Automatic Visual Plankton Identification

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ZPS 2011

Workshop format

- ***8:30 Introduction by Convenors***
- **8:35 Cabell Davis (Invited (W5-7254)**
  – presented by Mark Benfield
- **9:00 Lars Stemmann (W5-7009)**
- **9:15 Elvire Antajan (W5-7192)**
- **9:30 Harry Nelson (W5-7223)**
- **9:45 Catarina Marcolin (W5-7334)**
- 10:00 *Coffee/Tea Break*
  – **Posters: Xiaoxia Sun (W5-7149), Karen Manriquez (W5-7186)**
- **10:30 A beginners guide to automatic visual plankton identification**
- **11:30 Open Forum & Discussion**
- 12:30 Workshop ends

A beginners guide to automatic visual plankton identification

Summary

- The 1-D case flow cytometer
- 2D visual features
- Scanners and imagers
- Software
- Operational process
- An example
- Applications
- Machine performance

The 1-D case – flow Cytometry

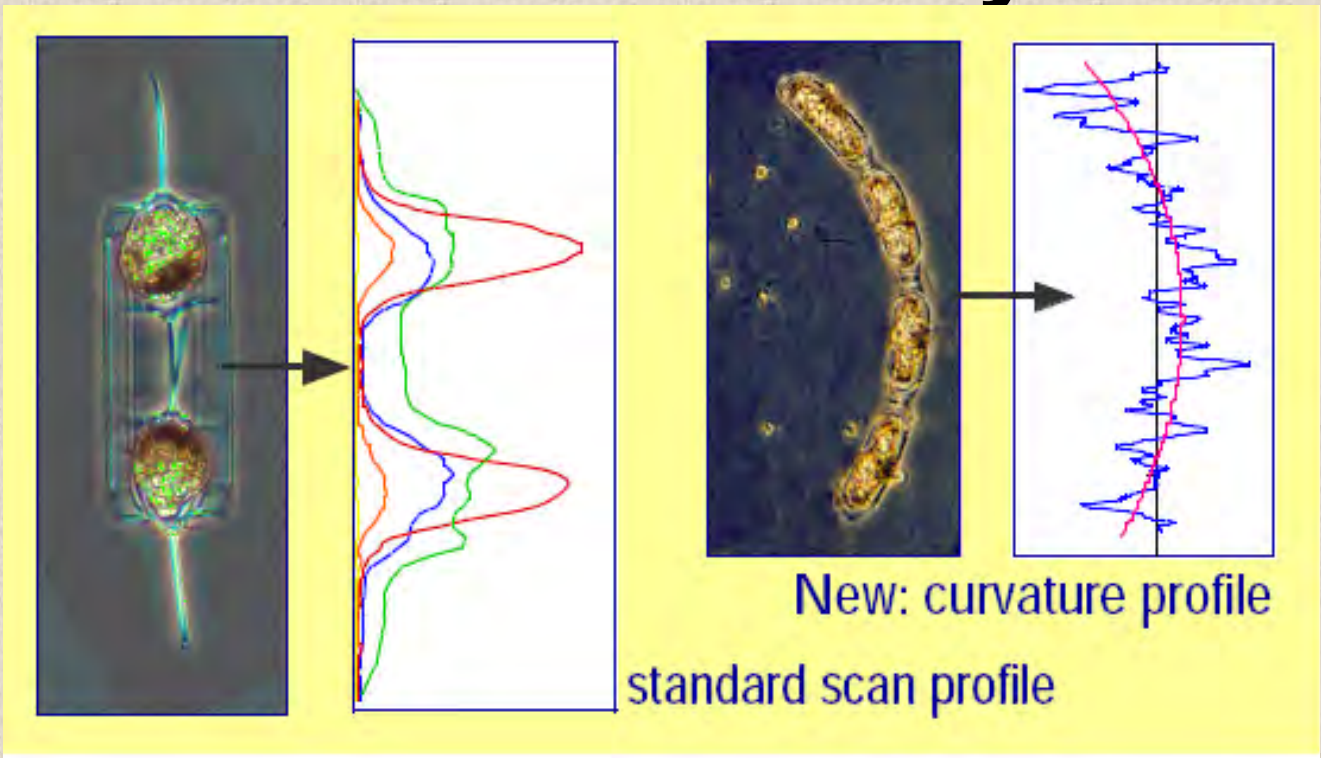


Fig.1 Cytosense image in flow examples of two phytoplankton and their accompanying laser fluorescence traces (source: G.Dubelaar Cytosense product flyer, 2006).

Scanning Flowcytometry

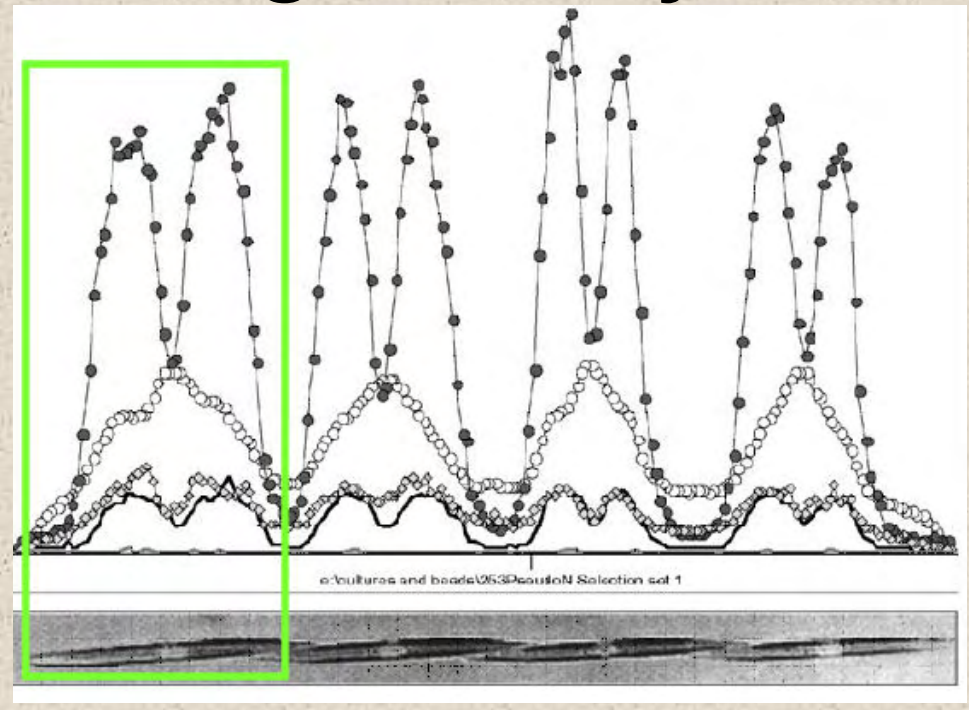
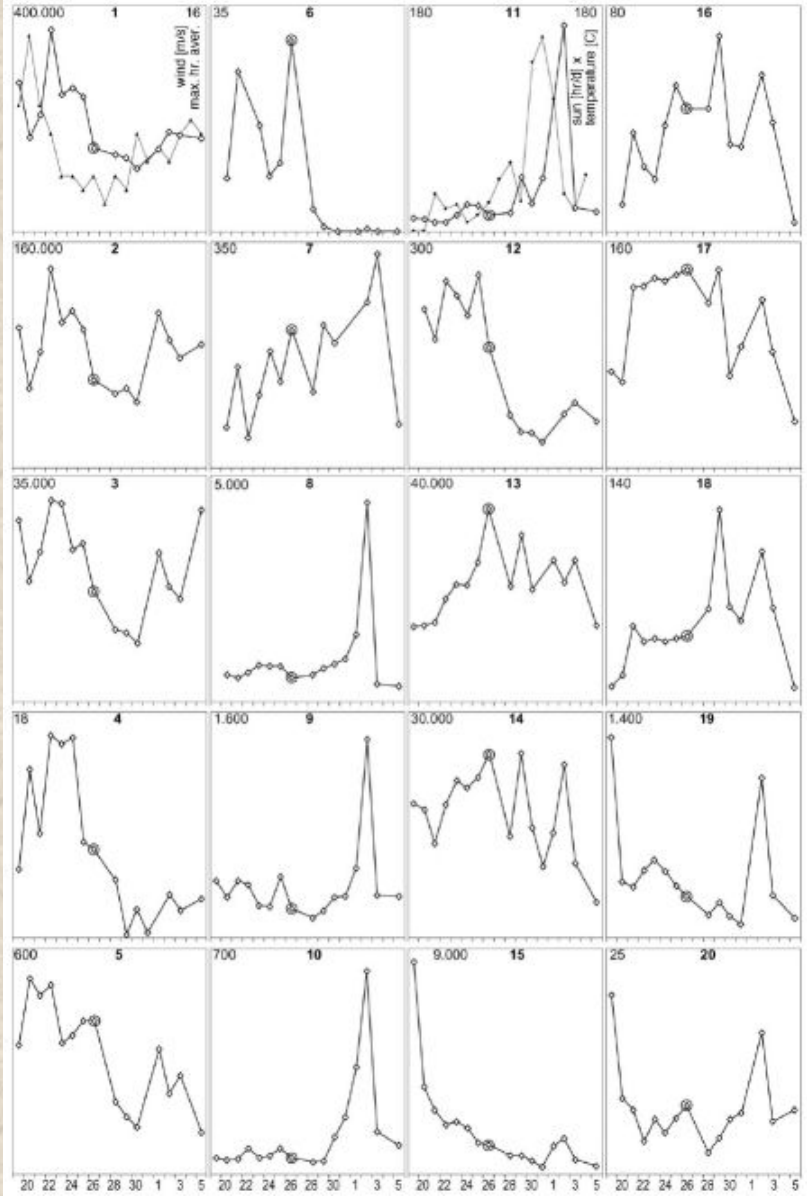


Fig. 2 Bottom: ‘‘Nitschia’’ type colony of 4 symmetrical cells (photo from: Gerhard Drebes, Marines Phytoplankton, 1974) Top: corresponding CytoSense 1D scan consisting of 5 signal profiles. Circles: scattered light captured at near forward angles; diamonds: scatter at sideward angles; dots: red coloured fluorescence (emitted by the phytoplankton basic pigment chlorophyll a); black line: orange fluorescence (predominantly from accessory pigments); grey line: green/yellow fluorescence (typically by some ciliates and cysts). (source: J. Env. Biol (2004) 6. 946-952). Note the green box encloses the ‘signature of one colony individual.



