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Increasing the quality, comparability and accessibility of phytoplankton species composition time-series data

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ABSTRACT

Phytoplankton diversity and its variation over an extended time scale can provide answers to a wide range of questions relevant to societal needs. These include human health, the safe and sustained use of marine resources and the ecological status of the marine environment, including long-term changes under the impact of multiple stressors. The analysis of phytoplankton data collected at the same place over time, as well as the comparison among different sampling sites, provide key information for assessing environmental change, and evaluating new actions that must be made to reduce human induced pressures on the environment. To achieve these aims, phytoplankton data may be used several decades later by users that have not participated in their production, including automatic data retrieval and analysis. The methods used in phytoplankton species analysis vary widely among research and monitoring groups, while quality control procedures have not been implemented in most cases. Here we highlight some of the main differences in the sampling and analytical procedures applied to phytoplankton analysis and identify critical steps that are required to improve the quality and intercomparability of data obtained at different sites and/or times. Harmonization of methods may not be a realistic goal, considering the wide range of purposes of phytoplankton time-series data collection. However, we propose that more consistent and detailed metadata and complementary information be recorded and made available along with phytoplankton time-series datasets, including description of the procedures and elements allowing for a quality control of the data. To keep up with the progress in taxonomic research, there is a need for continued training of taxonomists, and for supporting and complementing existing web resources, in order to allow a constant upgrade of knowledge in phytoplankton classification and identification. Efforts towards the improvement of metadata recording, data annotation and quality control procedures will ensure the internal consistency of phytoplankton time series and facilitate their comparability and accessibility, thus strongly increasing the value of the precious information they provide. Ultimately, the sharing of quality controlled data will allow one to recoup the high cost of obtaining the data through the multiple use of the time-series data in various projects over many decades.

1. Introduction

Phytoplankton time-series (PTS) data are important to assess the ecological health and status of water bodies and changes occurring under climatic and anthropogenic pressures. When

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supplemented with a suite of physical (e.g. temperature, salinity), chemical (nutrients), and biological variables (e.g. chlorophyll and zooplankton data), PTS can be used to evaluate long-term changes in pelagic systems and possible causes and consequences of changes on the marine ecosystem. Marked interannual differences in the response of phytoplankton to seasonal forcing factors are a common characteristic observed among and within coastal sites (Cloern and Jassby, 2010; Zingone et al., 2010). Therefore, time series over several decades are required to discern statistically significant climate-driven trends from random events (Henson et al., 2010). However, multi-decadal PTS, particularly those containing species composition data, are relatively rare (Edwards et al., 2010).

For many relevant issues such as biodiversity, Harmful Algal Blooms (HABs), food-web structure, the invasion of non-indigenous/alien species and ecological process studies, taxonomic information on plankton composition at the species level is required. In the case of HAB species, correct taxonomic identification is needed to inform decision-making in order to alert the public and possibly close areas to shellfish harvesting. Indeed, phytoplankton species composition is part of a suite of ecological indicators that are required to assess the ecological/environmental status of a water body as mandated by the EU Water Framework Directive 2000/60/EC (European Commission, 2000), Marine Strategy Framework Directive 2008/56/EC (European Commission, 2008), and other regional regulation, such as the State Oceanic Administration of China.

Presently, microscope-based identification and enumeration is the 'gold standard' to which other approaches to determine phytoplankton composition (e.g. HPLC, image analysis, flow cytometry) are compared. However, analysis by light microscopy (LM) is time consuming and difficult to standardise, and generally requires highly specialised taxonomic expertise. In addition, it may have limited resolution power for certain groups of phytoplankton species, such as the small flagellates. To study these taxa, molecular methods have been introduced in some time-series studies (e.g., Medlin et al., 2006; McDonald et al., 2007), also helping identify morphologically similar/identical species (cryptic species) which may have distinct ecological, biogeographic and phenological patterns (Degerlund et al., 2012; Ruggiero et al., 2015). Recent DNA metabarcoding approaches using high throughput sequencing (HTS) techniques represent an impressive advancement (e.g. Massana et al., 2002; Guillou et al., 2004). Rapid technological developments are expected to allow routine identification based on environmental DNA analyses, which will possibly be incorporated into automated detection systems in the future. Currently these methods are not quantitative, as they provide relative abundances that may be biased due to preferential sequence amplification, nor are they exhaustive, as reference molecular information is still lacking for most phytoplankton species. Verification and inter-calibration with microscope-based identification, and a coupling of molecular and morphological approach is recommended (McManus and Katz, 2009). Molecular methods are also diverse and have complex metadata for which a level of standardization comparable to other types of data does not currently exist (Sansone et al., 2012). This presents a considerable challenge and for the foreseeable future molecular surveys cannot entirely replace traditional taxonomic surveys.

