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AS WE SEE IT*

Automatic image analysis of plankton: future perspectives

Phil F. Culverhouse[1,], Robert Williams[2], Mark Benfield[3], Per R. Flood[4], Anne F. Sell[5], Maria Grazia Mazzocchi[6], Isabella Buttino[6], Mike Sieracki[7]**

[1]Centre for Interactive Intelligent Systems, University of Plymouth, Plymouth PL4 8AA, UK
[2]Plymouth Marine Laboratory, Prospect Place, The Hoe, Plymouth PL1 3DH, UK
[3]Louisiana State University, Coastal Fisheries Institute, Dept. Oceanology and Coastal Sciences, Baton Rouge, Lousiana 70803, USA
[4]Bathybiologica A/S, Gerhard Grans vei 58, 5081 Bergen, Norway
[5]Institute for Hydrobiology and Fisheries Science, University of Hamburg, Olbersweg 24, 22767 Hamburg, Germany
[6]Stazione Zoologica 'A Dohrn', Villa Comunale, 80121 Naples, Italy
[7]J. J. MacIsaac Facility for Individual Particle Analysis, Bigelow Laboratory for Ocean Sciences, 180 McKown Point, PO Box 475, West Boothbay Harbor, Maine 04575-0475, USA

ABSTRACT: In the future, if marine science is to achieve any progress in addressing biological diversity of ocean plankton, then it needs to sponsor development of new technology. One requirement is the development of high-resolution sensors for imaging field-collected and *in situ* specimens in a non-invasive manner. The rapid automatic categorisation of species must be accompanied by the creation of very large distributed databases in the form of high-resolution 3D rotatable images of species, which could become the standard reference source for automatic identification. These 3D images will serve as classification standards for field applications, and (in adjusted optical quality) as training templates for image analysis systems based on statistical and other pattern-matching processes. This paper sets out the basic argument for such developments and proposes a long-term solution to achieve these aims.

KEY WORDS: Natural object recognition · Object categorization · Zooplankton · Phytoplankton · Imaging · Taxonomy · Automatic identification · Image analysis

INTRODUCTION

The Darwin Declaration (Darwin Declaration 1998) states the requirement that: 'The governments of the world that recognize the Convention on Biological Diversity have affirmed the existence of a taxonomic impediment'. The 'taxonomic impediment' is the shortage of taxonomic expertise, information and capacity that is necessary to enable implementation of the Convention of Biological Diversity (the Rio 'Earth Summit' 1992). The Global Taxonomic Initiative has been set up to improve this capacity.

Unfortunately, this initiative does not acknowledge the magnitude of the task to systematically establish the means to measure biological diversity in the oceans and to monitor change. It is implicit that categorisation of species over basin scale can only realistically be carried out by automatic methods, underpinned by a large base of human taxonomic expertise. Our most comprehensive attempt to date is the current Continuous Plankton Recorder (Edinburgh Oceanographic Laboratory 1973, CPR Survey Team 2004) in the northern North Atlantic Ocean, which only collects sufficient data on the most abundant 20 species/groups of phytoplankton and zooplankton to carry out statistical analyses. This survey has essentially remained unchanged since the 1930's retaining the same sample acquisition and analysis techniques. Regional zooplankton surveys, carried out in the traditional way using nets and manual microscopical analysis, can be more detailed

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**Email: pculverhouse@plymouth.ac.uk

and often identify more than 300 distinct species. An example of this is seen in the 55 yr CalCOFI study and related programmes from the Pacific (Rebstock 2001, 2002, Brinton & Townsend 2003, Lavaniegos & Ohman 2003, Ohman & Venrick 2003, Rau et al. 2003).

Therefore, with the continuing loss of taxonomic expertise there is a gap between future requirements for basin scale studies and current scientific capability. This has led to the 'West' exploiting the taxonomic expertise still present in Poland and Russia as a 'stopgap' measure. This is giving a false sense of the degree of the taxonomic capacity residing in the marine community.

Practical applications of knowledge of plankton diversity and distribution in the oceans include ecosystem responses to climate change, food web modelling, and the detection of harmful algal blooms in coastal waters. Taking the plankton as an exemplar, progress has been made on automatic species identification of phytoplankton and mesozooplankton. Existing techniques are adequate for class/order-discriminations (e.g. chaetognaths, euphausiids, copepods, and hyperiid amphipods) for estimation of biomass and for ecological research of the major components of the plankton. Real time analysis for ecology and biomass (based upon equivalent species volume and not equivalent spherical diameter) will be feasible in the next 2 to 3 yr. However, to tackle the scientific questions relating to biodiversity, high volume throughput analysis of species abundance and morphological variation are needed. For these studies specific taxonomic detail is required and thus high-resolution images are necessary. Two-dimensional imaging is not sufficient for robust taxonomic categorisation of many species. We believe that real-time and large-scale biodiversity science will be feasible in a 5 to 15 yr time frame if appropriate initiatives are supported.

The reasons that it will take this long are: (1) not enough information is available in a 2D image format of the plankton species in the world's oceans; (2) the plankton represents an extremely wide range of individuals, which are represented by many complex 3D and semi-transparent objects; and (3) these individual organisms are free to rotate in 3D relative to the imaging sensor. The normal approach to imaging using multiple 2D views (the viewsphere approach) of the organism is not sufficient for *in situ* imaging and recognition. A final solution must be high resolution rotatable 3D or tomographic imaging of specimens in real-time *in situ*. While this is yet out of reach, a basis for the transition to automated 3D systems has been laid by the considerable progress during recent years to improve 2D imaging techniques. The current state or our imaging capability is reviewed below.

