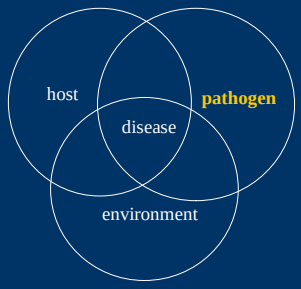
DIAGNOSTIC APPROACH

The OIE Aquatic Animal Health Manual: Disease of Fish: General information and Sampling



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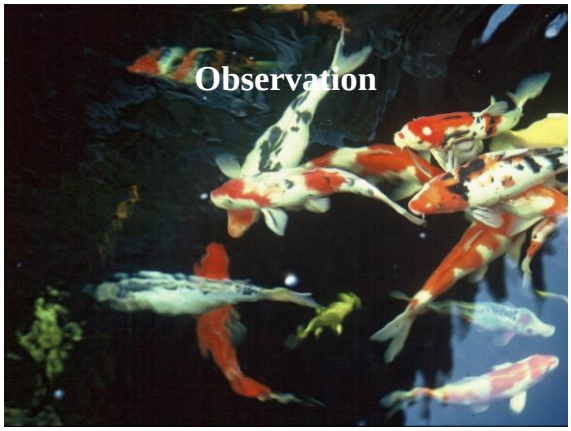
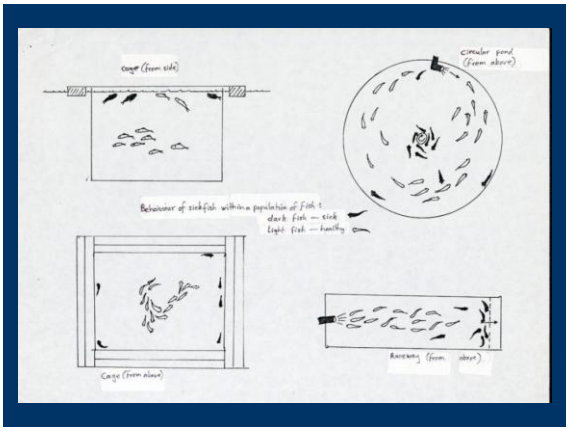
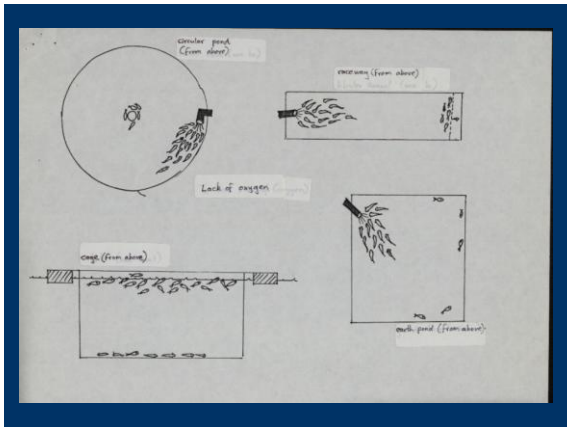
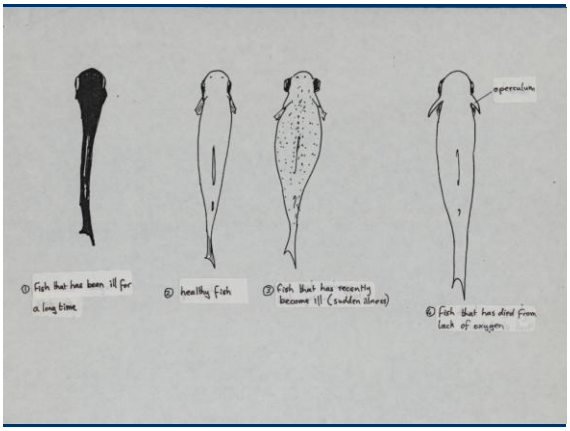
Anamnesis

- Size and type of pond and system
- Is it well established
- Frequency of water replacement
- Source of water and water temperature
- Visible water quality
- Mortality pattern
- Recent introductions
- Behavioural changes etc.



