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MEDITERRANEAN REGIONAL AQUACULTURE PROJECT

8

TRAINING COURSE ON DISEASE DIAGNOSIS AND PREVENTION

Bodrum, November 17-30 1991

MEDRAP II

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TRAINING COURSE ON DISEASE DIAGNOSIS AND PREVENTION

Bodrum, November 17–30 1991

United Nations Development Programme

Food and Agricultura Organisation of the United Nations

Edited by MEDRAP II Regional Center
Tunis - Tunisia

Preparation of this Document

This document is one of a series of documents prepared during the course of the Project identified in the title page. The conclusions and recommendations given were considered appropriate at the time it was prepared. They may be modified in the light of further knowledge gained at subsequent stages of the Project.

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Abstract

This report was edited by Bodrum Fisheries Research Institute with the papers provided by the trainers to the course.

The objective of the course on fish disease is to close the gap in knowledge on the diagnosis and control of diseases already affecting or posing a potential health hazard to cultured gild head sea bream and to give an introduction to the diagnosis and prevention of disease caused or induced by environmental conditions.

The participants reviewed the various aspects of fish pathology and considered the required measurements for the prevention against the frequent diseases in the Mediterranean.

It was noted that fish diseases can be genetic, nutritional, environmental, infections and parasitic.

The study of defense mechanises of the animal is essential. The internal defensive action is extremely elementary especially in bivalves, howerver, it is quite effective against microbial pathogenic agents.

It was emphasised that a proper nutrition is essential to the health of fishes as most of nutritional diseases are chronic in nature. To prevent these diseases, some precautions must be taken such as the conservation of food under a determined temperature, the use of cold storage rooms, the use of therapeutic dose of vitamin, etc.

The variation of Physico-Chemical factors in the site where the bivalves lives has a fundamental role in the sanitary status of the populations. The more or less intensive sedimentation of solid particles, the drastic changers in the salinity or temperature of the water changes on chemical parameters due to pollutants like pesticides, and overfishing and deficient management of commercial sea beds are frequently the cause of drastic diseases on productivity.

The participants suggested to organise longer general courses on pathology and more specialised courses on bacteriology, virology, parasitology, immunology, histopathology and microbiology. The recommended the establishment of a central library and a data base on pathology.

Acknowledgements

The Editor would like to thank the Turkish Authorities, namely the National Coordinator, Mr. Caglar Memisoglu, for the successful organisation of the course particularly for the availablity of the necessary facilities for practical and theoritical sessions.

The Editor would like also to thank the participants and the invited trainers for their positive contribution to the success of the course.

Note from the reviser

The revision and publication of this document could only be done a long time after the closure of the project. This has led to some difficulties in finalising the documents and implementing corrections, because authors and contributors as well as some of the original material or files were no longer available.

Therefore contributions from participants and session papers annexed to most of the documents were left in their original form. No language corrections were introduced, the content was not modified and left under their respective authors' responsibility.

Considering the above, we hope that the reader will understand that a standard of publication could not be maintained on a level as high as we would have liked it to be.

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11.30 to 13.00	:	Prevention actions. By. J. EDMONDSON (SCOTLAND)
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11.30 to 13.00	:	Crustacean disease and prophylaxy. By F. RUANO (PORTUGAL)
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26.11.1991		
9.00 to 10.00	:	Husbandry apsects of parasitic diseases. By C. SOMMERVILLE (SCOTLAND)
10.00 to 11.00	:	Parasitic diseases (I). By C. SOMMERVILLE (SCOTLAND)
11.30 to 13.00	:	Mollusc diseases and prophylaxy. By F. RUA (PORTUGAL)
15.00 to 16.00	:	Parasitic diseases (II). By C. SOMMERVILLE
16.00 to 17.00	:	Economic aspects of parasitic diseases. E C. SOMMERVILLE.
27.11.1991		
9.00 to 10.00	:	Fish as a source a human diseases. By C SOMMERVILLE
10.00 to 10.30	:	Mycotic diseases. By C. SOMMERVILLE.
11.00 to 13.00	:	Prevention in aquaculture. By S. DHAOUI
15.00 to 17.00	:	Laboratory work on parasitic diseases. B.C. SOMMERVILLE
28.11.1991	:	Departure to Izmir
29.11.1991	:	Discussions, recommendations
30.11.1991	:	Departure from Turkey

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SUMMARY OF REPORT

FOREWORD

This report was edited by Bodrum Fisheries Research Institute with the papers provided by the trainers to the course. The recommendations were prepared and approved by all the participants.

OPENING SESSION

All the participants except the representative of Bulgaria met on 17th. November at Otel Yumukoglu in Izmir. All course attendants came to Bodrum on 18th. November by bus, provided by Bodrum Fisheries Research Institute.

The session was opened by Mr. Nezih BILECIK, the manager of Bodrum Fisheries Research Institute. After that Mr. DOORENBOS, FAO Representative in Turkey welcomed the participants.

Mr. Othmen BEJI, MEDRAP II expert thanked the organisers and gave a short address on this matter.

The theoretical and laboratory sessions began on 19th. November in Bodrum Institute. All participants visited a floating net-cage farm and a hatchery during the training course. Morever, a field work was practiced on the earth-ponds of a farm o 20th. November.

CLOSING SESSION

Mr. BILECTIK, manager of Bodrum Institute, closed the training course and thanked the organisers and all participants. He expressed that the course was beneficial to all the participants.

- ENVIRONMENT AND FISH HEALTH WATER QUALITY FOR AQUACULTURE
By J. EDMONDSON
INSTITUTE OF AQUACULTURE UNIVERSITY OF STIRLING-SCOTLAND

- DIAGNOSIS OF BACTERIAL DISEASES
By M.T. HORNE
SCOTLAND

- BACTERIAL INFECTIONS IN GILT HEAD SEA BREAM (Sparus aurata)
By PROF. DR. HALUK ERYGUVEN
TURKEY

- DIAGNOSIS OF VIRAL DISEASESs
By M.T.HORNE
SCOTLAND

- LIMPHOCYSTIS DISEASE OF SPARUS AURATA IN MARINE CULTURE AT AEGEAN SEA AND MEDITERRANEAN COASTS OF TURKEY
By AKIN GANDAN
TURKEY

- A HISTOLOGICAL STUDY OF CARP POX (Viral epithelioma) DISEASE IN TURKEY
By G. TIMUR
TURKEY

LECTURES

- NUTRITIONAL DISEASES OF FISHES
BY DR. ATTIA EL HILI HEDIA
INSTOP - SALAMBO - TUNISIA

- SEA BASS, SEA BREAM AND FLAT OYSTER PREDOMINANT PATHOLOGICAL EASES IN NADOR LAGOON
BY TALBAOUT MUSTAPHA
MAROST - MOROCCO

- SOME IMPORTANT ASPECTS OF THE PATHOLOGY INVERTEBRATE SPECIES
BY FRANCISCO RUANO
PORTUGAL

- EXAMINATION OF FISH FOR PARASITES-EQUIPMENT
By Dr. C. SOMMERVILLE
SCOTLAND

- EXAMINATION PROCEDURE
By Dr. C. SOMMERVILLE
SCOTLAND

ENVIRONMENT AND FISH HEALTH WATER QUALITY FOR AQUACULTURE

By J. EDMONDSON
SCOTLAND

INTRODUCTION

This document is a collection of notes from lectures on environment and disease given on the MEDRAP II training project in disease diagnosis and prevention for technicians working in aquaculture. These notes are intended to act as a reference for the participants of the course.

The intention during the day is to give an introduction to the diagnosis and prevention of disease caused or induced by environmental conditions. Poor environmental conditions may promote disease in two ways; firstly by making fish more succeptible to infectious disease, and secondly, by direct toxicity. In the first case an autopsy will show the infectious disease which cases mortality but not the stress promoting factors which led to the disease outbreak.

In the second case an autopsy will provide little information as the symptoms of different environmental diseases are virtually identical. Therefore, because of the difficulties in diagnosing environmental diseases, emphasis during the day is placed upon knowledge of the environmental conditions and behaviour of fish before and outbreak of disease. This historical information is essential for accurate diagnosis and correct remedial action.

The day is separated into four lectures and a practical which is to be held in the afternoon:

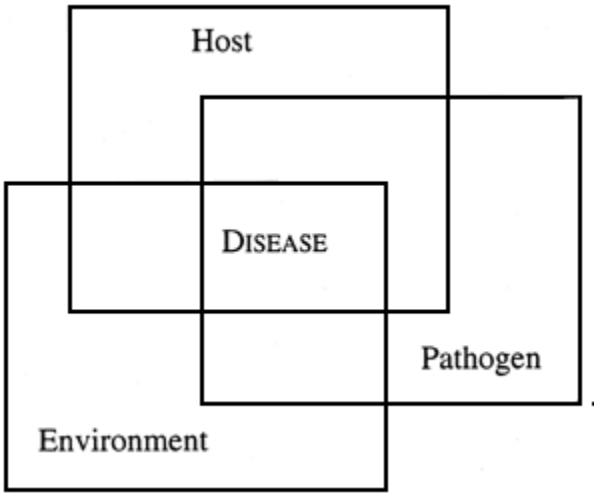
1. Introduction to water quality
2. Environment and health
3. Water quality management
4. Sampling and analysis methodology

1. INTRODUCTION TO WATER QUALITY

The aim of this lecture is to introduce and discuss some of the general concepts regarding water quality and aquaculture. it hopefully will prepare you for the following lectures which deals with tolerances and responses to particular water quality parameters.

GENERAL RELATIONSHIP

The relationships between an organism, its environment and disease are complex. This is summarised in the figure belows, which shows an organism in a state of equilibrium with its environment and with disease organisms, many of which are always present in the environment.



Disturbing this equilibrium by changing the environment may result in a stress (see Lecture 2) response in the fish, making it more vulnerable to fish disease. Some common stressors in farmed aquatic animals are:

1. poor water quality
2. overstocking
3. handling of fish
4. disease treatments

The most extreme response to stress is mortality, but below this level there may be several other responses, such as:

- changes in behaviour (e.g. hiding at the bottom of a tank, swimming near the inflow) or appearance (darkening or lightening of skin).
- poor appetite, poor food conversion, poor growth.
- reduced reproductive potential (eg low egg fecundity, spawning success)
- reduced tolerance to pathogens
- reduced ability to tolerate further stress

These last two are important because they emphasise:

1. That fish disease may follow any non-lethal but stressful environmental change.
2. that a combination of two or more factors, for example, high ammonia and handling, can be much more damaging to fish than one factor alone.

In many forms of aquaculture there is a fairly good general idea of the environmental factors which cause stress and will increase the likelyhood of disease outbreaks. However it is difficult to define what aspects of the environment cause specific stress to fish and to separate short term effects from the effects of long term exposure.

- In many ways, the aquatic environment is less stable than conditions on land (i.e. fluctuations can and do occur);
- Aquaculture is largely dealing with poikilotherms which are unable to regulate body temperature and thus maintain homeostasis;

- It is difficult to understand what aquatic animals need to keep them happy. As a farmer, it is also difficult to monitor and to react to changes in the aquatic environment.

(e.g. ammonia will affect the gill's structure with long term exposure, this may reduce the fish available area for gass exchange and in low oxygen conditions the combined effect will result in stress although the actual levels of ammonia and oxygen will not be toxic to the fish.)
When these sorts of combined effects are considered throught out the fishes life, it can be seen that it is a complex problem to resolve the different environmental factors.

Whilst long term effects are difficult to define, we have a fairly good idea about short term effects from short term toxicity tests (e.g. LC50 tests). These tests can help us estimate the king term toxicity effect but it is difficult to extrapolate to an aquaculture situation because of the complex combination of factors which are always acting upon the fish.

Variability of the environment is a problem in aquaculture. Whereas a fish may be able to adapt to long term exposure, a large fluctuation. Such as diurnal changes in oxygen, may be more critical. This is particularly important in the early life stages of the organism.

Water quality within an aquaculture system is determined by three principal factors:

- The name of the supply; (exogenous-dependent on water supply)
- The nature of the system; (endogenous - produced or affected by the fish)
- Management. (some control over above factors particularly the system)

Those factors largely determined by the water supply and those largely determined by the system and its management are shown below. The water supply depends on location (altitude, latitude, geology). Water quality in the farm depends on the quantities of uneaten feed, FCR, and the fates of these wastes (i.e. how quickly and gently they are removed from the system or processed into harmless compounds),

Water supply

The following parameters are largely determined by the nature of the water supply and are not significantly affected by most fish farm systems.

pH

temperature

alkalinity

salinity

biocides

suspended solids in inflowing water

disssolved gases in inflowing water

background nutrient levels

metals

hardness

Aquaculture systems and water quality

The following water quality parameters may be significantly affected by the aquaculture operation.

dissolved oxygen

ammonia

nitrite

biochemical oxygen demand (BOD)

carbon dioxyde

suspended solids

phosphorus

These factors are directly amenable to management (will be discussed later). They are related to the biomass of the stock, their activity, and the level of feed intake.

The gills of fish are a major interface with the environment where most of the exchanges between a fish and its environment take place. The surface area of the gills is approximately twice that of the skin. As such they play an important role in mediating environmental effects.

Gill pathology is important in diagnosis of water quality problems. However it is essential to have a clinical history (e.g. if fish are dying from lack of oxygen there may be no change in the gill because of the speed at which death is occuring, observations of water quality and fish behaviour, such as gasping, would be needed for diagnosis.)

Smears and squashes of fresh tissue or histology can be done. Smears and squashes may show over production of mucus or bacterial proliferation as well as parasites. If the appearance of the gill is uniform then it is likely that water quality is a problem (although extensive parasitic infection may give an uniform appearance.)

With histological examination it is very important to get good fixation from freshly killed fish. Briefly, the gill is composed of secondary lammellae protruding from primary lamellae. The secondary lamellae consists of a blood space covered by an epithelium. Pillar cells bridge the space to hold the two sides together. For gas exchange there is only a single or double epithelial distance for the gas to travel across. A thin layer of mucus covers the outer surface of the epithelium. Mucus cells, inflammatory cells, lymphocytes and salt cells are generally found embedded in the primary lamellae at the base of the secondary lamellae.

It is very easy to produce artefacts when looking at gill pathology. Common artefacts are separation of lamellae and epithelia, sloughing of epithelia. Telangiectasis may occur as an artefact due to killing a fish by a blow on the head. Haemorrhaging may occur when the fish is caught and killed. Debris on the gill surface may be due to vomited stomach contents.

