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FIELD GUIDE TO WARMWATER FISH DISEASES IN CENTRAL AND EASTERN EUROPE, THE CAUCASUS AND CENTRAL ASIA

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FIELD GUIDE TO WARMWATER FISH DISEASES IN CENTRAL AND EASTERN EUROPE, THE CAUCASUS AND CENTRAL ASIA

By

Kálmán Molnár[1], Csaba Székely[1] and Mária Láng[2]

[1]Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary

[2] National Food Chain Safety Office – Veterinary Diagnostic Directorate, Budapest, Hungary

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Preparation of this document

Recognizing that diseases cause severe damage to aquaculture production and fisheries of Central and Eastern Europe, the Caucasus and Central Asia, the Food and Agriculture Organisation of the United Nations prepared a comprehensive document that details the diseases that influence the outcomes of warmwater fish production in the region. The authors of this book are experts in diagnosis, prevention and treatment fish diseases in Hungary, as well as having extensive knowledge of fish diseases in the countries of Central and Eastern Europe, Caucusus and Central Asia. The main goal of this study is to provide information and easy to follow instructions / diagrams on identification ofthe diseases of the most commonly cultured cyprinid species (carps and their relatives) for the relevant region. However, the diseases of other cultured warmwater species (e.g. predators such as northern pike and wels catfish) are also detailed. This will be most beneficial to extension agents and research institutes of the targeted region in supporting aquaculture development in their respective communities.

The preparation of this publication was initiated by Mr Raymon Van Anrooy, Fishery Officer and funded through the FAO Sub Regional Office for Central Asia External editing was provided by Dr James Richard Arthur, FAO Consultant (English grammar and technical editing) and Nadav Davidovich, Fish Health Veterinary Officer, Ministry of Agriculture and Rural Development, Israel. Final editing and publication was facilitated by Dr Melba Reantaso, Fishery Resources Officer (Aquaculture), FAO; Dr Victoria Chomo, Senior Fishery and Aquaculture Officer and Ms Eva Kovaks, Fish Production Expert from the FAO Regional Office for Europe and Central Asia, Budapest and Dr Atilla Ozdemir, Aquaculture Expert from the FAO Subregional Office for Central Asia, Ankara.

Abstract

Due to the recent rapid development of freshwater aquaculture in the Caucasus Region, many new and previously known fish diseases have appeared. One of the most prominent features of the region's aquaculture is that it is mostly based on the rearing of cyprinids, mainly the common carp (*Cyprinus carpio*), as well as a few other predatory fish species. As a result, this book focuses on the diseases that affect these and other important warmwater fish species. Although this field guide covers the diseases of warmwater fish of Central and Eastern Europe, the Caucasus and Central Asia, it also draws upon the extensive knowledge base available for the countries of Central Europe and the former Soviet Union, as well as recent research findings from the Islamic Republic of Iran and from Turkey. The major warmwater fish species cultured in the region and their health status are discussed, and two major categories of disease are recognized: biotic and abiotic diseases. Although there are numerous biotic diseases, abiotic factors (e.g. lack of oxygen, temperature, feeding mistakes) remain the main cause of losses in aquaculture. The best practices for the field and laboratory examination of disease outbreaks are reviewed, and the importance of accurate and detailed data recording emphasized. Prevention as a key factor in avoiding the spread of disease is highlighted, and actions to prevent the spread of diseases between farms, regions, countries and continents are discussed. Possible methods for the treatment of each disease are reviewed; unfortunately, the chemicals available for use in aquaculture are now rather limited, as many of them are hazardous to both the environment and human health. Of the viral diseases discussed, spring viraemia of carp (SVC) and koi herpesvirus (KHV) pose the greatest threats to the world's carp populations. Of the bacterial diseases, ulcer disease is still the main problem in carp culture, while among the parasites, *Ichthyophthirius multifiliis*, the cause of white spot disease, is among the most important. Exotic parasites such as various *Thelohanellus* species, as well as tapeworms belonging to the genera *Bothriocephalus* and *Khawia*, are responsible for a considerable amount of damage. Some diseases of unknown aetiology are also discussed.

Key Words: Central and Eastern Europe, the Caucasus and Central Asia; Aquaculture; Fish Diseases; Identification; Prevention; Treatment

Contents

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Abbreviations and acronyms

BW	Body weight
CCA	Caucasus and Central Asia
CE	Carp erythrodermatitis
CEE	Central and Eastern Europe
CK	Carp kidney (cells)
CyHV1	Cyprinid herpesvirus1
CyHV2	Cyprinid herpesvirus2
CyHV3	Cyprinid herpesvirus3
DNA	Deoxyribonucleic acid
ECV	European catfish virus
EPC	Epithelioma papulosum cyprinid (cells)
EU	European Union
GBD	Gas-bubble disease
GCRV	Grass carp reovirus
GPS	Global positioning system
HVHN	Herpes viral haematopoietic necrosis
KHVD	Koi herpesvirus disease
OIE	World Organisation for Animal Health
PCR	Polymerase chain reaction (examination)
PFRD	Pike fry rhabdovirus disease
RNA	Ribonucleic acid
SBI	Swimbladder inflammation
SVC	Spring viraemia of carp
TAADs	Transboundary aquatic animal diseases

Glossary

Actinospore	A life-cycle stage of Myxosporea that develops in an oligochaete alternative host
Adhesion	An abnormal union of surfaces due to inflammation or injury
Aetiology	The cause or causes of a disease
Alternative host	A host in which a second, equally ranked phase of a parasite's development takes place
Annelid worm	A segmented worm of the Phylum Annelida, which includes earthworms, lugworms, rag worms and leeches
Anorexia	An abnormal thinness of a vertebrate due to lack of appetite
Anoxia	Absence or deficiency of oxygen
Ascites	The accumulation of fluid in the peritoneal cavity causing abdominal swelling (also referred to as dropsy or oedema)
Aseptate hyphal strands	Non-segmented filaments (hyphae) of fungi
Benthos	A collective name for organisms living on or in the pond bottom
Branchiura	A subclass of crustaceans, commonly known as "fish lice"
Cachexia	Weakness and decline of body condition due to severe chronic illness
Catarrhal	Referring to the excessive production of mucus
Cercaria	A larval stage of digenetic trematodes that is produced by asexual reproduction within a sporocyst or redia
Ciliate	A protozoan bearing hair-like peripheral organelles. (see also cilium)
Cilium	A short, microscopic, hair-like vibrating structure on the surface of certain cells (plural: cilia)
Clubbing	A change in the structure of the gills in which, due to proliferation of epithelioid cells, the ends of neighbouring lamellae grow together, the gill lamellae disappearing from the damaged filaments
Coccidia	Members of the Subclass Coccidia
Collagenous tissue	Tissue comprised of any of a group of insoluble fibrous proteins that constitute the main structural component of animal connective tissue
Commensalism	An association between two organisms in which one benefits while the other neither benefits nor is harmed
Copepod	A member of the Order Copepoda**,** a large group of tiny aquatic crustaceans which are important members of the zooplankton and which includes many parasitic forms
Copepodite	A developmental stage of parasitic copepods in which their structure resembles that of free-living copepods
Coracidium	The initial, ciliated larval stage of some cestodes
Cornea	The transparent layer forming on the front of the eye
Cyclopoid	A developmental stage of parasitic copepods in which their structure resembles members of the free-living genus *Cyclops*
Cytostoma	The mouth-like structure of some protozoans

Dactylogyrid	A monogenean worm belonging to the Family Dactylogyridae
Desquamation	Separation of scales or laminae from any surface
Digenean fluke	A parasitic worm belonging to the Subclass Digenea, members of which require from two to four hosts to complete their life cycle
Digenic	Referring to a developmental cycle in which at least two hosts (a final and an intermediate host) are needed to complete the life cycle
Dropsy	The accumulation of fluid in the peritoneal cavity causing abdominal swelling (also referred to as ascites or oedema)
Ectoparasite	A parasite living on the body surface of its host
Endoparasite	A parasite living inside the body or organs of its host
Enteritis	Inflammation of the intestine
Epidermal	Referring to the surface epithelium of the skin of an animal
Epithelium	The cellular tissue covering surfaces, forming glands and lining most cavities of the body. It consists of one or more layers of cells with only little intercellular material.
Epitheloid cell	A type of histiocyte which participates in reparation
Erythrodermatitis	Inflammation of the skin with associated redness
Eurythermal	Referring to the ability **t**o tolerate, survive and grow within a wide range of temperature
Eutrophic	Referring to a waterbody that is rich in plant nutrients and so supporting increased growth of plants in general, and phytoplankton in particular
Exophthalmia	Abnormal protrusion of the eyeballs (commonly referred to as "popeye") (also exophthalmos)
Facultative	Occurring optionally in response to circumstances rather than by nature
Family	A principal taxonomic category below an order and above a genus
Fingerling	A young fish of about 10–20 cm in length and 20–50 g in weight, which in some temperate areas is also termed a "one-summer-old fish"
Fission	Reproduction of a cell or organ by dividing into two or more new cells or organelles
Flagellate	A member of the Phylum Mastigophora, a group of flagellated protozoans, some of which are parasitic
Fry	The developmental stage of fish that starts when larvae gulp air and finishes when all organs are developed (or in case of the ovary and testes, when development is initiated). In the case of warmwater fish species, this life stage lasts about 20–40 days, depending on water temperature.
Gamete	A mature haploid female or male germ cell which is able to unite with one of the opposite sex in sexual reproduction and form a zygote (a fertilized female germ cell)
Gametogonic	Stages in the process during which cells undergo meiosis to form stages gametes
Gene	A unit of heredity determining the characteristics of the progeny through the sequence of DNA, and which is part of the chromosome

Genus	A principal taxonomic category that is below family and above species. The first part of the scientific (or Latin) name of species refers to the name of the genus, which always starts with a capital letter. (plural: genera).
Gill arch	The U-shaped cartilaginous structure that supports the gill filaments
Gill filaments	The filamentous parts of the gill, also called the primary lamellae
Gill lamellae	The subdivision of the gill filaments in which most respiratory and excretory changes take place
Granuloma	A mass of granulated connective tissue, typically produced in response to infection, inflammation or a foreign substance (plural: granulomas or granulomata)
Granulomatosis	The formation of multiple granulomas
Gyrodactylid	A type of monogenean worm belonging to Family Gyrodactylidae
Haematopoietic	Referring to the process of haematopoiesis, during which blood cells are produced
Haematopoietic tissue	A type of tissue which takes part in the formation of blood cells
Haemorrhage	Heavy bleeding
Histopathology	The branch of medicine dealing with tissue changes caused by a disease
Hydropic degeneration	A type of degeneration affected by dropsy (oedema)
Hyperaemia	Excess of blood in an organ or part of the body
Hyperplasia	Increase in volume of a tissue or organ caused by the growth of new cells
Hypertrophy	Increase in the volume of a tissue or organ produced entirely by the enlargement of existing cells
Hypha	A filament composing the mycelium of a fungus (plural: hyphae)
Hypotonic	Referring to a lower osmotic pressure than a given fluid, or the state of an abnormally low muscle tone
Inflammation	A specific tissue response to injury evidenced by vascular dilatation
Interlamellar	Located between two gill lamellae
Intermediate host	An animal in which an early developmental stage of a parasite takes place
Lamella	A thin layer, membrane or tissue; gill plates on gill filaments serving for gas exchange **(**plural: lamellae**)**
Lordosis	Curvature of the spine with abnormal concavity of the back
Macrogametes	The larger, female gametes
Macrophage	A large phagocytic cell, which engulfs and absorbs bacteria or other small particles. It is found in stationary form in the tissue as a mobile white blood cell, especially in infections.
Meiosis	Meiotic division, a type of cell division that results in daughter cells having half the number of chromosomes of the parent cell

Melano-macrophage centre	An accumulation of pigmented macrophage cells
Merogony	A series of stages in the life cycle of certain protozoans (Subphylum Apicomplexa) that forms merozoites and involves asexual reproduction by multiple fission
Meront	A uninucleate or multinucleate parent cell of certain protozoans (Subphylum Apicomplexa) that forms merozoites by a process of multiple fissions
Metacercaria	A stage between the cercaria and the adult in the life cycle of digenetic trematodes, usually encysted and quiescent (a stage of inactivity) (plural: metacercariae)
Metazoans	All organisms which are built up from more than one cell
Microgametes	The smaller, male gametes of a heterogamous organism (see also macrogametes)
Miracidium	The ciliated first larval stage of digenetic trematodes (plural: miracidia)
Monogenean	A member of the Class Monogenea, a group of parasitic flukes requiring only one host to complete their life cycle
Monogenetic	A type of development without intermediate hosts
Mucosa	The membrane that lines the gastrointestinal tract
Mycelium	The filamentary part of a fungus
Myofibrils	Bundles of contractile filaments that are arranged in parallel groups in the cytoplasm of striated muscle cells
Myxospores	Spore stages of myxosporidians developing in fish hosts
Nauplius	The first larval stage of a parasitic copepod
Necrosis	Death of most or all cells of an organ due to a disease (adj.: necrotic)
Nematode	A roundworm or threadworm belonging to the Phylum Nematoda which has a slender, unsegmented cylindrical body
Neoplasm	A new and abnormal growth of tissue in a part of a body
Oocyst	A cell in the ovary that undergoes meiosis to form ova (plural of ovum), which are the mature female reproductive cells. From this, after meiosis and fertilization by a male sex cell, develops the embryo.
Papillomatous	Referring to a process resembling a papilloma
Pathogen	A virus, bacterium or other organism which causes disease
Pathogenesis	The way a disease develops
Pathogenicity	Having the ability to cause disease
Periciliated	Referring to the whole body being covered by cilia
Peritonitis	Inflammation of the peritoneum, typically caused by bacterial infection
Petechia	A small haemorrhagic spot on the skin, mucous membrane, etc.
Photosynthesis	The process by which green plants (with the help of light and chlorophyll) produce their cells (their organic materials) from inorganic materials such as minerals and carbon dioxide. During this process they consume carbon dioxide and produce oxygen. The opposite of

	assimilation is dissimilation, when in the dark, plants respire, consuming oxygen and producing carbon dioxide.
Plasmodium	A form within the life cycle of some simple organisms that consist of a mass of protoplasm containing many nuclei. In myxosporeans, this is a life-cycle stage in which spores develop. (plural: plasmodia)
Plerocercoid	The larval stage of a cestode which develops from a procercoid, usually showing little differentiation
Poikilotherm	An organism whose temperature depends on and is equal to the temperature of its environment
Procercoid	The larval stage of a cestode which develops from a coracidium; usually having a posterior cercomer
Proliferation	Multiplication of cells
Propria	A tissue layer below the epithelium
Protozoans	Single-celled microscopic animals
Sclerotized	Hardened by conversion into sclerotin (dead thickened skin), which is a solid structural protein of, for example, the cuticle of insects
Scolex	The anterior end of a tapeworm (plural: scolices)
Scraping	A sample taken from the skin or gills by scraping with a scalpel blade or a glass microscope slide and examined as a wet-mount or stained preparation using a compound microscope
Septicaemia	A morbid condition due to the presence and reproduction of pathogenic bacteria in the blood
Serosa	The issue of the serous membrane which produces serum
Spore	A minute, typically single-celled reproductive unit of lower plants and protozoans, which is capable of giving rise to a new individual without sexual fusion
Sporocyst	The larval stage of a digenetic trematode developing after infection of the tissue of its molluscan intermediate host (typically a snail), and having a sack-like form
Sporogony	Multiple fission of a zygote (which is also called a sporont)
Sporozoite	A motile spore-like stage in the life cycle of sporozoans
Sporulated stage	The life-cycle stage of coccidians forming the spore or spores
Sporulation	The formation of spores
Stenothermal	Referring to the ability to tolerate, survive and grow within only a narrow range of temperature
Strobila	The body of an adult tapeworm behind the scolex and neck, consisting of a series of similar proglottids or segments (plural: strobilae)
Subepithelium	A tissue layer below the propria layer
Submucosa	A tissue layer below the mucosa
Surveillance	A systematic series of investigations of a given population of aquatic animals to detect the occurrence of disease for control purposes, and which may involve testing samples of a population
Tentacle	A slender whip-like organ of some protozoans
Tomite	A multiplying stage of *Ichthyophthirius* developing inside the tomont

Tomont	The stage in the life cycle of *Ichthyophthirius* in which tomites develop
Toxicosis	Poisoning
Trophont	The growing stage of *Ichthyophthirius* infecting under the superficial epithelium of fish
Ulcer	An open sore on the internal or external surface of a body caused by broken skin or mucous membrane, and which cannot heal
Vegetative stage	An asexual reproductive stage of protozoans (also termed merogonic stage)
Viraemia	The presence of viruses in the blood
Viviparous	Referring to those organisms that give birth to living young which are developed in the mother

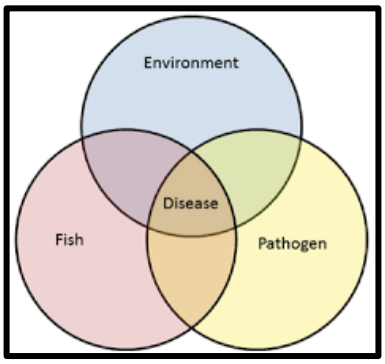
1. INTRODUCTION

According to the most recently available data from the Food and Agriculture Organization of the United Nations (FAO, 2018), in 2016, global production of finfish, crustaceans, molluscs and other aquatic animals (excluding aquatic mammals and reptiles) rose to about 171 million tonnes, with aquaculture contributing about half of this total (47 percent) and more than half (about 64 percent) of the value (USD 232 billion). While production from capture fisheries has remained more or less unchanged for several decades, production from aquaculture has continued to increase, with the sector continuing to grow faster than other major food production sectors (average annual growth of 5.8 percent during the period 2000–2016). In 2016, global aquaculture produced some 80.0 million tonnes of foodfish and 30.1 million tonnes of aquatic plants, as well as 37 900 tonnes of non-food products. Farmed foodfish production included 54.1 million tonnes of finfish, 17.1 million tonnes of molluscs, 7.9 million tonnes of crustaceans and 938 500 tonnes of other aquatic animals (FAO, 2018). Clearly, if fish production is to keep pace with the future growth of the human population, this additional supply will have to come mainly from increased aquaculture production.

The geographical area covered by this field guide includes Central and Eastern Europe (CEE), which is comprised of 20 countries (Albania, Belarus, Bosnia and Herzegovina, Bulgaria, Croatia, Czech Republic, Estonia, Hungary, Latvia, Lithuania, Moldova, Montenegro, Poland, Romania, Russian Federation, Serbia, Slovakia, Slovenia, the former Yugoslav Republic of Macedonia and Ukraine) and the Caucasus Central Asia (CCA), which includes the five countries of Central Asia (Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan and Uzbekistan) and the four countries of the Caucasus (Armenia, Azerbaijan, Georgia and Turkey).

Although none of these countries are among the world's top aquaculture-producing nations, warmwater finfish, mainly cyprinids (various carps) have been cultured in ponds for many centuries and are a traditionally and regionally important food item that is destined primarily for domestic consumption. More recently, other warmwater fish species, including catfishes (both native and introduced), northern pike, eels and perch have become locally important. In the rearing of warmwater finfish, the CEE and CCA countries still mainly utilize traditional pond-culture methods. There is thus a significant potential to increase fish production by using non-traditional methods (e.g. aeration, recirculating systems) that will allow faster growth with higher stocking densities. The most recently available FAO data (for 2016) shows the total freshwater fish production for the 29 countries covered by this field guide to be 485 174 tonnes, with cyprinids contributing 57 percent to this total (276 983 tonnes). Freshwater aquaculture production for the CEE countries was 323 559 tonnes during this period, with cyprinids contributing 74 percent (240 542 tonnes), while for the CCA countries, total freshwater aquaculture production was 161 615 tonnes, with cyprinids (36 441 tonnes) contributing 22.6 percent.

Disease in aquatic animals has long been recognized as a result of the interaction between the host (i.e. the aquaculture stock), its environment (e.g. the fish pond) and the pathogen, disease only occurring when these components overlap in a suitable manner. An intimate understanding of the the cultured species, the culture environment and the specific pathogen is thus critical to accurate disease diagnosis and treatment. The interaction of these three major players is shown in the accompanying"Szneisko circle".



Globally, diseases caused mainly by viruses and a few bacteria (see Bondad-Reantaso *et al.* 2005, OIE 2017a) are a major constraint to aquaculture production, causing billions of dollars of losses due to mortalities and decreased growth of cultured fish, shellfish and molluscs. As described in this field guide, two of the ten transboundary aquatic animal diseases (TAADs) of finfish that are listed as reportable to the OIE cause problems in warmwater fish culture in the CEE and CCA countries (i.e. spring viraemia of carp (SVC) and koi herpesvirus (KHV)). However this field guide also presents information on many other diseases, of both biological and environmental origin, that cause mortalities, poor growth and other problems in warmwater fish culture.

Recognizing that diseases cause severe damage to aquaculture production and fisheries of the CEE and CCA countries, the authors have provided a comprehensive document that details the diseases that impact warmwater fish production in the region. The main goal of this field guide is thus to provide information and guidance that will assist fish health specialists, veterinarians and aquaculturists to identify, treat and prevent the diseases of the warmwater fish species (primarily the carps and their relatives) that are most widely cultured in the CEE and CCA countries.

1.1 Fish species included

According to one of the widely used practical classifications of freshwater fish species, there are coldwater, warmwater and tropical fish. Coldwater fish species are usually stenothermal, and live in waters where the mean monthly temperature does not exceed 20 ^{o}C[1]. Tropical fish species are also stenothermal but require a relatively high water temperature (24–34 ^{o}C) and do not survive in waters where the temperature is constantly below 15–18 ^{o}C. Warmwater fish species are eurythermal, and hence tolerate a wide range of water temperature between 2 and 32 ^{o}C. However, all species have an optimal range of water temperature in which they grow and propagate the best.

[1]Source: http://pubs.usgs.gov/wri/wri984249/pdf/6ecological.web.pdf.

Typical culture systems for coldwater fish species are intensive tank and cage culture, while warmwater fish species are usually raised in pond polyculture systems.

In the countries and regions of Central and Eastern Europe (CEE) and the Caucasus and Central Asia (CCA), warmwater fish species include not only large cyprinids such as common carp and Chinese major carps, but other smaller carps and predators such as northern pike, pikeperch, wels catfish and brown bullhead. These all live in the same natural or manmade waters or are grown together in pond polyculture. Examples of some of the typical species discussed in this field guide are given in Box 1.

Box 1. Fish families and their typical species discussed in this field guide

- **Esocidae (pikes):** Northern pike (*Esox lucius*)
- **Cyprinidae (carps):** Common and koi carps (*Cyprinus carpio*); Chinese major carps, such as silver carp (*Hypophthalmichthys molitrix*), bighead carp (*H. nobilis*) and grass carp (*Ctenopharyngodon idella*); freshwater bream (*Abramis brama*); goldfish (*Carassius auratus*); Crucian carp (*C. carassius*); gibel carp (*C. auratus gibelio*); roach (*Rutilus rutilus*); ide (*Leuciscus idus*) and tench (*Tinca tinca*)
- **Siluridae (catfishes):** Wels catfish (*Silurus glanis*)
- **Ictaluridae (catfishes):** Brown bullhead (*Ameiurus nebulosus*)
- **Anguillidae (eels):** European eel (*Anguilla anguilla)*
- **Percidae (perches**): Pikeperch (*Sander lucioperca),* Volga pikeperch (*S. volgensis*)

1.2 Status of fish health in the region

Before the 1950s, in CEE and CCA countries, fish health services for warmwater fish species were based on knowledge obtained and disseminated by German specialists, while a branch of fish health science (fish parasitology) was dominated by Soviet, Polish and Czech scientists. The pathogenicity of most parasitic protozoans and helminths was well studied, but only a little was known about bacterial pathogens, and nothing about the viral diseases of carps.

The 1950s brought a general change, when wild common carp from the Amur River and Chinese major carps were introduced to CCA and CEE. Together with these fish species, several new pathogens were also introduced.
In the 1970s, a significant step was taken when the complex nature of infectious dropsy of common carp was revealed by separating this disease into spring viraemia of carp (SVC) and ulcer disease. In CCA countries, successes relied mostly on scientists of the former Soviet Union, who published several books on the parasites of fish in the former Union of the Soviet Socialist Republics (USSR).

Today, the fish health services in both the CEE and CCA countries are organized in two different ways. In some of the countries, fish health services are run by veterinarians specialized in fish disease, while in other countries, prevention and treatment of fish diseases is coordinated by specialized fishery and aquaculture engineers or biologists. Both options have advantages and disadvantages. In general, however, fish health services are under the veterinary services, regardless of the academic qualifications of those who provide it.

Due to the banning of residues of many chemicals and medications in fish and fisheries products by the European Union (EU), today only a few effective chemicals and medications are available for the treatment of diseases of foodfish. As a consequence, the number of chemicals and drugs allowed, licensed and legally used by the fish health services in the EU is very small. Many drugs which were previously widely used in European fish culture have been banned due to their carcinogenic effects (e.g. malachite green) or because of the lack of license (e.g. some of the organophosphates). Nevertheless, these drugs are also mentioned in this field guide, because in several countries fish (e.g. goldfish and koi carp) are cultured on a wide scale for non-nutritional purposes and hence, for these ornamental species, there are no restrictions on the use of substances banned for use on foodfish.

1.3 Guide to users

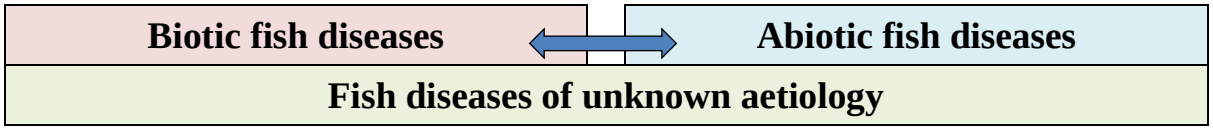
The structure of this field guide is the following:

- This publication aims to provide a concise guide for field personnel which, in addition to being a first reading on the subject, is also envisaged to serve as a technical book to which both unspecialized and specialized veterinarians can refer to in case they have to act and provide professional assistance.
- A Glossary providing definitions for many of the scientific and specialized veterinary terms used in this field guide is given at the front of this document.
- Section 1 provides a brief introduction to the field guide.
- Section 2 presents a short general introduction to the nature and main types of fish disease. This section aims to help readers find their way in the "maze" of fish diseases presented in Sections 6 to 24.
- In Section 3, the main aspects and protocols for field health inspection of fish are discussed and practical guidelines as to where, when and what should be done during routine and emergency fish health site inspections are given. This section is supported with relevant additional information presented in Annex 1.
- In Section 4, some administrative and practical measures of fish disease prevention are discussed. This section is supported by Annex 2, in which information and international regulations as presented by the World Organisation for Animal Health (OIE) can be found.
- Section 5 presents a concise inventory of the different methods of fish disease treatment, and is supported by Annex 3, which provides information on the chemicals, drugs and antibiotics used as disinfectants and curatives for warmwater fish diseases.
- Sections 6 to 24, which are arranged by major taxonomic groups, present detailed information on the all common diseases of warmwater fish mentioned in Section 2, including, for each disease, information, as appropriate, on its causative agent, the host species infected, optimal epizootic temperature, routes of transmission, pathology, clinical signs and methods for pathogen detection, prevention and treatment.
- Annex 1 presents information of the clinical signs of fish diseases based on macroscopic changes seen in the body and organs.
- Annex 2 presents fish health-related international recommendations, regulations and guidelines for measurements.
- Annex 3 presents a summary of the chemicals, drugs and antibiotics used for the prevention and treatment of warmwater fish diseases.
- Annex 4 provides a list of sources and recommended literature for further reading.

2. NATURE AND TYPES OF FISH DISEASE

One of the practical classifications of fish diseases divides them according to their cause, which can be either biotic or abiotic. Biotic fish diseases derive from living organisms, while abiotic diseases do not involve or derive from living organisms, but are related to factors such as water quality, the presence of poisonous materials or management problems, including incorrect feeding. As illustrated in Figure 1, these two main groups of disease have a complex interrelationship, both in natural waters and fish farms.

Figure 1. Practical grouping of fish diseases



In addition to the above-mentioned groups, there are also fish diseases of unknown aetiology, which may have either a biotic, an abiotic or a combined cause. As well as these three main groups of fish disease, there is a fourth group, tumours. These are also discussed in this field guide.

There are a few diseases which can be transmitted to humans from fish. These zoonoses are discussed in Section 24.

2.1 Biotic fish diseases

There is a wide range of organisms which cause fish disease when conditions are favourable for their development. These organisms can be categorized based on whether they are viruses, bacteria, fungi, plants or animals, as summarized below and discussed in detail in Sections 6 to 20. Biotic fish diseases are caused by the following major groups:

- Viruses
- Bacteria
- Fungi and algae
- Parasites
    - Protozoans
    - Myxosporeans
    - Parasitic worms
    - Molluscs (glochidia)
    - Crustaceans

2.1.1 Diseases caused by viruses

Viruses are extremely minute (maximum 300 nm) infectious agents which cannot survive and multiply outside the cells of the host organism. Although viruses are not considered as living organisms, they are biological systems because they have DNA and RNA. Therefore, as well as for thematic and didactical reasons, they are discussed with the group of biotic agents responsible for causing diseases in fish.

