COURSE CODE:	FIS508
COURSE TITLE:	Advanced Fisheries Microbiology and Pathology
NUMBER OF UNITS:	2 Units
COURSE DURATION:	Two hours per week

COURSE DETAILS:

Course Coordinator:	Dr. A.A. Akinyemi
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Office Location:	Room D204, COLERM
Other Lecturers:	Prof. G.N.O. Ezeri

COURSE CONTENT:

Morphological and biochemical methods of identification of fish parasites. Bacteria and viruses. Sensitivity test control and therapy.

COURSE REQUIREMENTS:

This is a compulsory course for all students in Department of Aquaculture & Fisheries Management. In view of this, students are expected to participate in all the course activities and have minimum of 75% attendance to be eligible to write the final examination.

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READING LIST:

• Akinyemi; A. A. (2009) Microorganisms associated with fish hatchery systems and their effects on
haematological parameters of cultured *Clarias gariepinus* (Burchell, 1822) broodstock. PhD thesis
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• Jay, J.M. (1986). Modern Food Microbiology. Van Nostrand Reinhold New York, pp 227

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• Ramalingam, S.T. (1987). Fish Biology. African FEP publ. Ltd. Pp 154-186

• Roberts, R.J. (ed) (1978). Fish pathology. First Edition Cassel Ltd. (Publ) pp 183-204 and 235-267

• Shewan, J.M. (1977). The bacteriology of Fresh and Spoiling fish and the biochemical changes

induced by bacteria action. In: Proceedings of the conference on the handling, processing and marketing of tropical fish London, Tropical products Institute pp 51-66
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Report of UNDP/WORLDBANK pp 55-62
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cytology Journal of Clinical Pathology 38: 293-294.

LECTURE NOTES

Microorganisms

• Microorganisms is the existence of every minute living organisms or they are living features that can be seen with the aid of microscope, most of them are normally singlecelled while some may exist in multicellular forms.
• These microorganism, though minute and microscopic, are a very powerful group of creatures.
• They can make healthy animals sick, destroy plantations, decay food (e.g fish) and cause world-wide epidemic.
• Some of them however, are very useful in that they can be used for the manufacture of drugs capable of destroying harmful microorganisms while a few can convert certain fruit juices into wine, vinegar and alcohol(Ramalingam,1987).
• Microorganisms are found everywhere except in a vaccum that is, they are ubiquitous; on and inside living organisms.
• These organisms vary in sizes from certain algae just large enough to be seen by the naked eye to viruses which are too small to be seen by normal microscope but can be discerned by an electron microscope.
• Microorganisms are chief agents of spoilage of food especially fish.
• These microorganisms responsible for food spoilage especially fish include bacteria, fungal and viruses.

Bacteria

• These are unicellular microscopic organisms smaller in size than mould and yeasts
• different from one another in size and shape,
• widely distributed in the environment-air,
• water and soil,
• on the surface of all living features,
• on the moist lining of the mouth, nostril and throat and
• inside intestinal tract of almost all animals.
• Their natural habitat is the soil.

• They are varied requirement for growth.
• Some bacteria are thermophilic; they grow best at a temperature range of 450C – 550C.
• Others are mesophilic which grow best at room temperature (200- 300C) and
• pyschrophilic which grow best at refrigeration temperature between 40C – 100C (Shewan,1977).
• Moreso, some bacteria will grow in the presence of oxygen (aerobic)
• while other grow in the absence of oxygen (anaerobic).
• Therefore, bacteria are classified based on temperature on oxygen preference.
• Bacteria do not generally grow at low pH especially below 3.5.
• Bacteria exist in four typical shapes:
• Spherical in shape (cocci),
• cylindrical or rod shaped (bacilli),
• long coiled thread or spiral shape (spirillae) and
• comma shaped (vibrio or filamentous (Ramalingam, 1987).
• They possess cell wall which sometimes may be surrounded by capsule or slime layer.
• Some bacteria are motile (capable of movement) through the use of flagella.
• They normally produce asexually by a process of binary fission.
• Bacteria spore with a very resistance bodies are produced for protection in unfavourable environment.