Current 2D imaging machines

The images shown in Figs. 1 to 4 highlight some of the successes in plankton imaging. They all provide sufficient resolution for class/order categorisation and estimation of organism size, which may be used to estimate biomass. Since the mid-1980s, approximately 8 different *in situ* 2D imaging systems have been developed (see

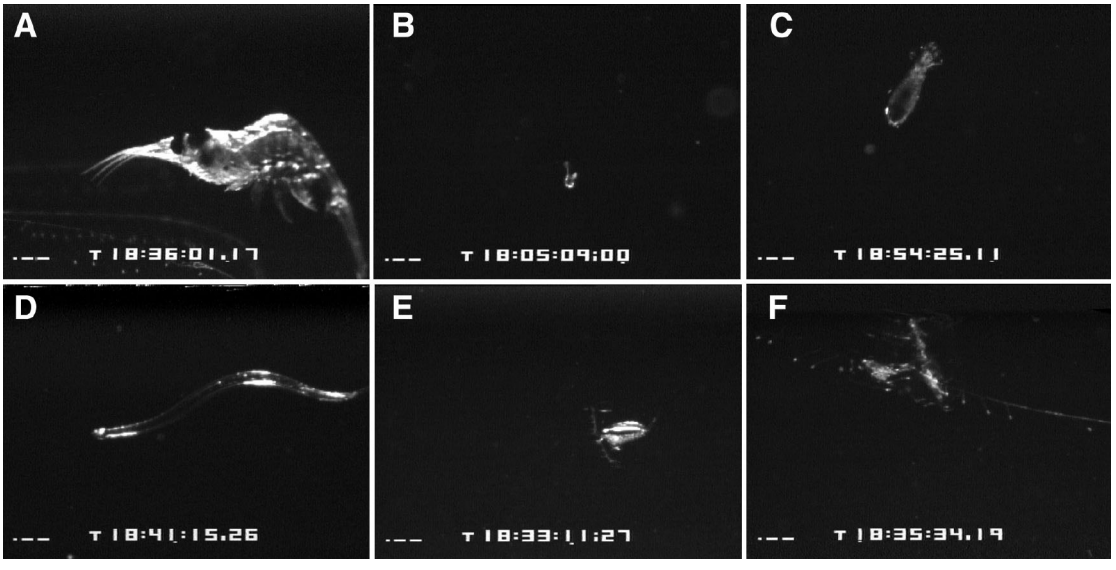

Fig. 1. Examples of images from the Video Planktion Recorder (VPR) taken *in situ*, showing a range of image capture qualities: (A) euphausiid *Meganyctiphanes norvegica*, (B) thecosomate pteropod *Limacina retroversa*, (C) an amphipod, (D) chaetognath, (E) copepod *Calanus finmarchicus*, and (F) part of a physonect siphonophore. The width of the field of view in each image is 17.5 mm and the image volume is 5.1 ml. (Source: M. C. Benfield, Louisiana State University, USA)

Fig. 2. Zooscan image, showing a mixture of mesozooplankton groups, 2003. (Source: G. Gorsky, Villefranche-sur-Mer, France)

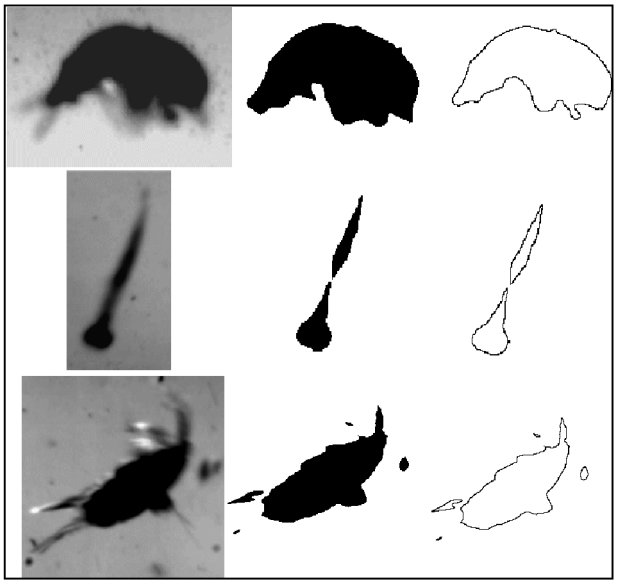

Fig. 3. Zooplankton for biomass and class/order recognition. Line scan camera images with blob detection and perimeter extraction prior to multi-dimensional clustering and object classification, 2003. (Source: C. Gallienne, Plymouth Marine Laboratory, UK)

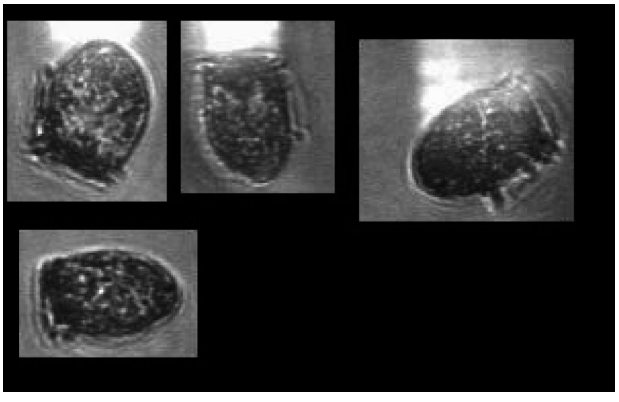

Fig. 4. A FlowCAM image of the dinoflagellate *Dinophysis acuminata*, 2003. (Source: M. Sieracki, Bigelow, USA, from www.fluidimaging.com)

Wiebe & Benfield 2003 for a review of imaging-optical systems). These include: hybrid optical-net systems such as the camera-net system (Ortner et al. 1981, Olney & Houde 1993) and the ichthyoplankton recorder (Welsch et al. 1991, Lenz et al. 1995); and stand-alone imaging systems such as the Video Plankton Recorder (VPR, Davis et al. 1992; Fig. 1), Zooscan (Gorsky et al. 1992, 2000; Fig. 2), *in situ* video profiler (Tiselius 1998), Shadowed Image Particle Profiling Evaluation Recorder (SIPPER, Samson et al. 2001, Remsen et al. 2004), zooplankton visualization and imaging system (ZOOVIS, Benfield et al. 2003; LISA, C. P. Gallienne, pers. comm.; Fig. 3), and the Flow-Cam (Sieracki et al. 1998, Fig. 4) In addition, several *in situ* holographic systems have been developed for 3D imaging of plankton (Katz et al. 1999, Hobson et al. 2000).

