Expanding Tara Oceans Protocols for Underway, Ecosystemic Sampling of the Ocean-Atmosphere Interface During Tara Pacific Expedition (2016–2018)

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Interactions between the ocean and the atmosphere occur at the air-sea interface through the transfer of momentum, heat, gases and particulate matter, and through the impact of the upper-ocean biology on the composition and radiative properties of this boundary layer. The Tara Pacific expedition, launched in May 2016 aboard the schooner Tara, was a 29-month exploration with the dual goals to study the ecology of reef ecosystems along ecological gradients in the Pacific Ocean and to assess inter-island and open ocean surface plankton and neuston community structures. In addition, key atmospheric properties were measured to study links between the two boundary layer properties. A major challenge for the open ocean sampling was the lack of ship-time available for work at “stations”. The time constraint led us to develop new underway sampling approaches to optimize physical, chemical, optical, and genomic methods to capture the entire community structure of the surface layers, from viruses to metazoans in their oceanographic and atmospheric physicochemical context. An international scientific consortium was put together to analyze the samples, generate data, and develop datasets in coherence with the existing Tara Oceans database.

Beyond adapting the extensive Tara Oceans sampling protocols for high-resolution underway sampling, the key novelties compared to Tara Oceans’ global assessment of plankton include the measurement of (i) surface plankton and neuston biogeography and functional diversity; (ii) bioactive trace metals distribution at the ocean surface and metal-dependent ecosystem structures; (iii) marine aerosols, including biological entities; (iv) geography, nature and colonization of microplastic; and (v) high-resolution underway assessment of net community production via equilibrator inlet mass spectrometry. We are committed to share the data collected during this expedition, making it an important resource important resource to address a variety of scientific questions.

Keywords: neuston/plankton genomics/taxonomy/imaging, aerosols, NCP, IOP, trace metals, microplastic

INTRODUCTION

Humans have accelerated the input of carbon dioxide into the atmosphere at unprecedented rates, and modern policy and governance in key countries suggests that there will be little effort to curb this tendency (Masson-Delmotte et al., 2018). About one-third of the anthropogenic carbon emissions is absorbed by the oceans which currently serve as a CO$_2$ sink, but this process is taking place at the cost of acidifying the oceans and further stressing its inhabitants (Gattuso et al., 2015). The ocean-atmosphere interface and its properties are undoubtedly critical to these processes and yet they remain understudied over scales as vast as the global oceans due to methodological and logistical challenges.

Ecosystems of the sea surface layers play an important role in the exchanges between oceans and the atmosphere (Cunliffe and Murrell, 2009; Wurl et al., 2017), impacting not only CO$_2$ fluxes via biologically derived surfactants (Pereira et al., 2018), but also the fluxes of aerosol particulates which are transported and deposited by winds. The ocean and the atmospheric boundary layers define an important coupled system that determines fluxes of gases, and acts as a key component of the climate system (Neukermans et al., 2018).

The uppermost layer of the ocean is the habitat of a specific community of organisms called neuston (Sieburth, 1983; Liss et al., 2005; Zaitsev and Liss, 2005). Neuston is closely connected to plankton communities and similarly comprises all domains of life, including prokaryotes (bacteria and archaea), unicellular eukaryotes (protists), fungi, metazoans as well as viruses. Interestingly, it also includes enhanced abundances of eggs and larvae of organisms living in the water column and on the seafloor (Zaitsev, 1971; Sundby, 1991; Wurl and Obbard, 2004), as well as insects, and floating organic and inorganic debris. This marine litter, including plastic, can be rapidly colonized by microbial and macrobial communities (Dussud et al., 2018a) and will then act as dispersive vectors for a variety of organisms (Barnes, 2002; Zettler et al., 2013; Viršek et al., 2017).

Aerosols are key players in the atmospheric radiation balance by absorbing and reflecting part of the incoming radiations back to space and by modulating cloud properties (Charlson et al., 1987; Boucher et al., 2013). Aerosolization can be a common mechanism for various elements aerial dispersal including iron
SAMPLING STRATEGY

The Tara Pacific expedition was based on the same multi-disciplinary, systems ecology approach that was pioneered during the Tara Oceans expedition (Karsenti et al., 2011; Pesant et al., 2015). The first distinctive feature of our approach was to combine state-of-the-art biogeochemical, imaging and genetic methods to assess the structure and functions of an ecological system. The second was to apply these multi-disciplinary methods across the full spectrum of system components. The third distinctive feature was to characterize this spectrum according to the size and classification of chemical and biological entities. Our approach provides both a conceptual framework that helps formulation of scientific questions, and a practical framework that helps making well-informed compromises toward a coherent set of protocols and a realistic sampling strategy. This blueprint ensured that in the present study, the entire spectrum of physical, chemical and biological entities was considered to characterize the atmosphere, the ocean surface and their interface, using both continuous and discrete measurements.

During the Tara Pacific expedition, instruments were deployed and recovered from the water at cruising speed (up to 9 knots), so that each sampling site represents a stretch of ocean of 5–20 nautical miles. The expedition completed a longitudinal sampling of the surface community and genomic compositions of the north Atlantic and of the southern and northern Pacific (Figures 1, 2). The western Pacific was also sampled latitudinally and a special focus was given to the north-eastern Pacific “Great Pacific Garbage Patch (GPGP)”.

Marine aerosols, defined as the aerosols present in the marine atmospheric boundary layer, consist of a mixture of natural and
anthropogenic components. Processes governing marine aerosols production and deposition are still not well understood, and can vary spatially and temporally depending on environmental forcing. Therefore, we used a methodology that couple continuous measurements with interval sampling to identify patterns, diversity and possible physical and biological drivers.
Continuous Atmospheric Measurements

A meteorological station (BATOS-II, Météo France) measured air temperature, relative humidity, and atmospheric pressure at \( \sim \)7 m above sea level. Wind speed and direction were measured at \( \sim \)27 m above sea level. In October 2016 a Photosynthetically Active Radiation (PAR) sensor (Biospherical Instruments Inc. QCR-2150) was mounted at the stern of the boat (\( \sim \)7 m altitude). Data were recorded continuously and binned by minute.

