

TiLV Diagnostics: Fish Necropsy and Clinical Signs (Level 1)

Ha Thanh Dong^{1,2}

¹Faculty of Science and Technology, Suan Sunandha Rajabhat University

²Fish Health Platform, Centex Shrimp (Mahidol University/BIOTEC), Thailand

Checklist

- tray
- necropsy kit
 - scissors
 - forceps
 - necropsy knife
- tissue paper
- ruler, scale
- camera/mobile phone
- clove oil
- alcohol 70%
- NBF or Bouin's (histology)
- alcohol 95% or Trizol or RNA later (PCR)
- Lab gown, glove, mask
- Microtubes
- Syringe, needle
- falcon 15 mL, 50 mL or plastic bottles
- bacterial loop
- media for bacterial culture
- fame
- disposable plastic bags
- permanent label maker
- sticker and pencil
- zip bags (to keep at -80)

Checklist



Necropsy Data Sheet

Date:

Case No.:

Name:

Phone:

Address:

History

Species affected

Species in the system

Average size

Age of affected fish

No. of fish in the system

Estimate mortality (%)

When mortality started/ended

Any new introduction? If yes, when
and what?

Behavioral changes

Appearance of fish

Appetite

Others

Necropsy Data Sheet (Cont.)

Environmental parameters

DO.....	mg/L	Nitrite.....	mg/L
T°.....		Nitrate.....	mg/L
pH.....		Salinity.....	ppt
Ammonia (total ammonia nitrogen).....	mg/L	Chloride.....	mg/L
Hardness.....	mg/L		

Physical Examination

Behavior

Appearance

Skin

Gills

Taking photo/video

Necropsy Data Sheet (Cont.)

Necropsy

Visceral cavity

Liver

Gall bladder

Stomach

Intestine

Spleen

Kidney

Heart

Brain

Others

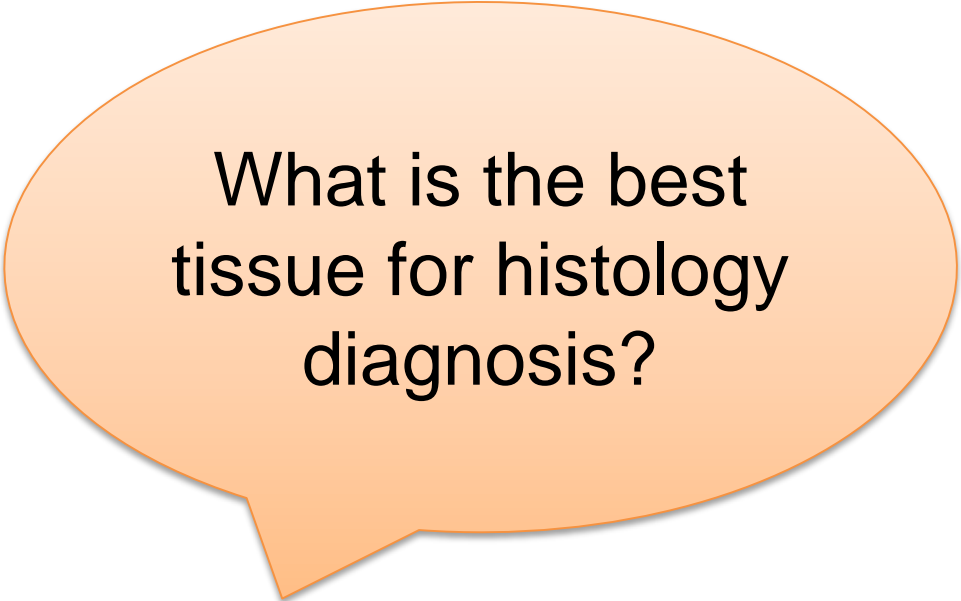
Taking photo/video

What need to be recorded?

