Experimental Study on Early Diagenetic Processes of Marine Phyto- and Zooplanktonic Lipid Materials.

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Kazuya YOSHIMURA

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Kazuya YOSHIMURA

Contents

Abbreviations

POM	Particulate Organic Matter
DOM	Dissolved Organic Matter
OC	Organic Carbon
TOC	Total of Organic Carbon
POC	Particulate Organic Carbon
DOC	Dissolved Organic Carbon
DIC	Dissolved Inorganic Carbon
LC	Lipids Carbon
LC-POM	Lipids Carbon in Particulate Organic Matter
LC-DOM	Lipids Carbon in Dissolved Organic Matter
P-OC	Photosynthetically-produced Organic Carbon
P-POC	Photosynthetically-produced Particulate Organic Carbon
P-DOC	Photosynthetically-produced Dissolved Organic Carbon
P-LC	Photosynthetically-produced Lipids Carbon
Chl *a*	Chlorophyll *a*
SEM	Scanning Electron Microscope
TLC	Thin Layer Chromatography

*Abbreviations of lipid classes are separately listed in Table 2-1 and 3-1.

Abstract

The elucidation of dynamics of marine organic matter is important to understand the carbon cycling in marine environments which control global environmental condition. While major part of marine organic matter is composed by dissolved organic matter (DOM), marine organic matter is mostly produced by photosynthesis of phytoplankton in the form of particulate organic matter (POM). Zooplanktonic organic matter is also one of the major sources of POM in the ocean. Since POM and DOM have different roles in the carbon cycling in the ocean, the elucidation of degradation/dissolution processes of planktonic organic matter is important to understand the dynamics of marine organic matter.

Lipids are one of the most important biochemical components in planktonic organisms, and specific lipid molecules in organic matter show their sources as biomarker. Therefore, molecular analyses of lipids are useful tool to elucidate the dynamics of marine organic matter. However, the diagenesis property of lipid materials has been poorly understood. Furthermore, exceedingly ^{14}C-depleted (i.e. very old) dissolved lipid materials were observed in various marine environments, but the sources of old lipid materials have been ambiguous. In the present study, phyto- and zooplanktonic organic matters were applied to degradation experiments in order to further understand the role of degradation/dissolution on dynamics of the planktonic organic matters and lipids during early diagenesis.

Phytoplankton population in the surface sea water of Tokyo-Bay was cultured with $NaH^{13}CO_3$ and nutrients under fluorescent light in order to label the photosynthetically-produced organic matters. The cultured sample was incubated under dark, and the photosynthetically-produced organic matters were degraded. In the degradation experiment of zooplanktonic organic matters, freeze-dried zooplankton

cells collected from the surface sea water of Tokyo Bay was added into artificial sea water with inoculum of bacterial population (0.7 μm filtered surface sea water). This sample was incubated under dark to decompose the zooplanktonic organic matter. The degradation/dissolution of the planktonic organic matter and lipid materials were followed during incubation, and the degradation properties of lipid materials were studied in lipid class level. In addition, the contribution of photosynthetically- produced lipid material to old lipid material in natural seawater was discussed from the lipid class composition.

In the degradation experiment of photosynthetically-produced organic matters, the photosynthetically-produced organic carbon (P-OC) were accumulated into dissolved organic carbon (DOC) fraction and remained stably, accompanying to the shift of the size distribution from particulate organic carbon (POC) to DOC dominated size distribution. On the other hand, phytoplanktonic lipids were preferentially remained as POM. These degradation/dissolution properties were also observed in the degradation experiment of zooplanktonic organic matters. Thus, it is suggested that planktonic lipids tend to remain as POM, while planktonic organic carbon tend to accumulate into stable DOM pool during early diagenesis regardless of the difference of planktonic sources (phytoplankton or zooplankton) in the ocean. These results indicate that planktonic lipids contribute to the vertical transport of organic matters rather than the formation of stable DOM pool, while planktonic organic carbon contribute to the formation of DOM pool more than planktonic lipids during early diagenesis. The stabilities of lipid classes showed that phospholipids (PL) were more stable than triglycerides (TG) in both phytoplanktonic- and zooplanktonic-lipids degradation. The different stabilities of PL and TG in the POM indicate that structural lipids are major materials which are transported toward deeper water and contribute to the vertical transport of lipids, and

that storage lipid of TG has high bioavailability and is readily remineralized during early diagenesis in the ocean.

In the degradation experiment of phytoplanktonic organic matters, the degradation constants of photosynthetically-produced lipid carbon (P-LC) was higher than that of P-OC. The remaining percentage at the end of incubation to the maximum concentration was lower in P-LC than P-OC. The lability of lipids was also confirmed in the degradation experiment of zooplanktonic organic matters. However, the degradation rates of phytoplanktonic- and zooplanktonic-lipids decreased after several days from the start of degradation in the dark incubation, indicating that planktonic lipids were composed by both labile and stable materials. In the lipid class analysis, particulate PL and waxes (W) showed high stability in the both degradation experiments. The quantitative relations between OC and biomarkers (i.e. ratios of PL/OC and W/OC) showed almost constant values. These results suggest that the contributions of OC derived from phytoplankton or zooplankton to the OC pools are able to be calculated from the concentration of biomarker organic molecules, possibly. However, the ratios of PL/OC and W/OC are likely affected by the degradation conditions such as organic composition, bacterial population and other environmental factors (i.e. temperature, nutrients and redox conditions). Thus, further studies are necessary on the degradation conditions for the quantitative estimation of each source to OC pools.

In the degradation experiment of phytoplanktonic organic matter, lipids carbon in the incubation bottles (LC) were composed by both P-LC (^{13}C-labeled lipids) and lipids originally existed in the sample sea water (not labeled lipids). The lipid class compositions of P-LC and LC at the end of degradation experiment were compared in order to elucidate the contribution of phytoplanktonic lipid materials to the dissolved lipid materials in natural seawater. From the lipid class composition, the presence of

fossil-like lipid materials such as petroleum in the natural sea water was indicated. However, the lipid class composition of major part of LC (about 80 %) was similar to the phytoplanktonic lipids. Thus, phytoplanktonic-lipids are possibly major source of dissolved lipids in the ocean. Although the presence of exceedingly ^{14}C depleted (i.e. old) dissolved lipid materials has been reported, it is possible that a little amount of fossil-like lipids lowered the apparent ^{14}C ages of dissolved lipids. Further researches are necessary to elucidate the origin of these non-bioreactive lipid materials and their variability in the environment.

Keywords: phytoplankton, zooplankton, organic carbon, lipid carbon, lipid class analysis, diagenesis, reactivity, size distribution

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1. General Introduction

Marine organic matter is one of the major organic matter pools on the earth. Thus, the elucidation of the dynamics of marine organic matter is important to understand the carbon cycling on the earth's surface. Although major part (>90%) of marine organic matter is composed by dissolved organic matter (DOM) which is stable and acts as carbon reservoir, marine organic matter is mainly produced as particulate organic matter (POM) by primary production of phytoplankton (Hedges, 1992; Libes, 1992; Benner, 2002). Secondary production of zooplankton is also able to be major source of POM, because the grazing rates of zooplankton exceeding the 100% of primary production rates have been reported sometimes in several regions (Sherr and Sherr, 2002 and references there in). Furthermore, zooplanktonic organic matters have been observed in sinking particles by the several studies (Burns et al., 2003, 2004; Parrish et al., 2005). Thus, zooplanktonic POM is also contributes to the POM pool. While most of planktonic POM is remineralized during early diagenesis, stable POM and DOM survived through early diagenesis likely have important roles in the carbon transportation and storage in the ocean. Stable POM settles to deeper water and contributes to vertical transportation of carbon, and stable DOM acts as carbon reservoir in the ocean (Libes, 1992; Benner, 2002). Thus, it is important to elucidate the stabilities and stabilization process of organic matters during early diagenesis for the understanding of the carbon cycling in the ocean.

The stabilities of organic matter have been studied intensively concerning the size of organic matter (Amon and Benner, 1996; Guo et al., 1996; Hama et al., 2004) and biochemicals such as carbohydrates (Amon et al., 2001; Hama et al., 2004), amino acids (Amon et al., 2001; Middelboe and Jørgensen, 2006; Kitayama et al., 2007) and lipids (Wakeham et al., 2003; Caradec et al., 2004; Moriceau et al, in press). Hama et al.

(2004) studied the changes in the size distribution of photosynthetically-produced organic matter on the carbon base, and demonstrated the shift of size distribution of phytoplanktonic organic carbon (OC) from particulate organic carbon (POC) rich distribution to dissolved organic carbon (DOC) dominant distribution during early diagenesis. Their results consist with other results of size/reactivity relation (Fig. 1) in the studies of degradation experiment and field observations (Amon and Benner, 1996; Guo et al., 1996; Carlson, 2002 and references there in), indicating that stable organic matters survived early diagenesis are accumulated as stable DOM. Hama et al. (2004) also showed that the size distributions of carbohydrates were sifted from dominating in POM to dominating in DOM fraction as well as the result of bulk OC. Thus, a part of planktonic organic matter survived through diagenesis has been considered to accumulate into stable DOM pool.

In the studies of stability of biochemicals, Aluwihare and Repeta (1999) studied the stabilities of carbohydrates produced by cultured phytoplankton using molecular analysis, and showed the stability of heteropolysaccharides. Since the heteropolysaccharides have been recognized to be major constituent of cell walls (Hama and Handa, 1992), this result indicates that structural carbohydrates are one of the stable material in the diagenetic process. The stability of the structural carbohydrate more than the storage carbohydrate (glucan) has been also showed by the studies of turnover rates of carbohydrate produced by natural phytoplankton populations (Hama and Yanagi, 2001; Hama et al., 2004). The monomer level studies of amino acids have also elucidated that structural amino acids are more stable than the amino acids contained in cell plasma during diagenesis (Cowie and Hedges, 1992; Laursen et al., 1996; Meckler et al., 2004). Thus, molecular analyses of biochemicals have shown that structural organic matters are more stable than storage organic matter, and the analyses of

biochemicals provide important information on the dynamics of marine organic matter during diagenesis.

Lipids are important biochemical components in plankton as well as carbohydrates and amino acids (Wakeham et al., 1997; Shin et al., 2000; Båmstedt, 1986 and references there in). Specific lipid classes, which were defined by their polarities and fundamental molecular structures, provide useful information about their sources with plankton or organelle levels (see reviews by Gurr and James, 1980, and Parrish, 1988). In order to assess the source of organic matter in marine environments, lipid class analysis has been applied effectively to the study of lipid dynamics such as production (Parrish, 1987; Parrish and Wangersky, 1987; Parrish et al., 2005), vertical flux (Parrish, 1998; Goutx et al., 2000; Parrish et al., 2005) and temporal /spatial distribution (Parrish, 1987; Parrish et al., 1988; Derieux et al., 1998). Thus, the lipid class analysis is a useful method to study the dynamics of marine organic matter

Although degradation rates of some lipid classes have been reported by the study of degradation experiment of algal cells under various conditions (Caradec et al., 2004; Sun et al., 2004; Goutx et al., 2007; Moriceau et al., in press), the degradation properties of lipid classes have not been well elucidated. Especially, the stabilities of storage and structural lipid classes such as triglyceride and phospholipid have not been compared. Since the storage and structural lipid classes have different function and distribution in the cells, it is possible that these lipid classes have different stabilities during early diagenesis. Furthermore, the comparison of the stabilities of lipid classes and bulk OC are important to understand the dynamics of planktonic OC in the ocean. Especially, waxes (W) and phospholipids (PL) have been widely used to estimate the sources of OC in the sediments and settling matters as biomarkers of zooplanktonic (Parrish, 1998; Parrish et al., 2005; Goutx et al., 2007) and phytoplanktonic organic matter (Brinis et al.,

2004; Boschker et al., 2005; Dijkman and Kromkamp, 2006), respectively. But the stabilities of these lipid class biomarkers have been barely compared with that of planktonic OC. The comparison of stabilities of planktonic OC and lipid class biomarkers (W and PL) probably elucidates the quantitative relation between biomarker and planktonic OC (i.e. biomarker/OC ratio) during diagenesis. This ratio makes it possible that quantitative estimation of the contributions of planktonic OC to the OC pools. In order to estimate the contribution of each source to OC pools, it is important to investigate the quantitative relations between biomarkers (W and PL) and planktonic OC.

In the past studies (Caradec et al., 2004; Sun et al., 2004; Goutx et al., 2007; Moriceau et al., in press), diagenesis of lipid classes have been studied focusing on only particulate lipids. However, lipids are mainly composed by dissolved lipids in the oceanic environments (Table 1). Furthermore, the dissolved lipids reportedly have exceedingly lower ^{14}C-ages (i.e. very old) than bulk DOC and other biochemicals such as carbohydrates and amino acids (Loh et al., 2004, 2006). These differences in ^{14}C-ages between dissolved lipid and other organic matter indicate that dissolved lipids have different source(s) and/or diagenetic process from that of other organic matter. Although Loh et al. (2004, 2006) inferred that the old lipid materials were derived from photosynthetically-produced organic matter (autochthonous input) and fossil-like material (anthropogenic input), the contribution of both sources to these old lipid materials dissolved in seawater has not been elucidated. The contribution of phytoplanktonic lipids to the dissolved lipid in the ocean will be able to be evaluated by tracing the fate of photosynthetically-produced lipid materials during diagenesis.

The overall objective was to understand the role of particle degradation/dissolution during decomposition of planktonic organic matter to the carbon cycling in the ocean,

especially on lipid dynamics in seawater. In order to evaluate the fate of planktonic lipids, the phytoplanktonic and zooplanktonic organic matters were applied to degradation experiment. The fates of planktonic organic matter and lipid materials were followed during their degradation/dissolution processes. The reactivity of bulk OC and biomarker lipids were also compared to assess the availability of OC/biomarker lipids on quantitative estimation of the contribution of sources to OC pools. In addition, the contribution of phytoplanktonic lipid materials to the dissolved lipid materials in natural seawater was investigated from the lipid class composition.

2. Degradation and dissolution of photosynthetically-produced organic matter and lipid materials

2. 1. Introduction

Marine organic matter mainly originates from primary production of phytoplankton. Major part (>50%) of OC fixed by phytoplankton is utilized by bacterial population through DOM during early diagenesis (Carlson, 2002 and references there in). Bacterial utilization, thus, is one of the major processes of diagenesis of photosynthetically-produced organic matter. It is known that about 90% of OC fixed by phytoplankton is POC, and the other 10% is DOC (Baines and Pace, 1991 and references there in). The extracellular production by phytoplankton is able to be considered as important production process of DOM (Carlson, 2002). The extracellulary produced DOC has high reactivity and is rapidly utilized by bacteria in a few days after the production (Cole et al., 1982; Norrman et al., 1995; Carlson, 2002). The POC constituting major part of primary products is processed further dissolution and decomposition, and the size distribution of photosynthetically-produced OC shifts from POC to DOC dominated size distribution during diagenesis (Benner et al., 1997; Hama et al., 2004). This shift of size distribution has been observed in not only bulk OC but also biochemical levels such as carbohydrates (Hama et al., 2004). These studies indicate that stable organic matter is accumulated into dissolved fraction through diagenesis of photosynthetically-produced organic matter. Studies of molecular analysis on the carbohydrates and amino acids revealed that the stable carbohydrates and amino acids were composed by structural materials constituting cell wall and membrane (Cowie and Hedges, 1992; Laursen et al., 1996; Meckler et al., 2004; Hama et al., 2004)).

