Sensors for ecology

Towards integrated knowledge of ecosystems

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Chapter 2

Assessing the spatial and temporal distributions of zooplankton and marine particles using the Underwater Vision Profiler

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1. Introduction

The last two decades, international multidisciplinary programs such as the Census Of Marine Life (COML), Joint GlObal Flux Studies (JGOFS), Global Ocean Ecosystem Dynamics (GLOBEC), Integrated Marine Biogeochemistry and Ecosystem Research (IMBER) conducted numerous cruises and sampled large areas of the oceans, often focusing on the first hundred meters of the water column. In parallel, advances in remote sensing technologies from satellites allowed synoptic descriptions of some physical and optical properties of the ocean surface used to assess epipelagic particle biomasses and primary production at a global scale (see III, 3). By contrast, pelagic ecosystems of mesopelagic water layers – also known as mid-water (100-1000m) – and deeper water layers remain widely unknown. Observing these pelagic ecosystems requires the use of large and often costly instruments launched from research vessels such as pumps, multinets, remotely operated vehicles (ROV), or submersibles. Furthermore, fragile zooplankton (ctenophores, medusae, siphonophores, appendicularians) or fragile aggregates are destroyed during collection with plankton nets, *in situ* water pumps, and/or sediment traps, which prevents the analysis of their spatial distribution. This challenge can partly be overcome by using non intrusive underwater optical and imaging technologies that appear to be promising tools for the study and quantifica-

tion of zooplankton community structures, diversity, as well as marine particles size spectra.

The description of the meso- and bathypelagic fauna began to emerge with the use of ship-tethered cameras hooked on ROV (Lindsay et al., 2004; Lindsay and Hunt, 2005; Robison, 2004; Robison et al., 2005a; Steinberg et al., 1997). However, the deployment of these cameras is time-consuming and financially expensive, which prevents their wide use. Smaller instruments hooked on conventional gears – such as a rosette – or on autonomous platform – such as gliders and profilers (see IV, 1), may be more cost efficient and would provide valuable dataset on the spatial and temporal distributions of organisms and non living particles. Relatively few available instruments allow simultaneous *in situ* measurements of oceanic particles and zooplankton. Particles can be detected and measured by the laser *in situ* scattering and transmissometry (Agrawal and Pottsmith, 2000) based on scattering intensity. However, this instrument does not provide information on the shape of the particles and limits its use for zooplankton identification. The laser optical plankton counter records a shape approximation of particles crossing an array of light beams and can hardly set one particle apart another among various classes of particles and organisms (Herman et al., 2004).

More recently, several instruments that employ image analysis to characterise and enumerate oceanic zooplankton have been developed and tested in the field (Benfield et al., 2007), including *i*) the video plankton recorder (Davis et al., 2005), *ii*) the shadowed imaged particle profiling and evaluation recorder (Sipper, Samson et al., 2001), *iii*) the *in situ* ichthyoplankton imaging system (Isiis, Cowen and Guigand, 2008), and *iv*) the zooplankton visualisation and imaging system (Zoovis, Benfield et al., 2007). Most of these instruments detect relatively large organisms (more than 100µm); however, there is an increasing interest in quantifying nano- and microplankton particles (Olson and Sosik, 2007; Sosik and Olson, 2007). Several systems using holographic imaging have been developed for this purpose (Alexander et al., 2000; Hobson *et al.,* 1997; Katz et al., 1999; Pfitsch et al., 2007). Whether designed for small or large plankton, all these instruments collect images of a defined volume of water that can be processed to obtain unique information about the distribution, abundance, and behaviour of plankton on scales that cannot be investigated by conventional sampling systems such as nets and pumps. Most of the time, these instruments were used to document the *in situ* behaviour, taxonomic diversity, spatial distribution, and relative abundance of planktons. They were also used independently to study the dynamic of non-living particles in the water column.

Ideally, both plankton and non-living particles should be studied simultaneously because of their interactions in the pelagic realm. These interac-

tions include for example zooplankton feeding on detritus produced at the surface leading to particle aggregation, fragmentation, and remineralisation in the water column. These interactions affect the transfer of large amounts of carbon from the surface to the deep sea – a process known as the "biological pump" – and contribute significantly to climate variability (Sarmiento and Le Quere, 1996; Volk and Hoffert, 1985). Therefore, in order to better understand the biological pump, it is crucial to evaluate simultaneously the distribution of the particulate matter and the zooplankton in the water column. The underwater vision profilers (UVPs) were designed and constructed in our laboratory at Villefranche-sur-Mer in order to achieve this goal (figure 1). Yet, particle and plankton-imaging systems present new challenges to the studies of aquatic biota. In this paper, we describe the fifth generation of the UVP (UVP5) design and calibrations. Moreover, we expose experimental results from different cruises showing the possibility of studying the biodiversity of zooplankton and the size spectra of particles.

