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

18–20 March 2014

Plymouth, UK



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Executive summary

The WGPME annual meeting took place in Plymouth, UK, 18–20 March 2014. The meeting was chaired by Xosé Anxelu G. Morán (xelu.moran@gi.ieo.es) and Alexandra Kraberg (Alexandra.Kraberg@awi.de) and was attended by 15 group members from 7 countries. In addition, there were 3 guests from Germany, UK and Canada (the latter by Skype conference) to guide specific topical discussions (Mirco Scharfe of AWI was invited to discuss the effects of hydrography on species distributions, John Bruun of Plymouth Marine Laboratory carried out statistical analyses and David Walsh reported on methods used for the study of microbial diversity, particularly prokaryotes).

The key aim of the meeting was to make progress on analyses discussed/commenced during the previous meeting on Helgoland. Therefore this year's meeting included a day's practical session to work on specific data analyses and on outlines for manuscripts. All meeting sessions were also discussed not only in the context of the group's ToRs but also those of the WKSERIES workshop.

The meeting began on 18 March with a discussion of methodologies and presentation of the results of a questionnaire on molecular methods. This had been distributed to a range of molecular ecologists, as well as the whole ICES group prior to the meeting. This revealed the great diversity of approaches used and therefore the need for harmonizing or at least summarizing for the available methodologies their advantages and disadvantages and to make this information openly available, especially useful for new time-series.

Following the discussions on methodologies, the contents and structure of the next Cooperative Research Report were discussed. Contributions are to be shortened. Todd O'Brien, Alexandra Kraberg and Xosé Anxelu G. Morán will edit this report.

19 March was devoted to the practical sessions. Several introductory talks on statistical and modelling approaches were given to start off discussions. In the morning the regions to be dealt with were re-affirmed (again taking into account the discussions at the Joint Phytoplankton/Zooplankton Workshop in Copenhagen) and data sets prepared for standardized analyses of long-term trends and seasonality of temperature, salinity and key phytoplankton species, including the genus *Leptocylindrus* and *Guinardia* represented in all time-series. In the next meeting to be held in Gothenburg manuscripts will be finalized.

In this session preliminary analyses of long-term current dynamics/prevaling current regimes were also shown and their potential explanatory power for phytoplankton distribution patterns was discussed.

While these discussions and analyses mostly dealt with eukaryotic phytoplankton a subgroup of WGPME members discussed progress with a manuscript on the distribution of *Synechococcus*.

1 Administrative details

Working Group name
Working Group on Phytoplankton and Microbial Ecology
Year of Appointment
2010
Reporting year within current cycle (1, 2 or 3)
2
Chair(s)
Xosé Anxelu G. Morán, Spain
Alexandra Kraberg, Germany
Meeting venue
Plymouth, United Kingdom
Meeting dates
18–20 March 2014

2 Terms of Reference a) – z)

ToR	Description	Background	Science Plan topics addressed	Duration	Expected deliverables
a)	Examine current marine microbial time-series sampling techniques with an effort towards harmonization if required.	WGPME can provide a summary of current methodologies used in microbial plankton time-series with the ultimate goal of achieving better comparability between sites.	112	3 years	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.
b)	Examine distribution and range patterns of microbial taxa and functional groups to discern significant change over time and to identify potential environmental drivers.	After finding examples of taxa and/or functional groups that have actually changed their distribution we need to know the environmental drivers underlying these changes before we can make sound projections.	113	2 years	Interim WG report; in 2014; to SSGEF

