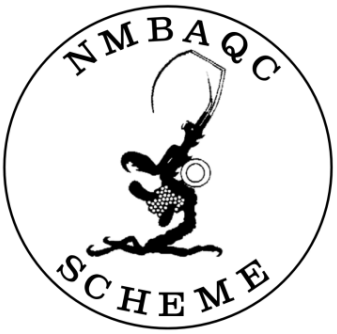

NMBAQC

Sir Alister Hardy Foundation for Ocean Science

NE Atlantic Marine Biological
Analytical Quality Control Scheme

Zooplankton UK Ring Test 2016/2017

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Introduction

In September 2016 SAHFOS, on behalf of the NE Atlantic Marine Biological Analytical Quality Control (NMBAQC) scheme, sent out a call of interest for the first official Zooplankton Ringtest to organisations known to be involved in zooplankton research.

Zooplankton are a Marine Strategy Framework Directive (MSFD) indicator group, however, there are no current standards for their sampling. As such, a quality control mechanism for the correct identification was identified by the Healthy and Biologically Diverse Seas Evidence Group (HBDSEG) to be one of the areas that NMBAQC should investigate. SAHFOS is a world leader in plankton research and has a unique plankton data set stretching over 80 years, which has been collected using the Continuous Plankton Recorder (CPR). SAHFOS has global zooplankton identification expertise from the major oceans and is therefore ideally placed to run the zooplankton component.

A questionnaire was sent out in 2013 to assess current zooplankton sampling, and need for quality control. A report on the results can be downloaded from the NMBAQC web site: http://www.nmbaqcs.org/media/1279/results-zoopktn-questionnaire.pdf. As a result from the questionnaire, a trial UK wide ring test was held in 2014/2015. The outcomes were that a zooplankton ringtest would be a useful exercise. The original format only included an identification component and written quiz: one of the recommendations from the trial test was that an enumeration component would be useful. The full report can be downloaded from the NMBAQC web site: http://www.nmbaqcs.org/media/1606/zooplankton-trial-ring-test-2015-report.pdf

A ring test comprising of 10, single taxon, tubed zooplankton specimens for identification (from the Northeast Atlantic); 10 written questions and a bead enumeration test were sent out in November 2016. 19 participants from 12 laboratories took part in the ring test. Most of these were UK competent monitoring agencies and contractor laboratories, but also one Italian laboratory, one Singapore laboratory and one Abu Dhabi laboratory signed up. Participants were given 8 weeks to complete their test, and results were judged by one of SAHFOS' senior taxonomists.

Materials and Methods

SAHFOS acquired various net mixed zooplankton samples from different areas of the North Atlantic. From these samples, single species were picked and verified by an analyst, and subsequently confirmed by the senior analyst. Single species were then transferred to centrifuge tubes and the success of the transfer was tested by checking the tube under the microscope to ensure the specimen was now in the tube.

The written quiz was prepared by the SAHFOS senior analyst.

For the enumeration component, as a trial, a collection of beads of different sizes and colours were included for sorting and counting. The rational for this was that having to classify, sort and count the beads might resemble the process in which zooplankton analysts tackle their own samples: there is natural variation in the beads, both in size and colour, but distinct 'species' or 'taxa' can be separated.

As what could determine a 'taxa' depended on bead colour, , a colour blindness test was also included (see Annex 3: Participants return form.), to ensure that participants were not unfairly judged.

To ensure that the level of the analyst was assessed correctly, participants were asked to provide an explanation for each of their identifications. Thus, if the explanation was correct, but the identification was not, the analyst could still get some points.

Results

Following on from participant feedback from the first NMBAQC zooplankton ring test in 2015, the ring test should be community driven and 'self-policed'. To help accomplish this, a results workshop (hosted by SAHFOS) was organised and took place in Plymouth on 7th March 2017. Thirteen participants from 10 laboratories attended the workshop where results were discussed and a consensus was reached.

The results from the zooplankton ring test are summarised in table 1-4 below, and discussed in detail in the remainder of this report. Correct answers are highlighted in green, incorrect in red, and partially correct and requiring discussion in amber.

For the specimen test, the worst identified species was *Pseudodiaptomus marinus*. Participants mistook this invasive species for *Aetideus armatus, Centropages hamatus, Pseudocalanus elongatus* and *Eurytemora affinis*. The overall score for this species was 55%, showing the great difficulty level. In contrast *Limacina retroversa,* a common coiled shell thecosome, was correctly identified by all the participants.

For the written test, the most poorly answered question was the identification of the order Spumellaria, with answers ranging from Globigerinida, Holacanthida, Rotaliida, Radiozoa and Heliozoa. The correct answer was Spumellaria, although Radiozoa or Radiolaria were marked as partly correct as the participant had correctly recognised that the specimen belonged to the radiolarian group. A question about the terminology and counting of spines protocol used in copepod leg descriptions was included in the test. Despite this detailed technique not being needed for the everyday identification of common copepods, and unheard of by many participants, all answered the question correctly and agreed it provided a useful training exercise during the workshop discussions.

To ensure fairness, a colour blindness test was part of the bead enumeration exercise to ensure participants could distinguish the different coloured beads. Three participants showed possible signs of colour blindness, but all analysts were able to distinguish the different coloured beads. The bead enumeration test results were not taken into account for the final scores of participants. This was a trial enumeration test, and after discussing the results in the workshop it was felt that additional information would be required to make this a permanent feature. The enumeration component would benefit from an identification key, higher numbers of beads for counting, and criteria when to disregard counts (e.g. count if more than 50% of specimen is present).

	Analyst code										
Overall score	Zo-2301-01	Zo-2301-02	Zo-2301-03	Zo-2301-04	Zo-2302-01	Zo-2303-01	Zo-2304-01	Zo-2304-02	Zo-2305-01	Zo-2306-01	Total (%)
per participant	34	35.5	35	28.5	35	33.5	22.5	22.5	35	29	
% of maximum	94.4%	98.6%	97.2%	79.2%	97.2%	93.1%	62.5%	62.5%	97.2%	80.6%	86.0%
per laboratory	33.25				35	33.5	22.5		35	29	
% of maximum	92.4%				97.2%	93.1%	62.5%		97.2%	80.6%	85.4%

	Analyst code									
Overall score	Zo-2307-01	Zo-2307-02	Zo-2308-01	Zo-2309-01	Zo-2310-01	Zo-2310-02	Zo-2311-01	Zo-2312-01	Zo-2312-02	Total (%)
per participant	30.5	34	27.5	22	35	31	34.5	33.5	29.5	
% of maximum	84.7%	94.4%	76.4%	61.1%	97.2%	86.1%	95.8%	93.1%	81.9%	86.0%
per laboratory	32.25		27.5	22	33		34.5	31.5		
% of maximum	89.6%		76.4%	61.1%	91.7%		95.8%	87.5%		85.4%

Table 1: Overall scores per participant and per lab

	Analyst code										
Question	Zo-2301-01	Zo-2301-02	Zo-2301-03	Zo-2301-04	Zo-2302-01	Zo-2303-01	Zo-2304-01	Zo-2304-02	Zo-2305-01	Zo-2306-01	Total (%)
1. Cnidaria terminology	3.5	4	4	4	4	3.5	4	4	4	3.5	94.7%
2. Ostracod/Cirripede cypris differences	3	3	3	3	2	3	2	2	3	0	82.5%
3. Spines terminology	2	2	2	2	2	2	2	2	2	2	100.0%
4.Match the ♀ and ♂	5	5	5	5	5	5	5	5	5	5	97.9%
5. Subclass Cerantharia	2	2	1	2	2	2	2	2	2	2	81.6%
6. D odd one out: Phylum Brachiopoda.	2	2	2	1	2	2	2	2	2	2	89.5%
7. Spumellaria	0.5	2	2	0.5	2	0	0	0	2	2	61.8%
8. Ammodytidae	2	2	2	0	2	2	0	0	2	0	78.9%
9. *Jaxea nocturna*	2	2	2	2	2	2	0	0	2	2	78.9%
10. Zoological classification	2	2	2	2	2	2	0	0	2	2	78.9%
Total score	24	26	25	21.5	25	23.5	17	17	26	20.5	
% of maximum	92.3%	100.0%	96.2%	82.7%	96.2%	90.4%	65.4%	65.4%	100.0%	78.8%	86.1%