Lipids are one of the major biochemical components in photosynthetically-produced organic matter as well as carbohydrates and amino acids, comprising about 10-30% of photosynthetic products (Wakeham et al., 1997; Shin et al.,

2000). Lipid class analysis is a useful method to study the dynamics of lipid in marine organic matter, because specific lipid classes provide useful information about their sources (see reviews by Gurr and James, 1980, and Parrish, 1988). Lipid dynamics have been studied widely on the production (Parrish, 1987; Parrish and Wangersky, 1987; Parrish et al., 2005), vertical flux (Parrish, 1998; Goutx et al., 2000; Parrish et al., 2005) and temporal/spatial distributions (Parrish, 1987; Parrish et al., 1988; Derieux et al., 1998) in the lipid class level. Parrish et al. (2005) studied the vertical transport process of lipid class from surface water to benthic food web by analysis of settling particle collected by sediment trap. In their study, lipid class analysis indicates that lipids produced by phytoplankton were major constituents of settling lipids. However, their study showed that the settling matter was also derived from several sources such as zooplankton and resuspension of sediment. Thus, the lipid materials derived from zooplankton and sediment contribute to the lipid in the settling matter in the ocean. The diverse sources of settling lipid lead the difficulty in the quantitative estimation of the contribution of photosynthetically-produced lipids to the settling lipids by the measurements of lipids in settling matter. In order to elucidate the transport process of photosynthetically-produced lipids in the ocean quantitatively, direct assessment of the stability of photosynthetically-produced lipids is important. On the other hand, the stabilities of lipid classes have been considered to be different among lipid classes (Caradec et al., 2004; Sun et al., 2004; Goutx et al., 2007; Moriceau et al., in press). Since the lipid class composition in phytoplankton is different depending on the nutritional condition (Parrish and Wangersky, 1987; Lombardi and Wangersky, 1991; Reitan et al., 1994), the stability of lipids must to be studied in lipid class level.

Although stabilities of photosynthetically-produced lipids have been studied widely in the fatty acids level (Hama, 1991; Hama et al, 1992; Sun and Wakeham, 1998;

Ding and Sun, 2005), the stabilities of lipid classes have not been elucidated completely. Furthermore, specific fatty acids constituting phospholipids (PL) have been applied to the estimation of the sources of settling particles and sediment in recent studies (Brinis et al., 2004; Boschker et al., 2005; Dijkman and Kromkamp, 2006). However, the stabilities have not been compared between PL and POC of phytoplankton, resulting in difficulties on the estimation of the contribution of phytoplankton to the OC in the sediment or settling matter quantitatively. In order to estimate the quantitative contribution of phytoplankton to OC pools, the elucidation of quantitative relation between PL and OC of phytoplankton (i.e. PL/ OC of phytoplankton ratio) during diagenesis is necessary.

The degradation of lipid classes of algae has been studied in various environments (Caradec et al., 2004; Sun et al., 2004; Goutx et al., 2007; Moriceau et al., in press), and the degradation rates of each lipid class have been determined. Moriceau et al. (in press) compared the degradation property of each lipid class of algal lipid associating with the biogenic silica-carbon interaction during degradation experiment of diatom *Skeletonema marinoi*. These studies, however, focused on only specific lipid classes in the particulate fraction. The stabilities of storage and structural lipid classes such as triglycerides (TG) and PL have not been compared in their study, although the storage and structural lipid classes have different functions and distributions in the cell and possible different stabilities. In addition, the stabilities of lipid classes have not been also compared with that of OC of phytoplankton.

In past studies of degradation experiment of phytoplanktonic organic matter (Caradec et al., 2004; Sun et al., 2004; Goutx et al., 2007; Moriceau et al., in press), dissolved lipids have not been assessed. Although the compositions of dissolved lipids in natural seawater have been reported in the studies of temporal/spatial distributions of

lipid classes (Parrish, 1987; Parrish et al., 1988; Derieux et al., 1998), the dynamics including production and decomposition processes of dissolved lipid have not been studied. Since major part of lipids is present as dissolved form in the ocean (Table 1), the elucidation of the dissolution process of planktonic lipids is important to understand the dynamics of lipid in the natural seawater.

In the recent studies, exceedingly ^{14}C-depleted (i.e. very old) dissolved lipid materials were observed in estuarine (Wang et al., 2006; Loh et al., 2006) and open seawater (Loh et al., 2004). In the studies of Loh et al. (2004, 2006), the apparent ages of lipids (5,300-6,900 and 6,400-17,100 years B.P. for estuarine and open seawater, respectively) obviously exceeded the values of bulk DOC (200-700 and ~2,000-6,100 years B.P. for estuarine and open seawater, respectively) and other biochemicals such as carbohydrates and amino acids (modern-1,500 and modern-4,200 years B.P. for estuarine and open seawater, respectively). Their studies indicate that the source(s) and/or diagenetic stage differ between dissolved lipid materials and other organic matters in the ocean. Although Loh et al. (2004, 2006) inferred that old/dissolved lipid materials are originated from both photosynthetically-produced organic matter (autochthonous input) and fossil-like material (anthropogenic input), the contributions of both sources to the old/dissolved lipid pool have not been elucidated. The direct evaluation of the fate of photosynthetically-produced lipid materials during diagenesis is important to understand the dynamics and the contribution of phytoplanktonic lipids to the old/dissolved lipids in the ocean. However, it is rather difficult because organic matter in natural seawater contains not only phytoplankton but also other materials such as bacteria and non-living organic matter.

In this study, natural phytoplankton populations were incubated with $NaH^{13}CO_3$ under light for 1 day to label the photosynthetically-produced organic matter with ^{13}C.

In addition to the assessment of the extracellular lipid production, the labeled phytoplankton was applied to the degradation experiment under the dark for 29 days in the presence of bacteria. In order to elucidate the role of lipids in the carbon cycling and the source of dissolved lipid in the ocean, the labeled organic matter and lipid materials were followed about their degradation/dissolution and lipid class composition through the incubation period. In addition, the stabilities of bulk OC and PL were compared to assess the possibility of quantitative estimation of the contribution of phytoplankton to OC pools using PL/OC ratio.

2. 2. Materials and Methods

2. 2. 1. Degradation experiment of phytoplankton

Surface seawater containing phytoplankton community was collected at the coast of Tokyo Bay, one of the most eutrophic bays in Japan on 6 August 2006. The concentration of chlorophyll *a* (Chl *a*) annually ranged between ~2 and over 50 μg L^{-1} (public data from Chiba Prefectural Fisheries Research Center). Seawater sample (136.2 L) was screened through a 100-μm mesh to remove macro zooplankton, and then 22.7 L of sample was transferred into acid-cleaned polycarbonate bottles (six bottles with a volume of 23 L each). The samples were then transported to the laboratory and $NaH^{13}CO_3$ and nutrients ($NaNO_3$, $NaH_2PO_4 \cdot 2H_2O$, Na_2SiO_3) were added with the ^{13}C atom% of dissolved inorganic carbon (DIC) of 16.2%, and the final concentrations of nitrate, phosphate and silicate of 72.1, 9.4 and 31.4 μM, respectively. The samples were incubated under fluorescent light (49 μmol m^{-2} s^{-1}) for 24 hours at 26°C in order to label the organic matter produced by natural phytoplankton population, and then incubated under dark at 20°C for 29 days. The sample in the incubation bottle was mixed by swinging the bottle when sub-sample was recovered. Sub-samples were recovered from the incubation bottles on Day 1, 1.5, 2, 4, 7, 15 and 30. The volumes of sub-sample were 10 L, 20 L and 40 L on Day 1-7, Day 15 and Day 30, respectively. Although sample was incubated without aeration, the organic matter was considered to be degraded under oxic condition by comparing the concentration of initial dissolved oxygen and organic matter decreased during degradation.

Sub-samples were filtered through pre-combusted (450°C, 6 hours) Whatman GF/F glass fiber filter (nominal pore size is 0.7 μm). The filtrates were subsequently filtered through Whatman Anodisc membrane filter (nominal pore size is 0.2 μm). Thus, organic matter was separated into three size fractions (particulate organic matter (POM) of

0.2-0.7 μm and 0.7-100 μm, and DOM with a size of < 0.2 μm). From scanning electron microscope (SEM) observations, POM with a size of 0.7-100 μm contained phytoplankton and bacteria, while POM with a size of 0.2-0.7 μm contained bacteria without phytoplankton. Non-living organic matter was contained in all fractions.

Filter and filtrate samples applied to analysis of Chl *a*, nutrient, carbon concentration and ^{13}C atom% were stored at -20°C until analysis. Filter samples applied to lipid class analysis were stored at -20°C in the chloroform until lipid extraction. Lipids in filtrate were extracted immediately after the filtration, and the extracts were stored at -20°C until analysis.

2. 2. 2. Lipid extractions

Lipids in samples were extracted with chloroform/methanol (2/1, v/v) using the modified method of Folch et al. (1957). The filters were cut into small pieces and transferred into a centrifuge tube with chloroform/methanol (5 ml/filter). Then the tube was exposed to ultra-sonication for 15 min in an ice-cold water bath, following centrifugation (1500 rpm, 5 min) and recovery of supernatant. This extraction process was repeated three times, and all supernatants were combined. The supernatant was washed three times with distilled water to remove water-soluble non-lipid content, and the chloroform phase was stored at -20°C until fractionation. Recovery of particulate lipid was 94% (±4%) in carbon concentration (n = 4).

Lipid in filtrate was extracted using a separating funnel with chloroform/methanol (35 ml/1 L of filtrate), and the chloroform phase was collected. This extraction was repeated three times, and the combined chloroform phase was stored at -20°C until analysis. Recovery of dissolved lipid was 87% (±6%) in carbon concentration (n = 4).

2. 2. 3. Fractionation of lipid extracts

Lipid extracts were separated by silica gel column chromatography using the modified method of Rouser et al. (1967), and neutral lipid fraction obtained by column chromatography was further fractionated by thin layer chromatography (TLC).

A gram of silica gel (Wakogel C-100) in chloroform was poured into a chromatographic tube (6 mm in diameter). The lipids loaded in the column were then successively eluted by chloroform (20 ml), acetone (80 ml) and methanol (60 ml). The eluate was collected for each solvent fraction. Lipid classes included in each solvent fraction were identified by TLC in comparison with results with standard materials; hydrocarbons (HC: HC mixture 11 (Larodan Fine Chemicals AB)), TG (tripalmitin), diglyceride (DG: dipalmitin), monoglyceride (MG: monopalmitin), free fatty acid (FFA: palmitic acid), alcohol (Alc: cethyl alcohol), sterol (St: cholesterol), glycolipid (GL: cerebroside) and PL (phospholipid Kit (DOOSAN Serdary Research Laboratories)) were used as standard materials. Lipid classes other than the above-mentioned standard materials such as waxes (W) and Chl *a* were inferred by *Rf* values. Lipids in the chloroform fraction were composed by neutral lipids (HC, W, TG, FA, St, DG and a small amount of MG). The lipid classes contained in acetone fraction were MG, Chl *a* and GL. Small amounts of phosphatidic acid and cardiolipin were also contained. Methanol fraction contained most of the PL. Acetone and methanol fractions were referred to the fractions of acetone mobile polar lipids (AMPL) and PL as listed in Table 2-1.

Neutral lipids were further fractionated by TLC into three fractions constituted by HC, TG and other neutral lipids (ONL) fractions. Neutral lipids spotted on silicagel coated plates (70 Plate-wako) were eluted with hexane/diethyl ether/acetic acid (6/4/1, v/v). Authentic neutral lipid standards listed above were co-developed at the adjacent

area of the sample lipids. Silica on the area of the sample plate corresponding with each standard of lipid classes were scraped, and transferred into a centrifuge tube with two milliliters of chloroform/methanol (2/1, v/v). The lipid extraction was repeated three times according to a particulate lipid extraction procedure (Section 2. 2.). Extracted lipids were stored at -20°C until analysis.

Lipid fractions obtained by column chromatography and TLC are listed in Table 2-1 with their major constituents of lipid classes. The lipid class of W are not or barely produced by photosynthesis of phytoplankton and mainly originated from zooplankton or terrestrial organic matter. Thus, the W fraction was not obtained in the degradation experiment of phytoplankton (Table 2-1). Overall recovery of fractionated lipid material through column chromatography and TLC was 81% (±12%) in carbon concentration (n = 21).

2. 2. 4. Measurement of photosynthetically-produced OC and LC

The concentrations of photosynthetically-produced OC (P-OC) and LC (P-LC) were analyzed according to Hama and Yanagi (2001).

Filter sample was exposed to vapor of hydrochloric acid to remove DIC and dried in a desiccator with sodium hydrate. Filtrate sample was desalted by electrodialysis (Microacilizer S-3, Asahi Chemical) and concentrated from 1 L to 30 ml by rotary evaporator. The HCl was added to the concentrate to adjust to pH 2, and the concentrate purged with N_2 gas to remove DIC. After the samples were neutralized by NaOH solution, part of the concentrate was absorbed and dried on quartz wool in a tin capsule in a vacuum desiccator. Lipid sample in the solvent was completely dried up in a tin capsule by nitrogen purge to remove the solvent.

The carbon concentration and ^{13}C atom% of OM and lipid class were analyzed

using a mass spectrometer (DELTA plus, Thermo Finnigan), combined with an elemental analyzer (EA 1108, FISONS Instruments). However, the possible loss of dissolved organic carbon (DOC) during concentration was reported to be 10-16% of DOC in the original filtrate (Hama and Yanagi, 2001), so that the DOC concentration determined by the high-temperature combustion method with Shimadzu TOC 5000A was used for the following calculation.

The concentration of P-OC and P-LC were calculated as follows:

$$\text{P-OC (or P-LC; } \mu\text{M)} = (a_{is} - a_{ns}) \times (a_{ic} - a_{ns})^{-1} \times \text{[OC] (or [LC]; } \mu\text{M)}$$

Where a_{is}, a_{ns} and a_{ic} are ^{13}C atom% of OC in incubation sample and ^{13}C atom% of OC in natural sample (i.e. non-incubated), and ^{13}C atom% of DIC in incubated seawater was enriched by adding $NaH^{13}CO_3$, respectively. Abbreviations [OC] and [LC] indicate their respective concentrations in the incubation sample.

The CV of the analysis of concentration in fractionated P-LC was determined for sample on Day 1 and 30 (n = 3 for each sample) and was generally less than 10% except for the value of P-LC in PL fraction on Day 1 (CV = 21%).

2. 2. 5. Measurements of inorganic nutrients and chlorophyll a

The concentrations of nitrate, phosphate and silicate were analyzed by spectrophotometry (Parsons et al., 1984). The concentration of Chl *a* was determined by fluorometry according to Suzuki and Ishimaru (1990).

2. 3. Results

2. 3. 1. Experimental conditions

The concentrations of Chl *a* and POC in the sample were 4.5 μg L^{-1} and 72 μM C at the beginning of incubation. The concentration of Chl *a* fell within the range of the concentration in natural seawater of Tokyo-Bay (~2.5-50 μg L^{-1}). The concentrations of Chl *a* and POC increased to 12.8 μg L^{-1} and 134 μM C during light incubation period. The ratio of POC/Chl *a* in the phytoplankton that increased during the light incubation period was calculated to be 7.4 by dividing the increasing concentration of POC (62 μM C) by that of Chl *a* (8.3 μg L^{-1}). Using this ratio and the initial Chl *a* concentration, the concentration of POC derived from phytoplankton was estimated to be 33 μM C, accounting for 53% of the total POC at the beginning of the incubation. At the end of the light incubation period, the phytoplankton population was mainly composed of diatoms such as *Chaetoceros* spp., *Haslea* spp., *Pseudonitzschia* spp. and *Leptocylindrus* spp., which have been generally observed in Tokyo-Bay.

