NMBAQC

NE Atlantic
Marine Biological Analytical Quality Control Scheme

Zooplankton Ring Test 2018/2019

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Introduction

Zooplankton are included in the Marine Strategy Framework Directive (MSFD) as an indicator group; however, at present there are no current standards for their sampling and identification. As such, a quality control mechanism for the correct identification of zooplankton was identified by the Healthy and Biologically Diverse Seas Evidence Group (HBDSEG). Thus, in 2014-2015 a zooplankton 'ring-test' component for the NE Atlantic Marine Biological Analytical Quality Control (NMBAQC) scheme was developed, by the Sir Alister Hardy Foundation for Ocean Science (SAHFOS), which was successfully adopted by the zooplankton research and monitoring community. The aim of the NMBAQC scheme, and therefore the ring-test, is to encourage consistency amongst zooplankton analysts, within and between different laboratories.

This is the second official NMBAQC scheme zooplankton component ring-test, with the test occurring biennially. In November 2018, the Continuous Plankton Recorder (CPR) Survey, with the Marine Biological Association (MBA)*[1], on behalf of the NMBAQC scheme, sent out a call of interest for the second official zooplankton ring-test, to organisations and individuals known to be involved in zooplankton research and monitoring.

A ring test comprising of: 10, single taxon, tubed zooplankton specimens for identification (from the North Atlantic); 8 written questions and a copepod (*Calanus*) enumeration test, were sent out in December 2018. 13 participants from 12 different laboratories took part in the ring test. Most participants were from the UK, as in the previous year's ring-test; in addition, four new laboratories from Denmark, Finland, Germany and Canada, were also welcomed in the Scheme.

Participants were given 10 weeks to complete their test, and results were assessed by the senior plankton analysts at the CPR Survey, Plymouth.

Materials and Methods

Specimen identification test: the CPR Survey acquired various mixed zooplankton net caught 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 taxa were then transferred to centrifuge tubes and the success of the transfer was checked. Where possible more than specimen of the same taxon, was placed in each tube.

Written quiz: this quiz was prepared by the CPR Survey senior analyst.

Enumeration component: counting and basic identification of copepods. For the copepod enumeration component, *Calanus finmarchicus* stage/sex-sorted specimens were supplied from culture by Biotrix, Norway and, together with *Metrida* specimens, from samples collected in the Norwegian Sea by the Institute of Marine Research, Norway. Specimens were sorted, counted and tubed according to sex and stage; prior to posting out to participants, contents were checked by the senior analyst with another experienced analyst as witness.

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[1] In April 2018, the Sir Alister Hardy Foundation for Ocean Science (SAHFOS) merged with the Marine Biological Association of the UK (MBA) and is now referred to as the 'CPR Survey' (within the MBA).

Results

Following on from participant feedback from the two previous NMBAQC zooplankton ring tests, the ring test should be community driven and 'self-policed'. To help accomplish this, a results workshop (hosted by the CPR Survey with the MBA) was organised and took place in Plymouth on 20th June 2019. Twelve participants (two remotely via Skype), from twelve different laboratories took part in the workshop, where results were discussed and consensus for marking of results was reached.   Four visitors (one remotely via Skype), from another two laboratories, also participated in the workshop.

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.   Answers to the test/quiz are also included below with a brief explanation.

The average result for the specimen identification section was 77.7%, with individual results ranging between 40% and 100%; the worst identified species were copepods *Oncaea scottodicarloi/waldermari* and *Acartia tonsa*. In contrast the anomuran decapod larva was correctly identified by all the participants, scoring 100% across the board.

For the written exercise, the average score was an impressive 86.8%, with individual marks ranging from 75% to 98%. The most poorly answered question concerned the counting protocol of siphonophores in samples. The overall score for this question was 59.6%; coupled with the wide range of answers given, this confirms the suspected lack of consistency amongst laboratories in recording this important gelatinous member of the zooplankton.
The question scoring highest marks amongst participants (97%) was a terminology-based question, with participants asked to label a diagram of a euphausiid.

This year, the enumeration section combined counting expertise together with basic copepod identification skills and an assessment of the ability to separate different copepods life stages.

Levels of accuracy varied between 0% and 100% for the various life stages, but the number of total copepods displayed a more encouraging range of 82%-100%.

Analyst Code	Lab Code	Overall per participant %	Overall per laboratory %
Zo-2301-03	Zo-2301	97.0	
Zo-2301-04	Zo-2301	92.7	94.8
Z0-2302-01	Zo-2302	93.5	93.5
Z0-2303-01	Zo-2303	81.5	81.5
Zo-2305-01	Zo-2305	87.4	87.4
Zo-2307-02	Zo-2307	80.9	80.9
Zo-2308-02	Zo-2308	70.0	70.0
Zo-3301-01	Zo-3301	99.5	99.5
Zo-2301-01	Zo-3302	88.6	88.6
Zo-3303-01	Zo-3303	84.0	84.0
Zo-3304-01	Zo-3304	82.2	82.2
Zo-3305-01	Zo-3305	89.6	89.6
Zo-2312-03	Zo-2312	77.3	77.3

Table 1: Overall scores per participant and per laboratory

	question number	1	2	3	4	5	6	7	8	Total score	
	max score	7	2	3	2	2	2	6	8		
Analyst Code	Lab Code									32	% of max
Zo-2301-03	Zo-2301	7	2	2	2	2	2	6	8	31	97
Zo-2301-04	Zo-2301	6.5	2	1	2	2	1	6	8	28.5	89
Z0-2302-01	Zo-2302	7	0.5	3	2	2	0.5	6	8	29	91
Z0-2303-01	Zo-2303	6.5	0.5	2	2	0	0.5	6	7	24.5	77
Zo-2305-01	Zo-2305	7	1.5	2	1	2	2	6	8	29.5	92
Zo-2307-02	Zo-2307	7	1	1.5	1	2	1	4	8	25.5	80
Zo-2308-02	Zo-2308	6.5	1	1	2	2	1.5	6	4	24	75
Zo-3301-01	Zo-3301	7	1.5	3	2	2	2	6	8	31.5	98
Zo-2301-01	Zo-3302	7	1.5	2	2	2	1	4	8	27.5	86
Zo-3303-01	Zo-3303	6	1	2	2	2	1.5	6	8	28.5	89
Zo-3304-01	Zo-3304	7	1	1	2	2	0.5	4	8	25.5	80
Zo-3305-01	Zo-3305	6.75	0.5	3	2	2	0.5	6	8	28.75	90
Zo-2312-03	Zo-2312	7	1.5	1	2	2	2	4	8	27.5	86