Unlike numerous diseases caused by viruses in coldwater fish, especially in salmonids, the number of viral diseases known in warmwater fish species is relatively small. Nevertheless, some of these are regarded as important pathogens. Table 3 provides a list of the most frequent diseases caused by viruses, while Chapter 6.1 presents their descriptions.

2.2.2 Diseases caused by bacteria

There are not too many fully described diseases of warmwater fish caused by bacteria. These are listed below and are discussed in detail in Section 7. The most frequent diseases caused by bacteria are:

- Ulcer disease (carp erythrodermatitis)
- Infectious dropsy (septicaemia) of carp
- Flexibacteriosis or columnaris disease
- Mucophilosis or epitheliocystis disease of common carp
- Fish tuberculosis

2.2.3 Diseases caused by fungi and algae

Fungal organisms are typically facultative pathogens that infect fish with low resistance. These are listed below, while their characteristics are described in Section 8.

In a strict sense, algae do not belong among the pathogenic organisms infecting fish; however, they may cause massive fish mortality for two reasons: they produce toxic materials or when they bloom, the oxygen content of water can be dangerously reduced. The most frequent diseases of warmwater fish caused by fungi and algae are as follows:

- Diseases caused by fungi
    - Saprolegniosis (dermatomycosis)
    - Putrefaction of the gills (branchiomycosis)
    - Infections caused by *Dermocystidium* spp.
- Diseases caused by algae
    - Toxicosis
    - Algal bloom

2.2.4 Diseases caused by parasites

Many fish diseases are caused by parasitic organisms. The concept of parasitism is rather extensive; in its wide meaning, all living organisms (viruses, bacteria, fungi, animals) which live in, feed from, and damage another organism are considered parasites. However, in its practical sense, only organisms, belonging to protozoans and metazoans**,** are real parasites. The main groups of fish disease caused by parasites are given below:

- Diseases caused by protozoans (flagellates, ciliates, apicomplexans)
- Diseases caused by myxosporeans
- Diseases caused by parasitic worms (helminths): monogeneans, cestodes, digeneans, nematodes, acanthocephalans, leeches)

- Diseases caused by parasitic larval molluscs
- Diseases caused by crustaceans

Some parasites live their entire lives in or on the same host, while others have more complex life cycles. Besides the final host in which they mature, they may have one or more intermediate hosts, in which they grow during their subsequent developmental stages. Of these hosts, the main or final host is that organism in which they reach sexual maturity**.**

2.2.5 Diseases caused by protozoans

Protozoan parasites, described in Sections 9 to 11 are single-celled microscopic animals, and can be flagellates (Section 9), ciliates (Section 10) or apicomplexans (Section (11). Some of them are obligate parasites of fish, which means they cannot live without a fish host. Others are facultative parasites, because they can survive without a host; however, these also frequently cause infections and disease in fish. Most of the flagellate and ciliate species belong to this latter group. These parasites grow on the fish body, causing changes in the fins, skin and gills, and frequently causing the death of fish. The majority of flagellates and ciliates infecting fish are ectoparasites. As they harm the epithelial surface of the gills and skin, they cause similar damage and clinical signs. For these reasons, the methods for their prevention and treatment are also similar; hence, they are often mentioned using the common name "ectoparasitic protozoans". White spot disease is often incorrectly grouped among the diseases caused by ectoparasitic protozoans. However, the ciliate that causes this disease lives under the epithelium, and its life cycle and pathogenicity also differ from that of ectoparasitic protozoans, and so the methods for its prevention and treatment are also different. A list of the most frequently encountered diseases of warmwater fish caused by protozoan parasites is given below:

- Diseases caused by flagellates
    - Veil disease or ichthyobodonosis (costiosis)
    - Gill cryptobiosis
    - Sleeping disease of fish
    - Spironucleosis
- Diseases caused by ciliates
    - Chilodonellosis
    - Trichodinosis
    - Apiosomosis
    - White spot disease (ichthyophthiriosis)
    - Balantidiosis
    - *Capriniana* infection
- Diseases caused by apicomplexans (coccidians)
    - Diffuse coccidiosis of common carp
    - Coccidiosis of silver and bighead carps
    - Nodular coccidiosis of common carp

2.2.6 Diseases caused by myxosporeans

The myxosporeans presented in Section 12 are common and pathogenic parasites of warmwater fish. For a long time, they were regarded as protozoan parasites, but new research has proved

that they are metazoan organisms. The development of all myxosporeans is complex: one of their developmental stages is in fish, and another one is in oligochaetes (annelid worms). They form two types of spores, namely myxospores in fish and actinospores in oligochaetes. Actinospores infect fish, while myxospores infect oligochaetes. The most important diseases of warmwater fish caused by myxosporeans are as follows:

- Swimbladder inflammation (SBI) of common carp
- Gill sphaerosporosis of common carp
- *Myxobolus cyprini* infection of common carp
- *Myxobolus pavlovskii* infection of silver and bighead carps
- *Thelohanellus nikolskii* infection of common carp
- *Thelohanellus hovorkai* infection of common carp

2.2.7 Diseases caused by parasitic worms (helminths)

The parasitic worms (helminths) described in Sections 13 to 18 are the most common and pathogenic parasites of fish. Some of them are ectoparasites, while others are endoparasites. Some infect fish in their adult stage; others, however, are parasites of aquatic birds and mammals and fish serve only as intermediate hosts for their developmental stages. Most of the known parasitic worms of fish belong to the Phylum Platyhelminthes: the Monogenea (Section 13), Cestoda (tapeworms) (Section 14) and the Trematoda (digenean flukes) (Section 15), but members of the Nematoda (roundworms) (Section 16), Acanthocephala (spiny-headed worms) (Section 17) and Hirudinea (leeches) (Section 18) are also common parasites of fish. Some of these worms are extremely pathogenic for fish. Of them, the monogenean gill worms, which include many host-specific species, are the best known pathogens of cyprinid fish, but tapeworms living in the intestine, as well as the larval stages of flukes can also cause mortality in fish stocks and economic losses for farmers. The most important diseases of warmwater fish caused by parasitic worms are given below:

- Diseases caused by gill worms (monogeneans)
    - Gill disease of common carp fry caused by *Dactylogyrus vastator*
    - Gill disease of common carp caused by *Dactylogyrus extensus*
    - *Dactylogyrus* infection of Chinese major carps
    - Gill disease of wels catfish caused by *Thaparocleidus vistulensis*
    - *Gyrodactylus* infection
    - *Diplozoon* infection of cyprinids
- Diseases caused by parasitic tapeworms (cestodes)
    - Infection with *Bothriocephalus acheilognathi*
    - Infection of common carp with *Khawia sinensis*
    - Infection of common carp with *Atractolytocestus huronensis*
    - Ligulosis
    - Other tapeworm infections
- Diseases caused by parasitic flukes (trematodes)
    - Sanguinicolosis of common carp
    - Diplostomosis of cyprinids
    - Blackspot disease
    - Tetracotylosis
    - Other metacercarial infections
- Diseases caused by parasitic roundworms (nematodes)

- Diseases caused by parasitic thorny-headed worms (acanthocephalans)
- Diseases caused by leeches (Hirudinea)

2.2.8 Diseases caused by parasitic larvae of molluscs (glochidia)

Certain species of freshwater bivalve mollusc use fish as hosts for developing their larvae, which are called glochidia. At first, female freshwater mussels incubate their fertilized eggs within their shells. However, after the incubation period, they release the glochidia into the water. These young larvae attach to the fins or gills of fish and remain parasitic for one or more months, while the young mussels develop. Although fish are able to sustain low levels of glochidial invasion without apparent harm, heavy infestations, especially in the gills of young fish, can cause injury and even death. Infection with glochidia is especially fatal in newly stocked fry populations. These parasitic larvae of bivalve molluscs are discussed in Section 19.

2.2.9 Diseases caused by crustaceans

The majority of aquatic crustaceans are free-living organisms, which means that they live independently, and not as parasites. However, some species have developed a parasitic lifestyle or a close association with fish. Many of them are responsible for disease, especially of farmed fish. There are a few dozen crustaceans that infect warmwater cultured fish, of which the most pathogenic species are those described in Section 20. These parasites cause severe economic losses in cyprinids and cultured predatory fish such as northern pike, pikeperch and wels catfish. Besides fingerlings, they also infect older fish, causing severe weight loss and death. The most common diseases of warmwater fish caused by crustacean parasites are as follows:

- Infection by *Ergasilus sieboldi*
- Other ergasilid infections
- Lernaeosis
- Infection by fish lice (argulosis)

2.3 Abiotic fish diseases

At present, there is an ever-increasing knowledge on diseases caused by viruses, bacteria, fungi and parasitic organisms. Still, both in natural waters and pond polyculture of carps, much more harm is caused by environmental factors, such as oxygen shortage, low and high water temperature, accumulation of poisons in the water, and by human activities, including unsuitable or poorly implemented fish production technologies, incorrect nutrition and rough handling. These abiotic diseases of warmwater fish are discussed in Sections 20 to 22 and include the following:

- Diseases induced by the physical and chemical qualities of water
    - Diseases caused by unfavourable water temperature
    - Problems in oxygen supply
    - Gas-bubble disease (GBD)
- Poisonings of fish
    - Poisonings of industrial origin
    - Poisonings of agricultural origin

- Poisonings of aquatic habitat origin
- Enteric inflammation caused by feeds

2.4 Diseases of unknown aetiology

Research on fish diseases is a fast-developing branch of animal pathology. While in the middle of the past century, we had only a scant knowledge of viral diseases, today dozens of these agents are known, first of all the pathogens of salmonids. On the other hand, swimbladder inflammation of common carp proved to be due to a myxosporean infection, although earlier it was thought to be a bacterial disease. Despite the great progress achieved in diagnosing fish diseases, the aetiology of some economically important fish diseases is still unknown. These are presented in Section 23 and are as follows:

- Winter skin disease of common carp
- Gill necrosis of common carp

2.5 Tumours

Tumours are widely reported in many families of fish. Fortunately, they are relatively rare in cultured cyprinids. This can be attributed to the fact that the rearing periods in culture systems do not allow the development of tumours, which characteristically occur in older fish. Consequently, tumours appear more frequently in wild fish populations and in ornamental fish such as goldfish and koi carp.

Of the neoplasms found in fish from natural waters, epidermal hyperplasia in the fins and skin of the common barbel (*Barbus barbus*) seems to be the most common.

3. FIELD INSPECTION OF FISH HEALTH

The diagnosis of fish diseases is based on proper observation, sampling and examination of both the fish and their habitat. The best practice is when both on-site and laboratory examinations are performed.

3.1 Examinations on site

The steps of field examination are as follows:

- Inspection of the waterbody
- Sampling of fish
- Examination of fish
- Collection of data and other information

3.1.1 Inspection of the waterbody

The examination of fish starts with the inspection of the affected waterbody and the behaviour of fish. Unlike healthy fish, sick animals neither feed nor hide. They slowly swim up to the oxygen-rich locations, such as the water surface or inflow. Dead and severely sick fish often drift with the currents or float in unusual positions and jerk irregularly. A description of a healthy fish is given in Box 2.

It is important to note that dead fish initially sink to the bottom, rising to the water surface only after a couple of days. The length of time during which dead fish remain on the bottom depends on the water temperature. In colder water, dead fish can appear on the surface after even a week, while in warmer water this happens faster, within two or three days.

Box 2. Appearance of a healthy fish

- The body is covered with a thin layer of mucus and is free of wounds, ulcers and parasites.
- The scales fit tightly into the dermis and their colour is characteristic for the species and age.
- The eyes are white and the pupils are black. The eye reflex is responsive; when the fish is turned, its eyes also move. The cornea of the eye is tight, shiny and reflective.
- The back is fleshy and rounded.
- The gill covers are undamaged. The gill rakers and filaments are also intact and free of wounds and parasites. They are covered with a thin layer of mucus and their colour is a deep red.
- The fins are also undamaged and free of wounds and parasites.

3.1.2 Sampling of fish

After studying the behaviour of the stock, fish samples should be taken from different parts of the waterbody. In waters where fish are fed, samples should be captured at feeding spots. Here healthy or relatively healthier specimens can be found. Examination of samples taken at inflows and outflows, together with fish captured at feeding points, will provide a more reliable picture about the stage and range of the illness.

3.1.3 Examination of fish

The examination of fish on site includes a close observation of the appearance of the fish, its body parts, and after dissection, its organs.

3.1.3.1 Observation of the body

The appearance of fish that differ from healthy individuals (see Box 2) allows the establishing of a first diagnosis. The most frequent changes in the shape, colour and intactness of the body are as follows:

- The **body condition** can be estimated from examination of the dorsal surface of the fish. An emaciated fish, viewed from this perspective, resembles the blade of a knife. Abnormal thinness may indicate many various sicknesses, including inflammation of the digestive tract or a high number of worms.

- The **body surface**, as well as the volume and quality of mucus on it, gives useful information about the possible cause of death. Poison provokes the skin to produce excess mucus, while with some diseases such as saprolegniosis (see Section 8.1) or winter skin disease of common carp (Section 23.1), mucus disappears from the skin.

- **Loss of scales** may be due to mechanical injury. However, scale loss also frequently occurs during bacterial and fungal infections; this is very common in Chinese major carps, whose scales are less resistant. With some diseases such as spring viraemia of carp (SVC) (Section 6.1) and carp erythrodermatitis (ulcer disease) (Section7.1), fluid accumulates in the scale pockets, causing the scales to protrude.

- **Wounds, ulcers and parasites** on the body and fins are evident signs of a problem. Often, parasites such as leeches, fish lice, larval flukes (metacercariae) and white spot disease (ichthyophthiriosis) (Section 10.4) can be observed on the skin, but hyphae of the fungus *Saprolegnia* and proliferation of the epithelium due to fish pox are also clinical signs of changes. External injuries on fish suggest mechanical effects; however, they can also develop due to infection of the epithelium by pathogens such as *Saprolegnia* (Section 8.1) or ulcer disease (Section 7.1). Less frequently, ulcers on the skin and black spots (metacercarial infections) on the fin rays call for attention.

- An **unusually large belly** can be due to viral or bacterial infection. Enlargement of organs, accumulation of secretions in the abdominal cavity (ascites), or the presence of parasites large in size and/or number can also cause an unusually large belly. This can also be a clinical sign indicating an accumulation of sera in the abdominal cavity due to infectious dropsy, swimbladder inflammation or abdominal metacercariae (Section 15.4), but the presence of large cestode plerocercoids (*Ligula* spp.) (Section 14.4) produces similar clinical signs. Enlargement of the belly can also be due to either a severe infection with tapeworms in the gut or because of the accumulation of gas in the intestines caused by bacterial infection.

In the latter case, fish often float belly up at the surface of the water, as they are unable to submerge.

- The **protrusion of the anus** indicates a possible inflammation, as well as being a sign of fluid accumulation in the digestive tract.

- **Rough handling**, as well different parasites can cause broken fins. As fins regenerate rather quickly, such wounds indicate recent problems.

- The **mouth and gill slits** are usually closed, but in the case of suffocation they remain open. After death, the gills become pale and the tissues lose their structure. The presence of excess mucus on the gills suggests poisoning or suffocation, while tissue erosion is a clinical sign of gill necrosis and putrefaction.

- **Crippled or dwarfed fish** are rare in nature. A deformed spine can be observed mostly in fish that have developed under unsuitable conditions during their larval stage.

Whether the fish is dead or alive can be judged by the presence or absence of the eye reflex. Stiff eyes are evidence of death. The white outer layer of the eyeball (sclera) of a recently dead healthy fish is tight and shining, while the sclera of a dead diseased fish is sunken, dim and wrinkled. In normal cases, the colour of the pupil is black but, for example, in the case of a severe infection by eye flukes (Section 15.2), it becomes white. Due to various parasitic infections, goggled, protruded eyes can often be observed.

3.1.3.2 Observation of the organs of fish

Before dissection, fish should be killed swiftly, as specified in the relevant regulations of the given country. The initial steps of dissection to expose the internal organs are illustrated in Figure 2.

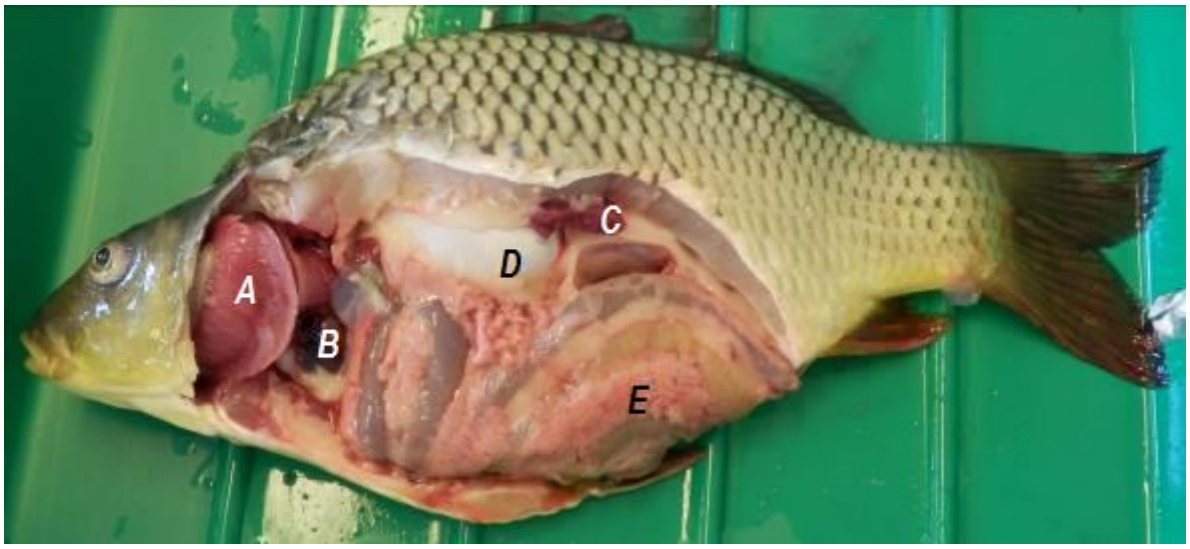
Figure 2. Initial steps in the dissection of a fish

1 2

3

4

1) The fish is killed by cutting through the backbone. 2–3) A cut starts from the head and the body is opened by a long cut in the medium line of the belly towards the anus. 4) Afterwards, a curved cut is made on the dorsal side in order to expose the internal organs. 5) Some of the internal organs: gills (A), heart (B), kidney (C), swimbladder (D) and liver (E).



5 Fresh preparation pictures

- **Sera** may be discharged and/or parasites may be observed upon opening up the fish; these are obvious signs of a potential health problem.

- **In the gut,** the mucous membrane of the intestine of a healthy fish is a light rose colour and its surface is flat. Inflammatory changes or thickening of the wall may indicate a bacterial infection.

- **Adhesion of the viscera and peritoneum** may also indicate bacterial infection. Large worms (cestodes and trematodes) may occur in the gut in large numbers. Some parasites, such as plerocercoids of the cestode *Ligula* or the nematode *Philometra*, hundreds of *Tetracotyle* metacercariae or large numbers of pseudocysts containing spores of *Myxobolus* draw the attention to infections that are already well established.

- The **swimbladder** is usually transparent and has no thick walls. Inflammatory changes in the swimbladder suggest sphaerosporosis or bacterial infections.

- **The heart** of a fish that is being necropsied is typically empty. However, in suffocated fish it may be filled with coagulated blood.

- A **highly swollen gall bladder** is a sign of starvation or of an illness of the digestive system.

- Normally, **the kidneys** are brownish-red in colour and easy to tear. However, bacterial (*Aeromonas*) infection degenerates them, and blood fluke infection (sanguinicolosis) causes them to become oedematous.

- **The liver** of a healthy fish is light brown, while **the spleen** is a dark-red elongated organ. When infected, these organs will become swollen.

- **The gills and gill cavity** can be best studied by cutting off the gill covers (see Figure 2). For detailed examinations, the gill rakers (cartilaginous gill arches) should be separated.

A table providing guidance for diagnosing fish diseases is found in Annex 1.

3.1.3.3 Collection of data and information

Collecting data and other information on background and the measures that have already been taken is the third step in on-site actions. This includes finding out:

- Whether there were similar earlier precedents and the date when mass mortality started.
- The number of dead fish.
- Whether and what actions have been taken so far.

Studying past management actions and situations on site gives useful information and data on:

- type and parameters of the waterbody;
- water supply (its source and whether there is any source of pollution or poisoning);
- physical (temperature, transparency/turbidity, colour), chemical (pH, oxygen content) and biological (aquatic weeds, number and size of the different fish species) parameters and qualities of the waterbody;
- dates of last stockings;
- quantity and quality of feeds given if the fish stock was fed;
- applied fishery and/or aquaculture management techniques;
- location of nearby industrial and agricultural plants; and
- possible agricultural sprayings in the vicinity.

3.2 Taking and sending samples for laboratory examinations

In cases where there is no specialized professional on the field, or if an on-site veterinary inspection does not result in a diagnosis, fish should be sent to a laboratory. In the laboratory, dissection and parasitological, bacteriological, virological and histological examinations will

support the establishment of an exact diagnosis. As soon as this is has been achieved, an official letter should be issued by the laboratory. It is important to note that a detailed, reliable investigation can be performed only by a well-equipped laboratory.

If there is a mass mortality, samples should include a minimum of ten fish. However, five fish should be taken for a routine control examination. The sample should be accompanied by an order or letter of request for examination signed by the owner or the responsible veterinarian. This order or letter should contain the information summarized in Box 3.

Box 3. Data and information on the order for examination fish in the laboratory

General information:

- Name and particulars of owner
- Name and particulars of veterinarian
- Name and address of payee of the examination

Particulars of the sample of fish:

- Species
- Age/age group
- Number of specimens
- Temperature of water (^{o}C)
- Location (name)
- Location (code or GPS)
- Requested examination
- Clinical signs (yes/no)

Sampling for koi herpesvirus (KHV) examination should be taken when water temperature is above 17 °C. On the other hand, the water temperature should be below 17 °C when taking samples for checking for spring viraemia of carp (SVC).

It is important to be aware that only live or freshly dead fish with undamaged organs should be sent for laboratory examination. Because their organs will show post-mortem changes, decaying fish that have died much earlier are unsuitable for laboratory examination. Fish captured alive, but transported dry, without water, are also unsuitable for examination, as parasites will dry and die. Even heavy infections of protozoans or monogeneans will remain undetected.

For the above reasons either just died or moribund live fish showing clinical signs should be sent to the laboratory. The best way is to place the sick but still live fish in a plastic bag of water with oxygen. Carrying the fish in water cooled with ice is also an acceptable solution. If there is no ice available, a third but not ideal solution is to pack the fish in fresh green, moistened plants.

If there is a possibility that the cause of mortality is environmental, water samples of one litre each should also be sent to the laboratory. If the container is a plastic mineral water bottle, it should not only be clean but also rinsed with the water from where the sample is taken. Sets of

Box 4. Data and information on the label of the sample of fish and water

Each sample of fish should be labelled as below:

- Name of farm and/or owner, address, phone and/or e-mail, case history, number of the fish pond or name of waterbody and species, number and age of sent fish.

Each sample of bottled water should be labelled as below:

- Name of farm and/or owner, address, phone and/or e-mail, case history, number of the fish pond or name of waterbody from which the sample was taken.

three samples from different typical points, such as inflow, should be taken. It is important to fill the bottles completely such that no air bubbles are left. The filled bottles should be properly labelled, as shown in Box 4.

If poisoning is suspected, an official person (veterinarian, notary, etc.) should be present when taking the samples, who by closing and signing the bottles can later testify as to the origin of the water, if the case goes to court.

In the frame of surveillance and eradication programmes, samples must also be taken and sent officially.

Sometimes it may also be necessary to transport tissue samples to a specialized laboratory. For virological examination, such samples can be frozen, but it is better to place them in transport media. Samples requiring bacteriological examination must not be frozen.

4. PREVENTING THE SPREAD OF FISH DISEASES

In fish ponds, especially when production is semi-intensive or intensive, prevention of fish diseases is particularly important. This can be due to the low or limited exchange of pond water relative to the density of fish. In this situation and in many countries, the treatment of disease using chemicals is uneconomical, and for human and environmental reasons, restricted in numerous countries. Hence, prevention is the best measure to obtain and maintain disease-free stocks in natural water bodies and fish farms. This can be achieved with a rigorous control of the trade of live fish, as well as with regularly scheduled monitoring of the health of the fish and water conditions.

4.1 Administrative measures for preventing the spread of diseases between continents, watersheds and farms

Natural barriers (i.e. oceans, mountains, deserts, isolated river systems) prevented the intercontinental spread of fish diseases for centuries. Nowadays, however, fish diseases from far biotopes can enter a new territory within a very short period of time. This is because today fish are transported by air and road.

Until the intensification of fish transfers in the middle of the last century, several diseases now commonly distributed worldwide (e.g. bothriocephalosis or khawiosis of common carp) were known only in the eastern part of Asia. Due to unconsidered introductions, these diseases were transmitted to Europe and the western part of Asia. Besides planned introductions, continuing imports of aquarium fish make the spread of fish diseases especially easy. Therefore, there is a need for strict regulation of fish transport, especially for those ornamental fish that are in close genetic relation to pond-cultured fish species.

Today there are well-established recommendations, guidelines, rules and regulations which support concerned decision-makers both in the field and in the administration and control of fisheries and aquaculture. This information is presented in Annex 2.

4.2 Practical measures for preventing the spread of diseases between continents, watersheds and farms

Effective veterinary control of fish transportation and the use of quarantine measures are necessary – as summarized in Annex 2 – to prevent the spread of fish diseases among regions. Previously, some widespread diseases, like SVC, were successfully contained by ordering the quarantine of infected farms. Unfortunately, ensuring prevention and maintaining effective measures for farmed fish species that also live in those waterbodies from which fish farms receive water is rather difficult.

4.3 Practical measures for preventing the outbreak and spread of diseases within a farm

Fish for stocking or further rearing should be purchased only from regularly checked fish farms. All purchased stock should be accompanied by a valid health certificate and documentation stating that the fish are free of specified diseases.

Some of the major points to be considered include the water supply and the disinfection of ponds, tools and water supply systems. It is also essential to use preventive treatment of fish stocks, not only when stocks are transported between different fish farms, but also when they are transferred from one pond or tank to another within the same farm.

Water supply

- Hatcheries should work with water that is free of pathogens.
- Fry-rearing ponds should receive the best quality water.
- Water supply of production ponds should come from natural waters carrying water free from toxic minerals and having only low numbers of pathogens.
- In order to prevent the entry of diseased wild fish with supply water, all waters entering a fish farm should be screened against fish, regardless of the actual size of the fish.

Management of fish ponds, water supply systems, equipment and tools

- New fish stocks should be released only in dried, possibly also previously frozen and disinfected ponds.
- Instruments and vehicles used for handling and transferring/transporting fish should be disinfected.

Management of fish stocks

- Preventive antiparasitic treatment should be administered to fish stocks received from outside or transferred within a fish farm.
- Because they facilitate the development of diseases, stress factors should be minimized; e.g. transfer and handling of fish should be done with care in order to avoid stress, trauma and wounds.
- Oxygen shortage in water during harvest and transport must be avoided.
- Thermal shocks are frequent when fish are transferred. For this reason, the temperature of the transporting water and the water in the tanks and/or ponds where they are to be placed should be equal.

5. TREATMENT OF FISH DISEASES

Diseased fish should be treated only with suitable, carefully selected products on the basis of a precise diagnosis. There are three different groups of treatment:

- Bath treatments
- Oral treatments
- Injection

Box 5 discusses the use of both certified and uncertified chemicals for treating fish diseases, while Box 6 provides information on the testing of new products for disease treatment.

Box 5. Use of certified and uncertified chemicals in treating fish diseases

There are countries where chemicals can be legally used for curing fish diseases, while in others there are serious restrictions. For this reason, it is important to check in officially approved lists whether the selected chemical is licensed for treating fish disease.

In cases where the selected chemical is not licensed for fish but only for use in other animals, the given product can be used, but only with certain restrictions and cautions. The minimum withdrawal period must be 500 degree days, which is considered as the required degradation time. The days required are calculated as follows:

No. days = 500 divided by the average daily water temperature (^{o}C)

Accordingly, the withdrawal period will be 100, 50, 25 or 20 days when the average daily water temperature is 5, 10, 20 or 25 ^{o}C, respectively.

5.1 Bath treatments

In fish medicine, when the objective is to remove ectoparasites or to heal lesions or wounds, in the majority of cases bath treatments are used. Depending on the length of the application time, four different types of bath treatment are used.

Box 6. Testing of a new product to be used for treatment of fish disease

The steps for testing new products are:

1. Three batches of 10 fish should be treated separately as instructed in the product description.
2. After finishing the treatment, fish should be observed for 48 hours. During this period, they should be properly kept, as improper conditions may cause mortality.

If there is no mortality observed, the product can be used.

5.1.1 Flush bath or dipping

This method is used when the chemical is effective within a very short period of time, i.e. from thirty seconds to about two minutes. Flush bath techniques are used in culture systems where eggs or fish are kept in flow-through tanks with continuous water exchange. For dip treatment, the fish to be treated are held in a net and dipped into a concentrated chemical solution. See Annex 3 for more details.

5.1.2 Short bath

This is usually the cheapest, most effective and most common way of using chemicals for curing fish diseases. The period of treatment is 5 to 40 minutes in tanks where the drug is precisely dosed. The reaction and state of the fish can be checked and if necessary, treatment can be modified or stopped by reducing the concentration, i.e. by adding clean water. In practice, fish are placed into a tank containing the precisely dosed chemical and are kept in this solution for the suggested time of treatment.

