



1 Metazoan zooplankton in the Bay of Biscay: 16 years of 2 individual sizes and abundances from the ZooScan and

- **3 ZooCAM imaging systems.**
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32 Abstract

33 This paper presents two metazoan zooplankton datasets obtained by imaging samples collected on the Bay of 34 Biscay continental shelf in spring during the PELGAS integrated surveys, over the 2004-2019 period. The samples were collected at night, with a WP2 200 µm mesh size fitted with a Hydrobios (back-run stop) mechanical 35 flowmeter, hauled vertically from the sea floor to the surface with a maximum depth set at 100 m when the 36 37 bathymetry is deeper. The first dataset originates from samples collected from 2004 to 2016, imaged on land with 38 the ZooScan and is composed of 1,153,507 imaged and measured objects. The second dataset originates from 39 samples collected from 2016 to 2019, imaged on board the R/V Thalassa with the ZooCAM and is composed of 40 702,111 imaged and measured objects. The imaged objects is composed of zooplankton individuals, zooplankton 41 pieces, non-living particles and imaging artefacts, ranging from 300 µm to 3.39 mm Equivalent Spherical 42 Diameter, individually imaged, measured and identified. Each imaged object is geolocated, associated to a station, 43 a survey, a year and other metadata. Each object is described by a set of morphological and grey level based 44 features (8 bits encoding, 0 = black, 255 = white), including size, automatically extracted on each individual image. 45 Each object was taxonomically identified using the web based application Ecotaxa with built-in, random forest and CNN based, semi-automatic sorting tools followed by expert validation or correction. The objects were sorted 46 47 in 172 taxonomic and morphological groups. Each dataset features a table combining metadata and data, at the 48 individual object granularity, from which one can easily derive quantitative population and communities 49 descriptors such as abundances, mean sizes, biovolumes, biomasses, and size structure. Each object's individual 50 image is provided along with the data. These two datasets can be used combined together for ecological studies as 51 the two instruments are interoperable, or as training sets for ZooScan and ZooCAM users.

52 Keywords

53 Zooplankton, ZooCAM, ZooScan, Bay of Biscay, imaging, PELGAS surveys.



55 1 Introduction

56 Metazoan heterotrophic planktonic organisms, hereafter referred to as zooplankton, encompass an 57 immense diversity of life forms, which have successfully colonized the entire ocean, from eutrophic estuarine 58 shallow areas to oligotrophic open ocean, from sunlit ocean to hadal depth. Their body sizes span five to six orders 59 of magnitude in length, from µm to tens of meters (Sieburth & Smetacek, 1978). Zooplankton plays a pivotal role 60 in marine ecosystem (Banse, 1995). It transfers the organic matter produced in the epipelagic domain by 61 photosynthesis to the deeper layers of the ocean (Siegel et al., 2016), by producing fast sinking aggregates (Turner, 62 2015), and by diel vertical migration (Steinberg et al., 2000; Ohman & Romagnan, 2016). Zooplankton therefore 63 participates in mitigating the anthropogenic carbon dioxide build up in the atmosphere responsible for climate 64 change. Moreover, zooplankton is an exclusive trophic resource for commercially important fish during their larval 65 stage, where a shift in zooplankton species or phenology can have dramatic effects on recruitment (i.e. North Sea 66 cod, Beaugrand et al., 2003). In addition, it is a major trophic resource for adult planktivorous small pelagic fish, 67 known as forage fishes (Van der Lingen, 2006). Recent studies suggest that zooplankton dynamics may have a significant effect on small pelagic fish population dynamics and individual body condition (Brosset et al., 2016; 68 69 Menu et al., 2023), and therefore impact wasp-waist ecosystem based fisheries and fisheries dependent socio-70 ecosystems, worldwide (Cury et al., 2000).

71 Despite zooplankton being of such global importance in both climate change effects on ecosystems and 72 management of fisheries (Chiba et al., 2018; Lombard et al., 2019), it is still technically difficult to monitor, with 73 respect to other marine ecological compartments. Zooplankton biomass, diversity and spatio-temporal 74 distributions cannot be estimated from spaceborne sensors as phytoplankton's does (Uitz et al., 2010), and 75 zooplankton commercial exploitation data do not exist yet, as fish data does. One noticeable exception is the CPR 76 surveys network that enables zooplankton data generation at decent spatio-temporal scales (Batten et al., 2019). 77 Yet, generating zooplankton data often requires dedicated surveys at sea, specific sampling instruments and trained 78 analysts. Moreover, besides actual observation, modelling zooplankton remains a challenging task due to the 79 diversity of traits such as life forms, life cycles, body sizes and physiological processes exhibited by zooplankton 80 (Mitra & Davis 2010; Mitra et al., 2014). However, over the past two decades the development of imaging and 81 associated machine learning semi-automatic identification tools (Irisson et al., 2022) have greatly improved the 82 capability of scientists to analyse long (Feuilloley et al., 2022), high frequency (Romagnan et al., 2016), or spatially 83 resolved (Grandremy et al., 2023a) zooplankton time series, as well as trait based data (Orenstein et al., 2022). 84 Imaging and machine learning have particularly enabled the increased development of combined size and 85 taxonomy zooplankton ecological studies (i.e. Vandromme et al., 2014; Romagnan et al., 2016; Benedetti et al., 86 2019). Yet, use of these machine learning tools is not trivial because those often require abundant, scientifically 87 qualified, sensor specific, training image data (i.e. learning set and test set, Irisson et al., 2022), and complex 88 hardware and software setups (Panaïotis et al., 2022). One good example of such image dataset is the ZooScanNet 89 dataset (Elineau et al., 2018), which features an extensive ZooScan (Gorsky et al., 2010) imaging dataset usable 90 as a training set for ecologists as well as for imaging and machine learning scientists.

91 The objective of this paper is to present two open zooplankton imaging datasets, originating from two 92 different instruments, the ZooScan (Gorsky et al., 2010), and the ZooCAM (Colas et al., 2018). These datasets 93 originate from the PELGAS integrated survey in the Bay of Biscay (Doray et al., 2018), a continental shelf





ecosystem supporting major European fisheries (ICES, 2021). Combined together, these datasets make up a 16 years time series of sized and taxonomically resolved zooplankton, along with context metadata allowing the calculation of quantitative data, covering the whole Bay of Biscay continental shelf, from the French coast to the continental slope, and from the Basque country to southern Brittany, in spring. These datasets can be used for ecological studies (Grandremy et al., 2023a), machine learning studies, and modelling studies.

