

Title	Vertical profiles of marine particulates : a step towards global scale comparisons using an Autonomous Visual Plankton Recorder (シンポジウム イメージング技法によるプランクトン研究)
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Citation	日本プランクトン学会報, 61(1), 72-81
Issue Date	2014-02
Doc URL	http://hdl.handle.net/2115/57089
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Туре	article
File Information	51)yama-pura-61-72(ocr).pdf



Vertical profiles of marine particulates: a step towards global scale comparisons using an Autonomous Visual Plankton Recorder

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Abstract An Autonomous Visual Plankton Recorder (AVPR) was used to record colour in situ images of plankton and other marine particulates at several oligotrophic stations around the world, including a northern hemisphere subtropical open ocean, a northern hemisphere subtropical marginal sea, a southern hemisphere tropical marginal sea, and a polar open ocean. Quantitative analyses and comparisons of particle concentrations, sizes and vertical profiles were possible after identification of optimal image enhancement settings and employment of a specially-developed macro routine in the off-the-shelf image analysis software Image Pro Plus. Such baseline data is invaluable for assessing the effects of surface or near-surface waste water or tailings disposal during deep-sea mining operations in oligotrophic areas. Marine particulate profiles, their relationships to oceanographic parameters and water mass structure, and the resolution-dependent limitations of the system are introduced and discussed.

Key words: VPR, marine snow, vertical profiles, global comparison, zooplankton

Introduction

The vast majority of the world's oceanic area centers around oligotrophic oceanic gyres. The water column in such gyres is usually highly stratified, with nitrogen being supplied by fixation of atmospheric nitrogen by organisms such as cyanobacteria (e.g. Trichodesmium), or through periodic injections of nutrients into the mixed surface layer through atmospheric forcing of the ocean surface in the form of momentum, heat, freshwater fluxes and cyclones (Ceccarelli et al. 2013). Such episodic spikes in the nutrient concentrations of the surface layer can lead to ephemeral blooms of phytoplankton and the zooplankton best adapted to take advantage of sudden increases in phytoplankton biomass-often pelagic tunicates such as larvaceans, pyrosomes and salps, or other animals adapted to prey on pico- or nano-sized plankton. A large proportion of the fauna in oligotrophic ocean gyres consists of gelatinous organisms such as tunicates, cnidarians and ctenophores (McKinnon et al. 2013). In-situ imaging is a powerful survey

technique for investigating both fine-scale structure in the vertical profiles of marine particulates and for surveys of highly fragile gelatinous organisms (Stemmann et al. 2008). The present study investigates the feasibility of using an Autonomous Visual Plankton Recorder (AVPR) to assess the type, quantity and distribution of marine particulates in oligotrophic oceanic waters.

Materials and Methods

Study Areas

The focus of this study being to assess the feasibility of the inter-comparison of data from different geographic areas, a highly divergent set of oligotrophic areas were studied (Table 1): a northern hemisphere subtropical open ocean, near Myojinsho Caldera in the Izu-Ogasawara Island Arc in southern Japan; a northern hemisphere subtropical marginal sea, the Balearic Sea in the north-western Mediterranean Sea; a southern hemisphere tropical marginal sea, near the Osprey Reef in

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04:48-06:00

04:50-06:30

03:05-04:00

Area	Cruise	Station No.	Latitude	Longitude	Date	Local Time
North of Southern Antarctic Circumpolar	CEAMARC	UM-17	63°30.48′ S	139°59.87' E	January 31, 2008	22:47-00:35
Front						
North of Southern Antarctic Circumpolar	CEAMARC	UM-21	65°30.71' S	139°48.42′ E	February 3, 2008	11:07-12:53
Front						
Myojin Caldera, Izu-Ogasawara Islands	KT11-29	7	31°53.06′ N	139°58.04' E	November 23, 2011	01:09-03:23
Off Barcelona, N–W Mediterranean Sea	FISHJELLY	4-1	40°58.42' N	02°03.09' E	June 20, 2012	07:10-09:00
Off Barcelona, N–W Mediterranean Sea	FISHJELLY	E4	40°47.74' N	01°33.57' E	July 16, 2012	05:30-06:40
Off Barcelona, N–W Mediterranean Sea	FISHJELLY	J2	41°19.25' N	02°29.65' E	July 18, 2012	04:00-05:00

K4

R4

2.2

41°17.7′ N

41°49.35' N

13°46' S

02°41.72' E

03°33.59' E

146°33' E

FISHJELLY

FISHJELLY

AIMS5441

Table 1. Autonomous Visual Plankton Recorder (AVPR) deployment records for data used in the present comparative analysis.

the Coral Sea off Australia; and a polar open ocean, in the Southern Ocean north of the continental shelf break off the French Antarctic base Dumont d'Urville.