During this treatment, aeration of the water is required. At the end of the treatment, the fish are either removed or the solution is diluted with freshwater, the latter being less stressful for the fish. For shorter periods (5–10 minutes), the use of a net is helpful to remove fish from the solution rapidly.

5.1.3 Transit bath

This is a type of short bath used on fish farms that is applied for the routine treatment of ectoparasites when fish are transferred from one pond to another during harvesting, wintering or stocking. This method is especially recommended when fry are transferred into a larger pond or before placing fish into wintering ponds.

If fish are transferred into tanks within a known period of time, a tank containing an antiparasitic or bactericidal solution serves the purpose well. The period of treatment ranges from 30 minutes to 4 hours, depending on the actual time of transfer. Accordingly, the concentration of chemicals or drugs depends on the treatment time. Combinations of drugs are available: a mixture of drugs effective against helminths and crustaceans and those effective against ectoparasitic protozoans is usually used, as detailed in Annex 3.

5.1.4 Long bath

This kind of bath treatment is used in ponds and aquaria. With this method, highly diluted drugs are applied for a period of between 24 and 48 hours. Before treatment, the level of the pond is usually lowered to about half, and the drug is continuously added to the inflowing water so that the dissolved drug will be evenly distributed throughout the pond. At the end of the application, the drug can be flushed out of the pond by opening the inflowing water. In order to save water, some farmers prefer spraying a concentrated solution of the drug from boats. Some drugs may lose their activity within one or two days, in which case there is no need to refresh the water.

In the past, this method was regularly used for treating fish stocks with malachite green and organophosphates. In wintering ponds, this method can still be applied for treatments with formaldehyde solution. For details, see Annex 3.

5.2 Oral treatments

Oral treatment is a practical way of administrating medicine, but only for those fish species which readily take feeds. Therefore, this method is not suitable for treating fish that feed only on plankton or vegetation.

This method is widely followed for treating sick fish with antibiotics or anthelminthics. Before treatment, the farmer must check whether the medicated feed will be consumed by the fish. Sick fish often either do not feed or consume less than the amount of feed needed to achieve the necessary dose of drug mixed into the feed.

The concentration of drug that must be contained in the feed depends on the ingestion of fish. In order to obtain the expected result, in spring and autumn, when fish feed less than in summer, the feed must contain the drug in higher concentration. Therefore, when calculating the

concentration of drug, body mass index should be considered. In some countries, there are already drug premixes, but in most cases farmers prepare the medicated feeds themselves. Before 1990, in CEE and in the former Soviet Union, drugs containing antibiotics and niclosamid were regularly fed at the period before the calculated time of outbreak of bacterial illnesses or cestode infections, respectively.

The most frequent on-farm methods for preparing medicated fish feeds are as follows:

- saturating wet grain with antibiotic solution;
- mixing the drug(s) into veterinary feed with a concrete-mixer; and
- spraying the feed with antibiotics dissolved in alcohol and coating the feed with cooking oil.

5.3 Injection

Because of economic considerations, using injection is practically restricted to protecting valuable warmwater brood fish or ornamental fish. Parenteral application with injections is done by inoculating the drug intramuscularly or intraperitoneally. The place of injection can be the massive musculature on the back or the caudal musculature. In the case of intraperitoneal injection, the abdominal wall should be pierced 4–5 cm in front of the anus. With a carp of 1 to 1.5 kg body weight, drugs in 5–10 ml isotonic solution should be injected. See Box 7 for details.

Box 7. Vaccination

In the farming of warmblooded animals, vaccination against contagious diseases is an accepted way of disease prevention. Similarly, vaccination against some viral and bacterial diseases of salmonids is frequently used, as only vaccination of the fish stocks effectively prevents outbreaks of such diseases.

In carp culture, only koi herpesvirus disease (KHVD) is treated by vaccination, and only if the given country is intensively infected. In most of the Central and Eastern European, Caucasus and Central Asian countries vaccination of carps seems to be practical only for disease prevention in broodstock. At present, because of the low value of carps, it not profitable to use vaccines in their culture.

As in other fields of animal health, the easiest way of vaccine production is the preparation of inactivated vaccines from the causative organisms. Most vaccines developed for fish belong to this group; however, there are also attempts to make vaccines from attenuated viruses and bacteria.

The most common way of vaccination is inoculation into the muscle or abdominal cavity. However, this method is labour intensive and thus can only be used for broodstock and precious ornamental fish. Nevertheless, it is recommended for preventing mass mortalities of fingerlings of predatory fish such as northern pike and wels catfish. In these cases, inactivated vaccines can be prepared from moribund fingerlings, and by inoculating the broodstock some weeks before breeding, the chance of them developing immunity and producing eggs free from pathogens is high.

For treating fish stocks, including fry, immersion vaccination is used. The simplest way is via direct immersion, where the fish are immersed into the solution containing antigen. The effectiveness of this treatment is improved by preceding immersion of the fish into the soluble antigen with a bath in a hyperosmotic solution, which causes a temporary disruption of the integrity of the epithelia of the gills and skin. This method effectively increases the uptake of vaccine and enhances the efficacy by which vaccine components are processed. The damage induced is slight and does not impose additional stress over the handling associated with immersion vaccination. This method has been used for preventing KHVD in

seriously affected countries, while in countries where only sporadic appearance of KHVD has been observed, eradication of the diseased stocks and veterinary quarantine are used.

A technology has also been developed for treating common carp for SVC and *Aeromonas hydrophila* infections, and there are also vaccines available for vaccinating grass carp and black carp against grass carp haemorrhage.

An extreme way of vaccination, developed by a Japanese scientist, involves dropping the inoculum directly onto the gills of fish.

Although there is a compound vaccine developed against *Aeromonas salmonicida* and *A. hydrophila*, it is surprising that no vaccine is available against the most common ulcer disease of carp, which caused by *A. salmonicida f. achromogenes*.

6. DISEASES CAUSED BY VIRUSES

Viruses are among the most important pathogens of finfish and other aquatic animals, causing billions of dollars in losses to global aquaculture annually. As viral infections are often difficult to detect, particularly in the case of emerging diseases, are untreatable, and are difficult or impossible to irradicate, they are responsible for many of the mass mortalities seen in aquaculture facilities, as well as for significant chronic losses.

Viruses are minute infectious agents that are characterized by a lack of independent metabolism and by the ability to replicate only within living host cells. There characteristics are discussed further in Section 2.2.1.

This chapter presents information on eight viral diseases of warmwater finfishes, two of which are listed by the OIE.

6.1 Spring viraemia of carp

Spring viraemia of carp (SVC) is caused by *Rhabdovirus carpio*. Earlier, this disease was known as infectious dropsy. SVC is an acute, systematic viral disease, primarily of common carp, which especially affects fish in their first and second years. Natural infections have been recognized in common and koi carps, Chinese major carps, Crucian carp, goldfish, roach, ide, tench and wels catfish. This disease is notifiable by the OIE.

Optimal epizootic temperature: SVC appears mostly under intensive culture conditions where losses are usually high. Disease typically occurs at water temperatures below 18 °C, and predominantly in the spring, but fry can be affected at temperatures as high as 22–23 °C.

Transmission: The reservoirs of SVC are clinically infected fish and covert viral carriers among cultured, feral or wild fish. The virus is spread via faeces, urine, gill and skin mucus and exudates of skin. The mode of transmission for the SVC virus is horizontal, but egg-associated transmission (vertical transmission) cannot be ruled out. Horizontal transmission may be direct by water or through animate vectors such as zooplankton, parasites (e.g. *Argulus foliaceus, Piscicola geometra*) and other fish.

Pathology: The virus propagates in the epithelial cells of the blood vessels, causing damage first of all in the capillaries. Due to damage to the circulatory system, haemorrhages appear on the skin, the serous membranes and the mucosa. Sera enters into the tissues and the abdominal cavity through the damaged walls of blood vessels. Organs such as the liver and kidney degenerate; less frequently, they become partially necrotic.

Clinical signs: Clinical signs (Figures 3 and 4) include enlargement of the belly, exophthalmos, and petechiae on the skin, in the eyes and under the gill covers. The gills are usually pale, while the anus is inflamed and protruded. Upon opening the belly, abundant sera empties. Carp in good condition can survive the disease; however, if the infection is exacerbated by co-infection of carp erythrodermatitis, the chance for recovery is slim.

Figure 3. Spring viraemia of carp in common carp

photo by Gy. Csaba

Figure 4. Elevated scales of a common carp with spring viraemia

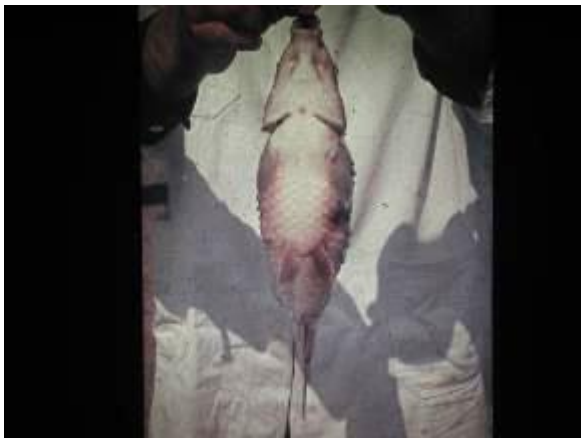


photo by Gy. Csaba

Prevention and treatment: Protection against the disease is based on prophylaxis as described in Box 8.

Pathogen detection: In the laboratory, SVC virus can be easily detected by viral isolation on epithelioma papulosum cyprinid (EPC) cells or by polymerase chain reaction (PCR) examination. It is important to note that SVC should be differentiated from other diseases characterized by swelling of the abdomen, such as *Aeromonas* infection, swimbladder inflammation, ligulosis, tetracotylosis and sanguinicolosis.

6.2 Haemorrhagic disease of grass carp

Haemorrhagic disease of grass carp is caused by grass carp reovirus (GCRV). It is the most serious infectious disease of this species and causes significant losses of fingerlings. Occasionally, infections also occur in older (one or two-year-old) fish.

Optimal epizootic temperature: 25–28 °C.

Transmission: The disease can be transmitted by water, infected fish and parasites.

Clinical signs: Clinical signs include exophthalmia and haemorrhages at the base of the fins, in the gill covers, on the gills, and in the liver, spleen, kidney and intestine.

Box 8. Prophylaxis applicable in the case of viral diseases

Viral diseases are impossible to control by chemotherapeutic methods. Keeping out pathogens seems possible only on small farms that are supplied by ground water and with drainage that prevents the entrance of fish from open recipient waters. On most large carp farms which are supplied by surface water, eradication by slaughter and disinfection has little chance of success. Prophylactic measures on farms should include:

- disinfection of eggs by iodophore treatment;
- chemical disinfection of ponds and equipment;
- careful handling of fish to minimize stress;
- safe and sterile disposal of dead fish;
- avoiding crowding of fish during winter and early spring; and
- purchasing fish from virus-free farms.

Prevention and treatment: Protection against the disease is based on the prophylactic measures described in Box 8.

Pathogen detection: In the laboratory, the virus can be easily detected by viral isolation on carp kidney (CK) cells or by PCR examination.

6.3 Carp pox

Carp pox is caused by cyprinid herpesvirus1 (CyHV1). Carp pox is one of the long-known viral diseases of common carp and is wide spread in most countries where cyprinids are cultured. It mostly infects common carp, but other cyprinids are also sensitive to this disease.

Optimal epizootic temperature: During colder winter periods below 14 °C.

Clinical signs: Presence of smooth, generally flat, white raised areas on the skin and fins are the signs of this disease. The lesions resemble wax melted from a white candle (Figure 5). These areas grow to 1–6 mm from translucent to milky-white plaques. They may be on isolated areas, but in severe cases they coalesce and cover large parts of the body. Sometimes the lesions progress from a simple hyperplasia to a papillomatous stage. In severe cases, this infection also reduces the immunity of fish and leaves the infected area prone to a secondary bacterial infection.

Figure 5. Clinical signs of carp pox on the head of common carp

photo by Gy. Csaba

Prevention and treatment: Protection against the disease is based on the prophylactic methods described in Box 8. There are no known treatments, but fish might recuperate at temperatures of over 15 °C. Mortalities are rare, although extreme infections can occur.

Pathogen detection: Diagnosis is easily made from external signs, and the virus can be easily detected by PCR examination.

6.4 Koi herpesvirus disease

Koi herpesvirus disease (KHVD) is caused by Cyprinid herpesvirus3 (CyHV3). It is one of the diseases of finfish listed by the World Organisation for Animal Health (OIE) and is the most important disease in carp farms. The virus infects both European and Asian common carp, including the coloured variations known as Koi carp.

KHVD affects fish of various ages, often resulting in 35–80 percent (can reach up to 100%). mortality in susceptible populations. However, as with other herpes viral infections, KHV may remain in fish that survived the disease for long periods; therefore, fish that recover from a KHVD outbreak should be considered as carriers of the virus.

Optimal epizootic temperature: Between 17 and 25 °C.

Transmission: The reservoirs of KHV are clinically infected fish and covert viral carriers among cultured, feral or wild fish. Virulent virus is spread via faeces, urine, gills and skin mucus. Water is the major abiotic vector. However, animate vectors (e.g. other fish species, parasitic invertebrates and piscivorous birds and mammals) may also be involved in transmission.

Clinical signs: Infected fish suffer from appetite loss, often remain near the surface, swim lethargically and may exhibit respiratory distress as well as uncoordinated swimming.

KHV infection may produce severe gill lesions in the form of gill mottling with red and white patches (Figures 6 and 7). The white patches are due to necrosis of the gill tissue. Other external signs of KHVD may include sunken eyes and pale patches on the skin. Some KHV-infected fish may have a notched nose.

Figure 6. Koi herpesvirus disease – relatively mild clinical signs on the gills of a common carp

Figure 7. Koi herpesvirus disease – necrotic changes on the gills of common carp

In some cases, secondary bacterial and parasitic infections may be the most obvious clinical signs, masking the damage caused by the primary viral infection. Microscopic examination of gill biopsies often reveals high numbers of bacteria and various parasites. Internal signs of KHVD are non-specific, but may include greater than normal adhesions in the body cavity and enlargement of internal organs.

Histopathology: Histopathological examination can reveal hyperplasia and hypertrophy of the branchial epithelium and fusion of secondary lamellae. Necrotic areas can also be detected. Occasionally, the branchial epithelial cells have nuclear swelling; margination of chromatin gives a “signet ring” appearance; and eosinophilic intranuclear inclusions can be seen.

Pathogen detection: Current diagnostic procedures include KHV detection based on typical clinical signs (as mentioned above), histological examination, and viral isolation in KF-1 cells, but the most efficient method is by PCR examination.

6.5 Herpes viral haematopoietic necrosis of goldfish

Herpes viral haematopoietic necrosis (HVHN) is caused by Cyprinid herpesvirus2 (CyHV2). The only known host is goldfish, but presumably gibel carp (*Carassius auratus gibelio*), a subspecies of goldfish, is also sensitive to this virus. The disease has been observed in all age groups, and can result in almost 100 percent mortality.

Optimal epizootic temperature: Outbreaks appear at water temperature between 15 and 25°C.

Clinical signs: Infected fish become lethargic, stay at the bottom of the pond, and may become anorexic. Clinical signs can include the following: ascites; pale colour of the gill, liver, spleen and kidney; and also enlargement of the spleen. Sick fish do not feed; hence the intestine is empty. At more detailed examination, necrosis in the haematopoietic cells of the liver, kidney, spleen and in the propria and submucosa of the intestine can be observed. Hypertrophy and hyperplasia of the gill filaments has also been reported.

Prevention and treatment: There is no effective method for treatment of this disease.

Pathogen detection: In the laboratory, the virus can easily be detected by PCR examination.

6.6 Herpes virus infections of silurids

This disease was first observed in Hungary in intensive fry and fingerling rearing ponds of wels catfish. Mortality can be 100 percent.

Optimal epizootic temperature: Outbreaks are likely to occur at water temperatures of about 25 °C.

Clinical signs: Externally, fish show signs of haemorrhagic disease. The abdomen is swollen, and the fins and abdomen are haemorrhagic. Internally, the peritoneal cavity contains yellowish fluid, the spleen is enlarged, and the kidneys and liver are also haemorrhagic.

Prevention and treatment: Successful but unpublished trials were made to prevent this disease by vaccinating broodstock with inactivated virus.

Pathogen detection: In the laboratory, the virus can easily be detected by PCR examination.

6.7 Pike fry rhabdovirus disease

Pike fry rhabdovirus disease (PFRD) is an aetiologically less studied *Rhabdovirus* infection. It is frequently seen in 2–3 week old pike fry (Figure 8). The infected stocks show 100 percent mortality. In old fish no disease was recorded.

Figure 8. *Rhabdovirus infection* in pike fry

photo by Gy. Csaba

Pathology: Disease develops in fry of which at least one of their parent fish has a latent infection. Hence, the parent fish is the vector of the virus.

Clinical signs: Infected young pike lose their schooling behaviour, move slowly near the surface or lie motionless on the bottom. The fry also show haemorrhages on the body. Due to this, the posterior part of the body is a scarlet colour.

Prevention and treatment: Egg transmission can be interrupted by disinfection with an iodophore (50 mg/litre for 10–15 minutes). Vaccination of broodfish with inactivated virus could also give good results.

Pathogen detection: Methods for the diagnosis of PFRD do not differ from those for SVC.

6.8 European catfish virus infection of the brown bullhead

European catfish virus (ECV) infection of the brown bullhead is caused by a ranavirus. In the waters of Central Europe where the brown bullhead, an exotic catfish, was introduced, mortality during disease outbreaks can be as high as 80–90 percent.

Optimal epizootic temperature: This disease occurs when water temperature is about 20–25 ^{o}C.

Clinical signs: Diseased fish totter in a slanted or vertical position near the water surface. On the skin and fins, a huge number of smaller or larger (0.5–1.5 mm) haemorrhages can be observed, and the anus is protruding and deep red in colour. Haemorrhages can also be found on the gills, as well as in the spleen, liver and kidney.

Pathogen detection: In the laboratory, the virus can be easily detected by viral isolation on EPC and BF2 cells, and by PCR examination.

7. DISEASES CAUSED BY BACTERIA

Bacteria are unicellular, prokaryotic microorganisms that multiply by cell division (fission) and that typically have a cell wall. They may be aerobic or anaerobic, motile or non-motile, free-living, saprophytic or pathogenic.

There are only a few bacterial diseases that are currently listed by the OIE, and none of these cause diseases of finfish.This chapter deals with five bacterial diseases of warmwater finfish, all of which affect mainly common carp and other cyprinids.

Although bacterial infections are often be successfully treated, the misuse of antibiotics in aquaculture, agriculture and human medicine, particularly those antibiotics important to treating disease in man, can result in the development of bacterial strains having antimicrobial resistance (AMR), a topic of growning global concern, as it may lead the ineffectiveness of antibiotics against bacteria causing human disease.

7.1 Carp erythrodermatitis

Carp erythrodermatitis (CE) or ulcer disease is caused by *Aeromonas* spp. It occurs mostly in cyprinids. It does not cause a general infection (septicaemia), but evokes acute epidermal ulcers (Figure 9). Mortality is usually low, not reaching 20 percent, but the consequences of secondary infections increase the mortality rate.

Figure 9. Ulcer disease of common carp caused by *Aeromonas salmonicida* forma *achromogenes*

Fresh mount picture

Optimal epizootic temperature: Water temperature plays an important role in the development of this disease. Below 12 °C the infection remains without clinical sign; however, at over 22 °C well-visible clinical signs always appear.

Transmission: In transmitting the disease from fish to fish, blood-sucking parasites might play role. However, epithelial lesions could develop to ulcers when infected water, fish or infected items carry the pathogen to an uninfected fish stock.

Clinical signs: Typical clinical signs are the following: small haemorrhages appear on the skin or fins, at the base of which these develop into deep, round ulcers of about 3 mm in diameter that are margined by a purple-coloured layer of living cells. The ulcers can deepen to the serosa covering the abdominal cavity. Ulcers can also develop in scaled carp, but their shape is not round. The changes start with the elevation of scales due to the accumulation of fluid in the scale pockets, which then is followed by scale loss. Very often exophthalmia, petechial bleedings on the gills and dropsy can also be observed, but these clinical signs seem to be the consequence of a secondary infection.

Prevention and treatment: Antibiotics mixed in the feed of the fish and fed for 7 days at a dose of 30–70 mg/body weight (BW) give good results (see Annex 3). Before feeding, the antibiotic should be tested for drug resistance.

Pathogen detection: Diagnosis of the causative agent is based on bacterial isolation. Isolation of the atypical *Aeromonas* strain can only be done from the freshly infected red zone of the ulcer. Only secondarily invasive water bacteria are isolated from other parts of the ulcer.

7.2 Infectious dropsy (septicaemia) of carp

Bacteria causing infectious dropsy occur worldwide, and all freshwater fish are prone to it.

This disease is mostly caused by mesophilic, motil *Aeromonas* spp., such as *A. hydrophila, A. caviae abd A. veronii.*

Optimal epizootic temperature: The disease develops when water temperature is above 10 °C, in most cases as co-infection of another pathogen.

Clinical signs: Septicaemia is marked by bleedings, exophthalmos, oedema in the scale pockets and ascites in the abdominal cavity, as well as petechial haemorrhages on the gills. Internally, there are varying amounts of pinkish-red fluid in the abdomen. Frequently, there are haemorrhages of the gastrointestinal tract and enlargement of the spleen (Figure 10). Co-infection with *Saprolegnia* is frequent.

Figure 10. Septicaemia caused by *Aeromonas hydrophila* in common carp. Note haemorrhages in tissues

Fresh-mount picture

Prevention and treatment: Prevention and treatment correspond to those suggested for CE.

Pathogen detection: Detection **i**ncludes finding bacteria in wet-mounts upon microscopic examination, isolation in blood agar substrate and identification with biochemical methods.

7.3 Flexibacteriosis or columnaris disease

This disease is common in salmonids but also occurs in stocks of common carp, Chinese major carps and wels catfish. The bacterium which causes this disease occurs in the water, soil and on the surface of fish, especially on the gills.

Optimal epizootic temperature: Outbreaks usually occur at water temperatures above 18 °C.

Clinical signs: The first external sign of the disease is the appearance of greyish-white spots on the body of the fish, often on the head, lips and fins (Figure 11). These spots are very similar to those caused by *Saprolegnia*. A localized lesion often develops on the gills too. In severe infections, the fish looks as if it is covered with a white cotton-wool layer. Groups of bacteria form white-coloured slopes (columns) on the surface of the skin and gills, which can be easily noticed upon microscopic examination (Figure 12). Unfavourable environmental conditions of keeping and feeding, stress and mechanical injuries support and accelerate the development of infection.

Figure 11. Flexibacteriosis in common carp; colonies of bacteria cover the skin on the head

Photo by Gy. Csaba. Fresh–mount picture

Figure 12. Columns built up by *Flexibacter* bacteria on the skin

Photo by Gy. Csaba.Wet- mount preparation

Prevention and treatment: For therapy, antibiotics mixed into the feed of fish are used. Improving water quality and living/rearing conditions of fish can also ensure a good result.

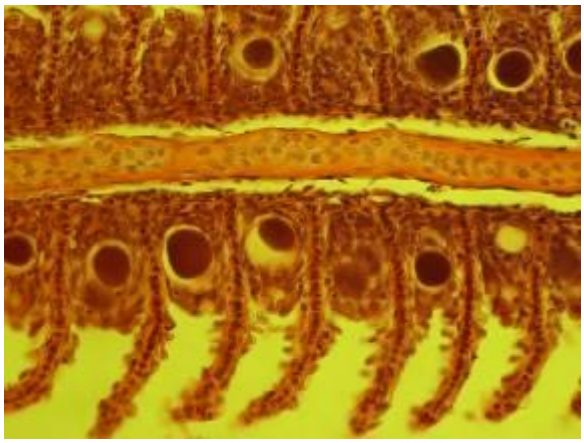
Pathogen detection: Infections can be easily diagnosed by examination of wet-mounts of tissue samples using a microscope.

7.4 Mucophilosis or epitheliocystis disease of common carp

This is a rarely diagnosed disease, with infection seeming to occur in different freshwater and marine fish. Besides common carp, intensive infection of the gills in silver carp is also known. Infections usually occur during summer.

Clinical signs: In cases involving intensive infection, the epithelial layer of the gill is full of small round spots (Figure 13). Due to proliferation of epitheloid cells, lamellae are no longer seen on the filaments. Changes are caused by *Chlamydia*-like organisms. Diseased fish show signs of suffocation.

Figure 13. Mucophilus (epitheliocystis) units in the gill filaments of common carp harbouring thousands of *Chlamydia*-like org.



Histological section, H & E staining

Prevention and treatment: No treatment is known, but drying and disinfecting the bottom of the pond and transferring the fish into another pond stops the intensification of infection.

7.5 Fish tuberculosis

Fish tuberculosis is a slowly developing chronic disease that is rather common in aquarium and marine fish. There are also reports from infected carps.

Clinical signs: Most fish species manifest few or no external signs of disease, but in advanced stages cachexia, exophthalmia, dermal ulcerative lesions and loss of scales can be observed. Internal signs include an enlarged and softened spleen, kidney and liver; development of nodules of different size; and formation of granulomas from epitheloid cells surrounded by a thick connective cell capsule.

Prevention and treatment: There is no treatment. As these bacteria can cause zoonosis in the form of skin granulomatosis, infected fish stocks must be destroyed.

Pathogen detection: The finding of bacteria in nodules stained by the Ziehl-Nelsen method supports diagnosis.

8. DISEASES CAUSED BY FUNGI AND ALGAE

These are two very different groups, which are placed together in this chapter for the sake of convenience. Fungi (singular: fungus) are members of the Kingdom Fungi, which includes single-celled or multinucleate organisms that live by decomposing and absorbing the organic material in which they grow. Algae (singular alga) is an informal term for a large and very diverse (polyphyletic) group of photosynthetic eukaryotic organisms that were formerly considered to be plants, and which range from microscopic unicellular organisms to large (multicellular) forms (seaweeds). While there is no generally accepted definition of algae, they are typically described as unicellular or multicellular organisms occurring in fresh or salt water or moist ground, and having chlorophyll and other pigments, but lacking true stems, roots and leaves.

This chapter deals with three diseases of warmwater finfishes caused by fungi, and with algal toxicosis and mortalities caused by algal blooms.

8.1 Saprolegniosis (dermatomycosis)

Fungal spores are present in all freshwater systems. This disease is caused by members of the genera *Saprolegnia* and *Achlya*, which are facultative pathogens of fish that usually live on putrid organic matter. Fish with vulnerable scales, like silver carp or grass carp, are especially sensitive to this infection.

Pathology: The fungus produces clumps of aseptate hyphal strands which project outwards from the infection site. These resemble white "cotton wool-like" tufts (Figure 14). Heavy infections develop when the resistance of fish drops due to a bacterial or parasitic disease, but may also occur at sites of mechanical injury. Less frequently, an unusual low temperature can be a predisposing factor.

Figure 14. *Saprolegnia* infection of an aquarium fish

Fresh–mount picture

Clinical signs: Infected fish are covered with "cotton" become emaciated and die. Diagnosis of the disease is easy. Sick fish are covered with the "cotton wool-like" hyphae of the fungi. Such heavily infected fish swim apathetically, trying to reach oxygen-rich water. When diagnosing the disease, it is necessary to consider the basic, causative problem, which can be mechanical injury, bacterial infection (e.g. *Aeromonas*), blood-sucking parasites (sanguinicolosis), etc.

Prevention and treatment: Treatment of infected young fish is worthwhile, while treatment of table fish usually is not. This is because seriously infected stocks no longer have any market value. Prevention is based on improving rearing conditions. In the past, malachite green was

used against fungal infections, but this chemical is now banned in many countries because of its carcinogenic characteristics. At present, formalin is used as presented in Annex 3.

8.2 Putrefaction of gills (branchiomycosis)

Infections appear mostly during the warm period of the year. Increased organic content of pond water promotes the development of this disease. In ponds of intensive duck culture or where excess phosphorous fertilizers are used, regular occurrences of branchiomycosis are recorded. The causative fungi, *Branchiomyces sanguinis* and *B. demigrans*, may attack the gills of different cyprinids, as well as those of predator fish such as northern pike and wels catfish. Infections are carried to uninfected ponds by the movement of sick fish or through inflowing water.

Pathology: Hyphae of these fungi develop in the blood vessels of the gills (Figure 15), and less frequently, in those of the liver or kidney. Spores develop inside the hyphae and are released through injury to the gills. It is still not known whether spores initiate infection through the gills or are taken up by the fish from the mud and carried to the gills by the blood stream.

Figure 15. *Branchiomyces* hyphae in the gills of a common carp

Wet- mount preparation

Clinical signs: Hyphae of the fungi block the arteries and veins of the gill, hence blood supply stops. Initially, a congestive hypertrophy develops which is followed by a partial decay of the gill tissue. Secondary infection by bacteria can make this process more severe. As a result, several gill filaments degenerate, die and fall off. An acute infection can pass within three days. In this case, the gills have a haemorrhoidal character. Gills may be mottled, showing a marbling appearance. Some parts of the filaments are red due to the presence of the stagnating blood, while other parts are pale due to the loss of blood supply. Mortality at this stage may be as high as 70–100 percent. In subacute cases, the disease process may take 1–2 weeks. Co-infections with bacteria and *Saprolegnia* are common, and besides the processes causing marbling, degeneration dominates. Clinical signs of the chronic form are less pronounced. Only the ends of the gill filaments seem to be thicker. The fish grow slowly, do not utilize food properly and sporadic mortality is observed. Identification of the disease is not difficult. The finding of hyphae in the gills helps to differentiate infections from gill necrosis and sanguinicolosis.