Paralleling the development of these systems has been a shift from photographic emulsion to charge-couple device (CCD) cameras of progressively higher pixel densities. The use of higher definition, digital formats has permitted a concomitant increase in image volume although most systems still record the contents of volumes of several ml to a few liters per image. Flowcam image volume is about 0.03 µl. In contrast to *in situ* zooplankton systems it uses triggered imaging. The quality of images produced by these systems is generally adequate for categorisation to the taxonomic level of class or order (e.g. Copepoda, Chaetognatha,

Decapoda, Pteropoda) or acoustical sound-scattering model categories (e.g. gas-filled inclusion, fluid-sphere). When organisms possess distinctive morphological features, categorisation to genus or species is possible. However, depth of focus imaging has a distinct influence on correct categorisation. An example is shown with the brachiolaria larva of the starfish *Asterias rubens*, Fig. 5, where the image has been degraded by successive matrix convolutions using MATLAB. Additional complications arise where the imaging method is intrusive (ichthyoplankton recorder) or deforms images beyond the capabilities of the recognition processes employed (SIPPER).

While 3D imaging is considered the ideal method, available technology has not yet advanced to a stage where we could easily switch from 2D to 3D imaging. For field applications with real-time imaging from a moving ship, 3D systems with confocal optics will not be available for some time. In the interim, we therefore need a database of images that allows us to link the information that we can get from 3D images in the laboratory with the large amount of 2D data that are available and will in future still be produced. This also makes provision for a smooth transition from 2D to 3D-based science in the future through a validated 2D and 3D database.

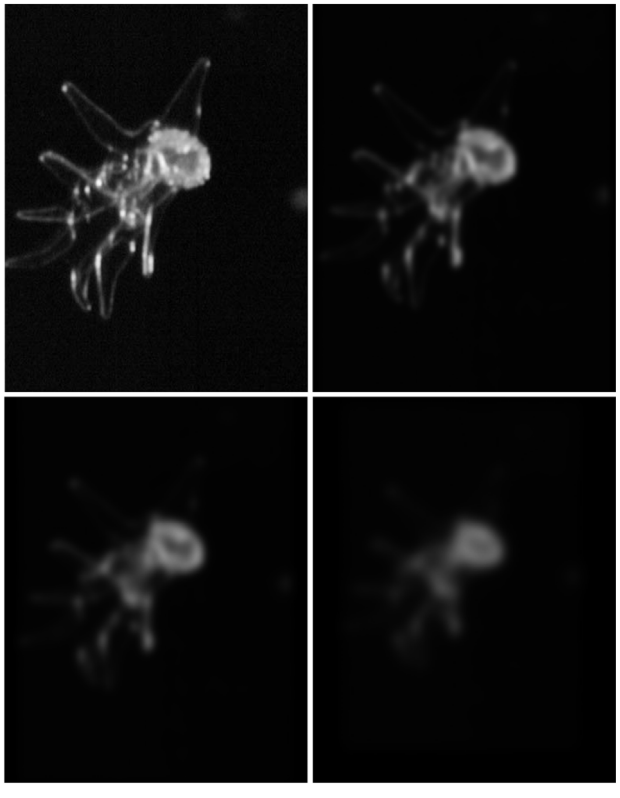

Fig. 5. Images of tunicate blurred with successive averaging using MATLAB. (Source: A. Sell, University of Hamburg, Germany)

A very high-resolution image database will allow experiments to determine the imaging resolution required for accurate classification. The image resolution can be degraded, and classification methods tested. Results of these experiments, conducted for different types of organisms, will inform the specifications for future imaging instrument development.

Current 3D imaging machines

Time-resolved 3D imaging is not new to field studies in marine science, stereo cameras have been used to track jellyfish in deep ocean over long time periods (Rife & Rock 2002) and holography has been used to view volumes of seawater *in situ* for zooplankton behavioural studies (Malkiel 1999, Hobson 2000). In practice stereo cameras can give sufficiently good images to be useful for a wide range of ecological and taxonomic purposes. But stereo images are not true 3D, and so view angle is still an important issue. Katz et al. (1999) and Malkiel et al. (1999) have used holography for *in situ* behavioural studies of plankton, demonstrating resolutions down to 10 µm. Particularly elegant experiments by Malkiel et al. (2003) reveal copepod-feeding flow-fields in 3D in the laboratory using digital in-line holography.

Tomography holds promise of true 3D volumetric imaging. It comes in several varieties, Positron Emission Tomography (PET) (Valk 2003), Magnetic Resonance Imaging (MRI) (Marinus et al. 1999) and X-ray Computerised Axial Tomography (CAT) (Lavrent'ev et al. 2001). Unfortunately all 3 techniques require either large magnets or hazardous radiation to operate and so are not yet used in marine science field studies.

Acoustic scanning has been demonstrated in the FishTV system (Mcgehee & Jaffe 1996), revealing good quality images at the mm scale of resolution.

A technique that is in widespread laboratory use is Confocal Microscopy. A small spot of laser light is drawn across a small volume of space in 3D and the optics of a high quality conventional microscope. The reflected or emitted light (in the case of fluorescence microscopy) is reconstructed into a 3D image using a computer. Confocal imaging perhaps offers most promise and is discussed below.

Confocal microscopy

Biological and ecological studies largely rely on light and electron microscopes, which have always been fundamental tools for analysing the structure, physiology and function of cells and microscopic organisms. However, many shortcomings are inherent to these

techniques, such as relatively low resolution, which prevent observation of ultrastructural details (for conventional light microscopy) and complicated fixation methods or sectioning artefacts, which damage the specimens (for electron microscopy). These limits have been reduced by the confocal microscope, which offers several advantages, including increased resolution, higher contrast, and more suitable depth of field.

In confocal microscopy, the illumination is scanned as a flying spot through the specimen. The light sensing detector follows the illumination; unwanted light is removed by placing a pin hole at the detector. The resulting imaging provides optical sections with exceptional contrast (Fig. 6), and highlights details previously not accessible (Wright et al. 1993). Specimens can be optically sectioned in both the horizontal and vertical planes. Series of optical sections taken at successive focal planes produce a 3D view of the specimen. The images are processed and stored in a digital format and can therefore be manipulated with image-analysis software. All sizes that are necessary for calculating the volume of the specimens can be precisely measured, and the composite images can be animated and rotated so that structures can be seen in 3D.

Many studies have shown that confocal microscopy offers a powerful means to address biological problems related to cellular structure and processes (Matsumoto 1993, Conn 1999, and others). In particular, this technique has been applied to: (1) determining the cellular localization of organelles, cytoskeletal elements, and macromolecules such as proteins, RNA, DNA, (2) tracing specific cells through a tissue, (3) following the temporal dynamics of cellular processes (4D).