Table 2: Written test scores per participant

Specimen:		1	2	3	4	5	6	7	8	9	10		
Analyst Code	Lab Code											Total score (out of 10)	% of max
Zo-2301-03	Zo-2301	1	1	1	1	1	1	0.5	1	1	1	9.5	95
Zo-2301-04	Zo-2301	0.5	1	0.5	1	1	1	1	1	1	1	9	90
Z0-2302-01	Zo-2302	0.5	1	1	1	1	1	0.5	1	1	1	9	90
Z0-2303-01	Zo-2303	0.5	1	1	1	0.5	0	0.5	1	1	0.5	7	70
Zo-2305-01	Zo-2305	0.5	0.5	0.5	1	1	0.5	0.5	1	1	0.5	7	70
Zo-2307-02	Zo-2307	0.5	0.5	1	1	0.5	0.5	1	1	1	0.5	7.5	75
Zo-2308-02	Zo-2308	0	0	0.5	1	0	0	0.5	1	1	0	4	40
Zo-3301-01	Zo-3301	1	1	1	1	1	1	1	1	1	1	10	100
Zo-2301-01	Zo-3302	0.5	1	1	1	1	0	0.5	1	1	1	8	80
Zo-3303-01	Zo-3303	1	0	0	1	1	1	0.5	1	1	0.5	7	70
Zo-3304-01	Zo-3304	1	0.5	1	1	1	1	0.5	1	1	0.5	8.5	85
Zo-3305-01	Zo-3305	0.5	1	1	1	0.5	1	1	1	1	1	9	90
Zo-2312-03	Zo-2312	0.5	1	0.5	1	0.5	0	0.5	0.5	0.5	0.5	5.5	55
	Total %	61.5	73.1	76.9	100.0	76.9	61.5	65.4	96.2	96.2	69.2		

Table 3: Specimen identification test, scores per participant

|	Counting category	*Calanus* adult female	*Calanus* adult male	*Calanus* CV	*Calanus* CIV	*Calanus* C I- III	Metridia	Total *Calanus*	Comments	% error of total *Calanus*
Analyst Code	Lab Code	**69**	**20**	**62**	**20**	**19**	**10**	**190**		
Zo-2301-03	Zo-2301	67	20	62	20	19		188		-1
Zo-2301-04	Zo-2301	68	20	62	20	18	10	188	18 Calanus CIII are included in the total Calanus count.	-1
Z0-2302-01	Zo-2302	69	20	62	20			190		0
Z0-2303-01	Zo-2303	69	19	61	18			186		-2
Zo-2305-01	Zo-2305	70	20	60	20	20	9	190		0
Zo-2307-02	Zo-2307	69	20	56	22			167		-12
Zo-2308-02	Zo-2308	0	171	11	18			200		5
Zo-3301-01	Zo-3301	69	20	62	20	18	10	190		0
Zo-2301-01	Zo-3302	69	20	63	19		10	190		0
Zo-3303-01	Zo-3303	65	37	86		16		204		7
Zo-3304-01	Zo-3304	71	26	40	12	7		156		-18
Zo-3305-01	Zo-3305	71	16	62	21			170	NB! Also some Calanus CIII in sample	-11
Zo-2312-03	Zo-2312	68	24	62	19		10	173		-9

Table 4: Copepod enumeration scores per participant

Q1. Please correctly label the euphausiid diagram below with the following terms: 7 points

A1F = Antennular flagellum
A2S = Antennal scale
As3 = Abdominal segment 3
**CD** = Carapace denticle
CG = Cervical groove

DK = Dorsal keel
ThEnp = Thoracopod endopod
GI = Gills
LAP = Lappet
PAS = Pre-anal spine

PH = Photophore
Pleop = Pleopod
TE = Telson
UR = Uropod

0.5 score for each correct answer
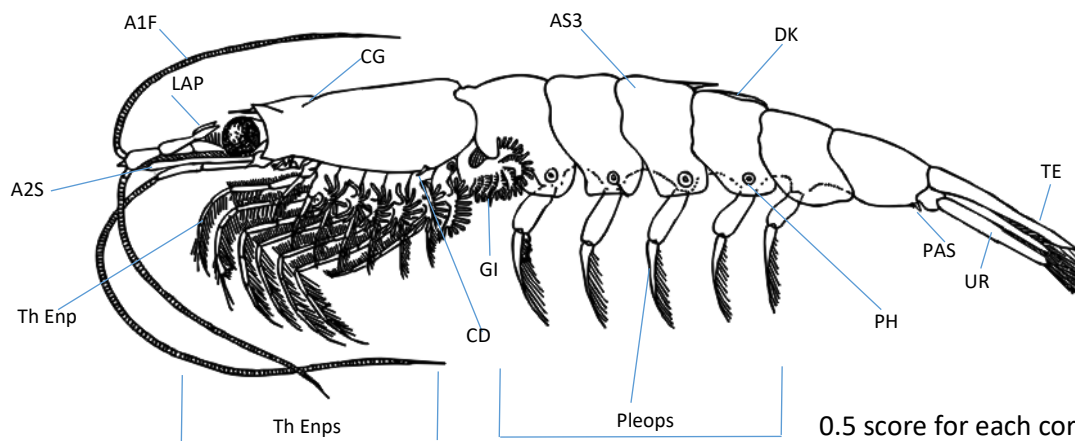
Most participants correctly identified the different features; two people forgot to label every item in the list of terms. The overall score was 97.0%, the best answered question in the quiz.