1	Non fluorescent particles of 1–3 μm (sediment-debris).
2	Non fluorescent particles of 3–10 μm (sediment-debris).
3	Non fluorescent particles of 10–50 μm (sediment-debris).
4	80–140 μm organisms with small local (12–18 μm) orange fluorescent section.
5	30–100 μm particles with localized weak to moderate chlorophyll fluorescence.
6	Filaments > 50 μm, cyano type.
7	Picoplankton cyano type.
8	*ca.* 8 μm cells, moderate accessory pigment.
9	20–35 μm Cryptomonas type cell.
10	50–70 μm Cryptomonas type cell.
11	Synura colonies (50–150 μm diam.)
12	*ca.* 7 μm cells, with accessory pigment fluorescence.
13	Picoplankton, green.
14	Green cells, 3–15 μm.
15	*ca.* 8 μm cells, high chlorophyll fluorescence.
16	Closterium-like, symmetrical cells, 50–80 μm.
17	Closterium-like, symmetrical cells, 70–100 μm, very thin.
18	Spindle shaped, 70–90 μm cell with double chloroplast (30–40 μm).
19	15–40 μm cells, high chlorophyll fluorescence.
20	Dinobryon colonies, 100–200 μm length.

(No taxonomic analyses were available for further identification of these groups.)

Fig.3 (a) distributions of 20 groups of particle in Cytosense analysed water sample, (b) group labels assigned to sample groups (source: J. Env. Biol (2004) 6. 946-952) vertical scale is individuals per ml.

2D analysis – scanners etc.

- Uses digitised images
- Several commercial products (FlowCAM, ZooSCAN, toolsets for microscopy)
- Several free toolsets (Zoo/PhytoImage, ZooProcess, & Weka, Tanagra for statistical analysis

Scanners

Silhouette photography (Ortner et al., 1979)

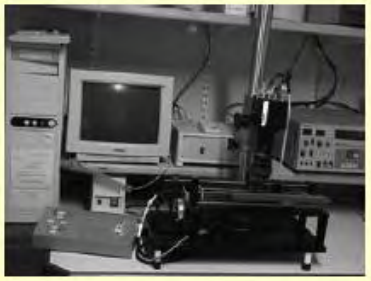
1979

1987

2003

2005

2001



2001

Bespoke Scanners (from Picheral, 2007)

Using a scanner

- Specimens are imaged on a scanner
  - Can be stained
- ZooImage (shown) & Zooscan automate identification

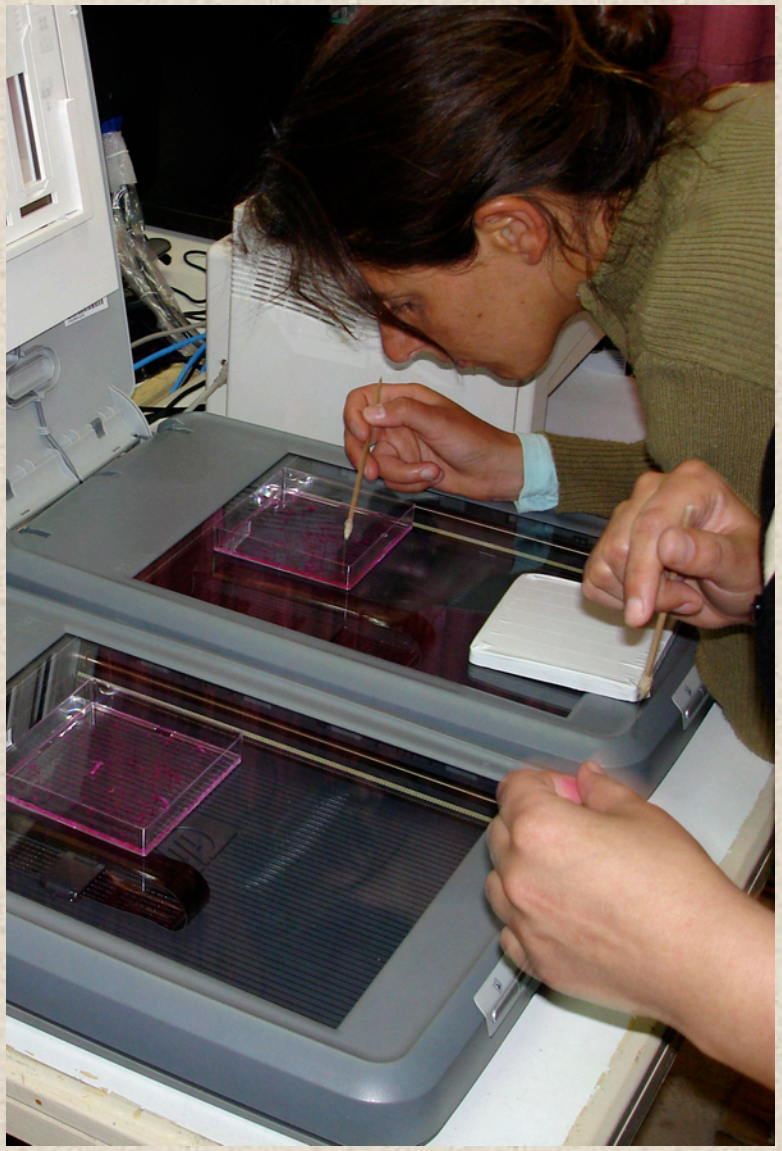
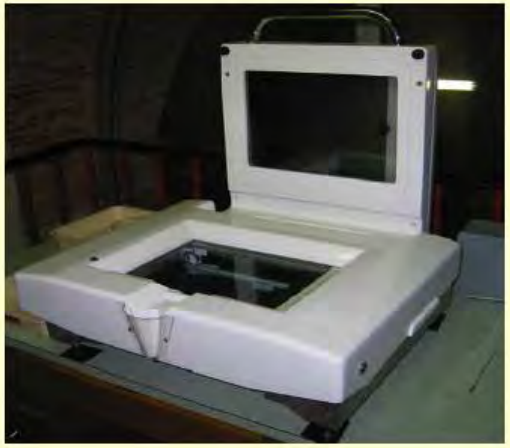


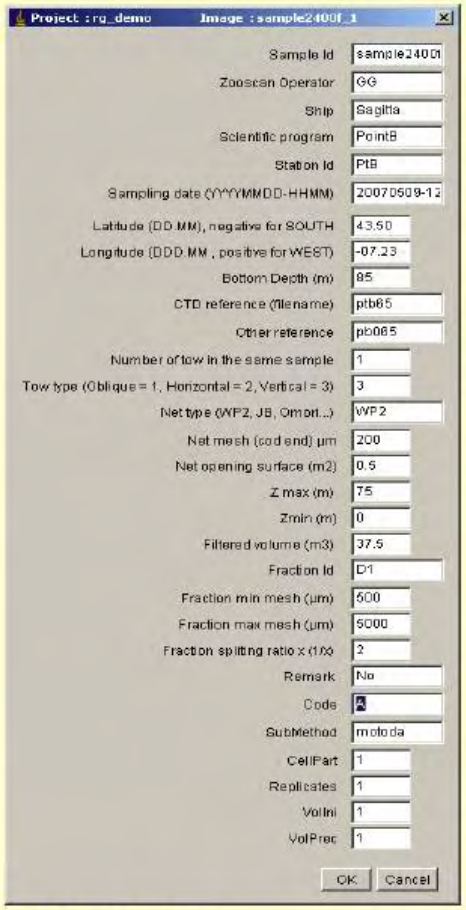
Image: Culverhouse 2007

Zooscan



It is essential to archive the best quality image and the associated metadata plus the scanning parameters.