99 2 Methods

100 2.1 Sampling

101 Zooplankton samples were collected during the successive PELGAS integrated surveys (Doray et al., 102 2018) carried out over the Bay of Biscay French continental shelf, in spring from 2004 to 2019 on board the R/V 103 Thalassa. The number of samples across years varied between 41 (2005) and 64 (2019), due to adjustments in the 104 sampling strategy and weather conditions, for 889 zooplankton samples collected in total. From 2004 to 2006, 105 samples were collected in the southern Bay of Biscay until the Loire estuary only (Fig. 1). Sampling was carried 106 out in vertical tows during night time using a 200-µm mesh size WP2 net, generally from 100 m depth (or 5 m 107 above the seabed) to the surface. In 2004 and 2005, the targeted maximum sampling depth was 200 m. In 2004, 108 fifteen samples were collected deeper than 100 m, among which eleven were deeper than 120 m; in 2005, twenty 109 samples were collected deeper than 100 m, among which thirteen were deeper than 120 m. Before 2014, the 110 sampled water volume was estimated by multiplying the cable length by the net opening surface (0.25 m²) whereas 111 since 2014, the net was equipped with a Hydrobios back-run stop flowmeter. The samples originating from 2004 112 to 2016 surveys were preserved in 4% formaldehyde (final concentration) and analysed on land in the laboratory 113 with the ZooScan in 2019, while since 2016 they were analysed live on board with the ZooCAM.

114 **2.2 Sample processing and analyses**

115 **2.2.1 Digitization with the ZooScan**

Preserved samples were digitized with the ZooScan (Gorsky et al., 2010), a flatbed scanner generating 117 16-bit gray-level high-resolution images (2400 dpi, pixel size: 10.56μ m, image size: 15×24 cm equivalent to 118 14 200×22 700 pixels). It is well suited for the imaging of preserved organisms ranging in size from 300 μ m to 119 several centimeters. The ZooScan is run by the custom made, ImageJ based, ZooProcess software which generates 120 one single large image for each scan that contains up to 2000 organisms depending on the size of the imaged 121 organisms.

122 Prior to digitization, the seawater and formaldehyde solution was filtered through a 180 µm mesh sieve 123 into a trash tank, under a fume hood. The organisms were then gently but thoroughly rinsed with freshwater over 124 the tank, in the sieve. They were then size-fractionated with a 1 mm sieve, into organisms larger and smaller than 125 1 mm size fractions. This size splitting step is recommended when using the ZooScan to address the possible 126 under-representation of large objects bias caused by the necessary subsampling. Each size fraction was subsampled 127 separately with a Motoda splitter to obtain two subsamples containing 500-1000 objects for the large organisms 128 size fraction, and 1000-2000 objects for the small organisms size fraction. Each subsample was imaged after 129 manual separation of objects on the scanning tray, to mitigate the number of overlapping objects as recommended 130 in Vandromme et al., 2012. Overall, 699 samples were digitized following this protocol, corresponding to 1397 131 scans (one sample was not size fractioned as it did not contained organisms larger than 1 mm).







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133 Figure 1: Metazoan zooplankton sampling locations during the PELGAS cruises in the Bay of Biscay from 2004

to 2019. The years with the poorest coverage are 2005 and 2006 with 41 and 43 sampling stations respectively;

and the years with the best coverage are 2015, 2017 and 2019 with 64, 64 and 65 sampling stations respectively.





136 **2.2.2 Digitization with the ZooCAM**

137 The ZooCAM is an in-flow imaging instrument, designed to digitize preserved as well as live zooplankton 138 samples, on board, immediately after collection (Colas et al., 2018). The ZooCAM features a cylindrical 139 transparent tank in which the zooplankton sample is mixed with filtered seawater. Depending on the richness of 140 the sample, and the subsampling (if necessary), the volume of seawater can be adjusted between 2-7 litres. The 141 organisms were pumped at a 1L.min⁻¹ from the tank to a flowcell inserted between a CCD camera (pixel size: 10.3 142 μ m) and a red LED flashing device where they were imaged at 16 fps. Given the flowcell volume, the size of the 143 field of view, the imaging frequency and the flowrate, all the seawater volume containing the organisms was 144 imaged (Colas et al., 2018). Before all the initial volume was imaged, the tank and the tubing were carefully and 145 thoroughly rinsed with filtered seawater to ensure the imaging of all the organisms poured in the tank. For each 146 sample, the ZooCAM generates a stack of small size (~1 Mo) raw images that are subsequently analysed with the 147 ZooCAM software. Depending on the initial water content of the tank and the rinsing, a ZooCAM run can generate 148 up to 10k raw images from which the individual organism vignettes will be extracted. A ZooCAM run on a live 149 sample often generates up to 5000-10000 vignettes of individual organisms. It is very important to subsample the 150 initial samples with a dichotomic splitter (here a Motoda splitter), to get subsamples with a quantity of objects that reduce the risk of imaging overlapping objects, and to break free from the dependency to the water volume imaged 151 152 to reconstruct quantitative estimates of zooplankton as the initial and rinsing volume are variable. Overall, 190 153 samples were digitized live on-board with the ZooCAM.

154 2.3 Images processing

155 Both instruments generate grey level working images (8 bit encoding, 0 =black, 255 = white). In both 156 cases, image processing consisted in (i) a "physical" background homogenization by subtracting an empty 157 background image to each sample image (1 for ZooScan, and as many as raw images for ZooCAM), (ii) a 158 thresholding of each raw image (threshold value: 243 for ZooScan, 240 for ZooCAM), (iii) the segmentation of 159 each object imaged. The ZooProcess software was set to detect and segment objects with an area equal or larger 160 than 631 pixels, whereas the ZooCAM software was set to detect objects with an area equal or larger than 667 161 pixels, which in both cases equals 300 µm ESD, or a biovolume of 0.014 mm³ (using a spherical biovolume model, 162 Vandromme et al., 2012).

163 Morphological features were then extracted on each detected object. Features generated by the ZooScan 164 are defined in Gorsky et al. (2010) and those generated by the ZooCAM are defined in Colas et al. (2018). ZooScan 165 images were processed with ZooProcess v7.39 (04/10/2020) open source software. ZooCAM images were 166 processed with the proprietary ZooCAM custom made software which uses the MIL (Matrox Imaging Library, 167 Dorval, Québec, Canada) as the individual object processing kernel. Each detected object was finally cropped from 168 the working sample images, and saved as a unique, labelled vignette, in a sample specific folder along with a 169 sample specific single text file containing the objects features arranged as a table with objects arranged in lines 170 and features in columns.

