Technical Note

SISI: A New Device for In Situ Incubations at the Ocean Surface

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Abstract: The sea-surface microlayer (SML) forms the uppermost boundary layer between atmosphere and ocean, and has distinctive physico-chemical and biological features compared to the underlying water. First findings on metabolic contributions of microorganisms to gas exchange processes across the SML raised the need for new in situ technologies to explore plankton-oxygen turnover in this special habitat. Here, we describe an inexpensive research tool, the Surface In Situ Incubator (SISI), which allows simultaneous incubations of the SML, and water samples from 1 m and 5 m, at the respective depths of origin. The SISI is deployed from a small boat, seaworthy up to 5 bft (Beaufort scale), and due to global positioning system (GPS) tracking, capable of drifting freely for hours or days. We tested the SISI by applying light/dark bottle incubations in the Baltic Sea and the tropical Pacific Ocean under various conditions to present first data on planktonic oxygen turnover rates within the SML, and two subsurface depths. The SISI offers the potential to study plankton-oxygen turnover within the SML under the natural influence of abiotic parameters, and hence, is a valuable tool to routinely monitor their physiological role in biogeochemical cycling and gas exchange processes at, and near, the sea surface.

1. Introduction

The sea-surface microlayer (SML) at the air–sea interface spans two-thirds of the global surface area. Despite having a maximum thickness of 1 mm, the SML has profoundly different physico-chemical and biological characteristics compared to the underlying water column [1–3]. In addition, due to the accumulation of organic matter and surface-active molecules [4,5], the SML constitutes a unique biofilm-like habitat for microorganisms [3,6]. Life within the SML is heavily influenced by a range of "extreme" conditions such as enhanced solar and ultraviolet (UV) radiation [7–9], strong wind-wave dynamics [10,11] and the accumulation of pollutants [12]. Heterotrophic microbes in particular benefit from the enrichment of organic substrates, allowing them to thrive and form distinctive communities within the SML compared to the underlying water [13,14]. Increased abundance of heterotrophic microorganisms within the SML is a typical observation [15], and is accompanied by remarkably enhanced respiratory activity [16,17].

The light/dark bottle incubation technique is the primary approach used to measure planktonic production and respiration in surface water [18]. For assessment of the plankton's metabolic potential, it is common practice to incubate sea water samples in closed containers on deck [19], although this method is less compatible with adequate temperature control, especially in tropical regions [20]. Other approaches involve incubations in bottles attached to a conductivity-temperature-depth (CTD)

device [21], in floating racks attached to the mother-ship [9,22], or incubations are replaced by performing consecutive water sampling over several hours or days from the same depth [23]. Although a practical incubation method for assessing in situ oxygen (O_2) turnover in SML samples exists [24], an incubation apparatus that additionally allows for incubations at two reference depths in subsurface layers is missing to date.

Therefore, the Surface In Situ Incubator (SISI) was built to enable in situ bottle incubations at the oceanic air–sea interface, and at two near-surface depths (1 and 5 m) for the purpose of comparison. It was designed on the basis of a similar incubation apparatus for coastal work under calm conditions [24]. Oceanic data on metabolic processes at the SML are scarce, due to the non-existence of incubation devices for routine and reliable deployments. The SML is a crucial but understudied component for air–sea exchange processes, for example, of climate relevant gases or organic matter. For instance, phytoplankton-derived surfactants accumulating within the SML are known to influence gas exchange [25]. Moreover, the direct metabolic control of gas exchange by the SML microorganisms has been previously suggested [26,27]. Such findings demonstrate that biology-driven gas turnover within the SML requires further attention, and stresses the need for new methodologies to improve our knowledge on the SML's biogeochemical nature and climate-related implications.

The aim of this technical note is to describe the SISI as a new and inexpensive research tool for oceanographers to carry out routine incubation studies in the SML and the near surface layer. We discuss first, results on planktonic O_2 turnover obtained from the Baltic Sea and the Pacific Ocean using the SISI. We additionally present a range of other possible applications in the field of environmental science.