RAW image :
16 bits
Up to 15 x 25 cm
Up to 2400 dpi
Up to 5000 objects per image

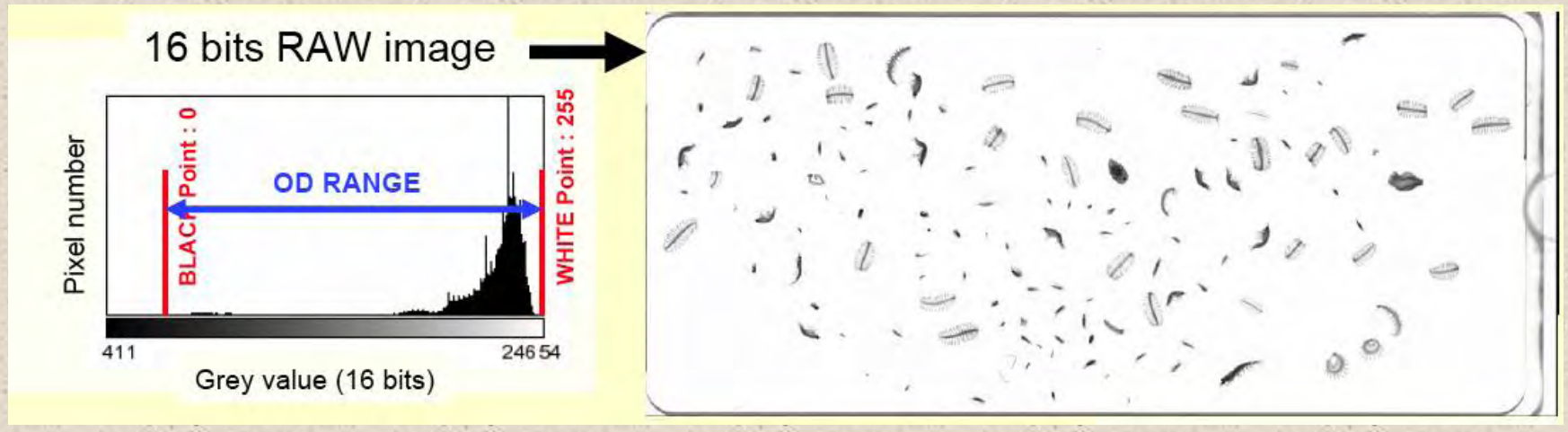


Metadata form

The Zooscan instrument (from Picheral, 2007)

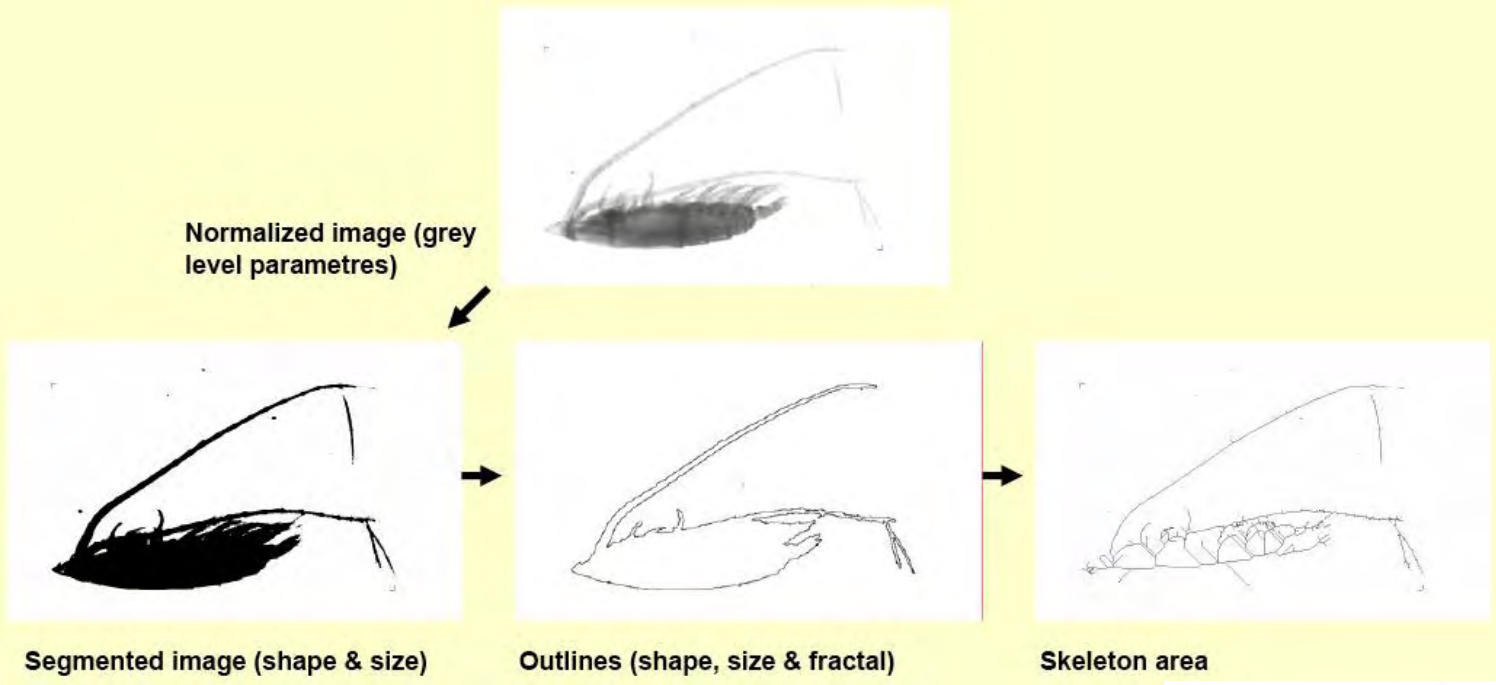
Image feature extraction

- Grey-level equalisation



Zooscan Normalised image (from Picheral, 2007)

Image feature extraction



ZooProcess feature extraction (from Picheral, 2007)

Image (from Picheral 2007) overlaid with bounding box and major/minor axes

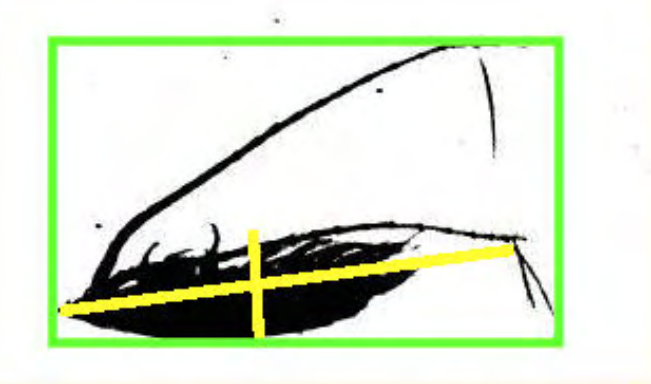
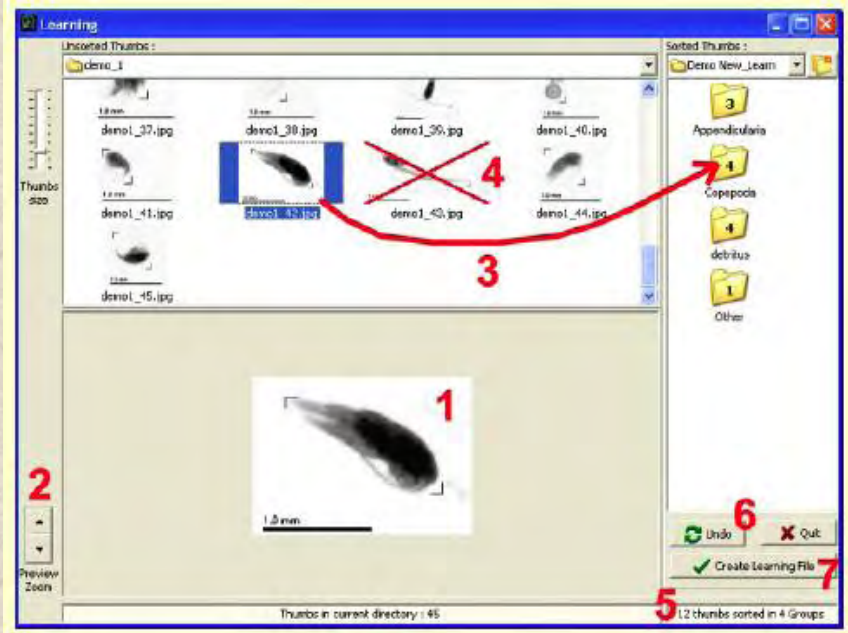
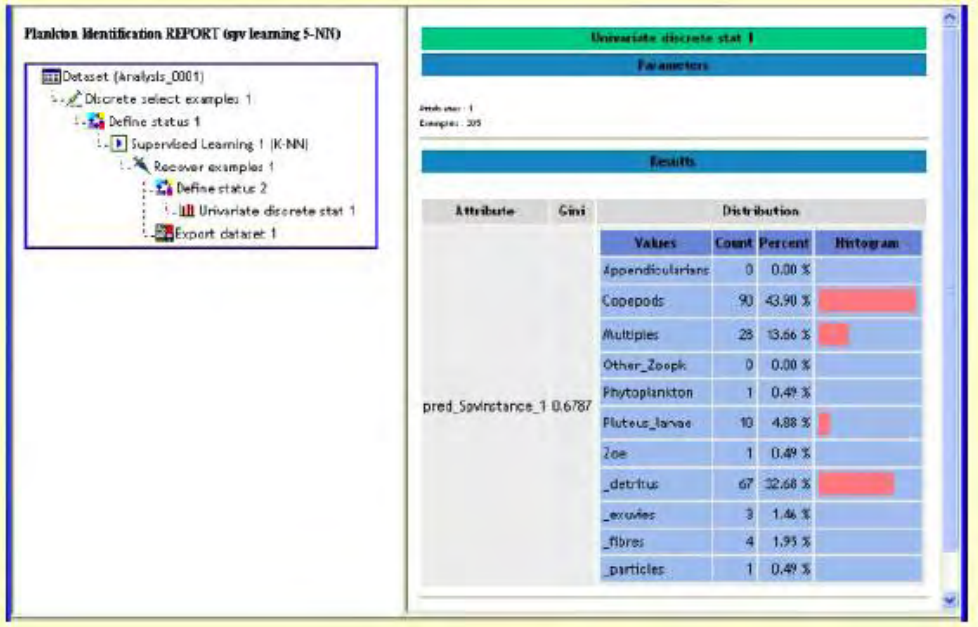


