Tasmanian Government

Field Sampling of Fish for Disease Investigation and Health Monitoring

Guidelines and Procedures Manual

Centre for Aquatic Animal Health & Vaccines
Department of Natural Resources and Environment Tasmania

May 2022

Tests and services available from the Centre for Aquatic Animal Health & Vaccines (CAAHV)

Pathology
Post mortem
Histopathology
Interpretation of results
Disease diagnosis

Parasitology
Parasite detection and identification

Virology
Culture and identification of Tasmanian salmonid viruses
Culture and identification of exotic or emerging salmonid viruses (Australian Centre for Disease Preparedness, CSIRO)

Clinical chemistry
Measurement of:
- Serum ions: Na, K, Cl, Mg, Ca
- Serum protein

Other analyses on request

Haematology
Measurement of haemoglobin and packed cell volume (PCV)

Microbiology
Isolation and identification of endemic bacterial pathogens of salmonids
Microscopy of tissues
Antibiotic sensitivity measurements
Detection of covert infections in fish for: *Aeromonas salmonicida, Yersinia ruckeri, Lactococcus garvieae, Tenacibaculum maritimum*
Supply of culture kits to farms for routine testing of fish

Molecular biology
Detection of specific aquatic animal pathogens in fish:
- Nodavirus
- Pilchard orthomyxovirus
- Tasmanian Atlantic salmon reovirus (TSRV-1 and TSRV-2)
- Tasmanian *Rickettsia*-like organism

Analysis of gene sequences for pathogen identification

Export certification of stock

Veterinary advice on aquatic animal diseases diagnosis

The results are only as good as the sample collected

Sampling

Why collect samples?

Laboratory testing of fish for the investigation of:

- sudden deaths
- signs of disease
- increased mortalities
- disease surveillance
- disease investigation

What tests can be done?

- Culture of bacteria
- Culture of viruses
- Molecular biology tests to detect fish pathogens
- Examination of fish tissues for pathological changes
- Blood chemistries

What types of fish should be sampled?

- Fish that are alive and with signs of disease
- Choose fish that have examples of key lesions or signs of disease

- Don't sample morts; these will be too decomposed for laboratory testing

How many fish should be sampled?

- At least five fish with signs of disease from each affected tank or cage and, where possible, a separate submission form for each tank or cage,

Sample planning

Planning

- The best samples are collected at a well prepared work station on-shore
- Collecting and preparing samples on a boat is difficult and should be avoided
- Give careful consideration to biosecurity. Will processing on a boat risk spreading disease through the lease? Do you have a disinfection plan for the work area on the boat?

Collecting fish for testing

- Ideally, collect fish from cages and place them in labelled plastic bags and place in bins with crushed ice
- Bring the fish back to shore within 3-4 hours of collection
- Keep the time between collection and sampling as short as practicable

Work area for sample collection

- The preferred area for sample collection is somewhere dedicated for the purpose
- Do you have a laboratory on-site? If not, set aside an area indoors that can be used for sample collection. Make sure you have good light
- Avoid sampling either outdoors or on board an open boat, particularly in summer. Wind, rain, spray or heat can affect samples badly, particularly those for bacteriology and virology
- Can you disinfect the work area? Do you have a biosecurity plan?
- Consider how you will dispose of waste, particularly carcases and blood

Storage of sample materials

- If using culture plates, check to see that they are in-date
- Have the culture plates been stored correctly?
    - Plates must be stored inverted
    - Keep plates away from cooling elements in the fridge, otherwise they will 'sweat' and can become contaminated
    - Store plates separately to any formalin pots
- Have tubes of transport medium been stored correctly? Are they in-date?

Instruments

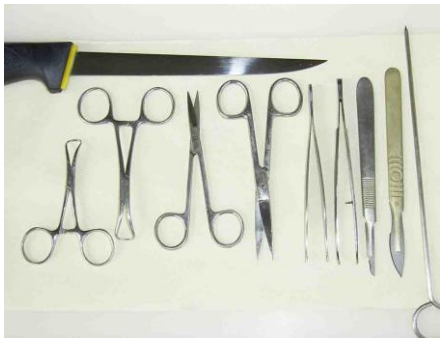
- Keep dissection instruments clean; use only for dissection
- Make sure you have duplicates of instruments
- Buy good quality instruments
- Look after them!