171 **2.4 Touching objects**

172 The ZooProcess features a tool that enable the digital separation of possible touching objects in the final 173 image dataset, for each sample. As touching objects may impair the estimations of abundances and size structure





174 (Vandromme et al., 2012), remaining touching objects were searched for on the individual vignettes from the

175 ZooScan and digitally manually separated with the ZooProcess separation tool to improve the quality of further

176 identifications, counts and size structure of zooplankton. ZooCAM does not offer such a tool.

177 2.5 Taxonomic identification of individual images

178 All individual vignettes from both instruments were sorted and identified with the help of the online 179 application Ecotaxa (Picheral et al., 2017), as two instrument-specific separated sets. Ecotaxa features a Random 180 Forest algorithm (Breiman, 2001) and a series of instruments specific tuned spatially sparse Convolutional Neural 181 Networks (Graham, 2014) that were used in a combined approach to predict identifications of unidentified objects. 182 First, an automatic classification of non-identified individual vignettes into coarse zooplankton and non-183 zooplankton categories was carried out. In both cases (ZooScan and ZooCAM), Ecotaxa hosted instrument specific image datasets, previously curated and freely available, were used as initial learning sets. These initial 184 185 classifications were then visually inspected, manually validated or corrected when necessary, and taxonomically 186 refined when possible. After a few thousand images were validated in each project, they were used as dataset 187 specific learning sets to improve the initial coarse automatic identifications. This process was iterated until all the 188 individual vignettes were classified into their maximum reachable taxonomical detail. It is worth mentioning here 189 that only a handful of taxonomists worked on identification of the two images sets.

190 **2.6 Intercalibration of the two instruments**

The two datasets are usable separately. However, considered together they build a 16 years long spatio-191 192 temporal time series. To ensure they are homogeneous and can thus be used together for ecological studies, we 193 conducted a comparison study using samples from year 2016 (61 stations over the whole Bay of Biscay continental 194 shelf, Grandremy et al., under review). In brief, all non-zooplankton and touching objects images were removed 195 from the initial datasets. Then, the interoperable size range was determined with an assessment based on the 196 comparison of Normalized Biovolume - Size Spectra (NB-SS) for each instrument. This size interval ranges 197 between [0.3-3.39] mm ESD. Finally, the zooplankton communities as seen by the ZooScan and the ZooCAM 198 were compared by taxa and by station using 27 taxonomic groups. Poorly represented taxa as well as non-199 taxonomically identified objects were not taken into account in the zooplankton variables computation and in 200 community structure analyses. Both instruments showed similar NB-SS slopes for 58 out of 61 stations; depicted 201 comparable abundances, biovolumes and mean organisms' sizes, as well as similar community composition for a 202 majority of sampling stations. They also estimated similar spatial patterns of the zooplankton community at the 203 scale of the Bay of Biscay. We therefore assume that the two presented datasets build a single, 16 years long spatio-204 temporal time series of abundances (Fig. 2) and sizes of zooplanktonic organisms (Fig. 3), from which biovolumes, 205 biomasses, Shannon index (Fig. 4), and zooplankton community size structure can be derived (Vandromme et al., 206 2012).







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Figure 2: Gridded maps of total zooplankton abundances expressed as individuals per cubic meters of sampled seawater, during the PELGAS cruises in the Bay of Biscay from 2004 to 2019. The abundances are well within the range of zooplankton abundances seen over other temperate continental shelves. They exhibit a marked coastal to offshore gradient, abundances being higher at the coast. Abundances also show an overall increase over the years.







Figure 3: Gridded maps of total zooplankton mean sizes expressed as mm Equivalent Spherical Diameter during the PELGAS cruise in the Bay of Biscay from 2004 to 2019. They exhibit a coastal to offshore gradient as well as a north-south gradient. Mean body sizes are smaller at the coast and usually smaller in the south. In general, mean body sizes show an overall decrease over the years.





218 3 Datasets

219 3.1 Taxonomic groups and Operational Taxonomic Units

220 The ZooScan dataset is composed of 1,153,507 zooplankton individuals, zooplankton parts, non-living 221 particles and imaging artefacts individually imaged and measured with the ZooScan and ZooProcess (Gorsky et 222 al., 2010), sorted in 127 taxonomic and morphological groups. The ZooCAM dataset is composed of 702,111 223 zooplankton individuals, zooplankton parts, non-living particles and imaging artefacts individually imaged and measured with the ZooCAM (Colas et al., 2018), sorted in 127 taxonomic and morphological or life stages groups. 224 225 The total number of different groups identified with both instruments combined is 170, among which 84 are in 226 common (Table 1), 43 belong to the ZooScan dataset only and 43 others belong to the ZooCAM dataset only 227 (Table 2). The identified groups were divided into actual taxa and Operational Taxonomic Units (OTUs). 228 Typically, OTUs are either non-adult life stages of taxa, aggregated morphological groups, or non-living groups 229 (see Tables 1 and 2). Among the groups common to both instruments, 45 are actual taxa, and 39 are OTUs (Table 230 1). Among the ZooScan only groups, 22 are taxa, and 21 are OTUs, and among the ZooCAM only groups, 18 are 231 taxa, and 25 are OTUs (Table 2).

232 The differences in identified groups, in the ratio taxa/OTUs, and in the associated counts arose from 233 several aspects of the data generation. Firstly, the two imaging methods differ in their technical set-up. The main 234 difference is that, on the one hand, fixed organisms are laid down and arranged manually on the imaging sensor 235 and digitized in a lab, steady 2-D, set-up when using the ZooScan. On the other hand, organisms are imaged live, 236 in a moving fluid, in a 3-D environment (the flowcell), on-board when digitized with the ZooCAM. Their position in front of the camera may not enable an identification as precise as when they are laid on the scanner tray 237 238 (Grandremy et al., under review; Colas et al., 2018). Secondly, the dataset are sequential in time, the ZooCAM 239 dataset follows the ZooScan's. Zooplankton communities in the Bay of Biscay may have changed over time, even 240 if their biomass as aggregated groups show a remarkable space-time stability (Grandremy et al., 2023a). Thirdly, 241 we cannot guaranty that there is no adverse effect on taxonomic identification, as validation involved several 242 experts (Culverhouse, 2007). Although we paid great attention to homogenize the final detailed datasets, we 243 recommend to aggregate taxa and OTUs and reduce the biological resolution for ecological studies (Grandremy et 244 al., 2023a, under review). Additionally, numerous identified and sorted taxa and OTUs do not belong to the 245 metazoan zooplankton, or are non-adult life stages, or parts of organisms. Those were included in the presented datasets because they are always found in natural samples. They need to be separated from entire organisms to 246 ensure as accurate as possible abundances estimations, as well as taken into account to ensure accurate biovolumes 247 248 or biomasses estimations. A good example is the siphonophore issue: numerous swimming bells of degraded 249 siphonophores individuals can be found and imaged in a sample. Determining an accurate siphonophore abundance 250 may not be easy, but this could be overcome by considering the biovolume or biomass of siphonophores by adding 251 up the numerous parts' biovolumes or biomass of the organisms imaged.