Table 2: Written test scores per participant

	Analyst code									
Question	Zo-2307-01	Zo-2307-02	Zo-2308-01	Zo-2309-01	Zo-2310-02	Zo-2310-01	Zo-2311-01	Zo-2312-01	Zo-2312-02	Total (%)
1. Cnidaria terminology	3.5	3.5	4	4	4	3	3.5	4	4	94.7%
2. Ostracod/Cirripede cypris differences	2	2	3	1	3	3	3	3	3	82.5%
3. Spines terminology	2	2	2	2	2	2	2	2	2	100.0%
4.Match the ♀ and ♂	5	5	5	3	5	5	5	5	5	97.9%
5. Subclass Cerantharia	1	2	2	0	2	1	1	2	1	81.6%
6. D odd one out: Phylum Brachiopoda.	1	2	2	0	2	2	2	2	2	89.5%
7. Spumellaria	0	2	0	2	2	2	2	2	0.5	61.8%
8. Ammodytidae	2	2	2	2	2	2	2	2	2	78.9%
9. *Jaxea nocturna*	2	2	0	0	2	2	2	2	2	78.9%
10. Zoological classification	2	2	2	2	2	2	2	0	0	78.9%
Total score	20.5	24.5	22	16	26	24	24.5	24	21.5	
% of maximum	78.8%	94.2%	84.6%	61.5%	100.0%	92.3%	94.2%	92.3%	82.7%	86.1%

Table 2 (continued): Written test scores per participant

	Analyst code										
Specimen	Zo-2301-01	Zo-2301-02	Zo-2301-03	Zo-2301-04	Zo-2302-01	Zo-2303-01	Zo-2304-01	Zo-2304-02	Zo-2305-01	Zo-2306-01	Total (%)
Specimen 1	1	1	1	0	1	1	0	0	0	1	55%
Specimen 2	1	0.5	1	1	1	1	0	0	1	0	68%
Specimen 3	1	1	1	0	1	1	0	0	1	1	76%
Specimen 4	1	1	1	1	1	1	1	1	1	1	100%
Specimen 5	1	1	1	1	1	1	1	1	1	1	95%
Specimen 6	1	1	1	1	1	1	1	1	1	1	92%
Specimen 7	1	1	1	0	1	1	1	1	1	0.5	82%
Specimen 8	1	1	1	1	1	1	0.5	0.5	1	1	89%
Specimen 9	1	1	1	1	1	1	0	0	1	1	89%
Specimen 10	1	1	1	1	1	1	1	1	1	1	92%
Total score	10	9.5	10	7	10	10	5.5	5.5	9	8.5	84%
% of maximum	100%	95%	100%	70%	100%	100%	55%	55%	90%	85%	

	Analyst code									
Specimen	Zo-2307-01	Zo-2307-02	Zo-2308-01	Zo-2309-01	Zo-2310-01	Zo-2310-02	Zo-2311-01	Zo-2312-01	Zo-2312-02	Total (%)
Specimen 1	1	0.5	0	1	0	0	1	1	0	55%
Specimen 2	1	1	0.5	1	1	0.5	1	0.5	0	68%
Specimen 3	1	1	0	1	1	0.5	1	1	1	76%
Specimen 4	1	1	1	1	1	1	1	1	1	100%
Specimen 5	1	1	0	1	1	1	1	1	1	95%
Specimen 6	1	1	1	0	1	0.5	1	1	1	92%
Specimen 7	1	1	0	0	1	1	1	1	1	82%
Specimen 8	1	1	1	0	1	1	1	1	1	89%
Specimen 9	1	1	1	1	1	1	1	1	1	89%
Specimen 10	1	1	1	0	1	0.5	1	1	1	92%
Total score	10	9.5	5.5	6	9	7	10	9.5	8	84%
% of maximum	100%	95%	55%	60%	90%	70%	100%	95%	80%	

Table3: Specimen test scores per participant

	Analyst code										
Bead	Zo-2301-01	Zo-2301-02	Zo-2301-03	Zo-2301-04	Zo-2302-01	Zo-2303-01	Zo-2304-01	Zo-2304-02	Zo-2305-01	Zo-2306-01	Total (%)
Bead 1	Correct	Correct	Correct	Correct	Correct	Correct	Correct	Correct	Correct	Correct	100.0%
Bead 2	Correct	Correct	Correct	Correct	Correct	Correct	Correct	Correct	Correct	Correct	100.0%
Bead 3	Correct	Incorrect	Correct	Correct	Correct	Incorrect	Correct	Correct	Correct	Correct	89.5%
Bead 4	Correct	Correct	Incorrect	Correct	Correct	Correct	Questionable	Incorrect	Correct	Correct	78.9%
Bead 5	Correct	Correct	Correct	Correct	Correct	Correct	Correct	Correct	Questionable	Questionable	89.5%
Bead 6	Correct	Correct	Correct	Correct	Correct	Correct	Correct	Correct			84.2%
Bead 7	Correct	Correct	Correct	Correct	Correct	Correct	Correct	Correct	Correct	Correct	100.0%
Bead 8	Correct	Correct	Correct	Correct	Correct	Correct	Correct	Correct	Questionable	Correct	89.5%
Bead 9	Correct	Correct	Correct	Correct	Correct	Correct	Correct	Correct		Correct	89.5%
Bead 10	Correct	Correct	Correct	Correct	Correct	Correct	Correct	Correct	Correct	Correct	100.0%
Bead 11	Correct	Correct	Correct	Correct	Correct	Correct	Correct	Correct	Correct	Correct	100.0%
Bead 12	Correct	Incorrect	Correct	Correct	Correct	Correct	Correct	Correct	Correct	Correct	89.5%
Total number of beads	39	39	38	39	39	40	40	40	39	39	

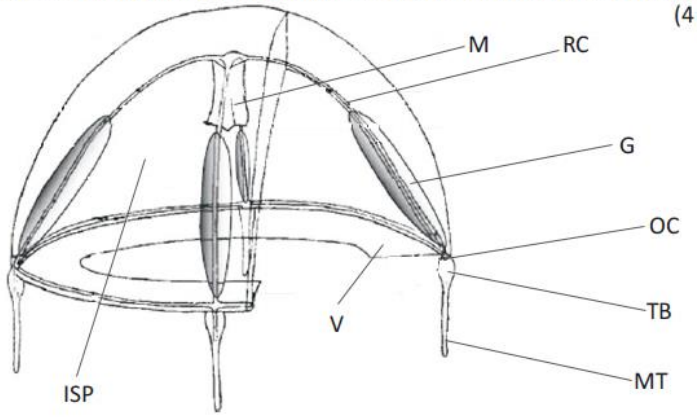
Table 4: Bead enumeration scores per participant

	Analyst code									
Bead	Zo-2307-01	Zo-2307-02	Zo-2308-01	Zo-2309-01	Zo-2310-01	Zo-2310-02	Zo-2311-01	Zo-2312-01	Zo-2312-02	Total (%)
Bead 1	Correct	Correct	Correct	Correct	Correct	Correct	Correct	Correct	Correct	100.0%
Bead 2	Correct	Correct	Correct	Correct	Correct	Correct	Correct	Correct	Correct	100.0%
Bead 3	Correct	Correct	Correct	Correct	Correct	Correct	Correct	Correct	Correct	89.5%
Bead 4	Incorrect	Correct	Correct	Correct	Correct	Correct	Questionable	Correct	Correct	78.9%
Bead 5	Correct	Correct	Correct	Correct	Correct	Incorrect	Correct	Correct	Correct	89.5%
Bead 6	Correct	Correct	Correct	Correct	Correct	Incorrect	Correct	Correct	Correct	84.2%
Bead 7	Correct	Correct	Correct	Correct	Correct	Correct	Correct	Correct	Correct	100.0%
Bead 8	Correct	Incorrect	Correct	Correct	Correct	Correct	Correct	Questionable	Correct	89.5%
Bead 9	Correct	Correct	Correct	Correct	Correct	Correct	Correct		Correct	89.5%
Bead 10	Correct	Correct	Correct	Correct	Correct	Correct	Correct	Correct	Correct	100.0%
Bead 11	Correct	Correct	Correct	Correct	Correct	Correct	Correct	Correct	Correct	100.0%
Bead 12	Correct	Correct	Correct	Incorrect	Correct	Correct	Correct	Correct	Correct	89.5%
Total number of beads	40	38	39	38	39	39.5	40	39	39	

Table 4 (continued): Bead enumeration scores per participant

Question 1

Please correctly label the cnidarian diagram below with the following terms: (4 points)



G = Gonad
V = Velum
ISP = Interradial space
M = Manubrium
OC = Ocellus/ocelli
TB = Tentacle bulb
MT = Marginal tentacle
RC = radial canal

Most participants correctly identified the different features, although some mistakenly took the circular canal for the radial canal;the interradial space was the other feature commonly misidentified. The overall score was 94.7%.