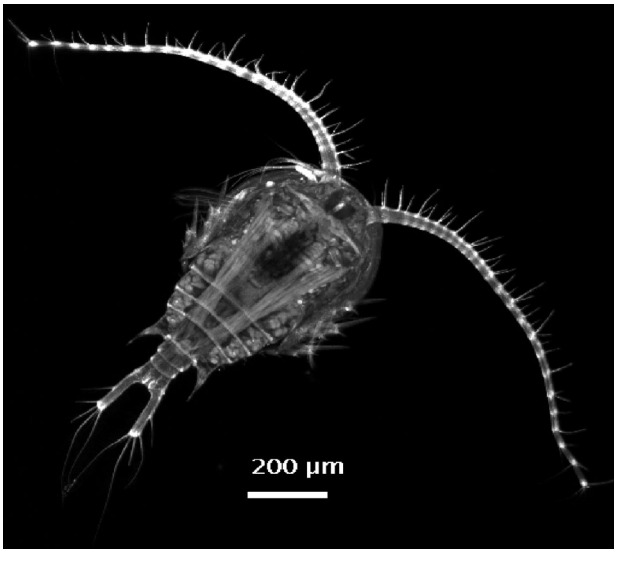

Fig. 6. Confocal image for morphological analysis of larval stages of *Temora stylifera* (Copepoda, Calanoida) from the Mediterranean Sea. (Source: I. Buttino, with permission. J Plankton Res 21(9):1613–1632)

Laser scanning confocal microscopy (LSCM) has recently been utilised in studies on planktonic organisms. By providing a means of observing external or internal structures in 3D, this instrument has advanced our understanding of the functional morphology of structures on microscopic organisms. The use of LSCM coupled with membrane-specific fluorescent carbocyanine dyes allowed rapid identification of sensory structures on the copepod antennules and provided insights into the mechanics of signal transduction from the environment to the organism (Bundy & Paffenhöfer 1993). LSCM was also used to study the morphology of the larval stages of *Temora stylifera* (Carotenuto 1999), *Calanus helgolandicus* (Buttino et al. 2003), and the decapod *Hippolyte inermis* (Zupo & Buttino 2001). The LSCM technique was also applied to rapidly assess embryo viability in the copepods *C. helgolandicus* (Buttino et al. 2004a) and *Clausocalanus furcatus* (Buttino et al. 2004b). Moreover, the LSCM technique has been also used to examine the undisturbed architecture and composition of marine snow (Holloway & Cowen 1997).

LSCM appears to be particularly valuable for morphological analyses in the field of taxonomy. To identify species (and their larval stages) on the basis of morphological characters is of prime importance in environmental research aimed at monitoring and identifying biological diversity in plankton ecology. LSCM is the only available instrument that shows the morphology of planktonic organisms with the high resolution for all details and simultaneously allows taking precise measurements of their body for a 3D image. Current 3D imaging techniques are not fast enough for rapid high quality imaging of large volumes in field studies.

Current automatic categorisation performance

Essential to these future aims are methods to automate the categorisation of specimens. To date, attention has mainly been devoted to the development of high speed or high resolution imaging systems for laboratory or *in situ* operation. This has created a bottleneck in the sampling and analysis of the plankton. Some laboratories now have very extensive archives of video tape recordings of cruise image data holding many hundreds of thousands of specimen images that still require manual inspection for identification, categorisation and further analysis.

Research in the field has been characterised by attempts to discriminate between either taxa (Berman et al. 1991) or species (Simpson et al. 1989). Measure-

ments, often derived from standard morphological analysis of specimens, are written into computer programs. The profile of the specimen is normally of primary interest here, providing detail of maximal X and Y dimensions. Additional Fourier-based analysis of the profile can allow shape categories to be robustly constructed and used for recognition. However, these descriptions are sensitive to camera viewpoint angle to the specimen. Partial views and rotations of objects may reduce machine performance. Enhancements to increase the number of parameters measured from each specimen have resulted in several useful tools for real-time use (Sieracki et al. 1998, Tang et al. 1998, Grossjean et al. 2004, Luo et al. 2004).

A system developed by Culverhouse et al. (1996a) called Dinoflagellate Categorisation by Artificial Neural Network (DiCANN), is a multi-channel machine that analyses surface texture in addition to shape descriptions. Texture analysis also has been shown to be of value in bivalve larval categorisation (Tiwari & Gallager 2003).

DiCANN is a software system capable of categorising marine biological groups, genera and species and has been developed to prototype (Culverhouse et al. 1996b, 2004). It has been used as a test-bed to understand how human experts make visual judgements of natural object categories and to develop machine methods that approach the performance of experts (Simpson et al. 1992, Culverhouse et al. 1994). We can use DiCANN to explore the issues of recognition. Dinoflagellate genera were chosen initially to test the automatic categorising software, as taxonomists know them as a 'difficult' group. The system's automatic categorisation performance has also been assessed on previously unseen images of zooplankton (Fig. 7) and fish larvae (Fig. 8). Some selected results are described below.

Looking at the images in Figs. 1–8, it is clear that the specimens in the fields of view are of variable quality and resolution. These types of data are acceptable to human analysers and so should be acceptable to analysis by machine categorisers. Studies using DiCANN have shown that specimen images of this quality can be processed and recognised. Fig. 7 shows a range of example images that were identified by DiCANN. The percent correct indicates the level of accuracy for each category (shown by column) for a trial involving Tintinnidae (Culverhouse et al. 1994, Williams et al. 1994) and mesozooplankton (unpubl. results, images supplied by G. Gorksy). Fig. 8 shows images from a test using fish larvae at 4 developmental stages (note that the optical magnification factor had not been recorded, hence all developmental stages appear similar in size). DiCANN performance at labelling the unseen images was: herring 40%, sprat 80% and sand eel 90%. Herring was mostly confused with sprat larvae (Toth & Culverhouse 1999).