Image feature analysis





- Feature sets are extracted for each object
- These can then be analysed for clusters (using multi-dimensional clustering tools such as SVM, Random Forest, LDA)

Plankton Identify (from Picheral, 2007)

An example

CEH.01-07-02.p7+B2.A showing scan of mixed zooplankton (source: Di Mauro, Mar del Plata, AR)

An example

CEH.01-07-02.p7+B2.A showing a small sample of copepods drawn from previous slide

An example

ID	major axis	Minor axis	Area	Centroid X	Centroid Y
50	49.847467	22.949748	773	820.274256	98.421734
51	38.66348	12.328536	359	405.041783	94.415042
52	36.453996	11.220497	303	212.277228	146.082508
55	54.676793	20.648836	816	616.53799	293.973039
58	42.046903	15.77498	501	1313.52495	309.854291
61	46.289936	14.698126	438	1215.51598	349.0570
62	34.615556	12.756711	320	62.78125	419.856
63	28.20686	7.952657	163	1108.36196	440.0184
65	39.420003	14.781337	446	250.210762	504.948
66	85.896223	21.420149	625	226.5472	557.14
67	50.898697	21.809834	746	1167.30027	541.2828
70	87.031542	23.041159	821	120.952497	608.6699
75	34.293377	14.11345	358	1197.28492	630.3212

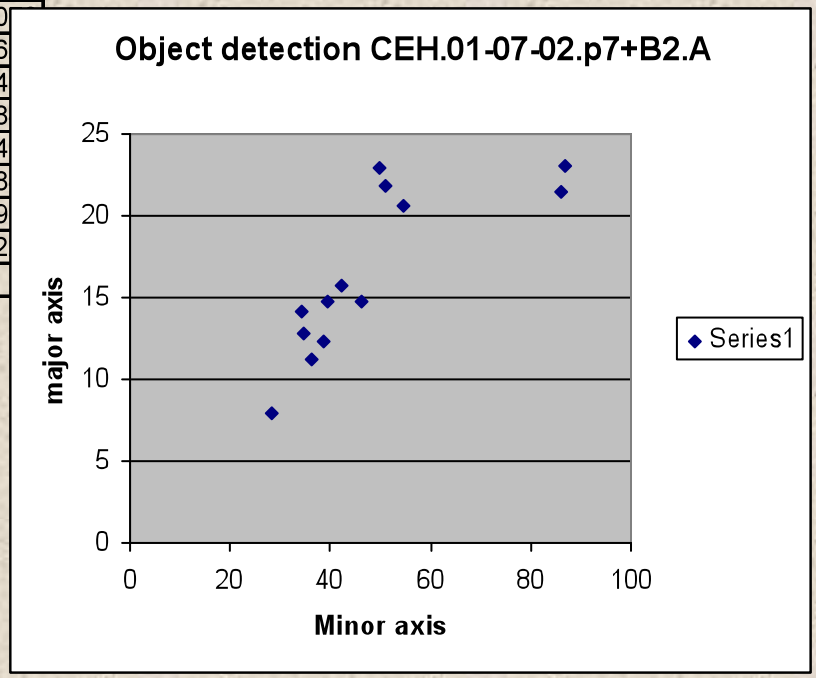


Fig.12 Morphological data extracted automatically from CEH.01-07-02.p7+B2.A (in Fig.11).

Training/Testing a classifier

- Expert selects specimens for training set
  - Identifies 20-50 examples of each class
- Choose classifier
    - Random Forest –fast to train, cannot over learn
    - Support Vector Machine – slow to train
    - ANN – moderate to train, can over learn
- Train and test cycle
  - Confusion table
  - Calculate recall & precision

Operational use

- Collect specimens, record ‘metadata’
- Fix, flush detritus or stain
- Subsample
  - to give ~200 specimens per aliquot
  - Size fractionate
- Scan, extract features, run classifier and record results
  - ESD, and other per specimen measurements
  - Identity (and confidence)
- Export to spreadsheet

Applications

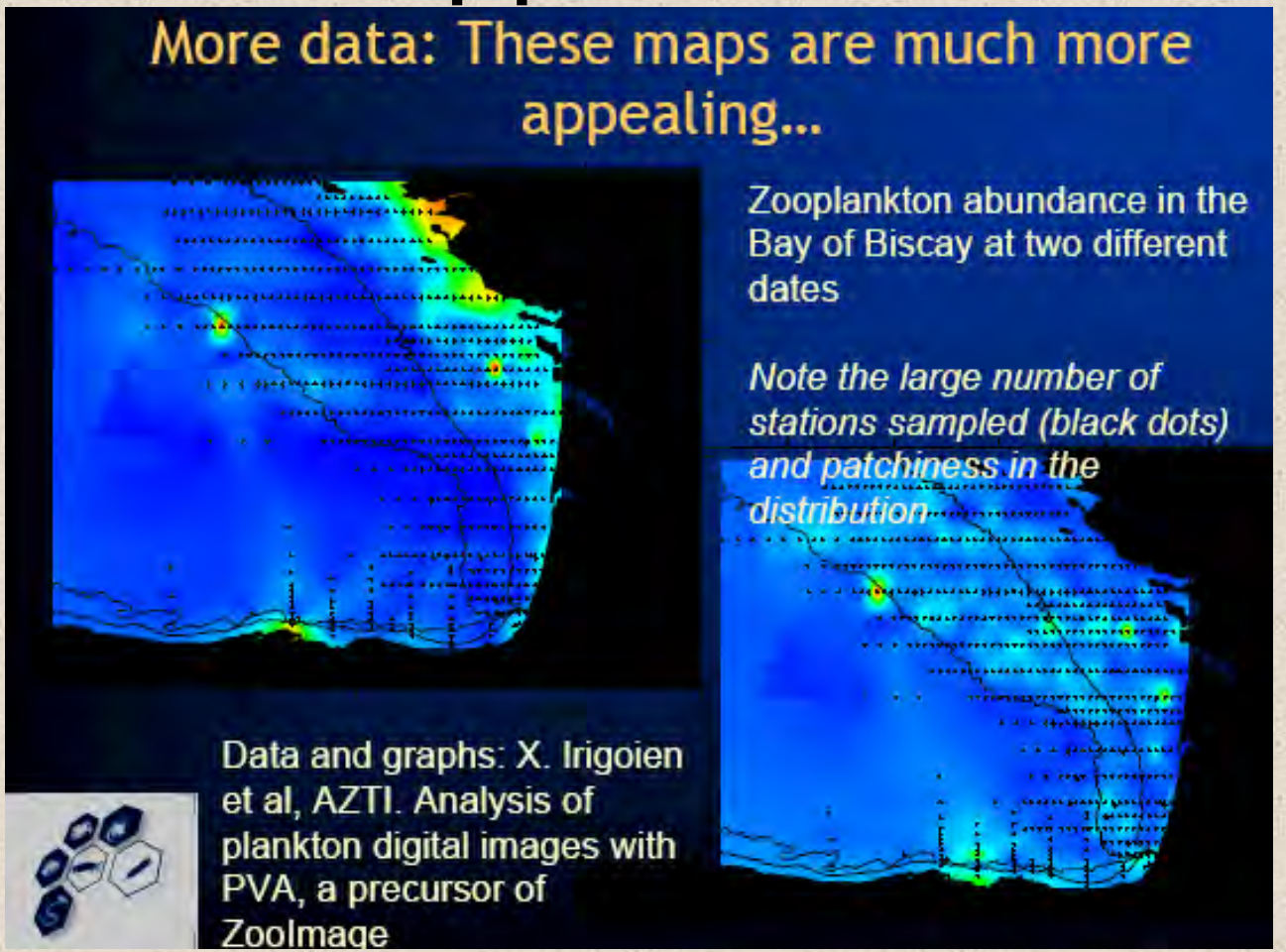


Fig.13 example analysis using ZooImage (from www.pices.int/publications/presentations/Zoopl%202007/Zoop%202007%20S9/S9_Grosjean.pdf)

Application examples (cont'd)