Question 2
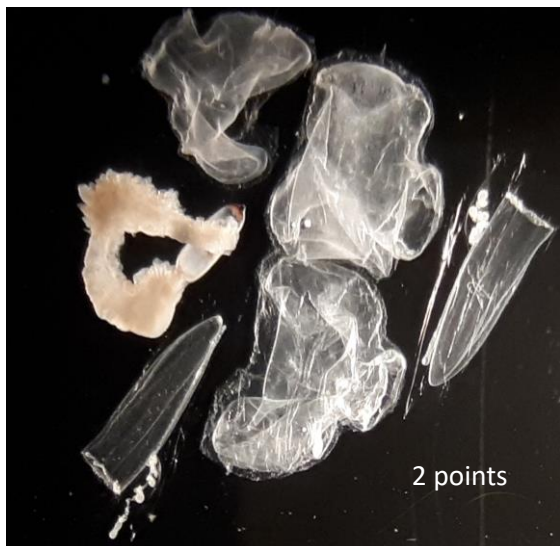
How do you record siphonophores?

Answer:
1 x pneumatophore of physonect;
3x physonect nectophores;
2x nectophores from Muggiaea atlantica (Calycophora, Diphyidae)
count of 3 in total

2 points

Question 2 was worth two points, indicating that two different aspects were required: a quantity identified and an explanation of the how the quantity was arrived at. This question was deliberately somewhat open to interpretation. The writer of the question has noticed for some time the apparent inconsistency between laboratories on how siphonophores are recorded and enumerated.

Thus, so as not to penalise individuals for adhering to their laboratory protocol, no answer was deemed fully incorrect, but full marks were awarded to those who answered the question fully and correctly.

As expected, answers were very varied with only two participants (from the same laboratory) receiving full marks. The variation in responses demonstrates the apparent lack of consistency amongst laboratories in the enumeration of these common organisms in the plankton. The overall score was 59.6%, the worst answered question.

Question 3

Ascidian tadpoles and appendicularians both belong to the subphylum tunicata.
They can be of similar size and shape and can co-occur in plankton samples.
Describe what features you would use to differentiate between them.
3 points

Answer:
Ascidian larvae: usually possess 3 adhesive papillae, a sensory vesicle (statocyst) and a pigmented ocellus (sensory organ). The tail is attached to the rear of the trunk longitudinally.

Appendicularians: do not possess the features above and their tail is attached mid-trunk (i.e. perpendicular).

This question was worth three marks, indicating that at least three features were required for a full answer. Most participants answered the question well, scoring half up to full marks (only three people scored full marks)

Question 4

Following a species name, what does the taxonomic authority Giesbrecht 1893 ["1892"] mean?
• Please circle the correct statement/s
• a.   The original description was published in 1893
• b.   The original description was published in 1892
• c.   The original description was published in 1892 and in 1893 a reprint was made
• d.   The original description was published in 1892, but in 1893 the genus name was changed
• e.   The original description was published in 1893, but was mistakenly printed as 1892
• f.   None of the above

2 points

One point was awarded for option 'a' and two points for option 'e', as this is the correct answer with a correct explanation. All participants gave a correct answer, either 'a' or 'e', reflecting a high overall score of 92.3%.

Question 5

100 µm

What organism is this?
Name the phylum and class it belongs too.
2 points

Answer:
Doliolaria larva:
Phylum Echinodermata;
Class Holothuroidea,
         or Crinoidea (would have a ventral adhesive pit)

Easily confused with:
Doliolids (Thaliacea) barrel shaped but have 8 muscle bands

This question was answered well, with most participants achieving full points. Only one person got an incorrect answer and mistook the doliolaria larva for a doliolid. The overall score was 92.3%.

Question 6

The image below is the P5 of a male *Calanus*, albeit somewhat damaged, taken from the northern North Sea. The prosome length of the specimen is approximately 3.0 mm
To what species of *Calanus* does the P5 belong to, please give reasoning for your answer?

2 points

a) *Calanus helgolandicus*
b) *Calanus finmarchicus*
c) *Calanus glacialis*
d) You cannot tell which species it belongs to
e) *C. helgolandicus* or *C. finmarchicus*
f) *C. helgolandicus* or *C. glacialis*
g) *C. finmarchicus* or *C. glacialis*

Answer is g) *finmarchicus* or *C. glacialis*

Not *C. helgolandicus* due to Ri spine/Re feature, spine is too short.
Prosome size overlaps for *C. fin/C. glacialis* and so does distribution

0.0 if (a) *C. helgolandicus*
0.5 score, if (b) *C. finmarchicus* (Ri/Re feature is of *C. fin* group)
0.5 score if (d) 'cannot tell' but not explanation of why e.g. overlapping size and distribution
1.0 score if (d) 'cannot tell' with explanation
1.5 score if correct answer (g) , but incorrect explanation given
2.0 score if correct answer (g), with correct explanation

Although this was the second worst answered question, no participant got the answer completely wrong. It is clear that there is perhaps some confusion on how a male *Calanus* should be identified, especially in regions with more than one species present, but encouraging that most analyst were cautious enough not to force an identification to species level. The overall score was 61.5%.