2. Materials and Methods

2.1. Description of the Research Tool

The SISI has a total weight of ~8 kg and consists of three rectangular frames of equal size (54 cm × 19 cm × 5 cm) made of polymethyl methacrylate (PMMA). The top frame floats 1–2 cm underneath the sea surface, the middle frame at 1 m depth, and the bottom frame at 5 m depth (Figure 1 and Supplement Video S1: Deployment and operation of the SISI). On both short sides of each frame, a metal bracket with two holes is mounted. Through these holes, ropes with a thickness of 3.2 mm are bowline-knotted to connect the four corners of the three frames with each other. Only on the top frame are ropes fixed to the bracket with shackles, which are additionally secured with cable ties. The bottom frame is weighted with two bricks (22 cm × 10 cm × 5 cm) attached to the underside of the frame. Each frame consists of a holder for eight test tubes, i.e., the inner side of the frame itself contains eight holes with a diameter of 25 mm. The test tubes (diameter = 22 mm, length = 200 mm) used are made of borosilicate glass type 3.3, have a wall thickness of 1.8 mm, a volume of ~50 mL, and feature a ground glass joint (Carl Roth, Karlsruhe, Germany). Glass bottles have been associated with metal contaminations affecting primary production [28], but according to the manufacturer's instruction, the DURAN® SIMAX material is inert and interactions between the treated glass surface and medium can be ruled out. For dark incubations, test tubes are wrapped with black polyvinyl chloride (PVC) electrical tape. Tubes positioned in the holder are affixed in the frame by gently screwing a mobile plastic plank (48 cm × 5 cm × 0.6 cm) tightly on top of the lids using lock nuts on three bolts. Foam material is glued on the inner side of the planks for shock protection and to prevent damage of test tubes. The frames are designed so they do not produce shade on the tubes. Within the frame, the test tubes are placed in a horizontal orientation, which is of particular importance in the incubation of the SML samples. The top frame floats at the water surface to mimic in situ incubation conditions at the SML, e.g., strong solar irradiance and temperature fluctuations. The SISI drifts freely with the current or is moored with an anchor. The floatation of the top frame is achieved by mounting four floats with shackles to its edges. On the top frame, a flashlight can be mounted to facilitate visual contact with the SISI during night deployments.

Underneath the bottom frame, four ropes fixed to the edges end in a large shackle that can be used to attach an anchor, e.g., if the SISI is moored in water of less than 30 m depth. For drifting freely, a global positioning system (GPS), SPOT Trace (SPOT LLC, Milpitas, CA, USA) allows constant monitoring of the SISI's position if the device is getting out of sight. The GPS device is kept in a waterproof cell phone case mounted on a bracket at the top frame and protruding 30 cm above the sea surface. The SISI was designed to be deployed from a small boat, and at least two persons are required to fill and fix the test tubes, and to release the SISI into the water. The SISI has been tested up to a sea state of 5 bft (Beaufort scale); even so, its deployment and recovery by a small boat requires calmer sea states for safety reasons. Preparation of the SISI prior to deployment, i.e., filling and fixing of test tubes, takes about two hours, and most of this time must be allocated to SML collection (see next paragraph). Recovery of the SISI can be achieved within 30 min, which includes time to remove and store the test tubes (see Supplement video S1: Deployment and operation of the SISI).
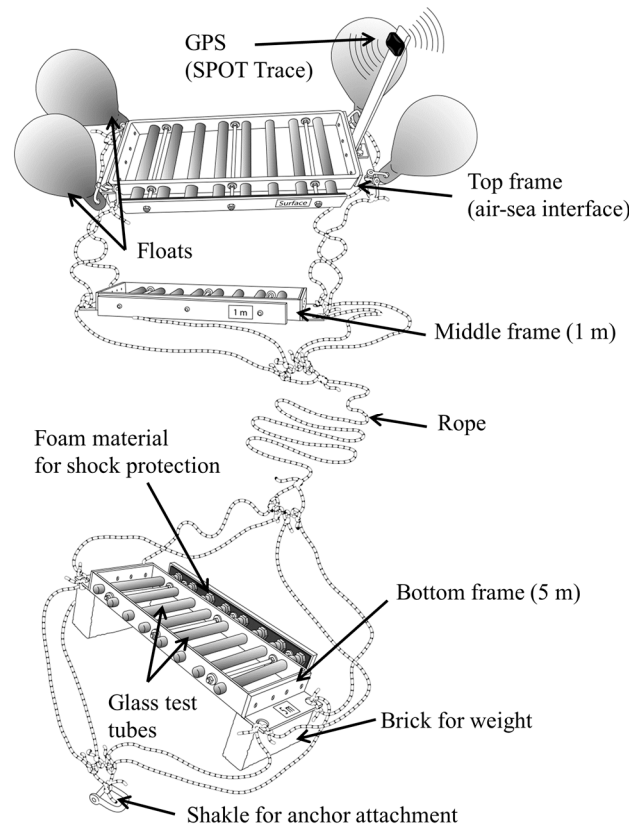
Figure 1. Scheme of the Surface In Situ Incubator (SISI).

