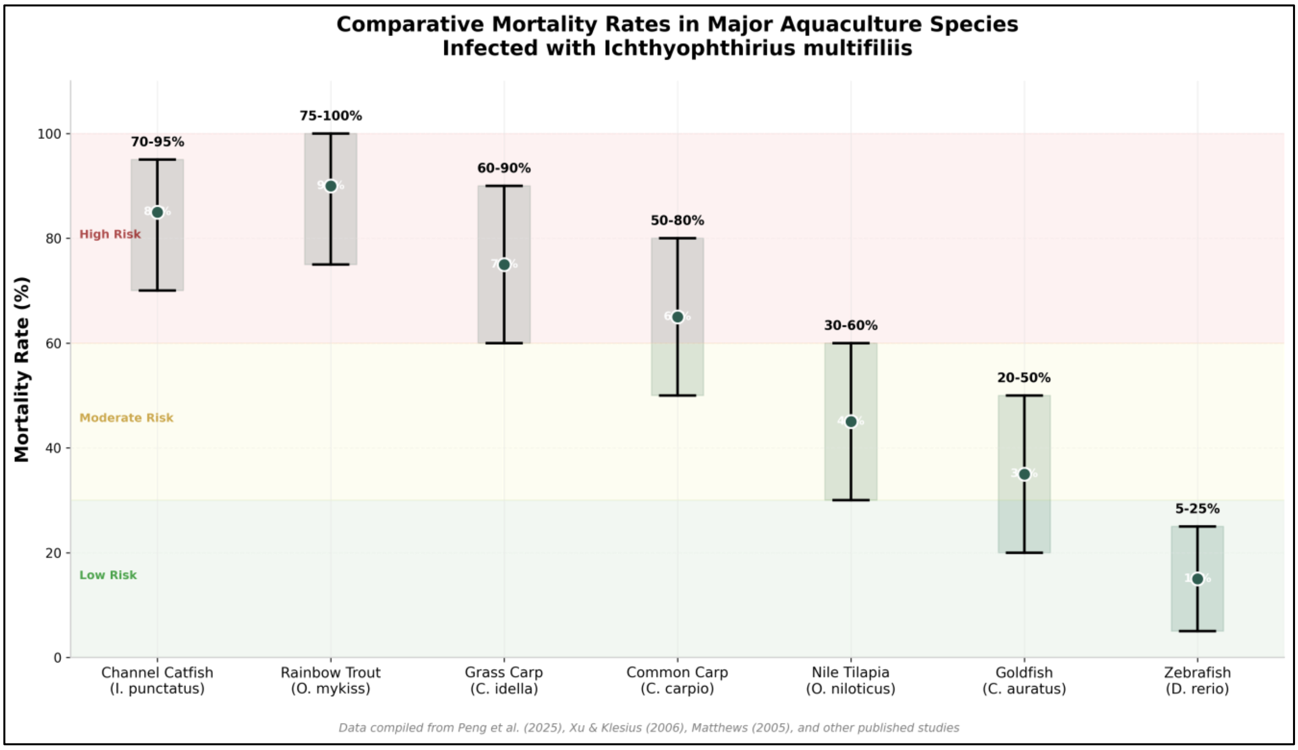
Definitive diagnosis can only be made with the help of microscopy of wet mount preparations made from skin scrapings, fin clips, or gill biopsy samples. The morphological features of trophonts can easily be recognized; their slow rotation, ciliation, and presence of horseshoe-shaped macronucleus is evident at 40 to 100 times magnification (Yanong, 2023). Theronts and tomites can also be detected in water samples and cyst preparations. This diagnostic technique continues to be the best available one.

7.3 Molecular Diagnosis

Various molecular diagnostic techniques have been shown to be useful in the detection of I. multifiliis infections, including asymptomatic carriers and environmental samples. The use of conventional PCR technique to amplify parasite DNA using small-subunit ribosomal DNA (SSU rDNA) is the earliest molecular diagnostic technique used to detect I. multifiliis, as proposed by Jousson *et al.,* (2005). However, due to the sequence conservation of the ciliate DNA, careful choice of primers should be considered to ensure non-cross reactivity.

A variety of techniques have been used to allow detection and quantification of parasite DNA. Hu *et al.,* (2023) came up with a TaqMan qPCR test using SSU rDNA which allowed the authors to detect and quantify parasite DNA and showed good correlation with standard plasmid DNA. Recently, Guo *et al.,* (2025) developed a novel TaqMan probe-based qPCR using the cathepsin L cysteine protease (ICP2) gene of the parasite with a detection threshold of 5.4 copies/μL and 4 theronts/L in aquaculture water.