Question 2

2. Ostracods and cirripede cypris larvae are both bivalved and can sometimes be misidentified for each other. Please describe how you would distinguish between the two. (3 points)

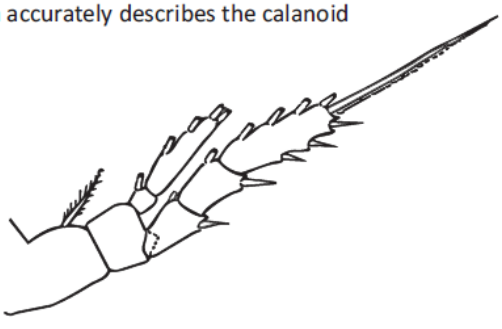
Cypris: oil globules, biramous limbs which are positioned to the rear, eyes clearly visible
Ostracod: antennal notch, no oil globules, most limbs uniramous and evenly spaced along body

The question was worth three points, which was an indication that three different aspects were required. Some participants only focussed on one aspect, others were not specific enough. Full points were given to those identifying the three points above. The overall score was 82.5%.

Question 3

3. Using the accepted numbering convention for describing spiny and setal elements on copepod swimming limbs (Sewell 1949), please indicate which formula accurately describes the calanoid copepod limb below? (2 points) .....

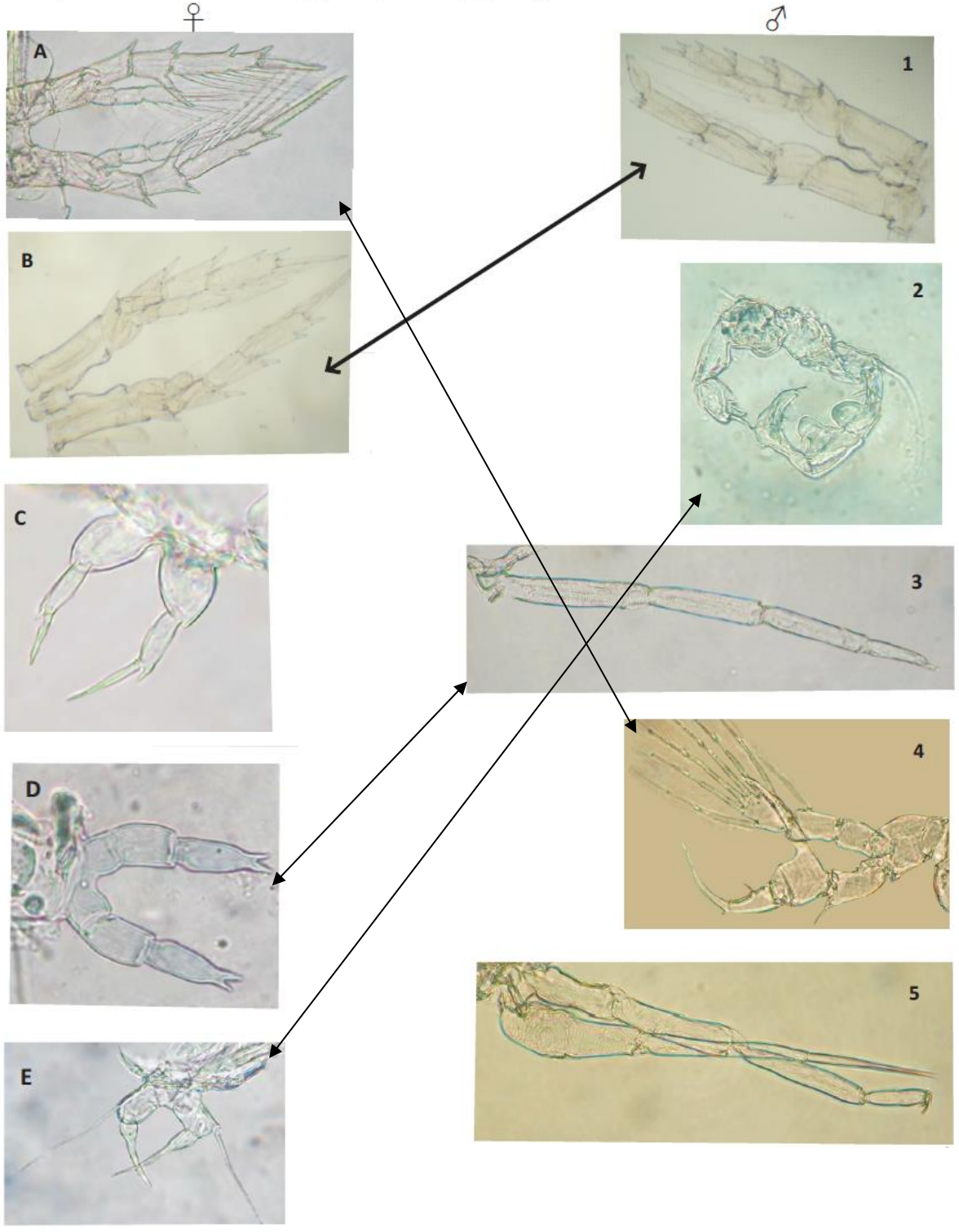
	coxa	basis	exopod	endopod
1	0-I	0-0	1-I; 1-I; 3, 1, IV	0-I; 0, II, II
✓ 2	0-1	0-0	I-1; I-1; III, I, 4	0-1; 0, 2, 2
3	1-0	0-0	1-I; 1-I; 4, I, III	1-0; 2, 2, 0
4	0-1	0-0	0-1; 0, 2, 2	I-1; I-1; III, I, 4



Roman numerals indicate spines, Arabic numerals indicate setae, and the number sequence starts from outer to inner. Everybody had correctly answered this question.

Question 4

4. Connect the matching copepod fifth pairs of limbs (P5), for example B matches 1.
Which pair are without a matching species partner?(4 points) .....



Most participants correctly linked the males and females. A few incorrectly matched the *Paracalanus* female P5 with the male *Clausocalanus* P5 (the number of segments in the P5 is important). The overall score was 97.9%.

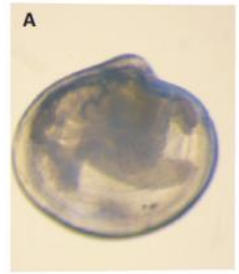
Question 5

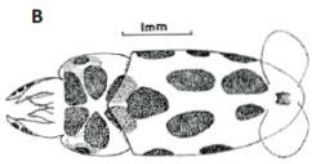
5. What type of larva is pictured in the photographs above and according to the World Register for Marine Species, to which subclass does this common larva belong to? (2 points)

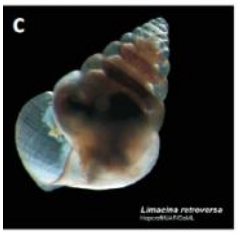
Class: Anthozoa
Subclass: **Ceriantharia**
(Cerianthus lloydii)

Most participants correctly identified these as anthozoan larvae. However, some participants did not look at the WoRMS site, as specified in the question, but checked Wikipedia instead, where it says the subclass would be Hexacoralia. At the time of the question, at www.marinespecies.org it was described as Ceriantharia, which was the correct answer. The overall score was 81.6%.

Question 6







6. Name the odd one out and why. (2 points)

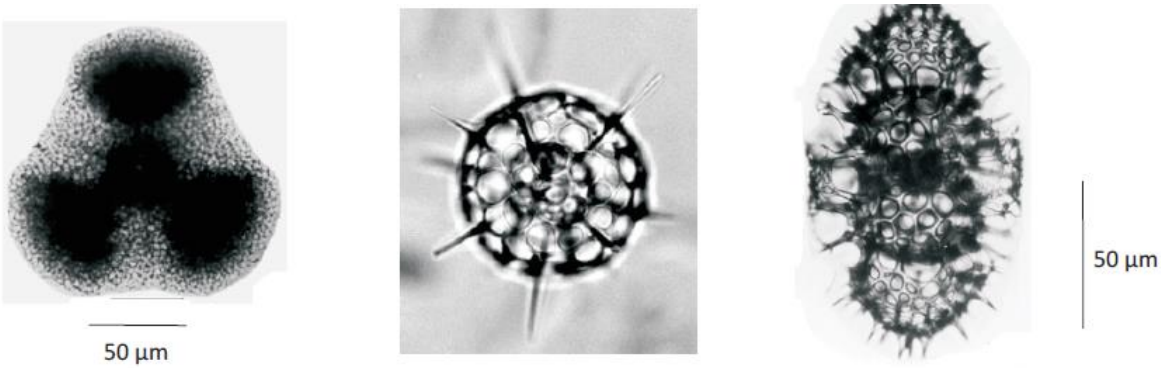
D is the odd one out as belongs to the Phylum Brachipoda, all others are Mollusca

.....

.....

Some participants came up with other answers, which were technically correct (e.g. C, because it is the only holoplanktonic specimen. All others are meroplankton), however, not the answer we were looking for. Those answers did get points, but not the full marks. The overall score was 89.5%.