A study by Culverhouse et al. (2003) attempted to discriminate between several morphologically similar species of dinoflagellates using DiCANN. Some of these species exhibit polymorphism (Fig. 9). Discriminant analysis of the data revealed 3 morphologically close species (*Dinophysis acuta*, *D. acuminata* and *D. fortii*). This is depicted in Fig. 10, where these 3 spe-

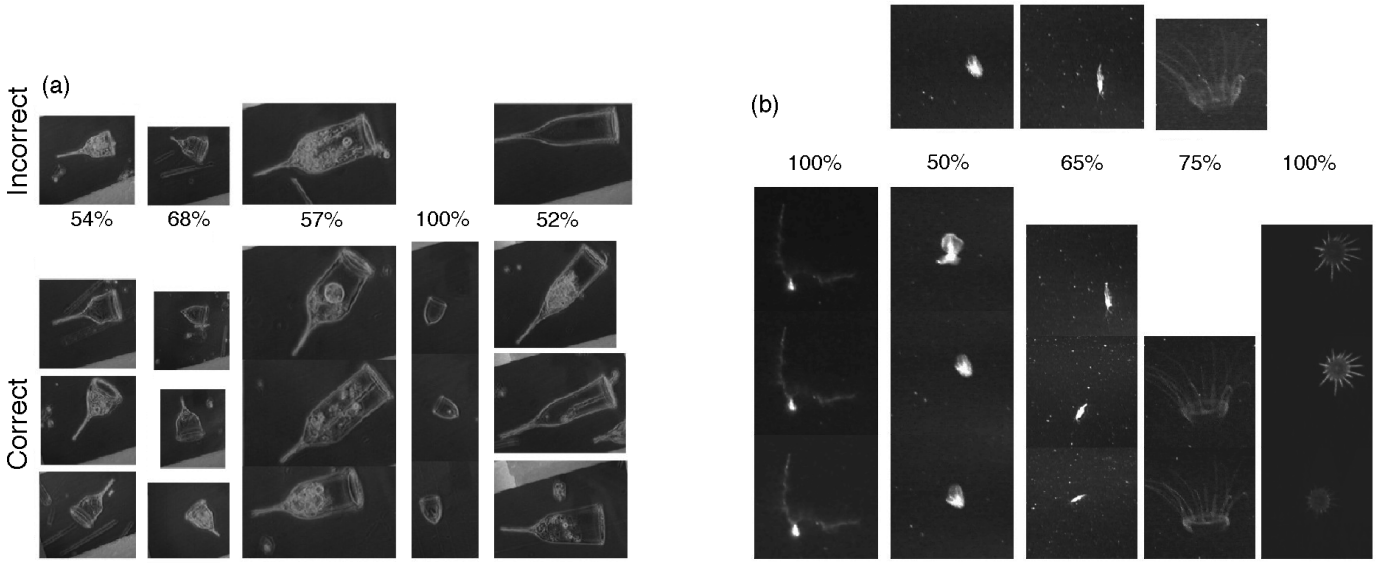

Fig. 7. (a) Images scanned from photomicrographs of net samples of Tintinnidae. (Source: J. T. Turner, R. W. Pierce, Univ. Massachusetts, USA, and R. Williams, Plymouth Marine Laboratory, UK), (b) Images of mesozooplankton direct from the Vertical Plankton Profiler. (Source: G. Gorsky, Villefranche-sur-Mer, France)

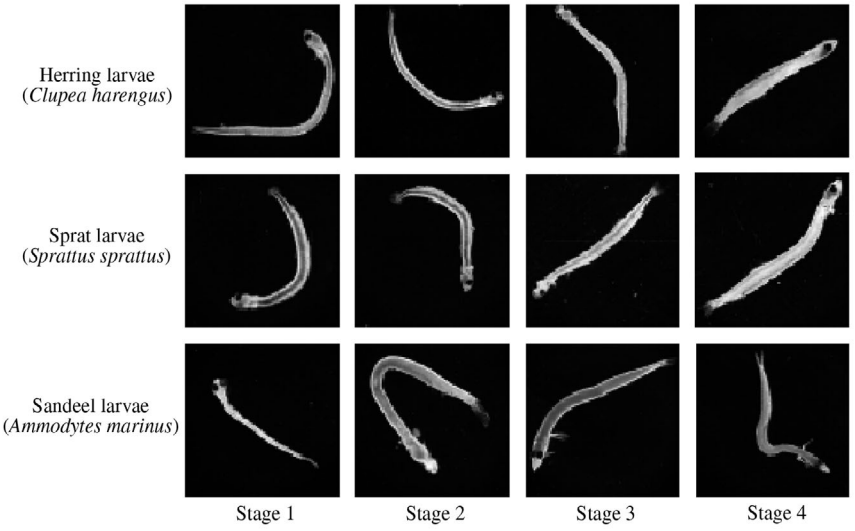

Fig. 8. Digital images of 3 species of fish, at 4 developmental stages. (Source P. Rankine, Aberdeen, UK, 1994)

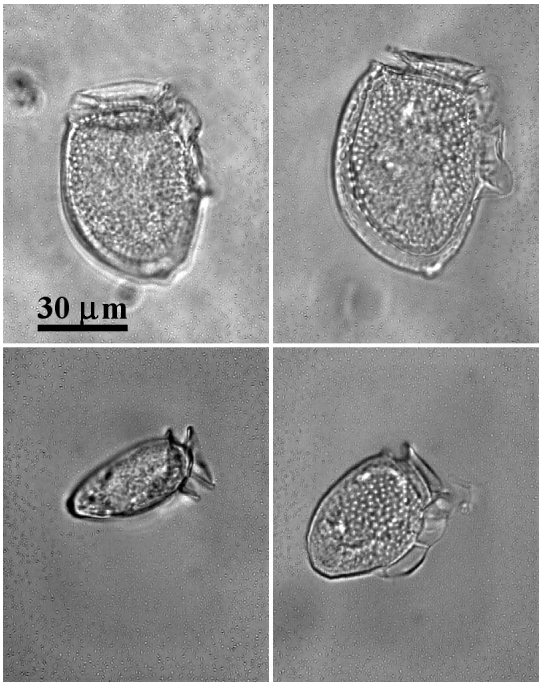

Fig. 9. Images showing morphological variation in incubated dinoflagellate *Dinophysis acuminata* cells. (Source: B. Reguera, Centro Oceanográfico de Vigo, Spain)

cies cluster adjacent to each other in the discriminant analysis. The morphology of specimens located at the boundaries of these 3 species will cause problems for automatic identification. In the study, an overall average of 72% accuracy of label was achieved using DiCANN (Culverhouse et al. 2003, their Table 1). This was similar to human performance in identifying the species. Human performance is affected by several psychological factors: human short-term memory limit of 5 to 9 items stored, fatigue and boredom, recency effects where a new classification is biased toward those in the set of most recently used labels, and positivity bias where categorising a specimen is biased by one's expectations of the species present in the sample (Evans 1987). Human experts also make their own rules up for categorisation tasks (Sokal 1974). All of these shortcomings mean that humans are not reliable as long-term visual categorisation instruments. These biases routinely affect the quality of taxonomic surveys that underpin marine ecology. There is also tacit assumption that an absolute standard of specimen categorisation exists (Solow et al. 2001). However, 100% accurate categorisation is not possible because of human error, which is compounded by morphological variation in the target species adding confusing information to the task (Culverhouse et al. 2003). Although the study used Harmful Algal Bloom (HAB) *Dinophysis* sp. to test human performance, the same problems will occur in zooplankton work (i.e. nauplii differentiation). Volume