Droplet digital PCR (ddPCR) is currently the most advanced molecular diagnostic technique available for detecting I. multifiliis. The comparative studies conducted by (Hu *et al.,* 2023) have proven that droplet digital PCR is more sensitive than qPCR and can detect sub-clinical infections early in the case of water samples from an environment. But qPCR has a larger dynamic range.



Graph 1: Comparative mortality rates reported in major aquaculture species infected with I. multifiliis (based on literature data)

8. Economic Impact on Global Aquaculture

The economic impact of I. multifiliis is not limited to mortalities but includes costs associated with treatment, poor growth performance, poor feed conversion ratio, and trade restrictions. However, economic analysis becomes difficult in light of underreporting and the effects of secondary diseases.

According to Peng et al. (2025), white spot disease causes mortality above 80% in intensive fish culture, causing losses amounting to over USD 1 billion globally annually. European trout farming incurs losses of about USD 140 million annually from I. multifiliis (Maezono *et al.,* 2025). China, which is a leading producer of freshwater aquaculture fish, incurs losses from deaths of 200,000 to 300,000 tons of fish annually, costing up to 2 to 3 billion RMB (around USD 280-420 million) (Peng *et al.,* 2025).

In the USA, I. multifiliis has been shown to affect 42% of channel catfish farms, causing individual producers to lose more than 2,000 pounds each (Peng *et al.,* 2025). In ornamental fish farming, there have been cases of infections among koi and aquarium species, causing loss in the pet trade industry on a global scale.

Apart from the immediate deaths caused by the pathogen, sub-clinical infections are known to be quite economically damaging due to slowed growth, poor feed conversion ratios, and poor product quality. Infected fish may suffer from scaring that lowers their economic value. Furthermore, continued chemical treatment may cause additional economic losses and the need for harvesting delay periods.

9. Prevention and Control Strategies

A proper management strategy of Ich requires an integrated approach involving the use of several strategies. No single strategy can provide total protection from Ich, and a proper sustainable management strategy entails using several strategies both for prevention and treatment.

9.1 Chemical Treatments

Formalin (37% formaldehyde solution) continues to be the most common chemical therapy for I. multifiliis Lahnsteiner and Weismann (2007) revealed that using 110 µL/L of formalin for 1–2 hours will successfully kill the parasite within a short period in repeat treatments synchronized to the life cycle of the parasite. However, this treatment is very harmful to humans and regulated in most countries.

Another treatment method that is efficient in ponds for raising channel catfish is the application of copper sulfate (CuSO4). The required amount is estimated as the result of total alkalinity (mg/L) divided by 100, but concentrations from 1 to 2 mg/L are used most often. Copper sulfate should not be used in low alkalinity water bodies (<50 mg/L), where it becomes highly toxic after the algae die-off (Francis-Floyd *et al.,* 2016).

The use of hydrogen peroxide (H2O2) is gaining popularity because of its environmental friendliness and breakdown products which are water and oxygen. In recirculating aquaculture systems, the effectiveness of H2O2 against ectoparasites such as I. multifiliis has been shown to be similar to formalin but concentration levels need to be carefully chosen to prevent the destruction of biofilters (Pedersen *et al.,* 2019).

Peracetic acid (PAA) is yet another oxidizing substance that has proven to be effective in treating fish disease. In a study done by Abu-Elala et al. (2021), treatment of I. multifiliis and Trichodina spp. using PAA treatment showed that it decreased Aeromonas hydrophila infection in Nile tilapia. (Sudova *et al.,* 2010) confirmed its effectiveness in the continuous treatment process for four days.

9.2 Salt Treatments

The usage of sodium chloride (NaCl) takes advantage of the limited tolerance to salinity by the different stages of the I. multifiliis life cycle. Continuous exposure to 10 ppt of salinity over 10 days in temperatures over 20°C will inhibit the formation of the tomonts, preventing the release of the theronts. Thus, the life cycle will be interrupted (Buchmann *et al.,* 2022; Li & Buchmann, 2001).

9.3 Biological Control

Biocontrol methods for I. multifiliis infections have been receiving greater emphasis owing to their environmentally sustainable nature. The past few years have seen studies that have shown that some copepod species attack the free-swimming forms of I. multifiliis. In-situ evidence of predation by copepods on I. multifiliis has been provided by Wang et al. (2024) using fluorescence labeling techniques. Although the theront's ability to move about makes their predation difficult, the use of copepod species could add up to the biocontrol approach.

