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First Interim Report of the Working Group on Integrated Morphological and Molecular Taxonomy (WGIMT)

28 March 2014

Reykjavik, Iceland



ICES

International Council for
the Exploration of the Sea

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Executive summary

The ICES Working Group on Integrated Morphological and Molecular Taxonomy (WGIMT) met in association with the 2014 WGZE meeting in Reykjavik, Iceland. The meeting included a plenary session with the Working Group on Zooplankton Ecology (WGZE) on 25 March 2014 and a dedicated meeting on 28 March 2014. The primary goals of the meeting were to: 1) hear suggestions and presentations from WGIMT members; 2) review the group's progress on ToRs, reports, and recommendations; 3) consider guidelines and suggestions from WGZE; and 4) review multiannual ToRs and any changes in focus resulting from our conversion from Study Group to Working Group status.

WGIMT membership currently totals 31 members from 11 countries; the continuing growth is consistent with WGIMT ToR (a) to expand membership and welcome new members who develop and use molecular and/or morphological approaches to taxonomic analysis of zooplankton. The WGIMT web platform for promotion and exchange of relevant scientific information [ToR (b)] was discussed, with reviews of progress to date for the morphological, molecular and optical elements, as well as plans for new elements, including a photo gallery of living zooplankton and literature database. WGIMT will continue work to initiate and support provision of standards, training materials, and taxonomy workshops through organized workshops [ToR (c)]. Two workshops are in various stages of planning: *SAHFOS-MBA Zooplankton Taxonomy Workshop* (Summer 2015) and *Future of Integrative Taxonomy* (Summer 2016). Both will be proposed as ICES Taxonomy Workshops through WGIMT Recommendations. Also for ToR (c), WGIMT will support the WGZE recommendation that Claudia Castellani and Anonina dos Santos be named as editors of the ICES Zooplankton Identification Leaflets. WGIMT will promote and encourage the continuing integration of molecular and morphological taxonomy by organizing special sessions at national and international conferences, including ASLO/TOS Ocean Sciences Meeting (2016), ICES ASC (2015), among others [ToR (d)]. WGIMT will advise on the implications of developments for marine science and management and plans to report via SSGEF and ACOM EGs on uses of integrative taxonomy (e.g., environmental sequencing or metagenetics) for integrated ecosystem assessment [ToR (e)]. WGIMT will cooperate with WGITMO and WGBOSV to encourage and facilitate application of molecular protocols for detection and identification of introduced and transported species in ballast water [ToR (f)]. WGIMT will publish peer-reviewed articles on diverse aspects of integrative (morphological and molecular) taxonomy of zooplankton [ToR (g)]. Finally, WGIMT members voted to nominate Ann Bucklin to serve as WGIMT chair for 2014–2017.

Next year, WGIMT proposes to meet on Plymouth, UK on 20 March 2015 in association with the annual WGZE meeting, which is planned for 16–19 March 2015. Additional work will be carried out by correspondence, including WeBex and Skype videoconferencing.

1 Opening of the meeting

WGIMT met in association with the 2014 WGZE meeting in Reykjavik, Iceland. The meeting included a plenary session with the Working Group on Zooplankton Ecology (WGZE) on 25 March 2014 and a dedicated meeting on 28 March 2014. The primary goals of the meeting were to: 1) hear suggestions and presentations from WGIMT members; 2) review the group's progress on ToRs, reports and recommendations; 3) consider guidelines and suggestions from WGZE; and 4) review multiannual ToRs and any changes in focus resulting from our conversion from Study Group to Working Group status. Of 32 current WGIMT members, 14 attended the 2014 meeting in person and another four joined via teleconferencing (Annex 1).

2 Adoption of the agenda

The agenda was circulated among WGIMT and WGZE members prior to the meeting to allow coordination and consideration of suggestions and comments. The WGIMT meeting was held from 9:00 am to 5:00 pm on Friday, 28 March 2014. The final version of the agenda was adopted by acclaim (Annex 2). The group gave careful consideration and discussed realistic planning for the multiannual WGIMT ToRs for 2014–2017.

3 ToR (a) Expand membership of WGIMT

Expected Deliverable(s): WGIMT will include experts in both morphological and molecular taxonomy for major zooplankton groups; 2 members in common with ACOM EGs (Year 1).

The WGIMT membership has grown from a total of 22 members as of March 2013 to a current total of 31 members from 11 countries (Annex 1). A WGIMT goal is to continue to expand membership by individual invitations; suggestions can be sent to Ann Bucklin. New members are welcome; we particularly encourage those who develop and use molecular and/or morphological approaches to taxonomic analysis of zooplankton. Membership in WGIMT extends to scientists working in any ocean region, not only the ICES region.

4 ToR (b) Develop a web platform for promotion and exchange of relevant scientific information

Expected Deliverable(s): WGIMT.net web portal designed, established (Year 1) and fully populated (Year 2); specially-designed elements and deep links created for WGAGFM, WGITMO, WGBOSV (Years 2, 3).

Discussion allowed review of progress to date and next steps in the several existing elements of the WGIMT web portal (morphological, molecular, and optical) as well as new elements to be developed. Primary responsibility for ToR (b) is Todd O'Brien with assistance from WGIMT members as indicated following.

Morphological methods: WGIMT members Claudia Castellani, Elaine Fileman, and Antonina dos Santos will assume responsibility for editing and updating this material on the website. A further request is for information and URL links to keys for morphological identification of zooplankton, so these new links can be added to the web portal.

Optical methods: The link to this section will be moved by Todd O'Brien from the main page to the Morphological Methods section, until and unless the material in this section can be updated and maintained with assistance from Mark Benfield.

Molecular methods: A comprehensive summary of PCR and sequencing primers and protocols and associated references will be prepared for posting by Ann Bucklin, Astrid Cornils, and Silke Laakmann. Todd O'Brien will translate the current flat XLS table into a more dynamic web-based interface.

Photo gallery: A new feature of the WGIMT portal is a photo gallery of high-quality images of living zooplankton. Software will be selected to allow posting and organizing the images for easy access and viewing; images will be cross-linked to WGIMT/WGZE taxonomic database entries (e.g., WGZE time series, geographic maps, and biovolume analyses). Photo galleries from the Census of Marine Zooplankton (www.cmarz.org) will be migrated to the new area of the WGIMT site.