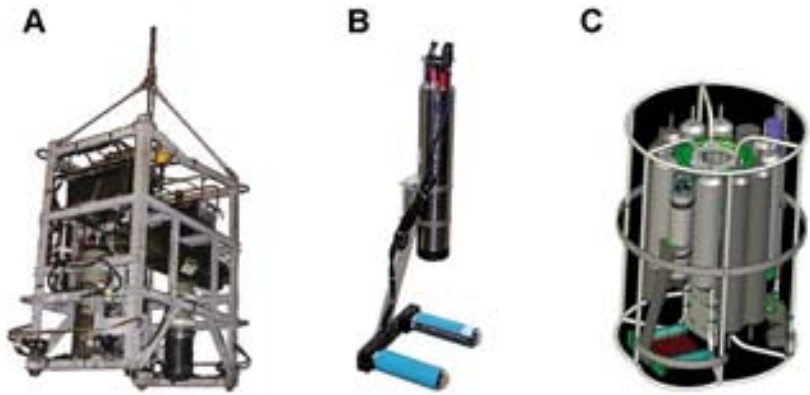

Figure 1: Pictures of the underwater vision profiler UVP4 (A) and UVP5 as stand alone (B) and picture of UVP 5 in a 24 bottles Rosette CTD system (conductivity, temperature and depth, C). UVP4 is a large stand-alone package of nearly 1 m^3 (300 kg) and incorporates a CTD, fluorometer and nephelometer sensors (Gorsky et al., 1992; Gorsky et al., 2000). The latest version called UVP5 (Picheral et al., 2010) is a smaller instrument (30kg) that can equip a standard rosette frame, interfaced with the CTD, and used down to 6000m deep instead of 1000m deep for UVP4.

2. Description of the underwater vision profiler (UVP)

2.1. Main characteristics

The underwater vision profilers (UVPs) were designed and constructed at the laboratory of Villefranche-sur-Mer to quantify simultaneously large particles (more 100 µm) and zooplankton in a known volume of water (Picheral et al., 2010). The UVP versions 2 to 4 had been operating since 1991 and they provided a database of more than 1300 inter-calibrated profiles of particle size distribution covering the global ocean. However these instruments required dedicated winch time on research ships, their maximum operating depth was 1000m, and the image acquisition at the ocean surface was limited because of daytime light saturation. In addition, their complexity required an onboard trained technician, which limited spreading their use over the oceanographic community. Nowadays, the UVP5 overcomes these limitations and can be set up for short or long-term deployments either as an autonomous system or as a complement to CTD (conductivity, temperature and depth) system. The UVP5 dimensions allow its incorporation into autonomous underwater vehicles (AUV), remotely operated vehicles (ROV), or drifting or geostationary mooring. In the near future, the ongoing miniaturisation of the sensors

Table 1: Underwater Vision Profiler 5 details

Housing	Camera housing pressure rated 6000 m 2 independent glass cylinders for the lighting
Data storage	Camera 8 with internal memory storage Optional external drive
Camera and image analysis	1.3 Megapixelup to 11fps processed images 9 mm fixed focal lens Pass band Filter centered on 625 nm
Lighting	Flash duration down to 100 µs
Piloting board	Persistor CF2 piloting processor Analog to digital conversion for external sensors Digital to analog output to CTD Power management
Connection (camera housing)	Serial interface 100Mb network
Embedded Sensors	Pressure digital sensor with 0.01% accuracy Pitch sensor Internal temperature sensor
Power	Rechargeable lithium-ion 6.3 A/29 V battery pack Continuous monitored during data acquisition

will lead to the development of autonomous camera systems that could be mounted on drifters and gliders working in network allowing real time "visual" monitoring of the biogeochemistry and the biology of the ocean (see IV, 1).

