Quantitative imaging datasets of surface micro to mesoplankton communities and microplastic across the Pacific and North Atlantic Ocean from the Tara Pacific Expedition

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18 Abstract. This paper presents the quantitative imaging datasets collected during the Tara Pacific Expedition 19 (2016-2018) on the schooner Tara. The datasets cover a wide range of plankton sizes, from micro-phytoplankton 20 $> 20 \,\mu\text{m}$ to meso-zooplankton of a few cm, as well as non-living particles such as plastic and detrital particles. It 21 consists of surface samples collected across the North Atlantic and the North and South Pacific Ocean from open 22 ocean stations (a total of 357 samples) and from stations located in coastal waters, lagoons or reefs of 32 Pacific 23 islands (a total of 228 samples). As this expedition involved long distances and long sailing times, we designed 24 two sampling systems to collect plankton while sailing at speeds up to 9 knots. To sample microplankton, surface 25 water was pumped onboard using a customised pumping system and filtered through a 20 µm mesh size plankton 26 net (here after Deck-Net (DN). A High Speed Net (HSN; 330 µm mesh size) was developed to sample the 27 mesoplankton. In addition, a Manta net (330 µm) was also used when possible, to collect mesoplankton and 28 plastics simultaneously. We could not deploy these nets in reef and lagoon stations of islands. Instead, two Bongo 29 nets (20 µm) attached to an underwater scooter were used to sample microplankton. In addition to describing and 30 presenting the datasets, the complementary aim of this paper is to investigate and quantify the potential sampling 31 biases associated with these two high speed sampling systems and the different net types, in order to improve 32 further ecological interpretations. Regarding the imaging techniques, microplankton (20-200 µm) from the DN 33 and Bongo nets was imaged directly on-board Tara using the FlowCam (Fluid imaging, Inc.) while the 34 mesoplankton (> $200 \mu m$) from the HSN and Manta nets was analyzed in the laboratory with the ZooScan system, 35 back on land. Organisms and other particles were taxonomically and morphologically classified using the web 36 application EcoTaxa automatic sorting tools, followed by taxonomic expert validation or correction. For micro-37 plankton smaller than 45 µm, a subsample of 30% of the annotations was 100% visually validated by experts. 38 More than 300 different taxonomic and morphological groups were identified. The datasets include the metadata 39 with the raw data from which morphological traits such as size (ESD) and biovolume have been calculated for 40 each particle, as well as a number of quantitative descriptors of the surface plankton communities. These include 41 abundance, biovolumes, Shannon diversity index and normalised biovolume size spectra, allowing the study of 42 their structures (e.g. taxonomic, functional, size structure, trophic structure, etc.) according to a wide range of

43 environmental parameters at the basin scale.

44 **1. Introduction**

46 Zooplankton serve as an important conduit for the transfer of energy from primary producers to higher trophic 47 levels (Ikeda, 1985). In this key position in the food webs, they also play an important ecological and 48 biogeochemical role (Turner, 2015; Steinberg and Landry, 2017), with associated ecosystem services. In particular, they are essential to Pacific fisheries management, as they influence fish productivity and ecosystem 49 50 dynamics (Balachandran and Peter, 1987; Chuanbo Guo et al., 2019; Hays, 2005). The datasets we present here, 51 cover a wide diversity of surface plankton, ranging from 20 µm to few cm, at the scale of the Pacific Ocean. The 52 vastness and unique characteristics of the Pacific Ocean make it a particularly interesting study area. From 53 nutrient-rich upwelling or islands zones to oligotrophic gyres, the diverse oceanic processes of the Pacific Ocean 54 present a wide range of environmental conditions that significantly influence plankton communities, making it a 55 key region for plankton research (Chavez et al., 2011; Longhurst, 2007). However, sampling efforts of 56 zooplankton in the Pacific Ocean largely focused on the temperate North Pacific, eastern and western boundary 57 currents in the North Pacific, leaving vast areas under-sampled (Drago et al., 2022). This gap is particularly evident 58 in the NOAA zooplankton dataset (https://www.st.nmfs.noaa.gov/copepod/atlas), where the under-sampling is 59 particularly true for the central subtropical and tropical Pacific where fisheries are important resources for the 60 thousands of pacific islands. We present a map (Fig. 1) overlaying updated zooplankton databases with samples 61 from the Tara Pacific expedition, illustrating how these new data address sampling gaps. Global mapping of 62 zooplankton in the Pacific is hindered by the highly expansive operational ship time face to this vast ocean. The 63 use of high-speed sampling, such as the Continuous Plankton Recorder (CPR, by Hardy in 1926), the LHPR 64 (Longhurst et al., 1966), the Gulf III OCEAN Sampler (Gehringer, 1958), the Gulf V plankton sampler (Sameoto 65 et al., 2000), as well as newer low-tech designs (CSN in Von Ammon et al., 2020; Coryphaena in Mériguet et al., 66 2022), including the one employed in our datasets, provides valuable opportunities to expand sampling coverage 67 and frequency and thus address this undersampling. In the hope of increasing similar cruising speed zooplankton 68 sampling efforts, we discuss the benefits, challenges and limitations of this high-speed sampling approach based 69 on the lessons learned from obtaining these datasets.

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73 distribution of zooplankton COPEPOD Figure 1. Spatial observations from the database 74 (https://www.st.nmfs.noaa.gov/copepod/; all groups) is represented by blue points. Plankton imaging data (> 20 µm) 75 from the Tara Pacific expedition are shown in grey.

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The aim of this paper is therefore to present and discuss this open-access quantitative plankton imaging datasets sampled during the Tara Pacific Expedition (2016-2018), conducted in the Pacific Ocean. In general, the effects of different environmental forcings on plankton are often focusing on one size range of plankton, or on a particular taxonomic or functional type to the exclusion of others. It is often difficult to reconcile different methods of 81 analysis (taxonomic, biogeochemical, genomic) to provide a coherent view of the plankton as a whole. In this 82 respect, quantitative imaging is complementary to other methods to study plankton community composition (e.g. 83 HPLC, flow cytometry, genomics) because it simultaneously provides quantitative measures of abundance, 84 morphology and biovolume (as a proxy for biomass) for different taxonomic groups of plankton organisms 85 (Lombard et al., 2019). The datasets represent a diversity of surface plankton analysed with the use of two 86 quantitative imaging instruments: 1. the FlowCam (Sieracki et al., 1998), which images microplankton from 20 87 to 200 µm, and 2. the ZooScan (Gorsky et al., 2010), which images meso-zooplankton (>200 µm). The dataset 88 also includes the plastics imaged by the ZooScan. Overall, it encompass a total of 2 356 231 images, including 89 both surface micro and mesoplankton, as well as non-living particles such as plastics, making a significant 90 contribution to improving the availability of plankton data.

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92 These datasets are of great value because of the relative rarity of sampling surface planktonic communities at the 93 oceanic scale. Potential limitations of the data presented here are discussed below. To ensure adequate spatial 94 coverage while considering navigation constraints, we designed two new sampling systems to collect surface 95 micro- and mesoplankton while sailing at a maximum speed of 9 knots. The 'Dolphin' sampler was designed to 96 pump seawater into a 20 µm net on board, the Deck Net (DN), while the 'High Speed Net' (HSN) was designed 97 and towed to collect surface plankton larger than 300 µm in size (see Gorsky et al., 2019 for details). In addition 98 to these high-speed sampling devices, but with less extensive spatio-temporal coverage, a Manta net (330 µm) 99 was also used whenever cruising speed made it possible (*i.e.* < 4 knots), to collect surface mesoplankton and 100 plastics. Two Bongo nets ($20 \,\mu$ m), towed by an underwater scooter, were also used by scuba divers around islands, 101 reefs, and lagoons. Thus, a complementary objective of this paper is study and quantify the potential sampling 102 biases of the different methods used during this expedition, in order to maximize the quality of the data offered to 103 the scientific community and promote similar high speed zooplankton sampling efforts which strongly enhance 104 the spatial coverage of samples. Another characteristic of these datasets is the daytime sampling of surface (0-1 105 meter) plankton communities. This offers the possibility of geographic intercomparisons and interdisciplinary 106 studies related to the ocean's surface layer, enabling direct comparisons with other surface measurements, such as 107 satellite and atmospheric data. However, this raises questions about the quantitative nature of the sampling itself, 108 particularly regarding the representativeness of the datasets. While these datasets provide quantitative accuracy 109 by offering all the necessary information to consistently calculate estimates of the sample contents, we must warn 110 that the data may not fully be 'quantitatively representative' of the broader ecosystem. Although the sampling 111 objective is the surface layer, daytime sampling alone cannot document the nocturnal intrusion of migrating 112 zooplankton and micronekton to the surface. It is worth mentioning that night sampling was also operated on 113 zooplankton alone (see Fig 10 in Gorsky et al 2021) but therefore does not reconcile in space and time with day 114 sampling and was therefore not analyzed in priority.

115 **2. Methods**

116 2.1 Sampling

117 We present a collection of FlowCam and ZooScan images acquired during the Tara Pacific expedition (2016-118 2018; Gorsky et al. 2019, Lombard et al. 2023). All samples and protocol names in this article follow Lombard et 119 al. (2023) in order to help the user match the samples and associated data presented here with other samples from 120 the expedition. Sampling was carried out generally at the daily frequency, every ~150-200 nautical miles, during 121 daytime, resulting in a total of 249 sampling events labelled [oa001] to [oa249] (Fig. 2). The first 28 sampling 122 events occurred during the trans-Atlantic crossing as the ship sailed from France to the Pacific. At the end of the 123 expedition, the schooner Tara acquired quantitative imaging samples at stations [oa232] to [oa249] across the 124 North Atlantic. Data are published on the SEANOE platform to allow for future updates and completion of 125 datasets. The plankton sampling covers a large latitudinal range (temperate, subtropical, and tropical) as well as a 126 diversity of environments associated with different oceanic regimes (equatorial upwelling, coastal upwelling, 127 eastern boundary current, subtropical gyres, and other provinces). We collected over 357 samples in the open 128 ocean and 228 samples close to the reef or in the lagoon. A selection of 32 coral reef islands systems (labelled

- [i01] to [i32]) in the tropical and subtropical Pacific Ocean were targeted for coral reef holobiont studies (Planes
- et al., 2019), including surface plankton sampling analysed by quantitative imaging. A summary of geological,
- 131 topological and human population characteristics of the different islands targeted (name, size, elevation, human
- 132 population, etc.) can be found in Lombard et al. (2023). Any sampling event that was conducted within the
- 133 Exclusive Economic Zone (EEZ) of an island (defined as the area that stretches 200 nautical miles or 370 km out
- of the coastline of an island in question) was considered as an island station and annotated with the island label
- 135 [i##_oa###]. All other sampling events were considered open ocean stations (high seas, 132 open ocean stations)
 - and were annotated [i00_oa###].