There are basically two types of change in the condition that can occur.

Firstly acute changes that can occur when there is a high level of irritants present in the water (e.g. treatment overdose, acid rainfall, toxic algal boom.) The acute changes that occur are normally: sloughing of epithelia, necrosis of individual cells in gill epitheleum, surface hypertrophy (oedema within the cells), spongiosis (oedema within and between

cells), telangiectasis (swelling of he ends of gill filaments and a filling up with blood following rupture of the pillar cells in the secondary lamellae.)

Secondly chronic changes will occur in the fill when subject to low levels of irritants (e.g. suspended solids, repeated chemical treatments chronic ammonia toxicity etc.) In chronic cases the first change is usually and increase in mucus production. This is damaging because gas exchange now has to take place through a much thicker layer and the excess mucus may cause secondary bacterial problems-**bacterial gill disease**. This is not a specific bacterial disease but a problem of water quality which then allows secondary bacterial proliferation, particularly with myxobacteria. over a period of time the epithelia in the gill may become fused together at the tips of the secondary lamellae and gradually the epithela along the secondary lamellae multiply a condition called **hyperplasia**. This is more serious than mucus proliferation because even after treatment a fish will never completely recover its normal gill structure.

Gill pathology may give some indication of whether a disease is due to water quality and if so what parameter is involved (e.g. algae or debris may be found on the gills, damage to tips of secondary lamellae may indicate water quality problems.) However, it is virtually impossible to distinguish between the effects of different water quality parameters, knowledge of water quality is therefore needed for accurate diagnosis.

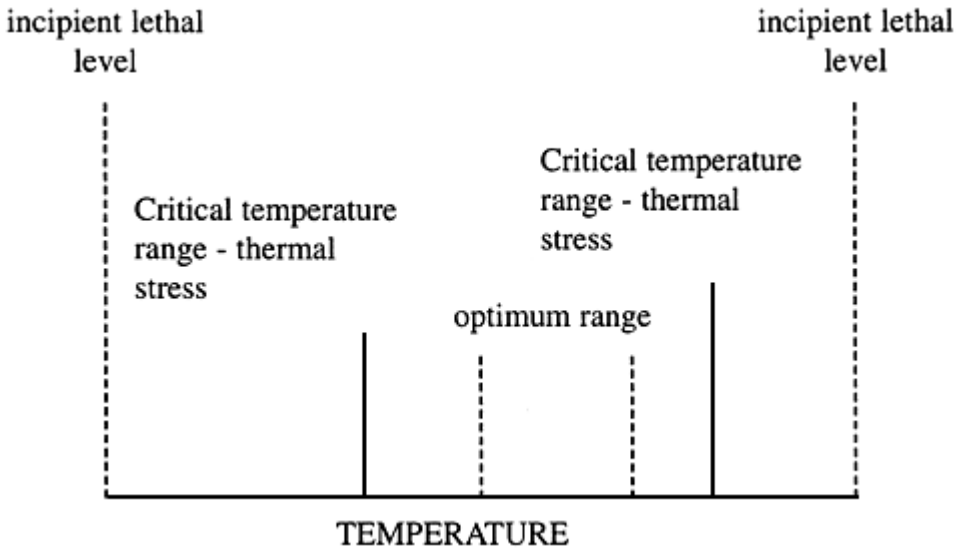
2. ENVIRONMENT AND HEALTH

The aim of this lecture is to outline in more detail the specific relationship between different water quality parameters and fish health. The first section discusses how stress by less than ideal environmental conditions can promote the onset of infections disease. The second discusses the action of different water quality parameters.

STRESS AND INFECTIOUS DISEASE

STRESS

All organisms have tolerance rages for environmental parameters. In figure below this is illustrated for temperature. There is an optimum range, outside of which is a zone of stress. Beyond this the animal crosses what is termed an incipient lethal level, beyond which death swiftly occurs.



These boundaries, although somewhat species-specific, are not fixed, but vary, depending on:

- age
- previous exposure
- genetic makeup
- other environmental parameters
- other factors (e.g. nutritional status).

What do we mean by stress? There are many definitions of biological stress, but all incorporate some notion of a stimulus acting on a biological system and the subsequent **reaction of the system.**

Stress may be **acute** or **chronic**. Acute stress is defined as one in which the duration of the stress-minutes or hours-is considerably shorter than the physiological response, components of which may last days or even some weeks. Chronic or continuous stress is unavoidable (thus making the stress response ineffective or even dangerous). The animal must acclimate to it, albeit at reduced performance capacity if it is to survive.

Acute stresses handling and disease treatment, both of which last only a few minutes, but fish have to be handled from time to (e.g. grading, transport), or treated for disease.

THE EFFECT OF WATER QUALITY ON PATHOGEN NUMBERS

20 genera of bacteria have been isolated from fish, of which at least 15 species are recognised as actual or potential pathogens (i.e. causitive agents of disease). Most of these organisms are naturally occurring and widely distributed, living on dead or decaying organic matter which is universally present in aquatic systems. An increase in the level of organic matter and an increase in the numbers of saprophytic organisms can follow.

The nature of water as medium allows easy transfer of pathogens between the cultured organisms and from wild fish. The high densities of culture organisms means a large of potential hosts.

In summary-an aquaculture system can induce stress (and hence reduce the effectiveness of the fishes immune system) whilst at the same time promoting an increase in the numbers of pathogens. It should therefore be remembered that environmental conditions have a vital role in the onset of communicable disease.

The following section discusses the action of different water quality parameters, how they cause stress and, if at toxic levels, mortality.

THE ACTION OF DIFFERENT WATER QUALITY PARAMETERS

Temperature and Salinity

Teleost fish are poikiolothermic and so their rate of metabolism immunological response, and reproduction changes along with changes in temperature. In addition other factors such as the solubility of gases in water, biological oxygen demand, toxicity of pollutants, and growth of fish pathogens changes with temperature. Different species have an optimum temperature range, outside of which is a zone of stress. Below and above certain temperature death occurs.

Sea bass are found in water between 5°C in the winter and 27°C in summer. Temperature is spawning areas are less diverse (10–12.5°C). Maturation does not occur above 18°C.

Salmonids will generally survive underneath thin ice cover and at temperatures up to 25°C. However, above about 18°C the solubility of oxygen becomes limiting and it is necessary to starve the fish to reduce oxygen consumption, both by the fish themselves and also as a result of breakdown of wastes. Incubation of eggs should take place a temperatures below 13°C. With time fish can aclimitise to temperature to a certain extent-a sudden increase therefore may be more stressful than moderately high temperature over a long period.

Fish such as sea bass and rainbow trout can grow in a wide range of salinities. e.g. Sea bass are found in all salinities from fresh to full seawater. Maturation does not occur in very low salinity. Rainbow trout can be grown in fresh water but higher salinities results in better grown in fresh water but higher salinities result in better growth (salmon need salinities >30ppt). Marine fanning has the advantages of more stable environmental conditions, higher winter temperatures, and greater water supply. However, young and sexually mature fish may suffer stress as they maintain their osmotic balance. This may result in disease.

Dissolved oxygen

Dissolved oxygen is one of the basic water quality requirements for fish. Most fish obtain oxygen from water, although some, such as the snakehead, Ophicephalus striatus and catfish, Clarias batrachus, can survive in waters without oxygen (anoxic) by breathing air. Such fish are known as air breathers. Although air breathers can survive in anoxic waters, experience suggests that they are vulnerable to dieseases in conditions where dissolved oxygen remains continuously low.

There are three main physical factors affecting the amount of oxygen a water can hold (ie the solubility of oxygen in water).

1. Temperature Water holds less oxygen at higher temperatures
2. Salinity Water holds less oxygen at higher salinities
3. Atmospheric Water holds less oxygen at low atmospheric pressure pressures (eg at high altitude)

Temperature (°C)	Solubility of oxygen in freshwater (ie at 100% saturation) (mg/l)	
	0 ppt	30 ppt
0	14.60	11.90
2	13.81	11.29
4	13.09	10.73
6	12.44	10.22
8	11.83	9.75
10	11.28	9.32
12	10.77	8.92
14	10.29	8.55
16	9.86	8.21
18	9.45	7.90
20	9.08	7.60
22	8.73	7.33
24	8.40	7.07
26	8.09	6.83
28	7.81	6.61
30	7.54	6.39
32	7.29	6.19
34	7.05	6.01
36	6.82	5.83
38	6.61	5.66
40	6.41	5.50

Other environmental factors which influence the dissolved oxygen in water include:

1. Phytoplankton blooms: during blooms dissolved oxygen will fluctuate during the day due to photosynthesis, with maximum concentrations during late afternoon and minimum concentrations at dawn. Dissolved oxygen will also decrease during the death of blooms due to bacterial respiration.
2. Organic loadings: bacterial oxidation of organic matter removes oxygen from water.
3. Respiration of fish and other aquatic vertebrates and invertebrates.

The basic requirement of fish for dissolved oxygen varies considerably, depending on several factors:

1. Species-oxygen requirements vary between species
2. Size-fry and juvenile fish normally require more oxygen per unit weight than adult fish
3. Activity-exercised fish require more oxygen than resting fish
4. Temperature-fish normally require more oxygen as temperature increases(this may cause problems as the water holds less oxygen at higher temperatures)
5. Feeding-oxygen requirements increases after feeding because oxygen is required to digest the food

6. Stress-stressed fish require more oxygen (this may cause problems if fish are stressed at times of low oxygen or poor water quality)

Typical oxygen requirements range from:

resting fish: 100–500 mg DO/kg wet weight/hour

active fish: 300–1500 mg/kg/hour

Some examples of times when the oxygen requirements of fish may exceed the available dissolved oxygen are given below:

1. After feeding-take care not to feed in the afternoon or evening in heavily loaded pond systems
2. After adding organic manure to ponds-organic material will consume oxygen during decomposition
3. Early morning in pond systems
4. During the death of phytoplankton blooms-decomposition requires oxygen
5. Increases in temperature-fish require more but there is less in the water (also bacterial respiration may increase at such times)
6. Decreased water flow in more intensive systems.

The first indication of possible oxygen stress may be change in fish behaviour, with fish crowding at the surface or near the pond inflow, gasping for oxygen. If sustained over a period of time, low dissolved oxygen may result in significant sublethal and lethal effects.

Guidelines for non-salmonids

0.3–0.8 mg/l	may be lethal to many fish species if sustained over a period of time (most pond fish species can tolerate near anoxic conditions for a short period at dawn provided such conditions occur for a short period of time)
1.0–5.0mg/l	may be sublethal effects, for example on growth, feed conversation, and tolerance to disease
>5.0mg/l	warm water fish reproduce and grow normally

Guidelines for salmonid fish

0.8–4.0 mg/l	may bed lethal to salmonids
4.0–6.0 mg/l	may be sublethal effects, eg poor growth, bad food conversion, reduced tolerance to disease
>6.0 mg/l	salmonids normally grow and reproduce normally
>7.0 mg/l	recommended for hatcheries

Nitrogen

Nitrogen is a very important element in aquaculture. As a nutrient, it is an important requirement for phytoplankton growth. Ammonia and nitrite, two inorganic forms of nitrogen, are also toxic to aquatic organisms.

Nitrogen originates in the atmosphere (80% of air is nitrogen), but can be found in several different forms in water nitrogen gas, ammonia, ammonium, nitrate, nitrate and various forms of organic nitrogen.

Ammonia

Ammonia is usually the second most important water quality parameter after dissolved oxygen. The total ammonia concentration in water consists of two forms:

NH_3 unionised ammonia (gaseous form)

NH_4+ ionised ammonia (ammonium ion)

These two are in equilibrium according to the equation:

$$NH_3 + H_2O \longleftrightarrow NH_4^+ + OH^-$$

The unionised ammonia (UIA) fraction is toxic to fish. The concentration of unionised ammonia in water depends on the pH and temperature of the water. As a general rule, the higher the pH and temperature, the higher the percentage of the total ammonia that is in toxic unionised from. This effect is illustrated below:

Percentage of unionised ammonia in water of different pH and temperature

pH	Temperaure (°C)	
	20	32
7.0	0.4	1.0
8.0	3.8	8.8
8.2	5.9	13.2
8.4	9.1	19.5
8.6	13.7	27.7
8.8	20.1	37.8
9.0	28.5	49.0
9.2	38.7	60.4
9.4	50.0	70.7
9.6	61.3	79.3
9.8	71.5	85.8
10.0	79.9	90.6
10.2	86.3	93.8

Alternatively, the percentage of unionised ammonia in a given sample of total ammonia can be calculated from the equation:

$$pKa = 0.09018 + \frac{2729.92}{(273 + T)} \quad \text{(where T = temp (°C)}$$

$$\%\,UIA = \frac{100}{(1 + \text{antilog}\,(pKa - pH)}$$

Amonia in water can originate from several sources:

1. Decomposition of organic matter-particularly after fertilising ponds with organic manure or inorganic ammonia based fertilisers. The decomposition of waste feed in intensive fish farming will also produce ammonia.
2. Industrial and domestic pollution
3. Excretion by aquatic organisms, particularly fish and shellfish in intensive aquaculture systems, Also, during fish transportation.

4. Denitrification-ammonia is oxydised to nitrite and harmless nitrate in oxygenated waters a (process known as nitrification). In deoxygenated waters, nitrate is converted to nitrite and ammonia (denitrification). Deoxygenation in heavily loaded pond system (such as intensive Clarias ponds in Thailand) can therefore lead to a build up of ammonia.
5. Death of phytoplankton blooms-high levels of ammonia in pond systems are commonly associated with the death of phytoplankton blooms.

The toxic effects of unionised ammonia on fish vary considerably depending on the fish species and environmental conditions. Some general guidelines are given below:

0.4–2.5 mg.l	Lethal to many fish species. Certain species, such *Clarias batrachus* have a very high tolerance to unionised ammonia with lethal concentrations around 3.4 mg/l
0.05–0.4 mg/l	sublethal effects depending on species, may include gill hyperplasia, reduced activity and growth, liver, kidney and brain damage.
>0.02–0.05 mg/l	safe concentrations for many tropical and temperate fish species (salmonids are more susceptible).

The toxicity of ammonia to fish is reduced at increasing salinity and at high dissolved oxygen and high carbon dioxide concentrations.

Nitrite

Nitrite is an intermediate product in the biological oxydation of ammonia to nitrate (nitrification). It is relatively low in most natural waters and healthly fish farming systems, but may reach high concentrations where there is organic pollution or oxygen is low.