The UVP5 instrumental package contains an intelligent camera and a lighting system encompassed into independent housings (figure 1). In addition, pressure and angle sensors are included to the system in order to monitor the UVP5 deployments and data acquisition. The hardware is also composed of an acquisition and piloting board, internet switch, hard drive, and dedicated electronic power boards whose details and characteristics are presented in table 1. Images can be recorded in fields of view ranging from 8 × 6 to 22 × 18cm at a distance of 40cm from the camera in red light environment in order to reduce zooplankton phototactic behaviour and to prevent contamination by the sunlight at the surface.

2.2 Calibration

The manufacturing process of the UVP5 produces light-emitting diodes (LED) lighting systems and glass housings with unique optical characteristics. Therefore, each instrument requires individual calibration. In order to be able to estimate accurate concentrations and sizes of *in situ* marine particles, calibrations of the water volume and the size of particle within an image have to be done prior to the first deployment. A short description of the method is presented below but details can be found in Picheral et al. (2010).

The calibration of the volume of the image has to be done independently for each of the two lights. A white sheet of paper, immerged in a tank with seawater, is placed at different distances from the LEDs. Pictures of the light field projected on the white paper are recorded and gathered in order to reconstruct the volume in 3D (Picheral et al., 2010, figure 2C). The size calibration protocol defines the equation and enables the conversion from a particle defined by a number of pixels to size (area) in metric unit. Due to light-scattering in the water, this relationship is not linear for small targets. It follows the rule

$$Sm = A \times Sp^B,$$

where Sp is the surface of the particle in pixels and Sm is the surface in squared-millimetres. The calibration and determination of A and B involves diverse objects sorted into three major qualitative optical groups (dark, transparent, and heterogeneous) in order to represent the diversity of natural particles present in the environment.

2.3. Zooplankton identification

Since 2001, the UVP4 and UVP5 have provided images of macrozooplankton over the globe. All profiles have been analysed following the same protocol and using custom software routines to extract large objects (i.e. 500µm in maximum length). This size threshold was selected because most of the organisms cannot be identified below that size due to current insufficient resolution of the images. The sorting of the objects is computer-assisted as for the laboratory Zooscan system (Gorsky, 2010) and the computer prediction is visually validated by specialists to identify taxa. The size of the organisms is reported as well as its area or major and minor axes of the best fitted ellipse. This measure is best suited for dark and opaque organisms such as chaeognathes, radiolarians, fish, and large crustaceans, but cannot be used for gelatinous organisms.

3. Study of particle dynamics and zooplankton community structures at different spatial scales

3.1. Marine particles

The UVPs were deployed more than 3000 times covering almost all oceans on Earth (figure 2). The first versions of the UVPs (2 and 3) were not able to efficiently distinguish the non-living particles from the zooplanktonic organisms. Therefore, earlier studies focused on the size spectra of all particles, assuming that most of them were nonliving particles. This hypothesis was then confirmed by the use of UVPs 4 and 5 showing that zooplanktons account for only 0.1 to 10% of the total number of particles in the water column (see next section).

The most important biogeochemical information provided by the UVPs consists on the size spectra of large particles (more than 100µm). These particles, in the form of aggregates of individual particles of different origins, are the main vector of the vertical flux of carbon to the deep sea. In order to correctly estimate this flux, the concentration of particle per size bin (number per centimetre) must be converted to biovolume ($cm^3.cm^{-3}$) and to biomass (mg DryWeight.cm^{-3}) assuming relationships between size and mass (Stemmann et al., 2008a). Then, the known relationship between size and settling speed can be used to estimate vertical flux (Guidi et al., 2008; Stemmann et al., 2004b).

The coupling between small and meso-scale (scales from 5km to 100km) physical and biological processes in highly dynamic environments such as frontal zones, filaments, and equatorial systems was shown to influence the spatial patterns of carbon export. Vertical profiles of particle flux can

be analysed in a spatial context in order to provide estimates of carbon sequestration by the oceans at different scales. Previous deployments of the UVPs at high spatial resolution revealed that particle spatial patterns can be observed at scales as small as 10 to 100 km (Gorsky et al., 2002a; Gorsky et al., 2002b; Guidi et al., 2007; Stemmann et al., 2008c). Particle size spectra were also used in time series to constrain mathematical models of particle flux to the interior of the ocean (Stemmann *et al.*, 2004a; 2004b). These analyses led to formulate the hypothesis that zooplankton organisms can detect large settling particles and can fragment them in numerous smaller parts that have slower settling speed. This process may generally affect carbon sequestration in the deep ocean.

