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Report of the Working Group on Phytoplankton and Microbial Ecology (WGPME)

10-12 March 2015 Gothenburg, Sweden



International Council for the Exploration of the Sea Conseil International pour l'Exploration de la Mer

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Executive summary

The WGPME 2015 meeting took place in Gothenburg, Sweden, 10–12 March. The meeting was chaired by Xelu Moran and Alexandra Kraberg and was attended by 14 group members from 7 countries, two of which attended by video conference or skype.

The meeting was mostly devoted to practical work on manuscripts (a molecular methods review, species specific long-term distribution patterns in relation to hydrography in the North Sea, Synechococcus distribution patterns) and the discussion of several items put to the group by ICES SCICOM, including a new draft of the OSPAR-JAMP eutrophication guidelines. The guidelines were discussed and several members commented on them (summarized in the section on ToR G).

One general session was also devoted to talks by group members in which they reported on progress in their local time-series.

Manuscripts were discussed jointly and also in breakout groups. Manuscript outlines have been produced for two of these (A review of molecular methods and their applicability to time-series research and species specific responses to physical drivers by phytoplankton species across the North Sea). We anticipate that at least one of these will be submitted by the end of 2015.

1 Administrative details

Working Group name

Working Group on Phytoplankton and Microbial Ecology

Year of Appointment

2013

Reporting year within current cycle (1, 2 or 3)

3

Chair(s)

Xosé Anxelu G. Morán, Spain

Alexandra Kraberg, Germany

Meeting venue

Gothenburg, Sweden

Meeting dates

10-12 March 2015

2 Terms of Reference a) - z)

ToR	Description	Background	Science Plan topics addressed Duration	Expected Deliverables
A	marine microbial time-series sampling techniques with an effort towards	summary of current methodologies used in microbial plankton time- series with the ultimate goal of achieving better	through establishment of	Best practice recommendations for microbial plankton time-series provided in the WGPME website (wgpme.net); in 2015 with regular updates; to biological oceanographers but especially phytoplankton and microbial ecologists.
В	range patterns of microbial taxa and functional groups to discern significant	After finding examples of taxa and/or functional groups that have actually changed their distribution we need to know the environmental drivers underlying these	historical baselines of population and community structure and production to be	Interim WG report; in 2014; to SSGEPD

	and to identify changes before we car potential make sound projections. environmental drivers.		
С	Report progress on By providing state of the discovery of novel art knowledge of nove lineages and cryptic microbial biota we wil taxa of be able to better phytoplankton and understand unexplained marine microbes. variation of current time series datasets.	l quantify the state l of North Atlantic r Ocean l regional systems.	Interim WG report; in 2014; to SSGEPD
D	to statistical researchers from analyses to provide different fields and ICES further WGs in order to understanding of disentangle the factors	d quantify the state f of North Atlantic Ocean regional systems O-Understand and f forecast the	Interim WG report; in 2014; to SSGEPD
E	Cooperative better establish the Research Report on climatologies and long ICES Phytoplankton term trends for and Microbial phytoplankton and other Plankton Status to planktonic microbes as be completed for well as introduce new June 2015. analyses, providing the	o quantify the state e of North Atlantic G Ocean regional f systems f -Understand and f forecast the impacts of climate variability and change on marine f ecosystems:	Second ICES CRR Phytoplankton and Microbial Plankton Status Report; in 2015; to research community and policy makers.
F	reviewed entering the position to manuscripts using provide multi datasets existing comparisons of microbia phytoplankton and time-series to a wide:	s of North Atlantic Ocean regional systems	Joint peer-reviewed articles with data across North Atlantic coastal waters on at least two of these issues: a) macroecological patterns of cyanobacteria, b) ratios of diatoms to dinoflagellates and c) comparison of drivers causing temporal dynamics of