*2.2. SISI Deployment and Sample Collection*

We carried out deployments of the SISI in the Baltic Sea in August 2015 (cruise M117 on R/V Meteor) and in the tropical Pacific Ocean in October 2016 (cruise FK161010 on R/V Falkor) (Table 1 and Figure 2). Wind speed (m s^{-1}) was taken from meteorological stations at 34.5 m and 10 m height on R/V Meteor and R/V Falkor, respectively. Wind speed from 35.5 m height was converted to wind speed at 10 m height by applying the equation as shown in Rahlff et al. [10]. The sea state in bft was calculated from the empirical formula

$$B = \left(\frac{V}{0.8360 \text{ m s}^{-1}} \right)^{\frac{2}{3}} \tag{1}$$

with *B* being the Beaufort number, *V* representing the wind speed in m s^{-1} at 10 m over ground [29].

Table 1. Time of incubation of seawater samples, wind speeds and sea states during operation of the Surface In Situ Incubator.

Location	Position (Decimal)		Date (UTC) Incubation Start	Station Name	Method of O_2 Measurement	Incubation Period (h)	Mean Wind Speed (m s^{-1})	Mean Beaufort	Max. Wind Speed (m s^{-1})	Max. Beaufort	Station Feature
	Latitude (East)	Longitude (North)									
Baltic Sea	56.1709	16.4275	03/Aug/2015 11:15	UP2	Winkler	12	4.3	3	5.4	3	Coastal, Upwelling
Baltic Sea	59.4609	23.3726	08/Aug/2015 10:30	UP5	Winkler	10.7	6.1	4	8.3	5	Coastal
Pacific Ocean	3.5109	137.5965	28/Oct/2016 00:34	Station 9	Microelectrode	29.5–35.5	8.1	5	16.6	7	Open ocean
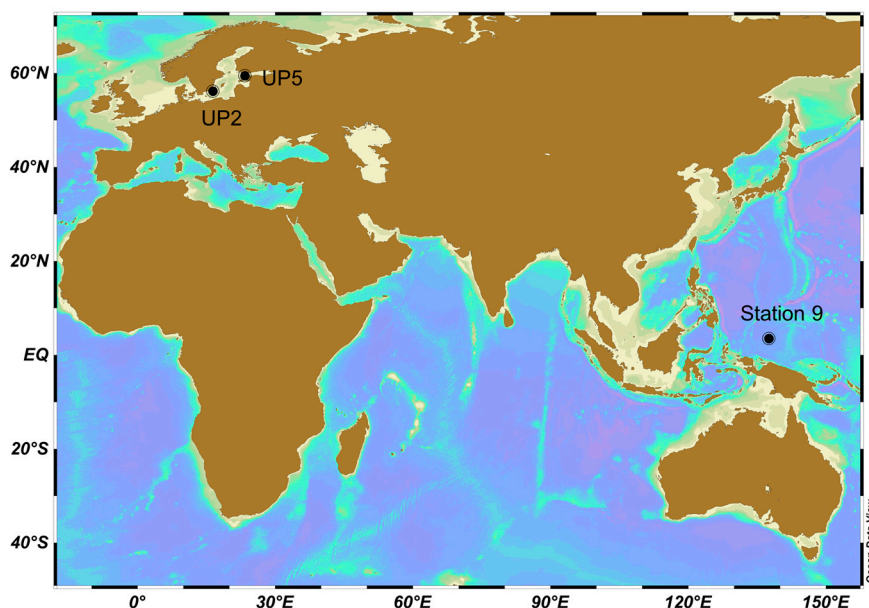
Figure 2. Station map for deployment locations of the Surface In Situ Incubator (SISI). Stations are presented using Ocean Data View [30].