The concentrations of nitrate, phosphate and silicate decreased to 24.1, 6.8 and 9.4 μM, respectively, during the light incubation period. Although nutrient limitation has been reported to affect the lipid concentration and lipid class composition in phytoplankton (Parrish and Wangersky, 1987; Lombardi and Wangersky, 1991; Reitan et al., 1994), nitrate, phosphate and silicate did not limit the phytoplankton photosynthesis in the present study.

2. 3. 2. Changes in the concentration of P-OC and P-LC

The concentrations of total P-OC (P-OC-total), P-OC in GF/F fraction (P-POC-GF) and P-OC in DOM fraction (P-DOC) increased during the light incubation period and reached maximum (82.2, 75.2 and 5.1 μM C, respectively) on Day 1 (Fig.

2-1-A). The concentration of P-OC in Anodisc fraction (P-POC-Ano) also increased from the start of incubation, but the maximum concentration was observed on Day 1.5 (2.7 μM C). The concentrations of P-OC-total and P-POC-GF decreased quickly after 1-day, followed by slow decreases until Day 30. The degradation constants (*k*) of P-OC-total and P-POC-GF were calculated by first-order kinetics for both early (1-4 days) and latter (4-30 days) degradation periods (Table 2-2), because the *k* was obviously decreased on Day 4. Although the degradation constants were calculated using the only 3-4 averaged data points, the most of r^2 were >0.9, indicating that degradation constants was sufficient to have a rough comparison each other. The degradation constants of P-OC-total and P-POC-GF showed higher values during early degradation period than latter degradation period.

After the concentrations of P-POC-Ano and P-DOC reached maximum, the concentrations decreased slowly until Day 4, followed by constant values until Day 30. P-OC-total and P-POC-GF concentrations decreased to 9.6 and 3.8% of the maximum concentrations, respectively at the end of incubation. On Day 30, P-POC-GF, P-POC-Ano and P-DOC accounted for 3.5, 1.4 and 4.7% of the maximum concentration of the P-OC-total observed on Day 1, respectively.

Concentrations of total P-LC (P-LC-total) and P-LC in GF/F fraction (P-LC-GF) increased rapidly in the light incubation period, and reached maximum (16.8 and 16.7 μM C, respectively) on Day 1 (Fig. 2-1-B). The contributions of P-LC to P-OC in each size fraction were 22.1% for GF/F fraction, 3.2% for Anodisc fraction and 2.2% for DOM fraction, at the end of the light incubation period. Maximum concentrations of P-LC of Anodisc and DOM fractions (P-LC-Ano and P-LC-DOM, respectively) were found on Day 1.5 (0.1 and 0.2 μM C, respectively). After the maximum concentrations were observed, the P-LC concentrations of each size fraction decreased rapidly until

Day 4, followed by a slow decrease until Day 30. The degradation constant of P-LC-GF was 0.48 (r^2 = 0.91) in the early degradation period (1-4day), and decreased to 0.08 (r^2 = 0.95) (Table 2-2). P-LC-total and P-LC-GF concentrations on Day 30 were 3.0 and 2.7% of the maximum concentrations, respectively. P-LC-GF, P-LC-Ano and P-LC-DOM on Day 30 accounted for 2.7, 0.1 and 0.2% of the maximum concentration of P-LC-total observed on Day 1, respectively.

2. 3. 3. Changes in the size distribution of P-OC and P-LC

The major part of P-OC was found in the GF/F fraction at the end of the light incubation period; P-POC-GF, P-POC-Ano and P-DOC accounted for 91.5, 2.3 and 6.2% of the P-OC-total, respectively (Fig. 2-2-A). The contribution of P-POC-GF to the P-OC-total decreased with the progress of incubation, whereas other fractions increased. P-POC-GF, P-POC-Ano and P-DOC accounted for 36.3, 14.9 and 48.8% of the P-OC-total at the end of incubation, respectively.

P-LC was exclusively composed of P-LC-GF (99.0%), and a small contribution of P-LC-Ano (0.4%) and P-LC-DOM (0.7%) was noticed on Day 1 (Fig. 2-2-B). The contribution of P-LC-GF slightly decreased with the progress of incubation, whereas that of P-LC-DOM increased. Although the size distribution of P-LC shifts to size distribution dominated by dissolved fraction as well as that of P-OC, the major part of P-LC was composed by P-LC-GF (91.0%), and the contributions of P-LC-Ano (2.5%) and P-LC-DOM (6.5%) were low at the end of incubation.

2. 3. 4. Changes in the concentration and composition of lipid fraction in P-LC

The concentrations of each lipid fraction in P-LC-GF showed rapid increases in the light incubation period except for P-LC-GF-HC (Fig. 2-3-A). P-LC-GF-HC was a minor

component of P-LC-GF throughout the incubation period (Fig. 2-4-A). Maximum concentrations were observed on Day 1 for P-LC-GF-TG and -PL (2.6 and 2.4 μM C, respectively) and on Day 1.5 for P-LC-GF-ONL and -AMPL (3.9 and 4.3 μM C, respectively). P-LC-GF-ONL and –AMPL were found to be the most important lipid fractions (29.2 and 30.8% to P-LC-GF, respectively), followed by P-LC-GF-TG and -PL (20.1 and 18.5% of P-LC-GF, respectively) on Day 1.

The concentrations of P-LC-GF-TG, -ONL and -AMPL decreased rapidly until Day 4, followed by slow decrease of P-LC-GF-ONL and -AMPL until Day 30 (Fig. 2-3-A). Most of P-LC-GF-TG disappeared during this period. On the other hand, the concentrations of P-LC-GF-PL decreased slowly throughout the whole incubation period. The concentrations of P-LC-GF-ONL, -AMPL and -PL decreased slowly after 4-day until the end of experiment. The degradation constant of P-LC-GF-TG during early degradation period (k = 1.05) was obviously higher than that of other lipid fractions (k = 0.29-0.47, Table 2-2). The degradation constants of P-LC-GF-ONL, -AMPL and -PL apparently decreased in the latter degradation period and showed similar values (k = 0.07-0.08) (Table 2-2). The major constituents of P-LC-GF were P-LC-GF-ONL (31.6%) and -AMPL (40.7%), followed by -PL (23.1%), while P-LC-GF-TG was a only minor fraction (2.0%) at the end of the incubation (Fig. 2-4-A). The concentration of P-LC-GF-TG, -ONL, -AMPL and -PL remained on Day 30 accounted for 0.3, 3.0, 3.7 and 3.5％ of each maximum concentration, respectively.

The concentrations of P-LC-Ano in each lipid fraction increased until Day 1.5 except for PL (Fig. 2-3-B); P-LC-Ano-PL was a only minor component throughout the incubation period (Fig. 2-4-B). The concentrations of P-LC-Ano-HC and -TG decreased rapidly after Day 1.5 and reached extremely low concentrations on Day 2. On the other hand, the concentrations of P-LC-Ano-ONL and -AMPL decreased slowly until the end

of incubation. The major components of P-LC-Ano were ONL and AMPL throughout incubation period, and the contributions of ONL and AMPL were 64.4% and 27.7% on Day 30, respectively.

The concentrations of P-LC-DOM-ONL and -AMPL increased until Day 1.5, reaching 0.05 and 0.09 μM C, respectively (Fig. 2-3-C). The maximum concentration of P-LC-DOM-PL (0.01 μM C) was observed on Day 2, after which the concentrations of P-LC-DOM-ONL, -AMPL and -PL decreased rapidly until Day 4 and remained constant until Day 30. Major fractions in P-LC-DOM were ONL (26.3-35.0%) and AMPL (58.1-67.4%) followed by PL (1.3-7.0%) (Fig. 2-4-C). P-LC-DOM-HC and -TG were exclusively minor components throughout the incubation period. There were no obvious changes in the composition of lipid fractions, and more than 85% of P-LC-DOM was composed of ONL and AMPL throughout the incubation period.

2. 4. Discussions

2. 4. 1. Extracellular lipid production and the contribution to the dissolved lipid in the ocean.

The release of organic matter by phytoplankton into the ambient waters has been known as the essential metabolic process (Biersmith and Benner, 1998; Hama and Yanagi, 2001) and one of the important production processes of DOM (Baines and Pace, 1991; Carlson, 2002). Carbohydrates are known to be major extracellular products (Hellebust, 1974; Hama and Yanagi, 2001), among which lipid material comprises a small part (2.8-10.3%) (Hellebust, 1974). On the other hand, Parrish and Wangersky (1987) observed that extracellular lipid production by the diatom *Phaeodactylum tricornutum* accounted for about 10% of the total (intracellular and extracellular) lipid production under the nitrogen-replete condition. However, little is known about the extracellular lipid production in natural phytoplankton populations.

The present study revealed that P-LC-DOM accounted for only 2.2% of P-DOC at the end of the light incubation period, which was considerably lower than the contribution of carbohydrates (66%, Biersmith and Benner, 1998; 28-49%, Aluwihare and Repeta, 1999; 47-59%, Hama and Yanagi, 2001). Thus, it is possible that extracellular lipid production of phytoplankton scarcely contributes to the dissolved lipids in the ocean. The ratio of P-LC-DOM to P-LC-total is calculated to be 0.7% at the end of the light incubation period, far lower than that of P-DOC (6.2%). This strongly suggests that the release of lipid material is not the essential metabolism of phytoplankton, and that most photosynthetically-produced lipids are retained as cellular constituents under an abundant nutrient condition.

After the concentrations of P-DOC and P-LC-DOM reached to each maximum concentrations (1 and 1.5-day, respectively), about 41% and 79% of maximum

concentrations decreased until Day 4, respectively. The degradation rate was higher in the P-LC-DOM than the P-DOC. These values indicate that dissolved lipid materials produced in the early incubation period were more labile than other organic matter.

2. 4. 2. Reactivities of P-OC and P-LC

The labile property of lipid materials more than the bulk organic carbon have been reported widely by the studies of degradation experiment of phytoplankton and vertical distribution of lipid (Wakeham et al., 1997; Hamanaka et al., 2002; Caradec et al., 2004). The degradation constant of P-LC-GF (0.48) was higher than that of P-POC-GF (0.38) during early degradation period（1-4 day) in the present study (Table 2-2). Furthermore, the remaining percentage of P-LC-total observed on Day 30 (3.0%) was less than one-third of that of P-OC-total (9.6%). Thus, the lability of P-LC comparing to P-OC was clearly shown in this study and consists with the results of studies reported so far.

On the other hand, the degradation constants of P-LC-GF decreased with progress of degradation and reached to the equivalent value with that of P-OC-GF during latter degradation period (Table 2-2). These results indicate that lipids produced by phytoplankton were composed by both labile and stable materials, and suggest that the stable lipid materials have same reactivity as P-OC-GF. These stable lipids probably contribute to the vertical transportation of organic matter.

2. 4. 3. Bio-degradability of photosynthetically-produced triglycerides and phospholipids

This study shows that lipids, which have been recognized as labile materials, are composed of both labile and stable materials. The different bio-degradability among lipid classes of algae has been reported (Sun et al., 2004; Caradec et al., 2004; Goutx et

al., 2007; Moriceau et al., in press). These studies, however, focused on only specific lipid classes and did not compare the reactivities between each lipid class and bulk organic carbon. In this study, the reactivities of lipid classes were studied focusing on structural (i.e. PL) and storage (i.e. TG) lipid materials, and compared with the reactivity of bulk organic carbon.

The structural lipid (P-LC-GF-PL) obviously exhibited a slower decrease than storage lipid (P-LC-GF-TG) (Table 2-2, Fig. 2-3-A). Furthermore, the remaining percentage at the end of the experiment to the maximum concentration of P-LC-GF-PL (3.5%) was more than ten times higher than that of -TG (0.3%). Since TG is the only minor component of bacterial lipids (Goutx et al., 1990), it is possible that most lipid of P-LC-GF-TG was a constituent of phytoplankton cells. The rapid decrease in P-LC-GF-TG was possibly caused by several processes such as release of P-LC-GF-TG into DOM fraction, consumption by phytoplankton as the energy source, hydrolysis by active lipase contained in algal cell, and degradation by bacteria. The minute amount of P-LC-DOM-TG throughout the incubation period (Fig. 2-3-C, 2-4-C) clearly shows that the storage lipids are difficult to dissolve and/or readily consumed by bacteria after the dissolution. On the other hand, the slow degradation of P-LC-GF-PL strongly suggests that structural lipid of phytoplankton may be resistant to bacterial attack. It is possible that attached bacteria during incubation period also produced PL by their growth using P-OC. However, the bacterial biomass was not determined in the present study. Thus, it is not able to estimate the contribution of bacterial PL to the P-LC-GF-PL at the end of incubation. Although the presence of bacterial PL might increase the apparent stability of P-LC-GF-PL, it is clear that structural lipid remained longer period than storage lipid during early diagenesis of photosynthetically-produced lipid.

The stability of structural materials has been widely established for other

biomolecules. Membrane proteins of phytoplankton (Laursen et al., 1996) and bacteria (Nagata et al., 1998) are reportedly more stable than cytoplasmic ones against to bacterial degradation. Preservations of amino acids associating with diatom cell walls have also been observed in sediments (Cowie and Hedges, 1992; Meckler et al., 2004). Furthermore, the bio-refractory property of structural carbohydrates (heteropolysaccharides) more than storage carbohydrates such as glucan has been repeatedly observed (Aluwihare and Repeta, 1999; Hama and Yanagi, 2001; Hama et al., 2004; Hanamachi et al., 2008). These studies are consistent with our observations and evidences of the stability of the structural materials in microorganisms.

It has been suggested that the high stability of membrane proteins results from association with other organic compounds like PL (Nagata et al., 1998; Borch and Kirchman, 1999). Because PL has hydrophobic and hydrophilic polarities, PL possesses not only hydrophobic bonds but also hydrogen and covalent bonds among other organic compounds such as integral membrane proteins in cell membrane (Alberts et al., 1994). Most TG, on the other hand, exists as oil droplets with hydrophobic bonds (i.e. surface tension) in the cytoplasm (Alberts et al., 1994). The different chemical properties between storage and structural lipids possibly affect their stability. Suzumura et al. (1998) reported that hydrophobic dissolved organic phosphorus resistant to enzymatic phosphohydrolysis was remineralized by enzyme after that dissolved organic phosphorus was extracted by chloroform. Their result also suggests that the stability of organic phosphorus under enzymatic hydrolysis results from interactions with other materials such as formation of macromolecules. Thus, the chemical interactions of the structural lipids among several organic compounds are possible reasons for the recalcitrant property of membrane compounds.

Specific fatty acids constituting PL have been used to estimate the sources of

sediments and settling matters as biomarker (Brinis et al., 2004; Boschker et al., 2005; Dijkman and Kromkamp, 2006). However, the difference in the reactivities between PL and OC has not been studied, so the quantitative estimation of the contribution of phytoplanktonic organic matter to the sediments and settling matters is not able to be elucidated. When the relation of reactivities between PL and OC is estimated, the contributions of phytoplanktonic OC to the OC in the sediments and settling matters should be estimated quantitatively. In the present study, the degradation constants of P-LC-GF-PL showed comparable values to that of P-POC-GF throughout experiment (Table 2-2). The similar reactivities of P-LC-GF-PL and P-POC-GF resulted in the constant values of PL/OC ratio (average: 3.2±1.0, n = 6). The constant values of PL/OC ratio suggest the possibility of the quantitative estimation of the contribution of phytoplanktonic organic matter to the sediments and settling matters by measuring PL associating to the biomarker (fatty acids) measurement. However, it is possible that degradation conditions such as sources of PL and OC, PL contents in the phytoplanktonic organic matter and bacterial population affect to the PL/OC ratio. Additional researches are necessary on several degradation conditions for the quantitative estimation of the contribution of phytoplanktonic organic matter to the OC pools.