Prevention and treatment: Treatment of the disease is not known and thus prognosis is poor. Reducing co-infections by bacteria and parasites helps survival of the diseased fish stocks. Prevention is based on improving water quality and changing the infected fish population.

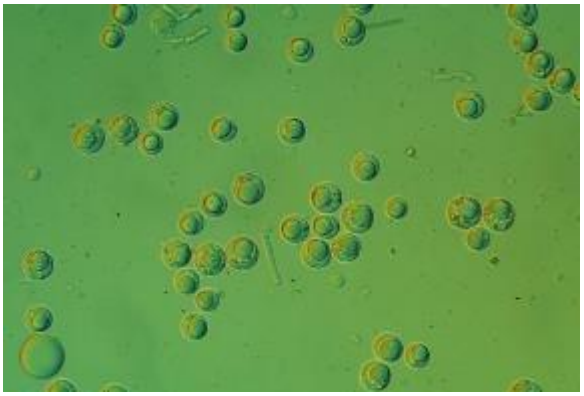
8.3 Infections caused by Dermocystidium spp.

Dermocystidium is an enigmatic genus with unknown taxonomic position. Dermocystidiosis is a rather frequent disease of cyprinids. In common carp, *Dermocystidium koi* (also known as *D. ershowi*) is the best-known species, but *D. cyprini* is also found on the gills of this species.

Clinical signs: Usually, white-coloured cysts located on the skin, fins or gills can be seen with the naked eye. They have hyphae-like strings filled with round spores (Figure 16). This parasite forms pea-sized protuberant nodules on the skin in which whirling hyphae are found. A specific form of infection of Crucian carp was reported where a *Dermocystidium* sp. infected the eye: a red ring-like iris surrounded the pupil (Figure 17). Although little is known about the pathogenicity of these organisms, their presence in the gills inhibits respiratory function, and wounds that develop on the skin provide open entries to bacterial pathogens. A less- known Dermocystidium infection occurs in common carp, causing granulomatosis in the internal organs. The center of the granuloma is necrotic and spores measuring 3–15 µm in diameter are located at the periphery of the nodules. In this infection no hyphae were found.

Prevention and treatment: Not known.

Figure 16. *Dermocystidium* spores



Wet-mount preparation

Figure 17. *Dermocystidium* infection in the eye of a Crucian carp

Photo by T. Müller. Fresh-mount picture

8.4 Toxicosis caused by algae

Some species of algae, especially members of the Cyanophytae produce toxins in the water that can cause mortality even in low concentration. This toxicosis occurs mostly in ponds where excess organic and chemical fertilizers are used. It is assumed that in natural waters toxins of a cyanobacterial alga, *Cylindropermopsis raciborski* cause poisoning of both the flora and fauna, including fish.

Prevention and treatment: Protection is based on killing the algae with lime or copper components (see Annex 3) and stopping the use of fertilizers until the water quality becomes acceptable. Upon noticing this problem, intensive aeration of the pond water may be helpful.

8.5 Algal bloom

Algal blooms in both natural and artificial waters, including fish ponds, are a frequent occurrence. In ponds that are rich in organic matter, excessive proliferation of algae can take place during hot summer days. These algae covering the water surface prevent light from entering the water, which causes oxygen depletion during the daytime. In addition, the increased biomass of algae will intensively consume oxygen during the night.

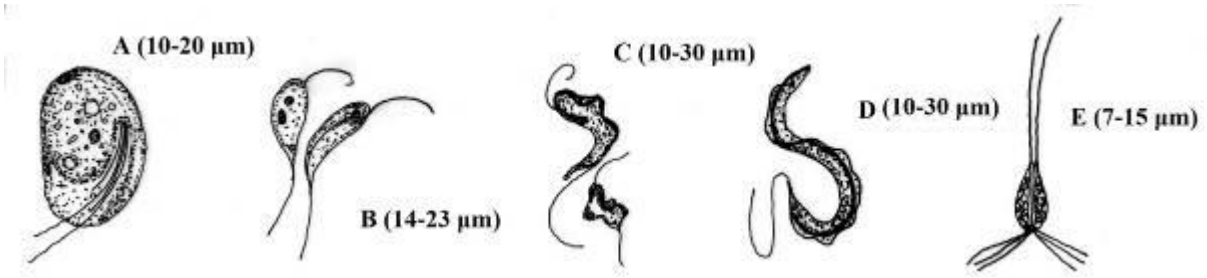
Clinical signs: In the early morning, stressed fish can be seen at the water surface where they gulp air. In severe situations, the fish may start to suffocate during the daytime and early night. In fry-rearing ponds, young fish can be damaged by algae covering the gill filaments.

Prevention and treatment: Protection is similar to that applied for toxicosis caused by algae. This includes killing algae with lime or copper components (see Annex 3) and stopping the application of fertilizers until the water quality becomes acceptable. Upon noticing the problem, intensive aeration of the pond water may be helpful.

9. DISEASES CAUSED BY FLAGELLATED PROTOZOANS

Parasitic flagellates are very small protozoans equipped with 1 to 8 flagella (Figure 18).

Figure 18. Flagellate parasites infecting fish. A) *Ichthyobodo necator*, B) *Cryptobia branchialis*, C) *Trypanoplasma borelli*, D) *Trypanosoma danilewskii*, E) *Spironucleus elegans*

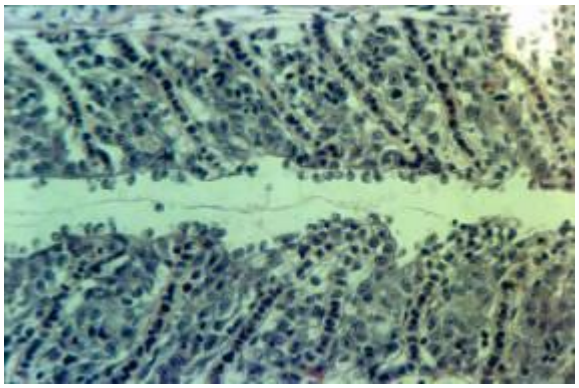


9.1 Veil disease or ichthyobodonosis (costiosis)

The disease is caused by a small flagellated parasite, *Ichthyobodo necator*, known better by its synonym, *Costia necatrix* (Figures 18A and 19). This small (10–20 by 6–8 µm) biflagellated bean-shaped parasite attaches to the epithelium of the skin or gills, or moves vigorously on them. It swims, so it can leave the already-infected host in order to find a new one.

Clinical signs: When infected with thousands of *Ichthyobodo*, the epithelium is damaged and a thick mucus that is full of dead epithelial cells covers its surface. The skin becomes rugged. Shreds of dead epithelium float on the skin surface, resembling a veil. Heavily infected fish stop feeding due to oxygen deficiency resulting from the damaged gill epithelium. Therefore, the fish swim up to the surface or gather at the inflow where there is oxygen-rich water.

Figure 19. Gill filaments of a common carp with large numbers of *Ichthyobodo* attached



Histological section, H & E staining

Prevention and treatment: Bath treatments used against protozoan infections (see Annex 3) are effective in curing veil disease.

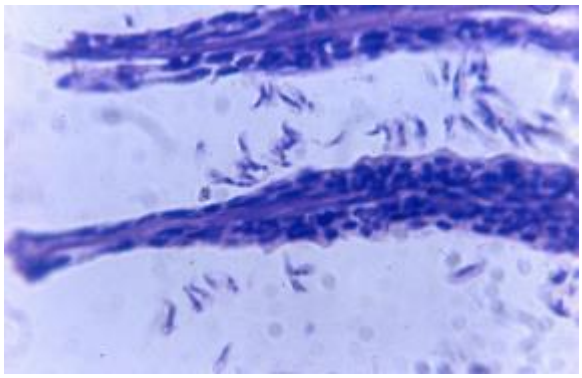
Pathogen detection: A 100–200 fold microscopic examination is needed in order to identify the infection. In a skin or gill scraping, specimens of *Ichthyobodo* showing a characteristic undulating movement can be easily recognized. In the case of intensive infection, hundreds of

parasites swarm in the sample, but even at moderate infections five to 20 protozoans are found in a microscope field.

9.2 Gill cryptobiosis

This disease is caused by *Cryptobia branchialis,* an elongated, biflagellated parasite with a size of 14–23 by 3.5 µm (Figures 18B and 20). Its body resembles the blood-dwelling *Trypanoplasma* spp., but it is an ectoparasitic organism. It can infect different cyprinids, but occurs most frequently on the gills of silver and bighead carps.

Figure 20. *Cryptobia branchialis* attached to the gill rakers of a silver carp



Histological section, Mallory staining

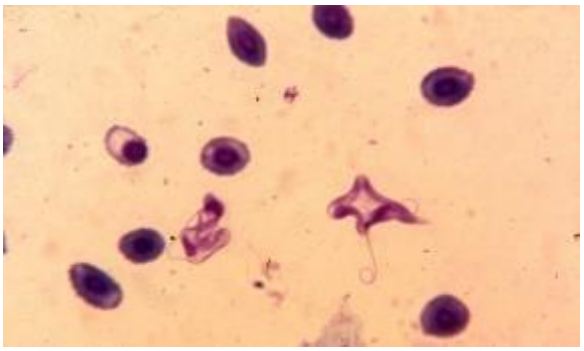
Clinical signs and pathology caused by this parasite, as well as treatment measures, correspond to those for infections by *Ichthyobodo*.

9.3 Sleeping disease of fish

This disease is caused by flagellates of the genera *Trypanosoma* and *Trypanoplasma* living in the blood of fish. *Trypanosoma* spp. have one flagellum, while *Trypanoplasma* spp. have two flagella. The latter group of parasites is often incorrectly called *Cryptobia*. Both genera have several species. *Trypanosoma danilewskii* and *Trypanoplasma borelli* cause infection in common carp (Figures 18C,D, 21 and 22).

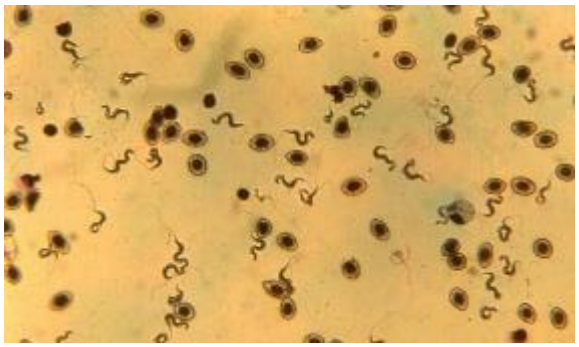
Pathology: Members of these two morphologically different parasite groups have a similar life cycle, and their pathogenic effect is also similar. They develop by leech intermediate hosts which carry infection from one fish to another during their blood-sucking activity. The 10–30 µm long and 6–9 µm wide protozoans develop in the blood by binary fission. In cases of intensive infection, their number is close to the number of the red blood cells. They feed on the components of the blood; therefore, infected fish become lethargic (sleepy) and emaciated. The number of red blood cells and the haemoglobin content in the blood drastically drops. Diseased fish gather at oxygen-rich inflows or gulp for air at the water surface. The main clinical signs include emaciation, sluggish movements, paleness of the skin and gills, and hollowness of the eyes. Diagnosis is done by microscopic examination of the blood.

Figure 21. *Trypanoplasma borelli* in the blood of common carp



Blood film, Giemsa staining

Figure 22. *Trypanosoma danilewskii* in the blood of common carp



Blood film, Giemsa staining

Prevention and treatment: Although these parasites are frequently found in the blood of fish, they rarely cause problems. However, there is no known treatment. Prevention by controlling or killing the intermediate host, the leech, is the only feasible action.

9.4 Spironucleosis

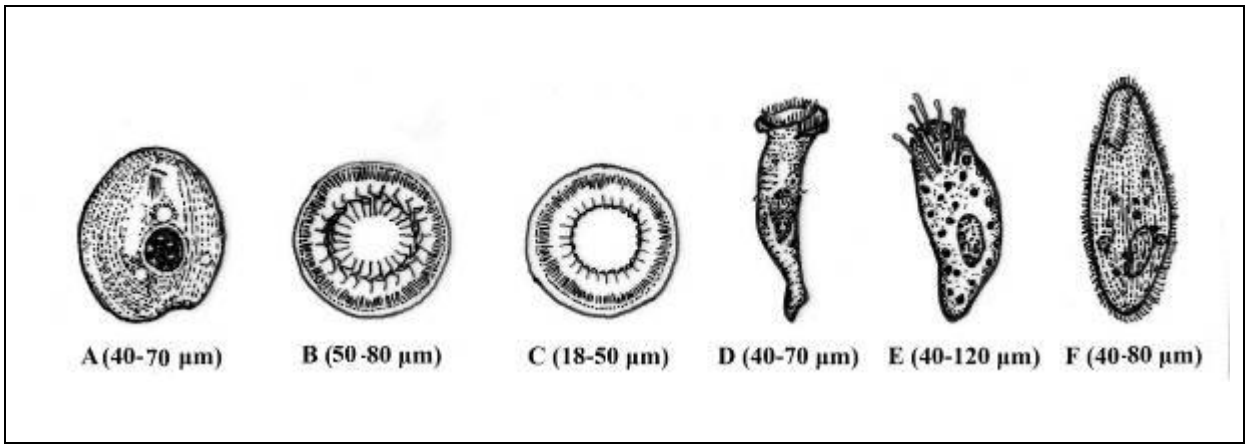
This disease is caused by *Spironucleus elegans* (Figure 18E), a protozoan with 8 flagella. It is 7-15 by 3–6 µm in size and can infect fish of various families. It most commonly occurs in aquarium fish, but also frequently infects the intestine of cultured cyprinids. This parasite has economic importance only in stocks of grass carp. It seems to be a facultative parasite, feeding on digested food in the gut. However, in fish stocks kept under poor conditions it enters the blood stream and the abdominal cavity, where it causes inflammation and haemorrhage of the intestinal wall, accumulation of fluid in the abdominal cavity and necrosis in the internal organs. Diagnosis is made by finding the vigorously moving specimens of *Spironucleus* in samples taken from the slime covering the epithelium.

Prevention and treatment: Treatment is not specified. Improving the health condition is usually enough. If necessary, metronidazole is used for medication as described in Annex 3.

10. DISEASES CAUSED BY CILIATED PROTOZOANS

Protozoans belonging to the Phylum Ciliophora are common parasites of fish. Their bodies are partially or completely covered by cilia, with the help of which they swim in the water or move on or in the gills, skin, intestine or urinary ducts of the fish (Figure 23). Some of them are highly pathogenic organisms.

Figure 23. Ciliate parasites of fish. A) *Chilodonella piscicola*, B) *Trichodina* sp., C) *Trichodinella* sp., D) *Apiosoma* sp., E) *Capriniana piscium*, F) *Balantidium ctenopharyngodoni*



10.1 Chilodonellosis

This disease is caused by *Chilodonella piscicola*, also known as *C. cyprini*, which is a junior synonym. It is a dorso-ventrally flattened, coffee-bean shaped, 40–70 by 38–57 µm periciliated protozoan (Figures 23A and 24). Due to its cilia, *Chilodonella* vigorously moves on the surface of the host's skin and swims in the water. It reproduces by binary fission.

Figure 24. *Chilodonella cyprini* from the gills of common carp

Wet-mount preparation

Temperature of infection: It propagates rapidly at both lower (5–10 ºC) and at higher (22–24 ºC) temperatures.

Pathology: Fish become infected when specimens of *Chilodonella* leave their host and swim to find another fish. Being a cosmopolitan parasite, *Chilodonella* can develop and cause infection in different fish species. Intensive infections and disease appear in the spring, when the water temperature warms up, in fish of poor condition that have been kept under crowded conditions in small ponds, especially if over wintering.

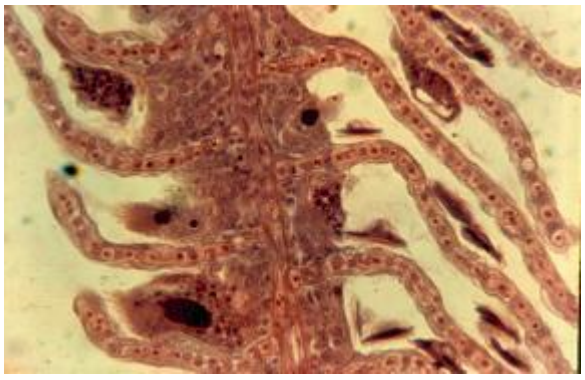
Clinical signs: Infected fish are restless, swim to the oxygen-rich part of the pond, gulp for air, and show constrained movements. The skin and gills are pale and ragged, and covered by excess mucus. Microscopic examination shows hundreds of slowly moving parasites in the scraping. These parasites are sensitive to desiccation; hence, they soon stop their movements, and so in desiccated smears even heavy infections can be missed.

Prevention and treatment: Evolvement of a disease in a less-infected population can be prevented by moving the fish into a larger pond. For treatment of sick fish, bathing in formalin or a salt solution is suggested, as described in Annex 3.

10.2 Trichodinosis

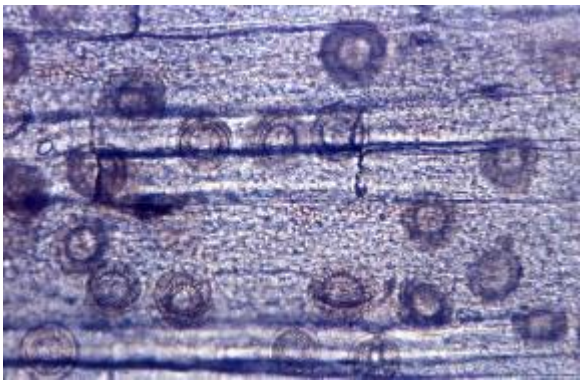
Trichodinosis is a collective name for diseases caused by ciliated parasites belonging to the genera *Trichodina, Trichodinella* and *Tripartiella* of the Family Trichodinidae (Figures 23B, 25 and 26). The great number of species of trichodinid parasites differ in their morphology and size. They are cap-like parasites with a size ranging from 18-50 to 50-80 µm. They are only partially ciliated, as they bear several wreaths of cilia on their bodies. The characteristic ring of denticles assists in the identification of species. Trichodinids propagate by binary fission. They are generally ectoparasites that move rapidly on the gills, fins and body surface, but some species live in the urinary tract. In general, they feed on bacteria and cellular debris, but because of their frequent movements and ciliary activity, they irritate the host tissues, causing micro-traumas, and consequently, tissue damage. Their tactile stimuli cause irritation, leading to the hyperproduction of mucus and hyperplasia, while their adherence and suction activity can damage and severely erode the surface tissues. Trichodinid infections often become extremely pathogenic, causing severe disease. In these cases, hundreds of specimens swarm on the gills or fins, which are covered by excess mucus and cellular debris. To compensate for this degeneration, proliferation of the interlamellar epithelium starts. The volume of epithelial surface drops, and diseased fish show clinical signs of suffocation similar to those of *Ichthyobodo* and *Chilodonella* infections. Without effective and timely treatment, high mortality rates can be expected.

Figure 25. Mixed infection with *Trichodina* (right) and *Capriniana* spp. (left) on the gill of a silver carp



Histological section, H & E staining

Figure 26. *Trichodinella* on the fins of a wels catfish fry



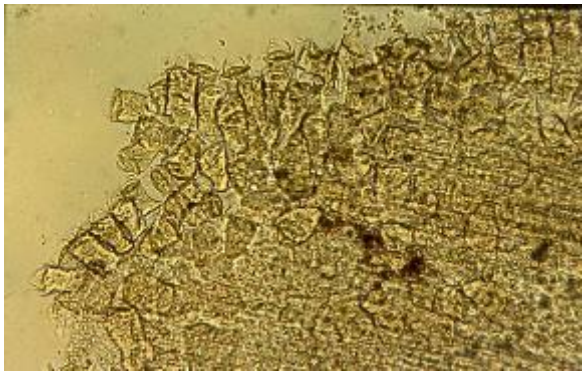
Wet-mount preparation

Prevention and treatment: Treatment of the fish is easy by a short bath in salt and formalin solution, or a bath in the pond as described in Annex 3.

10.3 Apiosomosis

Apiosoma spp. are 40–70 x 18–40 µm, large, bell-like, free-living ciliates (Figures 23D and 27), which with their tapering body end attaches to different objects in the water, very often to fish. They filter bacteria from the water by use of a ring of oral ciliature encircling their widened end.

Figure 27. *Apiosoma* sp. covering the fins of a common carp



Wet-mount preparation

Clinical signs: Although they are not regarded as dangerous parasites, they can still cause damage to fish by attaching to the fins, gills or skin surface, destroying the epithelium and hampering the functioning of these organs. Fry completely covered by these organisms die due to blocked oxygen intake and difficulties in feeding.

Prevention and treatment: *Apiosoma* infection can easily be treated with the products used to kill other ciliates listed in Annex 3.

10.4 White spot disease (Ichthyophthiriosis)

White spot disease is the best known and one of the most pathogenic diseases of warmwater fish. The infections are caused by a globule-shaped peritrichous ciliate, *Ichthyophthirius multifiliis* Figures 28 to 31). This protozoan can reach 1 to 1.5 mm in diameter, and can even be seen with the naked eye.

Figure 28. Skin of a common carp infected with trophonts of *Ichthyophthirius multifiliis*

Fresh–mount picture

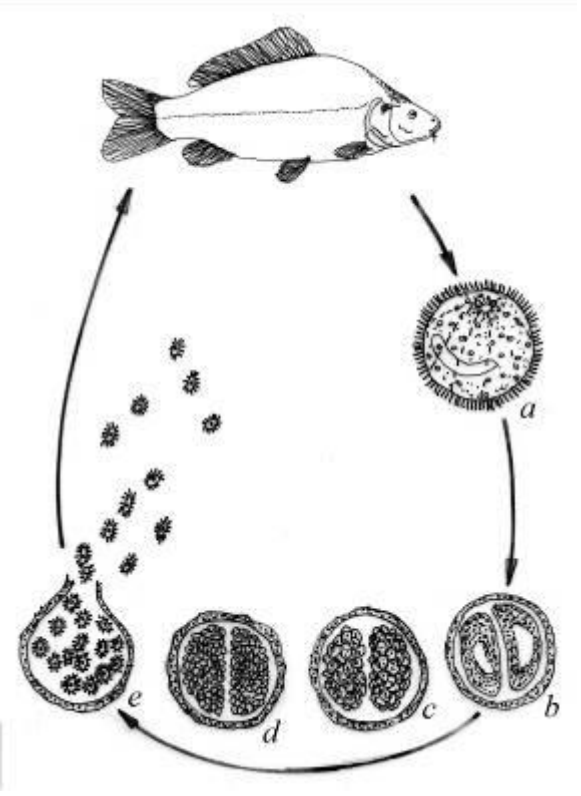
Pathology and life cycle: Although this parasite seems to be an ectoparasite, it has an endoparasitic way of life, with development in fish occurring in or under the epithelium, where it feeds and grows.

Ichthyophthirius ("ich") has a complicated life cycle (Figures 29, 30 and 31). During its growing stage inside the fish, the parasite is called a trophont. Mature trophonts leave the fish and drop to a substrate in the water, where they encyst and become tomonts. After several rounds of division inside the tomont,

hundreds of daughter cells or tomites develop. The infective ciliated small cells developing from the tomites (the theronts) break free from the tomont and swim, trying to find a fish. Theronts can survive in the water for no longer than three days. *Ichthyophthirius* has a wide host range and can infect all kinds of fish. The length of the developmental period depends on water temperature. At high water temperature, the cycle shortens, while at low temperature, it can last for months. The chance of a theront finding a host depends on fish density, the resistance of the fish population, and the fish species found in the waterbody. Intensive infections can develop in densely populated stocks of fish that are in poor physical condition. Although this parasite can invade all kinds of fish, some species such as wels catfish are especially susceptible, and hence threatened. Young age groups of wels catfish prefer to concentrate in certain parts of the waterbody, where theronts, originating from trophonts, concentrate in order to find hosts easily.

Theronts prefer to invade the gills, but they also infect the skin. In heavy infections, fish may suffocate due to damage to the gill epithelium that impairs gas exchange.

Figure 29. Developmental cycle of *Ichthyophthirius multifiliis* on carp



a) Mature trophont leaving the fish, b) Tomont in the bottom of the pond starting division, c) Tomont with dividing tomites, d) Tomont with developing theronts, e) Theronts releasing tomonts into the water for infecting fish.

Redrawn after Bauer, Musselius and Strelkov, 1969

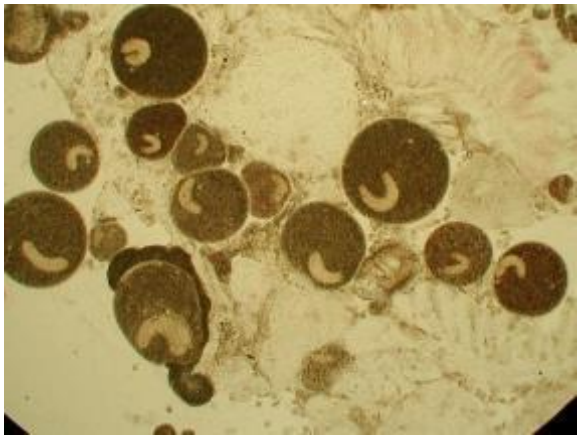
Clinical signs: These resemble those caused by other ciliates: fish gather at the oxygen-rich inflow, gulp for air at the water surface and swim listlessly. Heavy mortality is common. The gills and skin of infected fish are pale and are filled with small, grist-like bodies which are actually trophonts of different sizes. When the infection is intensive, the structure of the gill filament cannot be identified, the lamellae are occupied by large tomonts, and parts of gill filaments break off. Identification is easy, as the white spots on the skin can be seen with the naked eye. In scrapings from the gills or skin, the vigorously whirling trophonts of *Ichthyophthirius* with their characteristic large, bean-shaped nucleus give a trustworthy diagnosis.

Prevention and treatment: Prevention is based on interrupting re-infection and the intensive propagation of the parasite. Transferring fish into a new freshwater system or into another tank or pond where there are no infective theronts gives a chance for fish to recuperate.

For fish kept in intensive tank systems (e.g. for fry), one or more transfer to a tank or pond with clean water might ensure a parasite-free condition, but similar good effect can be achieved by transferring the infected fish stock into a larger pond. In this case, parasites can continue their development, but before the new theront generation develops, the fish may become resistant, and due to the lower stocking density, the chance of theronts finding fish to infect is lower.

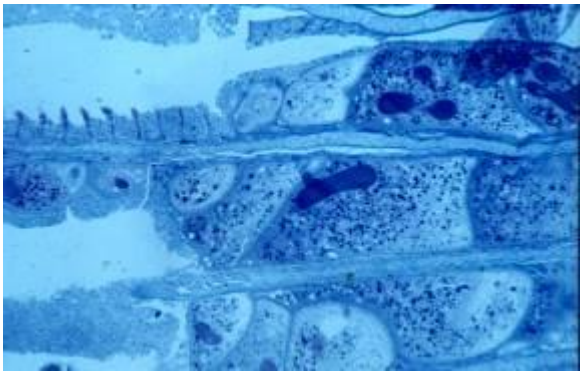
The only effective drug against white spot disease is malachite green solution, which is banned in many countries. Other chemicals (salt, formalin, etc.) that are used in bath treatments for killing other ectoparasites can damage extra-piscine stages (i.e. tomonts, theronts) but cannot kill parasitic stages (trophonts) developing under the epithelium of the fish. For the above reasons, fighting white spot disease is based on prevention. As infective theronts can enter the pond by inflowing water and with wild fish, low-level infections cannot be prevented in larger ponds. However, massive infection leading to disease develops only in densely populated stocks of fish having lowered resistance. Early diagnosis of infection is especially important. It is particularly important to check for infection before placing the fish into wintering ponds. In these densely populated systems where clinically healthy but infected fish are stored, there can be an outbreak. It is also important to check regularly for infections in fry-rearing ponds, where reduced natural food and increased density can enhance the development of white spot disease.

Figure 30. Trophonts of *Ichthyophthirius multifiliis* in scrapings of the skin



Wet-mount preparation

Figure 31. Heavy infection of the gill filaments with trophonts of *Ichthyophthirius multifiliis.* Gill lamellae are seen only in a small part of the filaments

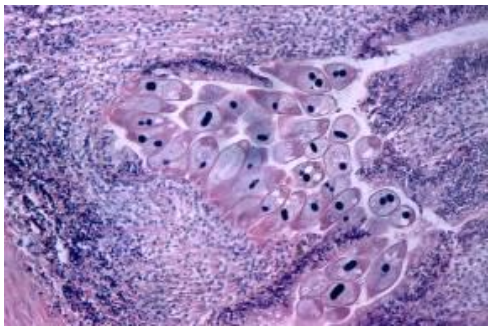


Histological section, H & E staining

10.5 Balantidiosis

Balantidium spp. are periciliated commensals that may become facultative pathogens. They infect the gut of different animals; for fish, *B. ctenopharyngodoni* is the best known (Figures 23F and 32). These 40–120 by 25–60 µm protozoans have a macro- and a micronucleus,

Figure 32. *Balantidium ctenopharyngodoni* in the intestine of the grass carp



Histological section, H & E staining

and a well-observable mouth (cytostoma). They move vigorously in the posterior part of the intestine of grass carp older than two years, feeding on digested particles. Less frequently, they attach to the intestinal epithelium with their mouth, causing the formation of ulcers. In these cases, enteritis with hyperaemia and inflammatory changes develops and mortality can occur. It is supposed that these changes are generated when the grass carp feeds on concentrated feed, instead of its natural diet.