Preparing to collect samples

- Think ahead: prepare your work area; be organised

- Will you be working alone or can someone assist you?
  Two people working on sample collection is ideal

- Have everything to hand:
  Dissection equipment: scalpel, forceps, scissors
  Paper towel
  Scrubbed clean cutting board
  Fish kit with culture plates, loops and slides
  Tubes of sterile viral transport medium
  Formalin fixative in sample pots
  Dissecting cradle for larger fish (see Appendix for details)
  Instrument holder with 100% alcohol (see Appendix for details)
  Flame source
  Alternatively, use isopropyl alcohol impregnated wipes for instrument disinfection



Pathology

Sample quality

- Moribund fish are the preferred sample
- Where possible sample at least 5 fish
- Maintain minimum tissue: fixative ratio of 1:10
- Standard sample sites:
  Internal – heart, liver, anterior and posterior kidney, brain, spleen, pyloric caecae, hind gut
  External – skin lesions, skin across the lateral line, gills, eye

Precautions

- Quality of sample affects quality of result
- Do not use morts as post-mortem tissue changes prevent diagnosis
- Do not use scissors to cut tissues when collecting samples
- Do not over fill sample containers with tissues
- Ensure that tissues are well immersed in fixative
- Take care when using buffered formalin; the fixative is toxic by inhalation, ingestion and skin contact

Examination

- Examine fish carefully and record any obvious abnormalities

Dissection

Small fish

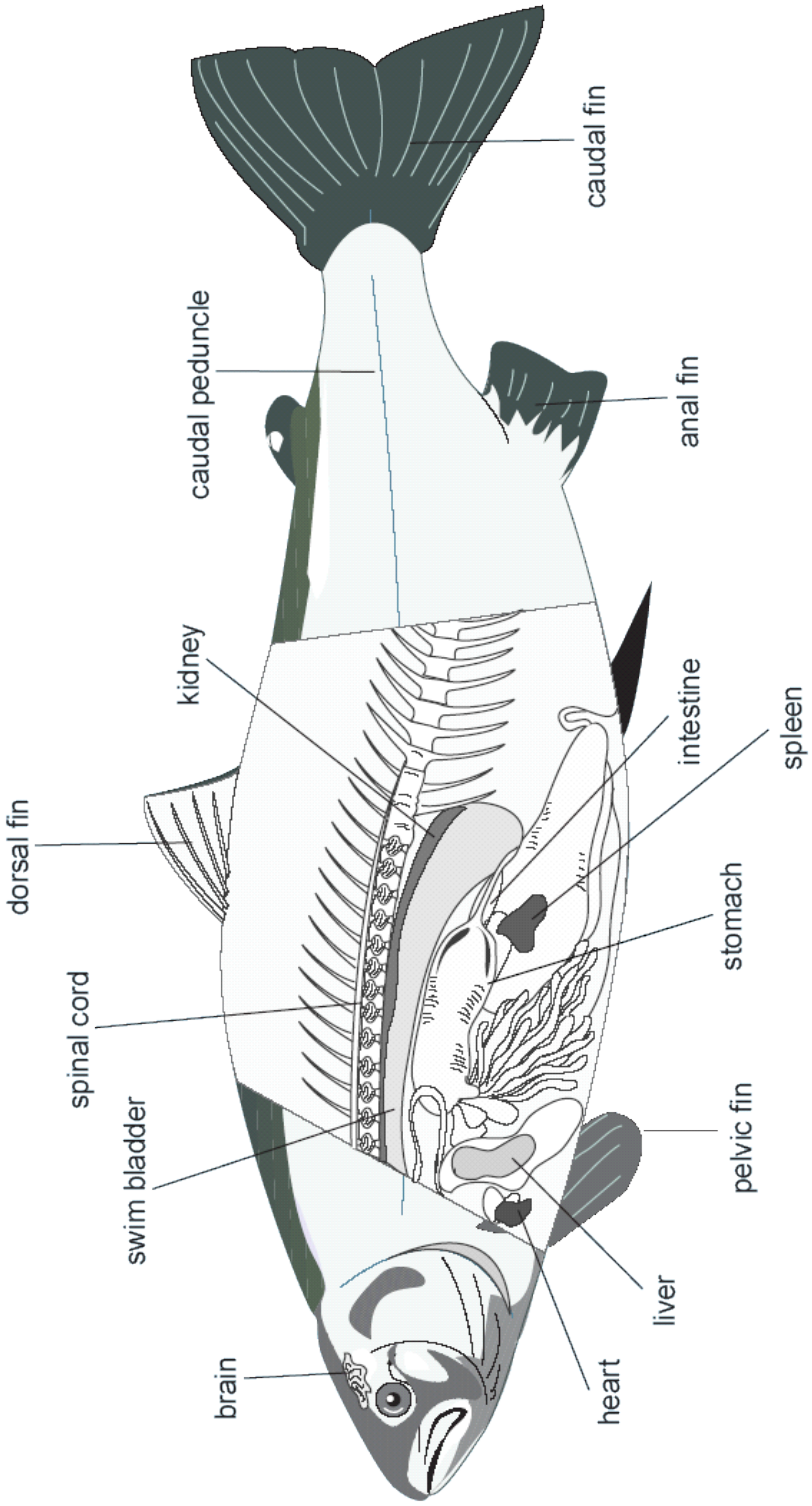
- Fish less than 3cm in length can be placed directly in fixative
- For fish between 4-10cm, make a slit along the abdomen, remove the opercula and place directly in fixative