- 252 Table 1: ZooScan and ZooCAM common taxa and OTU. Taxa are listed in the left column of the table, in italics;
- 253 OTU are listed in the right column of the table in non-italics. OTUs names are spelled as they appear in the dataset.
- 254 Numbers next to each taxa and OTU are the counts for each category for each instrument in the whole datasets.

i.

255 Non-zooplanktonic OTUs are highlighted in bold.

	ZooCAM	ZooScan		ZooCAM	ZooScan
taxa	counts	counts	OTU	counts	counts
Acartiidae	30403	66353	artefact	2643	60718
Actinopterygii	85	2113	Bivalvia <mollusca< td=""><td>1324</td><td>3766</td></mollusca<>	1324	3766
Aetideidae	15	75	bract <diphyidae< td=""><td>1315</td><td>386</td></diphyidae<>	1315	386
Amphipoda	68	853	bubble	32563	1112
Annelida	256	2434	calyptops is < Euphausiacea	1396	3246
Appendicularia	6724	34027	Cnidaria <metazoa< td=""><td>148</td><td>4974</td></metazoa<>	148	4974
Branchiostoma	15	210	Ctenophora <metazoa< td=""><td>94</td><td>126</td></metazoa<>	94	126
Calanidae	9578	91513	cyphonaute	684	2218
Calanoida	137536	149956	cypris	862	2363
Calocalanus	820	1196	dead <copepoda< td=""><td>13383</td><td>17151</td></copepoda<>	13383	17151
Candaciidae	70	2773	detritus	105751	219541
Centropagidae	4592	14651	Diatoma	36842	1084
Chaetognatha	624	7274	egg sac <egg< td=""><td>152</td><td>394</td></egg<>	152	394
Cladocera	5590	18213	egg unkn temp <engraulidae td="" temp<=""><td>61</td><td>192</td></engraulidae>	61	192
Corycaeidae	2021	4720	egg <actinopterygii< td=""><td>768</td><td>3596</td></actinopterygii<>	768	3596
Cumacea	4	180	egg <other< td=""><td>17</td><td>2281</td></other<>	17	2281
Decapoda	173	471	eudoxie <diphyidae< td=""><td>501</td><td>69</td></diphyidae<>	501	69
Doliolida	26	128	fiber <detritus< td=""><td>13379</td><td>25124</td></detritus<>	13379	25124
Echinodermata	24	253	gonophore <diphyidae< td=""><td>4395</td><td>1462</td></diphyidae<>	4395	1462
Eucalanidae	2	839	larvae <annelida< td=""><td>244</td><td>708</td></annelida<>	244	708
Euchaetidae	2643	12957	larvae <echinodermata< td=""><td>483</td><td>2200</td></echinodermata<>	483	2200
Euphausiacea	889	1195	larvae <porcellanidae< td=""><td>127</td><td>2838</td></porcellanidae<>	127	2838
Euterpina	1043	2870	megalopa	6	460
Foraminifera	1	384	multiple <copepoda< td=""><td>3740</td><td>961</td></copepoda<>	3740	961
Haloptilus	1	5	multiple <other< td=""><td>1928</td><td>10303</td></other<>	1928	10303
Harpacticoida	481	1697	nauplii <cirripedia< td=""><td>6766</td><td>6008</td></cirripedia<>	6766	6008
Heterorhabdidae	8	205	nauplii <crustacea< td=""><td>3422</td><td>10747</td></crustacea<>	3422	10747
Insecta	2	3	nectophore <diphyidae< td=""><td>839</td><td>14389</td></diphyidae<>	839	14389
Isopoda	1	123	nectophore <physonectae< td=""><td>106</td><td>696</td></physonectae<>	106	696
Limacinidae	8966	6423	Noctiluca <noctilucaceae< td=""><td>22165</td><td>20784</td></noctilucaceae<>	22165	20784
Metridinidae	2333	15081	other <living< td=""><td>15029</td><td>5861</td></living<>	15029	5861
Microsetella	116	1169	part <ctenophora< td=""><td>30</td><td>319</td></ctenophora<>	30	319
Mvsida	3	885	part <siphonophorae< td=""><td>279</td><td>12976</td></siphonophorae<>	279	12976
Neoceratium	2984	4830	pluteus <echinodermata< td=""><td>1623</td><td>1441</td></echinodermata<>	1623	1441
Obelia	459	1016	scale	2	53
Oithonidae	112977	110510	siphonula	1	20
Oncaeidae	11843	34651	tail <appendicularia< td=""><td>753</td><td>11349</td></appendicularia<>	753	11349
Ostracoda	55	341	tomaria larvae	21	83
Phoronida	90	163	zoea <decapoda< td=""><td>151</td><td>1405</td></decapoda<>	151	1405
Pontellidae	6	299			
Rhincalanidae	1	127			
Sapphirinidae	1	21			
Temoridae	13520	31335			
m	13320	51555			

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Thecosomata

Tomopteridae

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- 257 Table 2: ZooScan and ZooCAM not common taxa and OTU. Taxa and OTUs appearing exclusively in the
- 258 ZooCAM dataset are listed in the left column, those appearing exclusively in the ZooScan dataset are listed in the
- right column. For both instruments, taxa are written in italics and OTUs are listed below them in non-italics. OTUs
- 260 names are spelled as they appear in the dataset. Numbers next to each taxa and OTU are the counts for each
- 261 category for each instrument in the whole datasets. Non-zooplanktonic taxa and OTUs are highlighted in bold.