The Autonomous Visual Plankton Recorder (AVPR)

Off Barcelona, N-W Mediterranean Sea

Off Barcelona, N-W Mediterranean Sea

Osprey Reef, Coral Sea

The AVPR is composed of a digital camera (UNIC UC-1830CL, 2/3 inch interline CCD, Canon J10×10R-II Lensmodified) in a pressure housing rated to 1000 m depth. It records 1028×1024 pixel colour images, compressed and stored on a hard drive at a rate of ca. 15 Hz (Fig. 1). Darkfield illumination is provided by a ring strobe (Perkin-Elmer FX4400, 1 joule/flash) and photographs are linked to CTD (Citadel CTD-NV, Teledyne RD Instruments, FSI NIXC CTD Auto-7000 m) data to give environmental information on depth, water temperature and salinity for the plankton and particles in each photo. The field of view was set to 43 mm² during all deployments, resulting in an imaged volume of 518 mL-the entire volume illuminated by the strobe (Lindsay et al. 2013). Particles positioned close to the camera were out of focus and appeared much larger than their actual size as inferred by the size calibration done at the lighting and focus "sweetspot" where the field of view was 43×43 mm. These particles would hide other particles behind them that were further from the camera, resulting in an underestimate of true particle number per litre. Particles further from the camera would conversely seem smaller. The large number of photographs taken at each depth allowed such "noise" in the data values to be filtered out.

After each deployment of the system, data files were uploaded from the AVPR hard disk (80GB) to a shipside personal computer running Windows XP via USB2.0 and colour images (1028×1024 pixels) were extracted from the data file and resaved as separate JPEG2000 files (*.jp2) using full-frame extraction software (FFrExtr version 1.0.0.1, 20 June 2006 build)



July 19, 2012

July 24, 2012

July 20, 2012

Fig. 1. The Autonomous Visual Plankton Recorder (AVPR) used in the present study.

modified from the Auto Deck software (Seascan Video Plankton Recorder v3.01 CTD: SeaBird49 or FSI NXIC) by Teruki Tanaka, SEA Corporation (Japan).

Image Analyses

JPEG2000 files, extracted from the AVPR data files, were converted to JPEG files (*.jpg) using the software Advanced Batch Converter (Version 3.9, build number 3.9.76, Gold-Software Development). Full frame images were subject to particle analyses using the software package Image-Pro Plus 6.3J (Version 6.3.1.535, Media Cybernetics, Inc.) using a macro routine developed in-house.

Images were enhanced using the following settings: Bright-

ness 96, Contrast 98, and Gamma 100. These values were determined by constructing a matrix of brightness and contrast combinations and executing the "count" command with particle outlines displayed, to assess by eye the degree to which particles visible to the human eye were picked up or lost at each setting combination. False positives sometimes occurred due to either camera noise or image compression artefacts. In all cases these were 3 pixels or less in maximum dimension, so a size filter was applied within the macro routine to only count particles of maximum caliper length (feret) three pixels or greater. Preliminary profiles were determined and five "low" and five "high" particle concentration images were randomly selected from each of the oceanic areas to be compared. These images were used to determine the optimum settings for the comparative analyses.

In the first step of the macro routine, JPEG-decompressed colour images (Fig. 2a) were converted to 16-bit greyscale monochrome images (Fig. 2b) before being thresholded to black and white with a threshold minimum of 8192 and maximum of 65535 (Fig. 2c). A mask was then applied and a 2×2 pixel "close" filter was used to get rid of compression artefacts and fill holes in larger particles (Fig. 2d), before a 2×2 pixel "open" filter was used to filter out noise (Fig. 2e). Finally a size filter was applied to only count particles equal to or larger than

3 pixels in minimum caliper (feret) length (Fig. 2f).

Vertical profiles vs. depth of particle numbers and of total particle area per frame were obtained. Total number of particles per frame can be converted to particles per litre by dividing by 0.518 (Lindsay et al. 2013). No attempt was made to convert two dimensional area information into three dimensional volumes using Equivalent Spherical Diameters or into milligrams of carbon but this could presumably be done in the future if appropriate calibrations were carried out. A generalized additive model (GAM) with integrated smoothness estimation (mgcv package, R software: see Wood 2011) was used to visualize trends in average particle size vs depth.