Large fish

- Cut out several gill arches and place in fixative.
- Carefully open the fish
- Record any internal gross abnormalities (see drawing of fish)
- Excise the tissues as listed; each piece should not exceed 1cm cubed
- **Do not** crush tissues with forceps or scissors
- Place all tissues in the one sample container, maintaining a sufficient volume of fixative

Submission

- Label the samples clearly with fish number, date and serial number
- Check that sample container lids are secure and seal rim with paraffin tape.
- Pack **separately** from culture plates and surround with absorbent material
- Keep samples cool; refrigeration is not required
- Send to the CAAHV as soon as possible



Bondad-Reantaso, M.G., McGladdery, S.E., East, I., and Subasinghe, R.P. (eds.) (2001) Asia Diagnostic Guide to Aquatic Animal Diseases. FAO Fisheries Technical Paper No. 402, Supplement 2. Rome, page 48

Blood

Precautions

- Use moribund live or healthy live fish for collecting blood samples
- Dispose of blood collecting needles carefully; use puncture proof containers
- Do not submit blood samples in syringes with needles attached
- Use blood collection tubes as recommended

Blood collection tubes

- EDTA for haematology
- Plain blood tubes (no coagulant) for biochemistry

Syringe and needle

- Use a 26g needle and a 3 or 5ml syringe for general blood sampling

Procedure

- Insert needle at a 45 degree angle (towards the head) on the underside of the caudal peduncle until the vertebra is reached
- Pull back the needle slightly and withdraw plunger gently until blood begins to flow.
- Remove needle from syringe and gently expel blood into blood tube.
- If required for haematology, make a blood smear on a glass slide by placing a drop of blood at one end of a slide and making a film by drawing the blood across the slide with a second angled slide

Submission

- Label the samples clearly with fish number, date and serial number
- Check that sample container lids are secure
- Place in an Esky with ice bricks and send to the CAAHV immediately

Bacteriology

Sample quality

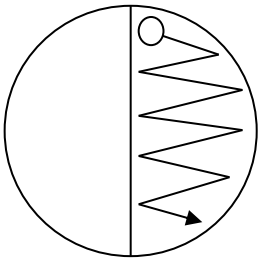
- Moribund fish are the preferred sample
- Where possible sample at least 5 fish
- Standard sample sites:
  - Internal – kidney, brain, eye
  - External – skin lesions, gills