Nitrite is highly toxic to fish. When nitrite is absorbed by fish it reacts haemoglobin to form methahaemoglobin. Methahaemoglobin is not as effective a carrier of oxygen as haemoglobin and therefore fish exposed to high levels of nitrite eventually die from lack of oxygen.

The main environmental factor which affect nitrite toxicity is chloride. The following guidelines have been developed for temperate water species by EIFAC :

Safe nitrite level (mg/l as N)

chloride	salmonid	non-salmonid
1 mg/l	0.01	0.02
5 mg/l	0.05	0.10
10 mg/l	0.09	0.18
20 mg/l	0.12	0.24

Lethal thresholds vary considerably Iron 1 mg/l for salmonids in low chloride waters to 152 mg/l for very tolerant species such as *Clarias batrachus* in high chloride waters.

Nitrate

Nitrate is the end product of the biological oxidation of ammonia and nitrite. It is effectively non-toxic to fish, except at concentration of <400 mg/l. Such concentration are unlikely in most water supplies.

Nitrogen gas

Water containing concentrations of gas above saturation levels known as supersaturated. Strictly speaking, all atmospheris gases can contribute to gas supersaturation, but because of its relative abundance in air, nitrogen gas in a major contributor to most gas supersaturation problems. These problems may occur under the following conditions:

1. Heating og water-the solubility of gases decrease with increasing temperature. Therefore, if saturated water is heated without allowing gas to escape, the water will become supersaturated. Mixing of waters of different temperatures can produce the same affect.
2. Ice formation-the solubility of gases is increased as water is cooled. As ice is formed, dissolved gases are expelled and concentrated in the remaining water. In shallow lakes, lethal dissolved gas levels may be formed under the ice.
3. Air entrainment-any time that air and water are in contact at pressure higher than aunospheric pressure, gas supersaturation may be produced (eg. spillways. air leaks in pipes).
4. Photosynthesis-dissolved oxygen production during algal blooms may result in lethal or sublethal gas supersaturation.
5. Pressure changes-reduction in pressure may result in gas supersaturation (eg in aircraft).

Gas bubble disease (or trauma) is classically characterised by gas bubbles in the blood, gills and other organs, resulting in various forms of tissue damage.

Safe levels: <105% saturation

Suspended solids

Suspended solids are normally defined as the solid material present in the water which is retained on a fine filter paper after filtration of the water sample. The mesh size of the filter influences the results and 0.45 um mesh is commonly used in most analyses. The suspended solids may also be measured indirectly using a Secchi dise (although this more correctly meaures turbidity).

Suspended solids may originate in the catchment area of a water supply through natural weathering of rocks and lend erosion or pollution. Within fish culture systems, suspended solids may come from phytoplakton blooms, uneaten food particles and fish faeces.

The effects of suspended solids depends on the nature of the solid. Abrasive particles such as wastes. from coal working or long-spind diatoms (algae) are more harmful to fish than soft materials.

The toxic effects of suspended solids are directed towards the sensitive gill tissues and gill damage, excessive mucus production and coughing and bacterial gill disease are all common responses to high suspended solids loads. The coating of eggs by suspended solids can also reduce efficiency of oxygen uptake and increase egg mortality.

Salmonids are extremely sensitive to suspended solids and he following safe levels are recommended:

<20 mg/l	acceptable for ongrowing
<5 mg/l	essential for hatcheries

Most tropical freshwater species such as the tilapias, many carps and catfishes are very tolerant of levels up to 10.000 mg/l, althoug effects will depend on the nature of the particle.

Suspended solids and turbidity may also be important in reducing the penetration of light into culture ponds, reducing the productivity and increasing the risks orf deoxygenation. Ponds with persistant turbidity problems (normally caused by fine clay mineral particles) should be treated with alum (25 – 45 kg/ha) or organic matter.

Acidity and alkalinity

pH

Definition

The pH of water is measure of the concentration of hydrogen icons in the water:

$$pH = - \log (H^+)$$

Note that because it is a logarithmic scale a one fold change in pH is equivalent to a 10 fold change in hydrogen ion concentration. pH is defined on a scale if 1 to 14; a pH <7 is acid and a pH >7 is alkaline.

Occurence of acid waters

Naturally acidic waters may be derived from waters draining peat swamps, acidic rocks or acid sulphate soils. Such waters may be particularly acid during floods, particularly in rain after a dry spell.

Pollution from mining and various industries (eg rubber and palm oil processing) may also be acidic. Effluents from such industries may also contain heavy metals and other acidic anions (eg chromic acid) which may themselves he extremely toxic to fish. Metal joins are more soluble in acidic waters and therefore all acid waters may contain metals which may be more toxic to fish than the acidity itself.

Occurrence of alkaline waters

Naturally occurring alkaline waters are usually derived from calcium and silica rich areas. In addition, legal blooms may result in very alkaline pH values.

Pollution from soft drink and brewing industries may also be extremely alkaline.

Physiological effects of alkaline waters

The optimum pH range for most freshwater fish species is from pH 6 to pH 9. Outwith this range, there are increasingly severe effects on fish production, because of increasingly toxic effects on the fish themselves and because, of adverse effect on pond productivity.

The direct toxic effects on fish at alkaline pH may star at pH 8 (artificial spawning trials of *Sarotherodon aureus* have failed at pH > 7.6) but continuous exposure to pH>9 is required before there are any toxic effects on most species. As a general guide:

pH	effect
>11	lethal to all fish species except sometimes in ponds with very high levels of dissolved oxygen
10–11	lethal to many fish species if exposed over a long period or time. Sublethal effects may include gill damage and damage to the lens and cornea of the eye (often seen as an opagueness in the eye)
9–10	sublethal effects on many species.

The toxic effects of high pH may also be made worse by the presence of metals (eg zinc) and by increasing toxicity of other compounds (eg ammonia) at high pH.

Physiological effects of acid waters

Acidic waters between pH 6.0 and 5.0 are not normally directly toxic to fish unless fish are acclimated to alkaline pH or the concentration of free carbon dioxide is greater than 20 mg/l or the water contains large amounts of iron or aluminium.

Increasing levels of H^+ result in a gradual breakdown of the gill and epidermis in fish which results in increasing loss of body salts and difficulties in taking up oxygen. Eventually fish may die due to osmotic disturbance and/or lack of dissolved oxygen. The effects on the gills can be seen as swelling or destruction of the epidermal cells and excessive mucus production.

As a general guide:

pH	effect
<4.0	direct mortality may occur in many fish species
4.0–5.0	sublethal effects may include a loss of body salts, gill damage, reduced spawning success, poor growth and lowered resistance to disease in many fish species
5.0–6.0	poor and productivity.

There are many factors affecting the toxicity of acid to fish. Some of the most important factors are outlined below.

1. Carbon dioxide: high free CO2 increases the toxicity of acids.
2. Calcium, magnesium, sodium and chloride: the primary effect of acidity is to disrupt the ionic balance of fish. Thus, an increase in the concentration of these cations will help to protect fish from harmful effects of acids. Calcium is particularly important
3. Species, size, age, acclimation of fish: the fry stages on hatching fish are normaly most vulnerable to acids. Some acid ponds can be successfully used for fish culture if fingerlings rather than fry are stocked. Fish may also be acclimated to low pH if exposure is gradual. Rapid changes in pH are most damaging to fish, particularly if the fish are acclimated to high pH.

Many of the effects previously attributed to H^+ ious are now known to be due to aluminium.

Alkalinity

Alkalinity refers to the concentration of bases in water ana capacity of the water to accept acidity (ie its buffering capacity). In most waters, bicarbonate and carbonate are the predominant bases.

Waters with a low alkalinity (total alkalinity < 20 mg/l as CaCO3) have a very buffering capacity and consequently are very vulnerable to fluctuations in pH (eg during rainfall and phytoplankton blooms). Such fluctuations amy be directly harmful to fish populations. Low alkalinity ponds also tend to be much less productive than high alkalinity ponds although ponds with alkalinities greater than 300 mg/l as CaCO3 may also be unproductive because of limitations to carbon dioxide availability at such concentrations.

The ideal range for alkalinity is 20 – 300 mg/l as CaCO3

Total hardness

The total hardness of water is made up of the cations of alkali earth metals, mainly calcium and magnesium ions. The total hardness concentrations should be similar to the total alkalinity in most waters because calcium and magnesium are commonly bound to the main alkalinity bases, bicarbonate and carbonate. When the total hardness of a water exceeds the total alkalinity, some of the calcium and magnesium is bound to anions other than bicarbonate and carbonate (eg sulphate and chloride). When the total alkalinity exceeds hardness, some of the bicarbonate and carbonate is associated with sodium and potassium rather than calcium and magnesium.

In most waters the alkalinity is more important than the total hardness. A total hardness of greater than 20 mg/l as CaCO3 is considered satisfactory for pond productivity and should help to protect fish against the harmful effects of pH fluctuations and metal ions.

Carbon dioxide

Carbon dioxide is a gas which is highly soluble in water, but because there is only a small amount in the atmosphere, the concentrations in most waters is low.

Carbon dioxide has an acidic reaction with water.

$$H_2O + CO_2 \longleftrightarrow HCO_3 + H^+$$

Because of this reaction, pure water in equilibrium with the atmosphere has an acidic pH. At 25°C. the pH of pure water is 5.7. As a general rule. Carbon dioxide will not cause the pH to drop below 4.5 and any pH below 4.5 must be due to mineral acidity.

Carbon dioxide can be found in three closely related forms in water:

CO_2 - free carbon dioxide

HCO_3 - bicarbonate ion

CO_3 - Carbonate ion

The concentration of each depends on pH:

CO_2	% of total CO_2 in each form in relation to pH							
pH	4	5	6	7	8	9	10	11
CO_2	99.5	95.4	67.7	17.3	2.0	0.2	-	-
HCO_3	0.5	4.6	32.2	82.7	97.4	94.1	62.5	14.3
CO_3	-	-	-	-	0.6	5.7	37.5	85.7

Free carbon dioxide is the form that is toxic to fish. Consequently high toxic concentrations are normally only found in natural or acidic waters.

Most natural waters contain low concentrations of free carbon dioxide (>6 mg/l). However, carbon dioxide may reach high levels in the following circumstances:

1. Acidic ground water
2. In ponds with large phytoplankton populations carbon dioxide may reach high levels during:
   - the death of a bloom
   - at night due to phytoplankton respiration
   - during cloudy weather
3. In ponds heavily loaded with organic manure of feed. Levels in Thai Clarias ponds commonly reach 30–40 mg/l.
4. Fish transportation - fish excrete carbon dioxide and consequently high concentrations may build up when a large biomass of fish is enclosed in a small volume of water. CO_2 build up is worse when fish are transported in enclosed bags containing oxygen. Open tanks with aeration are less likely to suffer from CO_2 problems.
5. In natural waters high concentrations may occur after herbicide treatments High concentrations of free dioxide can be harmful to fish. Carbon dioxide hinders the uptake of dissolved oxygen. Consequently the effects of high CO_2 are made worse at low dissolved oxygen concentrations. In air breathers, such as *Clarias* and *Ophicephalus*, 90% of carbon dioxide is excreted across the skin and gills into the water. It is thought that high external concentrations will interfere with this exchange causing respiratory problems and stress. Some general guidelines may be given as follows:

50–60 mg/l : lethal to many fish species with prolonged exposure
12–50 mg/l : sublethal effects may include respiratory stress and the development on kidney stones (nephrocalcinosis in some species).

Hydrogen sulphide

Hydrogen sulphide is produced by bacteria in anoxic waters (ie waters deficient in dissolved oxygen). It is common in aquaculture systems where there is a heavy organic load (eg it heavily fertilised ponds or below intensive cage farms).

Two forms of hydrogen sulphide exist in water:

HS ionised sulphide ion

H2S unionised hydrogen sulphide gas

Unionised hydrogen sulphide gas is TOXIC to fish

Analytical techniques measure TOTAL SULPHIDE (as ammonia). The proportion of this total sulhide which is in the toxic hydrogen sulphide gas form is related to pH:

% total hydrogen sulphide in the toxic gaseous form at 25°C

pH	%
5.0	99.0
5.5	97.0
6.0	91.1
6.5	76.4
7.0	50.6
7.5	24.4
8.0	9.3
8.5	3.1
9.0	1.0

Most fish species are extremely sensitive to H_2S gas. Toxic levels are:

0.002–0.4 mg/l	sublethal effects, including gill damage, depending on species
0.01–5.3 mg/l	lethal effects, depending on species

WATER QUALITY CRITERIA

	Salmonids	**Sea bass and bream**
Dissolved oxygen	>6 mg/l > 7 mg/l (hatcheries) > 5.5 mg/l (rainbow trout)	>3 – 4 mg/l
Ammonia (unionised form)	<0.02 mg/l (unionised form) 0.002 mg/l (hatcheries)	<0.1 mg/l
Nitrite	0.01 mg/l (soft water) 0.06 mg/l (hard water) (as NO2 - N)	
Nitrate	<40 mg/l	
Carbon dioxide	<6 mg/l free CO2	
pH	6.5–8.5 ideal	
Suspended solids	<5 mg/l (Hatcheries) <25 mg/l	found in very turbid Waters
Hydrogen sulphide	<0.002 mg/l	
Aluminium	<0.1 mg/l (pH 5–6)	
Cadmium	<0.001 mg/l	
Chromium	<0.025 mg/l	
Copper	<0.005 mg/l (soft water) <0.01 mg/l (hard water)	
Iron	<0.5 mg/l	
Lead	<0.001 mg/l (ideal)	
Mercury	<0.00005 mg/l	
Nickel	<0.01 mg/l (soft water) <0.05 mg/l (hard water)	
Zinc	<0.03 mg/l (soft water) <0.5 mg/l (hard water)	

SUMMARY - WATER QUALITY AND FISH DISEASE

factor	**disease link**
stress	all fish more susceptible to disease following one or more stress events
Wastes	myxobacteria, fungal infection, gill disease (chronic, acute), *Aeromonas*
solids	myxobacteria gill disease
hardness	soft water - chronic kidney disease hard - soft water - poor transfer
Carbon dioxide	nephrocalcinosis
temperature	high temp - more stress - less DO *Aeromonas* >16–18°C for salmon myxobacteria - low T, high wastes
nutrients	algal blooms site dependant, gill damage, direct toxicity

3. WATER QUALITY MANAGEMENT

Water quality within an aquaculture system is determined by three principal factors:

- The nature of the **supply**;
- The nature of the **system**;
- **Management.**

The water supply depends on location (altitude, latitutude, geology). Water quality in the farm depends on the quantities of uneaten feed, FCR, and the fates of these wastes (i.e. how quickly and gently they are removed from the system or processed into harmless compounds). The endogenous parameters (those produced or altered by the fish) can be controlled and managed to a certain extent. The different parameters are considered in turn below but first an outline of water quality management is given:

The essentials of good water quality management are:

Good site selection

Correct stocking biomass and density

Correct husbandry system

Monitoring

Planning

Remedial action

SITE SELECTION

Many factors are taken into consideration in site selection. Over emphasis on non-environmental factors such as price of land, road access and commercial opportunity may result in a site with a recurring environmental problem throughout the life of the farm which limits product on and causes stress to the cultured species. (e.g. land based farms with an inadequate water supply, cage farms in highly enclosed bays with poor water exchange).

