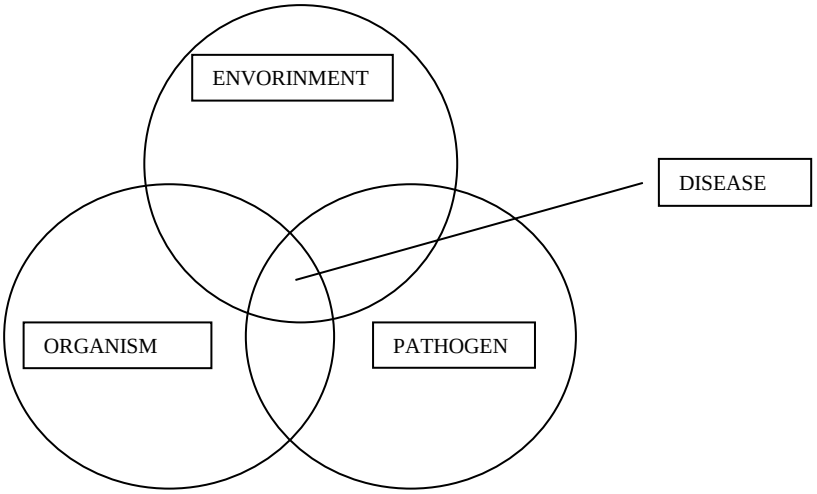
FISH DISEASES

Fish as living organisms, consistently, as is the case in lower and higher evolutionary animals, they exhibit diseases that are of various etiology.
Although fish live in water, the epidemiological aspects of the occurrence and spread of a disease are no different from terrestrial animals living in the ground/air interface. In the aquatic environment occur all those disorders that could create a predisposition to the appearance of a disease or cause some disease. This environment is not devoid of, on the contrary, it is full of microorganisms and parasites that can potentially and under specific conditions cause from a simple coexistence with fish to the appearance and development of a disease and end in death.
Diseases in fish have been observed for a long time, even before the appearance of environmental pollution from urban wastewater and from industries and before the development of fish farming. These diseases were usually caused by parasites in the aquatic environment for which fish were the intermediate or final host, and by natural processes in the aquatic environment leading to incompatible conditions with fish life. It is not excluded that diseases were caused by microorganisms such as viruses, bacteria and fungi, but due to lack of scientific observation and analysis the data available are scarce.
Fish diseases were at the center of scientific interest and prompted the creation of a whole specialized branch of veterinary medicine, when fish farming began to be applied, initially in the form of extensive rearing in freshwater aquifers and lagoons, then with the cultivation of salmonids and later in the form of more intensive forms of farming. At that time, the whole range of causes that can cause diseases in fish was identified and studied, which can be due to both abiotic and biotic factors.

As in terrestrial animals, the occurrence of diseases in fish is the result of many factors that more or less contribute to the disease. These factors can be classified into three categories. Environmental factors, factors stemming from the fish itself and factors related to the organism causing the disease.
The scheme presented in many pathology papers to represent the interaction of the above factors in the onset of a disease is considered very successful, and for this reason it is listed below in these notes.



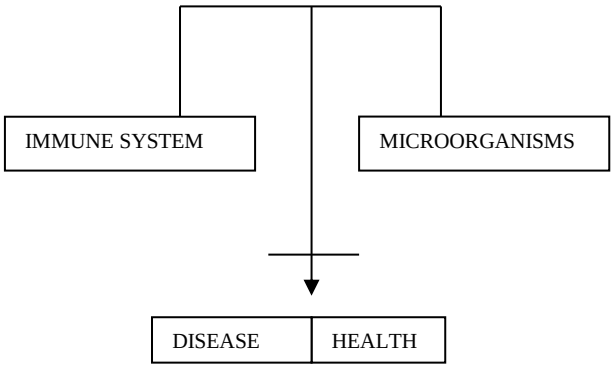
1. FACTORS CONTRIBUTING TO THE OCCURRENCE OF DISEASES

The environment

The aquatic environment, i.e. the physicochemical parameters that characterize it, plays a decisive role first of all in the appearance of a predisposition to the onset of a disease but also in causing a disease without the intervention of any other factor. Thus, when the physicochemical characteristics of the aquatic environment are not compatible with the life of aquatic organisms, then death occurs. This situation may be an extreme event, but it does not mean that it is not happening. Thus, mass and sudden deaths have been observed in fish populations, particularly in closed ponds, when the amount of dissolved oxygen in the water has fallen below compatible with life levels, when there has been an excessive amount of CO_2, or H_2S, following pollution of the aquatic environment by petroleum products, for example, after excessive production of toxins by phytoplankton, after pollution with toxic substances, to mention some of the causes of these sudden mass deaths. In all these cases, the aquatic environment alone is the cause of deaths.

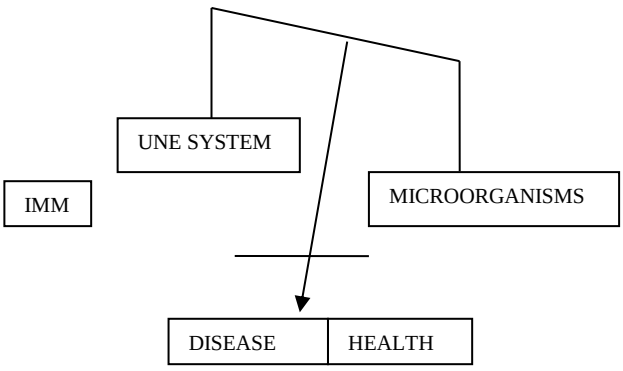
In most cases, however, environmental factors act predisposively to the development of a disease which needs the contribution of other factors in order to manifest itself. Thus, the low concentration of dissolved oxygen, the high concentration of CO_2 or H_2S or NH_4 or NO_2 or Cl, the excessively high or low pH, the abrupt changes in temperature and salinity (within the life range of some species), the presence of toxins and pollutants, heavy metals, the increased amount of suspended particles and others,

cause physiological and metabolic stress in fish resulting in a decline in the proper functioning of their immune system and creating an opportunity for pathogenic microorganisms to cause some disease. If we graphically illustrate the relationship of the immune system of fish with microorganisms in causing a disease, then the example of a balance is very typical.



When the immune system functions under normal conditions, then any attacks by microorganisms are constantly combated, resulting in balance. However, if it causes a decrease in the "weight" of the immune system in this balance, then microorganisms have a greater "weight" in this relationship, resulting in the balance tilting towards the onset of disease.

STRESS



Especially for farmed fish, the type of farming used influences and is directly influenced by the environmental factors of the aquatic environment in which the fish live, resulting in the occurrence of specific diseases with a higher frequency.

Type of rearing: relationship with the outbreak of diseases

Three types of farming are used for fish cultivation: a) intensive and hyper-intensive farming, which are characterized by high fish densities, full coverage of the nutritional needs of fish by feeding industrialized feed in the form of pellets and high yields in kilograms of marketable product per unit volume of water, b) semi-intensive farming, characterized by moderate fish densities, partial coverage of the nutritional needs of fish with industrialized feed and medium product yields per volume of water and c) extensive, in which natural fish densities are maintained, the nutritional needs of the fish are met by the natural food production of the water body and the yields are very small.

The increasing degree of intensification of rearing from extensive to intensive forms affects the type and severity of diseases that occur.

Thus, in extensive farming, there are very few problems dominated by parasitic diseases with very few incidents and low mortality, while the most significant problems are due to pollution of the natural water bodies where the fish are located and / or adverse environmental conditions that in combination with poor water renewal, eutrophic phenomena and algae growth can lead to anaerobic growing conditions and deaths from suffocation.

In semi-intensive farming, the disease problems that occur resemble those of extensive farming, only they are more serious and fungal and bacterial diseases can occur.

Finally, intensive farming is the one that presents the greatest problems of diseases that can be viral, bacterial, fungal, parasitic and nutritional. The specific problems (and their treatment) of these farms will be addressed in the following parts of this course.

The environmental causes that advocate intensive fish farming to be the ones that really suffer from all kinds of diseases are varied and concern the location of the farm with the physicochemical characteristics of the water that prevail but also the changes that occur due to the farming, the management of the farm and the impact it has on the environment but also on the farmed organism and the diet applied and its impact again on the environment and the farmed fish.

A. The location of the farm

The choice of location for the establishment of a fish farm is perhaps the most important first step for the subsequent future of the facility. The type of farming that presents the most problems in intensive fish farming in relation to the environment is fish farming in floating fish cages. The physicochemical characteristics of the water on the site selected for the placement of cages that need to be checked and that affect the occurrence of diseases are as follows:

1) Water quality

The location for the installation of the floating unit should be free from toxic waste, such as ammonia, nitrates, heavy metals and phenols, but also the physicochemical properties of the water at the specific site, pH, temperature, oxygen and salinity should be compatible with the species to be grown.

Temperature and salinity

Aquatic organisms other than cetaceans cannot regulate their temperature, so they are called poikilothermic animals, so changes in the temperature of the environment cause a change in their own temperature resulting in changes in their metabolism. For example, rising temperatures cause an increase in metabolism, greater food consumption and growth rates, but also greater consumption of oxygen and elimination of carbon dioxide and ammonia.

Water salinity is related to aquaculture through the control of osmotic pressure, which can affect the ion balance in the aquatic organism.

When choosing a location, ideal salinity and temperature conditions should prevail because even immediately outside them, behavior, food intake, feed convertibility and growth can be negatively affected. It can also cause stress that could lead to susceptibility to parasitic infections and immune impotence resulting in the development of diseases.

It should also be examined in detail whether acute changes in water temperature and salinity occur because such abrupt changes cause much more damage (e.g. severe stress) than slow seasonal changes.

Finally, areas close to estuaries should be excluded to avoid alterations in temperature, salinity and water purity (e.g. sudden and heavy rainfall can cause acute

freshwater ingress into a coastal marine area with a high number of particulate matter causing gill injuries, stress and entry of microorganisms).

Oxygen

Oxygen is required to produce energy. This energy is needed not only for the necessary functions of the body, such as digestion and use of food and osmoregulation, but also for activity. Oxygen needs vary depending on the type of aquatic organism, its stage of development and its size and are influenced by environmental factors such as temperature, water salinity, etc. If an animal's oxygen supply deviates from ideal then its food intake, convertibility, growth and health may be adversely affected.

Ammonia

Ammonia is usually the most important parameter of water quality after dissolved oxygen. The total concentration of ammonia in water consists of two forms: ammonia (NH_3) in gaseous form and ammonium salts (NH_4).

NH_3 is the most toxic form for fish.

Ammonia can come into water from several sources:

(a) the decomposition of organic matter. This material includes food lost in a unit and the feces of organisms.

(b) industrial and urban pollution

(c) Nitrification: ammonia is oxidized to nitrite and nitrate in oxygenated water during nitration. In unoxygenated waters, nitrates are converted to nitrite and eventually to ammonia (de-nitrification).

(d) After algae death.

Nitrite

Nitrite is an intermediate product of the biological oxidation of ammonia to nitrates. They are found in low concentration in nature and healthy aquaculture systems but can rise to high concentrations where there is organic contamination and low levels of dissolved oxygen.

Nitrite is very toxic to fish because it binds to hemoglobin to methaemoglobin, the molecule can no longer bind to oxygen, and fish can die of suffocation.

Particulate matter and water clouding

Water clouding situations can be caused by organic and inorganic solids in the water due to soil erosion, waste from industries, etc.

Some of these particles can be toxic (e.g. salts of various metals), while others such as organic waste can cause a decrease in available oxygen due to microbial decomposition of matter.

Although particulate matter can cause many problems in water systems, it is mainly their direct effects on cultivation. Thus, at high enough levels the suspended particles can cause mechanical damage to the gills of fish and their irritation can cause the thickening of the gill epithelium resulting in a decrease in respiratory capacity.

Infection

The number of pollutants entering the aquatic environment is huge.

The main categories of contaminants in the aquatic environment are shown in Table 1.

Table 1. Categories of pollutants in the aquatic environment

Acids and alkalis	Nutrients (phosphates and nitrates)
Anions (sulphites, cyanide)	Oils and oil solvents
Detergents	Organic toxic waste
Household and livestock waste	Pathogens
Waste from food processors (e.g. on fish farming)	Herbicides
Gases (chlorine, ammonia)	Polychlorinated biphenyls
Heat	Radioisotopes
Metals (cadmium, zinc, lead)	

Each of these contaminants can directly or indirectly predispose or cause disease in farmed fish.

Algae overgrowth

Algae overgrowth can negatively affect fish health, not only through its physical presence in water, it can injure the gills, but also through the reduction of available oxygen. Many phytoplanktonic species can impart unwanted odor to fish but also produce toxins that can cause fish to die or accumulate in their flesh and make them harmful for human consumption.

2) Water renewal

Good water renewal in a location is essential in an intensive form of floating cage farming in that this reduces the accumulation of waste and the problems associated with it. A good renewal of water allows its oxygenation with simultaneous removal of harmful metabolites of fish.

3) Macroalgae growth

The growth of macroalgae on cage nets reduces the size of their holes and increases their surface area. Reducing the size of the holes causes the flow of water through them to decrease, resulting in a decrease in the rate of oxygen supply and waste removal, which can negatively affect the fish. Increased resistance to water flow will often cause cages to deform, reduce their volume and cause additional stresses in their cages and moorings.

4) The currents

Good renewal of cage water is necessary both to renew the oxygen consumed by fish and to remove their excretions. Nevertheless, excessive currents will bring additional dynamic stress to cages, infrastructure and moorings, can negatively affect the behavior of fish, lead to loss of food, cause shaking of the bottom, cause stress, etc.

5) The depth

For most types of floating aquaculture, cages should be placed deep enough to maximize water exchange and keep the bottom of the cages at a distance from the bottom of the site.

Fish usually increase passive, exogenous currents that penetrate the cages, drawing water during their movement inside the cage and during feeding. Some of the internally generated currents, especially those produced during feeding, draw water into the cage through the bottom of the cages and as the cages are close to the bottom, food residues, faeces and water that is poor in oxygen, with toxic substances and gases (e.g. H_2S) and pathogenic microorganisms can enter along with the water.

6) The bottom

The bottom in a location can vary from rocks to mud and can significantly affect site selection. It is best to choose bottoms with rocks because there is a reduced risk of waste retention.

B. The management of rearing

Intensive farming makes full use of water bodies in terms of kilograms of living organisms that can survive per unit volume. Any change in the physicochemical parameters of the water as well as mainly in the available O_2, CO_2, ammonia, H_2S, particulate matter, which is not compatible with the number of fish farmed, causes stress to the fish and a drop in their ability to cope with pollution. More specifically, typical oxygen needs for resting fish are 100-500mg dissolved oxygen/kg live weight/hour or 300-1500mg dissolved oxygen/kg live weight/hour in active fish. In addition, an oxygen content in water greater than 5mg/l has been found to be satisfactory. Any drop in oxygen below the levels required at a particular time brings about a change in fish behavior; increase in the movement of the gill opercula (breathing) to take up a greater amount of oxygen in the unit of time and if the needs are not met causes hypoxia and eventually suffocation and death. The whole situation causes extreme stress and when the stress is long-term it affects both the growth of fish and the ability of the immune system to cope with infections.

The concentration of ammonia above which toxicity occurs in fish is above 0.02-0.05mg/l. However, levels below these cause stress with similar effects as those reported for O_2.

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Although particulate matter can cause many problems in water systems, it is mainly their direct effects on cultivation. Thus, at high enough levels the suspended particles can cause mechanical damage to the gills of fish and their irritation can cause the thickening of the gill epithelium resulting in a decrease in respiratory capacity. Particulates levels below 100mg/l have been found to have little effect on most fish

species. Salmonids, on the other hand, are very sensitive (they should be less than 20 mg/l).

In this type of farming, large fish densities are used and the large overcrowding makes the transmission of diseases easy.

The usual system of these farms is to fill the body of water with young fish Based on live weight ratios per volume of water and after fattening and changing the proportions, sorting the fish and distributing them into larger volumes. Depending on the management of the unit, these sortings can be done once or twice during the fish farming cycle. Also, several farms carry out fish vaccinations and dipping treatments. Such manipulations cause stress to farm animals and if not done correctly can lead to injuries and infections and then rapidly progressing and transmitting disease.

C. Nutrition

Intensively farmed fish are fed exclusively on industrialized feed. These are manufactured to meet the needs of fish in proteins, fatty acids, carbohydrates, celluloses, macro- and micro-elements and vitamins. However, these foods are not fully balanced and various deficiencies may arise which on the one hand can cause weight loss due to misuse of food and on the other hand nutritional diseases (will be mentioned below). And while most of the time some light deficiencies may go unnoticed, some others negatively affect the ability of fish to cope with infections. Also, many times, while the feed may be as described in detail on its packaging, its long-term storage under inappropriate conditions can lead to pulverization of the feed, oxidation of fatty acids and destruction of its vitamins, resulting in deficiencies during its administration to fish. These foods can also be carriers of microorganisms, especially when the temperatures developed during the production of pellets do not pasteurize their ingredients but can also be contaminated during transport or when stored or handled when some basic hygiene principles are not observed.

Directly related to the alteration of environmental parameters (water physicochemistry), the balance of the ecosystem prevailing in intensive farming (especially in floating fish cages at sea) and the development of sources of infection in intensive farming, is the loss of food that occurs during fish feeding. This loss is of varying etiology in a healthy fish population, but is usually due to poor study of the amounts required to feed the fish in a particular body of water and the failure to apply

many administrations during the 24-hour period, resulting in large amounts of food being administered during a limited number of feedings. Lost food is an excellent substrate for microbial growth and in combination with fish feces settles on the bottom under fish cages where it is a permanent source of infection for farmed fish, beyond the anaerobic conditions that can develop. Studies have even shown that excess food in an ecosystem can disrupt the delicate balance of the bacterial flora due to the increase in nutrients, promoting the growth of strains of bacteria resistant to a variety of antibiotics.

The organism that is reared is also one of the factors that contributes to the occurrence or not of diseases. The contribution of the environment to the ability of the reared organism through stress or other physiological and metabolic disorders to fight an infection through its immune system has already been mentioned. The organism itself influences the occurrence of diseases as described below.

The farmed organism

A. Genetic background

The fish raised in intensive farming units are individuals that derived from parents who have been selected for their fast growth rates, the good convertibility of the feed they receive, that is, for productive characteristics. Also, the conditions prevailing in hatcheries allow (and this is intended) the survival of individuals who under natural conditions may not have survived. The above can lead to the cultivation of individuals with reduced resistance to diseases. Such individuals, after being infected, quickly develop disease and transmit it to the other individuals of the group with which they are reared together.

All of the above, alone or in combination, are considered among the most basic factors that make intensive farming prone to the occurrence and rapid spread of diseases.

It is therefore understood that necessary conditions for the manifestation of pathological conditions or the predisposition to such a manifestation are: the lack of appropriate environmental/living conditions of the fish and their sensitivity. These

factors alone can cause disease, but diseases caused by microorganisms presuppose the existence of the latter.

Pathogenic microorganisms

For the development of an infectious disease, the presence of the infectious agent is necessary. The pathogens that cause mortality in fish have adapted to the aquatic environment as well as to different temperature ranges, thus differing from human pathogens. Many of them are found in the environment where the fish live (especially for diseases that are endemic in an area), but the occurrence or not of pathological conditions and mortality depends on many factors. Sudden rises or drops in temperature, especially when they are elevated, combined with eutrophication, cause on the one hand stress to fish and on the other hand a population explosion of microorganisms. The occurrence of the disease in even a small number of susceptible fish results in an increase in the number of infectious microorganisms and their transmission to other individuals of the group who, being under stress, are not able to fight the proliferation of microorganisms. In some cases, when the microorganism is very pathogenic, the disease will develop, even if the fish are not under the influence of stress. In these cases, the absolute number of pathogenic microorganisms is solely responsible for the occurrence of an epidemic or not. Some diseases have a slow progression and the fish, while they appear normal, are carriers of the microorganism and it is a matter of time and stress situations when the disease will be clinically perceived. As can be understood, the occurrence or not of the disease is a matter of a simple balance between the pathogen and the host's defense/resistance. Finally, often in cases where poor rearing conditions are combined with susceptible organisms, microorganisms that in other cases would be harmless bend the body's defenses and cause pathological conditions that lead to mortality (opportunistic pathogens).

The diseases observed in fish may be due to infectious or non-infectious agents. Pathogenic microorganisms that can cause infectious diseases in fish can be viruses, bacteria, fungi and parasites. Non-infectious diseases are due to abiotic causes, such as life-adverse physicochemical changes in water, nutritional causes and others.

Zootechnical/management measures to be taken to reduce the occurrence and spread of diseases

Before making specific reference to infectious agents causing disease, the general principles which should always be followed in order to reduce the risk of disease should be mentioned.

Thus, the physicochemical parameters of water should be checked thoroughly and frequently because any change can cause stress and reduce the resistance of farmed animals to microorganisms. Cage nets quickly fill with aquatic plants that prevent water renewal resulting in a reduction in available oxygen. Nets should be changed when there is excessive growth of aquatic plants causing reduced circulation and water renewal.

The location chosen for the installation of the production unit should have suitable conditions that will allow crowding of large masses of fish. Thus, the production unit should be located in areas with good water exchange and sufficient depth.

The amount of feed required for fish growth is given in water. Fish should be properly nourished and food should not be lost due to excessive administration. It is of great importance that food is administered in smaller quantities with an increase in the number of feedings in 24 hours.

Anything that enters the production area can be a source of contamination. The food used should be tested for its microbial load and under no circumstances should fish be fed raw fish or other animal remains. If for some reason the latter must be used, they should be given after being pasteurized. Changes in feed materials produced by feed factories should be communicated to producers in order to gradually replace one feed by another. Abrupt changes can cause digestive disorders and the appearance of diseases due to stress.

For various reasons, some fish on a farm die and can become sources of infection for healthy fish. Dead fish, fish injured either by mishandling or cannibalism as well as sick fish should be immediately removed and properly destroyed.

Fish grown in one unit may come from another area or production unit and may be contaminated. Fish entering a unit should carry certificates confirming that they are free from diseases, it is advisable to have been vaccinated for diseases that are endemic in the area they are transported and to apply preventive treatment by

bathing in antibiotic solutions during a quarantine period, which should always be applied.

In these farms fish are crowded in a very small space. Exaggerations should not be made, but proportions should be respected based on the physicochemical parameters of the existing water.

All handling of fish should be done gently and after light anesthesia to avoid injuries that are foci of entry of microorganisms.

Staff employed should be aware of the risks that can be caused by mishandling fish and should be informed about how to feed, handle and store fish properly. They should also take care of their personal hygiene.

2. INFECTIOUS DISEASES

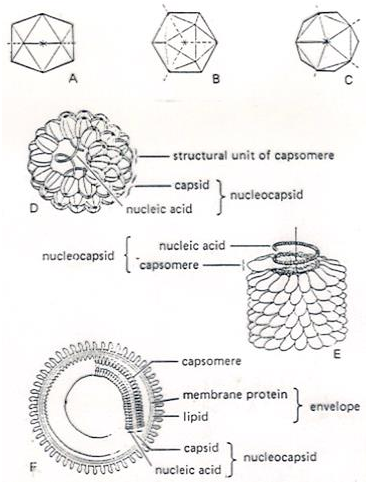
VIRUSES

Viruses are very small infectious agents that multiply only within living cells of a host. However, unlike some other intracellular parasites such as bacteria, rickettsias, chlamydia and mycoplasmas, they do not contain organelles and do not show metabolism. Viruses are obligate intracellular parasites that use the mechanisms of operation of the parasitized cell for their reproduction. Other characteristics include their small size that varies from 18-300 nanometers in diameter and the fact that their genetic material consists of either DNA or RNA but never both.

Morphology and structure of viruses

The virus particle or virion

The virus particle contains the genetic material of the virus which is surrounded by a protein cap or shell called a capsid. Some viruses also carry an additional outer envelope consisting of lipids and glycoproteins (Figure 1, 2).



Pict. 1. Geometry and structure of the virus particle, A, B, C: Eicosahedral virus particle visible from various angles. D: Subunits of an unencapsulated virus particle of icosahedral symmetry. E, F: Subunits of an unencapsulated virus particle with tubular nucleocapsid and helical symmetry.

Pict. 2. Morphology of animal viruses.

Nucleic acids of viruses

The genetic material of viruses consists of either DNA or RNA. Nucleic acids can be found either in the form of a double or single chain, divided into segments or cyclic and have a positive or negative polarity. The amount of genetic material varies between different viruses. The smaller picornaviruses contain $2x10^6$ daltons while the large poxviruses contain $2x10^8$ daltons. By comparison, the genetic material of a mammalian cell has a molecular mass of more than 10^{12} daltons. The molecular mass of the unique protein translated from the genetic material of a $2x10^6$ daltons picornavirus is about 250,000 daltons (about 1/9 of the molecular mass of the genetic material). This protein is then broken down into many smaller protein molecules that will form the structural proteins and enzymes of the virus.

The protein cover of viruses (capsule-capsid)

The capsule of viruses is formed by morphological units called capsomeres, which can be arranged by three types of symmetry: icosahedral, helical and complex.

Some viruses, when they leave the host cells after their proliferation, are covered by the cell membrane of the cell they parasitized. This process helps the released particles bind to other host cells and escape the host's immune defenses.

Viral enzymes

Some viruses need enzymes that are missing from host cells. These may be structural proteins which play a dual role such as influenza virus neuraminidase or may have a purely enzymatic function such as retroviral reverse transcriptase.

Viral antigens

Viruses can contain between 4 and 100 structural proteins, and each of them usually carries between one and four different epitopes.

About 66 viral infections concern fish but a more detailed description will be made only in viral infections that concern the Mediterranean region and/or fish species therein. Eleven major groups of viruses known for their infections in higher vertebrates have representatives that cause disease in fish. The most numerous references to viruses that cause disease in fish concern the following families of viruses: herpesviruses, rhabdoviruses, retroviruses, iridoviruses and rheoviruses. The viruses in the text below are recorded as DNA and RNA families of viruses and within each family the genera of viruses are divided into those isolated in cell culture and those that have only been detected by electron microscopy (EM).

Table 2. Fish viruses divided into families (generally farmed or wild fish around the world)

Group	Isolation	EM	Total
DNA-viruses			
Herpesvirus	7	4	11
Iridviruses	5	2	7
Adenoviruses	0	3	3
			21
RNA-viruses	1	0	1
Kalykovirus	10	0	10
Rhabdoviruses	3	0	3
Birnavirus	6	1	7
Rheovirus	2	0	2
Orthomyxoviruses	1	0	1
Retroviruses	0	8	8
Unidentified	4	8	12

1. DNA-viruses

Herpesviruses: features of the group

The herpesvirus family includes large envelope-surrounded DNA-viruses with a diameter of about 130 nanometers, in which the envelope is an integral part of the pathogenesis of the virus. The virus genome consists of a double helix of DNA of 176 kb and can express the production of up to 49 proteins.

Many of the herpesviruses have the ability to latent within the host and cause disease later. It has been found that herpesvirus cells can infect lymphocytes and therefore the infection of the latter may be a key point for the persistence of such infections that may be due to immunosuppressive phenomena. Known viruses in humans are the Epstein-Barr virus, which is associated with lymphomas and nasopharyngeal carcinomas, and herpesvirus-1, which causes lesions in the mouth and lips.

The herpesviruses of fish of interest, their host and the way they were found are presented below:

Table 3. Herpesviruses of fish

Virus name	Host	Finding	Researchers
Herpesvirus kyprini	(*Cyprinus carpio*)	A	Sano *et al* (1985)
Herpesvirus salmonis	(*Salmo gairdneri*)	A	Wolf & Taylor (1975)
Herpesvirus scophthalmi	(*Scophthalmus maximus*)	EM	Buchanan & Madeley (1978)
Herpesvirus of pike (EHV-1)	(*Esox lucius*)	EM	Yamamoto *et al* (1984)
Herpesvirus of sheatfish	(*Silurus glanis*)	EM	Bekesi *et al* (1984)
Pacific haddock herpesvirus	(*Gadus macrocephalus*)	EM	McArn *et al* (1978)

A: isolation
EM: electron microscopy

Iridoviruses: features of the group

The iridovirus family includes large isometric viruses with icosahedral symmetry, 130-300 nanometers in diameter, with a genome consisting of a double helix of DNA of molecular weight $130x10^6$ daltons. These viruses multiply only in the cytoplasm of the host cell.

The iridoviruses that have been reported to infect fish and are of interest are the following:

Table 4. Fish iridoviruses

Name of the virus	Host	Finding	Researchers

Lymphocyst virus	142 species of marine and river ceremonies	A	Wolf (1962)
Haddock iridovirus	Atlantic hake (*Gadus morhua*)	A	Jensen *et al* (1979)
The iridovirus of necrosis of the gills of carp	Carp (*Cyprinus carpio*)	A	Shchelkunov & Shchelkunova (1984)
Erythrocytic necrosis virus (ENV)	21 species of marine teleosteans	EM	Appy *et al* (1976)

A: isolation from cultivation
EM: electron microscopy

Adenoviruses: features of the group

In fish, three adenoviruses have been described. All three have been observed with EM and have not been isolated from cell culture. Two of them were observed in neoplasms and two are of interest to aquaculture.

Table 5. Fish adenoviruses

Name of the virus	Host	Finding	Researchers
Haddock adenovirus	Atlantic hake (*Gadus morhua*)	EM	Jensen & Bloch (1980)
White ling adenovirus	White ling (*Acipenser transmontanus*)	EM	Hedrick *et al* (1985)

HM: electron microscopy

2. RNA-viruses

Calycoviruses: features of the group

Calycoviruses are small isometric particles, possibly with icosahedral symmetry, 35-40 nanometers in diameter with characteristic diverticula in the form of a cup on their surface after negative staining. The virus virion contains a single helix RNA of molecular weight of 2.6-2.7 $x10^6$ daltons and a primary capsule protein of molecular weight of 60-70 kDa. Calycoviruses do not carry an envelope, are resistant to ether and multiply exclusively in the cytoplasm.
They are of no interest to fish farming.

Rhabdoviruses: features of the group

Rhabdoviruses are sphere-shaped and measure 130-380 nanometers long and 60-95 nanometers wide. They have an envelope bearing spines 5-10 nanometers long. These spines are an integral part of the virulence of viruses of this group. The genome consists of a single helix of negative polarity RNA. This genome expresses five main proteins of the virus. Two genera of rhabdoviruses have been found in animals: vesicular viruses and lysoviruses (to this genus belongs the well-known rabies virus).

The following viruses of interest to aquaculture have been found in fish:

Table 6. Fish rhabdoviruses

Name of the virus	Genus	Host	Finding	Researchers
EVX	V	European eel (*Anguilla anguilla*)	A	Sano *et al* (1977)
C30, B44, D13	V	European eel (*Anguilla anguilla*)	A	Castric *et al* (1984)
B12, C26	L	European eel (*Anguilla anguilla*)	A	Castric *et al* (1984)
Rhabdovirus salmonis	?	Trout (*Salmo gairdneri*)	A	Osadchaya & Nakonechnaya (1981)
Pike larvae virus (PFRV)	V	Pike (*Esox lucius*)	A	De Kinkelin *et al* (1973)
Spring viremia carp virus (SVCV)	V	Carp (*Cyprinus carpio*)	A	Fijan *et al* (1971)
Infectious haematopoietic necrosis virus (IHNV)	L	Salmon (*Oncorhynchus nerka*)	A	Amend *et al* (1969)
Viral hemorrhagic septicaemia virus (VHSV)	L	Trout (*Salmo gairdneri*)	A	Jensen (1963)

A: isolation from cell culture

Although viral haemorrhagic septicaemia virus (VHSV) was first described in salmonids, it should be mentioned that it has also been found in teleosteans of interest to Mediterranean marine aquaculture, such as sea bass larvae and juveniles in turbot (*Scophthalmus maximus*).