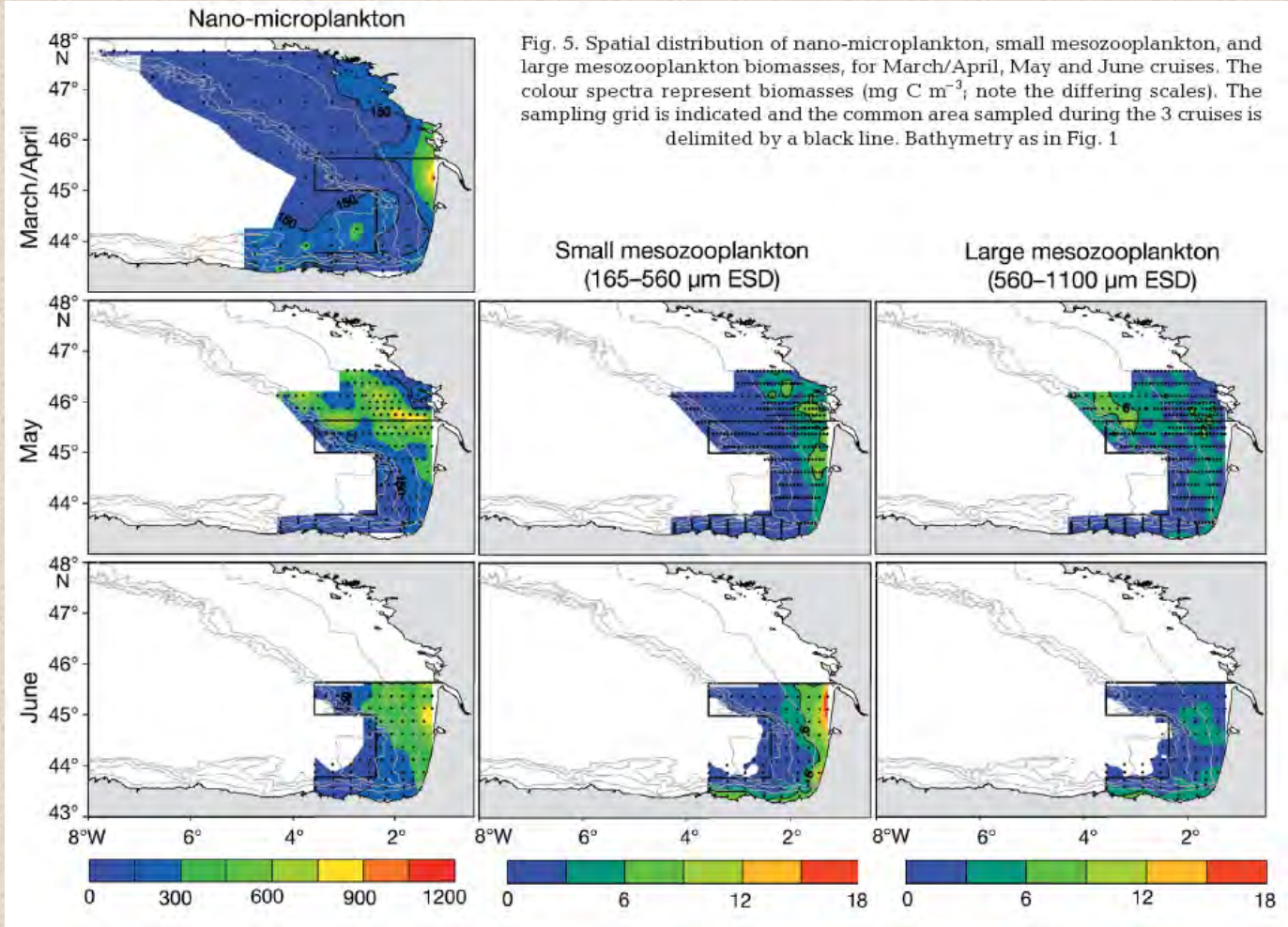


Fig. 5. Spatial distribution of nano-microplankton, small mesozooplankton, and large mesozooplankton biomasses, for March/April, May and June cruises. The colour spectra represent biomasses (mg C m^{-3}; note the differing scales). The sampling grid is indicated and the common area sampled during the 3 cruises is delimited by a black line. Bathymetry as in Fig. 1

Fig..14 Example analysis using ZooImage and FlowCam (from Zarauz et al. 2007).

Application examples (cont'd)

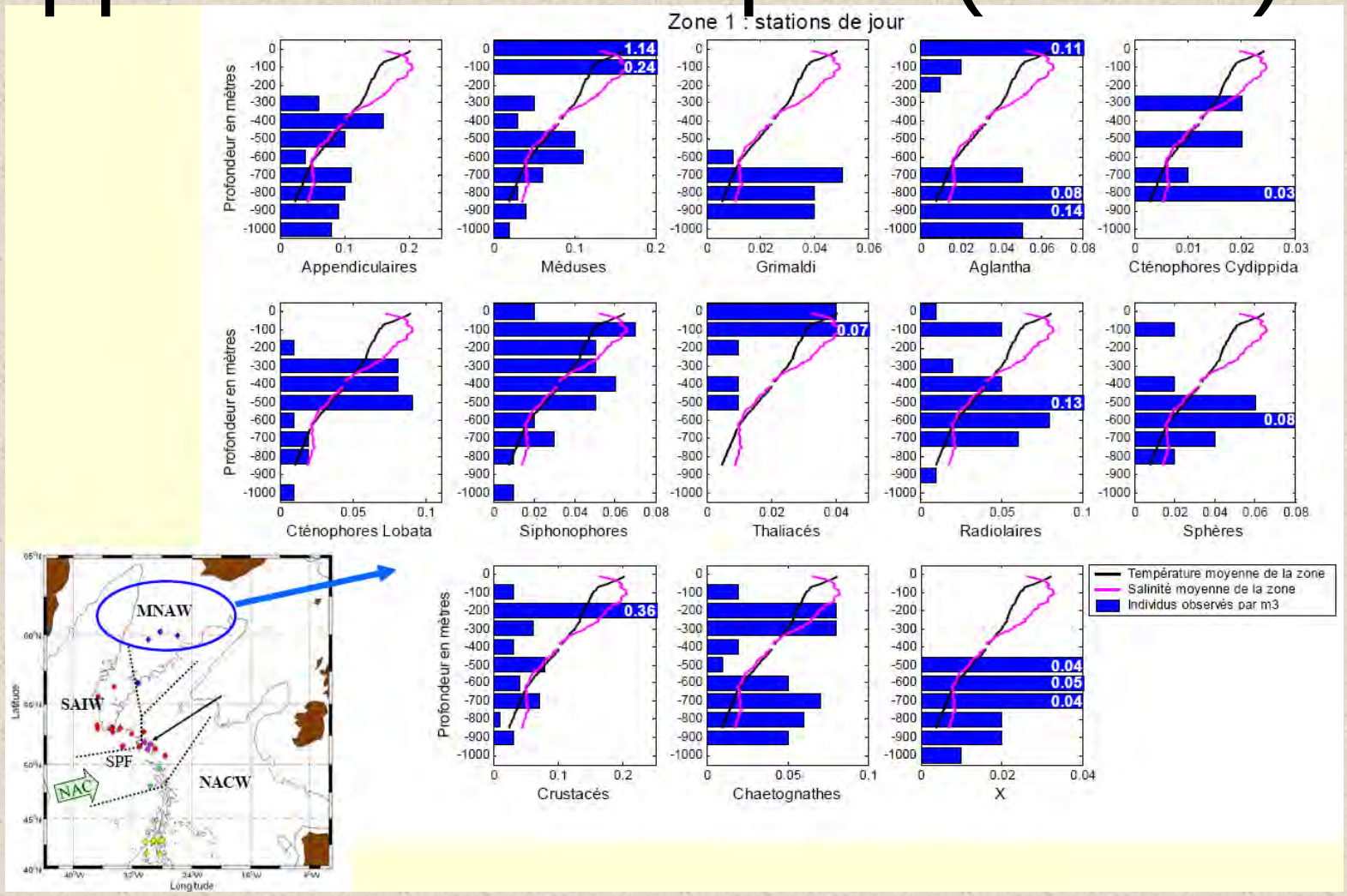
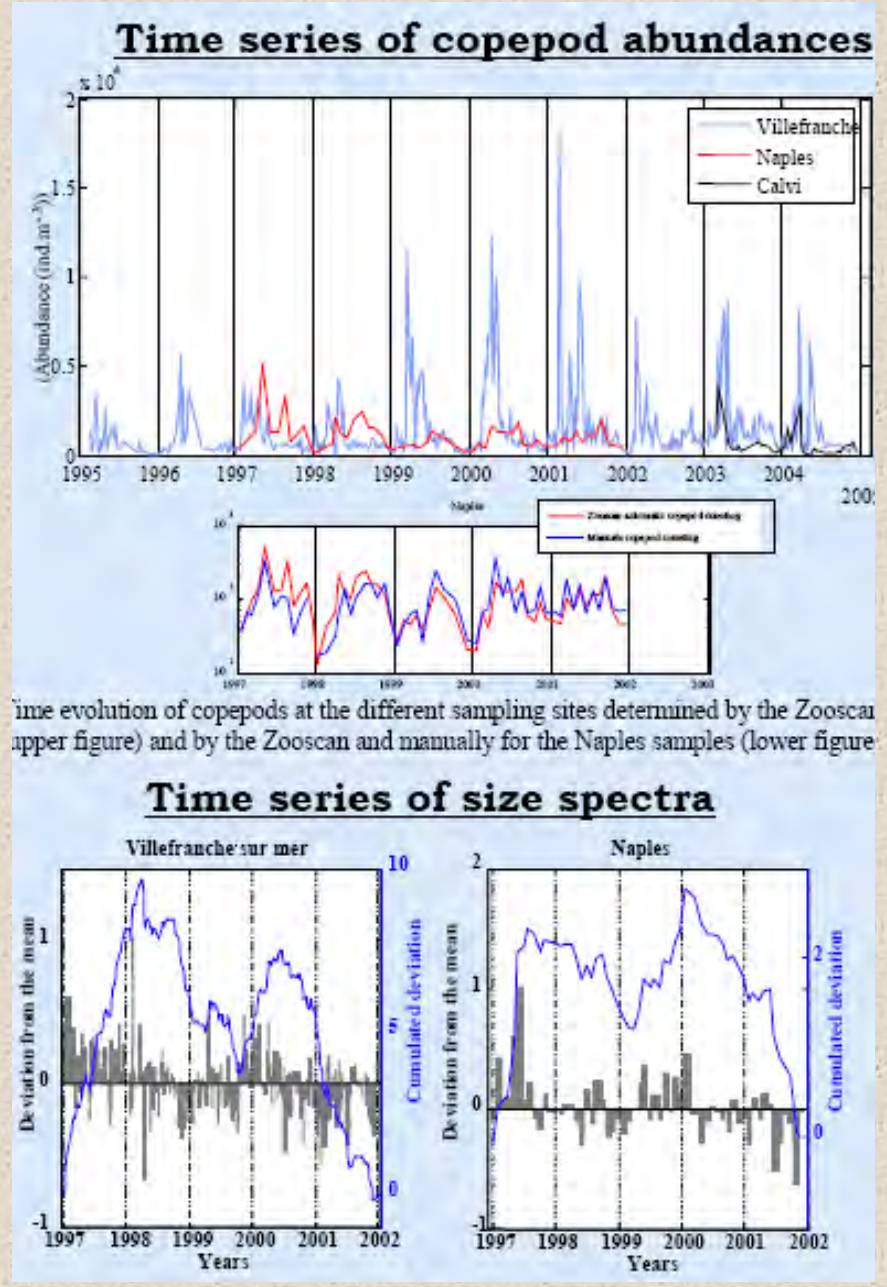