138 Figure 2. Tara Pacific expedition (2016–2018) sampling map for the 4 different datasets. Continuous sampling: (a) DN

139 (Deck-Net) – FlowCam (b) HSN (High-Speed-Net) – ZooScan. More discrete sampling, focus around islands: (c) Bongo

Net - FlowCam and (d) Manta - ZooScan (plankton and plastic samples). Island stations, station within 200 nautical
 miles of an island, are represented inside a yellow circle. The 'not yet analysed' stations in the figure legend mean that

- 142 the samples have not yet been scanned for the ZooScan dataset and have not been taxonomically validated for the
- 143 FlowCam dataset.





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146 Figure 3. Schematic overview of the sampling events and protocols used during the Tara Pacific expedition for 147 quantitative imaging. The top left panel corresponds to the sampling events with the deployed plankton nets: (1a) the 148 330 um High Speed Net (HSN) and the 333 um Manta net. (1b) the 20 um Bongo nets attached to the underwater 149 scooter and (1c) the 20 µm Deck Net (DN) on the deck of the Tara. Samples from DN (2c) and Bongo (2b) were imaged 150 live with the FlowCam (20-200 µm) and samples from HSN and Manta (2a) were imaged with the ZooScan (> 300 µm). 151 For the ZooScan analysis, samples were fixed using formaldehyde and stored on board and analysed on the Imaging 152 Quantitative Platform (PIQv) in the laboratory in Villefranche-sur-Mer, the protocols in this platform are detailed in 153 the section: "On the PIQv lab" (3a). Somes drawings were taken from Lombard et al. 2023 modified (credit N. Le 154 Bescot).

155 2.1.1 Deck-Net sampling

156 Surface water samples were collected using a custom-built water pumping system named "Dolphin". It consists 157 of a stainless-steel pyramidal frame with a front aperture of 0.04 m wide and 0.40 m high, deployed from the starboard side of the ship (see pictures in Gorsky et al., 2019). The Dolphin was used underway while sailing and 158 was connected to a peristaltic pump (max flow rate = $3 \text{ m}^3 \text{ h}^{-1}$) mounted on the deck of the schooner Tara. The 159 system was equipped with a flowmeter to record flow rates. The pumped water was filtered through a 20 µm net 160 161 (Deck-Net) that was mounted on the wall of the wet lab (Fig. 3; 1c and pictures in Gorsky et al., 2019). Before 162 entering the Deck-Net, the pumped water passes through a 2000 µm mesh filter. Deck-Net pumping lasted 1 to 2 hours, depending on plankton concentration. Samples were divided into subsamples, which included one 163 164 subsample for quantitative micro-plankton imaging analysis on live samples (LIVE20; Fig. 3; 2c) and the 165 remaining for specific protocols detailed in Lombard et al. (2023). Further information on the Dolphin system, the Deck-Net, and various protocols based on this sampling can be found in Gorsky et al. (2019) and Lombard et 166 167 al. (2023).

168 2.1.2 Bongo nets sampling

- 169 Plankton larger than 20 μ m were sampled at ~2 m below the sea surface using two small diameter Bongo plankton
- 170 nets with 20 μ m mesh size and an opening area of 0.071 m². These nets were towed by divers using underwater
- scooters (Fig. 3; 1b) and towed for about 15 min at maximum speed $(0.69 \pm 0.04 \text{ m s}^{-1})$. Each net was equipped
- 172 with a flowmeter rated to provide accurate measurements at speeds above 0.3 m.s^{-1} , but, the relatively low
- 173 maximum speed of the underwater scooter was insufficient to allow seawater to flow through the 20 µm mesh fast 174 enough to trigger the rotation of the flowmeter. Therefore, volume was estimated from the tow speed and tow
- 175 duration using the following Eq. (1):
- 176 Bongo volume = $0.071 \times \text{tow speed} \times \text{tow duration}$ (1)

177 2.1.3 HSN and Manta nets sampling

178 Simultaneously with the deployment of the Dolphin to collect microplankton, the High Speed Net (HSN) was 179 towed to sample the mesoplankton. The HSN was equipped with a 330 µm mesh and designed to be deployed 180 while sailing up to 9 knots (average speed deployment: 6.7 knots). The HSN features the same mouth opening as 181 the Dolphin system, consisting of a stainless-steel pyramidal frame with a front aperture measuring 0.40 x 0.04 m 182 (see zoom on the HSN mouth system on Fig. 3). The base opening of this pyramidal structure measures 0.34 x 183 0.34 m. This net was deployed from the starboard side and towed at a distance of 50–60 m behind the ship (to 184 avoid it being in the wake of the ship), for a period of 60–90 min (depending on plankton density). In addition to 185 the HSN, Manta net was also deployed in some locations (Fig. 2). The Manta net have rectangular frame of 186 0.16×0.60 m mouth opening with a 4 m long net with 333 µm mesh size, and was used at a maximum speed of 3 187 knots, for an average of 30-40 minutes.

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189 Flowmeters were mounted at half of the opening height above the bottom of the opening on both HSN and Manta 190 nets to ensure it was well submerged during deployment while measuring the filtered volume. Theoretical volumes 191 were calculated taking into account a 3/4 mouth opening of the HSN and Manta nets, 0.3×0.04 and 0.6×0.12 192 m, respectively (see Eq. (3), (4) and (5)). As these nets are surface nets, the water collected actually passed through 193 \sim 3/4 of the opening height (see photos of deployments in Gorsky et al., 2019). To calculate volumes from the flowmeter for the HSN, we considered an opening of 0.34×0.34 m, corresponding to the dimensions of the 194 195 pyramid base opening where the flowmeter was positioned inside the HSN (Eq. (2)). We compared the volume 196 estimated from the flowmeter readings with theoretical estimation using the towing distances. We computed the 197 towing distances using the minute binned latitude and longitude recorded with the Tara's GPS along each 198 deployment. We calculated the distance between the start-end latitude and start-end longitude for each minute, to 199 calculate the distance per minute covered by the boat. We then summed these 'per-minute' distances over the 200 duration of the deployment to obtain a calculated distance that is as close as possible to the true towing distance 201 and accounts for potential modification of the boat's heading during deployments. The equations for calculating 202 the filtered volumes are therefore as follows. The 0.3 factor in the flowmeter volume equation corresponds to the 203 impeller pitch, as recommended by Hydrobios, to convert the number of revolutions into towing distance.

- HSN flowmeter volume = flowmeter end flowmeter start \times 0.3 \times (HSN mouth opening area) (2)
- HSN theoretical volume = tow distance \times (HSN mouth opening area)
- 206 Manta flowmeter volume = flowmeter end flowmeter start \times 0.3 \times (Manta mouth opening area) 207 (4)
- 208 Manta theoretical volume = tow distance \times (Manta mouth opening area) (5)
- 209 Simplified Metadata in csv provides both flowmeters and theoretical volumes for HSN and Manta net, enabling
- 210 the user to select the filtered volume for the calculation of quantitative descriptors. A discussion of the biases
- 211 associated with each estimate is given in section 3.2. The filtered volumes uploaded as metadata in EcoTaxa
- 212 (EcoTaxa export table in tsv, see part 2.5) and used to compute quantitative descriptors (see part 2.5) are the
- theoretical volumes calculated from the distance (see the results of technical validation part 3.2.1).

(3)

- 214
- 215 Once recovered, samples collected both by the HSN net and the Manta net followed the same procedure (Fig. 3;
- 216 2a). The sample was divided into two 1 L fractions (details in Gorsky et al., 2019). One fraction was concentrated
- on a 200 µm sieve and resuspended in a 250 mL double-sealed bottle using filtered seawater saturated with sodium
- tetraborate decahydrate (borax), fixed with 30 mL of 37% formalin solution and stored at room temperature for
- taxonomic and morphological analysis by imaging methods in the laboratory (samples named [F300]). The other
- 220 fraction was used for omic analysis.

221 **2.2 Acquisition and treatment of plankton imaging data**

Sample labels were annotated by different users at different times during the expedition and are therefore not homogeneous. In order to avoid confusion or misunderstanding of the labelling of the samples, an additional column has been created in the csv Simplified Metadata (column "Homogeneous sample names") with homogeneous names for all datasets.

226 2.2.1 FlowCam analysis

Samples from the Deck-Net (250 mL) and Bongo net (50mL) were imaged live directly on board using a FlowCam Benchtop B2 series (Fluid Imaging Technologies; Sieracki et al., 1998) equipped with a ×4 objective and a 300 µm deep glass flow cell to examine the micro-plankton samples (size range 20-200 µm: Fig. 3; 2c). Each sample was first passed through a 200 µm sieve to remove large objects that could clog the FlowCam imaging cell. Samples were then diluted or concentrated to achieve optimum object flow. The auto-image mode was used to

image the particles in the focal plane at a constant flow rate.

233 2.2.2 ZooScan analysis

The ZooScan imaging instrument (Gorsky et al. 2010) was used to study the mesoplankton. Samples collected from the HSN and Manta nets ([F300]) were imaged at the Quantitative Imaging Platform (PIQv) of the Institut

de la Mer de Villefranche (Fig. 3; 3a). In addition, preserved zooplankton samples are stored in the Collection

Center for Plankton of Villefranche (CCPv). The formaldehyde solution was replaced by filtered seawater duringthe analysis.

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240 Plankton samples analysis from HSN and Manta nets on the ZooScan

242 Before scanning on the ZooScan, plankton samples were divided using a Motoda splitter (Motoda, 1959) to obtain 243 a concentration of approximately between 1000 and 2500 objects per subsample and scanned with the ZooScan. 244 This sampling strategy correctly accounted for the many small organisms as well as the large ones that might be 245 under-sampled when subsampling with the Motoda box. This limit ([1000- 2500] objects) was defined by the 246 PIQv platform to avoid the overlap of planktonic organisms, while retaining enough organisms to give a reliable 247 quantitative measurement of the sample. After each scan, a quality control was systematically carried out 248 concerning i) the quality of the scanned image and ii) the number of objects imaged, to ensure that that the number 249 of objects is within the limits given above. The quality control tool for imaging data is accessible on the PIOv 250 website: https://sites.google.com/view/piqv/. After treatment in the ZooScan, all samples were re-concentrated on 251 a 200 µm sieve and resuspended in a 250 mL double-sealed bottle using filtered seawater saturated with borax, 252 fixed with 30 mL of 37% formalin solution and returned to the CCPv.