Samples from the SML were collected using a glass plate sampler according to Harvey and Burzell [31], and a modified withdrawal rate of 5–6 cm s^{-1} as suggested by Carlson [32]. The glass plate was cleaned with 70% ethanol before use and rinsed with sample water. Two to three liters of SML were collected in a 5 L bottle and transferred into the test tubes by means of a 12 V gear pump (Barth Elektronik, Lengerich, Germany) and silicon tubing. Water for incubations at 1 m and 5 m depths was directly pumped from the respective depths into the test tubes. Each tube was filled to excess with three times its volume to prevent air bubble accumulation inside the test tubes. At each depth, up to four light and four dark incubations were performed, i.e., each frame held four light and four dark test tubes. The test tubes were incubated from 10.7 to 35.5 h (Table 1). Test tubes for measurement of initial O_2 concentrations at each depth were returned to the vessel's laboratory and immediately analyzed.

2.3. Oxygen Measurements

After recovery of the test tubes from the SISI, readings on O_2 concentration were performed using the commonly applied Winkler titration [33] during the Baltic Sea cruise, or by application of a Clark-type microelectrode (OX-50; Unisense, Aarhus N, Denmark) during the Pacific cruise (Table 1). Linear calibration of the OX-50 sensor was achieved by applying air-saturated seawater (100% O_2) and an anoxic 0.1 M sodium ascorbate solution (0% O_2). The O_2 concentration for the 100% signal was based on the salinity and temperature dependence of O_2 solubility [34]. The temperature sensor was calibrated to three random temperatures within the ranges of 0–10 °C, 10–30 °C, and 30–50 °C measured with a glass thermometer.

When measurements were performed with the microelectrode, the incubation was not stopped for all tubes via fixation, as per the Winkler method. Instead, the test tubes were kept at in situ-mimicking conditions, wrapped in a daylight-filtering foil (Lee-298; ZILZ direct, Pulheim, Germany) in a water bath until, and during, analysis. The extra incubation time was later considered when calculating the hourly rate. The temperature of the water bath was monitored during O_2 measurements by using an additional sensor (TP-200; Unisense, Aarhus N, Denmark). All O_2 readings were corrected for temperature if the temperature in the water bath was different from the temperature during calibration of the OX-50 (see Supplement Note 1: Microelectrode temperature correction).

O_2 concentration was measured at the start (n = 2 in the Baltic Sea, n = 3 in the Pacific Ocean) and end of the incubation period (n = 3 in the Baltic Sea, n = 4 in the Pacific Ocean) after recovery of the SISI. Constant stirring with a magnetic bar and complete submersion of the sensor ensured homogenous distribution of O_2 in the test tubes during measurements. After signal stabilization, the O_2 concentration was recorded for 90 s, from which the mean O_2 concentration was calculated. Rates of net community production (NCP) from light tubes, and respiration (R) from dark tubes, were defined as the difference in O_2 concentration between the start and end time points in the light and dark incubations, respectively, and were converted to µmol O_2 L^{-1} h^{-1} by dividing O_2 concentration changes by the period of incubation (Table 1).

