ParaFishControl

GUIDE 3 – FISH FARMER'S GUIDE TO COMBATING PARASITIC INFECTIONS IN COMMON CARP AQUACULTURE

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GOBIERNO DE ESPAÑA · MINISTERIO DE CIENCIA E INNOVACIÓN · CSIC

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A Series of ParaFishControl Guides to Combating Fish Parasite Infections in Aquaculture. **Guide 3**

ParaFishControl

“Wherever the fish are, that's where we go.”

Richard Wagner

Common carp is the third most cultivated freshwater species in the world. Carp aquaculture is usually performed in a semi-intensive manner, in earthen ponds, where parasitic diseases can easily compromise fish health, especially in the hot summer months, leading to production and economic losses. This guide provides useful information about the biological background of five parasites, their diagnostics and control measures.

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Introduction

Our relationship with common carp has a long history and tradition. Carp was considered luxury food in the middle and late Roman period. Later, fish ponds were constructed and maintained by Christian monasteries. Controlled semi-natural pond breeding and fry rearing of carp in Europe started in the 19th century. Today, common carp amounts to 4% of European aquaculture, and it is the favourite Christmas dish in Czech Republic, Slovakia and Poland, as well as in some regions of Austria, Germany and Hungary.

Originally native to temperate regions of Asia, common carp, *Cyprinus carpio* (Cypriniformes, Cyprinidae), is the most cultivated and refined carp species throughout the world. It represents the oldest domesticated species of fish for human consumption (identified in China, from the 5th century BC).

Carp production is comprised of ornamental fish industry, sport fishing and food production. Koi carp (*Cyprinus rubrofuscus*) represents one of the most expensive and widely used ornamental fish in garden ponds and public water bodies. However, the food fish industry is the largest sector of carp production. Common carp is the third most widely cultivated and the most commercially important freshwater fish species in the world. With approximately 4.6 million tonnes produced annually, carp contributes to 8% of the world's total finfish aquaculture production, with a gradual rise in production and economic value every year. EU production of common carp reached 85 thousand tonnes in 2016, which represents 4% of the global EU aquaculture production (Source: FAO). The leading producers within the EU are Poland and Czech Republic.

Globally, rearing systems of common carp are highly diversified. Carp production in the EU is accomplished in extensive or semi-intensive pond monoculture or polyculture systems with semi-intensive polyculture as the dominant form of management. In polyculture systems, common carp is the main species reared, associated with other cyprinids such as Chinese carps including grasscarp (*Ctenopharyngodon idella*), bighead carp (*Hypophthalmichtys nobilis*) and silver carp (*Hypophthalmichthys molitrix*), tench (*Tinca tinca*), roach (*Rutilus rutilus*), rudd (*Scardinius erythrophthalmus*) and various piscivorous fish including northern pike (*Esox lucius*), Eurasian perch (*Perca fluviatilis*), pikeperch (*Sander lucioperca*), or catfish. In Europe, reproduction takes place once a year between May and June, and marketable fish are produced over one or two summer seasons resulting in a three to four-year production cycle. Production typically reaches 600 kg/ha with maximum levels reaching 1000 kg/ha.

The features of carp aquaculture that make it a profitable industry – excessive stocking density in non-flowing, organically enriched waters with minimal nutrient food input – also represent high risk factors, promoting various pathogenic infections and enhancing disease development. Diseases are the major limiting factors for future carp aquaculture, and they are anticipated to emerge with increasing environmental temperatures. Alongside viral and bacterial pathogens, parasitic diseases are of particular importance, and development of targeted anti-parasite strategies is crucial to meet the constantly increasing demand for carp production.

The **ParaFishControl** project has investigated important carp parasites and developing new diagnostic tools and control systems for infections. In this manual, fish farmers can find important biological information on some of the main parasitic diseases of common carp in European countries. Profiles include the symptoms, correct identification of pathogens, the biology and life cycle of the parasite, and recommendations for control. This manual does not provide comprehensive scientific details but can serve as a practical guide that provides support during the daily handling and management of carp. The guidance provided for individual parasites reflects the current state of knowledge for these pathogens and has been informed, in part, by research conducted through the **ParaFishControl** project.

Guides are provided for five parasites: 1. *Sphaerospora molnari*, a myxozoan microscopic parasite that causes skin and gill sphaerosporosis in carp, 2. *Thelohanellus kitauei*, another myxozoan that is recognized as the agent of intestinal giant cystic disease, with insights on other thelohanellids commonly infecting carp, 3. *Ichthyophthirius multifiliis*, an economically important ciliate ectoparasite, 4. *Saprolegnia parasitica*, a fungal-like oomycete, and 5. zoonotic helminths.

Each guide provides a background on the parasite's biology, an examination of key risks for infection and disease progression and up-to-date guidance for the management and control of the parasite. Since the emergence of parasitic disease is dependent on many different factors including site, stock and environmental factors,instigation of any of the suggested control measures should be accomplished with the assistance and guidance of suitable fish health professionals, including veterinary practitioners and farm based health and welfare professionals. These guides also provide details for expert contacts within Europe who may be consulted for further support.

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1. Fish farmer's guide to combating *Sphaerospora molnari* infections

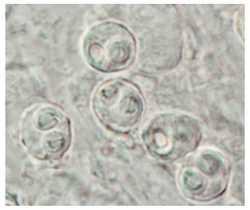

Figure 1. Mature spores of *Sphaerospora molnari* in a fresh squash preparation of gills from carp fingerlings (photo: A.S. Holzer, Biology Centre of the Czech Academy of Sciences).