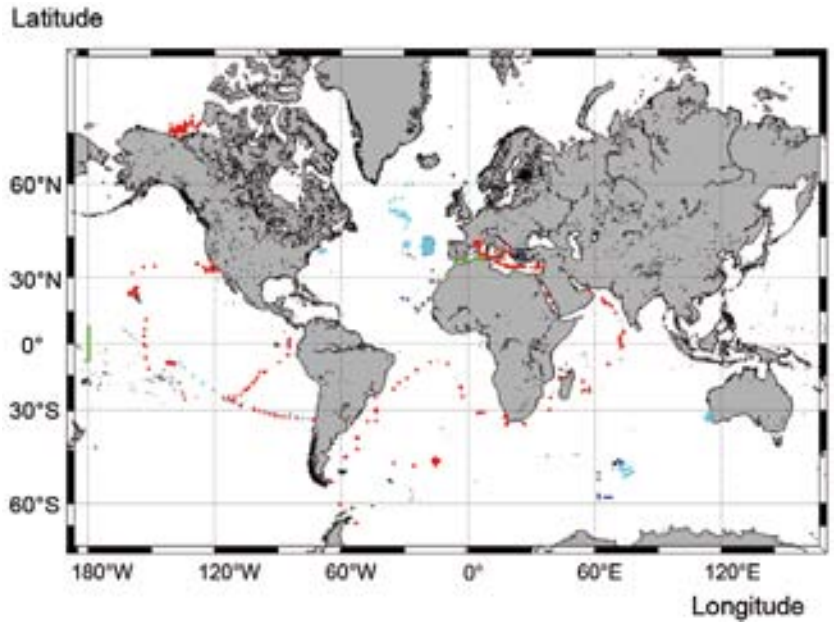

Figure 2: Global map showing the location of sites that were studied using the different versions of the UVP (dark blue = UVP2, green = UVP3, light blue = UVP4, red = UVP5).

3.2. Comparison between zooplankton and non living particle size spectra

The improvements of the optics and illumination of UVP4 and UVP5 enabled simultaneous estimations of the vertical distributions of both particles and zooplankton size spectra (figure 3).