Literature database: A new two-pathway online system for scientific literature relevant to WGIMT will be designed and developed by Todd O'Brien. Public access will entail display of a pathway showing only the reference. Password-controlled access will also have links to downloadable PDFs of the actual publication. The system will have a generic design to allow application for WGZE, WGPME, and others. A forms-based entry process will be used to reduce the administrator's (Todd O'Brien) workload.

WGIMT News: Several WGIMT members requested the addition of a "News!" bullet or link on the WGIMT.net homepage. This will allow members to post updates, announce new publications, and share highlights of group members' activities.

5 ToR (c) Initiate and support provision of standards, training materials, and taxonomy workshops

Expected Deliverable(s): SAHFOS-MBA Zooplankton Taxonomy Workshop (2015); ICES Taxonomy Workshop on: *Future of Integrative Taxonomy* (2016); support for updating ICES Zooplankton Taxonomy Leaflets

WGIMT will propose two ICES taxonomy workshops:

SAHFOS-MBA Zooplankton Taxonomy Workshop – 2015

In 2013, Claudia Castellani proposed a taxonomy workshop under the remit of ICES. SAHFOS management have agreed to run the workshop and it will go ahead in 2015; the event is now at the stage of planning and deciding which audience the workshop will target. Claudia asked the group for suggestions for specific taxonomic groups taxa that should be targeted. The workshop as envisioned will include a molecular component, with 1-1.5 days to be dedicated to molecular approaches to species identification and taxonomic analysis. Ann Bucklin, Pennie Lindeque, and others will be invited to contribute to the molecular approach elements. Ann Bucklin has run workshops on integrative taxonomy for high school teachers; she gave a brief overview with descriptions of these workshops.

Claudia Castellani will seek funding to support this activity and requested WGIMT support. Members of the group present at the meeting were all in support of this tentative recommendation; Ann Bucklin proposed that

those members not able to attend the meeting in person could be given the opportunity to comment in the draft report.

Action: All WGIMT members to provide feedback on the proposed SAHFOS-MBA Zooplankton Taxonomy Workshop, as discussed during the meeting and described in the draft 2014 WGIMT Report. Suggestions are welcome on the zooplankton taxa to be targeted.

Future of Integrative Taxonomy – 2016

In 2013, Janna Peters, Jasmin Renz, and Astrid Cornils recommended that an ICES Taxonomy Workshop be proposed for funding through a WGIMT recommendation. Accordingly, the following abstract was presented to SCICOM, which indicated their support and requested an official resolution (proposal). This action was delayed due to the schedule constraints of the workshop organizers. While the original proposed date for the workshop was 2015; the new date is summer 2016.

“Morphologically-based methods for species identification are increasingly combined with new methodologies in the field of integrated taxonomy and applied in ecological research, e.g. monitoring and biodiversity research, time series analyses, and assessment of marine communities. This workshop aims to review and discuss the combination and applicability of these approaches. Case studies focusing on integrated taxonomical and ecological research of marine organisms will be presented by invited experts and participants. Furthermore, this workshop will serve as platform to initiate future networking between specialists from different disciplines. WGIMT members have the expertise in both, morphological and molecular taxonomy as well as strong ecological backgrounds, and are thus able to evaluate the future potential of the different approaches.”

This is a conceptual workshop, not hands-on. Funding will be needed for invited experts, who will be critical to the success of the workshop as envisioned. The German Research Organization may be group to approach for financial support. Funding was discussed further and one suggestion was to link the workshop to the 2016 Zooplankton Production Symposium, now planned for Bergen, Norway. The workshop organizers will contact Astthor Gislason, who is a WGIMT member and WGZE representative for the Zooplankton Production Symposium. Ann Bucklin suggested to Janna Peters that she submit a proposal for a 1- or 2-day workshop once the official notification of the Symposium has been circulated by ICES/PICES. Alternatively or in addition, the organizers may wish to propose a special session in the Zooplankton Production Symposium that could be linked synergistically to the workshop.

Action: Janna Peters will ask whether German Research Foundation funding may be possible and develop a tentative list of experts whom the organizers would like to invite. Ann Bucklin will ask Pennie Lindeque (PML, UK) and/or Rowena Stern (SAHFOS, UK) whether they are able to assist Janna Peters with workshop planning and organization. Feedback from all WGIMT members is encouraged in response to the draft 2014 WGIMT Report.

WGIMT will support and facilitate updating and revision of ICES Zooplankton Identification Leaflets.

In conjunction with the WGZE, Antonina Dos Santos and Claudia Castellani offered to lead efforts to update the ICES Zooplankton Identification Leaflets. WGZE will propose the two individuals to ICES as new editors of the documents, which are currently stored on the ICES website. Among the challenges in this effort is that access to the leaflets is limited because of out-of-date PDF file formats. Good access to existing PDFs will be required; permission is needed from ICES to update and correct errors discovered in some of the keys.

Since revising all the keys will be a huge effort, it is proposed to start on the most common species in the ICES areas and the Mediterranean, and request assistance from taxonomic experts. Suggestions from WGIMT members are welcome for taxonomic experts who can be engaged to participate. Also, there are currently no impact factors associated with these publications; the group suggested that these be designated as data publications and be assigned a DOI, so they can be cited and more easily accessed. Another issue may be copyright of the leaflets and whether this can be transferred to WGZE.

Action: WGIMT to support WGZE recommendation to ICES that Claudia Castellani and Anonina dos Santos be named as editors of the ICES Zooplankton Identification Leaflets.

6 ToR (d) Promote and encourage the continuing integration of molecular and morphological taxonomy

Expected Deliverable(s): Organize special sessions at national and international conferences: Ocean Sciences Meetings (2014, 2016); ICES ASC (2015).

WGIMT members discussed plans to propose special sessions on integrative taxonomy at upcoming international conferences. Previously, the group organized special sessions at the 2014 Ocean Sciences Meeting (Honolulu, USA) and ICES 2013 Annual Science Conference (Reykjavik). The group noted that the ICES ASC 2013 special theme session was successful at drawing in participation.

Janna Peters and Claudia Castellani suggested that WGIMT propose a theme session for the 2015 ICES ASC on the functional importance of cryptic species and their geographic distribution. Further discussion resulted in the following tentative title: "Population specific differences at the molecular versus the population level: integrative approaches to understand diversity".