10.6 Capriniana infection

Infection by *Capriniana* (known also as trichophryosis) appears first of all in the gills of the Chinese major carps. The disease is caused by an interesting ciliate, *Capriniana piscium* (previously called *Trichophrya piscium*) (Figure 23E), which settles in great numbers between the gill lamellae. Instead of cilia, these ciliates are equipped with suctorial tentacles. They feed on other ciliates, and there is no firm evidence of feeding on host cells or mucus; however, in instances of massive infection, they cause irritation to the gill epithelium, hindering oxygen intake.

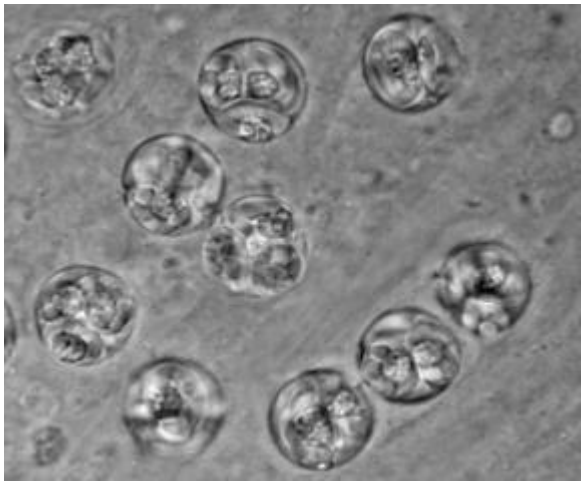
11. DISEASES CAUSED BY COCCIDIANS

Coccidians (Phylum Apicomplexa, Subclass Coccidia) belonging to the genera *Eimeria* and *Goussia* are common but highly host-specific parasites of fish. Their pathogenic effect is not as serious as that of some other coccidians of cultured mammals and birds. In pond-cultured cyprinids, there are three kinds of infection that should be mentioned: diffuse intestinal coccidiosis of common carp, diffuse intestinal coccidiosis of silver and bighead carps and nodular coccidiosis of common carp.

11.1 Diffuse coccidiosis of common carp

This disease is caused by *Goussia carpelli* (in the past, frequently reported as *Eimeria carpelli*), which is a specific parasite of common carp. *Goussia carpelli* has small, globular oocysts with a diameter of between 8 and 14 μm (Figure 33). It infects the intestinal epithelium. The oocysts leave the fish in the sporulated stage, each oocyst containing four sporocysts, with two sporozoites in each them.

Figure 33. Oocysts of *Goussia carpelli* from the gut of common carp



Wet-mount preparation

Fish become infected by ingesting sporulated oocysts from the mud or, more likely, by feeding on infected tubificid worms (oligochaetes). Oocysts are formed, after a complicated developmental stage of the parasite, by infecting and damaging several epithelial cells. When sporozoites get into the intestine of fish, they bore into epithelial cells where they start merogony by forming several meronts. Meronts entering a new epithelial cell start sporogony and form macro- and microgametes. When microgametes fertilize macrogametes, oocysts develop. After forming oocysts, the parasites remain for some days inside the host cell, from where they leave the epithelium incorporated with two or three oocysts in the dead host cells. These are called "yellow bodies". The name “diffuse coccidiosis” comes from the fact that oocysts are formed randomly in the different parts of the gut epithelium[2]. This coccidium infects fish and develops in all seasons of the year, causing a permanent infection. The infection cycle, however, is short. Intensive infection develops only in warm water. *Goussia carpelli* infects different age groups of common carp, but clinical signs of disease develop only in densely populated fingerlings or in overwintered fish stocks left crowded in wintering ponds for a long time.

Due to the damage caused the by the schizogonic and gametogonic stages of coccidia, infected epithelial cells die, local necrosis develops and sera enters into the intestine. Facultative bacteria can enter into the gut wall through this damage, and inflammation of the gut may develop.

[2] Contrary to “nodular coccidiosis” where they develop in some well-distinct nodules.

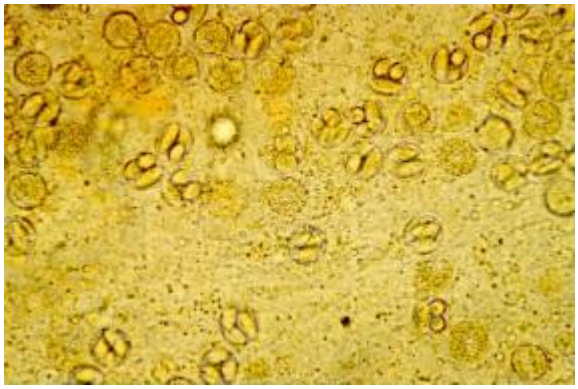
Infected fish become weak, are emaciated and often lay on the pond bottom. Their heads seem to be large. The intestinal epithelium is covered by a thick slime, and large numbers of oocysts inside yellow bodies are found in scrapings of the gut.

Prevention and treatment: Drugs used against coccidial infections at the poultry industry are effective, but this treatment is expensive and thus not suggested. Disinfection of pond bottoms with lime, combined with desiccation of mud and improving the body condition and rearing/holding conditions of fish is usually effective.

11.2 Coccidiosis of silver and bighead carps

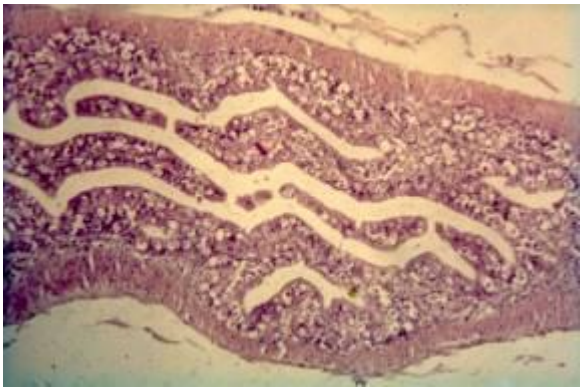
This infection is caused by *Goussia sinensis*, a species infective for both silver and bighead carps (Figures 34 and 35). The oocysts of this species measure 10–12 μm in diameter. In summer months, heavy infections, similar to diffuse coccidiosis of common carp, develop in fingerlings. Less frequently, heavy infections extending to the majority of epithelial cells develop, and in cases of co-infection with ectoparasitic protozoans in the gills, heavy losses may occur.

Figure 34. Sporulated and unsporulated oocysts of *Goussia sinensis* from the gut of silver carp



Wet-mount preparation

Figure 35. Heavy coccidian infection in the intestine of silver carp



Histological section, H & E staining

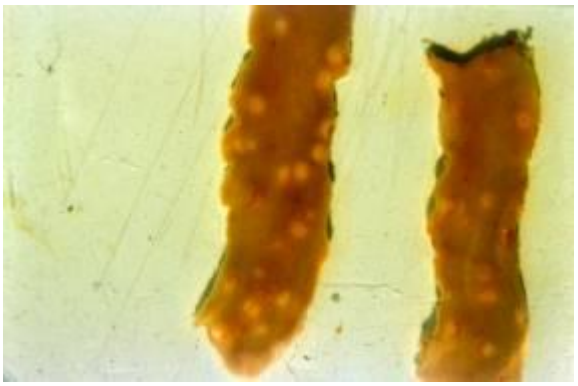
Prevention and treatment: Methods are similar to those suggested for diffuse coccidiosis of common carp.

11.3 Nodular coccidiosis of common carp

This infection is caused by *Goussia subepithelialis*, a coccidium with oocysts of 17–22 μm in size. Nodular coccidiosis is different from the diffuse type, as it appears seasonally in spring. Vegetative stages (merogonic stages) first appear in March in the epithelial cells, then oocysts are formed in April. Some of the parasites leave the fish in this month as unsporulated oocysts; however, the majority of them are captured by the regenerating epithelium, pressed to the deeper layer (subepithelium) and become sporulated.

These captured oocysts are only rejected from the host by a secondary host reaction in May. Both the vegetative and generative development of this species take place in some well-defined parts of the intestine where nodules of 10–14 mm in diameter are formed that can be easily seen with the naked eye (Figure 36). Nodular coccidiosis develops in fish stocks older than one year, mostly in the three-year-old generation. Despite severe clinical signs and local tissue changes, the economic importance of this disease is slight.

Figure 36. Nodular coccidiosis in the gut of common carp

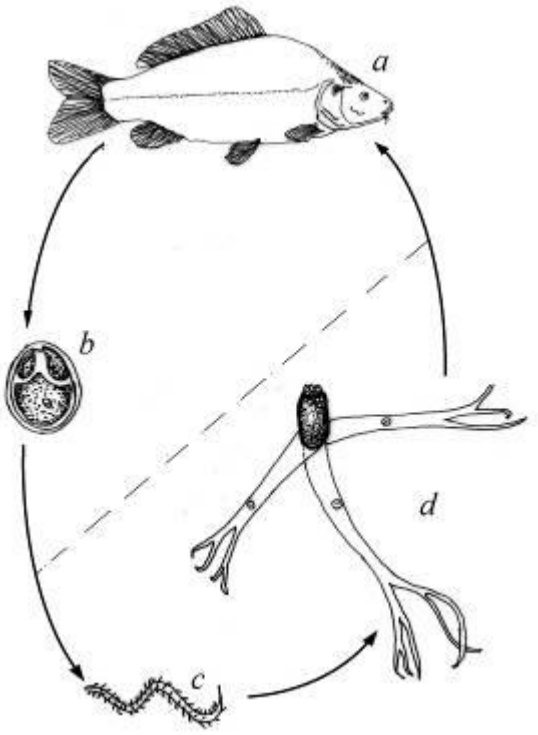


Fresh–mount preparation

12. DISEASES CAUSED BY MYXOSPOREANS

Myxosporeans frequently infect cyprinids. Fortunately, although a great number of cyprinid species are known in the CEE and CCA regions, only a few myxosporeans are known as pathogens in pond-fish culture. In the case of carps in Europe, severe clinical signs and heavy losses are rarely recorded. In Japan and the People's Republic of China, however, there are species (e.g. *Myxobolus artus, M. koi, Thelohanellus kitauei*) which cause horrifying clinical signs and high mortalities in fish farms rearing the Far Eastern subspecies of common carp (*Cyprinus carpio haematopterus*). For the cyprinids cultured in the CEE and CAA regions, five species (*Myxobolus cyprini, Sphaerospora dykovae, S. molnari, Thelohanellus nikolskii* and *T. hovorkai*) in common carp and one species (*M. pavlovskii*) in bighead and silver carps have economic importance. No effective protection is known against myxosporean diseases. Prevention is based on disinfection of the pond bottom, and if necessary, reduction of the oligochaete alternative hosts.

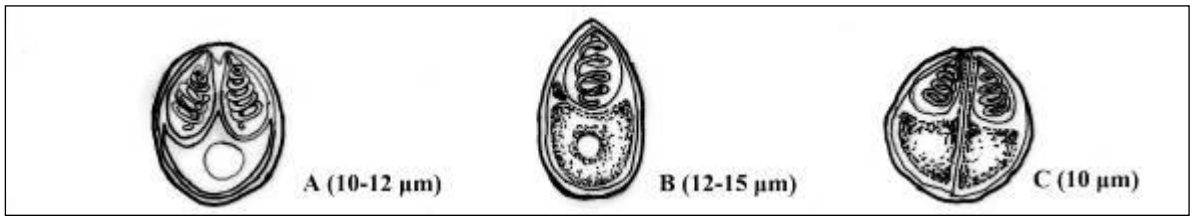
Figure 37. Developmental cycle of *Myxobolus dispar* of common carp



a) Infected fish, b) Myxospores released from the fish gills sink to the bottom, c) Tubificid oligochaetes become infected by myxospores and develop actinospores, d) Actinospores float in the water and infect the fish

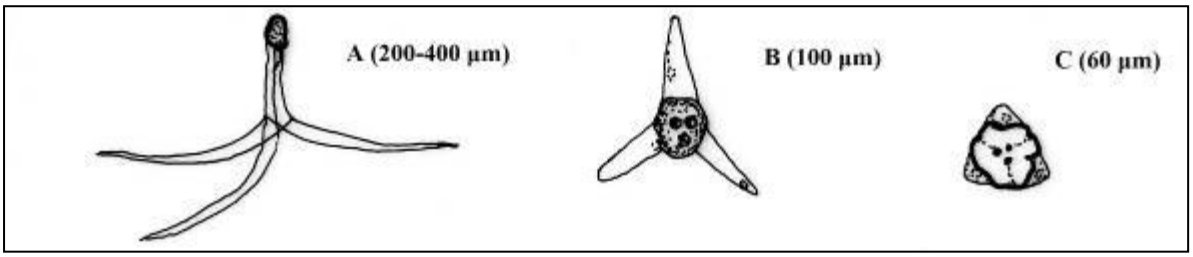
The developmental cycle of myxosporeans is quite unique (Figure 37). Myxospores develop in fish or less frequently, in other poikilothermic animals, with a rather complicated life cycle. Myxospores (Figure 38) released from fish infect the oligochaete alternate hosts in which another complicated phase, the actinosporean development starts (Figure 38). Mature actinospores (Figure 39) differing in shape and size from myxospores leave oligochaetes, float in the water and infect specific fish species by contacting them. Myxospores in fish develop mostly in large cyst-like plasmodia (called often as pseudoplasmodia), other spores develop coelozoically in the excretory channels of inner organs.

Figure 38. Myxospores of the most common myxosporean genera infecting fish. A) Spore of a *Myxobolus* sp., B) Spore of *Thelohanellus nikolskii*, C) Spore of *Sphaerospora dykovae*



Redrawn after Molnár and Szakolczai, 1980

Figure 39. Most common actinosporean types infecting oligochaete alternate hosts. A) Triactinomyxon type actinospore, B) Aurantiactinomyxon type actinospore, C) Neoactinomyxum type actinospore



12.1 Swimbladder inflammation (SBI) of common carp

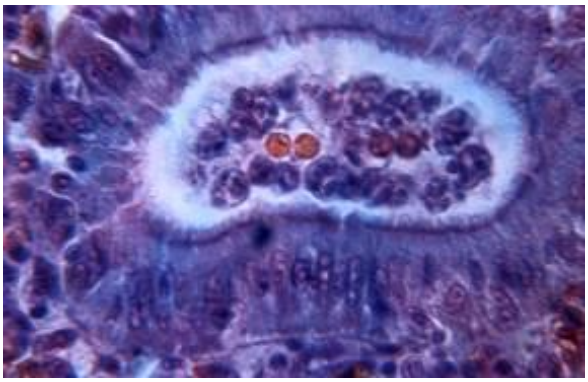
This disease is caused by the developing stages of *Sphaerospora dykovae* (best known under its synonym, *S. renicola*) (Figures 38C, 40 and 41), a parasite which forms spores in the kidney tubules of common carp fingerlings. During its development in the 1–3 month-old specimens, extra-sporogonic stages of the parasite develop by proliferating in the blood of infected fish, where multiple endogenic cleavage takes place. These stages obstruct the capillary network and cause a strong inflammatory response. The hyperplasia of connective and epithelial tissues results in the thickening of the swimbladder wall, and haemorrhage is also frequently present (Figure 40). In the acute phase, peritonitis and acute hypertrophy of the kidney, reddening of bulging eyes, and dropsy in the belly develops. Prognosis for SBI is poor.

Prevention and treatment: Prevention, as in all cases of myxosporean infection, relies on disinfection of the pond bottom. No drug treatment is known.

Figure 40. Swimbladder inflammation of the common carp. Note haemorrhages in the swimbladder wall.

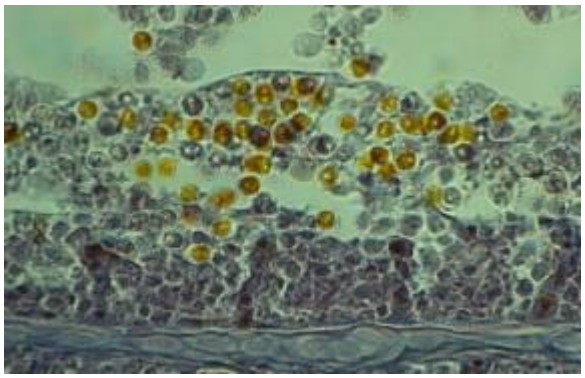
Histological section, H & E staining

Figure 41. *Sphaerospora dykovae* infection in the lumen of the renal tubules



Histological section, H & E staining

Figure 42. *Sphaerospora molnari* infection of the gill filament of common carp



Histological section, H & E staining

Figure 43. Spores of *Myxobolus cyprini*

Wet-mount preparation

12.2 Gill sphaerosporosis of common carp

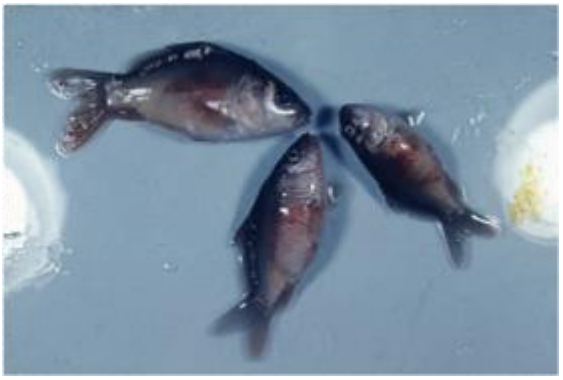
The disease is caused by *Sphaerospora molnari* in the young (1 to 2 month-old) common carp fry (Figure 42). Extra-sporogonic stages of this parasite invade the gills and form spores in the multilayered epithelium in small plasmodia. The prevalence of infection can reach 100 percent. Epithelial hyperplasia and necrosis develop. Fusion and rupture of the secondary lamellae takes place, and due to the damage in respiratory function, mortality of heavily infected fish occurs.

12.3 Myxobolus cyprini infection of the muscle of common carp

Most infections of *Myxobolus cyprini* in common carp remain latent and only subclinical damage is caused. In this case, large intracellular plasmodia in the muscle cells and scattered spores in the gill tissues and the melano-macrophage centers of the kidney draw attention to the infection (Figures 43–45). In severe cases, a large number of cells in the trunk musculature are infected, and these enlarged cells are filled with millions of spores. Infected myofibrils are destroyed.

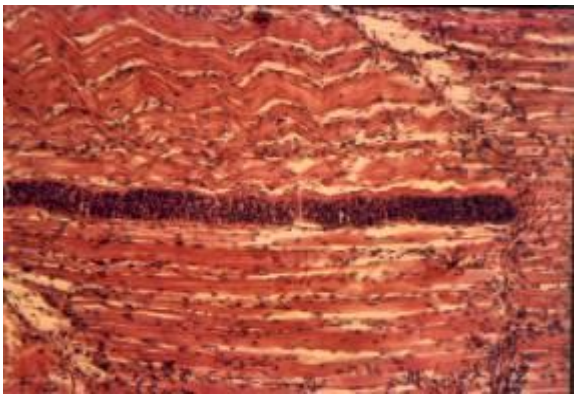
After the death of infected muscle cells, spores enter the blood stream and are carried to different organs, such as the liver, kidney, gills and intestine. Spores accumulating in obliterated capillaries of the gut, kidney and skin may be eliminated with the help of macrophages. However, the majority of spores block capillaries and cause hydropic degeneration in different organs. Dropsy may also develop. These latter clinical signs appear mostly in densely populated ponds. A similar disease caused by *M. artus* in Japan leads to lysis of the muscle fibres and results in mass mortalities of the hosts.

Figure 44. Haemorrhages in the skin caused by spores of *Myxobolus cyprini* obstructing capillaries



Fresh-mount picture

Figure 45. *Myxobolus cyprini* pseudocyst developing in a muscle cell of the common carp

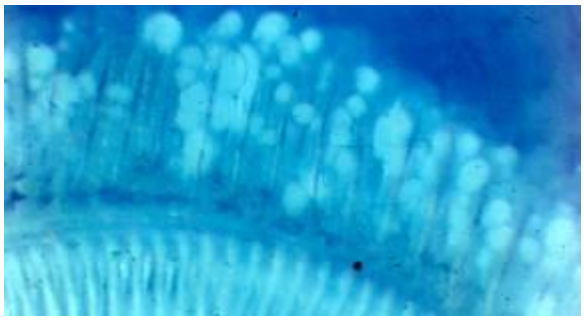


Histological section, H & E staining

12.4 Myxobolus pavlovskii infection of silver and bighead carps

This infection (Figure 46) is common in both fish species introduced to Europe from Chinese natural waters. The gills may be heavily infected by thousands of small plasmodia of 0.5 to 1 mm in size. Plasmodia develop between two neighbouring gill lamellae in the multilayered epithelium. They deform the filaments, reducing the volume of the epithelial surface of the gills, and produce breakages of gill lamellae. Therefore, heavily infected fish suffocate.

Figure 46. Gills of a silver carp infected by plasmodia of *Myxobolus pavlovskii*



Histological section, H & E staining

12.5 Thelohanellus nikolskii infection of common carp

Large plasmodia of this parasite, 2 to 3 mm in size develop in the cartilaginous tissues of the fin rays of common carp fingerlings in the late summer months (Figure 47). In older fish, they appear in the collagenous tissue of the scales during the spring months.

The relatively large spores in mature pseudocysts have only one polar capsule (Figure 38B), contrary to *Myxobolus* spp. which have two capsules.

Figure 47. Pseudocysts caused by *Thelohanellus nikolskii* in the fins of common carp fry

Fresh mount picture

In young fish, plasmodia develop on the surface of the rays. They are surrounded by a thick cartilaginous capsule and by a similarly thick dense connective tissue layer of host origin. The fins of heavily infected fish break; and these being a less crucial organ, the fish survive heavy infections but remain malformed.

In extensive culture systems, even heavy infections remain latent. They develop on fingerlings during late summer and at harvest time, to the horror of farmers; however, after rupture of the mature plasmodia and release of spores, only minor degenerations of fin rays resemble the past infection.

12.6 Thelohanellus hovorkai infection of common carp

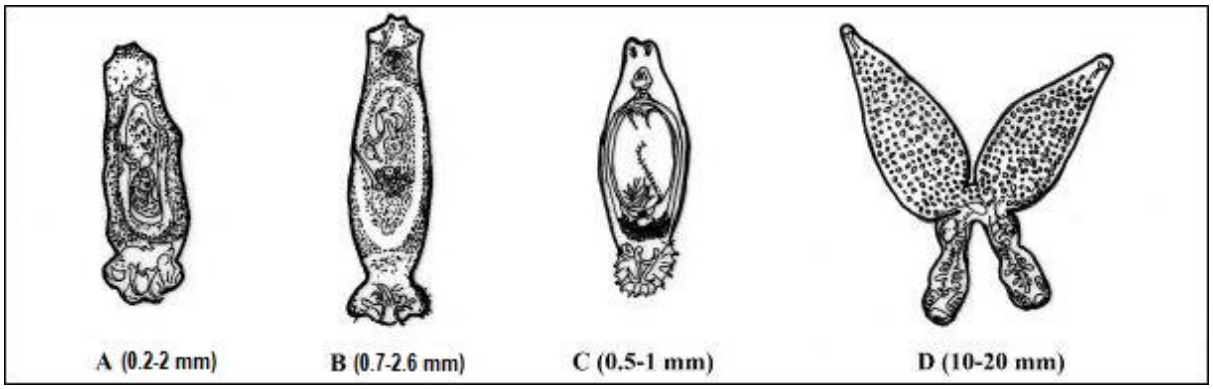
This parasite, which has morphologically somewhat different spores, infects the connective tissue in the abdominal cavity and muscles of common carp. Infections often remain hidden from the naked eye. Microscopic examination reveals large and easily noticeable pseudocysts in the serosa of the intestine. Its pathogenic effect is regarded as more serious than that of *T. nikolskii*; however, even heavy infections remain hidden. Although losses due to infection by this parasite have not been recorded in Europe, its resemblance to *T. kitauei* suggests its possible pathogenicity. *Thelohanellus kitauei* is an extremely pathogenic parasite in Far Eastern countries. It is also associated with the gut, but it forms its pseudocysts in the intestinal wall. The introduction of this species to the CEE or CCA region along with colour carp cannot be excluded.

13. DISEASES CAUSED BY MONOGENEANS (GILL WORMS)

Gill worms (Figure 48) are monogeneans; hence, they need only a single host to complete their life cycle. Members of the Class Monogenea mostly infect fish, and together with members of the Class Trematoda and Class Cestoda belong to the Phylum Platyhelminthes. Previously, monogeneans were erroneously called monogenetic trematodes.

Except for a few species, they are ectoparasites, and all have a direct developmental cycle without an intermediate host. Most are highly host specific, infecting only the gills or skin of a single host species. Of their hundreds of genera, dactylogyrids and gyrodactylids are significant sources of problems in freshwater fish farming. Dactylogyrid monogeneans (*Dactylogyrus, Ancyrocephalus, Thaparocleidus,* etc.) infect the gills of their hosts, while various species of the genus *Gyrodactylus* infect either the gills or the fins. Some species of dactylogyrid are regarded as highly pathogenic organisms causing gill disease, while severe clinical signs of disease on the fins and skin can be caused by *Gyrodactylus* spp.

Figure 48. Body structure of some monogeneans. A) *Dactylogyrus,* B) *Thaparocleidus,* C) *Gyrodactylus*, D) *Diplozoon*

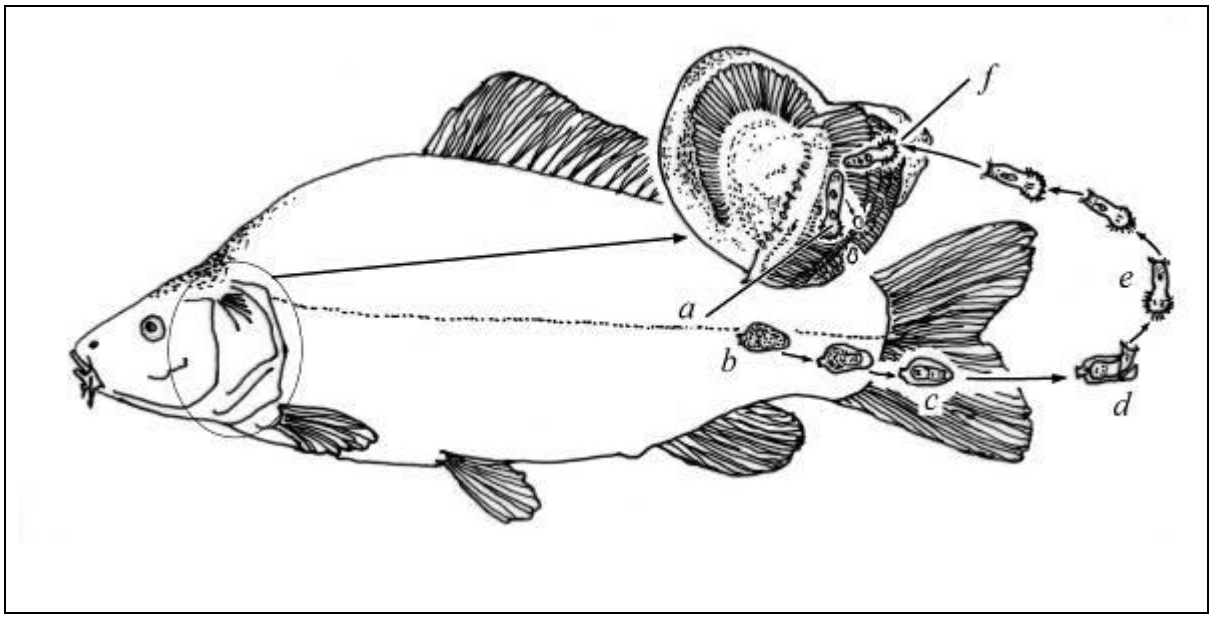


Redrawn after Molnár and Szakolczai, 1980

13.1 Gill disease caused by Dactylogyrids

Dactylogyrid monogeneans are hermaphroditic, strictly host-specific parasites with a length of from 0.2 to 2 mm. They are characterized by four pigmented eyes on the anterior end and a haptor (attachment organ) at the posterior end. In *Dactylogyrus* spp., the haptor has two sclerotized hamuli (anchors) and 14 marginal hooks, while *Ancyrocephalus* and *Thaparocleidus* spp. have four hamuli and 14 hooks. Their transparent body contains a cirrus and a vagina, sclerotized copulatory organs of various shape and size, which can help in identifying the species. Mature worms lay 1 to 30 eggs per day which sink to the pond bottom (Figure 49). Inside the egg, a ciliated larva, the oncomiracidium, develops which enters the water and actively swims for 4 to 20 hours. After finding a specific host, it attaches itself to the skin and then moves to the gills. At higher temperature, egg production and development of the oncomiracidium in the egg is faster, but at lower temperature, egg production and larval development may even stop.