Continuous Sampling of Aerosol Particles

Two instruments were installed aboard Tara to measure the size distribution and abundance of atmospheric aerosol particles. A scanning mobility particle sizer (SMPS, SMPS-C GRIMM Aerosol Technik Ainring GmbH & Co. KG, Ainring, Germany) measuring particles in the size range 0.025 – 0.70 \( \mu \)m, and an optical particle counter (OPC; EDM180 GRIMM Aerosol Technik Ainring GmbH & Co. KG, Ainring, Germany) measuring particles in the size range 0.25 – 32 \( \mu \)m. Aerosols were pumped through an inlet mounted on the rear backstay of Tara. During the initial crossing of the Atlantic Ocean in May-June 2016, the inlets extended half way up the backstay (\( \sim \)15 m altitude) and were relocated to the top of the backstay, near the mast (\( \sim \)27 m altitude) for the remainder of the expedition. The SMPS was set to perform a full scan of particle distribution every 5 min and the OPC produced a particle size distribution every 60 s; both instruments were set to measure continuously throughout the expedition. The analysis of the size distributions will help us to identify areas of interest that will be studied thoroughly using satellite measurements, reanalysis of meteorological data and other on-board oceanic measurements.

Interval Sampling of Aerosol Particles

A separate inlet was mounted on the rear backstay of Tara, next to the one used for continuous measurements (previous paragraph). Aerosols collected from this second tubing were vacuum-pumped (Diaphragm pump ME 16 NT, VACUUBRAND GmbH & Co KG, Wertheim, Germany) through four 47 mm filter holders mounted in parallel. Three filter holders contained m pore size PVDF membrane filters, and the fourth filter holder contained a 0.8 \( \mu \)m pore size polycarbonate filter. Filters were generally changed twice a day, collecting aerosols for a period of at least 12 h at flow rates of 20–30 Lpm through each filter holder. PVDF filters were packaged in cryotubes and immediately stored into liquid nitrogen, whereas polycarbonate filters were packaged in sterile PetriSlide preloaded with absorbent pads and stored dry at room temperature. Particles from the PVDF filters will be extracted to assess microbial diversity using metagenomics and 16S and 18S rDNA amplicon sequencing. A scanning electron microscope Energy dispersive X-ray analyzer (SEM-EDX) will be used to quantify the size, shape, quantity and elemental composition of all aerosols greater than 0.8 \( \mu \)m from the polycarbonate filters. Additionally, time-lapse cameras were installed to record the state of the ocean and clouds. See Flores et al. (accepted) for a detailed description of the aerosol measurements.

The aerosol inlet funnels were directed downward to prevent rain from entering the inlets and to not bias the sampling to one direction. To clean the data from possible contamination from the engines exhaust, the periods when the engines were turned on were recorded and the possible contamination periods detected. If increases in total aerosol concentration were identified in the transitions from sailing to engines on, the data was excluded. Additionally, contamination periods were also identified in the SMPS-OPC system as a single mode size distribution with a mode diameter of less than 0.04 \( \mu \)m and total particle concentrations of \( 10^5 \) cm\(^{-3} \). Contamination periods were identified only for the first Atlantic crossing and were excluded from the analysis. The particle loss through the inlets was calculated using the Particle Loss Calculator (Von der Weiden et al., 2009) to assess the collection errors. Due to the differences in height of the inlet for the first Atlantic crossing (the inlet height was \( \sim \)15 m) and the rest of the expedition (the inlet height was \( \sim \)27 m), two separate calculations were done. All size distribution measurements were corrected accordingly.

Discrete Sampling of Aerosol Optical Thickness

Additionally, daily measurements of atmospheric transmittance were performed using a five channel Microtops II Sunphotometer loaned from NASA (Smirnov et al., 2006) and a radiometer system called SIMBADA (Deschamps et al., 2004) when the sun disk was clear from any cloud. These parameters are all useful to link the in situ inherent optical proprieties to satellite-based measurements, in order to validate and improve the space-based algorithms for ocean and atmospheric products.

Model and Satellite Derived Data

Mercator Ocean expertise and daily operational service provided a complete set of environmental parameters in the form of oceanographic maps and data from Copernicus Marine Service and Mercator Ocean prediction models including various physical parameters, from the surface to 50 m depth with a spatial resolution of 1/12\( ^\circ \) (Lellouche et al., 2018), and chemical and biogeochemical parameters, from the surface to 100 meters deep, with a 1/4\( ^\circ \) spatial resolution (Perruchez et al., 2016). Reanalyses products from those models will be updated at latter stage.

Various satellites derived environmental parameters (e.g., SST, SSH, Chl \( \alpha \), productivity and other) will also be retrieved from NASA and Copernicus Marine Service databases.

Underway, Continuous Sampling of Surface Water

Surface seawater was pumped continuously through a hull inlet located 1.5 m under the waterline using a membrane pump (Shurflo), circulated through a vortex debubbler, and distributed to a number of flow-through instruments (Figure 3). The instruments comprise a thermosalinograph (TSG, SeaBird Electronics SBE45/SBE38), a spectrophotometer (WETLabs ACS), a CDOM fluorometer (WETLabs WSCD), a backscattering sensor (WETLabs ECO-BB3), and an Equilibrator Inlet Mass Spectrometer [EIMS] (Pfeiffer Vacuum Quadrupole 1–100 amu). The TSG was set to log time in coordinated universal time (UTC) synchronized with the ship's GPS. All instruments were then synchronized to the TSG.
**Temperature and Salinity**

The thermosalinograph (TSG, Seabird SBE45) was mounted 5.5 m downstream from the intake and measured sea surface temperature and conductivity at a sampling frequency of 0.1 Hz. A second temperature sensor (SBE38) was mounted 1.5 m downstream from the intake, upstream of the pump and the debubbler. Temperature and salinity measurements were quality checked throughout the expedition. The measurement errors of temperature are on the order of 0.01°C, whereas salinity records usually had uncertainties between 0.01 and 0.05. Salinity measurements were corrected after the analysis of unfiltered seawater samples and validated against Argo floats data in the vicinity of Tara. A systematic collocation of close Argo profiles in distance/time (±100 km and ±5 days) allowed a point-by-point comparison. The Argo data used were from 5 m depth while Tara data were from 1 m but excluding regions of strong vertical stratification (not sampled during *Tara* Pacific) the difference was negligible. The sensors were recalibrated by SeaBird at the end of the expedition. Their drifts were negligible.