c)	Report progress on discovery of novel lineages and cryptic taxa of phytoplankton and marine microbes.	By providing state of the art knowledge of novel microbial biota we will be able to better understand unexplained variation of current time-series datasets.	121	2 years	Interim WG report; in 2014; to SSGEF
d)	Explore the use of hydrographic models in addition to statistical analyses to provide further understanding of distributional patterns of phytoplankton and microbial assemblages	We need to incorporate other perspectives and the expertise of researchers from different fields and ICES WGs in order to disentangle the factors causing changes of distribution in microbial plankton groups.	111, 114, 115	2 years	Interim WG report; in 2014; to SSGEF
e)	Prepare sections for the second Cooperative Research Report on ICES Phytoplankton and Microbial Plankton Status to be completed for June 2015.	The CRR needs to be updated regularly to better establish the climatologies and long term trends for phytoplankton and other planktonic microbes as well as introduce new analyses, providing the basis for informed assessments of distributional changes at all organizational levels.	11,12	3 years	Second ICES CRR Phytoplankton and Microbial Plankton Status Report; in 2015; to research community and policy makers.
f)	Prepare peer- reviewed manuscripts using existing phytoplankton and microbial plankton time-series to describe large-scale and long-term patterns in the distribution and seasonality of phytoplankton communities and chosen key species	WGPME is currently entering the position to provide multi datasets comparisons of microbial time-series to a wider scientific community, potentially of use also by policy makers.	11,12	3 years	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; to oceanographic and marine ecology scientific community

Summary of Work plan

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).

3 List of Outcomes and Achievements of the WG in this delivery period

- Review of molecular and non-molecular techniques: in addition to a general questionnaire in 2013 a questionnaire has also been produced for molecular techniques. The results have been discussed and analyzed during the annual meeting in Plymouth.
- An online image library has been set up following the workshop in Helgoland. The goal of this image database is to provide a reference image of all the species in phytoplankton datasets delivered to the WGPME database.
- A standard set of statistical techniques has been agreed (based on the experiences from the joint phytoplankton-zooplankton workshop, WKSERIES) held in Copenhagen in 2013. The techniques are currently being applied to an extended set of WGPME datasets. All datasets analyzed using this set of techniques have been consistently formatted to facilitate analyses in R.
- A metadata file for all time-series used in the analyses has been compiled for easy reference during manuscript preparation. The file contains standard metadata such as location, duration, sampling frequencies, but also information on changes in methodologies, changes in sampling frequencies, data gaps, etc.
- Agreement on the environmental variables to be included in the analysis of *Synechococcus* time-series datasets was achieved during the meeting. Besides temperature, nutrients and total/size-fractionated chlorophyll we will include available information on cyanobacterial cell sizes and heterotrophic nanoflagellates abundance. Between 7 and 15 time-series will be analyzed for seasonal patterns and long-term trends. .

4 Progress report on ToRs and workplan

ToR A Examine current marine microbial time-series sampling techniques with an effort towards harmonization if required (Rapporteur Xelu Moran):

The short presentation by Veronique Créach (Analysis of phytoplankton functional groups in realtime) was cancelled since she finally could not attend this year's meeting.

The presentation and discussion on progress in methodology comparisons was split into two parts, with microscopy and flow cytometry lead by Glen Tarran and molecular methods by Katja Metfies and Rowena Stern.

Microscopy and flow cytometry

Glen summarized and analyzed the wide variety of responses he got to the questionnaire sent last year. The plan is to produce a set of guidance/best practice recommendations on the ICES website that could be used by people starting taking microscopy and/or flow cytometry (FC) planktonic samples. Since the comparability of different methods is hardly achievable we should better discuss whether differences are actually significant and concentrate on a few items for recommendations.

As a first practical example, Glen summarized the responses to several questions, first for flow cytometry (FC) sampling for bacteria, picoeukaryotes and nanoeukaryotes, and then microscopy for larger phytoplankton. In the questionnaire ancillary details on hydrography, starting date, sample preservation and storage, etc., were included.

Flow cytometry

Sampling: Niskin bottles or the CPR were used.

Time to analysis: it ranged from the same date to months, although Xelu Morán and Bill Li argued that after 1-2 years effects were minor in comparison with time 0. In the meanwhile, fixed samples are kept at -80 or -20°C. Although long-storage samples will suffer some degradation, it is probably very slow.

Preservative: glutaraldehyde (G) or paraformaldehyde (PFA), or a mixture of both. Glen expressed no preferences, except for the anecdotal lower background noise of the latter. Bill uses premade PFA, thus avoiding the tedious dissolution step. Eileen Bresnan also mentioned the problem with safety rules, favouring PFA compared with G.

Fixation time: between 10 and 30 min, either at 4°C or room temperature (Bill and Xelu, following Daniel Vaultot's protocol).

Flash freeze: either N₂ or -80°C are good choices, whenever it can be done. If not, -20°C for a short period probably does not harm samples.