Birnaviruses: features of the group

Birnaviruses are isometric viruses without an envelope and carry in their genome a double helix of RNA (two segments).

The most important representative of this group of viruses in fish is the virus of infectious pancreatic necrosis. Many serological groups and serotypes have been described, as listed below:

Table 7. Serological groups and serotypes of IPNV

Serological groups - serotypes	Host	Region
Serological group 1		
IPNV-Sp	Trout	Europe, N. America
IPNV-Ab	Pike	Europe, Asia
IPNV-He	Bivalve	Europe
IPNV-Te	Trout	Europe
IPNV-WB	Trout	N. America
IPNV-Ja	Trout	Canada
IPNV-C1	Salmon	Canada
IPNV-C2	Trout	Canada
IPNV-C3	Salmon	Canada
Serological group 2		
IPNV-TV1	Bivalve	Europe

As far as the geographical distribution of IPNV is concerned, it is around the world and concerns both salmonid and non-salmonid fish as well as cyclostomous fish, lampreys, bivalve molluscs, gastropods and crustaceans.
Another birnavirus that has been described by fish is the yellowtail ascites virus (YAV).

Rheoviruses: characteristics of the group

Rheoviruses are isometric particles 60-75 nanometers in diameter that carry a characteristic double-walled capsid and contain double RNA helices (10-12 segments) primarily. Rheoviruses do not carry an envelope.

The Rheoviruses that have been described in fish of interest to aquaculture are:

Table 8. Fish heathers

Name of the virus	Host	Finding	Researchers
Greens carp heather	*Ctenopharyngodon idella*	HM	Chen & Jiang (1984)
Reovirus 13p2	*Lepomis macrochirus* and *Salmo gairdneri*	A	Meyers (1980, 1983)

A: isolation from cultivation
EM: electron microscopy

Orthomyxoviruses: features of the group

Orthomyxviruses are spherical or elongated envelope-bearing viruses, 80-120 nanometers in diameter. The envelope has superficial projections of two glycoproteins, haemagglutinin and neuraminidase. The genome consists of single stranded (ss)-RNA in eight segments with negative polarity.

Possible viruses of this group isolated from fish are:
a) The eel 2 virus (EV-2) isolated from Japanese eels and b) the A1b virus isolated from the blood of European eels that had neoplastic lesions in the mouth.

Paramyxoviruses: features of the group

Paramyxoviruses are similar to orthomyxoviruses in morphology but contain a larger helical nucleocapsid. Their genome consists of a single helix of negative polarity RNA and the replication of viruses is resistant to actinomycin D. Paramyxoviruses are made of large particles, have a diameter of 150-200 nanometers and carry an envelope. The projections of the envelope 8-12 nanometers long cover

the whole envelope and to these projections have been attributed actions of the enzymes haemagglutinin and neuraminidase.

Paramyxoviruses are of no interest to aquaculture.

Retroviruses: features of the group

Retroviruses are a group of large spherical viruses containing single stranded RNA in a segment with positive polarity, while their most important feature is possession of the enzyme reverse transcriptase. The diameter of the particle can be up to 100 nanometers and virions have an envelope that encloses a nucleus of icosahedral symmetry inside which is a helical nucleocapsid. Viruses of this group when leaving the host cell also take part of the cell membrane with them.

Fish retroviruses are categorized into those where reverse transcriptase has been detected or not. Listed below are the possible fish retroviruses of interest:

Table 9. Possible fish retroviruses

Name of the virus	Host	Reverse transcriptase	Researchers
"Esocid" lymphosarcoma	*Esox lucius*	+	Papas et al (1976)
Sea bream virus	*Sparus aurata*	-	Gutierrez et al (1977)
The epidermal miltiplication virus of the fish "pike"	*Esox lucius*	-	Winqvist et al (1968) & Yamamoto et al (1984)

Unclassified viruses: features of the group

The viruses that will be mentioned below have problems identifying and placing in a family of viruses. Some have been isolated from cultivation while others have only been visible with EM.

Table 10. Unclassified fish viruses

Name of the virus Pathology	Host	Finding	Researchers
Eel virus – 1	*Anguilla anguilla*	A	Wolf & Quimby (1972)
Virus-like intraerythrocyte particles	*Salmo gairdneri*	SME	Landolt *et al* (1977)
Papilloma of pleuronectidae	*Hippoglossoides elassodon*	SME	Wellings *et al* (1965)

A: isolation
SME: virus-like particles

Viral spongiform encephalopathy virus and retinopathy VER
Viral necrosis (VNN).

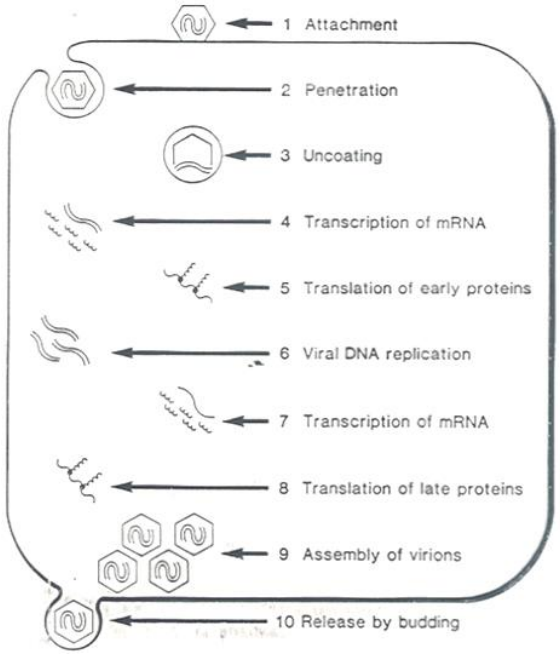
This virus is a relatively modern discovery and until recently was unclassified. It was originally thought to belong to the family of picornaviruses but was eventually classified as a nodavirus. The virus has been found in 18 species, including species related to Mediterranean mariculture such as sea bass, turbot, groupers and sea bream. The virus is isometric, without an envelope with a diameter of 30 nanometers. It has two structural polypeptides with a molecular weight of 40 and 42 kDa and an ssRNA consisting of two molecular weight fragments of 1.10 and 0.51 Da.

Physiology of virus infection

Replication of viruses

The process of producing new viruses includes the following stages (for DNA-viruses): a) Adhesion to the eukaryotic cell of the host, b) Penetration, c) Disclosure of genetic material (capsule removal), d) Transcription of messenger RNA, e) Translation into early proteins, f) Replication of virus DNA, g) Transcription of messenger RNA, h) Translation into mature proteins, i) The assembly of virions and j) The release of the latter after covering with the membrane of the eukaryotic cell (Figure 3). In more detail:

a) Adhesion to the eukaryotic cell: Adhesion is the critical first stage of infection. Strong attachment of the virus requires the presence of



Pict. 3. Stages during the proliferation of a DNA virus.

receptors for the virus on the cell membrane. There is often a high degree of virus specificity for a cell receptor, but alternatively there are also viruses that are opportunistic pathogens and may use a common receptor. Neither all cells of an organism nor all cells of an organ necessarily carry suitable receptors for viruses, and this may explain the frequently observed tropism of viruses for specific tissues and the pathology observed. Conversely, the infectiousness of some viruses may be associated with reduced adhesion to host cells because this allows the virus to be removed less from phagocytes and thus allows greater dispersion to target organs. Even if a cell carries the appropriate receptors for a virus, it does not necessarily mean that it will be infected, because after attachment the virus must reveal itself and begin the cycle of replication of its genetic material.

b) Infiltration: There are at least three input mechanisms: endocytosis, fusion and translocation.

Endocytosis follows a process similar to phagocytosis or pinocytosis. Most cells are able to endocell various macromolecules, such as hormones and others, by engaging receptors. During endocytosis, a small portion of the virus-carrying cell membrane attached to the receptor forms a diverticulum. The two closest ends of the diverticulum eventually come into contact and form an internal particle which enters the cytoplasm and binds to a lysosomal to form a lysosomal cyst. In some viruses, the virus envelope binds to the lysosome membrane, and then the virus particle is excreted into the cytoplasm.

During fusion, glycoprotein enveloped viruses fuse directly with the cell membrane and the virion is released directly into the cytoplasm.

Finally, during translocation, it is possible that some non-envelopeed icosahedral viruses pass directly into the cytoplasm through the cell membrane without the involvement of any of the above mechanisms.

c) Disclosure of genetic material: Little is known about this stage and it is likely that the mechanisms of revealing genetic material differ for DNA and RNA viruses. It is believed that viruses that fuse with the cell membrane or lysosome are revealed in the process.

d) Messenger RNA transcription: The transcription of viral DNA into messenger RNA typically occurs in the nucleus of the host cell.

e) Translation into early proteins: The messenger RNA of viruses is translated into proteins in the ribosomes of the host cell by the same process followed for host proteins. Proteins created by translating early in the virus genes include enzymes and other proteins thought to play an important regulatory role in transcription.

f) Replication of virus DNA: The rest of the virus genome is copied.

g) Messenger RNA transcription: As mentioned above.

h) Translation into mature proteins: These are the molecules needed to create virus particles.

i) Assembly of virions: With simple spherical viruses of icosahedral symmetry, structural proteins can automatically deduce and form capsomeres, which then self-assemble into pre-capsids around the genetic material of the virus. Randomly, many particles may not contain genetic material. The assembly of DNA viruses is often done in the nucleus of the host cell and for RNA viruses in the cytoplasm. The aggregation of simple spherical viruses or empty pre-capsids can create paracrystalline forms that can be seen under microscopy as encapsulated particles. Viruses covered by an envelope shall always contain glycoproteins of viral origin. The latter migrate from ribosomes through the endoplasmic reticulate, usually to the cell membrane. There these proteins are grouped into characteristic thorny forms. During the assembly of an envelope-bearing virus, the nucleocapsid is assembled under the cell or nuclear membrane and then the above glycoprotein spines attach. A layer of proteins enters the membrane around the nucleocapsid. This process causes the nucleocapsid inside the sheath of glycoprotein spines to pass through the cell membrane of the host and thus create a diverticulum.

(j) Release of virus particles: Viruses which do not carry an envelope are released after death and degeneration of the host cell caused by the closure of the metabolic processes of the cell due to virus products. File-bearing viruses are released with the aforementioned diverticula of the host cell membrane which is a gradual process.

Effects of virus infection on the host cell

These can be divided into the following:

a) Reversible changes, such as cloudy swelling of the cell observed in histological sections.

b) Irreversible changes leading to cell death. This is called the cytotoxic/pathic effect (CPE).
c) Irreversible changes that lead to the loss or failure of a particular function.
d) Conversion of the cell into neoplastic.
(e) Infection persisting after effect (a) and (c). Viral nucleic acids may remain in the genetic material of the host cell and function periodically.
Whatever the outcome, however, during the early stages of infection, various changes in the appearance of affected cells can be observed, such as:
a) Areas of the cell where staining differentiation occurs during histology.
b) The accumulation of virus antigens can take characteristic forms called encapsulated particles.
c) Fusion of two or more cells and formation of a multinucleated giant cell or syncyteum.

Effects of virus infection on host fish

Once the virus spreads further than some cells, various specific and non-specific defense reactions are triggered by the body. Depending on how well these reactions contain the virus and minimize damage to host cells and organs, the result of infection may be a) disease with mortality or b) infection without symptoms. Therefore, infection can be detected after finding the virus or its antigens or finding antibodies against the virus.
The outcome of a viral infection may be the following:
1) The host shows no symptoms and the virus disappears.
2) There are no symptoms but the disease persists (carrier).
3) The host develops clinical symptoms and dies.
4) The host develops symptoms, recovers and clears the virus and
5) The host recovers from the disease but the infection remains without clinical signs (carrier).

Diagnosis of viral infections

Cultivation

The most common method for diagnosing viral diseases of fish is culture of the virus in single-layer cell cultures. The cytotoxic effect (CPE) observed is a result

of infection of cells by the virus and means a first positive diagnosis of virus. CPE can be visible as cell rounding, shrinkage, necrosis, syncyteum growth and detachment. Different viruses cause different CPEs and these can be a first identification of the virus. But the diagnosis can only be confirmed after using immunological or molecular markers.

Microscopy

Simple microscopy cannot help observe viruses because they are too small in size. However, cellular alterations can be observed, such as impacted particles, syncytia and others. Electron microscopy can help in the observation of the virus, but it is used to study the morphology of the virus after cell culture and not before the detection of the virus due to the difficulty of the method.

Finding specific elements of the virus

As mentioned above, typically the only methods for confirming virus infection and identifying the pathogen are those using species-specific immunological and molecular markers.

Inactivation of viruses

Physical methods

Viruses can be inactivated by heating to 56^{o}C for 1-2 hours. X-rays, gamma rays and ultraviolet radiation can inactivate viruses very quickly.

Chemical methods

Most viruses remain stable at neutral pH. At pH less than 3 and greater than 11, most viruses are inactivated. For this reason, 2% sodium hydroxide solutions can be used for disinfection of many materials other than metal ones due to the intense oxidation that will be caused. Also, CaO powder can be used to disinfect earthen tanks where viruses were reported.

Hypochloric and iodinated solutions can also inactivate viruses as long as there is no large organic load in the body of water where they will be administered and allowed to act for a few hours. Finally, ozone can be used to disinfect water from viruses and bacteria, which acts very effectively.

Formaldehyde and glytaraldehyde inactivate viruses by acting on both their proteins and nucleic acids. In practice, however, they are used only in the laboratory.

Prevention and control of viral infections in fish

Avoiding virus entry

The general principles of prevention mentioned in the previous paragraph must be followed, in particular with regard to the purchase and use of eggs or fish certified to be free from viruses. Especially for hatcheries, the water entering the nursery should be disinfected.

Vaccines

Vaccinating fish against viruses could theoretically protect susceptible hosts from infection. This has been proven in the laboratory with the use of both live non-infectious viruses and inactivated viruses. However, it is coctly to produce viral particles for use in vaccinations due to the high cost of the procedure. An additional problem for non-virulent live viruses is that the use of such organisms is prohibited, especially when they are released into the environment because they can become virulent or promote mutations. A very promising way to produce vaccines for viral diseases is through genetic engineering. Thus, a protective antigen of the virus is analyzed, the gene of the virus responsible for its production is assembled and then placed in the genome of a bacterium which now expresses the antigen of the virus at a much lower cost. The bacterium is then inactivated or the viral antigen isolated and susceptible fish can be vaccinated with it. And this method is not devoid of problems that have to do with the protection provided by the expression of such antigens. The reason is that the "artificial" production of the antigen may result in the same characteristics (of the natural antigen) not being expressed and thus the antibodies produced are not protective.

Chemotherapy

Various preparations may be used, which are also used in humans. Their effectiveness has been proven in the laboratory, but due to cost and prohibitions of their use in animals for consumption, there is no practical application.

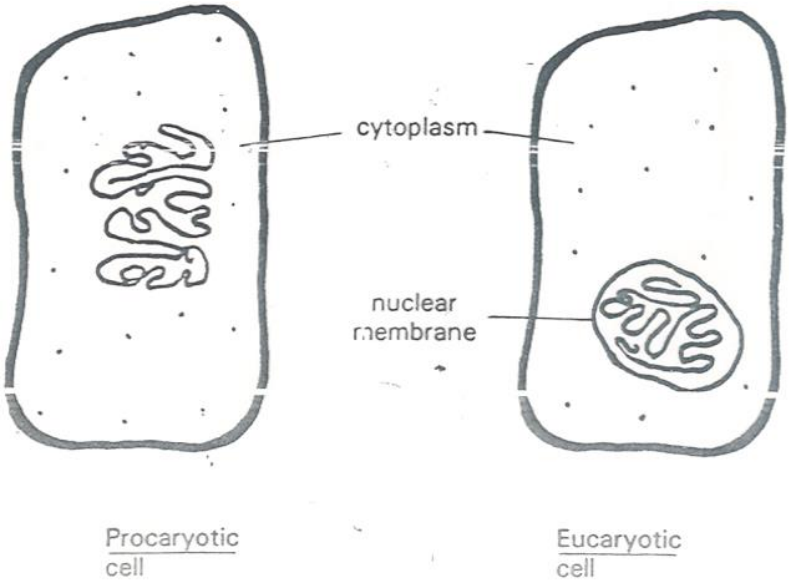
BACTERIA

Bacteria and cyanobacteria (blue-green algae) are single prokaryotic cells. Prokaryotic cells differ from eukaryotes mainly in that their nuclear material does not separate from the rest of the cytoplasm of the cell through any membrane. Other microorganisms (algae, fungi, protozoa) as well as the cells of higher plants and

animals show the cellular organization of eukaryotic cells by separating their genetic material from the cytoplasm (Figure 4).

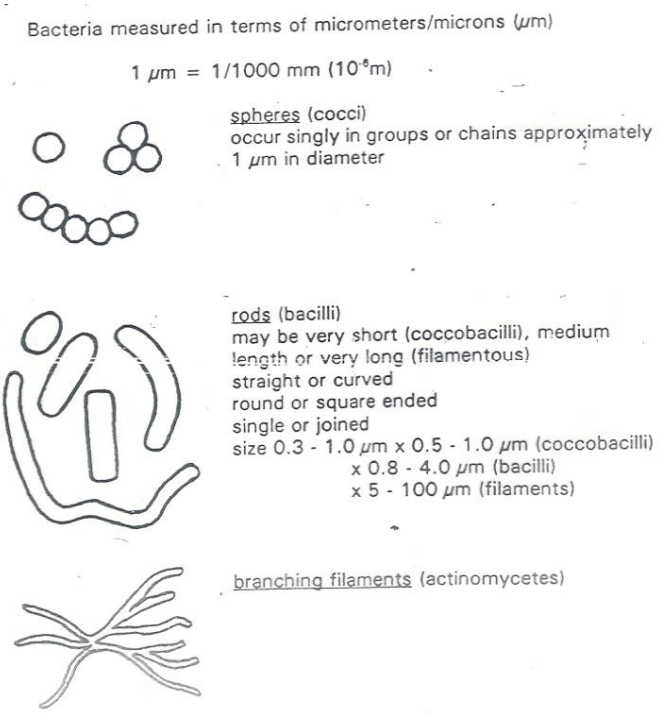
Bacteria carry a solid cell wall and usually multiply by splitting.

Size and shape



Pict. 4. Basic difference of a eukaryotic and prokaryotic cell.

Bacteria are measured on the micrometer scale. The shapes that can be observed vary from spherical particles (cocci) found either single or in chains and having a diameter of 1 micrometer to bacilli with various sizes from coccobacilli (0.3-1x 0.5-1 micrometers), medium length bacilli (0.3-1x 0.8-4 micrometers) and long bacilli (filamentous) (0.3-1x 5-100 micrometers). They may be straight, convex, round or with square edges and may appear single or connected (Figure 5).



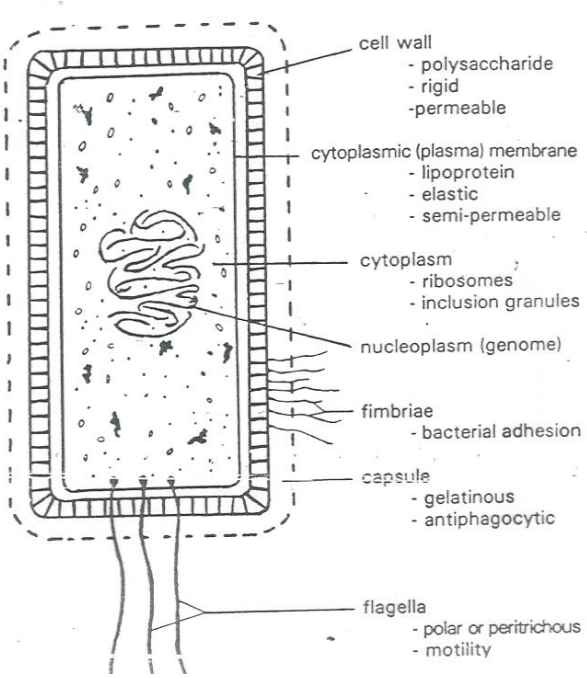
Pict. 5. Basic shapes and sizes of bacterial cells

Finally, bacteria in the form of branching filaments (actinomycetes) are observed.

Structure of the cell

The bacterial cell from the outside to the inside consists of:

A) From the cell wall or extracellular membrane. It contains polysaccharides which outwardly may expand as lipopolisaccharides, among which are various



Pict. 6. The structure of a bacterial cell.

proteins. The extracellular membrane is compact but permeable to various substances. It helps the bacterium maintain its shape and cope with changes in the osmotic pressure of the environment. The extracellular membrane may be covered by a gelatinous, mucous or polysaccharide capsule that helps the bacterium to attach to epithelial membranes, avoid phagocytosis and opsonization of the bacterium by antibodies and avoid the effect of complement and lysosomal enzymes. On the extracellular membrane, fibrils involved in the adhesion of the bacterium can also be observed. Finally, some mobile bacteria carry flagella that help them move, which may be located around the extracellular membrane (cuticle bacteria) or at the poles of the bacteria (Figure 6).

B) From the inner cell membrane which is lipoprotein in nature, elastic and semipermeable. Between the two membranes there is the mesosomal space in which the substances to be secreted by the bacterial cell are concentrated. These substances include enzymes produced by the bacterium to extract and use nutrients from its environment such as proteins, fats and saccharides. It would not be an exaggeration to say that the production of enzymes by bacteria has been described for almost every substance that can be enzymatically broken down in nature.

C) From the cytoplasm. This includes the genetic material of the bacterium, devices for producing bacterial products (ribosomes), encapsulated particles and others.

Substances required for bacterial growth

1) Water: water constitutes 80-90% of the bacterial weight and is the medium of all biochemical reactions. Its absence (free or active water) underpins a whole branch of natural food preservation.
2) Energy source: sunlight (phototrophic bacteria) or chemicals (chemotrophic bacteria). All pathogenic bacteria are chemotrophs.
3) Materials for biosynthesis: a) Carbon for the synthesis of all organic substances of the bacterial cell. Bacteria may use inorganic carbon (autotrophic bacteria) or carbon in organic form (organotrophic bacteria). All pathogenic bacteria require organic carbon, so they are organotrophs and according to the above chemo-organotrophs. (b) Nitrogen for the synthesis of proteins, nuclear acids and enzymes. Nitrogen can be in gaseous form or in the

form of an inorganic or organic compound. (c) Phosphorus and sulphur (components of phospholipids, nuclear acids, enzymes, amino acids and vitamins). (d) Development factors. Various macro- and micro-elements and vitamins of the complex B. Finally, e) Environment favoring their growth. The bacteria withstand a wide range of pH values with ideally 7.2-7.6. Oxygen may or may not be required. When bacteria require oxygen for their growth they are called aerobic, when they require lack of oxygen then they are called anaerobic, while when they can grow regardless of the presence or not of oxygen then called facultatively anaerobic (most pathogens). As far as temperature is concerned, here too there is a wide range of ideal growing temperatures. When the latter range below 20^{o}C, bacteria are characterized as psychrophilic, from 20-40^{o}C mesophilic and from 40^{o}C and above, thermophilic.

Identification of bacteria - Diagnosis

The identification of bacteria responsible for a disease is done by taking samples from the lesions observed and culturing them using nutrients as appropriate. After colony growth, the identification of the causative agent can be made. If the appropriate equipment is available, the diagnosis of the disease can be made directly on tissue samples by immunological or molecular methods. Typically, however, for many decades bacteria have been identified based on their morphological, cultural and biochemical characteristics.

1) Morphological features. a) The type of cell wall (positive or negative by Gram staining). Both types of bacterial cells carry a cell wall that covers the cell membrane. In Gram(+) bacteria, the cell wall consists mainly of a complex of peptidoglycan and teichoic or teichouronic acids. In Gram(-) bacteria outside the cell membrane there is the extracellular membrane consisting of lipopolysaccharides, phospholipids, glycolipids and proteins, while under the extracellular membrane there is the stable layer, another membrane, resembling the cell wall of Gram(+) bacteria. b) The form of the bacterium. (c) Its size. (d) If it forms spores, bears a capsule or intracellular granules. e) If he wears a flagella (polar or periwinkle) or fibrils.
2) Cultivation characteristics: a) The appearance of the colony. b) The production of pigment and its characteristics (diffusion, solubility, color). (c) The

mobility of the micro-organism. (d) Its oxygen requirement. e) Its resistance to temperature, pH, salt and sensitivity to chemicals.

3) Biochemical characteristics: Bacteria can be easily identified by a series of biochemical tests. These control the production of specific enzymes and the fermentation of sugars. These tests have even been standardized and marketed as ready-made tests for use.
4) Serological and molecular tests: These tests identify bacteria (and confirm the diagnosis) with the help of immunological and molecular markers.

Bacterial diseases of fish

They result in very large losses in both cultivated and wild fish. Many of the causative microorganisms are natural saprophytes of the aquatic environment which use organic substances and salts of the environment for their growth and multiplication. These microorganisms are essentially opportunistic pathogens that infect organisms that have become susceptible to infection by stress factors or some other disease. However, some bacteria appear to be major pathogens for fish and require contamination of the fish before they can grow. But even they infect susceptible organisms under the same conditions as opportunistic pathogens. Bacteria cause after establishment in the host either external disease involving all epithelium in contact with the aquatic environment or internal disease with a septic image. Localization of bacteria in all tissues and organs has been described depending on the disease, the pathogen responsible and the species of parasitized fish. Although most bacteria occur in extracellular spaces, there are also bacteria or parasites that live intracellularly (mycoplasmas, rickettsias, chlamydia) because they need to use nutrients or the energy metabolic pathways of host cells for their energy needs (they do not carry the appropriate mechanisms for energy production by their own means). Mycoplasmas are Gram(-) bacteria without a cell wall, very small in size, with an indeterminate shape (changes depending on what it is adjacent to) which cannot be cultured in common microbiological materials. They can survive in body fluids and live in eukaryotic cells from where they take up cholesterol, amino acids, fatty acids and DNA without causing cell death. Chlamydia are obligate intracellular bacteria because they need the metabolic pathways of eukaryotic cells to meet their energy needs. The same happens with rickettsias which are Gram(-) bacteria. Their size is

very small and they are grown only in cell cultures. In addition, there are bacteria that may be found intracellularly to avoid the action of the body's defenses, but only for this reason.

Effect of bacterial infection on fish

In order for a bacterium to cause infection and then the onset of disease, it must overcome a series of non-specific and specific defense mechanisms. As for most fish diseases, bacterial diseases are transmitted through water with only a few exceptions but also exceptional cases where the transmission of the microorganism occurs vertically from the broodstock to their offspring. Thus, the bacterium should penetrate the mucous layer of the external epithelium and intestine, avoid the action of secretory antibodies and eventually attach to epithelial cells and either cause local lesions or be picked up by leukocytes and through them enter the body. The infection and entry of the microorganism is easier when the fish are under the influence of stress because these conditions affect the production of mucus but also the secretion of antibodies and other non-specific antibacterial substances (such as lysozyme). Skin continuity interruption from various injuries open wide the way for microorganisms to enter.

Thus, an infection can be fought on the surface of the fish's body without disease progression, or a disease can develop with lesions visible externally. Depending on the severity of the infestation, the virulence of the infectious microorganism and the defensive capacity of the fish, the disease can be fought or spread either superficially or inwards resulting in the death of the fish. Similar is the development of an internal infection. This can result in the containment of the bacterium and its fight or the growth of the microorganism and sepsis resulting in death. In some bacterial diseases of fish the microorganism may exist even after fighting it without clinical symptoms, in which case the fish is considered a carrier.

Prevention and treatment of bacterial diseases of fish

The prevention of bacterial diseases, especially for endemic diseases, is not based on avoiding the entry of the micro-organism into the farm (as is done with viral diseases), but on reducing the further increase in the number of pathogenic microorganisms, observing general principles of hygiene. Because bacterial diseases

are primarily associated with stressful situations, the latter should be reduced as much as possible.

If, however, a disease occurs and the causative agent is identified as well as its sensitivity to antibiotics, then treatment is applied, as will be mentioned in later chapters in detail.

Prevention of bacterial diseases can also be done by carrying out vaccinations. Unlike vaccines against viruses, vaccines against bacterial diseases are much less expensive and easy to manufacture as long as appropriate laboratory studies have been carried out to identify the protective antigens of the pathogen. The prevention of bacterial diseases by carrying out vaccinations will be presented in detail in subsequent chapters.

PARASITES

Many phyla of the animal kingdom have representatives that parasitize on fish. The number of species parasitizing on fish is counted in the thousands, and much remains to be discovered. Few of them are serious pathogens of fish. Most wild and/or cultivated fish are parasitized, however, in most cases, no significant alterations are observed in the hosts. However, in some cases serious epizootic diseases have been reported in farmed fish. Having a large number of fish under confinement under certain environmental conditions can promote the growth of a parasitic species so that its population reaches high levels. The number of parasites required to cause harm to the host fish varies greatly depending on the species and size of the host and its health levels. Many species of parasites are specific to their host to some extent and are capable of infecting one or only a limited number of hosts.

The most important species of parasites of the Mediterranean

Although the isolation or observation of a large number of parasites has been reported, we will currently refer only to those that cause significant damage or are more commonly found in Mediterranean fish farming (salt and freshwater).

The species of parasites can be unicellular organisms such as protozoa or multicellular organisms such as worms and arthropods. The lesions they can cause are from simple irritations to intense lesions that can lead to death and the localization can be external to all epithelia or internal to all tissues and organs. Some parasites have a

complex biological cycle and require an intermediate host for its completion when the final one is the fish, while for some other parasites the fish are the intermediate hosts. The most important parasite species found and/or causing problems to Mediterranean euryhaline fish are:

1) Ectoparasites

1.1 Protozoa

Amyloodinium ocellatum, Cryptobia spp., Cryptocaryon irritans, Trichodina spp.

1.2. Monogeneous trematodes

Microcotyle spp., Lamellodiscus spp., Gyrodactylus spp., Furnestinia spp., Diplectanum spp., Serranicotyle spp.

1.3. Crustaceans

1.3.1. Isopods

Anilocra physodes, Nerocila obignyi

1.3.2. Copipods

Ergasilus spp., Caligus spp., Lernathropus spp., Larnaeocera spp.

2) Endoparasites

2.1. Protozoa

2.1.1. Microsporidia

Pleistophora spp.

2.1.2. Rhizopods

Paramoeba spp.

2.1.3. Myxosporides

Ceratomyxa spp., Myxidium leei n. sp., Sphaerospora spp., Polysporoplasma sparis, Henneguya spp., Kudoa spp., Zschokkela mugilis n. sp.

2.1.4. Coccidia

Eimeria spp.

2.1.5. Nematodes

Anisakis spp.

The most important species that cause problems for freshwater fish are:

1) Ectoparasites

1.1. Protozoa

Ichthyophtheirius multifilis, Trichodina spp. Myxidium spp. Dermocystidium anguillae

1.2. Trematodes

Pseudodactylogyrus spp.

2) Endoparasites

2.1. Nematodes

Capillaria spp., Anguillicola crassus.

Prevention and treatment of fish infestations

The prevention of parasitic infections in fish is complex and case-specific, particularly in those parasitic species that have an indirect biological cycle (require an intermediate host). In general, however, proper rearing and hygiene conditions should be observed and wild fish should be removed if possible. Prevention by vaccination does not exist, although for some infestations this would be legitimate and practically achievable.