Semi-automated image analysis of samples by a laboratory-based FlowCAM (Sieracki et al., 1998; Jakobsen and Carstensen, 2011), fluorescent image analysis with PlanktoVision (Schulze et al., 2013), submersed flow-cytometers such as the CytoBuoy (Dubelaar et al., 1999), the CytoSense scanning flow-cytometer (Malkassian et al., 2011) and the flow Cytobot with imaging options (e.g., Olson and Sosik, 2007) show promise for automatic classification of phytoplankton. These instruments can generate thousands of images per hour which preclude manual inspection to verify cell identification. Hence, new challenges in this field are in the development of techniques to analyse these large datasets of phytoplankton images (e.g., Álvarez et al., 2012), while expert taxonomists are required to 'train' automated systems for taxa recognition. On the other hand, these methods are consistent in the generation of their errors and do often provide precise information on the magnitude of their errors (e.g., Culverhouse et al., 2003; Culverhouse, 2007). Their imaging capability is most efficient in analysing individual cells from 10 to 100 μm (Olson and Sosik, 2007) which at times allows one to detect and track taxa of interest with distinct shapes (e.g., Campbell et al., 2010). However, limited image resolution and constraints in the window size observed often do not provide the species level detail that is needed to track changes in HABs, food-webs, and biodiversity in response to changes in climate. Switching from microscopy-based analysis to automated image analysis without considerable inter-calibration of the methods will likely result in inconsistent datasets.

Because species data over time are imperative for understanding changes in the phytoplankton community, most PTS continue to obtain data through LM analyses. Compared to abiotic oceanographic data, these analyses are more complex and difficult to standardise. Differences in sample collection, handling and observation techniques, and the diverse levels of taxonomic experience by the operators, may hamper the reliability of species composition and abundance assessments (Culverhouse et al., 2003; Jakobsen et al., this issue). In turn, the difficulty in controlling and assessing data quality may weaken statistical comparisons of phytoplankton diversity among different PTS datasets. Problems may exist even within a single PTS due to changes in methods, microscopes and analysts over time. For example, it may not always be possible to determine if a new species at a site is an alien/invasive species since it may have been previously misidentified or overlooked by a different person or method.

The development of standards for analysis, taxonomic identification and metadata recording, as well as quality assurance/quality control (QA/QC) procedures, are crucial in order to facilitate a reliable evaluation of the changes in species composition in PTS data, as well as the comparison among PTS collected at different sites. Despite the numerous efforts to standardise PTS data collection, different procedures are still followed to obtain these data. In addition, these procedures are not always reported in sufficient detail in scientific or dataset publications, where there is often a lack of detailed metadata and complementary information.

In an accompanying paper (Harrison et al., this issue), we examined the cell biovolume of 214 ecologically important species in 36 studies and found that incorrect or ambiguous species identification, taxa name changes, and lack of metadata presented limitations to using disparate datasets. That work highlighted the need for the current paper, which addresses metadata recording and QA/QC issues associated with phytoplankton species composition time series. Based on personal experience with time-series data and on information from the literature, we briefly review the different steps of PTS data collection. Our main objective is to identify the key methodological issues which may cause major differences among PTS data from different sites and geographic regions. With the aim of providing a first step towards a comprehensive manual on QA/QC of PTS, we offer some general recommendations which would allow a more reliable comparison among phytoplankton time-series datasets and ensure safe data storage and correct long-term use of these precious data.

2. Quality assurance/quality control elements for PTS data

QA/QC protocols for phytoplankton data should be based on clear procedures and exhaustive complementary information. Even when using a known standard method, for example the Utermöhl method (Utermöhl, 1958), it is necessary to have a comprehensive manual with a detailed description of the various steps for data production that should be archived electronically along with the phytoplankton data. Many unique datasets, like the North–Atlantic CPR (Continuous Plankton Recorder) and HELCOM PEG (Phytoplankton Group of Experts) monitoring in the Baltic Sea, have well documented procedures for all steps of phytoplankton data production (Richardson et al., 2006; HELCOM, 2014). In the latter case, additional, more detailed instructions are provided within each institute of the nine different HELCOM countries.

Following the classic "Phytoplankton Manual" by Sournia (1978), several manuals and book chapters have been published over the last decade with the aim of providing instructions on key steps of phytoplankton sample handling and counting (e.g., Andersen and Throndsen, 2003; Karlson et al., 2010; CEN, 2011). A number of alternative methods are reported in these manuals, with comments on the advantages and disadvantages for each choice. In some cases, the steps to validate and control the quality of the data are indicated. We refer the reader to these manuals for detailed and exhaustive descriptions of the methods for phytoplankton species analysis, whereas in this paper, we attempt to identify the main issues that may impair data quality and comparability within and among PTS, and the steps to allow QC/QA of the data obtained.

2.1. Sampling procedures

The first step of phytoplankton composition assessment, and therefore the first possible source of bias, is the sampling protocol/strategy. The choice of the sampling site within a given area and the sampling frequency are generally dictated by local, scientific and/or logistical needs and should be described in a clear and standardised way, with details of sampling time, coordinates and depth, along with the sampling device and methodology (Table 1).

Most phytoplankton samples are collected as whole seawater samples with Kemmerer/Van Dorn/Nansen/Niskin/GO-FLO type bottles, buckets or hoses. While the device used may not constitute the main source of variability, differences in the results may arise from where the sample was taken, either in the surface or subsurface waters, or by mixing samples from different depths (Majaneva et al., 2009). For example, collection systems avoiding the surface can ignore cyanobacterial blooms. In some cases, samples are taken as an integrated water sample from different depths (e.g., HELCOM, 2014). In automatic FerryBox sampling on ships-of-opportunity in the Baltic Sea area, the samples are taken with a hose from a fixed depth from the surface layer mixed by the moving ferry (Rantajärvi, 2003). The CPR survey tows the recorder at 7–10 m depth in the propeller wash of the ship which is comprised of waters mixed from the top 20 m of the water column (Richardson et al., 2006).