diatom species; in 2015; oceanographic and marine ecology scientific community. G of draft ICG-EUT 2014 conclud-Report this on Review OSPAR JAMP Eu- ed, and HASEC 2014 1/2015 OSPAR request by trophication Guide- endorsed, that these on guidelines were in need April 2015 lines phytoplankton spe- of a review. The guidelines should be revised to cies composition reflect new knowledge ICES is requested to about phytoplankton and advise OSPAR on needs within (directives the revision of the such as) the EU Marine OSPAR JAMP Eu-Strategy Framework Ditrophication Guide-rective (MSFD) and the lines which will be Water Framework Direvised by experts rective (WFD). from Germany, The It is the intention of the Netherlands and revision that the existing Sweden. WGPME is asked to aims described in the address the monitor- guidelines1 will be suping and measure- plemented with the folment techniques as lowing: mentioned in the to identify harmbackground inforful algae species and mation. blooms in line with MSFD Descriptor 5. to identify invasive (non-indigenous) species in line with MSFD Descriptor 2. to monitor effects of ocean acidification as e.g. on coccolithophorids (e.g. Emiliania huxleyi) in line with Descriptor 1 in MSFD. The revised guidelines should incorporate commonitoring measurement techniques such as (but not limited

¹ 1. to establish the spatial distribution and frequency of phytoplankton blooms; 2. to establish temporal trends, over periods of several years, in phytoplankton species composition and their relative abundance; 3. to identify key phytoplankton species

spectrofluorometry, flow cytometry and qualitative observations of foam production, and should make use of existing standards, such as EN 159722 and EN 152043 and reflect developments within OSPAR ICG - COBAM which is working on biodiversity monitoring and assessment. Data handling issues, such as the format required for reporting to ICES, should also be addressed.

Η

Produce four short Each paragraph should paragraphs for the be maximum 150 words **ICES** Ecosystem in length and can be sup-Overviews on the port by one figure. Parapelagic productivity graphs for each ecoregion (spatial variability, should be similar in style hot spots and sea- and address the overall sonality), one para-state and comment on the graph for each of the pressures accounting for following ICES changes in state. These ecoregions: Greater will go in section four of North Sea, Celtic the ecosystem overviews Seas, Bay of Biscay and not supposed to be & the Iberian coast long descriptions, but a and Baltic Sea. short synopsis of important points for man-

portant points for managers and policy developers.

(Template and Guidelines for Ecosystem Overviews)

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² Water quality – guidance on quantitative and qualitative investigations of marine phytoplankton

³ Water quality – guidance standard on the enumeration of phytoplankton using inverted microscopy (Utermöhl method)

3 Summary of Work plan

	1
Year 1	Gather and discuss methods used with WGPME (ToR a), find examples of microbial taxa and/or functional groups that have actually changed distribution (ToR b), analysis of data (ToR d), report on what is known (ToR e), review available modelling tools, statistical relationships and macroecological patterns (ToR f).
Year 2	Harmonize methods if required (ToR a), explore potential environmental drivers (ToR b), update existing time-series, include additional datasets and explore new analyses and presentations of data (ToR e), prepare and submit manuscripts (ToR f), explore geographical and recurring patterns, hindcast models and hypothesis testing using new datasets (ToR d).
Year 3	Presentation of best practice recommendations on a website (ToR a), delivery of second WGPME CRR (ToR e), provide an ecological syntheses and promote incorporation into existing time-series (ToR f), make projections under IPCC and other possible scenarios (ToR d).

4 Summary of Achievements of the WG during 3-year term

- An online image library has been set up following the workshop in Helgoland.
 The image library has been extended and this will be an ongoing process
 throughout the life time of the group. A dropbox has also been set up in which
 members can report unusual observations in their phytoplankton and microbial time-series.
- An updated summary of the results from the molecular methods survey presented in Plymouth was discussed in Gothenburg. A manuscript outline has been produced and a time table for further action (e.g. draft deadlines) has been agreed.
- An extensive literature review of molecular methods and their application in marine phytoplankton diversity assessments has been presented and discussed in Gothenburg. The results of both the questionnaire and the literature review will be incorporated into the current draft of the molecular survey review manuscript (probably to be submitted to PLOSOne).