STOCKING BIOMASS AND DENSITY

If a system is overloaded with an excessive biomass deterioration of water quality will result in an increase in stress and therefore disease. Holding capicity must be calculated for the particular system as some environments are more sensitive than others. In calculating holding capacity it may be possible to identify the parameters which will limit production and which may be the likely cause of health problems.

CORRECT HUSBANDRY SYSTEM

Some basic requirements for husbandry systems are:

- to provide for the environmental requirements of the stock
- to be able to observe and examine the stocks response in the production system
- control of the system so that conditions can be manipulated if possible
- failsafe in use i.e. if a breakdown occurs there is not an immediate fish kill
- easy as possible to operate e.g. feeding, cleaning, grading, disease treatment harvesting, removal of moribund/dead fish
- work economically-expensive systems may force the farmer to increase product leading to overstocking poor environment and disease problems
- hatchery systems should be compact with easy handling, observation and treatment eggs and fry

Without the correct system water quality problems will result, which will increase stress the incidence of disease.

some advantages and disadvantages of holding systems are listed below:

PONDS

Advantages	Disadvantages
Relatively low cost	Less easy to manage
Internal water reconditioning	Difficult to control water quality
Natural food supply	Stock handling relatively difficult
Easy to construct if location right	Possible dramatic changes in environment
	Difficult to clean
	Generally lower production/unit area
	Difficult to adapt or move

TANKS

Highly controllable environment	Relatively high cost
Good self cleaning	Concentrated wastes may be discharged
Relatively high production/area	Design problems with scale-up
Easy to handle/observe stock	Note normally fail-safe
More uniform environment	Raceways wasteful of water with small fish - may require specific land gradient

CAGES

Low cost	Difficult to control environment
Movable	Disease treatment often difficult
Relatively good water quality	Often difficult to observe fish
Easy stock management	Possible feed losses

Minimal need for land	More risk of physical damage
Rapidly developed	High labour input
Relatively high productivity	Wastes disposed around stock

MONITORING

It is important to monitor environmental parameters on a regular basis, particularly at certain times of the year (e.g. particularly warm water, high biomass). This is important for two reasons. Firstly so that remedial action is possible if a problem can be seen to be developing (e.g. algal bloom, falling oxygen levels, high temperature. The type and frequency of sampling is discussed in the next lecture.)

PLANNING

In order to protect stock from a developing water quality problem, or to overcome an existing problem, good planning is essential. It this context planning means avoiding causing additional stress to fish at important times (e.g. not grading or treating when oxygen levels are low.) and readiness for deterioration of water quality so that remedial action can be taken quickly. (e.g. cage salmon farms in south west Ireland which experience toxic blooms of *Gyrodinium aureolum* increase water sampling at critical times of the year and ensure that aeration equipement is ready for emergency use.)

REMEDIAL ACTION

Where a particular water quality parameter is posing a problem the following streps can be taken:

Dissolved oxygen

Dissolved oxygen concentrations may be controlled by:

1. Aeration (or oxygenation)
2. Correct stocking and fertilisation of ponds (amounts and timing). Fertilisation can also be used to stop phytoplankton blooms from dying.
3. Increasing water flow
4. Good pond design - deep sheltered ponds are more vulnerable to deoxygenation than shallow open ponds

Ammonia

The toxicity of ammonia to fish is reduced at increasing salinity and at high dissolved oxygen and high carbon dioxide concentrations. Several techniques may be used to reduce the effects of ammonia on fish populations.

1. Improve overall dissolved oxygen concentrations by aeration - will also tend to decrease pH (hence reduce toxicity) and may blow off some of the gaseous unionised ammonia from the water.
2. Good pond management - healthy phytoplankton populations will remove ammonia from water. Care should be taken when using fresh manures high in ammonia (these can be left to dry for a few days if required, to allow ammonia gas to escape)
3. Stocking and feeding control and improved water flows in more intensive systems

4. Chemical treatment - salt has been show to reduce the toxicity of ammonia to Clarias. 200–300 kg/rai (I rai = 1600 m^2) is commonly used to treat Clarias ponds in Thailand. Other forms of treatment for more intensive systems included ion exchange resins (zeolite) and addition of acid (commonly HCI) to reduce pH.
5. Biological filtration - may be used to treat water to convert ammonia to nitrite to harmless nitrate (nitrification). Essential ingredient of recycling aquaculture systems.

Nitrite

Problems with nitrite in fish culture can be avoided by:

1. correct stocking, feeding and fertilisation practices particularly by keeping ponds well oxygenated.
2. Addition of sodium chloride to ponds at 250 mg/l (succesfully used in *Clarias batrachus* culture in Thailand.
3. Biofiltration : biological conversion of nitrite to harmless nitrate.

Nitrogen gas

In order to avoid nitrogen gas supersaturation care should be taken in the following areas:

1. Heating of water - the solubility of gases decrease with increasing temperature. Therefore, if saturated water is heated without allowing gas to escape, the water will become supersaturated. Mixing of waters of different temperature can produce the same effect.
2. Ice formation - the solubility of gases is increased as water is cooled. As ice is formed, dissolved gases are expelled and concentrated in the remaining water. In shallow lakes, lethal dissolved gas levels may be formed under the ice.
3. Air entrainment - any time that air and water are in contact at pressures higher than atmospheric pressure, gas supersaturation may be produced (eg. spillways, air lakes in pipes).
4. Photosynthesis - dissolved oxygen production during algal blooms may result in lethal or sublethal gas supersaturation.
5. Pressure changes - reduction in pressure may result in gas supersaturation (eg in aircraft).

Acid and alkaline water

Treatment of alkaline water

Alkaline water can be treated in several ways:

1. Rapid fluctuation in pH caused by excessive phytoplankton blooms may be treated by ensuring the pond is welt limed and that the pond water has an alkalinity of greater than 20 mg/l as CaCO3.
2. Acid forming fertilisers
3. Addition of acid to water supplies (HCI and H2SO4 have been used: add small amounts and monitor pH to determine exact requirements)

Treatment of acid water

Acidic water can be treated in several ways:

1. Liming: the addition of calcium based material is preferable because calcium gives added protection to fish gills against the toxic effects of acidity
2. Salt water: sea water may be flushed through ponds in coastal waters to neutralise acidity.

Alkalinity

Ponds with low alkalinity can be treated with lime.

Hardness Total hardness can be improved by liming

Carbon dioxide

Carbon dioxide can be removed from water in several ways:

1. Vigorous aeration
2. Increasing pH by addition of calcium hydroxide (hydrated lime)

   $$Ca(OH)_2 + 2CO_2 \longrightarrow Ca(HCO_3)_2$$

   In ponds with low alkalinity, care must be taken not to overtreat because excess lime may cause the pH to rise which may be directly harmful to fish or increase the concentration of toxic unionised ammonia if total ammonia concentrations are high.

   Field trials have shown that approximately I mg/l of hydrated lime can remove 1.68 mg/l of free carbon dioxide.
3. Control of phytoplankton populations and organic loading by correct stocking feeding and fertilisation.
4. Pond design-open shallow ponds are less likely to suffer carbon dioxide problems that deep sheltered ponds.

Hydrogen sulphide

Sulphide is oxydised to sulphate in oxygenated waters. Keeping the system well oxygenated is the best way of stopping hydrogen sulphide being formed and of removing it from the system (particularly close to the sediments).

4. SAMPLING AND ANALYSIS

AQUACULTURE WATER QUALITY SAMPLING

AIMS OF SAMPLING

1. Evaluating suitability of a water supply for establishing a new fish farm (more important for sensitive species or sensitive stages of the life cycle, eg. salmonids, prawns, hatcheries).
2. Routine water quality monitoring, in order to ensure conditions are optimal for fish growth.
3. Assessing natural productivity of a lake (or marine site), as a basis for estimating fish yields, or for examination of the potential for cage culture (intensive or extensive).

4. Monitoring of effluent quality (increasingly important)

CHOICE OF PARAMETERS TO ANALYSE

1. Conservative parameters (eg major ions (hardness), conductivity, alkalinity, salinity) require less frequent sampling (although beware acidic catchments or marine and freshwater systems receiving large amounts of rainfall)
2. Variable parameters (eg dissolved oxygen, ammonia, carbon dioxide) may be very variable within aquaculture system and require more frequent sampling (weekly, daily, diurnal).

The parameters which should be investigated depend very much on the type of system and the critical parameters which you identified. In general, a more extensive sampling programme is used to identify critical parameters and subsequent sampling will concentrate on these. A good pre-development study should also identify future analytical requirements.

A guideline for preliminary sampling in different systems is given below:

SYSTEM	PARAMETERS	SAMPLING FREQUENCY
Heavily loaded flow through systems, recycle systems	DO.NH3.NO2 pH.T.CO2 solids (salinity, if SW)	daily at critical times plus 24h runs occasionally
Intensive ponds	DO.NH3CO2T pH. Secchi disc NO2 PO4 chlorophyll (salinity, if SW)	daily at critical times. plus 24h runs checks during high loading or algal blooms
Freshwater cages	DO, NH3, NO2, PO4 chloruphyll, Secchi, pH, (sediments), T	weekly, plus DO, NH3 daily
Seawater cages	as above, plus salinity and H2S	as above, plus salinity daily
Pumped intensive systems	as heavily loaded flow though, plus gas saturation	as heavily loaded flow through
Effluent checks	pH, BOD, NH3, solids, Total p(cages), DO	high loading, depends on consent conditions

SOURCES OF VARIABILITY

1. Spatial variability

   horizonal variability, eg in lakes and ponds (depending on wind, inflow, outflow configuration)

   vertical variability, eg during stratification

2. Temporal variability

   temporal variability varies depending on the type of water supply; eg in terms of stability, bore hole supply > lakes (sea) ponds > rivers > streams. Variability is also affected by season, tides, climatic conditions.

BIOLOGICAL INDICATORS

Critical fluctuations in water quality may be missed by water sampling. biological indicators sometimes give a better indication of overall water quality.

Why and who should monitor

(a) Statutory bodies

Most countries have some body or other concerned with regulating discharges from industry or agriculture. For legislative purposes either statuatory bodies or properly equiped and recognised water quality laboratories should carry out monitoring to ensure accuracy.

(b) The farm

Monitoring should be carried out to provide information on water quality for management.

Partly to fulfill legal consent obligations, partly to optimize conditions for stock. Some farms prefer an outside agency to carry our work.

The important parameters depend upon the system and any critical parameters which may have been identified. Usually the following should be monitored regularly and careful records kept as they are most important for fish health.

Dissolved oxygen
Temperature
Ammonia/nitrite
Transparency (cage sites)

These parameters can be monitored with probes or kits.

Sediments around water-based farms can be monitored for indicative changes in a number of parameters such as:

- macrobenthic community structure (most sensitive, although time-consuming)
- sedimentary redox potential (quick, easy; used in conjunction with macrofaona)
- organic C and N (useful, but patchy due to distribution of wastes and benthic inverts; may lead to complicated interpretation and thus not recommended for routime monitoring)
- photographs of the sediment surface

It is sometimes useful for the farm to monitor redox potential and sediment appearance to check for outgassing or excessive waste accumulation.

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DIAGNOSIS Of BACTERIAL DISEASES

By M.T. HORNE
SCOTLAND

Routine monitoring, isolation and identification is part of normal, goods farming practice. If a farm has a disease problem:

1. Obtain information, before visit, of type of farm, size of farm, species concermed, any previous history of discase and any indicators of the quality of the farming practice at the site.

2. At the farm, obtain information which might be relevant to the occurrence of disease, For example, any rise or fall in temperature before the disease outbreak, any change in water quality (oxygen level, flow rater, suspended solids or pollution).

   Any change in management , for example, grading, chemical treatments, fish movements, importation of new stocks, change in appetance or diet.

   The farm records and farm staff should have this information.

   This is all helpful because good quality fish, well managed, do not easily contract disease. Many discase problems are «secondary» caused by stress consequent to changes in the items listed above.

3. Look at the external sings of the fish behaviour abnormal swimming, «itchings» , clustering at outlets, jumping excessively. Chemical signs: haemorrhages, lession, gill conditions, parasites, binsters, fungal growth.

4. Similarly study the intenal appearance for these purposes and for sampling (below) use moribund or freshly dead fish. Do not forget to examine normal (non discased) stocs from the same farm.

5. Sampling:

   Where possible use a field sampling kit or transport suitable individuals to the laboratory. Transporting dead fish except for very short journeys, is not so good.

   Tissue samples, where live isolation of pathogen is not required, should be transported in a suitable fixative for either histopathology or electron microscopy.

   If tissue samples are transported for live isolation waterproff them in polyester and transport on ice.

   External sampling: The surface of a fish will always have many bacteria which will grow in culture media but which are not associated with disease. It may be necessary to sue a partially selective medium in addition to normal media.

   The central, necrosed part of a lesion will have many such bacteria: look for the pathogen at the periphery whwre new tissue is being necrose. Gills may be sampled by a direct «print» to a glass slide (for staining and microscopy) or by a print onto growth medium followed by spreading for single colonies.

Internal sampling: Swap the ventral surface with a sterilant of heat. Cut with a fresh scalpel through the sterilised are. Avoid puncturing internal organs. Sample any affected areas. Sample also «healthy» tissue. Good, «routine» sample areas are the anterior kidney and the spleen. Sampling is done by the use of a bacteriological loop, be careful to be clean. Surface: sterilise the organs for sampling if there is extensive haemorrhaging since the blood will probably be contaminated.

Remove samples for histopathology after sampling for bacteria has been completed.

6. Identification in the laboratory.

   For general growth use

   - Nutrient broth/agar

   ultra-violet microscope.