Question 7

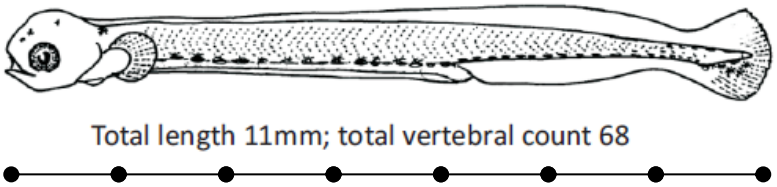


7. What taxonomic order do these single-celled organisms belong to? (2 points) ....Spumellaria.....

Many participants struggled with this question and answers ranged from Globigerinida, Holacanthida, Rotaliida, Radiozoa and Heliozoa. The correct answer was Spumellaria, although Radiozoa or Radiolaria was marked as partly correct. The overall score was 61.8%, the lowest of all the written quiz questions.

Question 8

8. Which family does this fish larva belong to? (2 points)



Ammodytidae
Position of anus, pre-anal and post anal body length is 4:3 (Pholidae 6:5, Clupeidae >4:1)
51-68 vertebrae

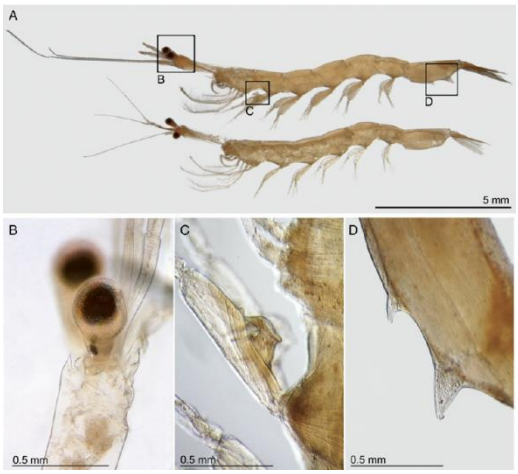
The correct answer was Ammodytidae, although participants incorrectly identified this as Engraulidae, Clupeidae and Pholidae. The pre-anal to post anal body length is different in these. The overall score was 78.9%.

Question 9

9. Please give the genus and species name of the organism opposite

(2 points)

Jaxea nocturna



For comparison (image on the left): These are pictures of *Lucifer hanseni*. Note the difference in the stalked eyes and limb structure

The correct answer was *Jaxea nocturna*. Some participants incorrectly identified this as *Lucifer hanseni*. The limb structure and stalked eyes are different in these, as illustrated above. The overall score for this question was 78.9%.

Question 10

10. Following recent phylogenetic analysis a species of copepod, *Qualiticontrol schemi*, has been reclassified and has been placed in the existing genus of *Analytica*.
The first person to originally describe this copepod was Spongebob in 1999. The author of the paper describing the recent changes is Squarepants, published in 2016.

Taking all of this into account, which of the options below is the correct way to refer this species? .....
(2 points)

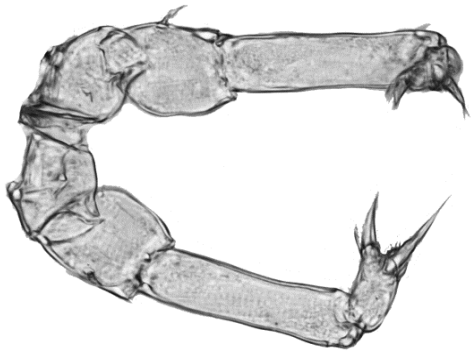
a) *Qualiticontrol schemi* Spongebob 1999
b) *Qualiticontrol schemi* (Spongebob 1999)
✓ c) *Analytica schemi* (Spongebob 1999)
d) *Analytica schemi* (Squarepants 2016)
e) *Analytica schemi* Squarepants 2016 sp. nov

The correct answer was C: the authority for the species does not change, but the genus name transfers to its original name. Most participants correctly answered this question; the overall score was 78.9%.

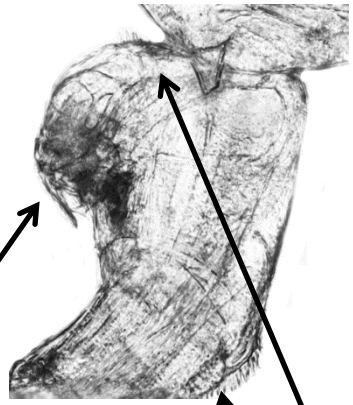
Specimen 1: Female *Pseudodiaptomus marinus*

- Small calanoid copepod (1-2mm)
- Metasome projection
- Elongated caudal rami
- Distinctive long fifth leg
- Flange and numerous denticles on ♀ genital segment

This was the most difficult identification. Participants mistook this invasive species for *Aetideus armatus, Centropages hamatus, Pseudocalanus elongatus* and *Eurytemora affinis*. The correct idenfication features are shown on this page. The overall score was 55%, the lowest score for any of the specimens.

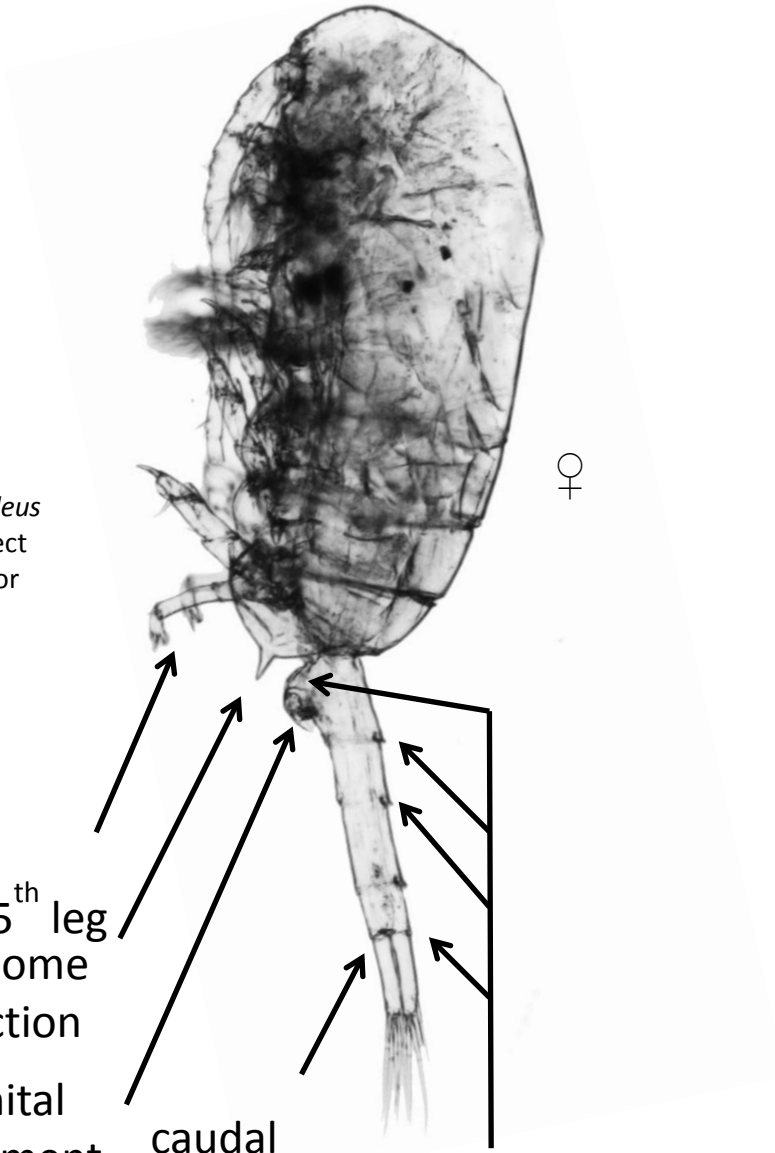


female 5th leg



flange

denticles



♀

5th leg

metasome projection

genital segment

caudal rami

denticles

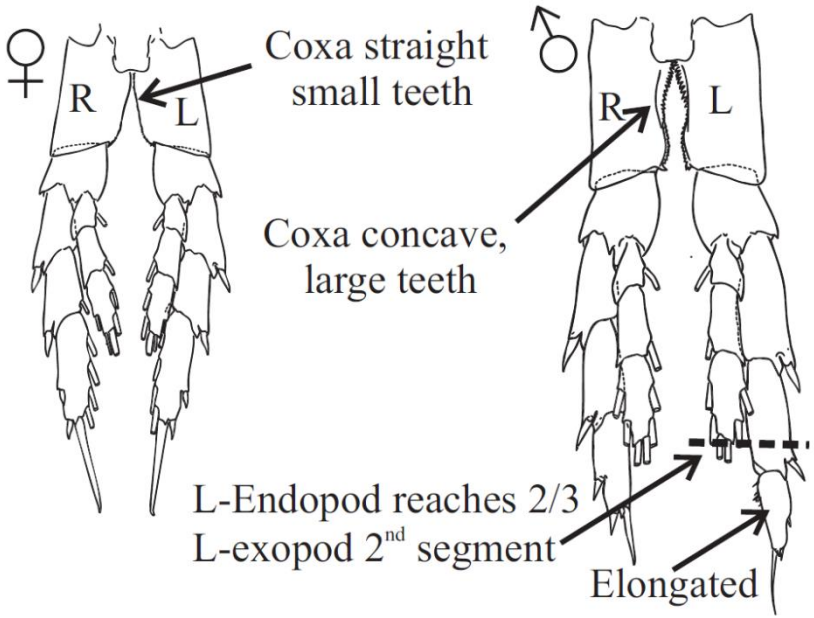
Specimen 2: Male *Calanus finmarchicus*

Many of the participants struggled with this male specimen. The identification features for male *Calanus finmarchicus and C. helgolandicus* are shown below. The overall score was 76%.