- General information of fish farm
- Fish species, sizes, source
- Environmental parameters
- Disease history
- Clinical signs (both external and internal), abnormal behaviors (plus pictures, video)
- Mortality rate
- Others

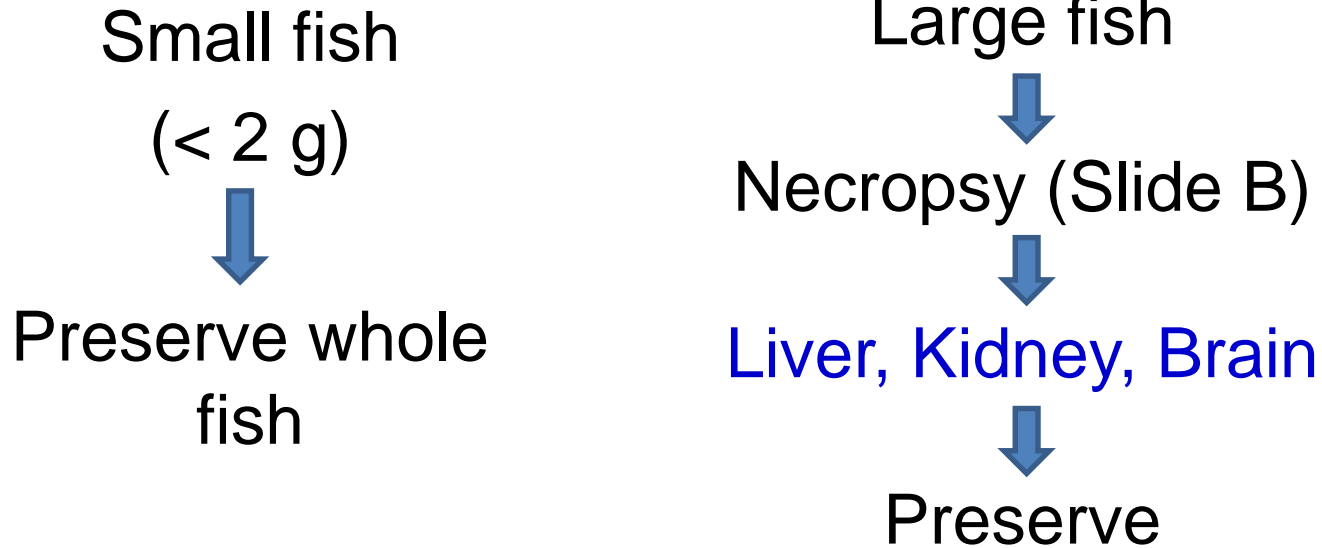
What are target tissues of TiLV?

- Liver
- Kidney
- Spleen
- Brain
- Blood
- Gills
- Mucus
- Muscle
- Heart
- Gut



What is the best tissue for histology diagnosis?