2.4. Statistical Analysis

A few outliers were removed from the original data set, which corresponds to the number of test tubes as mentioned above. Removal of outliers was based on the Dixon test, which is appropriate for small sample sizes, at the 95% significance level using the "outliers" package in R (version 3.3.2, Supplement Data S1: Rcode outlier test and dataset, [35]). For larger sample sizes (n > 20), the normal distribution of the data is an important requirement for this test, which has not been considered here due to the small number of observations. Outliers are indicated in blue in Figure 3. After outlier removal, the number of replicates was n = 2–3 for station UP2 and station UP5, and n = 3–4 for station 9. The limited number of replicates after outlier removal (n = 2 in some cases) precluded statistical analyses for differences in R, and NCP, between different depths at the Baltic Sea stations, and we applied more test tubes on the subsequent Pacific cruise. A non-parametric Kruskal–Wallis test with a post-hoc Dunn's multiple comparison test was performed to check for significant differences (95% significance level) of mean O_2 production rates between depths at Pacific station 9 using GraphPad Prism version 5.00 (GraphPad Software, San Diego, CA, USA). The complete data set has been deposited in the PANGAEA database [36].

3. Results and Discussion

For demonstrating that incubations with the SISI lead to reasonable results of O_2 turnover rates at different incubation depths, we applied the device at three stations of two different marine systems; in the enclosed Baltic Sea and in the open Pacific Ocean.

For the coastal Baltic Sea stations UP2 and UP5, we observed stronger differences in O_2 turnover rates between NCP and R (Figure 3a,b). NCP rates were generally positive in 1 m and 5 m water depth incubations in the Baltic Sea assuming the presence of photosynthesizing organisms in coastal proximity. At station UP5, we even measured a positive NCP rate for the SML, which matches the observation that phytoneuston productivity can sometimes be enhanced in sheltered regions [37,38].

In the tropical Pacific Ocean, rates of NCP and R were very similar at each depth (Figure 3c). Compared to the Baltic Sea, all samples in the tropical Pacific Ocean showed O_2 consumption. Nearly equal negative rates of NCP and R, as those observed for station 9, might indicate a lack of primary production or heterotrophic processes dominating O_2 turnover, which is typical for the oligotrophic Pacific open ocean [39]. According to the Kruskal–Wallis test, no significant differences of mean O_2 turnover rates among the different depths could be observed for station 9, with one exception: dark-incubated samples from the SML, and the 5 m water depth, showed a weak significant difference (Dunn's test, $p < 0.05$) in respiration rates.
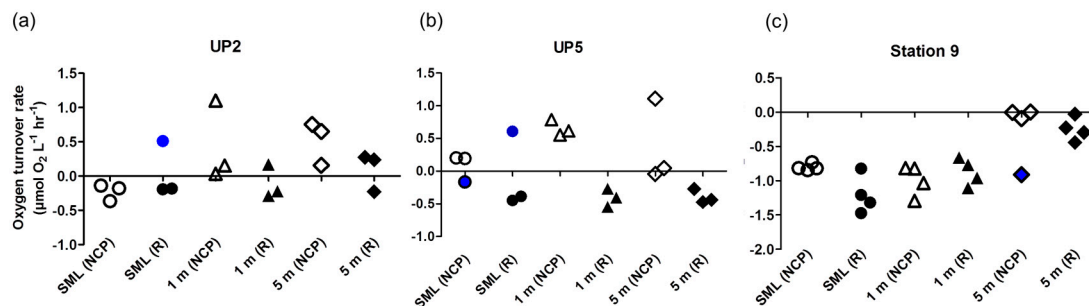
Figure 3. Oxygen turnover rates per hour recorded using the SISI and the light/dark bottle incubation technique for stations in the Baltic Sea (**a**,**b**) and the Pacific Ocean (**c**). Shown are single replicates and all outliers (blue symbols). NCP = net community production, R = respiration, SML = sea-surface microlayer.