Plankton identifications were made by DJL with reference to recent taxonomic guides (e.g. Chihara & Murano 1997) and/or reviews of specific taxonomic groups (e.g. Grossmann et al. 2012).

Results

Vertical Profiles of Marine Particulates

The dataset for the north-western Mediterranean was the most extensive, both temporally and geographically/oceanographically (Table 1). Waters of lower salinity tended to have higher particle concentrations and effects of the halocline or thermocline could also be observed, with average particle sizes



Fig. 2. Colour images (a) were converted to monochrome 16-bit greyscale images (b), before being thresholded to black and white (c), compression artefacts being removed using a "close" filter (d), and noise being filtered out using an "open" filter (e). Only particles greater than 125 μ m in minimum caliper (feret) length were counted (f).

and particle numbers being higher either above or within these clines (Fig. 3). At all stations apart from J2 and K4, particle concentrations decreased with depth. No signal was evident in the oceanographic parameters at these depths of higher particle concentrations—below 130 m depth at Station J2 and 165 m at Station K4. The particle concentrations at these depths were even higher than in the near surface layers, though the average size of the particles was smaller. Vertical profiles of particles at these two stations were otherwise broadly similar, with subsurface peaks in particle concentrations occurring below the surface mixed layer and being associated with lower salinity water masses. The highest particle concentrations in the Mediterranean dataset were approximately 270 particles per litre in a pronounced peak between 55 and 65 m depth at Station E4 on July 16, 2013 that occurred just above a pronounced halocline and seemed to be composed of diatoms (Fig. 4). The average size of the particulates within this peak increased with depth. On June 20 at Station 4–1 there were two pronounced peaks in particle concentrations—one peak (ca. 54 particles L^{-1}) of around 10 m thickness centred at 25 m depth and a second peak (ca. 68 particles L^{-1}), also of around 10 m thickness, centred around 70 m depth (Fig. 3). Although the upper peak seemed to be associated with waters of low salinity, the lower peak was not. The lowest overall particle concentrations occurred at Station R4, where the peak was broad and covered the entire surface mixed layer to the bottom of the halocline, with particle concentrations of the order of 15–48 particles L^{-1} .



Fig. 3. Vertical profiles vs. depth of salinity, temperature and particle count per frame (upper panel), average particle area running mean [line] and summed total particle area [points] (lower panel) for five deployments in the north-western Mediterranean Sea (see Table 1).



Fig. 4. Enhanced full-frame images captured by the AVPR at representative depths during each deployment in the north-western Mediterranean Sea (see Table 1).

In the Southern Ocean, Station UM17 lay north of the Southern Antarctic Circumpolar Current Front. Particle concentrations were high between 40 and 100 m depth with a maximum of 675 particles L^{-1} , and this peak seemed to be associated with a layer of cold remnant Winter Water below the summer-warmed surface mixed layer (Fig. 5). Average particle size was largest at the base of this Winter Water layer but remained high throughout the deeper layers, at least to 200 m depth, although the particles became much more heterogeneous in size below this remnant Winter Water. Station UM21 lay between Station UM17 and the Antarctic continental shelf break and was far enough away from the melting ice zone that warm sea surface and mixed layer temperatures above 0°C

were observed in the upper 40 m, while a steep thermocline was present below this to 60 m depth. At Station UM21, particle concentrations were highest in the upper 65 m with concentrations in the mixed surface layer around 145–215 particles L^{-1} and increasing to around 165–250 particles L^{-1} within the thermo/halocline. This was of a similar order of magnitude as that of particle concentrations within the peak at Station E4 in the N–W Mediterranean (Fig. 3), and was similar to values observed in the surface layer at Station UM17 (Fig. 5). Average particle size showed no clear trend with depth at Station UM21.

In the north-western Pacific Ocean at the Myojinsho Caldera in the Izu-Ogasawara Island Arc, a well-mixed warm sur-



Fig. 5. Vertical profiles vs. depth of salinity, temperature and particle count per frame (upper panel), average particle area running mean [line] and summed total particle area [points] (lower panel), and enhanced full-frame images captured by the AVPR at representative depths (right panel) during the two deployments in the Southern Ocean (see Table 1).

face layer was evident to 105 m depth, water temperature decreasing slightly below this before entering a marked thermocline at 118–122 m depth (Fig. 6). A homogeneous water mass occurred below this thermocline between 122–134 m

depth and then another pronounced thermocline occurred at 134–140 m depth. Below this depth, temperatures decreased gradually. Particle concentrations were highest and average particle sizes were largest within the warm surface mixed layer,