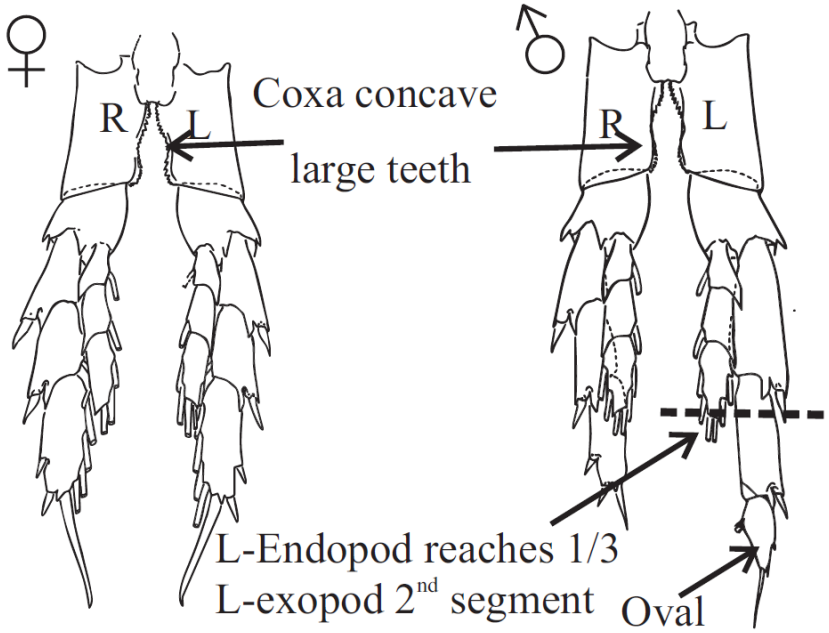
Specimen 3: Female *Calanus helgolandicus*

In contrast to the male *Calanus* species, 95% of the participants correctly identified the *Calanus helgolandicus* female.

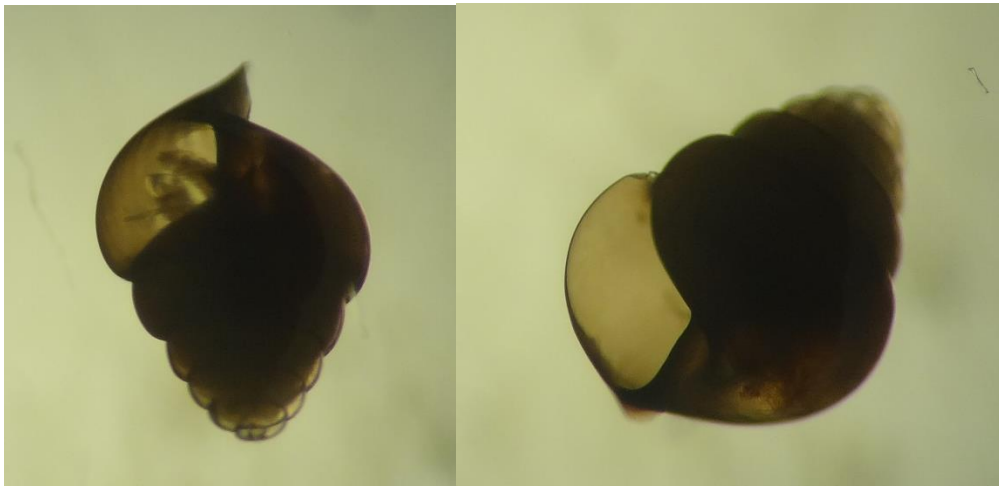
Calanus finmarchicus



Calanus helgolandicus



Specimen 4: *Limacina retroversa*



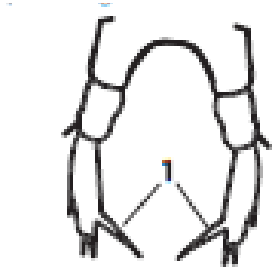
This specimen was correctly identified by all the participants.

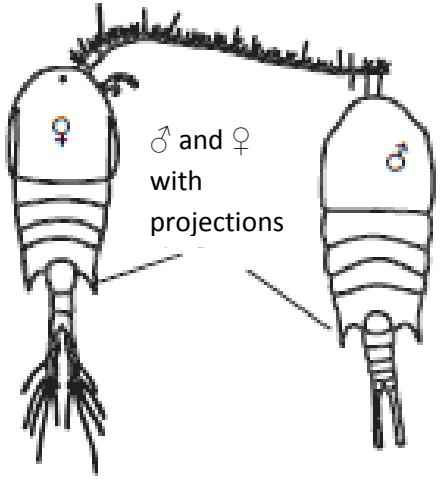
Specimen 5: Female *Temora stylifera*

The female *Temora stylifera* was correctly identified by 95% of the participants.

- body somewhat coffin-shaped with 4 segments
- End of metasome with projections
- P5 inner spine longer than terminal spines

Inner spine longer than terminal spine (1)

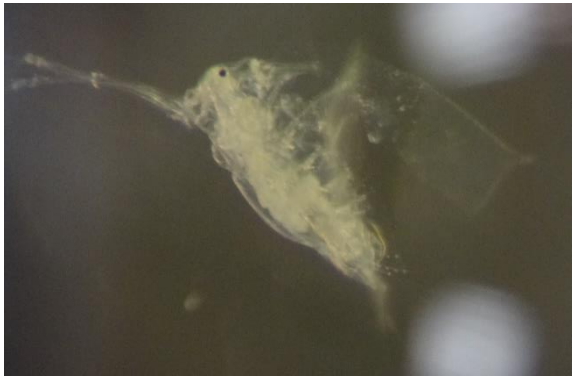




Specimen 6: *Penilia avirostris*

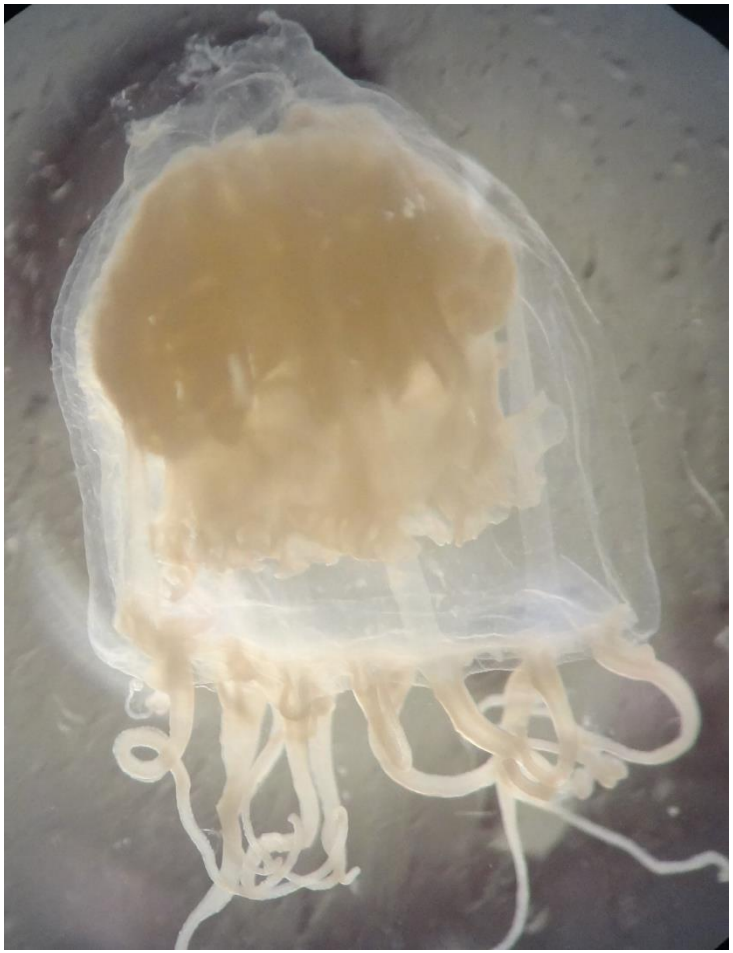
Of the participants, 92% correctly identified the cladoceran *Penilia avirostris*. One participant said the vial was empty, however, all vials were checked that they contained a specimen before they had been sent out, and upon receipt of samples analysts were given the opportunity to say if any of the samples had not been received via the return form.