Fungi

• These are microscopic plant, most of which are multicellular
• although yeast and some aquatic species are unicellular.
• Fungi living in a variety of habitat aquatic ranges to moist situation on land however,
• some of them are able to withstand drier/dried condition e.g *Aspergillus sp*, Penicillum spp).
• Fungi by nature are heterotrophic organisms that is they require an organic source of carbon for energy and depend upon this source for their life.
• Some fungi can be found feeding on non-living organic matter that is, dead or decaying organic matter called saprophytes (this is important for food spoilage).
• Many other fungi obtain their organic nutrient from the host and live as parasites.
• Some are also plants and animal pathogens they cause diseases (Young et al., 1984).
• The two main group of fungi important in food spoilage are mould and yeast which is made up of single cell.

Laboratory Investigation of Microorganisms Associated Fish Production

• 2 grams of a particular size/quality of sample will be aseptically taken and homogenized in 18mls of sterile distilled water using sterile mortar and pestle under hood.
• These will be taken as the original stock culture of the feed sample.
• The procedure will be repeated to get the original stock culture for other feed samples.

Laboratory Analysis

• **Preparation of Glass-Wares and working areas**
• Materials such as glass wares and media will be sterilized before use.
• The glass wares including test tubes, pipette's, petri dishes, bijou bottles will be washed with detergent, rinsed thoroughly with clean tap water and allowed to air dry before sterilizing in the hot air oven at 1600C of an hour.
• The media will be sterilized and bench working areas swabbed with cotton wool soaked in ethanol to sterilize before any microbiological analysis was carried out to avoid contamination.

Microbiological Analysis

• Samples from each category will be taken to the laboratory for microbiological analysis.
• 2ml of a particular section will be aseptically taken and homogenized in 20mls of sterile distilled water.
• These will be taken as the original stock culture of each category.
• The procedure will be repeated to get the original stock culture for other categories.

Preparation of the serial dilution of each fish sample stock culture

• Each of the original stock culture will be serially diluted as follows:
• 1ml of the original stock solution will be aseptically poured into 9ml sterile distilled water and mixed thoroughly to give 10-1 dilution of original stock culture. 1ml of dilution 10-1 of the original stock culture will be aseptically poured into another tube of 9ml sterile distilled water to give 10ml of 10-2 dilution of the original stock culture.
• Repeating the above procedure dilutions of 10-3, 10-4, and 10-5 will be obtained.

Viable Bacterial count on Nutrient Agar

• The pour plate technique will be employed.
• 0.5ml of dilution 10-1 of the stock culture will be introduced into each of three sterilized Nutrient agar plates.
• Sterile molten nutrient agar at about 450C will be added and then mixed thoroughly and allowed to set 45 C undisturbed.

• The set agar plates will be incubated at 370C for 24 hours. This procedure will be repeated using dilutions 10-2, 10-3, 10-4 and 10-5.
• Finally, the number of colonies per plate will be counted and recorded.

Isolation of Microorganisms from the stock culture

• A loopful each of the stock culture will be inoculated into a sterile Nutrient Agar and a sterile Potato Dextrose Agar plates.
• The Nutrient Agar plate will be incubated at 370C for 24 hours while the Potato Dextrose Agar plate will be incubated at 250C for 3-7 days for microbial growth.

Purification of micro-organisms

• Purification of bacteria: Characteristics colonies from original culture on Nutrient agar plates will be picked by sterile platinum wire loop and streaked to isolate on sterile Nutrient agar, MacConkey agar and Blood agar plates for purity.
• Distinct colonies will be gram stained to know the gram reaction and recorded.
• These isolates will be transferred to their appropriate agar slants, labeled and incubated at 370C for 24 hours for growth after which they will be kept in the refrigerator at 40C for identification.

Purification of mould

• Characteristic colonies of moulds will be picked with a sterilized wire loop
• and inoculated into a freshly prepared Potato Dextrose Agar incubated at 250C for 3-7 days for purification.
• Pure mould isolates will be observed for colonial characteristics and kept on Potato Dextrose agar slants for identification.

IDENTIFICATION

• Staining: All the isolates will be transferred from the slants into appropriate agar plates
• and incubated appropriately and used for identification.
• Identification of bacteria: each pure bacterium isolated will undergo the following tests.

