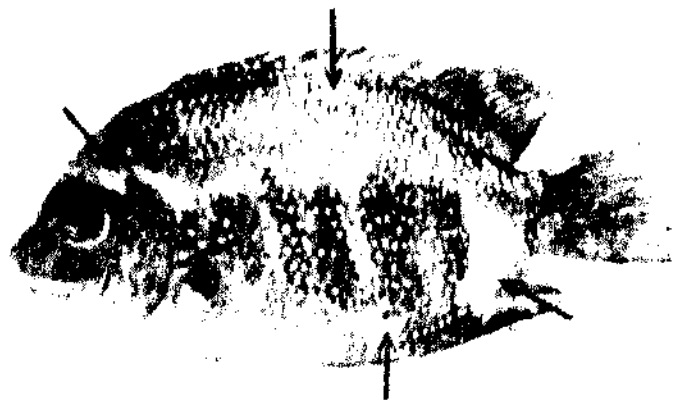
CMFRI SPECIAL PUBLICATION
Number 11

APPROACHES TO FINFISH AND SHELLFISH PATHOLOGY INVESTIGATIONS



Issued on the occasion of the Workshop on
APPROACHES TO
FINFISH AND SHELLFISH PATHOLOGY INVESTIGATIONS
Organised by
Centre of Advanced Studies in Mariculture
Central Marine Fisheries Research Institute
held at Cochin on 10-11 February, 1983

Approaches to Finfish and Shellfish Pathology Investigations

CMFRI SPECIAL PUBLICATION

Number 11

ISSUED ON THE OCCASION OF THE WORKSHOP ON APPROACHES TO FINFISH AND SHELLFISH PATHOLOGY INVESTIGATIONS ORGANISED BY THE CENTRE OF ADVANCED STUDIES IN MARICULTURE, CENTRAL MARINE FISHERIES RESEARCH INSTITUTE HELD AT COCHIN ON 10 - 11 FEBRUARY, 1983.

(LIMITED DISTRIBUTION)

Published by : **DR. E. G. SILAS**
Director
Central Marine Fisheries
Research Institute
Cochin 682 018

PREFACE

Several cases of mortalities and non-acceptability of fishes and shellfishes have been attributed to pathogens and diseases; many failures of aquaculture exterprises have also been indicated to be due to the same reason. To overcome this constraint and to save the crop from the disease hazards, concerted efforts are being made to study the pathological problems encountered both in the capture and culture fisheries, and to suggest ways and means of remedial and control measures.

In India, investigations on the pathology of the commercially important fishes are limited. This is particularly so in the case of marine fishes. However, with the rapidly increasing interest in the culture of fishes, often with high stocking densities and the increasing pollution of the aquatic regime, the incidence of diseases among the farmed fishes and shellfishes, causing their large scale production and economic loss, are being reported. In this context, it has become imperative to study the problems through an integrated approach.

Fish and shellfish pathology is one of the priority areas identified for intense research under

the Centre of Advanced Studies (CAS) in Mariculture. To aid in these research programmes and to train the scientists working in the field in the modern techniques of investigation, consultancies with experts in the subject are arranged. Under this scheme, Dr. A.L.S. Munro, Principal Scientist of the Marine Laboratory, Department of Agriculture and Fisheries, Aberdeen, Scotland and an internationally known Expert in fish pathology has been working with the Centre from 3rd January, 1983 for 6 weeks.

The present workshop on 'Approaches to finfish and shellfish pathology investigations' is organised by the CAS in Mariculture in close participation by Dr. Munro. The material presented in this publication is prepared by him in collaboration with Mr. S. Mahadevan, Scientist of this Institute. I am sure that the approach outlined and discussed in the Workshop would be immensely useful to the Scientists as guidelines in the identification of the problems and the rational approaches to be undertaken to tackle the same. I would like to record my deep appreciation to Dr. A.L.S. Munro, who within a short time available with him, concieved

the idea, prepared the material and led the discussions. I also wish to thank Mr. S. Mahadevan who ably assisted Dr. Munro and helped in the organisation and conduct of the Workshop.

E.G. Silas
Director
Central Marine Fisheries
Research Institute
Cochin 682 018

C O N T E N T S

I. ASSESSING REQUESTS FOR ASSISTANCE FROM MARICULTURISTS

1.1. What constitutes a problem in mariculture?

The following is a comprehensive list of the sorts of subjects which form the basis of enquiries:

- Mortality and morbidity of stock i.e., death and sickness.
- Abnormal appearance of stock.
- Abnormal behaviour e.g., not feeding, jumping.
- Reduced growth rate or poor yield.
- Precosity i.e., early sexual maturation.
- Loss of fecundity - sterlity of stock.
- Customer rejection of product.
- Predation, pests, theft, storm damage.
- Suspect water quality.
- Suspect quality of compounded feeds.
- Site selection parameters. i.e., how to choose and set up a farm.
- Where to buy ova, spat or grow-out stock.

Once a Pathology Team is established and regular contact made with farmers questions on these (and other) topics may be anticipated. As explained later the Team Leader and Field Officer will have to deal with most of these enquiries. General guidelines will have to be drawn up to assist them. However, they should answer immediately where possible if a general and brief reply can be given. Detailed advice should come from an expert on the subject. Enquiries of the former nature are commoner; where detailed advice is required the two officers should have had some prior guideance on who or what group to route the enquiry towards, if it is not their own specialism. Handling of enquiries, receipt of specimens, letters, field visits etc. are done on the following lines.

Telephone Enquiries:	Recorded, answered by staff verbally on the enquiries wanted to another name, in our lab or elsewhere.
Letters:	Filed, may be answered by letter or telephone; former preferred.

Specimens:	Recorded. Internal laboratory report, telephone conclusions as soon as possible. Formal report posted later.
Field investigations:	Verbal report on site. Internal laboratory report, telephone conclusions as soon as possible. Formal report posted later.
Field Advisory Visits:	Verbal report may be given. Internal laboratory report which will be the basis of a posted written report.

1.2. Who is likely to come forward with problems/ enquiries

- Commercial mariculture farmers.
- Prospective mariculture farmers.
- Research groups of State Government/Private Industry who are researching aspects of mariculture.

Our experience is that it is common for all such groups especially (3) above to present disease problems for diagnosis. Disease is a highly specialised subject and unless the disease is easily diagnosed and/or recurring such groups are unlikely to be able to diagnose it or proceed to control measures.