The treatment of infestations is done for ectoparasites with various disinfectants or antiparasitic baths and for endoparasites with the administration of oral antiparasitics. Many times, however, the alterations caused to the tissues of fish, both externally and internally, exclude the fish from consumption.

Below are the general principles that must be observed for the treatment and prevention of diseases.

3. GENERAL PRINCIPLES OF TREATMENT AND PREVENTION

Therapy

From the very first cases of diseases in farmed fish, especially in marine aquaculture, the situation was successfully treated with antibiotics or other chemotherapy substances. This situation persisted for less than a decade because due to the application of poor treatments and due to the excessive use of the above substances, disease-causing microorganisms became resistant. The antibiotics approved and are legally available for aquaculture are limited in number and for all these resistance to disease microorganisms in fish has been described. Therefore, in principle, they should be used in rotation and treatments should always be therapeutic

and not preventive, with the exception of the entry of new stock into a business and/or area and antibiotics administered by immersion. Treatments are usually applied with food in fattening units and bathing in hatcheries. In particular, the administration of oral chemotherapy contributes even more to increasing the resistance of disease agents for the following reasons: treatment is done when it is found that there is a disease and after the identification and antibiogram of the disease agent. Even if the latter have not been done, the determination of the morbid condition is made relatively late in relation to the progression of the disease. One of the first symptoms that appear in almost all fish diseases is anorexia. Therefore, when an attempt is made to administer an antibiotic with food, a lot of it is wasted and ends up at the bottom under the fish cage, while most fish do not receive the appropriate amount of food and therefore the appropriate therapeutic dose of the antibiotic. The presence of an increased amount of antibiotic in the environment contributes to the development of resistance to natural bacterial flora and pathogens found near farms, while the sub-therapeutic dose of antibiotic eventually received by diseased fish does not inactivate microorganisms but makes them more resistant to the administered substance. For these reasons it is a common European belief that the use of antibiotics should be reduced to a minimum and only in exceptional cases, while disease prevention measures should be increased.

As far as treatments are concerned, treatments are divided into antiparasitic and antimicrobial. Some of the substances used to treat protozoa and metazoan parasites are formaldehyde, potassium permanganate and copper sulfate. The use of antiparasitic treatments is done by bathing fish for a short or longer period of time and in some cases using the feed. The use of antimicrobials, with oxolinic acid and oxytetracycline being the main examples, has been widespread in aquaculture for the treatment of bacterial septic diseases fish. The methods of applying antimicrobial treatments are the addition of the antibiotic to the food or immersion in a solution of the substance.

The main methods of treatment are:

- Immersion therapy (external therapy)
- Systemic treatment through food
- Combination of the two above
- Sponge therapy

- Parenteral therapy.

The last two are rarely applied and only to broodstock (or aquarium fish).

a) Immersion therapy

Its success depends on three factors: the physicochemical parameters of the water, the relationship of toxicity / potency of the drug and the physical condition of the fish. For this purpose, water temperature, dissolved oxygen levels, ammonia levels, pH, water hardness and dissolved particles are checked before and during treatment. To reduce oxygen consumption and ammonia production, fasting of fish for 12-48 hours before treatment is applied. Bathing is usually done in the morning hours. Toxicity directly depends on the drug, dosage, physicochemical conditions of the water and the burden on the organism of the fish. Immersion therapy is carried out in the following forms: bathing, prolonged immersion, short immersion, pouring and flow therapy.

b) Systemic therapy

Mixing the drug in food is used to treat systemic bacterial diseases and digestive infestations. Treatment should be applied in a timely manner before the onset of anorexia for the reasons mentioned above. The temperature of the water decisively affects the absorption and metabolism of the drug as well as the period of its stay in the tissues of the fish. The failure of systemic treatment may be due to late application, incorrect choice of antibiotic and dosage, insufficient mixing of the active substance with food, etc.

In order to allow antibiotics to be used in the treatment of bacterial infections in fish, the maximum permitted residue limit of the antibiotic in fish flesh must first be established and published. The maximum residue limits (MRLs) of antibiotics in fish are presented in Regulation EC 2377/26-6-1990 as converted on 16-6-2008 by Regulation EC 542/2008.

The antibiotics currently allowed for use in aquaculture in Greece are: oxolinic acid, oxytetracycline, flumequine and sulpfadiazine-trimethoprim.

Prevention

The prevention of diseases has always been the main concern in any systematic rearing of animal organisms. It is widely accepted that disease prevention

rather than treatment is what gives greater benefits both economically and environmentally.

Prevention may be specific and non-specific. The latter includes measures aimed at improving the general condition of farmed species either by genetically increasing disease resistance or by improving management conditions. Specific prevention is mainly achieved through the use of vaccination programmes.

(a) Non-specific prevention

These are the measures to be taken for the farming method, the feeding of fish and their genetic background in order to reduce a) the chances of disease agents entering a farm and b) the susceptibility of farmed animals to diseases and increase the capacity of non-specific reactions of the immune system.

(b) Specific prevention

It is the specific prevention against diseases achieved by carrying out vaccinations and inducing an immune reaction by immunized animals against the disease agent. The aim of vaccinations is therefore to prepare the immune system of fish to deal with a possible disease. We will talk about this system a little more extensively below.

3.1. The immune system of teleost fish (general)

Fish, like other vertebrates, carry a wide variety of defense mechanisms to fight microorganisms that threaten their health. Natural (scales, skin, mucus) and biochemical (lysozyme) barriers prevent most infectious agents from entering the host. However, when the homeostasis of the latter is disturbed by a variety of stress factors then there can be infection and disease. But then the immune defense mechanisms are activated that reduce the duration of infection by fighting the infectious agent, preventing the death of the fish. In general, immune responses are divided into two main systems: the internal (innate) and the adapted immunity systems. The adapted immune response is characterized by greater specificity and memory creation. Both immune systems include cells (leukocytes) and humoral factors responsible for a successful immune response. Thus, while phagocytes (macrophages / monocytes), granulocytes (neutrophils, eosinophils, basophils) and nonspecific cytotoxic cells are characteristic of the innate immune system,

lymphocytes (T and B lymphocytes) are essential for all adapted immune reactions. Lysozyme, complement, interferons (α, β and γ), C-reactive protein, transferrin, lectins, hemolysin, proteinases, α2-macroglobulin, chitinase and α-precipitin are characteristic molecules of the innate immune system, while immunoglobulin can be considered as the final acting molecule of an adapted immune response. In practice, both systems interact to a significant extent, mainly through the release of soluble factors (cytokines and inflammatory mediators) involved in communication between cells of the systems. The presentation of antigens by phagocytes and easier identification of antigens that have been opsonized with an antibody are other examples of the interaction of the two systems. Most immune responses consist of a variety of factors from both systems with dominance of the innate during the early stages of infection and emphasis of the adapted system in the final stages.
Important for the prevention of fish diseases through vaccinations has the adapted immune system, and on this we will dwell a little more below.

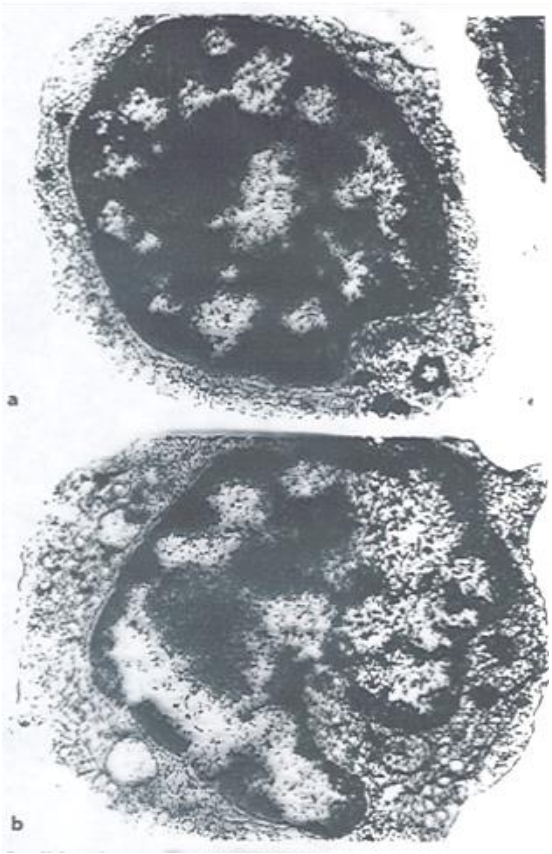
Cells involved in the adapted immune response

All immune reactions of vertebrates are made by leukocytes. As in mammals, fish leukocytes can be classified into T and B lymphocytes, monocytes / macrophages and granulocytes. Lymphocytes are the cell type necessary for the adapted immune response because they express heterodimeric immunoglobulin or T lymphocyte receptors that act as units of specific recognition of an antigen. From the literature, the adapted immune system is present in all jaw-bearing vertebrates and is missing from cyclostomous fish, lower chordates and protostomes. We can distinguish two types of adapted immune responses: cellular immunity, which is provided by cells derived from the thymus, and humoral immunity, which includes the production of antibodies by plasmocytes.

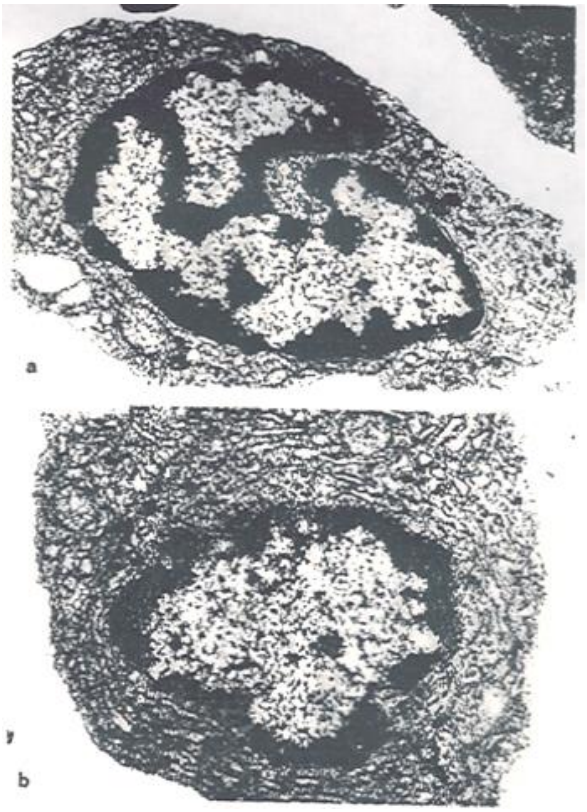
T-lymphocytes

T-lymphocytes (Figure 7a, b) are characterized by the presence on the outer surface of the cell membrane of specific antigenic receptors and the expression of molecules involved in communication between cells and determine the subgroups of T-lymphocytes that are involved in different functions. Due to the lack of specific markers to identify these receptors and molecules, it is difficult to identify these subgroups.

Thus, their separation according to their function into cytotoxic T cells and T helper cells has been done indirectly after studies of their function under experimental conditions, such as: reactions of mixed lymphocyte populations, transplant rejection, experimentation with haptenes-carriers and through the production of macrophage activation factor. Further studies have concluded that fish T-lymphocytes have an amplifying or regulatory role similar to that of higher vertebrates, playing a central role in immune responses against proteins



Pict. 7. (a): Small lymphocyte that can develop into a T or B lymphocyte. Before lymphocyte activation, it is not easy to separate into T or B lymphocytes. 60,000x magnification. (b): Lymphocytes with a little granular



Pict. 8. (a): B lymphocyte containing a large amount of endoplasmic reticulum. 28,000x magnification. (b): Plasma cell (stimulated B lymphocyte) containing a high amount of endoplasmic reticulum (in arangement due to the antibodies produced). 28,000x magnification.

antigens (T-dependent) by synthesizing and releasing the necessary interleukins for the differentiation of B-lymphocytes after the treatment and presentation of the antigen by accessory cells carrying major histocompatibility antigens.

B-lymphocytes

As in higher mammals, fish B-lymphocytes (Figure 8a, b) express immunoglobulin molecules on their surface, which acts as a specific antigenic receptor. Electron microscope studies have shown that surface immunoglobulin occurs mainly regionally grouped on the outer membrane of B-lymphocytes. As in mammals, fish B-lymphocytes reorganize heavy chains of immunoglobulins and block sequences during their formation. Most B-lymphocytes express on their surface the Class II of major histocompatibility antigens which play an important role in interactions (antigen presentation) with T-lymphocytes. Monoclonal antibodies that recognize the immunoglobulin of various fish species showed that there is heterogeneity between B-lymphocytes. In addition, it has been shown that in adult fish the proportion of cells carrying immunoglobulin molecules on their outer membrane is greater in lymphocyte populations on blood leukocytes, followed by the spleen, anterior kidney and intestine, while their amounts in the thymus are negligible. The production of B-lymphocytes can be stimulated by polysaccharides (T-independent antigens) and these can differentiate into antibody-producing cells with the help of an interleukin-1 produced by helper cells such as monocytes or macrophages.

Cells presenting antigens

As in mammals, monocytes/macrophages and B-lymphocytes are the main cells involved in the presentation of the antigen playing an important role in the adapted immune response. These cells are rich in molecules of the main histocompatibility class II antigens which are important for presenting antigenic peptides, which are derived from exogenous proteins, to T-helper lymphocytes thus helping to initiate adapted immune responses. The main histocompatibility antigens in fish work similarly to those in mammals. In the case of antigens entering the cells presenting the antigens with the cooperation of class II major histocompatibility antigens, they are initially localized within the endosomes / lysosomes of these cells. Then the antigens are degraded and redistributed to the cell membrane for their presentation to T-lymphocytes.

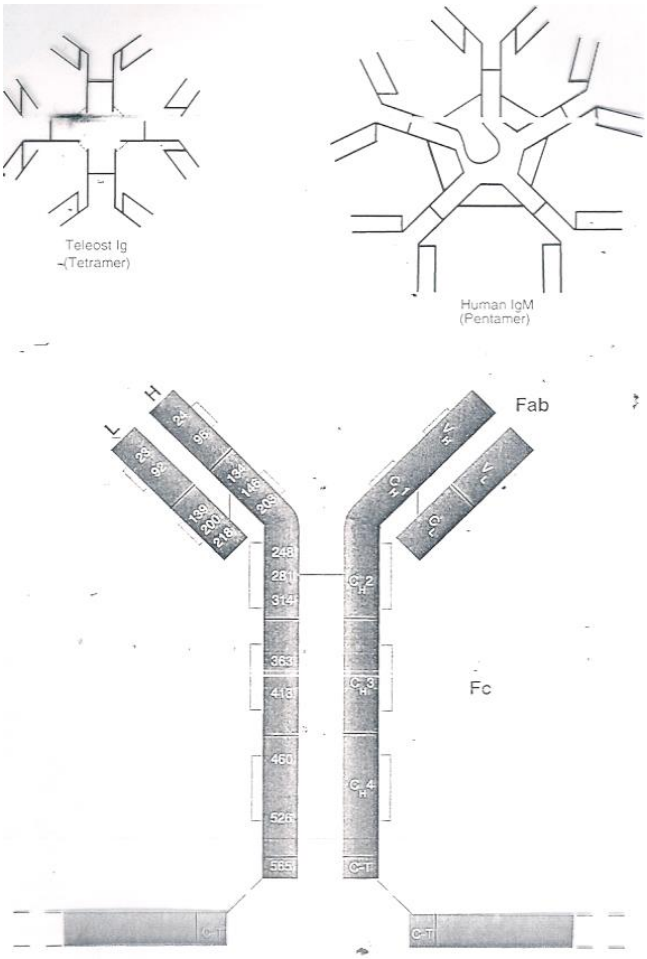
Teleost fish immunoglobulins

The immunoglobulin (antibody) molecule is responsible for many processes during specific immunity against a pathogen. It occurs in all jawed vertebrates and belongs to a type of proteins that are biochemically defined as immunoglobulins due to the characteristic structures of their stereochemistry. Serum immunoglobulin in fish ranges from very small amounts in salmonids (about 1mg/ml, 2-6% of total serum protein) to relatively high concentrations in other fish (14-17mg/ml, 40-50% of total serum protein). Lower concentrations are found in mucus, bile and eggs.

Molecular and biochemical characterization

In fish there are few classes of antibodies with the main type corresponding to the immunoglobulin M of mammals.

The main structure of the antibody molecule appears to be tetramer (Figure 9)



Pict. 9. The teleost fish immunoglobulin molecule in relation to human immunoglobulin M. Detailed structure of heavy and light chains of fish immunoglobulin subunits.

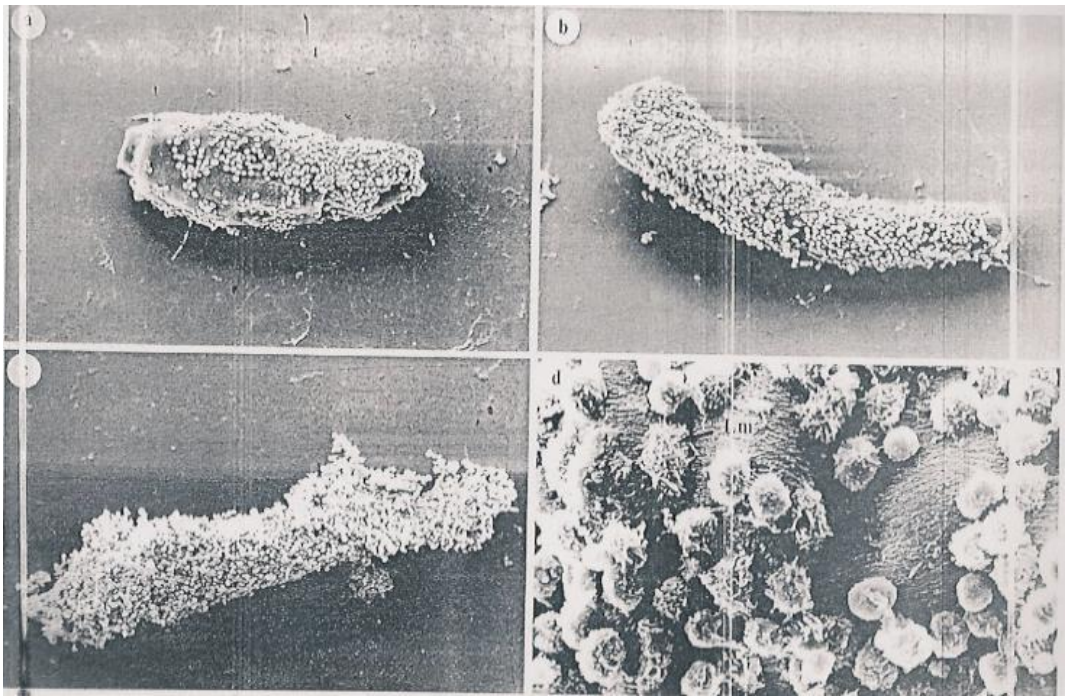
Its molecular weight ranges from 600-900 KD and contains up to 16% sugars. The molecule consists of four Y-shaped monomer units that in total form 8 binding sites with an antigen. Unlike mammals, fish have the potential for significant structural variation of the immunoglobulin molecule by creating various bonds between the

parts of the molecule ("hemimers", monomers, dimers and trimers) of the tetramer antibody. Thus, instead of using different genes of heavy chain immunoglobulin to create the various isotypes (classes) observed in mammals, fish use processes after the creation of the antibody parts to secrete the above hemers, monomers, dimers and trimers, including the tetrameric form. Although low molecular weight immunoglobulins have been described in fish that may be related to M, some structural or functional relationship to mammalian immunoglobulin G has not been demonstrated. However, under normal conditions a dimer form of immunoglobulin in the mucus and bile of some fish different from plasma immunoglobulin has been described. Recently, a new chimeric heavy chain immunoglobulin that bears similarities to mammalian immunoglobulin D was described in catfish and Atlantic salmon.

Humoral immunity

Antibody-initiated mechanisms

Antibody molecules can trigger the initiation of various mechanisms that can, directly or indirectly, influence the destruction of a foreign antigen. Neutralization of the antigen after blocking a key function (an important receptor, an enzymatically active region, or a toxicity determinant) appears to be the most direct effect of the antibody on the antigen. The creation of macromolecular antigen-antibody complexes large enough to be desolutized (precipitation when the antigen is soluble and aggregation when the antigen is a whole cell) is another direct effect of the antibody on the antigen. In addition, this insoluble matter can more easily be phagocytosed, and the coexistence of many antibodies in the antigen-antibody complex also facilitates complement activation or adhesion of antibodies to their specific receptors on phagocytes. The latter procedure is an example of opsonization, which usually refers to covering with specific antibodies or other related immune molecules the cells of bacteria, fungi and parasites, which often promotes their phagocytosis (Figure 10).



Pict. 10. *White blood cells from the anterior trout kidney attaching to procercoids of the tapeworm Diphyllobotrium dendriticum (a) lack of trout serum (x 148), (b) in the presence of normal trout serum (x 143), (c) in the presence of immune trout serum (carrying antibodies against the parasite) (x 130) or (d) greater magnification of the image (c) showing the pseudopodia of leukocyte adhering to the surface of the parasite.*

The specific activation of the complement system by the antibody (classical pathway) is an indirect but very active effect of the antibody on its antigen. Binding the antibody to an antigen results in a change in the stereostructure of the antibody which allows it to bind to and activate the first complement factor, which in turn triggers the sequence of reactions of the complement system. At each stage of these reactions, proteolytic products are produced that can opsonize or lyse pathogens, as well as act pharmacologically to carry out changes in the vascular and muscular systems of the host as well as immunological changes in tissues. Finally, in fish, both classical (antibody-dependent) and alternative (non-antibody-dependent) mechanisms of complement system activation have been described.

Humoral immune response and immunological memory

The culmination of humoral immunity is the production of specific antibodies by plasmocytes, which initiate a number of processes that eventually lead to the destruction of the foreign antigen. As mentioned above, B-lymphocytes can be produced directly or indirectly and differentiate into antibody-producing cells,

depending on whether the antigen is a polysaccharide (T-cell independent) or protein (T-cell dependent). If the antigen is of different chemical composition (glycolipid or nucleic acids), data from mammals indicate that the antigen will be treated as either a dependent T-cell or an independent antigen. However, in most cases the immune system is called upon to react to chemically complex antigens to the detriment (suppression) of other antigens found in the same or another molecule. In fish, both of these suppressions to T-lymphocytes dependent antigens have been described.

A first and important line of defense in fish is mucous surfaces. While in mammals specific immune organs appear on the mucous membranes and a secretory immunoglobulin A, in fish no such organized structures appear nor any form of immunoglobulin A (could be the dimer mentioned earlier). However, fish carry lymphoid cells dispersed in layers of the intestinal epithelium with specialized functions related to the uptake and processing of antigens. In addition, it has been shown that fish carry a different immune system in mucous membranes compared to that of mammals, since both antibodies and cells that produce them can be produced, especially, in mucous organs.

Immune memory is related to the ability to elicit a more effective response after repeated contact with an antigen. Immunological memory has been studied based on mammalian reactions to proteins. These reactions are characterized by spread and changes in the properties of antigen-specific lymphocyte populations during the primary reaction. These changes essentially result in an increased, faster and longer secondary reaction as well as a change in the isotype (class of antibodies) and maturation of the affinity of the antibody to the antigen. All these specific phenomena either do not occur in fish or occur to a much lesser extent, with the secondary reaction of fish resembling the immunoglobulin M production reaction in mammals (primary reaction). Although forms of immunoglobulin isotypes have recently been confirmed in fish, immune maturation similar to that occurring in mammals cannot occur because the relevant genetic information is lacking. So immune memory in fish has more to do with a higher and faster production of antibodies at the secondary level. However, secondary reactions in fish do not always show the last two commemorative effects. This is related to the age of the fish, temperature, type and dose of antigen, route of administration and intervals between immunizations.

Studying the ontogeny of the humoral reaction is very important to determine the age of the fish at which it can be inoculated without inducing immune tolerance, since it has been shown that exposure to antigens too early can cause tolerance. In addition, the ability to react to bacterial T-lymphocytes independent antigens predates the ability to react to protein-related antigens (dependent T-lymphocytes). Immune tolerance in juveniles has been established in T-lymphocytes dependent antigens administered by injection but not in similar fish where the antigen was given by direct immersion. Thus, the immune response of young fish depends not only on the type of antigen but also on the route of administration. In fish species that have free-living nymphs, the latter encounter a plethora of antigens in their environment at a stage in their lives when their immune systems are still immature. It is possible that these fish do not ingest antigens that can trigger immune tolerance during this sensitive stage of their lives.

Vaccination

The principle of vaccination is based on the ability of memory development by the immune system. In addition, it is necessary that the produced antibodies are specifically directed against important virulence determinants of the pathogen in order to be able to induce adequate protection. Thus, it is very important to examine the mechanisms of pathogenesis and develop a vaccine containing the appropriate antigens or epitopes, which will trigger immune reactions suitable to inactivate the mechanisms involved in the virulence of the pathogen. Molecules that assist the adhesion (adhesins), the entry into some cells (invasins), toxins, proteins of the outer membranes and proteases are good examples of molecules for inclusion in vaccine mixtures. At the same time, it is also important to know the conditions that promote ideal immunization. As mentioned in a previous section, antigen type and dose, route of administration, water temperature, fish age and stress factors can all influence fish immune responses and appear to be important factors for a successful vaccination. The fact that the immune response of fish is subject to regulatory and suppressive agents involving antigens to both the same molecule and another molecule raises questions about how this may affect the specificity of immune responses against vaccine antigens and the achievement of immune protection, particularly when using synthetic vaccines and vaccines containing peptides. Thus, the specificities of an

immune response in fish as well as in mammals may preferably concern immunodominant determinants for B- and T-lymphocytes, which do not necessarily concern antigens that are involved in the pathogenesis of the pathogen and therefore do not protect the immune organism from it. The immune response may also be suppressed for other epitopes on the same or another antigen that may be important to protect against the microbe.

As for vaccinations, many ways have been developed for mass immunization of fish, including: injection, hyperosmotic immersion, direct immersion, bathing, spraying and oral administration.

Intraperitoneal injection: it has been shown to be the most efficient method of vaccine administration in terms of systemic antibody development. In addition, it allows the use of adjuvants to improve protection. Nevertheless, it requires high labor and time, causes stress and cannot be applied to small-sized fish. Most importantly, this method of vaccination does not cause the secretion of antibodies to the epithelium, so the host is not protected against contamination through water (the most common route of infection);

Hyperosmotic immersion: it has been shown to be an efficient method of administering the vaccine to fish. It is based on increasing the permeability of the gills to antigens. It is not used because it can damage fish and because direct immersion can have similar effects.

Direct immersion: exposure of fish to the vaccine mixture even for only 20 seconds is sufficient for adequate uptake of antigens, while increasing exposure time does not result in higher uptake as long as antigen concentration is not the limiting factor. Although it causes less stress than hyperosmotic immersion, the method is limited by the number of fish that can be vaccinated per unit volume of the vaccine and is quite expensive when relatively large fish need to be vaccinated.

Bathing and spraying: they are considered variants of direct immersion. Fish are exposed to diluted antigen solutions for long periods or simply sprayed with the

vaccine, respectively. It has been found that long periods of exposure increase antigen uptake and can compensate for high vaccine dilution.
In the last three methods of vaccine administration, no significant production of systemic antibodies has been observed, only the development of the latter locally in epithelium. Thus, while fish are better protected from infections through water, in case the pathogenic microorganism enters inside the body, there they remain unprotected.

Oral administration: it is theoretically the most ideal method for vaccinating a large number of fish. It does not cause stress, does not interrupt production routine, and as for immersion, it can be used on all sizes of fish. However, in practice, various and unstable immune responses have been found using this route. These are most likely due to the degradation of antigens in the stomach and anterior part of the intestine, since when antigens are administered by the anal route, stable reactions can be measured. New oral delivery systems have been developed that protect antigens from low stomach pH by allowing the uptake or release of the antigen into the second section of the intestine. In addition, oral administration of antigens, when successfully administered, has been shown to trigger immune responses both systemically and on mucous surfaces. Immune stimulation of epithelium is of great importance because most infections start there, especially in the aquatic environment where fish live. However, further studies are needed to determine the doses of antigens and their timing of administration, particularly for proteins, to avoid the development of immune tolerance. An additional problem associated with the latter is the different amounts of food that individuals receive within a population.

Recently, vaccines developed with DNA recombination technology offer significant help in the fight against diseases caused by viruses and parasites. The potential of this technology is constantly growing, and vaccines based on "recombinant" antigens could in the future significantly help in the fight against several diseases of cultivated fish. "Recombinant" antigens/proteins, expressed in prokaryotic or eukaryotic cells produced under strictly controlled laboratory conditions, live inactive pathogens, non-pathogenic "recombinant" microorganisms, carrying foreign genes from pathogens and genetic or DNA vaccines have all been tested in vaccinations with satisfactory

results to a certain extent, in laboratory conditions. Vaccines containing "recombinant" proteins do not provide sufficient immunity, possibly due to poor antigenicity. In a more advantageous position are vaccines containing live "recombinant" microorganisms which can also be given by immersion. In conclusion, however, their use means the release of genetically modified microorganisms into the environment with indeterminate consequences for both vaccinated animals and the ecosystem, and for this reason research into such vaccines is not important. Most experiments with DNA vaccines have been conducted on mammals, so little data exists on the expression of foreign pathogen genes by fish. Although their use theoretically has many advantages in terms of production costs, compared to vaccines of subunits or "recombinant" organisms, in that they raise both humoral and cellular immunity that lasts for a long time and in that they are non-infectious and stable, it is not known how DNA will behave in terms of transmission to the offspring of vaccinated fish or other organisms and others. In other words, these vaccines follow the same philosophy and restrictions as genetically modified products, and if there is no concrete proof of their safety in the future, they will probably not be used.

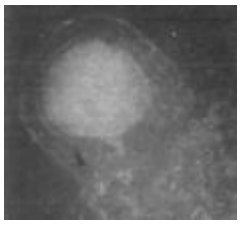
Below are presented the most important diseases of fish of Mediterranean aquaculture.

4. DISEASES OF EURYHALINE FISH

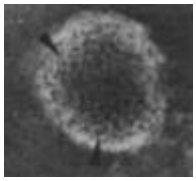
Viral diseases

Lymphocystis

Etiology: It belongs to the family of iridoviruses. Iridoviruses have been implicated as the causative agents of severe disease, mortality and economic losses in cultivated fish, ornamental as well as wild fish. Different groups of histopathological lesions after iridovirus infection have been identified, which may be localized or systemic. Up to 10 glycoproteins have been isolated from the surface and inside of the viral particle.



Outer cover and membrane of iridovirus x156.000.



The virus capsomers that form the capsid x 156,000

Sensitive species: Infects higher evolutionary species such as salmonids. Iridoviruses have been isolated from both white sturgeon and cod. Sea bream appears to be the most sensitive of the widely cultivated species. On the contrary, sea bass is very little sensitive and under normal conditions is practically not affected.
Temperature of onset of the disease: It is observed in a wide range of temperatures, but cases are more intense during the warmer months of the year.
Clinical signs: Localized lesions include those of the lymphocyst of the skin and fins, iridovirus infection of the skin and gills of white sturgeon, and erythrocytic necrosis in cod. Systemic iridovirus infections are found in a plethora of freshwater fish, both edible and ornamental, and in marine edible fish. Systemic iridovirosis is characterized by the presence of hypertrophic cells in the spleen, kidney and intestine. Localized iridoviroses of interest to Mediterranean aquaculture are characterized by cytomegaly of dermal fibroblasts and systemic involvement is rarely observed. Small (0.5-2 mm) whitish nodules are observed, either individually or in groups on the surface of the body, on the fins and more rarely on the gills and/or internal organs (Figure 11).