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The borax (sodium tetraborate decahydrate) used as a buffer may form crystals grains, forming white crystals. If the borax solution was not filtered sufficiently, crystals would end up in the plankton samples, be digitised and counted as objects. Thus, if Borax was not filtered sufficiently, white crystals may represent a large proportion of the objects within the 1000-2500 limit and thus bias the quantitative measurement of the plankton. We identified 24 samples containing borax crystals during the analysis. Therefore, prior to scanning, these samples were

thoroughly rinsed with filtered seawater through a 300 μ m mesh sieve to remove a maximum of borax crystals

from the sample. A 200 μm mesh sieve was placed below the 300 μm sieve in order to conserve the initial sample
 in the collection (CCPv). Analysis on the ZooScan was performed from the 300 μm sieve.

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263 Plastic sampling from Manta net

265 Samples from the Manta nets were gently transferred to a Petri dish. Plastic-like particles were manually separated 266 from other components such as wood, zooplankton, and organic tissues (Fig. 3; 3a). Entangled pieces of plastic were picked up manually from zooplankton and aggregated under a stereoscopic dissecting microscope, using 267 268 forceps. The visual criteria used to classify a microfiber as synthetic were the absence of cellular structures and 269 scales on the surface, a curved shape with a uniform surface, a uniform thickness along the entire length of the 270 filament, spots, and strong strands (Barrows et al., 2018; Hidalgo-Ruz et al., 2018). Each sample was examined 271 twice to ensure the detection of most of the plastic particles. Isolated plastic particles were then imaged with 272 Zooscan. To minimise the plastic contamination of the samples, a quality control approach was undertaken 273 following the protocol described by Pedrotti et al. (2022).

274 2.3 Images processing

275 For FlowCam and ZooScan, the full methodology used can be found in their respective manuals (https://sites.google.com/view/piqv/piqvmanuals/instruments-manuals; for the ZooScan the protocol is also 276 277 available on zenodo by Jalabert, 2022). Images generated by FlowCam and ZooScan were processed using the 278 ZooProcess software in ImageJ (Gorsky et al. 2010) which extracts segmented objects as vignettes. During this 279 process, each vignette was associated with a set of 46 morphometric measurements for object characterization, 280 including grey levels, fractal dimension, shape and size, which were imported into the EcoTaxa web application 281 (Picheral et al. 2017) for taxonomic classification. For ZooScan, the ZooProcess software includes a tool that 282 enables the digital separation of potentially touching or overlapping objects in the original image. If two objects 283 (possibly two plankton organisms) are touching, they will be considered as a single vignette and assigned a single 284 label, which could therefore biais estimates of abundance and size, as described in Vandromme et al. (2012). 285 Objects that were still touching after the application of the ZooProcess automatic tool were identified and 286 separated using the ZooProcess manual separation tool to improve the quality of the subsequent taxonomic 287 annotation, counts and size structure analysis of the zooplankton. For each ZooScan dataset, this quality control 288 step was systematically performed during taxonomic annotation.

289 2.4 Taxonomic identification

290 Using image recognition algorithms on EcoTaxa, predicted taxonomic categories were validated or corrected by 291 trained taxonomists. For the majority, the taxonomic classification effort was possible up to the genus and only in 292 rare cases up to the species. A number of organisms could not be reliably taxonomically identified due to a lack 293 of identification criteria and were therefore grouped into temporary categories (t00x) following similar 294 morphological criteria. Nine different trained taxonomists from the PIQv platform annotated FlowCam and 295 ZooScan vignettes on these datasets. Annotations of FlowCam and ZooScan vignettes from the different nets were 296 also done by different taxonomists but the list and the global criteria to identify a group were common. To reduce 297 operator bias between taxonomists and to ensure taxonomic consistency, a final stage of homogenisation was 298 carried out by two taxonomists after all vignettes had been validated. At the time of publication of these datasets, 299 copepod genera had not been homogenised for ZooScan, but homogenisation will be pursued in the future and the 300 published SEANOE dataset will be updated accordingly. Overall, these datasets are published on the SEANOE 301 flexible platform that allows updates and corrections, so that taxonomic annotations can be improved over time. 302 All vignettes with taxonomic annotations are visible on the open access project in EcoTaxa (section 4).

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304 2.5 Case of FlowCam taxonomic identification for objects smaller than 45 μm

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The Tara Pacific settings for the FlowCam live analysis generates many more images than the ZooScan. For example, for station oa140, the ZooScan counts 1 435 images compared to 42 915 images for the FlowCam. Given 308 that taxonomists annotated images on an image-by-image basis, the validation or correction of the automatic 309 classification on these numerous FlowCam images would require a much higher investment of time than for the 310 ZooScan samples. In addition, the resolution of the FlowCam images of the smallest organisms does not allow us 311 to classify them properly and at a sufficient precision. Therefore, we validated only 30% of the total images 312 smaller than 500 pixels (equivalent to ~45 µm in ESD), randomly picked, assuming that this 30% random 313 subsample leaves a statistical count that is sufficiently representative of the population. Prior to this choice, a 314 series of tests were conducted to assess the impact of different fraction of image validation at varying object size 315 thresholds. Samples were randomly selected and 100% of the images were taxonomically validated. Subsequently, 316 a series of simulations (three times for the four samples, random sampling each time) were conducted to assess the impact of varying size thresholds (i.e. from 200 to 600 pixels, equivalent to 18 to 55 µm, with a step of 50 317 pixels) on the proportion of total images to be annotated (fractions from 5% to 50%, with increments of 5%). We 318 319 compared the results of these simulations by using the relative Root Mean Square Error (RMSE). The RMSE 320 values were divided by the total number of 100% validated values and multiplied by 100 to express the cumulative 321 error as a percentage. Results are shown in Fig. 4 and illustrate the cumulative error across the absolute abundance 322 values. For our chosen threshold of 500 pixels and subsets at 30% (highlighted in bold on the Fig. 4), we observed 323 induced errors of 0.02%. In Figure 3d, we present the absolute abundance and taxonomic group composition of 324 plankton from the four samples that were 100% taxonomically annotated, alongside the same four samples that 325 were only 30% (< 500 pixels) annotated. These samples show highly comparable results in both absolute 326 abundance and taxonomic composition (data not shown). We carried out the same analysis as described in Figure 327 4 for the total size spectrum, slope of the NBSS, and for the taxonomic composition (relative abundance). They 328 showed an induced error of 20% and 12%, respectively. This supplementary analysis can be found in appendix C.

329 The software ZooProcess 8.27, available on the PIQv website, now includes the capability for subsampling on

330 Flowcam data.



³³¹

333 Figure 4. (a) Estimated cumulative error associated with partial validation of particles below a size cut-off threshold 334 ranging from 200 to 600 pixels and validated fractions ranging from 5% to 50%. Errors are computed as the percentage 335 Root Mean Squared Error (RMSE) between fully validated samples and partially validated samples in three different 336 metrics for cumulative error in absolute abundance. RMSE values represent the outcomes of simulations, each 337 conducted three times for the four samples, with random sampling. (b) Cumulative error according to the Fractions 338 chosen. The threshold is fixed at 500 pixels. (c) Comparison between the absolute abundance (ind.m⁻³) and plankton 339 group composition for samples taxonomically annotated at 100% and for the same samples annotated at 30% below 340 the threshold of 500 pixels, equivalent to 45 µm.

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341 2.5 Datasets

342 **2.5.1** Plankton images on EcoTaxa and the associated tsv.

343 The datasets include 4 datasets of microplankton imaged by the FlowCam and sampled by the Deck-Net and the

Bongo Nets, and mesoplankton imaged by the ZooScan sampled by the HSN and the Manta. All of the sorted

images of plankton, plastic and particles are visible on the open-access projects on the EcoTaxa web application.

346 The *.tsv files exported from the EcoTaxa platform are provided. Readme tables for FlowCam and ZooScan *.tsv

347 are also provided to facilitate their use.

348 2.5.2 Quantitative descriptors to study the micro- and meso-plankton community

349 For each dataset, we designed a table combining the metadata and data from which we have calculated quantitative 350 descriptors of planktonic communities: abundance (ind/m³), biovolume (mm³/m³; proxy of biomass) and Shannon diversity Index. Abundance (ind/m³) and biovolume (mm^{3}/m^{3}) were calculated taking into account the volume of 351 352 water filtered by the plankton samplers (see formula in Table 1). Biovolumes (in mm^3/m^3) were computed using 353 area, riddled area, and ellipsoidal measurement of each object, and are available in the *.csv table (following 354 Vandromme et al., 2012; formula in Table 1). For analysis shown here, major and minor axes of the best ellipsoidal approximation were used to estimate the biovolume of each object, following the recommendations of 355 356 Vandromme et al. (2012). Size was expressed as equivalent spherical diameter (ESD, µm, see formula Table 1). 357 Diversity was calculated using the Shannon index (H: see formula Table 2). It is important to note that Shannon's 358 diversity index is dependent on the number of taxonomic categories, as defined by Shannon and Weaver (1949), 359 it assumes that individuals are randomly sampled from an independent large population and that all species are 360 represented in the sample. However, in the majority of cases, taxonomic classification was possible up to genus 361 level using quantitative imaging methods. This must be taken into account in these Shannon diversity indices, 362 which therefore differ from more commonly used taxonomic categories. The individual biovolumes of the 363 organisms were arranged in Normalised Biomass Size Spectra (NBSS), as described by Platt & Denman (1978), 364 along a harmonic range of biovolumes such that the minimum and maximum biovolumes of each class are linked 365 by: $B_{vmax} = 20.25 B_{vmin}$. The NBSS was obtained by dividing the total biovolume of each size class by its biovolume 366 interval (Bv_{range}=Bv_{max}-Bv_{min}). The NBSS was representative of the number of organisms (abundance within a factor) per size class. This can provide insight into ecosystem structure and function through the 'size spectrum' 367 368 approach, which generalises Elton's pyramid of numbers (Elton, 1927, Sheldon, 1972, Trebilco et al., 2013). The 369 NBSS size spectra of each sample (in abundance/µm) is provided in a separated zip files (.csv). Plankton 370 abundance and biovolume were calculated for each taxonomic annotation and for different levels of grouping: 371 living or nonliving, plankton groups and trophic association. The full list of these groups linked to all EcoTaxa 372 taxonomic annotations is given in the Table A1 to A4 (appendix A) of the taxonomic list and groups in each 373 dataset.