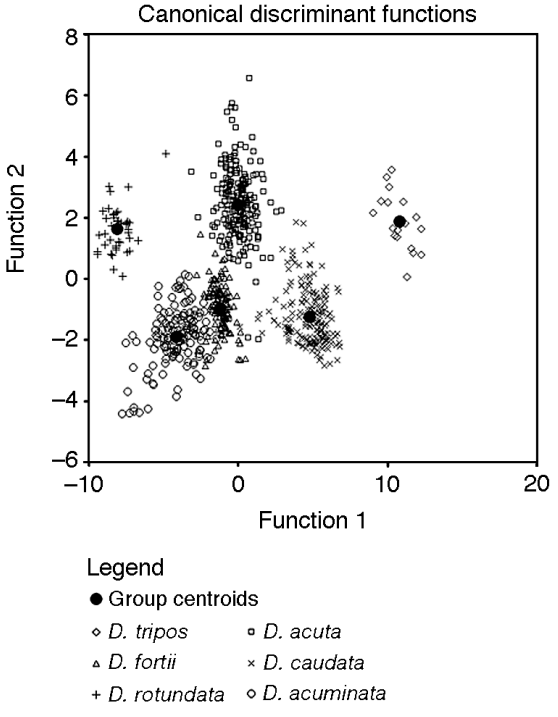

Fig. 10. Canonical discriminant analysis plot of morphological variation across 310 images of 6 species of the dinoflagellate genera *Dinophysis*

processing of samples will result in errors through the human factors of fatigue and bias. Data obtained from human recognition and categorisation is therefore suspect. This has no significant impact on current methods because each human analyser is idiosyncratic and therefore the errors are not accumulative across laboratories. However, if machines that are capable of very high volume analysis are trained with data obtained from a small number of human experts, then systematic biases can be expected. With 'difficult' species and specimens, it has been estimated that errors resulting from these biases can be as high as 28%. These must be removed. Any reference database, or standard, proposed for future work and calibration, will need to eliminate this large source of error.

FUTURE SCIENCE

To improve our knowledge of biodiversity in the marine pelagic environment we can suggest 3 issues that will be important for the 5 to 15 yr time frame: *In situ* non-invasive monitoring of zooplankton on an ocean basin scale; less than 1 km resolved sampling and categorisation of species for fine-scale science; ocean basin-scale biodiversity estimates at the species/population level.

Enabling activities

It is suggested that these goals can only be achieved if the following 3 activities are implemented:

(1) Rapid automatic categorisation of species in an oceanographic region. The term real-time is often used in this context, but it is open to interpretation. However, since automation is intended to augment human experts, automation must be at least as fast as an expert, which could be taken as faster than 1 specimen per minute. Operation faster than a human is very desirable. This can therefore be considered to be rapid and not real-time.

(2) Construction of high-resolution (3D) specimen image database as a reference source for global scientific work in recognition and ecology. Multiple images will be required for each species, to provide a range of data that somehow capture the natural variance present in field specimens.

(3) Routine worldwide access to the database for the development of new 3D real time imagers/analysers via the World Wide Web (WWW). Access is also required for the day-to-day operation of the automatic categorisation systems. Expert scientist panels must validate the contents of the database, which also can be achieved via the WWW.

The first activity would allow timely zooplankton ocean basin scale monitoring. Technically this is a difficult task. The second activity would support basin-scale biodiversity estimates at the species/population level. The global taxonomic initiative can only operate effectively if the knowledge required to understand species diversity is in the public domain and is easily accessible to scientists across the world. The WWW as described in activity 3 above can mediate this. The physics research community already operates in a similar and highly successful manner. Physical models and data sets are published, sharing data from high cost experiments. This is seen to promote co-operation, consensus and debate across the world, which is perhaps an important lesson for large-scale marine ecology.

These enabling activities could be achieved through a variety of large-scale projects, but one which is applicable to some of the authors and demonstrates the complexity of the work, would be: (1) to obtain a large number of confocal images of zooplankton and harmful algal bloom specimens in the laboratory and (2) to assess morphological variation of species using automation techniques. More specifically the following tasks would be required: (a) Create a distributed database of these images available in the public domain. (b) Engage a network of experts for image specimen – species validation. (c) Establish standards for imaging and data formats for image storage, image viewing and validation protocols (Culverhouse et al. 2003). (d) Develop transduction models of existing 2D imaging systems to allow extraction of match-data from the 3D confocal archive. This would enable existing 2D images to be used too. The library of 2D field observations could be used to cross-link between 2D and 3D systems. (e) Build Internet interfaces for existing 2D instrumentation to enable real-time access to database for training/matching with images of live specimens. (f) Develop new sensors for high resolution 3D operation in the field. A diagrammatic overview of the project operational processes is given in Fig. 11.