Fig.15 Example Zooscan analysis (from: Picheral 2007a)

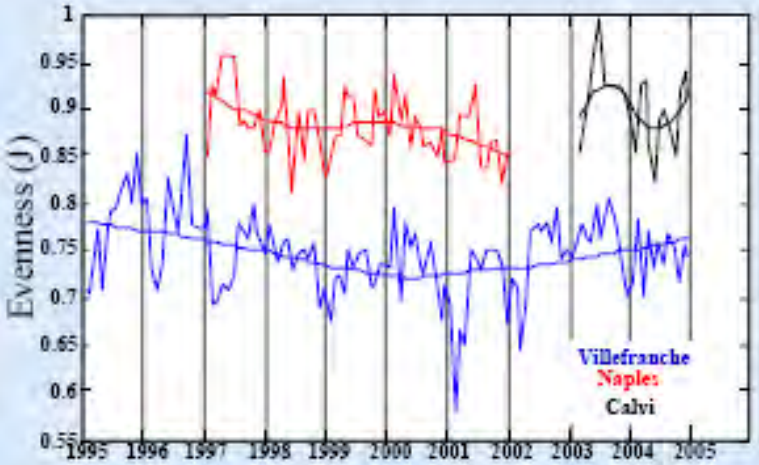
(cont'd)

Fig.16 Example Zooscan time series analysis (from: Gorsky et al 2005)

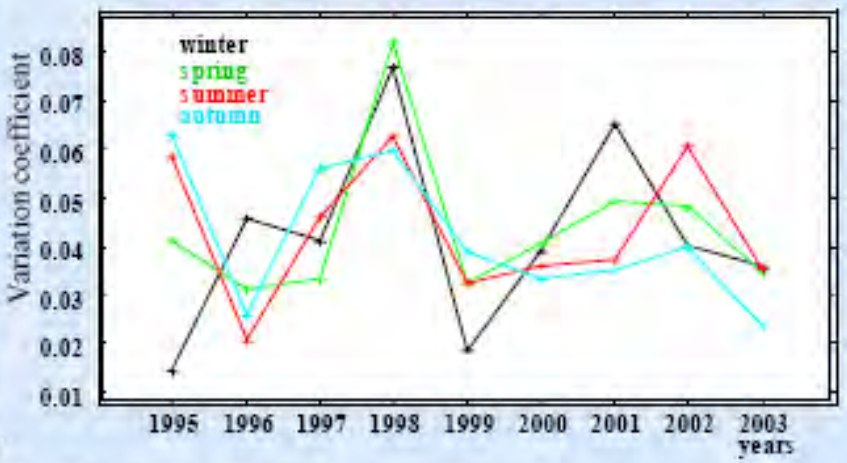


ime evolution of copepods at the different sampling sites determined by the Zooscan
pper figure) and by the Zooscan and manually for the Naples samples (lower figure

Application examples (cont'd)



Evenness (Pielous's J) estimated from Shannon's diversity index on size classes for the series from Villefranche, Naples and Calvi (Zooscan results).
General trends (bold lines) correspond to local polynomial filtering.
Pielous's J:



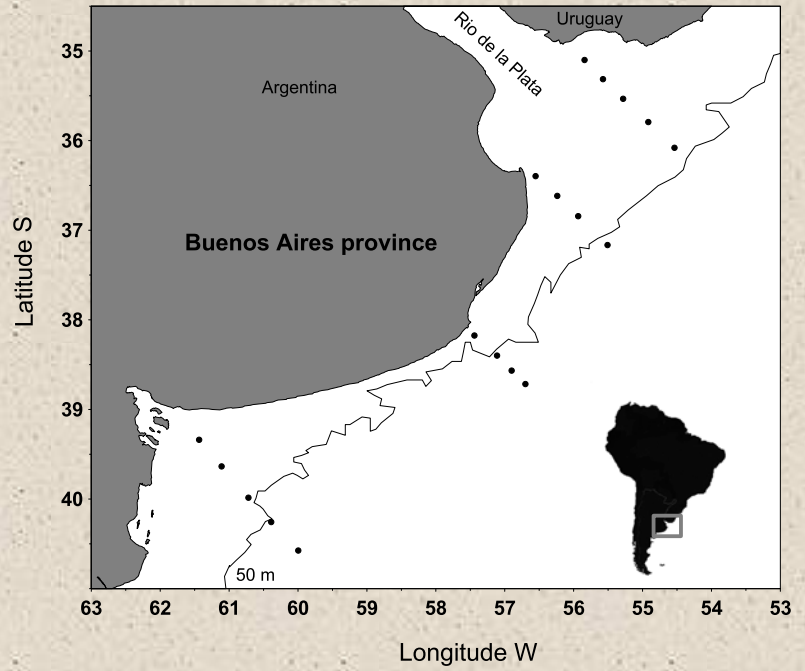
Variation coefficient for seasonal size diversity.
Data: 403 size spectra obtained using the Zooscan from weekly sampling in Villefranche were transformed into 108 monthly spectra by the Jackknife method.

Fig.17 Further examples of Zooscan time series analysis (from: Gorsky et al 2005)

Zooimage run through

Data set example

Roxana di Mauro,
INIDIEP,
Mar del Plata,
Argentina.



- Sample EH0606 was taken from waters off the Buenos Aires province in 2006 using a 200um net.
- email:rdimauro@inidep.edu.ar

Machine performance

- *HAB Buoy, 26 species 65-90%*
- *Zooscan, 40 groups (semi-automatic) 75-85%*
- *SIPPER, 5 groups 75-90%*
- *Video Plankton Recorder,>7 groups 72%*
- *Cytosense, 20-100 groups,*

- *All can process many 1,000 objects per hour*

Conclusions for automatic visual plankton identification

- Performance OK for ecology
  - Flow cytometry – 60 groups
  - Zooscan – semiautomatic >40 groups
  - Zoo/Phyto Image - 20-30 groups
- Clutter and detritus can cause problems
- Can make useful tools for ecology
- Semi-automatic
  - Keeps ecologist in the loop
  - Reduces false positive/negative rates

Open Forum & Discussion

So how about sorting manually?