7. Frequently diagnosis is of lesser importance than recommendation of therapy. Here the antibiotic sensitivity of the isolate s required. Use commercially available testing discs which contain relevant levels of antibiotic and will give zones of lysis, indicating sensitivity, when placed on an agar plate culture of the pathogen. Rarely the MIC (Minimal Inhibitory Concentration) may be required. This is asseyed by growth in a range of concentration of the antibiotic.

   Bacterial groups discussed:

   VIBRIOS
   AEROMONADS
   GRAM NEGATIVE+PIGMENTED RODS
   EDWARDSIELLA

   - Tryptic Soy broth/agar

   If marine bacteria are being isolated, ensure that the sodium chloride is a minimum of 0.5%. 1% salt is normal for initial isolation. It may be necessary to add some serum or blood, 1 – 5%.

   There are many specialist agars such as Marine Broth/Agar or Cytophaga Agar which may be preferred. Some bacteria are more fastidious and may grow slowly for example Renibacterium., Where not bacteria are cultured, histopathology may indicate whether this is genuinely negative or is result od culture conditions. Incubation temperature may sometimes be a problem. For example, *vibrio salmonicida* grows poorly or not all above 14°C. It may also be useful in diagnosis, for example, *vibrio alginolyticus* and *parahaemolyticus* grow at > 35°C.

   Selective media are not extensively use for priminary isolation but may be useful for diagnosis later or where there is heavy, non-specific contamination. Examples are Rimmler-Shotts medium for Aeromonas, Thio-citrate, bile salts medium for vibrio species and SKDM for Renibacterium.

Identification is made from:

a. Gross morphology of colonies on media.

b. Gross morphology of individual cells (microscopy) such as shape, size, motility, gram reaction etc,. The electron microscope is easy to sue and informative at this stage. At this stage a presumptive diagnosis is likely to be possible based upon the clinical signs, the farm history, gross cell morphology and rapid serological tests.

c. Biochemical tests based on the detection of enzymes, ability to utilise different carbon substrates, tolerance of physical and chemical conditions. Use proper diagnostic tables such as those in Bergey's Manual of determinative Bacteriology.

d. Serological tests depend on the availability of sera containing specific anti bodies. The commonest tests are simple, slide-agglutination, Ochterlony reaction and EIA. For detection and diagnosis of pathogens in histo-pathological tissue section immunofluorencense is valuable but requires the use of an.

BACTERIAL INFECTIONS IN GILT HEAD SEA-BREAM (*Sparus aurta)*

By Prof. Dr. Haluk ERGUEN
TURKEY

Bacterial disease are responsible for heavy mortality in both wild and cultured fish.

The majority of fish pathogens are Gram-negative rods but there are some Gram-psoitive pathogens, including a few which are acid-fast.

With the growing interest in the development of fish culture, there is the importance of diseases as one of the major limiting factors in culturing of fish. In many countries, there has been considerable recent development of fish farming in warm sea water. From that reason, aquaculturists must be carefull for diagnosis and control of diseases.

In many countries, wild fry are still main source of culture material especially for extensive farming. We know that, this is in spite of the increasing prospects for hatchery reared culture stocks, since production of this stocks will remain limited by practical and socio-economic factors (the high cost of hatchery construction and operation and the expertise requirements).

The purpose of the studies of fish diseases, is to close the gap in knowledge on the diagnosis and control of diseases already affecting, or posing a potential health hazard to cultural gilt head sea-bream.

One of the important diseases of the Gilt head Sea-bream (*Sparus aurata*) is Vibrio spp.

Vibrio disease is a serious and economically important disease occurring during the warmer months in salt water.

The first external signs were small petechiae in the mouth region and on the opercula and at times on the ventral body surface immediately anterior to the pectoral fins. From that reason we called «Red pest» or «Red boil disease».

The incubation period varies with temperature, strain virulence and the degree of stress under which the fish are living.

They are Gram-negative curved rod 0.5 × 1.0–2.0 um.

Clinical pathology:

First signs of losses, affecting most susceptible fish, are often anorexia, darkening and sudden death.

Acutely affected fish show swollen, dark skin lesions which ulcerate to release blood coloured exudate. Ulcers may be very deep and necrotic.

Internally: main feature is enlargement and liquefaction of the spleen and kidney and petchlation of visceral and parietal peritoneum. Focal haemorrhages may also be seen on the surface of the heart and the gills are usually paler.

Histological examination of peracute cases, cardiac myopathy, renal and splenic necrosis and periorbital oedema. Acute cases show less severe cardiac lesions but are characterized by the skin lesion which comprise acute hypodermal inflammatory foci extending deep into the muscle.

Although these lesions are limited by the stratum compactum for some time, they eventually ulcerate. There is severe myofibrillar necrosis with the center of the lesion comprising an agglomerate of sarcoplasmic debris, macrophage nuclear baseophilic remanants and fibrin, with bacteria. In the liver there is focal necrosis and spleen and

kidney. In the kidney the necrosis extends to the renal glomerulus, tubules and often to the endocrine cells of the internal tissue.

In chronic cases the severe haemolytic anemia induced by the lytic toxin of the vibrios results in heavy deposition of haemosiderin in the melanomacrophage centres of the remaining splenic and renal haemopoietic tissue.

Control of the disease:

Immunization and genetic selection have been show to improve the resistance of the fish. Vaccination is possible

We can be use Oxyletracycline, Suplhonamides and Nitrofuraus, but anorexic fish do not receive the drug.

Isolation of bacteria:

The two media routinely used for the first isolation were TSA (tryptic soy agar) and TCBS (thiosulfate citrate bile salts sucrose) agar.

Due to salt requirements of all the isolated, the media for isolation and for biochemical tests were prepared with 25% filtered natural sea water: The pH was adjusted to 7.2 – 7.4 with a 1 M NaOH solution.

Pseudomonas spp. and *Aeromonas spp*, are also identified in a few cases. They have also mentioned assoication of Myxobacteria with morbid gillrot and skin and fin necrosis in all cultured species of fish.

Pseudomonas spp.: it is found in soil and water and can often be isolated from decaying fish and spoilage-vulnerable foods.

It is usually motile, multitrichous polar flagella this organism grows well on ordinary nutrient media and usually produces diffusible fluorescent pigments, especially in iron-deficient media colonies are round and glistening and a fluorescent yellow colour may be noted in the adjacent medium.

This bacteria is usually associated with haemorrhagic bacterial septicaemia. The condition is usually clinically indistinguishable from aeromonad septecaemias.

Clinical pathology:

The haemorrhagia septicaemia may be acute or chronic. Large haemorrhagic skin lesions are the most commonly observed signs and heavy mortalities may ensure very shortly after the advent of lesions. At necrosis, in chronic cases fibrinous peritonitis have been described, and may be we can see ascites.

Histopathology:

Main foci of pathological change are the skin and haemopoietic tissues. In the skin the earliest changes, hyperaemia of dermal vessels with severe oedema extending into the lower epidermis. But ulceration follows and the lesions also extend down into the underlying muscle.

Spleen and kidney lesions are primarily interstitial and comprise rupture of melanomacrophage centers, necrosis of haemopoietic elements and presence of large number of melanin-granule-bearing macrophages within the renal blood sinuses.

Treatment:

Poor environmental conditions is the affective on the fish and this is the because of the pscudomonad infections.

We can be use Oxytetracycline with food but usually such infected fish will not feed.

If it is possible we can use kanamycin injection intraperitoneally. Also we must be change to possitive the environmental conditions.

Aeromonas spp: This organism is a Gram-negative red 0.7–0.8 um × 1.0–1.5 u. It is motile by means of polar flagella.

Culture:

This organism is readily isolated from the kidney or blood of affected fish on relatively unsophisticated media and by use of the Rimmler-Shotts selective medium it can usually be isoleted and typed in 24 hours.

It is cytochrome oxidase positive, chemo-organotrphie fermentative and respiratory.

This organism is usually associated with haemorrhagic septicemia of fishes, which are under stress for some other reasons.

The clinical feature and pathology are similar to those of psecudomonad septicaemia.

Clinical pathology:

Affected fish are usually under stress from some other factor and show darkening in colour, with large and irregular haemorrhages on the body surface and base of fins and ascites.

Internal organs are seen to be congested with haemorrhages over the viscera. Incision of the kidney and swollen spleen usually results in the semifluid contents dripping out.

Histopathology is indistinguishable from that of pseudomonad infection with the renal and splenic haemopoietic tissue reduced and the remaining cells necrotic. The intenstinal mucous membrane is usually necrotic and sloughed into the lumen. Focal necrosis is found in cardiac muscle, liver, gonad and pancreas.

The skin lesions beg in as severe oedema of the dermis and hyperacmia of the stratum reticulare, leading to spongiosis and ulceration of the epidermis followed by extensive hacmorrhagic necrosis down to the level of the muscle, but usually the lesions are more superficial than those of vibricosis.

Treatment:

Enviornmental improvement, reduction of the organic pollutants.

The condition can usually be controlled by treatment with antibiotics or polentied sulphonamides. But affected fish usually anorexic, paranteral treatment may be necessary.

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DIAGNOSIS OF VIRAL DISEASES

By M.T.HORNE
SCOTLAND

Viruses are genctic material, RNA or DNA, protected by a protein coat made up of identical sub-unit called capsomeres. They reproduce by invading a host cell and controlling its metabolism with their own genetic material. Because the infected cell is functioning in an approximately normal fashion there are no chemical treatment available for treating disease routinely: control is by avoidance, vaccination and culling.

Sampling procedures and field work :

Similar to those given for bactriology.

Laboratory procedures : (Sample preparation)

Samples for storage may be frozen at-20°C or-70°C although occasionally some viruses may be damaged by this. Samples for histopathology are fixed in formalin: samples for electron microscopy in glutaraldehyde. Other fixatives are available some of which have dual purpose.

For the isolation and growth of the virusis, homogenise the tissue sample in phosphate buffered saline (PBS), centrifuge or filter to remove cell debris. If the sample is not to be used immediately add antibiotics, for example, 100 i.e. Pencillin, 100 mg/ml Streptomycin and Nystation. If lonf term storage is planned also add 50% by volume glycerol and store at-20°C or-70°C.

Laboratory Procedures : (Host cells)

Several cell times are available commercially Rainbow trout gonad (RTG-2) and Fathead Minnon (FHM) are the commonest. Failure to isolate virus may be to the choice of an unsuitable host. It is also possible to prepare cell lines for other species of fish if required. In outline, tissue is homogenised in PBS and the integrity of the tissus destroyed by incubation with trypsin, Cells are harvested and re-trypinised. After final harvesting they are seeded into growth medium.

Laboratory Procedures : (Media)

There are many commercially available growth media and manufacturer's manuals should be consulted to determine which are most suitable for your purpose. In general terms such media are based on: balanced mineral salts, essential and non-essential amino acids, essential vitamins, antibiotics foctal calf serum and glutamine.

Laboratory Procedures : (Isolation of virus from fish samples)

Select the host cells and grow as a monolayer to 60–80% confluence. Change the mediaum and add the tissue extract. Allow the virus to absorb for 1 hour at 20°C. discard the medium and the sample and add fresh growth medium. culture and examine the cells daily for cutopathic effects (CPE). The most important of these are rounding of the cells, the formation of multinucleated cells and the formation of plaques due to cell lysis. These may be seen directly with a suitable substage microscope or by staining for normal, light microscopy.

Laboratory Procedures : (Indentification and quantification of virus)

Each of these should be studied in detail to obtain protocols and technique.

1. Serum neutralization:
   Serum specific for the virus suspected, sometimes together with other viral sera, are added to the wells of a tissue culture, microtiobic plate at an unform concentration Samples of the supernatant from the cultures under scrutiny are diluted across the plate. Positive and negative controls are incorporated. The plates are incubated and examined daily for signs of CPE.

   There are many variations on this procedure designed to investigate or quantify other parameters. For example, the use of an uniform virus those against sera taken from fish suspected of disease may be used to determine the presence of virl-specific antibodies in blood samples.

2. Enzyme immunoassay (EIA) :

   Virus specific antibodies are coated onto microtitre plates-normally 96 well or strips-and a postcoat added to prevent non-specific adsorption to the plastic. Tissue extracts from tissue samples are added to the well and incubated for 1–3 hours. Positive and negative controls are used. The sample is discarded and the wells washed. At this stage any antigen (virus) present will have adhered to antibody. A second virus specific antibody, conjugaled with an enzyme, (the «conjugate») is added and this will also combine with viral antigen if it is present. This is then detected by the addition of the enzyme's substrate with a colour dye (or «chromogen») coupled to it. The reaction of the enzyme with its substrate relases colour indicating a positive (virus present) result. By diluting the reagents across the plate and comparing with the control (virus positive) rows the concentration of virus in the fish tissue sample may be quantified.

3. Complement Fixation (CF)
   This test which, like the others, may be modified to show different parts of the virus host interaction, depends, on the utilization of complement by the antigen/antibody reaction. Complement is the name given to a cascade system of enzymes working as a unit equimolar proportions in the blood. Viral antigen and antibody are allowed to react, in the presence of complement, in a microtitre plate. The use of the complement gives no direct visual effect. The amount of complement used is measured indirectly by the use of a second antigen/antibody reaction which does give a visible effect i.c. Sheep red blood cells and their specific antibody

Viruses discussed

Infections Pancreatic Necrosis (IPN)
Viral Haemorrhagic Septicaemia (VHS)
Infectious Haematopoietri Necrosis (IHN) - Lymphocystis.

LIMPHOCYSTIS DISEASE OF *SPARUS AURATA* IN MARINE CULTURE AT AEGEAN SEA AND MEDITERRANEAN COASTS OF TURKEY

By Akin CANDAN
TURKEY

Lymphocystis disease has been reported in almost one hundred marine and freshwater fish species, since Lowe (1874) described it in ***Platichthys flesus*** from Britain.

Lymphocystis disease is the only viral disease of ***Sparus aurata*** that has been reported until now. This first case in Turkey is the third report of the disease in ***Sparus aurata*** (Paperna et.al., reported the disease in Israel in 1982 and Menezes et.al., reported in Portugal in 1987).

Lymphocystis disease in a chronic, slowly developing viral disease of connective tissue cells. Only cells infected with the virus become hypertropic others near or attached to affected cells, remaining unaffected. The tumorous growths are not malignant. The disease usually is not fatal to infected fishes.