Gram's staining

• A very small drop of distilled water will be placed on a clean slide.
• The inoculating wire loop will be sterilized by flaming it until it is red hot in the blue flame of a Bunsen burner.
• The loop will be allowed to cool and a small portion of distilled water on the slide and spread into a thin smear along the slide.
• The smear will be allowed to air dry and heat fixed briefly over flame.
• The smear will be stained with 1% crystal violet for 60 seconds
• and washed with distilled water

• this will be stained with Lugols iodine for 60 seconds and washed with distilled water.
• The slide will be decolourised rapidly with 75% alcohol and washed off immediately with distilled water.
• The slide will finally be flooded with counter stain-Safranine for 30 seconds and washed off with distilled water and air dried.
• This will then be observed under the microscope with the aid of oil immersion lens (100 objective lens).
• Capsule staining: Grown micro-organisms will be picked with sterile needle and will be mixed with a drop of sterile distilled water to make a smear which will be allowed to air dry.
• The dry smear will be fixed by passing over Bunsen flame.
• The smear will then be covered with crystal violet stain.
• Heat will be applied gently with the steam just began to rise, and then left to stain for 1 minute.
• The stain will be washed off with copper-sulphate solution.
The back of the slide will • be wiped clean and placed on a draining rack for the smear to air dry.
• The smear will then be observed microscopically under x 100 objective lens to look for capsules which appeared pale blue with bacteria cells stained dark purple.
Spore staining
• A drop of distilled water will be placed on a clean slide and a small loopful of organisms will be added, aseptically using sterile wire loop and mixed then spread out into a thin smear.
• It will then be covered with 2 drops of Ziel-Nelson Carbol Fuschin solution for 5 minutes after which it will be decolourised with 95% alcohol for 2 minutes and
• counter stained with Noeflers methylene blue for 1 minute.
• Thereafter, it will be washed and air dried.
• The spores appeared red and the bacteria bodies (vegetative form) appeared blue.

Mould Staining (Methylene-Blue Staining)

• A drop of methylene blue stain will be put on a clean slide and with the aid of an inoculating needle, a small portion of mycelium will be removed from the mould culture and placed on the drop of methylene blue.
• The mycelium will be spread very well on the slide with the aid of two mounted needles.
• Gently, a cover slip will be lowered on it, excess liquid wipe off by putting the slide between two filter papers and applying a gentle pressure around the cover slip.

Biochemical Tests

• Catalase Test
• Coagulase Test

• Citrate Utilization Test
• Urease test
• Nitrate Reduction Test
• Indole Reaction
• Oxidase reaction
• Hydrogen Sulphide Production
• Gelatin Hydrolysis
• Voges Proskauer
• Sugar utilization
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University of Agriculture, Abeokuta, Nigeria 116pp
Introduction
• Higher stocking densities call for the
introduction of large quantities of
concentrated feeds and mineral fertilizers.
• This affects the environmental factors in the
aquatic environment
• environmental changes are stressful and lower
body resistance of fish to disease
• Infections and other diseases therefore sets in
• Such unfavourable conditions include
– crowding,
– temperature fluctuations,
– inadequate dissolved oxygen,
– excessive or rough handling,
– sublethal levels of toxic materials.
• Fish diseases are
the end result of
interactive of the
etiologic agent,
the susceptible
the fish and the
environmental
condition
Physiological And Biochemical Changes
• These are non-specific physiological and
biochemical changes which take place in three
phases:
– The alarm reaction
– The stage of resistance during adaptation to
achieve homeostasis under the changed
circumstances taking place.
– The stage of exhaustion when adaptation has
ceased to be adequate and homeostasis is not
achieved.
Classification of diseases
• Generally, diseases are classified as
– infections,
– parasitic,
– non-communicable and
– diseases of unknown etiology.
• **Infections diseases** are caused by viruses,

bacteria, fungi and less frequently algae.
• **Parasitic diseases** are caused by protozoa, helminthes and parasitic crustacean.
• **Non-communicable disease** include
– nutritional fish diseases (e.g goiter or hyper plasia of the thyroid gland)
– diseases associated with physical or physicochemical abnormalities of water e.g gas bubble disease
– low temperature disease, water borne irritants
• **Diseases of unknown aetiology** are diseases for which the causative agents have not been identified.
• It includes proliferative kidney disease

BACTERIA DISEASES

• Bacterial diseases are responsible for heavy mortality in both wild and cultured fish. The actual role of these micro-organisms may vary or differ from that of a primary pathogen to that of an opportunist invader of a host rendered moribund by some other disease process.