Most importantly for demonstrating the benefit of using the SISI, the absolute O_2 turnover rates in the Baltic Sea and Pacific Ocean were in a range comparable with those previously reported. Namely, 4.9 ± 0.23 mmol O_2 m^{-3} d^{-1} and 3.3 ± 0.15 mmol O_2 m^{-3} d^{-1}, for surface water <10 m in coastal, and open ocean areas, respectively [18]. At all three stations, both in the Baltic Sea and the Pacific open ocean, SML samples showed the strongest respiration rates in dark incubations compared to underlying water depths; which is in line with previous observations on enhanced heterotrophic respiration within the SML [16,17].

Previously mentioned containment errors, e.g., by prevention of grazing pressure and the associated increase of bacterial abundances in closed-bottle incubations [40], could not be circumvented by using the SISI. Containment errors supporting increased bacterial cell numbers might be especially relevant for SML samples, which are particularly substrate-rich and, thus, attractive to bacteria [6]. Our observations fit with findings from Pomeroy, Sheldon and Sheldon [40] of a 23% increase in bacterial cell numbers in dark-incubated SML samples from the start to end of the incubation period for Pacific station 9 (Supplement Data S2: Cell counts St.9 SISI). One reason for this might be that SML microorganisms in closed-bottle incubations particularly benefit from low turbulence and minimal impact from waves and wind, which are crucial factors that would naturally affect them [10,11]. We suggest that incubation times should be adjusted to the minimum, which produces reasonable rates of O_2 turnover but reduces containment errors, such as enormous increases in cell numbers. It has been argued that primary production should be measured within a 24 h diel cycle, because abundant phytoplankton cells have a lifespan limited to hours or days [20], and the likelihood for containment-associated errors increases with time. However, we extended the incubation periods >24 h due to previously reported low productivity in the oligotrophic Pacific Ocean [39] and assumed little changes of O_2 concentration from the start to the end of the incubation period.

Furthermore, incubations using the SISI account for effects from temperature, whose fluctuations are more extreme within the SML [37], but also for impacts from solar and partial UV radiation on SML organisms [7,9]. Even if the construction of a rigid upper frame is not ideal regarding its conformation to the wave crests resulting in unwanted elevations above and submersions beneath the surface, we assume that the effects due to variations in light exposure will, on average, balance each other out. Although the upper frame might temporarily sink to a depth of approximately 5 cm due to wavy sea conditions and the rigid make-up of the upper frame, light conditions will be very similar to those at the immediate air–sea interface, but profoundly different from 1 m. According to the Beer–Lambert law, and applying a typical oceanic light attenuation coefficient of 0.0384 m^{-1} [41], losses of irradiances at 5 cm are minor compared to 1 m depth, namely 0.2% and 3.8%, respectively.

The test tubes made of borosilicate glass type 3.3 allowed for transmission of UV radiation in the 310–400 nm wavelength range, whereas UV radiation in the lower 280–310 nm range was blocked (manufacturer's specification). Alternatively, customer-made test tubes of quartz glass can be used to

cover a wider range of UV radiation, but could not be used in this study due to the high costs of these tubes. To make intermediate readings with the microelectrode during an incubation period, test tubes with a resealable rubber septum as a lid could be used.

The advantages and disadvantages of different methodologies to measure dissolved O_2 concentrations have been extensively compared and discussed in the literature [42–44]. With Winkler titration, we used a highly accurate standard method that was, however, not very field-friendly and, as it was conducted manually, required experienced analysts to obtain high quality data. Clark-type microelectrodes require thorough calibration and temperature compensation. Using both analytical approaches, incubations with the SISI led to reasonable results on plankton metabolism in terms of O_2 turnover in surface water [18] of the two different marine systems.