Q7. Match the larvae to its corresponding adult. See example below.   6 points
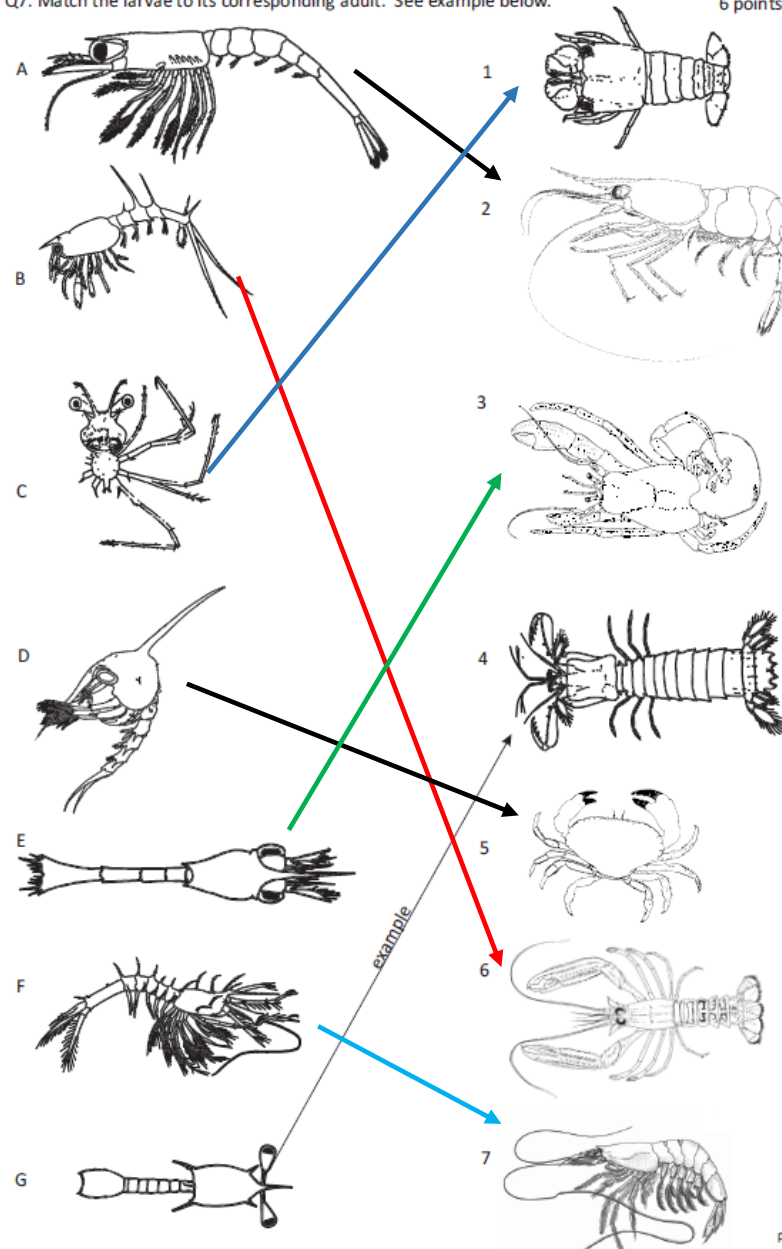
Answer:

A= 2 (*Pandalus borealis,* caridean shrimp/prawn)

B = 6 (*Nephrops norvegicus,* Norway Lobster/Dublin Bay prawn)

C = 1 (*Scyllarus* sp., slipper lobster)

D = 5 (*Cancer pagurus,* edible crab)

E = 3 (*Pagurus bernhardus*, common hermit crab)

F = 7 (*Sergestes arcticus*)

Over two-thirds of participants achieved full marks for this question. Most of those who dropped marks confused the two shrimp-like decapods, *Pandalus* and *Sergestes*.   In carideans such as *Pandalus*, the second segment of the abdomen overlaps those of the first and third segments; this is not the case in *Sergetes*. The overall score was 89.7%.

Question 8

The boxes below contain limbs and descriptions belonging to various common species of female *Oithona*: *O. atlantica*, *O. plumifera* and *O. similis*.

For each species, please list four boxes which best match their species description.

You will have boxes left over, the majority of these boxes match the description of another common species of *Oithona*.

Please insert the missing species name together with its corresponding four boxes.
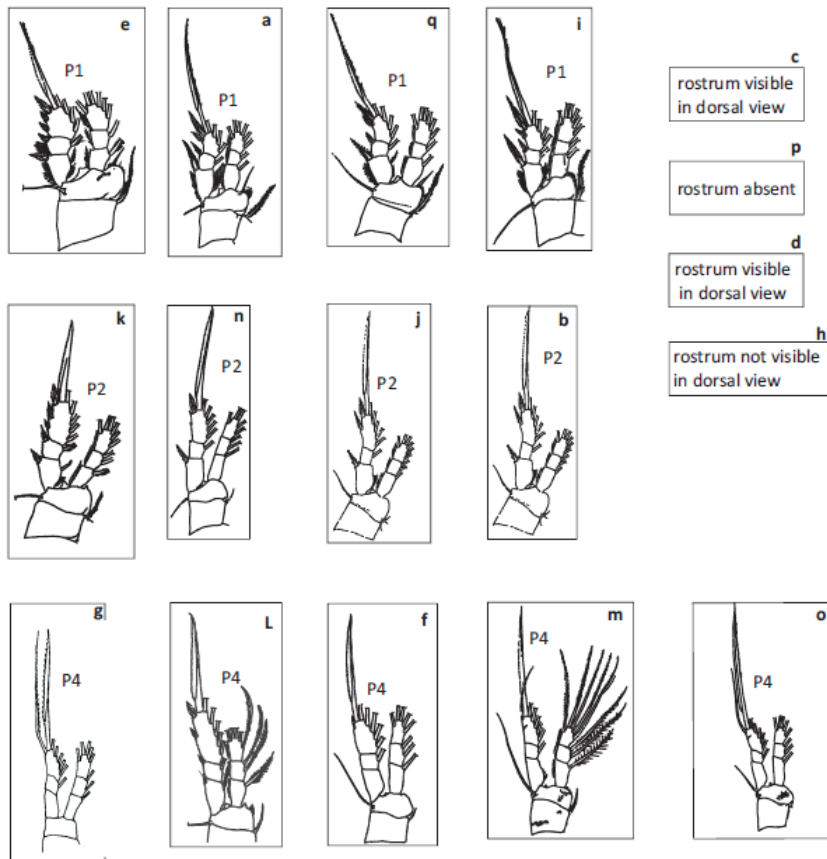
Oithona atlantica, boxes: ....

Oithona plumifera, boxes: ....

Oithona similis, boxes: ....

Oithona? ...., boxes: ....

O. atlantica: P1 = a; P2 = b/j; P4 = o; rostrum = c/d

O. plumifera: P1 = q; P2 = b/j; P4 = m; rostrum = c/d

O. similis: P1 = i; P2 = n; P4 = f; rostrum = h

O. nana P1 = e; P2 = k; P4 = l; rostrum = p

Spare box = g (P4 of *O. longispina*)

Question 8 was answered really well with 11/13 participants achieving full marks. The overall score was 95.2%.