1.3. <u>What information should be sought on receipt of enquiry/request</u>

- Name, address and telephone No. of enquirer.
- The situation concerning the request:
- when, where, how many affected relative to the relative to the total stock size?
- how often has it happened before?
- what is the economic significance of the incident?
- general description of incident/pathology- officers tries to relate any similar past experiences.
- a brief record should be made and annually these requests analysed for the teams annual report.

1.4. <u>A decision should be made at this stage of the enquiry on how to proceed e.g.</u>

- answer briefly
- refer to more experienced group/officer
- initiate a pathology team investigation
- how will the pathology team proceed
- by advice
- by seeking receipt of live specimens/or chemically fixed tissues

- by on site investigation

- referred to others e.g., a remote location might be visited by a local Fisheries Officer who would relay information/ specimens to the pathology team laboratory

If the problem sounds serious and/or if no immediate clue to the identity can be gained by questioning them, an on site investigations is preferable by a field officer. If from the description a tentative diagnosis can be made then receipt of live specimens showing the condition may be the most economical approach assuming some method of delivery is available.

The following team may rely on:

- farmers bringing live animals in plastic bags by their own transport.

- farmers sending by a direct train, plane or bus service and telephoning about the expected arrival of the specimens at the station/airport where they can be collected.

1.5. Who in the pathology team will conduct primary investigations of field material i.e., in laboratory or in the field,

In the DAFS Marine Laboratory there are two field officers servicing some 170 farm sites and some river situations as well. Both are trained parasitologists who have been given additional

in house training in the diagnosis of bacterial diseases of aquaculture species, a period of experience visiting fish farms, and learning about farm systems and their operation. Previously one field officer was a bacteriologist given in house training on parasitology. Part of their training is familiarisation with the treatments available for recurring diseases. Section leaders are responsible here for maintaining an uptodate schedule of treatments for common recurring diseases. Strategies for disease control are usually the prerogative of the team or section leaders. However, this is a flexible area and once a particular method is well proven field officers may be quite capable of explaining its use to farmers.

It is advisable that field investigations are staffed with full time officers, initially one is recommended. This system has the advantage that the research staff are not constantly interrupted and can plan their major activities on this premise. The DAFS Team will have an average of 3 calls a day which the field officers or the team leader will handle although in their absence as often happens other team members must respond.

1.6. Some of the ways field problems have been solved.

- The farmer recognises and knows how to deal with theproblem. He has achieved this competence through past experience, training and hand-outs describing the disease and methods for its control

- The farmer recognises the problem and telephones for advice or assurances on the latest or most appropriate methods of control. Usually the field officers will request specimens for confirmation of the doagnosis. Often the sample will be pieces of key organs in fixative. Many farmers have a supply of bottles and fixative and can therefore dissect out and send material to the laboratory. Simple dissection of pieces of key organs should be part of the training courses for farmers.

  Of course the farmer will be advised of the recommended treatment there and then. A written report on the specimen will be issued when available.

- The farmer does not recognise the problem and telephones the laboratory. Either a visit is arranged within 24-48 hours or samples are

sent as discussed. The investigation of the samples is the same in the field or the laboratory. In the field, however, the officer has the advantage of seeing the affected populations, choosing samples for himself and questioning all staff.

It is obligatory that if specimens are received/taken a written report be issued. Similarly if a visit is made a written report should be sent recording the findings.

1.7. Field Officers Investigations:

7.1. Morbid and abnormally behaving animals of grow-out size and bigger:

- examine not less than 6 and upto 20 apparently affected animals to obtain an average picture of the range of any abnormality. If necessary examine an equivalent number of apparently healthy animals to establish the differential picture.
- examine exterior and interior organ and record abnormalities including whether feeding or not and appearance of faeces.
- wet mounts of scrappings of skin, gills and gut contents plus squashes oforgans for parasites or fungi. Can either be found? A high power microscope is necessary and a dissecting microscope as well. If it is a fungal or parasite

problem tell the farmer and recommend any appropriate treatment. If a parasite/fungal cause cannot be established proceed -

- signs of anaemia in fish may indicate a bacterial disease i.e., pale gills. If gills are pale measure the haematocrit value of 6 fish. Values of 30% or less indicate poor health status. Note if buffy coat, the layer of leucocytes on top of the red cells is deeper than normal also a sign of some bacterial diseases.
- make smears of blood and impression smears of kidney and spleen. Gram stain for evidence of bacteria or fungi.

-If a bacterial or fungal problem is suspected tell the farmer and recommend any treatment. If such investigations are not conclusive either way the investigation should proceed -

- by asceptic techniques plate onto nutrient agar medium (2% Nacl content) from kidney, spleen and blood. These plates should be carried for field investigations. They are for bacterial culture.

If a specialised medium is indicated by the exploratory studies e.t. Saborouds medium for fungi or a richer medium for some bacterial species then either live specimens or tissues in asceptic conditions in ice,

will have to be taken and examined in the laboratory. Isolates are processed by normal keys for bacterial identification.

- Except in the case of obvious ectoparasitic disease small pieces of tissue from the major organs should always be preserved in a histological fixative appropriate to the animal under study e.g., 20 - 20% buffered formal saline for fish tissues. Where no obvious cause of disease is found it may be appropriate to fix material from many organs as well.

  Major organs: kidney; liver; spleen; caeca/gut.
  Other organs: gill; brain; heart; stomach; hindgut; skin/muscle; eye; swim bladder.

- If from past experience it is clear that electron microscopy will be important in achieving diagnosis then approximate steps should be set in hand to fix such material; otherwise material for EM is not routinely taken.

- If a virus cause is suspected samples are taken asceptically of kidney and possibly other organs like spleen, liver and pancreas, stored in ice in a vaccum flask until processing for tissue culture in the laboratory.
- If no cause of morbidity or abnormality is found the report should say so. The features of unresolved investigations should be remembered and comparison made with any similar occurrence in future. A significant frequency of similar events will require careful scrutiny by the team leader for any overall clues. It may be that such a problem will be taken into the research programme and specific staff allotted to its study. (See research projects for the further study of such problems).

7.2. Eggs, larvae, etc. from hatcheries.

The problems facing the investigating pathologist in hatching situations are significant. However the following procedures should always be carried out:

- Examine affected animals in the dissecting microscope for signs of abnormality.
- Make squashes and smears for signs of invasive parasites and fungi.
- Filter water and examine either the filter paper or the culture water contents, concentrated for signs of other animals.
- Preserve specimens of abnormal animals (not dead) for histology.