The etiological agent of Lymphocustis disease belongs to the lridovirus family. The Lymphocystis disease virus is a large. complex virus with a deoxyribonucleic acid genome. The nucleocacid is icosahedral shaped. The viral diameter is with a rang of 200 to 300 u.m. The virus is located in the cytoplasm of infected cells, where it stimulates formation of bars of inclusion substance. virions can be found in large numbers in contact with the inclusion substance.

The prevailing water temperatures in the Acgea Sea water range annually from 17 to 28°C and the salinity is about 33%. In a period of 18 months, 14 fish farms were checked our periodically.

For the diagnosis, tissues with lesions were analysed under light and electron microscope and the existance of the virus particles in the cells were observed.

In March 1990, Lymphocystis infection began from one farm in Bodrum Yalikavak and spread to Marmaris Bozburun within 12 months. The clinical picture of the disease is chjaracterised by whitish nodules, which aggregates of hypertropic cells, scaltared on the fins and skin of affected fishes.

In heavily infected fish aggregates of hypertorphic cells, 2–3 mm. in diameter, covered a large portion of the body and the entire surface of the caudal and pectoral fins.

For light and electron microscopy studies, isolated, nodules of hypertrophic cells were fixed in 3% glutar aldehyde for 6 h.at 4°C washed extensively in buffer and postfixed in 1% OSO4 for 1 h. After dehydratation the tissue was embedded and blocked.

In size (150–250) mm. in diameter) and structure, Lymphocystis infected cells from *S. aurata* were in agreemant with data from other hosts such as flatfish (Russel 1974) sciaccids (Christmas and Howse 1970 and Paperna et al., 1982)

All organelles are enlarged in the lymphocystis cell: a large vesiculated nucleus with irregular ring and large nucleolus and large ribbonshaped basophilic inclusions at the peripheral zone of the cytoplasm.

The virus particles were 180–230 um. in diameter. the capsid was hexagonal and was surrounded by amorphous layer of fuzzyhalo.

Mortality levels of lymphocystis disease are quite low, but in heavy culture conditions of some farms in Bodrum peninsula the mortality was more than 10% among the small fish.

Maintenance of fish in high densities, frequent handling, transfer and netting, which always results in minor injuries and abrasions to fish skin, could undoubtedly promote transmission of the virus and at the same time stress the fish, hence reducing their tolerance to infection.

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A HISTOLOGICAL STUDY OF A CARP POX (Viral epithelioma) DISEASE IN TURKEY

By TIMUR. G
TURKEY

Carp pox was recognised and described as a disease of cultivated carp more than 400 years ago but suspected virus has only been demonstrated by electron microscope in 1952 but has not isolated (Roberts, 1978).

Electron micrographs show viral-like genome bound in a protein coat within nucleus and cytoplasma of the infected cells. The process of replication and general morphology (complex icosahedral) and size of the particle suggests the carp pox virus belongs in the herpes virus group (Amlcher, 1986; Roberts, 1978). A similar condition also has been reported from other cyprinids such as, common bream and tench (Amlacher, 1986; Roberts, 1978).

The lesions usually begin as small white nodules on most parts of the body. They grow, spread and coalesce to produce milky or gelatinous masses (Amlacher, 1986; Roberts, 1986; Roberts, 1978). Where the lesions are large, an extensive capillary network may give the lesions a pink tinge (Roberts, 1978).

Argulus is believed to play a role as a vector of a virus causing the epithelioma. In many cases, after eradication of Argulus, affected fish recovered from the epithelioma (Amlacher, 1970; Snieszko and Axelrod, 1970).

A low mortality of mirror carp occured in a private fish farm in Antalya in April 1988. The sick animals showed milky white or transparent gelatinous lesions on most parts of the body, fins and opercula. The weight of the affected carp was between 250–500 g.

Carp pox was also seen as a second case in another private carp farm in Denizli in 1991. The infected fish were in 50–60 gram weight and 7–8cm. in total length. Similar lesions were also observed on affected fish.

A histological study was carried out to diagnose the disease in two case which induced low mortality.

For histopathological examination, the skin lesions were dissected and fixed in 10% formal saline and embedded in wax. Sections (5 um) were stained with haematoxylin and eosin (H + E), Masson's trichrome or Periodic-acid-shiff (PAS).

Affected fish exhibited an ugly appearence with milky or gelatinous masses on most parts of the body, fins and opercula. The colour of large lesions was pink rather than white. Fish were also infested with Argulus.

Histopathological examination of the skin lesions revealed multilayered neoplastic epihelium. The folds or layers were separated by thin connective tissue. Mucus cells were few but occasional groups of 4–5 cells were seen amongst neoplastic epithelium cells. The neoplastic cells were small and darkly stained in the upper folds of the lesions and large and vacuolated in the deeper folds close on the dermis. Darkly stained (basophilic) inclusion bodies were occasionally seen within the vacuolted neoplastic cells. An inflammatory infiltration and haemorrhagie was also observed among these cells and also in the dermis. Different stages of mitosis were observed in the neoplastic epithelial cells.

Although carp pox disease was recognised and described as a diseases of cultivated carp more than 400 years ago the present report is the first to describe the disease in carp farms in Turkey.

While verrucose plagues on most of the body of the infected fish are common finding of the other workers (Amlacher, 1986; Roberts, 1978).

The presence of Argulus on the body of the infected fish support the idea that Argulus plays a role as a vector of a virus cusing the viral epithelioma (snieszko and Aqelrod 1971).

Observation of basophilic inclusion bodies within the cytoplasm of some neoplastic epithelial cells also suggests the disease has a viral aetiology (Amlacher 1970), 1986; Roberts 1978).

The reduction of mucus cell numbers was also similar to the reports o other workers (Amlacher 1986).

SUMMARY

An outbreak of carp pox in two private carp farm was associated with low mortality. Milky white or transparent gelatinous lesions were observed on the body, fins and opercula of line infected fish.

Histopathological examination of the skin lesions showed them to consist of neoplastic epidermis. An inflammatory infiltration and haemorrhagie were present among neoplastic cells. Darkly stained (basophilic) inclusion bodies were ween in some neoplastic epithelial cells.

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NUTRITIONAL DISEASES OF FISHES

By Dr. Attia El Hili HEDIA
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Proper nutrition is essential to the health of fishes. There are many diseases among fishes directly related to nutritional deficiencies and excesses. By another way, malnutrition is implicated in many diseases involving pathogenic organisms. Estimating the role of malnutrition in diseases is essential for diagnosis.

Most nutitional diseases are chronic in nature. Usually, diseases signs are masked by secondarily invading infectious organisms. This is one of the principal reasons why it is important to know the compound diet in point of view quantitative (rates of proteins, carbohydrates, lipids, minerals and vitamins) and qualitative (availability of the components and probable presence of toxin or antimetabolite.

PROTEINS

Proteins are large complex organic compounds which perform an essential role in the structure and functioning of plants and animals.

Proteins are composed mostly of amino-acids. Some amino acids can be synthesised by animals and others can not be synthesised and are called essential) (essential in the diet) amino acids or EAA's.

For fish a crustaceans, the EAA's are: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine.

To synthesise the non essential amino acids, animal must have available a source of nitrogene.

The quantitative EAA content of different species varies. The EAA content of different feed ingredient varies even more widely, for exemple:

- the most plant proteins are deficinentin the sulphur-amine acids (methionine and cystine)
- meat meal, because of it poor levels isoleucine and methionnine and cystine, is a poor quality protein compared fish meal
- fish silage is poor in tryptophan

This is one of the principal reasons why a compound diet made from several ingredients is potentially more efficient than a single ingredient which may be too high or low in one or more essential amino acids.

Even when an EAA is shown by chemical to be present in sufficient quantities, it may not be biologically available to the animal. For example, the free amino group of lysine may become bound to other molecules during processing of the fatstuff, rendering it unavailable of the target animal.

Reduced protein, or the amino acids therein, in the diet of fishes affects biosynthesis of many essential nitrogenous compounds, including all enzymes, hormones such as thyroxin or adrenalin, melanin pigments, histamine, creatine and other cofactors and many others vital substances.

In fish, often proteins of amino acids deficiencies show very few pathological symptoms, mortality rates are low and the most commun sign is reduction or cessation of growth. Feeding a diet in which an indispensable amino acid has been removed will cause

growth to cease until the amino acid is restored to the diet. The fiew amino acids which does show pathological symptoms is tryptophan causes scoliosis, lordosis and renal calcinosis.

CARBOHYDRATES

The carbohydrates include starches, sugars, cellulose and gums. They contain only the elements carbon, hydrogen and oxygen.

Fish and shrimp vary in their ability to digest carbohydrate effectively but they digest and metabolize them at a lower rate than higher animals. Many fish appear to be able to utilize simple carbohydrates such as sugars, more effectively than complex starches; the reserve appears to be true for shrimp and pawns. Many fish don't have the enzyme cellulase, and fibre is usually regarded as unavailable as an energy source. Cellulase, however is produced by the gut bacteria of many fish, as is chitinase in crustacea, and herbivorous fish are able to digest fibre.

Excess digestible carbohydrate in the diet of most fishes increases blood sugar, liver glycogen storage and increased liver mass, sometimes to pathological levels. Excessive glycogen in the liver of fishes, depending on severity, tends to cause liver malfunction and many contribute to kidney mal-function. Both liver and kidney malfunction may contribute to impairment of health.

LIPIDS

The lipids or fats are complex chemicals and the simpler forms are esters of glycerol and fatty acids.

Lipids are a source of energy and important elements of cell structure. In fact, the main lipid constituent of cellular membranes is phospholipids which constitute a barrier for exterior elements but can admit certain specific elements; thanks to the globular proteins found in the membrane.

Phospholipids are formed by a molecule of glycerol by 2 fatty acids in position 1 and 2 a phosphate having 4 possible bases; choline, serine, ethanolamine and inositol.

Triglycerides are forms of energy storage, in the case of fish, they are stored in the muscle and in the liver. Tiglycerides are formed by a molecule of glycerol and by 3 fatty acides. Certain of the fatty acids are essential (can not by synthesised by the animal itself) for growth and normal appearance of fishes.

Animals have 3 series polyunsaturated fatty acids (PUFA); the n-9 series (oleic series), the n-6 series (linoleic series) and the n-3 (linolenic series); only the oleic series is synthetizable by animals.

- oleic acid has 185 atoms of carbon and one double in position 9, the 9 carbon from he methyl
- linoleic acid (18:2n-6) has 2 double bounds and the first occurs on the sixth carbon atom.
- linolenic acid (18:3n-3) has eighteen carbon atoms and three double bounds, the first of which appears on the third carbon atom

The animal and fish synthetize both palmitic (16 atoms of carbon) and stearic acid (18 atoms of carbon). The latter can be converted into oleic acid. This bioconversion (desaturation) is possible in all animals.

The essential fatty acid EFA requirement of different species vary but are not yet fully understood.

- aquatic animals have a higher requirement for the n-3 series of fatty acids the terrersterial animals, for which the n-6 series is more important.
- EFA deficiencies are more noticeable in sea water than in freshwater conditions (for trout), thus salinity affects EFA requirements.
- marine fish appear to have a greater requirement for HUFA's than fresh water or anadromous species
- cold water species appear to have a greater requirement for the n-3 series fatty acids than warm water species

The best sources of essential acids for fish diets are fish oils. Vegetable oils are low in the m^3 acid but generally high in w6 acid.

The more highly unsaturated fatty acids, linoleic, linolenic and arachidanic, are regarded as essential fatty acids and have a vitamine like action in the body, for example: deficiency of linolenic series family (18 : 3n-c3) causes reduced growth, skin depigmentation, fin erosion and feinting in rainbow trout.

The necessity of high dietary levels of PUFA's in aquatic animals diets makes the possibility of fats becoming rancid. These may be toxic or growth depressive. To prevent the autoxydation products, all fats and oils which contain unsaturated falls should be stabilized the time of manufacture. Antioxidant such as ethoxiquin or santoquin can be used to prevent autoxidation.

It must be underlined that food influences the fatty acid composition of both phospholipids and triglycerides. A good diet formulation concerning the required EFA supply by food is very important, particularly in vitellogentic females and all early stages of fish development. A deficient diet produces important desorders in embryo and can result in a decreased hatching ratio and larva abnormalities.

Feeding of high fat diets may cause fatty infiltration of the liver and excessive obesity. The liver of fishes with fatty infiltration of the liver are yellowish to ochre in color. Treatment of fatty infiltration is by reduction of dietary fats of the diet and increasing choline in the diet may assist the fishes in metabolizing intracellular fat as dietary is being reduced.

VITAMINS

Vitamins are complex organic compounds required in trace amounts for normal growth, reproduction, health and general metabolism, Marry vitamin deficiency symptoms have been described for fish and a few (notably vitamin C deficiency) for shrimp.

There are two types of deficiency symptoms of each vitamin in fishes:

- non specification symptoms which are: anozrexia, apathy, ;lack of growth exophtalmia and dark pigmentation
- specific symptoms which are summurized in Table 1.

The most common vitamin deficiency in fish nutrition is that of vitamin B1 or Thiamine, In fact, the feeds which contain raw aquatic animal products, notably if not fed contain raw aquatic animal products, notably if not fed immediately after manufacturer, contain enzymes called thiaminases. Theses enzymes may partially or completely inactivate the thiamine. Thiaminase levels in freshwater fish are higher than in that of marine fish.

There are also two others importants vitamins for fishes: vitamin E and C.

- the vitamin E has anti-oxidative properties and may be required at higher levels in fish and shrimps diets which are high in PUFA's as these are suscptible to rancidity. This vitamin has also immunostimulating effect.
- The vitamin C intervenes in a large number of enzymatic reactions and in the most varied cases: synthesis of collagen, cartilages, synthesis of adrenalin from where it has an antistress effect, reproduction system, immunostimulating effect,…

With salmoneids. The most spectacular manifestations are: scoliosis, Iordosis, dwarfish, gib bosity and shortening of the operculums.

With turbot and sea bream, deficiency in vitamin C provokes a granulomatous deasease (figure 1).
With eel, deficiency in vitamin C provokes haemorragies in the head region.

MINERALS

Mineral elements are important in many aspects of fish and shrimp metabolism. They provide strength and rigidity to bones in fish and the exoskeleton of crustacea.

There are two types of minerals:

- Macro elements which are the architectural elements of the organisms (P, Ca, Mg, Na, K, S)
- Micro elements or oligo-elements which intervene as enzyme molecule compounds and as boicatalyzers. Their role can be compared to that vitamins, whith which they interfere (Fe, Cu, Mn, Zn, Co, I, Se).