Plankton nets filtering large quantities of seawater can provide comprehensive samples of species including sparsely occurring taxa. This approach was the one originally applied in all phytoplankton studies, and basically is still used in the case of the CPR (Richardson et al., 2006). However, the use of plankton nets has been dismissed in routine sampling in most of PTS studies, or rarely applied to obtain only qualitative or semi-quantitative data. This has led to a paucity of information on larger phytoplankton taxa that do not attain high abundance. For example, in the Mediterranean Sea, information on the long term trends of the colonial *Halosphaera viridis*, *Phaeocystis globosa*, the large diatoms such as Rhizosoleniaceae and the dinoflagellates of the genera *Tripos*, *Ornithocercus*, *Histioneis* and *Citharistes* is largely missing despite the relevant role that these species play in biogeochemical cycles (Siokou-Frangou et al., 2010). In fact for microplankton (>20 μm), at least the relative proportion of species can be obtained through cell counting of net samples. Based on net sample analysis, a comparison was possible among recent and older distribution data of *Tripos* (previously called *Ceratium* and *Euceratium*) species collected in distinct Mediterranean areas (Tunin-Ley et al., 2009). The flow through the net determined using a digital flow-meter is prone to errors since waves, different tow speeds and ejection may all affect the assessment of the volume of water filtered. However, when nets are towed vertically from a given depth to the surface, the water volume filtered through the net can be calculated, allowing one to obtain cell concentrations of species larger than the mesh size. The net mesh-size and the range of the depth of sampling are critical details. A mesh size of 20 μm fits the delimitation of the microplankton, whereas a 10 μm mesh-size, e.g. recommended within HELCOM (HELCOM, 2014), would allow for a more complete collection of the >10 μm size fraction, but may be subject to clogging during algal bloom conditions. The 270 μm mesh-size used in the CPR is more suited for larger and robust phytoplankton species; however, smaller taxa are routinely retained mainly due to clogging produced by larger species (Richardson et al., 2006).

2.2. Sample preservation

Sample fixation required for subsequent analyses is a critical step for both the preservation of specific plankton groups and their identification. Some species are completely destroyed by specific fixatives. For example, many naked nanoplanktonic species or even larger organisms are lost in the fixation with either formaldehyde or Lugol's solution, or they lose flagella or markedly change their shape, impairing identification.

A saturated formaldehyde solution (37%) at variable dilutions (1–3.7% final formaldehyde concentration) is often used for preservation. The solution must be neutralised with different methods (Andersen and Throndsen, 2003) to avoid the dissolution of the calcitic plates of coccolithophores and still allow for diatom silica preservation. As this fixative cannot be used without precautions, it has been replaced by Lugol's solution in many cases. Acid Lugol's solution is normally used in areas where coccolithophores may be less abundant or absent (HELCOM, 2014). As Lugol's solutions decays with time, the amount of fixative should be checked by the sample colour, and analyses must be performed within a few months from sampling. On the other hand, neutral Lugol's or formaldehyde is needed for those samples which are used to identify dinoflagellates through their plate pattern using epifluorescence and Calcofluor (Andersen and Throndsen, 2003). Different fixatives, or a mixture of some of them, may be used for different areas, to answer special research questions or for long term storage (Mukherjee et al., 2014); therefore PTS data producers and users must be aware of the different performance of each of these preservatives and clearly report them in their metadata.

The time between the collection and the examination of the samples may have a great but unpredictable impact on the phytoplankton samples. Although specimens collected more than one century ago may still be retrieved in liquid samples, preservation time may have different effects on a mixture of species in a natural sample. Normally, naked species are more delicate and sensitive to preservation time. However, even silica diatom frustules may dissolve with time, possibly due to specific bacteria present in the original sample (Bidle and Azam, 1999). Occasionally, even formaldehyde-fixed samples may show growing bacteria, which makes the length of preservation time presumably dependant on

Table 1
List of the suggested minimum metadata to be included in publications and archived PTS datasets.