- body and legs covered by bivalve carapace
- sensory setae terminal
- distinctive serrated edge of carapace



Specimen 7: *Leuckartiara octona*

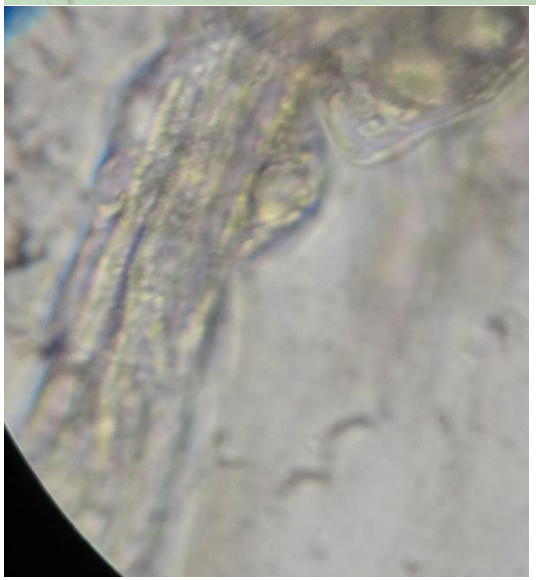
Leuckartiara octona was generally easily recognised, with 82% of the participants identifying it correctly. As with all cnidarians, features to look at are the outline shape of manubrium, shape of mouth, radial canals and tentacles. One participant thought it was a *Trichydra*, however, these have >30 tentacles.



- 4 broad radial canals, often with jagged outline, joined to manubrium by long mesenteries
- gonads are placed inter-radial on whole manubrium
- manubrium is flask shaped and not longer than the umbrella, without gastric peduncle and with crenulated lips
- usually 16 (varying from 8-23) hollow tentacles, with tentacle bulbs clasping umbrella to form a spur and with 1 ocellus. One to three rudimentary tentacle bulbs between adjacent tentacles
- Umbrella with a round/conical apical process.

Specimen 8: Female *Microcalanus pusillus*

Micocalanus pusilus was identified correctly by 89% of the participants. One participant identified it as *Bestiolina* spp, however, this species belongs in a different family (Paracalanidae) where the exopodite only has 2 spines, not three as in the Clausocalanidae.

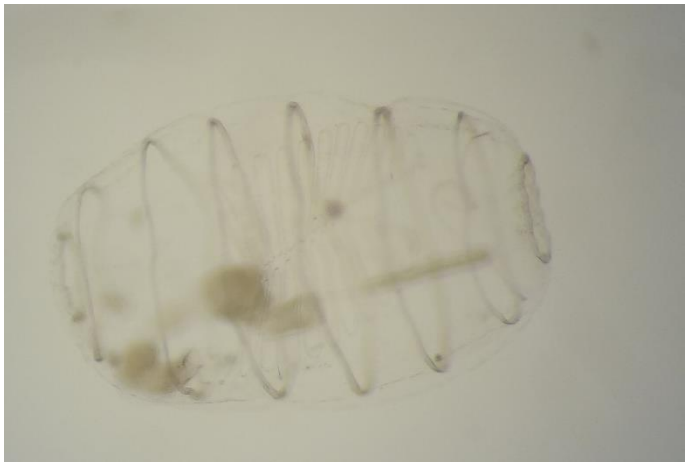


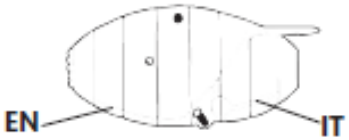
- small: 0.6-1.1 mm
- head rounded, rostrum with filaments. ♀ and ♂ A1 length variable, reaching from GS to end of caudal rami.
- metasome has 3 segments. Body small and compact, end of metasoma rounded
- Legs: P1 endopod with 4 setae. P2-P4 with 3 outer-edge spines equally spaced on 3rd segment of the exopod
- ♀ P5 absent

P2 with 3 outer edged spines

Specimen 9: *Doliolum nationalis*

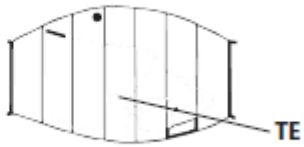
Doliolum nationalis was correctly identified by 89% of the participants. Two participants from the same laboratory thought it was *Metridia lucens*.





Genus Doliolum
EN between MB_II-V

Phoro/ Gonozooid (Blastazooid)



Size: up to 4 mm
GB: strongly arched, from MB_II dorsally to MB_V ventrally forming an arch at MB_V (2/3)
EN: short, between MB_II-IV
TE: short, horizontal, swollen behind MB_IV

EN- endostyle
IT- intestinal tube
GB- gill bar
MB- muscle band
TE-Testis

Specimen 10: Caridean decapod

The caridean decapod was correctly identified by 92% of the participants.

- **Carapace flattened**
- **Telson flattened, antennal exopod unsegmented or segmented near the distal end only**
- **Second telson spine usually with seven (zoea I) or eight setae (late zoeas) on each half of the posterior margin**
- **Telson posterior margin without median spine**
- **Abdominal segments without:**
  - **small dorsal spines on first segment,**
  - **third abdominal segment with long median dorsal spine,**
  - **fifth abdominal segment with ventral hook shaped spine**

Bead 1

One clear and blue large round bead was included in the test.

Bead 2

One blue glass medium round bead was included in the test.

Bead 3

Three pearlescent blue small doughnuts were included in the test. Bead 2 and bead 3 are different in size and colour.

Bead 4

Two small blue bugle beads were included in the test. A fragment of <50% of total size of this bead was also include in the enumeration test to find out how participants deal with broken beads.

Bead 5

Four white pearlescent medium round beads were included in the test.

Bead 6

Two white clear medium round beads were included in the test. Bead 5 and 6 have a different inner core, making bead 5 look slightly more pearlescent, and bead 6 slightly more white.

Bead 7

Four turquoise/ green medium round beads were included in the test.

Bead 8

Five brown/red medium beads were included in the test.

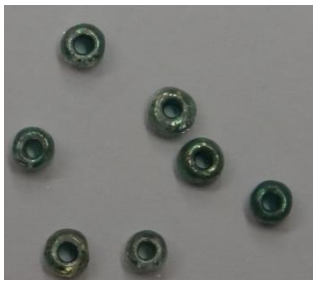
Bead 9

Three brown/red small beads were included in the test. Bead 8 and 9 are of similar colour, but are a different size (compare *Calanus* spp with *Pseudocalanus* spp.).

Bead 10

Five green small doughnut beads were included in the test.

Bead 11



Three green pearlescent small doughnut beads were included in the test. Bead 10 and 11 are of similar size, but have a slightly different colour

Bead 12

Six clear pearlescent with hinge of green doughnut beads were included in the test. Bead 12 was a bead that showed a lot of natural variation.

Total number of beads

There were 39 beads and one fragment in this test.

Conclusions/recommendations

Overall, the zooplankton ring test was deemed a success. It showed that the level of zooplankton identification amongst participants overall is very good, and that it was a useful training exercise.

The competent monitoring agencies all achieved a level of at least 89% in both tests. For the specimen test, the most difficult to ID proved to be the invasive species *Pseudodiaptomus marinus*. For the written test the most difficult question was to specify the taxonomic order of the single celled organisms, Spumellaria. The participants enjoyed the test, saying that it challenged them and that it was gauged at the right level of expertise.

The bead enumeration test was considered to be challenging, but it was unclear to the participants how a species was defined. In future test it was recommended that the bead enumeration would have a taxonomic key that defined bead species. The results of the test did show that most analysts agree on the differentiation in the beads, however, for the individual scoring of participants the enumeration was not taking into account.