Action: WGIMT will propose at least one theme session proposal; Claudia Castellani and Janna Peters will prepare a draft session proposal for WGIMT review. Additional suggestions for sessions at the ICES 2015 ASC and other meetings are welcome.

7 ToR (e) Advise on the implications of developments for marine science and management

Expected Deliverable(s): Report via SSGEF and ACOM EGs on uses of integrative taxonomy (e.g., environmental sequencing or metagenetics) for integrated ecosystem assessment (Years 2, 3); report on uses of metagenetic indicators to WGAGFM (Year 2).

Ann Bucklin introduced ToR (e) and stated that there is no expected deliverable for this ToR for Year 1, given the change in focus and the new multi-year deliverables. The discussion focused on considering this new focus on metagenetic analysis and specifying appropriate and useful WGIMT deliverables. Suggestions from all WGIMT members were sought.

Panagiotis Kasapidis (Hellenic Centre for Marine Research) presented results of his project, "MetaCopepod: Designing an integrated DNA metabarcoding and image analysis approach to study and monitor biodiversity of zooplanktonic copepods" (see <http://metacopepod.hcmr.gr>). The aim of this effort is to develop a novel methodology, based on the combination of next generation sequencing technologies and image analysis, to assess and monitor the biodiversity of planktonic marine copepods and cladocera in a fast, high-throughput, reliable, and quantitative way. This project is based in the Mediterranean and Black Seas and aims to collect images, train image analysis software, and provide material for barcoding (16S and COI). The methods are tested using pseudo-samples and bulk zooplankton samples. All the information is publicly available in a format that is compatible with other larger databases. The aim is to produce a network of marine observatories similar to the European Marine Ecosystem Observatory (EMECO) in the North Sea. Panagiotis Kasapidis then asked for feedback from WGIMT members.

A useful exchange followed between Elena Gorokhova and Panagiotis Kasapidis:

EG: Do you have any suggestions for a positive control strategy? How many organisms do you need for a reliable signal? How do you deal with phylogenetically close and distant species?

PK: The pseudo-samples were sorted by taxonomists, so the actual composition is known. However, the strategy for positive controls needs to be designed so that it can be seen whether primers amplify what is in the sample or whether there is under- or over-replication.

EG: Is the positive control used to adjust the method so it reflects what is in the sample or to find the optimal number of organisms in the sample?

PK: The positive control is used to determine the bias in a method, but is not necessary in every analysis once a technique is standardized.

EG: Please clarify whether the positive control should be universal with as many species as possible in uneven amounts.

PK: Real samples could also be used if they are counted first. So far they have had very good results for PCR but other methods still need to be tested. This is a very important step, but results seem to be reliable so far (with some over/under bias).

Panagiotis Kasapidis then stated that he would like advice about what information to put in the MetaCopepod database. Some species already have a lot of information available so new information is not necessary. However, this project aims to fill in the gaps which would be a good opportunity to collaborate with WGIMT.

Next, Pennie Lindeque (PML, UK) gave a presentation via SKYPE based on her recent paper on next-generation sequencing (Lindeque *et al.*, 2013, PLoS ONE 8: e81327). She emphasized the need for a reference library of sequences from accurately-identified species is recognized as being of high importance. Different laboratories work on different parts of the genome so reference libraries need to

reflect this. This could be addressed as part of a WGIMT ToR. There are discrepancies on how NGS can be used and specific questions need to be answered for NGS to be useful for management needs, e.g., what level of taxonomic identification is required and what are the associated threshold similarity values? For bivalve larvae, there is one family that has a 1% difference between species that is very hard to resolve using 18S. WGIMT could provide valuable information and guidance on these issues, perhaps through a published paper or report. Also, WGIMT might develop a comprehensive reference database of DNA barcodes for identified specimens, including especially those species occurring in the ICES areas of particular interest and concern.

Ann Bucklin thanked Pennie Lindeque for acknowledging WGIMT in her publication.

Discussion followed:

Pennie Lindeque asked Panagiotis Kasapidis whether, for a positive control, it is a good idea to have a known concentration of organisms so that you can quantify bias and errors. Panagiotis Kasapidis replied that if you assume no bias with eDNA, the values would be proportional to biomass rather than abundance. Image analysis can be used to get an idea of the biomass of a species in a sample, so eDNA analysis can be corrected with respect to abundance in the sample. An inherent problem when using PCR is that the results are semi-quantitative. It would be good if there was a general correction factor for the size of a species to correct sequencing results.

Pennie Lindeque noted that a natural community has different developmental stages so there will be a lot of variation in individual biomass within a species, but the barcoding results would be the same. Panagiotis Kasapidis noted that taxonomic analysis of one zooplankton sample from an area or season will also have a lot of unknown variability so a bit of error in eDNA analysis might not make much of a difference and could still be sufficient for analysing the community.

Ann Bucklin reiterated a question from WGBOSV/WGITMO raised in an earlier session (ToR f) about the suitability of metagenetics for estimating abundance and biomass making this an important discussion.

Naiara Rodriguez-Ezpeleta stated that she is constantly asked whether barcoding can give quantitative results. She agreed with Pennie Lindeque that barcoding won't give the number of individuals in a sample, but feels that it could eventually provide biomass measurements. This would rely on having information such as DNA extraction efficiency, the number of gene copies per cell etc. At the WGAGFM meeting last year there was a Tor (c) to review the use of metagenomics and metatranscriptomics as an approach for marine ecosystem management. The WGAGFM 2013 report (<http://www.ices.dk/sites/pub/Publication%20Reports/Expert%20Group%20Report/SSGHIE/2013/WGAGFM13.pdf>) includes a review of factors that limit quantitative abundance and biomass estimates. Some of these problems are not easy to address. Pennie Lindeque noted that this is exactly what she had been suggesting that WGIMT do for this ToR. Naiara Rodriguez-Ezpeleta replied that WGAGFM's work may not have covered everything, but could be used as a starting point for WGIMT. Ann Bucklin said that she was very impressed by what had already been done and would like to refer all WGIMT members to the WGAGFM report.