taxa/OTU	counts	taxa/OTU	count
Calocalanus tenuis	17	Actiniaria	13
Calycophorae	30	Aglaura	1113
Centropages hamatus	8	Atlanta	43
Chaetoceros sp.	9	Cavoliniidae	21
Diphyidae	144	Cephalopoda	3
Evadne	1889	Creseidae	7
Halosphaera	193	Cymbulia peroni	3
Hydrozoa	1674	Euchirella	239
Isias	51	Eumalacostraca	9815
Jaxea	2	Fritillariidae	3635
Podon	162	Harosa	374
Poecilostomatoida	1094	Liriope tetraphylla	121
Pyrosoma	1	Lubbockia	1
Rhizaria	13347	Monstrilloida	1
Sphaeronectidae	4	Nannosquillidae	2
Thalassionema	4	Nemertea	31
Thaliacea	7	Paguridae	4
Trichodesmium	265	Peltidiidae	133
Aggregata	253	Penaeoidea	7
chainlarge	114	Pleuromamma	695
Copepoda X	6727	Salpida	470
dead <harpacticoida< td=""><td>528</td><td>Solmundella bitentaculata</td><td>178</td></harpacticoida<>	528	Solmundella bitentaculata	178
egg 1 temp <engraulidae td="" temp<=""><td>65</td><td>actinula<hydrozoa< td=""><td>49</td></hydrozoa<></td></engraulidae>	65	actinula <hydrozoa< td=""><td>49</td></hydrozoa<>	49
egg 1 temp <sardina td="" temp<=""><td>100</td><td>badfocus<artefact< td=""><td>34507</td></artefact<></td></sardina>	100	badfocus <artefact< td=""><td>34507</td></artefact<>	34507
	3	badfocus <copepoda< td=""><td>11656</td></copepoda<>	11656
egg 2 3 temp <s ardina="" td="" temp<=""><td>49</td><td>ephyra<scyphozoa< td=""><td>64</td></scyphozoa<></td></s>	49	ephyra <scyphozoa< td=""><td>64</td></scyphozoa<>	64
egg 4 6 temp <engraulidae td="" temp<=""><td>6</td><td>larvae<crustacea< td=""><td>114</td></crustacea<></td></engraulidae>	6	larvae <crustacea< td=""><td>114</td></crustacea<>	114
egg 4 6 temp <s ardina="" td="" temp<=""><td>15</td><td>larvae<mysida< td=""><td>73</td></mysida<></td></s>	15	larvae <mysida< td=""><td>73</td></mysida<>	73
egg 7 8 temp <engraulidae td="" temp<=""><td>13</td><td>larvae<squillidae< td=""><td>4</td></squillidae<></td></engraulidae>	13	larvae <squillidae< td=""><td>4</td></squillidae<>	4
egg 9 11 temp <engraulidae td="" temp<=""><td></td><td>like<laomediidae< td=""><td>36</td></laomediidae<></td></engraulidae>		like <laomediidae< td=""><td>36</td></laomediidae<>	36
egg 9 11 temp <s ardina="" td="" temp<=""><td>26</td><td>part<annelida< td=""><td>121</td></annelida<></td></s>	26	part <annelida< td=""><td>121</td></annelida<>	121
egg unkn temp <s ardina="" td="" temp<=""><td>23</td><td>part<cnidaria< td=""><td>692</td></cnidaria<></td></s>	23	part <cnidaria< td=""><td>692</td></cnidaria<>	692
Enteropneusta <hemichordata< td=""><td>12</td><td>part<crustacea< td=""><td>7530</td></crustacea<></td></hemichordata<>	12	part <crustacea< td=""><td>7530</td></crustacea<>	7530
feces	227	part <thaliacea< td=""><td>44</td></thaliacea<>	44
fluffy <detritus< td=""><td>3589</td><td>pilidium<nemertea< td=""><td>12</td></nemertea<></td></detritus<>	3589	pilidium <nemertea< td=""><td>12</td></nemertea<>	12
gelatinous	348	phyllosoma	8
head <crustacea< td=""><td>9</td><td>pluteus<ophiuroidea< td=""><td>640</td></ophiuroidea<></td></crustacea<>	9	pluteus <ophiuroidea< td=""><td>640</td></ophiuroidea<>	640
arvae <ascidiacea< td=""><td>1</td><td>protozoea<mysida< td=""><td>229</td></mysida<></td></ascidiacea<>	1	protozoea <mysida< td=""><td>229</td></mysida<>	229
arvae <gastropoda< td=""><td>116</td><td>protozoea<penaeidae< td=""><td>28</td></penaeidae<></td></gastropoda<>	116	protozoea <penaeidae< td=""><td>28</td></penaeidae<>	28
light <detritus< td=""><td>38126</td><td>protozoea<sergestidae< td=""><td>12</td></sergestidae<></td></detritus<>	38126	protozoea <sergestidae< td=""><td>12</td></sergestidae<>	12
			251
0	857		
Rhizaria X Rhizosol enids	857 761	tail <chaetognatha trunk<appendicularia< td=""><td>1210</td></appendicularia<></chaetognatha 	1210





- 263 OTUs' names are mainly in the form of two words separated by a "<" character. Although we tried to name them
- as most explicitly as possible, a few potentially needed clarifications can be found in Table 3.
- 265 Table 3: Non-exhaustive list of prefixes, their types (morphological, developmental stage, taxonomical, non-living
- and imaging artefact), and content.

prefix	type	content of category
bract	morphological	single siphonophore bracts
eudoxie	morphological	single siphonophore Eudoxia zooids
gonophore	morphological	single siphonophore gonozooids
nectophore	morphological	single siphonophore swimming bells
trunk	morphological	single appendicularian trunks detached from their tails
tail	morphological	appendicularian's or chaethognath's tail shaped part of the body
head	morphological	individual organisms' heads detached from the body
part	morphological	unidentified body part
egg sac	morphological	detached copepod egg sacs
like	morphological	look alike, without absolute certainty
multiple	morphological	two or more objects touching each other in the same vignette
other	morphological	non-identified living object
actinula	developmental stage	undefined hydrozoa actinula larval stage
calyptopsis	developmental stage	Euphausiacea calyptopsis larval stage
egg	developmental stage	egg larval stage
ephira	developmental stage	ephira hydrozoa larval stage
larvae	developmental stage	undefined larval stage
nauplii	developmental stage	crustacean nauplius larval stage
pilidium	developmental stage	free-swimming larva of nemertean worm
protozoea	developmental stage	crustacean protozoea larval stage
pluteus	developmental stage	Echinodermata pluteus larval stage
zoea	developmental stage	crustacean zoea larval stage
egg 1 temp	developmental stage	clupeid fish embryo developemental stage 1*
egg 23 temp	developmental stage	clupeid fish embryo developemental stages 2 and 3 aggregated
egg 46 temp	developmental stage	clupeid fish embryo developemental stages 4 to 6 aggregated*
egg 78 temp	developmental stage	clupeid fish embryo developemental stages 7 and 8 aggregated
egg 911 temp	developmental stage	clupeid fish embryo developemental stages 9 to 11 aggregated*
egg unknown	developmental stage	clupeid fish unidentified embryo developemental stage*
Bivalvia	taxonomical	small bivalve larvae of unidenfified mollusca
dead	non_living	copepod's exuvia, carcass or part of dead body
fiber	non_living	fiber like detritus
fluffy	non_living	vey porous detritic particles
light	non_living	very transparent detritic particles
badfocus	imaging artefact	out-of-focus objects

267

268 * clupeids fish embryo developmental stages according to Ahlstrom (1943) and Moser & Ahlstrom (1985).