For future ring tests it was recommended that the results of the tests get sent out before the workshop, so that participants can prepare better for the workshop and ask questions on the day. Other suggestions made were:

- Include juveniles of common copepods
- Include two of the same species
- Include more species in the specimens' test
- Include Echinodermata
- More focus on copepods other than Calanoida, include e.g. Cyclopoida
- Develop a taxonomic discrimination protocol (to which level should a species be taken)
- Include higher numbers in the enumeration test
- Have a two-day workshop with more time for participants' specimens
- Have better quality microscopes at the venue
- Hold the workshop at a venue that has better travel connections.

The zooplankton community felt a yearly zooplankton ring test would be too frequent, but going forwards a zooplankton ringtest once every two years was suggested.

There was one clear case of participants from one lab working together. This was discussed at the workshop, and, as a result, for this laboratory only one certificate was issued. We would like to remind participants that results are individual, and it is of benefit to do the test individually and not to confer with colleagues also taking the same test. The instructions clearly state that all results must be the analysts' own work.

All recommendations from the workshop will be taken into account in the next zooplankton ringtest.

We thank all the participants and their constructive feedback.

Annex 1: Participants information.

Sir Alister Hardy Foundation for Ocean Science
Scheme

NE Atlantic Marine Biological Analytical Quality Control

Zooplankton Ring Test 2016/2017

1. Introduction

In January 2013 SAHFOS, on behalf of the Northeast Atlantic Marine Biological Analytical Quality Control (NMBAQC) scheme, sent out a questionnaire to organisations known to be involved in zooplankton monitoring and research. The questionnaire was aimed at gauging current quality control mechanisms, as well as identifying possible interest in a zooplankton ring test, similar to the other NMBAQC components. Zooplankton are an MSFD indicator group and, as such, a quality control mechanism for the correct identification will be of crucial importance.

Subsequently a small UK trial ring test was carried out as a follow-on from the questionnaire, to assess current identification levels and to determine the best way forward for zooplankton quality control. The UK Trial Ring test concluded that a Zooplankton Ring Test including an enumeration component would be ideal. The final report of the UK Trial Ring Test can be downloaded from the NMBAQC web site.

2. Preliminary checks and deadlines

Upon receipt of the samples, every analyst must make sure that they have received everything listed in the Return Slip and checklist form (Return slip form.docx). Make sure that all the samples are intact and sealed properly and check that you have received the identification results log sheet (log form.xls) as an Excel workbook. Please complete the return slip and checklist form and send it by fax to (+44 1752 600015) or scan it and send it via e-mail to acfi@sahfos.ac.uk . A receipt of fax/e-mail is necessary for SAHFOS to ensure all samples have been received properly.

Once samples have been received, analysts have 8 weeks to complete the exercise and return the results to Astrid Fischer, NMBAQC/SAHFOS, The Laboratory, Citadel Hill, Plymouth, PL1 2PB; by e-mail (acfi@sahfos.ac.uk), fax, or post. If you decide to post your results, make sure first to make a copy of

them and then send the originals to the address above. The enumeration and identification results log sheet must be received by SAHFOS by Monday 9th January 2017.

Please note: Results received after this date will not be included in the final report. Also, if you are posting your results make sure to make a copy for your records before sending the originals.

3. Samples

The set consists of ten samples. The samples are preserved in 'sorting fluid', a mixture of 2% v/v propylene phenoxytol/18 % v/v propylene glycol in 80% v/v water, which can be irritating to eyes and skin. You will therefore need appropriate personal protection (gloves, laboratory coat and goggles).

You will need to use a dissecting microscope and possibly need to dissect parts of the zooplankton for identification. We recommend using forceps and or needles where appropriate. You are entitled to use any reference books available. For comparison purposes of this test, please use taxonomic names as accepted by the World Register of Marine Species and identify to the highest taxonomic level that you feel confident with. Please also provide a brief note on how you arrived at your identification and the feature/s used: for example, *Calanus helgolandicus*- was an adult female with curved inner margin on p5 coxa. Please ensure that scientific names and terms are spelled correctly.

Analysts will need to identify all ten samples to complete the test. Specimens selected for this test represent taxa which can be found in the North Atlantic and its marginal seas. Specimens have been picked from net haul samples and Continuous Plankton Recorder Survey samples.

4. Written quiz

In addition to the practical test, there is also a written quiz for you to complete. The quiz consists of 10 questions, all of which need to be answered. The results for the written test should be submitted by Monday 9th January 2017. Please ensure that scientific names and terms are spelled correctly.

5. Enumeration test

To test your enumeration skills, a sample containing beads is included. Like zooplankton, these beads will have some natural variety in their appearance, see figures below. You will need to describe the different types of beads. There are less than 15 different 'species' in the sample.

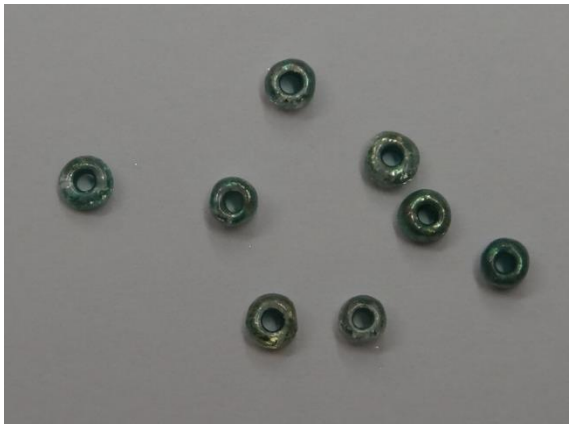


Figure 1: Examples of natural variation in the beads. Left: species A, possible descriptor: small pearlescent dark green doughnut beads. Right: species B, possible descriptor: small clear/green pearlescent doughnut beads.

This may be difficult for those with colour blindness, so this will be taken into account in the results, a colour blindness test is included in the return form for you to complete.

6. Workshop

A workshop will be held Tuesday 7th March 2017, date to be confirmed to inform the outcomes of the test and to discuss a way forward. The workshop will be held at SAHFOS, The Laboratory, Citadel Hill, Plymouth, PL1 2PB. There will be microscopes available and specimen samples from the ring test. If you have any samples of your own that you feel are of interest to the wider zooplankton community or zooplankton samples you struggle to identify, you are encouraged to bring these with you, for discussion at the workshop.

SAHFOS will analyse the results of the trial ring test, and participants of the workshop will be informed on these beforehand in a preliminary results report. After the workshop, a final report for NMBAQC will be produced.

7. Points to remember

1. All results must be the analysts' own work. Conferring with other analysts is not allowed.
2. Please ensure that scientific names and terms are spelled correctly.
3. The excel work sheet Log form.xls must be received by SAHFOS by Monday 9th January 2017

Annex 2: Participants checklist.

NMBAQC

Zooplankton Ring Test 2016/2017

RETURN SLIP AND CHECKLIST

Please ensure to complete the table below upon receipt of samples, then fax to + 44 1752 600015 or scan and e-mail to acfi@sahfos.ac.uk	
Analyst Name:	
Laboratory Name:	
Analyst Code Assigned :	
Contact Tel. No. / e-mail	

CHECKLIST OF ITEMS RECEIVED	**(Please circle the relevant answer)**	
Please enter Sample numbers received _____	YES	NO
Set of Instructions	YES	NO
Enumeration Test	YES	NO
Identification result log sheet **(Log form.xls)**	YES	NO
Written Quiz	YES	NO

I confirm that I have received the items as detailed above and that the materials were received in good working order.

(If any of the above items are missing, please contact acfi@sahfos.ac.uk)

SIGNED: _____

DATE: _____

Annex 3: Participants return form.

Specimen test

Analyst name	
LAB Code	
Analyst Code	

	Identification (scientific name)	**Reason for identification made**	**Additional comments**
Specimen 1			
Specimen 2			
Specimen 3			
Specimen 4			
Specimen 5			
Specimen 6			
Specimen 7			
Specimen 8			
Specimen 9			
Specimen 10			

Bead enumeration test

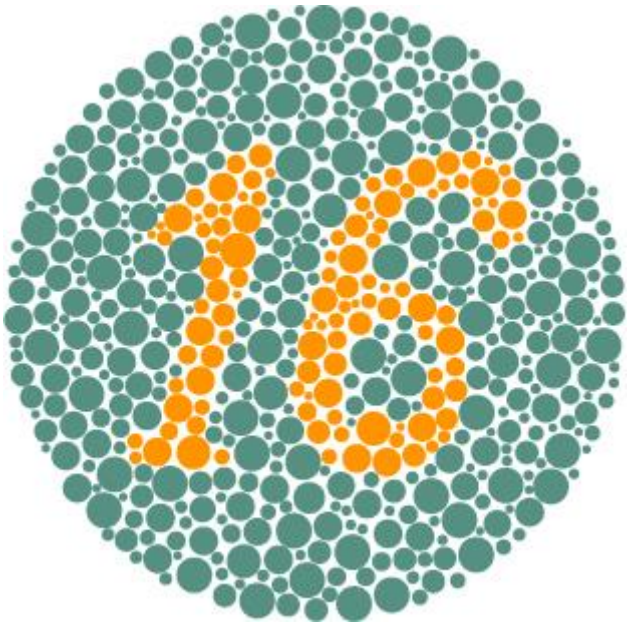
Analyst name	
LAB Code	
Analyst Code	

	Description of bead	**Number of beads of this 'species'**	**Additional comments**
Species 1			
Species 2			
Species 3			
Species 4			
Species 5			
Species 6			
Species 7			
Species 8			
Species 9			
Species 10			
Species 11			
Species 12			
Species 13			
Species 14			
Species 15			
Total number of beads			