9.4 Immunostimulants

Several immunomodulatory substances have been researched for improving the resistance of fish to I. multifiliis infections. Beta-glucan, nucleotide, and herbs have shown good effects in increasing the innate immunity; however, the reliability of their protective effect in field trials has yet to be confirmed. Vitamin C and its analogs have been tested for reducing the impact of infection, and the silicone-coated formulation is promising in rainbow trout (Wahli *et al.,* 1995).

9.5 Vaccination Approaches

Vaccination is one of the most promising means to deal with I. multifiliis sustainably. The i-antigens of I. multifiliis have been studied extensively as vaccine candidates. According to the research by Xu *et al.,* (2020, 2021), the DNA vaccines against the IAg52b i-antigen induce antibodies against Ich, and confer partial protection (survival between 35-49%) in channel catfish; increased doses (20 µg/fish) and double vaccinations were more protective than single vaccines (10 µg).

Recent studies by Ye et al. (2024) have focused on alternative antigenic targets, which include the use of a β-tubulin DNA vaccine (pVAX1-Bt) for grass carp, resulting in 70% relative percent survival. The vaccine improved the production of antibodies, antioxidant enzymes, and gene expression in the immune system, indicating that even non-surface antigens can offer protection.

However, many challenges are still associated with the commercial application of vaccines. Variations between isolates of I. multifiliis in terms of serotype specificity may hinder cross protection, and the best means of delivering vaccines for mass vaccination is not yet known (von Gersdorff Jorgensen *et al.,* 2012).

9.6 Biosecurity and Farm Management

The use of preventative biosecurity procedures represents the cornerstone of any successful management plan for I. multifiliis. Critical procedures include quarantine and health checkups of new stock, using all-in/all-out farming methods, disinfection of equipment and facilities between production cycles, and maintaining good water quality parameters that minimize host stress (Buchmann *et al.,* 2022).

The use of integrated control methods including various measures has proved to be more efficient than single measures. The best practice in trout farming includes mechanical filtration using micro-sieves to clean out tomonts, treatment with hydrogen peroxide or peracetic acid for theront elimination, maintaining low infections in order to develop resistance against infection, and using disease-resistant stock if possible (Buchmann *et al.,* 2022).

Table 4: Comparison of available treatment and control methods for I. multifiliis, including advantages and limitations

Treatment	Application	Advantages	Limitations
Formalin (37%)	Bath: 110 µL/L, 1–2 h; repeated 3–5 times	Highly effective; well-documented; rapid action	Human health hazard; carcinogenic potential; regulatory restrictions; water quality impact
Copper sulfate	Pond: 1–2 mg/L (alkalinity/100)	Effective and inexpensive for ponds	Toxic in low alkalinity; oxygen depletion; environmental persistence; residue concerns
Hydrogen peroxide	Bath: 6.5–35 mg/L	Degrades to water and oxygen; environmentally benign	Narrow therapeutic window; biofilter effects; repeated applications needed
Peracetic acid	Bath/flow through: 1 mg/L continuous	Rapid degradation; low environmental impact; broad antimicrobial activity	Concentration maintenance difficult; corrosive; limited long-term data
Salt (NaCl)	10 ppt for 10 days at >20°C	Safe for food fish; no residues; environmentally friendly	Species tolerance limits; infrastructure requirements; limited efficacy data
Potassium permanganate	2 mg/L; repeated based on water color	Effective oxidant; broad spectrum	Strong oxidizer; gill/skin damage with repeated use; deactivates in organic water
Biological control	Copepod augmentation in ponds	Environmentally sustainable; no chemical residues	Limited efficacy data, theront behavior may evade predation; unpredictable outcomes
DNA vaccine	Intramuscular injection	Potential for long-term protection; no environmental impact; species-specific development	Partial protection only; serotype specificity; delivery challenges; not commercially available

10. Emerging Research and Recent Advances

Contemporary research on I. multifiliis encompasses diverse disciplines including genomics, transcriptomics, immunology and novel therapeutic development. These investigations are generating critical insights with direct implications for improved disease management.