Sampling program	
Name	Full name, acronym
Type of program	International, national or local monitoring program, scientific project
Funding	Institutional, by funding agencies, national or international programs
Sampling site	
Geographic coordinates	Latitude and longitude
Site description (name and area)	Site name and general description (e.g., depth and coastal geomorphology)
Site description (hydrographic properties)	Is the site tidally influenced, in an area of strong upwelling or currents, near a river outlet, near a busy shipping lane, or near a water treatment plant, etc.?
Sampling procedures	
Sampling frequency	Daily, weekly or monthly or other specified time period
Sampling time	As recorded (time provided), at "night" or "noon", at high or low, spring or neap tides
Collecting device(s) and method(s)	For net samples, mesh size, towing time and speed. For sea-going surveys, kind of platform, ship, boat or moored buoy
Sample type	Spot sample, composite sample, integrated sample or *in situ* measurement
Sampling depth(s)	Minimum and maximum depth values. For integrated or composite samples, provide specific method and depth information
Name(s) of collector(s)	
Sampling notes	Note any gear problems, observable water conditions (blooms) or weather events (strong wind, sun/clouds, or rain while sampling)
Sample preservation[b]	
Fixative type	Chemical, pH, neutralizing method
Fixative concentration/amount added	ml of fixative/100 ml sample
Fixative time since collection	How long between sampling and fixation
Fixative maintenance schedule	How maintained and how often (e.g., for Lugol-fixed samples, based on visual check of colour)
Storage container type	Dark or transparent glass bottle, plastic jar, etc.
Storage temperature	°C
Storage in light/dark	Light/dark
Sample preparation[b]	
Time since collection and preservation	How long since field sampling and/or preservation
Acclimatization method	How long the sample was kept at room temperature
Homogenization method	Automatic or manual, number of rotations, gentle
Pre-concentration	If applied, specify the method (decantation, sedimentation, centrifugation, filtration)
Sub-sampling method	Volume of the sample in the counting chamber (ml)
Type of chamber	Compound chambers, other chambers
Calibration of the chambers	By the manufacturer, or by users
Chamber cleaning procedures	Bio-detergents are recommended
Sedimentation time	Hours
Sedimentation temperature	°C
Name(s) of sample preparer(s)	
Additional preparation notes	Any observations or deviations from standard protocol
Sample storage after analyses	Yes/no. Left over sample volume and storage place, or waste disposal
Counting procedures[b]	
Uniform sedimentation	Yes/no. In the latter case, a new sample should be prepared or the whole chamber should be sampled
Microscope (brand, type, equipment)	
Microscope viewing parameters	Bright field, phase contrast
Magnification[a] (objective x eyepiece x optovar)	
Surface counted[a]	Transects (1, more), fields, all chamber bottom
Special software for count records	Yes (specify software)/no
Cell count record sheets	Yes/no
Total volume examined[a]	Area observed/chamber area x subsample volume
Type of cells counted[a]	All, most abundant taxa, small taxa, single cells/colonies, life stages (e.g. cysts, resting spores, dividing cells, conjugating cells)
Detection limit	Based on volume examined
Special techniques[a]	Staining, tilting, differential interference contrast, epifluorescence
Name(s) of analyst(s)	
Counting notes	Problems with preservation or observations (detritus, bacteria, etc.)
Taxa identification	
Literature[a]	
Confirmation by special techniques[a]	Squashing, staining, others
Check-list availability	Yes/no. Specify the check-list used
Photo-archives[a]	Yes/no. Give the web address if available
Additional names of experts for identification[a]	
Data management	
Method of raw data and counts archiving	
Method of final cell counts archiving	
Quality control methods (numeric)	
Taxonomic name storage and validation	Check for spelling mistakes, taxonomic and nomenclatural changes (e.g., using WoRMS)
Name(s) of data preparer	
Data storage, availability and publications	DOI's, papers, datasets, local websites, public websites
Data sharing policy	

[a] Information should be given for individual samples or species in case specific methods or resources were used.
[b] References to specific methods or resources used should be included.

the bacterial community present at the time of collection. The recommendations in these cases are that samples should be kept in tightly sealed bottles in cold (4 °C) and dark conditions before analysis, and that they should be observed within a year from collection, or at least at comparable times, to avoid any possible bias within a single time series. Unfortunately, the time between collection and analysis (i.e. time in storage) and preservation conditions are rarely mentioned in phytoplankton studies or in the accompanying metadata.

2.3. Sample preparation and counting procedures

In order to obtain comparable and reliable results, detailed counting procedures should be followed, which should be explained in the methods section of published papers and included in the metadata of published datasets. In addition, a description of the preparation of the sample for analysis, the microscope and magnifications that were used, the counting method, the volume of seawater examined and the number of cells counted, must be included in the information accompanying PTS datasets (Table 1). The latter information is needed in order to calculate the error in the cell densities for individual species which is 20% or less for counts of at least 100 cells (Lund et al., 1958). The error can be much higher for rarer species, which should be taken into account in statistical analysis and inter-comparison exercises. In addition to species-specific errors of cell densities, species richness estimates are also strongly affected by sampling effort (Rodríguez-Ramos et al., 2014; Olli et al., this issue). The name of the analyst should be noted in order to exclude that changes recorded in species composition might be associated with changes of personnel. Differences in the settled sample volume, settling time (e.g., Hasle, 1978; Rott, 1981), counted sample area, and the number of cells that are counted (Lund et al., 1958) may hinder the comparability of the results.

Generally, LM analysis on fixed material does not allow the identification of naked dinoflagellates to the species level, and often not even to the genus level, while for small flagellates and coccoid species even the assignment to any algal class may be doubtful. These uncertain taxa are often counted under supra-specific, supra-generic or non-taxonomic group names. In such cases, as for all cases of supra-specific taxa, indications about their size and morphology, e.g. 'flagellates <5 μm' or 'coccoids <3 μm', pennate naviculoid diatoms >20 μm, as well as possible trophic behaviour, are useful. Whether small organisms were included in counting procedures is not always clear in published papers and datasets, even though this information is clearly relevant for inter-comparison among datasets from different provenances.

Phytoplankton counting requires high proficiency and identification skills (see section 2.4) from the analyst (Dromph et al., 2013; Jakobsen et al., this issue). The analyst should always perform a quality check of the results directly after counting, since it is difficult to identify and remove anomalous results afterwards. Ideally, quality checking should be performed by an independent analyst and may only be possible in places with several taxonomists nearby. For example, in the case of the CPR survey, samples flagged as anomalous are redistributed to other CPR analysts for quality verification; yet some discrepancies still exist even when these precautions are taken (Culverhouse et al., 2003).

2.4. Taxa identification

Taxonomic expertise is a key foundation for any sustained phytoplankton sampling program; without such skills, it is not possible to identify, name or enumerate phytoplankton, much less describe and explore relationships between taxa, identify appearances of invasive/alien species and detect changes in biodiversity or in food-web structure. However, taxonomy has been recognised as a 'science in crisis' as the number of taxonomists is steadily declining worldwide and vital taxonomic knowledge is being lost (Agnarsson and Kuntner, 2007).