**Net Community Production**

The Equilibrator Inlet Mass Spectrometer (EIMS, Cassar et al., 2009) was mounted 3.5 m downstream from the intake and provided high-resolution measurements of the ratio of dissolved oxygen and argon (i.e., O₂/Ar). While oxygen and argon have a similar solubility in seawater, argon is biologically inert, whereas oxygen is involved in biological processes such as photosynthesis and respiration. The O₂/Ar ratio is therefore a measure of oxygen saturation associated with biological processes (i.e., biological O₂ saturation), providing an estimate of net community production (NCP) (Cassar et al., 2009). As described in Cassar et al. (2009), errors in the biological oxygen anomaly as measured with the EIMS are on the order of ±0.3%. The uncertainty in NCP calculations, c. 30%, is mostly associated with the wind speed parameterization of the gas exchange velocity. However, additional biases may be associated with effects of a lack of steady state and vertical mixing on the biological oxygen anomaly at the ocean surface (see Cassar et al., 2014).

**Inherent Optical Properties (IOPs)**

High frequency (1–4 Hz) measurements of IOPs were performed to derive numerous biogeochemical parameters. A WetLabs ACS (Figure 3) measured hyperspectral (~4 nm resolution) attenuation and absorption in the visible and near infrared; a WetLabs BB3 mounted in a ~4.5 L BB-box measured the volume scattering function (VSF) at 3 wavelengths (470, 532, 650 nm) and a WetLabs WSCD measured fluorescent colored dissolved organic matter (CDOM). We used a calibration independent technique (Slade et al., 2010) to obtain particulate absorption (ap), attenuation (cp), and particulate backscattering (bbp) by subtracting measurements made without filter by measurements with a 0.2 μm filtered seawater, switching between those periods was done every 60 min (50 min total water, 10 min filtered). Instruments were cleaned and filters were changed weekly or before starting a new leg. The datasets were manually checked, periods removed were mainly associated with saturated sensors, accumulation of particles in the BB-box, extended periods without filtered times (failure of switching), and strong changes in flow rate. The quality-checked IOPs can be used to retrieve proxies of phytoplankton biomass (chlorophyll a, with a detection limit of 0.03 mg m⁻³ and a maximum relative error of 38%, see Boss et al., 2013) and phytoplankton carbon
(Graff et al., 2015) concentrations, particulate organic carbon (with about 20% of variability due partly to the phytoplankton community composition; see Cetinić et al., 2012), relative FDOM (fluorescent dissolved organic matter), with a relative error ranging between 4 and 40% depending on CDOM concentration as a proxy for dissolved organic matter concentrations (Belzile et al., 2006), particle size index (y, 11% of variability as given in and Slade and Boss, 2015), and major phytoplankton pigments (30–50% of variability; Chase et al., 2013, 2017). These parameters provide key information about ecosystem structure, function (Falkowski et al., 1998, 2003; Le Quéré et al., 2005; Hood et al., 2006), and carbon export (e.g., McCave, 1975; Eppley and Peterson, 1979; Falkowski et al., 1998; Buesseler et al., 2007).

**pH**

Seawater was collected once a week from the outlet of flow-through to determine pH. Surface seawater pH on total scale was determined using meta-Cresol Purple, a precise pH indicator dye (Clayton and Byrne, 1993; Dickson et al., 2007). Surface seawater samples were acclimated to 25°C for 2–3 h in closed, 5-ml polypropylene tubes. Absorbance at specific wavelengths was read, and after of 40 μL meta-Cresol Purple dye addition using an Agilent Technologies Cary 60 UV-Vis Spectrophotometer equipped with an optical fiber. TRIS buffer solutions (Nemzer and Dickson, 2005) were measured regularly along the cruise to validate the method. This spectrophotometric pH measurement is about ±0.001 pH units precise (Dickson et al., 2007). However, a bias of pH measurements can occur if the temperature is not well controlled during the spectrophotometric pH reading. For the *Tara* Pacific expedition, due to often-difficult conditions, analytical uncertainty on pH values have been reassessed to ±0.01 pH units.

**Discrete Sampling of the Ocean-Atmosphere Interface**

Two sampling devices were developed to collect seawater and organisms from the ocean-atmosphere interface while sailing at a maximum speed of 9 knots. The “Dolphin” sampler was designed to collect seawater and neuston of size < 2000 μm (Figure 4), whereas the “High Speed Net” (HSN) was designed to collect plankton and neuston of size > 300 μm (Figure 4). The two devices are made of stainless steel and have the same front aperture (4 cm wide and 40 cm high). The back end of Dolphin is fitted with a 4 cm diameter reinforced tubing connected to a large volume industrial peristaltic pump (max flow rate = 3 m³ h⁻¹) on the deck, whereas the HSN is 200 cm long, equipped with a nytrel net of 330 μm mesh size and with a cod-end collector. A mechanical flowmeter is mounted in the inlet opening of the HSN net. The two sampling devices were deployed simultaneously, the Dolphin near the deck for ~ 120 min and the HSN for 60–90 min, 50 – 60 m behind the ship on starboard, to avoid the wake (Figure 4). Their detailed description will be given elsewhere.

In addition to the high-speed sampling devices, near the islands and in the Great Pacific Garbage Patch a Manta net was used for surface sampling of neuston, plankton and microplastics (Figure 5). The Manta net used during *Tara* Pacific expedition had a 16 × 60 cm opening and a 400 cm long nytrel net of 330 μm mesh size. A Mechanical Flow Meter was mounted in the inlet opening. The maximum towing speed of the Manta net is rated to 3 knots, speed which allows for a wider mouth opening, six times that of the HSN.

The water inlet design of the HSN decreases the pressure of the entering water flux. Soft-bodied plankton species are more damaged when sampled by the HSN at 5 to 9 knots speed than crustaceans, but are still identifiable (Figures 5, 8). When comparing samples from the two nets, community composition and normalized biovolume size spectra are similar.