Specimen Identification Test:

Specimen 1:

Female *Oncaea scottodicarloi/waldermari* (Copepoda)

Id features:
- Small cyclopoid copepod (<1mm)
- Female, genital apertures on dorsal side of genital segment
- Strong claw-like maxillipeds (cf. *Oithona*)
- No eye lenses (cf. *Corycaeus*)
- P4 without cone-shaped terminal process on 3rd endopodal segment (cf. *Triconia*)

	Genital segment length/width	Genital segment length/ urosomite segs 3-5 length	Gonopore sclerotisation
O. scottodicarloi	1.27-1.5	2.2-2.5	present
O. walderami	1.70	1.9	not present
O. media	1.9	3.9	present

Together with specimen 6, this proved to be the most difficult identification to make; however, 92% of participants correctly identified the specimen as belonging to the family Oncaeidae. One participant mistook this species for *Oithona nana*. The overall score was 61.5% (joint lowest).

It is clear from the range of answers submitted that there is some uncertainty in identifying oncaeids to species level. Participants were reminded it is best not to force an identification to species if they are not confident to do so.

Specimen 2:

CV female *Paracalanus parvus* (Copepoda)

Id features:
- Small Calanoid copepod <2mm
- 4 segments in urosome, 1st segment without seminal receptacles and not swollen
- Small, uniramous, symmetrical P5 present on both sides
- P2-P4 with smooth terminal swimming blades
- A1 not reaching beyond caul rami
- P4, 3rd exopod seg serrated
- P4 endopod with spinules on 2nd segment only

10/13 participants identified this common copepod correctly to genus, and many were correct to species. Half a mark was deducted if the participant identified the copepod as a male, even if the genus/species name was correct (males have an extra segment in the urosome and their P5 would be strongly asymmetrical). Misidentifications included *Calanus*, *Pseudocalanus* and *Acartia*. The overall score was 73.1%.

Specimen 3:

Ophiopluteus larva (Echinodermata)

Id features:
- Characteristic 'coat-hanger'-like shape
- Long posterolateral arms (absent in echinoid larva)

All participants correctly identiffied the specimen as an enchinoderm larva of some sort. Full marks were given if the specimens were correctly identified as larvae belonging to the class Ophiuroidea (ophiopluteus); half a mark was deducted if the participant identifeid the specimens to species level (even if within the Ophiuroidea) without providing evidence of how they had arrived at their identification. The deduction of half a mark highlights that one should not assume that a specimen belongs to a common species for a region, unless a thorough identifcation using taxonomic literature has been undertaken. Zero points were given if an identifcaiton was made to an incorrect class e.g. Echinoidea. Again this cautions against forcing an identification to a more detailed level; if there is any uncertainty in identification, assignment to a higher taxonomic level should be sought. The overall score was 76.9%.

Specimen 4:

Anomuran (Pagurid) larvae (Decapoda)

Id features:
- No median telson spine
- Carapace spines/denticles
- Rostrum longer than antennal scale

This specimen was correctly identified by all the participants as decapod larvae belonging to the group Anomura. . The overall score was 100%, the best identified specimen in the test.

Specimen 5:

Parasagitta elegans (Chaetognatha)
Id features:
- Firm body
- Vacuoles/alimentary diverticula present
- Eye shape round (not star shaped)
- Two pairs of lateral fins

All particiants identified the specimens as belonging to the Chaetognatha. Full marks were given for the correct assignment to species (specimens possessing vaculoles were deliberatley chosen to support id to species level). Half a mark was awarded to those identifying to the correct genus, and zero marks given to an incorrect genus. The overall score was 76.9%.

Specimen 6:

Acartia tonsa female (Copepoda)

P5
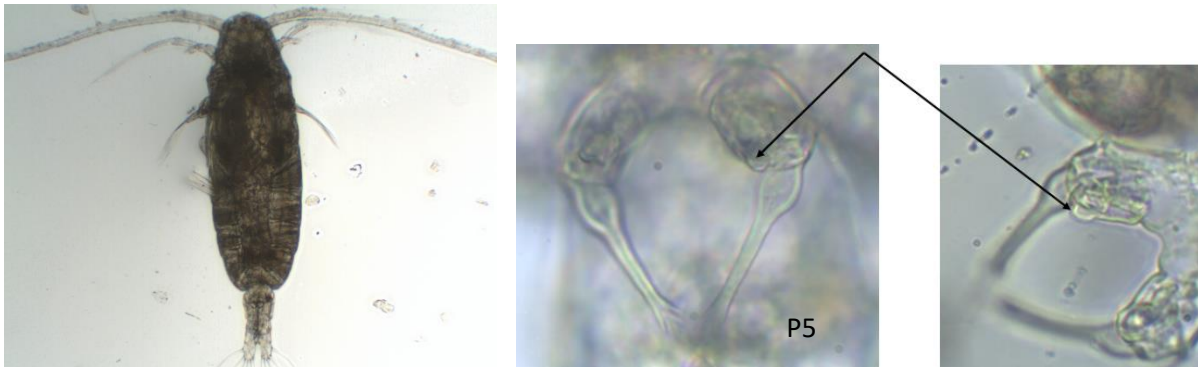
Id features:
- Similar to *Acartia clausi*
- has rostral filaments (unlike *A. clausi*)
- fine spinules on last prosome segment
- ♀ P5 with inner process

Acartia tonsa is considered a non-native species to European waters, thus its correct identification is of importance.   Full marks were awarded for correct identification to species level, half a mark for correct identification to genus level only and zero marks for an incorrect species given (even if genus level was correct). These specimens were all taken from an ongoing culture of *A. tonsa* so there is absolute certainty, on behalf of the organisers, that all specimens supplied in the test were of the same species. One participant correctly identified the specimens, but labelled them as males, again the organisers are confident that females were selected, thus half a mark was deducted. Zero marks were given for assignment to an incorrect genus; this was the case for two participants who assigned their specimens each to *Calanus* sp. and *Scolecithricella* sp. The overall score was 61.5%.

Specimen 7

Calanus marshallae stage CV (Copepoda)

Id features:
- *Calanus*, has teeth on coxa of P5
- Urosome with 4 segments, first segment without seminal receptacles and not swollen
- Inner margin of P5 coxa curved
- RE1/RI1 P5 feature of *C. finmarchicus* group
- P5 coxa average number of teeth approximately 26 (c.f. *Calanus finmarchicus* ~34.7; Calanus glacialis ~ 30.2)

Although endemic to North Pacific waters, *C. marshallae* has been found in the North Atlantic, perhaps linked to ice melt and an opening up of the Northwest Passage. Inclusion of this species in the ring test was to highlight to participants that our oceans are changing (due to climate change and ballast water introductions) and that it is wise to not become complacent, even when dealing with such common genera as *Calanus*.