The identification of individual taxa is the most critical step in the analysis of phytoplankton samples, and the one that is hardest to standardize by specific QA/QC procedures. While sampling, preservation and counting methods can be standardised or described in detail, the classification of organisms observed in the microscope is prone to a high amount of uncertainty and subjectivity. Wide variability in the effective identification of unicellular organisms may stem from differences in the time devoted to this activity, the quality and accessories of the microscope and objectives used, the illumination conditions, the availability of adequate literature and, most importantly the ability, experience and knowledge of the analyst (Culverhouse et al., 2003; Dromph et al., 2013; Jakobsen et al., this issue).

Similar to the other steps of phytoplankton data analysis, detailed information related to species identification should emerge from the metadata accompanying a dataset (Table 1). Ideally, the identification of each species should be annotated with the specific methods and criteria applied, including the evaluation of distinctive characteristics, besides shape and morphology, preservation state, magnification and other optical settings, and the specific literature base on which a given species was identified. The reason why given taxa were not identified to the species level should also be documented and possibly accompanied by image metadata for the specimen. Making the identification process more transparent would allow the *a posteriori* judgement of the data quality, which would be useful to other users and analysts, and also increase the internal consistency within the PTS.

The reliability of species identification depends on whether all the tools needed to identify any species were actually applied, and therefore varies largely among species. For some taxa, shape evaluation is sufficient for a correct identification (Fig. 1). In many other cases, specific techniques should be applied (e.g. frustule cleaning in diatoms, thecal plate staining in dinoflagellates, electron microscopy and molecular techniques). For example, the diatom *Thalassiosira*, the dinoflagellate *Scrippsiella,* the prymensiophyte *Chrysochromulina*, among others, often appear in PTS datasets as identified to the species level. However, these taxa generally require special techniques or electron microscopy for species, and at times even genus identification. In recent years, progress in the application of molecular tools to taxonomic identification has led to the discovery of more than one species being included under one single species name. A classic case concerns one of the most widespread phytoplankton taxon, *Skeletonema costatum*, whose taxonomic identity has been redefined in recent years leading to the recognition of at least seven species previously identified under this name (Fig. 1 and Table 2) (Zingone et al., 2005; Sarno et al., 2005, 2007). In other taxa, for example in the diatom genus *Pseudo-nitzschia*, a large number of species have been discovered which show minor or no morphological differences (e.g., Lundholm et al., 2012) and which are referred to as cryptic and pseudo-cryptic species. In these cases, only molecular tools can provide a reliable identification. Examples of recent taxonomic and nomenclatural changes affecting some of the most widespread phytoplankton taxa are reported in Table 2. A general recommendation in these cases is the use the term 'sp.' when the organism can be identified only to the genus level ('spp.' in case of more than one species), or 'cf.', from the Latin *confer* (compare to) when the species or genus identification is provisional (Bengtson, 1988). The addition of 's.l.' (from the Latin *sensu lato*, in the broad sense) is also used for names of taxa known to include cryptic species, e.g., *Skeletonema costatum* s.l.