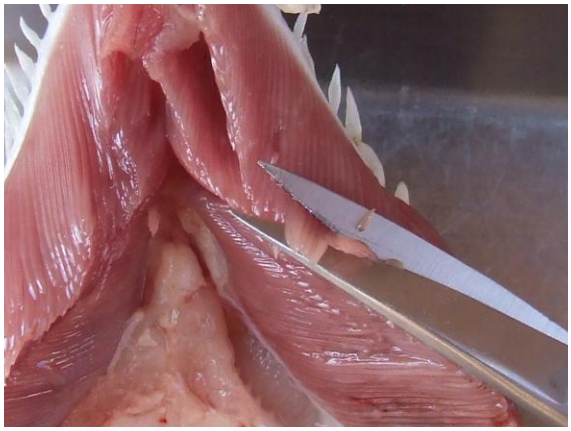


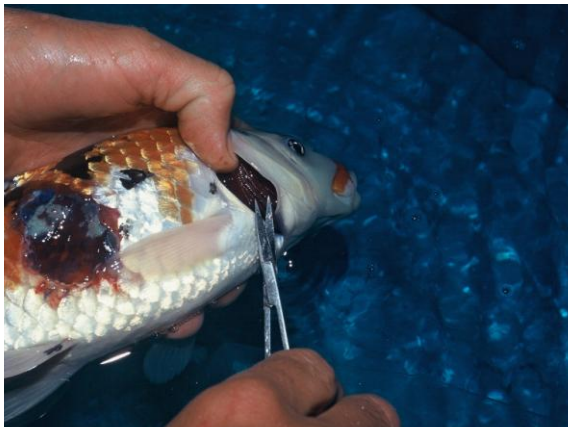


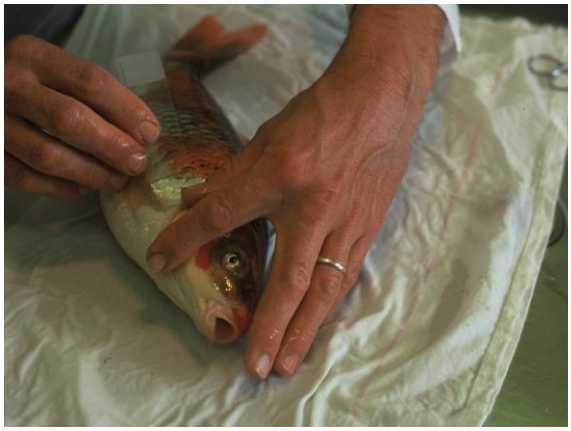


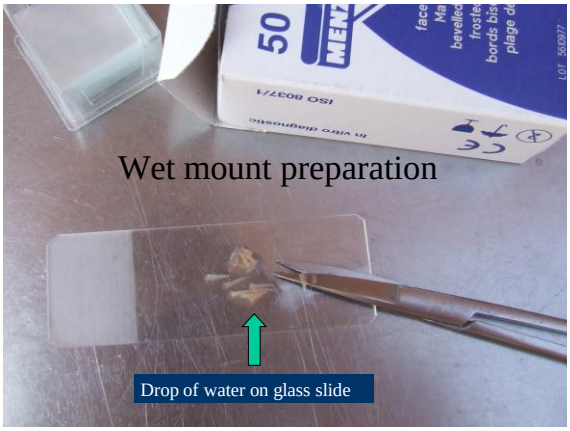
Examination of fish material

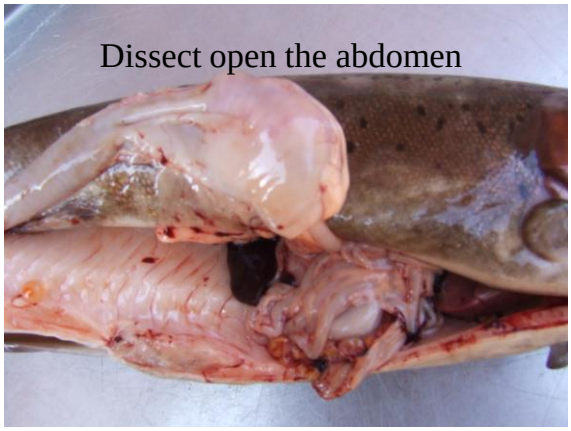
- Fish tissues rapidly autolyse after death.
- Only on freshly dead or sacrificed fish
- Freezing and thawing renders fish useless for bacteriological and histological examination.
- Frozen material may be suitable for virological and toxicological examination.





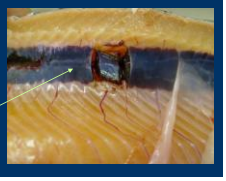






Tissue sampling

- Transport medium swab from liver or kidney for bacteriology
- Small tissue samples from organs in 10 % buffered formalin for histology
- Small samples from organs in PBS for virology
- Swab from gills or kidneys for PCR

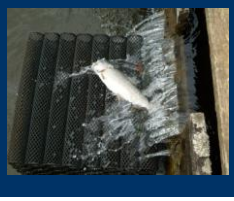


Toxicological sampling

- Tissue – Muscle, fat, kidney and liver. Minimum of 200 gram fresh tissue, preferably not less than 25 gram from each organ. Keep frozen.