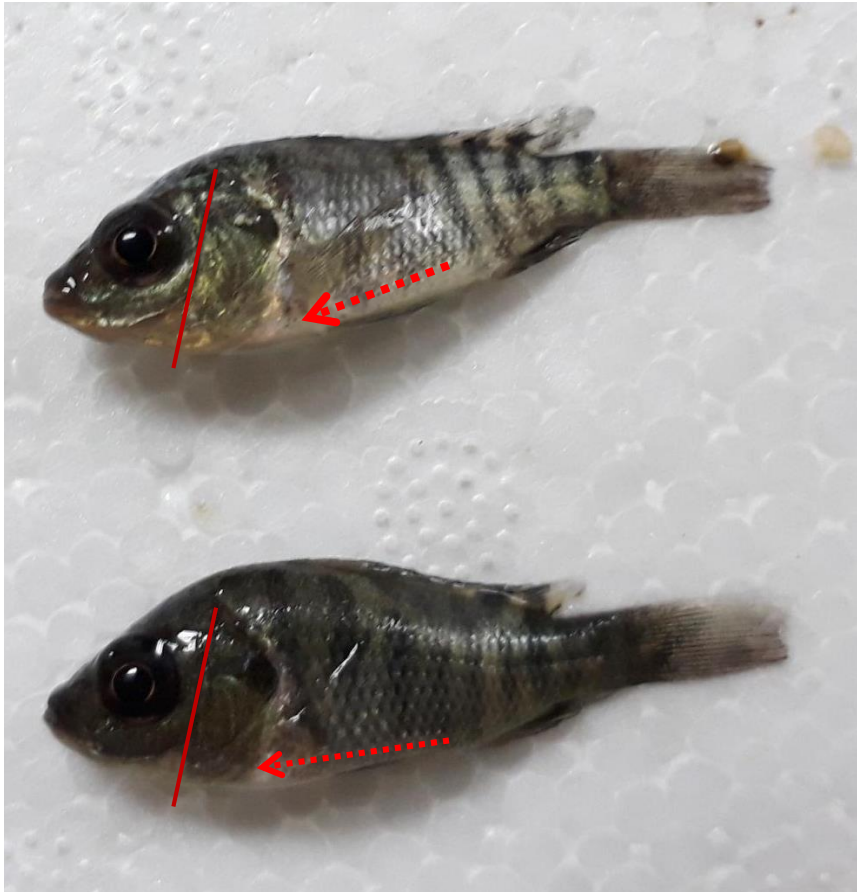
Sampling for TiLV diagnosis



Note: Liver, kidney and brain are recommended organs for TiLV diagnosis. However, other organs such as gills, spleen, muscle, mucus, etc. may be possible for diagnosis as well