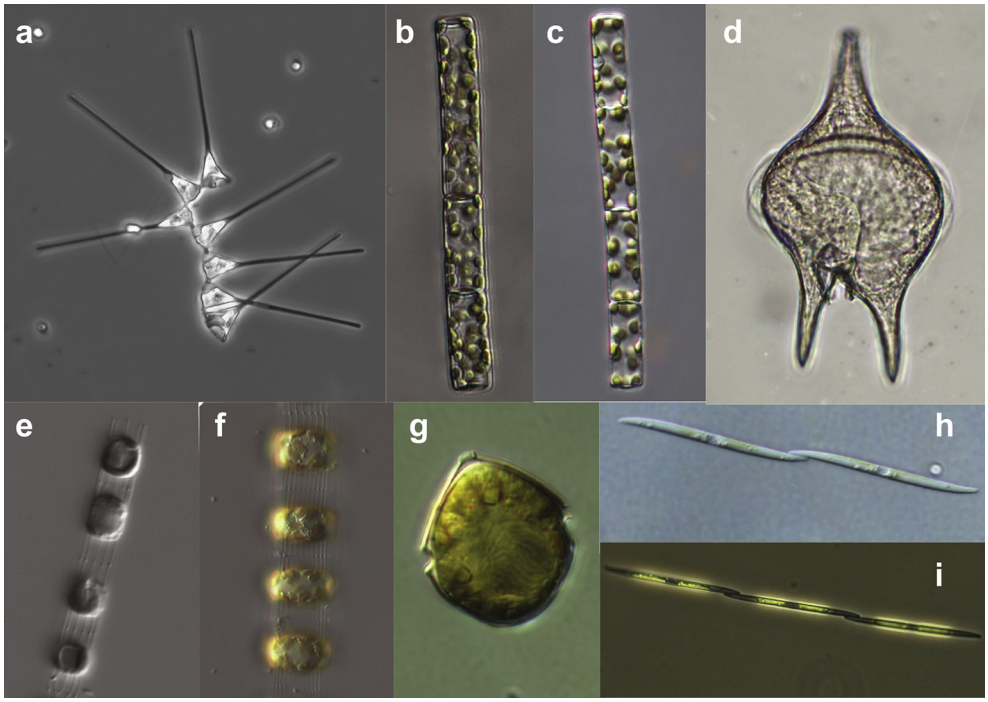

Fig. 1. Phytoplankton species for which LM identification presents different levels of uncertainty (Tables 2 and 3). a) *Asterionellopsis glacialis*; b) *Leptocylindrus danicus*; c) *Leptocylindrus hargravesii*; d) *Protoperidinium* cf. *oceanicum*; e) *Skeletonema marinoi*; f) *Skeletonema tropicum*; g) *Scrippsiella* sp.; h) *Pseudo-nitzschia multistriata*; i) *Pseudo-nitzschia* cf. *delicatissima*. While light microscopy is sufficient for a reliable identification of *A. glacialis*, *S. tropicum* and *P. multistriata* (Flag 1 in table 3), electron microscopy or molecular confirmation are needed in all other cases shown here.

An alternative solution to limit the uncertainty of identification data is the provision of annotated check-lists for each long term dataset. The check-lists should explain for each species the criteria on which it was identified, and the literature used for the identification. Ideally, names in a list should be linked to a photographic archive that has pictures of each species in different views and with different techniques and microscope types, as well as drawings of critical details not easily seen in pictures and, when applicable, to diagnostic sequences used in the molecular identification of the species. In the case of nomenclatural changes, the annotation should also allow one to trace back to other names under which it was identified previously. This voucher collection could be published in specialised journals and/or dedicated online repositories, and would be extremely useful for future confirmation of data identification, as well as for other initiatives related to biodiversity (e.g., Costello et al., 2013).

With all this information available, it would be possible to assign a quality flag to PTS data, reflecting the probability that a given identification was accurate or uncertain (Table 3). This latter parameter depends on whether the tools applied were adequate for the species, as well as on the number of similar species and the background information on the phytoplankton diversity in the PTS geographic area. The quality flagging procedure should also be part of the metadata. Compared to the previous suggestion to use 'sp.' or 'cf.', a quality flag would allow the assignment of a species name even in uncertain cases, which would increase the precision while decreasing the accuracy. A species name, in spite of its uncertainty, at least provides an indication of the shape/size of the specimen, which is useful for the conversion of cell abundance into carbon biomass through the calculation of biovolume (Harrison et al., this issue). In addition, if a species identified with a low quality flag was enumerated separately, subsequent information (with EM or molecular tools, or from the literature) could improve accuracy, thus allowing the recovery of the abundance values recorded.

New information on species morphology and phylogeny may often change the assignment of known species to different genera (taxonomic changes), or the substitution of their name (nomenclatural changes). In some cases, the substitution of an invalid or wrong name with the actual valid ones is straightforward, provided that all literature updates are available to the identifiers. Nowadays, thanks to efforts to standardize species identification, there are at least two web resources that can help update species lists. One is the World Registry of Marine Species (WoRMS) (Boxshall et al., 2014) which includes the semi-automatic 'match taxa' tool which allows one to obtain correct taxonomic information on various marine species, including phytoplankton. This tool is also useful to identify trivial misspellings in species names, and provides information on species authorship, pointing to cases with different authorships for the same name, for which a species name cannot be immediately converted into the accepted one (i.e. an ambiguous case). The WORMS 'match taxa' tool is extremely useful in such cases when different datasets are combined and analysed, as in the cases of the SCOR datasets used by Olli et al. (this issue) or the biovolume data from different time series analysed by Harrison et al. (this issue). To keep pace with taxonomic changes, WoRMS relies on another important web resource, AlgaeBase (Guiry and Guiry, 2014), which is frequently updated to reflect published literature. This is a never-ending work in progress, and it should be noted that some taxonomic changes may require time to be incorporated into these databases, while contradictory information may emerge from that literature based on controversial taxonomic issues. Therefore, the information provided should be carefully checked, as warned on the respective websites.

Primary taxonomic information on phytoplankton species, including that needed for correct species identification, is spread over a large number of papers. Several general manuals have been published over the years (e.g., Tomas, 1997), while other manuals gather phytoplankton images and information for certain areas, or

Table 2
Examples of worldwide distributed/abundant taxa which have undergone taxonomic changes, either because of the discovery of species diversity within a single recognised taxon, or because of nomenclatural changes (not included in Tomas, 1997). See also Table 2 in Harrison et al. (this issue) for a list of common taxa in various coastal time-series.

