Comparative studies on zooplankton community between the sea surface microlayer and the subsurface microlayer in Daya Bay, China*

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Abstract Characteristics of the zooplankton community inhabiting the sea surface microlayer (SM) and the sub-surface microlayer (SSM) are compared at six sampling stations in Daya Bay, near Shenzhen City of China during 2 cruises in 1999. This is the first study on zooplankton community in the SM in China. Results show that protozoans and nauplii are the most dominant components, accounting for 80.71% (SM) and 89.15% (SSM) of the total zooplankton in the average abundance, respectively. The densities of copepods (adult + copepodid) are higher in the SSM than in the SM. The size-frequency distributions indicate that the frequency of micro-zooplankton (≤0.2 mm) is higher in the SM (0.8235, n=290) than in the SSM (0.6768, n=306). Enrichment phenomenon of zooplankton is detected in the SM at the sampling stations excluding two stations near nuclear power plants (NPP). The enrichment factor is from 1.516 to 3.364 with the average value of 2.267. The SM zooplankton community structure revealed in the present study is quite different from previous investigations in the Bay. Typical sea water characteristics such as turbidity, biological oxygen demand (BOD), chemical oxygen demand (COD), total nitrogen (TN), total phosphorus (TP) and chlorophyll a (Chl-a) indicate that the water quality is poorer in the SM than in the SSM.

Keywords: **zooplankton, density, sea surface microlayer, subsurface microlayer, Daya Bay.**

Historically, study on the sea surface has been restricted basically to the content of the mixed layer of the water column and to the sea surface dynamics. Less attention has been paid to the air-water interface, which is constituted by the first few tens of microns and defined as the sea surface microlayer (SM)[1]. SM is an important boundary between atmosphere and the ocean, as an area of exchanging matter and energy. The SM plays a key role in the global biogeochemical cycle because all gaseous, liquid and particulate materials must pass through this interface between the ocean and the atmosphere. The SM has often been operationally defined roughly as the top several tens to several hundreds micrometers of the water surface depending upon the collection method used. More than 20 different sampling techniques have been applied to sample the sea surface microlayer[2]. The SM is generally enriched in organic substances, particularly those which are surface active metal ions, bacteria, and other microorganisms, relative to subsurface microlayer (SSM). Although increasing effort has been made to characterize chemical and biological properties of microlayer over the past decade, there are still some gaps (e.g. knowledge about the zooplankton community structure in SM) which may be of environmental significance, but we have incomplete or even no information about that[3,4]. Actually, some microzooplanktons, such as protozoans, constitute a potentially significant source of surface-active material. Plankton and bacteria presented in the SM can affect the concentration and composition of dissolved organic matter (DOM) by directly releasing DOM and also indirectly via their predatory activities which can take up and release DOM[3,5].

Daya Bay (114°29′42″—114°49′42″E, 22°31′12″—22°50′00″N) is near the Shenzhen City of Guangdong Province. It is located at the left side of the Zhujiang River estuary and the north part of South China Sea. The surface area of the bay is about 600 km^2, the maximum depth is 21 m and the mean depth is 11 m. Length of the coastal line is about 92 km. The annual average air temperature is 22 ℃. The maximum monthly air temperature is about 28 ℃ in July, and the minimum temperature is about 15 ℃, usually in

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January or February. Annual average precipitation varies from 1500 to 2000 mm. During May-September, monthly precipitation is up to 200—400 mm. Daya Bay is a bay of multiple uses: nuclear power supply, mariculture and sightseeing. From the 1980s to now, great changes have taken place in the bay: over-developing marine fish culture industry and operation of nuclear power plants resulted in great changes in water quality. Due to the man-caused increasingly nitrogenous pollution accompanying the development of industry and aquaculture in the bay, the content of total inorganic nitrogen (TIN) in seawater has increased, and the NH_4-N content has increased significantly. Eutrophication is serious in coastal waters such as mariculture zone in the bay[6—8].

Although study on zooplankton has a long history in bulk water of Daya Bay[6,9], study on zooplankton in SM is poorly reported. The aim of the present study is to compare the difference of zooplankton community and water quality between SM and SSM, to fill some of the gaps in the knowledge of zooplankton in the air-sea interface, as well as evaluate the influence of environmental parameters on them in Daya Bay.