HAB buoy images:

Rià Arousa, N. Spain

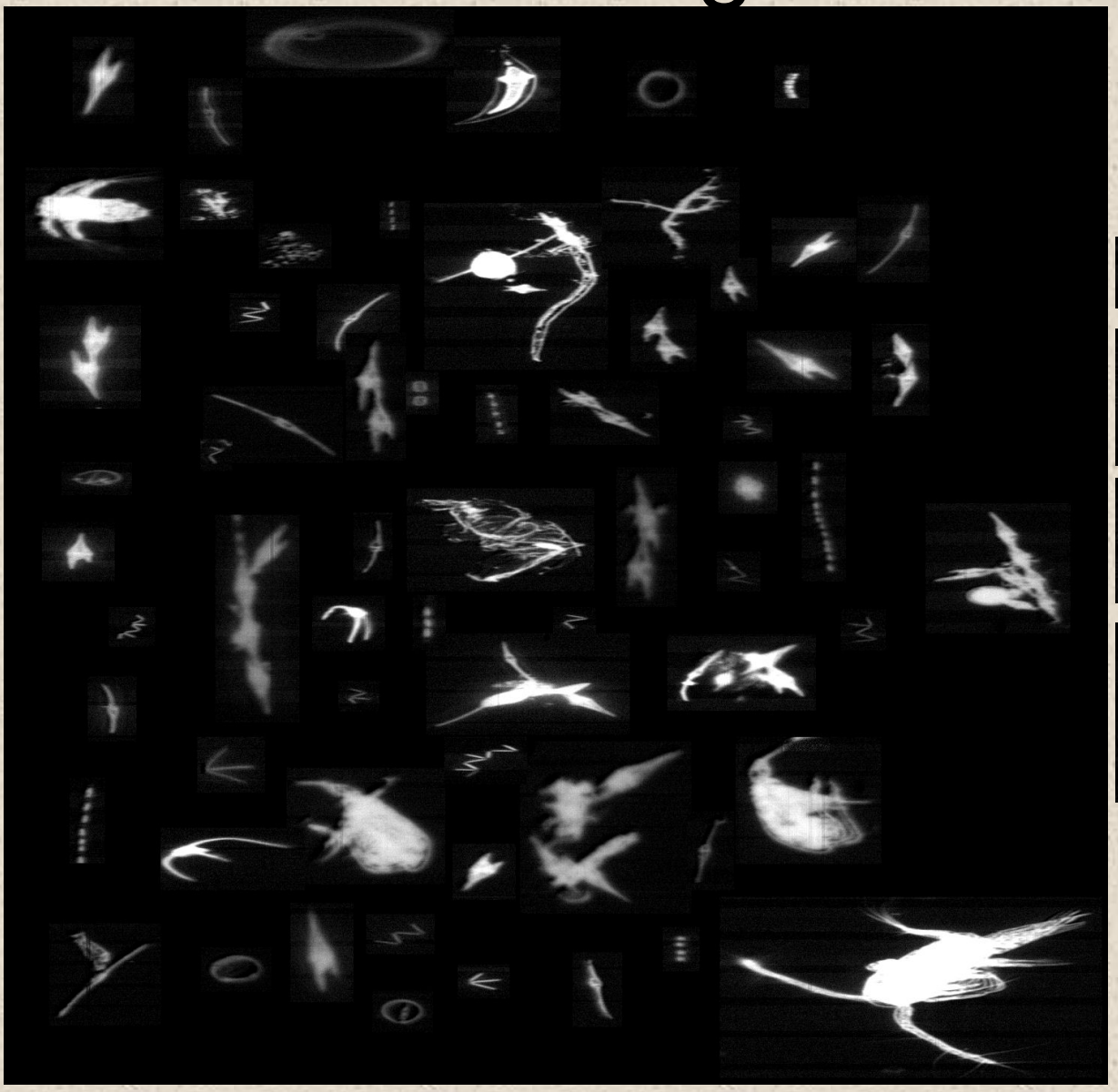
June 2005

Microplankton

(composite)

70 micron

Its hard



11

8

13

6

N

Human factors

Human performance in identifying and sorting organisms is affected by several psychological factors:

(a) Human short-term memory limit of five to nine items,
(b) Fatigue and boredom: severe loss of categorisation performance (> 50% error!!)
(c) Recency effects where a new classification is biased toward those in the set of most recently labels and
(d) Positivity bias, where specimen identification is biased by one's expectations of the species likely to be present in the sample.

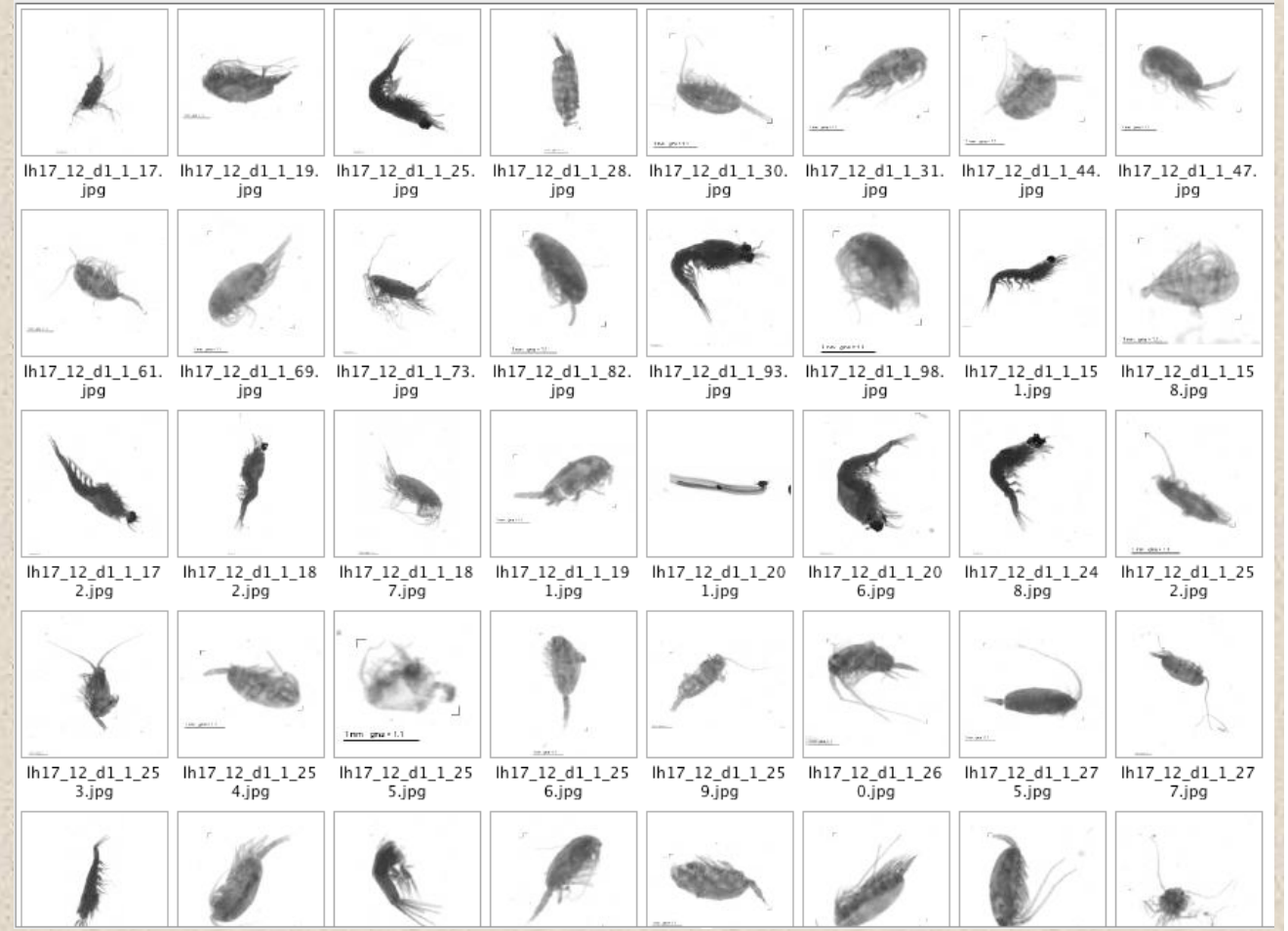
Context and other prior cues to category speed recognition significantly.