The pathologist will have to recognise that many causes of death and mortality at the hatchery stage are imperfectly understood. Among these are:

- Poor health status of brood stock e.g. because they have infections or are fed unbalanced diets.
- Lack of knowledge of correct conditions for egg fertilization resulting in improper development in the embryo.
- Inadequate nutrition for the developing animal.
- Lack of proper knowledge and control of the environment, especially water quality and bacterial load for the hatchery animal.

The diagnosis of such problems solely by examination of hatchery juveniles is often not feasible and much more information may have to be sought on parent animals, food supplies, methods of husbandry and water quality.

II. ACTIVITIES OF THE PATHOLOGY TEAM

2.1. Advice on the significance and control of diseases in mariculture

The team and the particular team leader will have to collect the information received from his Field Officers, from discussions with farmers and other researchers on the current status of particular disease problems, noting regional variations, the efficiency of any control measures and the status of any ongoing research on the problems. In all the probability this material will be presented in an annual report.

2.2. Field investigations of diseases in mariculture

- The team will have the capability of monitoring field investigations on problems occurring at mariculture sites. In general a specific officer(or officers) will be engaged in this work which will be their major activity.
- The response to problems should be rapid. There is little point in arriving after an epidemic is over and all stock are dead. The implication here is that transport is readily available - transport for which the field service has priority over other matters.

.3. Establishing priorities for research

Again this is a major duty of the team leader, with the information available in section 2.1 and with his knowledge of the strengths and weakness of his team (and other teams if a collaborative effort is indicated) he will have to choose between in all probability more potential projects than he has resources to study.

The availability of students is advantageous and as long as the student's interests are safeguarded it will be an invaluable asset to assist in data collection.

The developmental status of an industry will influence choice of project. For example prawn monoculture seems poised to start growth. Prawn diseases should therefore receive high priority. Similarly pearl and edible oyster culture have signs of commercial promise and must also be high on the priority list.

.4. Areas for research

Diseases of unknown aetiology

Here the team leader has a crucial role to play in assembling the evidence from field officers, farmers, bacteriology, histopathology etc. Where evidence is lacking he will have

to try and obtain it through efforts of his team or by co-operating with other teams. He will have to set up hypothesis testing known facts, assembling new data which confirm or reject the hypothesis, but working to a pattern to establish if it is:

- infections and if so of what nature
- of toxic origin and if so the nature of the toxin and, how is the animal exposed.
- of nutritional origin.
- of an environmental nature.

Adequacy of diagnostic methods : Can improvements be made in the speed of diagnosis or in reducing its cost? Can new diagnostic methods be developed?

Parasite diseases:

- Taxonomy : establish what is known in the scientific literature including control measures of significant parasite diseases.
- Life cycle studies if little is known and if considered relevant. A convenient control measure may be possible from this knowledge.

- Trials with recommended or probable chemotherapeutants to determine their efficiency.
- Prophylaxis - chemical or other avoidance strategies.
- Epidemiology - are there other hosts of the parasite in the vicinity to assess the abundance of such reservoirs.
- Experimental pathogenesis - description by histopathology and pathophysiology of progression of the disease on the host.

Bacterial Diseases:

- Taxonomy and culture - Literature review including control measures. Are diagnostic methods adequate?
- Antibiotic sensitivity - leading to trials with oral/baths treatments, or other strategies.
- Experimental pathogenesis studies - as in parasitology.
- Maintain a collection of isolates of pathogens for comparative studies and for use by students.
- Studies of any toxins produced by bacterial pathogens, their isolation, purification and study of their properties in vitro and in vivo.

Fungal diseases.

- Taxonomy and culture - Literature review including control measures. Are diagnostic methods adequate?
- Chemotherapy trials - efficiency to treatments by malachite green and other chemicals.
- Prophylactic methodologies.
- Experimental Pathogenesis - as in parasitology.
- Maintain a collection of isolates of pathogens for comparative studies.

Host studies:

- National collection of histopathology material on diseases of mariculture species.
- Experimental pathogenesis studies - description of the histopathology and pathophysiology of diseases of commercial importance in mariculture - cooperative projects with other sections of the team.
- Description of the cellular components of inflammatory responses in different hosts.

Field Studies.

- Some will be cooperative projects with other sections of the teams and with other

teams e.g. chemotherapy, epidemiology, development and assignment of strategies for disease control.

2.5. Teaching Role

The team leader and section leaders will be expected to teach their respective disciplines and others.

Artisanal training should also be part of the team's work e.g. at the KVK Centre. The field officers should figure prominently here not only as a means of instructing but in order that they become known and the services they can provide well understood.

III. THE PATHOLOGY TEAM STRUCTURE

3.1. Section Divisions:

The team should be headed by an individual and divided into sections based on discipline or activity as follows:

	Team Leader			
Section Leaders	Bacteriology	Pathology	Parasitology	Field Investigations
Scientists				
Technicians				

It is suggested that fungal problems are divided between bacteriology and parasitology.

3.2. Staffing

Team Leader	2nd technician shared by all these.
Pathologist and technician	
Bacteriologist	
Parasitologist	
Field Officer (and driver?)	

Initially the Team Leader, if suitably qualified, might take on one of the Section Leader roles.

IV. JOB DESCRIPTION OF TEAM MEMBERS

4.1. Team Leader

- Co-ordinates and leads his staff's activities in field investigations, research and in assisting coordination of joint inter team studies.
- Advises on the significance of particular diseases as they affect the mariculture industry, the efficacy of control measures and on research priorities.
- Prepares Annual Reports, Staff Reports, Estimates, Field Reports etc.
- Provides instruction for students and staff.

4.2. Section Leaders (Bacteriology/parasitology)

- Assemble evidence for team leader on the significance of particular diseases.
- Assists field officers as appropriate in field investigations.
- Research diseases appropriate to their discipline.
- Teach students as required.
- Keep such collections of pathogens as is appropriate to their discipline.

4.3. Pathologist

- Provides pathology reports on the material submitted by the field investigation service.
- Maintains a registry of histopathological material as a national record of diseases of mariculture species.
- Assists bacteriologists/parasitologists in their studies of the pathogensis of diseases.
- Teaches students on the principles of pathology and familiarises them with the pathology of the diseases of nationally cultured aquatic animals.
- Conducts comparative studies of the responses of mariculture species to disease thus advancing knowledge of his subject.
- Supervises technician.

4.4. Field Officer

- Answers enquiries on mariculture in general.
- Investigates reports of mortality, morbidity etc. at mariculture sites and provides reports on such investigations.
- Assists research staff when field duties allow especially in epidemiological studies,

in field trials with chemotherapeutic agents and in the investigation of diseases of unknown aetiology.

- Assists in teaching at artisanal courses on disease recognition and control.