Chemical preservation of samples was done outside the wetlab (Figure 6, left side, facing the stern) and seawater pumped from the Dolphin was collected and pre-filtered on the outer wall of the wetlab as shown in Figures 6, 7 (right side, facing the bow). Chemical preservation of samples was done outside the wetlab (Figure 6, left side, facing the stern). Inside the wetlab, two filtration systems were used, one for 142-mm-diameter filters, and the second for 25- and 47-mm-diameter filters. The first system was used for the serial filtrations of small size fractions (<0.22, 0.22–3, and 3–20 μm) for genomic analyses. The second system was used to concentrate particulate matter in the large size fractions (20–2000 and 300–2000 μm) for genomic analyses, and to collect material on glass fiber filters (0.7 μm) for pigment analyses.
FIGURE 6 | Surface water from the Dolphin was distributed to different outlets installed on the external wall of the wetlab. (1) Unfiltered sea water, used mainly for biogeochemical sampling; (2) pre-filtered seawater on a 20-µm-pore-size filter mounted inside a 142 mm diameter filter holder; (3) 50 L carboys for the collection of 20 µm prefiltered seawater; (4) 20-µm-mesh-size decknet. Water was collected from one outlet at a time and the volume of water was measured using a flowmeter; in the case of excess pressure, water was evacuated through an overpressure valve.

Finally, an extendable carbon-fiber pole equipped with a sturdy PVC bottle holder was used to sample surface seawater uncontaminated by the ship. The pole was hand-held off the bow of the ship while sailing at up to 9 knots. The uncapped bottle attached at the end of the pole was rinsed 4–5 times with surface seawater, then filled, and re-capped using polyethylene gloves.

Macronutrients
Seawater was collected from the unfiltered seawater circuitry [Figure 6(1)] connected to the Dolphin at each sampling site to determine the nitrate (NO$_3$), nitrite (NO$_2$), phosphate (PO$_4$) and silicate (Si(OH)$_4$) concentrations. Seawater was immediately filtered through a 0.45 µm pore size cellulose acetate membrane with a syringe, dispensed into duplicate sterile 20 mL polyethylene vials, and stored at −20°C until analysis at the Laboratory of Microbial Oceanography (LOMIC-UMR 7621) in Banyuls-sur-Mer, France. The precision of the measurements and the detection limits respectively were ±0.02 and 0.02 µM for nitrate (NO$_3$), ±0.005 µM and 0.01 µM for nitrite (NO$_2$), ±0.005 and 0.01 µM for phosphate (PO$_4$), and ±0.01 µM and 0.03 µM for silicate (Si(OH)$_4$). The concentrations were measured by colorimetry on a continuous flow Seal-Bran Luebbe$^\text{®}$ AutoAnalyzer III (Aminot and Kérouel, 2007).

Carbonate Chemistry
Unfiltered seawater was collected once a week from the seawater circuitry [Figure 6(1)]. Glass bottles (500 mL) were filled with
unfiltered seawater, poisoned with Hg₂Cl₂, and stored on board at room temperature until later analysis at the SNAPCO2 facility at Sorbonne University in Paris, France. Total Alkalinity (TA) and Total Inorganic Carbon (TC) were measured, and all carbonate chemistry parameters were computed following the SNAPCO2 protocol (Edmond, 1970; DOE, 1994). External reproducibility obtained for standards solutions for both parameters, TA and TC, is about 3 μmol/kg (0.15%). Deduced seawater pH values will be compared to the underway surface seawater pH measurements previously described.

Trace Metals
The task of quantifying extremely low levels of trace metals in seawater is complicated by sea vessels being a source of metal contamination, and therefore specialized oceanographic equipment and sampling procedures have been developed for collection in the water column (Measures et al., 2008). However, these procedures are typically specific to crane vessels, and sampling onboard the Tara schooner therefore required a basic surface-sampling protocol with similar metal-free handling precautions. Unfiltered seawater was collected using a custom-made hand-held bow-pole at each sampling site to determine total dissolvable iron (Fe), zinc (Zn), cobalt (Co), copper (Cu), manganese (Mn), nickel (Ni), and lead (Pb). Samples were collected off the bow of the boat while oriented toward the wind to minimize contamination, and polyethylene gloves were used during bottle handling. Low-density polyethylene (LDPE) bottles (125 mL) were cleaned on land by soaking overnight in 1% Citranox detergent, rinsed thoroughly, then soaked for at least 1 week in 10% HCl, followed by at least 8 rinses in ultrapure water, and individually enclosed in plastic bags to reduce contamination during transport and storage. Samples were stored in separate plastic Ziploc bags on-board at ambient temperature in the dark. At the University of Southern California (USC), samples were stored and processed in a class −100 clean room. Seawater was acidified with 0.1% 12 N HCl for 4–6 months to liberate the ‘total dissolvable’ metals (Sedwick et al., 2011). This approach captures total dissolved metals which might have precipitated during storage, in addition to acid labile particulate metals. However, this method does not dissolve or include metals from refractory particles (Sedwick et al., 2011). 15 mL subsamples were then analyzed. Samples were amended with a mixture of isotope spikes to facilitate analysis by isotope dilution, concentrated and purified from major seawater salts using a seaFAST system, and analyzed for elemental concentrations using an Element2 ICPMS. Samples were run alongside GEOTRACES standards to confirm accuracy, with concentrations within the community consensus range. The trace metal concentrations were found to have an average error of 5% (Supplementary Figure 1).
Pigments
Unfiltered seawater was collected with the Dolphin for the determination of pigment concentrations. Two liters of seawater were filtered on a 25-mm-diameter, 0.7-µm-pore-size glass fiber filter (Whatman GF/F). Filters were immediately stored in liquid nitrogen until later analysis by High Performance Liquid Chromatography for pigment analysis (Ras et al., 2008) at the Laboratoire Oceanographique in Villefranche-sur-Mer, France.

Bioluminescence
Stimulated bioluminescence was measured at night, starting in Taiwan (2017/04/30), using an Underwater Bioluminescence Assessment Tool, UBAT (WETLabs). The surface water was pumped from the Dolphin at a rate of 20 L/min into a 5 L open reservoir that was continuously overflowing. The U-BAT was pumped from water this reservoir at a flow rate of 20 L/min and measured the bioluminescence for ~50 min while the bioluminescence was determined for pigment concentrations. Two liters of seawater were filtered on a 25-mm-diameter, 0.7-µm-pore-size glass fiber filter (Whatman GF/F). Filters were immediately stored in liquid nitrogen until later analysis by High Performance Liquid Chromatography for pigment analysis (Ras et al., 2008) at the Laboratoire Oceanographique in Villefranche-sur-Mer, France.