Colour blindness test for bead enumeration

Analyst name	
LAB Code	
Analyst Code	

Please write the number you can see on the image next to the image



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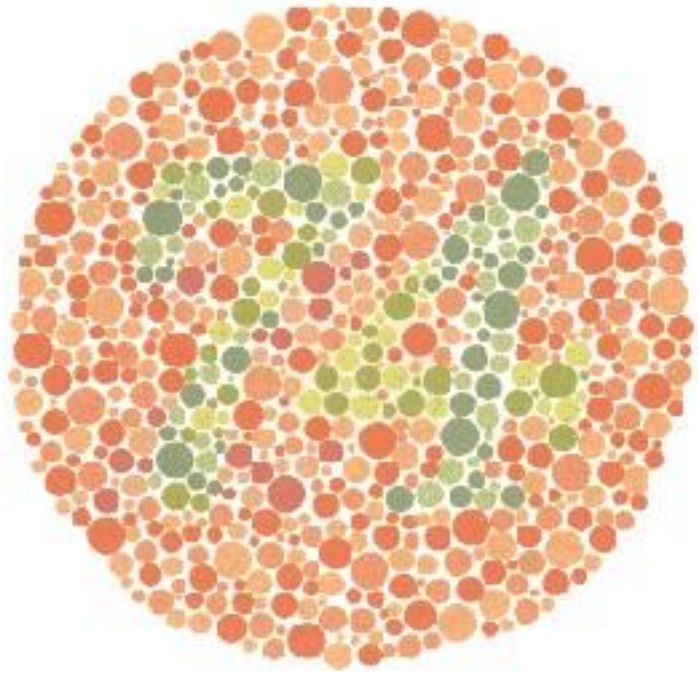

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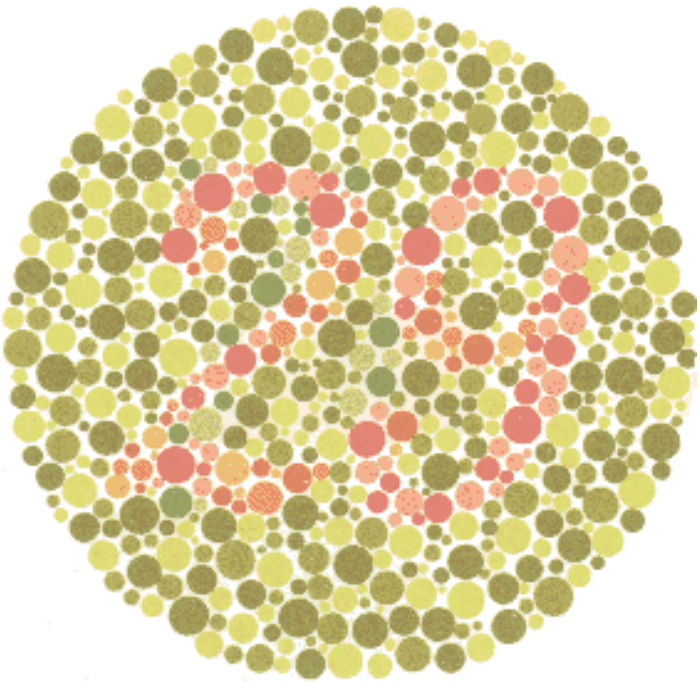

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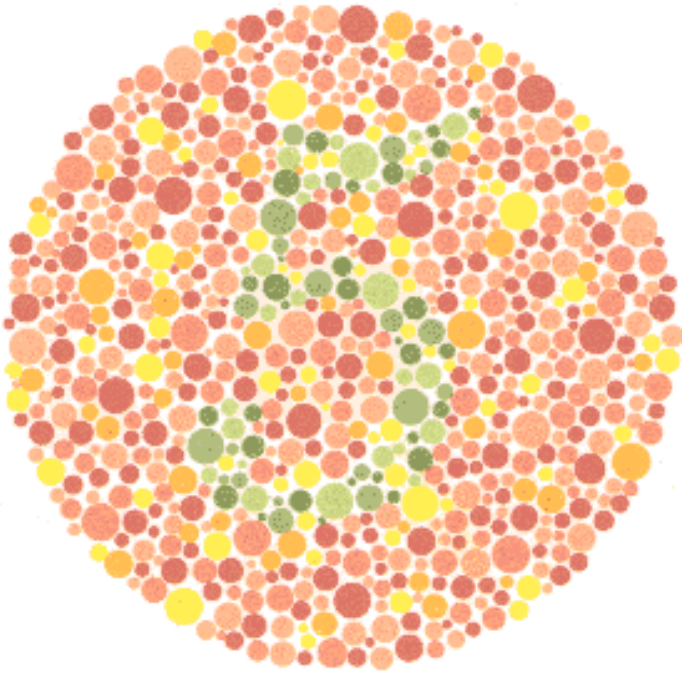

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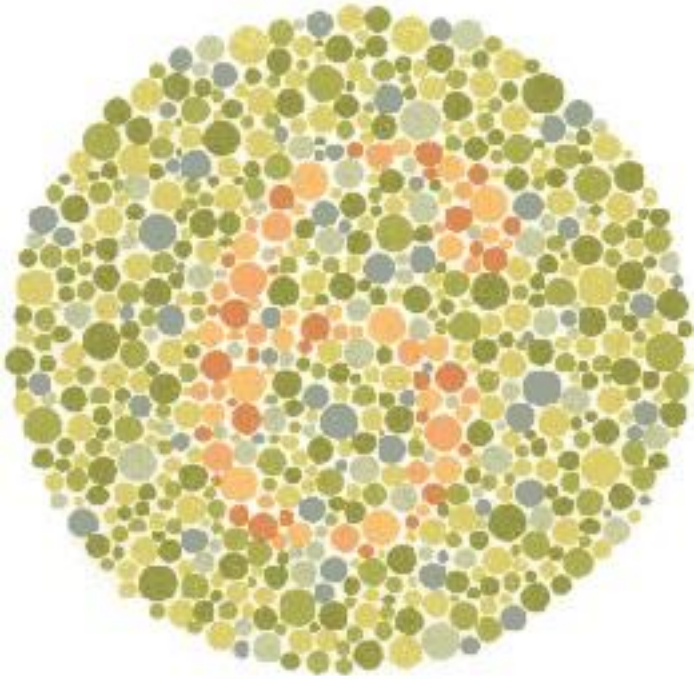

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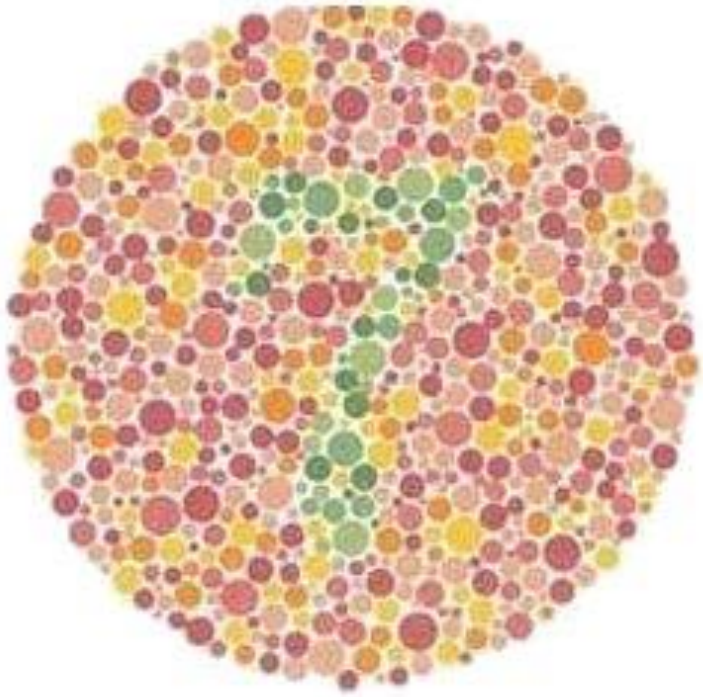

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