10.1 Genomics and Transcriptomics

The genome of I. multifiliis has been sequenced and there are unique chances to study parasite biology and discover new drug targets. Genomics analysis has shown the presence of many gene families that participate in the process of host invasion, immunity evasion and development of particular stages (Coyne *et al.,* 2011). Transcriptomic analysis has discovered differentially expressed genes in various life stages, with the emphasis on cysteine proteases and i-antigen variants.

The study of host transcriptomics has provided important knowledge on mechanisms of disease resistance. The comparative study of the response of gills in zebrafish and rainbow trout in infection of I. multifiliis by Noethiger *et al.,* (2025) showed that the early activation of the immune system is an important factor in determining the outcome of the disease. In resistant zebrafish, the immune responses were quickly activated through the activation of Toll-like receptor, NOD-like receptor, and C-type lectin pathway. On the other hand, in susceptible rainbow trout, the immune response was delayed and weaker.

10.2 Immunological Studies

Modern immunological studies have provided new insights into the process of mucosal immunity toward I. multifiliis. Immunological research on rainbow trout has uncovered the immune responses of the eye, buccal and pharyngeal mucosa and found that IgT is the main antibody isotype responsible for mucosal immunity against I. multifiliis (Kong *et al.,* 2019; Yu *et al.,* 2019).

Non-immunological aspects of the disease immunity have been studied as well. Immune mechanisms of complement activation, involvement of phagocytes and their respiratory burst play an important role in parasite elimination, especially in the early stages of infection. Cytokine networks consisting of interleukins, interferons, and chemokines control these responses, and the profile of pro-inflammatory cytokines correlates with increased resistance (Buchmann *et al.,* 2001).

10.3 Novel Therapeutics

Alternative treatments for parasitism have led to the discovery of some potential compounds. A synthetic isoquinoline derivative named BHTCA synthesized by Peng *et al.,* (2025) was selective in terms of its effectiveness on different stages of I. multifiliis theronts (EC50 = 0.10 mg/L at 4 hours) and tomonts (EC50 = 0.40 mg/L at 24 hours). The in vivo studies showed a 78.1% reduction in parasites' load and host mortality by 66.7% with good therapeutic index (LC50 = 16.75 mg/L).

Another potential treatment is based on antimicrobial peptides isolated from fish hemoglobin that were found to be effective against I. multifiliis theronts in vitro. According to the study by Ullal and Noga (2010), a beta-hemoglobin peptide HbβP-1 had strong antiparasitic effect.

Table 5: Recent advances in molecular diagnostics, vaccine development and disease management for I. multifiliis (2018–2025)

Year	Research Area	Key Finding	Reference
2025	Novel therapeutics	Synthetic isoquinoline derivative BHTCA: 78.1% parasite load reduction, 66.7% mortality reduction	(Peng *et al.,* 2025)
2025	Transcriptomics	Comparative transcriptomics reveals early immune responses determine disease outcome; zebrafish clears infection via rapid immune activation	(Noethiger *et al.,* 2025)
2025	Molecular diagnosis	TaqMan qPCR targeting ICP2 gene: detection limit of 5.4 copies/μL and 4 theronts/L water	(Guo *et al.,* 2025)
2025	Histopathology/microbiome	I. multifiliis infection alters gill microbiota; increases opportunistic pathogens Aeromonas and Achromobacter	(Zhang *et al.,* 2025)
2024	Vaccine development	β-tubulin DNA vaccine (pVAX1-Bt) achieves 70% RPS in grass carp; enhances specific immunity and antioxidant activity	(Ye *et al.,* 2024)
2024	Biological control	In-situ confirmation of copepod predation on I. multifiliis theronts in fish-farming ponds	(Wang *et al.,* 2024)
2023	Molecular diagnosis	ddPCR vs qPCR comparison: ddPCR higher sensitivity for environmental samples; qPCR wider detection range	(Hu *et al.,* 2023)
2023	Mucosal immunity	Transcriptomic characterization of ocular mucosa immune responses in rainbow trout	(Zhang *et al.,* 2023)
2021	Alternative therapeutics	Peracetic acid treatment reduces I. Multifiliis and Trichodina, improving survival in Nile tilapia	(Abu-Elala *et al.,* 2021)
2021	DNA vaccine optimization	Higher dose (20 μg) and two-dose protocol improves protection (48.9% survival) vs single dose (15.6%)	(Xu *et al.,* 2021)
2020	Environmental biology	pH tolerance study: theronts survive pH 4–10; tomonts release theronts at pH 5–10; theront size increases with alkalinity	(Tange *et al.,* 2020)
2020	Vaccine development	DNA vaccine encoding IAg52b elicits anti-Ich antibodies and partial protection in channel catfish	(Xu *et al.,* 2020)

11. Challenges and Future Perspectives

Despite the tremendous progress made in studying and treatment of I. multifiliis, there is still an array of difficulties that need innovative approaches and further scientific work.