Neuston and Plankton in the <20 µm Size Fraction
Seawater was collected at each sampling site from the Dolphin and prefiltered through a 20 µm-pore-size nylon membrane mounted in a 142-mm-diameter filter holder installed on the wetlab's outer wall [Figures 6(2), 7]. The filtrate was used to prepare the samples of the 3–20, 0.22–3, and <0.22 µm size fractions. Two replicates of this filtrate (1.5 mL) were fixed with Glutaraldehyde (0.25% final volume) and Poloxamer (0.1% final volume) for 15 min at 4°C and then flash-frozen for later analyses using a FACS Canto II Flow Cytometer equipped with a 488 nm laser (Marie et al., 1999) at the Station Biologique in Roscoff, France. Two replicates of 4 mL filtrate were mixed with 600 µL of 48% betaine aliquot and preserved in liquid nitrogen for later single cell genomics.

Two 50 L replicates of 20 µm prefiltered seawater were size fractionated in the wetlab into the 0.22–3 µm and 3–20 µm fractions using 142-mm-diameter, stainless-steel filter holder “tripods” (Millipore) and a peristaltic pump (Masterflex). Each replicate was filtered through a 142-mm-diameter, 0.22-µm-pore-size polyethersulfone Express Plus membrane filter and a 142-mm-diameter, 3-µm-pore-size polycarbonate membrane filter placed on top of a woven mesh spacer Dacron 124 mm (Millipore). The two filters were mounted in series to collect neuston and plankton in the 0.22–3 and 3–20 µm size fractions. To ensure that at least one of the two replicates yielded high quality RNA for transcriptomics analysis, the filtration of the first replicate was stopped after 15 min, whereas the second was allowed to go on for up to 60 min. Filters were packaged into 5 mL cryovials and preserved in liquid nitrogen immediately after filtration, until later sequencing at Genoscope, CEA, France.

Two 10 L replicates of the previously 0.22 µm filtered seawater were flocculated using iron chloride solution at room temperature for 1–12 h. The flocculated particulate material containing viruses (John et al., 2011) was concentrated onto a 142-mm-diameter, 1-µm-pore-size polycarbonate membrane filter placed on top of a woven mesh spacer Dacron 124 mm (Millipore). Filters were packaged in 5-mL cryotubes and stored at +4°C for later sequencing analysis at Ohio State University in Sullivan lab.

Neuston and Plankton in the 20–2000 µm Size Fraction
The seawater pumped from the Dolphin was prefiltered through a 2000 µm metal sieve and concentrated with 20 µm decknet suspended on the outside wall of the wetlab [Figures 6(4), 7]. The volume of water concentrated in the net was measured using a flowmeter and, after 60–120 min of continuous pumping, the net was rinsed thoroughly from the outside with filtered seawater (<0.22 µm). The sample concentrated in the cod end was diluted to 2 L and divided into six subsamples. (1) 4 × 250 mL were filtered onto a 47 mm diameter, 10-µm-pore-size polycarbonate membrane filter, packaged in 5 mL cryotubes and stored in liquid nitrogen for later sequencing analysis, (2) 250 mL volume concentrated on a 20 µm mesh was preserved with acidic lugol in a 50 mL Falcon tube and stored at 4°C for enumeration and identification of microplankton using traditional inverted microscopy as detailed in Villar et al. (2015), (3) 45 mL was preserved with 5 mL of buffered formaldehyde (4% v/v) in a 50 mL Falcon tube and stored at room temperature for enumeration and identification of microplankton using traditional inverted microscopy as detailed in Malviya et al. (2016), (4) 250 mL concentrated on a 20 µm mesh was resuspended in a 50 mL Falcon tube with pure ethanol. After 24 h the sample was concentrated again and preserved in a 15 mL Falcon tube with pure ethanol for genomic and morphological analyses. (5) 45 mL from the 2 L cod-end content was transferred to a 50 mL Falcon tube and preserved with 5 mL aliquot of buffered 1% monomeric formaldehyde and 0.25% EM grade glutaraldehyde (Marie et al., 1999) for later environmental High-Content Fluorescent Microscopy analysis (eHCFM; Colin et al., 2017), (6) 250 mL was sieved through a 200 µm filter and the size fraction between 20 and 200 µm was analyzed live on-board using the FlowCam imaging system (Fluid Imaging Inc.; Sieracki et al., 1998).

In the Great Pacific Garbage Patch, two decknet (20–500 µm size fraction) samples were collected and preserved in buffered formaldehyde (4%v/v) and stored at room temperature for later analysis of plastic fragments.

Neuston, Plankton and Microplastics in the >300 µm Size Fraction
Organisms and floating debris of size >300 µm were collected using the HSN and additionally in the vicinity of Pacific islands and in the Great Pacific Garbage Patch (GPGP) using the Manta net.

The daytime HSN cod-end was resuspended in 2 L of filtered seawater and split into two equal subsamples. The first was concentrated and preserved in a 250-mL plastic bottle with 25 mL tetraborax buffered formaldehyde (4%v/v) and stored at room temperature for later analysis with the ZooScan imaging system (Figure 8, Gorsky et al., 2010). The second subsample was sieved through a 2000 µm metal sieve and the filtrate, 300–2000 µm size fraction, was concentrated on four 47 mm diameter, 10 µm pore size polycarbonate membrane filters,
packages in a 5-mL cryotube and stored in liquid nitrogen for later sequencing analysis.

Fragments of plastic collected on the metal sieve (>2000-µm size fraction) were packaged individually in cryovials and stored in liquid nitrogen for genomic analysis of organisms attached to the fragments.

The night-time HSN cod-end was not split but entirely preserved with tetra borax buffered formaldehyde (4%v/v) for imaging and taxonomic analyses. The ZooScan analysis is not destructive (Figure 8), the samples remain entire and are stored in the Collection Center for Plankton in Villefranche-sur-Mer, France.

Genomic Protocols
Genomic analyses from all size fractions are conducted at the Genoscope, CEA, France. The protocol applied for nucleic acid extractions from the size fractions <0.22, 0.22–3 µm, 3–20 µm, 20–2000 µm, and 300–2000 µm is based on simultaneous extraction of DNA and RNA as previously described (Alberti et al., 2017).

DNA extracts are sequenced to analyze metagenomes and amplicons for eukaryotic (18SV9) and prokaryotic (16SV4V5) metabarcoding. The already analyzed DNA data show that 99% of the extracts are above the 500 ng threshold that allowed library construction with the standardized procedures used in the Tara Oceans project (Supplementary Figure 2). Symbiodiniaceae diversity (ITS2) (LaJeunesse et al., 2018) will be analyzed for the 3–20 µm size fraction only according to Hume et al. (2018, 2019).