Figure 49. Developmental cycle of *Dactylogyrus vastator* on common carp fry**.** a) Worms infecting the gills, b) Eggs laid down sink to the bottom, c) Oncomiracidia develop in the eggs, d) Larvae hatch from the eggs, e) Oncomiracidia swim in the water and look for a common carp fry, f) Finding the host, they invade the gills



Until the Second World War, there were only three species (*Dactylogyrus vastator, D. minor* and *D. anchoratus*) known to infect common carp in Europe and the western part of Asia. Of these, *D. vastator* and *D. anchoratus* infected both common and Crucian carps. After the intensive transcontinental transfer of fish that occurred during the 1950s and 1960s, first *D. extensus* and then six other *Dactylogyrus* spp. previously known only from wild carp of the Amur River were introduced and infected common carp in Europe. Fortunately, of the known ten species only two large-sized (over 1 mm in length) *Dactylogyrus* spp. (*D. vastator* and *D. extensus*) have economic importance/

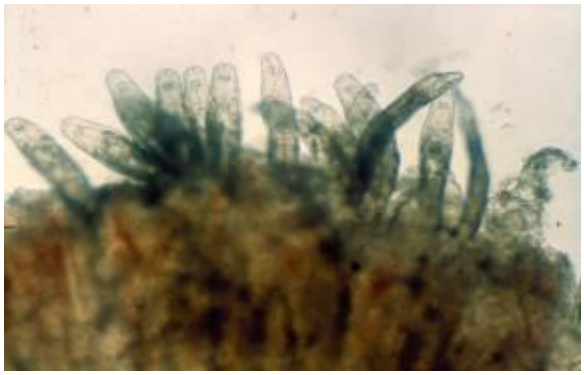
13.1.1 Gill disease of common carp fry caused by *Dactylogyrus vastator*

Dactylogyrus vastator is a relatively large monogenean, with a body that can be 1.1 mm long and 0.4 mm wide (Figures 49 and 50). The marginal hooks are 29 to 33 μm in length, while the hamuli measure 29–33 μm. They are connected by a 32–38 μm long dorsal bar. The length of the copulatory organ is 44–58 μm.

Dactylogyrus vastator typically infects the gills of young (2–6 cm long) fingerlings, and is always located at the tip of the gill filaments.

It occurs in warm summers and disappears in autumn. Two types of eggs are laid by the

Figure 50. Intensive infection with *Dactylogyrus vastator* in the gills of a common carp fingerling



Fresh-mount picture

worm. Some eggs start their development immediately, and at 28–29 ºC oncomiracidia develop within 2 or 3 days. These oncomiracidia can produce an intensive infection in the fry population. The other type of egg (a resistant egg) over-winters and starts its development only when the water warms up in the following year. In this way, an infection may occur in less-disinfected ponds without direct or indirect contact with older carp generations.

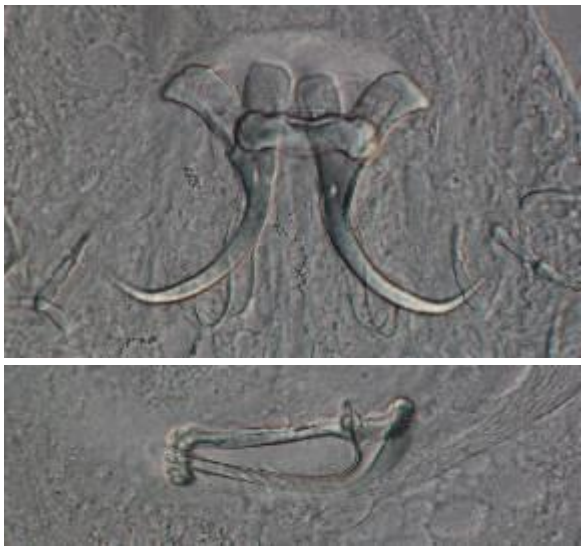
13.1.2 Gill disease of common carp caused by *Dactylogyrus extensus*

This is a very common parasite found in all generations of common carp, and in all seasons. Despite its frequent occurrence, it rarely causes intensive infections.

Dactylogyrus extensus, a specific parasite of common carp, is one of the largest *Dactylogyrus* species. Its body length measures 1.5–1.7 mm, and its width is 0.3–0.4 mm. The length of the marginal hooks is 27–36 μm and the length of the hamuli is 62–89 μm (Figure 51).

In unfavourable conditions, it evokes similar clinical signs as *D. vastator*. Although 20 to 30 specimens of *D. extensus* can kill a 4–4.5 cm long fingerling, intensive infections are less frequent in the summer because of the specific ecology of this worm. Some *D. extensus* specimens, however, together with some small-sized *Dactylogyrus* spp. (i.e. *D. anchoratus, D. achmerovi* and *D. molnari*) commonly occur on healthy carp year-round.

Figure 51. Sclerotized structures of *Dactylogyrus extensus*. Above: Hamuli, Below: Copulatory organ



wet-mount preparations

13.1.3 *Dactylogyrus* infection of Chinese major carps

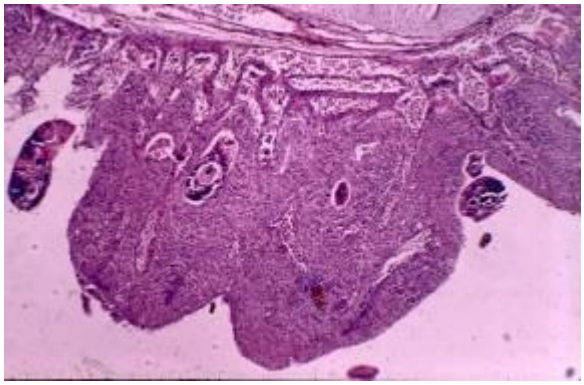
As with common carp, Chinese major carps introduced to Europe and Central Asia are infected by their specific *Dactylogyrus* spp.: *D. lamellatus* and *D. ctenopharyngodonis* infect grass carp, *D. suchengtaii* and *D. hypophthalmichthys* infect silver carp, and *D. nobilis* and *D. aristichthys* infect bighead carp. Of these, the best known is *D. lamellatus*, which can cause high mortalities of grass carp fry. The development and pathology of these parasites, and also the methods of prevention are similar to those for species infecting common carp.

13.1.4 Gill disease of wels catfish caused by *Thaparocleidus vistulensis*

This infection is caused by a relatively large dactylogyrid monogenean that is better known by its synonym, *Ancylodiscoides vistulensis*. Of the three *Thaparocleidus* spp. which commonly infect wels catfish in natural waters, *T. vistulensis* is the most pathogenic. It causes fatal infection in populations of wels catfish fry, but it is also a common pathogen in intensively cultured older generations.

About 2 mm long, this worm differs from *Dactylogyrus* spp. by having two pairs of hamuli in its haptor, which serve for attachment on the gills. These worms produce damage to the gills similar to that caused by *Dactylogyrus* spp., and the host reaction also corresponds to that for these species. In intensive infections, almost all gill filaments are diseased, and entire stocks of fingerlings and even one-year-old fish may die. The devolvement of massive infection is enhanced by the specific behaviour of wels catfish, which prefer to gather in a small area, even in a large pond.

Figure 52. Clubbing of the gill filaments of a wels catfish caused by *Thaparocleidus vistulensis* (cross-section)



Histological section, H & E staining

13.1.5 Pathologic changes on the gills caused by Dactylogyrids

Worms attached to the gill filaments pierce their hamuli into the lamellae and fix themselves there. Their marginal hooks damage the gills mechanically. These monogeneans also produce enzymes that dissolve tissues. They feed on damaged epithelial cells, tissue sera, slime and red blood cells. Several hundred worms may attach to the gills of a small fingerling. In order to repair damaged tissues, a proliferous process starts. The proliferating tissue, composed mostly of epitheloid cells, fills up the degenerated parts of the filaments. Due to this process, two or more filaments may grow together, forming clubs which lack the respiratory lamellae. Large parts of the filaments may break off and the gills become covered by a thick slime. At this stage of infection, very often worms can no longer be found, but because of the previous damage, the gills are unable to take up oxygen and the fish suffocates. A carp fry of about 2 cm in length dies if infected with about 20–40 worms, while a 4–5 cm long fry can be killed by 140 to 160 worms.

Clinical signs: Fish are irritated and they gather at the water inflow. Moribund specimens float on the water surface with their bellies upward.

Diagnosis: The gills are pale, covered with abundant slime and the filaments break off; alternatively, clubbing of other filaments may be observed. During a microscopic examination, a great number of vigorously moving worms proves the final diagnosis.

Prognosis: Fish in good condition can survive even relatively severe infections. However, infection with large numbers of larvae may damage the greater part of the gills, and losses can be 90–95 percent. After successful medication, fish can recuperate quickly.

Prevention and treatment: Prevention is easy in situations where artificial propagation occurs. Fry and fingerlings should be cultured separated from older age groups. In addition, young fish should not be exposed to a water supply from infected ponds that carries larvae of monogeneans. In the case of infections by *D. vastator*, the soil of the pond bottom should also

be disinfected. If the infection is only with a moderate number of worms, transferring fish to a larger pond is enough to prevent outbreaks.

For treating *Dactylogyrus* infection of cyprinids, a short bath in salt solution usually gives good results, most of the worms dying or falling off the fish. Some species, such as *D. extensus* and *T. vistulensis* are more resistant. They can be killed only with ammonia solution or organophosphates as described in Annex 3.

13.2 Gyrodactylus infection

A large number of *Gyrodactylus* spp. are common parasites of freshwater and marine fish (Figure 48C). Some of them prefer infecting the gills, but others live on the fins and body surface. There are devastating species among them, such as *G. salaris*, which is a salmon parasite. Species infecting cyprinids are less important, but those living on goldfish can cause losses similar to those seen in infections by *Dactylogyrus* spp.

Gyrodactylus spp. are usually small and transparent viviparous worms. They lack pigmented eyes on the head end; instead, they have two glandular extensions. Their haptor is armed with two hamuli and 16 marginal hooks. Their identification is difficult, as they have no sclerotized copulatory organs and only the size and shape of the hamuli and hooks can be used for species identification. Their way of propagation is unique. Within the body of a worm, four consequent generations may be located, each with developed or semideveloped hooks and hamuli. In common carp, three species are most common: *G. katharineri*, a parasite of the skin; *G. sprostonae*, a parasite of the gill filaments; and *G. schulmani*, a parasite of the gill rakers.

13.3 Diplozoon infection of cyprinids

Diplozoon is a relatively large and interesting parasite (Figure 48D). After mating, two individuals fuse together and live out the rest of their lives as a remarkable creature called a twin worm. There are several species of these twin worms that infect the gills of cyprinids, one of them being *D. paradoxum*, which is found on the freshwater bream. Recently, another species, *Eudiplozoon nipponicum*, which is a specific parasite of the common carp was introduced to Europe and Central Asia. Despite its common occurrence, no significant pathogenic changes have been recorded.

14. DISEASES CAUSED BY TAPEWORMS (CESTODES)

Although tapeworm infections are rather common in freshwater fish, in cultured cyprinids only a few of these parasites cause disease of economic importance (Figure 53). Of these, intestinal infections by *Bothriocephalus*, *Khawia* and *Atractolytocestus* spp. and intra-abdominal infections with the larval stage of *Ligula intestinalis* can evoke severe damage or death of hosts.

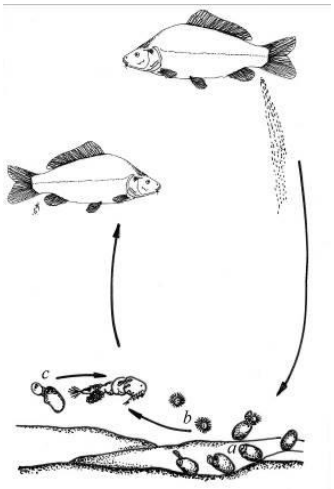
14.1 Infection with Bothriocephalus acheilognathi

This worm was introduced to Europe and Central Asia together with grass carp after the Second World War. This 10–20 cm long tapeworm spread rapidly and is now found in several countries on different continents. It mainly infects common carp, but may also infect other cyprinids and some non-cyprinid fish. Worms have a characteristic "viper-like" scolex with two long attachment grooves (Figure 53A). Its elongated body (strobila) is segmented, the last segments being continuously detached and leaving the host's digestive tract with the faeces. The life cycle consists of two hosts (Figure 54). Adult parasites live in the intestine of fish. Eggs on the pond bottom develop to ciliated coracidia, which leave the eggs and remain floating in the water. Coracidia consumed by copepods continue their development in the haemocoel, where depending on water temperature, they develop to the procercoid stage, which is infective to fish. Fish become infected by consuming infected copepods.

Figure 53. Tapeworms of carp

A) Head (scolex) of *Bothriocephalus acheilognathi*, its body (strobila) is segmented, B) Head region of *Khawia sinensis*, its body is unsegmented.

Figure 54. Developmental cycle of *Bothriocephalus acheilognathi*



a) In the bottom, coracidia develop in eggs discharged from infected fish, b) Coracidia hatched from the eggs swim in the water, c) *Cyclops* captures coracidium and becomes infected by procercoid larva. Fish eating infected *Cyclops* gets tapeworm infection. Redrawn after Bauer, Musselius and Strelkov, 1969

In some fish farms, 100 percent infection of common carp fingerlings is common. Clinical signs associated with bothriocephalosis include sluggish movement and emaciation; but despite poor body condition, the abdomen is enlarged due to the mass of tapeworms which fill the intestine (Figure 55).

The lumen of the intestine is blocked with worms, and mechanical injuries appear at the attachment point of scolices. Here, local inflammation and haemorrhages occur. Less frequently, accumulation of serous discharge into the intestine and abdominal cavity occurs. Heavily infected fish become exhausted and swim close to the water surface; their gills and skin are usually co-infected by a series of facultative pathogenic protozoans.

Figure 55. Strobila of *Bothriocephalus acheilognathi* leaving the intestine of the common carp through a rupture in the gut

Fresh preparation

Prevention and treatment: Infections can be easily treated with drugs containing niclosamid or praziquantel components. Unfortunately, these drugs, which are used worldwide for treating mammals and birds, have not been tested for fish, and their use in Europe is not approved. Earlier in Hungary and in the former Soviet Union, niclosamid (Yomezan or Devermin) included in fish feed at 0.1–0.2 g/kg body weight was 100 percent effective in treating infections. Similar good results can be achieved by using praziquantel as described in Annex 3.

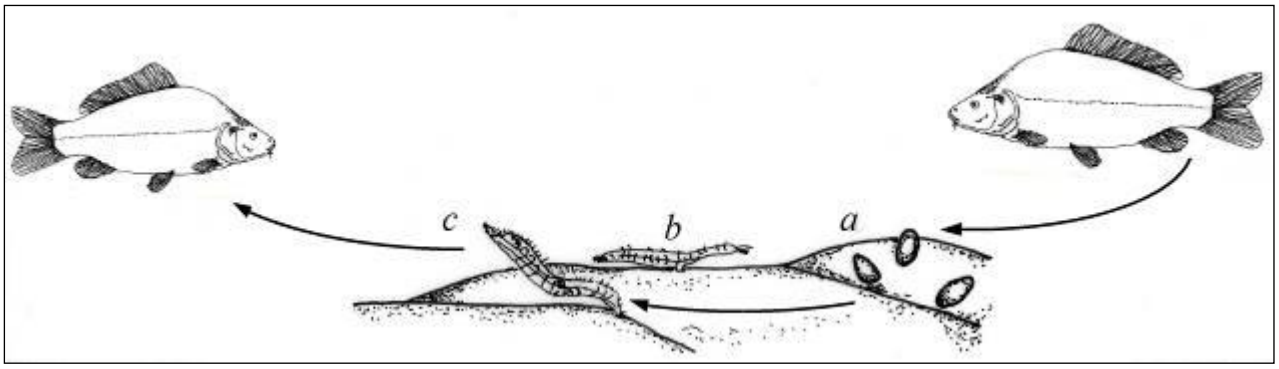
14.2 Infection of common carp with Khawia sinensis

Khawia sinensis, a specific parasite of the common carp is an unsegmented caryophyllid tapeworm (Figures 53B and 56). It has a widened scolex which resembles a carnation. Mature worms can reach 10–17 cm in length and 4–5 mm in width. This tapeworm was introduced to the European part of the former Soviet Union and some Central European countries along with Amur wild carp in the 1970s and 1980s. It is an economically important pathogen which mostly infects large fish. It has tubificid (oligochaete) intermediate hosts, which consume the eggs of the worm, which settle onto the bottom (Figure 57).

Figure 56. *Khawia sinensis* released from the opened intestine of a common carp

Fresh preparation

Figure 57. Developmental cycle of *Khawia sinensis.* a) Eggs of parasite emptied from fish sink to the bottom, b) Eggs are eaten by tubificid oligochaetes in which large plerocercoid larvae develop, c) Fish become infected by eating infected oligochaetes.



Depending on water temperature, the embryonic development in the intermediate host takes 16–57 days, at which point the procercoid stage reaches its infectivity. Carp becomes infected by feeding on infected oligochaetes. This worm can infect carp year round, but reaches its maturity only between April and June, when mature worms lay eggs and then die. The rate of infection in fish depends on the number of oligochaetes in the mud and the age and size of fish. Older carp are more frequently infected than younger ones, due to their benthic feeding habits.

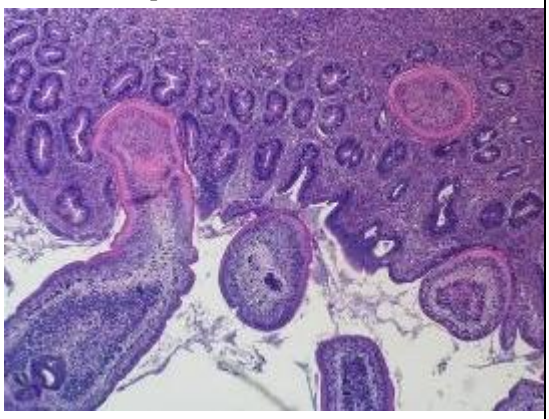
Khawia can be found throughout the intestine. In cases of intensive infection with 35–50 worms, the parasites completely obstruct the intestinal lumen and mortalities occur. Pathology of the gut includes lesions, blood loss, inflammation, proliferation of gut mucosa and increased mucus production.

Prevention and treatment: Accumulation of worms and losses can be prevented by drying and disinfecting the pond bottom, and by culturing young fish separately. Treatment of infections is as described for bothriocephalosis in Annex 3.

14.3 Infection of common carp with Atractolytocestus huronensis

This worm is a small (22–27 by 3–7 mm) caryophyllid cestode which was only recently introduced to Europe from the Far East. In the former Soviet Union, it was known and described as *Markewitschia sagittata*. In Europe, it only infects common carp, although in the United States of America, it was first described in a catostomid fish. It infects mostly the first part of the intestine where, differing from *Khawia,* it bores its spear-like head into the mucosal layer (Figure 58). Development of *A. huronensis* resembles that of *K. sinensis*, procercoids of this parasite developing in oligochaetes. Although these worms cause more severe pathological changes to the infected part of the intestine, due to their small size their pathogenic effect on fish is less pronounced.

Figure 58. *Atractolytocestus huronensis* boring its head into the intestinal wall of a common carp



Histological section, H & E staining

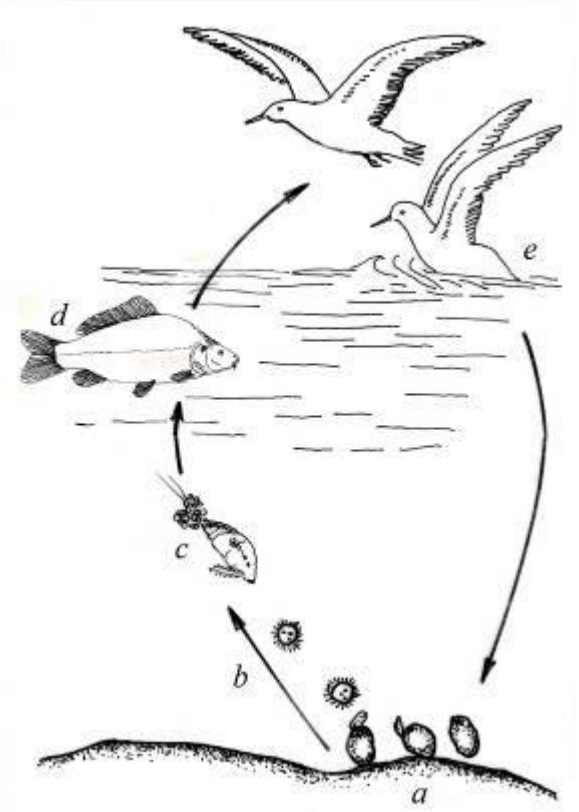
14.4 Ligulosis

Ligulosis is caused by the plerocercoid stages of large tapeworms of water birds belonging to the genera *Ligula* and *Digramma*. *Ligula intestinalis* and *D. interrupta* are known from cyprinid fish. The large plerocercoid developing free in the abdominal cavity of fish can reach 30–60 cm in length and 0.7–1.2 cm in width (Figure 59). The final host, a fish-eating bird is usually infected after no longer than three days. The worms in the bird's gut produce eggs that are voided into the water with the faeces (Figure 60). Eggs hatch within 5–8 days as a free-living larval stage, the coracidium. Coracidia are consumed by copepods, in which the procercoid stage develops. When the infected copepod is eaten by a fish, the parasite burrows through the gut wall and develops into the plerocercoid stage in the abdominal cavity. The plerocercoid must spend about 425 days in the fish to achieve infectivity. During this time, the worm grows considerably, and the weight of one to three worms in the abdominal cavity can reach one third of the fish's weight. Infected fish lose weight and have difficulty swimming. They become emaciated, but the belly is swollen due to the presence of the worms. Fish may survive heavy infections, but they are easily captured by fish-eating animals. The flesh of infected fish is of low quality. No treatment for this infection is known.

Figure 59. Two-year-old grass carp infected with plerocercoids of *Ligula intestinalis*

Fresh-mount preparation

Figure 60. Development of *Ligula intestinalis*



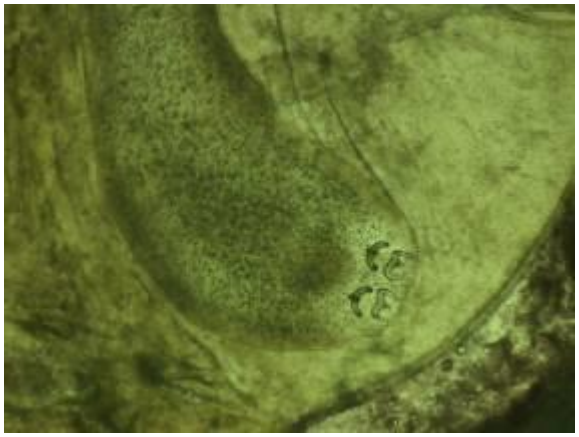
a) Eggs voided by water birds sink to the bottom, b) Coracidia developed in the eggs swim, c) Procercoid larvae develop in *Cyclops*, d) In the body cavity of fish, a large plerocercoid larva develops which becomes infective for birds after developing for more than one year, e) Water birds are infected by eating infected fish.

Redrawn after Bauer, Musselius and Strelkov, 1969

14.5 Other tapeworm infections

A great number of relatively host-specific proteocephalid cestodes commonly infect freshwater fish. However, they rarely occur in pond-cultured fish. Of them, the long strobilae of *Proteocephalus osculatus* and *Silurotaenia siluri* are often found in the gut of wels catfish. The two species differ, the scolex of *P. osculatus* being unarmed, while the scolex of *S. siluri* is surrounded by spines. In the gut of the northern pike, *Triaenophorus nodulosus* is common. This worm can be easily recognized by the trident-shaped hooks on its scolex. It develops in the liver of percid fish, large nodules with skeins of this worm being frequently found in the liver of pikeperch (Figure 61).

Figure 61. Head end of a plerocercoid of *Triaenophorus nodulosus* from the liver of pikeperch



Fresh-mount preparation

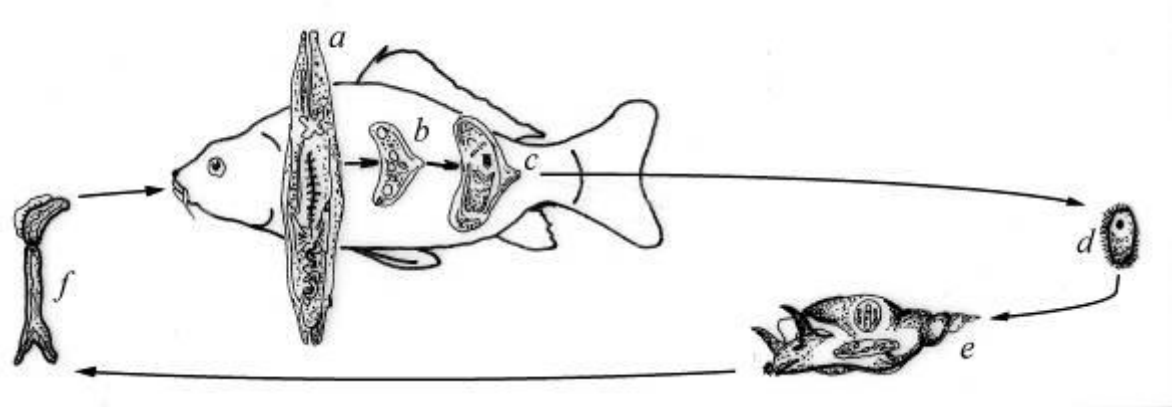
15. DISEASES CAUSED BY PARASITIC FLUKES (DIGENEANS)

Digeneans are flatworms (Phylum Platyhelminthes, Class Trematoda, Subclass Digenea) that develop by molluscan first intermediate hosts, which in most cases are snails or clams. After a complicated development in the first intermediate host, the 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, ready to infect a final host, such as a piscivorous fish, bird or mammal. Although some adult-stage trematodes infecting fish are regarded as serious pathogens (e.g. blood flukes), the most well-known trematode diseases of fish are caused by their metacercariae.

15.1 Sanguinicolosis of common carp

This disease is caused by a blood fluke, *Sanguinicola inermis*, which inhabits the blood vessels of common carp. This, about 1 mm long worm produces characteristic triangular eggs, which are carried by the blood stream to different organs. During the first larval stage, the miracidia hatch from eggs that are already in the blood, but they can only exit the fish through superficial organs, like the gills (Figure 62).

Figure 62. Developmental cycle of the blood fluke *Sanguinicola inermis* in common carp.



a) Mature fluke lives in the heart and gill arteries of common carp, b) Eggs are carried to capillaries by the blood stream, c) Miracidia break through the capillaries and enter the water, d) Ciliated miracidia swim in water to find a snail, e) In the body of the snail, sporocyst, redial and cercarial stages develop, f) Cercaria leaving the snail infect fish.

Redrawn after Kocylowski and Myaczynski, 1960.

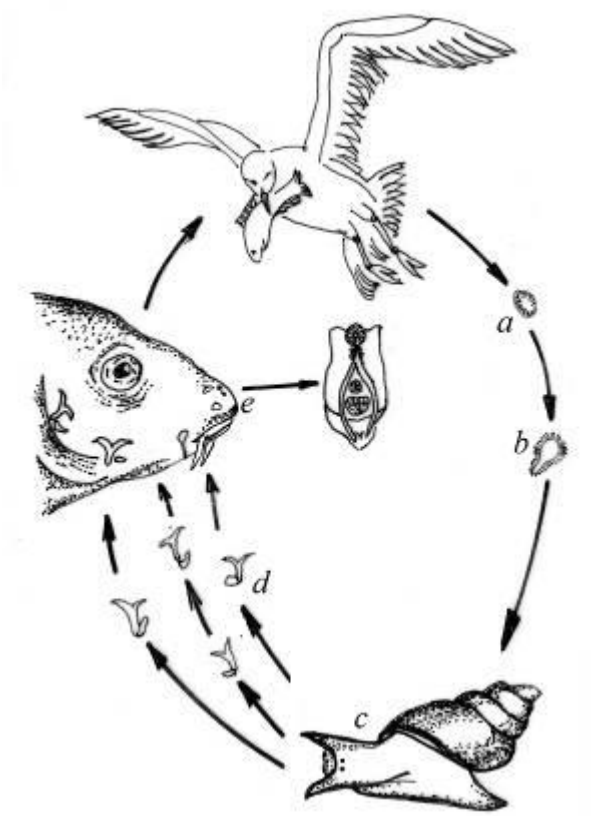
Other larvae found in the capillaries of parenchymatous organs die and became small nodules surrounded by a capsule of connective tissue. Entering the water, larvae bore themselves into snails of the genus *Limnaea*, and within some weeks cercariae develop by multiplying in this mollusc. Cercariae swimming in the water actively invade common carp and develop to adults in the blood vessels. Miracidia obstruct capillaries, and cause stasis and localized degeneration. In intensive infections, the gills are pale or marbled and the gill filaments break off. Malpighi bodies in the kidney die, and sera accumulate in the abdominal cavity, under the scales and in the eyes.

Clinical signs: The gills show clinical signs resembling gill rot caused by fungi or dactylogyrosis. In cases involving changes to the kidney, sanguinicolosis must be differentiated from spring viraemia of carp (SVC). With microscopic investigation, the characteristic triangular eggs of *S. inermis* can be easily recognized.

Prevention and treatment: Prevention is based on reducing the number of intermediate-host snails in the pond.