Precautions

- Quality of sample affects quality of result
- Use aseptic procedures for collecting samples
- Do not leave plates uncovered or exposed to the sun
- Check media expiry date & condition of media
- Have the media been stored refrigerated?
- ✘ **Do not use media if expired, contaminated or has been frozen**

Culture

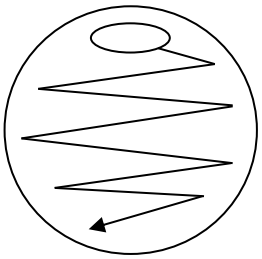
- For more information on sampling, see sample guidelines in fish kits
- Use appropriate media for sample site (see picture guide)
- ! It is important to streak the plates properly



Simple streak pattern for half-plates

✓ Marine farms
  BA/TCBS[1] for **internal** sites: kidney, brain, gut, eye
  BA2%/SS **external** sites: skin and gill lesions

✓ Freshwater hatcheries
  BA for **internal** sites: kidney, brain, eye
  BA and FO **external** sites: skin and gill lesions



Simple streak pattern for whole plates

- ! **Make a smear for microscopy from each culture site**

Labelling

- Label **all** smears & plates with **date**, **fish number**, **sample site** & **serial number**
- Use supplied labels or **permanent** marker for plates
- Label plates on the agar side; not the lid
- Label slides with a **pencil**

Returning to the CAAHV

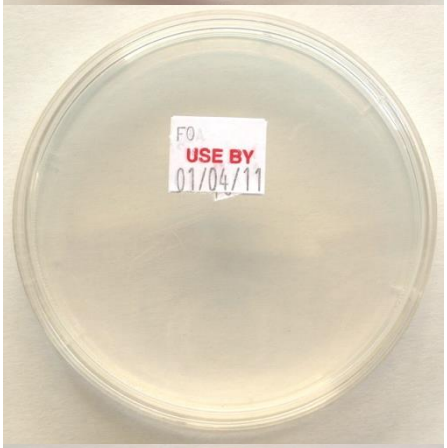
- Send back immediately
- If delayed, store plates in fridge (Summer) or at room temperature (Winter)

[1] BA: blood agar; TCBS: thiosulphate citrate bile sucrose agar for Vibrios; BA2% blood agar with additional salt; SS: selective Shieh's medium for marine flavobacteria; FO: freshwater Ordal's medium for freshwater flavobacteria

Culture plates

Name: BA/TCBS
Use: Marine fish
Samples: internal sites
kidney, brain, eye

Name: BA2%/SS
Use: Marine fish
Samples: external sites
skin lesions, gills



Name: FO
Use: Freshwater fish
Samples: external sites
skin lesion, gills

Name: BA
Use: Freshwater fish
Samples: internal sites
kidney, brain, eye

Virology

Sample planning

- Viruses are grown on fish cell lines, which must be prepared at least 7 days before inoculation
- Book-in your samples by contacting the CAAHV Virologist and discuss the best type of samples to be collected: whole fish (preferred) or tissues collected in the field
- Even if cell lines are not immediately ready for inoculation, submit samples anyway and they will be held by CAAHV until the cell lines are ready

Precautions

- Great care is required if samples are to be collected for virus isolation in the field
- If samples are contaminated virus isolation is unlikely to be achieved
- Use **strict** aseptic procedures for collecting samples
- Do not attempt sample collection on board a boat, use a dedicated field laboratory or station
- Keep samples chilled at all time
- Do not expose samples to direct sunlight

Sample quality

- Moribund fish are the preferred sample
- Do not use morts
- Where possible sample at least 5 fish

Sampling

- The preferred type of sample is whole fish on ice. CAAHV staff will collect the samples at the laboratory
- If you are confident and have access suitable facilities, samples can be collected in the field. This may be required if sampling large fish
- Use paired sets of sterile instruments to dissect fish; one set for exposing the abdominal cavity, the other for collecting tissue samples
- Disinfect and clean instruments frequently using iso-propyl alcohol wipes
- For routine testing, collect samples of liver, kidney and spleen
- Tissue size should be around 5-10 mm^3, about the size of a pea
- Place each piece of tissue in a tube of sterile viral transport medium, supplied by CAAHV at the time samples are booked-in
- Do not pool tissues from several fish in the one tube
- Clearly label the tube with the date of collection and a fish identification number