4.5. Scientist

- Assists his section leader in section's activities.
- Conducts a research project of his own or assists in an aspect of a research project.

4.5. Technician (Pathology)

- Prepares material from field service for histopathology examination.
- Provides material for instruction of students.
- Assists in teaching students techniques in histology.

Technician - 2

- Assists pathology technician as appropriate.
- Assists bacteriology section in preparation of media.
- Assists team in reagent preparation etc.

V. REQUIREMENTS OF THE PATHOLOGY TEAM

5.1. Laboratories and equipment

On the basis of the team composition and structure the following are recommended:

Bacteriology Laboratory:

A separate laboratory to provide space for instruments and staff.

Area : 6 x 7 m = 42 m^2

(including office space for section leader)

Equipment:

- High power microscope with phase contrast and incident and transmitted UV light for fluorescence microscopy. This requires to be housed in low intensity light room for u.v. work e.g. windows fitted with black venetian blinds.
- Centrifuges:
  - High speed refrigerated at 60,000 rpm.
  - Low speed refrigerated at 8,000 rpm. capable of 4 L bulk loads.
  - Low speed bench centrifuge 10,000 rpm.
- Laminar flow cabinet for performing certain essential asceptic procedures

- Spectrophotometer–visible range 350 - 650 mm
- Balances
  - Rough balances 3 kg.
  - Fine balances 200 g.
- Autoclave - 2 small autoclaves e.g. pressure cookers
- Fridge - 10 - 14 cft 4° C unit. Upright Model
- Deep freeze - 10 - 14 cft - 15 C unit upright model
- Incubators : 30°, 37° and 42° C
- Water baths - (two)

Laboratory services: Hot and cold water
vacuum pump
Gas supply for bunsen burners and autoclaves; airconditioning
Compressed air

Bacteriology Kitchen and Preparation area

Principally for bacteriological and mycological preparation of media but also to serve as glassware cleaning centre for team.

Area 6 x 7 m = 42 m^2

one half devoted for media preparation, storage of chemicals for media and pouring of agar plates and the second half devoted for glassware washing and sterilisation of materials.

Equipment and services : Sinks for wash up; Distillation units for distilled water; Hot air oven for dry heats; Sterilisation; pH meter; Balances (500 g. to 10 mg. sensitivity); Steamer for 100° C sterilisation; Autoclave (larger) for bulk sterilisation (Air extractor to remove steam and heat).

Services: Hot and cold water adjacent to kitchen; but not part of 42 m^2 one walking 4° C room for storage of bacteriological media (2 x 3 m = 6 m^2 room).

Pathology and Parasitology Laboratory

One large laboratory for Pathology and Parasitology is recommended. Histopathology processing uses some noxious chemicals. Therefore a fume cupboard and air extractor over histokinette bench is required.

Area: 10.5 m x 6 m = 63 m^2

Equipment:

- Microscopes: high power photomicroscope with planar objectives and large field vision for histopathology. Facility for dual viewing and for projection of image to a wall screen (e.g. Leitz orthoplan) - one;

- High power photomicroscope with phase contrast for parasitology - one
- Dissecting microscopes - two
- Balances : Rough balance 2 kg.
  Fine balance 200 g.
- Centrifuge, low speed (5000 rpm)- one
- Fridge 4.C (10 - 14 cft) - one
- Deep freeze 15 C (1- - 14 cft)- one
- Water bath - one
- Incubator - one
- Electrophoresis apparatus - (e.g.) LKB make-one
- Fraction collector LKB make - one
- Histokinettes - two
- Microtomes - two
- E.M. Section cutting equipment comprising glass cutting knife maker, block preparer and glass knife microtome
- Small water bath - two
- Hot plates - two

Services: Fume cupboard 2 m long (one)
(Area over histokinettes with extraction head)
Hot and cold water

Compressed air
vacuum pump
Gas for burner
Air conditioning

Post mortem room

For receipt and examination of diseased specimens
Area 3.5 x 6 m = 21 m^2

Equipment

Benches to be Terrazzo (Mosaic) and well shaped in order that water from a wall tap can easily flush the bench water draining into a sink integral with bench.

- Fridge (10 - 14 cft)
- Dissecting microscope - one
- High power with phase microscope - one
- Balance - 2 kg. - one
- Pressure cooker - one

Services: Gas supply
Compressed air
Hot and cold water

Electron microscope suite

Area : E M room 3.5 x 6 m = 21 m^2
Dark room 3.5 x 6 m with sinks, benches and enlarger
Office 3.5 x 3 m for team leader.

Storage Areas:

The team will have special storage requirements. These can be met proving an area of 3,5 x 6 m divided equally into three parts for use as follows:

Cold Store: An insulated room held at 4° C to store bacteriological media. No fan driven ventilation should be used because of the risk of infecting media.

Alcohol Store: Significant quantities of ethanol and methanol are routinely used in histological processing. To ensure the continuous operation of the Team Histopathology service it is recommended they have their own safe store of these materials.

Formalin and formalised specimen Store: As the National Pathology Register a safe adequate store for the sage keeping of some specimen is required.

National Pathology Registry: A room of 3,5 x 6 m should be kept for the purpose of determination and storage of slides and blocks (wax and resin embedded) of pathology material.

5.2. Other requirements of pathology team

Field Investigators: They require ready access to transport, to meet calls from farmers and to

conduct field work. They will have to carry a high power microscope, a dissecting microscope, a small table and chair and an awning from the back of the vehicle. They will need to carry sample bottles, fixatives (2 l.), water (2 l.) bacteriological media (6 - 12 plates) and a small gas burner. Vacuum flasks and an oxygen cylinder with plastic bags for live specimens.

5.3. Field studies

To conduct certain field studies it may be necessary to hire the use of ponds for grow-out trials.

VI. RELATIONSHIP OF PATHOLOGY TEAM TO OTHER TEAMS AND PROJECTS WORKING ON MARICULTURE RESEARCH

5.1. Co-ordination of research projects requiring significant collaboration with other teams

Such project requiresa project leader, preferably one of the team leaders. However, the project leader will have to keep his team leader and other colleagues well advised of the progress and about manpower requirements. Therefore committee work is necessary for both review and planning. Alternatively, but less satisfactorily, a committee of team leaders may be the project leader.

(See item 8 for full details)

5.2. Field investigations

Such investigations may call for periodic assistance from other specialised groups e.g. for chemistry of pond water, phytoplankton analysis, stomach content analysis. Some formal arrangements for the work and indeed for minor assistance in research projects should exist.