15.2 Diplostomosis of cyprinids

Figure 63. Developmental cycle of *Diplostomum spathaceum*



a) Fluke eggs in faeces are voided by birds, b) On the bottom, miracidia develop from eggs and swim, c) Further stages develop in snails, d) Cercaria swim in the water, e) Cercaria infecting fish develop to metacercariae in the lens of the eye. Birds become infected when infected fish are consumed.

Redrawn after Bauer, Musselius and Strelkov, 1969

This is a commonly occurring disease, almost all cyprinids cultured in ponds having some metacercariae of *Diplostomum spathaceum* in the lens of their eyes (Figure 63). Adult worms live in the gut of water birds, the eggs entering the water with their faeces. Miracidia hatched from the eggs infect snails of the genus *Limnaea* in which cercarial stages develop. Cercariae swimming in water actively attack fish, boring into the skin and migrating towards the eyes. If the number of invasive cercariae is high, heavy infection develops and the migrating larvae damage tissues and cause bleeding.

In heavily infected fry and fingerlings, haemorrhage develops in the muscles, primarily at the dorsal part of the head, causing death of the fish. In less severe infections, cercariae sporadically migrate to the lens, where they accumulate without encystation, being located in the vitreous humor. In this chronic form of infection, the vigorously moving cercariae cause the lens to become opaque, and this change can be easily seen with the naked eye. In silver and grass carp stocks, extremely intensive infections may develop, but fingerlings of common carp are less frequently affected. In severe cases, fish become blind and may lose one of their eyes.

Although blind fish can survive, their condition is poorer than that of less-infected specimens. Within some months, infected fish can recuperate.

Prevention and treatment: For killing metacercariae, Mebendazol is effective if mixed in the food or administered in bath solution. Prevention relies on killing the snails in the pond by desiccation, wintering or disinfecting the bottom. Chasing off water birds is also useful.

15.3 Blackspot disease

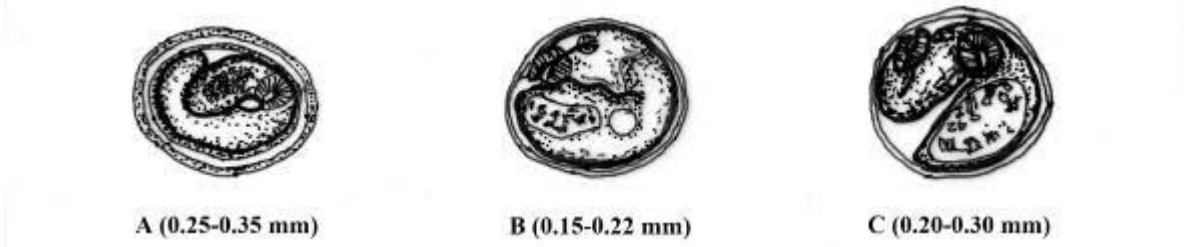
This disease is caused by metacercariae of trematodes of the genera *Posthodiplostomum* and *Apophallus* (Figures 64 and 65A). Both types of fluke infect fish with their encysted metacercariae. Mature flukes live in the gut of water birds, and cercariae develop in snails, but when infecting fish they encyst and cause a host reaction, the formation of a black pigment layer around them.

Metacercariae of *Posthodiplostomum cuticola*, (also reported under the synonym *Neascus cuticola*), form relatively large cysts (about 1.5 mm in diameter) in the muscle of fish (Figure 64). However, the large (approximately 0.2–0.3 mm) metacercarial cysts of *Apophallus muehlingi* (Figure 65A) infect mainly the fin rays, and less frequently, the skin. In small fish, they can cause serious deformation of the body. Fish may survive heavy infections but because they become unsightly, they are not marketable.

Figure 64. Heavy black spot infection in the skin of freshwater bream caused by *Posthodiplostomum cuticola* metacercariae

Fresh-mount preparation

Figure 65. Encysted metacercariae



A) *Apophallus muehlingi*, B) *Metagonimus yokogawai*, C) *Opistorchis felineus*

Prevention and treatment: Prevention is similar to that suggested for diplostomosis. No treatment is known.

15.4 Tetracotylosis

A great number of strigeid-type flukes (*Apharyngostrigea, Cotylurus, Apathemon,* etc.) form encysted metacercariae, called *Tetracotyle* in the abdominal cavity, inner organs or the muscle of fish. In heavy infections, these transparent metacercariae, which are surrounded only by a thin wall, can cause dropsy that resembles infectious bacterial dropsy. Hundreds of cysts attached to the pericardium hamper the movement of the heart and can cause the death of the host. The presence of cysts in the serous membranes makes diagnosis easy.

Prevention and treatment: Prevention is similar to that suggested for diplostomosis. No treatment is known.

15.5 Other metacercarial infections

In Siberia and some Far Eastern countries, metacercariae of *Metagonimus yokogawai* (Figure 65B) are common the muscles of cyprinid fish, while *Opisthorchis felineus* often occurs on the scales (Figure 65C). Both species are of zoonotic importance, causing human infections in these regions. Their intermediate host snails are not common in ponds; therefore, their occurrence in pond farms is less probable.

16. DISEASES CAUSED BY ROUNDWORMS (NEMATODES)

In natural waters and fish ponds, the economic importance of nematodes infecting fish is low; only one roundworm, *Philometroides cyprini* may have significance in some carp-farming regions (Figure 66).

Figure 66. Infection of common carp by *Philometroides cyprini*. Mature female worm is leaving the scale pocket.

Fresh-mount picture

Philometroides cyprini, a specific parasite of the Far Eastern subspecies of common carp was introduced to the European part of the former Soviet Union in the 1950s and has subsequently spread to several countries in Europe and Asia.

The female of this parasite is a large, red-coloured worm reaching 9–10 cm in length. The male, however, is small, not longer than 3–3.5 mm. *Philometroides cyprini* has a one-year developmental cycle. Mature viviparous female worms developing in the scale pockets reach full maturity in May and June, when, protruding one of their body ends to the external environment, they burst in the hypotonic pond water and release millions of their larvae. Third-stage larvae are captured by copepods, in which larvae infective to carp develop. Carp become infected during summer by feeding on these copepods. The larvae enter the body cavity of the fish by boring through the intestinal wall. After copulation in the swimbladder wall, female worms wander to the scale pockets and grow enormously. Infections in fish can first be recognized in the autumn, when only a small nodular deformation of the scales is observable. However, in the spring, scale deformation and the presence of nodules are obvious. Two-year-old fish can die if they are infected with 30 or more nematodes, while older fish can survive intensive infections. Ulcers may appear at locations where the worms develop. In infection with a low number of worms, total recuperation can be expected. The economic damage is large, as infected fish are disgusting; hence, they cannot be sold for human consumption.

Prevention and treatment: Prevention can be easily accomplished by separating infected fish and by raising new generations in water free of infected copepods. No reliable drugs are available for treatment.

17. DISEASES CAUSED BY SPINY-HEADED WORMS (ACANTHOCEPHALANS)

Acanthocephalans are common endoparasites of fish in natural waters. They are easily recognized by their cylindrical bodies bearing a proboscis that is equipped with a series of hooks (Figure 67). Worms bore their proboscis into the intestinal wall and feed on the digested food (Figure 68). They develop through intermediate hosts like amphipods. Their occurrence in pond fish is rare, as pond farming is not favourable for most of the intermediate hosts.

Figure 67. Head of an acanthocephalan

Figure 68. Heavy infection by *Pomphorhynchus laevis* in the gut of a common barbel

Fresh-mount picture

18. DISEASES CAUSED BY LEECHES

Leeches are annelids: parasitic segmented worms belonging to the Class Hirudinea. They are blood-feeders and are equipped with suckers at both ends of the body, enabling them to maintain a grip on their host. Some leech species are specialized to live on fish. *Piscicola geometra* is one of the best-known species infecting cyprinid fish. *Piscicola geometra*, which grows to 30–50 mm in length, is a temporary parasite, and only sucks the blood of the fish (Figure 69). This parasite has a wide host range and is able to infect different fish species. Its life cycle is direct. Eggs in cocoons are placed on aquatic plants. Young worms are ready to suck blood.

Figure 69. Fish leach (*Piscicola geometra)*

Fresh-mount picture

In cases of heavy infection (hundreds of worms), fish become restless and make unusual movements. In wintering ponds, fish stop their rest and gather at the inflow. They grow thin, and their eyes and body become pale due to blood loss. In unresistant stocks, other diseases, caused by ectoparasitic protozoans or bacteria also develop. Leeches can directly cause the death of fish. However, they can also transmit fungi, bacteria and blood parasites, which can be just as lethal. It has also been shown that leeches can transmit viruses, including the virus causing spring viraemia of carp (SVC). Less frequently, in some fish stocks another leech, *Hemiclepis marginata,* can cause infections similar to *Piscicola*.

Prevention and treatment: For prevention, the suggested action is to stop the reproduction of leeches by reducing water weeds in the pond. Organophosphates can be effectively used to treat infections. Although salt and formalin solutions are less effective, good results can be achieved by bathing fish, because the worms leave the fish and sink to the bottom of the treatment tank.

19. DISEASES CAUSED BY PARASITIC LARVAE OF BIVALVE MOLLUSCS (GLOCHIDIA)

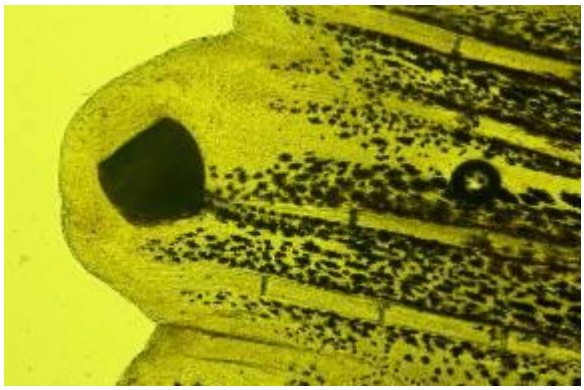
Mature freshwater mussels release their larvae, called glochidia Figures 70 and 71), which attach to the fins or gills of fish and remain parasitic for one or more months while they develop into young mussels. Several cyprinids can serve as hosts.

Glochidia range in size between 0.05 and 0.45 mm shell length depending on the species. Glochidia of *Unio* spp. are generally smaller than those of *Anodonta* spp. Fish are able to sustain low levels of glochidial invasion without apparent harm, but heavy infestations, especially in the gills of young fish, can cause injury and even death. Infection with glochidia is especially fatal in fry populations in freshly stocked rearing ponds. Cases have been reported where glochidia, with their clamps, have closed the mouth of fry, causing their starvation and death.

Prevention and treatment: For preventing severe infections with glochidia, drying and disinfecting the soil of fry-rearing ponds is suggested.

Figure 70. Glochidium of a *Unio* sp.

Figure 71. Glochidium of a *Unio* species attached to the fin of a perch, causing proliferation of the epithelium



Wet-mount preparation

20. DISEASES CAUSED BY CRUSTACEANS

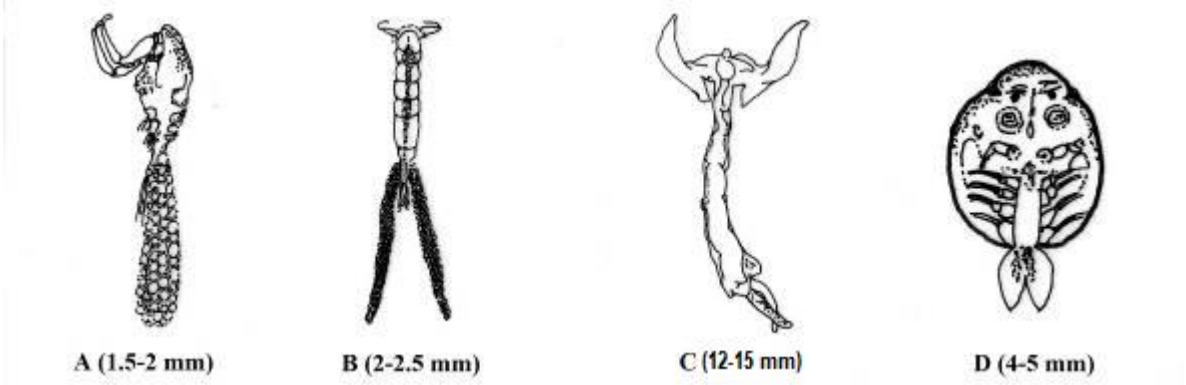
Crustaceans are aquatic animals belonging to the Phylum Arthropoda, a large group of animals that are characterized by their chitinous exoskeletons and jointed appendages. The Class Crustaca includes many important marine organisms, such as crabs, lobsters, crayfish, shrimps, prawns, isopods, ostracods, amphipods and copepods. Among these are many species that are parasites of finfish, belonging mainly to the Order Copepoda and the Order Arguloidea (Subclass Branchiura).

Parasitic copepods are frequently found on the skin and gills of warmwater finfish, and are typically highly modifed to their parasitic mode of life. Several, such as the "anchor worm" (*Lernaea* spp.) can be serious pests in freshwater aquaculture facilities, causing impaired growth and occasional mortalities and, through their feeding and attachment activities, opening wounds that provide entry points for other pathogens, such as bacteria and fungi.

This chapter deals with four diseases of warmwater finfish, three caused by parasitic copepods and one caused by a branchiuran (*Argulus* sp.)

20.1 Ergasilus sieboldi infection

Figure 72. Some parasitic crustaceans infecting fish



A) *Ergasilus sieboldi*, B) *Sinergasilus lieni*, C) *Lernaea cyprinacea*, D) *Argulus foliaceus*

Infection with ergasilid copepods is common both in fish farms and natural waters. Of the large number of *Ergasilus* spp., *E. sieboldi* plays the most important role (Figure 72A). The body of the *Cyclops*-like, pear-shaped female copepod measures 1,5-2 mm in length. The anterior part of its body broadens, while the posterior part narrows and ends in a tapered tail. The body is composed of segments. At the head end, a pair of large antennae have robust claws. These serve to thrust into each side of a primary gill lamella. For most of its life cycle, *E. sieboldi* is free-living, with six naupliar stages and five copepodite stages, which all lead to free-swimming adult males and females. After mating, the male dies, while the female seeks a host fish. After attaching to a fish, it loses the ability to swim, but its ability to move on the surface of the gills remains.

This parasite feeds by damaging the epithelium and ingesting cellular debris and mucus. Each female has two egg sacs which contain 100–300 eggs, from which nauplius larvae hatch into the water. In temperate climates, *E. sieboldi* has three generations per year. Some members of

the last generation can over-winter and cause a new infection the next year. The speed of development depends on water temperature. At 16 °C, the development in the eggs takes 10–12 days, at 20 °C it takes 6 days, while at 25 °C it lasts only 3.5 days. The egg production in females usually starts in April and ends in September.

Figure 73. Heavy infection of *Ergasilus* on the gills of pikeperch.

Fresh mount preparation

Of the different cyprinids, infection by *Ergasilus* is most common in tench. For predatory fish, ergasilosis is common in northern pike and pikeperch. In heavy infections in the latter fish, besides the gills, these crustaceans also invade the outer surface of the gill cover, the grooves.

Infected fish are emaciated, restless and swim to the inflow. When the infection is severe, death is common. These crustaceans, as well as the changes they cause, can be easily seen with the naked eye, as they appear as white spots. Therefore, this disease can easily be diagnosed.

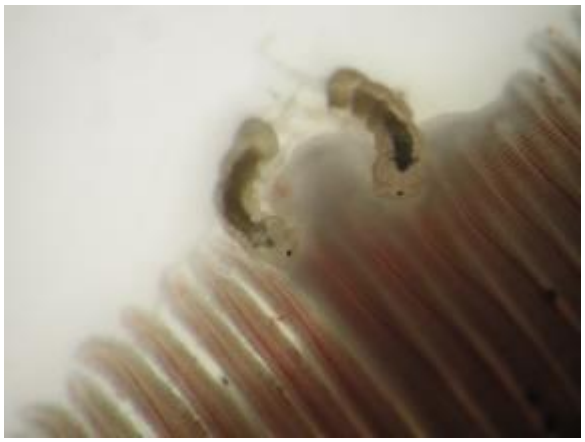
Prevention and treatment: The wide host range of this parasite enables infections to be spread by wild fish. Its free-living larval stages can also been carried into ponds by inflowing water. Nevertheless, a preventive bath before placing fish into a new pond helps. Bathing fish in organophosphates is an effective treatment. In countries where these chemicals are not allowed, a short bath in potassium permanganate solution is suggested.

20.2 Other ergasilid infections

Sinergasilus major and *S. lieni* (Figures 72B and 74), common in the Far East and specific parasites of Chinese major carps, were introduced to Europe and Central Asia. These parasites, which are characterized by their elongated bodies and egg sacs, cause severe infections on their hosts.

Pathology: Damage to the host is caused by the aggressive attachment mechanism and by feeding. At their attachment points, gill filaments can break off due to necrosis, and the gills become rugged (Figure 73). On other areas of the gill, heavy proliferation of gill epithelium starts and epitheloid cells grow over the lamellae, which then become unable to take up oxygen. These parts of the gills appear as white-coloured nodules with female copepods in their centers, from which their two elongated egg sacks emerge

Figure 74. *Sinergasilus lieni* clubbing the gill filaments.



Fresh-mount preparation

20.3 Lernaeosis

This disease is caused by *Lernaea cyprinacea*, which is a copepod parasite that produces characteristic clinical signs on the skin (Figures 72C and 75). The parasite can cause severe infections in intensively reared and densely kept common and Chinese major carp populations.

Figure 75. *Lernaea cyprinacea* infection in silver carp

Local haemorrhages appear at attachment points of the copepods.

Fresh-mount picture

Some specialists think that the causative agent is *L. cyprinacea*, which has been known in Europe for more than 100 years, but others believe that the introduction of a morphologically similar parasite, the Far Eastern species *L. elegans*, can be attributed to the intensification.

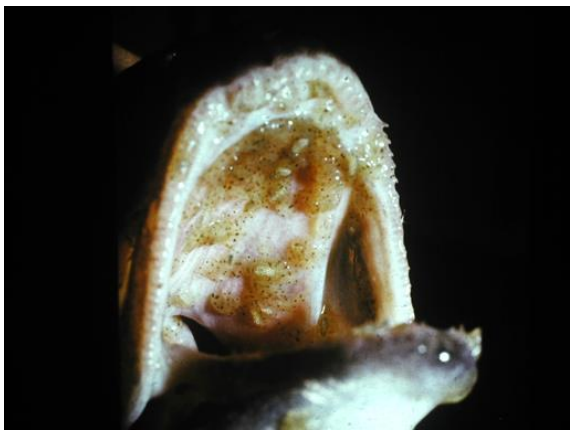
In its life cycle, just as for *Ergasilus, L. cyprinacea* has a free-living and a parasitic stage. Naupliar stages live free in the plankton, while copepodite stages, however, are parasitic on the gills of fish, but still remain mobile. *Lernaea cyprinacea* shows sexual dimorphism. Its males have a typical *Cyclops* shape. Its females, however, after the copepodite stage, when attaching onto the host, completely change their morphology. The body of the female is elongated and unsegmented, and without egg sacks, measures 12–15 mm in length. At the head end, it has a four-armed sclerotic attachment organ, which it buries deep into the host tissue to fix the parasite in the spot. They usually bore through scales. The posterior part of the copepod floats on the surface of the skin in the water. In heavy infections, this gives a "hairy" appearance to the fish. At the attachment point, the parasite causes a puncture wound. The scale is often lost, and there is also damage to the underlying muscle tissue in which the cephalic horns of the anchor grow. Muscle necrosis, haemorrhaging, inflammation and suppuration are common. Due to the necrosis of tissues, wounds become secondarily infected by bacteria and fungi.

Prevention and treatment: The best method of control is to avoid infestation by preventing the introduction of infected fish into the system. No effective treatment for killing mature females is known. Organophosphate insecticides can be used, but they are only effective against the copepodite stages, so treatment must be repeated every seven days for at least a month. Biological control of the larval stages of *L. cyprinacea* is available by using predatory free-living cyclopoid copepods.

20.4 Fish lice (Argulosis)

This disease is caused by *Argulus* spp., which belong to the crustacean Order Branchiura. In carp farms, only one species, *A. foliaceus*, plays an important role (Figures 72D and 76). This parasite, infects all age groups of fish.

Figure 76. Specimens of *Argulus foliaceus* located in the mouth of a fish



Fresh-mount picture

Argulus foliaceus is a leaf-like, dorso-ventrally flattened crustacean which has a large carapace covering most of the organs and partially, the legs. This translucent crustacean has two pigmented eyes in its head and two strong attaching discs (suckers) on the abdominal surface. The mouth tube or proboscis with a pre-oral stylet is also located on the ventral surface between the suckers. When feeding, the stylet is inserted into the epidermis of the fish.

The parasite has a wide host range. It finds fish by actively swimming. In most cases, it stays on one fish, but it can also change hosts. For propagation, the female leaves the fish and lays about 250–300 eggs on water weeds or rocks when the water temperature is over 10 °C. Depending on the water temperature, the eggs hatch within 15–55 days. The larvae are similar to adults, and they also have a parasitic life style. They need to find a host within 3 days. During its stay on the fish, *Argulus* feeds by penetrating the fish with its stylet. The stylet is thought to inject digestive toxins into the host, which help the crustacean to suck blood and tissue sera. At its attachment point, a small groove is formed with a small protrusion around it. At the place of sucking, ulcers can develop in which bacteria and fungi propagate. *Argulus foliaceus* can also transmit viruses (e.g. *Rhabdovirus carpio*). Being intermediate hosts for some parasites, they are also known to inject larvae of the nematode *Skrjabillanus* spp. (e.g. *S. cyprini*), into common carp.

Both young and old fish can become sick when the infection is intensive. Young fish are more sensitive and often die when infected.

Clinical signs: Infected fish are restless, stop feeding and their body weight drops. Parasites can be easily recognized in the abundant slime covering the body surface. Reddened spots at the site of feeding and small ulcers also call attention to this infection.

Prevention and treatment: Prevention is based on separating young fish from parasite-infected older wild fish. Drying, freezing and disinfecting the pond bottom with lime or bleach-powder promises good effect. For treatment against fish lice, a bath in potassium permanganate solution is suggested, but a bath in salt solution can also help, because most of the parasites will leave the fish and remain in the solution.

21. DISEASES INDUCED BY THE PHYSICAL AND CHEMICAL QUALITIES OF WATER

Maintaining a water quality that is optimal for the species of finfish that is being cultured is a fundamental aspect of successful fish culture. Recognizing the diagnostic signs of distress and mortality caused by the unfavourable physical characteristics of the water in which fish are kept (e.g. unsuitable temperature, salinity or dissolved oxygen content) is an important first step in disease diagnosis, as these basic parameters should be ruled out before moving on to consideration of possible toxicological and biological causes.

In this chapter, problems associated with unfavourable water temperature and oxygen supply are discussed.

21.1 Diseases caused by unfavourable water temperature

Although common carp can survive high water temperatures (up to 35 °C), low water temperature (0–1 °C) can support the development of winter skin disease, saprolegniosis, etc., and hence can be a contributing factor to disease outbreaks. A sudden 10–15 °C difference of water temperature at transfer shocks large fish, causing them to lose equilibrium, stop swimming and cease gill movements. This may last for minutes, but the fish may also die. At higher temperatures, fish usually die of oxygen shortage, rather than due to the direct effect of the high temperature.

It is a general rule that the younger the fish, the less tolerant it is to differences in water temperature when transferred. Larvae may die if the difference between the water temperature in the hatchery and the pond is more than 1 ^{o}C, while advanced fry and fingerlings will swim away without considerable stress.

21.2 Problems in oxygen supply

An adequate oxygen level in the water is a basic factor for fish health. Some species are more tolerant of low oxygen content, while others are less so. Common carp is an ideal fish in this respect, as it is able to endure relatively low water oxygen concentrations. Some predator fish (e.g. pikeperch), however, can only be raised in polyculture with carp if the pond water is rich in oxygen.

The amount of dissolved oxygen in water increases as temperature reduces, and decreases when altitude increases. During sunny days, the oxygen content of water rich in phytoplankton and algae increases because their photosynthesis produces oxygen. Dissolved oxygen often becomes limited during nights due to the combined respiration of fish, phytoplankton and mud-dwelling organisms. This is especially true during late summers when the level of phytoplankton biomass is high. Therefore, a late night–early morning oxygen shortage may develop. If this early morning deficit is not offset with aeration, oxygen shortage causes stress, loss of appetite, and may also kill fish. The critical level of oxygen concentration in carp ponds is about 3-4 mg/litre. Below this concentration aeration of the pond is necessary.

Clinical signs: During acute dissolved oxygen shortage fish start to gulp air at the water surface. Dead fish having open mouths and gill covers is an obvious sign of acute oxygen shortage. A sign of constant low oxygen is the elongated/enlarged reddish lower lip of fish, which, being webbed with fine arteries provides support to the uptake of oxygen.

21.3 Gas-bubble disease (GBD)

At higher temperature, water can dissolve less oxygen. In eutrophic waters, including fertilized fish ponds, excess oxygen accumulates during warm summer afternoons due to the intensive photosynthesis of phytoplankton. However, this accumulation is natural, as well as of short duration, because after dark, when oxygen production by phytoplankton stops, oversaturation of oxygen also stops. However, in the case of hydropower turbines or too intensive aeration, the water becomes mechanically oversaturated with air. Other causes of gas supersaturation can be when thermal effluent water or deep bore-hole water is used for aquaculture.

Figure 77. Gas bubble disease

Bubbles accumulated in the capillaries of the serous membranes of the fish.

Fresh-mount preparation

Due to excess gas coming out of solution within the blood, fish that have been exposed to water supersaturated with one or more gases are at risk of sustaining gas embolus. This can cause "gas-bubble disease" (GBD) (Figure 77). Nitrogen is widely believed to be the major gas responsible for GBD. However, oxygen can also be responsible for GBD, especially downstream of hydropower plants.

22. POISONINGS OF FISH

Although a more frequent cause of finfish mortalities in natural waters, poisoning due to toxic compounds in industrial effluents (e.g. heavy metals, chlorine derivatives, petroleum derivatives) entering natural waterways, and to agricultural products (mainly pesticides) applied to crops that are washed into rivers and streams sometimes occur. Such contaminants may occasionally enter aquaculture facilities that draw their intake waters from local rivers and streams, or affect fish that are raised in cages placed in freshwater lakes. Such toxic events often resemble fish kills due to environmental problems, such as toxic algal blooms and oxygen depletion, with large numbers of fish rapidly dying. To recognize these rare events, aquaculturists are well advised to monitor regularly the chemical composition of the water used in their culture facilities.

Contamination of waterways with heavy metals such as mercury, even at very low levels may cause fish to become unsafe for to human consumption, through their bioaccumulation of these products via the food chain.

In addition to poisoning of industrial and agricultural origin, this chapter also deals with poisonings due to the breakdown of aquatic vegetation in fish ponds, and with contaminants that may occur in poorly manufactured fish feeds.

22.1 Poisonings of industrial origin

Different metal compounds (e.g. compounds containing iron, manganese, lead, copper or mercury) often enter the water from industrial plants, polluting natural waters and killing fish in large rivers and lakes. In cases of mercury poisoning, besides directly killing fish, fish exposed to low concentrations can accumulate mercury in their flesh, reducing or even destroying its quality. Moreover it can cause disease in humans. In fish ponds, direct effect of these pollutants is rare, but accumulation of inorganic components in the musculature of older predatory fish can negatively affect meat quality.

Chlorine enters the water in the process of bleaching the water to disinfect it of organic materials. In addition, in some cities, it is often used to disinfect tap water. These waters usually arrive at the fish farms diluted, but fish fry are sensitive to even small quantities.

The water of some polluted rivers contains petroleum derivates, e.g. phenol. Phenol poisoning is reflected in clinical signs associated with impairment of the nervous system, but small quantities of this chemical can also cause fatty degeneration in the liver. In fish farms which receive water from such polluted sources, fatty degeneration and the unpleasant odour and taste of fish flesh call the attention to this problem. Table fish from these farms should be held in clean water before being marketed. Depending on water temperature, this rancid odorous flavour leaves the flesh of fish after 3 to 6 weeks. Cyanide, which is used in gold mining is also a well-known pollutant.

22.2 Poisonings of agricultural origin

Different ingredients or wastes used in agriculture and animal husbandry can cause mortalities of fish, either by causing anoxia or toxicosis. Sewage water carrying rotting organic matter

may reach waterbodies from agricultural plants, animal colonies, slaughter-houses, etc. The rotting of the organic material causes oxygen depletion, and fish die of suffocation. Lime used for fertilization or disinfection can produce high water pH and damage the respiratory epithelium. Organic fertilizers in excess can cause direct damage to the epithelium, and by changing the ion-balance of the water, indirectly damage the fish. Drugs used for plant protection in agriculture are mostly nerve agents. They frequently run off from fields into waters. Spasmodic swimming, rapid mortality and indications of suffocation on dead fish are the major clinical signs. When the exposure is chronic, hydropic degeneration in the liver is the characteristic clinical sign. Chemicals (e.g. organophosphates**)** that are used for treating fish diseases are poisonous in larger doses, although the sensitivity of different fish species to these drugs varies.