The creation of a marketable vaccine is the key challenge to address. Although the DNA-based vaccines based on i-antigens proved to have a certain level of protection, their efficacy of 35-70% is too low for its practical application in a case when more effective protection is needed. Moreover, the diversity in serotypes of the parasite isolates makes it difficult to develop a vaccine because protection is likely to be serotype-dependent (Wang *et al.,* 2002).

The emergence of drug resistance is another challenge despite the few records of drug resistance among I. multifiliis in comparison to other aquaculture parasites like sea lice. The repetitive use of drugs to control I. multifiliis leads to selective pressure, which promotes drug resistance in the parasites. In order to ensure sustainability, it is important to employ therapeutic drugs and other methods of control without chemicals (Picon-Camacho *et al.,* 2012).

Climate change can have both positive and negative consequences on the incidence of I. multifiliis in the aquatic environment. Warmer waters due to climate change can promote faster growth of the parasite life cycle, leading to longer transmission periods and increased incidence of the disease in temperate climates. On the other hand, severe weather conditions can affect the dynamic of the aquaculture systems.

Sustainable aquaculture that is environmentally friendly requires reduced use of chemicals. Research and development of biological control techniques, probiotics, feeding additives to stimulate immune response and disease-resistant breeds are some of the promising directions that can help achieve this goal.

Research Gaps and Future Research Priorities

The following research priorities were highlighted by the review:

Vaccine Limitations: The current protection offered by DNA vaccine candidates is not good enough and ranges between 35 percent and 70 percent. Improvements should be made with regards to the development of antigens, use of adjuvants, and mass administration of vaccines. Scientists should investigate the possibilities of developing multi-antigen vaccines, delivery of conserved epitopes, mucosal delivery methods, and mRNA vaccines.

Drug Resistance Concerns: The detection of evolving drug resistance among I. multifiliis populations has not been systematically monitored. There is no baseline data available on the susceptibility of field isolates to commercially available drugs. Standardization of procedures for resistance testing needs to be addressed urgently to develop guidelines for effective treatment.

Climate Change Impacts: Effects of climate change on I. multifiliis epidemiology have not yet been well documented. Areas to be explored include effects of temperature on development rate of parasites, range expansion, effects of altered immune response of hosts and effect of warming on existing control measures.

Sustainable Disease Management Approaches: Integrated approaches to disease management using biosecurity measures, biological control agents, genetic resistance and specific therapies need to be developed and validated through commercial production systems. It is necessary to undertake economic evaluations of traditional chemical-dependent systems versus integrated systems to provide incentives to companies to adopt sustainable practices. Precision technologies for aquaculture that enable detection of disease problems and automation of treatments could be a way forward.

12. CONCLUSIONS

I. multifiliis remains one of the major economically significant and clinically difficult diseases for the freshwater aquaculture sector globally. The high adaptability of the parasite, in terms of a wide host range, rapid reproduction rate, and resistance to the environment throughout different life cycle stages, necessitates monitoring and integration into management strategies.

Some of the progress that has been achieved in terms of biology of the parasites, host-parasite interactions and disease management has been presented in this review. There is the possibility of early diagnosis using molecular diagnostic methods. Peracetic acid and hydrogen peroxide are environmentally friendly substitutes compared to traditional chemicals. There have been some promising antigens developed from vaccine studies.

In the future, sustainable management of I. multifiliis would need the application of a combination of several approaches that could work best in particular environments of production. The reduction of chemical use in the process of disease control through biosecurity measures, biological control enhancement, genetic improvement of hosts' resistance, and precision aquaculture techniques provides a promising approach for environmentally friendly disease management. It is essential to fill in some of the existing research gaps concerning vaccine development, monitoring drug resistance, climate change adaptation, and integrated management approach testing.