Fig. 6. Vertical profiles vs. depth of salinity, temperature and particle count per frame (left panel), average particle area running mean [line] and summed total particle area [points] (centre panel), and enhanced full-frame images captured by the AVPR at representative depths (right panel) during the deployment near Myojinsho Caldera in the Izu-Ogasawara Island Arc in southern Japan (see Table 1).

with concentrations being relatively stable in the range of between 19–48 particles L^{-1} —an extremely similar value to that observed in the offshore warm surface mixed layer at St. R4 in the north–western Mediterranean (Fig. 3). The anomalous spike in particle concentrations around 82 m depth was due to sudden high concentrations of small, spherical particles of unknown origin. Particles of large areas included medusae such as *Aglaura hemistoma*, colonial radiolarians, hyperiid amphipods and oikopleurid larvaceans (Fig. 6).

The dataset from Osprey Reef in the Coral Sea off the north–east coast of Australia, was problematic in that analysis with the same brightness and contrast values as used for other oligotrophic areas gave images that were so bright that the image analysis treated them as single particles of size 1028×1024 pixels when a threshold minimum of 8192 was employed. The

threshold minimum was increased by increments of 4096 until a value was found (49152) where particle counts done by eye were most similar to those done automatically using the Image Pro macro routine. A subsurface maximum in particle concentrations was observed centred at approximately 40–65 m depth (120–170 particles L^{-1}) although particle concentrations remained high to around 100 m depth (Fig. 7). A deep thermocline was present at 100–108 m depth with a wellmixed warm, lower salinity layer above it. Maximum particle concentrations did not occur above or within this thermocline but rather as a broad peak at subsurface depths that were not associated with nutrient supply. Slightly elevated particle concentrations were observed as deep as the bottom of the secondary thermocline at 135–140 m depth. No information on the vertical profiles of photosynthetically active radiation



Fig. 7. Vertical profiles vs. depth of salinity, temperature and particle count per frame (left panel), average particle area running mean [line] and summed total particle area [points] (centre panel), and enhanced full-frame images captured by the AVPR at representative depths (right panel) during the deployment near Osprey Reef in the Coral Sea off Australia (see Table 1).

(PAR) or photosynthetic pigments is available for this station. The average sizes of particles showed no clear trend with increasing depth.

In general, broadly similar vertical profiles to those outlined above for each station at each geographic locality, with respect to the positions of peaks within the water column, were obtained by graphing the total area in mm² per frame made up of particulates vs. depth (Figs. 3–7). A log axis for total area was found to be most appropriate due to the wide range of particle sizes, with marine snow or phytoplankton particulates being very small, while zooplankton were sometimes quite large. Generally speaking, deeper depths were characterised by larger particles within a background of only sporadically occurring smaller particles, while in the upper layers such larger particles occurred within a background of many smaller particles.

Taxonomic Identification of Marine Particulates

Attempts were made to identify the types of marine particulates contributing to the observed peaks in abundance and/or area. The resolution, in terms of pixels, necessary to identify organisms based on images to various taxa was dependent on several factors:

1) the existence or not of morphologically similar species in the study area

2) the presence or not of morphologically distinctive structures on the animal e.g. perfectly spherical or bilobed eyes on euphausiid species. This includes significantly different sizes as a morphologically distinctive character

3) the existence of coloration differences between morphologically similar species.

Areas with relatively large zooplankton such as the Southern Ocean stations allowed identification to species or genus level of a much higher proportion of the imaged plankton than did the oligotrophic tropical or subtropical areas. The exception to this general rule was the gelatinous plankton, where large sizes were the norm both in the Southern Ocean and also in tropical/subtropical areas. Larvacean appendicularians were extremely difficult to identify based on the morphology of their bodies per se, but house morphology seems to be a good proxy to identify them at least to genus level (R. Hopcroft, personal communication).

In general it was found that images of at least 40 pixels in maximum dimension were needed to identify animals to meaningful taxa. Under the current settings of 43 mm image width at 1028 pixels this translates to a minimum size for identifiable zooplankton of approximately 1.67 mm. Reducing the imaged volume by adjusting the field of view of the AVPR to 10 mm image width would therefore allow identification of animals of approximately 390 µm maximum dimension.

Discussion

Even with such low overall concentrations of particles as were observed in the present dataset for oligotrophic waters worldwide, the AVPR and the present method of quantifying particle number using off-the-shelf software were able to resolve the fine structure of particle distributions in the water column.