Introduction

Sphaerospora molnari (Fig. 1) is a myxozoan obligate parasite that belongs to the Cnidaria (relatives of jellyfish). It is a significant pathogen of juvenile common carp causing skin and gill sphaerosporosis / inflammation, haemolytic anaemia, and may contribute to swim bladder inflammation. The disease has mainly been reported from Central Europe (Czech Republic, Poland, Hungary, Germany) and less frequently from south-eastern European countries (e.g. Bulgaria and Serbia). First year carp fingerlings and, to a lesser extent, second year fish are affected. The disease occurs predominantly in the summer months but in April / May outbreaks may also occur since the fish immune system is compromised during colder winter months.

Biological life cycle

The complete life cycle of myxozoans typically includes an invertebrate (often an annelid worm) and a vertebrate (usually fish) host, but the invertebrate host has not yet been elucidated for *S. molnari*. Direct transmission of spores from fish to fish has been attempted several times, unsuccessfully. Therefore, the infective spore and its fish invasion site are unknown, though the gills are believed to be the portals of entry into fish. The development of *S. molnari* in its fish host is well documented (Figure 3). The parasite is first detected in the vascular system about 3-4 weeks after infection or exposure to *S. molnari-enzooic* waters. The parasite can easily be detected in blood smears, where multicellular stages from 2 to 20 cells (most commonly 2) can be found, which show a typical cell-in-cell composition (Figure 2). These stages rapidly increase in number in the blood. Blood stages are highly motile. They move in a characteristic twitching or 'dancing' mode, attaching to, and feeding on, red blood cells. The peak of blood stage infection occurs around 4 weeks post-infection, after which blood stages invade the gills. In natural infections, spores are known to form between the epithelial cells of the gills (and areas of the skin that are adjacent to the gills), 6-8 weeks after infection. In the laboratory model, large stages have been detected in the liver, which may present a parasite reservoir. Sporogonic stages in the gills settle in the intercellular space along the gill filaments, between the gill lamellae, and less frequently at the gill arch and branchial cavity. Myxospores are formed and released into the environment by rupture of host epithelium, and measure about 10 µm in length and thickness. These myxospores produced in carp likely infect a benthic annelid worm, but cannot be transmitted from fish to fish.

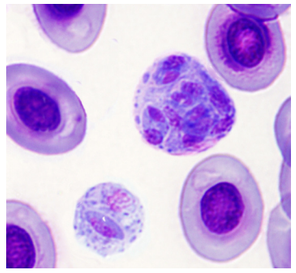

Figure 2. Quick-Diff stained blood smear of carp showing multicellular blood stages of *Sphaerospora molnari* amongst red blood cells (photo: A.S. Holzer, Biology Centre of the Czech Academy of Sciences).

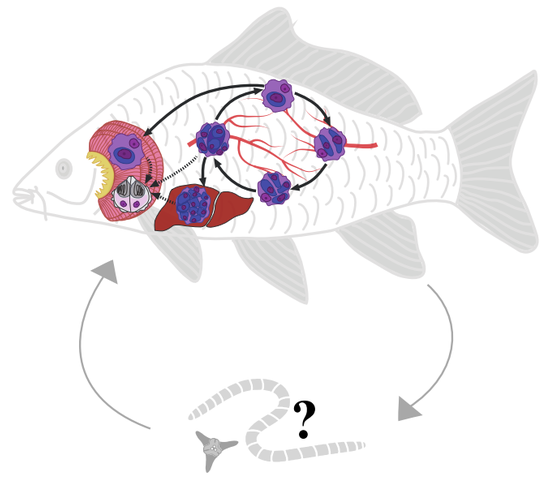

Figure 3. Life cycle of *Sphaerospora molnari.* Multicellular blood stages invade the gills and the liver. Liver stages are assumed to re-enter the blood stream to target the gill. Spore formation takes place exclusively in the gills and adjacent areas of the skin, from where spores are released into the environment. These spores likely infect an annelid worm alternate host, which is presently unknown. (Illustration: P. Bartošová-Sojková and M. Lisnerová, Biology Centre of the Czech Academy of Science).

Seasonality

S. molnari first infects fry in July / August. In overwintered fish, blood stages peak once again in early spring, presumably from intrapiscine reservoirs such as the liver. Parasite prevalence decreases in the winter months. Gill sphaerosporosis occurs in late spring (1-year-old fish) and summer time (fingerlings).

Age / mean weight susceptibility

S. molnari is strictly host-specific and infects predominantly common carp fry and fingerlings in the first summer, and possibly again in the second summer, but not later. Laboratory models have demonstrated that infected fish become immuno-competent, hence mortalities in pond cultures rarely occur after the second summer. However, the parasite is never completely cleared from the fish and may well emerge under unfavourable conditions later in life.

Risk predisposing factors

Low oxygen levels and high levels of nitrite are the main risk factors. Nitrite binds preferably to haemoglobin when oxygen levels are low, resulting in a reduced capacity for oxygen transfer. This is especially critical in summer when the dissolved oxygen levels are generally low and algal blooms frequently occur. Increased water temperature is one of the main factors linked with accelerated proliferation of *S. molnari* and disease severity. At 20 °C, parasite numbers peak at 28 days, while it takes 70 days to reach a peak at 10 °C.

A) What clinical signs should alarm me?

External signs

Severe anaemia (pale gills) and hyperventilation, slow reaction speed of fish to disturbance. Macroscopically visible white foci on gills during spore formation.

Internal lesions

Visible enlargement of head kidney, trunk kidney and liver. Pale appearance of blood and internal organs due to reduction of erythrocytes.