REFERENCES

- Abdel-Hafez, E. S., Fawzia, A., Nadia, A., & Abdel-Rahman, A. M. (2014). Protozoal parasites and symbionts of freshwater fish in Egypt with special reference to their intensity and pathogenicity. Egyptian Journal of Aquatic Biology and Fisheries, 18(2), 125–142.
- Aihua, L., & Buchmann, K. (2001). Temperature-dependent expression of heat-shock proteins in the ciliate Ichthyophthirius multifiliis. Diseases of Aquatic Organisms, 46(3), 213–219.
- Buchmann, K., Sigh, J., Nielsen, C. V., & Dalgaard, M. (2001). Host responses against the fish parasitizing ciliate Ichthyophthirius multifiliis. Veterinary Parasitology, 100(1–2), 105–116.
- Buchmann, K., Jaafar, R. M., & Kania, P. W. (2022). Adaptive immune responses and vaccine-induced protection against Ichthyophthirius multifiliis. Fish & Shellfish Immunology, 122, 68–76.
- Chapman, G. B., & Kern, R. C. (1983). Ultrastructural aspects of the somatic cortex and contractile vacuole of the ciliate Ichthyophthirius multifiliis Fouquet. Journal of Protozoology, 30(3), 481–490.
- Clark, T. G., Lin, T.-L., & Dickerson, H. W. (1995). Surface immobilization antigens of Ichthyophthirius multifiliis: Their role in protective immunity. Annual Review of Fish Diseases, 5, 113–131.
- Clark, T. G., McGraw, R. A., & Dickerson, H. W. (1992). Developmental expression of surface antigen genes in the parasitic ciliate Ichthyophthirius multifiliis. Proceedings of the National Academy of Sciences, 89(14), 6363–6367.
- Clark, T. G., Gao, Y., Gaertig, J., Wang, X., & Cheng, G. (2001). The I-antigens of Ichthyophthirius multifiliis are GPI-anchored

proteins. Journal of Eukaryotic Microbiology, 48(3), 332–337.