Next, Naiara Rodriguez-Ezpeleta (AZTI) gave a presentation via SKYPE from an earlier conference about using metabarcoding to calculate an AMBI (AZTI's Marine Biotic Index) for benthic macrofauna. The results showed that presence/absence data was enough to calculate the AMBI accurately, although there were not enough barcodes available to calculate the AMBI using metabarcoding. Calculations showed that barcodes for 10% of the most frequent species would give an accurate AMBI. This work is being continued with an experiment using mock samples to test how the results compare using different species assemblages, biomass ratios, primers, PCR conditions etc. to find the main biases in the process. Jon Corell Saiz (PhD student at AZTI) explained via SKYPE the results of his metagenetic experiments with zooplankton communities, showing that complete metagenetic analysis of zooplankton diversity requires a cocktail of consensus and group-specific primers.

Then, Ann Bucklin showed two slides. One summarised work by John Pearman (KAUST, Saudi Arabia) that was presented as a poster at the Ocean Sciences 2014 meeting titled, "High throughput amplicon sequencing to determine zooplankton diversity in the Red Sea". The second summarised another poster presented at the same meeting by Ann Bucklin and Leocadio Blanco-Bercial titled, "Toward metagenetic analysis of biodiversity of zooplankton communities" that concluded that metagenetics has realistic and cost-effective applications for fisheries and ecosystem monitoring and management.

More discussion ensued:

Panagiotis Kasapidis stated that the 18S gene is quite robust and able to resolve most species, although there were some problems with bivalve larvae, as Pennie Lindeque had pointed out. The 18S region targeted is too long a sequence for analysis on some sequencing platforms (e.g., Illumina), so a shorter region should be evaluated to allow full analysis of species. Ann Bucklin asked Pennie Lindeque which 18S region she had used; Pennie replied that she used primers from Fonseca *et al.* (2010). Leocadio Blanco-Bercial stated that his is the V1-4 region of 18S and that he had used this same region; he noted that at least semi-quantitative analysis is possible on Illumina platforms. Each species may have a number of 18S copies, but copy number is pretty conserved within a species. Pennie Lindeque described work to convert morphological measurements to biomass, which can then be compared to metagenetic results. However, there is a huge range of individual sizes among species, so this could only be done in very general terms. There needs to be good integration with morphology to get conversion factors. Elena Gorokhova expressed some doubt that ribosomal genes can be converted to biomass or abundance. Pennie Lindeque noted that 18S is easier as it is a nuclear gene. Naiara Rodriguez-Ezpeleta commented that the variation is due to biological factors, which we can try to understand and correct for; she is more concerned about technical factors, e.g., primers and DNA extraction are not equally effective for different species or even different individuals of the same species.

Naiara Rodriguez-Ezpeleta noted that some WGIMT members are doing taxonomic analysis of samples and enquired whether it would be possible to do this and metabarcoding on the same sample. Taking one sample in formaldehyde for taxonomic analysis and one in ethanol for metabarcoding means samples are not directly comparable, but is it possible to get DNA from samples that were originally preserved in formaldehyde for taxonomic analysis and

then transferred to ethanol for DNA analysis? Ann Bucklin agreed that this suggestion could perhaps be implemented among WGIMT members pursuing this type of research. They could identify samples and address issues of comparison ethanol- and formalin-preserved material. Antonina dos Santos stated that this is an important question, and asked if Naiara Rodriguez-Ezpeleta knew how long samples could be/had to be in formaldehyde before going in ethanol. This question will be added to the remit of a small group.

Pennie Lindeque asked WGIMT members which points are most important for the WGIMT report. She suggested the first issue is making ICES aware of the opportunity that metagenetic analysis of diversity may provide. Next is a recommendation on which gene to use for metagenetics, to encourage expansion of the reference database. Ann Bucklin noted that the expected deliverables of this ToR are due in years 2 and 3, and a first step would be the report of this discussion and recommendations on applications of the research for management, as specified in the title of the ToR. She stated that it would be useful to identify key WGIMT members to take this discussion forward by correspondence. Volunteers among those present at the meeting included: Pennie Lindeque, Naiara Rodriguez-Ezpeleta, Leocadio Blanco-Bercial, and Panagiotis Kasapidis. Additional WGIMT members are welcome and encouraged to volunteer.

Ann Bucklin summarized that the message from WGIMT is that technological advances in this area are useful and contribute to integrating molecular techniques with and taxonomy. There are a number of people within WGIMT exploring metagenetic approaches to analysis of zooplankton species diversity, but there is a need to continue growing the reference barcode database. She welcomed and encouraged suggestions from WGBOSV/WGITMO in this regard, saying that WGIMT could focus on invasive species and/or organisms from particular geographical locations; a subset of WGIMT members could decide on which genes to use for metagenetics (e.g., COI and/or 18S). Barcode data should be submitted to GenBank; specimens barcoded for COI could also be analyzed for an agreed-upon region of the 18S rRNA gene. WGIMT could propose metagenetic best practices.

Action: Ann Bucklin, Pennie Lindeque, Naiara Rodriguez-Ezpeleta, Leocadio Blanco-Bercial, Panagiotis Kasapidis will coordinate via correspondence to prepare plan of action to implement this ToR on the designated time-frame.

8 ToR (f) Cooperate with WGITMO and WGBOSV to encourage and facilitate application of molecular protocols for detection and identification of introduced and transported species in ballast water

Expected Deliverable(s): Define (Year 1), carry out (Year 2) and publish (Year 3) results of collaborative activities with WGITMO/WGBOSV. Participate in WGITMO-WGBOSV annual meetings (Years 1, 2, 3). Provide identifying DNA sequences for invasive species listed on AquaNIS (Year 2).

Maiju Lehtiniemi presented a summary of discussions held at the 2014 WGBOSV and WGITMO meetings about areas where collaboration with WGIMT would be useful. Early detection of invasive non-indigenous species (NIS) is a high priority for management of new introductions. Methods for detection of aquatic NIS have relied on microscopic examination of environmental samples and traditional taxonomy. How-

ever, invading species may first occur at very low abundances and, given the sampling efforts typically used in such studies, NIS may be missed. Accurate and rapid identification of target species from a sample or community (metagenetics) or even from water (eDNA) would be useful.

Maiju Lehtiniemi asked whether WGBOSV/WGITMO members might assist in collecting certain species/taxa for WGIMT analysis to expand barcode reference libraries, which, as discussed under ToR (e), will allow rapid analysis of biodiversity in environmental samples by metagenetics. Ann Bucklin had initially responded to this question by email during the WGBOSV/WGITMO meeting saying that this might be cautiously planned to design an agreement for a certain number of specimens with a schedule built around available resources in participating laboratories. She suggested that WGIMT might consider this cooperation and seek to develop a realistic plan.