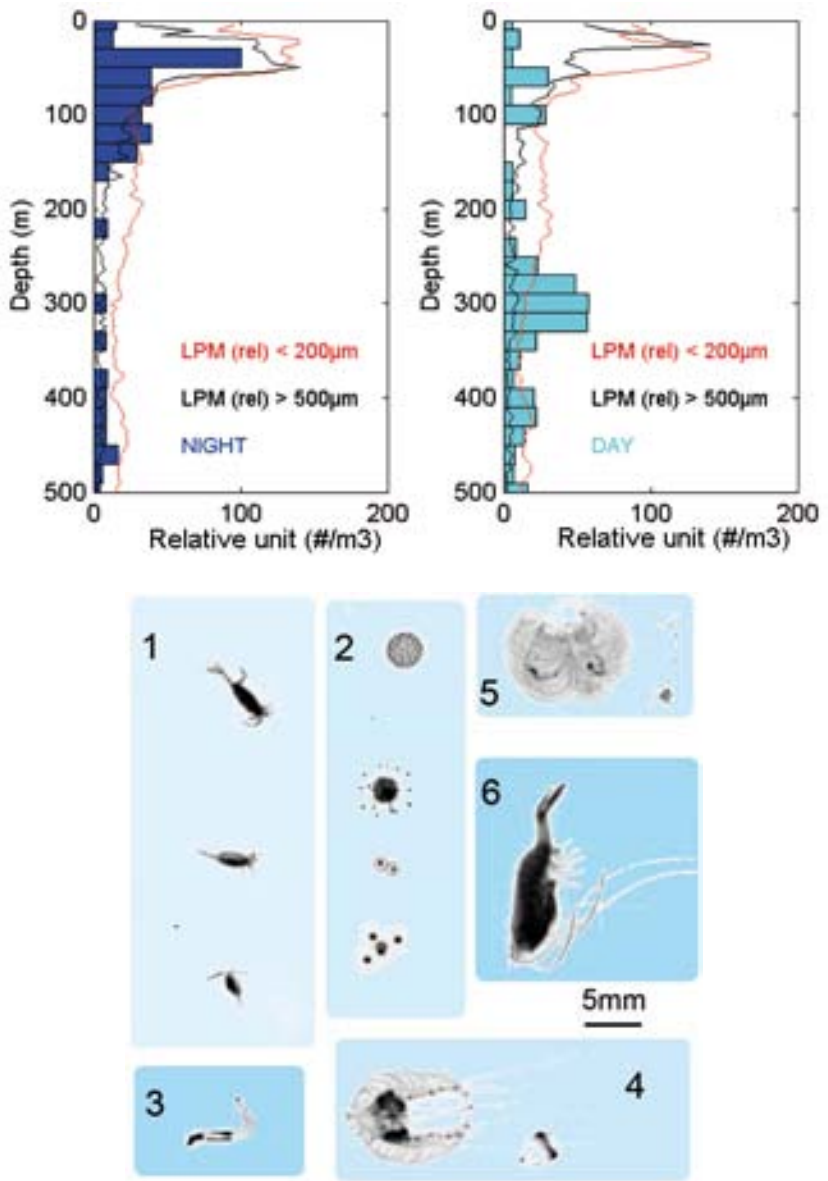

Figure 3: A. Vertical abundance (relative units) of two size classes of large particulate matter (LPM lines) and vertical day (upper right) and night (upper left) distributions of copepods during the California current ecosystem long-term ecological research (CCELTER) cruise off the Californian coast in autumn 2008. B. Typical UVP5 images of individuals from different macrozooplankton groups including copepoda (1), radiolarian (2), chaetognate (3), medusae (4), appendicularia (5), and euphausid (6).

Acoustic, optical, and imaging systems all face the same challenge when trying to distinguish between plankton and other particles in the water column. Plankton larger than 500µm includes crustacean (e.g. copepods and euphausiids), gelatinous taxa (e.g. medusae, tunicates), and eggs and fish larvae. Other particles of the same size range include aggregates, abandoned houses of larvaceans, mucous webs of pteropods and all associated material, including living (protozoa and bacteria) and dead materials. Many of these "other particles" are fragile and are not retained and/or preserved by filters or nets meshes (Gonzalez-Quiros and Checkley, 2006). Therefore, the contributions of organisms to the total number or the biomass of particles is not well known. Misrecognition between organisms and particles can have deep implication for the estimation of available biomass for higher trophic levels and for the estimation of vertical carbon fluxes. The laser optical plankton counter (LOPC) potentially distinguishes automatically zooplankton from particles based on the opacity and size of the recorded objects (Checkley et al., 2008; Gonzalez-Quiros and Checkley, 2006; Jackson and Checkley, 2011). However, results provided by this instrument consist in a proxy for zooplankton since the recognition cannot be validated nor the taxa recognised. The UVP's distinction is based on the automatic sorting of particles larger than 500µm followed by manual image analysis and visual verification of the plankton identifications by experts (Stemmann et al., 2008b; Stemmann et al., 2008d).

During the Boum cruise on the Mediterranean Sea (summer 2008), the UVP was deployed on a longitudinal transect from the East to West basin for short-term stations and 3 sites were selected for their oligotrophic characteristic (figure 4). The comparison between particles and zooplankton size spectra for the same size range (500µm-few mm) shows that the dominant zooplankton in abundance were radiolaria. More interesting, the results show almost for the first time that living organisms were only 1-15% of total particles detected by the UVP in the more than 500µm size range. These ratios are slightly lower than those reported earlier for the OPC (25%) and LOPC (20+/-14%) in the Californian Current system (Gonzalez-Quiros and Checkley, 2006; Jackson and Checkley, 2011). More data of such type should be acquired in different oceans to test whether the strong dominance of non-living particles is a common feature of pelagic ecosystem.