5 Final report on ToRs and workplan

ToR A: As data on general phytoplankton collection and analysis methods had been dealt with in a previous meeting discussions concentrated on the molecular tools and the review of these methods that is to be submitted to a peer-reviewed journal. The aim was to assess whether/how molecular tools are used for routine monitoring in the context of phytoplankton time-series. The assessment was carried out using two different methods: A questionnaire distributed among scientists, and additionally a literature review. Updated results of the questionnaire as well as first results of the literature review (based on 100 individual articles) were discussed in Gothenburg in preparation of the review manuscript. The results revealed a considerable methods diversity in terms of sampling methods, primer sets, fixation and extraction methods. Software tools and bioinformatics platforms used for the analysis of sequence data also differed greatly. A key result was also that very few time-series operators as yet use molecular methodologies routinely although they hold great promise for increasing the taxonomic resolution of phytoplankton time-series. Further analyses of existing workflows for molecular surveys used by different teams will lead to recommendations on which set-ups could be incorporated into routine monitoring. These can be published online on the ICES website along with the general methods review.

ToRs B and D (and F): were discussed jointly and where individual manuscripts were in breakout groups. The Review of molecular methods included a skype discussion between the 3 principal authors (Kraberg, Stern, Metfies). The molecular methods review is based on a questionnaire of current practices in the use of molecular methods by taxonomic time-series operators. Additionally a literature review has been carried out and the outcomes of both review and questionnaire will be discussed jointly to produce a set of recommendations for the incorporation of molecular methods into routine time-series methods.

Tor C (Novel lineages): This was discussed again informally, based on previous discussions of a possible joint study on oomycetes. A. Kraberg reported on the occurrence of diatom parasites at Helgoland during 2014, where several diatom species showed high incidences of infection by parasites (mostly oomycetes e.g. *Lagenisma coscinodisci* and an unidentified oomycete infecting the pennate diatom *Pseudo-nitzschia*). During a previous meeting it had been discussed whether WGPME members collecting molecular data could re-check any existing sequence information for the presence of oomycetes. Two members did, and only one detected oomycetes in their existing data. However, most members have very little information on plankton parasites and they are rarely regularly quantified during routine monitoring. Therefore in Gothenburg it was decided to make a start with collecting information on known parasites but also any other unknown structures or cells found in samples during routine counting. Such information will be collected in a dropbox initially, but will also eventually be published online.

ToR E Prepare sections for the 2. Corporate Research Report

This report has been pushed back from May to 30 October 2015. The last report was 200 pages reporting on 61 sites with 1–3 pages dedicated per site. Bill Li's special chapter was

10 pages. This year's report should also have approximately 200 pages, reporting on 65 sites with a maximum of 2 pages per site to keep it compact. ICES is only publishing edited electronic copies, with high quality pdfs for printing by users. By comparison, the WGZME report in 2014 was 212 pages with no spatial chapter, 2–3 pages dedicated per site.

The following timeline was adopted by participants: 1. April 2015 to submit any remaining data up to 31/12/2012 and a 150 word summary of key work/projects done in individual time-series. 2. In May–July site authors should populate their site text, which should be written as an update to the last report. Todd also suggested a special topic section. 3. In September/October, WGPME editors should assemble the final draft to submit to ICES in November (actually 30/10/15): 4. ICES editors will carry out the final review and printing layout. Electronic co-products are encouraged as a dynamic elements e.g. IROC interactive graph tool, which facilitates downloads of ICES annual anomalies.