Descriptors		Formulas for FlowCam	Formulas for ZooScan	
Abundance (ind/m ³): Number of individus in the sampling/ m ³		(object_annotation_category x sample_conc_vol_ml) / (acq_fluid_volume_imaged x sample_initial_col_vol_m3)	(object_annotation_category x acq_sub_part) / sample_tot_vol	
Biovolume (m m ³ / m ³): Volume biomass of individus in the	Plain biovolume	(4/3 x ∏ x (√ (object_area) / ∏)) ³ x sample_conc_vol_ml) / (acq_fluid_volume_imaged x sample_initial_col_vol_m3)	((4/3 x $\prod x (\sqrt{\text{object}_\text{area}}) / \prod$) ³) x acq_sub_part) / sample_tot_vol	

sampling/ m ³ Riddled biovolume		(4/3 x ∏ x (√ (object_area_exc (mm2) / ∏)) ³ x sample_conc_vol_ml) / (acq_fluid_volume_imaged x sample_initial_col_vol_m3)	$((4/3 \text{ x} \prod \text{ x} (\sqrt{(\sqrt{(\sqrt{(object_area_exc / \prod)})})3) \text{ x}})$ acq_sub_part) / sample_tot_vol	
	Ellipsoid biovolume	(4/3 x ∏ x [(object_major/2) x (object_minor/2) x (object_minor/2)] x sample_conc_vol_ml) / (acq_fluid_volume_imaged x sample_comment_or_volume)	((4/3 x ∏ x [(object_major (mm)/2) x (object_minor (mm)/2) x (object_minor (mm)/2)]) x acq_sub_part) / sample_tot_vol	
Diversity S	hannon Indice (H)	- \sum (abundance relative (%) / 100) *	log(abundance relative (%) / 100)	
Equivalent (ESD, μm)	Spherical Diameter	2 x √ (object_area× p	rocess_pixel $^{2}/\prod$)	
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object_area : object_area_ object_mino	: surface area of the ob exc : surface area of the r : length of secondar	vject [pixel ²] he object excluding holes (object_area*(1) y axis of the best fitting ellipse for the ob	l-(object_%area/100)) [pixel ²] ject [pixel]	
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object_area : object_area_ object_mino object_major process_pixe Data descri J See Export F object_annot	: surface area of the ob _exc : surface area of the r : length of secondary r : length of the primar el : dimension of the si ption for FlowCam EcoTaxa FlowCam rea tation_category : taxor	vject [pixel ²] he object excluding holes (object_area*(1) y axis of the best fitting ellipse for the ob ry axis of the best fitting ellipse for the ol de of a pixel in the scanned image [mm] d me.csv h display_name in Ecotaxa	l-(object_%area/100)) [pixel²] ject [pixel] bject [pixel]	
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Table 1. Formulas used to calculate quantitative variables in datasets. The variable names correspond to the real names
 of the variables in the exports (tsv files) and are described in the table.

3. Technical validation and discussion

3.1 Limitations of Bongo net micro-plankton sampling for quantitative estimations

Both the Bongo nets and the Deck Net consisted of a 20 μm mesh to collect surface micro-plankton throughout
 the expedition. A key difference between these two nets lies in their deployment locations, which correspond to

- distinct environments: Bongo nets were deployed near islands, reefs, or within lagoons, while the Deck Net was deployed in the open ocean. These environments are characterized by differing chlorophyll a concentration, with a clear increase observed near islands and within lagoons, as highlighted in Bourdin et al. (2024). As such, we expected higher plankton concentrations in the reef and lagoon areas, and consequently, in the Bongo net samples. However, the majority of Bongo net samples showed lower concentrations than nearby open ocean samples from the Deck Net, as evidenced by the NBSS size spectra (Fig. 5a).
- 388

389 This discrepancy raises concerns about our reliability of the volume-filtered estimates, whether based on 390 flowmeters or theoretical calculations, which are critical for consistent quantitative plankton sampling. Regarding 391 the flowmeters, as mentioned in the methods section, Bongo nets were equipped with flowmeters rated for speeds 392 above 0.3 m·s⁻¹. However, the relatively low towing speed of the underwater scooter was insufficient to generate 393 enough water flow through the 20 µm mesh to rotate the flowmeters reliably. For the theoretical volume, the 394 deployment time of the Bongo nets by divers was highly uncertain. The uncertainty surrounding the theoretical 395 volume stemmed from inconsistent deployment times recorded by the divers and methodological biases associated with using an underwater scooter, which made the filtered volume estimates unreliable. Moreover, the suspended 396 397 particle concentrations were very variable for different sampling sites which complicated the correct prediction 398 of the towing time required to obtain reasonable concentrate in the net and avoid clogging.

399

400 Overall, the lack of correlation of total chlorophyll a and total phytoplankton biovolume from FlowCam, as shown 401 in figure 5b, indicates that the Bongo net sampling was not quantitative. The chlorophyll a (chla) values obtained 402 from the HPLC measurements do not represent the same size classes of phytoplankton as those observed with the 403 FlowCam, but we were interested in whether or not there were likely to be similar trends in phytoplankton biomass 404 changes measured for the same station (Fig. 5b). The correlation between chlorophyll a and total phytoplankton 405 biovolume of the Bongo being lower than for the Deck-Net samples. This suggests that phytoplankton biovolume 406 was underestimated relative to chlorophyll a in the Bongo samples. Given the methodological limitations of the 407 Bongo net filtration volume estimation, our most plausible hypothesis is an overestimation of the theoretical 408 volume likely due to clogging. Therefore, as a conclusion, it is highly recommended to use Bongo net samples for 409 qualitative analysis only.





412 Figure 5. (a) Comparison of Normalised Biovolume Size Spectra (NBSS; in log-log) of the live plankton between the 413 Bongo nets (34 samples) and the Deck Net (207 samples). (b) Phytoplankton biovolume (mm³.m⁻³) estimated from the 414 FlowCam samples, which were collected using the Bongo nets and the Deck Net, according to the Chla values obtained 415 from the HPLC measurements at the same station. The selection of phytoplankton organisms was made possible by 416 taxonomic validation of FlowCam images from these two nets.

417 **3.2 Benefits and limitations of high-speed deployment**

418 During the Tara Pacific open ocean transects, we decided to take on the challenge of collecting plankton samples 419 while sailing at speeds of up to 9 knots. This high-speed sampling provides valuable opportunities to expand and optimise the coverage of our sampling with a daily frequency. Initially, the Tara Pacific expedition was designed 420 421 to focus on coral reefs (Planes et al., 2019). The addition of high-speed sampling allowed for the opportunistic 422 use of transit periods, covering a significant spatial area of the expedition. As a result, one of the most valuable 423 aspects of the Tara Pacific plankton samples is the daily collection of samples approximately every 150 to 200 424 nautical miles, covering a wide range of oceanic structures across the Pacific basin. However, it is important to 425 note that, given the patchy spatial distribution of plankton (Robinson et al., 2021), this sampling scale is somehow 426 discrete rather than continuous. This designed sampling is also valuable as we aimed for 'end-to-end' sampling of 427 surface waters (Gorsky et al., 2019) with the micro to macroplankton fractions presented in this article. However, 428 the constraint of surface sampling and of deploying and retrieving the instruments at cruising speed forced us to 429 develop new robust, relatively small and user-friendly devices adapted for the Tara schooner. The combined 430 deployment of the Dolphin system and the High-Speed Net (HSN) designed to this purpose and present in this 431 article, represents, to our knowledge, the first system enabling discrete sampling of the entire surface planktonic 432 ecosystem with deployment and retrieval at cruising speeds < 9 knots.

433

434 The development of the high-speed plankton samplers began in the early 20th century with the well-known 435 Continuous Plankton Recorder (CPR), developed by Alister Hardy in 1926, which is designed to be towed under 436 the surface over long distances at speeds up to 25 knots. Following the CPR, other high-speed net systems 437 emerged, including the Longhurst-Hardy Plankton Recorder (LHPR: Longhurst et al., 1966), Gulf III OCEAN 438 Sampler (Gehringer, 1958), and Gulf V plankton sampler (Sameoto et al., 2000) as well as newer low-tech designs 439 (CSN in Von Ammon et al., 2020; Coryphaena in Mériguet et al., 2022). All high-speed zooplankton samplers 440 face the challenge of maintaining filtration efficiency at higher towing speeds. Thus, higher speeds require a larger relative filtration area to optimises filtration efficiency while minimising excessive pressure on the net and 441 442 mitigating the pressure wave that pushes organisms away from the net (Harris et al., 2000; Keen, 2013; Skjoldal 443 et al., 2013). A critical design principle is therefore to obtain a sufficiently high ratio of mesh filtering area to net 444 opening area (Smith et al., 1968b; Skjoldal et al., 2013). To achieve this, high-speed zooplankton samplers often 445 employ a small initial opening area that widens internally (e.g. CPR has an 1.27 cm² entrance aperture expanding 446 to 5cm x 10cm; the use of conic noses on the Gulf-V and LHPR). This design trade-off essential for pressure 447 reduction, comes at a cost. The small surface area of the mouth opening means a smaller volume filtered, reducing 448 the probability of collecting less abundant, larger organisms (Skjoldal et al., 2013). The avoidance of active 449 swimming zooplankton, net opening area size dependent, is also described as the bias affecting the catch of 450 mesoplankton by Harris et al., 2000. This may be discussed, as increasing tow speed may improve the capture efficiency of zooplankton capable of active avoidance (Skjolad et al. 2013). Therefore, high-speed sampling 451 452 methods have the advantages of increasing sampling coverage and frequency, but they also introduce bias due to 453 the pressure generated by high speeds, resulting in even greater undersampling compared to traditional nets (Harris 454 et al., 2000; Cook and Hays, 2001).

455 **3.2.1 Impact on filtered volumes estimation**

456 One of the primary challenges in quantitative plankton sampling is the estimation of the filtered volume. Because 457 the immersion depth of surface nets changes constantly with waves, wind and boat movement, it is difficult to 458 accurately calculate the volume of water being filtered (reviewed in Pasquier et al., 2022). Results obtained by 459 different studies show that a surface sampling with a difference in immersion depth of a few centimeters can lead to a large difference in the sampled volume (Pasquier et al., 2022). Overall, the impact of high-speed deployment 460 461 on filtered volume remains largely unexplored in the literature with the exception of Jonas et al (2004). They 462 tested the relationship between CPR filtered volumes estimated by a flowmeter or by theory, and their relationship 463 to CPR deployment speed. Their findings revealed overestimations by the flowmeter compared to theoretical 464 values. This raises concerns about the effectiveness of flowmeters in measuring volumes during high-speed deployments. We therefore investigated whether our high-speed surface sampling approach had an effect onfiltered volume measurements.