As expected, all participants correctly identified the specimens as belonging to the genus *Calanus*, however species ranged from *C. helgolandicus*, *C. finmarchicus* to *C. glacialis*, so it is clear there was some confusion; again, highlighting the prudency of not committing to a species level identification if there is uncertainty.

None of the participants correctly assigned the specimens to *C. marshallae*. Those who recorded *Calanus* sp. were awarded a full mark and those who correctly identified the genus, but mistook the species were awarded half a mark. The overall score was 65.4%.

Specimen 8:

Obelia sp. (Cnidaria, Hydrozoa)

Id features:
- flat
- 4 radial canals with gonads positioned half way along
- Numerous short marginal tentacles
- **Not possible to Id to species**

Most participants correctly identified the specimens as belonging to the genus Obeila; no one fell in to the trap of attempting to identify to species.   The overall score was 96.2%; the joint second highest scoring specimen together with specimen 9.

Specimen 9:

Oikopluera dioica (Appendicularia)

Id features:
- Body comprised of clear trunk and tail (tail emerges mid-trunk)
- Trunk compact
- Fol's oikopalst present
- 2 subchordal cells in tail

Specimens were of *Oikopluera (Vexillaria) dioica*, however it was difficult to spot the 2 subchordal cells in some specimens due to damage. Those submitting an answer of *Oikopluera* sp. or *O. dioica* were given a full mark. Half a mark was given for 'appendicularia' only.   Most participants achieved a full mark, generating an overall score of 96.2%; joint second highest scoring specimen together with specimen 8.

Specimen 10:

Merlangius merlangus (fish larva: whiting)

Id features:
- Short gut
- Pigment runs to end of body, not in distinct bands (c.f. *Gadus morhua*)
-

Merlangius merlangus was correctly identified by 69.2% of participants. Two participants incorrectly recorded the specimens as *Gadus morhua*; although very similar in appearance to *M. merlangus*, *G. morhua* has distinct pigment banding (unlike *M. merlangus*). One participant identified the specimen as belonging to Ammodytidae; however, these have a different body shape, possessing 51-68 vertebrae whereas *M. merlangus* is recorded as possessing 49-53.

Enumeration exercise

In response to feedback from the enumeration component included in the 2016/2017 ring test,   the 2018/2019 enumeration exercise involved the counting of copepods.

Participants were given a vail containing a mix of *Calanus* life stages and were asked to count the different sexes and stages of *Calanus* into the following categories: adult female; adult male; copepodite stage V; copepodite stage IV; total *Calanus*.   Included in the vial were also a number of *Metridia* copepods.   Thus, the enumeration exercise was a test of proficiency in basic copepod identification (separating *Calanus* from *Metridia*), the ability to separate different life stages and enumeration skills.

Most participants were able to separate out *Metrida* from *Calanus* well and staged the *Calanus*, generally, to with an accuracy of 80-100%. One participant in particular scored 0% for correct identification and enumeration of adult male and female *Calanus*, however their total *Calanus* score was 95% accurate.

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.

For the specimen identification test, the most difficult to identify proved to be the oncaeid copepod and the non-native copepod *Acartia tonsa*. However, most participants correctly identified the genus for both of these specimens. It is not uncommon for oncaeids to be routinely identified to genus or even family level in the North Atlantic; however, *A. tonsa* is included in the Marine Strategy Framework Directive non-indigenous species watch-list, so its correct identification is of importance. For the written test the most difficult question concerned the recording of siphonophore taxa and their correct enumeration.

Recommendations from the previous ringtest were taken on board by the organisers and, where possible, were tried to be employed in this current test (e.g. include: juvenile copepods, non-calanoid copepods, Echinodermata; higher numbers in the enumeration test; results sent out to participants prior to workshop; host a two day workshop).

At the end of the results workshop, participants were again given an opportunity to give feedback on the ring test and training, both verbally and anonymously via feedback form (see Feedback Forms in Annex 4). The overall feeling was that participants found the test useful and enjoyable, saying that it challenged them at the right level; also the length of time it took to complete the test was about right.

Suggestions made to further improve the ringtest included:

- Include polychaetes
- Include several species belonging to the same family (e.g. Paracalanidae)
- Include fish egg identification
- Give a region of locality for specimens to help with ID
- Clearer instructions in the enumeration test needed
- Have better quality microscopes at the venue and have one microscope per person
- Fine dissecting equipment should be made available during practical session of the workshop.

Once again, the zooplankton community felt a yearly zooplankton ring test would be too frequent, and that every two years was appropriate.

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

We thank all the participants and their constructive feedback.
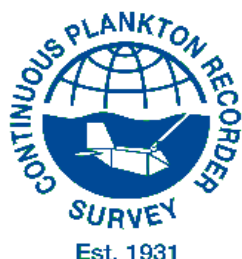
Zooplankton Ring Test
2018/2019

1. Introduction

In January 2013 the Sir Alister Hardy Foundation for Ocean Science (SAHFOS), on behalf of the National Marine Biological Analytical Quality Control (NMBAQC) scheme, sent out a <u>questionnaire</u> to organisations known to be involved in zooplankton 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 a Marine Strategy Framework Directive indicator group and, as such, a quality control mechanism for the correct identification is of 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.

Following on from the trial Ring Test in 2014/2015, and the first official Ring Test in 2016/2017, the 2018/2019 Ring Test represents the second official test to be sent out to interested parties.

As mentioned above, SAHFOS on behalf of the NMBAQC scheme organise and run the Ring Test. SAHFOS operates the Continuous Plankton Recorder (CPR) Survey, the longest running most geographically extensive marine biological survey in the world. In April 2018, SAHFOS was taken over by the Marine Biological Association of the UK (MBA), also based in Plymouth, UK. The CPR survey is still in operation but the organisational name of SAHFOS has now gone. Thus any correspondence previously linked to SAHFOS should now be referred to the CPR Survey Department within the MBA.