2. 4. 4. Differences in accumulation of P-OC and P-LC in dissolved fraction during early diagenesis

The concentration of P-POC-GF decreased throughout dark incubation period, whereas that of P-DOC maintained a constant value in the latter dark period (4-30 days, Fig. 2-2-A), resulting in the increase in percentage of P-DOC in the P-OC-total; P-DOC accounted for the major part (49%) of the P-OC-total on Day 30 (Fig. 2-3-A). This

result is consistent with the results of Hama et al. (2004), and strongly suggests that relatively stable organic carbon tends to accumulate as dissolved organic matter during early diagenesis. Experimental studies strongly suggest that formation of recalcitrant DOM is accompanied by bacterial degradation of organic matter (Ogawa et al., 2001; Hama et al., 2004).

The contribution of P-LC-DOM in the P-LC-total was very small (6%, Fig. 2-3-B) on Day 30. This high contribution of particulate fraction to the P-LC-total on Day 30 is quite different from the results of P-OC. These results indicate that planktonic lipids contribute to the vertical transport of organic matters rather than the formation of stable DOM pool, while planktonic organic carbon contribute to the formation of DOM pool more than planktonic lipids during early diagenesis. The high contribution of P-LC-GF to the P-LC-total throughout the experiments possibly resulted from the difficulty of solubilization of lipid materials into ambient seawater. Lipids are chemical groups soluble in nonpolar solvents such as chloroform, whereas most carbohydrates and amino acids are water-soluble. Hydrophobic molecules are preferentially insoluble in water and tend to form micelles or be adsorbed on/into the POM in the water as a result of their hydrophobic property (i.e. van der Waals adsorption). The adsorption of lipid-like materials to the POM was suggested by the studies of the source of materials constituting sinking POM using stable carbon and radiocarbon isotope signatures (Hwang and Druffel, 2003; Hwang et al., 2006). Thus, it is probable that the insolubility of lipid materials in ambient water is one of the causes of the low contribution of P-LC-DOM to the P-LC-total.

Another factor preventing the accumulation of P-LC-DOM is the susceptibility of dissolved lipids possibly supplied by lysing phytoplankton cells. The concentration of P-LC-DOM rapidly increased during Days 0-1.5, indicating a part of the

photosynthetically-produced lipid was dissolved during the early incubation period. But about 80% of the maximum concentration of P-LC-DOM was readily decomposed by Day 4 (Fig. 2-2-B), while P-DOC decreased slowly during Days 1-4 (Fig. 2-2-A), thus, only 40% of the maximum concentration of P-DOC was degraded by Day 4. This result implies that P-LC-DOM is one of the most susceptible components among the DOM released during the lytic process of phytoplankton cells.

While P-LC-DOM is supposed to be susceptible to bacterial activity due to the rapid decrease in P-LC-DOM from Days 2 to 4, the concentration of P-LC-DOM was constant after Day 4. Furthermore, the contribution of P-LC-DOM to the P-LC-total increased together with the progress of incubation the same as for P-DOC. These results indicate that at least a few stable lipid materials were accumulated in the dissolved fraction during the early diagenesis. Accumulations of less bioreactive carbohydrates and amino acids during bacterial degradation have already been reported (Aluwihare and Repeta, 1999; Amon et al., 2001; Hama et al., 2004). Thus, it is possible that a relatively stable fraction of lipid materials is also able to accumulate as dissolved fraction during early diagenesis, though the accumulation rate is much slower than that of stable DOC.

2. 4. 5. Size distributional changes in lipids and source of marine dissolved lipid

In order to estimate the source of marine dissolved lipid, the size distribution and composition of dissolved lipid classes were compared between P-LC and LC. In this study, the concentrations of OC and LC were measured as well as those of P-OC and P-LC (See Materials and Methods). The OC and LC are composed of not only P-OC and P-LC but also organic matter which was already contained in the water sample collected from Tokyo-Bay. On Day 30, the contributions of P-OC to OC and P-LC to

LC were very low (4.6% and 11.9%, respectively). The size distributions of OC and LC at the end of incubation (Fig. 2-2-A, -B) could therefore reflect that these materials existed in natural seawater. The present study thus makes it possible to compare the size distributions and molecular compositions between P-OC (P-LC) and OC (LC).

The contribution of P-DOC to the P-OC-total increased throughout the incubation period and approximated that of OC on Day 30 (Fig. 2-2-A). The size distribution of OC on Day 30 was comparable with the knowledge accumulated for oceanic waters (Williams, 1971). Thus, the size distribution in the P-OC readily approximated that of OC in the ambient seawater during 30-day diagenesis, demonstrating the size change in phytoplankton OM to the size distribution of OM in natural environments.

In contrast to P-DOC, the P-LC-DOM contributed only 6.5% of P-LC on Day 30, although the size distribution of LC was dominated by the DOM fraction (Fig. 2-2-B). This size distribution of LC is almost comparable with that of lipid materials in natural environments (Table 1), though the contribution of dissolved lipid shows a variation with a rather wide range depending on the phytoplankton biomass. From these results, it is obvious that the size distribution of P-LC obtained after 30-day diagenesis did not reflect the size distribution of LC in natural seawater. The predominance of dissolved lipids in natural samples indicates that sources other than early plankton diagenesis fed the dissolved fraction, which is not demonstrated by the 30-day incubation experiment. Thus, one may hypothesize that the photosynthetically-produced lipid-material survived longer term of diagenesis than that of present study and/or refractory allochthonous lipid material constitute the dissolved lipid materials in the ocean.

In this study, about 80% of dissolved LC (LC-DOM) was due to the lipid classes in -ONL and -AMPL on Day 30 (Fig. 2-4-C), and this lipid class composition was comparable with the composition of P-LC-DOM. This compatibility implies that

phytoplanktonic lipid materials remain as the stable lipid materials in the dissolved fraction in natural environments. Loh et al. (2004) suggested that the aged (i.e. stable) lipid materials in the open ocean may be dominated by planktonic materials rather than fossil materials from the measurements of $\delta^{13}C$, $\Delta^{14}C$, elemental ratios and biomarkers. In the present study, the percentages of P-LC-DOM slightly increased throughout the 30-day incubation (Fig. 2-2-B). This result also indicates that photosynthetically-produced lipid materials accumulate in the dissolved fraction as stable lipid materials, although the production rate of stable lipids is much slower than that of bulk organic carbon. Thus, it is possible that the longer period of diagenesis more than this study makes the size distribution of P-LC approximate to that of LC in natural seawater.

On the other hand, lipid materials in -HC and -TG were noted as one of the main components of LC-DOM (totally accounting for 21%) on Day 30, though they accounted for only a minor portion (0.5-4.1%) in photosynthetically-produced lipids (P-LC-DOM). The major constituents of -HC are likely originated from two sources; fossil-like lipid materials and/or biological products through biological reactions such as decarboxylation of fatty acids. Since little P-LC-DOM-HC was observed on Day 30 (Fig. 2-3-C, 2-4-C), hydrocarbons produced by biological degradation of photosynthetically-produced lipids are unlikely to remain through early diagenesis. This result indicates that the LC-DOM-HC collected from Tokyo Bay mostly originated from the fossil-like lipid materials. Previous studies (Wang et al., 2004, 2006) pointed out the possible contribution of fossil-like materials to the dissolved lipid in the estuary waters by measurements of carbon isotopes. In this study, the water sample was collected at near an urbanized region, so it is possible that the fossil-like materials such as petroleum were included in the dissolved fraction. Thus, recalcitrant allochthonous lipid materials

such as fossil-like materials may indeed be one of the contributors of stable and dissolved lipid materials in natural seawater.

It is likely that the non-bioreactive lipid materials in the dissolved fraction of natural seawater derived from inputs of both allochthonous fossil materials and photosynthetically-produced lipid material processed during a diagenesis period longer than one month. The contribution of phytoplanktonic lipids to dissolved lipids pool in the ocean is probably higher than that of the fossil like lipids. Although it is true that extremely old lipid materials present in the DOM in the ocean because of the studies of Loh et al. (2004, 2006), the apparent ^{14}C ages were probably obtained from the mixture of old and relatively fresh lipids. Thus, a little of old (^{14}C deplete) lipids such as petroleum possibly lower the apparent ^{14}C ages. Although it is inferred that the major part (80%) of this lipid material in natural seawater is derived from phytoplanktonic lipids, judging from the lipid class composition, more research are necessary to elucidate the origin of these non-bioreactive lipid materials and their variability in the environment.

3. Degradation and dissolution of zooplanktonic organic matter and lipid materials

3. 1. Introduction

The biomass of zooplankton is large organic carbon pool and follows to that of phytoplankton which is the largest organic carbon pool in the bio-facies of the marine environment (Libes, 1992). Although primary production of phytoplankton is major source of particulate organic matter (POM), zooplanktonic organic matter is also one of the major sources of POM. Zooplanktonic grazing rates exceeding the 100% of primary production rates have been sometimes reported in several regions (Sherr and Sherr, 2002 and references there in). Furthermore, organic matter originated from zooplankton has been observed in the sediments and settling matters (Burns et al., 2003, 2004; Parrish et al., 2005). These studies indicate that zooplanktonic organic carbon (OC) has unignorable contributions to the carbon cycling in the ocean. Since the stabilities of zooplanktonic OC during diagenesis affect their roles in the carbon cycling in the ocean, it is important to elucidate the diagenesis of not only phytoplanktonic but also zooplanktonic OC for the understanding of the dynamics of marine organic matter.

The biogeochemical properties of zooplanktonic organic matter have been studied by the degradation experiment focusing on the morphological observation (Harding, 1973; Poulicek and Jeuniaux, 1991) and biochemical analysis especially concerning chitin (Seki and Taga, 1963; Seki, 1965 [1], [2]; Poulicek and Jeuniaux, 1991). Poulicek and Jeuniaux (1991) assessed the stabilities of the major biochemicals constituting body of zooplankton such as carbohydrates, proteins, lipids and chitin, and reported that lipids, carbohydrates and proteins were degraded more rapidly than chitin. However, the reactivities of zooplanktonic organic matter have not been poorly understood in molecular levels.

Zooplankton has considerable quantity of lipids materials in their body being comparable with other biochemicals such as carbohydrates and amino acids, and the

lipid materials constitute 3-73% of zooplanktonic biomass (Båmstedt, 1986 and references there in). This high contribution of lipids to the constituents of the body of zooplankton indicates that lipid materials produced by zooplankton have quantitatively large contribution to the dynamics of zooplanktonic organic matter. Triglycerides (TG) and phospholipids (PL) constitute zooplanktonic lipid as storage and structural lipid materials as well as phytoplankton. In addition, waxes (W), which are specific storage lipid class of zooplankton, also constitute zooplanktonic lipids as storage lipids and sometimes exceed 80% of total lipid of zooplankton (Sargent and Henderson, 1986; Hagen et al., 1995; Richard et al., 2006 and references there in). Although W and TG are found as storage lipids of zooplankton, the distribution and existing state in the body are different between W and TG. While TG is scattered in the body in the form of minute oil drops, W is collectively preserved in oil sac (Miller et al., 1998; Richard et al., 2006). This difference probably contributes to the difference in stabilities of storage lipids of phytoplankton and zooplankton followed by the difference in lipid dynamics. In order to understand the role of zooplanktonic lipids dynamics in the carbon cycling in the ocean, the reactivities of zooplanktonic lipids have to be elucidated in lipid class level and compared to that of phytoplanktonic lipid materials.

On the other hand, W has been applied to the estimation of the contribution of zooplanktonic organic matter to sediment or settling matter widely (Burns et al., 2003, 2004; Parrish et al., 2005). However, these studies have been limited to estimate the qualitative contributions of zooplanktonic organic matter to sediment or settling matter, because the quantitative relation between W and particulate organic carbon (POC) of zooplankton during diagenesis has not been elucidated. Since the stabilities of W and POC of zooplankton probably differ, the quantitative relation between W and POC should change during diagenesis. When the quantitative relation between W and

zooplanktonic POC (i.e. W/POC of the zooplankton ratio) during diagenesis is elucidated, the contribution of zooplanktonic POC to the OC pool will be able to be estimated quantitatively.

In this study, degradation experiment of zooplanktonic organic matter was carried out to assess the decomposition/dissolution of zooplanktonic organic matter and lipids with lipid class level. The reactivities of OC and lipids were compared, and the roles of zooplanktonic organic matter and lipids in the carbon cycling in the ocean were elucidated. Furthermore, the ratio of W/OC of the zooplankton was investigated to assess the possibility for the quantitative estimation of the contribution of zooplanktonic organic matters to the organic matter pool.

3. 2. Materials and Methods

3. 2. 1. Degradation experiment of zooplankton

Natural zooplankton population (size = 100 μm - 2 mm) applied to degradation experiment was collected by plankton net (100 μm-mesh) from pre-screened (2 mm-mesh screen) surface water of Tokyo-Bay on 13 December 2007. By the microscopic observation, the plankton net sample was mainly composed of copepods such as *Acartia* spp., *Paracalanus* spp. and *Oithona* spp., which have been generally observed in coastal waters. Zooplankton sample was transported to laboratory and washed by artificial sea water (ASW), which was prepared according to Seki (1976), to remove DOM and POM of less than 100 μm. Rare abundance of POM other than the zooplankton in the plankton net sample was ascertained by microscopy.

The washed zooplankton sample was freeze dried and added by 2L of ASW to the concentration of 10mg dry weight (DW)/L in the acid-cleaned polycarbonate bottle (twenty-six bottles with a volume of 2.5 L were used). Filtered seawater using pre-combusted (450°C, 6 hours) Whatman GF/F glass fiber filter was inoculated into incubation bottle with a ratio of 1:50 as inoculum.

The inoculated sample was applied to degradation experiment of zooplanktonic organic matter. The degradation experiment was carried out under dark at 20°C, and the degradation of zooplanktonic organic matter was followed for 120 days. The sample in the incubation bottle was mixed by swinging every day during first seven days. Sub samples were recovered; the volumes of the subsample were as follows, 4L for on Day 3, 11 and 30, and 20 L for on Day 71 and 120. Although the degradation experiment was conducted without aeration, the zooplanktonic organic matter were theoretically assumed to be degraded under oxic condition by the calculation of the oxygen demand from the decrease in the concentration of organic carbon.

Sub-samples were filtered through pre-combusted (450°C, 6 hours) Whatman GF/F glass fiber filter, and the filtrates were subsequently filtered through Whatman Anodisc membrane filter (nominal pore size is 0.2 μm). Although the filtration was carried out as well as the methods in degradation experiment of phytoplankton, two POM fractions obtained by filtration were combined as a POM fraction with size of >0.2 μm in this experiment. The filter and filtrate samples applied to OC and lipid carbon (LC) measurement were stored until analysis according to the method described in the degradation experiment of phytoplankton.

3. 2. 2. Lipid extractions

Lipids in samples were extracted with chloroform/methanol (2/1, v/v) using the modified method of Folch et al. (1957) as well as those written in the Chapter 2. Filter sample was cut into small pieces and homogenized in chloroform/methanol (5 ml/filter) using glass homogenizer. The cocktail composed by homogenized filter and solvent was transferred into a centrifuge tube. The sample remained in homogenizer was washed by chloroform/methanol (5 ml), and the solvent was combined in a centrifuge tube. Then the lipid extraction was carried out according to the methods described in Chapter 2. Lipids in filtrate were also extracted using the methods in Chapter 2. The chloroform phase obtained by the lipid extraction was stored at -20°C until fractionation.