1 Materials and methods

Ecological investigations including zooplankton and water quality were made at six stations (Stations 1-1, 3-2, 3-3, 4-1, 5-1 and 8-2) in Daya Bay during 2 cruises from May 20—22 to June 6, 1999 (Fig. 1). Stations 4-1 and 5-1 are near the inlet and outlet of nuclear power plants (NPP), respectively. Stations 3-2, 3-3 and 8-2 are located in the marine fish culture zones. Station 1-1 is far away from NPP about 4000 m. Samples from the SM were collected with a plate sampling device. The device is a 40 cm × 60 cm × 0.5 cm glass plate. A microlayer of the water surface was collected by submerging the glass plate into the water vertically and then drawing up water surface at a constant velocity (about 20 cm/s). The water film adhering to the glass plate was wiped off into a sample bottle with a silicone wipe blade. This procedure was repeated until 1 L water sample was accumulated. The thickness of the sample ranged from 30 to 90 μm, depending on the withdrawal speed[4]. The mean sampling thickness of the SM was 50 μm[10]. Samples from the sub-surface microlayer were collected with a 5 L plexiglass sampler, and 20 L of sea water was collected at depth 5—50 cm (underlying SM water). At each station, zooplankton samples were obtained by filtering 1 L (SM) and 20 L (SSM) sea water through a plankton net (mesh size 35 μm), and then preserved with 5% formalin. In the laboratory, larger-sized zooplanktons were counted totally under a microscope, while protozoans, rotifers and nauplii were subsampled before counting. Immediately after counting, animals were sorted randomly from each sample for measurement of body length. Lengths were arbitrarily assembled into 0.1 mm size subsections. The frequency of occurrence of zooplankton in these size subsections was calculated separately. About 30 individuals were measured per sample. The water temperature and salinity were measured by YSI-85 (USA). Other water quality data were provided by the Marine Biology Research Station of Daya Bay, Chinese Academy of Sciences.

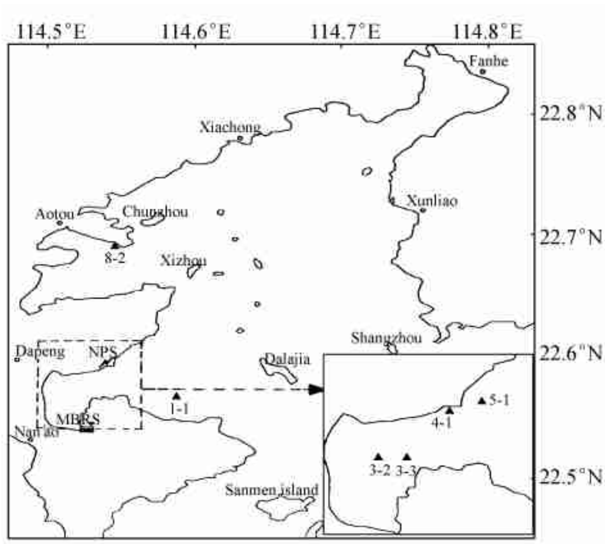

Fig. 1. Map of Daya Bay showing the position of sampling stations (▲), Marine Biology Research Station (MBRS), and Nuclear Power Station (NBS).

The enrichment factor (A_{EF}) in the microlayer is

$$A_{EF} = C_m / C_s,$$

where C_m is the concentration of any substance in the SM, and C_s is its concentration in the SSM[11].

2 Results

2.1 Characteristics of sampling stations and water quality

During the investigation period, water depth of the sampling stations varied from 3.7 m to 17.0 m (Table 1). The maximum water depth and transparency were recorded at Station 1-1, while the minimum value at Station 3-3. Because transparency is one of the important characteristics of water quality, the results show that water quality in fish culture zone is poorer than other sea areas. Water temperature in SM was higher at Stations 4-1 and 5-1 than at other sampling stations. Temperature of SM at other stations ranged from 24.6 ℃ (Station 3-2) to 25.4 ℃ (Station 1-1), but temperature of inlet (Station 4-1) and outlet (Station 5-1) of NPP reached 27.1 ℃ and 26.8 ℃, respectively. Temperature measurement suggested that thermal pollution was present near NPP. Salinity varied from 32.434‰ to 34.872‰ at the sampling sites and values were higher in SM than in SSM. The values of pH and temperature were higher in SSM than in SM at the stations.