467

For the Deck Net, the water intake was identical in design and mouth opening to HSN but a flowmeter was integrated into the water circuit downstream of the pump as well as two de-bubblers (pictures Fig. 6 in Gorsky et al., 2019). This allowed for reliable estimation of water volumes that were pumped into the Deck-net based on

flowmeter recordings (Gorsky et al., 2019). Both HSN and Manta nets were equipped with mechanical flowmeters
 mounted in the inlet frame, while the towed distance, time and speed were recorded on board to also estimate the

- mounted in the inlet frame, while the towed distance, time and speed were recorded on board to also estimate the
 theoretical volume filtered. While the HSN was towed between 3.9 and 9 knots, the Manta net was towed at lower
- 474 speed, between 1.2 knots and maximum speed of 3.6 knots (Fig. 6).







Figure 6. (a) and (b) Linear regression between volumes filtered estimated from the tow distance (theoric volumes; m3) and estimated from the flowmeters respectively for the HSN and Manta. The range of 95% confidence intervals is represented in orange for the HSN and in blue for the Manta. The 1:1 dotted line represents the linear regression obtained if both volumes were similar. The colour of the dots represents the deployment speed of the net in knots.

482

483 Figure 6 shows a clear discrepancies in the slope of the estimated volumes between the HSN and the Manta, 484 meaning that the theoretical and flowmeter filtered volumes of the Manta are closer to each other than for the 485 HSN. Manta theoretical volumes tend to be higher and thus potentially overestimated compared to flowmeter 486 measurements (Fig. 6b), but the difference remains largely small compared to the HSN. For this one, flowmeter 487 estimation methods provide volumes in the same order of magnitude as the theoretical volume for HSN, yet exhibit 488 considerable differences between stations (mean difference between flowmeter and theoretical volumes per station 489 = 90.5, standard deviation = 172.6; Fig. 6a). Linear regression analysis between this volume differences per station 490 (flowmeters - theoretical volume) and speed deployment showed a significant relationship with a slope coefficient 491 of 91.168 (standard deviation = 11.86, t-test = 7.69 and p-value < 0.001), indicating that higher speeds are 492 associated with greater differences. Consistently with the results of Jonas et al (2004) described before, the high-493 speed deployment is thus associated with the overestimation of the flowmeters volumes compared to theoretical 494 ones (Fig. 6a). These results indicate that the use of the flowmeters is not appropriate in high-speed conditions. 495 The pressure increase caused by the high speed generates turbulence and could affect the flowmeter rotation and 496 explain the overestimation of the filtered volume for the high-speed that we found. Globally, the turbulence 497 generated could explain the malfunction of flowmeters which are designed and calibrated by the manufactures to 498 accurately measure flow speed in a laminar flow. This result is highlighted by Skjoldal et al. (2019), who assume 499 the use of flowmeters being complex because of their position in relation to the cross-sectional flow field or 500 functioning in a turbulent system.

- 502 In addition to the speed, we tested the HSN's immersion depth varied when the sea state was high. The HSN was
- designed to sample the surface ocean, at the air-seawater interface, thus the upper part of its mouth opening was
- rarely completely submerged during the deployment (see images Fig. 4 in Gorsky et al. 2019). The relationships
- between wind strength (as a proxy for sea state) recorded by Tara's navigation instruments and the two estimates
- 506 of HSN sampling volumes showed no correlation ($R^2 = 0.00$ for flowmeter volumes and for theoretical volumes; 507 data not shown). While the flowmeter does not provide accurate flow measurements under turbulent conditions,
- 508 it appears that the sea state does not affect its volume estimates.
- 509
- 510 Therefore, we recommended using the theoretical volume for the HSN. The towing distance used is relative to
- 511 ground, not to the seawater, therefore there is a potential bias in the theoretical volume estimation due to the non-
- 512 consideration of the surface current speed. This bias is likely negligible for the majority of our samples located in
- 513 the subtropical gyres, mostly characterised by relatively low geostrophic currents (Tara Pacific data available
- 514 Bourdin et al. 2022 in 'at current_speed_copernicus').

515 **3.2.2** Quantitative comparison between HSN and Manta

516 The Manta net was designed to study neuston and floating particles, such as microplastics. Thus, it is the most 517 commonly used net for studying surface plankton and widely recognised as a reference system for investigating 518 surface ocean (Eriksen et al., 2018; Karlsson et al., 2020; Pasquier et al, 2022). Both HSN and Manta nets were 519 deployed at the same stations when approaching islands and in the Great Pacific Garbage Patch. The Manta net 520 was deployed in closer proximity to islands than the HSN net. Given that the HSN net was towed for a duration 521 of 60-90 minutes, while the Manta net was towed for approximately 30-40 minutes, the decision was taken to 522 sample with the Manta net in the immediate vicinity of the island, in order to capture the variability associated 523 with the island mass effect.

524

We conducted a comparison of the Normalized Biovolume Size Spectra (NBSS; Fig. 7a) obtained from the two 525 526 nets. The analysis follows the analysis presented in Lombard et al. (2023), incorporating data from 31 additional 527 samples collected by the HSN. The NBSS of both nets was of the same order of magnitude, with Manta 528 biovolumes appearing higher in each NBSS size class (Fig. 7a), suggesting an underestimation by the HSN. 529 Considering the principle that, when represented on a logarithmic scale (as in Fig. 7c), the intercept of NBSS 530 spectra reflects the total abundance of organisms in the studied ecosystem (Platt & Denman, 1978), and assuming the same water masses were sampled, we compared the NBSS intercepts, which support the underestimation by 531 532 the HSN, as higher intercepts were observed for the Manta (with the NBSS intercept of HSN showing 0.2 533 compared to 0.8 for the Manta). This difference was expected due to the undersampling at high speed compared 534 to traditional plankton sampling discussed above. In contrast to the HSN net, which has a smaller mouth opening 535 leading to a smaller sampling volume, the Manta net benefits from a larger opening and lower towing speed. This 536 combination reduces turbulence and allows for a larger sampling volume, resulting in potentially lower loss. This is reflected in Fig. 7a, where the Manta net captures a wider range of sizes, including larger and rarer fragile 537 538 organisms. Skjoldal et al. (2019) measured less biomass in the large size fraction and more biomass in the small 539 and medium size fractions at the higher towing speeds. The opposite effect might have been expected for the small 540 fraction due to extrusion (Skjoldal et al., 2019), suggesting that the HSN net may be more effective at capturing 541 smaller organisms. However, this is not clearly demonstrated, as the slopes of the HSN's NBSS are largely 542 equivalent to those of the Manta (mean NBSS slope for HSN = -0.35, std = 0.30 and mean NBSS slope for Manta 543 = -0.30, std = 0.23; Fig. 7a). This also suggests that both nets capture the same trophic plankton ecosystem 544 structure, while the HSN underestimates plankton in each size class.





Figure 7. (a) Comparison of Normalized Biovolume Size Spectra (NBSS) of living organisms sampled with HSN in
 yellow dots and Manta nets in blue dots. Only stations where both were deployed are included in this figure. Average
 taxonomic composition of the 'plankton groups' in biovolume (mm³/m⁻³) for all stations by size class (in μm) for samples
 collected with HSN in (b) and Manta net in (c).

552 All these observed differences may therefore introduce differences in species composition. Investigating the 553 taxonomic composition, the HSN and the Manta show on average relatively similar community compositions 554 (Fig. 7c and 7d; the dinoflagellates are almost entirely composed of the genus Noctiluca). Investigating the 555 taxonomic composition in terms of biovolume, the five most represented groups in the Manta dataset are Cnidaria 556 (59%), Copepoda (13%), other (11%), Crustacea (9%), and Mollusca (3%). In contrast, the HSN dataset shows a 557 more even distribution, with other taxa contributing 33%, followed by Cnidaria (28%), Copepoda (19%), Tunicata 558 (10%), and Crustacea (6%). Although there is a general difference in the sampled plankton community, the 559 greatest discrepancies are observed for gelatinous organisms. Thus, HSN net undersampled larger and more fragile organisms such as cnidarians and tunicates (Fig. 7c). This aligns with the limitations of high-speed deployments, which have been shown to damage delicate organisms (Harris et al., 2000; Keen, 2013). This damage to large and fragile plankton could cause the higher concentrations of smaller size classes we found in HSN compared to Manta samples. In contrast, the HSN consistently sampled more robust organisms such as copepods and chaetognaths than the Manta (Fig. 7c and 6d).

565

566 For the quantitative and qualitative comparison of plankton community sampling, we only considered stations where both nets were deployed sequentially (first the Manta, followed by the HSN). Although small, this temporal 567 568 and spatial difference remains a limitation in our comparison between the two nets. In terms of location, this 569 combination of Manta-HSN deployments was primarily conducted near islands, where plankton concentrations 570 and composition are known to be highly variable (Bourdin et al., 2024; Kristan et al., in prep). Given that the 571 Manta was deployed before the HSN, i.e., closer to the islands, we also expect part of the HSN underestimation 572 signal to be explained by this small spatial difference. Therefore, while our primary hypothesis attributes these 573 differences mainly to the high-speed deployment of the HSN (up to three times greater than that of the Manta), 574 these spatial and temporal factors, in addition to the patchiness distribution of plankton (Robinson et al., 2021), 575 may also play a role in our comparison of the two plankton sampling systems.

576

577 4. General discussion

578

579 In conclusion to our investigation of sampling biases associated with the high-speed sampling, the HSN must 580 therefore be considered as semi-quantitative. The use of the HSN introduces an undersampling bias that is also 581 found in other high-speed samplers, as described for the CPR. Nevertheless, we highlight the usefulness of the HSN for sampling surface zooplankton when it is not possible to stop or slow the boat, and its value in extending 582 sampling coverage and frequency. Consistent with the CPR, HSN captures a roughly consistent fraction of the in-583 584 situ abundance reflecting the main patterns observed in plankton. Consistent with expected ecological trends, 585 higher plankton abundances and biovolumes are observed in nutrient-rich regions such as coastal and upwellings, 586 whereas oligotrophic gyres exhibit significantly lower biomass (see abundance, biovolume, and diversity maps 587 for each sampling device in appendix B). For example, the trend of increasing plankton abundance due to 588 California upwelling (Checkley and Barth, 2009) appears to emerge regardless of the sampling method used 589 (appendix B: Fig. B1 to B4). Each net is a filter through which we sample the ocean, but if the overall patterns 590 they show are consistent, we can conclude that they are likely to be robust patterns. This is true for many types of 591 sampling nets, as many previous studies have shown (Herdman, 1921; Barnes and Marshall, 1951; Anraku, 1956; 592 Wiebe and Holland, 1968).