B) How to detect the parasite at farm level

1. Monitoring plan (what to measure and how often) and trigger level for action

Routine biweekly monitoring of juvenile fish (checking of skin and gills) may reveal some of the external signs listed above. Dissection of moribund fish following euthanasia will show clear clinical internal signs. Presence of the parasite can be confirmed in gill scrapes and blood smears, with molecular confirmation by PCR. Using PCR, the parasite can also be detected in water samples.

2. Recommendations for the submission of samples to be diagnosed

Prepare air-dried or methanol-fixed imprints of carp gills and blood smears. Eventually, gill tissue or a drop of full blood (weight/volume 1:10) can be collected and fixed in ethanol for molecular analysis.

3. Contact laboratories

- Fish Protistology Lab, Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, **České Budějovice**, Czech Republic.
- Fish Pathology and Parasitology Research Team, Institute for Veterinary Medical Research, Centre for Agricultural Research (former Hungarian Academy of Sciences), Hungary

C) Action plan after diagnosis

1. Prevention and farm management

Preventive strategies and farm management include: i) ensuring good water quality standards, especially with regard to nitrates / ammonia and oxygen (ensure flow through pond and aeration of water), ii) avoiding temperature stress by providing shading and deep water areas, iii) elimination of spores from the water by ozonation, and / or decreasing invertebrate numbers in sediments by annual draining, drying and liming of ponds, iv) daily removal of dead and moribund fish from the system.

Vaccine developments are under way and may contribute to efficient disease prevention in the future.

2. Treatment

Effective, legal treatments against *S. molnari* infections in carp destined for human consumption are not available. The commonly used antibiotic, fumagilin from *Aspergillus fumigatus* can be used in-feed to prevent and reduce natural infections of the congener *S. dykovae* and also effective also against *S. molnari* (laboratory trial), at an early stage.

3. Management of co-infections

Common co-infection with *S. dykovae* can lead to swim bladder-infection of carp, a serious disease, which is managed in the same way as *S. molnari* infection. *Dactylogyrus spp.* (ectoparasitic flatworm) and *Ichthyophthirius multifiliis* (ectoparasitic ciliate) commonly occur in co-infections with *S. molnari* and worsen a host carp's condition by immunocompromising its health status. Novel biological compounds, investigated in **ParaFishControl**, such as microbial surfactants can kill external life cycle stages of *I. multifiliis* and likely the monogeneans (see section on *I. multifiliis*).

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2. Fish farmer's guide to combating *Thelohanellus* infections, with special emphasis on *Thelohanellus kitauei*

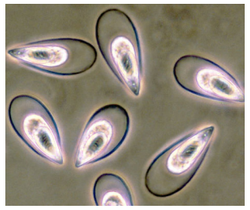

Figure 4. Mature spores of *Thelohanellus kitauei* in a fresh squash preparation of a cyst from the intestine (photo: M. Lisnerová, Biology Centre of the Czech Academy of Sciences).

Introduction

Thelohanellus is a myxozoan genus of endoparasites infecting different fish tissues. Most *Thelohanellus* species that affect common carp are described from Asia. In European carp stocks, the occurrence of two species is common: *Thelohanellus hovorkai* and *T. nikolskii*. The presence of a third species, *T. kitauei* (Figure 4), was recently reported in water and invertebrate samples from the natural waters of Czech Republic, Germany and Hungary, as an outcome of **ParaFishControl** research. In Asia, *T. kitauei* infection of carp results in intestinal giant cystic disease, causing a pathology whereby large parasite cysts in the intestinal tract obstruct the alimentary canal, leading to large mortalities. It is the most pathogenic species of *Thelohanellus* in carp described so far, however, in Europe, intestinal giant cystic disease has not been reported. A different strain of *T. kitauei* has been shown to infect the skin of common carp.

Biological life cycle

The complete life cycle of myxozoans usually includes a fish and an invertebrate host (Figure 5). During research as part of the **ParaFishControl** project, the life cycle of *T. kitauei* was discovered and shown to involve the oligochaete worm, *Branchiura sowerbyi*. Myxospores, produced in the intestinal tract of carp, are released into the environment via faeces or upon death of the fish. These myxospores lack appendages and sink to the ground where the worm host lives and takes them up during sediment feeding. In the worm, actinospores are produced in the digestive epithelium and are also released with faeces. Actinospores have appendages which allow them to float in the water column and hence infect the fish host by attaching to skin and gills. Other *Thelohanellus* spp. have a similar life cycle, though they have different host target organs. *T. hovorkai* is a parasite of the connective tissue, and its plasmodia are found inter-muscularly and on the serosa of the intestinal wall (Figure 6). *T. nikolskii* infects the fins and skin of common carp.

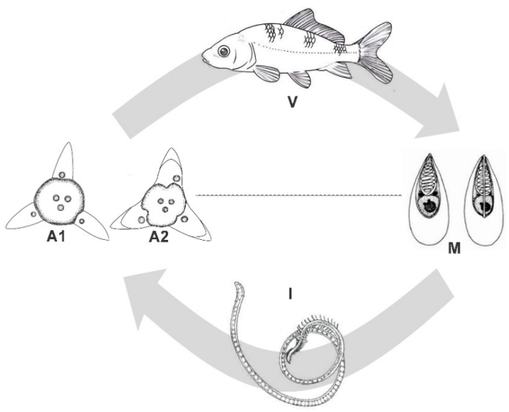

Figure 5. Schematic illustration of the life cycle of *Thelohanellus kitauei*, alternating between fish and worm hosts. Sporogonic stages in common carp develop in large intestinal cysts or in scale pockets in the skin and myxospores are released and infect the invertebrate, *Branchiura sowerbyi*. V=vertebrate host (common carp), M=myxospospores, I=invertebrate host, A1/A2=actinospores. (Drawing by H. Borkhanuddin, Fish Pathology and Parasitology Research Team, Institute for Veterinary Medical Research, Centre for Agricultural Research (former Hungarian Academy of Sciences), Hungary).