Table 1. Location, sampling date, water depth and transparency of sampling stations and main properties of sea surface microlayer in Daya Bay during May-June, 1999[a)]

Stations	Date	Location	Depth (m)	Transparency (m)	Salinity (‰) (SM/SSM)	pH (SM/SSM)	Temperature (℃) (SM/SSM)
1-1	22/5	114°35′21″E 22°33′89″N	17.0	4.5	33.970/33.912	7.59/8.09	25.4/25.5
3-2	20/5	14°31′31″E 22°33′88″N	5.5	2.5	33.798/33.474	7.11/7.83	24.6/25.8
4-1	22/5	114°32′43″E 22°35′26″N	6.0	2.5	34.031/33.676	7.86/8.10	27.1/28.8
5-1	22/5	114°33′13″E 22°35′52″N	12.0	2.5	34.027/33.705	7.89/8.11	26.8/29.0
8-2	20/5	114°33′01″E 22°41′89″N	7.0	2.0	33.340/32.861	7.58/7.95	25.5/26.1
3-3	6/6	114°33′36″E 22°33′63″N	3.7	1.0	34.872/33.942	8.06/8.12	27.9/30.5
4-1	6/6	114°32′55″E 22°35′26″N	4.5	2.5	no data	no data	no data/30.80
5-1	6/6	114°33′10″E 22°35′67″N	9.0	2.1	no data	no data	no data/34.30
8-2	6/6	114°33′10″E 22°41′80″N	4.8	2.1	32.507/32.434	8.13/8.18	29.30/30.0

a) The sampling data of Station 1-1 on June 6 is not given because of strong wind wave.

Data presented in Table 2 indicate that concentrations of turbidity, biological oxygen demand (BOD), chemical oxygen demand (COD), total nitrogen (TN), total phosphorus (TP) and chlorophyll a (Chl-a) were much higher in SM than in SSM (except for value of Chl-a at Station 3-2) at all research sites. There is 1.07—20.6 fold increase in SM. The highest A_{EF} of COD reached 20.6 at Station 1-1. Maximum value of turbidity was recorded at Station 8-2 (9.08°), followed by Station 3-2 (8.12°). Concentration of COD was the highest at Station 3-2 (5.80 mg/L), followed by Station 8-2 (4.89 mg/L). The highest concentrations of TN and Chl-a occurred at Station 3-2 (95.55 mg/L) and Station 8-2 (12.05 μg/L), respectively. These results indicate that the water quality at Stations 3-2 and 8-2 positioned in fish culture zone was poor. Lower contents and ranges of turbidity, COD, TN and Chl-a appeared at Station 1-1, which indicates good water quality. However, we do not know why the highest concentration of TP (2.87 mg/L) appeared at this station. Six water quality indicators (excluded BOD) were ranged in middle at Stations 4-1 and 5-1 near NPP.

Table 2. Typical sea water characteristics of the surface microlayer (SM) and the subsurface microlayer (SSM) in Daya Bay in May, 1999 (Data from Marine Biology Research Station of Daya Bay)

Stations	Layers	Turbidity (°)	COD (mg/L)	BOD (mg/L)	TN (mol/L)	TP (mol/L)	Chl-a (μg/L)
1-1	SM/SSM	0.92/0.32	2.06/0.10	2.94/0.55	49.13/29.94	2.87/0.78	1.53/1.43
3-2	SM/SSM	8.12/0.92	5.80/0.91	3.50/0.54	95.55/39.25	0.58/0.55	1.22/1.67
4-1	SM/SSM	1.27/0.47	1.62/0.44	5.13/0.72	37.85/30.86	1.19/0.50	1.92/1.67
5-1	SM/SSM	1.43/0.32	2.23/0.44	5.66/0.54	70.84/25.03	0.86/0.52	1.43/1.12
8-2	SM/SSM	9.08/1.48	4.89/0.70	3.71/0.69	60.32/29.52	1.11/0.41	12.05/2.01