VII. RELATIONSHIP OF PATHOLOGY TEAM TO THE MARICULTURE INDUSTRY

It cannot be too clearly emphasised that the team exists to service the Mariculture Industry. Its first priority should always be to servicing that industry's requirements. Therefore the diagnostic service will, on the establishment of a mariculture industry, be of crucial importance because primarily the industry will judge the team on their service, and not on research or teaching.

The choice of research projects is also crucial. Research on diseases affecting the industry will receive praise albeit sometimes critical whereas research on projects which are not perceived in the industries' interests will be criticised. In this context, drafting i.e. the wording of research projects will be of significant importance.

The team leader and the field officers will be in significant contact with the industry. Their abilities and performance will therefore be of critical importance.

VIII. IMMEDIATE PRIORITIES

At present only an embryo pathology team and mariculture industry exists. Prawn and oyster rearing and to a lesser extent fish culture seem poised for significant commercial development. That development could be hindered, even frustrated by biological problems. The phrase'biological problems' is used because it has been evident from discussions and visits to field stations that the word pathology has a broader meaning to fishery scientists than it may have in a medical or veterinary context. Scientists may consider not only the investigation of frank disease but also of poor growth and survival and the prevention on control of such problems as within the gambit of pathology. This broadened definition of pathology extends the area for study to population performance, the ecosystem, its management and the sequlae which may be significant.

A small pathology team can be expected to investigate morbidity of the cultured host whereas a conclusion on the cause and/or control or prevention of the causes may require investigation of population performance and study of ecosystems, management priorities and rearing methods.

A second conclusion is that at present little is known of the occurrence of causes of mortality and morbidity in the proposed mariculture systems. This should be remedied by engaging in the study of the performance of populations undergoing trials. Such work will give the team unvaluable experience of the commonly occurring diseases and parasites and of the general biological problems faced by the culturist.

Priority should be given to prawn grow-out and to both oyster grow-out and larval oyster rearing in that order.

Schemes for these studies are as follows:

Fish, clam and seaweed culture should receive less attention.

8.1. Prawn grow-out : The following illustrates the role of the pathology team in overall trials to establish field performance data:

i. Aim: To establish a commercially viable methodology for the grow-out of prawns in extensive field culture objectives:

(a) To establish the range of performance of prawns in unmodified pond systems at both east and west coasts stocked at different densities with hatchery reared juveniles by assessing growth and survival.

(b) To study the food composition of prawn stocks and availability of such food organisms in the pond ecosystem.

(c) To study mortality and morbidity in the prawn population.

ii. To experiment by modifying the pond ecosystem to establish if management practices influence prawn performance, e.g.

(a) by fertilizing the pond waters with water soluble inorganic fertilisers.

(b) by fertilising the pond bottom with organic particulate wastes.

(c) by raking/ploughing the pond bottom either before introduction of seed or periodically during the grow-out period.

(d) by chemical treatment e.g. lime chip, clacium carbonate chip or other mineral additions to ponds, perhaps followed by raking.

iii. If food is found to be a significant factor limiting growth it should be established.

(a) if there is a simple procedure for diagnosing an imminent food shortage or loss of prawn condition e.g. length, weight, measurements of the population.

(b) when during the growth cycle it may be most commonly expected?

(c) if a supplementary feeding regime is effective and when should it be implemented?

iv. If an infectious aetiology is a significant factor affecting growth and survival:

(a) determine the nature and if bacterial whether it can be controlled by antibiotic addition to supplementary feeds.

(b) determine if factors such as population density, chemical treatments i.e. lime chip addition influence the occurrence or ploughing or raking the pond bottom influence the occurrence of the disease.

(c) review accumulated data and if necessary by trials establish if fallow periods influence occurrence and severity. Control strategies may be developed from such an approach.

v. If neither food nor an infectious aetiology is indicated then other following possibilities should be examined:

(a) Algal toxin. Correlate the disease onset with the dominant flora in the water and on the pond bottom.

(b) Chemical toxicant e.g. chlorinated hydrocarbons. Analyse prawn tissues for the presence of DDT, dieldrin, PCB's etc.

(c) Mineral or trace element deficiency- Analyse prawn tissues or body fluids for Ca, Mg, Cu, Fe, Mn etc. A mix of minerals could be added to test ponds or supplementary diet to determine their effectiveness in prevention.

i. If no cause is rapidly determined it is probably more important to establish a prevention measure if any is indicated. If this works, it can be used as an interim/longer term solution. For example, supplementary feeding will prevent or minimise the occurrence of soft shell disease if given early enough. The efficacy of such a remedy and the minimum effective dietary additions, if it works, should be established.

Methods: The principal methods involved will be the measurement of the length, weight and determination of sex, estimates of survival rate, examination of gross signs of dead and morbid animals, histopathology of morbid animals and where necessary pathophysiological measurements of haemolymph, identification and/or culture of any pathogens.

8.2. Oyster grow-out: The following illustrates how the pathology team may participate in trials to establish field performance data:

Aim: To recommend grow-out sites for edible and pearl oyster culture and to provide data on probable performance.

Objectives:

(i) To establish the range of values for growth and survival in grow-out populations of both edible and pearl oyster at different sites.

- measure growth parameters and record survival of oysters in different culture systems, densities of stocking, at different sites etc.

- record the number of pests, commensals, fouling organisms and parasites for all populations.

- Sample morbid or otherwise abnormal live oysters for study of gross appearance of organs, preserve tissues for histology and record occurrence of infectious and other disease conditions and also abnormalities. Isolate, culture and identify, if possible, pathogens.

  For serious infectious diseases establish if adjacent wild molluscan populations are reservoirs of infection.

- record main physical parameters of each site, and the presence, abundance and proximity of other populations of plants and animals.

(ii) Comparision of oyster performance, pearl formation etc. with physical and biological factors of different sites to determine if site assessment prior to grow-out trials can be made.

8.3. <u>The oyster hatcheries at Tuticorin:</u> The proposed scheme is designed to show how the pathology team may participate in developing methods for the mass production of oyster spat.

<u>Aim:</u> To develop a methodology for the mass production

of spat of the edible and pearl oyster.

Objectives:

- To produce on a regular basis large numbers of spat of both species for grow-out trials.
- To establish factors important for good growth and survival.
- To record growth and survival in each batch of spat produced.
- To investigate the causes of mortality/ poor growth in spat production.
- To keep, maintain and select brood stocks for spat production.