Original description (single species)	Actual possible species (multiple-species misidentifications)	Valid name	Literature
Diatoms			
Skeletonema costatum	*S. costatum*		Zingone et al., 2005;
	S. pseudocostatum		Sarno et al., 2005;
	S. marinoi		Sarno et al., 2007
	S. dohrnii		
	S. grevillei		
	S. grethae		
	S. ardens		
Pseudo-nitzschia delicatissima	*P. delicatissima*		Quijano-Scheggia et al., 2009;
	P. dolorosa		Lundholm et al., 2006
	P. arenysensis		
	P. micropora		
	P. decipiens		
Pseudo-nitzschia pseudodelicatissima	*P. pseudodelicatissima*		Lundholm et al., 2012; Lundholm et al.,
	P. mannii		2003; Priisholm et al., 2002;
	P. calliantha		Amato and Montresor, 2008; Lim et al., 2013
	P. caciantha		
	P. cuspidata		
	P. hasleana		
	P. fryxelliana		
	P. batesiana		
	P. lundholmiae		
	P. fukuyoi		
Chaetoceros compressus	*C. compressus*		Rines, 1999
	C. contortus		
Chaetoceros socialis	*C. socialis*		Chamnansinp et al., 2013
	C. gelidus		
Leptocylindrus danicus	*L. danicus*		Nanjappa et al., 2013
	L. hargravesii		
	L. aporus		
	L. convexus		
Leptocylindrus minimus	*L. minimus*		Nanjappa et al., 2013
	Tenuicylindrus belgicus		
Odontella sinensis		*Trieres chinensis*	Ashworth et al., 2013
Odontella mobiliensis		*Trieres mobiliensis*	Ashworth et al., 2013
Odontella regia		*Trieres regia*	Ashworth et al., 2013
Dinoflagellates			
Alexandrium catenella/tamarense	*A. tamarense*		John et al., 2014
	A. fundyense		
	A. pacificum		
	A. mediterraneum		
	A. australiense		
Ceratium spp. and *Neoceratium* spp.		*Tripos* spp.	Gómez, 2013
Gymnodinium sanguineum		*Akashiwo sanguinea*	Daugbjerg et al., 2000
Gymnodinium breve		*Karenia brevis*	Daugbjerg et al., 2000
Katodinium rotundatum		*Heterocapsa rotundata*	Hansen, 1995
Prorocentrum minimum		*P. cordatum*	Velikova and Larsen, 1999
Scrippsiella trochoidea	*Scrippsiella* spp.		e.g., Lewis, 1991
Prymnesiophyceans			
Chrysochromulina polylepis		*Prymnesium polylepis*	Edvardsen et al., 2011
Chrysochromulina minor		*Prymnesium minus*	Edvardsen et al., 2011
Chrysochromulina hirta		*Haptolina hirta*	Edvardsen et al., 2011

for algal categories such as potentially harmful species (e.g., Bérard-Therriault et al., 1999; Hoppenrath et al., 2007; Throndsen et al., 2007; Hallegraeff et al., 2010). All these manuals are excellent aids for species identification worldwide. Yet, the information they provide reflects the state of knowledge at the date of their publication, while none of them includes all described species. In controversial identification cases, one often needs to go back to original descriptions, or to papers including images and descriptions of local microflora. In addition, more recent literature should be considered to incorporate the progress in taxonomy into identification protocols. To this end, specific websites collecting classic and recent literature on phytoplankton species would be a very useful tool, but often they are left to individual initiatives and are not sustained over the long term.

3. Data management

There is a clear need to arrange, manage and store all data and metadata from PTS in a standardised way and secure them for future use. Metadata, or information about how the data were collected and treated, should be recorded continuously by respective analysts, so that data can be interpreted correctly during the numerical analysis process and comparisons among datasets become feasible and meaningful. Several metadata standards exist to support standardized archival of biodiversity data. A commonly used metadata standard is DARWIN Core, which is used by GBIF (Global Biodiversity Information System), OBIS (Ocean Biogeographic Information System) and other major repositories for biodiversity data. Standard terms for metadata which could be

Table 3
Examples of quality flags indicating the reliability of the identification of individual species, based on the tool used. While for some species, light microscope (LM) observation is enough to guarantee a 100% certain identification (Flag 1), in other cases, LM can only give a good probability that a species is correctly identified (say, 70%, Flag 2), unless special techniques are applied. In other cases, i.e. cryptic species, the level of certainty is lower (50% or less, Flag 3) even using both LM and electron microscopy (EM). See Fig. 1 for species images.

Species	LM	EM	Molecular data	Flag[a]
Asterionellopsis glacialis	✓			1
Leptocylindrus danicus	✓			3
Leptocylindrus danicus	✓	✓		2
Pseudo-nitzschia delicatissima	✓	✓		3
Pseudo-nitzschia delicatissima	✓		✓	1
Pseudo-nitzschia multistriata	✓			1
Skeletonema costatum	✓			3
Skeletonema tropicum	✓			1
Skeletonema pseudocostatum	✓	✓		1
Protoperidinium oceanicum	✓			2
Protoperidinium oceanicum	✓	✓		1
Scrippsiella trochoidea	✓	✓		3
Scrippsiella trochoidea	✓	✓	✓	1

[a] 1: good (100%); 2: probable (>50%); 3: uncertain (<50%).

applied to phytoplankton datasets are also found in the Ecological Metadata Language (EML) developed initially within the Ecological Society (Michener et al., 1997) and used, for example, by the United States Long Term Ecological Research (LTER) Program. An example of the implementation of EML is provided by the Knowledge Network for Biocomplexity (KNB, https://knb.ecoinformatics.org). The utility of metadata standard terms for PTS data is that they allow both the standardized and therefore comparable description of all PTS components, such as taxonomy and nomenclature of the recorded organisms, and detailed georeferencing, along with the description of fixation and analytical methods. While such standards are useful, they are only as good as the information they are populated with and it is therefore important to enter correct and complete information and adopt controlled vocabularies and short–codes to describe methods in a consistent and synthetic way. Examples of controlled vocabularies are those developed within SeaDataNet by the BODC (British Oceanographic Data Centre) and ICES (International Council for the Exploration of the Sea) (http://vocab.ices.dk/). The choice of metadata standards and vocabularies is nonetheless a complex matter that requires clear instructions and good communication between data managers and the analysts responsible for entering data into a database.

