



# AUSRIVAS

## TRAINING & ACCREDITATION

### Macroinvertebrate Sample Processing





# Sample processing

- ✦ Samples collected with D-net
- ✦ Rapid biological assessment - large sample requires sub-sampling
- ✦ Largely two methods used:
  - Live-sorting in the field
  - Sub-sampling in the laboratory
- ✦ Individual state & territory protocols may be variations on these two methods





# Live-sorting - NSW

- ✦ Aims to recover as many taxa from the sample as possible
- ✦ Minimum picking period is 30 mins & the maximum is 60 mins
- ✦ At 30 mins, if the number of animals collected exceeds 200, then stop picking





## Live-sorting cont.



If by 40 mins no new taxa found within the last 10 mins, stop picking even if 200 animals are not collected. If new taxa are found, picking will be continued until 200 animals are collected or 50 mins have lapsed.







If new taxa were collected between 40 & 50 mins, continue picking for 60 mins or 200 animals










## Guidelines for live-sorting procedure

-  Empty all or part of the sample into a sorting tray & pick using forceps &/or a pipette - examined all material in first 30 minutes
-  In first 5 mins, collect the active, common taxa - try to avoid bias towards the larger, colourful ones
-  For next 20 mins, concentrate on finding new taxa
-  If new taxa not found by 25 mins, concentrate on collecting more animals for the next 5 mins



# Guidelines for live-sorting procedure

## Cont.

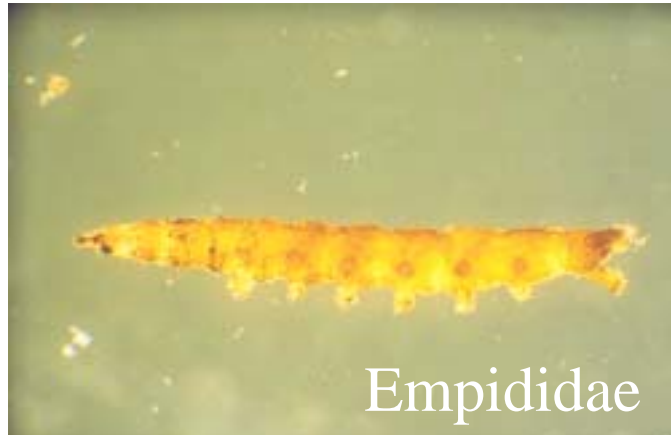
-  Place picked animals into a jar of ethanol (with 2% glycerol)
-  Record the number of animals collected
-  Examine the sheet showing cryptic &/or small taxa & actively search for them during the sorting procedure
-  Attempt to pick at least 20 Chironomidae - to ensure adequate representation of subfamilies
-  When emptying the tray check for cryptic animals stuck to the bottom of the tray



# Cryptic Taxa



Oligochaeta



Empididae



Sphaeriidae



Chironomidae



Hydracarina



Hydroptilidae



# Lab-sort - ACT



Wash sample in a 250  $\mu\text{m}$  mesh sieve to remove preservative & fine sediment



Evenly distribute the sample in the sub-sampler box



200-organism sub-sample required



Use a vacuum pump to remove the contents of cells randomly selected





# Lab-sort - ACT

- ✦ Removed sand from cell contents using a saturated  $\text{CaCl}_2$  solution
- ✦ Sort the samples with the aid of a microscope
- ✦ Remove & count all macroinvertebrates in the cell - check list of organisms counted
- ✦ Calculate the number of cells needed to obtain 200 organisms & extract these from the sub-sampling box
- ✦ Extracted cells must be completely sorted even if the 200-organism count is reached



# Macroinvertebrate Identification



Family level ID except Oligochaeta (class), Hydracarina (order) and Chironomidae (sub-family)