RNAs extracts will be sequenced to analyze metatranscriptomes using different cDNA synthesis protocols depending on the size fractions (Alberti et al., 2014; Carradec et al., 2018).

Imaging Methods
The imaging methods used to study the entire size range of the surface community structure include standard inverted microscopy, high-throughput confocal imaging, FlowCam and ZooScan imaging for quantitative sorting and classification of respectively micro- and zooplankton, and flow-cytometry to monitor bacteria and small protists (Figure 9). The FlowCam and ZooScan methods allow a rapid classification of organisms to order, family, or functional type levels, and sometimes to the species level. Validation by experts allows a finer taxonomic tuning. Preserved samples of micro- and zooplankton including the neuston are stored at the CCPV (Collection Center of Plankton at Villefranche-sur-Mer, France) managed by the Laboratoire d'Oceanographie de Villefranche (LOV), France and EMBRC-France (European Marine Biological Resources Center), and are available for detailed taxonomic work to the scientific community.

Images generated by the FlowCam and the ZooScan are processed using the same Zooprocess software and the classification of each object is predicted automatically by the Quantitative Imagery Platform and validated with taxonomy experts using the web-based Ecotaxa Application (Picheral et al., 2017), a tool for the taxonomic classification of images which offers the world's largest database of classified planktonic images following the UniEuk taxonomic tree.

Data Management
Tara Pacific endorses the FAIR principles for scientific data management (Wilkinson et al., 2016) as well as ethical and responsible use of data. With a few exceptions, all physical samples were preserved on board Tara and sent back to partner laboratories for subsequent analysis. Controlled vocabularies describing sampling devices and sample preparation protocols were used throughout the expedition to capture provenance metadata on customized log sheets, and samples were assigned unique identifiers to facilitate their traceability (Table 1). Rich environmental context will be generated for each sample, using in situ measurements as well as climatology and remote sensing data obtained from NASA Ocean Color, the European Copernicus Marine Service and Mercator Ocean products. Legal documents regulating the collection, export and import of samples, as well as links to the CBD’s access and benefit-sharing clearinghouse1 will be provided for each sample as a first step toward ethical and responsible use of the Tara Pacific data. The detailed registry of all samples, including their provenance, environmental and legal context, is curated manually using simple semantics that enables machine and human-readable data discovery services. The structure of all data sets and metadata will be consistent and interoperable with that of the Tara Oceans expedition, with improvements when needed. Genomics data will be deposited in free, open access at the European Nucleotides Archive2, and environmental data will be deposited in free, open access at PANGAEA3. The two archives

1http://sites.google.com/view/ccpv/home
2https://ecotaxa.obs-vlfr.fr/
3https://unieuk.org/
4https://absch.cbd.int/
5https://www.ebi.ac.uk/ena
6https://www.pangaea.de/
Gorsky et al. Tara Pacific Underway Surface Sampling

FIGURE 9 | Schematic summary of the systems approach and “end-to-end” plankton sampling adopted during the Tara Pacific expedition. The size spectrum and classification of entities that characterize the ocean-atmosphere interface are depicted in the left section. The various devices and protocols used to sample the atmosphere (top section) and the ocean surface (bottom section) are shown, indicating which, biological physical or chemical entities are targeted by each protocol.

will be interlinked via sample provenance metadata registered at BioSamples.

ANTICIPATED RESULTS AND DISCUSSION

Wide-scale integrated studies such as the Tara Pacific expedition are rare. In order to allow for cross-comparison of diverse environments, the protocols have to be consistent. The major constrain we encountered during Tara Pacific was the lack of ship-time available for work at “stations”. This constraint forced us to develop two high-speed sampling devices. The High Speed Net for the sampling of plankton and neuston > 300 µm and the “Dolphin” for surface water pumping.

The first high-speed zooplankton samplers were developed in the early 1900s, and the best known is the Hardy continuous plankton recorder (see the review in Wiebe and Benfield, 2003), the latest is the AVANI trawl (Eriksen et al., 2018). However, the constraint of deployment and retrieval of the instruments at cruising speed forced us to design a robust, relatively small and user-friendly instrumentation. Hull and deck pumping were also used in the past (Harris et al., 2000), mainly for chemistry and phytoplankton studies. Here we present the first system, to our knowledge, adapted for discrete sampling of the whole, end-to-end, planktonic ecosystem, from viruses to metazoaa, deployed and recovered at cruising speed (5–9 knots). This system can be easily adapted to ships of different size and propulsion.

The combined deployment of the High-Speed Net (HSN) and the Dolphin allowed us without major modifications of the cruise timing to reach a reasonable sampling density and collect the full set of parameters (Figure 2) nearly every cruising day and almost every night (Figure 10). Several large-scale hydrological features were sampled, including currents (Kuroshio, East Australian current, California and Equatorial currents, Gulf-Stream, North Atlantic drift, Labrador current), gyres (North and South Pacific, North Atlantic) and upwellings (equatorial, California). Moreover, the study of Pacific coral reef ecosystems during the Tara Pacific expedition (Planes et al., 2019) provided a rare opportunity to study small-scales hydrologic features such as the wake of Pacific islands.

The Tara Oceans expedition was set up for the generation of open science resources linking plankton diversity, genomics and the environmental context in the subsurface, deep chlorophyll and mesopelagic layers. The surface ocean study undertaken in the framework of the Tara Pacific expedition followed the same cross-disciplinary approach that was used during Tara Oceans.

Our set of multi-disciplinary (physical, chemical, biogeochemical, morphological, and genomic) and end-to-end (from viruses to metazoan eukaryotes) protocols (Figure 9 and Table 1) were realized at 249 sampling stations during daytime (Figure 2), whereas only the morphology of organisms > 300 µm was assessed during nighttime at 185 stations (Figure 10). A total of 89 Manta net tows and 13 Decknet samples were dedicated to sample microplastics at specific locations close to South Pacific

https://www.ebi.ac.uk/biosamples/
islands and in the North Pacific (Figure 2). More than 20 ocean biogeographical provinces (Longhurst, 1998) were sampled day and night during the Tara Pacific expedition (Figure 10).