Only seven elements: Ca, P, Mg, Fe, Zn, I and Se have been shown to be required or utilized by salmonids. Their role are summarised in table 2.

The water notably the sea water contain calcium and the requirement rate in fish is feeble. However, both sea water and freshwater contain very little phosphorus. On the other hand some types of phosphorus are unavailable to fish and an assessment of the availability of phosphorus in the diet is essential. As this mineral is important, it seems advisable to supply phosphorus to the diet of fish under the form of monosodic or monocalcic phosphate.

The deficiency in Zn may be induced by an excess of calcium which blocks the Zn available.

OTHERS COMPONENTS OF FEED

Feeds contain many other types of substances:

1. Synthetic substances such as hormones, antibiotics, pellet binders and pigment.
2. Natural substances such as mycotoxins, enzyme. inhibitors, vitamin destroying enzymes, haemagglutanins, products of oxidative racidity, pesticides, herbicides,…

These substances decrease the quality of the feed. The tab:3. provide a summary of some toxic and anti-metabolite elements.

CONCLUSION

To prevent nutritional diseases of fishes, some precautions must be taken:

1. Fishes confined under intensive fish culture conditions must be suppled a ration containing all required nutritents each day.
2. Conserve the food for not more than one month in a place which have 20°C of temperature. The use of a cold storage room advisable in the mediterranean climate.
3. Don't mix premix of vitamins with oligo-elements premix with because theses latters accelerate the process of vitamin degradation.
4. Add an antioxidant like ethoxiquin to the diets to prevent the formation of the peroxide.
5. Add vitamin C to fishes each time a decrease in performance after a treatment or after a stressing operation.
6. Add a therapeutic dose of vitamin when we use some nutriments or medicaments such as raw fish (anti-vitB1), sulfaguanidi (anti-vitk).

Figure 1. PRINCIPAL FUNCTIONS OF VITAMIN C

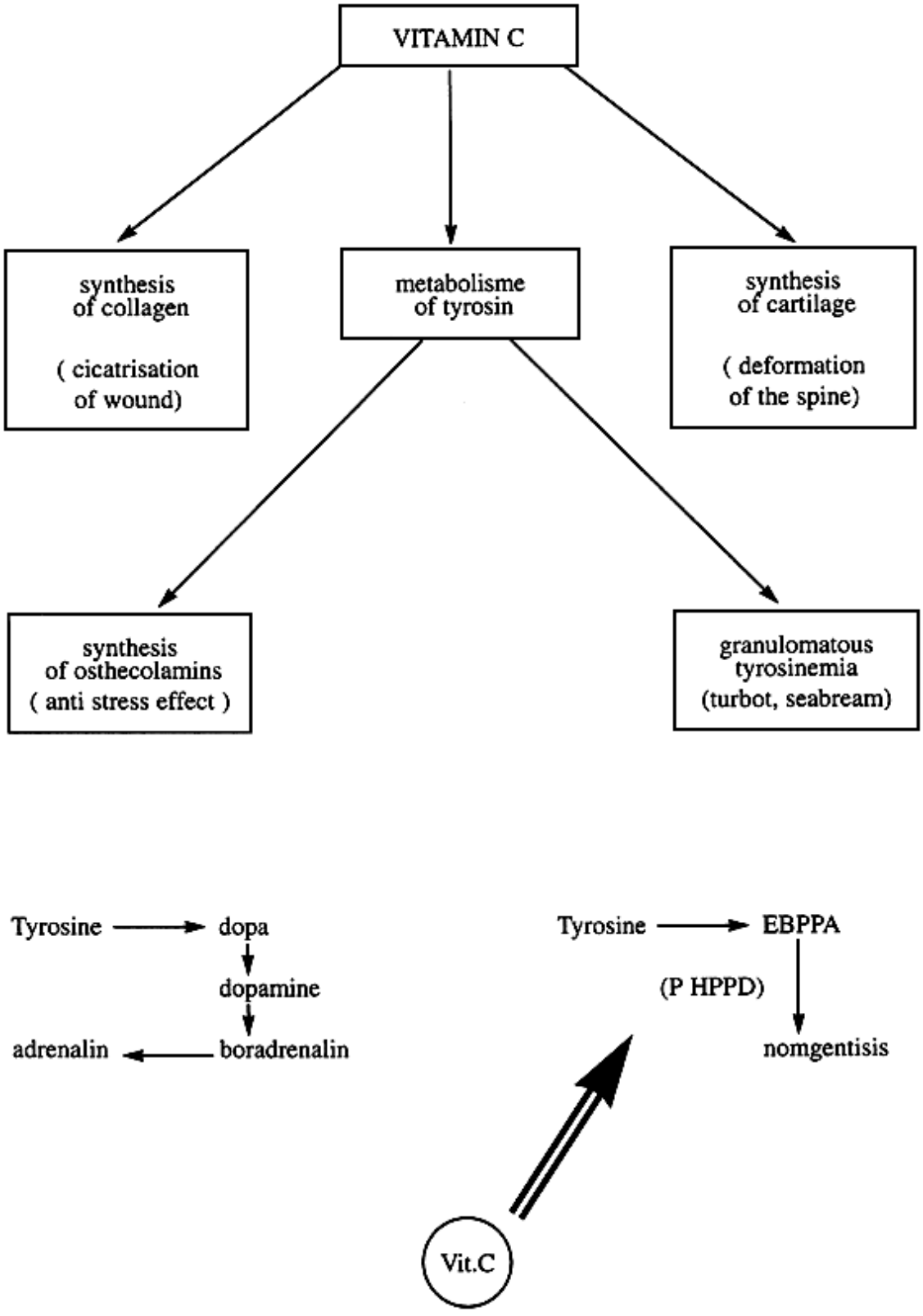

Table 1. MAJOR SIGNS OF VITAMIN DEFICIENCIES IN FISHES

Vitamin	Signs
Fat-soluble	
A	Retinal alterations
D	Tetany
E	Muscular dystrophy - Fatty livers
K	Skin hemorrages
Water-soluble	
B1	Loss of equilibrium convulsions
(thiamine)	Hyperirritability
B2	Cataract, keratitis

Table 2. SYMPTOMS ON SOME MINERAL DEFICIENCIES OF FISHES

Mineral	Symptoms
Ca	loss of appetite, feeble growth poor consumption index
P	poor growth, low consumption index, bone deficiencies, frontal swelling, increase muscular fat
Mg	poor growth, anorexia, apathy muscular relaxation
Fe	microcytic hypochromic anemia
Zn	reduced growth, cataract, dwarfism wasting away of the fine, mortality

Table 3. SOME TOXIC AND ANTI - METABOLITE SUBSTANCES OCCURING IN FEEDS

TYPE	FACTOR
Fungal toxins	Aflatoxins
Bacterial toxins	Botulism
Chemical contaminants	Organe chlorine and polychlorine
	biphenyls
	Volatile N nitrosamines
Natural feed	Cyclopropenoid acids
components	Glucosides
	Oxalic acid
	Gossypol
	Haemagglutinins
	Alkaleids
	Toxic amine acids
	Vitamin antagonists
	Thiaminase
	Linatine
	--
	--
Peroxides	Oxidised oils

SEA BASS, SEA BREAM AND FLAT OYSTER PREDOMINANT PATHOLOGICAL CASES IN NADOR LAGOON

By Mustapha TALBAOUI
MOROCCO

INTRODUCTION

The important development of marine aquaculture help in knowing more about diseases and its incidences.

Even though, many technical problems have been salved, others, such as fish pathology, are not well known and are obstacles to development of aquaculture. More research on this subject and diagnosis are needed for the viability of aquaculture projects.

Fish diseases can be genetic, nutritional, environmental (physical, chemical, pollution related), infectious and parasitic.

Larval Pathology

General information: The inflation of swim bladder in sea bream (*S. auratu and P. major*) occurs with contact of air on water suface. The bubbles of air pass through the ((pneumatic)) canal which degenerates when the larva is 4–5 mm

Two syndromes in larval rearing in sea bass and seabream are:

1. Non inflation of the swim bladder.

   The swim bladder is formed normally during the first ten days of the larvae: between 5th–10th days for sea-bream and 7th–10th days for sea bass.

   The non inflation of the swim bladder constitues the major obstacle in larval rearing for many marine species

   In such cases swim bladder does not develop and is replaced by a compact tumoural tissue or degenerative tissue

   Symptoms and lesions:

   - Skeletal deformations (lordosis)
   - Slow growth and big susceptibility to stress

   Possible etiology:

   - Lipid layer on water surface prevents air contact
   - Big aeration and turbulances.

The elimination of the lipidic film on the surface is achieved with a low level blower on the surface and elimination of this film. We can also use food grade emulsifier agent (TWEEN 80).

2. Hyperdilatation of the swim bladder in the sea bass.

   Enlargement of the swim bladder occurs between the 20th–25th day after hatching of the larvae and involves 10 to 20% of the total population.

   Stress due to manipulations, change of food, rapid temperature variation are the possible etiology. This anomaly can be solved with good environmental conditions and high quality of live food.

Mateiliosis of the flat Oyster
(Marteilia refringens)

Definition:

Marteiliosis is a parasitic disease of the digestive gland in flat oyster. It is due to the development of a haplosporid, ***Marteilia refringens***.

M. refringens has been isolated for the first time by COMPS (1970) and HERBAACH (1971) in the flat oyster along the French Atlantic coasts.

Symptoms, lesions and diagnosis:

The sings are not specific and are similar to those of malnutrition.

- **retarded growth**
- **transparent shell**
- **discoloration of the genital gland (brown to yellow)**

Diagnosis is possible by histological examination which show refringent sporanges in the profond canal and in digestive cells.

Stomach epithelium and secondary canalicules are not affected. This is related to particular biochemical conditions favourable to the development and maturation of the sporanges.

Polydora sp.

They are annelids of the family Sipiorids with different species: ***Palydora haplura*** and ***P. ciliata*** in Europe ***P. websteri*** and ***P. ligni*** in North America, Australia and Japon Reproduction period: Spring and summer.

Note : Translated from the Frensh text.

SOME IMPORTANT ASPECTS OF THE PATHOLOGY OF INVERTEBRATE SPECIES

By Francisco RUANO
PORTUGAL

INTRODUCTION

Considering the marine invertebrates with economic interesting aquaculture two main groups BIVALVES and CRUSTACEANS are the most important species.

Marine cephalopods and gasteropods are also important species for fisheries however the methodology for his culture at least for commercial proposals still is in improvement.

The bivalves oysters, mussels, clams and cockles are the main cultivated species on Mediterranean region. The culture of crustaceans is very recent in this region and it starts in the early eighties with the introduction of Indo-Pacific species of prawns in south of France.

THE ROLE OF PATHOLOGY

A. STUDY OF DEFENSE MECHANISMS OF THE ANIMAL

Pathology, using basic sciences looks for a deep and correct knowledge of each species behaviour in the presence of an external aggression: what kind of defense action (s) is involved on the control and selection of each action. The understanding of this, is crucial for our correct procedure in the presence of a diseased situation.

Let see the different defense mechanisms that both bivalves and crustaceans have and how they act.

1. IMMUNITY-Includes 2 different components

Humoral.

This internal defensive action is extremely elementary specially in bivalves however it is quite effective against microbial pathogenic agents.

Both bivalves and crustaceans have in the serum of its hemolymph specific proteins with a hight molecular weight such as **LYSINS** on bivalves and **OPSONINS** on crustaceans.

In crustaceans, the action is crucial, which maintains the internal milieu absolutely sterilized. It is also so powerful that in some cases, when a pathogenic bacteria e.g. reaches the hemolymphatic vessels the reaction frequently causes itself the dead of the animal provoked by a generalised congestion in hemolymphatic circulation.

In bivalves, this action is less intense and specific. Although the agglutination and lytic actions has been well proved against strange substances, it is not clear his effectiveness against certain bacterial virus or even some internal parasites.

Cellular

Closely related with humoral mechanisms, the haemocytes activity in terms of protection, is basic a **phagocytosis** action. The capapility for extending cytoplasm (pseudopodia), for moving in intersticial spaces and for recognise nonself substances, are it's main characteristics when

phagocytosis as related to molluscan immunity, is dissected into its component parts, three major phases may be recognised (1) attraction between phagocyte and the invading material (2) surface attachment of nonself material to the phagocyte, and (3) internalization (on endocytosis).

One other defensive action very common but apparently more related with the inflammatory reaction than an humoral immune mechanism is the **encapsulation** of strange material. This mechanism usually involves both the haemocytes and connective tissue cells in order to confine and eventually destroy the aggressive agent.

2. **Immune memory versus genetic selection**

The existence of the immune memory in molluscs, is still a controversial question. So far the immune protection of populations challenged in laboratory with modified (attenuated, strains or several microbial agents have not been success.

In nature however exists several examples of increasing resistance in wild or cultivated populations during a second epizootic occurrence comparing with the first occurrence. As an example we can mention the increasing resistance in oysters at Chesapeake Bay against *Minchinia nelsoni* (MSX), during the second outbreak of the disease.

Several authors considers a genetic mechanism of the specie which creates a resistant strain against to «MSX» and not an acquired immunity.

B. **OTHER DEFENSE MECHANISMS**

CRUSTACEANS

SHELL - It's shield very effective against external aggressions. the became vulnerable to the pathogenic agents every time the protection loses resistance, for example during the moulting period after intensive handling or fishing and whenever some wounds are caused to the shell.
MOBILITY - Less vulnerability against predators
MUCUS - Besides the mechanic and lubricate action carry out by the mucus, it is also a powerful bactericidal and bacteriostatic agent.

BIVALVES

SHELL - The same action of the shell on crustaceans

MANTLE - It's a very large organ, composed by two membranes of connective tissue which separates, internally, the soft parts of the body from the internal face of the shell. Its external border is covered by tactile villosities, very sensitive organs that collects all kind of information form outside allowing the animal to against different aggressions

MUCUS - This same action than in crustaceans.

GILLS - This organs have an intensive filter activity which select and cleans all the particies carried by the water.

Besides all those defensive mechanisms it is possible to find in the internal organs of bivalves different aggressive agents in hight concentrations apparently

without any trouble for the animal. Usually it is during a stress situation than morbid processes occurs in bivalves namely during the increasing of chemical pollution, rapid changes in water salinity and temperature, spawning seasons etc.