In the north-western Mediterranean Sea, a correlation was observed between lower salinity values and higher particle concentrations, with water temperatures in those layers remaining high (Fig. 3). The possibility that these water masses are of recent Atlantic origin is extremely unlikely and it would seem more likely that the water masses are of continental origin with entrained river discharge (Salat 1995) —hence the higher particle concentrations. The deep abundance peaks in particle concentrations with no correlation to oceanographic parameters that were observed at station J2 and K4 in the north-western Mediterranean may be associated with such water masses subducting, be turbidity currents penetrating the midwater at the depths of their isopycnals or sefloor shelves, or they may have originated in pulses of production that were imaged as they were sinking out of the euphotic zone. In the Southern Ocean, vertical profiles of particle concentrations versus depth were extremely different north and south of the Southern Antarctic Circumpolar Current Front (Fig. 5). When determining and interpreting particle profiles it seems imperative to also gather information from satellites and in situ probes such as CTDs in order to correctly characterize and extrapolate the data obtained.

North-western Pacific particle profiles conformed to the generally-accepted paradigm for subtropical gyres -a warm, surface mixed layer extending deeper than 100 m depth and with low particle concentrations overall (Fig. 6). Somewhat surprisingly there was no peak in particle density associated with the thermocline. Primary producers in this system are probably too small to have been captured in the present analysis where particles smaller than 125 µm were effectively excluded as "noise". Total particle area was also largest in this upper mixed layer and frames with large area values often contained gelatinous organisms such as oikopleurid larvaceans, jellyfishes and chaetognaths, as well as many hyperiid amphipods-a group often associating with gelatinous zooplankters. Taxonomic identification was only possible to genus level at most with hyperiids such as Vibilia and Phronima, and jellyfishes such as the siphonophore Sphaeronectes (non koellikeri), although the trachymedusa Aglaura hemistoma was easily recognizable. Chaetognaths and oikopleurids were unable to be resolved to species, or in many cases genus, level.

Although the Coral Sea dataset was analysed with a different threshold minimum value than that employed at the other stations worldwide, the same methodology was used to determine the optimum threshold value: i.e. manual checks by eye of the efficiency of automatic particle counts. The brightness of the in situ light field seems to have been caused by the oceanographic winch lights, which are 100% downward-pointing lights, being left on during deployment of the AVPR due to a miscommunication issue. The origin of the subsurface particle concentration maximum at Station 22 (Fig. 7) is somewhat enigmatic. Because low tide was at 03 : 36 on 20 July 2012, just 30 minutes after deployment of the AVPR, it was thought that tidal runoff from Osprey Reef might have led to the peak in particle concentrations. However, no salinity or temperature anomalies were evident so it is more likely that this subsurface peak was instead determined by the photosynthetically active radiation (PAR) vertical profile and its effect on phytoplankton of maximum dimension greater than 125 µm.

Plankton imaged by the AVPR included Acantharians, lar-

vaceans, siphonophores, ctenophores, copepods, amphipods, chaetognaths and krill at all the oligotrophic stations analysed during the present study. Comparatively high numbers of pelagic polychaetes were imaged at Station UM21 in the Southern Ocean, while *Trichodesmium*, colonial radiolarians, pteropods, salps and pyrosomes were comparatively abundant at the Coral Sea station. The Mediterranean stations had comparatively high numbers of larval fish. Although component species differed between the different oceanographic areas surveyed, the overall trend was towards high numbers of gelatinous zooplankton and single-celled protists such as Acantharians being recorded by the AVPR.

Acknowledgements

We are grateful to the reviewers for critical and constructive comments on the manuscript. We also thank Drs. Katsunori Fujikura of the BioGeos Research team, JAMSTEC, for his support, and the captains, crews and scientific teams of the CEAMARC cruise aboard the T/V Umitaka-maru, of the KT 11-29 cruise aboard the R/V Tansei-maru, of the 2012 FISHJELLY cruises aboard the R/V García del Cid, and of the 2012 cruise to the Coral Sea aboard the R/V Cape Ferguson. This study is a contribution to the Census of Marine Zooplankton (CMarZ) and the Census of Antarctic Marine Life (CAML), ocean realm field projects of the Census of Marine Life, and the International Network for Scientific Investigations of Deep-Sea Ecosystems (INDEEP). This work was partially funded by Japan Society for the Promotion of Science (JSPS) KAKENHI, grant number 24248032, MEXT Grant-in-Aid for Scientific Research on Innovative Areas TAIGA (20109003), FISHJELLY project (MAR-CTM2010-18874) and JST grant CREST, the fund for Interdisciplinary Collaborative Research by the Atmosphere and Ocean Research Institute, University of Tokyo (AORI, UT).

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Received: August 23, 2013; Accepted: October 30, 2013