593

594 In addition to the unique characteristic of high-speed sampling, these datasets are also distinguished by their focus 595 on surface plankton communities during daytime, offering both advantages and limitations. These surface 596 plankton data enrich interdisciplinary studies of ocean's surface layer, in direct associations with other surface 597 measurements (satellite and atmospheric data; Lombard et al., 2019). This surface ecosystem, hosting a uniquely 598 diverse planktonic community, remains largely unexplored, but appears to play an essential role in ocean-climate 599 feedbacks (Helm, 2021; Hunter, 2023) as a critical interface between atmospheric and oceanic process and 600 contributing significantly to biogeochemical cycles (Falkowski et al., 2008). Processes controlling the abundance 601 and diversity of the surface plankton communities may be significantly different from those in deeper layers (Ibarbalz et al., 2019, Santiago et al., 2023). The surface is also on the frontline of climate change and pollution. 602 603 Thus, these particular communities face increasing challenges such as rising temperatures, stratification and 604 nutrient stress (Bopp et al., 2013; IPCC, 2022) and floating contaminants ranging from plastics, metals and toxins 605 to petroleum (Helm, 2021). However, surface plankton sampling has limitations regarding the "quantitative representativeness" of the broader plankton ecosystem in the water column. The Tara Pacific sampling was 606 607 conducted under stable daytime conditions, minimizing variability from diel vertical migration (Lampert, 1989). 608 As a result, zooplankton concentrations do not reflect deeper-dwelling organisms, particularly those migrating to 609 the surface at night, leading to potentially higher abundances within the water column (Lampert, 1989). This is 610 also valuable for phytoplankton communities that are known to be heterogeneously distributed from the surface

to deeper waters into the euphotic zone, especially in the transparent oligotrophic waters of the Pacific gyre, where

- Deep Chlorophyll Maxima can occur tens to hundreds of meters below the surface (Mignot et al., 2014). In terms
- of comparison with non-surface plankton data, this limitation must be carefully considered by future users.
- 614

615 Conclusion

616

617 The Tara Pacific Expedition is part of the first initiatives aiming to implement a system for discrete sampling of the planktonic ecosystem while operating at cruising speed (5–9 knots), covering viruses to metazoa at the scale 618 619 of the whole expedition (Gorsky et al., 2019) and focusing on micro- to mesoplankton in this paper. The use of two new sampling systems highlights some biases that lead to undersampling, which is important to consider in 620 621 subsequent ecological analyses. However, the simultaneous high-speed sampling of the different components of 622 the surface ecosystem may contribute to address the issue of undersampling of the open ocean at difficult-to-reach 623 spatial and temporal scales, a major challenge for marine science. These systems can be improved and adapted to vessels of different sizes and propulsion systems, opening the way to complementary initiatives, such as plankton 624 collection by citizen sailors. (De Vargas et al., 2022; Mériguet et al., 2022). 625

626

In conclusion, using these new sampling methods covering the North and South Pacific and North Atlantic basins, 627 we provide an important dataset focusing on the surface plankton rarely sampled as a whole. Our large-scale 628 analysis reveals an important taxonomic and functional diversity within the surface planktonic communities, 629 630 encompassing approximately 370 different taxa, primarily identified at the genus level, spanning across 12 major 631 plankton groups and 5 trophic levels. We hope that the dataset presented here, will stimulate further studies (i.e., 632 biodiversity, biogeochemistry, modeling studies...) using the different environmental imprints recorded during the Tara Pacific expedition (data available in Lombard et al., 2023) to highlight the processes influencing this 633 634 particular plankton ecosystem, from large scale to mesoscale levels, from taxonomic scale to trophic scale, or 635 from species barcodes to genomes. Such an important dataset will not only serve as a starting point for many 636 studies to deepen our understanding of planktonic ecosystems, their biogeochemical roles, and their socio-637 economic importance, but could also serve as a reference state of the ecosystem in the context of environmental 638 changes.

639 4. Data availability

- 640 The referenced datasets related to figures are available at:
- https://doi.org/10.17882/102537 Mériguet et al., (2024a) (EcoTaxa link: <u>https://ecotaxa.obs-vlfr.fr/prj/1344</u> and https://ecotaxa.obs-vlfr.fr/prj/1345),
- 643 https://doi.org/10.17882/102336 Mériguet et al., (2024b) (EcoTaxa link: https://ecotaxa.obs-vlfr.fr/prj/11292),
- https://doi.org/10.17882/102694 Mériguet et al., (2024c) (EcoTaxa link: https://ecotaxa.obs-vlfr.fr/prj/11370 and
 https://ecotaxa.obs-vlfr.fr/prj/11369)
- 646and https://doi.org/10.17882/102697Mériguet et al., (2024d) (EcoTaxa link: https://ecotaxa.obs-vlfr.fr/prj/11341).647vlfr.fr/prj/11353and https://ecotaxa.obs-vlfr.fr/prj/11341
- 649 The imaging datasets are also summarized in Table 2.
- 650

- 651 A key strength of this quantitative imaging dataset is its complementarity with a wide range of environmental data
- 652 collected during the Tara Pacific expedition. This expedition is described in detail in Lombard et al. (2023), where
- the full set of environmental datasets is available and referenced: <u>https://doi.org/10.1038/s41597-022-01757-w.</u>
- Environmental data were collected station by station, making it possible to link them directly to our dataset using
- the station name. Each station is identified by a unique [0a###] code, where the "0a" label is the key identifier for
- associating environmental measurements with our imaging data. When looking at data at this 'station' level, all
- environmental data are already compiled and compatible for easy analysis and cross-analysis, and when linked to
- sample barcodes, they could be further linked to any other associated data (e.g. genomic) by linking them to the sample registry available in Lombard et al 2023, with sample and event registry at:

660 https://doi.org/10.1594/PANGAEA.944548. In addition to station-based data, continuous environmental 661 measurements from the Tara Pacific expedition (Lombard et al., 2023) can also be linked to our dataset. These 662 measurements can be linked to plankton net sampling events using date, time and GPS coordinates, all of which 663 are available in both the plankton and in line environmental datasets. This ensures a robust integration of imaging 664 and environmental data, facilitating large-scale ecological analyses.

	Datasets			
Name	FlowCam Tara Pacific DN 20 microns	FlowCam Tara Pacific Bongo 20 microns	ZooScan Tara Pacific HSN 330 microns	ZooScan Tara Pacific Manta 333 microns
DOI	10.17882/102697	10.17882/102694	10.17882/102336	10.17882/102537
Sampling Location	Open-ocean and islands sampling	Islands, reef and lagoon sampling	Open-ocean and islands sampling	Open-ocean (Great Pacific Garbage Patch) and islands sampling
Plankton size imaged	(20-200 μm)	(20-200 µm)	(> 300 µm)	(> 300 µm)
	Subset 30% < 500 pixels:	Subset 30% < 500 pixels:		Subset Plankton images
Link to open EcoTaxa	<u>https://ecotaxa.obs-</u> <u>vlfr.fr/prj/11353</u>	<u>https://ecotaxa.obs-</u> <u>vlfr.fr/prj/11370</u>	https://ecotaxa.obs-	https://ecotaxa.obs- vlfr.fr/prj/1344
project	Subset 100 % > 501 pixels:	Subset 100 % > 501 pixels:	<u>vlfr.fr/prj/11292</u>	Subset Plastics images
	https://ecotaxa.obs- vlfr.fr/prj/11341	<u>https://ecotaxa.obs-</u> <u>vlfr.fr/prj/11369</u>		<u>https://ecotaxa.obs-</u> <u>vlfr.fr/prj/1345</u>
	Subset 30% < 500 pixels:	Subset 30% < 500 pixels:		Subset Plankton images
ZIP files with one tsv	Export EcoTaxa FlowCam Tara Pacific DN 20 microns < 500 pixels.zip	Export EcoTaxa FlowCam Tara Pacific Bongo 20 microns < 500 pixels.zip	Export EcoTaxa ZooScan Tara Pacific	Export EcoTaxa ZooScan Tara Pacific Manta 333 microns plankton.zip
export from EcoTaxa	Subset 100 % > 501 pixels:	Subset 100 % > 501 pixels:	HSN 330 microns.zip	Subset Plastics images
	Export EcoTaxa FlowCam Tara Pacific DN 20 microns > 501 pixels.zip	Export EcoTaxa FlowCam Tara Pacific Bongo 20 microns > 501 pixels.zip		Export EcoTaxa ZooScan Tara Pacific Manta 333 microns plastics.zip

CSV files with ab, bv (x3: area, riddled and ellispoidal), shannon	Descriptors FlowCam Tara Pacific DN 20 microns.csv	Descriptors FlowCam Tara Pacific Bongo 20 microns.csv	Descriptors ZooScan Tara Pacific HSN 330 microns.csv	Descriptors ZooScan Tara Pacific Manta 333 microns.csv
ZIP files with 1 table csv / sample for NBSS (1 NBSS / sample)	NBSS FlowCam Tara Pacific DN 20 microns.zip	NBSS FlowCam Tara Pacific Bongo 20 microns.zip	NBSS ZooScan Tara Pacific HSN 330 microns.zip	NBSS ZooScan Tara Pacific Manta 333 microns.zip

668 Table 2. Summary of data availability, description and useful link for each dataset.

669 Appendices

FlowCam Tara Pacific DN 20 microns			
Taxonomic list	Plankton groups	Trophic type	
Bacillariophyceae			
Asterionellopsis			
Asterolamprales			
Bacillariaceae			
Climacodium			
Climacodium inter. Crocosphaera			
chainlarge			
chainthin			
multiple < Diatoma			
Pseudo-Nitzschia chain			
Thalassionematales			
Corethron			
Coscinodiscophycidae	bacillariophyta	phototroph	
Coscinodiscids			
Bacteriastrum			
Chaetoceros			
Chaetoceros protuberans			
Chaetoceros peruvianus			
Ditylum			
Eucampia			
Hemiaulus			
Fragilariopsis			
Nitzschia			
Planktoniella sol			
Rhizosolenids			

Dactyliosolen		
Guinardia		
Rhizosolenia inter. Richelia		
pennate < Bacillariophyta		
Helicotheca		
	•	
Cyanobacteria		
UCYNA like		
cyano a	avenabestaria	autotroph
cyano b	cyanobacterra	
Richelia		
attached		
	·	
Codonaria		
Ciliophora		
Amphorides		
Codonellidae		
Codonellopsis		
Codonellopsis orthoceras		
Cyttarocylis		mixotroph
Dictyocysta		
Epiplocylis		
Eutintinnus		
Lacrymaria		
Metacylis	ciliophora	
Poroecus		
Rhabdonella		
Rhabdonellopsis		
Salpingella		
Steenstrupiella		
Tintinnida		
Undellidae		
Amplectella		
Xystonellidae		
Dadayiella		
Zoothamniidae		
Dictyochophyceae	dictyochophyceae	phototroph
	•	
Gonyaulacales		
Dinophyceae	1	
Amphisolenia	dinoflagellata mixot	
Dinophysis	1	