Fish Necropsy

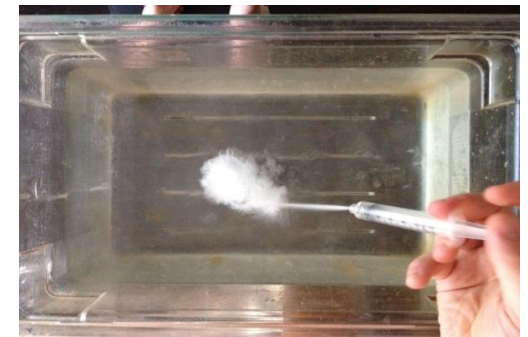
Small fish < 2 g



- ✓ Remove gill opercula
- ✓ Open fish belly
- ✓ Pull out internal organs

Fish Necropsy

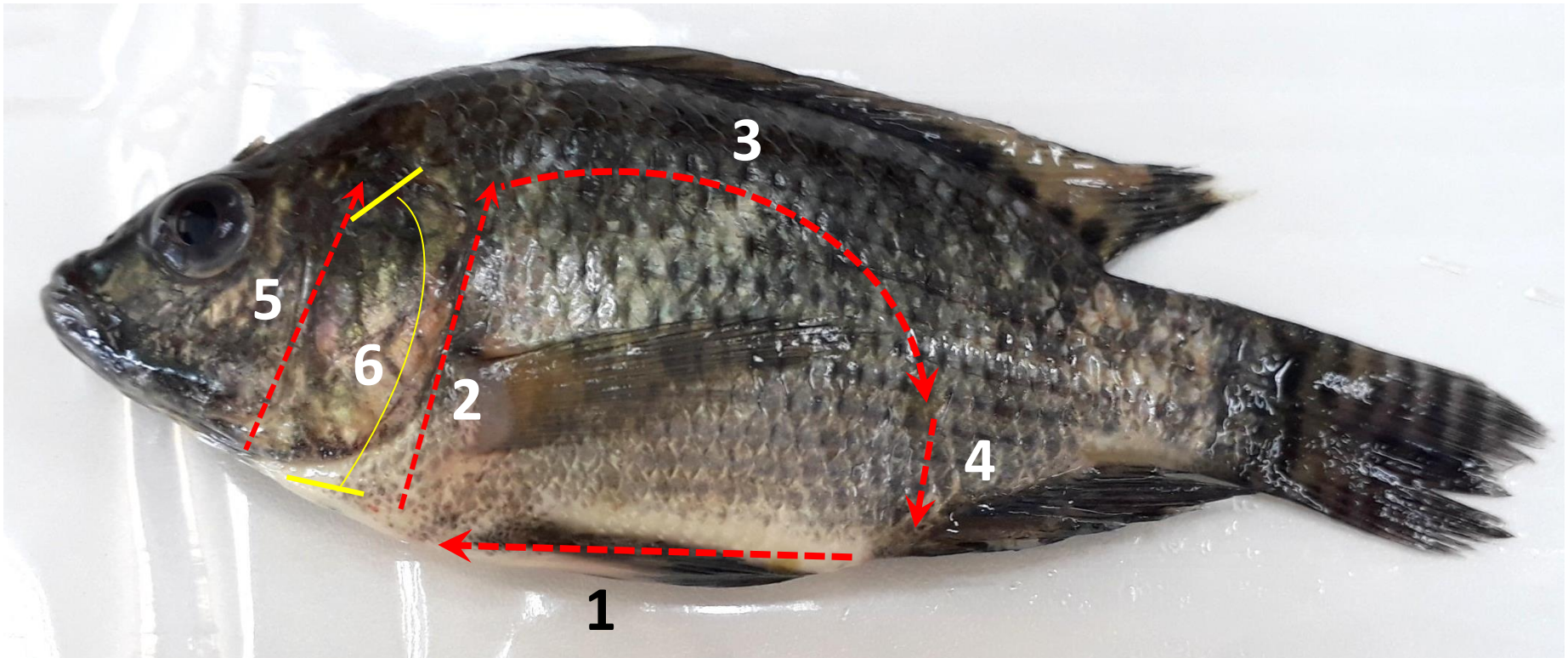
1. Terminate the fish by an overdose of clove oil (≥ 100 ppm) or ice
2. Disinfect the fish body surface with alcohol 70%
3. Dissect the fish (see picture below)
4. Collect target tissues for different purpose (histology, molecular analysis, TEM or virus isolation)



add clove oil into water

Fish Necropsy

Large fish



How to dissect a fish?