Ann Bucklin further suggested that WGIMT could produce a summary of laboratories that are actively barcoding species. There is high demand for reference DNA libraries, but it is hard to get funding for this work. This request from WGBOSV/WGITMO would be an excellent justification for WGIMT to pursue this area. WGIMT members noted laboratories that are actively barcoding species:

- Silke Laakmann is actively barcoding North Sea species
- Panagiotis Kasapidis is focused on the Mediterranean and invasive species
- Antonina dos Santos is working along the Portuguese coast
- Ann Bucklin has an active barcode laboratory and accepts identified specimens
- Elena Gorokhova has determined barcodes for several taxa; data are in GenBank

Ann Bucklin said that the report should state that there is considerable interest from WGIMT to cooperate with WGBOSV/WGITMO. The hardest part is to get specimens with a trusted identification, which could be supplied by WGBOSV/WGITMO taxonomic experts for invasive species/NIS, and a resource supplied by WGIMT would be a list of barcoding laboratories. Maiju Lehtiniemi noted that this work would need a list of priority species, and could use the AquaNIS list of known alien species in the Baltic, Mediterranean, and North Sea as a starting point. The work would have to focus on zooplankton, but the inclusion of meroplankton would make the information useful in other areas.

Antonina dos Santos noted a call on Horizons 2020 for Atlantic cooperation (see: <http://ec.europa.eu/research/participants/portal/desktop/en/opportunities/h2020/topics/2457-bg-14-2014.html>), for project that support EU, USA, and Canada partners.

Maiju Lehtiniemi continued to report on outcomes from the 2014 WGITMO/WGBOSV meeting:

Questions were raised about whether it is possible to know if the organism detected with molecular methods is alive or dead. The Ballast Water Management (BWM) Convention requires ballast water treatment in order to kill all organisms transported in the ballast water, so ships are allowed to transport dead animals but not live ones. Also, DNA sequencing of environmental samples has been demonstrated as an effective method of identifying rare species, which is particularly pertinent in regards to invasive species that occur at low abundances. Could that work for ballast water or ports?

Naiara Rodriguez-Ezpeleta suggested RNA analysis as a potential method of discriminating between live and dead animals. RNA is unstable, so as soon as an organism dies the RNA is degraded; if RNA is detected it can be assumed that it comes from a live organism. However, there is still much work needed to really understand if this method would be applicable. Elena Gorokhova felt that this method would not work, as it may not account for resting stages. These stages are not metabolically active, so their RNA is unlikely to be detected, and they are more prone to being transported.

Elaine Fileman noted that the current process to discriminate live/dead organisms is to use vital staining after the treatment process. Elena Gorokhova commented that this method detects esterase activity and may still miss resting stages with low metabolic activity. Ann Bucklin asked whether you could test for the RNA specific for esterase activity. Elena Gorokhova replied that staining would be much quicker and cheaper and so more cost effective. Ann Bucklin concluded that, as RNA is produced before the esterase protein, the use of RNA to distinguish live/dead organisms was a good idea, but probably impractical and may have sensitivity issues.

Ann Bucklin then posed the question of how resting stages could be detected. Claudia Castellani explained that it is difficult because different cues trigger the activation of resting stages in different organisms. You can distinguish some resting stages by visual identification as they have special external morphology. Alexandra Chicharo noted that there are a lot of other organisms with resting stages for which molecular approaches are not available.

Ann Bucklin said that WGIMT would keep thinking about this question, but that it seems unlikely that molecular approaches would be the best and most cost-effective approach to discriminating between live and dead organism in ballast water.

Maiju Lehtiniemi asked another question from the 2014 WGITMO/WGBOSV meeting: Could WGIMT members recommend or use molecular approaches help to identify areas of origin of non-indigenous species by comparing the genetic material from Europe and the area of origin? Another subject of potential mutual interest could be the genetic analysis of wide spread non-indigenous species to determine whether the species arrived in Europe from their area of origin by one introduction (primary introduction) and thereafter spread further in Europe (secondary introduction), or whether the NIS was introduced multiple times to Europe from its native area (e.g. *Mnemiopsis*). WGIMT might set up a structure – or incorporate this goal into an existing structure – or portal to identify researchers studying distribution and phylogeography of species of interest.

Ann Bucklin reminded the group that the identification of active zooplankton barcoding laboratories has already been agreed. She noted that phylogeography or population genetics is rather outside the scope of WGIMT, which is focused on the use of molecular techniques to identify organisms to species.

Next, Maiju Lehtiniemi suggested that eDNA methodology could be focused on target species or "black lists", i.e., NIS of particular concern. Each country could update the target list, and specific genetic markers could be developed. WGIMT could help by giving attention to the development of Aquatic Invasive Species (AIS) phylum-specific markers based on our target lists. Ann Bucklin replied that indeed WGIMT could do this, and could make sure that these species are included in the WGIMT portal, with primers, protocols, and reference sequences for zooplankton species.

Another question concerned metabarcoding: what would be an appropriate sample volume if you want to sample either small water bodies (e.g., marinas/ports) or larger water bodies (e.g., seas)? What are the limitations of these methods? How many DNA sequences do you need before the sample is representative? These questions were addressed during discussion of ToR (e); (see above).

Action: WGIMT will prepare a summary listing of active barcoding projects by taxon and region; identify high priority species (e.g. invasive species); and assist with identifying funding for barcoding, especially any new resources.

Action: One or more members of WGIMT will join the WGBOSV/WGITMO annual meeting next year, planned for March 16, 2015 in Bergen, Norway. Interested participants include: Panagiotis Kasapidis, Pennie Lindeque, Naiara Rodriguez-Ezpeleta (and her PhD student Jon Corell Saiz), and Ann Bucklin.

9 ToR (g) Publish peer-reviewed articles on diverse aspects of integrative (morphological and molecular) taxonomy of zoo-plankton

Expected Deliverable(s): Publish two papers related to WGIMT ToRs (Years 2, 3); publish review article on integrative taxonomic approaches to characterizing zoo-plankton communities (Year 2).