Key novelties of our sampling protocols compared to Tara Oceans' global assessment of plankton include the measurement of (i) neuston vs. plankton biogeography and functional diversity; (ii) bioactive trace metals distribution at the ocean surface to explore metal-dependent ecosystem structures and metabolism; (iii) marine aerosols, including biological entities and plastic, to model their biogeography, (iv) high-resolution underway assessment of NCP via equilibrator inlet mass spectrometry and (v) the characterization of surface microplastic and attached communities.

The resulting datasets will complement and extend the Tara Oceans database, and constitute a new data collection for surface Atlantic and Pacific. Furthermore, they will add an oceanic context to the coral studies conducted during the Tara Pacific expedition. We outline here a few scientific questions that can be addressed using data collected during this expedition.

**Bioavailability of Micro- and Macronutrients**

Life in the sunlit ocean depends not only on the macronutrients nitrogen, phosphorus and silicon, but also on several trace elements needed to carry out biochemical processes including iron (Fe), zinc (Zn), manganese (Mn), nickel (Ni), copper (Cu), cadmium (Cd), and cobalt (Co). The distribution of these metals in the ocean is controlled by various biological, geochemical and physical processes influencing their chemical state and bioavailability (Bruland and Lohan, 2004). Iron is a key micronutrient required for photosynthesis, cellular respiration and nitrogen assimilation, and is therefore a major micronutrient influencing the biological cycling of carbon. However, iron is directly limiting to phytoplankton growth in approximately one-third of the ocean (Moore et al., 2001); it is generally low in concentrations in the South Pacific and Northeast Pacific Oceans (Behrenfeld et al., 2006; Sunda, 2012) while being relatively high in the North Atlantic due to aerosol input from the African continent (Jickells et al., 2005). Other trace metals similarly play critical roles in cellular functions, such as manganese and copper being utilized in the photosynthetic electron transport chain, zinc and nickel required for the assimilation of macronutrients such as phosphorus and nitrogen, and cobalt serving as the central coordinating ion of the micronutrient vitamin B₁₂ (Twining and Baines, 2013). The regional biogeochemical processes controlling the bioavailability of these metals and their role in regulating plankton community composition, metabolic functionality and ecological interactions has not yet been fully explored across large surface gradients of the North Atlantic, North Pacific, and South Pacific Oceans.

During the Tara Pacific expedition, samples were collected from low iron, phosphorus, and nitrogen regions where primary growth limitation and/or co-limitation due to these elements is expected (Moore et al., 2013). Locations were targeted where locally sourced additions of nutrients and metals could influence microbial ecology and metabolic processes, such as in the vicinity of islands (Doty and Oguri, 1956; Caputi et al., 2019) or along upwelling zones. Combining trace metal data along with “omic” information, including microbial community composition and inferred functionality, will ideally provide high resolution insights into the strategies adopted by plankton when responding to various growth limiting factors, at multiple spatial scales and ecosystem levels.

**Ecosystem Connectivity**

Wind, waves and surface currents are known to accelerate biological dispersion to new locations (Zaitsev, 1971; Fraser et al., 2018; Onink et al., 2019). However, we do not know to what extent these processes may impact the survival and dispersion of organisms such as coral larvae or enhance
**TABLE 1** | Summary of the Tara Pacific “ecosystemic sampling of the ocean-atmosphere interface” protocols, targeted entities and analyses of samples from above, below and the sea-surface.

| Environment                  | Altitude or Depth | Mode of sampling events | Frequency of sampling events | Duration of sampling events | Device of sampling events | Targeted entity | Size fraction of targeted entities | Classification of targeted entities | Targeted analysis                           |
|------------------------------|-------------------|-------------------------|------------------------------|-----------------------------|---------------------------|------------------|------------------------------------|--------------------------------------|----------------------------------------|
| Atmosphere (A)              | 5 m               | Continuous              | 1 every 1 min                | Instantaneous               | PAR sensor               | Apparent optical properties | na                   | na                                   | Photosynthetic active radiation        |
|                              | 5–26 m            |                         | 1 every 60 min              | Instantaneous               | BATOS                     | Atmospheric conditions    | na                   | na                                   | Air temperature and relative humidity, wind speed and direction, atmospheric pressure |
|                              | 26 m              | Semi-continuous         | 1 every 5 min               | SMPS                        | Aerosol particles        | 0.025–0.45 µm           | Inorganic, organic, virus, prokaryotes and pico-eukaryotes | Size distribution and abundance          |
|                              |                   |                         |                              |                             | EDM1S0                    | 0.25–32 µm              | Inorganic, organic, virus, prokaryotes and pico-eukaryotes | Size distribution and abundance          |
|                              |                   |                         | 1 every 12 h                | Air pump, intake on the mast| Aerosol particles        | >0.45 and >0.8 µm       | Inorganic, organic, virus, prokaryotes and pico-eukaryotes | Biogeochemistry, imaging and genomics  |
|                              |                   |                         | 12 h (day and night)        |                             |                           | Diffuse reflectance     | na                   | na                                   |                                        |
|                              |                   |                         | 1 every 24 h                | SIMBADA                     | Apparent optical properties | Carbonate ions, and lithium (Li) and bromine (Br) isotopes | Concentration                                    |
|                              |                   |                         | (daytime)                   |                             |                           | Aerosol optical thickness | Na                   | Na                                   |                                        |
|                              |                   |                         | 10 min                      | Dolphin                     | Whole water               | Macronutrients <0.45 µm | Nitrate (NO₃⁻), nitrite (NO₂⁻), phosphate (PO₄³⁻) and silicate [Si(OH)₄⁻] | Concentration                                    |
|                              |                   |                         |                              |                             |                           | Trace metals >0.7 µm    | Fe, Zn, Cu, Mn, Ni, Pb | concentration                                  |
|                              |                   |                         |                              |                              |                           | Plankton and neuston <3 µm | Inorganic, organic, virus, prokaryotes and pico-eukaryotes | Size distribution and abundance imaging and genomics |
|                              |                   |                         |                              |                              |                           | <0.22 µm               | Viruses                 | Genomics                                           |
|                              |                   |                         |                              |                              |                           | 0.22–3 µm              | Nano-eukaryotes¹,²     | Imaging and genomics (flow cytometry & single-cell genomics) |
|                              |                   |                         |                              |                              |                           | 3–20 µm                | Nano-eukaryotes¹,²     | Genomics                                           |