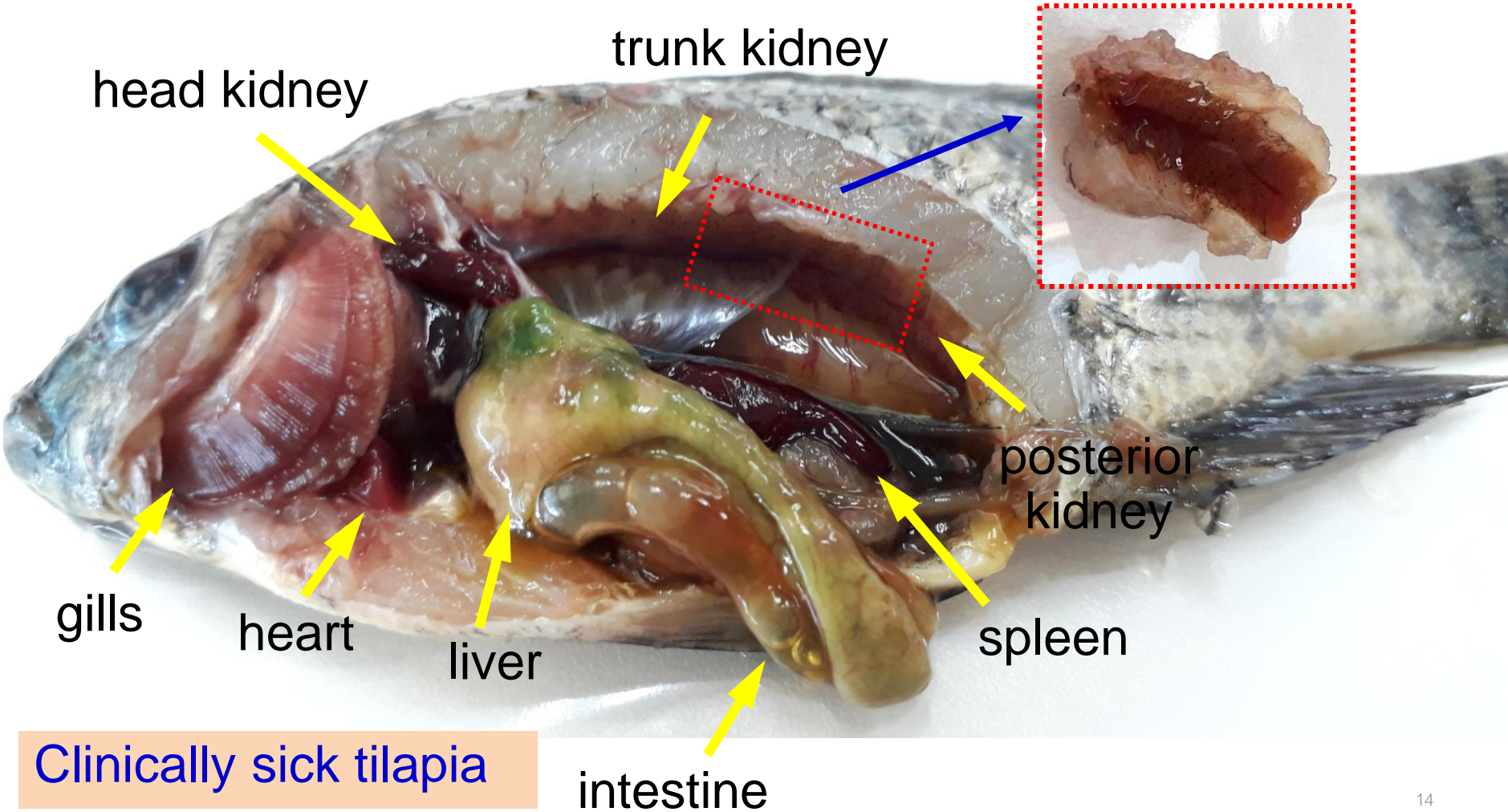
Fish Necropsy



Clinically healthy tilapia

Fish Necropsy

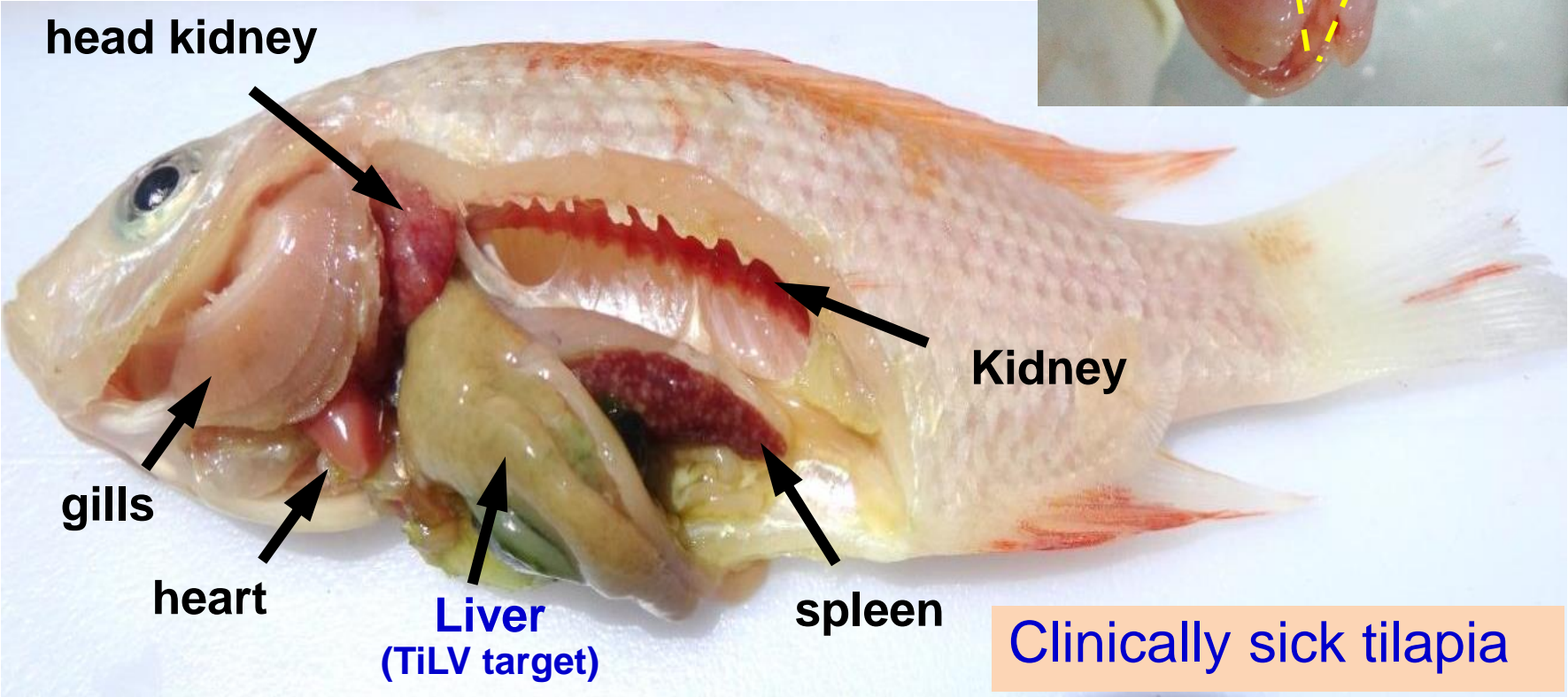
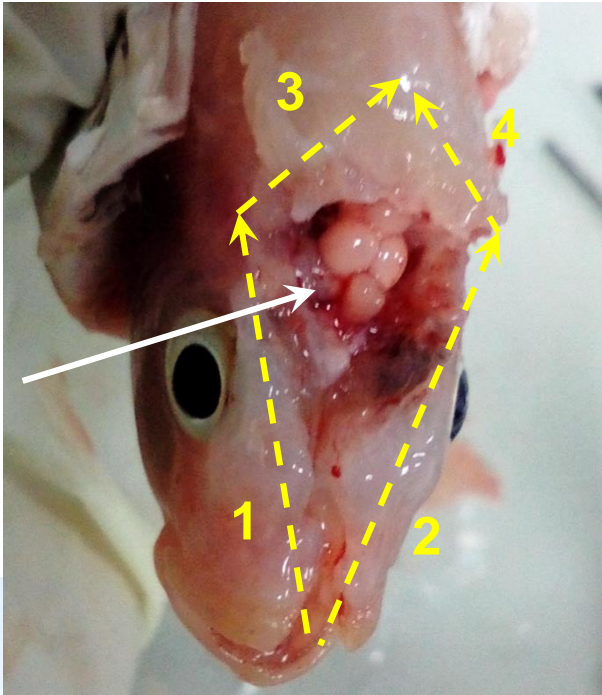
How to collect kidney?



Clinically sick tilapia

Fish Necropsy

How to collect brain?



Clinically sick tilapia

Necropsy Data Sheet (Cont.)