3. 2. 3. Fractionation of lipid extracts

Fractionation of lipid extracts was carried out using the modified methods shown in Chapter 2. Lipid extracts were separated by silica gel column chromatography, and neutral lipid fraction obtained by column chromatography was further fractionated by thin layer chromatography (TLC). Zooplankton have considerable amount of W.

Furthermore, substantial free fatty acids (FFA) were assumed to be produced by the hydrolysis of W and acyl lipids such as TG. In order to follow the dynamics of W and FFA during early diagenesis, neutral lipids were fractionated into five fractions (hydrocarbon (HC), W, TG, FFA and other neutral lipids (ONL)) as listed in Table 3-1. Lipid fractions were stored at -20°C in the chloroform until analysis.

3. 2. 4. Measurement of OC and LC

The concentrations of POC and LC were analyzed by elemental analyzer (EA 1108, FISONS Instruments), and dissolved organic carbon (DOC) was analyzed by Shimadzu TOC 5000A according to the procedures described in Chapter 2.

3. 3. Results

3. 3. 1. Changes in the concentration of OC and LC

The initial concentration of POC in the incubation bottle was calculated to be 304 μM C using the dry weight of zooplankton applied to the incubation bottle (10 mg DW/L) and the carbon content of dried zooplankton sample (about 36.4%). The initial concentration of particulate LC (LC-POM) in the incubation bottle was 54 μM C calculated by using the dry weight of zooplankton applied to the incubation bottle and the LC content of dried zooplankton sample (about 6.4%). The filtered sea water inoculated into incubation bottle as bacterial population also contained OC and LC, which was mainly in dissolved fraction, with carbon concentration of 7.8 μM C and 0.3 μM C, respectively. However, the inoculum was inoculated into sample water with a ratio of 1:50, and the contributions of OC and LC derived from inoculum was negligible to the concentration of zooplanktonic OC and LC throughout the incubation period.

46.5% of the initial concentration of POC (141 μM C) decreased rapidly in the early incubation period (0-3 days), and then decreased slowly until the end of incubation (Fig. 3-1-A). The concentration of POC reached to 104 μM C (34.3% of the initial concentration) on Day 120. The incubation sample contained 0.2 μM C of DOC derived from the inoculum at the start of incubation. The concentration of DOC increased to 50 μM C on Day 30 when the rapid decrease in POC was observed. The concentration of DOC decreased to 31 μM C on Day 11, then the concentration of DOC increased to 46 μM C again on Day 30 and a little change was observed until the end of incubation (48 μM C). POC and DOC accounted for 34.1% and 15.6% of the initial concentration of the total OC on Day 120.

The changes in the concentration of LC-POM showed rapid decrease from the start of incubation, and 76.6% of the initial concentration of LC-POM (41 μM C) was

decreased by Day 3 (Fig. 3-1-B). The concentration of LC-POM decreased slowly after Day 3 and reached to 4 μM C (8.2% of the initial concentration) on Day 120. The concentration of dissolved lipid (LC-DOM) increased from 0 to 3 μM C during early incubation period (0-3 days) and decreased slowly from 3 to 120-day. Finally, the concentration of LC-DOM reached to 1 μM C on Day 120. LC-POM and LC-DOM on Day 120 accounted for 8.2% and 1.9% of the initial concentration of the total LC observed on Day 1.

The degradation rates of POC and LC-POM were obviously different between early incubation period (0-3 days) and later incubation period (3-120 days) (Fig. 3-1). Although only two data points of each POC and LC-POM were obtained during early incubation period (0-3 days), the degradation rates are calculated for the rough comparison of the rates between early and later incubation period. The degradation rates of POC and LC-POM during early incubation period were 0.209 and 0.484 (r^2 is not shown because the rates are calculated from only two data points), respectively. The degradation rates obviously decreased after Day 3, and the values were 0.004 (r^2 = 0.94, n = 5) for POC and 0.008 (r^2 = 0.90, n = 4) for LC-POM. The degradation rates of LC-POM were two times higher than that of POC throughout the incubation.

3. 3. 2. Changes in the size distribution of OC and LC

The contribution of DOC to the total organic carbon (TOC) increased rapidly from 0.6% to 23.5% during the first 3 days (Fig. 3-2-A). After the contribution of DOC to TOC decreased on Day 11, the contribution continued to increase slowly until on Day 120. The contributions of POC and DOC to TOC were 68.6% and 31.4% on Day 120, respectively. Although the contribution of DOC to TOC increased throughout incubation period, major fraction of OC was observed in POM fraction.

The changes in the contribution of LC-DOM showed rapid increase same as the changes in the contribution of DOC to TOC during Day 0-30 (Fig. 3-2-B). After the rapid increase of the contribution of LC-DOM, a little variation of the contribution of LC-DOM to total LC was noticed until on Day 120. The contributions of LC-POM and LC-DOM to total LC on Day 30 were 81.6% and 18.4%, respectively.

3. 3. 3. Changes in the concentration and composition of lipid fraction in zooplanktonic lipid.

Changes in the concentration of each lipid class and the contribution in the POM fraction are shown in Fig. 3-3 and Fig. 3-4. At the start of incubation, the highest concentration was observed in PL fraction (LC-POM-PL; 17 μM C) (Fig. 3-3), and LC-POM-PL showed the high contribution in LC-POM (32%) (Fig. 3-4). W (LC-POM-W; 9 μM C), TG (LC-POM-TG; 8 μM C), FFA (LC-POM-FFA; 8 μM C) and ONL (LC-POM-ONL; 8 μM C) were found as next important groups accounting for 17%, 14%, 15% and 15% of LC-POM, respectively. On the other hand, the concentration of LC-POM in HC fraction (LC-POM-HC) showed insufficient value to determine the concentration quantitatively throughout the incubation period. Thus, the concentration of LC-POM-HC is not shown in Fig. 3-3. These lipid class compositions in the zooplankton (Fig. 3-4) differed from that of photosynthetically-produced lipids by phytoplankton (Fig. 2-4-A), especially in the existence of W and a little contribution of AMPL. All fractions of LC-POM shown in Fig. 3-3 decreased rapidly until on Day 3 and followed by slow decrease until the end of the incubation. During the incubation period, the decreases in contribution of LC-POM-TG and –FFA to total LC-POM were obvious, although the contributions of other fractions increased or did not change (Fig. 3-4). The concentration of LC-POM-TG disappeared on Day 120, while LC-POM-W,

-ONL, -AMPL and -PL were apparently remained at the end of the incubation. The remaining percentages of LC-POM-W, -TG, -FFA, -ONL, -AMPL and -PL on Day 120 to the respective initial concentrations were 7%, 0%, 2%, 14%, 29% and 8%, respectively.

3. 4. Discussions

3. 4. 1. Reactivities of OC and LC

Although the lability of lipid materials has been recognized about the phytoplanktonic organic matter, the reactivity of zooplanktonic lipid has not been compared with their OC. The lipids composition were different between phytoplankton and zooplankton especially for the existence of W and low contribution of AMPL in the lipids of zooplankton (Fig. 2-4-A and Fig. 3-4). These differences possibly affect to the reactivities of zooplanktonic lipid materials. In this study, the reactivity of zooplanktonic lipids was compared with their OC; 50.0% of the initial concentration of the OC (sum of POC and DOC) remained, while only 10.1% of the initial concentration of the LC (sum of LC-POM and -DOM) remained on Day 120. Furthermore, the degradation rate of LC-POM was more than twice of that of POC. These remaining percentages and degradation rates indicate that zooplanktonic lipids are labile more than the bulk organic carbon as well as the lability of phytoplanktonic lipids. Thus, lipids are one of the most susceptible organic materials irrespective to planktonic sources (zooplankton or phytoplankton).

On the other hand, the degradation rates of POC and LC-POM obviously decreased after Day 3 (Fig. 3-1). This result indicates that POC and LC-POM in the zooplankton were composed by both labile and stable materials as well as that of phytoplanktonic organic matter.

3. 4. 2. Bio-degradability of triglycerides, phospholipids and waxes in the zooplanktonic lipids

The reactivity of lipids of zooplankton has not been studied with lipid class levels so far. In this study, the degradation rates of lipid classes during early degradation

period could not to be compared because the insufficient data points make the calculation of degradation rates impossible. However, the reactivities of TG, PL and W were able to be compared using the remaining percentages obtained at the end of experiment (on Day 120). LC-POM-PL was remained stably until the end of experiment, while most of LC-POM-TG was disappeared on Day 30 (Fig. 3-3-A, -B). These results strongly indicate that zooplanktonic structural lipid is more stable than TG as well as the result obtained for phytoplankton. On the other hand, LC- POM-W, which is one of the major storage lipids of zooplankton as well as TG, was preserved stably during experiment and 7% of the initial concentration remained on Day 120. The remaining percentage was nearly equal to that of LC-POM-PL (8%), indicating that W is also one of the stable lipid materials.

Although the zooplanktonic W has been reported to be transported to deeper layers in the ocean (Burns et al., 2003, 2004; Parrish et al., 2005), the stability of zooplanktonic W has been rarely studied. On the other hand, W was also intensively used to estimate the contribution of terrestrial plants to the organic matter in the estuarine and coastal sediments as biomarker (Prahl et al., 1994; Makou et al., 2007; Medeiros and Simoneit, 2008). Since these terrestrial W has been acknowledged to be well preserved in marine sediments (Prahl and Muehlhausen, 1989; Meyers, 1997), zooplanktonic W is likely preserved stably during early diagenesis.

About 7% of initial concentration of LC-POM-W was preserved until on Day 120. This value was obviously lower than that of POC (34%), indicating that zooplanktonic W was more labile than zooplanktonic POC. In fact, the contributions of LC-POM-W to POC readily decreased from 3.1% to 1.0% during first eleven days. However, LC-POM-W was preserved stably after 11-days, and the LC-POM-W/POC ratios on Day 11, 30 and 120 showed constant values (0.6-1.0%). This result indicates that the

stability of W observed during latter degradation period was equal to that of POC. W has been used to estimate the source of organic matter in the sediment as biomarker of zooplankton (Parrish, 1998; Parrish et al., 2005; Goutx et al., 2007). These studies, however, have been limited to the qualitative estimation of the contribution of zooplankton to the organic matter in the sediment, because the quantitative relations between W and bulk organic carbon have not been elucidated. If the reactivities between W and bulk organic carbon were comparable, the zooplanktonic organic carbon in the settling matter is able to be calculated from the concentration of W. Since organic matter in the settling matter is processed through diagenesis of comparable period with this study, the narrow range of the LC-POM-W/POC ratios (0.6-1.0%) during latter degradation experiment suggests that the ratio was usable to the quantitative estimation of the contribution of zooplankton to the organic matter in the settling matter. However, the W/OC ratio probably changes under different degradation condition such as source of organic matter and bacterial population. Although additional studies are necessary on the W/OC ratio during diagenesis of zooplanktonic organic matter, the stability of W comparable to that of OC strongly indicates that W are usable index for the study of the dynamics and role of zooplanktonic organic matter in the carbon cycling in the ocean.

3. 4. 3. Differences in accumulation of OC and LC in dissolved fraction during early diagenesis

The accumulation of DOC accompanied with the degradation of POC was observed during first three days in the degradation experiment of zooplanktonic organic matter (Fig. 3-2-A). The contribution of DOC to total-OC tended to increase through degradation period indicating that recalcitrant DOM accumulated during experiment as well as the result of degradation experiment of phytoplanktonic organic matter (Chapter

2). However, the contribution of DOC to total OC was 31% at the end of experiment, and major fraction of total OC was composed by POC. Although the degradation conditions were different between the degradation experiments of phytoplanktonic- and zooplanktonic organic matters, this size distribution obviously differs from that of result of degradation experiment of photosynthetically-produced organic matter (Chapter 2). This difference was seemed to be affected by the difference of constituents of planktonic organic matters. It is known that about 2-10% of the dry weight of copepods, which was major constituents of zooplanktonic population in this study, was composed by chitin (Ramont, 1983; Omori and Ikeda, 1984; Båmstedt, 1986; Postel et al., 2000 and references there in). The slow degradation of chitin more than the other biochemicals such as carbohydrates, amino acids and lipids have been reported (Seki and Taga, 1963; Poulicek and Jeuniaux, 1991). Thus, these stable crustaceous organic matters probably inhibit the dissolution/degradation of POC. However, the contribution of DOC to total OC kept increasing even during the latter degradation period. This result indicate that the size distribution of zooplanktonic OC tend to shift to the DOM dominated size distribution during diagenesis as well as the results of the degradation experiment of phytoplanktonic organic matter.

The accumulation of dissolved materials accompanied with the degradation of particulate materials was also observed for the lipid during first three days (Fig. 3-2-B). This result indicates that the dissolved lipid was formed by degradation of particulate lipids as well as the formation of DOC. However, the LC was conserved mainly in particulate fraction as well as the result of degradation experiment of phytoplankton. The contribution of dissolved fraction to total (dissolved and particulate) concentration was lower in the LC (18%) than OC (31%), indicating that LC preferentially remained as POM more than OC. These properties of lipid materials found in the degradation

experiment of zooplankton were comparable with the results of degradation experiment of phytoplanktonic organic matter.

The contribution of LC-DOM to total LC did not change after Day 3, although the concentration of LC-POM continued to decrease (i.e. production of LC-DOM continued) until the end of incubation. These results indicate that production rate of stable LC-DOM accompanying to the dissolution of LC-POM was low comparing to that of OC, and it is possible that the most part of dissolved lipid was labile and readily decomposed during early diagenesis. Thus, lipid materials were conserved in particulate form independently with their planktonic sources (phytoplankton or zooplankton). This property of size distribution of lipids during early diagenesis was possibly affected by the lability of dissolved lipid and difficulty of dissolution of lipid materials independent with their planktonic sources.

4. Conclusions

Planktonic lipids preferentially remained in POM fraction, while planktonic OC tend to be accumulated into stable DOM pool independently with the planktonic sources (phytoplankton or zooplankton) during early diagenesis. These degradation/dissolution properties indicate that planktonic lipids preferentially remain as POM and are vertically transported, while planktonic OC tends to contribute to the formation of DOM pool. From the results of lipid class analysis, the stabilities of PL more than that of TG were confirmed in both phytoplanktonic- and zooplanktonic-lipids degradation. The preferential preservations of PL in the POM indicate that structural lipids constituting the cell membrane are major lipid materials to be transported toward deeper water and contribute to the lipids vertical transport. On the other hand, storage lipid of TG is one of the most labile materials and is readily remineralized during early diagenesis.

In the analysis with the total lipids and total OC levels, planktonic lipids were more labile than planktonic organic matters as well as past studies. However, planktonic lipids were composed by both labile and stable materials. In the lipid class analysis, the stabilities of particulate PL and W are high and equal to that of OC during latter degradation period (after several days from the start of degradation). The quantitative relations between OC and biomarkers (i.e. ratio of PL/OC or W/OC) suggest the possibilities of quantitative estimation of the contributions of phytoplankton or zooplankton to the OC pools. Although more researches are needed on the quantitative relations of OC and biomarkers under several degradation conditions (i.e. sources of organic matters, contents of biomarkers and bacterial populations), the stabilities of biomarkers comparable to OC strongly indicate the availability of biomarker/OC ratio to the study of the dynamics of planktonic organic matter in the ocean.