- Corrêa, L. L., Tavares-Dias, M., & de Melo, F. R. (2019). An outbreak of Ichthyophthirius multifiliis (Ciliophora) in fingerlings of Steindachneridion parahybae in Brazil. Annals of Parasitology, 65(4), 417–422.
- Coyne, R. S., Hannick, L., Shanmugam, D., et al. (2011). Comparative genomics of the pathogenic ciliate Ichthyophthirius multifiliis, its free-living relatives and a host species provide insights into adoption of a parasitic lifestyle and prospects for disease control. Genome Biology, 12(10), R100.
- Dickerson, H. W. (2006). Ichthyophthirius multifiliis and Cryptocaryon irritans (Phylum Ciliophora). In P. T. K. Woo (Ed.), Fish diseases and disorders: Volume 1. Protozoan and metazoan infections (2nd ed., pp. 116–153). CABI Publishing.
- Dickerson, H. W., Clark, T. G., & Leff, A. A. (1993). Serotype variation among isolates of Ichthyophthirius multifiliis based on immobilization. Journal of Eukaryotic Microbiology, 40(6), 816–820.
- Abu-Elala, N. M., Abdelaziz, M., Marouf, S., & Sallam, M. E. (2021). Peracetic acid treatment of Ichthyophthirius multifiliis (Ciliophora: Ichthyophthiriidae) and Trichodina spp. reduces the infection by Aeromonas hydrophila and improves survival in Nile tilapia (Oreochromis niloticus). Aquaculture, 536, 736439.
- Everett, K. D. E., & Dickerson, H. W. (2002). Ichthyophthirius multifiliis and Tetrahymena thermophila tolerance of cryopreservation. Journal of Parasitology, 88(1), 41–46.
- Ewing, M. S., & Kocan, K. M. (1992). Invasion and development strategies of Ichthyophthirius multifiliis, a parasitic ciliate of fish. Parasitology Today, 8(6), 204–208.
- Food and Agriculture Organization. (2024). The state of world fisheries and aquaculture 2024. FAO.
- Francis-Floyd, R., Yanong, R., & Klinger, R. (2016). Ichthyophthirius multifiliis (White Spot) infections in fish. University of Florida IFAS Extension, FA006.
- Guo, S. Q., Zhang, Q. Z., Fu, Y. W., Hou, T. L., & Huang, S. L. (2025). Establishment and application of TaqMan probe-based quantitative real-time PCR for rapid detection and quantification of Ichthyophthirius multifiliis in farming environments and fish tissues. Veterinary Parasitology, 325, 110270.
- Hines, R. S., & Spira, D. T. (1974). Ichthyophthirius multifiliis (Fouquet) in the mirror carp, Cyprinus carpio L. V. Acquired immunity. Journal of Fish Biology, 6(4), 373–378.
- Hu, G., Zheng, T., Zhai, Y., et al. (2023). Comparison of droplet digital PCR and real-time quantitative PCR for quantitative detection of the parasitic ciliate Ichthyophthirius multifiliis in the water environment. Journal of Fish Diseases, 46(4), 357–367.
- Jousson, O., Lechenne, B., Bontems, O., et al. (2005). Non-invasive detection and quantification of the parasitic ciliate Ichthyophthirius multifiliis by real-time PCR. Diseases of Aquatic Organisms, 65(3), 253–256.
- Kong, X., Qiao, K., Wang, J., & Liu, Y. (2019). Mucosal immunoglobulin T in teleost fish: A review. Fish & Shellfish Immunology, 92, 718–727.
- Lahnsteiner, F., & Weismann, T. (2007). Treatment of ichthyophthiriasis in rainbow trout and common carp with common and alternative therapeutics. Journal of Aquatic Animal Health, 19(3), 186–194.
- Leao, J. A., da Silva Junior, E. A. N., & de Moraes, F. R. (2015). Outbreaks of Ichthyophthirius multifiliis in farmed fish in Brazil. Revista Brasileira de Parasitologia Veterinaria, 24(3), 355–361.
- Li, A. H., & Buchmann, K. (2001). Temperature-dependent development of Ichthyophthirius multifiliis: A role for saline environments? Parasitology Research, 87(9), 751–753.
- Lin, Y., Wang, X., Clark, T. G., & Dickerson, H. W. (2002). Variation in primary sequence and tandem repeat copy number among i-antigens of Ichthyophthirius multifiliis. Molecular and Biochemical Parasitology, 119(2), 213–220.
- Lom, J., & Dykova, I. (1992). Protozoan parasites of fishes. Elsevier.
- Maezono, M., Ueki, N., Masukane, T., et al. (2025). The current state of knowledge of the economic impact of diseases in global aquaculture. Reviews in Aquaculture, 17(1), e70039.
- MacLennan, R. F. (1935). The morphology and life cycle of Ichthyophthirius multifiliis, a ciliate parasitic on fish. PhD thesis, University of Toronto.
- Maharsi, S. (1979). Studies on Ichthyophthirius multifiliis. PhD thesis, University of Indonesia.
- Matthews, R. A. (2005). Ichthyophthirius multifiliis Fouquet 1876: Infection and protective responses within the fish host. In P. T. K. Woo (Ed.), Fish diseases and disorders: Volume 2. Protozoan and metazoan infections (pp. 116–153). CABI Publishing.
- Meyer, F. P. (1969). Ichthyophthirius multifiliis: A study of the reproductive and life cycle. PhD thesis, University of Minnesota.
- Noe, J. G., & Dickerson, H. W. (1995). Life cycle of Ichthyophthirius multifiliis. In P. T. K. Woo (Ed.), Fish diseases and disorders: Volume 1. Protozoan and metazoan infections (pp. 135–153). CABI Publishing.
- Noethiger, M. T., von Gersdorff Jorgensen, L., et al. (2025). One dies, the other survives – A comparison of the early transcriptomic immune responses in zebrafish and rainbow trout infected with Ichthyophthirius multifiliis. Fish & Shellfish Immunology, 152, 109987.