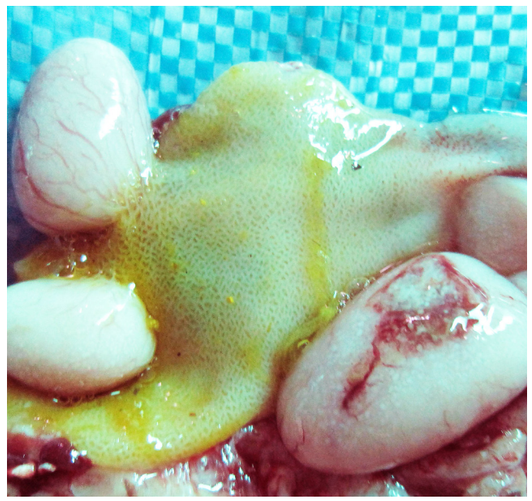

Figure 6. *Thelohanellus kiatuei* giant cysts in the digestive tract of common carp (photo: Dr. Yang Liu, Huazhong Agricultural University).

Seasonality

T. nikolskii has a specific seasonality. Plasmodia with myxospores appear on the fins of 4 to 6 weeks-old fingerlings in July and August and persist until September. In older age groups of carp, plasmodia develop in May. After bursting, they release myxospores, and from the start of June signs of infection are no longer observable. During the period from October to March and from June to July, development of actinospores takes place inside the oligochaetes, a process dependent on water temperature. The seasonality of *T. hovorkai* infection shows the same pattern. In young fish intramuscular cysts are predominant, in old fish abdominal cysts are more frequent. No data is available on seasonality of *T. kitauei* in Europe, but actinospores have been found in *B. sowerbyi*, during summer time.

Age / mean weight susceptibility

All age groups are susceptible to infection. Infection of fingerlings can reach 100 %, in older fish infection appears to be limited to selected stocks. Year-old fish are the most affected age group for *T. kitauei*.

Risk predisposing factors

High stocking density of fish farms and high organic load in ponds are the primary predisposing factors, due to the accumulation of faeces and uneaten food in the sediments providing an optimal niche for oligochates and their reproduction. Intensity of infection depends on the number of infected oligochaete hosts.

A) What clinical signs should alarm me?

External signs

In the case of *T. nikolskii*, large numbers of cysts on fins or the fragmentation of fins are easily recognized (Figure 7). Signs on the scales are less obvious. In *T. hovorkai* and *T. kitauei* infections, the external signs are rare, but haemorrhages may occur.

Internal signs

In *T. hovorkai*, plasmodia may cause haemorrhages with fluids in the abdominal cavity. Less severe infections show no observable clinical signs. In *T. kitauei*, large intestinal cysts packed with spores are easily detectable.

Figure 7. Cysts of *T. nikolskii* on the skin of common carp (photo: C. Székely, Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungary).

B) How to detect the parasite at farm level

Monitoring plan (what to measure and how often) and trigger level for action

Routine biweekly monitoring of juvenile fish (checking of skin and gills) will reveal infections of the skin, scales and fins (*T. nikolskii, T. kitauei*). Dissection of moribund fish following euthanasia will show clear clinical internal signs (*T. kitauei, T. hovorkai*). Presence of *Thelohanellus* spores can be confirmed using a light microscope for fresh skin scrapes or for smears of cyst contents. Molecular species confirmation is performed by PCR, which can also be used for detection of the parasites in water or annelid worms.

2. Recommendations for the submission of samples to be diagnosed

Prepare air-dried or methanol-fixed smear preparations of cysts and skin scrapes. Cysts and tissue pieces can be collected and fixed in ethanol for molecular species identification.

3. Contact laboratories

- Fish Pathology and Parasitology Research Team, Institute for Veterinary Medical Research, Centre for Agricultural Research (former Hungarian Academy of Sciences), Hungary
- Fish Protistology Lab, Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, **České Budějovice**, Czech Republic.

C) Action plan after diagnosis

1. Farm management for prevention

Prevention and control of the disease may be achieved through adoption of a tailored farm management strategy and development of an appropriate production plan. The reduction of the number of invertebrate hosts can cause a decrease in fish-infective spore stages. Annual draining, drying and liming of ponds is recommended.

2. Treatment

Effective treatments against *Thelohanellus* infections in carp are not available.

3. Management of co-infections

Co-infections with other myxozoans infecting gills and other internal organs are common, but also other ectoparasites, such as *Dactylogyrus* spp. (flatworm) and *Ichthyophthirius multifiliis* (ciliate), which can settle in large numbers on the gills. For management of these ectoparasites, microbial surfactants may be effective (see section on *I. multifiliis*).

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3. Fish farmer's guide to combating *Ichthyophthirius multifiliis* infections

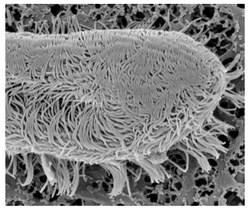

Figure 8. Scanning electron microscope image of *Ichthyophthirius multifiliis* (photo: Ole S. Møller, University of Copenhagen).