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 2018.docx). Please make sure that all the samples are intact and sealed properly. Also please check that you have received the identification results log sheet (log form 2018.xls) as an Excel workbook. Please complete the 'Return slip and checklist' form and send by e-mail to mawo@mba.ac.uk . A receipt of e-mail is necessary for us 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 Marianne Wootton, NMBAQC/MBA, The Laboratory, Citadel Hill, Plymouth PL1 2PB; by e-mail (mawo@mba.ac.uk), or post. If you decide to post your results, please make a copy of your results and send the original documents to the address above. The enumeration and identification results log sheet (Log form 2018.xls) must be received by the CPR Survey by Friday 8th February 2019.

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. If this deadline is unachievable for some reason, please contact mawo@mba.ac.uk .

2. Samples

The identification test consists of ten taxa for identification, (each tube is labelled with a number 1-10). In each tube you should find at least one planktonic organism, where possible we have tried to supply more than one specimen per tube (multiple specimens in a tube will be of the same taxa).

The samples are preserved in 4% formaldehyde solution. You will therefore need appropriate personal protection (gloves, laboratory coat and goggles, with fume hood extraction or a well ventilated area).

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 helgolandicu*s- was an adult female with curved inner margin on p5 coxa.

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.

3. Enumeration test

To test your enumeration skills, a sample tube containing copepods is included in your package and is labelled 'Enumeration'. Please count the abundance of the various copepod stages as indicated in the log form.

4. Written quiz

In addition to the practical test, there is also a written quiz for you to complete. The quiz consists of 8 questions, all of which need to be answered. The results for the written quiz should be submitted by end Monday 11th February 2019.   The quiz will be emailed out to participant's week commencing 17th December 2018. Again, please email or post your quiz results to the address above.

 Workshop

Following on from the Ring Test a results, discussion and training workshop will be held at the MBA, Plymouth, UK.   A 'doodle poll' will be emailed out to participants in order to select the most suitable date.

Microscopes will be available at the workshop and specimen samples from the ring test. If you have any problem specimens of your own, you are encouraged to bring these with you for discussion at the workshop.

The CPR Survey will do some statistical analysis on the results of the Ring Rest, and participants of the workshop will be informed of these beforehand in a preliminary results report. After the workshop, a final report for NMBAQC will be produced.

5. Points to remember

1. Please send your completed 'Return slip and checklist' upon receipt of the test samples.
2. All results must be the analysts' own work. Conferring with other analysts is not allowed.
3. The Excel work sheet Log form 2018.xls must be received by the CPR Survey by Friday 8th February 2019 and answers to the written quiz by Monday 11th February 2019.
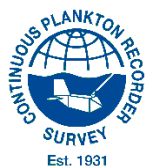
Zooplankton Ring Test 2018/2019

RETURN SLIP AND CHECKLIST

Please ensure to complete the table below upon receipt of samples, then scan and e-mail to mawo@mba.ac.uk	
Analyst Name:	
Analyst Code Assigned :	
Laboratory Name:	
Laboratory 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

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 mawo@mba.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			

Enumeration

Analyst name	
LAB Code	
Analyst Code	

Taxon	Abundance
Calanus adult female	
Calanus adult male	
Calanus CV	
Calanus CIV	
Total Calanus	

NMBAQC Zooplankton ringtest 2018/2019 DELEGATE FEEDBACK FORM Please send to Claire.Taylor@mba.ac.uk			
Name (optional)			
Support Administration			
Was the Zooplankton Ringtest information satisfactory, useful and accurate?		Yes	~~No~~
If no, why? What could have been included, excluded or changed?			
Content Zooplankton Ringtest			
Was the specimen test at the right challenge level?		Yes	~~No~~
If no, why? What could have been included, excluded or changed?			
Was the zooplankton written test at the right challenge level?		Yes	~~No~~
Was the copepod enumeration test at the right challenge level?		Yes	~~No~~
If no, why? What could have been included, excluded or changed?			
Do you have any other comments/ suggestions for the ringtest and/or the results & training workshop?			
I think the standard of both parts of the test is very high. Combined with the ring test workshop it highly valuable as a training exercise and helps me maintain good skills. This is especially true for me as I work on zooplankton irregularly through the year and am a team of one! One thing I would find helpful would be to have 4-5 similar species of the same family – e.g. Paracalanidae in a ring test to get a good chance to compare tiny differences.			
Workshop Venue			
Please rate the following			

	poor	average	good	excellent
Overall, how would you rate the training venue? Consider the training rooms, food, customer service etc.	~~poor~~	~~average~~	~~good~~	excellent
Was there enough lab equipment of suitable quality to use?	~~poor~~	~~average~~	good	~~excellent~~
Were there enough zooplankton samples and of suitable condition and type?	~~poor~~	~~average~~	~~good~~	excellent
Any further comments?				

NMBAQC Zooplankton ringtest 2018/2019
DELEGATE FEEDBACK FORM
Please send to Claire.Taylor@mba.ac.uk

Name (optional)

Support Administration		
Was the Zooplankton Ringtest information satisfactory, useful and accurate?	**Yes**	No
If no, why? What could have been included, excluded or changed?		

Content Zooplankton Ringtest		
Was the specimen test at the right challenge level?	**Yes**	No
If no, why? What could have been included, excluded or changed?		
Was the zooplankton written test at the right challenge level?	**Yes**	No
Was the copepod enumeration test at the right challenge level?	**Yes**	No
If no, why? What could have been included, excluded or changed?		
Do you have any other comments/ suggestions for the ringtest and/or the results & training workshop?		

Workshop Venue				
Please rate the following				
Overall, how would you rate the training venue? Consider the training rooms, food, customer service etc.	poor	average	good	**excellent**
Was there enough lab equipment of suitable quality to use?	poor	average	**good**	excellent
Were there enough zooplankton samples and of suitable condition and type?	poor	average	good	**excellent**

Any further comments?
The only things I missed was fine dissection equipment. As a beginner in zooplankton, I had big difficulty to dissect the specimens (to get the P5 for example). I would have liked to have the opportunity to do it with appropriate equipment during the workshop.