Methods: Growth and survival of oyster veliger and post veliger populations in many parts of the world has been found to be variable even where hatchery conditions are considered good. Causes of poor survival other than infection have been discussed previously. The diagnosis of these causes based on pathology examinations is difficult at best and often impossible with currently developed techniques and knowledge. It is usual in these cases where infection is considered absent to resort to empirical methods for resolving

the problem e.g. changing one or two parameters at a time to learn if improvements occur. Where infection is proven or suspected first principles suggest the source of infection be sought and then rendered free of infection. Avoidance of future infection is the principle here and this leads to the conclusion that rapid destruction of infected populations is the best course of action unless very effective and cheap control methods are available.

The main sources of infection are water, food and brood stocks. However residual infection on tanks and other hatchery equipment should be considered. It should be possible to produce food free of infection and to either do the same for brood stock or select brood stock free of infection. Hatchery equipment can be disinfected after thorough cleaning with agents such as, 1% sodium hydroxide with 0.1% detergent hypochlorite solutions and where delicate treatment is necessary the use of proprietory iodine preparations such as Vanodine FAM, Pov. Iodine, Buffodine. Providing sea water supplies free of pathogens is a major undertaking and requires careful study of hatchery sites and the developing technologies which caters for this area.

IX. NATIONAL REGISTER OF PATHOLOGY SPECIMENS AND REPORTS

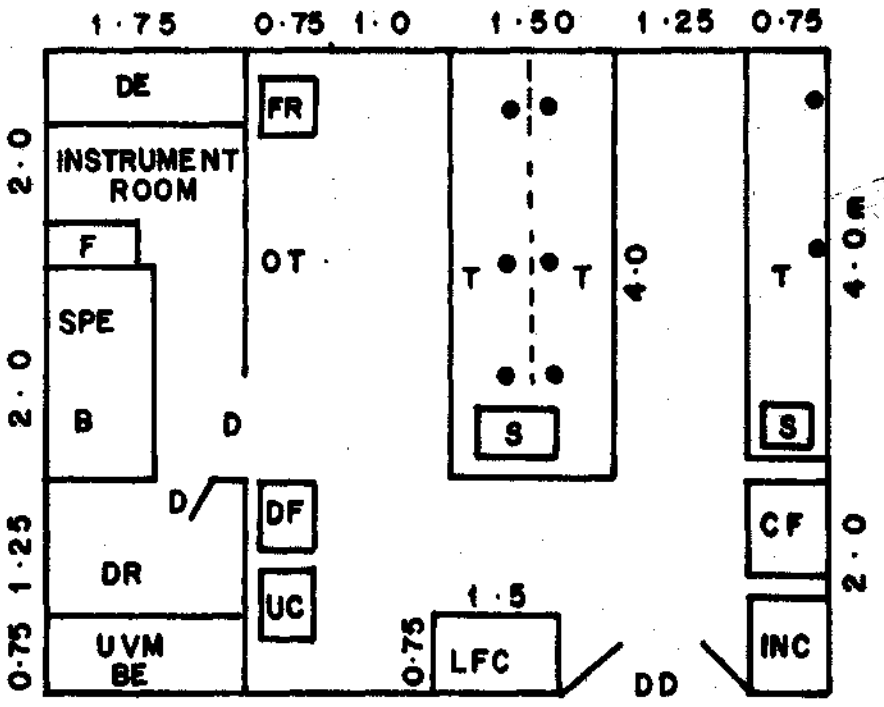
The setting up and maintenance of a register of pathology specimens should be one of the duties of the team. As the CMFRI is the national fisheries research organisation it would be appropriate if the registers were the national one for all marine species, cultured and wild. Indeed it would be best if the register included all aquatic specimens from the Indian subcontinent thus keeping them in one place.

The register would concentrate on storing wax or resin blocks of preserved material and mounted stained sections from them. These specimens would be unequivocal examples of particular conditions. The stored material would be used for teaching purposes and limited supplies made available to teaching institutes, research organisations, both national and international. An informal exchange system between national registers already exists and the Indian Register would be expected to participate in this exchange system.

It is possible the register might act also as a centre for receiving and storing reports on

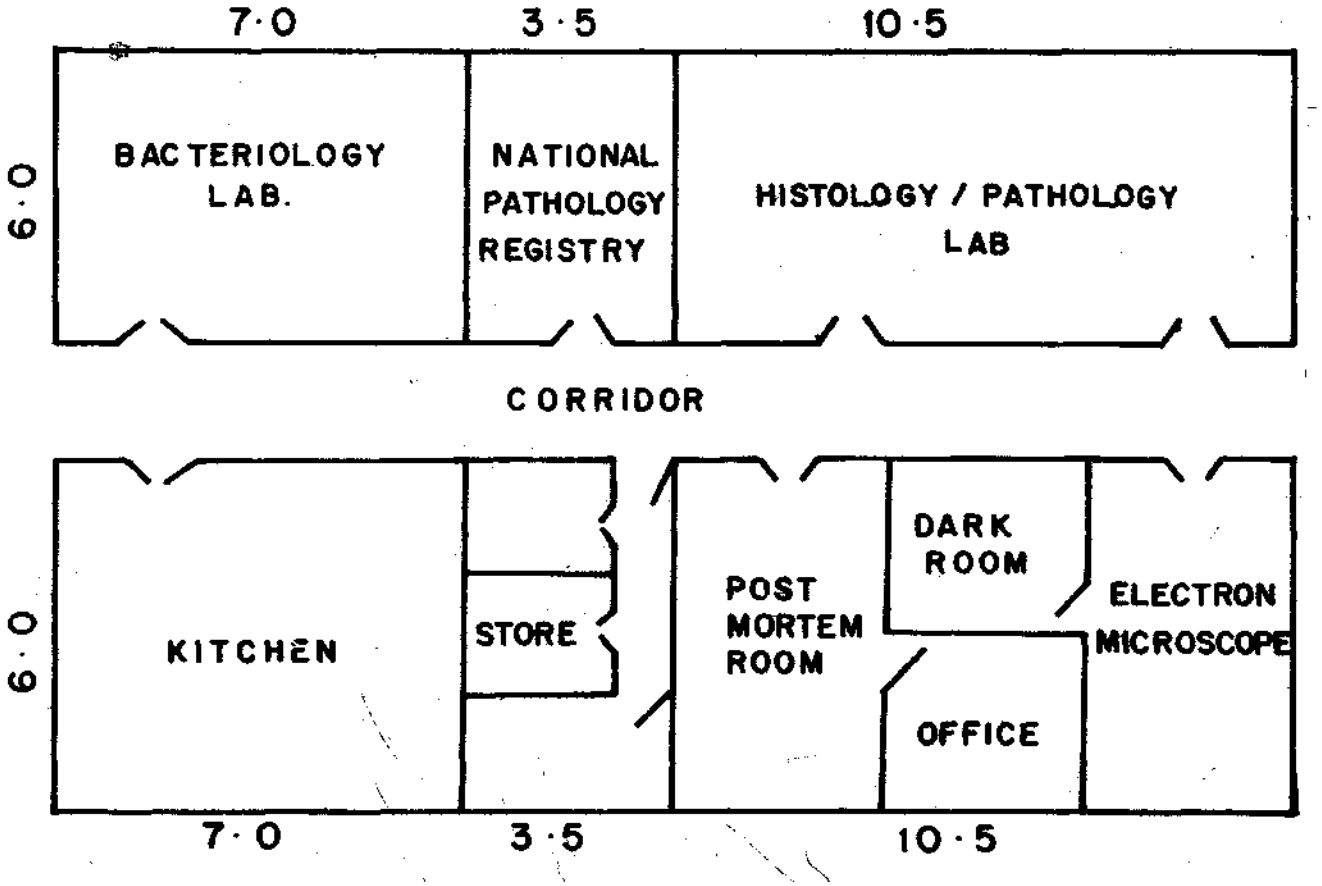
the nature and prevalence of disease and natural mortality in cultured and wild fish and shellfish. Computor facilities are available which could process information concerning a register of both specimens and reports.