Introduction

I. multifiliis is a freshwater parasite able to infect the skin and gills of all freshwater fish tested so far. It causes problems both in flow-through systems and in recirculated systems. It causes a disease commonly referred to as white spot disease due to the macroscopically visible trophonts in the skin and fins. It can survive in a temperature range from 1 to 30 °C, but, as a thermophilic species, it needs temperatures above 15 °C to propagate fast and efficiently.

The main species affected in European aquaculture are rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*), perch (*Perca fluviatilis*), pikeperch (*Sander lucioperca*), European eel (*Anguilla anguilla*), common carp (*Cyprinus carpio*), and European catfish (*Silurus glanis*). Although the infection has been considered one of the worst parasitic diseases in these species due to the frequent use of earth pond systems for culture, it is now even more serious due to the use of recirculation systems in which infective parasitic stages become continuously recirculated. This can cause a major increase in fish exposure to the parasite.

Biological life cycle

I. multifiliis is a single-celled parasite. It is covered with numerous hair-like cilia (Figure 8), firmly attached to its external cell membrane, and belongs to the taxonomic group termed Ciliophora (organisms carrying cilia). The beating of these cilia allow the parasite to move and swim. The parasite has a characteristic horseshoe shaped nucleus and several micronuclei. The genus comprises only one species and it has its own family, Ichthyophthiriidae, which also includes its marine counterpart *Cryptocaryon irritans* (which is strictly marine and needs salinities near 30 ppt). *I. multifiliis* is unspecific in its host choice and can infect all freshwater fish species tested so far. The life cycle of the parasite is direct, which means it can be transmitted from fish to fish. It includes a trophont stage residing in the fish surface (gill epithelia, skin and fin epidermis). This stage is the feeding stage which continuously ingests cellular debris and live host cells in its epidermal location, making the parasite able to grow rapidly over a short time - depending on temperature.

Figure 9. *I. multifiliis* on common carp: Large numbers of trophonts (white spots) in the skin of common carp (photo: Fish and Bee Diseases Laboratory, Central Vetarinary Institute, Hungary).

When the trophont has reached a certain size (100-1000 µm), it will break out of the host epidermis and swim freely as a tomont (also covered by cilia). After minutes to hours, the tomont attaches to any surface in the fishpond or fish tank and produces a thick, gelatinous cyst wall. This is termed the tomocyst stage. Within the tomocyst, a series of mitotic cell divisions take place and, depending on temperature, up to 1000 resulting daughter cells (tomites) are produced. These escape the tomocyst by penetrating

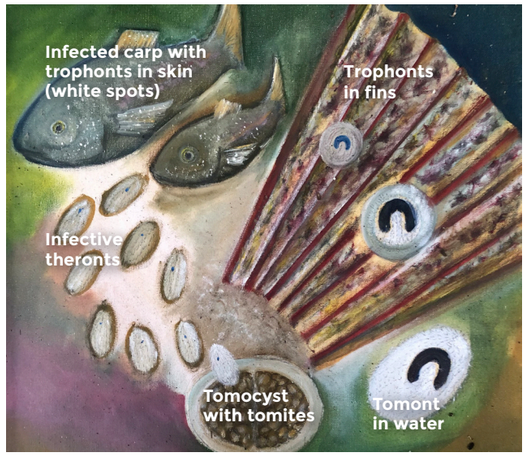

Figure 10. Life cycle of *Ichthyophthirius multifiliis* showing the trophont in the skin and on the fin of common carp, the released tomont, the encysted tomocyst and the tomites which are released as infective theronts (artwork by K. Buchmann, University of Copenhagen).

the cyst wall, whereafter they swim in the fish tank water searching for a fish host, which they will penetrate fast and efficiently if it is naïve and non-immunized. Numbers of trophonts on skin and gills can be very high (Fig. 9) and cause osmotic and respiratory problems. The life cycle of *I. multifiliis* is illustrated in Figure 10, showing the trophont, the tomont, the tomocyst, the tomites and the infective theronts.

Seasonality

The life cycle is highly temperature dependent. In open earth pond systems in Central and Northern Europe, this means that the main disease problems appear from April, when water temperatures increase until October, when temperatures decrease. However, the use of recirculated systems, also at higher latitudes, ensures a rather high mean temperature throughout the year and therefore the parasite is a major concern across all seasons.

Age / mean weight susceptibility

All age classes, from the yolk sac larva via the fry to adult fish, are susceptible to infection. However, a fish surviving a moderate infection is able to develop immunity against re-infection.

Risk predisposing factors

Water temperatures between 15 and 30 °C increase the risk of spreading the disease but even 10 °C facilitates infection. High density of hosts allows efficient transmission and increases the likelihood of parasite infection.

A) What clinical signs should alarm me?

External signs

White spots (diameter 0.5 to 1.0 mm) are visible on fins, skin and gills. Infected fish are clearly affected by the presence of the parasites in the fish surface and may rub their surface against firm objects in the fish pond. Highly infected fish become emaciated, lethargic, anorexic and discoloured (dark).

Internal lesions

The infection induces a strong systemic stress response.

B) How to detect the parasite at farm level

1. Monitoring plan (what to measure and how often) and trigger level for action

During high-risk periods, fish should be monitored daily. All fish tanks must be monitored. Any sign of epidermal spots should alert the personnel. Observation of one trophont on the fish surface is a trigger level for action.

2. Recommendations for the submission of samples to be diagnosed

Microscopic evaluation of skin scrapings at farm level is necessary, as the disease can spread rapidly. If neither sufficient equipment nor skilled personnel are available, contact laboratories for rapid diagnostic aid.