Water quality examination

- Temperature
- pH
- Dissolved oxygen (DO)
- Ammonia
- Hardness
- Alkalinity
- Nitrite



Water testing equipment

- Simple calorimetric test kits (e.g. HACH test kit)
- Hand held electronic meters – pH, temperature and DO – regular calibration
- Specialized testing equipment for dissolved gas pressures – gasometer or tensionometer (expensive)

Feed analysis

- Observation – lumps indicating mould growth - aflatoxin
- If suspect dietary imbalance:
  - sample in presence of commissioner of oaths. Record batch numbers
  - take duplicate sample
  - deposit one sample with local police

Assessing the health of the epidemiological unit

- Sampling material depends on:
- The pathogen
- Size of the animal
- Objective of testing:
  - diagnosis of overt disease,
  - detection of subclinical pathogen carriers or
  - targeted surveillance to demonstrate freedom from a specific pathogen

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For listed viral diseases

- Alevin and yolksac fry: entire fish excluding the yolksac
- Fish 4 – 6 cm: entire viscera including kidney plus encephalon
- Fish over 6 cm: kidney, spleen, heart and encephalon or as per specific pathogen
- Adult fish: ovarian fluid and/or tissues appropriate for the specific pathogen

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Other listed diseases

- EUS – any size fish: muscle tissue, kidney, liver, spleen
- *Gyrodactylus salaris*: skin and fins
- KHV: fish 4 cm to adult: gills, kidney, spleen, encephalon, gut

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Processing of samples

- Macroscopic examination: EUS and *G. salaris* – followed up with histological examination for EUS and wet prep examination for *G. salaris*
- Virological sampling: fresh on ice in sterile containers – must reach laboratory for virus extraction within 48 hours or in suitable transport media (cell culture medium, Henks balanced salt solution or phosphate buffered saline) with added antibiotics. One volume organ in at least five volumes transportation fluid.

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For detecting subclinical carriers

- Organ samples can be combined as pools of up to 10 fish per one sample pool.
- In the case of brood fish, ovarian fluid from no more than 5 fish can be pooled. Each sample should not exceed 1 ml of ovarian fluid from each fish giving a total of 5 ml.

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Fish cell lines for virus isolation

- Epithelioma papulosum cyprini (EPC)
- Bluegill fry (BF-2)
- Fathead minnow (FHM)
- Rainbow trout gonad (RTG-2)
- Chinook salmon embryo (CHSE-214)
- Salmon head kidney (SHK1)
- Atlantic salmon kidney (ASK)
- Atlantic salmon (TO)
- Grunt fin (GF)
- Koi fin (KF-1)
- Cyprinus carpio brain (CCB)

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Virus nomenclature

- Epizootic haemotopoietic necrosis virus (EHNV)
- Infectious haematopoietic necrosis virus (IHNV)
- Infectious salmon anemia virus (ISAV)
- Koi herpesvirus (KHV)
- Red sea bream iridovirus (RSIV)
- Spring viraemia of carp virus (SVC)
- Viral haemorrhagic septicaemia virus (VHSV)

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For OIE listed diseases

- Macroscopic examination
- Virological examination
- Parasitic examination
- Fungal examination

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Laboratory diagnostic techniques

- Macroscopic examination
- Histopathology - haematoxylin eosin (routine) and Grocott- Gomori (specific for EUS)
- Oomycete culture
- Virus isolation
- Serology – immunofluorescence and enzyme linked immunosorbent assay
- Molecular techniques – polymerase chain reaction (PCR) – highly sensitive but prone to laboratory contamination and yielding false positives.
- (Electron microscopy)

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Preservation of specimens for PCR

- Use new plastic sample bags or bottles
- Freshly collected live specimens packed on ice must reach the laboratory within 24 hours
- Frozen whole specimens selected from live specimens. Use dry ice to quick freeze to –20ºC or lower
- Alcohol preserve in 90 to 95 % ethanol. Ship to laboratory in alcohol.

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Sample information

- Use water and alcohol resistant labelling (pencil rather than ball point pen)
- Geographical origin and coordinates or location along water course
- Collectors name
- Organisation
- Date and time
- Water body
- Description of location
- Species sampled e.g. Nile tilapia
- Type of specimen e.g. liver, spleen, kidney, etc.
- Preservation method e.g. 10 % formalin