Ceratocorys			
Cladopyxis			
Neoceratium			
Neoceratium limulus			
Neoceratium candelabrum			
Neoceratium furca			
Neoceratium fusus			
Neoceratium pentagonum			
Neoceratium geniculatum			
Pyrocystaceae			
Pyrophacus			
Gymnodiniales			
Ornithocercus			
Ornithocercus heteroporus			
Ornithocercus magnificus			
Ornithocercus quadratus	7		
Ornithocercus steinii			
Oxytoxum			
Phalacroma			
Podolampas			
Protoperidinium			
polar view			
Hemidiscus cuneiformis			
Tunicata	tunicoto		
Appendicularia	tumeata		
Copepoda	copepoda		
Ostracoda	crustacea	grazers	
nauplii < Crustacea	erustaeea		
Rotifera			
trochozoa	other		
larvae < Annelida		omnivorous	
veliger	mollusca	grazers	
Pterosperma	other	phototroph	
	-	1	
Rhizaria	4		
Retaria	4		
Amphibelone	rhizaria	mixotroph	
Acantharia	mzana	inixouopii	
Foraminifera			
Nassellaria			

Spumellaria			
cyst		_	
egg	other		
egg sac			
multiple < other	-	_	
othertocheck			
darkrods < othertocheck			
lightrods < othertocheck	other unidentified	unidentified	
othersphere			
1			
t001			
t003	other unidentified	unidentified	
t004			
tail < Appendicularia			
part < Crustacea			
spines < Acantharea			
part < Ciliophora			
artefact			
badfocus < artefact			
bubble	non-living		
detritus	non nying	—	
dark < detritus			
fiber < detritus			
light < detritus			
pollen			
duplicate			
t002			

673 Table A1. List of EcoTaxa taxonomic annotations and associated groups: plankton groups and trophic type for the FlowCam DN 20 microns dataset.

FlowCam Tara Pacific Bongo 20 microns			
Taxonomic list	Plankton groups	Trophic type	
Trichodesmium			
UCYNA like	ovanobacteria	autotroph	
Cyanobacteria <proteobacteria< td=""><td>eyanobacteria</td><td rowspan="2">autonoph</td></proteobacteria<>	eyanobacteria	autonoph	
Richelia			
Ciliophora	ciliophora	mixotroph	

Lacrymaria <lacrymariidae< td=""><td></td><td></td></lacrymariidae<>		
Vorticella		
Codonellidae		
Cyttarocylis		
Epiplocylis		
Dictyocysta		
Metacylis		
Rhabdonella		
Rhabdonellopsis		
Tintinnida		
tintinnid-diatom		
Amphorides <tintinnidiidae< td=""><td></td><td></td></tintinnidiidae<>		
Eutintinnus		
Salpingella <tintinnidiidae< td=""><td></td><td></td></tintinnidiidae<>		
Steenstrupiella		
Tintinnidae X		
Poroecus		
Undellidae		
Xystonellidae		
part <ciliophora< td=""><td></td><td></td></ciliophora<>		
Dinophyceae		
Dinophyceae X		mixotroph
Amphisolenia		
Ornithocercus		
Ornithocercus magnificus <ornithocercus< td=""><td></td></ornithocercus<>		
Ornithocercus steinii		
Phalacroma		
Neoceratium		
Neoceratium candelabrum		
Neoceratium furca <neoceratium< td=""><td>dinoflagellata</td></neoceratium<>	dinoflagellata	
Neoceratium fusus <neoceratium< td=""><td>unionagenata</td></neoceratium<>	unionagenata	
Neoceratium pentagonum		
Cladopyxis		
Ostreopsis		
Pyrocystaceae		
Pyrophacus		
Peridiniales		
Oxytoxum		
Podolampas		

Rhizaria		
Retaria		
Acantharea		
spines <acantharea< td=""><td></td><td></td></acantharea<>		
Foraminifera		
Nassellaria <polycystinea< td=""><td>rhizaria</td><td>mixotroph</td></polycystinea<>	rhizaria	mixotroph
Spumellaria		
Radiolaria		
aggregate <radiolaria< td=""><td></td><td></td></radiolaria<>		
part <rhizaria< td=""><td></td><td></td></rhizaria<>		
spines <rhizaria< td=""><td></td><td></td></rhizaria<>		
		•
Bacillariophyceae		
Asterionella		
Coscinodiscophycidae		
Asterolamprales		
Hemidiscus cuneiformis		
Hemidiscus		
Cylindrotheca		
Diatoma		
chainlarge		
chainthin		
multiple <diatoma< td=""><td></td><td></td></diatoma<>		
Licmophora		
Naviculales		
Nitzschia	bacillariophyta	nhototronh
Pseudo-nitzschia	bacmanophyta	phototroph
Striatella		
Synedra		
Thalassionematales		
Amphitetras		
Bacteriastrum <mediophyceae< td=""><td></td><td></td></mediophyceae<>		
Biddulphia		
Chaetoceros <mediophyceae< td=""><td></td><td></td></mediophyceae<>		
Chaetoceros inter ciliate		
Chaetoceros inter. Calothrix		
Ditylum		
Eucampia		
Hemiaulus		
Odontella sp.		

Odontella <mediophyceae< td=""><td></td><td></td></mediophyceae<>		
Planktoniella		
Corethron		
Coscinodiscus		
Stephanopyxis		
Rhizosolenids		
Dactyliosolen		
Guinardia		
Rhizosolenia		
Rhizosolenia inter. Richelia		
rhizosolenia inter richelia tmp i		
rhizosolenia tmp i		
centric		
chain <centric< td=""><td></td><td></td></centric<>		
pennate <bacillariophyta< td=""><td></td><td></td></bacillariophyta<>		
part diatom		
Dictyochophyceae		
Dictyochales	dictyochophyceae	phototroph
Dictyocha		
Annelida		
larvae <polychaeta< td=""><td></td><td></td></polychaeta<>		
trocophora	others	grazers
larvae <annelida< td=""><td></td></annelida<>		
trochophore		
Copepoda <maxillopoda< td=""><td></td><td></td></maxillopoda<>		
Calanoida		
Cyclopoida		omnivorous
Oithonidae	aananada	
Harpacticoida	copepoda	
Corycaeidae		
Oncaeidae		
part <copepoda< td=""><td></td><td></td></copepoda<>		
nauplii <crustacea< td=""><td>omistecco</td><td>arozoro</td></crustacea<>	omistecco	arozoro
part <crustacea< td=""><td>ciustacea</td><td>grazers</td></crustacea<>	ciustacea	grazers
Bryozoa	other	arozoro
trochozoa	outer	grazers

larvae <echinodermata< td=""><td></td><td></td></echinodermata<>		
Mollusca	mollusca	
veliger	monusea	
larvae <living< td=""><td></td><td>unidentified</td></living<>		unidentified
other <living< td=""><td>other</td><td>undentified</td></living<>	other	undentified
egg <other< td=""><td>otiler</td><td></td></other<>	otiler	
egg sac <egg< td=""><td></td><td>_</td></egg<>		_
multiple <other< td=""><td></td><td></td></other<>		
duplicate	-	_
othertocheck		
crumple sphere	other unidentified	unidentified
darkrods <othertocheck< td=""><td>other undentified</td><td>undentified</td></othertocheck<>	other undentified	undentified
lightrods <othertocheck< td=""><td>_</td><td></td></othertocheck<>	_	
t001		
t002		unidentified
t003		
t004		
t005		
t006		
t007		
t008		
t010	other unidentified	
t011		
t012		
t013	_	
t014	_	
t015	_	
t016	_	
t017		
		•
part <other< td=""><td></td><td></td></other<>		
part <seaweed< td=""><td>7</td><td></td></seaweed<>	7	
Micracanthodinium quadrispinum	7	
artefact	non-living	_
badfocus <artefact< td=""><td rowspan="2"></td><td></td></artefact<>		
bubble		
detritus		

aggregates	
dark <detritus< td=""><td></td></detritus<>	
fiber <detritus< td=""><td></td></detritus<>	
light <detritus< td=""><td></td></detritus<>	
feces	
darkrods <rods< td=""><td></td></rods<>	
lightrods <rods< td=""><td></td></rods<>	

675Table A2. List of EcoTaxa taxonomic annotations and associated groups: plankton groups and trophic type for the676FlowCam Bongo 20 microns dataset.

ZooScan Tara Pacific HSN 330 microns		
Taxonomic list	Plankton groups	Trophic type
Actinopterygii	other	predators
egg < Actinopterygii	onici	
		I
Annelida		
Spirorbis	other	omnivorous
larvae < Annelida		
Appendicularia	4	
Oikopleuridae	tunicata	grazers
		T
Bryozoa	other	grazers
cyphonaute		
Chaetognatha	chaetognatha	predators
Undrozoo		
Reunhazaa		
Scyphozoa	_	
Porpita	_	
larvae < Porplidae	_	
Sipnonophorae	_	
bract < Abylidae	_	
gonophore < Abyndae	cnidaria	predators
nectopnore < Abylidae	_	
	_	
bract < Diphyidae	_	
eudoxie < Diphyidae		
gonophore < Diphyidae	_	
nectophore < Diphyidae	_	
nectophore < Hippopodiidae		

Abylopsis tetragona		
bract < Abylopsis tetragona		
eudoxie < Abylopsis tetragona		
gonophore < Abylopsis tetragona		
nectophore < Abylopsis tetragona		
bract < Bassia bassensis		
nectophore < Bassia bassensis		
Physonectae		
nectophore < Physonectae		
Velella		
polype < Leptothecata		
polype < Anthozoa		
Cirripedia		
cirrus		
cypris	omistação	arozore
nauplii < Cirripedia	ciustacea	grazers
Evadne		
Podon		
Calanoida		
Acartiidae		
Calanidae		
Calocalanus pavo		
Candaciidae		
Centropagidae		
Eucalanidae		
Euchaetidae		
Heterorhabdidae		
Metridinidae		
Pontellidae	copepoda	omnivorous
Pontellina plumata		
Monstrilloida		
Temoridae		
Oithonidae		
Harpacticoida		
Corycaeidae		
Oncaeidae		
Sapphirinidae		
Copilia		
Lubbockia		