Necropsy

Visceral cavity	normal
Liver	pale, some green areas, watery, white nodules 1-2 mm
Gall bladder	disappear/ very big (swollen)
Stomach	
Intestine	yellow liquid, gas
Spleen	bigger than normal
Kidney	N/A
Heart	
Brain	hemorrhage
Others	
Taking photo/video	Yes

TiLV Diagnostics: Clinical signs (Level 1)

TiLV Diagnostics: Clinical signs (Level 1)



Ferguson et al. J Fish Dis 2014, 37, 583–589

- Darkening,
- Abdominal distension,
- Scale protrusion
- Exophthalmia
- Few developed a progressively emaciated appearance.
- The fluid in the abdominal cavity was watery and colourless
- Gill pallor

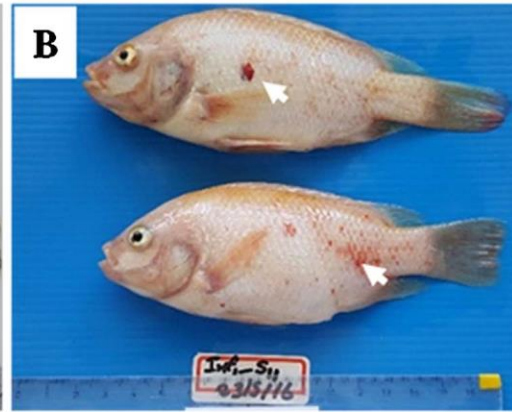
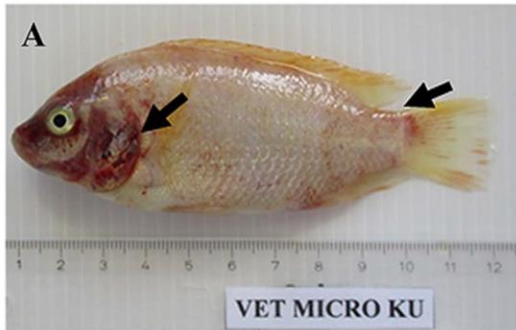
TiLV Diagnostics: Clinical signs (Level 1)



- Shrinkage of the eye and loss of ocular functioning
- Dermal erosions and ulcers

Eyngor et al. 2014

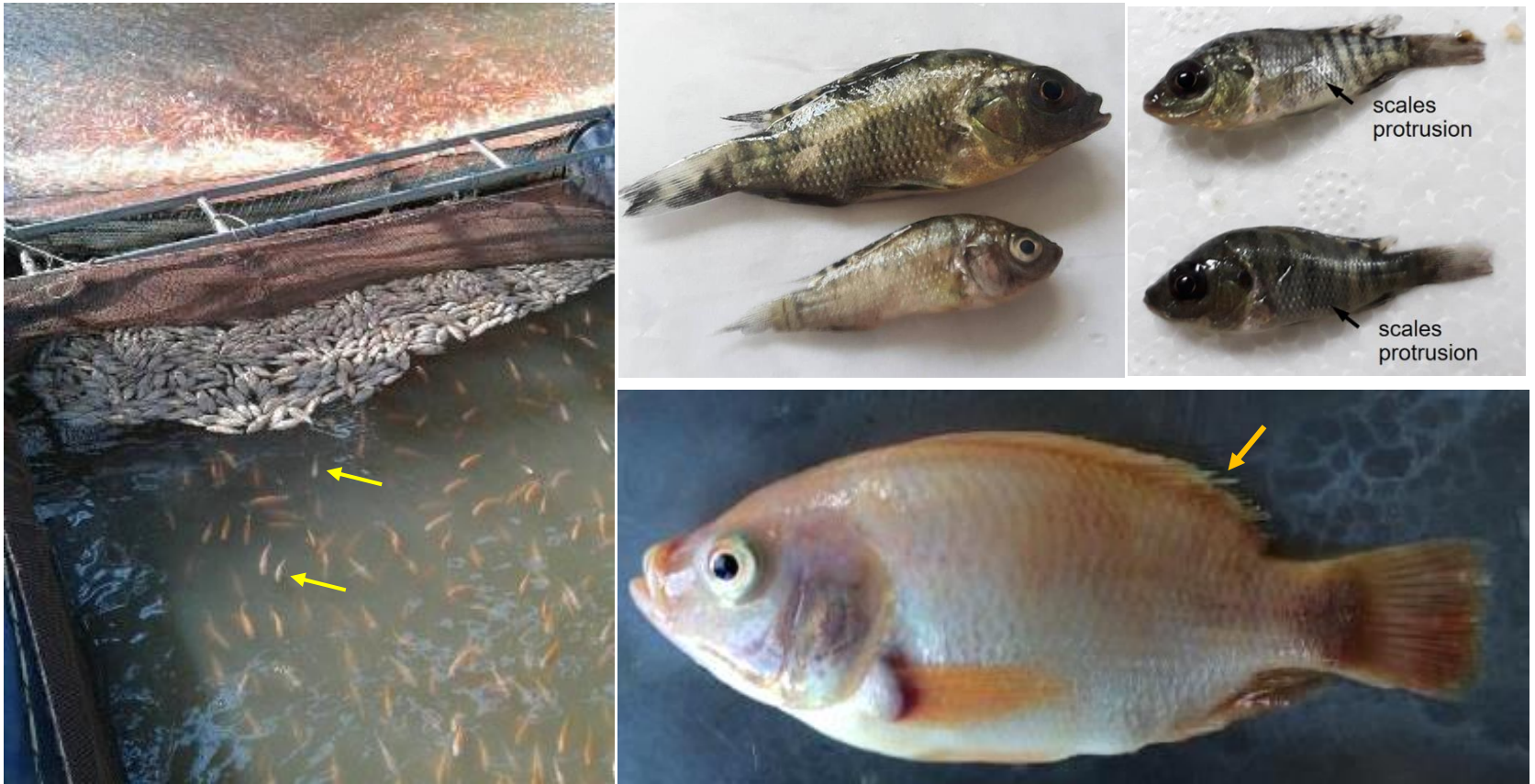
TiLV Diagnostics: Clinical signs (Level 1)