BACTERIOLOGY LAB.

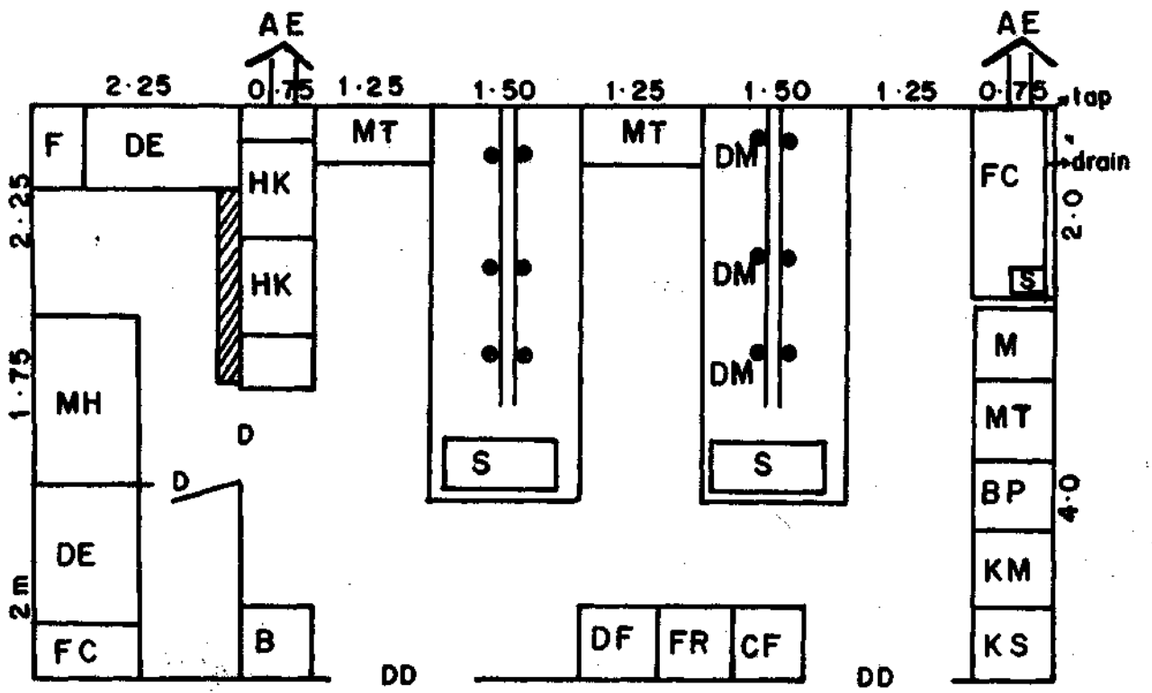


B _ balance
BE _ bench
⊕ _ comp. air
CF _ centrifuge
D _ door
DD _ double door
DE _ desk
DF _ deep freeze
DR _ darkened room
F _ filing cabinet
FR _ fridge
INC _ incubators
LFC _ laminar flow cabinet
OT _ optional table
S _ sink
SPE _ spectrophotometre
T _ table
UC _ ultra centrifuge
UVM _ U.V. microscope

OVERALL PLAN FOR PATHOLOGY LABORATORY



HISTOLOGY AND PARASITOLOGY LAB.