Figure 4: Gridded maps of total zooplankton Shannon index (calculated on spherical biovolumes) during the PELGAS cruise in the Bay of Biscay from 2004 to 2019. Shannon index exhibit a coastal to offshore gradient as well as a north-south gradient. Shannon index is larger at the coast and in the south, except in 2014 where it is smaller in the south, offshore.



274 3.2 Data and images

275 **3.2.1 Data**

276 The data is divided into two datasets available as tab separated files, one for each instrument. Within each 277 dataset the data is organized as a table containing text data as well as numerical data. Each dataset combines 278 together actual data and metadata at the individual object granularity. For each object, the user will be able to find 279 descriptors originating from the image processing (i.e. features), and sampling metadata (i.e. latitude and longitude of sampling station, date and time of sampling, sampling device, etc.) and sample processing metadata (i.e. 280 281 subsampling factor, seawater sampled volume, pixel size), in columns, and individual objects in lines. The columns 282 headers are defined in Tables A1 and A2 for ZooCAM and ZooScan datasets respectively. The following prefixes 283 enable the segregation of types of data and metadata: (i) "object_", which identifies variables assigned to each 284 object individually; (ii) "sample ", which identifies variables assigned to each sample; (iii) "acq ", which 285 identifies variables assigned to each data acquisition for the same sample (note here that this type of variable is 286 found only in the ZooScan dataset as ZooScan samples were splitted in two size fractions corresponding to two 287 acquisitions); (iv) "process ", which identifies variables describing key image processing features (i.e. pixel size). 288 Those prefixes originate from the use of the Ecotaxa web application to sort and identify the images (Picheral et al., 2017) that promote this specific formatting. The ZooCAM dataset is shaped as a 72 columns (variables) x 289 290 702,111 rows (individual imaged objects) matrix and the ZooScan dataset is shaped as a 71 columns (variables) x 291 1,153,507 rows (individual imaged objects) matrix.

- Among the 70+ variables it is worth noticing the following ones:
- (i) objid: it is a unique individual object numerical identifier that enables to link single data line to a
 corresponding single image in the image dataset;
 (ii) taxon: it is the taxonomic or OTU identification of the imaged objects written as they appear in the
- (ii) taxon: it is the taxonomic or OTU identification of the imaged objects written as they appear in the
 Tables 1 and 2;
- (iii) lineage: it is the full taxonomic lineage of the taxon. Lineage may be used to aggregate taxa at a higher
 taxonomic levels, respecting taxonomic lineages;

299 (iv) classif_id: it is a unique, numerical, taxon identifier;

300 (v) sample_sub_part / acq_sub_part: those are the subsampling ratios, for ZooCAM and ZooScan

301 respectively, needed to reconstruct the quantitative estimates of the samples' abundances;

- (vi) sample_fishingvolume / sample_tot_vol: those are the total seawater sampled volumes for ZooCAM
 and ZooScan respectively, needed to normalize the samples' concentrations by seawater volume.
- 304 One can therefore calculate quantitative abundances estimates for a taxon in a sample as follow:

305 ZooCAM:
$$Ab_{taxon} = \frac{n_{taxon} \times sample_sub_part}{sample_fishingvolume}$$
 (1)
306 ZooScan: $Ab_{taxon} = \frac{(n_{taxon_{acq1}} \times acq_sub_part_{acq1}) + (n_{taxon_{acq2}} \times acq_sub_part_{acq2})}{sample_tot_vol}$ (2)

307 Where Ab is the abundance in ind.m⁻³ and n is the number of individuals for "taxon".



308 3.2.2 Images

309	Two sets of individual images sorted into folders by categories (Tables 1 and 2) come along with each
310	dataset. For the ZooCAM only, the associated images from years 2016 and 2017 contain printed Region Of Interest
311	(ROI) bounding box limits and text at the bottom of each image, and non-homogenised background within and
312	around the ROI bounding box; images from year 2018 contain non-homogenised background within the ROI
313	bounding box only; images from 2019 have a completely homogeneous and thresholded background around the
314	object. The differences arose from successive ZooCAM software updates that do not modify the calculation of
315	object's features. The ZooScan images have all a completely homogeneous and thresholded background around
316	the object, no bounding box limits nor text printed in the images. All images for the two instruments datasets have
317	a 1 mm scale bar printed at the bottom left corner.

318 4 Data availability

The ZooScan dataset can be found as the *PELGAS Bay of Biscay ZooScan zooplankton Dataset (2004-2016)* in
the SEANOE dataportal following the link: <u>https://www.seanoe.org/data/00829/94052/</u> (Grandremy et al., 2023b).
The ZooCAM dataset can be found as the *PELGAS Bay of Biscay ZooCAM zooplankton Dataset (2016-2019)* in
the SEANOE dataportal <u>https://www.seanoe.org/data/00828/94040/</u> (Grandremy et al., 2023c).

- 323 Each dataset comes as a .zip archive that contains:
- One tab separated file containing all data and metadata associated to each imaged and identified object.
- One comma separated file containing the name, type, definition and unit of each field (column)
- One comma separated file containing the taxonomic list of the dataset, with counts and nature of the
 content of the category
- A directory "*individual_images*" containing images of each object, named according to the object id
 objid and sorted in subdirectories according to their taxonomic identification, across years and sampling
 stations.