3. Contact laboratories

- Laboratory of Aquatic Pathobiology, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark
- Institute of Aquaculture, University of Stirling, Scotland, UK
- Veterinary Medical Institute, Budapest, Hungary
- Fish Pathology and Parasitology Research Team, Institute for Veterinary Medical Research, Centre for Agricultural Research (former Hungarian Academy of Sciences), Hungary
- Bioloxía Celular, Dpto. de Bioloxía Funcional, Santiago de Compostela, Spain
- Università degli Studi di Udine, Dipartimento di Scienze AgroAlimentari, Ambientali e Animali, Sezione di Patologia Veterinaria, Udine, Italy
- Instituto de Acuicultura Torre de la Sal (IATS), CSIC, Castellón, Spain

C) Action plan after diagnosis

1. Prevention

Daily addition of hydrogen peroxide containing auxiliary products such as peracetic acid or sodium percarbonate will kill infective theronts and thereby decrease infection pressure. If these more environmentally friendly products are unavailable, formalin may be applied. Recirculated fish farm systems may sustain a high NaCl concentration (10 g/L) over 14 days in order to prevent production of theronts in tomocysts and thereby new infections.

2. Farm management

For Koi carp in fish tanks, mechanical filtration of water with mesh sizes 40-80 µm will continuously remove a part of the tomonts in the fish tanks and prevent their subsequent attachment and proliferation.

3. Treatment: No treatment is available for trophont stages in the fish skin. Novel biological compounds, investigated in **ParaFishControl**, such as microbial surfactants, can kill all external life cycle stages.

4. Management of co-infections

The use of hydrogen peroxide containing compounds and formalin reduce the bacterial exposure of skin wounds that are caused when trophonts leave the fish surface.

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4. Fish farmer's guide to combating *Saprolegnia parasitica* infections

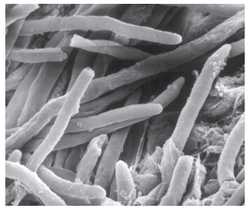

Figure 11. *Saprolegnia* spp. hyphae. Scanning electron micrograph (photo: Kurt Buchman, University of Copenhagen).

Introduction

Saprolegnia parasitica is a fungal-like oomycete (order Saprolegniales, family Saprolegniaceae) infecting a wide range of freshwater fish species (eggs, yolk sac larvae, fry, juveniles, adults including spawners). Susceptible aquaculture host species include rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*) and common carp (*Cyprinus carpio*). All types of aquaculture hatchery and production systems may be affected, including ponds, flow-through systems and recirculation systems.

S. parasitica is a fungal-like filamentous coenocytic (cytoplasm with many nuclei) oomycete consisting of aseptate branching mycelia, giving a furry appearance to external lesions (Figure 11).

Biological life cycle

Mycelia produce terminal sporangia releasing biflagellate zoospores which subsequently encyst to cytospores. These will excyst and release secondary zoospores which may give rise to extensive branched mycelia. Sexual reproduction occurs with male and female sexual structures (oogonia and antheridia) on the same mycelium leading to production of oospores. These may germinate and establish new mycelia. Sporangia, oogonia and antheridia are separated from the remaining mycelium with septae (Figure 12).

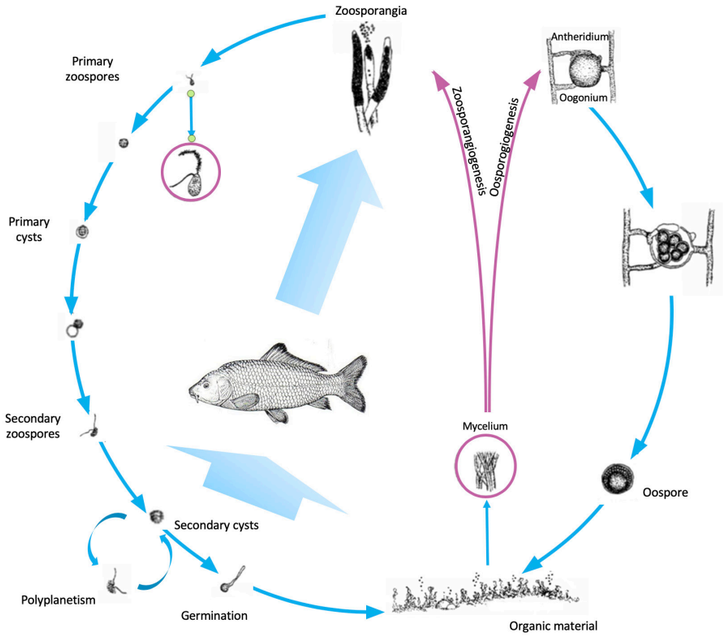

Figure 12. Involvement of fish in the life cycle of *Saprolegnia* spp.: the secondary zoospores are the most important dispersive phase and can encyst on a suitable submersed substrate or on a fish host, giving rise to mycelium made of hyphae that will produce zoosporangia. Secondary zoospores can repeat cycles of encystment and release (termed "polyplanetism") if a new appropriate substrate is not found. Fish infection and development of lesions are influenced by several environmental, intrinsic and/or management factors. Drawings by Dr Roberta Galuppi, Univerity of Bologna.

Seasonality

In laboratory conditions, this oomycete can grow at temperatures between 5 °C and 37 °C. The infection occurs throughout the year, but low temperatures are predisposing as they tend to impede hosts' immune functions.

Age / mean weight susceptibility

All stages of freshwater fish species (eggs, yolk sac larvae, fry, juveniles, adults including spawners) are susceptible to infection. Heavy infections are seen at all stages, but the larvae and fry are more delicate due to their vulnerable epithelia.