- Noga, E. J. (1996). Fish disease diagnosis and treatment. Mosby-Year Book.
- Pedersen, L. F., Meinelt, T., & Malte, H. (2019). Hydrogen peroxide in recirculating aquaculture systems. Global Seafood Alliance.
- Peng, X., Wang, Y., Li, J., et al. (2025). Effects of a synthetic isoquinoline derivative against Ichthyophthirius multifiliis in grass carp. Aquaculture, 624, 111937.
- Picon-Camacho, S. M., Marcos-Lopez, M., & Shinn, A. P. (2012). An assessment of the use of drugs and non-drug interventions in the treatment of Ichthyophthirius multifiliis Fouquet, 1876, a protozoan parasite of freshwater fish. Parasitology, 139(2), 149–190.
- Qasim, M., Manzoor, S., Nabeel, M. I., Hussain, S., Waqas, R., Joseph, C. G., & Suazo-Hernández, J. (2025). Harnessing High-Valent Metals for Catalytic Oxidation: Next-Gen Strategies in Water Remediation and Circular Chemistry. *Catalysts*, *15*(12), 1168.
- Qasim, M., Manzoor, S., Nabeel, M. I., Ullah, Q., Sair, Y. Z., Khomphet, T., ... & Gulzar, T. (2026). Waste-derived nanocomposites as dual-function materials for water and energy sustainability. *Frontiers in Sustainability*, *7*, 1777462.
- Shinn, A. P., Pratoomyot, J., Bron, J. E., et al. (2015). Economic impacts of aquatic parasites on global finfish aquaculture production. Journal of Fish Diseases, 38(10), 831–875.
- Straus, D. L., & Meinelt, T. (2009). Acute toxicity of peracetic acid (PAA) formulations to Ichthyophthirius multifiliis theronts. Parasitology Research, 104(6), 1237–1241.
- Sudova, E., Piackova, V., Kroupova, H., & Papezikova, I. (2010). Evaluation of continuous 4-day exposure to peracetic acid as a treatment for Ichthyophthirius multifiliis. Parasitology Research, 106(2), 539–542.
- Tange, N., Buchmann, K., & Kania, P. W. (2020). pH tolerance of Ichthyophthirius multifiliis and the influence on future distribution. Parasitology Research, 119(8), 2487–2495.
- Ullal, A. J., & Noga, E. J. (2010). Antiparasitic activity of the antimicrobial peptide HbβP-1, a member of the β-hemoglobin peptide family. Journal of Fish Diseases, 33(8), 657–664.
- Ventura, M. T., & Paperna, I. (1985). Histopathology of Ichthyophthirius multifiliis infections in fishes. Journal of Fish Biology, 27(2), 185–203.
- von Gersdorff Jorgensen, L., Sigh, J., Kania, P. W., et al. (2012). Approaches towards DNA vaccination against a skin ciliate parasite in fish. PLoS ONE, 7(11), e48129.
- Wahli, T., Schmitt, M., & Meier, W. (1995). A comparison of the effect of silicone coated ascorbic acid and ascorbyl phosphate on the course of ichthyophthiriosis in rainbow trout, Oncorhynchus mykiss. Journal of Fish Diseases, 18(4), 347–355.
- Wang, X., & Dickerson, H. W. (2002). Immunization of channel catfish Ictalurus punctatus with Ichthyophthirius multifiliis immobilisation antigens elicits serotype-specific protection. Fish & Shellfish Immunology, 13(5), 337–350.
- Wang, L., Cao, Y., et al. (2024). In-situ investigation of copepod predators of Ichthyophthirius multifiliis theronts from fish-farming pond. Microorganisms, 13(1), 38.
- Xu, D.-H., & Klesius, P. H. (2006). Susceptibility of Nile tilapia to infection of the parasite Ichthyophthirius multifiliis. Journal of the World Aquaculture Society, 37(3), 260–263.
- Xu, D.-H., Shoemaker, C. A., Klesius, P. H., et al. (2020). Dose effects of a DNA vaccine encoding immobilization antigen on immune response of channel catfish against Ichthyophthirius multifiliis. Fish & Shellfish Immunology, 103, 47–54.
- Xu, D.-H., Shoemaker, C. A., & Klesius, P. H. (2021). Enhanced protection of channel catfish against Ichthyophthirius multifiliis using a higher dose DNA vaccine. Aquaculture, 531, 735875.
- Yanong, R. P. E. (2023). Ichthyophthirius multifiliis (White Spot) infections in fish. University of Florida IFAS Extension, FA006.
- Ye, J., Zhang, J., et al. (2024). Preparation and immunological study of Ichthyophthirius multifiliis β-tubulin DNA vaccine in grass carp (Ctenopharyngodon idella). Aquaculture Reports, 37, 102231.
- Yu, Y., et al. (2019). Mucosal immune response in buccal and pharyngeal cavities of rainbow trout following Ichthyophthirius multifiliis infection. Fish & Shellfish Immunology, 94, 752–761.
- Zhang, Q., et al. (2023). Mucosal immune responses to Ichthyophthirius multifiliis in the ocular mucosa of rainbow trout. Marine Life Science & Technology, 5(4), 452–466.
- Zhang, X., et al. (2025). Effect of ectoparasite Ichthyophthirius multifiliis on the histopathology and gill and gut microbiota of goldfish (Carassius auratus). Frontiers in Veterinary Science, 12, 1539446.
- Zhao, X., et al. (2009). Molecular cloning and characterization of a cathepsin L-like cysteine protease from Ichthyophthirius multifiliis. Parasitology Research, 105(4), 1183–1190.