The SISI is a new tool that allows in situ incubations in surface water and at the SML; the immediate boundary between the atmosphere and hydrosphere. The SISI has several advantages:

(1)   It leads to reasonable results on O_2 turnover rates of plankton communities as reported here.
(2)   It allows for in situ bottle incubations close to the SML and in near-surface layers.
(3)   Incubation periods up to several days are possible.
(4)   The SISI is applicable under typical oceanic conditions (tested and recommended for sea states up to 5 bft), and under these conditions shallow submersion of the upper frame (approximately 5 cm; see Supplement Video S1: Deployment and operation of the SISI) ensures incubation under surface radiation.
(5)   It is inexpensive with material costs of approximately \$950 USD, which includes 33 test tubes.
(6)   It allows applications without environmental impacts.
(7)   It can be deployed by being anchored in shallow water, or GPS-tracked in the open ocean, allowing for other operations on the R/V.

However, the deployment of the SISI requires three hours of ship time and the operation of a small boat and coxswain, as well as two additional persons for the deployment. Despite the SISI's seaworthiness up to 5 bft (Table 1), temporarily even standing short periods of 7 bft (station 9), its deployment and recovery require moderate weather conditions with sea states $\leq$4 bft (wind speeds of 5.5–8 m s^{-1}) for the operation of a small boat. In oligotrophic water, long incubation periods of up to 48 h might be required to observe appreciable changes in O_2 concentration between the start and end of the incubation. Taking all these factors into account, the SISI is a useful device for routine assessment of planktonic O_2 turnover rates within the SML and comparative near-surface depths, which are poorly described in the literature. In addition, the SISI and the test tube volume of ~50 mL allow for other parameters to be assessed such as microbial cell counts and community composition. We suggest using the SISI for long-term monitoring studies, for instance, during different seasons, which could be useful to improve biogeochemical, or gas exchange models of a defined area.

Possible improvements for a future version of the SISI could include larger frames to allow for a higher number of replicates (more test tubes) to be installed, and to facilitate incubations of larger sample volumes for more analyses to be made (e.g., measurements of pigment composition and concentration). Although it is of strong interest to study processes within the SML as close to in situ conditions as possible, the here presented SISI does not allow for incubations of the SML in its natural integrity and surface-to-volume ratio, i.e., as a thin film in atmospheric contact. Technically, it is very challenging to sample and incubate the SML at exact in situ conditions as this would require expensive technology such as the application of sensor spots. Our design simulates the closest conditions keeping the device practical for routine measurements.

Future Research Directions

Apart from the purpose presented here, we envision using the SISI for various future applications. Since long-term incubations are possible, the SISI could be applied to study the fate of marine pollutants, which frequently accumulate within the SML [12], and which are probably exposed to different rates

of photochemical or microbial transformation at different depths. This could be relevant to the study of the biodegradation of oil products forming oil slicks at the sea surface [45], and associated impacts on marine biota and ecosystem functioning [46]. Another application for the SISI might be the incubation of water samples containing microplastics, whose higher abundance in the SML, compared to underlying water, has been recently described [47]. Bacterial colonization of polymer-based materials and biofilm formation [48], as well as microbial degradation of microplastic particles [49], constitute highly topical research areas that could be further investigated using the SISI.

In addition, the photochemical and microbial transformation of dissolved organic matter (DOM), which is enriched in the SML, compared to the underlying water [17], and plays a profound role in marine biogeochemical cycling, could be explored using incubations in the SISI. Photolysis transforms recalcitrant DOM into bioavailable, labile carbon compounds, a process that occurs even at low intensities of visible light [50]. Such labile compounds can be further utilized by heterotrophic microorganisms [51]. On the other hand, labile DOM components under the influence of UV radiation can also undergo crosslinking and, in turn, contribute to the refractory DOM pool [50].

For ecological questions, the SISI could help to elucidate the short-term adaptation potential of bacteria, phyto- and microzoo- plankton when exposed to different temperatures, UV, and light regimes. As sea-surface temperature and UV radiation are predicted to increase with ongoing climate change [52,53], incubation experiments can contribute to a better understanding on how planktonic organisms may adapt to environmental changes and influence food webs in a future ocean.

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