In regards to making monthly/annual anomalies downloadable with a citable format, some members had experience where data was misused if delivered in poor formats or else not acknowledged, when downloaded through a third party site and were concerned about this happening again. Most members said some of the data was already available with or without a time lag and could be used without acknowledgement but were more comfortable for data requesters to approach them directly through a link to the provider and/or to have some form of data requester identification through registration, linking or URI tracking. BK said ICES data policy was that all data should be open access so registration may not be possible- but this needs to be verified. After discussion, it was agreed that as a group WGPME was not happy about releasing annual anomalies and to maintain a two year time lag. Should we consider downloaded monthly/annual anomalies with citable format?

Special topic features

Todd O'Brien suggested 1–2 page special topic features for new ideas- members came up with the following suggestions:

- 1) An introduction to "Phytoplankton" e.g. compared to algae.
- 2) Abundance v. biomass discussion and how this alters data interpretation. A change in abundance may not equate to a change in biomass if the cells are small. This would tie in with SCOR137 biovolume initiative led by Jacob/Mike Michaellson.
- 3) What satellites and fluorometers cannot tell us- e.g. don't replace in situ with electronic sensors.
- 4) The usefulness of long-term phytoplankton and microbial data.
- 5) Discussion on phyto contribution to total ocean carbon/biomass. Norbet: Carbon or total biomass unit is important methodologically.
- 6) Hydrography as special topic-currents, tides.
- 7) Todd O'Brien requested last year a species list as an additional online effort to create basic species information. This could be viewed on a distribution map based on participant sites. If something interesting is found it could be report-

ed on the interface for example the *Calanus finnmarchicus/Calanus Helgolandicus* distribution map.

8) Science behind creating trend maps.

ToR G Comments on OSPAR JAMP Eutrophication Guidelines on phytoplankton species composition (Rapporteur A. Kraberg).

This ToR arose from a request by OSPAR to update the current guidelines which were adopted in 1997. The update was deemed necessary as it still contains indicator species which might not be accepted anymore by the scientific community, e.g. the links between Noctiluca blooms and eutrophication.

A new draft has been devised that discusses issues not previously considered, including biodiversity and invasive species. The guidelines also consider the whole microplankton community which includes the microzooplankton.

The new draft was introduced by Bengt Karlson (SMHI) and then discussed by the whole group. One item that was discussed was the paragraph on fixation methods. These vary greatly in different surveys and if microzooplankton is to be included in the guidelines, the need for stronger Lugol fixation (up to 5%) for microzooplankton assessments should be included in the guidelines. However, there as yet no commonly agreed let alone accepted fixation protocols and this should be remedied as this hampers the comparability of results in different studies

At the end of the session it was agreed, that members of the group should report any comments in writing to A. Kraberg for incorporation in a set of WGPME recommendations for further discussion by ICES/OSPAR.

Comments provided by group members after the session

Page 4 (Introduction): "These guidelines on phytoplankton include the microzooplankton" and in other sections where the term "micro-zooplankton" occurs, e.g. page 5, number "8" in Section 2 "Objectives" and Section 4...

The microzooplankton (20–200 µm; e.g. rotifera) cannot be reliably counted by the Utermöhl method as the volume of the sedimentation chambers is too small for representative countings of these species of low abundancy. It is already problematic for ciliates and other large unicellular species like Noctiluca. I would accept instead of "microzooplankton" the term "unicellular microzooplankton, which includes flagellates and ciliates. If the last sentence of section 7.1 is taken as a definition of "microzooplankton" within this guideline, it is acceptable, but this kind of preliminary definition should stand in the introduction.

However, it has to point out, that in reality we count only the heterotrophic flagellates and not the heterotrophic ciliates in routine phytoplankton monitoring.

The next point is the picoplankton. If the Utermöhl method is the only recommended method (section 7.1), picoplankton has to be excluded because it cannot reliably be counted by that method. Alternatively, it should be pointed out earlier in the text that a special technique is suggested for picoplankton, described in section 10.1

Page 4: 5th line from the bottom of section 1: there is written "and and meso-plankton". Delete one "and".