Microscopic examination

• Can provide information on the size and shape of the bacteria
– Rods (1)
– Cocci (2)
– Spiral (3)
• It cannot provide enough information to enable bacteria to be identified

Taxonomy

• The taxonomic position of the currently recognized bacterial pathogens of teleosts includes the following families and genera

Cytophagaceae

• The cytophagaceae are single or filamentous rods which are characteristically motile by a sliding movement.
• They are gram-ve with orange, yellow or red colonies.
• They are usually associated with mucus of normal or diseased fish.
• They measure about 0.5um wide by 12um long.
• e.g *F. columuans, flexibacter* (cytophge) *Phychrophile*
• Causes skin infections which may affect lateral line, fin edges, gill lesions,etc

Pseudomonadacea

• They are gram-ve non-sporing rods that may be straight or curved, motile by potafeaells, oxidaline chemo-organotroph
• They are strict aerobes.
• They may produce a fluorescent pigment.
• They commonly occur in soil and water. The rods measure about 3um x 0.5um
• e.g *P. fluorescens.*

• They with aeromones cause a disease known as Bacterial haemorrhagic septicemia e.g fin-rot,

Enterobacteriaceae

• This include various genera of small, gram-ve non-sporing rods that germent dexterose to produce acid or acid of gas and one oxidase negative may species are motile by peritrichons flagella.

• They measure -0.75um x 2.5um e.g E. tanda which cause conferen of external lesions to the head of affected fish e.g rainbow front.

• There may be erosion of lower jaw resulting in a need haemorrhagic ulcer and necrosis of the intestinal mucosa but the urtnal confinement of external lesions to the head of affected fish is a clinical feature for RM bacterium (Enteric red month).

Vibrionaceae

• These are gram-ve, non-sporing, straight or curved rods

• usally motile by polar oxidase +ve, catalase positive.

• They are usually found in both fresh of salt water.

• Some species are pathogenic to man, some to fish while others are of economic interest in the food industries.

• They cause a disease known as vibriosis which is the most significant disease of cultured and mild marine fish

• V. arguillanum which includes strain of V. piscium and V. Ichthyodernis have been isolated from diseased fish in both sea or river waters and are pathogenic for fish.

• First signs of infection are anonexia, darking of sudden death.

• Acutely affected fish show swollen, dark skin lesions which ulcerate to release blood coloured excidates. Gills are pace

Streptococacea

• These are gram +ve spherical or ovoid cells arranged in pairs or chains.

• They are usually less than 1.0um in diameter and non-motile.

• They are facultative anaerobic

• some species that infect fish include *S.taecalis, ad S. boris.*

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Bacillaceae

• These are gram +ve endospore-forming bacilli.
• They are non-acid-fast and measure up to 6ug (*C. botulinum)* and 1.7-3.5ug long (*C.tertrum),*
• Bacillus coagulans, *B. subtilis. B. coagulars* is Gram +ve, but gram –ve in old cultures while *B. subtilis* is Gram +ve.
• Both are motile clost.
• Botilium leads to nervous inbalance with infected fish swimming up of down in water and twitching.

Corynebacteriaceae

• Are gram +ve non-motile, non-sporing rods with characteristic "splitting morphology, with clubshaped swellings and arranged in palisades.
• Affected fish are usually darker in colour with occasional exophthalmos and small haemorrhages at the bases of the pectoral fins.
• At recrosopy, lesions are use found in the kidney, they are whitish with red hyperaemicrim.
• Organisms may be found in spleen and liver.

Mycobacteriaeae

• The mycobacteriaceae are slightly curved or straight bacilli measuring 1-10um.
• They may occasionally have filamentous or branching forms.
• They are Gram positive but are acid-fast positive.
• They are non-motile, acerbic
• species include M. Marium, *M. fortunitum, M. chelonei.*
• Symptoms include darker colour, swelling of abdomen.
• They cause, skin infection and or hypersensitivity especially in aquanists.

Bacterial diseases of eggs

• Bacteria can completely destroy an egg mass in a very short time.
• First indication of a bacterial in section is an opaque or whitish appearance in small areas of the egg mass.
• *Flavourbacterium* and *Acinetobacter* have been isolated from diseased eggs and are considered to pathogen of catfish eggs.
• Aeromones, a common fish pathogen, has also been isolated several times