Pict. 11. Lymphocyst lesions in sea bream juveniles.

Diagnosis: The macroscopic image is characteristic. Histologically, the "giant lymphocyst cells" are distinguished in the lesions, which are actually fibroblasts that have been overgrown.

Indirect immunofluorescence and flow cytometry have been identified as the best methods for detecting the virus. Also very sensitive has been characterized an immunoblot test using specific antiserum against a viral protein of molecular weight 60kDa. Monoclonal antibodies specific to virus antigens located on the outer surface of the viral capsid have also been produced.
Finally, primers for PCR have been manufactured with excellent results in terms of sensitivity to virus detection.
Mortality: Low and mainly due to injuries and secondary infections. In Greece, recent cases of lymphocystis have been observed in juvenile sea bream with high mortality.
Transmission: Horizontally from diseased fish to their cohabitants. The virus spreads after detachment and rupture of nodules. Vector fish without clinical symptoms have also been described. The onset of symptoms is associated with stress factors. The virus has been found in Artemia's nymphs and larvae.
Prevention: Quarantine of newcomers and their destruction if they develop the disease are measures to limit the transmission of the latter. In general, fish injuries and stress should be avoided.
Treatment: None. Antibiotics may be given to fight secondary bacterial infections. Fish usually recover automatically after a few weeks or months.

Viral Encephalopathy and Retinopathy VER

Etiology: Viral encephalopathy and retinopathy (VER) is an important disease of larvae, but also of larger fish. Responsible for the disease are Nodaviruses that infect fish (class Osteichthyes) and belong to the family *Nodaviridae* and the genus *Betanodavirus* (Carstens et al., 2000). Fish nodaviruses have been categorized into four genotypes, SJNNV, TPNNV, RGNNV and BFNNV. In Greece, RGNNV is found, which generally affects thermophilic fish species.
Sensitive species: The disease is found in almost the entire world, except Africa. To date, it has been reported in more than 40 species of fish. The virus has been isolated from cod in the UK and Canada, Asian sea bass in Asia and Australia, Japanese sea bass in Japan, European sea bass in the Caribbean, France, Greece, Italy, Malta, Portugal and Spain, red spotted grouper in Japan and Taiwan, yellow grouper in Taiwan, orange-spotted grouper in China and the Philippines, blackspotted grouper in Taiwan, dragon grouper in China, malabar star in Thailand, dusky grouper in the

Mediterranean, kelp grouper in Japan, sevenband grouper in Japan and Korea, greasy grouper in Malaysia and the Philippines, humpback grouper in Indonesia, striped trumpeter in Australia, bone in Japan, amberjack in Japan, pompano in Taiwan, red drum in Korea, sea bream in Italy, mill in Italy and France, white seabass in the USA, Japanese parrot fish in Japan, rock porgy in Japan, sleepy cod in Australia, cobia in Taiwan, barfin flounder in Japan, winter flounder in Canada, halibut in Norway and UK, japanese flounder in Japan, turbot in Norway, language in UK, firespot snapper in Taiwan and tiger puffer in Japan.

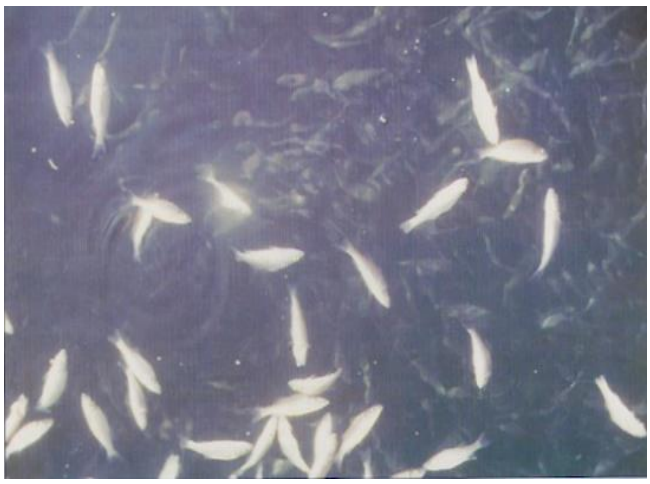
Also in 2002, isolation of the virus from freshwater fish was first reported, although initially the virus was thought to contaminate fish that lived exclusively in seawater. The virus has been isolated from European eel in Taiwan, Chinese catfish in Taiwan and sturgeon in Greece. Another example is the occurrence of clinical infection in sea bass in Greece, 8 months after their transfer to fresh water.

In 2004, Gomez and colleagues tested, with PCR and nested PCR, 30 species of healthy cultivated and wild fish collected from two remote areas of Japan. Of the species found infected with the virus, mullet also exists in Greece, while the Japanese jack mackerel and red seabream are related to horse mackerel and red seabream, respectively.

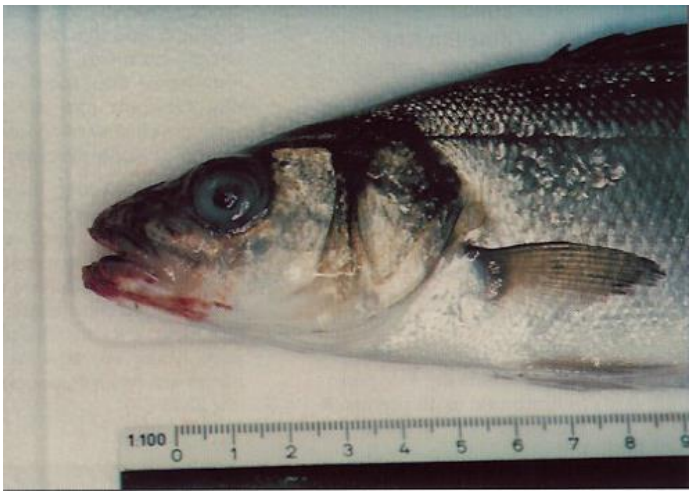
Temperature of manifestation: The disease depends on the age of the fish or temperature. In sea bass, the disease causes significant mortality in larvae at 18°C. Fish under one year old are more susceptible than two-year-old fish, and the disease manifests itself when the temperature reaches 22-25^{o}C.

Clinical symptoms: The symptoms are nervous in nature manifested as swimming abnormality (circular, spiral), body flexion and lethargy (Figure 12).

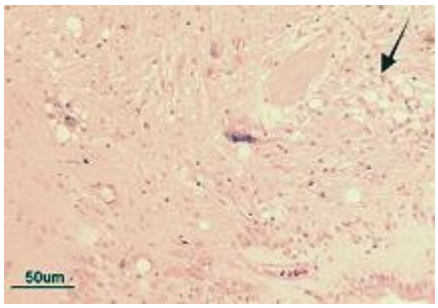
When aroused, fish react with hyperactivity. In the subacute form, darkening resulting from blindness and hemorrhagic ulcerations in the anterior part of the snout are observed (Figure 13). The latter are observed at low temperatures, while at high temperatures nervous symptoms predominate. Internally, congestion of the brain and meninges and dilatation of the swim bladder are observed.



Pict. 12. Viral encephalopathy and retinopathy in juvenile sea bass.

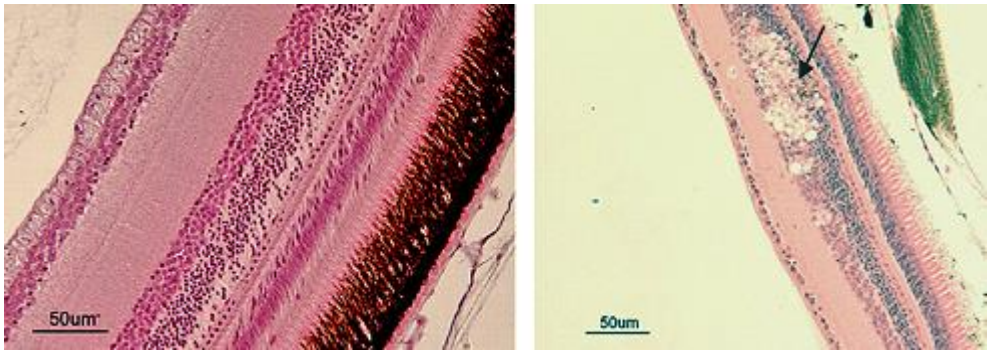


Pict. 13. Ulcerations of the upper and lower jaw in sea bass with viral encephalopathy and retinopathy.



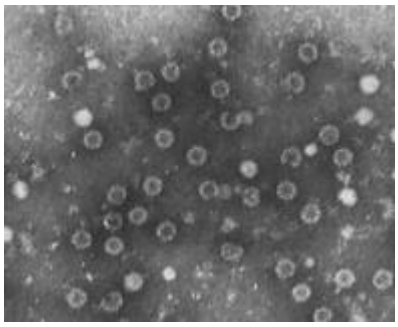
Vacuolar degeneration in the brain

Turbot.



Left normal and right infected retina where vacuolar damage is visible.

Diagnosis: A hypothetical diagnosis can be made after histological examination where vacuolar degeneration is observed in the brain and retina, while confirmation can only be made after culture of the virus in cell lines and its isolation, with immunological methods (immunohistochemistry, ELISA, immunofluorescence) and PCR.



Incomplete and complete virus particles

Laboratory diagnosis is based on the detection of the virus by classical or molecular methods and the detection of antibodies specific against Noda-viruses. Molecular methods for detecting the virus include (a) RT-PCR, (b) nested RT-PCR and (c) real-time PCR (real-time quantitative PCR). From the findings of measurements so far with the method of real-time PCR, it is found that this method is very sensitive since very small amounts of virus were detected in the tissues of the fish, something that is not possible with the existing molecular methods.

Mortality: In nymphs it can reach 90-100% in just a few days. In larger fish it ranges from 15-60% depending on the age-size of the fish and the duration of the disease. Most fish are attacked in the larval stage or in young stages of development, so losses are usually very large. According to OIE (2000) data, in Asian sea bass, Japanese parrot fish, red spotted grouper and turbot, the mortality rate reaches 100% in fish less than one month old. In recent years, however, significant losses have been reported in older fish (commercial size). Specifically for sea bass, the OIE reports that the disease occurs from fish aged 10 days to fish aged ≥12 months. Le Breton et al. report that in two regions of Greece a total mortality rate of 60% was observed in sea bass 10-30 g, and 11% mortality rate in sea bass 400-580g.

Annually there are losses of many tens of tons of sea bass of various sizes in Greek aquaculture due to the Nodavirus, mainly during September and October.

Transmission: Horizontal transmission has also been proven experimentally. Vertical transmission is strongly suspected due to the early onset of the disease in sea bass larvae.

In more detail, the virus is transmitted between different species of fish, which is important for the management of fish farms in the open sea. Munday and colleagues report that when the temperature is favorable, the virus has no specificity in a

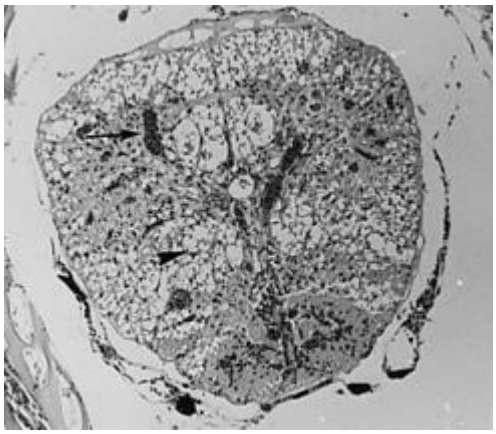
particular species of fish. Also, a species of Nodavirus that causes acute infection in some species of fish can cause asymptomatic infection in other species of fish. This was demonstrated in sea bream when, from a unit where the disease occurred in sea bass living with apparently healthy sea bream, samples were taken from the sea bream and inoculated in healthy sea bass of the same unit. then these bass became ill. On the contrary, when the samples were inoculated in sea bream, they did not get sick but could transmit the virus to sea bass with which they lived.

Apart from natural occurrences, experimental studies have shown that several species of fish are susceptible to the virus, but without showing clinical symptoms. In these fish, the concentration of the virus is often low, so its detection requires the use of sensitive methods, such as nested RT-PCR (nested RT-PCR) and real-time PCR (real-time PCR). Therefore, it is likely that a plethora of wild fish populations are asymptomatic carriers of the disease and thus be a major source of infection of farmed fish.

Transmission from a patient to healthy fish is influenced by fish density, virulence of the viral strain and water temperature. The resistance of the virus to environmental factors, such as pH values of 2-9 or seawater of 15^{o}C for more than a year, increases the likelihood of transmission from fish to fish.

Horizontal transmission of the virus can also take place through contaminated food since, in an infected farm, its presence in live bait was found. Transmission of the virus can also occur through contaminated water coming from neighboring infected farms.

In the context of the study of the pathogenesis and transmission of the virus, Arimoto and colleagues confirmed that the causative agent of infection was Nodavirus and that the virus is transmitted horizontally. Nguyen and colleagues immersed larvae in a water solution containing homogenized infected tissue and found that vacuolation of the nervous system first started in the spinal cord.



Vacuolar degeneration in the spinal cord

They also observed hyperplasia of the epithelium of the skin associated with the presence of viral antigen. Boonyaratpalin et al., in order to determine the causative agent of a disease with high mortality rates that occurred for a long time in young malabar, inoculated, by the intraperitoneal route, pathological material from diseased fish, in juvenile malabar fish. Based on the results of inoculation, the authors identified the presence of viral particles in the tissues of fish and studied the pathogenesis of the disease in this species.

Many experimental studies have been conducted on sea bass to date. In a study by Thiery and colleagues, intramuscularly infected fish of 3 g size and showed an overall mortality rate of 28%. The fish were in a tank with salinity water of 35‰. In another experimental study, the fish were of different ages and were infected in three different ways, namely intramuscularly, intraperitoneally and through symbiosis of healthy fish with fish contaminated in the first two ways. Another study was done by Peducasse and colleagues and the fish were contaminated with five different ways. The results showed that: (a) the younger the fish, the higher the mortality rates, (b) mortality rates increase as water temperature rises, and (c) intramuscular infection causes acute infection with high mortality, while intraperitoneal and oral infection, as well as infection by immersion and through symbiosis, causes subacute infection.

Another study was done by Breuil and colleagues on sea bass broodstock. The broodstock were inoculated intramuscularly with a live strain of the virus, and during the virological examination that followed, it turned out that both they and their larvae were infected. It also proved that nested RT-PCR is the most suitable method for detecting the virus in broodstock, since it was more sensitive than classical RT-PCR

and ELISA. Another study was done to investigate the effect of salinity on the pathogenicity of the virus in sea bass, which is a widespread species. Three groups of sea bass 3 g were infected. by the intramuscular route which were maintained at salinities of 0, 18 and 33‰. The overall mortality rates observed were 49, 27 and 30%, respectively. The transmission of the virus from asymptomatic infected sea bream to sea bass 11g was also studied, through eating by sea bass of infected sea bream tissue. Although there were no clinical symptoms, only mortality (total mortality 20%), it was found by real-time PCR that the virus was transmitted from one species to another. In this case, it was also found that real-time PCR is a more sensitive method than cell cultures, since in samples negative with this method, the virus was titrated with real-time PCR.

There have also been experimental studies on sea bream. Specifically, Castric and colleagues reported that sea bream is an asymptomatic carrier of the disease, but the strain isolated from the apparently healthy sea bream they tested was highly pathogenic to sea bass. Aranguren and colleagues inoculated intramuscularly sea bream of average weight 0.7, 2 and 4 grams with strain 475-9/99, which had been isolated from clinically ill sea bass. They also inoculated sea bream intraperitoneally with the same strain and finally contaminated sea bream by immersion. Clinical signs were not observed with any of the three methods, but with intramuscular injection mortality was observed, which decreased as the size of the inoculated sea bream larvae increased. Finally, sea bream was infected with two different doses of virus but showed no clinical symptoms for 21 days during which the experiment lasted, although the method of real-time PCR revealed the presence of a large amount of virus in the tissues of both groups of sea bream.

Although asymptomatic infected fish are considered the most likely carriers of virus transmission, little is known about the pathogenicity of the disease in these fish and its impact on their health.

The lack of knowledge about the epidemiology and pathogenesis of Nodaviruses makes control measures difficult and losses continue on a consistent basis.

In addition, it should be mentioned that there is a report of isolation of the virus from mussels (*M. galoprovincialis*) in Italy. The survival of the virus in mussel tissues is a very important epidemiological element that needs further investigation, especially in Greece where no research has been done in this area.

To date, there have been few studies on identifying the gateway of the virus into the body and how it spreads to the fish's CNS. In 1996, Nguyen and colleagues detected the virus in both the CNS and degenerated skin cells of infected fish. Thus, the question arose whether the skin was the entry point of the virus into the body or whether the detection of the virus in the skin was a consequence of systemic infection. Subsequently, several authors identified the virus in the olfactory epithelium or olfactory lobes of the brain. The detection of the virus in the olfactory lobes of the brain indicates that it probably enters through the nasal cavity. Finally, Grotmol detected with the method of immunohistochemistry, virus-positive cells in the epithelium of the skin, intestines and gills, while Mladineo with the same technique, observed virus-positive cells in a vein of the liver. From the above it is obvious that at the moment nothing is confirmed about the entry gate of the virus and that is why the investigation continues.

Regarding the way the pathogen spreads in the CNS, it is considered that this is achieved in three main ways: a) by local spread through neighboring tissues, b) by spreading through nerves and c) through blood. In most samples from diseased fish, involvement of the brain and retina of the eye is found. Johansen and colleagues and Grove and colleagues found that the visual lobe of the brain and the inner layers of the retina are the places where the attack is most intense, possibly because of the contact between them, through the optic nerve, is closer.

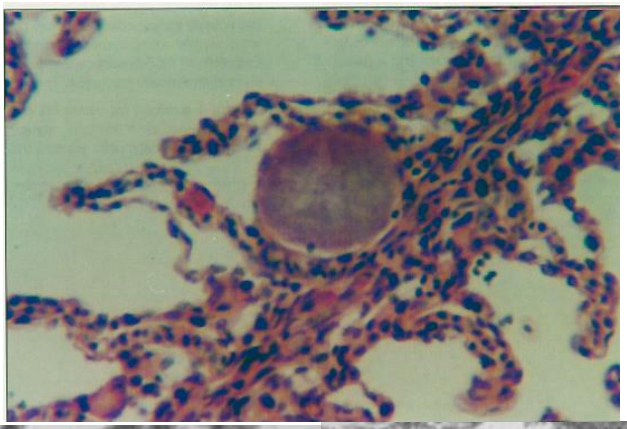
Prevention: In hatcheries, detection and extermination of broodstock carriers and sterilization of incoming water can prevent the spread of disease. As far as fattening plants are concerned, prevention is related to the presence of virus-free zones. In the latter, the newcomer fry should always be checked. There are also available vaccines which provide satisfactory protection.

Treatment: None. It is only possible to improve management by eliminating stress factors, such as high stocking density and handling, as well as taking sanitary measures, such as removal and sanitary destruction of dead and infected fish, disinfection of devices, facilities and vehicles, etc.

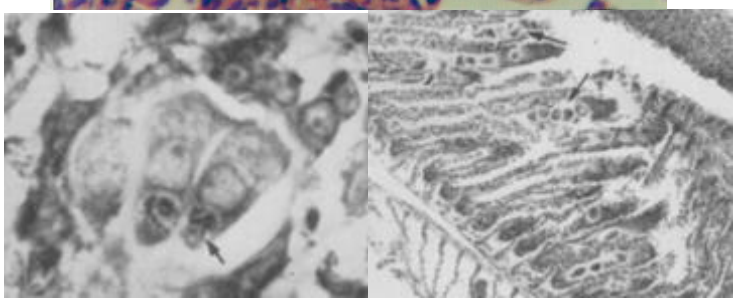
Diseases due to intracellular parasites

Epitheliocystis

Etiology: It is due to chlamydia. Chlamydia are small coccoid prokaryotic microorganisms that multiply intracellularly.
Sensitive species: It is observed in many species, including fish of the salmonid family, sea bream and sea bass, sea bream, sharpsnout bream.
Temperature of manifestation of the disease: The disease manifests itself mainly during spring and summer.
Clinical symptoms: In light infections there are no symptoms and the behavior of the fish is normal. In severe infestation, respiratory symptoms (tachypnea) and lethargic behavior occur. The level of contamination of fish depends on the age, temperature and quality characteristics of the water. Thus, the problems are more pronounced at 0+ ages, at low temperatures and when water quality is poor.
The gills are pale, like pseudobranchs, abnormal swimming and emaciation, slight discoloration of the fins and even clouding of the cornea are observed.
Diagnosis: It is performed macroscopically with the presence of small white cysts on the gills and microscopically by examining fresh gill swabs. Confirmation of the diagnosis is established by examination of histological sections (Figure 14).



Pict. 14. Epitheliocyst in histological section of gills of sea bream (HE x 400).

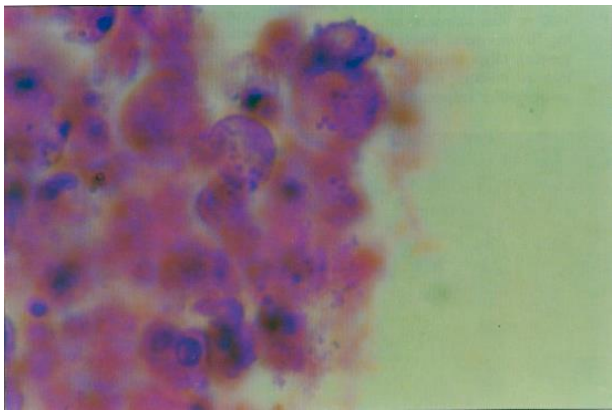


Epitheliocyst in the cytoplasm of two epithelial cells (HE x 1400) and cells of the organism free between gill filaments (HE x 36)

Transmission: Not fully known. The indications point to horizontal transmission between individuals of the same species. There is no transmission data between different species.
Mortality: High mortality rates have been reported in bream juveniles. In high infestation, negative effects on osmoregulation, respiration and food intake due to damage to the skin, epithelium and gills are the causes of mortality.
Mortality under favorable conditions can reach up to 85% of the affected population.
Prevention: Sterilization of incoming water with UV in hatcheries is recommended. In fattening, prevention is carried out by avoiding the entry of the disease.
Treatment: None. The literature reports an improvement in the condition with chloramphenicol administration, but this substance is not approved for use in fish.

Necrosis of the nervous system

Etiology: It is due to involvement of the nervous system by coccoid intracellular organisms resembling rickettsia.
Sensitive species: The disease has only been described in sea bass. It first appeared in France and in 1993 in Greece. It is observed in bass weighing up to 10-15 grams (juveniles).
Temperature of manifestation: The disease occurs during winter and early spring when the water temperature ranges from 12-15^{o}C. The disease seems to subside when the temperature exceeds 19^{o}C.
Clinical symptoms: Nervous symptoms manifested by swimming disorders (spiral swimming). Macroscopically during autopsy, hemorrhages on the surface of the brain and meningitis lesions are observed.
Diagnosis: It is performed by histological examination and the detection of basophilic intracellular coccoid microorganisms (Figure 15). The disease is similar to a disease



Pict. 15. Intracellular basophilic microorganisms RLO in sea bass brain (HE x 1000).

caused by *Piscireckettsia salmonis* (another rickettsia) in trout in Chilean aquaculture. The above microorganism carries antigens similar to those of sea bass rickettsia and antibodies against *P. salmonis* could be used with caution to perform immunohistochemistry or other immunological techniques.

Mortality: Ranges from 6-20% of the affected fish population.

Transmission: Unknown

Prevention: Avoid disease entry by controlling new entrant fish. In areas where the disease is endemic, newcomers should be placed when the water temperature is higher than 17^{o}C.

Treatment: Rickettsias are considered sensitive to tetracyclines and penicillins. Attempts to control the disease with tetracyclines have had varying results.

This disease is no longer a major disease for Mediterranean aquaculture.

Diseases caused by bacteria

Vibriosis

Aetiology: It is due to bacteria of the genus Vibrio. Historically, for all cases of fish diseases caused by Vibrio, the disease was attributed to *the species Vibrio anguillarum* (renamed *Listonella anguillarum*) and 10 serotypes were reported. The serotype most commonly isolated in Mediterranean farmed species was serotype O1. Serotype O3 occurred in sea bass larvae.

This classification of marine pathogens of Vibrio eventually proved to be oversimplified. For many years efforts were made to systematically classify marine pathogens of Vibrio based on biochemical and serological analyses and the description of new species, but these were not appropriate methods because for the classification of species they were based on phenotypic characteristics that proved too unstable for such environmental and pathogenic strains. The solution to the systematics of marine Vibrio pathogens was given with molecular biology and more specifically, it was based on sequences of nucleotide markers such as 16s rRNA, 5s rRNA and DNA.

These studies separated *V. anguillarum* from other Vibrio, led to its renaming to *L. anguillarum*, and helped attribute various diseases to the actual Vibrio pathogens.

Data from recent years show that more and more new species of Vibrio cause vibriosis in euryhaline fish. These species include: *V. harveyi, V. alginolyticus, V. splendidus, V. vulnificus* and others.

Vibrio are gram-negative mobile bacteria in the form of rods, mesophiles, chemoorganotrophs and facultative anaerobes.

Sensitive species: Vibrio *harveyi* isolated from sea bream and sea bass, *Vibrio alginolyticus* isolated from red porgy, sea bream, bivalve and sea bass, *Vibrio fischeri* isolated from sea bream, *Vibrio splendidus* isolated from sea bream, bivalve larvae and sea bass, *Vibrio mediterranei, Vibrio pelagius, Vibrio ordalii, Vibrio aestuarianus, Vibrio cambellii, Vibrio nereis* and *Vibrio tubiashii* have been isolated from sea bream and *Vibrio vulnificus* has been isolated from sea bass and red sea bream.

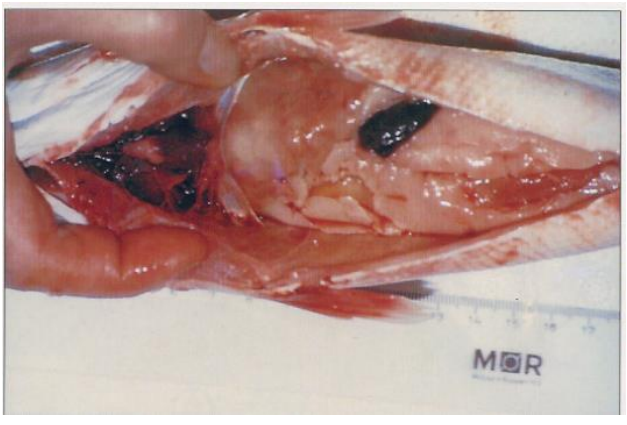
Temperature of the disease: The disease occurs especially during autumn and spring when the water temperature changes from 20 to 15^{o}C and vice versa. In recent years in Greece the disease appears during winter at temperatures lower than 15^{o}C.

Pathogenesis of vibriosis

The pathogenesis of marine Vibrio pathogens is due to the production of toxic extracellular products that include proteases, caseinases, lipases, phospholipases, hemolysins and siderophores. Toxicity has also been attributed to their lipopolysaccharide O chains. Finally, an important factor in the pathogenesis of vibriosis is considered to be the ability of many species to produce agglutinins with which adhere to the mucus and mucosal surfaces of fish.

Clinical signs: It is a classic bacterial septicemia but the symptoms depend on the form of the disease. In the hyperacute form (usually in juveniles) high mortality occurs without clinical signs except anorexia and darkness.

Pict. 16. Vibriosis in sea bass – hemorrhagic lesions and ulcerations on the skin.



Pict. 17. Vibriosis in sea bass – splenomegaly and bowel hyperemia.

In the acute form, externally, redness of the perioral region, exophthalmos, redness at the base of the fins and anus, hypodermic cysts are observed. In some cases, ulcerative lesions on the skin are observed in various parts of the body (Figure 16). Macroscopically during autopsy, hemorrhages and necrosis are observed in most organs characteristic of hemorrhagic septicemia. The spleen is swollen, and the intestine shows pronounced hyperemia, enteritis, distension, necrosis and ulcerations with a characteristic picture in the area of the anus; (Figure 17). In *V. ordalii* infections, microcolonies of the organism are observed in the skeletal muscles and heart, gills and intestine and leukopenia in the blood.
In an attempt to group the clinical symptoms that have been described for diseased by Vibrio fish, the following tables are listed.

Table 11. External signs of disease (modified after Austin and Austin 2007).

External disease signs														
PATHOGEN	1	2	3	4	5	6	7	8	9	10	11	12	13	14
V. alginolyticus		+	+		+						+			
V. fischeri						+					+			
V. harveyii		+	+								+			+
V. ordalii	+			+	+			+				+		
V. pelagius							+	+						
V. splendidus	+							+	+		+	+	+	
V. vulnificus										+				

1= catatonic behavior, 2= dark-chromia, 3= eye damage / exophthalmos, 4= bleeding in the oral area, 5= gill lesions, 6= white nodules on the skin / gills, 7= fin erosion, 8= bleeding at the base of the fins, 9= abdominal distension, 10= surface / muscle bleeding, 11= ulcers / ulcerations, 12= hemorrhages around the anus, 13=anorexia, 14= spiral swimming, bursts, swimming disorders.

Table 12. Internal signs of disease (modified after Austin and Austin 2007).

PATHOGEN	1	2	3	4	5	6	7	8	9
V. alginolyticus		+		+	+	+	+		
V. fischeri									+
V. harveyii						+	+		
V. ordalii			+		+		+		
V. pelagius						+	+		
V. splendidus					+	+		+	
V. vulnificus									

1= bowel necrosis, 2= ascites, 3= petechiae in muscle walls, 4= fluid in the bladder, 5= hemorrhages and/or bloody exudate in the peritoneal cavity, 6= bowel dilatation, 7= hemorrhages over/inside internal organs, 8= pale brittle/discoloured liver, 9= presence of tumours

Diagnosis: It is performed by isolating and identifying the responsible microorganism in appropriate nutrients. Marine agar and selective thiosulfate-citrate-bile salt-sucrose

agar (TCBS) are commonly used. The identification of the pathogen is carried out by various biochemical tests.

Classic methods can be used in laboratory tubes or ready-made tests such as API 20E and API ZYM systems, and more recently API 20NE API50CH, API 50L, Biolog-GN, Enterotubes and RapidID 32 systems.

Below are tables with the results given by some of the aforementioned systems and for different types of Vibrio.

Table 13. Profiles of fish pathogenic Vibrios using the API20E system.

	API20E TEST No																				
Pathogen	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
V. alginolyticus	-	-	+	-	v	-	-	-	+	-	V	+	+	-	-	-	+	-	-	-	+
V. harveyii	-	-	+	+	-	-	-	-	+	-	+	+	-	-	-	-	+	-	-	-	+
V. ordalii	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	+	-	-	-	+
V. splendidus	-	+	-	-	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	-	+
V. vulnificus	+	-	v	-	+	-	-	-	-	-	+	+	+	-	-	-	-	-	+	-	+

1= b-galactosidase, 2= arginine dehydrolase, 3= lysine decarboxylase, 4= ornithine decarboxylase, 5= citrate utilization, 6= H2S production, 7= urease production, 8= tryptophan deaminase, 9= indole production, 10= Vogues Proskauer reaction, 11= gelatine hydrolysis, 12= acid from glucose, 13= acid from mannitol, 14= acid from inositol, 15= acid from sorbitol, 16= acid from rhamnose, 17= acid from sucrose, 18= acid from melibiose, 19= acid from arabinose, 20= oxidase production.
+, - and v correspond to >80, >20 and 21-79% of positive results, respectively.
( ) indicates weakly positive results.