2.2 Species composition of main zooplankton

The species composition of zooplankton was determined from 2 cruises in Daya Bay during May—June, 1999. The author detected that protozoa and copepod nauplii were the most dominant components of zooplankton in SM and SSM at all stations (Table 3). The main species consisted of Protozoa (*Tintinnopsis radix*, *T. tocantinensis*, *Codonellopsis ostenfeldi*, *Favella amoyensis*), Rotifera (*Synchaeta* sp.), Cladocera (*Penilia avirostris*), Copepods (*Paracalanus crassirostris*, *Microsetella norvegica*), nauplii, and others (*Oikopleura*, Balanus larva, Polychaeta larva). Among animals, *Tintinnopsis radix*, *T. tocantinensis* and nauplii were the dominant species group in both SM and SSM at all sampling stations. *Synchaeta* sp. appeared only in June, while *Penilia avirostris* was found only in SSM in May. Copepods in SM almost consisted of nuplii and copepodid at the stations. Large-sized zooplankton such as *Oikopleura*, Balanus larva, Polychaeta larva were recorded only in SSM.

Table 3. List of main zooplankton species in the surface microlayer (SM) and the subsurface microlayer (SSM) in Daya Bay during May-June, 1999[a)]

Species groups	SM (May)	SSM (May)	SM (June)	SSM (June)
Tintinnopsis radix	++	++	++	++
T. tocantinensis	++	++	++	++
Codonellopsis ostenfeldi	+	+	+	++
C. rutunda	+	+	+	+
Leprotintinnus nordquisti	+	++	+	+
Favella amoyensis	+	++	+	++
Synchaeta sp.	—	—	+	++
Penilia avirostris	—	+	—	—
Paracalanus crassirostris	+	+	+	++
Microsetella norvegica	+	++	+	++
nauplii	++	++	++	++
Oikopleura sp.	+	++	+	++
Balanus larva	+	++	+	++
Polychaeta larva	+	+	+	++

a) — not present; + present; ++ abundance.

2.3 Zooplankton densities

During the period of investigations, the quantitative values of zooplankton densities were very variable in SM and in SSM at 6 stations (Fig. 2). The highest and lowest zooplankton densities were all found in SM, with values 502 ind./L at Station 8-2 and 87.5 ind./L at Station 4-1 in May, 512.5 ind./L at Station 3-3 and 55 ind./L at Station 5-1 in June, respectively. Zooplankton were more abundant in SSM than in SM at Stations 4-1 and 5-1 near NPP, while zooplankton in SSM were lower than in SM at other stations. Investigation results showed that the maximum abundance of animals in SM appeared in marine fish culture zones (Stations 3-3 and 8-2), while the minimum abundance appeared in the areas near NPP.

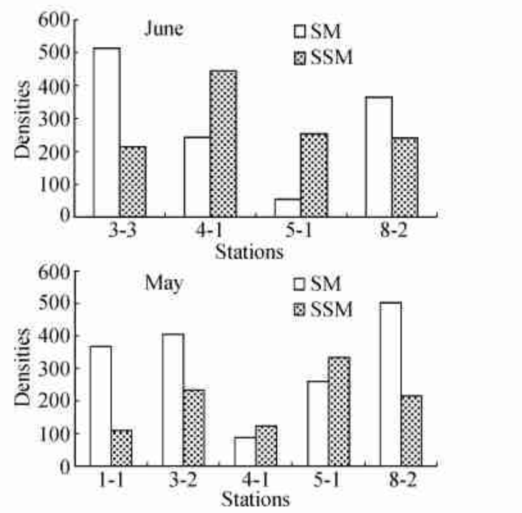

Fig. 2. Changes in zooplankton (ind./L) of the surface microlayer (SM) and the sub-surface microlayer (SSM) at sampling stations in Daya Bay during May-June, 1999.

Enrichment phenomenon of zooplankton was detected in SM at the sampling stations near the nuclear power plants excluding 2 stations. The A_{EF} of zooplankton varied from 1.516 (Station 8-2 in June) to 3.364 (Station 1-1 in May) with the average value of 2.267.

Investigation showed that protozoans and nauplii were the most dominant groups during the study period, comprising 80.71% (SM) and 89.15% (SSM) of the total zooplankton, respectively. Percentages of copepods (adult + copepodid) were only 2.7% in SM and 5.38% in SSM, respectively. The maximum values of protozoans (300 ind./L) and nauplii (303 ind./L) were found in SM of Station 3-3 in June, 1999 and in SSM of Station 5-1 in May, 1999, respectively. The maximum abundance of copepods (adult + copepodid) was 45.45 ind./L in SSM of Station 8-2 in May, 1999.