These activities are discussed in more detail below:

(a) Distributed database: High-resolution 3D images are likely to be very large. Initially a few researchers who have access to confocal microscopes will generate them, but a larger group will wish to have image access. A distributed database is proposed as a good solution to this access issue. The database could operate by having pointers to the images that are held on a variety of computers across the world. An Internet interface to this database would allow access from anywhere. The database would require a minimum set of functions, which include: (i) unique identifier for each image, (ii) a pointer to the WWW location of that image, (iii) a complete textual description of that image

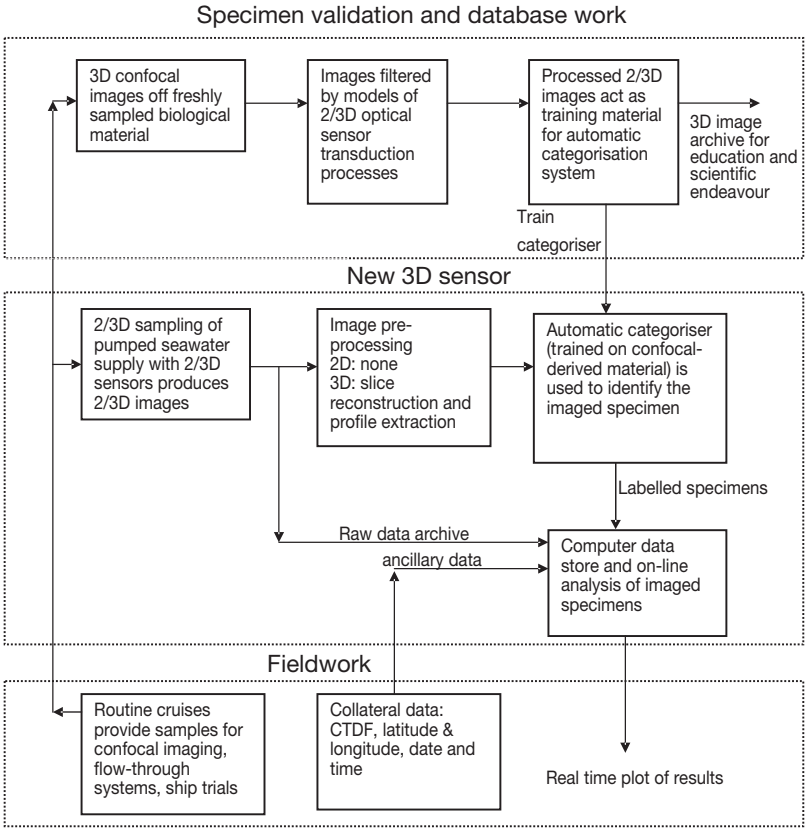

Fig. 11. Proposed project flow diagram

specimen detailing originator of image, of specimen, (iv) the normal oceanographic metadata (i.e. a curated entry in the 'library', including data like location of collection, time, depth, salinity) and (v) a 'flag' declaring that the specimen has been validated (see b below). Registered users can add such database entries to ensure a complete audit trail for each specimen. The image originator can also ascribe a probable taxonomic species label to the specimen. Mirror sites can exist to maintain copies of this database. The database will also track expert judgements (see below) and highlight to the database enquirer specimen images that need to be validated by panel members. Specimens from the imaged source material could also be submitted for DNA analysis with the ZooGene project (www.zoogene.org). This additional validation would result in specific DNA signatures that could be held with the above information.

(b) Expert panels: Each specimen in the database will possess a species label assignment, which will be of unknown, and likely variable, reliability. The task of the expert panel is to form a consensus of opinion on this label. Any registered user can become a panel member. The Internet interface to the database will need to track the categorisation activities of each panel member. It has already been shown (Simpson et al. 1992, Culverhouse et al. 2003) that self-consistency of an expert and mutual consistency between experts can expose inadequacies in human performance. Registered users may self-rate themselves as 'taxonomy expert' (perhaps with a number of years standing) or ecologist. Machine analysis of their categorisation performance will automatically accrue information on their actual competence. This could be fed back confidentially to each panel member. Once personal data has been removed it can also published to aid the community. To remain an expert one could perhaps participate in regular 'tests' of competency run online to assess self and mutual consistency. This procedure may have the following properties: (i) Only panel members and image contributors will have write-access to the database. All other users will have read-only access. (ii) A specimen is declared 'validated' if a predefined number of competent panel members agree on a species label for it. (iii) A further suggestion for a later database complement: Link to taxon lists for specific oceanic regions/basins as previously identified through traditional sampling and taxonomic identification.

(c) Standards: The database should contain high-resolution 3D images of specimens that have been unequivocally identified using morphological and potentially, genetic criteria. They will constitute the 'gold standard' for taxonomy and ecology. It is quite possible that the database will have to contain sets of images that demonstrate particular features and variations of a type. It could be that for low variance features one image may be sufficient, while others may require a larger number to adequately record the range of characteristic patterns that can exist. The ideal observer in this situation is still a trained person; unfortunately expert performance is largely invariant to view angle, image contrast, staining method, whereas machine systems are not as robust. Standards may have to reflect this more limited operation of machine recognition.

(d) Models of imaging processes: The database will eventually hold a large enough set of images across a wide range of species. At this point it will be possible to use selected images to train automatic categorisers, which could be done automatically through online access. However, the imaging processes within the

categorisers may not be capable of resolving specimen features that are exposed by 3D microscopy. In this situation a model of the lower resolution or instrument distortion and transduction processes would be needed. Each image can then be filtered through this model to selectively reduce the image from confocal resolution to a lower resolution. It could be used to convert a full 3D view of a specimen to a constrained view for a 2D image analysis system. This image manipulation could include morphing of the original 3D image to generate images for iterative image matching. It must also take into account any imaging depth of field limitations that might result in out of focus image data from 2D imaging devices. In the future, as an alternative to modelling the focal characteristics of imaging devices it may be possible to have a sufficiently good understanding of the human visual system to allow the categorisation software to accept lower quality image data created by limited depth of field blurring.

(e) Internet instrumentation: Any image gathering and analysing instrument can make free reference to the 'gold standard' images in the database. They can be used for training as with the image filtering process described above. This can take place automatically when the instrument is not in use to allow it to become an 'expert' labeller for species as the task demands.