For comparison, a table is provided where classical biochemical tests were used.

Table 14. Profiles of fish Vibrio isolates originating from farmed gilthead seabream using conventional methods (modified after Balebona *et al*. 1998b)

TEST	*V. aesturianus*	*V. campbelii*	*V. fischeri*	*V. nereis*	*V. tubiashii*
Swarming	-	-	-	-	-
Indole production	+	+	-	-	+
Acetoin production	-	-	-	-	-
Growth at C					
4	+	-	-	-	-
18	+	+	+	+	+
22	+	+	+	+	+
30	+	+	+	+	+
35	+	+	-	+	+
40	-	NT	-	NT	-
Growth in NaCl %					
0	-	-	-	-	-
1.5	+	+	+	+	+

6	+	+	+	+	+
8	V	-	-	+	-
10	-	-	-	+	-
Production of:					
Alginase	-	-	-	-	-
Amylase	+	+	+	-	+
Arginine dihydrolase	V	-	-	+	+
Catalase	+	+	+	-	+
b-Galactosidase	+	-	-	-	+
Gelatinase	+	+	-	-	+
Lipase	+	+	+	-	+
Lysine decarboxylase	-	+	+	-	-
Nitrate reduction	+	+	+	+	+
Omithine decarboxylase	-	-	-	-	-
Hydrolysis of:					
Aesculin	+	-	+	-	-
Casein	+	-	+	+	V
Urea	-	-	+	-	-
Utilization of:					
Acetate	V	-	-	+	+
Aconitate	-	+	-	+	-
D-Alanine	+	+	-	+	+
L-Alanine	+	+	-	+	+
g-Aminobutyrate	+	-	-	+	+
Amygdalin	-	-	-	-	-
L-Arabinose	-	-	-	-	-
L-Aspartate	+	-	+	+	-
Cellobiose	+	+	+	-	+
Citrate	+	+	+	+	+
D-Fructose	+	+	+	+	+
D-Galactose	V	-	+	-	+
D-Galacturonate	-	-	-	-	-
D-Gluconate	+	-	-	+	+
a- D-Glucose	+	+	+	+	+
D-Glucuronate	-	-	-	-	-
L-Glutamate	V	-	+	+	+
Glycerol	+	+	+	+	+
Glycine	V	-	-	+	+
L-Histidine	-	-	-	+	+
b-Hydroxybutyrate	-	-	-	+	+
myo-Inositol	-	-	-	-	-
a-Ketoglutarate	+	+	-	+	+
DL-Lactate	+	+	-	+	+
Lactose	-	-	-	-	-
L-Leucine	-	-	-	+	-
DL-Malate	+	+	-	+	+
Maltose	+	+	+	+	+
D-Mannitol	+	+	+	-	+
D-Mannose	V	-	+	-	+
Melibiose	V	-	-	-	+
N-Acetyl-glucosamine	+	+	+	+	+

L-Ornithine	-	-	-	+	+
L-Proline	+	+	+	+	+
Propionate	+	+	-	+	-
Putrescine	-	-	-	+	+
Pyruvate	+	+	-	+	+
Quinate	V	-	-	-	+
L-Rhamnose	-	-	-	-	-
L-Serine	+	+	-	+	+
D-Sorbitol	-	-	-	-	-
Succinate	+	+	-	+	+
Sucrose	-	-	-	+	+
D-Trehalose	+	+	-	+	+
L-Threonine	+	+	-	+	+
Tween-40	+	+	+	+	+
Tween-80	+	+	-	-	+

Table 15. Differential characteristics of *V. alginolyticus* obtained with the API20NE (Results after incubation at 30C for 24-48h).

	API20 NE TEST No																				
Pathogen	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
V. alginolyticus	+	+	+	-	-	v	+	-	v	-	v	v	v	v	v	-	-	+	-	-	+

+, - and v correspond to >80, >20 and 21-79% of positive results, respectively.
1= b-galactosidase, 2= arginine dehydrolase, 3= lysine decarboxylase, 4= ornithine decarboxylase, 5= citrate utilization, 6= H2S production, 7= urease production, 8= tryptophan deaminase, 9= indole production, 10= Vogues Proskauer reaction, 11= gelatine hydrolysis, 12= acid from glucose, 13= acid from mannitol, 14= acid from inositol, 15= acid from sorbitol, 16= acid from rhamnose, 17= acid from sucrose, 18= acid from melibiose, 19= acid from arabinose, 20= oxidase production.

Table 16. Characteristics of Vibrio species by BIOLOG-GN (modified after Austin and Austin 2007).

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
V. alginolyticus	-	+	+	+	+	v	-	+	-	-	v	-	-	+	-	-	+	+	-	-
V. harveyi	-	+	v	+	+	-	+	+	-	-	-	+	-	+	-	+	-	+	-	-
V. vulnificus	-	-	v	+	-	v	v	+	-	-	-	+	-	+	-	v	+	+	-	-

+, - and v correspond to >80, >20 and 21-79% of positive results, respectively.
1= water, 2= a-cyclodextrin, 3= dextrin, 4= glycogen, 5= Tween 40, 6= Tween 80, 7= N-acetyl-D-galactosamine, 8= N-acetyl-D-glucosamine, 9= adonitol, 10= L-arabinose, 11=D-arabitol, 12= cellobiose, 13= i-erythritol, 14= D-fructose, 15= L-fucose, 16= D-galactose, 17= gentiobiose, 18= a-D-glucose, 19= m-inositol, 20= a-lactose.

Table 16. continued

Species	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
V. alginolyticus	-	+	+	+	-	v	+	-	-	+	+	+	v	-	+	+	+	-	-	V
V. harveyi	-	+	+	+	-	-	+	-	-	v	+	+	-	-	+	-	-	-	-	-
V. vulnificus	-	+	v	+	-	v	+	-	-	v	v	+	-	-	+	v	v	-	-	-

+, - and v correspond to >80, >20 and 21-79% of positive results, respectively.

21= lactulose, 22= maltose, 23= D-mannitol, 24= D-mannose, 25= D-melibiose, 26= b-methyl glucoside, 27= psicose, 28= D-raffinose, 29= L-rhamnose, 30= D-sorbitol, 31= sucrose, 32= D-trehalose, 33= turanose, 34= xylitol, 35= methyl pyruvate, 36= mono-methyl-succinate, 37= acetic acid, 38= cis-aconitic acid, 39= citric acid, 40= formic acid

Table 16. continued

Species	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
V. alginolyticus	-	-	+	-	-	v	-	-	-	-	v	v	-	+	-	+	-	-	-	+
V. harveyi	-	-	+	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
V. vulnificus	-	-	v	-	-	-	-	-	-	-	-	-	-	v	-	-	-	-	-	+

+, - and v correspond to >80, >20 and 21-79% of positive results, respectively.
41= D-galactonic acid lactone, 42= D-galacturonic acid, 43= D-gluconic acid, 44= D-glucosaminic acid, 45= D-glucuronic acid, 46= a-hydroxy butyric acid, 47= b-hydroxy butyric acid, 48= b-hydroxy butyric acid, 49= p-hydroxy phenylacetic acid, 50= itaconic acid, 51= a-keto-butyric acid, 52= a-keto-glutaric acid, 53= a-keto-valeric acid, 54= D,L-lactic acid, 55= malonic acid, 56= propionic acid, 57= quinic acid, 58= D-saccharic acid, 59= sebacic acid, 60= succinic acid

Table 16. continued

Species	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
V. alginolyticus	v	+	-	v	+	+	+	+	+	+	+	+	+	v	v	v	-	+	-	V
V. harveyi	-	-	v	-	-	+	+	-	+	+	+	V	-	-	-	-	-	-	-	+
V. vulnificus	-	v	-	-	-	v	v	v	v	+	v	v	-	-	-	-	-	-	-	-

+, - and v correspond to >80, >20 and 21-79% of positive results, respectively.
61= bromosuccinic acid, 62= succinamic acid, 63= glucuronamide, 64= alaninamide, 65= D-alanine, 66= L-alanine, 67= L-alanyl-glycine, 68= L-asparagine, 69= L-aspartic acid, 70= L-glutamic acid, 71= glycyl-L-aspartic acid, 72= glycyl-L-glutamic acid, 73= L-histidine, 74= hydroxyl-L-proline, 75= L-leucine, 76= L-ornithine, 77= L-phenyl-alanine, 78= L-proline, 79= L-pyroglutamic acid, 80= D-serine

Table 16. continued

Species	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96
V. alginolyticus	+	+	-	-	+	+	+	+	v	-	v	-	+	v	+	+
V. harveyi	+	+	+	+	-	+	+	+	-	-	-	-	+	+	+	+
V. vulnificus	v	-	-	-	v	+	+	v	-	-	-	-	v	-	v	v

+, - and v correspond to >80, >20 and 21-79% of positive results, respectively.
81= L-serine, 82= L-threonine, 83= D,L-carnitine, 84= g-aminobutyric acid, 85= urocanic acid, 86= inosine, 87= uridine, 88= thymidine, 89= phenylethylamine, 90= putrescine, 91= 2-aminoethanol, 92= 2,3 butanediol, 93= glycerol, 94= D,L-a-glycerol phosphate, 95= plucose-1-phosphate, 96= glucose-6-phosphate

There are immunological markers specific to the different serotypes of *L. anguillarum*, *V. alginolyticus* ATCC 17749, *V. fischeri* NCIMB 1274, *V. anguillarum*

O2 CECT 522, *V. anguillarum* O1 R82 and *V. anguillarum* O3 6064A (polyclonal antibodies) and monoclonal antibodies to *V. alginolyticus, V. carchariae, V. harveyi, V. ordalii* and *V. vulnificus* and based on such markers all immunological identification methods ELISA, western blot analysis, immunofluorescence and immunohistochemistry can be used.

There are also molecular markers and the use of techniques such as AFLP, DNA:DNA hybridization, and 16S rDNA sequencing, as mentioned above, have been widely used in the systematics of Vibrio.

Mortality: In the hyperacute form can reach 80%. In the acute form, mortality rates of up to 40% of the affected population have been observed. Usually, however, in cases that occur in Greece, even if antibiotics are administered early, mortality ranges between 10 and 20%.

Transmission of the disease: Horizontally through the water from infected wild fish, equipment, from pathogens surviving in the bottom sediment under the fish cages and from invertebrate organisms of the water. At the establishment of the disease, transmission occurs from diseased to healthy fish.

Prevention: The disease is endemic, pathogenic microorganisms are present everywhere in the marine environment and therefore there is no reason to avoid the entry of the pathogen into a farm. Its manifestation is directly related to factors that cause deterioration of the qualitative environmental characteristics in the breeding area and stress on fish. Thus, they should be avoided and the general principles of prevention mentioned in a previous section of the Notes.

Experimentally, the following have been found:

Vibrio harveyi

A bivalent vaccine containing formalin-inactivated whole cells and extracellular products of *Vibrio harveyi* and *Ph. damselae* subsp. *piscicida* provided protection of up to 88% for 4 months to experimental Vibrio *harveyi* infection.

Vibrio ordalii

The LPS of this bacterium has given good signs of antigenicity and protection. Bivalent vaccines with *Vibrio ordalii* and *L. anguillarum* are commercially available.

Vibrio vulnificus

There are various reports of vaccine mixtures containing capsule antigens and toxins of the bacterium and both serotypes, R & D with very good results in terms of

immune response and protection after experimental infection. But there are no vaccines on the market.

Vaccines containing the serotypes O1, O2a, or O1 biotype I, O2 biotype I, O2 biotype II of *Listonella (Vibrio) anguillarum*, which are multivalent and may also contain the pathogen *Photobacterium damsela* subsp. *piscicida*. are marketed. All these products contain formalin-inactivated whole cells of bacterial species. One of these multivalent vaccines contains whole cells of *V. ordalii*.

In conclusion, there are no vaccines on the market (at least in Greece) against the other species of Vibrio that are increasingly isolated from fish vibriosis.

This creates a significant gap. Although some of the antigens between Vibrio are common and so a vaccine for one species may provide some relative protection for another species, this is not enough to protect farmed fish from new challenges.

The prevalence of increasingly non-classical vibrio in relation to classical species (i.e. *L. anguillarum* and *V. ordalii*) as causative agents of vibriosis in marine farmed fish is probably the result of the long-term and effective application of vaccinations against vibriosis due to classical species. The fish were not diseased, the disease was not maintained or evolved, resulting in the reduction of the dynamics of classic Vibrio. This, however, caused the observed flowering and evolution of species newly described from diseases.

Therefore, development in the fight against vibriosis must include the development of vaccines for the newly involved species as well.

Treatment: Antibiotic administration is usually effective. However, the emergence of resistant strains requires an antibiogram before treatment and the alternating use of antibiotics in repeated cases. Usually the disease relapses.

Antibiotics to be used must have published maximum residue levels (MRLs) in fish (Reg. EC 542/2008).

From the published data of various researchers, Oxolinic acid and Flumequine are antibiotics to which most species of marine pathogens Vibrio are sensitive.

Photobacteriosis of fish

Photobacteriosis of fish, originally called pasteurellosis and attributed to the bacterium *Pasteurella piscicida*, has been described and researched for more than 5

decades. Reports of new fish species showing the disease and new localization areas in the Northern or Southern Temperate zone are common in the literature.
The disease once it appears in an area becomes endemic, impossible to eradicate and is almost always associated with fish farming activities.

History

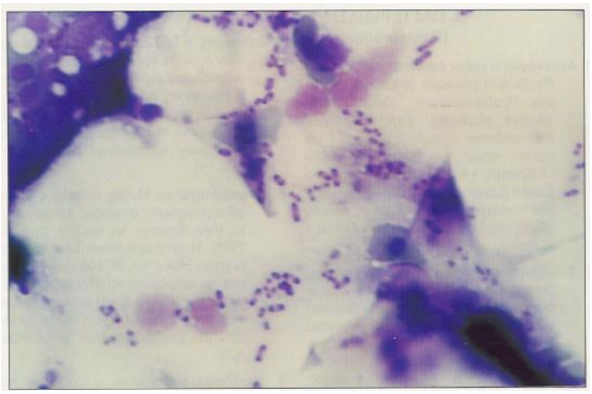
Photobacteriosis was first reported in 1990 in Italy (as far as Europe is concerned because cases of the disease had previously been described from Japan) and affected sea bass, mullet, sea bream, atherina, pagrus and sole. Similar fish species were reported in France, Greece and Spain in the following years. In 1995 the disease was described in Turkey and Israel and in 1997 in Malta.

Sensitive species

Species from which the bacterium has been isolated include: yellowtail (*Seriola quinqueradiata*), mullet (*Mugil cephalus*), beetle (*Acanthopagrus schlegeli*), Japanese porgy (*Pagrus major*), Japanese ayu (*Plecoglossus altivelis*), Japanese grouper (*Epinephelus akaara*), sea bass (*Dicentrarchus labrax*), Atlantic sole (*Solea solea*), sea bream (*Sparus aurata*), sea bream (*Atherina buoyeri*), turbot (*Scophthalmus maximus*), muskrat (*Diplodus puntazzo*), sea bream (*Diplodus sargus sargus*), sea bream (*Dentex dentex*), Atlantic sea bream (*Pagrus auriga*), Senegalese sole (*Solea senegalensis*), amberjack (*Seriola dumerili*), bluefin tuna (*Thunnus thynnus*).

Aetiology

The causative agent of photobacteriosis is a gram-negative bacterium, non-motile, showing bipolar staining and polymorphism from cocci to long rods and filamentous forms. For many years this bacterium was called *Pasteurella piscicida* but after DNA homology studies in 1995, it was proved to belong to the genus Photobacteria, the species *damsela* and because it is ichthyophonic, was named *piscicida* to distinguish it from *Photobacterium damsela* which is an environmental bacterium. Thus, the species that causes the disease in fish was called *Photobacterium damsela* subspecies *piscicida*, and for short, Phdp.



Pict. 18. Spleen smear – bacteria Pasteurella piscicida with obvious bipolar staining (Stain May Grunwald – Giemsa x 1000).

Laboratory characteristics

Requirements for development

The bacterium can grow in many simple bacteriological culture materials as long as salt is administered at the optimal rate of 2%. Often for the first isolation blood agar is used, but it is not necessary and materials such as tryptone soya, marine and brain heart infusion with a final content of 2% NaCl, have proven very useful. Special mixtures with interesting results, such as yeast extract and fish peptone, have also been used in the cultivation materials. The best growth is achieved at a temperature of 22^{o}C and neutral pH.

Morphological and biochemical characteristics

In addition to the morphological features mentioned above, it has been established that there is a polysaccharide capsule that surrounds the bacterium and that may interfere with its protection from the defence mechanisms of fish. This polysaccharide capsule is not related to the lipopolysaccharide of the bacterial cell and has been established by electron microscopy. Although it was initially thought that its production was related to the presence of glucose in the culture material, more recent data point to the fact that the capsule is also produced in materials that do not necessarily have glucose and that its production is related to how pathogenic and freshly isolated the strain of the bacterium.

Fish photobacterium is a relatively inactive biochemical bacterium. Using Biomerieux's API 20E system, a reaction is observed only in the arginine hydrolase test and in aerobic and anaerobic fermentation of glucose. The use of conventional tests (particularly to determine the use of other carbohydrates) revealed that the bacterium can also use the sugars galactose, mannose and fructose and that it is positive in oxidase testing (but not always – strong reactions are associated with having large amounts of cytochrome c).

The phenotype of photobacterium

The phenotype of bacteria is mainly analyzed by electrophoresis and concerns total bacterial cells, extracellular membranes, enzymes and extracellular products and lipopolysaccharide.

Comparison of the phenotypes of whole cell proteins and extracellular membrane and lipopolysaccharide of different strains isolated from different fish species and

geographical areas, revealed that there is a phenotypic homogeneity regardless of species and geographical area.

The extracellular products and enzymes produced by the bacterium have been analyzed and compared for many strains. Most extracellular products have a lipopolysaccharide nature and only a few protein molecules have been identified. Using Biomerieux's API ZYM system, enzyme reactions such as acid and alkaline phosphatases, esterases, lipases, caseinases and amylases were identified, but heterogeneity between strains was also found. In addition, pure extracellular products of the bacterium have been found to be toxic to fish and fish cell lines and to have haemolytic properties.

The pathogen produces additional proteins in its extracellular membrane in response to iron deficiency in the culture environment, while the production of siderophore substances is not clear. Under such conditions, changes in the enzymatic activities of extracellular products were observed.

Serotypes-antigenic types

Various serological studies have shown that there is a homogeneity in terms of antigenic characteristics of the pathogen regardless of the species and geographical area isolated. The most pronounced reactions to the sera produced involved the bacterium's lipopolysaccharide, but no different serotypes were found. However, two different ribotypes for the bacterium have been found using RNA markers that could, if expressed, have antigenic implications for the existence of two serotypes.

Epidemiology

Sensitive organisms

There is a negative relationship between the age of the fish and the disease. Young fish are more sensitive than older ones. Some fish species appear to be more susceptible to the bacterium than others, but there is no comprehensive study with the same methods that could be reported. In general, however, the LD50 for Phdp ranges between 10^3 and 10^6 cells/ml for intraperitoneal injection and 10^5-10^6 cells/ml for immersion.

Seasons of appearance

Generally, the disease occurs from late spring to mid-autumn at temperatures of 18-29^{o}C. However, outbreaks of the disease have also been reported at lower temperatures (15^{o}C).

Sources and reservoirs of infection

Although water has been implicated as a reservoir of the pathogen, it has been found that the bacterium does not survive in water for more than 4-6 days. However, it can survive in benthic sediments for up to 12 days depending on the type of benthic microcosm. However, longer survival times in benthic sediments or surfaces in nutrient-rich areas, such as near aquaculture farms, cannot be excluded. In addition, forms of the bacterium that are alive but not cultivable and in a state of "hypnosis" have been reported.

Since the disease is endemic, as is the case in Mediterranean aquaculture, we must consider that the environment with the living beings present in it is polluted and can contaminate sensitive organisms at any time.

Incubation period

The incubation period varies and depends on the dose of the bacterium, its pathogenesis, the species and age of the fish, and environmental conditions. For example, sea bream 4 and 20g and turbot 5g died after 2-3, 3-5 and 1 day after intraperitoneal infection, respectively, with $5x10^7$ Phdp cells at 18-20^oC.

Pathogenesis of photobacteriosis

In general, the pathogenesis of a bacterial infection involves a complex series of biochemical interactions between the pathogen and the host. The outcome of an infection depends on a combination of factors including the pathogen's virulence, the state of the host's immune system, and its internal defences.

Important stages in the pathogenesis of bacteria and their virulence are the ability of the invading bacterium to attach to and enter the host, multiply rapidly *in vivo* overcoming both non-specific and specific defence mechanisms of the host causing disease and damage to the host.

Attachment and entry into the host

Phdp exhibits a strong ability to adhere to the gut of natural hosts, but this is not due to fibrils or other components of the extracellular membrane of the bacterium, nor to protein molecules. Because the bacterium's lipopolysaccharide is not involved, a candidate molecule that could be involved is the bacterium's polysaccharide capsule. Unfortunately, there is no study on the possibility of adhesion of the pathogen to the gills of natural hosts.

Invasion and multiplication in the host

After experimental infection and attachment of the pathogen to the skin, gills and epithelium of the mouth, rapid spread and multiplication is observed in almost all internal organs. The tissues of the gastrointestinal tract are not the target of the bacterium for proliferation as much as the gills, liver, spleen, kidneys and heart. In another study, within 10 hours after infection by immersion, bacteria could be found in the heart, spleen and gills, and by day 2 the pathogen was visible in all organs except the brain. In conclusion, in sea bass, after the entry the pathogen is localized and multiplied in the spleen and kidneys, and then it is transferred through the bloodstream to all other organs.

Many bacteria enter the host's cells to survive defence mechanisms, and this has been found for photobacterium. Phdp enters host macrophages where not only is resistant to lytic enzymes, but also it proliferates and eventual ruptures the macrophage and bacterial cells are released.

Clinical symptoms & pathology

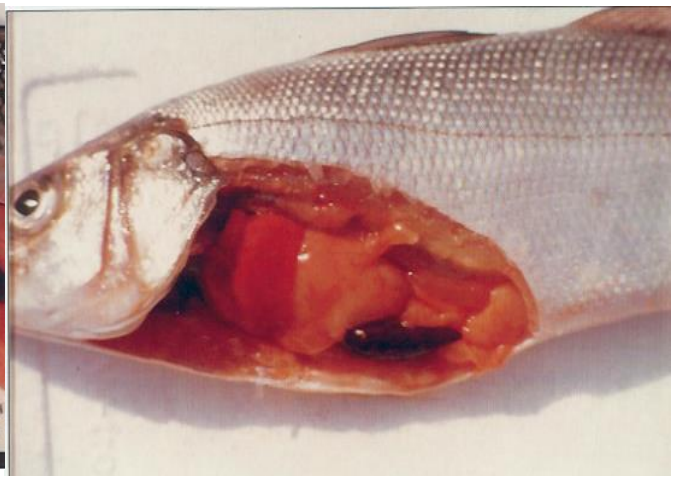
Clinical signs

Photobacteriosis is a septic disease and therefore has no characteristic symptomatology that differentiates it from other fish septic diseases. The hyperacute, acute and chronic forms have been described.

In the hyperacute form, only sudden deaths are observed without any other symptoms. In the acute form, discoloration or dark pigmentation and anorexia and swelling of the abdominal cavity and, of course, mortality are observed. In the chronic or subacute form, a continuous low mortality is observed usually in older individuals or in fish that survived the acute form of the disease.

Pathological & histopathological findings

Pict. 19. Pasteurellosis in sea bass – focal gill necrosis and splenomegaly.



Pict. 20. Pasteurellosis in sea bass – liver hyperemia and splenomegaly.

In the acute form, pale liver and kidneys, enlargement of the spleen and kidneys are observed, microerythemas and petechiae in organs and tissues and sometimes white nodules in the spleen, 1-2mm. These nodules characterize the chronic form of the disease.

Histopathologically, during the acute phase, necrosis in the spleen and kidneys, infiltration by erythrocytes, bacterial aggregates that clog capillaries and bacteria inside and outside macrophages are observed. The characteristic nodules in the spleen observed in the chronic form of the disease are granulomatous lesions consisting of bacteria, epithelioid cells, macrophages in various degenerative stages and fibroblasts.

Diagnosis

The diagnosis is based on the isolation and identification of photobacterium from the spleen, kidneys and liver of diseased fish using routine bacteriological methods. The presence of whitish nodules in the spleen is considered pathognomic of the disease, but these nodules are observed only in chronic and rarely in acute forms of the disease.

Differential diagnosis

Photobacteria usually need to be differentiated usually from Vibrio and more recently from Aeromonas infections. The tables below provide a guide to differentiate Phdp from other bacteria with similar morphological, physiological and biochemical characteristics. However, it should be emphasized that characteristics such as motility,

bipolar staining, colony morphology and others may vary depending on growing conditions.

Table 17. Biochemical characteristics of Vibrio and Pasteurella spp. grown at 25oC for 48h on API 20E test strips and Baumann's media (Kent 1982).

Species	*Ph. Damsel* subsp. *piscicida*	*P. plecoglosaccida*	Vibrio (atypical)	*V. anguillarum*	*V. ordalii*	*V. alginolyticus*	*V. parahaemolyticus*
Na+ requirement	+	+	+	+	+	+	+
Oxidase	+	+	+	+	+	+	+
O/129	+	n.r.	+	+	+	-	-
Penicillin	+	n.r.	-	-	-	-	-
Novobiocin	+	n.r.	+	+	+	+	+
NO3-NO2	-	-	+	+	+	+	+
ONPG	-	n.r.	-	+	-	-	-
Arginine dehydrolase	+	-	-	V	-	-	-
Lysine decarboxylase	-	-	-	-	-	+	+
Ornithine decarboxylase	-	-	-	-	-	+	+
Citrate utilisation	-	n.r.	-	+	-	+	+
Urease	-	n.r.	-	-	-	-	-
Tryptophan deaminase	-	n.r.	-	-	-	-	-
Indole	-	-	+	V	-	+	+
Vogues-Proskaer	-	-	-	+	-	+	-
Gelatinase	-	-	-	+	+	+	+
Acid from: Glucose	Weak +	+	+	+	+	+	+
Mannitol	-	-	+	+	+	+	+
Inositol	-	n.r.	-	-	-	-	-
Sorbitol	-	n.r.	+	+	-	-	-
Rhamnose	-	n.r.	-	-	-	-	-
Sucrose	-	+	+	+	+	+	-
Melibiose	-	-	-	-	-	-	-
Amygdalin	-	n.r.	-	+	-	-	-
Arabinose	-	-	-	V	-	-	+
Growth at 40oC	-	-	-	-	-	+	+

+ : positive , - : negative, V : variable, n.r. : not reported

Table 18. Characteristics useful for the identification of piscine Pasteurella spp. and Vibrio obtainable using the API 20E system (Kent 1982).

Species	*P. piscicida*	*P. plecoglosacida*	Vibrio (atypical)	*V. anguillarum*	*V. ordalii*	*V. alginolyticus*	V. parahaemolyticus
Gelatinase	-	-	-	+	+	+	+
Sorbitol	-	-	+	V	-	-	-
Sucrose	V	+	+	+	+	+	-
Arabinose	-	n.r.	-	V	-	-	+
V-P	-	-	-	+	-	+	-
Penicillin	+	n.r.	-	-	-	-	-
Motility at 20-25oC	-	-	+	+	+	+	+
Growth at 40oC	-	-	-	-	-	+	+

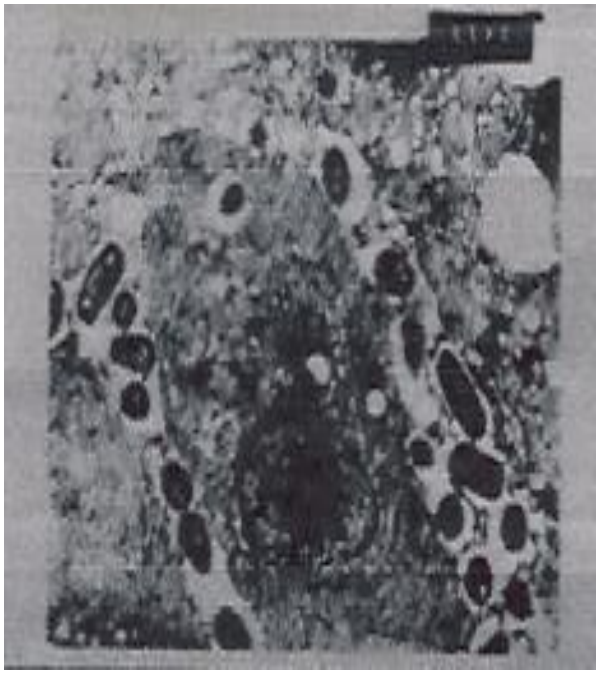
Oxidase	+	+	+	+	+	+	+

+ : positive , - : negative, V : variable, n.r. : not reported

The use of Biomerieux's API 20E is widespread, but the fact that the bacterium reacts only to the arginine dehydrolase test and the use of glucose, makes the use of this test unprofitable. It is therefore preferable to use conventional methods. However, the definitive identification of the pathogen cannot be based only on its biochemical characteristics.

Final identification

Definitive identification can only be done using specific antibodies against Phdp or specific DNA or RNA markers. On the market there are both polyclonal sera and monoclonal antibodies and industrialized tests for the identification of the pathogen using various serological methods such as ELISA or its variants, immunofluorescence, etc. Molecular markers can be used in PCR, immuno-PCR and other molecular techniques. The only requirement from all indicators is to be specific to the pathogenic photobacterium of fish.



Pict. 21. Live bacteria inside a leukocyte (no degeneration of bacteria is observed). EM magnification x 18,000

Pict. 22. *Sea bass macrophage with phagocytosed a bacterial cell Pasteurella piscicida. The microorganism is in a lysosome. EM magnification X 22,000.*

Therapy

The treatment is applied only when the pathogen has been isolated and an antibiogram has been made.

There are dozens of reports on the sensitivity or resistance of photobacteria to antibiotics. As with Vibrio, the only antibiotics that can be given are those for which MRLs have been established.

For flumequine, a dose of 10mg/Kg live weight for 10 days at a temperature of 19-20^{o}C in sea bream is considered a good treatment regime. For oxolinic acid the dose is slightly higher, 15-20mg/Kg live weight.

Prevention

Since the disease is endemic throughout the Mediterranean, we cannot talk about avoiding the entry of the microorganism into the farming environment or avoiding contact of fish with the bacterium.

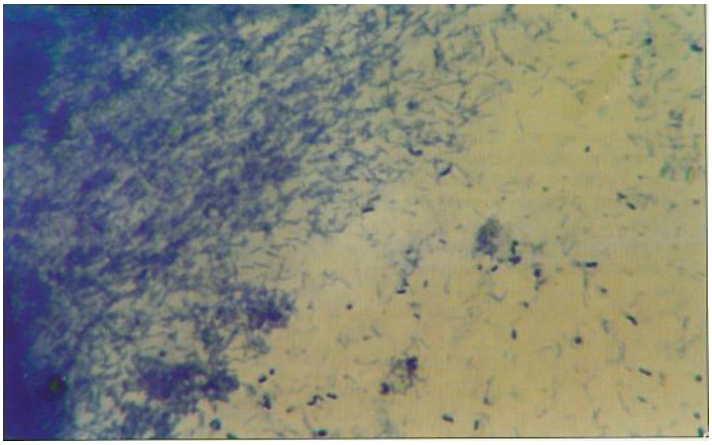
General prevention measures, as mentioned above, should be observed to avoid an increase in the number of bacteria in the environment and the entry of a more virulent strain of the bacterium into a farm.