Separate into order & place in separate vials - label each of the vials



ID problem? Consult the decision tree

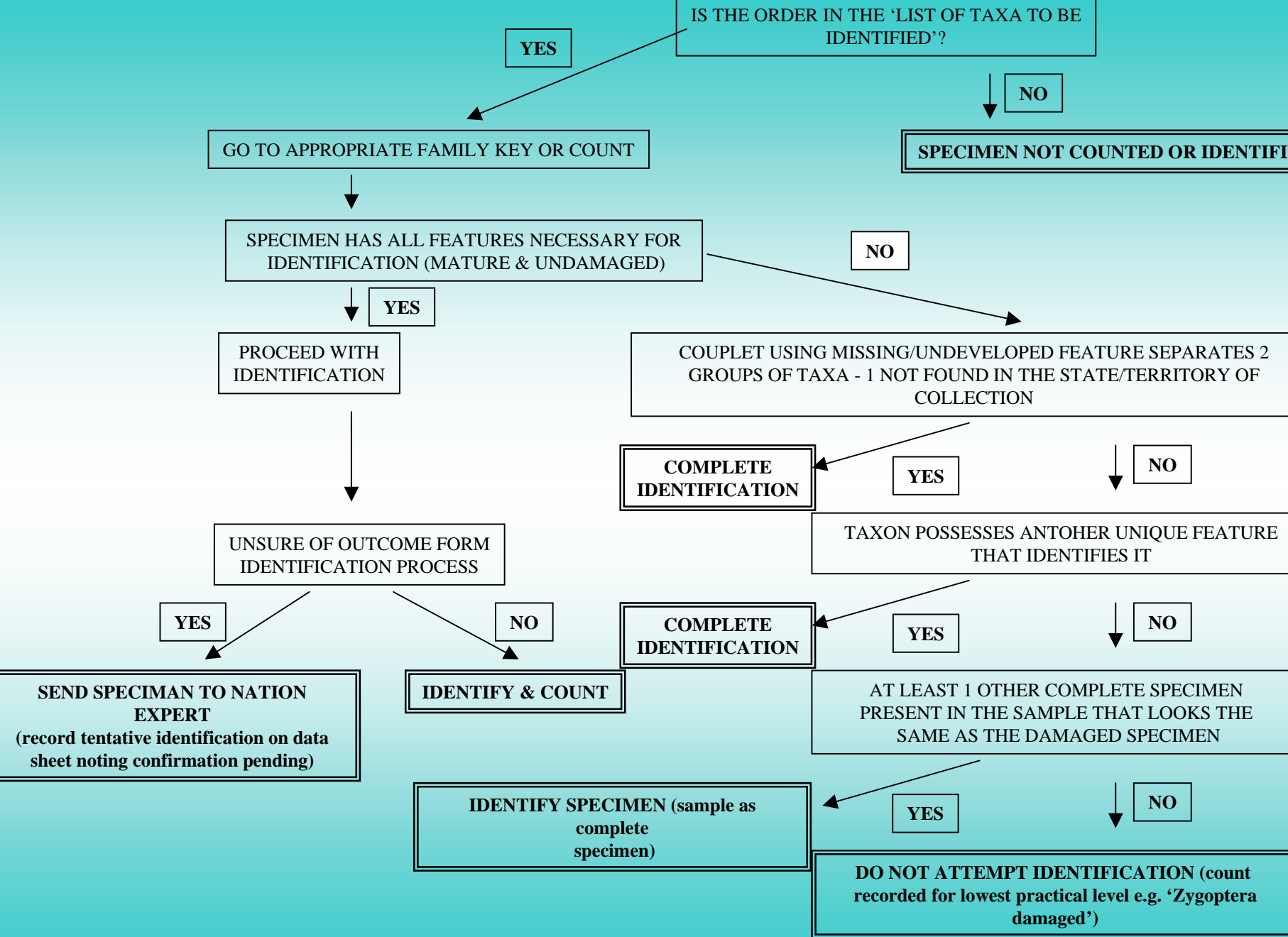


Note very small, damaged, early instars or pupae that cannot be identified (e.g. Trichoptera juvenile).



Damaged animals should be identified if possible





YES

IS THE ORDER IN THE 'LIST OF TAXA TO BE IDENTIFIED'?