Control of therapy

• Sooner or later, the fish culturist arrives at a situation where he must treat his fish for one disease condition or another.
• The treatment of fish is always a dangerous undertaking and every possible precaution must be taken to avoid disastrous results.
• the hatchery man must treat his fish enmasses
• The problem is complicated because the environment in which fish live plays such an important role in

determining the type and effectiveness of the treatment.
• Prevention, rather than treatment, should be the goal of every fish culturist
• Some disease preventive measures are;
– Use of water that is free of wild fish e.g well or spring water.
– Good quality water with no harmful substances.
– sand-gravel fitters can be installed to prevent the introduction of with fish and most parasites from stream or river water.
– Avoid introduction of fish from other hatcheries but if this is not possible, obtain fish from hatchery without history of serious disease problems. Treatment for especially ectoparasites prior to stocking is advisable.
– Avoid crowding of fish at any time especially during hot weather.
– Maintain good environmental conditions in order not to include stress.
• The general methods of treatment that are commonly adopted.
– Introduction of chemical or drug directly into the pond water is external treatment.
– Incorporation of medicine in feed of fish i.e systemic treatment via diet.
– Parenteral treatment i.e injection of drugs.
External treatment
• Various methods of treatment and drug application have been used in the control of disease.
• They include
– dip,
– flush and
– bath (short and prolonged).
Dip Method
• The dip method involves using a strong solution of a chemical for a relatively short period of time.
• The difference between an effective dose and a lethal one can be very narrow.
Fish are usually placed in a net • and dipped into a strong solution of the chemical for 15-45 seconds depending on the type of chemical.
• This is most effective while treating small number of fish.
Flush Method
• The flush method is fairly simple and consists of adding a stock solution of a chemical to the upper and of the unit to be treated and allow it to flush through the unit.
• This is a popular method in trout and salmon hatcheries but is rarely used in catfish hatcheries.
• It is only applicable with raceways, tanks or egg incubators.
• Adequate water flow must be available in order to completely flush the chemical through the unit

or system within a predetermined period of time.
Bath Method
• The bath method is of two types, namely
– a short term bath and
– indefinite prolonged treatment;
• Bath method involves adding chemical directly to the unit or system and lift for a specified period of time after which it is then quickly flushed with freshwater.
• Although a treatment of 1hour may be recommended, the fish should always be observed throughout the treatment period for any fish of distress e.g gasping at the surface.
• When noticed, freshwater should be added quickly
• Bath treatment often lead to reduced Water concentration and aerators should be used to ensure adequate oxygen supply to fish
• **Prolonged bath**
• Is used in treating ponds where a low concentration of a chemical is applied to dissipate naturally.
• This is generally one of the safest methods of treatment, but the large quantities of chemicals regard may be expensive if not prohibitive.
Other forms of treatments
• Systemic treatment via diet or oral application
Parenteral treatment (i.e • injection of drugs)
• Drugs and chemicals introduced directly into the water
• Some drugs and chemicals used in control of infectious diseases include;
– Pyridylmeranic acetate (PMA): (i.e Phenyl mercuric acetate)
– Formalin
– Malachite Green
– Potassium permanganate (KMNO4)
– Oxytetracycline
– Copper sulphate
– Salt (sodium chloride)
– Furacin and Nitrofurazone
• Drugs added to the feed
– Sulfamethazine-Commonly called sulmet
– Terranyain
– Furox-50 i.e a product containing 11% furazolidone
– Erythromycin thiocynate: (in form of Gallimycin 50) contains 50gms erythomyin thiocynate /1lb
– Vit. B1 (Thiamine hydrochloride)
– Furacin and Nitrofurazone
VIRAL DISEASES
• The majority of fish virus diseases occurs in cultured species with high economic value.
• In most instances these virus diseases are acute, with high mortality
• Viral diseases of fish are impossible to control with chemotherapeutics, since no drug or chemical has any effect on the course of the

viral disease of fish because of this prevention instead of treatment is a sine qua non.
• Some viral diseases include;
– Channel catfish virus disease (CCUD)
– IHN-infections hematopoietic recrosis
– IPN- infections pancreatic necrosis
– VHS-viral hemorrhagic septicemia

FUNGAL DISEASE

• Fungal species of the genera saprolegmia and Achylya are usually implicated in fungus infections of cultured catfish with saprolegnia being more common.
• Aphanomyces pythium and leptomitus lactenus individual saprolegna is generally considered to be a secondary invader, taking advantage of a lesion or abrasion in order to establish itself and may cause mortalities to fish but to injection is possible.

PROTOZOAN PARASITES

• Nearly all losses of fish due to parasites are caused by protozoans.
• While many protozoans are obligate parasites requiring fish loss, some are facultative and constitute a problem as a result of poor water quality or poor nutrition.
• The most important parasitic protozoans of fish include the following
– Costia (ichthyobodi)
– Trichodina
– Ichtyophthrins nultifihs (ICH)
– Spistylis perifrichous i.e hair-like cilia