Siphonostomatoida		
badfocus < Copepoda		
damaged < Copepoda		
multiple < Copepoda		
Crustacea		
Eumalacostraca		
Amphipoda		
Caprellidae		
Gammaridea		
protozoea		
Hyperiidea		
Brachyura		
Phronimidae	crustracea	predators
megalopa		
zoea < megalopa		
Euphausiacea		
calyptopsis < Euphausiacea		
Isopoda		
Laomediidae		
larvae < Porcellanidae		
phyllosoma		
nauplii < Crustacea		
metanauplii < Crustacea	crustracea	grazers
Ostracoda	erustruceu	grazers
larvae < Squillidae		
Cyanobacteria < Bacteria	cyanobacteria	autotroph
Echinodermata		
echinopluteus		
pluteus < echinoidea	other	grazers
ophiuroidea	outer	grazers
ophiopluteus		
pluteus <ophioroidea< td=""><td></td><td></td></ophioroidea<>		
Harosa		
Acantharia	rhizorio	mixotroph
Collodaria	rnizaria	ппхоцорп
Globorotalidae		

Orbunila		
Foraminifera		
Spumellaria		
Pyrocystaceae	dinoflagellata	mixotroph
multiple < Pyrocystaceae	unionagenaa	mixouopii
Insecta	other	predators
Halobates		1
Mollusca		
Bivalva		
Gymnosomata		
Cavolinia inflexa		
Diacria		
Atlanta		
Cavoliniidae		
Cephalopoda	mollusca	grazers
Creseidae		
Creseis acicula		
Creseis virgula		
Firola		
Limacinidae		
part < Mollusca		
veliger		
Doliolida		
Salpida	4	
juvenil < Salpida	tumcata	predators
nucleus < Salpida		
egg < other		
egg sac < egg	other	_
gelatinous	other	predators
nudibranchia	other	_
multiple < other	other	_
othertocheck	other unidentified	unidentified

darksphere		
othersphere		
t001		
t002	other unidentified	unidentified
t003		undentified
t004		
part < Actinopterygii		
scale < Actinopterygii		
trunk < Appendicularia		
head < Chaetognatha		
part < Annelida		
tail < Appendicularia		
tail < Chaetognatha		
part < Thaliacea		
part < Siphonophorae		
part < Copepoda		
part < Cnidaria	non-living	
part < Crustacea	non nying	-
part < Ctenophora		
wing < Halobates		
empty < Ostracoda		
artefact		
badfocus < artefact		
bubble		
detritus		
borax		
dark < detritus		
fiber < detritus		

679 Table A3. List of EcoTaxa taxonomic annotations and associated groups: plankton groups and trophic type for the

ZooScan HSN 330 microns dataset.

Tara Pacific 2016 2018 Manta 300 plankton		
Taxonomic list	Plankton groups	Trophic type
Actinopterygii	other	
egg < Actinopterygii		predators
Annelida	other	omnivorous
larvae < Annelida		ommvorous

Alciopidae		
Tomopteridae		
Spirorbis		
Terebellidae		
Fritillariidae	tunicata	grazers
Oikopleuridae		0
Chaetognatha	chaetognatha	predators
Cnidaria		
polype < Anthozoa		
Hydrozoa		
larvae < Porpitidae		
Porpita porpita		
Velella		
polype < Leptothecata		
bract < Abylopsis tetragona		
eudoxie < Abylopsis tetragona		
gonophore < Abylopsis tetragona		
nectophore < Abylopsis tetragona		
bract < Bassia bassensis		
gonophore < Bassia bassensis		
nectophore < Bassia bassensis	anidaria	mudatana
bract < Diphyidae	Cindana	predators
Chelophyes		
eudoxie < Diphyidae		
eudoxie < Eudoxoides spiralis		
gonophore < Eudoxoides spiralis		
nectophore < Eudoxoides spiralis		
gonophore < Diphyidae		
nectophore < Diphyidae		
nectophore < Hippopodiidae		
Physalia		
nectophore < Physonectae		
Aglaura		
Rhopalonema velatum		
ephyra		
Ctenophora	other	predators

cirrus		
cypris	-	
nauplii < Cirripedia		
Evadne	- crustacea	grazers
larvae < Crustacea	-	
metanauplii < Crustacea	-	
Eumalacostraca		
Amphipoda		
Gammaridea		
Hyperiidea	-	
Oxycephalidae	-	
Phronima		
protozoea < Penaeidae	1	
protozoea < Sergestidae		
zoea < Galatheidae	crustacea	predators
larvae < Porcellanidae		
Brachyura	-	
megalopa	7	
zoea < Brachyura	7	
like < Laomediidae	7	
calyptopsis	7	
protozoea < Mysida		
Crustacea		
nauplii < Crustacea		predators
metanauplii < Crustacea	crustacea	produtors
Ostracoda		
larvae < Squillidae		grazers
Copepoda		
Calanoida	-	
Acartiidae	-	
Haloptilus	-	
Calanidae	-	
Candaciidae	cononoda	omnivorous
Centropagidae		omnivorous
Eucalanidae	-	
Euchaetidae	-	
Metridinidae	-	
Calocalanus pavo	4	
	1	

Pontellidae		
Pontellina plumata	-	
Temoridae	-	
Oithonidae	-	
Harpacticoida	-	
Miraciidae	-	
Corycaeidae		
Lubbockia		
Oncaeidae		
Sapphirinidae		
Copilia		
badfocus < Copepoda		
multiple < Copepoda		
damaged < Copepoda		
Insecta	other	predators
Gerridae		F
Bruozoa		
curbonauta	other	grazers
cyphonaute		
Branchiostoma lanceolatum	other	grazers
	other	gruzens
Doliolida		
Doliolida Pyrosomatida	-	
Doliolida Pyrosomatida Salpida	tunicata	omnivorous
Doliolida Pyrosomatida Salpida chain < Salpida	tunicata	omnivorous
Doliolida Pyrosomatida Salpida chain < Salpida juvenile < Salpida	tunicata	omnivorous
Doliolida Pyrosomatida Salpida chain < Salpida juvenile < Salpida	tunicata	omnivorous
Doliolida Pyrosomatida Salpida chain < Salpida juvenile < Salpida Mollusca	tunicata	omnivorous
Doliolida Pyrosomatida Salpida chain < Salpida juvenile < Salpida Mollusca Bivalvia	tunicata	omnivorous
Doliolida Pyrosomatida Salpida chain < Salpida juvenile < Salpida Mollusca Bivalvia Cephalopoda	tunicata	omnivorous
Doliolida Pyrosomatida Salpida chain < Salpida juvenile < Salpida Mollusca Bivalvia Cephalopoda Atlanta	tunicata	omnivorous
Doliolida Pyrosomatida Salpida chain < Salpida juvenile < Salpida Mollusca Bivalvia Cephalopoda Atlanta Firola	tunicata	omnivorous
Doliolida Pyrosomatida Salpida chain < Salpida juvenile < Salpida Mollusca Bivalvia Cephalopoda Atlanta Firola Gymnosomata	tunicata	omnivorous
Doliolida Pyrosomatida Salpida chain < Salpida juvenile < Salpida Mollusca Bivalvia Cephalopoda Atlanta Firola Gymnosomata Cavoliniidae	tunicata	grazers
Doliolida Pyrosomatida Salpida chain < Salpida juvenile < Salpida Mollusca Bivalvia Cephalopoda Atlanta Firola Gymnosomata Cavoliniidae Diacavolinia	mollusca	grazers
Doliolida Pyrosomatida Salpida chain < Salpida juvenile < Salpida Mollusca Bivalvia Cephalopoda Atlanta Firola Gymnosomata Cavoliniidae Diacavolinia Diacria trispinosa	tunicata	grazers
Doliolida Pyrosomatida Salpida chain < Salpida juvenile < Salpida Mollusca Bivalvia Cephalopoda Atlanta Firola Gymnosomata Cavoliniidae Diacavolinia Diacria trispinosa Creseidae	mollusca	grazers
Doliolida Pyrosomatida Salpida chain < Salpida juvenile < Salpida Mollusca Bivalvia Cephalopoda Atlanta Firola Gymnosomata Cavoliniidae Diacavolinia Diacria trispinosa Creseidae Creseis acicula	mollusca	grazers

Limacinidae		
Nudibranchia		
egg < Mollusca	other	_
pluteus < Echinoidea	other	omnivorous
pluteus < Ophiuroidea	other	ommvorous
Harosa	other	
Neoceratium	dinoflagellata	
Pyrocystaceae	unionagenata	mixotroph
Foraminifera		mixouopii
Orbulina	rhizaria	
Spumellaria		
Diatoma	diatoms	phototroph
egg < other	other	-
living < other	other	-
multiple < other	other	_
othertocheck	other unidentified	unidentified
seaweed	other	phototroph
t002		
1003		
1004		
1005		
t007		
1008		
1010	other unidentified	unidentified
1012		
1013		
1014		
1015		
t016		
t017		
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detritus		
artefact		
badfocus <artefact< td=""><td></td><td></td></artefact<>		
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fiber <detritus< td=""><td></td><td></td></detritus<>		

683 Table A4. List of EcoTaxa taxonomic annotations and associated groups: plankton groups and trophic type for the ZooScan Manta 333 microns dataset.



685 686 Figure B1. FlowCam DN 20 microns: (a) Map of plankton abundance (ind.m⁻³). (b) Map of plankton biovolume (mm.m⁻³). (c) Map of Shannon diversity Index.



Figure B2. FlowCam Bongo 20 microns: (a) Map of plankton abundance (ind.m⁻³). (b) Map of plankton biovolume (mm.m⁻³). (c) Map of Shannon diversity Index.

692 693 Figure B3. ZooScan HSN 330 microns: (a) Map of plankton abundance (ind.m⁻³). (b) Map of plankton biovolume (mm.m⁻³). (c) Map of Shannon diversity Index.

(a)

(b)

(c)

695 696 697 Figure B4. ZooScan Manta 333 microns: (a) Map of plankton abundance (ind.m⁻³). (b) Map of plankton biovolume (mm.m⁻³). (c) Map of Shannon diversity Index.

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Figure 4. (a) and (b) Estimated cumulative error associated with partial validation of particles below a size cut-off threshold ranging from 200 to 600 pixels and validated fractions ranging from 5% to 50%. Errors are computed as the percentage Root Mean Squared Error (RMSE) between fully validated samples and partially validated samples in three different metrics for cumulative error in respectively, NBSS slope and communities composition (relative abundance). RMSE values represent the outcomes of simulations, each conducted three times for the four samples, with random sampling. (c) and (d) Cumulative error according to the Fractions chosen in respectively, NBSS slope and communities composition. The threshold is fixed at 500 pixels.

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753 Competing interest

The authors declare that they have no conflict of interest.

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