NO

GO TO APPROPRIATE FAMILY KEY OR COUNT

SPECIMEN NOT COUNTED OR IDENTIFIED

SPECIMEN HAS ALL FEATURES NECESSARY FOR IDENTIFICATION (MATURE & UNDAMAGED)

NO

YES

PROCEED WITH IDENTIFICATION

COUPLET USING MISSING/UNDEVELOPED FEATURE SEPARATES 2 GROUPS OF TAXA - 1 NOT FOUND IN THE STATE/TERRITORY OF COLLECTION

YES

COMPLETE IDENTIFICATION

NO

UNSURE OF OUTCOME FORM IDENTIFICATION PROCESS

TAXON POSSESSES ANOTHER UNIQUE FEATURE THAT IDENTIFIES IT

YES

SEND SPECIMAN TO NATION EXPERT (record tentative identification on data sheet noting confirmation pending)

NO

IDENTIFY & COUNT

YES

COMPLETE IDENTIFICATION

NO

AT LEAST 1 OTHER COMPLETE SPECIMEN PRESENT IN THE SAMPLE THAT LOOKS THE SAME AS THE DAMAGED SPECIMEN

IDENTIFY SPECIMEN (sample as complete specimen)

YES

DO NOT ATTEMPT IDENTIFICATION (count recorded for lowest practical level e.g. 'Zygoptera damaged')

NO



## Recording Macroinvertebrate Identification Data

Check that:

- All tallies have been transferred to the count column
- Counts have been summed & the total count is correct
- Number of taxa have been summed & is correct. Note: If a taxon is represented in the sample by both juvenile & adult forms, count them as a single taxon when recording the total number of taxa (some exceptions apply)





## Recording Macroinvertebrate Identification Data cont.

Check that:

- Number of vials used is recorded
- Percentage of sample used is recorded (lab-sort)
- Lab-sort scan data kept separate from the sub-sample data





# Lab-sort Scan - ACT

Place the unsorted sample residue into a large tray & scan for 15 minutes, collecting any taxa not found in the sub-sample





# Macroinvertebrate

## Quality Assurance/Quality Control Procedures

QA/QC procedures are designed to establish an acceptable standard of macroinvertebrate sorting & identification.

**Lab Sorting:** Internal QA/QC checking procedures are carried out by experienced persons for the first 5 samples sorted by new personnel &/or from each new sampling run

- Following the completion of sample sorting the QA/QC personnel are to check the sample remains for missed organisms
- In order for a sample to pass,  $\geq 95\%$  of the total number of organisms in the sub-sample must be recovered.



# Quality Control/Quality Assurance

<b>Error codes</b>	
CC	Number of organisms recovered from the sub-sample represents less than 95% of the total number of organisms in the sub-sample
IE	Identification error (i.e., Percent Taxa Error” and/or the “Percent Incorrect Identifications”) greater than 5%.
LE	Labelling error
SE	Sub-sampling error – eg if the sample was stored in more than one sample container and only one container was sorted.
WE	Washing error – some sample bypassed washing sieve
DE	Data entry error on data sheet
CE	Calculation error – mathematical error on data sheets
<b>Action codes</b>	
LC	Labels corrected – contact person who collected the sample if error is on the original sample label
SC	Sample re-subsampled, processed, re-checked and data sheets corrected
WC	Material bypassing the sieve caught in washbasin, sample combined and rewashed
WI	Material bypassing sieve lost, partial sample processed
DC	Data entry corrected (strike out incorrect entry with one line and write in the correct entry, initial)



# Identification QA/QC

- ✦ For all new persons, projects, or sampling runs, QA/QC staff should check the first 5 samples identified
- ✦ A “Percent Taxa Error” of  $\leq 5\%$  is acceptable at family level
- ✦ A “Taxa Error” is defined as a mis-identification resulting in the loss or addition of a taxon
- ✦ The “Percent Taxa Error” is the “Number of Taxa Errors” divided by the “Total Number of Original Taxa”, multiplied by one hundred





## Identification QA/QC cont.



### Percent Taxa Error

- Number of Taxa Errors (a)
- Total Number of Original Taxa (b)
- Percent Taxa Error ( $[a/b] \times 100$ )
- Pass or Fail? (Pass if  $\leq 5\%$ )

### Example

1  
15  
6.67  
Fail



After checking the initial 5 samples, a random selection of 2 samples in the following 10, 2 samples in the following 30 and two samples in the following 50, will be checked