(f) New 3D sensors: Light microscopy images (Fig. 12), although essential to taxonomists, have limitations. Manipulation of the specimen and focal plane is often necessary to reveal features critical for identification. Scanning electron microscopy (SEM) (Fig. 13) and confocal digital microscopy (Fig. 5) are preferred as they offer significantly higher resolution. Confocal imaging has become important as images are gathered in 3D and can be viewed from any angle. For these reasons, new high-resolution real-time 3D sensors, for laboratory and fieldwork, are required to replace existing 2D imagers. Four technologies are currently available: (i) confocal technology, (ii) optical tomography (iii) optical holography and (iv) acoustic tomography. However all 4 technologies have problems. Current confocal scan rates and depth of field for full 3D large field applications do not approach the speed and resolution required, for example: to resolve the setae of a copepod when the sensor is run at ship cruise speeds. Optical tomography is still experimental. Sensors have high noise levels because signal transit times across the specimen are in the femto and pico-second transit times, making imaging an ill-posed problem for reconstruction (see Shimizu & Kitama 2000). Holographic images suffer from speckle noise and generate very large data files, which can be many gigabytes in size, depending on resolution and image field of view). The on-axis holographic technique demonstrated by Hobson et al. (2000) has pixel resolutions of about 10 μm^3. This is not sufficient for high-resolution feature analysis. In addition the speckle patterns inherent in laser imaging systems introduce high levels of noise that make analysis difficult. Acoustic signals for tomography are easier to work with but the conduction velocity of signals in water places a limit on the imaging aperture for underwater towed operation. The issue is that acoustic signals have a relatively slow velocity in seawater, with transit times across a sampling aperture of several tens of microseconds. Although this slow transport of acoustic signals simplifies the measurement electronics of an acoustic tomographic sensor, it also ensures that the signal path through the water is warped by the flow of water through the sampling aperture and thus complicates the signal reconstruction process required for 3D imaging.

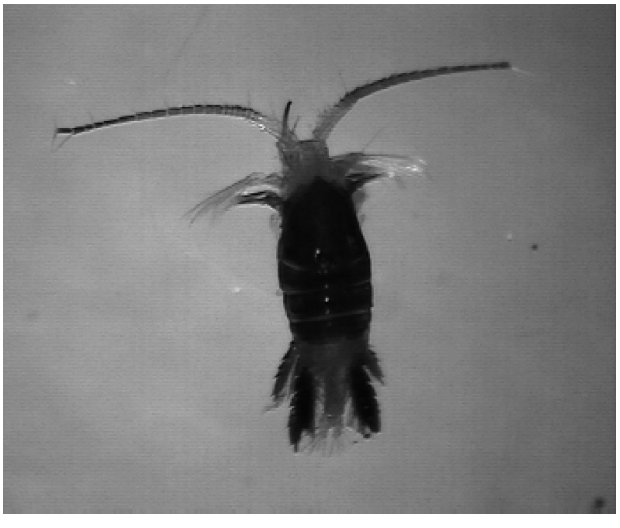

Fig. 12. A digital scan of a photomicrograph of *Candacia armata*. (Source: R. Williams, Plymouth Marine Laboratory, UK)

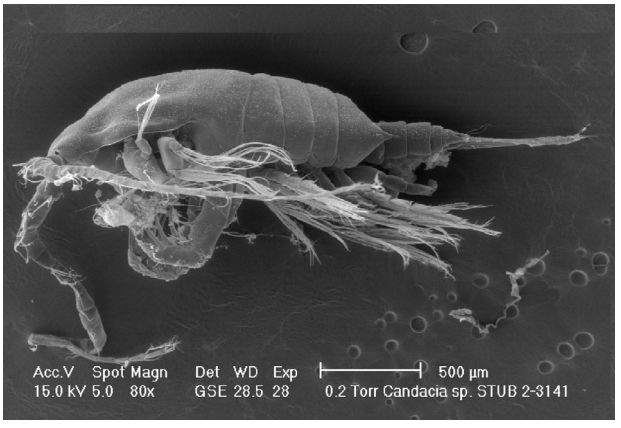

Fig. 13. Scanning electron microscope image of *Candacia* sp. (Source: M. Osore, Kenyan Marine Fisheries Research Institute)

DISCUSSION

Zooplankton encompasses a wide range of species (Protozoa and Metazoa) with very different morphologies that frequently change drastically through ontogeny. These include transparent and non-rigid organisms, which confound many automatic recognition systems. This is often because the software relies on shape profile characteristics, which may be insufficient for recognition or may not be constant for the species in question. Their recognition is further confounded by the various developmental stages of the species. The demands of science as defined by the Darwin Declaration (1998) and the Global Taxonomic Initiative have been contrasted to current imaging methods and automatic recognition performances. A set of future activities arises from these comparisons, which are needed to sustain future biodiversity science. A new large-scale proposal is suggested to provide the underpinning technology. Partners would be required to build a consortium in 3 areas: Basic technology developers, internet-based specimen validators (expert panels), and confocal imagers or similarly capable and (2D and 3D) database creators.

The need and benefits of co-operation must be appreciated since one person or laboratory cannot complete the suggested work. The work can only be achieved by a worldwide co-operative initiative. We must harness the expertise of the remaining taxonomists to achieve this Gold Standard reference database. Adoption of such a Gold Standard approach would facilitate technological cooperation and taxonomic standardization among researchers, which would greatly advance attempts to meet the challenge of conducting automated, basin-scale enumeration of plankton. The database would also be of great value as a teaching tool for the training of new taxonomists, where additional taxonomic key features could be added by experts and placed in an overlay for each image. The freely available distributed web-database would supply the basis for internet-based training courses on taxonomy and marine ecology.

Aims and objectives

(1) To establish a standard of taxonomic quality images of specimens. These will be validated for use in automatic categorisation machines and for other scientific endeavours.

(2) To identify 'holes' in taxonomic expertise in basic technology developers. For example, it has been estimated that there are only 5 or 6 competent experts worldwide on Appendicularia (larvaceans) identification, 3 of whom have already reached retirement age. Through validation of the database contents, experts for the taxonomy of specific groups would become more 'visible' and approachable to the wider community.

(3) To disseminate images of unknown species to experts across the world, through the World Wide Web. This would provide the widest possible access to expert taxonomic opinions.

Goals

(1) Short-term goals would be to establish the database and canvas image gatherers and expert panel members. The database and its use would then need to be promoted worldwide. Additional short-term goals could include the sharing of existing validated 2D image data sets for laboratory inter-calibration tests, which would promote co-operation and sharing. 2D models would be developed for filtering 3D images in preparation for use in training automatic categorisers. Image morphing techniques would allow confocal images to be used in morphological variance studies and in studies comparing genetic and morphological identification methodologies.

(2) Medium-term goals would be to demonstrate real-time 3D and tomographic imaging.

(3) Long-term goals would be to construct a very large dataset with over 100 examples of all designated zooplankton species in each oceanographic region. Users of the database could include modellers and ecologists using the oceanographic data in conjunction with its genetic signature data.

(4) Operational use of 3D imaging in the field. This is a separate goal, because it is a major change in the way in which marine ecology is carried out. The widespread use of fast 3D imaging and analysis instruments would be required before this goal could be realised.

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