Submission

- Keep the sample tubes of viral transport medium refrigerated at all times
- Pack them immediately in an Esky with enough ice bricks to the keep the samples at 2-4°C
- If sending whole fish, pack them well with plenty of crushed ice
- Send samples immediately to the CAAHV
- For further information on sampling for virology, see Lab Facts at http://nre.tas.gov.au/AHLabFacts

Molecular Biology

Precautions

- Use aseptic procedures for collecting samples
- Keep collected samples cool at all times to conserve nucleic acids
- Use the correct medium for the samples (see below)
- Make sure all sample containers are closed securely
- **Do not pool samples from several fish in the one container**
- **Do not store samples for later submission: send immediately**

Sampling for Tasmanian *Rickettsia*-like organism (RLO), Tasmanian Atlantic salmon reovirus (TSRV) and pilchard orthomyxovirus (POMV)

- Samples from one fish for each specimen container.
- Aseptically open the fish.
- Collect from each fish: liver, kidney and spleen (tissues should be <5mm in size) into RNAlater[2].
- Clean and disinfect the dissecting instruments between fish to prevent sample carry-over. (Use alcohol impregnated wipes, or if safe and practical, dip instruments in ethanol and ignite over a flame.)
- Label sample containers clearly with tank/cage number, date of sampling and farm name.
- Place in an Esky with ice bricks and send samples **immediately** to the CAAHV.
- For further information on sampling for molecular tests see LabFacts at http://nre.tas.gov.au/AHLabFacts

[2]Available from the CAAHV – stable for 1 year refrigerated or at room temperature.

Submission

Labelling

- Ensure that all samples are well labelled with information such as fish no., sample site, serial no. & date

Accession form

- Fill out as many details as possible
- Include the Property Identification Code (PIC)
- Include information on disease history, population and observations
- Note samples submitted and tests required
- A separate submission form for each tank or cage is preferred

Transport

- To limit sample degradation, pack samples in a cool box with ice bricks.
- Address: Post PO Box 46, Kings Meadows, Tasmania 7249

  Courier CAAHV, Animal Health Laboratory
  165 Westbury Rd, Prospect, Tasmania 7250
- Send to the laboratory with 24 hours of sampling

Turnaround times (guide)

- Microbiology: 4-7 working days depending on complexity of case and the types of microorganisms in culture. Some bacteria may require at least two weeks of incubation
- Virology: Culture incubation time is at least three weeks for fish viruses; reports will be issued within 3-4 weeks of submission
- Molecular Biology: 4-5 working days
- Pathology: Necropsy reports will be sent the same day if samples arrive before 3pm and histopathology reports provided within 5 days

Contact

If you require information on sampling or submitted cases, or need more fish kits, please email us specimenreception@nre.tas.gov.au or call specimen reception on 03 6777 2111 and you will be transferred to the relevant staff member.

Appendix

Where possible, using some additional equipment will make sampling of fish easier and can give improved results.

For larger fish, a cradle as shown, simplifies opening up the fish to collect samples. If a cradle is not available, it is still possible to open up the fish cleanly; the simplest approach is to remove the abdominal flap on the left side of the fish to expose the viscera.