Human Performance

Categorisation task	**Self consistency within panel individuals**	**Panel consistency across individuals performance**	**Machine**	**Specimen Data size**
Ceratium *longipes* & C. *arcticum*	expert: 94-99% 'book' expert 67-83% - 8 experts	95% to 43%	99%	60 epidiascope tracings (Yarranton, 1967)
5 spp. Tintinnidae	N/A	91% - 6 experts	87%	100 printed photomicrographs
3 spp. Fish larvae	N/A	N/A	70%	1562 computer analysed photomicrographs
5-9 spp. Dinoflagellates	N/A	91-95% - 6 experts	67%	30 computer displayed photomicrographs
23 spp. Dinoflagellates	N/A	83-86% - 6 experts	83%	310 computer displayed photomicrographs

Ocean Weather Station India 1975

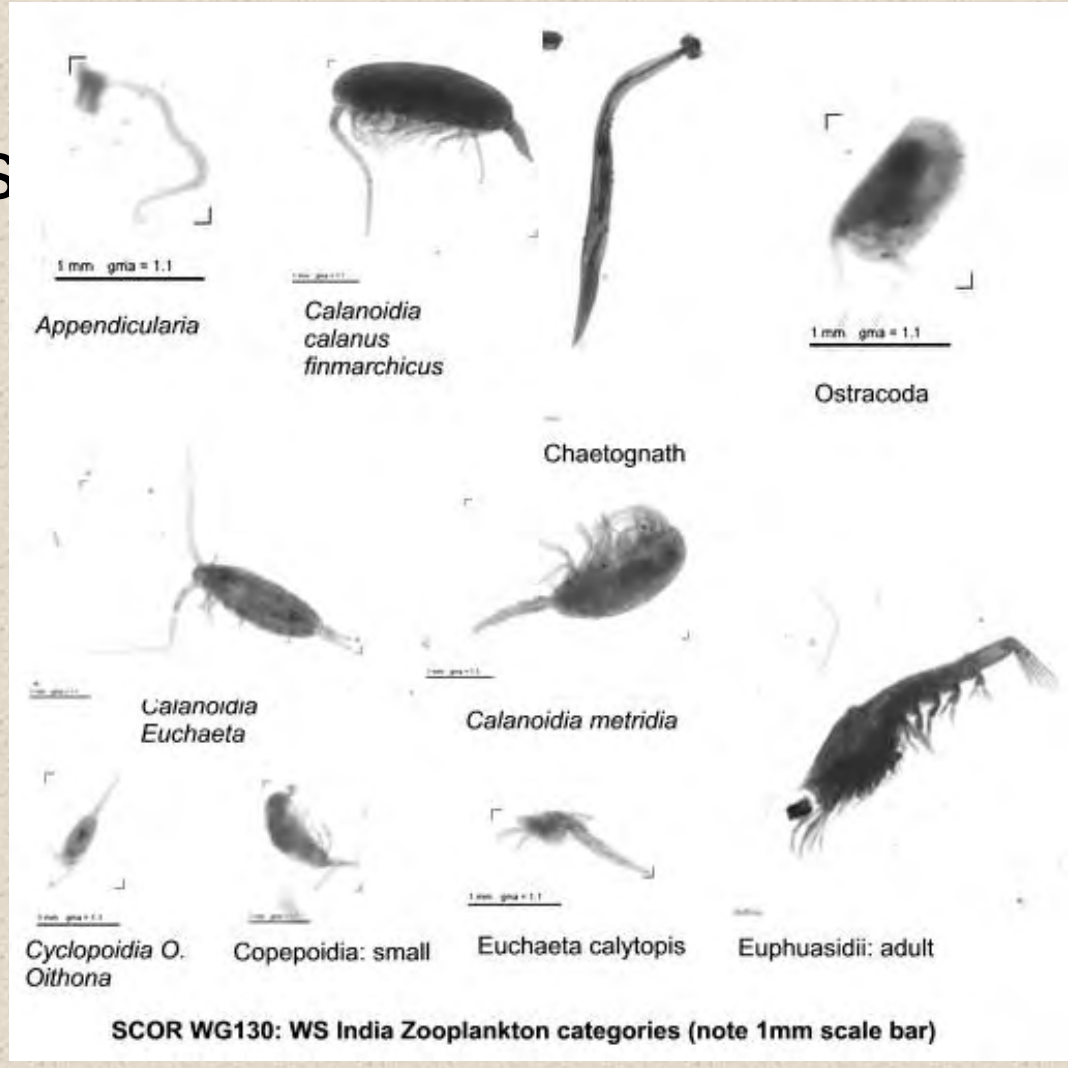
- ✓ SCOR WG130 experiment
- ✓ Zooplankton identification
  - ✓ by human analyst and
  - ✓ by Zooscan machine
- ✓Original data Bob Williams from OWS India May 1975
  - ✓0-500m LHPR trawl
  - ✓22 net samples
  - ✓6 for humans & machine comparison



Source: SCOR WG130: Automatic Plankton Identification

WS India categories

- Fixed samples in inspection trays
- Mixture of taxa and genera
- Discrimination
  - Some easy
  - Some hard



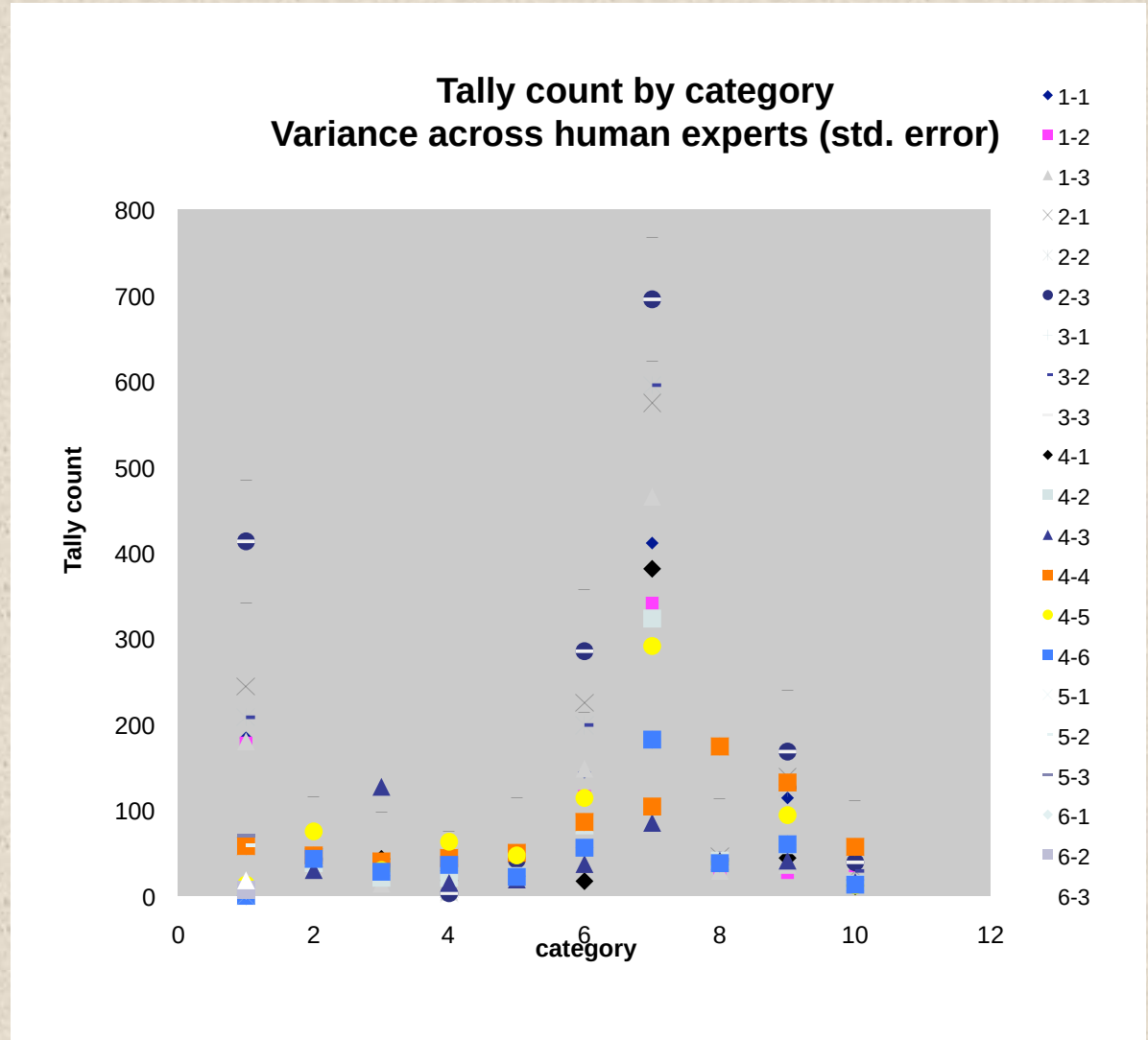
SCOR WG130: WS India Zooplankton categories (note 1mm scale bar)

OWS India: Analyst tally count plot

21 experienced analysts
over 700 specimens,
in 6 samples

Categories:

1. Appendicularia
2. Chaetognatha
3. *Calanus finmarchicus*
4. *Euchaeta norvegica*
5. *Metridia lucens*
6. Oithona spp.
7. Copepoda: small
8. Euphausiacea: Adults + furcilia
9. Euphausiacea: Calyptopis
10. Ostracoda



Source: SCOR WG130: Automatic Plankton Identification - unpublished

Human Performance Conclusions

- People are not perfect identification machines
  - Usually good at tallying (specimen counting)

    15 of 21 > 90% repeatable [mean 700 specimens]
  - Can be inconsistent at binning (identifying)

    13 of 21 > 90% self-consistent
    - Experts are highly self-consistent >0.9 ICC (Intraclass correlation coefficient)
    - Novices are not self-consistent 0.03 – 0.76 ICC
- Inter-analyst variation is high

Open Forum & Discussion

- Machine performance
  - Throughput > 1,000 specimens per hour
  - false positive/false negatives (confusions)
    - 50% to 95% binning by category
    - 100% tally count
- Human performance
  - Throughput <300 per hour
  - 70-96% self consistency at tally counting
  - Can be poor (<80%) at binning consistency

The Future

- Challenges include
  - Validating training data using scarce human expert resources
  - Widespread Uptake of automation
- Funding research in this area
  - Cross disciplinary
  - Difficult for referees to review
  - Needs more support

Please join the RAPID group

Discuss!

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