Instruments: A wide variety of instruments are adequate, including Accuri, FACSCan, FACSsort, FACSCalibur, etc.

Groups: Most of researchers use FC for counting heterotrophic bacteria, *Synechococcus*, *Prochlorococcus*, autotrophic picoeukaryotes and nanoeukaryotes and Cryptophytes. A few of them count also Coccolithoformids and heterotrophic nanoflagellates.

Flow rate: This is a critical step, which can be done with beads of known concentration, external standard or weighing samples of water before and after running the

sample. The latter, preferable method, becomes complicated on a ship, where micropipettes can be used instead. Marta Varela mentioned that 3 years ago at A Coruña they changed beads to weighing.

Microscopy

Sampling: Niskin bottle, although a simple bucket can be also used for strictly surface samples. Eileen mentioned integrated photic layer sampling while Malin Mohlin uses the Lund tube, well-established in HELCOM.

Preservative: Lugol and acidic lugol. Malin said that acid works well for long-term storage while neutral is good for 3-6 weeks for coccolithoforids. Claire Widdicombe and Alex Kraberg use formaldehyde for coccolithophorids.

Settling volume for the Uthermöhl chamber: From ca.1 mL to 100 mL, this is not prescriptive. It makes a difference though the size of the chamber according to Alex, i.e. in a large chamber they very small cells may not settle (or only very slowly). Certain consensus around 25–50 mL except for the deep blue ocean.

Microscope: There are no inherent clear advantages for using different microscope set-ups, it rather depends on the focus of the investigation and the detail required. However for Uthermöhl samples inverted microscopes are clearly necessary. Individual labs often use different magnification protocols for enumerating the plankton samples. Often magnifications of 5X, 10X 20 X, are used to scan the entire Uthermöhl chamber to count species of certain sizes, 40X is required for the smallest taxa. In very dense samples only a portion (e.g. one horizontal or vertical track) is enumerated at a certain magnitude and the result extrapolated to the area of the whole chamber. Minimum needed is 1 transect /field of view. Although myriads of individual protocols are in common use, attempts to produce standardized protocols exist. Malin and Norbert Wasmund suggested to check the HELCOM website for an example. In any case, volumes need to be properly justified according to the purpose of the analysis. Dominique Soudant, after a bibliographic search, warns about potential bias in the counting process. Using 10 or 50 ml chambers for instance can result in significant differences.

In the context of discussing sampling programmes quality assurance programmes such as BEQUALM (<http://www.bequalm.org/about.htm>) were also mentioned as one means for intercalibrating analyses.

Net tows: Taking discrete water sample with net tows allows for a fast, qualitative analysis at the sampling site. Different mesh sizes (Claire, XX; Norbert, 20-25 µm) and vertical (Alex) or surface hauls. Samples thus taken can be examined live, and then preserved with lugol or formalin. As for recommendations, net tows are only qualitative but it is good to do it, especially for groups such as radiolarian (Alex). It will take roughly 10 min sampling plus 1.5 h analysis.

To close this section, other issues were mentioned, including fixation time and temperature. It was also suggested to conduct a pre-WGPME annual meeting workshop of 1–2 days. We should agree where, with Plymouth as a possible venue.

Should be Molecular Methods for environmental samples using Next Generation Sequencing (NGS)

Rowena and Katja first stated that with regard to molecular sampling methods, there is no such thing as a standard, everybody does it their own way. For instance, sample

volume ranges from <1 to 100s of litres. In regards to high throughput sequencing of environmental samples, the use of replicates is rare because of the tremendous costs.

There is a large size range in the organisms addressed by these methods, from bacteria to protists. The advantage of capturing cryptic species is hampered by the risk of introducing biases, e.g. at the PCR amplification and sequencing stage, which can generate more erroneous sequences than older methods of Sanger sequencing of clone libraries due to the sheer scale of output- thousands to millions in NGS compared to hundreds from clone libraries.

In a survey study sent to people conducting molecular research, Katja and Rowena noted that there are few, if any molecular time-series over 5 years dating back earlier 2004. Problems with funding stopped some of these earlier molecular time-series. In summary, the results of this survey were:

Frequency: Wide range, from every other day to year basis.

Geographical range: From single-station to cruise data.