(Continued)
| Environment | Altitude or Depth | Mode of sampling events | Frequency of sampling events | Duration of sampling events | Device of sampling events | Targeted entity | Size fraction of targeted entities | Classification of targeted entities | Targeted analysis |
|-------------|------------------|--------------------------|-----------------------------|----------------------------|--------------------------|-----------------|-----------------------------------|------------------------------------|------------------|
| Ocean surface (0) | 1.5 m            | Continuous               | 1 every 1 min               | Instantaneous              | Thermosalinograph (TSG)  | Hydrographic conditions | na                  | Chromophoric dissolved organic matter (CDOM) | Concentration            |
|              |                  |                          |                             |                            | WCSD sensor              | Inherent optical properties | <0.2 µm            | Prokaryotes$^1$ and pico/nano/micro-eukaryotes$^1$ | Chi a, accessory pigments, POC, gamma |
|              |                  |                          |                             |                            | AC-s (spectral absorption and attenuation) | Pico-microplankton | 0.2–20 µm | Prokaryotes$^1$ and pico/nano/micro-eukaryotes$^1$ | Backscattering coefficient (at 470, 532 and 650 nm wavelengths); POC, phytoplankton carbon concentration (Cphyto) |
|              |                  |                          |                             |                            | BB3Backscatter sensor     | Pico-microplankton         | 0.2–20 µm | Prokaryotes$^1$ and pico/nano/micro-eukaryotes$^1$ | | |
|              |                  |                          |                             |                            | Equilibrator Inlet Mass Spectrometer (EIMS) | Dissolved gas | na | Oxygen and argon | Net primary production |
|              |                  |                          |                             |                            | Underway system outlet   | Seawater pH | na | | pH |

TABLE 1 | Continued
basin scale productivity across the tropical Pacific Ocean. The sampling design of the Tara Pacific expedition will help address the connectivity (a) between islands, looking for example at the distribution of coral and other larval stages; (b) between coastal and open ocean communities; (c) between coral holobiomes and pelagic microbiomes; (d) between atmosphere, land and sea surface, zooming on the role of sea spray aerosols as vectors of biotic and abiotic dispersion; and (e) between the ocean-atmosphere interface and the deeper ocean by comparing daytime and night-time community composition and the intrusion of vertically migrating plankton into the surface layer.

**Diurnal Cycles**

Diel vertical migration of plankton is one of the key features to understand the fate of the primary production (Steinberg et al., 2008). Zooplankton transfers matter and energy from primary production to large predators, including fisheries (Mackas and Beaugrand, 2010). The diel vertical migration contributes significantly to the biological pump by transporting carbon to depth (Giering et al., 2014) but also to the sequestration of CO₂ (Boyd et al., 2019). At the level of the ecosystem, this daily cycle is generally governed by the photosynthetic biomass production during the daylight and the nighttime respiration and biomass use through grazing. NCP reflects the balance between photosynthesis and respiration in the mixed layer. An excess of photosynthesis over respiration leads to a net production of organic matter available for export. The NCP therefore reflects the potential organic carbon export of the ecosystem (Li and Cassar, 2017). It may be expected that the community composition and interactions autotrophs vs. heterotrophs, the processes influencing carbon and energy fluxes in metabolic pathways in particular photosynthesis and respiration, and the balance between them should drive the spatial variability in NCP along Tara’s route. The systematic day and night net sampling carried out during the Tara Pacific expedition combined with the continuous acquisition of physical, chemical and biological parameters should stimulate studies on surface ocean and water column interactions on a variety of scales.

**Microplastic**

Surface microplastics represent a novel ecological habitat with a clear niche partitioning among bacteria living on plastics, organic particles and surrounding seawater (Dussud et al., 2018b). The sampling realized during Tara Pacific will complete the existing databases of surface microplastics spatial distribution and contribute to evaluate the interaction between the microplastics, neuston and plankton from individuals to communities. The geography of plastic fragments is not well understood, and ocean scale datasets like those assembled during the Tara Pacific expedition are important for realistic modeling of the fate of plastic pollution (Van Sebille et al., 2015; Onink et al., 2019). Plastic fragments are rapidly colonized and become a new artificial habitat for pelagic and benthic communities. Among other topics, the identification of species associated with plastics will help to establish colonization zones and spatial distribution of exotic, invasive and pathogenic organisms (De Tender et al., 2017; Jacquin et al., 2019).

**CONCLUSION**

We have entered a period of time where humans have greater impact on the Earth System than any geochemical cycles. The ocean-atmosphere interface represents a critical chokepoint for gas and nutrient exchange between these global engines of geochemistry. These surface boundary layer ecosystems are subject to myriad stressors in an already intense and strained environment. Such added stressors will undoubtedly alter the physico-chemistry of the ocean-atmosphere interface in ways that will impact the ocean’s ability to absorb the atmosphere’s (elevated) CO₂ levels. These responses will be complex, and their study requires cross-disciplinary and large-scale surveys to begin to map the community changes and possible mechanisms underpinning ocean-atmospheric processes. The underway sampling protocols presented here offer a first step toward these goals, and the coming data will be invaluable for catapulting the ocean-atmosphere science into the modern ‘big data’ era.

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DATA AVAILABILITY STATEMENT

The datasets generated for this study are available upon request to the corresponding author.

AUTHOR CONTRIBUTIONS

GG, FL, MLP, EBos, NCo, JF, and RT designed the study with input from SPE, DA, and EK. GG and FL directed the project. GB, FL, MLP, RT, and GG directed the field work. SPE, GB, and FL directed the data management and organized the field protocols. SPE, NCA, and EBoi managed the samples. GB, SRo, SA, NB, LC, DC, AE, JF, JG, NH, MH, YL, YM, MP, and MTRA collected samples at sea. GC and MTRA supplied the satellite data. GB, FL, MLP, EBos, NCA, NCo, JD, ED, AE, JF, JG, NH, SJ, RK, YL, DM, JP, MP-P, GR, SRe, MS, MTRA, and PW are involved in samples and data treatments. GG organized the manuscript with substantial input from GB, FL, MLP, CB, JD, and SPE. All the authors contributed significantly to the manuscript and Tara Pacific coordinators provided constructive criticism throughout the study and discussed the results and commented on the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2019.00730/full#supplementary-material
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