In order to study the contribution of phytoplanktonic lipid materials to the

dissolved lipid materials in natural seawater, the lipid class compositions were compared between P-LC and LC at the end of degradation experiment. The lipid class composition in the LC suggested the presence of fossil like lipid materials such as petroleum. However, the contribution of fossil like lipids was about 20 %, and the rest of lipids (80 %) showed comparable composition of lipid class to that of phytoplanktonic lipids. Although the presence of exceedingly ^{14}C depleted (i.e. old) dissolved lipid materials have been reported, major part of dissolved lipid in the ocean is possibly originated from the phytoplankton, and a little of fossil like lipids possibly lower the apparent ^{14}C ages of dissolved lipids.

The reactivity of organic matter is important factor controlling the carbon cycling in the ocean. In this study, most part of planktonic lipids was decomposed during early diagenesis (monthly time scale), and the degradation rates were faster than that of OC. Although the stabilities of certain lipid classes are comparable with that of bulk OC, it is appeared that lipid is one of the most labile organic materials compared to bulk organic matter. Thus, the contribution of lipid materials produced by plankton is likely quantitatively small to the vertical transportation of carbon and accumulation of stable DOM.

On the other hand, certain lipid materials such as PL and W have been widely applied as biomarkers to the studies of estimation of the source of organic matter in the settling matter and sediment. However, these biomarkers have been applied only to qualitative analysis, because the reactivities and quantitative relation between biomarkers and bulk OC during diagenesis have not been elucidated. The present study is the first attempt to assess the quantitative relation between biomarkers (i.e. W and PL) and bulk OC during early diagenesis, and the results of the quantitative relation obtained in this study indicate the possibility of the quantitative estimation of the

contributions of each planktonic organic matter to the organic matter pools.

Marine organic matter is originated from diverse sources. Since the reactivities of organic matter originated from each source are different, the subsequent dynamics of organic matter in the water column and the sediment are possibly different depending on their sources. In order to understand the dynamics of organic matters derived from different sources, the quantitative relation between lipid biomarkers and OC assessed in this study is useful to study the dynamics of organic matter originated from several sources separately. Progress in the study of the reactivities of lipid biomarkers and OC under the different diagenetic conditions will contribute to the understanding of the details of the dynamics of organic matter in the oceanic environments.

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Tables

Table 1. Percentages of dissolved lipid to total (sum of particulate and dissolved) lipid in natural seawater.

Study	Data set	DOM size	Percentages
Parrish (1987)	Bedford Basin (during spring bloom)	<1.2 μm	44-81%
Parrish et al. (1988)	Scotian slope, Bedford Basin (Vertical distribution)	<1.2 μm	66-88%
Andersson et al. (1993)	Surface water from northern Baltic Sea (Seasonal distribution)	<1.0 μm	Pre-bloom: 66-79% Bloom: 26-43% Post-bloom:
Liu et al. (1998)	Surface water from Conception Bay (Seasonal distribution)	<10 kDa	68-93%
Derieux et al. (1998)	North Adriatic Sea (Spatial distribution)	<0.7 μm	30-53%

Table 2-1. Lipid classes in the lipid fraction obtained by column chromatography and TLC on the degradation experiment of phytoplanktonic organic matter.

Abbreviations of lipid fractions	Lipid classes contained in lipid fraction
HC	Hydrocarbons
TG	Triglycerides
ONL	Other neutral lipids composed by free fatty acids, sterols, alcohols and diglycerides.
AMPL	Acetone mobile polar lipids composed by glycolipids, monoglycerides, pigments and other undetectable lipid materials.
PL	Phospholipids

Table 2-2. Degradation constants (*k*) of P-POC-GF, P-LC-GF and P-LC-GF in each lipid fractions. The degradation constants of P-LC-GF-HC throughout degradation period and P-LC-GF-TG during latter degradation period are not calculated, because their concentrations are too low to calculate degradation constants accuracy. The determination coefficients (r^2, n = 3-4) are in parentheses.

Sample	*k* (r^2)	
	Early degradation period	Latter degradation period
	1-4 day	4-30 day
P-POC-GF	0.38 (0.81)	0.08 (1.00)
P-LC-GF	0.48 (0.91)	0.08 (0.95)
P-LC-GF in-TG	1.05 (0.97)	-
-ONL	0.45 (0.95)	0.08 (0.97)
-AMPL	0.47 (0.83)	0.07 (0.91)
-PL	0.29 (0.95)	0.08 (0.93)

Table 3-1. Lipid classes in the lipid fraction obtained by column chromatography and TLC on the degradation experiment of zooplanktonic organic matter.

Abbreviations of lipid fractions	Lipid classes contained in lipid fraction
HC	Hydrocarbons
W	Waxes
TG	Triglycerides
FFA	Free fatty acid
ONL	Other neutral lipids composed by sterols, alcohols and diglycerides.
AMPL	Acetone mobile polar lipids composed by glycolipids, monoglycerides, pigments and other undetectable lipid materials.
PL	Phospholipids

Figures

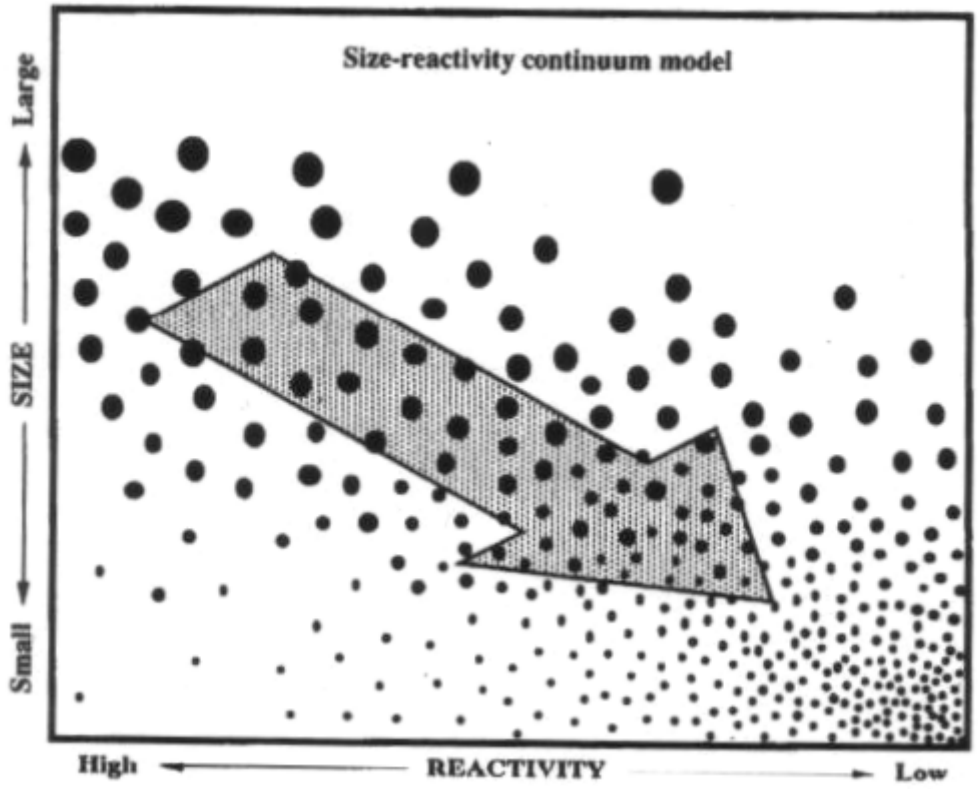

Fig. 1. Schematic diagram of the size-reactivity continuum model for organic matter decomposition in aquatic environments reported by Amon and Benner (1996). Arrow indicates the major pathway of degradation from bioreactive organic particles and macromolecules to refractory dissolved compounds. The size of the dots is representative of the size of organic matter from the large dots for particulate organic matter to small dots for dissolved organic matter. The distribution of dots indicates that most larger sized organic matter is more reactive than most smaller sized organic matter.

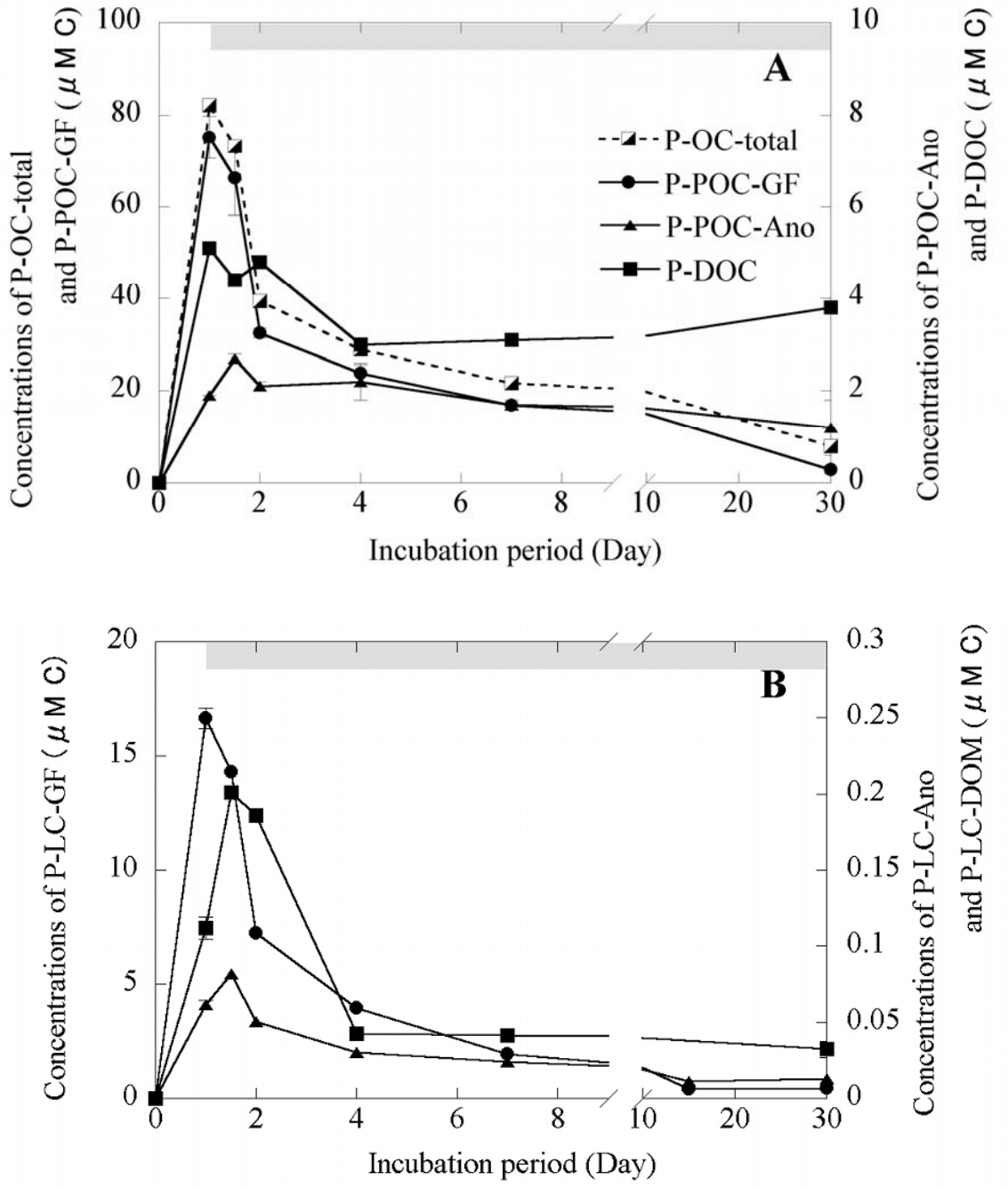

Fig. 2-1. Changes in total P-OC (A) and P-LC (B) concentrations and in each size fraction (GF/F, Anodisc and DOM fractions). Concentration of P-LC-total changed mostly as in P-LC-GF, so that the graph of concentration in the P-LC-total is not shown. Incubation period in the dark is indicated by gray. Data are mean ± SD, n = 3 for P-OC throughout incubation and for data of P-LC obtained on Day 1 and 30.

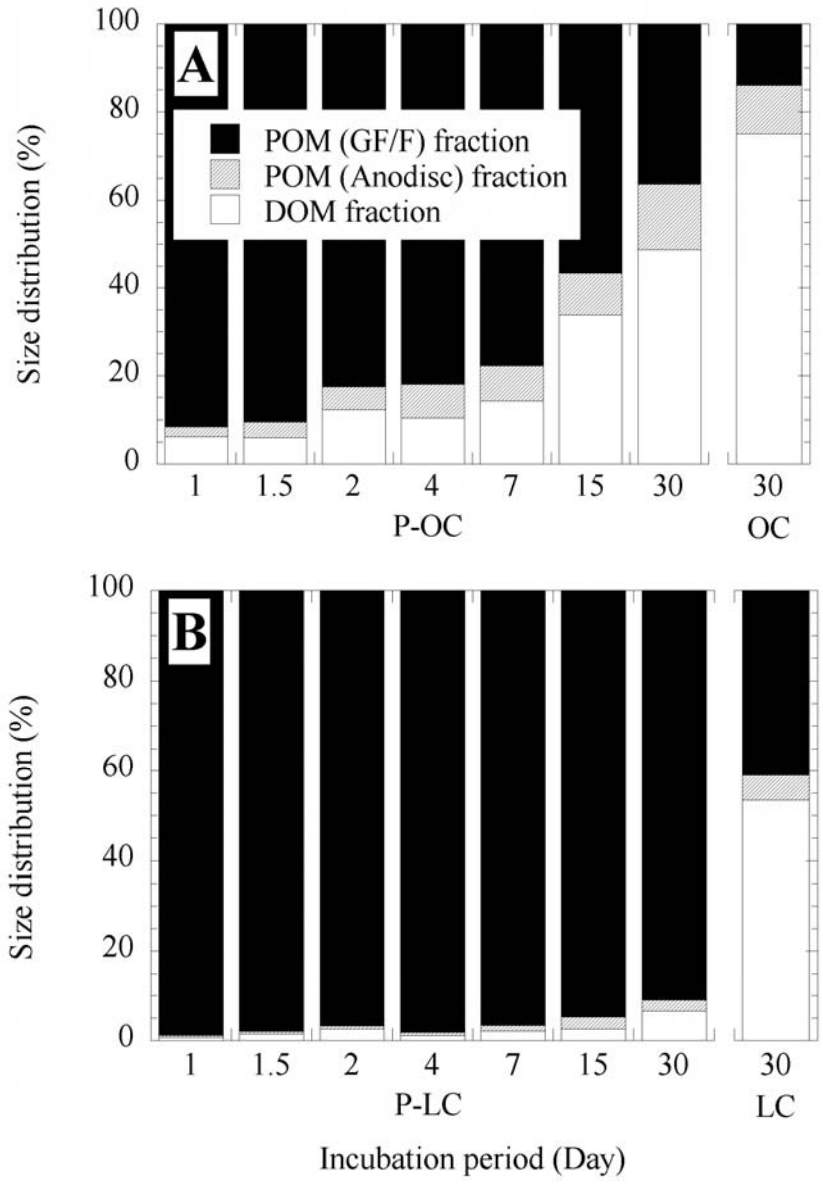

Fig. 2-2. Changes in size distributions of P-OC (A) and P-LC (B). The size distributions of OC and LC on Day 30 are also shown. In this study, the concentrations of OC and LC were measured in order to calculate P-OC and P-LC (See Materials and Methods). The OC and LC were composed of not only P-OC and P-LC but also organic matter which was already contained in the water sample collected from Tokyo-Bay. Since the contributions of P-OC and P-LC to the OC and LC were very low (4.6% and 11.9%, respectively) at the end of experiment, the size distributions of OC and LC obtained on Day 30 could reflect those of OC and LC existed in natural seawater.