Volume: 0.5–6 L, the question is to have enough samples without clogging.

Storage: As long as sterile equipment is used, up to 5 years. DNA is stored in a buffer. Quantification is usually made with the NanoDrop®, although PicoGreen® dye is also a recommended alternative.

Most of the time-series target ribosomal genes, via PCR or next-generation sequencing (NGS) technologies Qiime to become one of the standards of NGS, although most researchers customised the Qiime bioinformatic pipeline".

Katja stressed the benefits of molecular time-series, but a serious problem is that they are detached from the traditional taxonomic or other microbial surveys. We should seek to get the "traditional" and molecular time-series communities to collaborate more closely. According to Pep Gasol's response to the survey, this is not the case yet. Xelu mentioned that they continue to sample DNA at their time-series and suggested that at least collecting the DNA could be a good procedure whilst agreed molecular procedures and funding have been established. At Roscoff, for example, the continuing effort of sampling DNA is analysed only certain years. We should include at least basic variables in these "potential" molecular time-series. Bill commented on the additional value for cryptic species and rare taxa, not accessible by microscopy. Norbert raised the question of quantitative versus qualitative results. Although quantitative issues are open we are working towards standard qualitative DNA methodology for NGS. This is not really the case for RNA, which is a problem in itself. The group is reluctant to do RNA until we reach a more mature developmental stage.

Katja stated that close agreement between microscopic and molecular analyses at identifying higher taxonomic levels such as diatoms became increasingly difficult when you come down in cell size. The misidentification increases because frequently there are no taxonomical annotations. Culturing is for most species a dead end. These issues have been raised in past ICES meetings, but efforts to improve our culturing capabilities are frustrating.

David Walsh called to our attention the major issue that while microbial eukaryotes share a universal database, for bacterial diversity, there are multiple taxonomic databases coexisting, with different clades making it very difficult to compare results. After identifying the need for the community to produce a review article, taxonomically unified, as has been done for freshwater bacteria (with much easier taxonomy), he wondered whether it could be something deliverable by ICES. Whilst major efforts

have been achieved for microbial eukaryotic taxonomy and ribosomal databases, much needs to be done for alternative DNA markers. This should also apply to understudied marine fungi.

We finally agreed that we should try to hold a meeting with traditional time-series researchers, with the final outcome of producing a harmonized paper, combining ideas from the two sides.

After discussing the sampling techniques, Alex Kraberg gave us a short update on the images reference collection for the WGPME. In this webpage you can look at the methods, the image library, together with all relevant images, observational and taxonomic metadata all within a common library. Material is published under a creative commons non-commercial licence. Since www.planktonnet.awi.de already exists, it can create new collections easily. Images include live and preserved organisms. It contains links to other databases such as WORMS and ALGAEBASE Pangaea for relevant numerical datasets.

PLANKTONNET also involves an element of quality control. The sequence usually goes: register on the database, upload image, review by administrator, publication. Only then become images visible. So far 4 WGPME sites have been uploaded. Before closing ToR A, Malin briefly described how they dealt with microalgae in the Baltic Sea. A list of all nordic algae is available at www.nordicmicroalgae.org after subscription. There is also a smartphone app. Synonyms are sent to WORMS. There is a standard list of what people supply, after filling some required fields. The app is under Creative Commons licence and used mostly for educational purposes with different languages available.

ToR B Examine distribution and range patterns of microbial taxa and functional groups to discern significant change over time and to identify potential environmental drivers

ToR D Explore the use of hydrographic models in addition to statistical analyses to provide further understanding of distributional patterns of phytoplankton and microbial assemblages

ToR B was addressed jointly with ToR D in a practical session, in which a set of standard techniques is to be deployed to investigate an agreed set of phytoplankton species and physical variables. Multivariate analyses (including multidimensional scaling) were used as an exploratory technique to investigate whether there are sites or years more similar to each other than others. This was followed by the calculation of climatology indices (Box Jenkins model) on the whole dataset once all the data (monthly averages) have been assembled. As a further step, once the entire dataset has been treated in this manner, we will also explore non-linear techniques to further drill into the data. We will keep expanding the database of standardized species data. One additional dataset for Ireland has already been delivered.