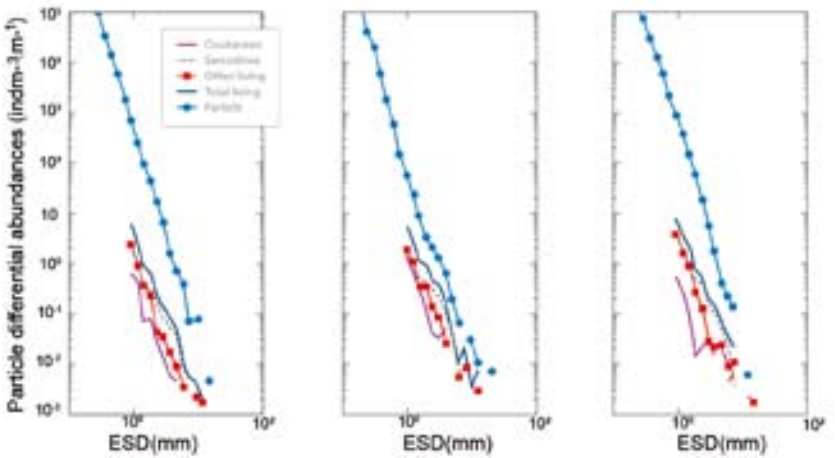

Figure 4: Particles and zooplankton normalised number spectra obtained by the underwater vision profiler at 3 locations in the Eastern (left), Central (middle) and Western (right) Mediterranean Sea during the BOUM cruise in July 2008 (adapted from Stemmann and Boss, 2012). Particles were counted automatically from 60µm in equivalent spherical diameter (ESD) and thus include non living particles and zooplankton organisms. The different taxa were counted manually on the images only for size larger than 500µm from which they can be identified.

3.3. Appendicularians and the biological pump

Appendicularians are zooplanktonic pelagic tunicates. They produce a mucous external filtration device called "the house" which allow them to filter small particles (0.2-50µm, see Lombard et al., 2011) from the seawater. Up to 26 houses can be produced within a day by a single individual (Sato et al., 2003), and once clogged, are discarded contributions to marine snow (Alldredge, 2005; Alldredge and Silver, 1988). Thus, the biogeochemical action of appendiculiarians includes mostly "repackaging" by filtering small particles and producing large ones. This effect on the biogeochemistry of particles and therefore on carbon fluxes was shown to be potentially important (Berline et al., 2011; Robison et al., 2005b). However, these organisms have been largely understudied until now mainly because of instrument limitations.

Imaging systems such as the UVP overcome these limitations and provide simultaneous observations of their distribution and relation to particle stocks and fluxes. Appendicularians repackaging action were estimated from observations in the northeastern Atlantic Ocean by the UVP4. Combined data of appendicularians and associated fluxes from UVP observations and from sediment traps suggested that the estimated pro-

duction of particulate matter by sub-surface appendicularians exceeded the observed total sinking flux at 200m (Lombard et al., 2010). This study supports the hypothesis that appendicularians play an important role in the production of particle fluxes (Alldredge, 2005). In addition, laboratory observation on discarded houses showed that empty appendicularian houses undergo a rapid deflation and compression process, decreasing their size and increasing their sinking speed (Lombard and Kiørboe, 2010). This process, combined with the previous estimation of discarded houses production, leads to the conclusion that up to 20-40% of the 300-500µm particles observed by the UVP in the upper 100m of the water column may be of appendicularian origin.

In addition to producing discarded houses in the epipelagic layers, appendicularians are also supposed to be efficient at repackaging small particles by grazing into larger aggregates (more than 1mm) in the deep ocean (Alldredge, 2005). Using the UVP4 observations, the relationship between the changes in the vertical distributions of particles and zooplankton, including appendicularians, was investigated during the Mareco cruise in the North Atlantic (Stemmann et al., 2008b). The gelatinous fauna were consistently the most numerous between 400-900m and in particular the appendicularians, that occurred mostly below 300m (figure 5). Particles vertical profiles showed that the equivalent spherical volume of particles (100µm$<d<$1mm) generally rapidly decreased with depth, down to 150m in the North Atlantic central water (NACW) and down to 300-400m in the other regions of the investigated area by the cruise (figure 5). A mid-water peak of small particles was observed in the Modified North Atlantic water (MNAW) and the Sub-Arctic intermediate water (SAIW) regions. In contrast, the decrease in biovolume of the larger particles (1-5mm) with depth was smoother and an increase in concentrations with depth below 300-400m was also observed in the SAIW and NACW regions. This increase in large particle biovolume was associated with an increase in appendicularians abundance. Moreover, in the MNAW region a peak in the biovolume of large particles (400-500m) is clearly associated with a peak in appendicularians concentrations. The observed close vertical association between the large particles and the appendicularians at the three sites could result from the small particles aggregation by appendicularians into feces or discarded houses. These small particles, which are food for appendicularians, may not be detected by the UVP because of their typical size, smaller than 30 µm (Lombard et al., 2011)