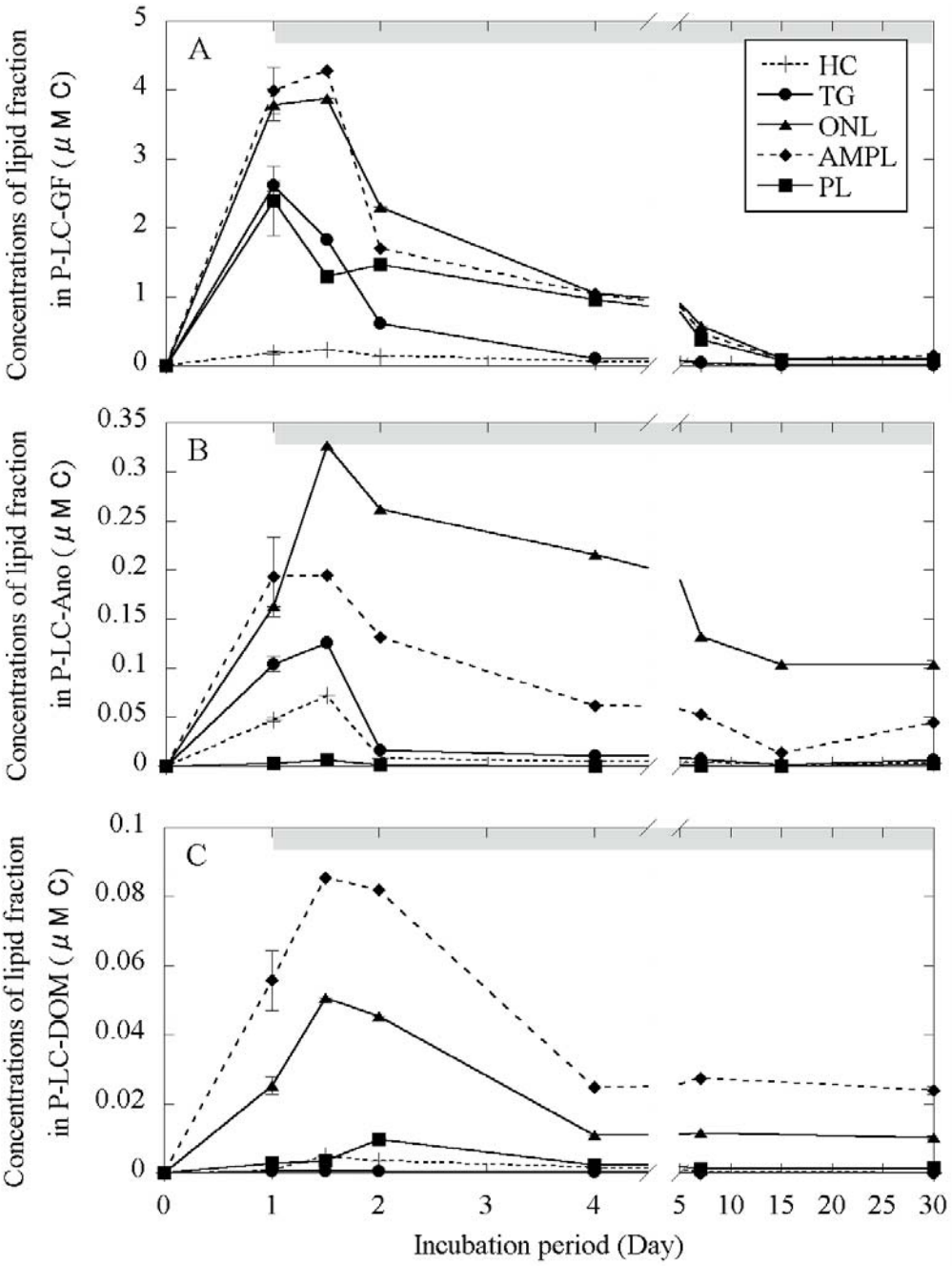

Fig. 2-3. Changes in concentrations of lipid fractions in P-LC-GF (A), P-LC-Ano (B) and P-LC-DOM (C). Standard deviation of the data was determined for samples on Day 1 and 30 (n = 3 for each sample).

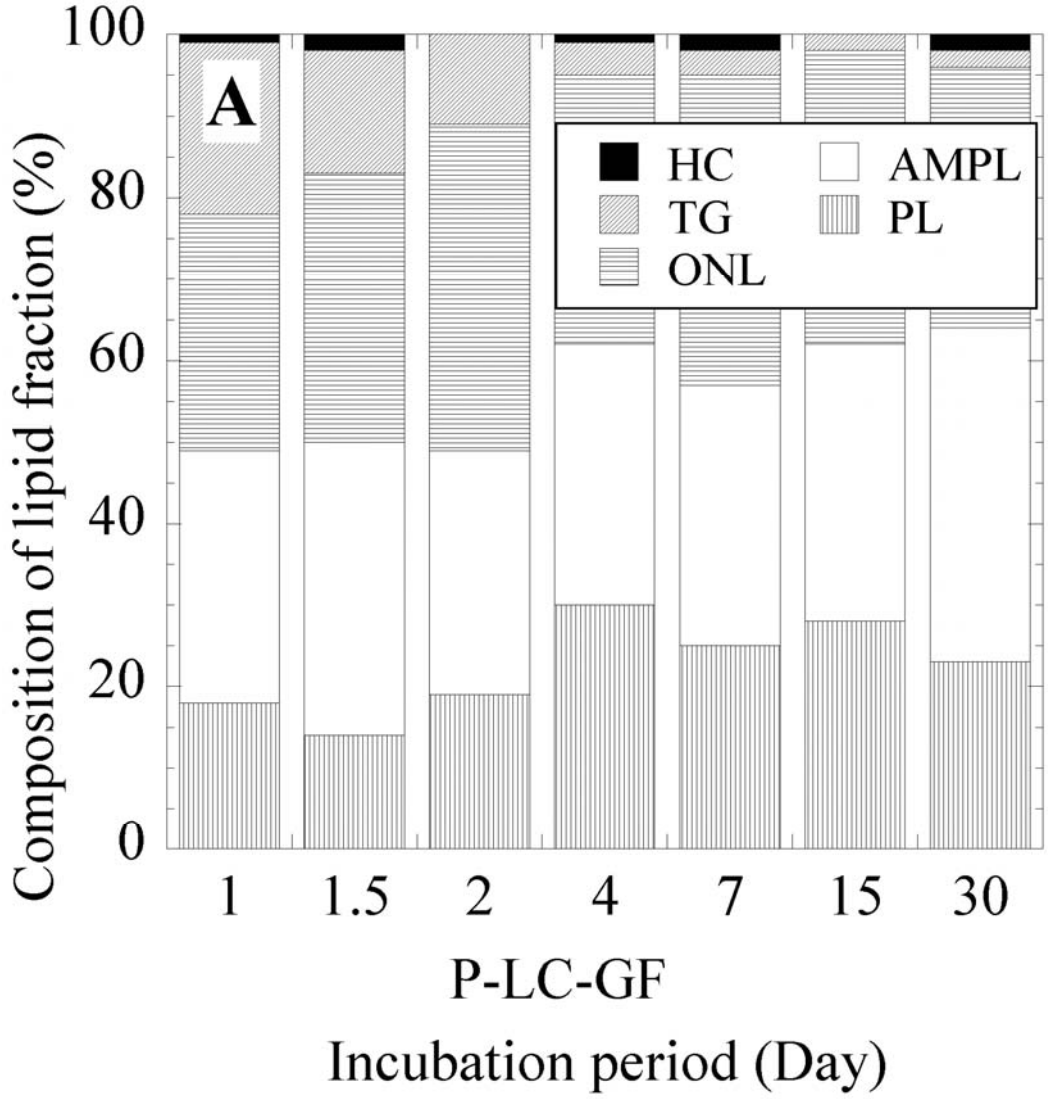

Fig. 2-4-A. Composition of lipid fraction in P-LC-GF throughout the incubation period.

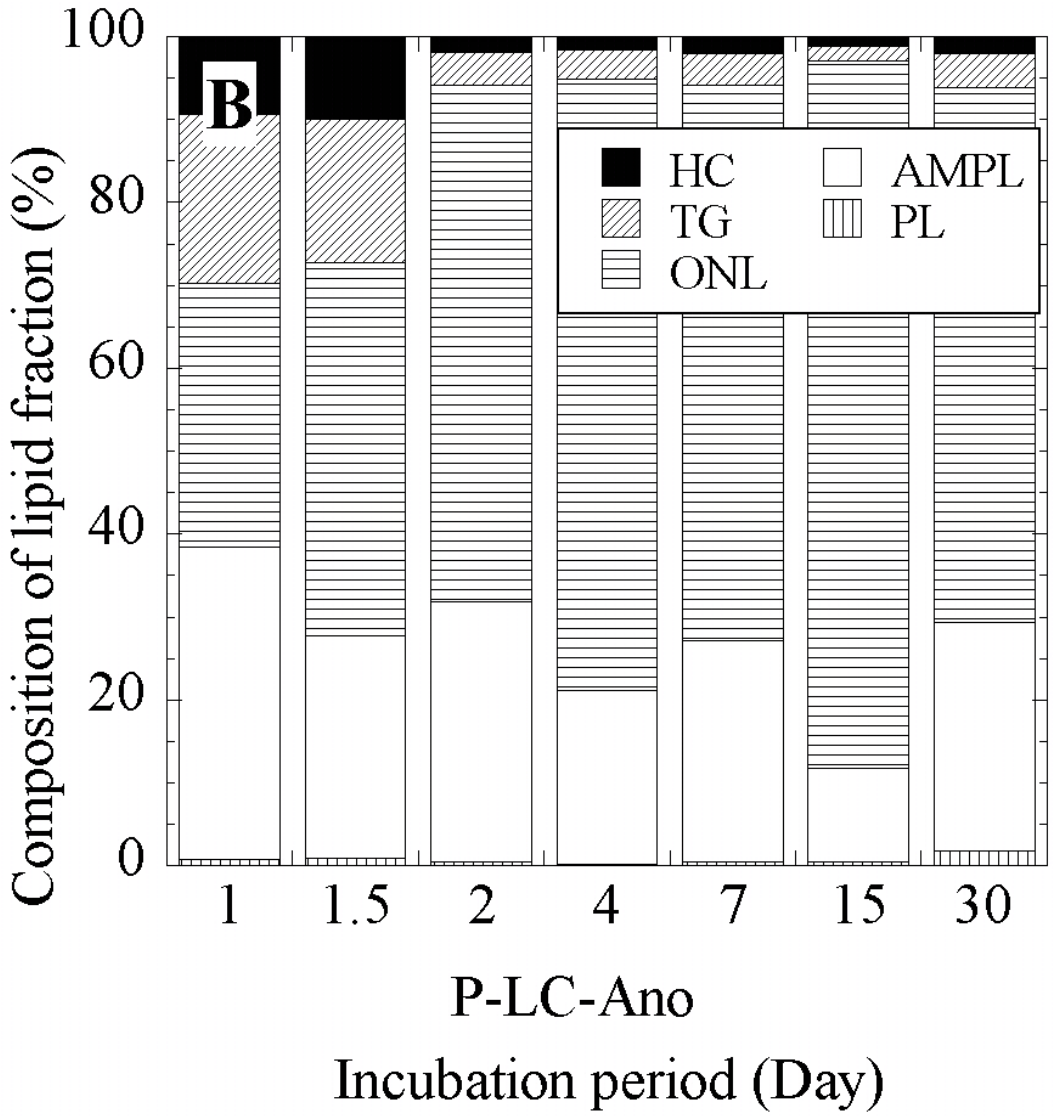

Fig. 2-4-B. Composition of lipid fraction in P-LC-Ano throughout the incubation period.

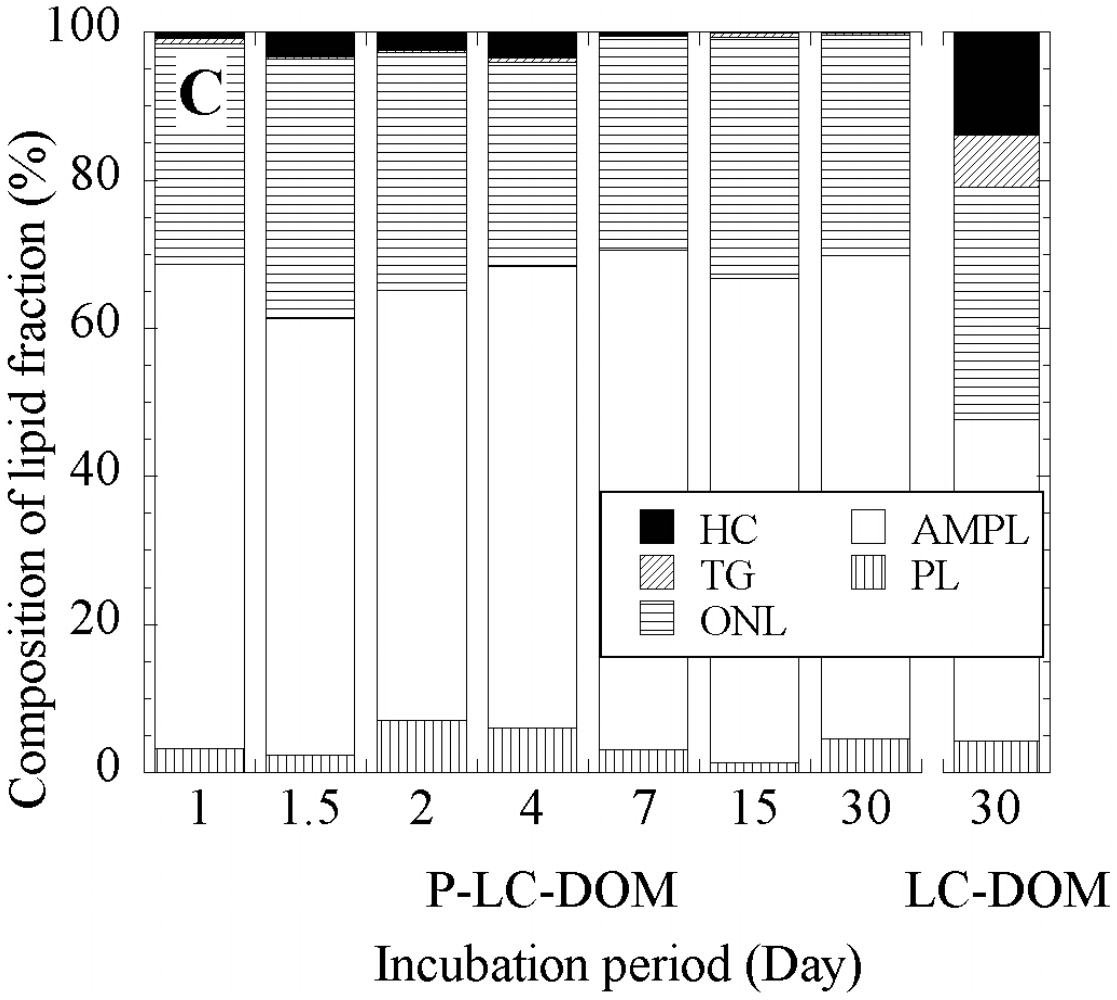

Fig. 2-4-C. Composition of lipid fraction in P-LC-DOM throughout the incubation period. LC-DOM on Day 30 is also displayed.