WGIMT has a goal – consistent with ICES guidance, as we understand it – to publish in the peer-reviewed scientific literature. WGIMT members do publish individually, and there is at least one recent WGIMT acknowledgement (Lindeque *et al.*, 2013). Ann Bucklin suggested a WGIMT goal to gather a list of publications by WGIMT members for the WGIMT homepage and web portal [see discussion of ToR (b), above].

With reference to the review article, it was suggested that we write a Short Communication article (approximately 2000 words), with a possible focus on the need for a barcode reference library, metagenetic analysis of species diversity, or other topic relevant to WGIMT.

Action: Ann Bucklin will ask Roger Harris whether these or similar topics may be appropriate for a Horizons article in the Journal of Plankton Research. Depending on the answer, a short review article for this or another journal would be planned. Ann Bucklin will query the WGIMT members to identify individuals interested in being on the writing team; the writing team would then select a lead-author to begin manuscript preparation.

10 New Business

WGIMT 2015 Annual Meeting

The group expressed an opinion as to the venue for the next meeting and proposed a recommendation to WGZE that we meet in association with them and we support them in their decision making process between Plymouth or Faro. The group would like to meet for one day and this would be following the WGZE meeting.

In addition, the group suggested a recommendation to WGIMTO/WGBOSV that one or more members from WGIMT participate in the WGIMTO/ BOSV meeting, which will take place around March 2015.

Election of WGIMT Chair for 2014–2017

ICES guidance on chair selection states that a member of the group can be chair for up to 6 years. Janna Peters proposed that Ann Bucklin continue in the role as chair for up to the next three years, this was seconded by Astthor Gislason and a show of hands from all those present was unanimous in accepting the proposal. The group is delighted that Ann is happy to continue in this role and are extremely grateful for all the hard work that she has done for the group and her efforts in promoting it to Working Group status. The group stressed that a new chair would need to be 'groomed' over the next year or so to be ready to take over at the end of the chair period.

Appreciation to WGIMT 2014 Meeting Hosts

On behalf of WGIMT, Ann Bucklin thanked Astthor Gislason, Hildur Pettursdottir, and staff of the Marine Institute for their hospitality and hosting of the meeting.

Annex 1: List of participants

WGIMT Membership 2014: WGIMT members as of 28 March 2014.

Participants in the 2014 annual meeting are indicated in the right-hand column: 14 members attended in person (Y); an additional four members joined via teleconference (V).

	First / Last Names	Email address	Function	Start date	Nomination	2014 Mtg
1	Christina Augustin	christina.augustin@io-warnemuende.de	Chair-invited Member	3/13/2013	Other	Y
2	Dorte Bekkevold	db@aqu.dtu.dk	Member	1/1/2011	DK	
3	Mark Benfield	mbeffie@lsu.edu	Chair-invited Member	2/19/2013	Other	
4	Leocadio Blanco-Bercial	leocadio@uconn.edu	Chair-invited Member	3/19/2014	Other	V
5	Ann Bucklin	ann.bucklin@uconn.edu	Chair	1/1/2012	Other	Y
6	Claudia Castellani	cxc@sahfos.ac.uk	Chair-invited Member	3/13/2013	Other	Y
7	Maria Alexandra Chicharo	mchichar@ualg.pt	Chair-invited Member	3/19/2014	Other	Y
8	Kathryn Cook	kathryn.cook@scotland.gsi.gov.uk	Chair-invited Member	2/19/2013	Other	Y
9	Astrid Cornils	Astrid.Cornils@awi.de	Chair-invited Member	2/19/2013	Other	
10	Elaine Fileman	ESE@pml.ac.uk	Chair-invited Member	2/19/2013	Other	Y
11	Asthor Gislason	astthor@hafro.is	Chair-invited Member	3/19/2014	Other	Y
12	Xabier Irigoien	Xabier.irigoien@kaust.edu.sa	Chair-invited Member	2/19/2013	Other	
13	Panagiotis Kasapidis	kasapidi@hcmr.gr	Chair-invited Member	3/19/2014	Other	Y
14	Silke Laakmann	slaakmann@senckenberg.de	Chair-invited Member	2/19/2013	Other	
15	Maiju Lehtiniemi	maiju.lehtiniemi@ymparisto.fi	Chair-invited Member	3/14/2013	Other	Y
16	Pennie Lindeque	PKW@pml.ac.uk	Chair-invited Member	2/19/2013	Other	V
17	Amy Maas	amaas@whoi.edu	Chair-invited Member	2/19/2013	Other	
18	Sanna Majaneva	Sanna.majaneva@gmail.com	Chair-invited Member	3/14/2013	Other	V
19	Piotr Margonski	pmargon@mir.gdynia.pl	Chair-invited Member	2/19/2013	Other	
20	Maria Grazia Mazzocchi	grazia.mazzocchi@szi.it	Chair-invited Member	3/19/2014	Other	
21	Vijayalakshmi R. Nair	vijayalakshmi40@hotmail.com	Chair-invited Member	2/19/2013	Other	
22	Einar E. Nielsen	een@dfu.min.dk	Member	1/1/2011	DK	
23	Todd D. O'Brien	Todd.O'Brien@noaa.gov	Chair-invited Member	2/19/2013	Other	Y
24	Janna Peters	janna.peters@uni-hamburg.de	Chair-invited Member	2/19/2013	Other	Y
25	Hildur Petursdottir	hildur@hafro.is	Chair-invited Member	3/19/2014	Other	Y
26	Uwe Piatkowski	upiatkowski@geomar.de	Chair-invited Member	2/19/2013	Other	
27	Jasmin Renz	jrenz@senckenberg.de	Chair-invited Member	2/19/2013	Other	
28	Naiara Rodriguez-Ezpeleta	nrodriguez@azti.es	Chair-invited Member	3/19/2014	Other	V
29	Antonina Santos	antonina@ipma.pt	Chair-invited Member	2/19/2013	Other	Y
30	Robertas Staponkus	ichtiandrus@gmail.com	Member	1/21/2013	LT	
31	Lidia Yebra	lidia.yebra@ma.ieo.es	Chair-invited Member	2/19/2013	Other	V
32	Peter H. Wiebe	pwiebe@whoi.edu	Chair-invited Member	3/19/2014	Other	Y

Annex 2: Agenda

Annual Meeting March 28, 2014

Meeting place*: Marine Research Institute, Reykjavik, Iceland

***Video-conferencing access will be available via SKYPE**

8:30 COFFEE and TEA

9:00 **Introductions and welcome to new WGIMT members**

9:15 **ToR (a) Expand membership of WGIMT**

Expected Deliverable(s): WGIMT will include experts in both morphological and molecular taxonomy for major zooplankton groups; 2 members in common with ACOM EGs (Year 1).