22.3 Poisonings of aquatic habitat origin

Fish mortalities are often caused by the rapid decay of vegetation or by the abnormal putrefaction of chemicals (e.g. hydrogen sulphide and ammonia) occurring in the water or in the mud. Hydrogen sulphide (H_2S) accumulates in the mud and during fast cooling or pressure drop, is released into the water, killing fish. Hydrogen sulphide becomes dangerous to fish at a concentration of 1–10 mg/litre. Its lethal effect is higher at low oxygen content and high acidity (lower pH). Free ammonia (NH_3) and ammonium ion (NH_4^+) together represent the total ammonia (NH_3 + NH_4^+) content of water, which is always present, and is one of the most frequent toxicants. It forms as the end product of metabolism, as well as when protein-rich organic matter and urine degrade. It can also enter the water with sewage or with rain water from fertilized agricultural fields. Fish can survive concentrations of 5–10 mg/litre total ammonia in neutral water (i.e. at pH 7). However, in alkaline waters (over pH 7), it is gradually more and more toxic and already causes poisoning at a very low concentration (0.2–0.5 mg/litre). Ammonia is a neural poison; affected fish become restless and show spasmodic movements at the water surface. The mouth of dead fish is open and blood exudates from the gills.

22.4 Enteric inflammation caused by feeds

There is a very adverse view that feeds unsuitable for other animals can be given to fish without causing problems. The old concept that fish in ponds can be fed with supplementary feeds of reduced quality is a mistake. Feeding fish with unsuitable, poor or bad quality feeds is the reason for most of the losses of fish in pond culture. Its direct effect is the development of inflammation in the intestine, which can appear as general inflammation or as the loss of appetite due to catarrhal changes in the epithelium. Of the bad-quality supplementary feeds, treated seeds are the most dangerous. When treating seeds before sowing, mercury components or gamma-hexachlorocyclohexane (gamma-HCH) is often used. These may not kill fish, but they reduce their appetite and by accumulating in their flesh, make it toxic for humans. When fish feeds are improperly stored, they may decay and become mouldy, and bacteria and their toxins will accumulate. Micro-organisms present in the feed ingredients (e.g. albumen, fats, carbohydrates) form poisonous compounds, such as amines and peroxides or they change the normal bacterial flora of the gut. Similar to the situation for warm-blooded animals, moulds and toxins of specific fungi are dangerous for fish. Aflatoxin, which is a product of *Aspergilus*

flavus, damages the liver, while F2 toxin of *Fusarium graminearum* is harmful to the genital organs. It is known that feeding mouldy corn significantly drops sperm production.

A specific type of feeding causing intestinal inflammation occurs in fish harvested during high water levels in the summer. In this case, fish are usually attracted with feeds to the place where they are captured. Therefore, their intestines are usually full of feeds. If these fish are transported alive before the feed has emptied, digestion stops because of stresses, and inflammation starts in the intestine. Consequently, the feed starts fermenting and decaying, and gas accumulates in the gut. The belly of the fish swells or even ruptures. Rotting material is soaked through the mucosa and causes the death of the fish. Carps with enlarged bellies are unable to submerge and they float belly up on the water surface.

Prevention and treatment: Diseased fish cannot be treated, but resting the fish before transport helps.

23. DISEASES OF UNKNOWN AETIOLOGY

Diseases of unknown aetiology include emerging diseases that have not been sufficiently researched to understand their etiology (initially all new diseases are of unknown etiology), and a few enigmatic diseases of sporadic occurrence for which the cause has yet to be clearly identified.
This chapter discusses two diseases of common carp in the CEE and CAC countries that are of unknown aetiology.

23.1 Winter skin disease of common carp

This disease first appeared in the 1980s in some Middle European countries. It develops during the winter months, from December to February, when the water temperature is 1–2 °C. This disease causes considerable economic losses.

Figure 78. Map-like changes on the skin of a common carp suffering from winter skin disease

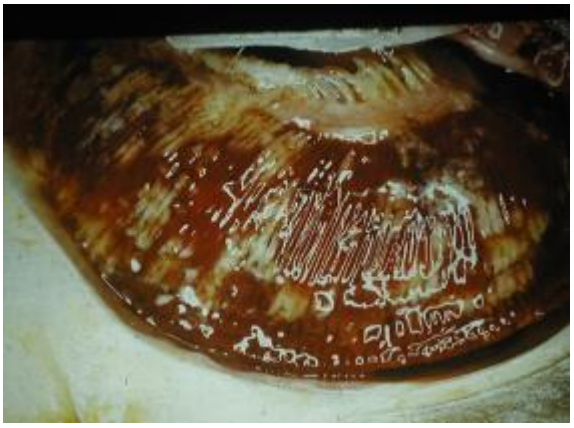
Fresh-mount picture

Lesions are located on the back skin and fins of adult common carp (Figure 78). The first visible lesions are a discernible proliferation of the epidermal areas. Later, the proliferation becomes extensive and epidermal hyperplasia appears generally as "opaque glass". Later this opaque epidermal layer sloughs off. Meanwhile, the mucus disappears from the skin surface and the skin becomes reddish and ulcerated. Together, all these pathological changes result in a "map-like" pattern. There are no lesions or histopathological changes in the internal organs. On the surface of the affected skin area, a 4–7 µm large, colourless, refractive, pear-like organism can be observed, which has root-like appendages (rhizoid) on its narrower end.

23.2 Gill necrosis of common carp

This disease appeared in late 1940s in the European part of the former Soviet Union. By the 1970s and 1980s, it had become the most important carp disease in Central and Eastern Europe, but the Central Asian Republics of the USSR were also affected by it. In these years, the disease caused 60–80 percent losses in the Soviet Union, but about 30–40 percent losses were also recorded in Hungary.

Figure 79. Gill necrosis of common carp



Fresh-mount preparation

The aetiology of the disease is still unknown. Some specialists consider this disease an auto-intoxication, while others think that it is caused by an unknown virus.

Researchers promoting the auto-intoxication aetiology have stated that in highly alkaline waters that are rich in ammonia, fish cannot get rid of ammonium ions produced in their organs, and these ions are the cause of cell and tissue damage in the gills.

It seems to contradict this aetiology that in the ponds of Israel, where the water is highly alkaline, this disease has never been observed. Researchers proposing a viral aetiology received confirmation when in 1998 a new disease showing clinical signs similar to gill necrosis, koi herpesvirus disease (KHVD) appeared (see Section 6.4). They think that when cases of gill necrosis occurred in Europe, a new disease was also appearing in Israel and other parts of the unaffected world, causing heavy losses, but only moderate fatalities in Europe.

Gill necrosis affects every age group of fish, but it is most common in one and two-year-old common carp. The disease appears in the summer months in densely populated, highly eutrophic ponds.

Pathology: The disease has both an acute and a chronic form. Gill necrosis in its acute form passes off within 10–15 days, and losses are high, while in its chronic form the infection persists for 3–4 months and losses are sporadic. In the acute form, the gill lamellae become damaged, the nuclei of epithelial cells degenerate, and the respiratory epithelium dies. The place of epithelium is substituted by proliferating amoeboid cells containing acidophilic granules. This granulation tissue fuses the neighbouring filaments, which lose their ability to support oxygen exchange. In chronic infections, lymphocytes and goblet cells appear in the granulation tissue. In the filaments, necrotic and haemorrhagic areas are formed (Figure 79). The damaged filaments break down and the gills become characteristically serrated.

Clinical signs: Diseased fish show signs of suffocation and stop feeding. Diagnosis of the disease based on the obvious gill damage is easy, but its separation from KHVD is possible only by isolating the virus.

24. ZOONOTIC DISEASES

Diseases transmitted to humans from fish are rare. Viral pathogens of fish do not cause infections in humans. However, some bacteria and fish parasites can cause human infection.

Bacteria belonging to the genus *Mycobacterium* are common in fish, in which they cause fish tuberculosis. Once infected, humans usually develop granulomas on the skin. Fish such as barramundi (*Lates calcarifer*) cultured in warmer water can transmit *Streptococcus* septicaemia to humans through superficial wounds. They usually cause localized inflammation, but can also enter into the lymph system.

In ponds fertilized with pig manure, the bacteria *Erysipelothrix rhusiopathiae* and *Salmonella suis* commonly occur. They are found in the mucus covering the body surface of the fish, and although unlikely, it is theoretically possible that they could infect humans or their food.

Through consumption, some fish parasites may infect people. Of these parasites, the metacercarial stages of trematodes (e.g. members of the genera *Opistorchis*, *Heterophyes* and *Metagonimus*) that use fish as intermediate hosts develop in the liver or gut of humans, causing heavy infections and occasionally, death. Metacercariae belonging to other genera (e.g. *Cryptocotyle*, *Posthodiplostomum*) do not develop in humans, but from the gut, they may make shorter or longer migrations though the host tissues (larval migration), in the process causing microdamages. In addition, after dying, they remain in the tissues as sclerous (hard) nodules. These flukes are common parasites of fish-eating birds and mammals, but can also be a source of disease in humans, for example in Siberia, Korea and Japan, where eating raw freshwater fish is a tradition. It should be noted that consumption of infected fish, even with dead parasites, can cause allergic reactions in humans.

In a similar way, through consumption of uncooked fish, some cestodes and nematodes that use fish as second intermediate hosts can cause human infections. The broad tapeworm, *Diphyllobothrium latum,* grows to a very great length in the intestine of humans eating raw fish. Ascaridoid nematodes such as *Anisakis and Pseudoterrranova* (*Phocanema*), whose larvae parasitize marine fish are the best known for infecting humans, but larval *Contracaecum* and *Eustrongylides* infecting freshwater fish are also able to develop in the human gut. Infected humans show gastrointestinal signs.

Besides living pathogens, toxins transmitted by fish can also cause illnesses. During the reproductive season, the eggs of the common barbel (*Barbus barbus*) are poisonous, and remain toxic even when cooked.

"Ciguatera fish poisoning" cause by exotoxins of blue algae and dinoflagellates accumulated in the flesh of fish are also able to cause human death. However these cases are rather rare in freshwater systems. Cases of toxins of *Clostridium botulinum* transmitted to humans from fish have also been reported. These bacteria can propagate anaerobicly on fish feeds and produce toxins at the bottom or in pond mud.

Among the dangers to humans, drugs used for treating fish diseases represent a special case. Some years ago, it became known that malachite green, a widely used chemical for treating protozoan diseases of fish, could persist in the flesh of fish for a very long time. As malachite green is a known carcinogen, it endangers human health. Due to this, the use of drugs (i.e. antibiotics, oxolinic acids, nalidinic acids, nitrofurantoin, etc.), the treatment of fish diseases needs to be done carefully. Because these drugs may also persist in fish and be transmitted to humans, unwanted resistance toward these drugs may occur.

Annex 1. Diagnosis of fish diseases by changes found on the body and in the organs

Changes in the fins	
Fragmentation	Bacterial infection, ectoparasitic protozoans, gyrodactylosis, mechanical injury
Desquamation of epithelium	*Ichthyobodo, Trichodina* infection
Accretion	Saprolegniosis, carp pox
Nodules	Saprolegniosis, carp pox
White patches	Trematode metacercariae
Black colouration	Trematode metacercariae
Scale damage	
Loss of scales	Mechanical injury
Fluids in the scale pocket	Spring viraemia of carp (SVC), *Aeromonas* infection
Changes in the skin	
Discolouration	Winter skin disease
Ulcers	Ulcer disease, lernaeosis
Accretions	Saprolegniosis, carp pox
Desquamation	*Ichthyobodo* infection
Lumps	*Lernaea* infection, larval trematodes
Large-sized parasites	*Lernaea, Piscicola, Argulus*
Changes to the eyes	
Whitening of the lens	Hatching problems, fungal infection, diplostomosis
Whitening of the cornea	Hatching problems, fungal infection
Deformations in the body	
Swelling of the abdomen	SVC, *Aeromonas* septicaemia, swimbladder inflammation, tetracotylosis, ligulosis, granulomatosis
Lordosis, loss of gill covers	Oxygen deficiency during hatching, chronic bacterial infection
Emaciation	Khawiosis, bothriocephalosis, coccidiosis
Changes in the gill structure	
Loss of gill filaments	Dactylogyrosis, sphaerosporosis, branchiomycosis, gill necrosis, koi herpesvirus disease, protozoan infections
Pale gill colour	Trypanosomosis, dactylogyrosis
Excess mucus on the gill	Protozoan diseases, sphaerosporosis, water problems, dactylogyrosis
Nodules on the filaments	Myxosporean pseudocysts, larval trematodes

Changes in the abdominal cavity	
Excess fluid	SVC, *Aeromonas* septicaemia, swimbladder inflammation, tetracotylosis
Adhesions	SVC, *Aeromonas* septicaemia, swimbladder inflammation, tetracotylosis
Nodules and worms	Tuberculosis, granulomatosis, *Tetracotyle* and *Ligula* infections
Changes in the swimbladder	
Bleeding	SVC, *Aeromonas* septicaemia, swimbladder inflammation
Thickening of the wall	Swimbladder inflammation, tetracotylosis
Accumulation of sera or pus in the lumen	Swimbladder inflammation, *Aeromonas* septicaemia
Changes of the gut	
Worms in the lumen	Tapeworms: *Bothriocephalus, Khawia*
Haemorrhages in the lumen and the wall	Bacterial infections, SVC, food problems, poisonings
Epithelial damage, excess mucus	Bacterial infections, SVC, coccidiosis

Annex 2. Fish Health-related International Recommendations, Regulations and Guidelines for Measurements

World Organisation for Animal Health

The World Organisation for Animal Health (OIE) (http://www.oie.int/) regularly updates and issues guidelines, manuals and reports for monitoring and controlling both terrestrial and aquatic animal health internationally. These include the *Aquatic Animal Health Code* (OIE 2017a), the *Manual of Diagnostic Tests for Aquatic Animals* (OIE, 2017b) and the Annual Reports of the OIE.

The *Aquatic Animal Health Code*[3] (OIE, 2017a) contains the following chapters that discuss various aspects of fish health.

SECTION 1.	**NOTIFICATION, DISEASES LISTED BY THE OIE AND SURVEILLANCE FOR AQUATIC ANIMALS**
Chapter 1.1.	Notification of diseases, and provision of epidemiological information
Chapter 1.2.	Criteria for listing aquatic animal diseases
Chapter 1.3.	Diseases listed by the OIE
Chapter 1.4.	Aquatic animal health surveillance
Chapter 1.5.	Criteria for listing species as susceptible to infection with a specific pathogen
SECTION 2.	**RISK ANALYSIS**
Chapter 2.1.	Import risk analysis
SECTION 3.	**QUALITY OF AQUATIC ANIMAL HEALTH SERVICES**
Chapter 3.1.	Quality of Aquatic Animal Health Services
Chapter 3.2.	Communication
SECTION 4.	**DISEASE PREVENTION AND CONTROL**
Chapter 4.1.	Zoning and compartmentalisation
Chapter 4.2.	Application of compartmentalisation
Chapter 4.3.	Disinfection of aquaculture establishments and equipment
Chapter 4.4.	Recommendations for surface disinfection of salmonid eggs
Chapter 4.5.	Contingency planning
Chapter 4.6.	Fallowing in aquaculture
Chapter 4.7.	Handling, disposal and treatment of aquatic animal waste
Chapter 4.8.	Control of pathogenic agents in aquatic animal feed
SECTION 5.	**TRADE MEASURES, IMPORTATION/EXPORTATION PROCEDURES AND HEALTH CERTIFICATION**
Chapter 5.1.	General obligations related to certification
Chapter 5.2.	Certification procedures
Chapter 5.3.	OIE procedures relevant to the Agreement on the Application of Sanitary and Phytosanitary Measures of the World Trade Organization
Chapter 5.4.	Criteria to assess the safety of aquatic animal commodities
Chapter 5.5.	Control of aquatic animal health risks associated with transport of aquatic animals
Chapter 5.6.	Aquatic animal health measures applicable before and at departure
Chapter 5.7.	Aquatic animal health measures applicable during transit from the place of departure in the exporting country to the place of arrival in the importing country
Chapter 5.8.	Frontier posts in the importing country
Chapter 5.9.	Aquatic animal health measures applicable on arrival
Chapter 5.10.	Measures concerning international transport of aquatic animal pathogens and pathological material

[3]Available at: http://www.oie.int/international-standard-setting/aquatic-code/access-online/.

The *Manual of Diagnostic Tests for Aquatic Animals*[4] (OIE 2017b) contains the following chapters which can be accessed via on the home page of OIE:

[4]Available at: http://www.oie.int/international-standard-setting/aquatic-manual/access-online/

Reference Experts and Laboratories for diseases of aquatic animals
List of Collaborating Centres for diseases of aquatic animals

The Annual Report of OIE[5] contains all key reports of OIE collaborating centres.

Regulations of the European Union

The rules, regulations on control and reporting measures for fish diseases in the European Union can serve as guidelines for considering the importance and observing key aspects of fish disease related international and national measures. These regulations are listed below and can be found online[6]:

- **Council Directive 2006/88/EC** on animal health requirements for aquaculture animals and products thereof, and on the prevention and control of certain diseases in aquatic animals.
- **Commission Regulation (EC) No1251/2008** of 12 December 2008. implementing **Council Directive 2006/88/EC** as regards conditions and certification requirements for the placing on the market and the import into the Community of aquaculture animals and products thereof and laying down a list of vector species.
- **2009/177/EC** implementing **Council Directive 2006/88/EC** as regards surveillance and eradication programmes and disease-free status of Member States, zones and compartments.
- **Commission Decision 2010/221/EU** approving national measures for limiting the impact of certain diseases in aquaculture animals and wild aquatic animals in accordance with Article 43 of Council Directive 2006/88/EC.
- **2008/392/EC Commission Decision** implementing **Council Directive 2006/88/EC** as regards an Internet-based information page to make information on aquaculture production businesses and authorised processing establishments available by electronic means.
- **2001/183/EC Commission Decision** laying down the sampling plans and diagnostic methods for the detection and confirmation of certain fish diseases and repealing Decision 92/532/EEC.
- **Commission Decision 2008/896/EC** on guidelines for the purpose of the risk-based animal health surveillance schemes provided for in Council Directive 2006/88/EC.
- **Commission Decision 2008/946/EC** implementing **Council Directive 2006/88/**EC as regards requirements for quarantine of aquaculture animals.
- **Commission Regulation (EC) No 1250/2008** of 12 December 2008 amending **Regulation (EC) No 2074/2005** as regards certification requirements for import of fishery products, live bivalve molluscs, echinoderms, tunicates and marine gastropods intended for human consumption.

[5]http://www.oie.int/our-scientific-expertise/collaborating-centres/annual-reports/
[6]http://eur-lex.europa.eu/homepage.html?locale=en

Annex 3. Chemicals, Drugs and Antibiotics used to Prevent and Treat Fish Diseases

An essential consideration in the use of drugs and antibiotics for treatment of aquatic diseases, is that it should be used based on correct diagnosis and administered by a licensed aquatic veterinarian or an aquatic animal health professional recognized by the government.

A. List of chemicals, drugs and antibiotics used to prevent and treat fish diseases[1]

Name of Product	Disinfection without Fish	Chemicals				Drugs	
		Dip[2]	Short bath	Transit bath	Long bath	Oral	Injection
Most common disinfectants, antiseptics and chemicals used against ectoparasites							
Benzalkonium chloride	Yes				Yes		
Calcium hypochlorite ($Ca(OCl)_2$)	Yes				Yes		
Copper oxychloride ($3Cu(OH)_2\ CuCl_2$)					Yes		
Copper sulfate ($CuSO_4$)					Yes		
Diflurobenzuron (Dimilin)					Yes		
Formalin (32%)	Yes	Yes	Yes		Yes		
Hydrogen peroxide (H_2O_2) (3%)	Yes	Yes					
Hypo ($Na_2S_20_3$)	Yes						
Divasan forte (15%)	Yes						
Levamisol						Yes	
Malachite green (banned in the European Union)			Yes	Yes	Yes		
Mebendasol			Yes		Yes		
Methylene blue			Yes	Yes	Yes		
Metronidazole				Yes			
Organophosphates			Yes		Yes		
Potassium permanganate ($KMnO_4$)					Yes		
Praziquantel			Yes		Yes	Yes	
Quick lime	Yes						
Salt (NaCl)	Yes		Yes	Yes			
Salt and potassium permanganate				Yes			
Most frequently used antibiotics							
Enrofloxacin						Yes	Yes
Erythromycin						Yes	Yes
Florphenicol						Yes	Yes

Flumequin					Yes	Yes	Yes
Neomycin					Yes	Yes	
Oxytetracycline					Yes	Yes	Yes
Sulphamethoxazole-trimethoprim						Yes	Yes

[1] It is important to note that all chemicals and drugs unknown to or not yet tested by users must be tested before use. In instructions and calculations, frequent measures are:

PPM: parts per million (1 PPM = 1 mg/m^3)
PPT: parts per thousand (1 PPT = 1 mg/litre)
Per thousand (‰): 1 ‰ = 1mg/litre or 1ml/litre
Percent (%): 1% = 10 mg/litre or 10 ml/litre

[2]Dip=flush bath or dipping

B. Disinfectants, antiseptics and other chemicals used for treating parasites

Before using a chemical, not only its effects on the fish to be treated, but also the possible danger that it poses to the health of the user should be considered by carefully reading the product description. In order to be well prepared for immediate action in case of an accident, the following points must be clarified before use:

- Identification of hazards
- First aid measures
- Accidental release measures
- Handling and storage
- Exposure control and personal protection

During the short transport of harvested fish, a mixture of different chemicals, such as salt, methylene blue (earlier, malachite green) and organophosphates is sometimes used as a bath before releasing the harvested fish in wintering or rearing ponds. Although skilled fish farmers may follow this procedure, it is not recommended for general use. Only salt solution as indicated below is recommended.

Benzalkonium chloride

- Used as a disinfectant for nets, tools, tanks, etc. in concentration: 1:50 000 –1:20 000
- Used in tanks as long bath against protozoan parasites in concentration: 1–2 mg/litre for a period of 0.5–1 hour/day
- **Note that benzalkonium chloride is less effective in hard and saline waters**

Calcium hypochlorite ($Ca(OCl)_2$)

- Used as a disinfectant on empty pond bottoms or in discharged water in concentration: 30 mg available chlorine/litre final concentration on pond surface or in the water
- Used as bath treatment in fish ponds against gill necrosis and algal bloom in concentration: 7–10 kg/ha distributed in strips over the water surface, repeated a maximum 3 times every 4^{th} or 5^{th} day

Copperoxychloride ($3Cu(OH)_2\ CuCl_2$)

- Used against infection by protozoan ectoparasites in ponds in concentration: 4 g/m^3; the well-dissolved material is distributed evenly on the water surface

Copper sulfate ($CuSO_4$)

- Used against fungi and protozoan ectoparasites in concentration: 1 ppm
- Used against branchiomycosis in a yearly total quantity: 8–10 kg/ha which is divided and distributed evenly over the pond water surface in 3 equal portions in the warmest months of the season at an interval of 3–4 weeks

Diflurobenzuron (Dimilin)

- Used against crustacean ectoparasites (*Lernaea* spp. and other copepods) in concentration: 0.5 kg/ha; the well-dissolved material should be evenly distributed over the pond water surface

Formalin (32%)

- Used as a disinfectant for nets, tools, equipment, tanks and water pipes in concentration: 50–200 ml/litre for a period of 20 minutes
- Used for treatment of protozoan and metazoan ectoparasites in concentration: 1–2 ml/litre for a period of 15 minutes
- Used for constant flow disinfection of water of fish hatcheries and in intensive flow-through systems in concentration: 0.015 ml/litre
- Used as a bath treatment in concentration: 1:5 000–10 000 dilution for a period of 15–20 minutes; it can be repeated several times
- Used for treatment of protozoan and metazoan ectoparasites in concentration: 0.1–0.2 ml/litre for a period 60 minutes
- Used for treatment of protozoan and metazoan ectoparasites in concentration: 15 ppm for a period of 24 hours
- Used for treatment of eggs in concentration: 0.23 ml/litre for a period of 60 minutes
- **Note that formalin is more toxic in soft and acid waters, as well as at higher temperature**

Hydrogen peroxide (H_2O_2) (3%)

- Used as a disinfectant in concentration: 0.02–0.06 %.
- Used for disinfecting bacterial infections of the body surface in concentration: 0.5–1 ml/litre for a period of 15 minutes
- Used against protozoan parasites in concentration: 10 ml/litre for a period of 10–15 minutes
- **Note that many fish species do not tolerate this treatment**

Hypo ($Na_2S_20_3$) (8–10%)

- Used as a general disinfectant for tools, nets, equipment and tanks in concentration: 10 ml/litre
- Used for disinfecting effluent water in final concentration: $10g/m^3$
- Used for disinfecting water wells of fish hatcheries in concentration: 250 ml/m^3

Divosan forte (15%)

- Used as a general disinfectant for tools, nets, equipment and tanks; concentration as on the instruction sheet of the product, which can be 0.04–2%
- Used for disinfecting effluent water in final concentration: 10g/m^3
- Used for disinfecting water wells of fish hatcheries in a concentration: 250 ml/m^3

Levamisol

- Used against nematodes (e.g. *Philometra* infection) in concentration: 2–10 mg/kg body weight (BW)/day mixed into the feed of fish

Malachite green

- Used against saprolegniosis and protozoan parasites in ponds in concentration: 0.1–0.2 mg/litre
- Used against white spot disease in ponds in concentration: 0.1–0.2 mg/litre at least 2 times within 1 week
- Used against saprolegniosis and protozoan parasites in wintering ponds in concentration: 0.1–0.2 mg/litre; at a minimum interval of 1 week in a total of 4 occasions per winter
- Used in fish hatcheries for treating common carp eggs in concentration: 1:200 000 for the period while water exchanges in the jar; treatment can be repeated 4–5 times per day
- **Note that malachite green is highly carcinogenic and therefore its use is banned in the European Union (EU); importation of products containing traces of this chemical is also banned in the EU.**

Mebendasol

- Used against *Dactylogyrus* spp. in concentration: 100 mg/litre for a period of 10 minutes
- Used against *Dactylogyrus* spp. in concentration: 1 mg/litre for a period of 24 hours

Methylene blue

- Used against ectoparasites in concentration: 100 mg/litre for a period of 10 minutes
- Used against ectoparasites in tanks in concentration: 2 ppm 2 mg/litre for a period of 24 hours

Metronidazole

- Used against protozoan parasites in concentration: 50 mg/100 litre for a period of 24 hours

Organophosphates

- Used against *Dactylogyrus* spp., *Argulus* spp. and *Lernaea* spp. in ponds in concentration: 1–1.5 g/m^3
- Used in bath treatment against *Dactylogyrus* spp., *Argulus* spp. and *Lernaea* spp. in concentration: 0.5 mg/litre for a period of 6 hours
- Used in bath treatment against *Ergasilus* spp. in concentration: 1g/litre for a period of 0.5–2 hours

Potassium permanganate ($KMnO_4$)

- Used in bath against protozoan ectoparasites in a concentration: 20 mg/litre for a period of 1–2 hours or 1 g/litre for a period of 30 minutes but only in acid water

Praziquantel

- Administered orally against monogenean ectoparasites in concentration: mixed 50 mg into the feed of fish, which quantity is for 1 kg BW. This medical feed is given once
- Administered orally against *Diplostomum* infection of the eye at a dose of 330 mg kg-'body mass for 1 wk
- Administered orally against cestodes (*Bothriocephalus, Khawia*) 50 mg/kg-'body mass for 1 x
- Used in bath against monogenean ectoparasites in concentration: 10 mg/litre for a period of 3–48 hours

Quick lime

- Used as a disinfectant on pond bottom in concentration: 0.5-2.5 tonnes/ha.
- Used in ponds as a general disinfectant of water and against gill necrosis and algal bloom in concentration: 200 kg/ha distributed in strips over the water surface. Do not use at high pH.

Salt (NaCl)

- Used in bath against protozoan ectoparasites in concentration: 1 050 mg/litre (1–5%) for a period of 5–15 minutes

C. Antibiotics used to treat bacterial diseases of fish

Antibiotics should only be used to treat bacterial infections – they are not effective against other pathogens. It is important to do sensitivity testing before using antibiotics and to avoid using those antibiotics that are important for use in human medicine. They are used in oral, bath or injected formulation. In oral formulation, the duration of treatment is usually 5 days, unless instructed otherwise in the description of the purchased product. **Enrofloxacin**

- Oral treatment in concentration: 10 mg/ kg BW mixed into feed
- interperitoneal (IP) injection in concentration: 10 mg/kg

Erythromycin

- Oral treatment in concentration: 75–100 mg/kg BW mixed into feed
- IP injection in concentration: 10–20 mg/kg

Florphenicol

- Oral treatment in concentration: 10 mg/kg BW mixed into feed
- IP injection in concentration: 10 mg/kg

Flumequin

- Bath treatment in concentration: 50–100 mg/litre for a period of 3 hours
- Oral treatment in concentration: 12 mg/kg of BW mixed into feed
- IP injection in concentration: 30 mg/kg

Neomycin

- Bath treatment in concentration: 66 mg/litre for a period of 1–2 hours
- Oral treatment in concentration: 100 mg/kg BW mixed into feed

Oxytetracyclin

- Bath treatment in concentration: 50–100 mg/litre for a period of 1 hour
- Oral treatment in concentration: 77 mg/ kg BW mixed into feed
- IP injection in concentration: 25–50 mg/kg

Sulphamethoxazole-trimethoprim

- Oral treatment in concentration: 50 mg/ kg BW mixed into feed
- IP injection in concentration: 50–70 mg/kg

Annex 4. RECOMMENDED READING

General information related to fish diseases

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