331 5 Concluding remarks

332 Recent studies showed that the small pelagic fish (SPF) communities have suffered from a drastic 333 decrease of condition in the Mediterranean Sea and in the Bay of Biscay (Van Beveren et al., 2014; Doray et al., 334 2018b; Saraux et al., 2019) over the last 20 years. This loss of condition was especially expressed by the constant 335 decrease of SPF size- and weight-at-age (Doray et al., 2018b; Veron et al. 2020), and possibly explained by a 336 change in SPF trophic resource composition, size and quality (Brosset et al., 2016; Queiros et al., 2019; Menu et 337 al., 2023). Identifying and measuring zooplankton at appropriate temporal and spatial scales is not an easy task, 338 but can be addressed with imaging. These datasets were assembled as an effort to make possible the exploration 339 of the relationship between SPF observed dynamics in the Bay of Biscay and their main food resource's dynamics, 340 the metazoan zooplankton. This zooplankton imaging data series is a significant output of Nina Grandremy PhD 341 (2019-2023), that is currently being exploited (Grandremy et al., 2023a), and is intended to be continued and 342 updated on a yearly basis in the framework of the PELGAS program, to better understand the underlying processes 343 presiding to long-term SPF dynamics. Moreover, those two zooplankton datasets can be associated with the 344 PELGAS survey datasets previously published in 2018, also in the SEANOE dataportal, featuring hydrological, 345 primary producers, fish and megafauna data arranged as gridded data (Doray et al., 2018a). Together, all these





346 datasets allow to study simultaneously all the pelagic ecosystem compartments, with coherent spatial domain (the 347 Bay of Biscay continental shelf), resolution and time series. Nevertheless, a spatial gridding of the data is highly 348 recommended (as represented in the Fig. 2, 3 and 4), since the spatial coverage of the sampling protocols can vary 349 between years (Fig. 1), within and between each pelagic ecosystem compartment. A procedure for such batch data 350 spatial smoothing is presented e.g. in Petitgas et al. (2009, 2014). See also Doray et al. (2018b) and Grandremy et 351 al. (2023a) for application examples. As several descriptors of the spring zooplankton community (abundances, 352 sizes, biovolumes, biomass) can be derived from this 16 years long spatially resolved time series at several 353 taxonomic levels, these datasets are intended to be used in various ecological studies including the zooplankton 354 compartment, especially modelling studies, where zooplankton is usually underrepresented (Mitra, 2010; Mitra et 355 al., 2014). Finally, these datasets can also be used for machine learning applied to plankton studies serving, for 356 example, as consequent learning sets.

357 Disclaimer

Data are published without any warranty, express or implied. The user assumes all risk arising from his/her use of data. Data are intended to be research-quality, but it is possible that the data themselves contain errors. It is the sole responsibility of the user to assess if the data are appropriate for his/her use, and to interpret the data accordingly. Authors welcome users to ask questions and report problems.

362 Authors' contributions

363 GN scanned and validated most of the ZooScan dataset, assembled the datasets, and led the drafting. BP collected 364 and managed the samples since 2004, and participated in the manual validation of identifications. DE scanned a 365 substantial fraction of the ZooScan samples and participated in the initial sorting of vignettes. DMM participated 366 in the collection of samples, and was involved in the ZooCAM development. DM was chief scientist on the 367 PELGAS surveys and participated in the drafting. DC supervised GN work and participated in the drafting. FB developed, improved and maintained the ZooCAM software. JL curated a substantial fraction of the ZooScan 368 369 dataset manual validation of identifications. HM participated in the collection of samples, lead the DEFIPEL 370 project, and participated in the drafting. LMS participated in the collection of samples, and managed the ZooCAM. 371 NA curated a substantial fraction of the ZooScan and ZooCAM dataset manual validation of identifications. PP 372 supervised GN work and participated in the drafting. PPh participated in the collection of samples and participated 373 in the drafting. RJ supervised the development and improvement of the ZooCAM. TM developed and improved 374 the ZooCAM, and participated in the collection of samples. RJB supervised GN work, participated in the collection 375 of samples, curated a substantial fraction of the ZooCAM dataset manual validation of identifications, and lead 376 the drafting.

377 Competing interests

378 The authors declare that they have no conflict of interest.

379 Acknowledgements

The authors acknowledge receiving funding from the 'France Filière Pêche' DEFIPEL project. NG acknowledges the funding of her PhD by Region Pays de la Loire, FR and Ifremer. The authors wish to thank Jean-Yves Coail, Gérard Guyader and Patrick Berriet (Ifremer – REM-RDT-SIIM) for their contribution to the hardware assembly of the ZooCAM. The authors acknowledge the work of Elio Raphalen for scanning year 2005 samples. The authors





- thank the EMBRC platform PIQs for image analysis. This work was supported by EMBRC-France, whose French state funds are managed by the ANR within the Investments of the Future program under reference ANR-10-INBS-02. Finally, the authors wish also to thank the many other students, technicians and scientists who participated in the sampling and samples imaging on board, and the successive crews of the R/V *Thalassa* involved
- in the PELGAS surveys from 2004 to 2019.
- 389