Therefore, hygiene rules should be observed for anything that enters the unit, quarantine and preventive baths with antibiotics for new fish, hygiene of employees, avoidance of movement from "dirty" to "clean" areas of the farm, etc. Especially these measures should be taken for hatchery facilities where the young age of the fish maintained makes them much more susceptible to attacks by the bacterium. In addition, due to the fact that hatcheries are usually terrestrial facilities, the implementation of hygiene rules and avoidance of entry of the bacterium is easier than in floating facilities.

These are passive prevention measures. Active prevention is the one that shields fish against the bacterium and can concern both the strengthening of the innate immune system of fish by administering immunostimulants, and stimulating the adaptive immune system of fish by conducting vaccinations.
Studies on the production and application of inoculant mixtures in various species of sensitive fish are numerous. Whole bacterial cells, broken down cells, lipopolysaccharide, polysaccharide capsule, proteins of the outer membrane, extracellular products of the bacterium after inactivation with formalin or heating alone and in any combination that can be done have been used and have been found to provide immunity to vaccinated fish according to some researchers small and others greater, but which is not maintained for a long time.
It is important to know that unlike Vibrio where immunity is based on antibodies against their lipopolysaccharide and outer membrane components, immunity against Phdp is mainly based on proteins and extracellular products. In addition, while in Vibrio it is not necessary to significantly involve cellular immunity (lipopolysaccharide = T-non-dependent), in Phdp this is required (photobacterium survives within macrophages). Another factor affecting the success or failure of a vaccine mixture is whether the pathogenic bacterium used in the vaccine expresses antigens that are also expressed naturally.
Vaccines against photobacterium are available on the market alone or in combination with Vibrio.

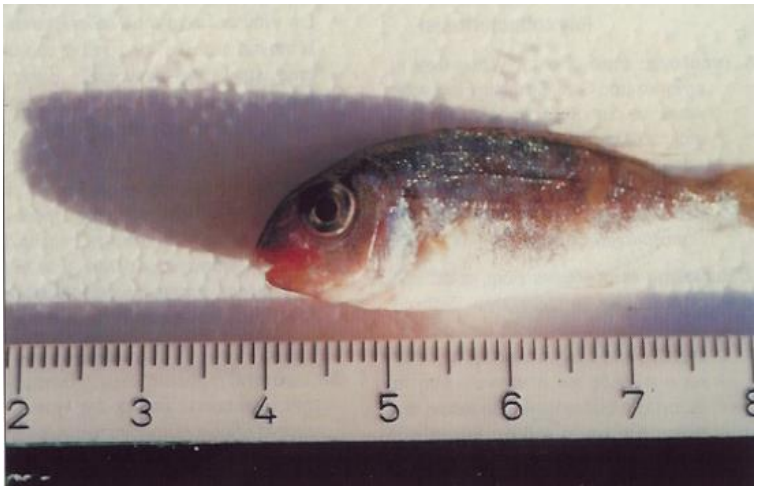
Myxobacteriosis

Aetiology: The main cause of the disease is the gram-negative bacterium *Flexibacter maritimus* (Figure 23) which is now called *Tenacibaculum maritimum*. Other related species of microorganisms involved in causing skin lesions in widely cultivated species, such as Flavobacterium spp., are also reported in the literature.



Pict. 23. Myxobacteria in gill smear (Methylene blue x 400 staining)

Sensitive species: turbot, sole and Atlantic salmon.
Mediterranean species, sea bream and sea bass, are considered sensitive.
Temperature of onset of the disease: The disease occurs throughout the year in a wide temperature range. In Greece it has been observed that, especially for newly introduced bass juveniles, the most critical period is from April to June.
Clinical symptoms: Ulcerative skin lesions at various locations of the body as well as necrosis of the fins and tail. In juveniles, redness of the mouth is observed (Figure 24), which can even lead to erosion of the jaws. Often lesions are coated by a layer of viscous yellowish mucus. In some cases it is possible to observe focal necrosis on the gills.



Pict. 24. Redness of the mouth in juvenile sea bream from myxobacteria.

Diagnosis: By observing fresh smears from lesions and the presence of elongated bacilli. It is possible to isolate the microorganism and on special selective substrates.
Mortality: Quite high. In newly introduced bass juveniles, mortality reaches up to 10-20% of the affected fish population, as it has been reported.
Transmission: Unknown. Between fish it is transmitted through water.
Prevention: The onset of the disease is intertwined with the existence of factors that cause stress. Avoiding the latter and reducing the causes that can cause injuries can help prevent the disease.
Treatment: The administration of antibiotics either with bath or food gives satisfactory results.

DISEASES DUE TO PARASITES

ECTOPARASITES

Protozoa

Amyloodinium ocellatum - Amylodiniasis

Sensitive species: The parasite has been identified in many species. For Mediterranean cultivation, the infection of sea bream and sea bass is important.

Manifestation temperature: >20ºC.

Clinical signs: Weight loss, swimming disturbance and loss of orientation. Grayish lesions on the lateral walls of the body and gills with or without local bleeding and with respiratory problems. In pronounced parasitosis, increased hyperplasia of the gills with bleeding and necrosis and high mortality are observed.

Diagnosis: Microscopic observation of oval trophozoites (diameter 150m) in fresh skin or gill preparations (Figure 25).

Εικ. 25. Oodinium sop.

Transmission / Biological cycle: Direct from fish after cell division of trophozoites in the environment.

Prevention: None. There are signs of developing immunity after infection.

Treatment: Copper sulfate in the form of a continuous bath, for 12-14 days helps in the fight against forms of the parasite. Also bath lasting 3-5 minutes with fresh water helps to dispose of trophozoites from skin and gills.

Cryptobiosis

Etiology: It is due to the protozoan Cryptobia. The disease manifests itself mainly in two forms, depending on the presence of the parasite in the blood or gills. In rare cases the parasite can also be found in the liver, pancreas, gallbladder and gonads. The disease is of little interest to mariculture.

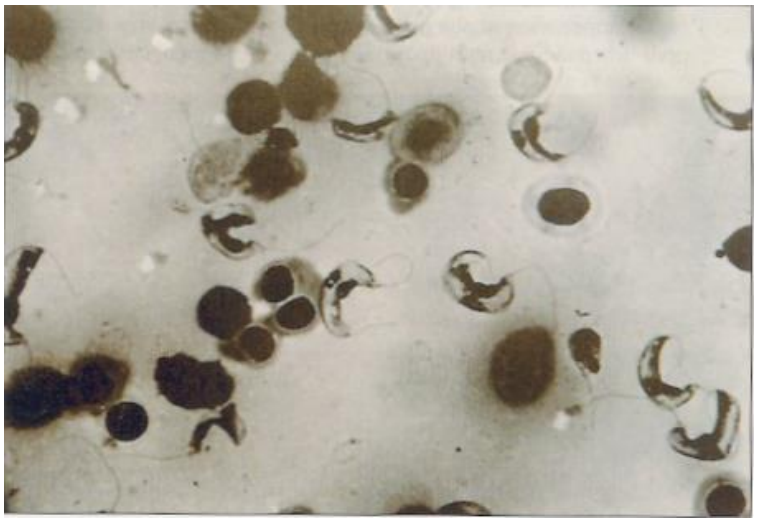
Blood form

Sensitive species: Infects many species of the family Sparidae. Farmed species affected are sea bream and sea bass.

Temperature of onset of the disease: There is no correlation with temperature.

Clinical symptoms: Anorexia, increase in mucus in the skin and local bleeding and thickening in the gills accompanied by general emaciation and high anemia. Mortality is low and chronic.

Diagnosis: Microscopic observation of parasites in fresh gill or mucus preparations, or in blood smears after Giemsa staining (Figure 26).



Εικ. 26. *Cryptobia sp. Blood smear.*

Transmission/biological cycle: The parasite is transmitted by leeches or similar marine organisms.

Prevention: Organisms responsible for the transmission of the parasite must be removed. There is no effective way of prevention.

Treatment: Bathing with formalin has proven effective.

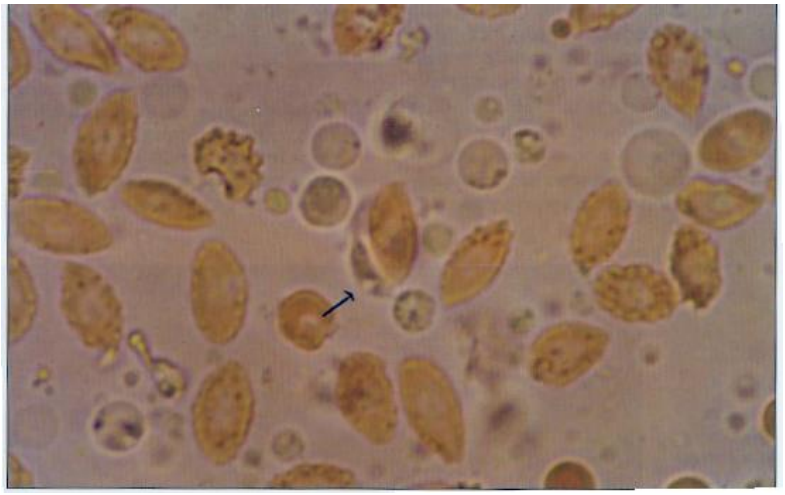
Gill form

Sensitive species: Sea bream and sea bass from the farmed species.

Temperature of onset of the disease: No specific temperature is reported.

Clinical signs: Shortness of breath, gill hyperplasia, inflammation of capillaries and thrombosis. Anorexia and darkness. Acute or chronic mortality ranging between 0.5-10%, depending on the parasitic load and breeding conditions.

Diagnosis: Microscopic observation of parasites in fresh gill or mucus preparations, or in smears stained with Giemsa (Figure 27).



Εικ. 27. *Cryptobia sp in gills (fresh smear).*

Transmission/biological cycle: From fish to fish.

Prevention: Good farming conditions combined with periodic formalin prophylactic baths.

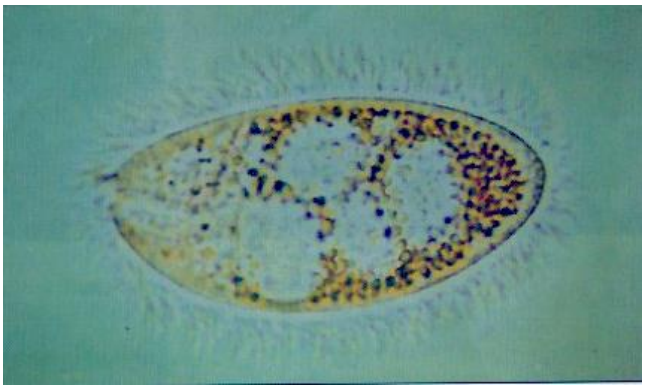
Treatment: Bathrooms with formalin.

Cryptocaryon irritans

Sensitive species: Many species of fish. Of the farmed species, it affects sea bream, amberjack and sea bass. In rearing conditions the problem is more pronounced in broodstock.

Disease onset temperature: >20ºC.

Clinical signs: Shortness of breath, emaciation, swimming disorders, loss of orientation. Small white lesions on the lateral walls of the body and gills with or without local bleeding and an increased amount of mucus. Often the lesions develop into ulcers resulting in secondary infections. Major mortality can be caused by shortness of breath and osmotic problems originating in the skin.



Εικ. 28. *Cryptocaryon sp. From gills*

Diagnosis: By microscopic observation of skin scrapings and mainly gills. The parasite has a cytosome and a large nucleus consisting of four spherical particles arranged in the shape of a horseshoe (Figure 28).
Transmission/biological cycle: Direct from fish to fish after multiple cell divisions of trophozoites/cysts in the environment. The final stage of divisions is mobile and affects the fish entering through the epidermis.
Prevention: Observance of good hygiene and rearing conditions.
Treatment: It should be done quickly after the disease is detected. A combination of malachite green / formalin is used with good results. Also effective are baths in water of reduced salinity.

Trichodiniasis

Aetiology: It is due to *Trichodina* spp. and concerns the skin and gills of fish.
Sensitive species: Parasitize on most fish. Their number on the skin and gills in healthy fish is small, while in weakened or immunosuppressed animals the infection progresses very quickly.
Temperature of manifestation of the disease: It does not depend on temperature.
Clinical signs: In healthy individuals parasites do not adhere to the surface of the body and do not cause serious problems except for an increase in mucus and grayish pigmentation of the skin. In weakened, young or immunosuppressed individuals, parasites grow rapidly on the skin, where they attach to epithelial cells. Mechanical damage caused by severe infections in the epithelium of the skin and disruption of osmotic homeostasis lead to large mortality.
Diagnosis: Microscopic observation of parasites in fresh skin and gill preparations.
Transmission/biological cycle: Direct from fish to fish, with contaminated tools, plants and live food.
Prevention: Observance of good hygiene conditions, especially in hatcheries.
Treatment: Baths with a combination of malachite green and formalin.

MONOGENEAN TREMATODES

Microcotyle sp., Lamellodiscus sp., Gyrodactylus sp., Furnestinia echeneis, Diplectanum sp., Serranicotyle sp, Zeuxapta seriolae.

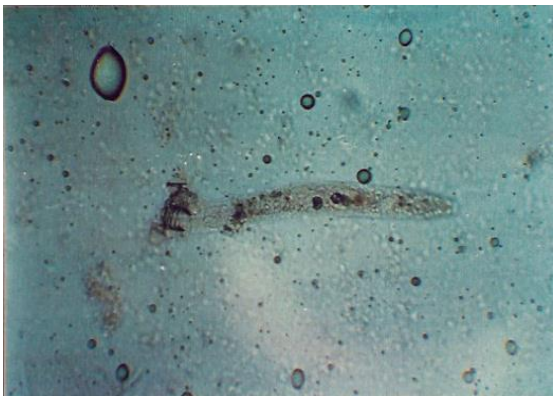
Sensitive species: Many species of fish are affected. Farmed fish been infested with are: sea bream, *Diplodus sargus*, sea bass, mullet, amberjack and red porgy.

Temperature of manifestation of the disease: The duration of hatching and maturation of parasite eggs decreases as the water temperature rises. At 20^{o}C it lasts 7 days and at 10^{o}C, 11-19 days. The biological cycle ranges from one to two months and depends on the temperature of the water. Usually outbreaks occur during the autumn or winter months and these are associated with substandard hygiene conditions.

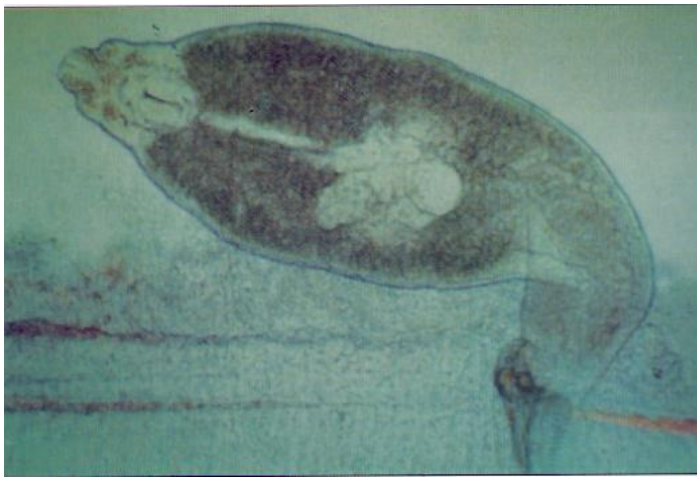
Clinical signs: Parasites irritate the gills, and the fish's organism reacts with abundant mucus production and hyperplasia of the epithelium. A large number of parasites cause suffocation and respiratory symptoms (dilatation of gill opercula, confluence of fish on the surface and in places with better oxygenation). Mortality ranges from 0.5-20% and can be very sudden. In chronic cases there is anemia and mortality is due to secondary bacterial Infections.

Diagnosis: Microscopic observation of parasites in fresh gill preparations or histological values (Figures 29, 30, 31).

Εικ. 29. Microcotyle sp. From gills.



Εικ. 30. Diplectanum sp. From gills.



Εικ. 31. Lamellodiscus sp. From gills – fresh specimen.

Transmission/biological cycle: Direct from fish to fish through the young hatching parasites that find the host. Eggs from adult individuals fall and hatch at the bottom.
Prevention: Parasitosis is associated with poor hygienic conditions, especially in hatcheries. In closed systems, eggs may be recycled if the filtration system is incomplete. Sterilization of incoming water, improvement of hygiene conditions and reduction of fish density help. In cages, systematic periodic parasitological examination of fish is needed to determine the increase in parasitic load and to carry out preventive treatment.
Treatment: Baths with formalin are the most effective. However, when the lesions caused to the fish are extensive, fish may be sensitive to treatment.

CRUSTACEANS

ISOPODS

Anilocra physodes, Nerocila orbignyi

Adult parasites are found mainly on the caudal fin, while nymphs (pulli II) are localized in the oral cavity and gills.
Sensitive species: They parasitize on many wild fish. The main host for *Nerocila* is cephaloid fish while for *Anilocra Salpa sarpa* and *Diplodus sargus*.
Temperature of onset of the disease: Usually when it is high, in summer.
Clinical signs: Parasites irritate the gills, mucus is produced and hyperplasia of the epithelium progresses. A large number of parasites cause suffocating phenomena. The larvae of the parasite seriously injure the epithelium, causing necrosis that are then

infected with bacteria (*Flexibacter*) (Figure 32). Mortality can exceed 20% in juvenile, especially sea bass.

Diagnosis: Direct observation of parasites (Figure 33).

Pict. 32. Lesions by pull II stages of the parasite Anilocra physodes in sea bass.

Transmission/biological cycle: Direct from fish to fish through water. The first stage of development of nymphs occurs inside an adult sac. The biological cycle of these isopods can be completed on the same fish.

Prevention: Difficult. Small diameter netting around fry cages can reduce the load. In areas where the parasite is endemic, the introduction of new fry should be planned accordingly.

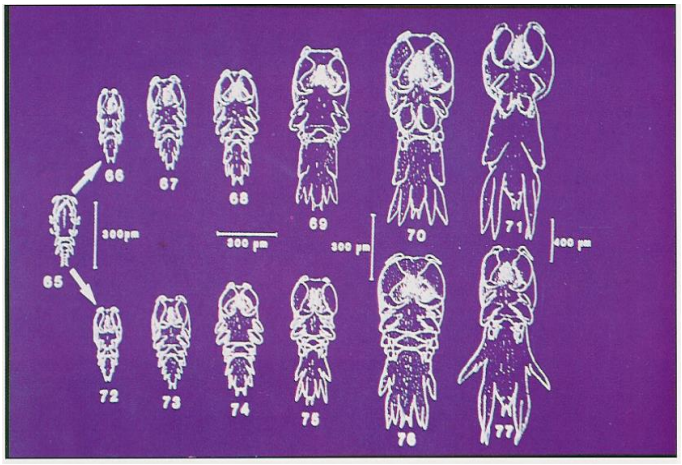
Treatment: Organophosphates had been used. Experimentally, administration of ivermectin with food gave good results. Also hydrogen peroxide.

Εικ. 33. *Anilocra physodes.*

COPEPODS

Ergasilus sp., Caligus sp., Lernathropus sp., Lernaeocera sp.

Sensitive species: Parasites are mainly found in the gills of fish and from farmed species mainly sea bream, sea bass and sharpsnout bass are parasitized.
Temperature of manifestation of the disease: Not related.
Clinical symptoms: Mucus production and hyperplasia of the gill epithelium that can lead to the manifestation of suffocating symptoms when parasitosis is intense. Especially the parasite *Lernathropus kroyeri* causes extensive damage to semi-intensive or intensive farming systems. Necrosis is then complicated with bacteria. Mortality can exceed 50% in bass less than 10 grams.



Εικ. 34. *Biological cycle of Lernathropus kroyeri.*

Diagnosis: Direct observation of parasites.
Transmission/biological cycle: Direct from fish to fish through water (Figure 34).
Prevention: Difficult. Small diameter netting around fry cages can reduce the load. In areas where the parasite is endemic, the introduction of new fry should be planned accordingly.
Treatment: Organophosphates and hydrogen peroxide. Experimentally, administration of ivermectin with food gave good results.

ENDOPARASITES

PROTOZOA

Microsporidia

Pleistophora spp.

Sensitive species: They parasitize on most fish, and each type of parasite is typically specific to certain fish species. Sea bream and halibut in particular are sensitive mainly to *P. senegalensis* (intestine). In sea bream muscles, a new species from the latter genus has been reported, as well as species of the genus *Glugea*.

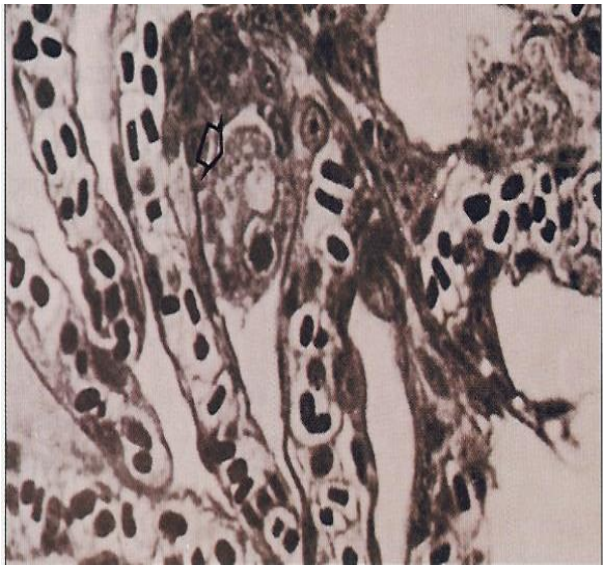
Temperature of manifestation of the disease: Not related.
Clinical symptoms: Mainly in the final stage of fattening, lesions are usually localized in the muscles where intracellular infection by parasites leads to hypertrophy of muscle cells which leads to rejection of fish for human consumption. In rarer cases, 50-gram juveniles are also affected. In some species of fish, parasites can be found in the wall of the intestine, forming white nodules that are filled with its characteristic piriform spores of the parasite. Mortality is usually small but depends on the type of parasite.
Diagnosis: Microscopic observation of the spores inside the nodules. Without staining, the seeds strongly refract light and have a greenish color. After Giemsa staining, the glomerulus is best observed, and at the other pole the vacuolar particle. The spores are also gram-positive and PAS.
Transmission/biological cycle: Direct by ingestion of spores.
Prevention: None.
Treatment: Long-term (1-3 months) treatment with fumagillin in food or with the new synthetic analogues (TNP-470) reduces mortality but muscle lesions do not disappear. It is important that diagnosis and treatment are made early because on the one hand the substance is more active in early stages of sporogenesis and on the other hand there are fewer lesions in the muscles.

RHIZOPODS

Paramoeba spp.

Sensitive species: Most species are affected and each parasite species is generally specific to certain fish species.
Temperature of onset of the disease: Parasites can be in the gills of fish at lower temperatures, but clinical signs appear at temperatures above 12-14^{o}C.
Clinical signs: Parasites are usually localized in the gills where they cause pronounced respiratory symptoms due to destruction of the gill filaments by hyperplasia of the respiratory epithelium. In acute forms, mortality is high if accompanied by mixed infection with monogeneans and anemia, increased mucus and grayish coloration by regions. The nutritional status of the fish is good. The chronic form presents with few symptoms and mainly lethargy and anorexia.

Diagnosis: Microscopic observation of parasites on fresh gill scrapings (Figure 35) may reveal the presence of parasites (20-30m), but the identification is very difficult and requires specialized techniques. Thus, the diagnosis is mainly histological with the characteristic image of extensive thickening and agglutination of secondary lamella with the formation of empty areas. Special staining techniques help identify pests.



Εικ. 35. *Amoeba-like parasite in gills (arrow). Histological section.*

Transmission / biological cycle: Direct from water and from fish to fish. Parasites are part of the seabed fauna and decomposing states of organic and plant matter and are found at temperatures of 12-14ºC and salinity above 32-35‰. Usually exacerbations of the disease occur in the first summer or spring after the transfer of juveniles to cages.

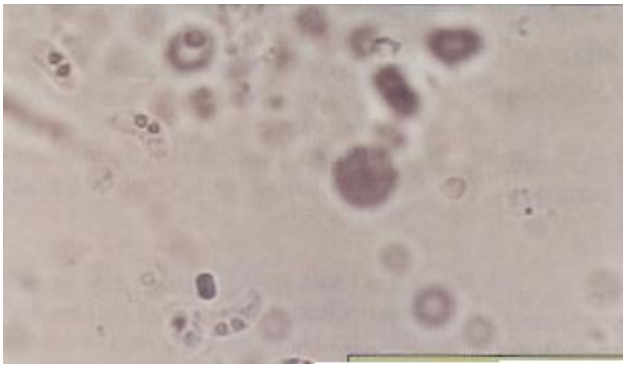
Prevention: Improvement of rearing conditions. Sometimes resistance to reinfection is observed.

Treatment: Baths with low salinity water (5‰) seem to act against osmotic stress in the gills. Hydrogen peroxide reduces the number of parasites or, depending on the dose, parasites are completely killed.

MYXOSPORIDIA

Ceratomyxa spp.

Sensitive species: Several species have been found in marine fish of the families Sparidae, Serranidae, Mugillidae etc. The most important for farmed fish are: *C.*



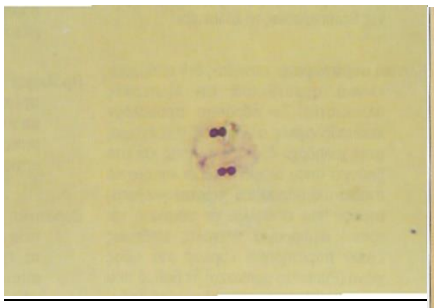
Pict. 36. Ceratomyxa labracis. Fresh preparation from bile.

sparaurati in sea bream and species of the genus *Pagrus*, *C. diplodae* in the species *Dentex*, *Puntazzo puntazzo* and sea bass and *C. labrakis* in sea bass.

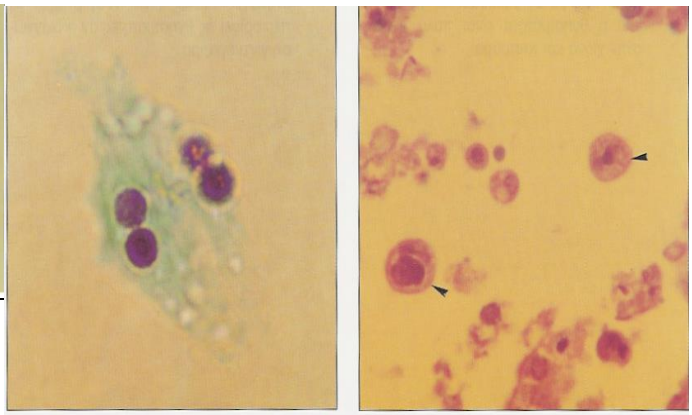
Temperature of onset of the disease: Not related. But they usually occur at low temperatures.

Clinical symptoms: There are no clinical signs and external lesions. Parasites cause histopathological lesions in the gallbladder mainly in larger fish and can spread to other organs in intense infection. In these cases, *C. sparaurati* infects the intestine and causes mortality in sea bream of both small and commercial size fish. In mixed infections with *C. diplodae* and *Myxidium leei* mortality is very high in sharpsnout bream and sea bream.

Diagnosis: Microscopic observation of parasites in fresh smears or organ contents where they parasitize (Figures 36, 37).



Pict. 36. Ceratomyxa labracis (two-seeded spore). Histological preparation from bile.



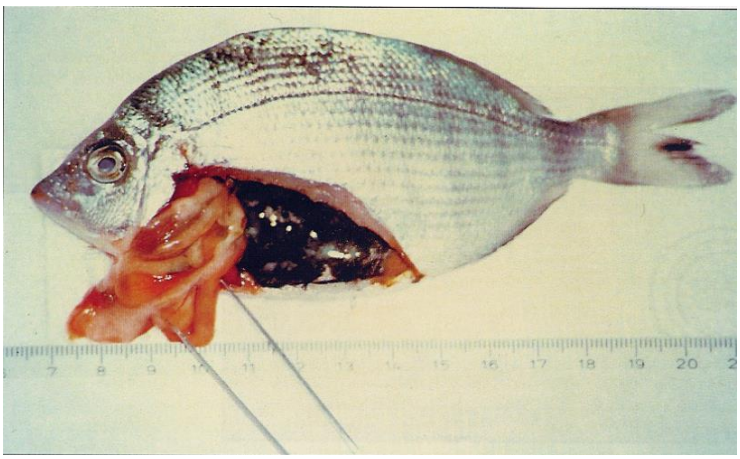
Pict. 37. Ceratomyxa labracis (two-spore cyst). Histological preparation from bile.

Transmission/biological cycle: Direct from fish to fish after ingestion of spores. The biological cycle of myxosporids of marine fish in general has not been ascertained, while there are bibliographic data indicating the possible existence of intermediate hosts (worms) where parasites carry a different form.

Prevention: None. In hatcheries should filter or sterilize incoming water.
Treatment: Long-term (1-3 months) treatment with fumagillin in food or with the new synthetic analogues (TNP-470) reduces mortality. It is important that diagnosis and treatment are made early because the substance is most active in early stages of sporogenesis.

***Myxidium leei* n. sp.**

Sensitive species: Sharpsnout bream, sea bream, *Diplodus sargus* and species of the genus Pagrus. The disease is of great interest for mariculture. In fact, the attacks on the newly farmed species sharpsnout bream were so great that they almost stopped any attempt to cultivate them.

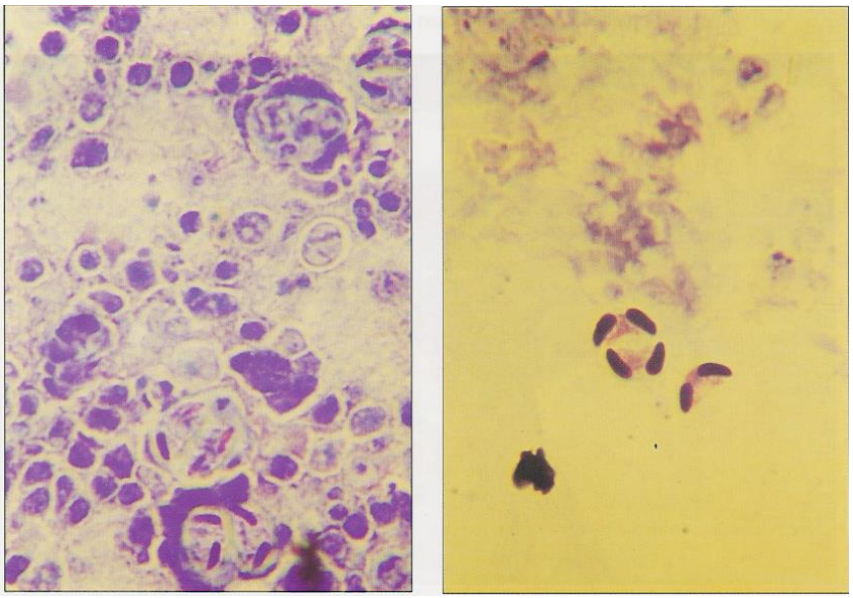


Pict. 38. Sharpsnout bream infected with Myxidium leei. Swelling of the intestine with cheesy contents (tip of forceps) is evident.