ToR C

The session started with an introductory talk by Dr David Walsh (see abstract, in italics, below).

Integrating molecular approaches into a microbial plankton time-series using archival samples

Time-series observations have been essential in assessing environmental variability and discerning ecosystem responses to global change. However, for the microbial communities that sustain Earth's ecosystems, we still only partially understand their long-term response to

environmental forcing. In part this is due to (1) the logistical difficulty of frequent and prolonged sampling campaigns, particularly in remote locations like the ocean and (2) the trouble in accurately describing the composition of exceptionally diverse microbial communities. Here, we address these issues by applying 16S rRNA gene deep-sequencing technology to a multi-year microbial sample archive from the coastal ocean, specifically Bedford Basin, Nova Scotia. Previous studies in Bedford Basin have demonstrated climate-driven changes in phytoplankton communities and propagation of this response to bacterioplankton. Our analyses reveal the temporal dynamics of bacterioplankton populations in the coastal ocean. Moreover, we have identified links between physiochemical conditions (e.g. temperature, nutrients, oxygen), and biotic factors (e.g. phytoplankton composition) and bacterial community structure. Using the extensive microbial time-series data, we aim to identify microbial species that may serve as sentinels of environmental change, or those that may provide insight into environmental perturbations undetectable by physicochemical analyses (i.e. bio-indicators).

The ensuing discussion dealt with the diversity of different taxa (groups that are not necessarily routinely counted). For instance new fungal species are brought to the English channel by shipping (comment Katja Metfies).

A suggestion was made to add the yet scarce information on parasites within this ToR (e.g. chytrids as parasites of dinoflagellates). Also, we should pay specific attention to the growing literature on viruses associated to phytoplankton blooms. The oomycete question also still requires further discussion. A joint sampling campaign had been discussed in Helgoland and we still need to follow up whether even from existing samples we could organize some re-analyses or molecular work. A potential collaborator at the Senckenberg Institute, Frankfurt has already been identified and would be willing to participate in oomycete sample and data analyses.

ToR E (Rapporteur Claire Widdicombe): The next Phytoplankton and Microbial Plankton Status Report, printed as an ICES Cooperative Research Report, has to be delivered in 2015. The report will be edited by Todd O'Brien, Alexandra Kraberg and Xosé Anxelu G. Morán. The bulk of the report should be mostly written before the next WGPME meeting (March 2015) so that the trans-basin results and general topic sections can be discussed with the larger group. Todd reported that for the last Zooplankton Status Report the number of printed copies requested by the participating authors was very high (50+ copies) and that this was causing production cost concerns with the ICES printing office. In WGPME requests would probably be fewer, but some members commented that they would still find it useful to have a hard copy for their institute libraries. For our next report, only ten free copies will be given to WGPME as a whole.

The report structure and other possible means of communicating/presenting WGPME data and resources were discussed. It was proposed to reduce the length of the site summaries, produce a spatial summary, but also to include topical summary pages. This proposal was accepted but the exact topics for the topical summaries will be discussed later.

New ideas for inclusion in the Status Report were discussed and agreed in order to appeal to a wider audience:

- Introduction to Phytoplankton, including descriptive text, glossary and schematic information (Alex Kraberg and Norbert Wasmund to lead)
- Contribution of phytoplankton taxa to total carbon/biomass (Xelu Morán to lead)

- The use of abundance *versus* biomass data, as discussed in HELCOM (Norbert Wasmund and Malin Mohlin to lead)

“What satellites cannot do?” Importance of *in-situ* data to complement and calibrate large-scale information e.g. satellite data (Bill to lead)

In addition to the standard plots included in previous reports it was also discussed whether the report should include summary plots of baseline data from remote sensing and that these reports might be presented at monthly frequencies or as seasonal plots. Again, if relevant data can be made available to the group, these plots can be included in the report.

In addition to reducing printing costs, the ICES printing office feels that the future of reports like this are best found in an electronic and interactive medium (versus paper sitting on a shelf). They presented IROC (<http://oceans.ices.dk/iroc>) as one mechanism for visualizing data that might be transferable to WGPME activities as well. IROC (ICES report on ocean climate) has an online facility in which time-series data can be graphically presented as annual means or anomalies and the data can also be downloaded. This interface was created in addition to a printed report by IROC, as a supplement. The advantages and disadvantages of this were discussed briefly (an advantage would be a greater visibility of WGPME resources, a disadvantage might be versioning problems). It was decided that this would remain an option but required a bit more thought (i.e. there will be no immediate steps to implement such a resource).