2.4 Size-frequency distribution of zooplankton

Size-frequency distributions are very important to reveal characteristics of zooplankton community structure. In the present studies, body length of some 600 animals ($n=290$ individuals from SM and $n=306$ from SSM) was measured from 18 samplers during 2 cruises. The frequencies of zooplankton (< 0.2 mm) were 0.8235 in SM and 0.6737 in SSM, respectively. Frequency of zooplankton (< 0.2 mm) was higher in SM than that in SSM, and it was reversed (> 0.2 mm) (Fig. 3). In SM, very few individuals (> 1 mm) were found. In contrast, more zooplankton (> 1 mm) were detected, and the biggest animal was over 4 mm in SSM. The results presented here indicated that micro-zooplankton in SM were more dominant than in SSM. More large-sized zooplankton appeared in SSM. Here, we must point out that although the sampling mean thickness of SM is 50 μm, several larger individuals (> 1 mm) present in the SM sampling. This is probably due to the fact that larger animals can easily adhere to the glass surface.

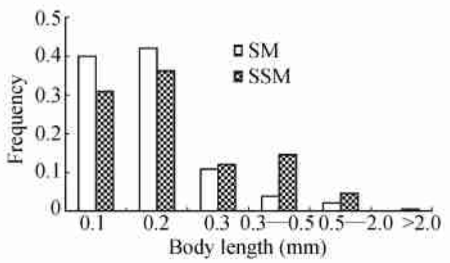

Fig. 3. Size-frequency distributions of zooplankton of SM (n = 290) and SSM (n = 306) in Daya Bay during May-June, 1999.

3 Discussion

The sea surface microlayer is a thin layer at the sea surface which has special physical, chemical and biological properties. Because of the sampling difference, the thickness of SM ranges from 30 μm to 400 μm[2, 3]. The thickness of the SM samples is changed by controlling the withdrawal speed of the plate. In general, the microlayer sampling thickness increases with the increasing withdrawal speed. The microlayer sampling thickness is also influenced by water temperature. The sampling thickness slightly decreases with the increasing temperature, presumably due to the decreasing viscosity and surface tension[4, 12].

SM is an important boundary with some special environmental properties. Many researchers demonstrated that SM had higher enrichment factors for nutrients, metals and microbiological components[2, 3, 7]. In addition, Rumbold and Snedaker reported that the SM samples were more toxic compared with the subsurface water collected[13]. Yang demonstrated that DMS concentrations were higher in microlayer samples, compared with subsurface samples[11]. In the present studies, it is detected that the water quality was poorer in SM than that in SSM. Salinity, turbidity and concentrations of BOD, COD, TN, TP and Chl-a were much higher in SM than in SSM (except for the value of Chl-a at Station 3-2) at all research sites. However, the values of pH and temperature were higher in SSM than in SM at the stations. The results demonstrated that the environmental characteristics are different between SM and other water layers in the sea.

Because SM has a thickness from several tens to several hundreds micrometers, the microlayer waters could be suitable for some small animals. Prior to this study, no report had been presented on community structure of zooplankton in SM in Daya Bay. In previous studies, many scientists focused on physical and chemical properties of SM, meanwhile, they demonstrated the presence of microorganisms such as bacteria, phytoplankton and zooplankton[3, 5]. In this study, it was found that zooplankton of SM mainly consisted of small-sized micro-zooplankton in Daya Bay, and the densities of zooplankton were lower in SM than in SSM at Station 4-1 (near inlet of NPP) and Station 5-1 (near outlet of NPP). A possible reason for this is that discharge of heated water from nuclear power plants can have harmful effects on aquatic organisms, and result in decrease in animal abundance[14, 15]. Under a microscope, dead protozoa, such as empty shell of *Tintinnopsis radix* and *T. tocantinensis* were often observed. These results can lead to a conclusion that some zooplankton did not survive after passing through the NPP cooling system, which resulted in a decrease of zooplankton densities near NPP areas.