Temperature of the disease: It is not related, but cases are increased during the warm months. Recent studies have shown, however, that the development of the parasite essentially stops at a temperature of <15^{o}C.
Clinical signs: There are usually no clinical signs, except for emaciation, and external lesions. Parasites cause histopathological lesions in the intestine (Figure 38), gallbladder, liver and gills where mature spores and initial stages of the parasite cause destruction of the epithelium with necrosis, catarrhal enteritis and local bleeding. Dilatation of the bladder has also been observed, discoloration and loss of scales; Large epidemics have been observed in sharpsnout bream to the extent that they have made this species unprofitable for intensive cultivation. Losses can reach up to 70%, the disease is endemic and seems to be associated with poor nutrition (overfeeding

and high fat in food). In mixed infections with *C. diplodae* and *Myxidium leei* , mortality is very high in sharpsnout bream and sea bream.

Diagnosis: Microscopic observation of parasites in fresh swabs or organ contents where they parasitize (Figure 39).



Εικ. 39. *Various developmental stages of Myxidium leei.*

Transmission / biological cycle: In sea bream it has recently been shown to be direct, from fish to fish, after ingestion of the parasitic forms contained in fish excretions.

Prevention: None. In hatcheries incoming water should be filtered or sterilized. Frequent parasitological examinations should be performed to start treatment early.

Treatment: There is no satisfactory treatment, especially for sharpsnout bream. Long-term treatment with fumagillin in food or new analogues can reduce mortality if diagnosed early.

Sphaerospora spp.

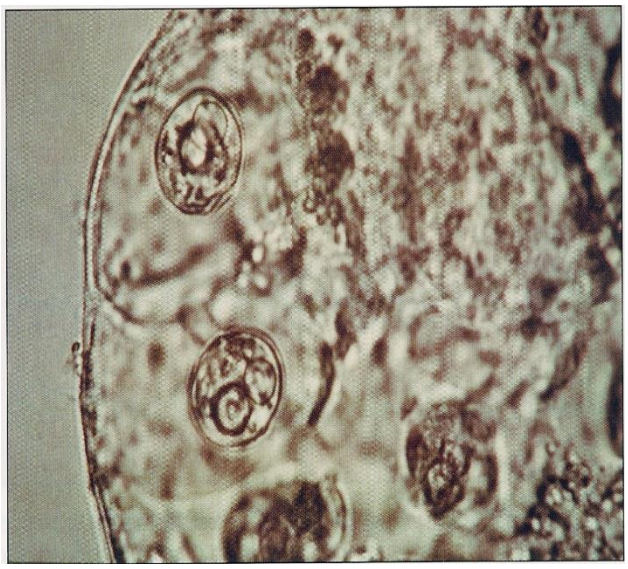
Sensitive species: Various species of parasites have been found in farmed fish. Two species have been found in sea bass: *S. dicentrarchi* and *S. testicularis*.

Temperature of manifestation of the disease: Not related.

Clinical signs: There are usually no clinical signs and external lesions. The histozoic parasite *S. dicentrarchi* parasitizes in the intestine, gallbladder, liver and gills where mature spores and the initial stages of the parasite do not cause serious lesions unless found in large numbers. The parasite *S. testicularis* is coilozoic, usually parasitizing on the gonads of fish without an inflammatory reaction, but when the infection is

heavy it can cause total destruction of the gonads. The clinical symptoms then are ascites, testicular hypertrophy with replacement of tissue by fibrous tissue and parasitic forms.

Diagnosis: Microscopic observation of parasites in fresh swabs or organ contents where they parasitize (Figure 40).



Pict. 40. Sphaerospora sp. in glomerulus of sea bream. Fresh preparation.

Transmission/biological cycle: Not established, rather direct.

Prevention: None. Preventive examination of broodstock before breeding and removal of infected ones.

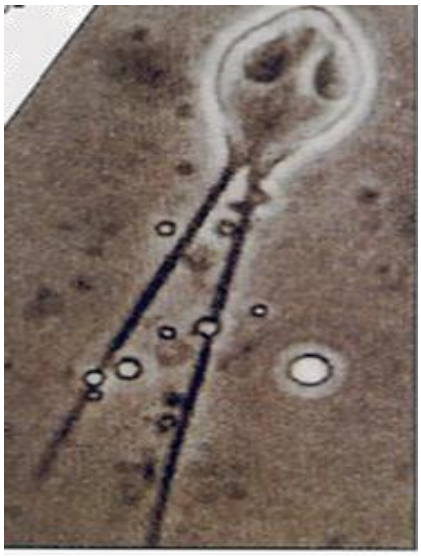
Treatment: There is no satisfactory treatment, especially in sharpsnout bream. Long-term treatment with fumagillin in food or new analogues can reduce mortality if diagnosed early.

Polysporoplasma sparis

It causes severe lesions in the kidney of sea bream in intensive and semi-intensive cultures.

Henneguya sp.

It is usually found in wild fish and mainly sea bream, in the gills. The



Pict. 41. Henneguya sp. Fresh preparation, Phase contrast.

morphology of the parasite is characteristic (Figure 41).

Kudoa sp.

Sensitive species and symptoms: It usually affects the kidney glomerulus and mesentery of sea bream, causing serious lesions.

Transmission / biological cycle: Contaminated wild fish are the main cause of infestation of farmed fish in cages. The biological cycle is unknown and there may be intermediate hosts as in freshwater fish (Figure 42).

Pict. 42. Kudoa sp. from sea bass muscles, Fresh preparation, Phase, contrast.

Prevention: None.

Treatment: There is no satisfactory cure. Long-term treatment with fumagillin in food or new analogues can reduce mortality if diagnosed early.

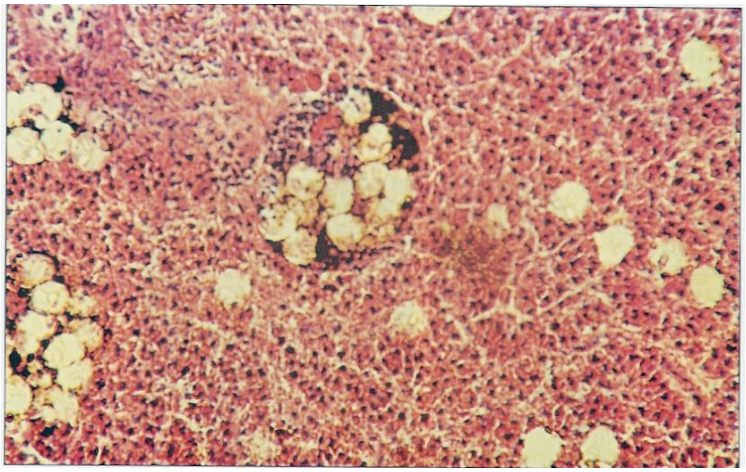
***Zschokkella mugilis* n. sp.**

It causes lesions (necrosis and apoptosis of epithelial cells) in the epithelium of the gallbladder due to early stages (trophozoites) of the parasite in Mugilids.

COCCIDIA

Eimeria sp.

They are found in various organs of fish but their pathogenesis is disputed. Small mortality from their presence in the gut has been reported in sea bream (Figure 43).

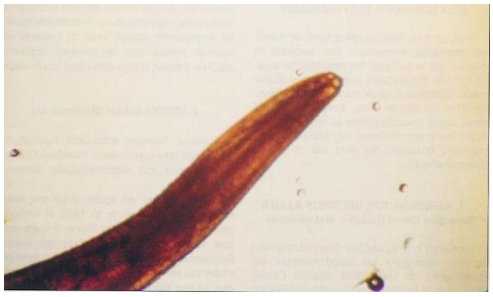


Pict. 43. Eimeria sp. in sea bream liver – Histological preparation.

NEMATODES

Anisakis sp.

They are common parasites of marine fish and rarely parasitize on farmed fish, but the parasite infects humans.



Pict. 44. Anisakis sp. (front). Fresh preparation.

DISEASES DUE TO NUTRITIONAL/METABOLIC CAUSES

Systemic granulomatosis (granulomatous hypertyrosinemia)

Aetiology: This disorder was initially attributed to the consumption of foods that have been preserved for a long time under inappropriate conditions. Recent research has shown that vitamin C deficiency may be involved in the aetiology of the disease

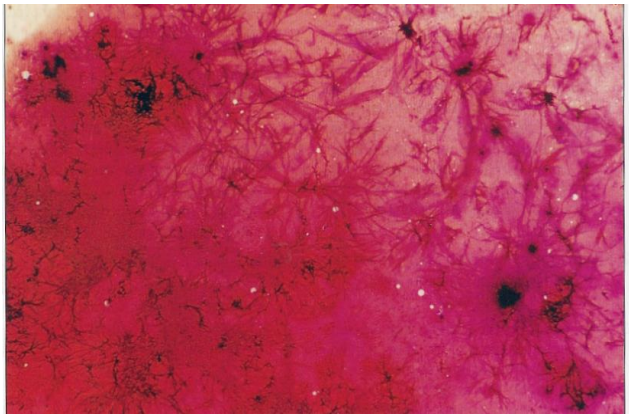
causing inhibition of tyrosine catabolism and its deposition in the form of crystals in various tissues and the formation of granulomas.

Sensitive species: This disorder has been described in sea bream, turbot and sharpsnout sea bream.

Temperature of the disease: The disease manifests itself during the summer months and disappears in early autumn.

Clinical signs: The affected fish show darkness, lethargy and bilateral swelling of the body at the height of the kidneys. Clouding of the cornea and exophthalmos, usually unilateral, are often observed. At necropsy, hypertrophy of the kidneys and spleen is characteristic (Figure 45) with the presence of characteristic nodules on the surface of the latter.

Pict. 45. Systemic granulomatosis in sea bream – kidney hypertrophia.



Pict. 46. Systemic granulomatosis in sea bream – tyrosine crystals in kidney smear (Gram staining, X 100).

Diagnosis: The diagnosis can be established from clinical findings. Confirmation is established by observation of fresh smears from the kidneys (Figure 46) and where tyrosine crystals are observed that refract light and histologically where the presence of granulomas in the kidneys and spleen is characteristic.

Mortality: It can reach 10-15% of the affected population. Mortality automatically subsides when the temperature drops in early autumn and stops at the end of October.

Prevention: The administration of foods with sufficient amounts of vitamin C that have been preserved under the right temperature and humidity conditions (shady and dry place) can prevent the onset of the disease.

Treatment: Administration of large doses of vitamin C in the early stages of the disease can reduce biochemical changes and prevent further progression of the disease.

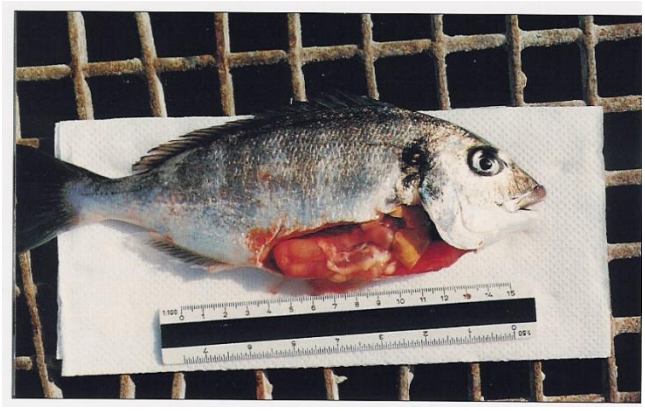
Winter syndrome

Aetiology: Many factors have been implicated such as overfeeding, high concentration of fatty acids in food, low temperature, bacteria of the genera *Aeromonas, Pseudomonas* and others.

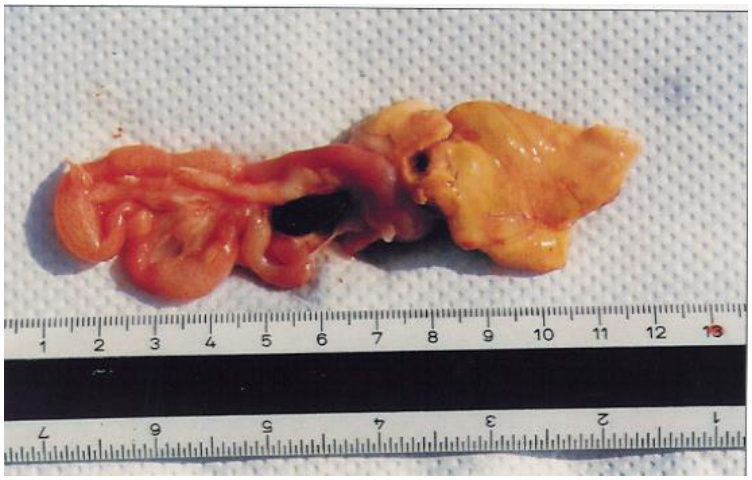
Sensitive species: Sea bream.

Temperature of the disease: The syndrome occurs during the winter period when the water temperature drops below 13^{o}C and disappears when the temperature exceeds 16-18^{o}C.

Clinical signs: Darkness, lazy behavior and peeling on the skin. Characteristic is often the swelling of the abdomen due to ascites and the presence of necrosis in the gills. During autopsy (Figures 47, 48) fluid concentration is observed in the abdomen, the liver is discolored and brittle, distension and hyperemia of the intestine accompanied by watery contents and in some cases splenomegaly of varying degrees.



Pict. 47. Winter sea bream syndrome – presence of peeling and small ulcerative lesions on the skin.



Pict. 48. Winter Sea Bream Syndrome – liver discoloration, intestinal distension and hyperemia, splenomegaly.

Diagnosis: It is set on the basis of clinical symptoms and the period of onset of the disease. First-year fish are usually affected in cages.
Mortality: It varies depending on the severity of the syndrome and usually ranges from 15 to 20% of the affected population.
Prevention: Better management of nutrition and stock densities may prevent the onset of the syndrome.
Treatment: Various antibiotics and vitamins had moderate results. It is usually recommended to apply a low nutritional factor in combination with vitamin preparations.

5. DISEASES OF FRESHWATER FISH

DISEASES CAUSED BY VIRUSES

Viral hemorrhagic septicaemia

Pathogenic cause - Susceptible species: The viral haemorrhagic septicaemia virus is widespread in Europe and is endemic to wild fish in infected areas. In rainbow trout, which is the main affected species, the very early stages of juveniles as well as broodstock appear to be resistant, while a clear disease picture is observed up to a weight of 200-300 grams.
Clinical symptoms - Mortality: The onset of the disease occurs when the temperature fluctuates between 7 and 11^{o}C during spring and autumn. Symptoms of the disease are rarely observed when the temperature ranges between 14-16^{o}C and do not appear at all when the temperature exceeds 16^{o}C. Three main stages-phases of the disease are observed:
a) Acute phase: Weak fish are apathetic and stand out from the rest. Externally, darkness, exophthalmos, hemorrhages around the eyes and bases of the fins, discolored gills with mottled hemorrhages are observed. Internally, ascites, hemorrhages in the intestine, liver, bladder, intra-abdominal fat and skeletal muscles. It is accompanied by rapid and high mortality.
b) Subacute or chronic phase: It is a continuation of the previous one. Fish perform rotational movements around the elongated axis of their body, show more pronounced darkness and exophthalmos. The gills are gray. The fish are anemic, and there are few hemorrhages in the internal organs and muscles. Mortality is noticeably reduced.

c) Nervous phase: All the above symptoms become subtle, the swimming disorder that becomes more intense is maintained. Mortality is negligible. In general, the latter depends on the type of virus, the general state of health of the fish and stress factors. Overall mortality ranges from 10-50% and in exceptional cases reaches 80%.
Transmission - Spread of the disease: Through water, carrier fish, fish-eating birds, gear, contaminated fish transport water and through bloodsucking parasites. Vertical transmission has not been proven. Various stress factors can lead to the onset of the disease in infected but also apparently healthy populations. The responsible virus does not survive high temperatures. Thus, after successive hot summers, eradication of the disease is possible in contaminated farms.
Diagnosis: By isolating the virus and identifying it with molecular or immunological markers.
Prevention - Treatment: In areas where the disease is not endemic, the fish or eggs entering should be certified free from the disease. Where the disease is endemic and control and sanitation programmes are not in place, older animals that have survived the disease should be purchased for breeding because they are more resistant to infection. All stress factors should be observed to a minimum. There is no cure. But they can combat secondary bacterial infections by administering antibiotics after identifying the pathogen and testing its susceptibility. Viral hemorrhagic septicaemia is a notifiable disease (Class II).

Infectious haematopoietic necrosis (IHN)

Pathogenic cause - Sensitive species: It is due to a virus and the most sensitive fish is rainbow trout. The critical temperature of the onset of the disease is 10^{o}C during spring and autumn. Cases are rare above 15°C.
Clinical signs - Mortality: They are observed in very young individuals of trout and salmon. They consist of pronounced hemorrhages in the yolk sac, darkening, external hemorrhages in the abdominal cavity and eyes around the pupil, exophthalmos, swollen abdomen due to ascites, the presence of pseudofeces, hemorrhages at the base of the fins, anemia in the internal organs, and sometimes in the skeletal muscles. The stomach is swollen, while the intestine lacks food and is full of gelatinous contents. The above picture in hyperacute situations may be missing and there may be only immediate mortality. Necrotic lesions in the hematopoietic organs (anterior kidney,

spleen) characteristic of the disease are observed only by histological examinations. At 8-12ºC mortality reaches 80-100% in juveniles 8-15 days old. In fish 1 year old, mortality does not normally exceed 10%. In fish >100 grams, the disease has a chronic route with a total mortality not exceeding 10-20%.

Transmission-Spread of disease: Through water and gills from fish carriers, fish-eating birds, contaminated gear, contaminated transport water, eggs and bloodsucking parasites. Stress situations promote the onset of the disease in individual carriers. Fish that survive the disease are carriers of the virus for life.

Diagnosis - Prevention - Treatment: The diagnosis is made after isolation and identification of the virus with immunological or molecular markers. Prevention is done by avoiding the entry of the virus into the farm, disinfecting and avoiding stressful situations. There is no cure. The disease is compulsorily declared (Class II).

Infectious pancreatic necrosis (IPN)

Pathogenic cause - Susceptible species: The responsible virus causes mortality in juveniles mainly of rainbow trout between the 1st and 3rd week of absorption of the yolk sac and the start of feeding with artificial food. The disease rarely manifests itself after the 20th week from the start of artificial nutrition, but fish are carriers of the virus. The temperature of manifestation of the disease varies between 6-16ºC.

Clinical signs - Mortality: Irregular spiral swimming. Swelling of the anterior part of the abdomen and internal hemorrhages on the surface of many intra-abdominal organs. The liver, spleen and kidney are very discolored, and there is a large swelling of the gallbladder.

The intestine is swollen and empty of food but filled with milky - gelatinous liquid colorless to yellowish. Mortality ranges from 10-90%.

Transmission - Spread of the disease: The transmission of the disease occurs from infected adult fish, carriers, contaminated water, birds, tools, bloodsucking parasites, infected eggs and sperm (the disease is also transmitted vertically).

Diagnosis - Prevention - Treatment: Diagnosis is made after isolation of the virus and its identification with immunological or molecular markers. There is no cure. Prevention consists in taking measures similar to those mentioned for previous diseases. The disease is included in the category of diseases III where disease control is left to the discretion of the Member States.

Spring viraemia of carp (SVC)

Pathogenic cause - Susceptible species: It mainly affects carp.

Clinical signs - Mortality: Exophthalmos, swollen abdominal cavity, pronounced ascites, hemorrhages in the skin, gills, intra-abdominal fat, necrotic gallbladder, other intra-abdominal organs and muscles. Mortality can reach 30%, depending primarily on environmental conditions and the general health of the fish.

Transmission of the disease: With water, gills of infected fish or carriers, from contaminated tools, transport water, undisinfected eggs and bloodsucking parasites. Stress factors can lead to the onset of the disease in fish carriers.

Diagnosis: By isolation of the virus and identification with molecular or immunological markers.

Prevention - Cure: There is no cure. In case of secondary bacterial infections, the secondary factor can be treated. Prevention is carried out only by avoiding the introduction of the disease. The disease is included in the Category III diseases of the European Union.

DISEASES CAUSED BY BACTERIA

Trout erythrostomatitis (ERD)

Pathogenic cause - Susceptible species: It is caused by the bacterium *Yersinia ruckeri*. It mainly affects trout, but all salmonids should be considered susceptible.

Clinical signs - Mortality: Characteristic redness in the oral cavity (hence its name), hemorrhages in the jaws and bases of the fins. Internally, hemorrhagic enteritis is observed mainly in the last part of the intestine. The disease is more severe in young trout and at a temperature of 15-18^{o}C. Below 10°C the disease is rarely observed. In larger fish the disease is less severe and chronic.

Transmission - Spread of disease: Through water from weak to healthy fish. The contribution of stress factors is always decisive.

Prevention - Treatment: Prevention In addition to observing hygiene principles, it can also be done with the use of vaccines. Treatment is carried out using antibiotics in food. The development of resistance has been reported.

Erythrodermatitis of carp

Pathogenic cause - Susceptible species: The pathogen is the bacterium *Aeromonas salmonicida* spp. nova while similar lesions can be caused by *A. hydrophila*. Sensitive fish is carp.

Clinical symptoms - Mortality: The disease is directly associated with skin lesions caused by ectoparasites or injuries of various etiology. Factors that favor infection are long winter or a previous infection with the spring viremia virus. Central necrotic areas are observed on the surface of the body surrounded by hemorrhagic halos (Figure 49). Later ulcers develop (up to 4 cm in diameter) penetrating deep into the muscles.

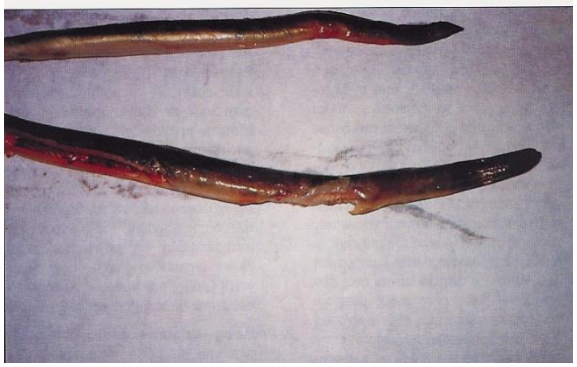
Prevention - Treatment: Prevention is done by observing proper and hygienic breeding conditions. Treatment is done by administering antibiotics.

Pict. 49. Carp erythrodermatitis.

Diseases due to infection by genera *Aeromonas, Pseudomonas*

Pathogenic causes - Sensitive species - Clinical symptoms: Pathological conditions characterized by the presence of externally large skin lesions (peeling, hemorrhages) (Figures 50, 51), while internally there is a picture of sepsis with the presence of ascites and diffuse hemorrhages in all intra-abdominal organs. Pathogenic causes can be *A. hydrophila, A. anaerogenes, Ps. fluorescens* and *Ps. aeruginosa*. These microbes are part of the normal flora of farmed water. Stressors, however, can promote fish infection and disease manifestation.

Prevention - Treatment: Prevention is done by observing proper farming conditions and hygiene. Treatment is done by administering antibiotics in food.



Pict. 50. Infection with Aeromonas hydrophila in eels.

Pict. 51. Lesions on the skin of sturgeon (Acipenser gueldeastaedit) infected with Aeromonas hydrophila.

Myxobacteria infections

Pathogenic causes: Species of the genus *Flexibacter* that cause Columnar disease and bacterial disease of the gills mainly in trout and eel.

Clinical symptoms - Lesions: Columnar disease concerns lesions on the outer surface of the body, while gill disease concerns the gills. In the skin, lesions consist of intense local hyperemia, which can develop into necrotic ulcerative lesions, while gill involvement leads to hyperplasia, discoloration and obstruction of respiratory function. Stress factors and poor rearing conditions contribute decisively to the appearance of these morbid conditions.

Prevention - Treatment: Observance of proper rearing conditions and hygiene decisively help prevent diseases. Treatment is done by administering antibiotics with food.

DISEASES DUE TO FUNGI

Ichthyosporidiosis - Ichthyophoniasis

Pathogenic cause - Susceptible species: It is due to the fungus *Ichthyosporidium (Ichthyophonus) hoferi* and mainly affects trout presenting a fairly complex biological cycle.

Clinical signs - Lesions: It affects the internal organs of the fish and is often observed when the fish are fed with seafood residues. Weak fish show imbalance when swimming. Darkening and exophthalmos, slimming, anemic gills and spinal abnormalities are observed when fish are affected at a young age before ossification

of the skeleton. Macroscopically in the liver, kidney, spleen, heart, white nodules the size of a pinhead are observed, as well as small gray nodules that are purulent colonies of the fungus.
Prevention - Cure: There is no cure. As a precaution, feeding fish with seafood residues should be avoided. In case of occurrence of the disease, after the end of rearing should be carried out radical disinfection of water ponds.

Saprolegnia

Pathogenic cause - Sensitive species: It is due to species of the genus *Saprolegnia* and affects almost all freshwater fish regardless of their age as well as their eggs.
Clinical symptoms - Lesions: Externally, whitish spots are observed on the skin. These foci develop on wounds and continuity resolutions of the skin. Then they grow, join together and cover the entire surface of the body. In very severe cases, the lesions can extend to the muscles, destroying them.
Prevention - Treatment: Prevention is done by observing proper rearing conditions, handling and hygiene. In case of establishment of the disease in fish and eggs, treatments are made with antifungal baths.

DISEASES DUE TO PARASITES

Ichthyophthiriasis

Pathogenic cause - Sensitive species: This is the most common external parasite of freshwater fish (trout, carp, eel) and is due to the parasite *Ichthyophthirius multifilis*.
Clinical signs - Lesions: Whitish spots are observed on the skin, fins and gills. Stagnant and warm waters favor the appearance of parasitosis, which is why it is very often observed in eel farms. Poor rearing conditions and farms fed with river water are more easily affected. Water temperature plays an important role in the onset and especially in the severity of the disease. Diseased fish show anxiety, anorexia, progressive slimming and in case of gill involvement, respiratory symptoms.
Mortality: It mainly concerns young fish. Sources of contamination of a farm can be carrier fish, contaminated tanks and water, plants, aquatic animals, etc.
Prevention - Treatment: Prevention is done by observing the appropriate rearing and hygiene conditions and avoiding the entry of the parasite into the farm.
Therapeutically solutions of formalin, malachite green and sodium chloride are used.

Trichodiniasis

Pathogenic cause - Sensitive species: Frequent infestation of fresh, brackish and marine fish. In the first fish the skin localization is more common, while in the latter the gill.

Clinical symptoms - Lesions: In its typical form it affects young fish and is characterized by mild dermatitis. In severe involvement, lesions on the gills and respiratory symptoms may occur. The disease is associated with poor rearing conditions.

Prevention - Treatment: Improvement of rearing conditions and treatment with formalin.

Pseudodactylogyrus *infestation*

Pathogenic cause - Sensitive species: Frequent infestation of eels, especially in farms with recycled water. Adults (1.5 mm) produce eggs and the ideal temperature for their development is 22-25^{o}C. At such temperatures eggs hatch in 1 week, and young parasites infect other eels.

Clinical symptoms - Lesions: A small number of parasites do not cause symptoms. In severe attacks there is an inability to eat, respiratory symptoms and severe losses.

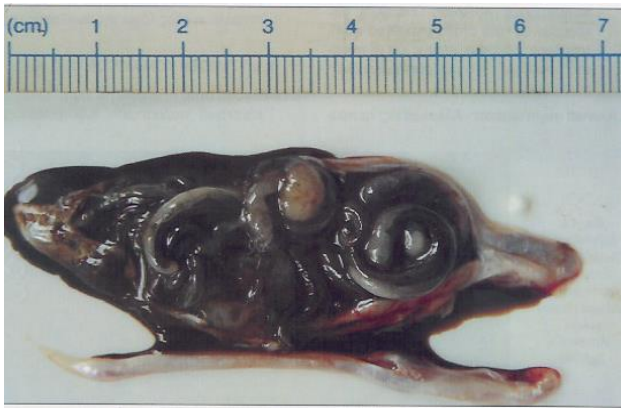
Prevention - Treatment: Treatment is quite difficult and is done with mebendazole.

Anguillicola crassa *infestation*

Pathogenic cause - Susceptible species: It is due to the above nematode. It attacks eel. To complete the biological cycle of the parasite, the presence of small copepods as intermediate hosts is required. The presence of parasites inside the necrotic bladder of eels is easy to establish (Figure 52).

Clinical symptoms - Alterations: In severe cases, the bladder swells considerably and presents symptoms of inflammation. Then there is a 20-30% decrease in the growth rate and a mortality of 10-20%, which is mainly due to secondary bacterial infections.

Prevention - Treatment: Prevention by avoiding entry into the unit of infected eels. In practice it is difficult to rid the plant of the pest. Long-term treatment of sick animals

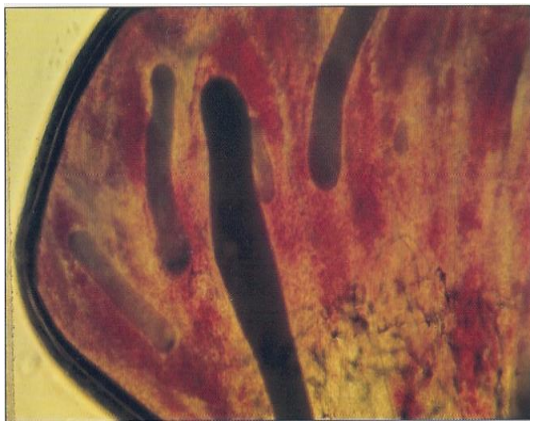


Pict. 52. Anguillicola crassa in eel. Nematode parasites in the swim bladder of eels.

with levamisole baths has some positive results.

Dermocystidiosis

Pathogenic cause - Susceptible species: Parasitosis of the gills of young eels weighing up to 10 grams due to haplosporidium *Dermocystidium anguillae*. In essence, these are cysts of whitish color 1x2 mm which have the shape of a handle (Figure 53). The number of cysts per fish may vary as well as the rate of population involvement.



Pict. 53. Desmocystidosis in eel. Elongated formations on the gills of young eel.

Clinical symptoms - Lesions: There is varying degrees of respiratory failure. Losses are generally minimal. In eel dermocystidiosis, the disease seems to heal itself and the cysts disappear suddenly as they appear after about 2 months.

Prevention - Treatment: It is not a significant disease to take special prevention measures other than the observance of proper rearing and hygiene conditions. Therapeutically salinization (10‰) of the farmed water can be used.

Myxidiosis

Pathogenic cause - Susceptible species: It is an external parasite of eels due to the protozoan *Myxidium giardi*.

Clinical signs - Lesions: Numerous skin lesions are observed (150-200 per fish). They are localized in the posterior part of the body but can extend forward to the gill area. The lesions are slightly raised, gray, elliptical or spherical in shape, 1-2 millimeters in diameter.

Prevention - Treatment: As for the previous disease, no special prevention measures are required due to the lightness of the disease. There is no cure, while the disease heals itself.

Hepatic capillariasis

Pathogenic cause - Sensitive species: Various freshwater fish, especially trout, are affected. It is due to the parasite nematode *Capillaria (Hepaticola) petruschewskii*. The biological cycle of the parasite is indirect, and trout infection occurs after eating aquatic worms, which as intermediate hosts carry the infected larvae of the parasite.

Clinical symptoms - Lesions: Externally, darkening and exophthalmos are observed. Internally, lesions appear in the liver. The organ is swollen, anemic, yellow, and on its surface numerous nodules resembling those of ichthyophoniasis are observed.

Treatment: Not reported.