The need for an improvement of data infrastructures that facilitate data sharing and the design of more detailed integrated data products has been recognized by many international initiatives (e.g., EMODNET). At the European level, the most important one is the INSPIRE Directive (Directive 2007/2/EC), whose goal is the creation of a data infrastructure that can support environmental policy. The directive makes several recommendations for standards for environmental and chemical/biological data, and also on good practice for data archival. The directive aims at facilitating the sharing/analyzing of data on the basis of their geography and is therefore applicable to PTS data as well.

Publication of the datasets on websites, specific dataset journals, repositories (e.g. PANGAEA, http://pangaea.de) should be encouraged. For years, journals would not publish species lists along with ecological papers. For this reason, information on species present for many geographical areas has remained in the scientist's desk drawers. Fortunately, many journals now publish check-lists and datasets. Accordingly, there is a greatly increased effort to standardise methods and metadata description and QA/QC assessment, which will allow more straightforward and confident use of these resources by other investigators and an effective retrieval of the data also by automated methods. Thus, the sharing of QA/QC'ed data allows one to recoup the high cost of obtaining the data through the multiple use of the time-series data in various projects over many decades.

4. Harmonisation and inter-calibration among PTS

Phytoplankton identification is a costly component of time-series sampling programs. If a common set of protocols is followed which allow inter-comparisons with other datasets and future automated data retrieval and usage, while still preserving the original time series, then this cost can be better justified. Ideally, in order to increase the comparability of PTS data, the same procedure should be applied worldwide for all steps of phytoplankton counting and identification. However, many countries or institutes have used their own methods for decades and may be reluctant to make changes. An obstacle to harmonization could be due to practical or legal reasons, as many PTS might be tied to different requirements (e.g., by Institutes or Authorities) of the procedures for data collection and reporting. For example, formaldehyde requires the use of fume hoods and special disposal procedures, and its use is not allowed in some institutes, while the quality of microscopes is proportional to the research budget and the priority given to the importance of species data in the monitoring program. One of the main hindrances to harmonization is the impact that changes in the methods would have on the internal consistency of a PTS dataset. Nonetheless, changes aimed at increasing inter-comparability should be encouraged, especially if those changes involve an improvement in the quality of the data produced. In these cases, it is important to run auto-calibration procedures, i.e. comparing the old and new method for a number of samples, and note the date of the change. This is similar to QA/QC inter-calibration procedures between a new and old instrument used in chemical analysis.

International and national harmonization workshops, inter-calibrations and proficiency tests are extremely important for the quality control of both identification and methodology. For example, in Europe, the Biological Effects Quality Assurance in Monitoring (BEQUALM) program, using the scheme developed by UK National Marine Biological Analytical Quality Control (NMBAQC), develops appropriate quality standards for community structure analysis and organizes proficiency tests for phytoplankton sample analysis. The HELCOM PEG organizes yearly harmonization workshops among the Baltic Sea countries. Participation in inter-calibrations and proficiency tests is recommended for increasing QA/QC of phytoplankton species composition data.

While tests can assess the level of taxonomic proficiency, taxonomy courses represent an effective way to increase, standardise, harmonise taxonomic knowledge and train new taxonomists. Courses should be organised at all levels of expertise, while teaching material, including identification slides and dedicated web resources, should constitute a common patrimony to be constantly updated and shared free on the web.

5. Summary and final recommendations

Due to the many different constraints placed on individual time series, ranging from methodological to financial and political issues, it would be extremely challenging to establish a globally agreeable and consistently applied single protocol for processing phytoplankton samples in PTS. However, much can be done to increase and improve the information about a PTS dataset to ensure that data can be compared in detail to other PTS and remain internally consistent at the same time. To this end, and to facilitate

the open use of the data and automated data retrieval, the following guidelines are recommended:

- produce detailed procedures and protocols for PTS data production and archiving. This documentation should outline the general time-series data collection process and possible variations occurring over the PTS life-span, which is important also for the internal consistency assessment;
- provide and archive regular metadata information for each of the sample counts. Metadata archives should include complementary information for sampling, fixation and storage, analyses, and taxonomic/nomenclatural issues. In order to facilitate data sharing, metadata should make use of standardized vocabularies;
- organise and participate in inter-calibration exercises and ring tests among laboratories, institutes and neighbouring countries involved in phytoplankton counting and identification and move towards harmonization of counting procedures;
- promote and participate in training courses for taxonomy, from basic ones for initial training, to advanced courses to stay updated with recent progress in taxonomic research that may have important implications for species identification;
- support and contribute to web resources collecting taxonomic information;
- manage PTS data, adopting internationally agreed standards and depositing them in public repositories, to encourage wide usage.

The challenges in increasing the consistency, comparability and accessibility of PTS data outlined in this paper are a consequence of the complexity of this kind of data, which is even higher when their intended use is tracking species of interest that can be rare or difficult to identify. Nonetheless, the effort needed is worth it, since long-term trends, alien/invasive species and significant changes in time and geographic distribution have already been demonstrated in many cases (Suikkanen et al., 2013; Olli et al., 2014). Reports of these changes in phytoplankton composition have also illustrated their potential impact on ecosystem functioning, human health and other relevant societal issues. Therefore, PTS sampling programs must continue their important ecosystem watchdog role. Following the recommendations outlined above will greatly enhance the scope and even cost effectiveness of the PTS which, in the age of climate change, are invaluable treasure troves of historic and recent findings that are key to understanding the link between phytoplankton species and subsequent climate-induced changes in the food-web.

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