Risk predisposing factors

Mechanical injuries of fish surfaces are highly predisposing as *S. parasitica* spores readily germinate in wounds. Low temperature and stressful stimuli are predisposing factors due to the associated lowering of host immunity. Stripping of eggs and milt from spawners causes skin injuries and stresses fish, such that these fish may often develop the disease within days to weeks.

A) What clinical signs should alarm me?

External signs

Hyphal overgrowth of incubating eggs in hatching trays. Woollen coats and tufts on skin, fins and gills of fish (Figure 13).

Internal lesions

Hyphae may penetrate internal organs including the gastrointestinal tract and elicit inflammatory reactions.

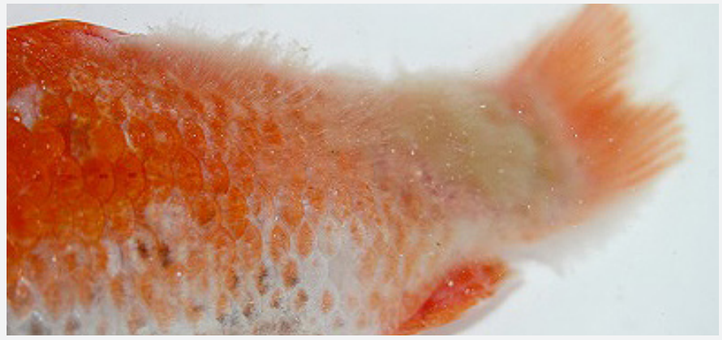

Figure 13. *Saprolegnia* infected Koi carp (photo: O. **Cheníček).**

B) How to detect the parasite at farm level

1. Monitoring plan (what to measure and how often) and trigger level for action

Fish should be surveyed on a daily basis and any sign of the disease should trigger action, including instigation of preventive measures and water cleaning.

2. Recommendations for the submission of samples to be diagnosed

Saprolegnia infections are easily detected based on the macroscopically visible tuft of hyphae but specific diagnosis may be performed by diagnostic laboratories. Tissue sampled from infected fish (containing mycelium) should be conserved in 96 % ethanol and submitted to a diagnostic laboratory for molecular identification.

3. Contact laboratories

- Centre for Environment, Fisheries and Aquaculture Science (Cefas), Weymouth, UK
- Institute of Aquaculture, University of Stirling, Scotland, UK
- University of Aberdeen, International Centre for Aquaculture Research and Development (ICARD), UK
- University of Bologna, Fish Pathology Laboratory of DIMEVET-UNIBO, Italy
- Laboratory of Aquatic Pathobiology, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg C., Denmark
- Real Jardín Botanico, CSIC, Madrid, Spain

C) Action plan after diagnosis

1. Prevention

Keep the temperature at the optimum for the cultured species, helping to secure optimal immune status of the host. Avoid stressing conditions, survey and secure high water quality. No commercial vaccines are available.

2. Farm management

Ozonisation of fish tank water, regular treatment with hydrogen peroxide containing compounds and continuous water filtration using fine meshed screens.

3. Treatment

Ozonisation of fish tank water, treatment with hydrogen peroxide containing compounds. Eggs in hatching trays can successfully be treated by addition of hydrogen peroxide, formalin, sodium chloride, copper compounds or iodophores. Use of the effective compound malachite green is banned and various anti-fungal drugs must be used with caution.

4. Management of co-infections

Co-infections should be diagnosed and specific treatments should be instigated.

References: Alderman, D. J. (2008). Fungal diseases of fish. In: Eiras, J. C., Segner, H., Wahli, T., Kapoor, B. G. (eds.). Fish Diseases. Vol. 1. 279-349.

Bruno, DW & Wood, BP (1999). *Saprolegnia* and other oomycetes. In: PTK Woo and DW Bruno (eds): Fish Diseases and Disorders, Vol. 3. CABI Publishing, Oxon, UK.

5. Fish farmer's guide to combating infections by zoonotic helminths

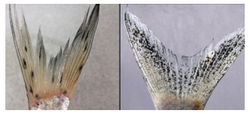

Figure 14. Blackspot disease by *Posthodiplostomum* (left) and by *Apophallus* (right) species (photo: Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungary).

Introduction

Fish-borne zoonotic trematodes affect the health of millions of humans worldwide. The life cycle of these parasites depends on aquatic snails, fish, and definitive hosts like humans, pigs or chickens. Humans, either as definitive or accidental hosts, can become infected by eating raw or undercooked fish. Common carp can harbour fish-borne zoonotic trematodes (flukes), therefore it is important to avoid them raw, not only for fish health issues, but also for human food safety. At harvesting of fish, the percentage of infected fish and parasite burden depend on previous gain and subsequent survival of the parasites. In a survey perform under the framework of the **ParaFishControl** project, no zoonotic helminths were found in carp sampled in Hungarian carp ponds.

In carp, zoonotic flukes that can be found in the muscle belong to *Opisthorchis*, *Metorchis* and *Metagonimus* spp. In contrast, *Apophallus* spp. encyst in the body surface or fins, producing black spots due to a host reaction that leads to the formation of a black pigment layer around them (Figure 14). Another commonly found fluke genus, *Posthodiplostomum*, is not zoonotic.

Biological life cycle

Trematodes are flatworms with complex life cycles (Figure 15). They first develop in an intermediate host, which are usually snails or clams. After a complicated development inside the first intermediate host, the released cercariae either infect the final host directly or find a second intermediate host, such as a snail, a crustacean or a fish, in which they become metacercariae. These developmental stages are ready to infect the final host which is a piscivorous bird or mammal. Zoonotic diseases are generally caused by metacercariae from fish.