Name (optional)

Support Administration		
Was the Zooplankton Ringtest information satisfactory, useful and accurate?	Yes	
If no, why? What could have been included, excluded or changed?		

Content Zooplankton Ringtest		
Was the specimen test at the right challenge level?	Yes	
If no, why? What could have been included, excluded or changed?		
Was the zooplankton written test at the right challenge level?	Yes	
Was the copepod enumeration test at the right challenge level?	Yes	
If no, why? What could have been included, excluded or change		
Do you have any other comments/ suggestions for the ringtest and/or the results & training workshop?		
It might be good to have a microscope per participant during the practical sessions and examine specimens/samples while an invited speaker gives information about the organisms.		

Workshop Venue				
Please rate the following				
Overall, how would you rate the training venue? Consider the training rooms, food, customer service etc.	poor	average	good	excellent
Was there enough lab equipment of suitable quality to use?	poor	average	good	excellent
Were there enough zooplankton samples and of suitable condition and type?	poor	average	good	excellent
Any further comments?				

NMBAQC Zooplankton ringtest 2018/2019 DELEGATE FEEDBACK FORM Please send to Claire.Taylor@mba.ac.uk				
Name (optional)				
Support Administration				
Was the Zooplankton Ringtest information satisfactory, useful and accurate?		Yes		~~No~~
If no, why? What could have been included, excluded or changed?				
Content Zooplankton Ringtest				
Was the specimen test at the right challenge level?		~~Yes~~		No
If no, why? What could have been included, excluded or changed? I found the practical element of the ring test quite challenging, but I feel that was because I'm very new to zooplankton. For a more experienced person I think the ring test is probably the right level. The ring test could give a bit more information such as regional locality information for each specimen e.g. South West UK.				
Was the zooplankton written test at the right challenge level?		Yes		~~No~~
Was the copepod enumeration test at the right challenge level?		Yes		~~No~~
If no, why? What could have been included, excluded or changed?				
Do you have any other comments/ suggestions for the ringtest and/or the results & training workshop?				
Workshop Venue				
Please rate the following				
Overall, how would you rate the training venue? Consider the training rooms, food, customer service etc.	~~poor~~	~~average~~	~~good~~	excellent
Was there enough lab equipment of suitable quality to use?	~~poor~~	~~average~~	~~good~~	excellent
Were there enough zooplankton samples and of suitable condition and type?	~~poor~~	~~average~~	~~good~~	excellent
Any further comments?				

Name (optional)		

Support Administration		
Was the Zooplankton Ringtest information satisfactory, useful and accurate?	<mark>Yes</mark>	No
If no, why? What could have been included, excluded or changed?		

Content Zooplankton Ringtest		
Was the specimen test at the right challenge level?	Yes	No
If no, why? What could have been included, excluded or changed? NA – Did not participate within specimen test.		
Was the zooplankton written test at the right challenge level?	<mark>Yes</mark>	No
Was the copepod enumeration test at the right challenge level?	Yes	No
If no, why? What could have been included, excluded or changed? NA – Did not participate within the copepod enumeration test.		
Do you have any other comments/ suggestions for the ringtest and/or the results & training workshop?		

Excellent range of zooplankton specimens covered overall. Whilst there was a question regarding the identification of an ichthyoplankton, it may perhaps be useful to also include egg identification. I enjoyed the talks which covered various image analysis techniques, though including an overview of ZooScan and potentially a comparison between it and FlowCam and PIA would be beneficial. It would also have been interesting to have received information relating to examples of the types of projects they are used within and what parameters were measured for use within such projects.

Workshop Venue				

Please rate the following				
Overall, how would you rate the training venue? Consider the training rooms, food, customer service etc.	poor	average	good	<mark>excellent</mark>
Was there enough lab equipment of suitable quality to use?	poor	average	good	<mark>excellent</mark>
Were there enough zooplankton samples and of suitable condition and type?	poor	average	<mark>good</mark>	excellent
Any further comments?				

Name (optional)		

Support Administration		
Was the Zooplankton Ringtest information satisfactory, useful and accurate?	Yes	~~No~~

If no, why? What could have been included, excluded or changed?

I found the instructions very clear (with the exception mentioned below) and I like how the ringtest comprises of three sections - specimen identification, a written element and an enumeration exercise.

Content Zooplankton Ringtest		
Was the specimen test at the right challenge level?	Yes	~~No~~

If no, why? What could have been included, excluded or changed?

I like how participants are encouraged to only identify the specimens as far as they are comfortable so we would not necessarily get marked down for not identifying the organism as far as species level if we are not confident to do so.

Was the zooplankton written test at the right challenge level?	Yes	~~No~~

I thought there was a broad range of questions covering different organisms and I found the siphonophore question particularly useful

Was the copepod enumeration test at the right challenge level?	Yes	~~No~~

If no, why? What could have been included, excluded or changed?

I found this section of the test the most challenging as I do not routinely stage copepods but I understand the importance of this and found it a useful exercise and at the right challenge level. However, I felt that the way this question was worded was perhaps a bit misleading. In my total Calanus count I included adult females, adult males, CV and CIV copepodites. I did not include earlier Calanus copepodite stages in my final count as this was not asked for on the spreadsheet. Perhaps I just didn't read the question properly but I understood it to mean the total count of what was asked for on the spreadsheet and not including anything else. Aside from that, I thought it was good to have the Metridia in the sample as well as the Calanus as this added an extra level of challenge to the exercise.
  I thought that the enumeration exercise was an improvement from two years ago where we counted the breads (although I still thought that the bead exercise was useful).

Do you have any other comments/ suggestions for the ringtest and/or the results & training workshop?
Overall I found the ringtest extremely useful and I am very appreciative of all the hardwork and effort made by everyone who makes this happen. I think the layout of the ringtest is very good by having the three components to the test and I hope that the ringtest continues for many years to come.

Workshop Venue				
Please rate the following – N/A				
Overall, how would you rate the training venue? Consider the training rooms, food, customer service etc.	poor	average	good	excellent
Was there enough lab equipment of suitable quality to use?	poor	average	good	excellent
Were there enough zooplankton samples and of suitable condition and type?	poor	average	good	excellent
Any further comments? I was unable to attend the workshop so cannot comment on this aspect.				