WGIMT member(s): Ann Bucklin

9:30 **ToR (b) Develop a web platform for promotion and exchange of relevant scientific information**

Expected Deliverable(s):

A) WGIMT.net web portal designed, established (Year 1) and fully populated (Year 2).

WGIMT member(s): Todd O'Brien, Janna Peters

B) Coordination with WKSERIES

WGIMT member(s): Lida Yebra (Skype)

C) Specially-designed elements and deep links created for WGAGFM, WGITMO, WGBOSV (Years 2, 3). Coordination with new database for Aquatic NIS; see <http://www.corpi.ku.lt/databases/index.php/aquanis/>

WGIMT member(s): Maiju Lehtiniemi

11:00 BREAK

11:15 ToR (f) Cooperate with WGITMO and WGBOSV to encourage and facilitate application of molecular protocols for detection and identification of introduced and transported species in ballast water

Expected Deliverable(s): Define (Year 1), carry out (Year 2), and publish (Year 3) results of collaborative activities with WGITMO-WGBOSV. Participate in WGITMO-WGBOSV annual meetings (Years 1,2,3). Provide identifying DNA sequences for invasive species listed on AquaNIS (Year 2). WGIMT member(s): Maiju Lehtiniemi, Ann Bucklin

12:30 LUNCH

1:00 ToR (e) Advise on the implications of developments for marine science and management

Expected Deliverable(s): Report via SSGEF and ACOM EGs on uses of integrative taxonomy (e.g., environmental sequencing or metagenetics) for integrated ecosystem assessment (Years 2,3). Report on uses of metagenetic indicators to WGAGFM (Year 2).

WGIMT member(s): Penny Lindeque (Skype), Leocadio Blanco-Bercial (Skype), and others

2:00 ToR (c) Initiate and support provision of standards, training materials, and taxonomy workshops

Expected Deliverable(s): SAHFOS-MBA Zooplankton Taxonomy Workshop (2015); ICES Taxonomy Workshop on: *Future of Integrative Taxonomy* (2016)

WGIMT member(s): Claudia Castellani, Janna Peters

3:00 ToR (d) Promote and encourage the continuing integration of molecular and morphological taxonomy

Expected Deliverable(s): Organize special sessions at national and international conferences: Ocean Sciences Meetings (2014, 2016); ICES ASC (2015).

WGIMT member(s): Elaine Fileman (ICES 2013); Ann Bucklin (Ocean Sciences 2014); Everyone (Future Plans)

3:30 BREAK

3:45 ToR (g) Publish peer-reviewed articles on diverse aspects of integrative (morphological and molecular) taxonomy of zooplankton

Expected Deliverable(s): Publish two papers related to WGIMT ToRs (Years 2, 3).
Publish review article on integrative taxonomic approaches to characterizing zooplankton communities (Year 2).

WGIMT member(s): Ann Bucklin and everyone

4:00 pm Action Items and Next Steps for WGIMT

WGIMT Chair (see guidance from ICES), Next meeting, Suggestions

5:00 pm ADJOURN

Annex 3: Terms of Reference for 2014–2017

The **Working Group on Integrated Morphological and Molecular Taxonomy** (WGIMT), chaired by Ann Bucklin, USA, will meet in Plymouth, UK during March 2015 to work on ToRs and generate deliverables as listed in the Table below.

WGIMT will report on the activities of 2015 by 15 July 2015 to SSGEPD.

ToR descriptors

ToR	Description	Background	Science Plan topics addressed	Duration	Expected Deliverables
a	Expand membership of WGIMT	a) Integrative taxonomy requires experts in both morphological and molecular taxonomic approaches. b,c) Members in common will facilitate coordination between WGIMT and ACOM EGs and help ensure advisory goals are met.	1.1.3, 1.2.1, 1.2.3, 3.1	Year 1	WGIMT will include experts in both morphological and molecular taxonomy for major zooplankton groups; 2 members in common with ACOM EGs. (Year 1).
b	Develop a web platform for promotion and exchange of relevant scientific information	a) Locating and accessing morphological and molecular taxonomic information can be difficult: some classical taxonomic references are out-of-print; molecular data are not released prior to publication. b,c) Easier access to data and information will encourage use of integrative taxonomic approaches.	1.1.3, 1.2.1, 1.2.3, 1.6.1, 3.1	Years 1,2,3	WGIMT.net web portal designed, established (Year 1) and fully populated (Year 2). Specially-designed elements and deep links created for WGAGFM, WGITMO, WGBOSV (Years 2, 3).
c	Initiate and support provision of standards, training materials, and taxonomy workshops	a,b) ICES Taxonomy Workshops are very effective in engaging the target audience and ensuring trained technicians and researchers for applications in fisheries and ecosystem management. c) Co-sponsored workshops and meetings with ACOM EGs will provide cross-training and establish organic links between science and	1.2.1, 1.2.3,	Year 2	ICES Taxonomy Workshop on: <i>Future of Integrative Taxonomy</i> (2015); <i>SAHFOS-MBA Zooplankton Taxonomy Workshop</i> with added molecular component (2015). <i>Baltic Zooplankton Expert Network</i> with WGITMO (2015).