C. THE PRINCIPAL BIOAGRESSOR AGENTS

When we speak about parasites or microbes, pathogenic agents, we must separate two different situations:

1. In culture (Artificial environment)

The causes of mortality in these conditions usual are very similar both to bivalves and to crustaceans and basically they are related with cyclical outbreaks of pathogenic strains of bacteria, namely *vibrio sp.* and some halofic strains of *Aeromonas*.

Also the metamorphosis and larval stages on bivalves and larvae and juveniles, on crustaceans are the more affected phases during the production cycle.

Some fungi, namely the *fusarium sp.* In crustaceans and a *phycomycete* strain in bivalves are described as the cause of epizootics that killed most of the cultured larval populations in 2–4 days. Also in crustaceans, chitin-destroying bacteria affects all the evolutive phases of its culture occurring usually after a deficient handling.

2. In Nature

In nature the situations are different with bivalves and crustaceans.

BIVALVES

These species, and of course its habitat, determines a different nosological map for each one, not totally coincident with all of them. A mussel population, for example, that lives in the inter-tidal zone, is much more exposed to the variations occurred on the water collumn than a clam, permanently buried in the sediment. And the clam is also more exposed to the qualitative and quantitative variations occurred on the interface water-sediment, than other species that lives in the water collum.

One other important aspect, for the understanding of the evaluation of mortality rates on these species, in the difficulty to establish a correct correlation between dead and alive and sick animals. Due to the extreme fragility of he soft parts of its body that degrades rapidly in the water after the death of the animal the only relationship possible to be establish is between empty shells and alive animals.

This fact can change drastically the conclusions if we don't have the necessary precaution when we evaluate a massive mortality in field.

The density of each population modifies the impact of a specific disease. As an example, the Focal Necrosis in oysters caused by a Gram positive bacteria, that we will be talking about later on in this work, causes necrotic focus in the Vacuolar Connectivetissue of the mantle, and consequently the death of affected animals. This disease, is much more virulent and the mortality rate is also higher in old oyster beds not harvested frequently, with high desity and hight percentage of old empty

shells, however this disease has no expression in mussel or clam populations.

Later on I will mention, in synthesis and according its importance, the different aggressor agents.

a. **MICROBIAN**

- **Bacteria:**
  Gram negative bacteria, probably *Achromobacter*, have been related with massive mortalities in Japan during 1960.
  Vibrio sp - Bacillar necrosis in oysters
  Achromobacter sp. - Focal necrosis in oysters
  Aeromonas sp.

- **Virus:**
  Iridovirus - Gill disease in Portuguese oyster *Crassostrea angulata.*

  Herpesvirus- Hemocytic infection viroses of bivalves.

  More than twelve different virus strains was identified in bivalves, however their virulence is very reduced and don't causes any disturbance to the host.

- **Fungi:**
  Monilia sp. - Causal agent of «shell disease» of portuguese oyster

- **Protozoa:**
  Haplosporidians (Minchinia nelsoni, M. costialis on Crassostrea virginica;
  Martelia refringens on flat oyster Ostrea edulis; on Minchinia tapetis on Ruditapes decussatus).
  Urosporidium sp - In oysters
  Bonamia ostreae - Causes hight mortality in european and North America flat oysters populations, also recognised as «Microcell disease» by American authors.
  Steinhausii mytilovum - Causes losses on the reproductive capacity of mussels. Ciliates, like *Ancistrum* sp.; *Flagellates-Hexamita* sp. and Gregarines-Nematopsis sp. are very common in wild and cultivated populations of bivalves. Mortalities caused by these agents are also related with stress factors originated both internally (loss of resistance caused by spawning, for example) or externally (hight densities, pollution etc.).

- **Helminths**
  Trematodes-*Bucephalus* sp. *Proctoeces* sp. *Himasthla* and *Gymnophallus* sp. are very common in different bivalves species, its pathogeny varies according the incidence of he agent.
  Cestodes-Larval forms of *Tylocephalum.*

- **Parasite crustaceans**
  Mytilicola sp. - Parasite the digestive tract of oyster and mussels. *Pinnotheres* sp. - Inhabit the shell cavity of several moluscs, where their activities and effects suggest that they are parasites rather than commensals.

b. **PREDATORS**

- **Sea stars**
  One single animal of this specie eats 7–9 young oysters per day.

- **Anemones**
  It is an important predator of planktonic larvae of bivalves.

- **Gasteropods**
  The **Muricidae** family is the most active and dangerous for bivalve beds. They drill the shell and suck the meat through the hole, selecting the young oysters. Besides that, some evolutive phases of several parasites (*metacer cariae* of the genus *Himasthla*), are transmitted to bivalves, using these gastropods as carriers.

- **Crustaceans**
  Several species of crabs, depending the geographic region we are talking about, are the main predators of bivalves fiddler crab *Uca tangeri* and the Green crab *Carcinus maenas* causes heavy losses on the culture beds of clams and oysters for example, in the south coast of Portugal. blue crab *Callinectes sapidus.*

- **CBV**
  (Cheasapeak Bay Virus) - Isa Picornavirus which affects specially the ectoderm.

- **Baculavirus**
  Affects the tubules hepathopancreas of crustaceans

- **Bacteria**

  - the chitin-destroying gram-negative bacteria, usually cause the «Shell disease» of several species of crustaceans.
  - *Vibrio* sp. causes frequently acute infections in larval phases, during the hatchery stage of crustaceans.
  - Gaffkemia-Caused by *Aerococcus viridans* a gram-positive bacteria, a tetraforming encapsulated coccus, is the most virulent bacteriosis found in crustaceans.

- **Fungi**

  - *Lagenidium callinetes* affects the eggs mass of blue crab females, reaching 95% of prevalence. This infection causes eggs failure during the hatch or gave rise to abnormal subsequent larval stages.

- *Fusarium* sp. also affects several species of crustaceans, both from fresh and salt water, causing heavy losses in ponds of Kruma shrimp in Japan. The disease promotes the blackened exoskeletal spots, namely in the gills region.
- *Aphanomyces astaci* cause the crayfish plague in the european populations. This fungal disease, almost vanish the indigenous species of freshwater crayfish in France in the last century. Carried by the Red crayfish *Procambarus clarkii*, introduced at that time in France from USA, the fungi spread out rapidly, killing the indigenous populations highly sensitive to the disease. American specis, resistant to that agent, acts as a carrier, spreading that agent through almost all european countries including Turkey.

- Protozoan:

- Microsporean :
  Nosema sp. causes important lesions in gills and muscular tissues. Thelohania sp. is the causative agent of the ((cotton disease)), affecting the muscular tissue and also the sexual organs, reducing the reproductive capacities of several shrimp general namely *Penaeus*, *Pandalus* and *Crangon*.
- Greegarines - The specie, *Nematopsis sp.* is common in the digestive tract of some Penaeid shrimps.
- Amoeba - The genus *Pleistophora* parasites specially the muscular tissue.
- *Haplosporidium*, *Urosporidium*, *Sarcomastigophora* and several ciliates are also causative agents of diseases in crustaceans.

- Metazoan:

- Trematodes, *Metacercarean* from the specie *Microphallus* sp.
- *Nematodes-Ascarididea*

C. **OTHERS :**

Predators, competitors, changes on the environmental conditions, overfishing, etc., all this factors, like I said on the Bivalves have a very negative action on the crustaceans species.

D. **PROPHYLAXIS**

1. In Intensive Culture Systems, several procedures are very important to maintain an healthy situation of animals.

- Preserving an healthy situation of animals.
- Preserving the good quality of water, on the facilities used for rearing the first stages of development, and for the food

production it is fundamental to do the sanitary control of all production.

- The mechanic filtration of the water, (wheel water supplies, sandfilters, etc.), combined with its treatment-Sterilization- using a UV radiation system, it's a very effective proceeding to maintain a good water quality.
- The maintenance of all facilities, perfectly clean.
- The cleaning of the water supply and sewage system, as well as tanks of production must be frequent and periodic.

NOTE: The use of antibiotic drugs in a preventive dosage, it's a very common practice in several hatcheries. The ((control)) of the development of several pathogenic strains of bacteria, it is not a correct procedure from a sanitary point of view. The risks involved in that method are grater than the benefits. The great probability to produce resistant strains to future antibiotics therapies, is one of them.

2. In extensive production systems we must pay attention to :

- Control of the predators and competitors.
- The correct control and constant surveillance of the environmental conditions on earth ponds, ((long line)) systems, floating platform etc. in order to prevent natural or men caused damages.
- In natural or artificial sea beds of bivalves a correct management and the regulation of the fishing effort it's important for the maintenance of its productivity.

E. **GENERAL MEASURES**

- Legislation: The creation of protection areas, and specific seasons for fishing the different species, for example, are important measures to protect an endangered species.
- The implementation of artificial collectors for bivalves seed and the restocking of natural bed populations it's also important.

CONCLUSION

Massive mortalities of invertebrate species in the different regions where its cultivation takes place are explained by several adverse factors that I mentioned before: Diseases, pests, poisonings, parasites, environmental changes etc. However, it's very rare, that a single cause have been found as sufficient to explain such mortalities.

So, the different factors can be combined in order to promote the outbreaks of severe mortalities in wild populations.

On the other hand the reduction of population, under a very low level, even if the aggressive conditions has been stopped, can itself promote the extinguishment of that population, of bivalves for example.

- An explanation for this phenomena, is closely related with genetical capacity of each species that, after reaching the limit of its adaptation on adverse conditions, can not produce enough genetic varieties (strains) to survive in the new environment.

The consequence can be decreasing of a certain species from a particular ecosystem and the occupation of its ecological niche by a better adapted species. An example for this is the substitution of portuguese oyster *Crassostrea angulata*, by a non commercial *C. Stentina*, in the Tagus estuary.

EXAMINATION OF FISH FOR PARASITES-EQUIPMENT

By Dr. C. SOMMERVILLE
SCOTLAND

The instruments required for the practical examination of fish are typical of those used in any surgical dissection. It is important to have, in addition, a bottle of physiological saline to keep internal tissues moist, otherwise evaporation will cause the destruction of fragile parasites such as the protozoa.

Ideally, you should use a good compound microscope preferably with internal light source. Many small protozoan parasites are more easy identify under Phase Contrast and a microscope with this system should be used if available.

A stereo or dissecting microscope is also necessary as many small worms, cysts etc are not quite visible to the naked eye. If a stereo microscope is not available use a powerful hand lens.

Glass slides and coverslips should be spotlessly clean otherwise details of small parasites will be obscured and identification impossible.

Other items of equipment are as follows:

Dissecting board
Petri dishes
Pasteur pipettes
Paper towels
Paper tissues
Spare scalpel blades

ENSURE ALL ITEMS ARE PREPARED BEFORE KILLING THE FISH AS SOME PARASITES DIE VERY QUICKLY FOLLOWING THE DEATH OF THE FISH

ROUTINE SCREENING OF FISH FOR PARASITES

1. Ponds, tanks, cages etc. or other sites under study should be sampled regularly.
2. Whenever possible examine fresh material. Fish should be freshly killed, without anaesthetic, and kept moist throughout examination.

   Fish should be obtained live, if possible, and killed immediately prior to examination.

   There are several good reasons:

   i. Parasites are more easily recognised and identified.

   ii. Parasites, especially ectoparasites, may leave the host or die after death.

   iii. Collection of blood parasites is nearly impossible after death.

   iv. Decomposition starts immediately after death and internal parasites may be destroyed by hosts enzymes.
3. Handle fish as little as possible.
4. Kill fish by cutting though cranium or though spinal cord immediately behind the head.
5. Fish should be kept WET at all times during examination.

6. If the fish is already dead, refrigerate, but keep moist. Do not freeze as most small parasite become unrecognisable and only large helminths and crustacea can be recovered. If examination is to be delayed, fix in 10% Formal saline and slit open the body cavity to allow fixative to penetrate internal organs.
7. It is essential to examine skin and gills for ectoparasitic protozoa immediately after death as these may die or leave the host within a short time, e.g. flagellates.

EXAMINATION PROCEDURE

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SCOTLAND

Kill fish quickly by cutting through the spinal cord with a sharp scalpel in the region immediately posterior to the gills.

Blood can be collected at this stage from the heart of major vessels using a Pasteur pipette. Place a few drops on a slide and allow to clot. A smear can also be made, fixed in methanolfor 10 minutes and stained later.

1. **Examination of skin**

   i. Take «scrapings» for HP examination (several, if fish is large). Scrape with a sharp scalpel in an anterior to posterior derection and place mucus and epithelial cells on a slide in a drop of water. Avoid scraping scales as these reduce the visibility of small protozoa. Thin preparations are essential. Spread scrapings thinly and cover with a coverslip. Examine under HP.

      NB. Scrapings should be made along the dorsum in an anterior to posterior direction including head, from the fins and from any discoloured areas or lesions.

   ii. Examine the entire fish under low power using a stereo microcope. Be sure to examine under fins as well as other areas. Large metazoan parasites and *Argulus* can be seen in this way.

2. **Examination of gills**

   i. Remove operculum and examine inside.

   ii. Remove a whole gill and place on a slide or in a petri dish (add water if necessary and examine under low power on the stereo microscope. Separate the primary lamellae with needles to observe large monogenea and crustacea. Examine any lesions in detail.

   iii. Cut off lamellae and remove gill arch. Place lamellae on a slide and spread thinly-chop if necessary and cover with coverslip. Examine under High Power.

3. **Other organs**

   i. Make incision along ventrum from vent of head. Remove abdominal wall to expose viscera.
   Examine visceral surfaces, abdominal cavity and pericardial cavity carefully under low power using stereo microscope. Examine any abnormalities or cysts, spots etc. in detail under Hight Power.

   ii. Remove alimentary canal and associated organs by cutting across oesophagus and around anus. Divide alimentary canal into stomach, pyloric caeca, fore,-mid and hind-intestine, and rectum. Examine surface and scrape contents onto a slide. Examine contents under High Power.
   Compress sections of alimentary canal between slides and examine under High Power.

   iii. Dissect and make squash preparations from heart, liver, gallbladder, spleen, kidney, gonads, urinary bladder and swim bladder.

iv. Dissect out eyes and open nares. Examine under low Power and High Power for hel minths.
Squash lens and examine for eye flukes. Dissect out the separate tissues of the eyes care fully to determine the location as the site is helpful for identification.

v. Remove skin and slice muscle for helminth larvae and protozoan cysts. Squash muscle bet ween slides or glass plates.

vi. Open cranial cavity, examine and make smear of brain tissue.