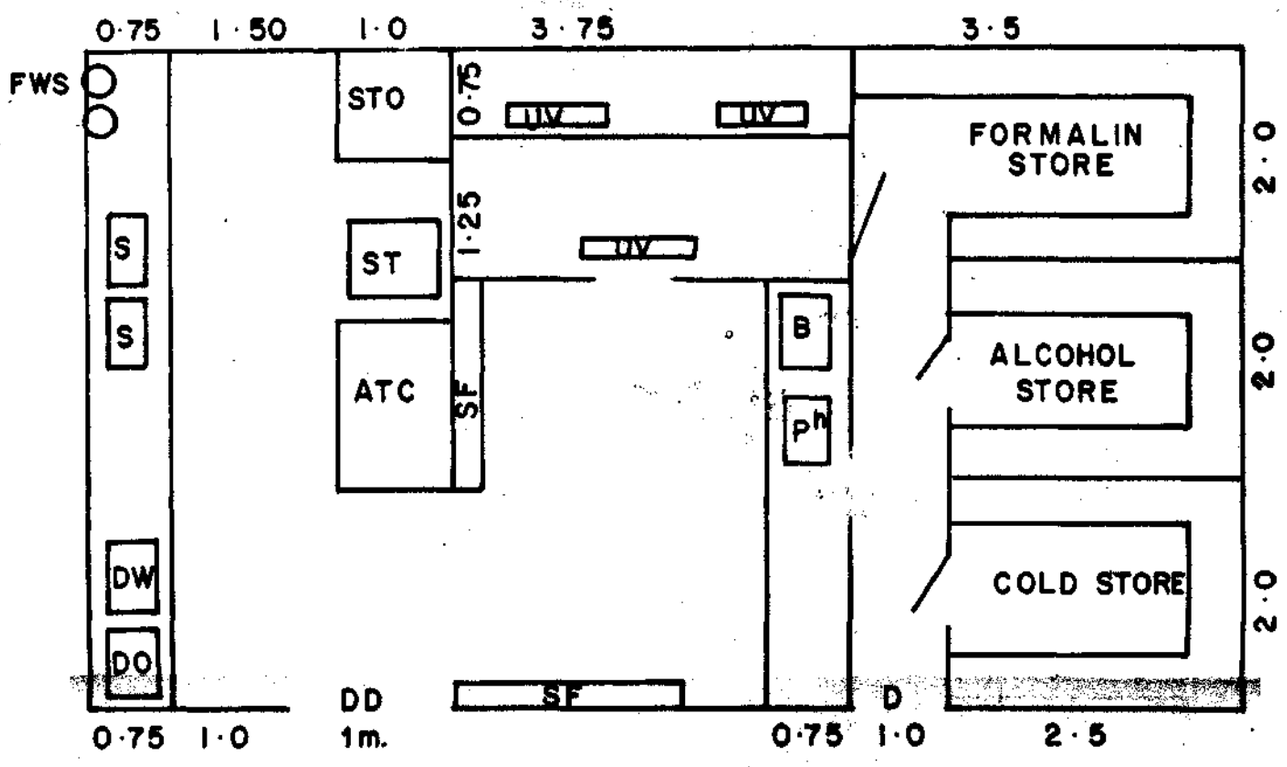


AE _ air extractor
B _ balance
BP _ block preparer
CF _ centrifuge
⊕ _ compressed air
D _ door
DD _ double door
DM _ dissection microscope
DE _ desk
DF _ deep freezer
F _ filing cabinet
FC _ fume cup-board
FR _ fridge
HK _ histokinette
KM _ knife maker
KS _ knife sharpner
M _ microscope
MH _ microtome (hist.path.)
MT _ microtome
S _ sink

E R R A T A

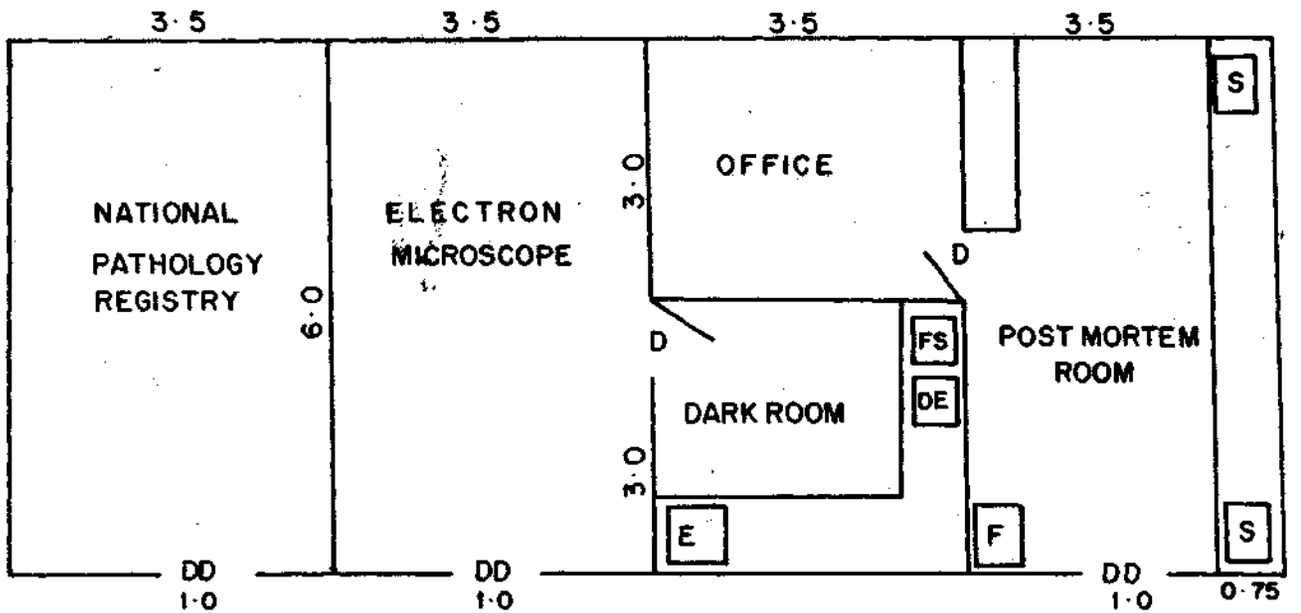
		Read as	Instead of
Preface	4th line	Enterprises	exterprises
Contents	IV	members	member
Page 1	line 10	Sterility	sterlity
2	18	on	on
	19	routed	wanted
	20	the	owe
3	17	state/	state
4	7	(to the relative)	to be deleted
4	12	Officer	Officers
5	22	Proceed?	Proceed
5	8	Investigation	Investigations
5	14	Pathology team	Following team
9	(last but 2 lines)	e.g	e.t
10	8	10-20%	10-20%
10	(last but 3 lines)	Appropriate	Approximate
14	3	in	the
15	3	With	with
15	11	they	it
18	[illegible]	of	to
19	6	to	and
26	3	heat	heats
		Sterilisation	sterilisation
26	5	large	larger
	8	Adjacent	Adjacent
	9	Walk in	Walking
31	5.1(1)	Projects require	Project requires
33	13	and	on
34	13	Scheme for these studies are as follows - to be transposed to after ---- less attention	
34	20	the word objectives should be delected after culture and to be pasifioned before (q)	
35	19	Calcium	Calcium
42	6	Ones	One
Common all figures		The 'Measurements in meter' should be inserted.	

KITCHEN



ATC – autoclave
B – balance
D – door
DD – double door
DO – drying oven
DW – dish washer
FWS – fresh water still
P^h – P^H meter
S – sink
SF – shelf
ST – steamer
STO – sterilising oven
UV – U.V. lamp

ELECTRON MICROSCOPE, POST MORTEM AND NATIONAL PATHOLOGY REGISTRY ROOMS



D_ door DD_ double door DE_ developer E_ enlarger F_ fridge FS_ fixer(sink) S_ sink

Manuals of research methods issued under the Centre of Advanced Studies in Mariculture, Central Marine Fisheries Research Institute, Cochin

1. Manual of Research Methods for Crustacean Biochemistry and Physiology - CMFRI Special Publication No. 7, 1981, 172 pp.

2. Manual of Research Methods for Fish and Shellfish Nutrition - CMFRI Special Publication No. 8, 1982, 125 pp.

3. Manual of Research Methods for Marine Invertebrate Reproduction - CMFRI Special Publication No. 9, 1982, 214 pp.

4. Approaches to Finfish and Shellfish Pathology Investigations - CMFRI Special Publication No. 11, 1983, 43 pp.