- ✓ skin erosion and hemorrhage
- ✓ skin redness
- ✓ mild exophthalmos and abdominal swelling

Tattiyapong et al. Vet Microbiol 207 (2017) 170–177

TiLV Diagnostics: Clinical signs (Level 1)



Photographs were taken in conjunction with the outbreaks described in Dong et al. *Aquaculture* 476 (2017): 111-118

TiLV Diagnostics: Clinical signs (Level 1)



Naturally diseased fish

- ✓ discoloration
- ✓ loss of scales
- ✓ skin erosion
- ✓ skin hemorrhage



Experimentally diseased fish

- ✓ exophthalmia
- ✓ abdominal swelling
- ✓ scale protrusion

Behera et al. Aquaculture 484 (2018): 168-174

Jansen et al. Review in Aquaculture 2018

TiLV Diagnostics: Clinical signs (Level 1)



Red hybrid tilapia juvenile that positive to TiLV and *Aeromonas veronii* showing skin redness and haemorrhages on the body surface

Amal et al. Aquaculture 485 (2018) 12-16

TiLV Diagnostics: Clinical signs (Level 1)



Cases in 2018

TiLV Diagnostics: Clinical signs (Level 1)



- Skin: discoloration, scale protrusion
- Liver: pale, green, watery
- Spleen: swollen
- Gills: pale
- Gut: ascites
- Gallbladder: shrunken



Case in 2018

TiLV Diagnostics: Clinical signs (Level 1)



- Liver: pale, green or hemorrhage
- Muscle: hemorrhage
- Gallbladder: shrunken

TiLV Diagnostics: Clinical signs (Level 1)



Subclinical/Inapparent infection

All gross signs are unreliable

- darkening
- abdominal distension
- scale protrusion
- exophthalmia
- skin discoloration
- shrunken eye
- hemorrhage
- liver watery
- green or pale liver
- spleen swollen
- shrunken gallbladder
- empty gut
- ascites

No gross signs can be taken as conclusive for any disease

Thank you for your kind attention