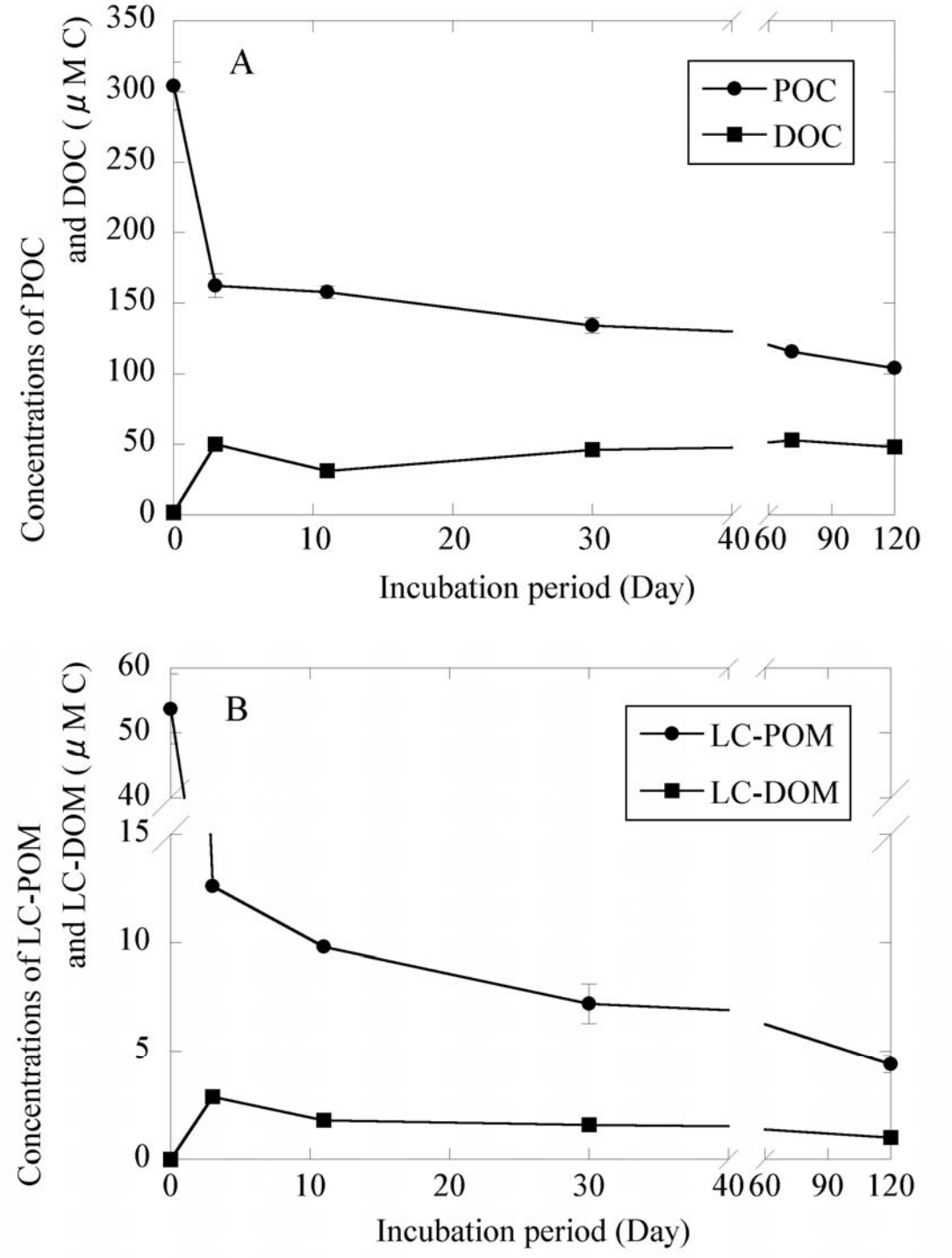

Fig. 3-1. Changes in OC (A) and LC (B) concentrations in each size fraction (POM and DOM fractions). Data are mean ± SD, n = 3 for OC throughout incubation and for data of LC obtained on Day 0, 30 and 120.

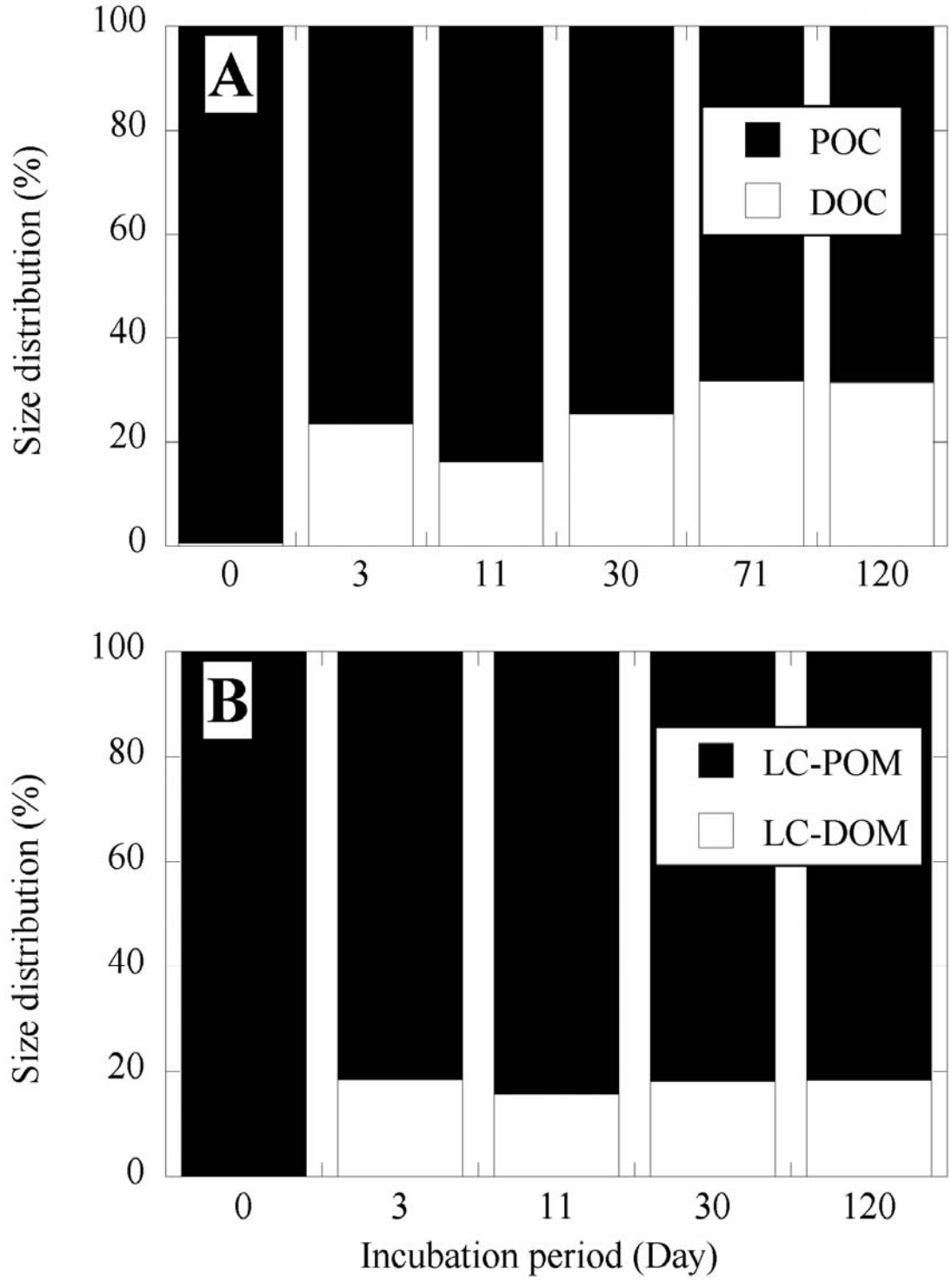

Fig. 3-2. Changes in size distributions of OC and LC throughout the incubation period.

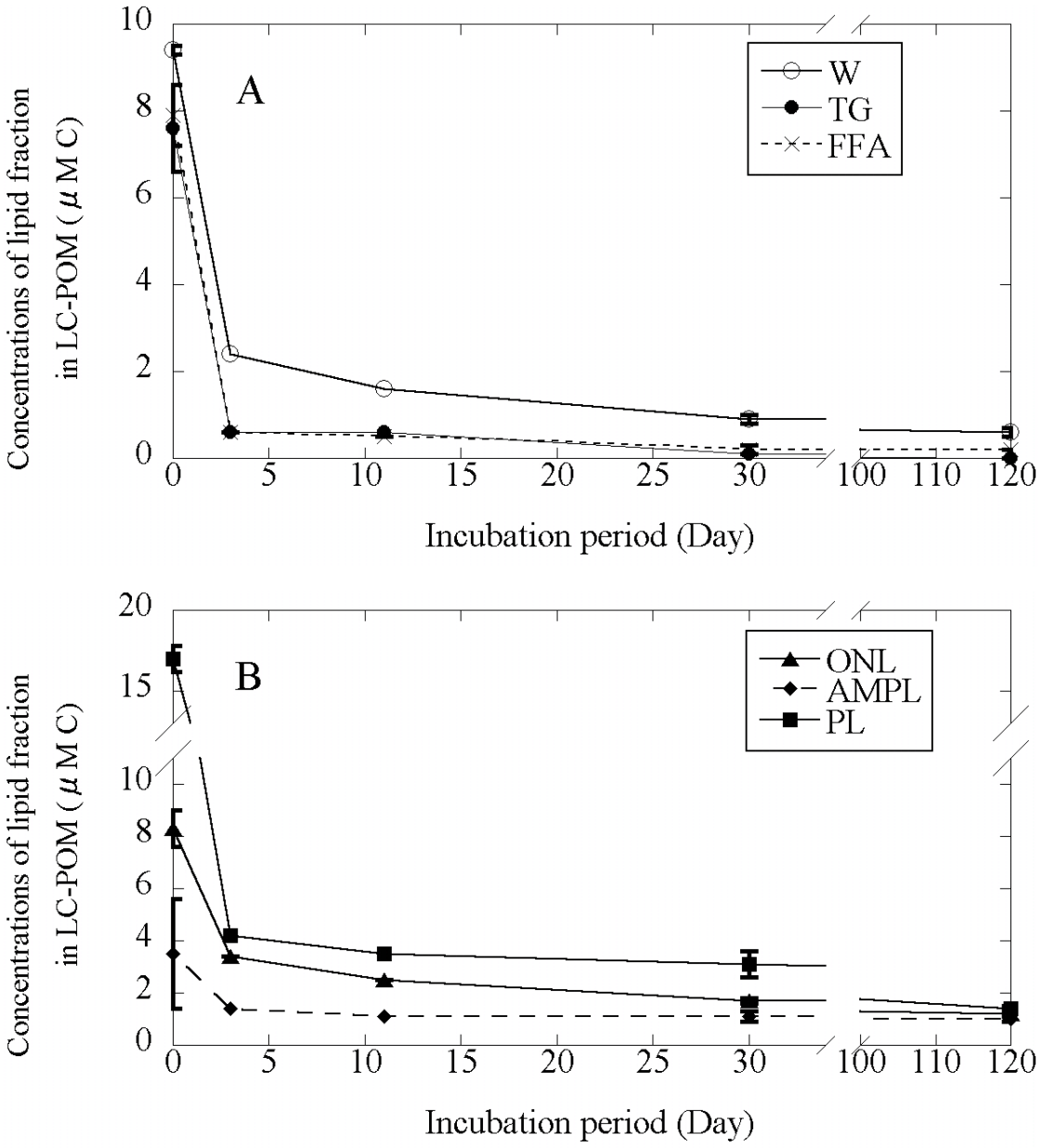

Fig. 3-3. Changes in concentrations of lipid fractions in LC-POM. The changes in concentration of W, TG and FFA are shown in A, and that of ONL, AMPL and PL are shown in B. Since the concentration of LC-POM in HC fraction showed insufficient value to determine the concentration quantitatively throughout the incubation period, the concentration of LC-POM-HC is not shown. Standard deviations of the data were determined for samples on Day 0, 30 and 120 (n = 3 for each sample).

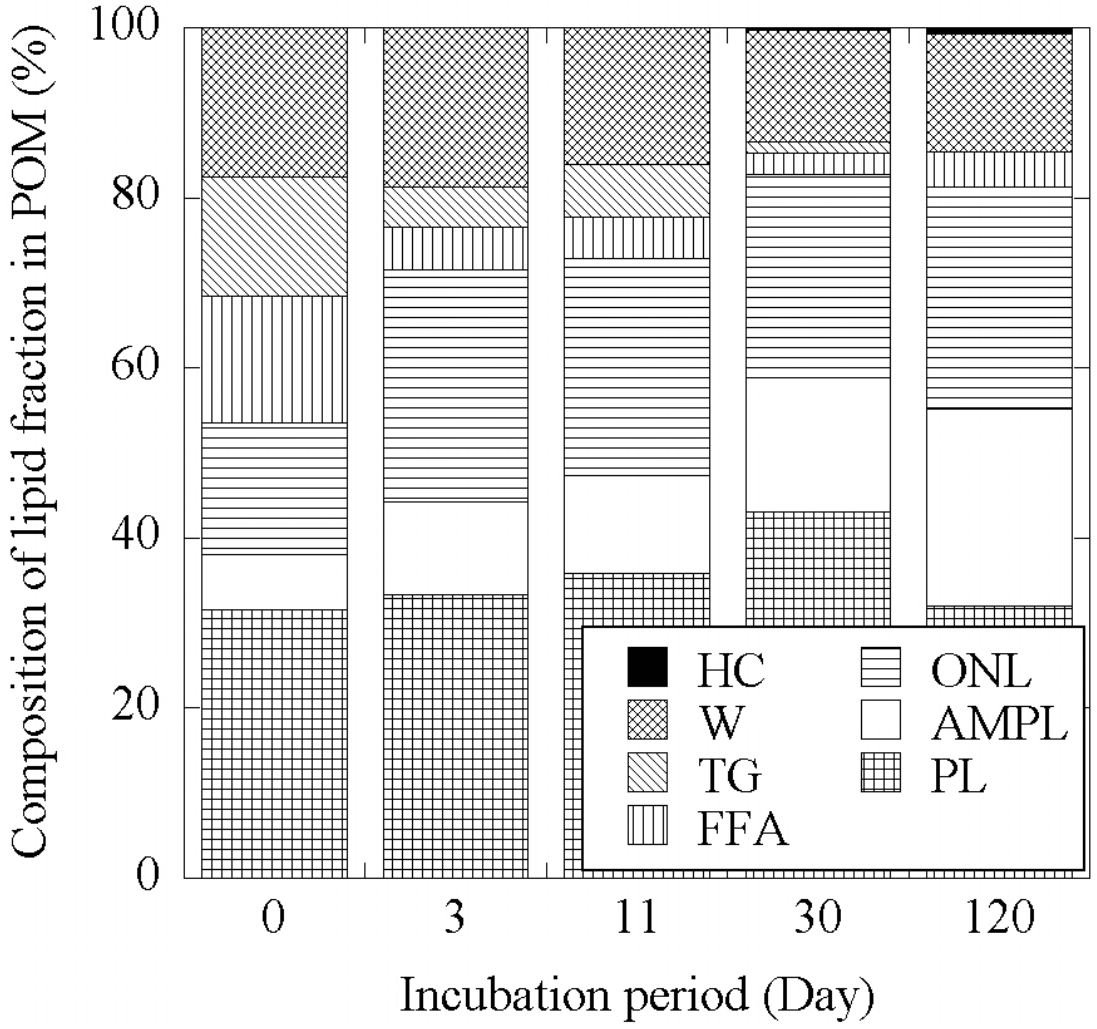

Fig. 3-4. Composition of lipid fraction in LC-POM throughout the incubation period.