An additional discussion item was the IGMETS initiative (International group for marine ecological time-series). This IOC-UNESCO initiative will produce a global scale “plankton and biogeochemical status report” that will include plankton (micro- to zoo-) as well as nutrients, carbon elements, and alkalinity. This report will not compete with the ICES report series, as it will (by necessity of volume) be fairly general in its discussions. This report will also be beneficial to WGPME, as it is already drawing in new North Atlantic-area time-series that could be invited to participate in future WGPME endeavors. Todd O’Brien, as member and analysis lead for both IGMETS and WGPME, will ensure that the two reports do not duplicate or contradict each other.

ToR F: Prepare peer-reviewed manuscripts using existing phytoplankton and microbial plankton time-series to describe large-scale and long-term patterns in the distribution and seasonality of phytoplankton communities and chosen key species

Rowena Stern and Katja Metfies presented a draft outline for a paper detailing molecular procedures including the question “What constitutes a time-series?” Key factors in success are: repeatable, sustained, consistent, regular, good practice/protocols, long-term, abundance element, availability with or without genetic database, good strategy, financial support.

Rowena and Katja aim to hold a workshop with other molecular scientists to:

- Define what a time-series is
- Review current molecular datasets
- Bring and combine available information and practices into the future

Following discussions it was concluded that in an ‘ideal world’ scenario molecular techniques would concentrate on taxa not/rarely seen by microscopy and therefore increase taxonomic resolution and added value for ICES.

Xelu Morán presented progress on the *Synechococcus* time-series paper which currently includes 7 time-series datasets but aims to also include BATS. Any other datasets, e.g. Roscoff-Astan or Mediterranean sites would be welcome, especially data up to the end of 2013. The outline paper is progressing well and relationships with temperature, nutrients and total chlorophyll *a* (only a few sites with size-fractionated data) will be tested. The question of changes in cell size distribution was noted as important but again only a few sites have data available. It was also agreed between the ad-hoc subgroup of WGPME members discussing on this paper (Bill Li, Marta Varela, Glen Tarran and Xelu Morán) that data on heterotrophic nanoflagellates abundances should be included when available in order to address top-down controls on cyanobacterial distributions. The first draft of the paper will be prepared by the end of 2014.

Alex Kraberg presented progress on a third paper which addresses the spatial and long-term patterns of bulk phytoplankton e.g. diatoms and dinoflagellates using CPR data as well as 3 key (and easily identified) taxa using abundance data from microscopy counts from 7+ time-series stations. Multivariate analyses (e.g. Primer) and climatology indices (Box Jenkins model) will be performed on the whole dataset once all the data (monthly average) have been assembled.

A detailed metadata file describing collection and analysis methods of the different time-series will be collated (by Claire Widdicombe) to accompany the papers above.

John Bruun (statistician, PML) presented a first 'look see' of the combined time-series results (*Synechococcus* and microscopy data) using the Box Jenkins model and TSA analyses using R. This provided the group with an opportunity to compare climatology and harmonic regression patterns between individual time-series. John is to adapt the R script and run the finalised datasets and provide the lead authors with plots and data for the papers outlined above.

5 Revisions to the work plan and justification

None.

6 Next meetings

Annual WGPME meeting will be held in Gothenburg, Sweden, March 2015 (dates to be confirmed).

Annex 1: List of participants

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Annex 2: Recommendations

Recommendation	Adressed to
1. The outcomes of the numerical analyses will be shared through the production of peer reviewed publications the reviews of methodologies should additionally be made available on as a regularly updated web resource . A general methods questionnaire and one dedicated to molecular tools have already been prepared and the return analyzed and this information should be made available on dedicated ICES web resources.	
2. Hold a molecular methods workshop (to be organized by WGPME molecular ecologists Rowena Stern and Katja Metfies) to discuss the current diversity of data options for better standardization/better availability of information on different techniques.	
3. A general methods workshop is sought prior to the next meeting in Gothenburg.	