Page 5: Section 3.2 is empty as contents for this section is already contained in section 3.1 and 3.3.

Section 3.2 can be deleted and Section 3.3. should be named: "Temporal and spatial resolution".

I support reference to the existing European standards, e.g. concerning preservation, storage and biovolume and carbon calculation. Carbon calculation according to Menden-Deuer and Lessard (2000) should not be questioned as it was agreed in extensive phytoplankton expert discussions on the basis of 7 publications on non-linear calculations. The calculation formula of Menden-Deuer and Lessard were considered the best. It is used world-wide.

I can imagine that research institutes that do not regularly report the monitoring data to international data banks will complain that they should be accredited and use standardized species lists. However, I fully support the original draft that laboratories contributing to data banks have to cope with regulations demanded in section 8.1 and 8.2.

General session

As WGPME is a very dynamic group, in each annual workshop members are invited to provide updates on the work with their individual time-series. Four presentations were given:

- 1) Elisa Cappuzzo and Veronique Creach: Analysis of phytoplankton functional groups in realtime
 - The use of Flow cytobuoy and ferrybox systems for routine monitoring purposes at CEFAS were described and calibration/QA methods explained (the flow cytobuoy for instance is calibrated with beads every day). The instrument was described as robust, but one big challenge is the data management due to the large amount of image and numerical data that need to be archived and logically linked. N. Poulton commented that having images as metadata linked to the numerical data was a big advantage
- 2) Xelu Moran: More, smaller bacteria in a future ocean?
 - A study on the long-term trends in heterotrophic bacteria in Xelu's time-series was described. Long-term (Decadal?) signals were not only found in abundance but also size (decrease in size over time). Bacterial trends were investigated for two groups of bacteria, those with high and low nucleic acid content (HNA and LNA bacteria respectively) Laboratory experiments supported the hypothesis that a temperature increase might drive such changes.
- 3) Karen Wiltshire: The latest analyses from the Helgoland Roads time-series

 The Helgoland Roads time-series and associated data and data management processes were introduced. Recent work on the underlying drivers of long-term changes in biodiversity were described.

4) Norbert Wasmund: "The diatom/dinoflagellate index: a potential indicator for the Water Framework Directive"

An update of the use of the index was provided (based on a previous presentation in an earlier WGPME meeting). The utility of the diatom: dinoflagellate ratio was discussed. N. Wasmund stated that this ratio is mostly a general indicator of change in a planktonic community but does not point to particular drivers of change. However, it is still useful also because the ease of calculation of the index.

6 Summary of Working Group self-evaluation

To be submitted.

Annex 1: List of participants

Name	Address	Phone/Fax	Email
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Annex 2: Recommendations

1. Results of methodological reviews should be published on ICES website? This would not take the form of strict recommendations to be adhered to (in preference over other existing methods), but should provide more general guidelines (based on investigations used by many different teams) outlining pros and cons of different methodological set-ups for phytoplankton and microbial monitoring. This would be done for the first time for molecular methods.

Annex 3: Technical minutes from RGJAMP

Review of ICES Working Group on Phytoplakton and Microbial Ecology, 10-12 March 2015, regarding its report on ToR G (Comments on OSPAR JAMP Eutrophication Guidelines on phytoplankton species composition)

28 May 2015

Reviewers: Harri Kuosa, Finland (chair) and Donald Boesch, USA

WGPME Chairs: Xosé Anxelu G. Morán, Spain, and Alexandra Kraberg, Germany

ICES Secretariat: Sebastian Valanko

The WG gives a number of comments in their report. From the comments it appears the definition of microzooplankton includes also small rotifers in the used terminology. The discussion ends up with the term 'unicellular microzooplankton', which can be accepted.

The comment on picoplankton and its enumeration is relevant.

The comment on the use of existing European standards in preservation, storage and biovolume and carbon calculations is valid, and the standards could be mentioned in the Guidelines.