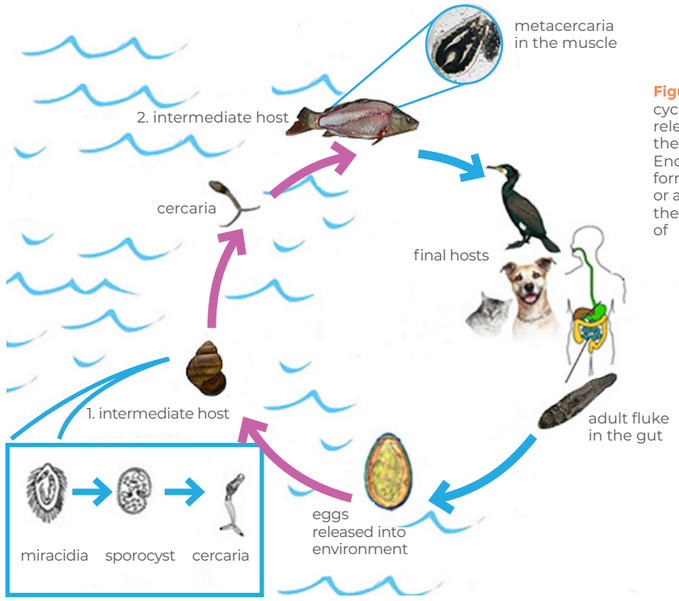

Figure 15. Schematic description of the general life cycle of (zoonotic) trematodes. Swimming cercariae are released from the snail host and actively swim to infect the second intermediate host, in this case common carp. Encapsulated parasite stages, termed metacercaria are formed in this host which are consumed by the definitive or accidental (human) host. Adult trematodes parasitize the gut. Schematic drawing modified from Center of Disease Control and Prevention, www.cdc.gov.

Seasonality

Fish are usually infected by cercariae between April and June, due to the increasing activity of molluscs during spring. Metacercariae develop within 4-6 weeks. Thereafter, the encysted metacercarie in the fish might be present during the whole year.

Age / mean weight susceptibility

All age groups of carp are susceptible to infection. Fingerlings might show serious deformation of the body. Fish may survive heavy infections but become undesirable for consumption due to aesthetic aspects, rendering them non-marketable.

Risk predisposing factors

The abundance of water snails and water birds facilitates completion of the life cycle of trematodes, therefore their presence increases the risk of infection. Theoretically, proximity of protected natural areas represents a high risk factor, as species diversity in culture correlates with the number and diversity of parasites in the habitat. However, in a recent survey carried out in Hungarian fish farms, metacercaria on the skin of 348 carp were analysed, but no zoonotic helminths were detected, even at sites close to such protected areas.

A) What clinical signs should alarm me?

External signs

Metacercaria encysted in the skin appear black due to melanisation by the host. Relatively large cysts are signs of *Posthodiplostomum* infection. Smaller cysts, especially on fins, are caused by *Apophallus* species.

Internal signs

In muscle-infecting species, cysts of metacercariae can be visible as small dots on the surface of the muscle. Metacercariae are easily visible under the stereomicroscope when muscle tissue is compressed between glass slides or petri dishes.

B) How to detect the parasite at farm level

1. Monitoring plan (what to measure and how often) and trigger level for action

Routine biweekly monitoring of juvenile fish (checking of skin and gills) can reveal black spots without magnification. Their appearance is conspicuous whenever they are present. Deformities of fingerlings can be a warning sign of trematode infection.

For monitoring of muscle infections, muscle pieces are pressed between two-glass slides, observed by stereomicroscope, then the detected metacercariae are manually freed. Pepsin-digestion of larger muscle pieces may improve detection of parasites.

2. Recommendations for the submission of samples to be diagnosed

Isolated metacercariae or pieces of the infected skin/fin tissue or muscle should be preserved in ethanol and sent to the reference laboratory. Photographs of the infected fish can also be very useful. Precise species identification is needed to ensure that no zoonotic helminths are involved at the harvest point. This should be evaluated by experts.

3. Contact laboratories

- Fish Pathology and Parasitology Research Team, Institute for Veterinary Medical Research, Centre for Agricultural Research (former Hungarian Academy of Sciences), Hungary.

C) Action plan after diagnosis

1. Prevention

Prevention is based on reducing the number of intermediate-host snails in the pond by collecting them manually, and by drying and liming the ponds in winter. Keeping away water birds can be also a part of solution. This can be achieved by covering with nets or by sound / movement.

2. Treatment

Anthelminthics (e.g. praziquantel) are effective drugs against helminths and can be mixed in food or added as a bath solution. However, effective legal treatments against zoonotic helminths in carp destined for human consumption are not available.

3. Management of co-infections

On the skin, important co-infections are monogeneans worms (*Dactylogyrus* spp.), *I. multifiliis*, or other ectoparasites. For their management, please refer to the previous sections.

References: Boerlage, A.S., *et al.,* (2014). Transmission of fish-borne zoonotic trematodes (*Heterophyidae*) to common carp (*Cyprinus carpio*) is independent of density of fish and trematodes. *J Helminthol.* 88, 183-188.

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Other ParaFish Control Resources

1. Integrated Pest Management Strategies for *Neoparamoeba perurans*: bit.ly/2XWqbx0

2. Integrated Pest Management Strategies for *Saprolegnia*: bit.ly/2VKDs9c

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