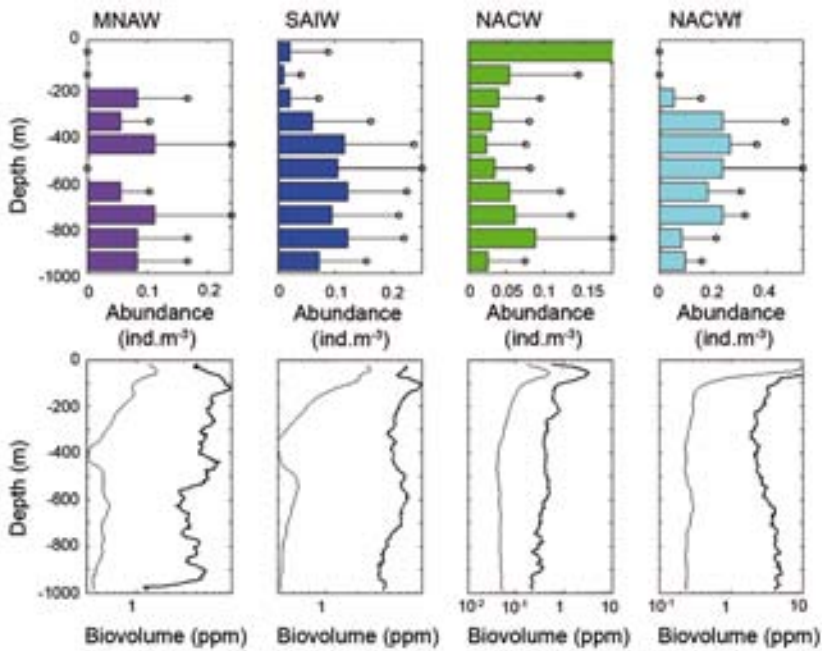

Figure 5: Vertical distribution of appendicularians (upper panel, bars are mean abundance and the stems are the standard deviation) and particles (lower panel, 100µm <d< 1mm thin line and 1mm <d< 5mm bold line) in the 4 sites sampled during the Mareco cruise (Sub-Arctic intermediate water (SAIW), modified North Atlantic water (MNAW), North Atlantic central water (NACW) and North Atlantic central water front (NACWF) which is a modified water mass from NACW).

3.4. Macrozooplankton spatial distribution in the mesopelagic layer

The mesopelagic layer of oceans is located between the photic zone (the illuminated surface zone, where light penetrates the water down to a depth of 100m) and a depth of 1000m. It is bathed in half-light, which is why it is often referred to as the "twilight zone". The mesopelagic zone represents one of the largest habitat on Earth, yet it is still widely unknown, especially when it comes to its biological composition. Since 2001, we have studied the *in situ* vertical (0-1000m) distribution of macrozooplankton during 12 cruises in 6 oceans (Mediterranean Sea, North Atlantic shelves, Mid-Atlantic ridge, tropical Pacific Ocean, eastern Indian Ocean, and sub-Antarctic Ocean). Nine regions were identified based on the hydrological properties of the water column. They correspond to nine of the biogeochemical provinces defined by Longhurst (1995).

We tested if the zoogeography of macrozooplankton in the mesopelagic layer corresponds to these biogeochemical provinces (Stemmann et al., 2008d). The zooplankton community was sorted in 21 morphotypes and more than 5000 organisms were identified in the 100-1000 m depth layer. The numerically dominant groups were crustaceans (24%) followed by the medusae (18%), appendicularians (14%) chaetognathes (11%), fish (7%) and single-cell sarcodines of the group Star (6%, see figure 6). The other taxonomic groups were less than 5% of the total count each. However, pooling all single-cell sarcodines moved this group to second rank (23%) in term of frequency of occurrence. From a trophic perspective, the assemblages