Recently, marine fish culture industry has rapidly developed in Daya Bay. In the bay, with cages suspended from rafts in the sea, the fish culture zones are all located in relatively sheltered sea areas with low depth. The fish farming has a considerable pollution impact on the marine environment. Some researches have demonstrated that intensive cultivation of fish generates large amounts of organic and inorganic wastes from uneaten food, fish faeces and excretory matter. These materials are continually produced and released at single point sources into the culture zone, resulting in an increase in the organic and inorganic contents of the water body and sediments. Therefore, it can be concluded that water quality is poor at the sampling stations in marine fish culture, which is in good agreement with Lam's result[16].

He et al. indicated that serious organic and nutrient enrichment occurred in marine fish culture zones in Daya Bay[17]. Lam reported that higher nutrient concentrations could result in an increase in phytoplankton growth[16]. Enhanced phytoplankton may provide sufficient food to zooplankton, especially small-sized individuals. Nutrient enrichment may be the reason why the maximum abundance of zooplankton occurred in marine fish culture areas. Higher values of turbidity, BOD, COD, TN, TP and Chl-a were recorded in SM. Since enrichment of organic and nutrient materials may supply enough food for animals, higher densities of zooplankton were recorded in SM but not in SSM.

Most of the information on coastal zooplankton is based on sampling in bulk water. The information about zooplankton from special water layers (e.g. boundary layers of sea-air and sea-sediment) has not been assessed properly. Up to now, we have not read any special papers on zooplankton community in SM. Some sampling methods have their shortcomings. In previous studies, most Chinese scientists collected zooplankton sampling with plankton net I (mesh size 505—507 μm) or net II (mesh size 160 μm) in bulk water. A lot of small-sized animals escaped by passing through the mesh of plankton net, which resulted in a quite low record of zooplankton densities in Chinese seas. Xu et al. reported that copepods were the dominant component, comprising 32% of the total zooplankton in abundance, but the total densities were less than 1 ind./L[6]. Similar results of zooplankton from the bay were reported by Shen et al. in the 1990s[9]. The samples probably underestimated the quantity of zooplankton in Daya Bay. Because of their inconspicuous size, and rare dominance in biomass, micro-zooplankton usually received less attention than larger zooplankton in previous studies. As a matter of fact, micro-zooplankton are potentially significant as an alternate link in the marine food webs. They generally feed on the smaller sized particulates, which are not utilized efficiently by larger consumers. Thus, the micro-zooplankton, as trophic intermediates, make considerable contributions to higher level consumers[18, 19]. In the present study, zooplankton sampling was filtered using a net with mesh size of 35 μm, with densities ranging from 55.0 to 512.5 ind./L (Fig. 2). Small-sized individuals were the main component of zooplankton in SM in the bay. The significant difference in species composition, standing stock of zooplankton between previous and present studies in Daya Bay mainly resulted from the difference of sampling methods.

Fluctuations of environmental factors are more significant and complex in coastal areas due to the combination of land and ocean influences. As a result, the study of the spatial and temporal variability of coastal zooplankton communities seems to be important for a better understanding of the function of coastal ecosystems[20, 21]. SM has special physical, chemical and biological properties and has great importance for gaseous exchange at the sea-air interface. Neustonic organisms (zoo-, phyto- and bacterioplankton) living in SM may influence transfer of O_2 and CO_2, and thus play a role in respiratory CO_2 production[22, 23] and constitute a significant source of surface-active materials[5]. For these reasons, the MS zooplankton community analysis is very important and necessary to assess sources of organic matter, transfer of O_2 and CO_2, and relationship among microorganisms in the marine ecosystem.

4 Conclusions

(1) Protozoa and nauplii were the most dominant groups of zooplankton during the study period, comprising 80.71% (SM) and 89.15% (SSM) of the total abundance, respectively.

(2) Enrichment of zooplankton in SM was obvious at sampling stations apart from 2 stations near NPP. The enrichment factor of zooplankton varies from 1.516 to 3.364 with the average value of 2.267.

(3) Size-frequency distributions showed the frequency of micro-zooplankton was higher in SM (0.8235) than in SSM (0.6768). Small-sized zooplankton were the most dominant zooplankton component of the sea surface microlayer in Daya Bay.

(4) Nuclear power plants cooling system has thermal pollution effects on the community structure of zooplankton.

(5) Difference of sampling methods may result in significantly different results of zooplankton community structure.

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