DISEASES DUE TO ENVIRONMENTAL/NUTRITIONAL CAUSES

Vesicular rash - Gas disease

Aetiology: It is observed mainly in trout and eels and is characterized by the appearance of small air bubbles on the skin, eyes, mouth and gills. It is due to the abundance of various gases in the water. It is usually found in aquariums supplied with pressurized air, in water areas rich in aquatic vegetation and brightly lit, but mainly in reservoirs fed with artesian waters supersaturated in dissolved gases.

Conditions of occurrence: In eel cultures, oversaturation of water with nitrogen gas may be due to the following:

- In recirculating systems, liquid oxygen under pressure is added to the water. When it reaches saturation levels of 150-200% then gases such as nitrogen lead to intense bubble formation.

- Oversaturation of water in nitrogen can occur when atmospheric air enters the system under pressure through holes in the pipes.
- Oversaturation of gases in water can also occur when the latter overheats because then the solubility of gases is reduced.

Clinical signs - Lesions: The disease is usually of slow progression. Subcutaneous rash occurs in fish regardless of age. Under the skin, bubbles are formed which can also be seen on the fins, head, gill opercula, oral cavity as well as inside the eyes, resulting in intense exophthalmos.

Fatty liver degeneration

Aetiology - Sensitive species: It is found relatively often in trout cultures in individuals aged 7-12 months. It is observed mainly during the winter months in farms supplied with cold water. The disease is associated with diet (administration of larger than normal amounts of fat, oxidized food low in vitamin E).

Clinical signs - Lesions: There is reduced mobility and stay of fish in the calm areas of farming, bilateral exophthalmos, anemia and discoloration of the gills especially during the late stages of the disease. The course of the disease is chronic with low mortality unless there are stress factors. Internally, fluid is observed in the abdominal cavity, the stomach and intestine are empty of food, and the latter carries a large amount of mucus. Characteristic lesions are observed in the liver, which is swollen, pale yellow and pulpy. Death occurs from liver dysfunction and fragility of red blood cells.

Prevention: The disease is associated with poor diet planning and administration of altered fatty acids in food. The disease does not occur if the diet is qualitatively and quantitatively appropriate. Therapeutically, in order to ascertain the situation, it is necessary to stop the given food, change the diet by giving rational and balanced food as well as the additional addition of vitamins.

6. NUTRITIONAL DISEASES

INTRODUCTION

In contrast to extensive and semi-intensive farming, where fish receive all or part of the nutrients they need from the organisms in the environment in which they live, fish farmed in intensive farming depend solely on the supply of complete and balanced feeds throughout their lives to meet their nutritional needs. For many cultivated fish species, the development of complete artificial feeds (for use in intensive farming conditions) has progressed despite a lack of information on basic nutrient needs. It is therefore not surprising that various nutritional diseases develop in cultivated species due to lack of specific nutrients or their imbalance.

Below we will refer to:

A) Eating disorders due to food proteins

Lack of essential amino acids (AA)

Toxic non-AA

B) Eating disorders due to food fats

Lack of essential fatty acids (EFA)

EFA toxicity

Toxic non-EFA

Oxidation of food fats

C) Eating disorders due to mineral salts in food

Lack of essential mineral salts

Toxicity of mineral salts

D) Eating disorders due to vitamins in food

Lack of vitamins

Vitamin toxicity

E) Anti-dietary agents in raw materials for fish feed production

F) Adventitious toxic agents in feed production raw materials

It should be emphasized that deficiency in any of the components needed for the development of an organism sooner or later lead to a drop in the feed convertibility index, weight loss and cachexia, conditions observed as general symptoms in all deficiencies regardless of the cause that causes them.

Food protein eating disorders
Deficiency of essential amino acids (AA)

In addition to the general symptoms observed in deficiency, the following symptoms (Table 19) have been observed in young fish fed synthetic experimental foods lacking AA.

TABLE 19. Symptoms of lack of essential amino acids

Restrictive AA	**Fish species**	**Symptoms**
Lysine	Trout	Erosion of the dorsal and caudal fin
	Carp	Increased mortality
Methionine	Salmonids	Cataract
Tryptophan	Trout	Scoliosis, lordosis, nephrocalcification, cataracts, caudal fin erosion, decreased fat concentration, increased concentration of Ca, Mg, Na, K in the flesh.
Miscellaneous AAs	Carp	Increased mortality and cases of lordosis have been observed in leucine, isoleucine, lysine, arginine and histidine pennies.

Under actual farming conditions, AA deficiency can be observed under the following four cases:

- Poor selection of raw materials for the manufacture of artificial feed and use of disproportionate percentages of materials with natural deficiency in AA.

As mentioned earlier, some raw materials are deficient in AA. Such deficiencies occur mainly in materials of plant origin, which cannot be used as the only source of protein in the diet, while the raw material that contains the best ratio of protein and AA is fish meal. Characteristic is the lack of methionine in plant-based proteins in yeasts, meat and bone meal, bone meal, blood meal and hydrolysed feathermeal, lack of lysine in oily fruits, hydrolysed feathermeal and algae, lack of threonine in some oilseeds and legumes and lack of tryptophan in fish silage.

AA imbalance in food can also come from the presence of disproportionate levels of specific amino acids-antagonists such as leucine / isoleucine and to a lesser extent

arginine / lysine and cystine / methionine. For example, blood meal is a rich source of valine, leucine and histidine, but it is poor in methionine and isoleucine. However, regarding the competitive effect of a large amount of leucine on isoleucine, animals fed large amounts of bloodmeal suffer from isoleukinopenia caused by an overabundance of leucine. Although similar antagonisms have been reported between cystine/methionine and arginine/lysine in terrestrial animals fed combinations of synthetic amino acids in their food, this has not been observed in fish.

- AA deficiency in food can occur due to overheating of raw material proteins during the preparation of artificial feed. The overheating of proteins results in the change of their stereostructure (denaturation), the reduction of their digestibility, their binding to other components of food (such as polysaccharides), etc.

- AA deficiencies in food may occur due to their loss in water during feeding. For example, losses of all free amino acids and 1/3 of free and protein-bound amino acids from frozen and lyophilized, respectively, zooplankton (Artemia, Moina) have been reported after 10 minutes in water at 9°C.

Toxic non-essential amino acids

Although no cases have been recorded, dietary diseases can result from eating foods containing toxic amino acids. Raw material proteins known to contain toxic amino acids are soybeans after treatment with alkali (toxic amino acid lysinoalanine), legumes *Leucaena leucocephala* (toxic amino acid mimosin) and fava beans (toxic amino acid dihydroxyphenylalanine).

Eating disorders due to food fats
Essential fatty acid deficiency (EFA)

In addition to the general symptoms of deficiency, the following (Table 20) pathological conditions have also been observed following the use of food in young fish lacking EFA:

TABLE 20. Symptoms of lack of essential fatty acids.

Fish species	*Symptoms of deficiency in EFA*
Salmonids	Increased mortality, increased water content in muscles, increased susceptibility of the caudal fin to erosion by *Flexibacter* sp., fainting syndrome, decreased haemoglobin and red blood cell volume, fatty infiltration/liver degeneration, enlarged and pale liver, decreased ability to produce eggs (drop in hatchability and survival rate), high mortality.
Carp	Fatty liver infiltration, increased mortality.
Eels	Increased mortality.
Tilapia	Liver pale, swollen, with fatty infiltration.
Pagrus	Reduced ability to produce eggs (hatchability/survival of nymphs).
Turbot	Increased mortality, decreased growth, degeneration of the gill epithelium.

EFA deficiency in general is the result of poor selection of raw materials.

Toxicity of essential fatty acids in food

Under laboratory conditions, it has been shown that excessive amounts of EFAs in food can inhibit the growth of fish and the use of food by the body.

Toxic non-essential fatty acids

Cyclopropenoic acid is a toxic fatty acid found in cottonseed oleoderivatives. Experimentally, this acid reduces growth and acts synergistically on the carcinogenic activity of aflatoxins. Other pathological findings of intoxication from this fatty acid in trout include liver destruction with increased glucagon deposition and reduced protein content, as well as a drop in the activity of several important enzymes.

Oxidation of feed fats

When there is a lack of proper antioxidant protection, fats rich in polyunsaturated fatty acids (PUFA, including EFAs) are very prone to auto-oxidation when exposed to atmospheric oxygen. Under these conditions, the dietary value of EFAs is destroyed and EFAs become detrimental to fish health. PUFA-rich raw

materials that are particularly prone to oxidative degradation of fats include fish oils, fish meal and oilseed pies which contain little or no natural antioxidant protection. During the process of auto-oxidation of fats, various chemical degradation products are created including free radicals, peroxides, hydroperoxides, aldehydes and ketones which in turn react with other components of the food (vitamins, proteins and other fats) reducing their biological value and availability during digestion. At present, rancidity is considered one of the biggest degenerative changes that occur in stored raw materials and ready-made foods.

Various pathological conditions have been described (Table 21) in fish fed with foods containing oxidized fats without the presence of antioxidants (vitamin E).

TABLE 21. Pathological conditions caused after consumption of oxidized fish oil.

Fish species	**Effects of consumption of oxidized fish oil**
Tilapia	Pronounced congestion with hemorrhages in the skin vessels of the snout and bases of the thoracic and dorsal fins, lordosis, exophthalmos, abdominal edema and abdominal distension, cataracts, dark pigmentation of the liver, pronounced dilatation of the cholagogue, steatites in the adipose tissues of the abdomen, ceroid deposition intracellularly in the liver, spleen, kidney and increased mortality.
Salmon	Dark skin pigmentation, anemia, lethargy, brownish-yellow liver pigmentation (ceroid deposition), abnormal kidney and lesions on the gills.
Carp	Poor growth, anorexia, muscular dystrophy, high mortality, impaired absorption of dietary fats.
Catfish	Poor growth and conversion index, muscular dystrophy, high mortality, discoloration and fatty infiltration of the liver.
Yellowtail	Decreased growth, dilated liver, decreased fat deposition, anorexia, dorsal muscle slimming and muscular dystrophy.
Trout	Decreased growth and convertibility of food, microcytic anemia with a drop in hematocrit and hemoglobin concentration, fatty liver degeneration, muscle damage and increased mortality.

The pathological effect of oxidized fats, with few exceptions, can be prevented by supplementing tocopherol dl-acetate (vit. E) in food. When there is a lack of appropriate antioxidant protection, the rate of autooxidation of fats in stored raw materials increases in the presence of: lipoxidase (present in soybeans), heme derivatives (hemoglobin / myoglobin are pre-oxidizing substances present in any flour of animal origin), peroxides (autooxidation product of fats), light (ultraviolet light promotes the production of oxygen radicals and free radicals), increased temperature (the rate of autooxidation increases) and the presence of trace elements (Fe, Zn and Cu accelerate the oxidation of fats);

Eating disorders due to minerals in food
Deficiency of essential minerals

The following symptoms (Table 22) have been observed in young fish fed mineral-poor foods:

TABLE 22. Pathological conditions observed during the lack of minerals from food.

Element/ Fish species	**Symptoms of deficiency**
Phosphorus	
Carp	Decreased growth, low food conversion, osteoporosis, skeletal abnormalities, abnormal calcification of the ribs and soft rays of the pectoral fins, dysplasia in the skull, increased deposition of visceral fat.
Catfish	Impaired growth, low feed conversion, osteoporosis.
Pagrus	Decreased growth, low food conversion, osteoporosis, increased fat deposition in muscles, liver and vertebrae, curved, swollen and spongy vertebrae, decreased glycogen deposition in the liver.
Eel	Anorexia, decreased growth.
Salmonids	Impaired growth, low feed conversion, osteoporosis.
Calcium	
Catfish	Decreased growth, low content of ash, calcium and phosphorus in the body (these symptoms were observed after eating food poor in vit. D).
Trout	Anorexia, low growth, low feed convertibility.
Eel	Anorexia, low growth, low feed convertibility.
Pagrus	Anorexia, low growth, low feed convertibility.

Magnesium	
Carp	Decreased growth and mobility, anorexia, spasms, high mortality, low magnesium content in the bones, cataracts.
Catfish	Decreased growth and mobility, anorexia, spams, high mortality, low content of magnesium in the body, bones and blood serum.
Eel	Anorexia, decreased growth.
Trout	Anorexia, decreased growth and mobility, cataracts, nephrocalcification, high mortality, flexion of vertebrae, degeneration of muscle fibers and epithelial cells of pyloric cecum and gill filaments, decreased ash and magnesium in bones and increased calcium concentration.
Iron	
Carp, Pagrus, Eel	Hypochromic, microcytic anemia.
Zinc	
Catfish	Decreased growth and appetite, reduced content of calcium and zinc in the bones and blood.
Carp	Decreased growth, cataracts, anorexia, high mortality, erosion of fins and skin, increased concentration of iron and copper in the intestine and hepatopancreas.
Trout	Decreased growth, increased mortality, cataracts, dwarfism, erosion of fins.
Manganese	
Tilapia	Decreased growth and appetite, loss of balance, mortality.
Carp	Impaired growth, dwarfism, cataracts.
Trout	Decreased growth, dwarfism, cataracts, abnormal development of the tail.
Copper	
Carp	Decreased growth, cataracts.
Selenium	
Salmon	Increased mortality, muscular dystrophy, suppression of the action of glutathione peroxidase.
Carp	Decreased growth, cataracts, anemia.
Catfish	Reduced growth.
Iodine	
Salmonids	Hyperplasia of the thyroid gland.

Despite the presence of macro- and trace elements in almost all raw materials used to prepare fish feed and the ability of fish to absorb some trace elements from the environment, mineral deficiencies can occur under conditions of intensive rearing in the following cases:

- Lack of some macro- or trace element in the premix in the formed feed.

- Reduced bioavailability of minerals due to imbalances in the diet. The availability and use of trace elements of feed by fish depends on the origin of the raw material and the form of the absorbed element, the adequacy of the body's stores, the interaction with other minerals present in the gastrointestinal tract and within the tissues (antagonisms) and finally on the interactions of minerals with other components food or their metabolites (vitamins, fiber and phytic acid).

For certain fish species, the availability and absorption of P and other macroelements (Ca) from fishmeal and meat, bone-flour is further complicated by the lack of stomach acid secretion, which is necessary for bone breakdown. In fish that do not have a stomach, soluble monobasic minerals or bioavailable organic salts should be supplied with food. In addition, in proteins of plant origin a large percentage of P is present as organically bound P in phytases. The P of phytates is not only non-bioavailable but also phytic acid has the ability to bind other trace elements (Fe, Cu, Zn, Co, Mb) making them non-bioavailable for fish during digestion.

Under farming conditions, mineral deficiencies often result from disproportionate levels of Ca in feed due to the competitive effect of large amounts of Ca on absorption of P and trace elements Zn, Fe and Mn. For example, the bioavailability of Zn and to a lesser extent Mn in fishmeal from white-fleshed fish is much lower than in fishmeal from dark-fleshed fish (which contains less ash and Ca). Thus, fish fed experimental diets containing only fishmeal from "white" fish without supplementation of trace elements, the fish showed reduced growth, dwarfism and cataracts.

Mineral toxicity of food

A major risk that may be associated with the use of "exotic" raw materials in fish food is the presence of heavy metals including accumulated minerals such as Cu, Pb, Cd, Hg, Ar, F, Se, Mb and Va. For example, Cu contamination can be caused by products fermented in copper-plated containers (such as beer factories) or pig and bird excretion products where growth stimulants and antifungal agents were used. Other food ingredients that may contain minerals in large quantities are: poultry waste → Ar, Zn, pulp waste → Pb, Fishmeal → Hg, Se, Ar, Cd, Pb, Shellfish → Zn, Antarctic shrimp → F.

Various symptoms of metal intoxication have been described under experimental conditions (Table 23).

TABLE 23. Pathological conditions observed after experimental intoxication in fish.

Metal element	**Kind Fish**	*Symptoms of toxicosis*
Zinc	Carp	Reduced growth (concentration of metal in food >300mg/kg)
Copper	Catfish	Reduced growth (concentration of metal in food >15mg/kg)
Selenium	Trout	Decreased growth and convertibility of food, high mortality (mineral concentration in food >13mg/kg), nephrocalcification.
	Catfish	Reduced growth (concentration of metal in food >15mg/kg)
Cadmium	Trout / Carp	Scoliosis, hyperactivity, reduced concentration of calcium in the bones.
Lead	Trout	Scoliosis, lordosis, dark coloration of the tail, anemia, degeneration of the caudal fin.
Chromium	Trout	Reduced growth and convertibility of feed.

Eating disorders due to vitamins in food

Vitamin deficiency of food

The following pathological symptoms (Table 24) have been reported in fish fed diets lacking vitamins:

TABLE 24. Pathological conditions caused by vitamin deficiencies in fish.

Vitamin/ Fish species	**Pathological symptoms**
Riboflavin	
Salmonids	Anorexia, decreased growth, vascularization of the cornea, cloudy lenses, erosion of the snout, dysplasia of the spine, increased mortality, severe fin erosion and hemorrhages, rapid movement of gill opercula, apparent muscle weakness, abnormalities in skin pigmentation, contractions of the abdominal wall, photophobia, loss of orientation, lethargy, anemia.
Carp	Anorexia, decreased growth, high mortality, hemorrhages in the skin and fins, nervousness, photophobia.
Catfish	Dwarfism, anorexia, decreased growth, cataracts.
Pagrus	Reduced growth.
Eel	Anorexia, decreased growth, hemorrhages in the fins, photophobia, lethargy.
Pantothenic acid	
Salmonids	Anorexia, decreased growth and mobility, necrosis of the gills, anemia, hypersecretion of mucus in the gills, dilatation of gill opercula.
Carp	Anorexia, impaired growth and mobility, anemia, bleeding in the skin, exophthalmos.
Catfish	Anorexia, gill abnormalities, erosion of the skin, lower jaws and head, anemia.
Pagrus	Decreased growth, mortality.
Eel	Decreased growth, abnormal swimming, skin lesions.

Niacin	
Salmonids	Anorexia, impaired growth and convertibility of food, darkening of the skin, jerky movements, muscle spasms, stomach swelling, susceptibility to UV burns.
Carp	Hemorrhages in the skin, high mortality.
Catfish	Hemorrhages and lesions on the skin / fins, dysplasia of the jaws, anemia, exophthalmos, high mortality.
Pagrus	Reduced growth.
Eel	Hemorrhages and lesions on the skin, decreased growth, ataxia, darkening of the skin.
Thiamin	
Salmonids	Anorexia, decreased growth, nervous disorders, defective carbohydrate metabolism, increased susceptibility to shock after a blow or bright light.
Carp	Congestion / hemorrhages of the fins, nervousness, anorexia, decreased growth.
Catfish	Anorexia, decreased growth, darkening of the skin, mortality.
Pagrus	Anorexia, decreased growth.
Eel	Anorexia, decreased growth, ataxia, bleeding of the fins.
Pyridoxine	
Salmonids	Nervous disorders, hyperirritability, anorexia, indifference to light, quick establishment of rigor mortis, ataxia, edema of the peritoneal cavity, pronounced dilatation of the gill opercula, spasmodic and rapid swimming, lesions in skin pigmentation, anemia, rapid breathing with the mouth open out of water.
Carp	Anorexia, decreased growth, nervous disorders.
Catfish	Anorexia, nervous disorders, convulsive swimming, distension of gill opercula, tetanus conditions, abnormal pigmentation of the dorsal surface of the body.
Pagrus	Reduced growth.
Eel	Anorexia, decreased growth, nervous disorders.
Turbot	Reduced growth.
Sea bream	Anorexia, impaired growth and convertibility of food, high mortality, hyperirritability, jerky swimming.
Yellowtail	Reduced growth.

Chanos	Anorexia, decreased growth, ataxia, hyperirritability, muscle spasms, spasmodic swimming, loss of scales, edema, abnormal skin pigmentation, clouding of lenses and blindness.
Biotin	
Salmonids	Anorexia, decreased growth and food conversion, increased mortality, lesions in the colon, muscle atrophy, spastic contractions, thickening of secondary gill filaments and pale pigmentation of the gills.
Carp	Decreased growth and activity.
Catfish	Anorexia, decreased growth, discoloration, anemia, hypersensitivity.
Pagrus	None
Eel	Decreased growth, dark coloration, abnormal swimming.
Folic acid	
Salmonids	Macrocytic anemia, decreased growth, anorexia, lethargy, skin darkness, gill paleness, exophthalmos, abdominal distension, ascites.
Eel	Decreased growth, anorexia, darkening of the skin.
Carp	They have not been reported.
Pagrus	They have not been reported.
Catfish	Anorexia, increased mortality, lethargy.
Vit. B12	
Salmonids	Microcytic hypochromic anemia, impaired growth and convertibility of food, anorexia, brittle red blood cells, dark skin.
Carp	They have not been reported.
Catfish	Decreased growth, low hematocrit.
Eel	Reduced growth.
Pagrus	Reduced growth.
Choline	
Salmonids	Decreased growth, fatty infiltration of the liver, decreased food convertibility, hemorrhagic kidney and intestine.
Carp	Impaired growth, fatty infiltration of the liver.
Catfish	Decreased growth, liver dilatation, hemorrhagic kidney and bowel.
Pagrus	Decreased growth, mortality.
Eel	Anorexia, decreased growth, gray-white intestine.

Inositol	
Salmonids	Decreased growth, distended abdomen, darkening of the skin, increase in bowel emptying time.
Carp	Decreased growth, lesions / hemorrhages in the skin and fins, loss of the mucous layer of the skin.
Catfish	They have not been reported.
Pagrus	Reduced growth.
Eel	Impaired growth, gray-white intestine.
Vit. C	
Salmonids	Decreased growth, effect on collagen synthesis, scoliosis, lordosis, internal hemorrhages, bleeding in the fins, distortion and twisting of the gill filaments, low hematocrit, poor wound healing, increased mortality.
Catfish	Decreased growth, scoliosis, lordosis, increased susceptibility to disease, decreased levels of collagen in the bones, "broken back" syndrome, internal and external hemorrhages, erosion of the fins, darkening of the skin, anorexia.
Pagrus	Reduced growth.
Eel	Impaired growth, erosion of the fins, head and lower jaw.
Tilapia	Scoliosis, lordosis, impaired growth, poor wound healing, internal and external hemorrhages.
Vit. A	
Salmonids	Decreased growth, exophthalmos, skin discoloration, thickening and whitening of the corneal epithelium, retinal degeneration.
Carp	Anorexia, discoloration, bleeding in the skin and fins, exophthalmos, dysplasia of the gill opercula.
Catfish	Discoloration, exophthalmos and whitening of the cornea, atrophy, edema, bleeding in the kidneys, increased mortality.
Vit. D	
Salmonids	Decreased growth and convertibility of food, anorexia, tetanus, increased concentration of fat in muscle and liver, increased concentration of T3 in the blood.
Catfish	Reduced growth, low concentration of ash, phosphorus and calcium in the body.

Vit. K	
Salmonids	Increase in blood clotting time, anemia, hemorrhages in the gills, eyes and vascular tissues.
Catfish	Skin hemorrhages.
Vit. E	
Salmonids	Decreased growth, exophthalmos, ascites, anemia, gill lesions and paleness, epicarditis, ceroid deposition in the spleen, increased mortality, increased concentration of moisture and ash in the body, brittle red blood cells, muscle damage and degeneration, decreased egg hatchability.
Carp	Muscular dystrophy, mortality, exophthalmos.
Catfish	Decreased growth and convertibility of food, hypersecretory diathesis, muscular dystrophy, discoloration, fatty liver infiltration, anemia, atrophy of pancreatic tissue, mortality, deposition of ceroid in the vessels of the liver, splenic hemosiderosis.

Under intensive breeding conditions and lack of natural food, vitamin deficiencies can occur in the following cases:

Food preparation and storage

I) <u>Riboflavin</u> : Generally stable in dry multivitamin mixtures. Losses of up to 26% have been observed for pellets. Foods containing riboflavin should be protected from bright light/ultraviolet radiation (sensitive to oxidation) and alkaline conditions.

(II) <u>Pantothenic acid</u> : Generally stable in dry premixes. Losses of up to 10% have been found during the manufacture of pellets.

(III) <u>Niacin</u> : Generally stable in dry premixes. Losses of up to 20% during the manufacture of pellets. It remains stable only if the feed is kept in a cool and dry place.

IV) <u>Thiamine</u> : Stable in dry premixes that do not contain choline or trace elements. It quickly destroys under alkaline conditions or in the presence of sulfides. Pellet preparation and storage (7 months at room temperature) lead to losses of 0-10% and 11-12%, respectively.

V) Pyridoxine : Stable in dry premixes without trace elements. Foods with pyridoxine need protection from light (UV), heat and humidity. Preparation and Storage (10 months) losses of 7-10%.

VI) Biotin : Generally stable. Losses of 10% during the manufacture of pellets.

VII) Folic acid : 43% drop in activity after 3 months at room temperature. Preparation and storage → losses of 3-10%. Sensitive to oxidation during storage at high temperatures and during exposure to light.

VIII) B12 : Increased temperatures reduce its action, especially in the presence of slightly acidic conditions.

IX) Choline : Stable. May reduce the action of other vitamins.

X) Vitamin C : Low stability. It oxidizes easily in the presence of moisture, trace elements, increased temperature, light and oxidation products (fat peroxides). Its stability depends on the product in which it is contained and the method of its preparation. Losses of up to 90% have been reported during pellet preparation and drying even at low manufacturing temperatures. 70% losses have been reported when adding water to the raw material mixture for preparation of food before creating pellets.

During preparation and storage under farming conditions, losses of up to 95% have been reported. However, these losses can be reduced by using more stable forms of vitamin C.

XI) Vitamin A : It oxidizes quickly at high temperatures and in the presence of fat peroxidation products. Losses of up to 20% have been reported during pellet manufacturing. Storage for 6 months at room temperature → losses of 53%. Its stability can be increased with appropriate antioxidant therapy.

(XII) Vitamin D : High stability.

(XIII) Vitamin K : Good stability in premixes in the absence of trace elements. Under certain conditions of preparation, increased temperature and humidity, alkaline pH and some trace elements achieve its destruction. Ready-made foods must be protected from light to reduce losses from oxidative processes.

XIV) Vitamin E : Stable at temperatures lower than the room. Its stability increases when used in oxide form, but it is prone to oxidation during storage at high temperatures and the presence of oxidizing agents.

Losses of water-soluble vitamins in water

Unlike fat-soluble vitamins (A, D, E, K), water-soluble vitamins can easily be lost from food before it is ingested by fish. In general, the smaller the size of the food given and the longer the time it stays in water before ingestion, the greater the losses of water-soluble elements. Thus, losses of 30-50% for vitamin C, 5-20% for pantothenic acid, 0-27% for folic acid, 0-17% for thiamine, 3-13% for pyridoxine have been described by this route, after 10 seconds of food in water.

Disorders due to the presence of anti-vitamin agents in food

I) Avidine : Heat-sensitive anti-biotinic agent in uncooked egg white. It is easily destroyed by temperature.

(II) Thiaminase : Heat-sensitive anti-thiamine agent found in uncooked fish, shellfish, etc. Increasing the temperature of raw materials renders it inactive, or a more stable form of thiamine can be used in food.

III) Anti-vitamin A, E, D, and B12 agents present in raw soy trunk. They are inactivated by heating.

IV) Anti-pyridoxine agents present in linseed. They are inactivated by heating.

Disturbances due to the addition of antibiotics to food

The use of antibiotics in food for the treatment of diseases can consequently lead to the destruction of the gut microflora of fish which in omnivorous / vegetarian fish species is believed to play an important role in vitamin synthesis and meeting the needs of fish (carp, tilapia, catfish → B12, folic acid and possibly biotin, thiamine and vitamin K).

Toxicity of feed vitamins

Unlike water-soluble vitamins, fish accumulate fat-soluble vitamins (A, D, E, K) under conditions of oversupply. Under certain conditions the accumulation is such that hypervitaminosis can be caused. Although such a condition is rare in farming conditions, hypervitaminosis has been caused experimentally, with the following symptoms (Table 25):

TABLE 25. Pathological conditions caused by hypervitaminosis.

Vitamin/ Fish species	**Symptoms**
Vit. A	
Salmonids	Decreased growth and hematocrit, severe necrosis and erosion of the fins, scoliosis, lordosis, increased mortality, liver pale yellow and brittle, reduction of fat in the body.
Vit. D	
Salmonids	Decreased growth, lethargy, darkness.
Catfish	Reduced growth and feed convertibility.
Vit. E	
Miscellaneous species	Decreased growth, toxic reactions in the liver, mortality.

Anti-dietary agents in vegetable raw materials

The presence of endogenous anti-dietary agents in plant raw materials is the most important factor limiting their use in large quantities in artificial foods. The table below (Table 26) summarizes the main categories of these factors in vegetable raw materials.

TABLE 26. Categories of anti-dietary agents in vegetable raw materials.

Type of agent	**Substances**
Proteins	Protease inhibitors
	Hemagglutinins
Glycosides	Substances that cause hyperthyroidism
	Substances that cause cyanide poisoning
	Saponins
	Estrogen
Phenols	Gosypol
	Tannins
Various	Anti- macro- and trace- elements
	Anti-vitamins
	Anti-enzyme

	Allergens
	Carcinogens of microbial / plant origin
	Toxic amino acids

Although the toxicity of these agents varies, a large percentage can be destroyed or inactivated by heating.

Unfortunately, toxicological tests have not been carried out for the majority of these substances. In general, however, their presence in unprocessed raw materials usually results in anorexia, growth decline and low food conversion rates when used in large quantities in food.

Accidental toxic agents in raw materials

Food diseases can develop from the presence of specific random toxic agents or cross-contamination in specific raw materials. These factors include:

(a) Legitimate additions:

- Agglomerates and stabilizers (mastics, carboxymethyl alcohol).
- Chemotherapeutics (antibiotics, sulfonamides, nitrofurans, arsenilic acid).
- Growth accelerators (as above and anabolic, synthetic androgens).

(b) Toxic agents arising from the manufacture of foodstuffs:

- Solvent residues present after treatment of oil seeds with solvents (methylene chloride, ethylene dichloro, trichloroethylene, acetone, isopropyl alcohol).
- Fats damaged by oxidation or heating (peroxidation, oxidizing products).

(c) Infections by micro-organisms:

- Protozoa toxins from spoiled fish
- algae toxins from shellfish / fish
- fungal toxins in stored foods (e.g. aflatoxins)
- bacteria toxins from contaminated raw materials
- pathogens (live bacteria, viruses and fungi).

(d) Contamination by synthetic substances:

- residues of insecticides (chlorinated hydrocarbons)
- organochlorates (polychlorinated biphenyls)
-Mineral oils
- heavy metals

In conclusion, the most serious and frequently encountered pathological conditions due to dietary factors are presented in the table below (Table 27):

TABLE 27. Nutritional etiology of main pathological conditions and their causes.

Pathological condition	Dietary imbalance
Scoliosis / lordosis	
Shortage	Tryptophan, magnesium, phosphorus, Vit. C
Intoxication	Lead, cadmium, Vit. A, oxidized fish oil.
Cataract	
Shortage	Methionine, tryptophan, zinc, magnesium, copper, selenium, manganese, Vit. A, riboflavin.
Intoxication	Choline, oxidized fish oil.
Fin erosion	
Shortage	Lysine, tryptophan, zinc, riboflavin, inositol, niacin, Vit. C.
Intoxication	Lead, Vit. A.
Fatty liver infiltration	
Shortage	Choline, essential fatty acids.
Intoxication	Oxidized fish oil.
Exophthalmos	
Shortage	Pantothenic acid, niacin, folic acid, Vit. A and E.
Intoxication	Oxidized fish oil.
Skin/fin bleeding	
Shortage	Riboflavin, pantothenic acid, niacin, thiamine, inositol, Vit. A, K and C.
Intoxication	Oxidized fish oil.