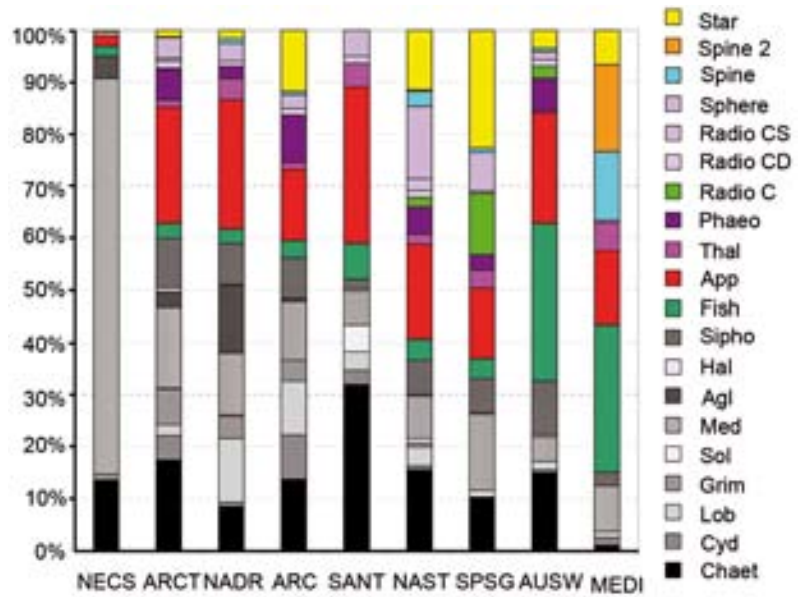

Figure 6: Frequency of occurrence for the 20 taxonomic groups in the 9 regions. Note that the numerically dominant group of Crustacean has been removed from the list to increase the details in the other groups. Appendicularians (App.), Thaliacae (Thal.), Fish, *Haliscera spp. medusa* (Hal.), *S. bittentaculata* (Sol.), *Aglantha spp.* (Agl.), *Aeginura grimaldii* (Grim.), "other medusae" (Med.), chaetognath (Chaet.), lobate ctenophore (Lob.), cydippid ctenophore (Cyd.), siphonophore (Sipho.), single-cell sarcodine grouped by four (Radio CS.), colonial radiolarians (Radio C.), colonial radiolarians with double line (Radio CD.), Phaedorian (Phaeo.), single-cell sarcodine with spines (Spine.), double-cell sarcodine with spines (Spine 2.), spheres (Sphere.), and sarcodine with hairs (Star.). The regions are defined as: Northeast Atlantic shelves (NECS), Atlantic Arctic (ARCT), North Atlantic drift (NADR), Atlantic Subarctic (ARC), Subantarctic, Ocean (SANT), North Atlantic Subtropical Ocean, (NAST), South Pacific Subtropical Gyre (SPSG), Western Australia (AUSW), Mediterranean Sea (MEDI). The order of the region is set so the proportion of carnivorous organisms (in grey from Chaet. to Sipho.) decreases from left to right (modified from Stemmann et al., 2008).

of zooplankton could be lumped into three categories: gelatinous carnivores (cydippid stenophores, lobate ctenophores, medusae, siphonophores, chaetognathes), filter feeder detritivores (appendicularians and salps) and omnivores (sarcodines, crustaceans and fish). Interestingly, the proportion of carnivores decreased from 95% to 15%, from the high latitude regions (Northest Atlantic shelves, Atlantic Arctic, North Atlantic drift, Atlantic Subarctic, Subantarctic ocean) to the low latitude regions (Mediterranean sea, western Australia, South Pacific subtropical Gyre). The similarity in the community assemblages of zooplankton in the layer between 100 and 1000m was significantly higher within regions than between regions, for most cases. The regions with comparable compositions were located in the North Atlantic with adjacent water masses, suggesting that the assemblages were either mixed by advective transport or that environmental conditions were similar in mesopelagic layers. The data suggest that the spatial structuring of mesopelagic macrozooplankton occurs at large scales (e.g. basin scales) but not necessarily at smaller scales (e.g. oceanic front).

4. Conclusion

Results obtained using the UVP but also several other *in situ* imaging instruments have shown that bio-imagery techniques can provide useful data on plankton and particles spatial and temporal distribution in the upper kilometre of the ocean. In the next decade, rapid technological evolution toward miniaturisation in the optical sensors is expected, and will make possible the use of these sensors on autonomous platforms. Their extensive use may set a revolution in ocean plankton sciences equivalent to the revolution in medical practices for the last 15 years. Broader spatial and longer temporal coverage of plankton size spectra will soon be possible for global monitoring programs (see chapter IV, 1). Mathematical models for individual physiological and population change rates, biomasses flow between trophic levels, and functions of organisms or particle size, were also developed in the last decade. The new sets of data obtained by the wide use of imaging instruments are well adapted to calibrate and validate these models.

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