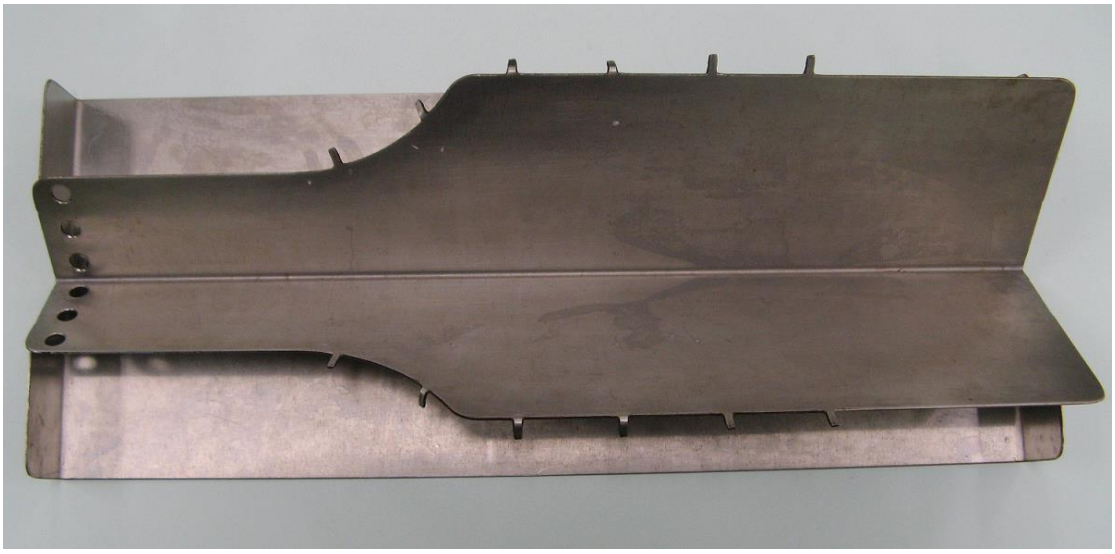


Fig. 1 Stainless steel cradle for holding large fish for dissection

Ideally, the best practice for collecting samples aseptically is to use sterile instruments. Cleaning instruments with isopropyl alcohol impregnated wipes (IsoWipes or similar) helps with disinfection, but the best approach is to dip the instruments in ethanol and ignite the residue in a naked flame. Instrument holders made from stainless steel should be used for this purpose; for safety, a cover for the instrument holder should be close by in case the ethanol is accidentally set alight.

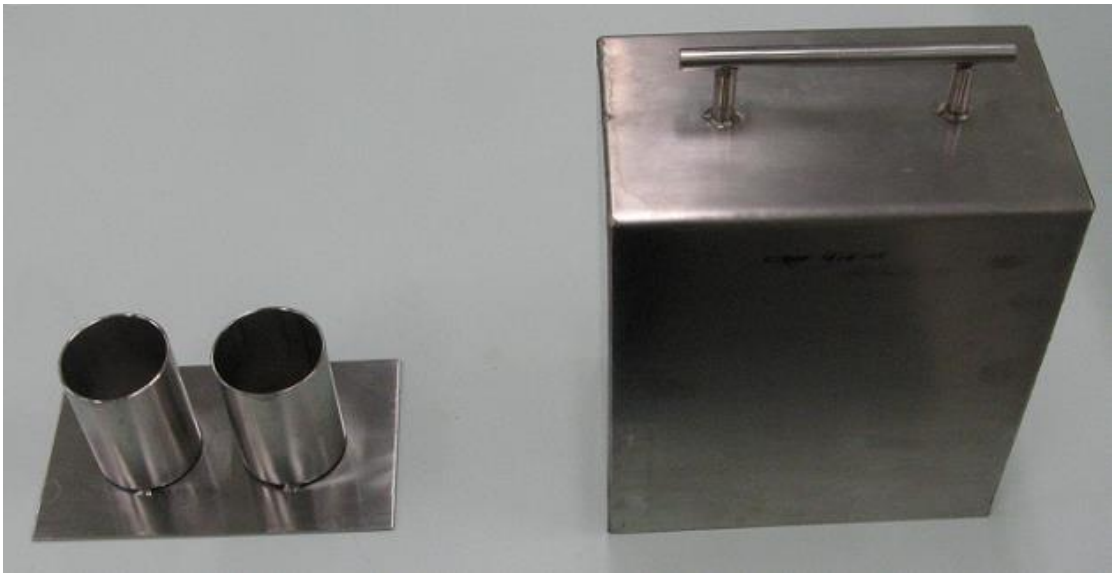


Fig. 2 Holder for flaming instruments and safety cover