		advice.			
d	Promote and encourage the continuing integration of molecular and morphological taxonomy	a,b,c) Integrative taxonomy is an emergent field; uses and applications for fisheries and ecosystem management should be explained in high-visibility settings in ICES and other organisations through special sessions	1.2.1, 1.2.3, 1.6	Years 1,2,3	Organize special sessions at national and international conferences: Ocean Sciences Meetings (2014, 2016); ICES ASC (2015).
e	Advise on the implications of developments for marine science and management	b,c) Integrative taxonomy (e.g., 'library' of DNA sequences for accurately-identified species) can provide a foundation for genetic methods for assessing species, diversity and abundance in integrated ecosystem assessments. c) Standardized metagenetic data can fulfill requirements of indicators defined in the Marine Strategy Framework Directive (WGAGFM).	1.2.1, 1.2.3, 1.6	Years 2,3	Report via SSGEF and ACOM EGs on uses of integrative taxonomy (e.g., environmental sequencing or metagenetics) for integrated ecosystem assessment (Years 2,3). Report on uses of metagenetic indicators to WGAGFM (Year 2).
f	Cooperate with WGITMO and WGBOSV to encourage and facilitate application of molecular protocols for detection and identification of introduced and transported species in ballast water	a,b,c,) Integrative morphological-molecular taxonomic approaches will provide uniquely sensitive and accurate methods for detection of invasive species in natural assemblages and ballast water. Sources of origin and pathways of transport can be inferred from genetic analysis. These advanced will enable and facilitate better enforcement of regulations.	2.5	Years 1, 2, 3	Define (Year 1), carry out (Year 2), and publish results (Year 3) of collaborative activities with WGITMO-WGBOSV. Participate in WGITMO-WGBOSV annual meetings (Years 1,2,3). Provide identifying DNA sequences for invasive species listed on AquaNIS (Year 2).
g	Publish peer-reviewed articles on diverse aspects of integrative (morphological and molecular) taxonomy, of zooplankton.	a) Stronger foundation and visibility in primary research literature is needed to establish the field of integrative taxonomy. b) Publication in peer-reviewed literature needed to demonstrate validity of data, protocols, and results for application to	1.1.3, 1.2.1, 1.2.3, 3.1	Years 2, 3	Publish two papers related to WGIMT ToRs (Years 2, 3). Publish review article on integrative taxonomic approaches to characterising zooplankton

fisheries and ecosystem management.	communities (Year 2).
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Summary of the Work Plan

Year 1	Focus on increasing membership (ToR a); design and establish web portal (ToR b). Advise WGAGFM on metagenetic approaches for taxonomic analysis of analysis (ToR e). Define partnership activities with WGITMO and WGBOSV (ToR f).
Year 2	Focus on integrative taxonomy workshops (ToR c). Carry out collaborative activities with WGITMO and WGBOSV (ToR f). Publish overview review article summarizing integrative approaches to zooplankton taxonomy (ToR g).
Year 3	Facilitate, encourage and enable use of integrated morphological and molecular taxonomic analysis of zooplankton in integrated ecosystem assessments in ICES area seas (ToRs d,e,f,g).

Supporting information

Priority:	This Working Group will assist ICES and its Expert Groups with issues related to the development, dissemination and application of taxonomic knowledge and skills in support of Integrated Ecosystem Understanding. Accurate identification of species and characterization of species-level diversity are and will remain foundations of integrated ecosystem assessments of function and state. Integrated taxonomic approaches – including morphological, molecular, optical, and other – may enhance and accelerate progress toward rapid, automatable, and near-real-time identification of species for fisheries and integrated ecosystem assessments; detecting of the impacts of climate change on species diversity, distribution, abundance; and understanding alterations in food web structure and function, and associated biogeochemical cycles. The availability of and need for new technology and techniques in taxonomic analysis make WGIMT's goals and activities important and high priority.
Resource requirements:	The research programs and Expert Group activities that provide input and are stakeholders for this group are already in place. ICES support will be requested for the proposed ICES Taxonomy Workshop: <i>Future of Integrative Taxonomy</i> (2015). No additional resources are required for planned activities.
Participants:	The Expert Group now includes 23 members from 11 countries. Membership is expected to grow in near future through engagement of members from partner ICES Working Groups and other scientists with needed expertise and knowledge. The goal is to ensure balance and coverage of varied taxonomic approaches (including morphological taxonomists for the full range of taxonomic groups) and ICES geographic regions.
Secretariat facilities:	None.
Financial:	No financial implications.
Linkages to ACOM and groups under ACOM:	Cooperative partnerships have been established with WGITMO and WGBOSV as defined in ToR (f); additional activities will be planned and implemented. A recommendation from WGAGFM was accepted and the requested cooperative activities will be carried out.
Linkages to	WGIMT arose as a Study Group from the WGZE in response to

other committees or groups:	perceived need, meeting in association with WGZE during 2012 and 2013. WGIMT will remain in close partnership with WGZE, while promoting and supporting integrated morphological and molecular taxonomy science for the benefit of other ICES Expert Groups and marine science generally.
Linkages to other organizations:	The work of this group relates to and is connected to a diversity of other projects and organisations, e.g., EU MARBEF, EDIT, GBIF, PESI, GOBI, and others.

Annex 4: Recommendations

RECOMMENDATION	ADDRESSED TO
1. WGIMT recommends that the 2015 meeting be held in association with the WGZE 2015 meeting, which is being planned in coordination with WGPME and will meet at the Plymouth Marine Laboratories (Plymouth, UK) during 16-19 March 2015. WGIMT proposes to meet at the PML on 20 March 2015 immediately following the WGZE meeting.	WGZE
2. Develop a coordinated, collaborative activity plan together with WGIMT, WGITMO and WGBOSV.	WGITMO, WGBOSV
3. WGIMT recommends that WGZE adopt SGIMT ToR (d), to assist in the development, revision and updating of zooplankton species identification keys and ensuring their availability via the web, including especially ICES Zooplankton Identification Leaflets.	WGZE
4. WGIMT recommends that an ICES Taxonomy Workshop be established entitled, <i>SAHFOS-MBA Zooplankton Taxonomy Workshop</i> , be to provide practical training in integrated morphological and molecular taxonomic analysis, with a primary focus on copepods. The workshop is planned for Summer 2015 in Plymouth, UK with leadership from WGIMT member Claudia Castellani and Rowena Stern (SAHFOS); WGIMT members Ann Bucklin and Pennie Lindeque will contribute as instructors.	SCICOM
5. WGIMT recommends that an ICES Taxonomy Workshops be established entitled, <i>Perspectives on the Future of Integrative Taxonomy</i> , with a focus on identifying species boundaries using molecules and morphology. A multi-day workshop is planned for Summer 2016 using a theoretical case-studies approach, with presentations and discussions by invited distinguished researchers, organized by WGIMT members Janna Peters, Jasmin Renz, Silke Laakmann, Astrid Cornils. The proposed venue is the University of Hamburg or Senckenberg Institute (Hamburg, Germany).	SCICOM
6. WGIMT will propose an ICES 2015 ASC Theme Session.	SSGEPD
7. WGIMT nominates Ann Bucklin as Chair for 2014-2017.	SCICOM