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536 Appendix A

537 Table A1: ZooCAM dataset columns header – definition of data and metadata fields.

column name	definition
object id	name of object and associated image
objid	unique ecotaxa internal object identifier
object_lat	latitude of samp ling
object_lon	longitude of sampling
object_dat e	date of sampling
object_time	time of sampling
object_depth_min	minimum sampling depth
object_depth_max	maximum sampling depth taxonomic name
object_taxon object lineage	full taxonomic lineage corresponding to the taxon
classif id	unique ecotaxa internal taxon identifier
object area	object's surface
object area exc	object surface excluding white pixels
object_%area	proportion of the image corresponding to the object
object_area_based_diameter	object's Area Based Diameter: 2 * (object_area/pi)^(1/2)
object_meangreyimage	mean image grey level
object_meangreyobjet	mean object grey level
object_modegreyobjet	modal object grey level
object_sigmagrey	object grey level standard deviation
object_mingrey object_maxgrey	minimum object grey level maximum object grey level
object_maxgrey object_sumgrey	maximum object grey level object grey level integrated density: object mean*object area
object_sungrey object_breadth	breadth of the object along the best fitting ellipsoid minor axis
object length	breadth of the object along the best fitting ellipsoid majorr axis
object elongation	elongation index: object length/object breadth
object_perim	object's perimeter
object_minferetdiam	minimum object's feret diameter
object_maxferetdiam	maximum object's feret diameter
object_meanferetdiam	average object's feret diameter
object_feretelongation	elongation index: object_maxferetdiam/object_minferetdiam
object_compactness	Isoperimetric quotient: the ratio of the object's area to the area of a circle having the same perimeter
object_intercept0	number of times that a transition from background to foreground occurs a the angle 0° for the entire object
object_intercept45 object_intercept90	the number of times that a transition from background to foreground occurs a the angle 45° for the entire object the number of times that a transition from background to foreground occurs a the angle 90° for the entire object
object_intercept135	the number of times that a transition from background to foreground occurs a the angle 135° for the entire object
object convexhullarea	area of the convex hull of the object
object_conv exhullfillratio	ratio object_area/convexhullarea
object_convexperimeter	perimeter of the convex hull of the object
object_n_number_of_runs	number of horizontal strings of consecutive foreground pixels in the object
object_n_chained_pixels	number of chained pixels in the object
object_n_convex_hull_points	number of summits of the object's convex hull polygon
object_n_number_of_holes	number of holes (as closed white pixel area) in the object
object_transparence object_roughness	ratio object_sumgrey/obejct_area measure of small scale variations of amplitude in the object's grey levels
object_rectangularity	ratio of the object's area over its best bounding rectangle's area
object_skewness	skewness of the object's grey level distribution
object kurtosis	kurtosis of the object's grey level distribution
object fractal box	fractal dimension of the object's perimeter
object_hist25	grey level value at quantile 0.25 of the object's grey levels normalized cumulative histogram
object_hist50	grey level value at quantile 0.5 of the object's grey levels normalized cumulative histogram
object_hist75	grey level value at quantils 0.75 of the object's grey levels normalized cumulative histogram
object_valhist25	sum of grey levels at quantile 0.25 of the object's grey levels normalized cumulative histogram
object_valhist50	sum of grey levels at quantile 0.5 of the object's grey levels normalized cumulative histogram
object_valhist75	sum of grey levels at quantile 0.75 of the object's grey levels normalized cumulative histogram number of objects after thresholding at the object_valhist25 grey level
object_nobj25 object_nobj50	number of objects after thresholding at the object_values 25 grey level
object_nobj55	number of objects after thresholding at the object_values of grey level
object_symetrich	index of horizontal symmetry
object_symetriev	index of vertical symmetry
object_thick_r	maximum object's thickness/mean object's thickness
object_cdist	distance between the mass and the grey level object's centroids
object_bord	tag for object touching the frame edge
sample_id	name of the sample from where the object originates
sample_ship	name of the ship used to collect the samples
sample_campaign	name of the cruise where samples were collected
sample_station sample_depth	name of the station where samples were collected bottom depth at station
sample_depth sample_device	net used to collect the sample
sample_device sample_fishingvolume	seawater volume sampled
sample_nsningvolume sample_sub_part	subsampling elevation factor
process_id	name of software/software version used to analysed digitized sample images
process_resolution_camera_micron_	





539 T	Table A2: ZooScan	dataset columns	header –	definition o	f data and	metadata fields
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column name	definition
object_id	name of object and associated image
objid	unique ecotaxa internal object identifier
object_lat object_lon	latitude of sampling longitude of sampling
object_ion object_date	date of sampling
object time	time of sampling
object depth min	minimum sampling depth
object depth max	maximum sampling depth
object_deptii_max	taxonomic name
object lineage	full taxonomic lineage corresponding to the taxon
classif id	unique ecotaxa internal taxon identifier
object area	object's surface
object mean	mean object grey level
object_incur	object grey level standard deviation
object mode	modal object grey level
object min	minimum object grey level
object max	maximum object grey level
object perim.	object's perimeter
object major	lenght of major axis of best fitting elipse
object minor	lenght of minor axis of best fitting elipse
object_circ.	circularity: 4*pi(object_area/object_perim.^2)
object_feret	maximum feret diameter
object_intden	object grey level integrated density: /object_mean*/object_area
object_median	median object grey level
object_sk.ew	skewness of the object's grey level distribution
object_kurt	kurtosis of the object's grey level distribution
object_%area	proportion of the image corresponding to the object
object_area_exc	object surface excluding white pixels
object_fractal	fractal dimension of the object's perimeter
object_skelarea	surface of the one-pixel wide skeleton of the object
object_slop e	slope of the cumulated histogram of the object grey levels
object_histcum1	the number of times that a transition from background to foreground occurs at the angle0°
object_histcum2	grey level at quantiles 0.5 of the histogram of the object grey levels
object_histcum3	grey level at quantiles 0.75 of the histogram of the object grey levels
object_nb1	number of objects after thresholding at the object_histcum1 grey level
object_nb2	number of objects after thresholding at the object_histcum2 grey level
object_symetrieh	index of horizontal symmetry
object_symetriev	index of vertical symmetry
object_symetriehc	index of horizontal symmetry after thresholding at the object_histcum1 grey level
object_symetrievc	index of vertical symmetry after thresholding at the object_histcum1 grey level
object_convperim	perimeter of the convex hull of the object
object_convarea	area of the convex hull of the object
object_fcons	object's contrast
object_thickr	maximum object's thickness/mean object's thickness object's Equivalent Spherical Diameter: 2 * (object area/pi)^(1/2)
object_esd	
object_elongation	elongation index: major/minor range of grevs: max-min
object_range object_meanpos	range of greys: max-mm relative position of the mean grey: (max-mean)/range
object_meanpos object_centroids	distance between the mass and the grey level object's centroids
object_centroids	coefficient of variation of greys: 100*(stddev/mean)
object_sr	index of variation of greys: 100*(stddev/mean)
object_perimareaexc	index of variation of greys. Foo (stude nange)
object_feretareaexc	another elongation index : object feret/object area exc
object_perimferet	index of the relative complexity of the perimeter: object_perim/object_feret
object_perimmajor	index of the relative complexity of the perimeter: object_perim/object_major
object_perminujor	circularity of object excluding white pixels: 4*pi(object area exc/object perim.^2)
object_cdex.c	distance between the mass and the grey level object's centroids calculated with object area exc
sample id	name of the sample from the object originate
sample ship	name of the ship used to collect the samples
sample_program	name of the cruise where samples were collected
sample_stationid	name of the station where samples were collected
sample_bottomdepth	bottom depth at station
sample_net_type	net used to collect the sample
sample_tot_vol	seawater volume sampled
sample_comment	comments associated with sampling/sample treatment
process_id	name of software/software version used to analysed digitized sample images
process_particle_pixel_size_mm	pixel size
	name of subsample if any
acq_id	
acq_min_mesh	minimum sieve size of subsample
	minimum sieve size of subsample maximum sieve size of subsample subsampling elevation factor