STUDY OF BIOGEOCHEMICAL FACTORS AFFECTING ORGANIC MATTER (LIPID BIOMARKERS) DEGRADATION: STRUCTURAL ASSOCIATION, REDOX CONDITION, ENZYMATIC RESPONSE, AND BENTHIC MACROFAUNAL ACTIVITY

by

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(Under the Direction of Ming-Yi Sun)

ABSTRACT

Major portion of this dissertation focused on comprehensively examining the degradation processes of three classes of lipid biomarkers derived from alga *Emiliania huxleyi* in three simulated microcosms (oxic/anoxic seawater systems, oxic/anoxic sediment-water interface, and sediment cores with and without presence of macrofauna). The results obtained from these studies demonstrated that both internal factors, which arise from the structural associations of different molecules within cellular components, and external factors, which result from different environmental conditions, affect the fates of various algal biomarkers in marine systems significantly.

This dissertation includes five chapters. Chapter 1 described the research background. Chapter 5 summarized the major new results and conclusions.

Chapter 2 focused on how the intracellular structural associations affect degradation of Chl-a. By calculating degradation rate constants of Chl-a based on a 90-day incubation of *E. huxleyi* cells in natural oxic and anoxic seawaters, the effects of the structural complexes on Chl-

a degradation were evaluated. By following the variations of Chl-a derivatives, a concept model was established to describe the degradation pathways of Chl-a from various pigment complexes.

Chapter 3 described a 56-day incubation experiment of *E.huxleyi* in simulated oxic/anoxic sediment-seawater interface microcosms. The structural effects on the degradation of algal fatty acids bound in intracellular and membrane components under various redox conditions were discussed based on the experimental results. The complicated relationships among enzymes, microbial community and fatty acid degradation were discribed by following time-dependent variation of lipase activities and bacterial abundance.

Chapter 4 was concentrated on how crustacean (*P. pugio*) affect the fates of algal lipids by incubating *E. huxleyi* cell in simulated sediment-seawater microcosms with or without presence of crustacean for six weeks. Experimental results indicated that crustacean's activities caused differential degradation of algal fatty acids and alkenones and minimized the structural effects of intracellular and membrane components on fatty acid degradation, but had little influence on $U_{37}^{k'}$. Moreover, a conceptual model was proposed to assess the fates of various algal lipids in surface sediments.

Overall, this study added new knowledge on the degradation of organic biomarkers, which is helpful in understanding global carbon cycling and in paleoceanographic research.

INDEX WORDS: *Emiliania huxleyi*, chlorophyll-a, phaeophorbide-a, redox conditions, bacterial abundance, biochemical degradation, pigment complexes, membrane and intracellular fatty acids, lipase, *Palaemonetes pugio*, lipid degradation, alkenones, algal fatty acids, bacteria-specific fatty acids, $U_{37}^{k'}$ index

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DEDICATION

This work is dedicated to my beautiful wife, Chengjun Sun, who has been giving me her selfless support for my study and research all these five years. This work is also dedicated to my pretty son, Feifei (Neal), who has been bringing tremendous amount of happiness in helping me to overcome all kinds of difficulties.

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TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

GENERAL INTRODUCTION AND LITERATURE REVIEW

Background

Organic matter degradation and its controlling factors. The biochemical degradation of organic matter in marine systems is a key process controlling global carbon cycling, which substaintially affects global climate changes (Berger and Keir, 1984; Hedges and Keil, 1995; Ikeda and Tajika, 2002; Ogrinc et al., 2003). The biogeochemical cycling of organic matter also influences nutrient cycling and metal redox chemistry in various environments (Cowie and Hedges, 1994; Keil et al., 1994; Hedges and Keil, 1995; Sun et al., 1999). The degradation of organic matter is controlled by external and internal factors. External factors include sedimentation rate, redox conditions, exoenzymes, microbial activity and bioturbation. (Aller, 1984; Meyer-Reil, 1991; Canfield, 1994; Harvey and Macko, 1997; Green et al., 2002). Internal factors are dependent on organic matter sources, compositions and structures (Blair, et al., 1996; Harvey et al., 1995; Hedges et al., 1997; Wakeham, et al., 1997; Sun and Wakeham, 1998). The interactions among these factors determine the fates of different organic matter in various environments.

Some of control factors have been well studied. For instance, the roles of high sedimentation rate and high productivity event on organic matter degradation and preservation have been well examined (Aller and Mackin, 1984; Henrichs and Reeburgh, 1987; Pederson and Calvert, 1990). However, other factors such as effects of oxygen on organic carbon preservation

and degradation have not been well understood (Lijmbach, 1975; Emerson and Hedges, 1988; Pedersen and Calvert, 1990; Lee, 1994; Canfield, 1994; Cowie et al., 1995). Early experimental studies (Foree and McCarty, 1970; Jewell and McCarty, 1971; Otsuki and Hanya, 1972a; 1972b) showed little difference between the aerobic and anaerobic decompositions of algal materials. Lately, more field and laboratory work (Henrichs and Doyle, 1986; Canfield, 1989; Blackburn, 1991; Lee, 1992) indicated that degradation rates of some organic compounds under oxic and anoxic sediments were comparable. However, this conclusion was challenged by further composition analysis of organic matter during aerobic and anaerobic degradation (Harvey et al., 1995) and by examining organic matter degradation rates according to the multi-G model (Berner, 1964, Westrich and Berner, 1984). In the multi-G model, organic matter is assumed to have multiple fractions, which have different reactivities and different responses to redox conditions, and organic matter degradation rate is the sum of individual degradation rate of each fraction (G_i). Based on the G model estimation, a series of studies indicated that various organic compounds had different degradation behavior under different redox conditions. For example, Harvey et al. (1995) found that there were remarkable differences in degradation rates of major biochemical components under oxic and anoxic conditions (Table 1.1). Other studies have suggested that oxygen effects are complicated and related to the bioturbation by animals, the different responses of organic matter to hydrolytic enzymes and the compositions of organic matter (Aller, 1982; Emerson and Hedges, 1988; Meyer-Reil, 1991; Harvey and Macko, 1997). Therefore, for understanding how redox conditions affect organic matter degradation in variable environments, more studies are still necessary.

Lipids as biomarkers. To investigate organic matter degradation, biomarker approach is a powerful tool. As typical biomarkers, lipids have been widely used to track the sources and

fates of organic matter in water column and sediment (Welschmeyer and Lorenzen, 1985; Volkman et al., 1987; Penry and Frost, 1991; Wakeham and Lee, 1993). Lipids, which are insoluble in water, are a chemical diverse group of compounds, including storage lipids, structural lipids, pigments, sterols and some unusual lipids with specific structures (Harwood and Ressell, 1984; Lehninger, et al., 1993). Lipids comprise about 5-20% of organic matter in phytoplankton (Parson et al., 1961; Cranwell, 1982). Since they are less reactive than proteins and carbohydrates (Harvey et al, 1995), they are not only used as biomarkers in studies of organic matter cycling, but also as indictors in paleoceanographic records (Gagosian et al., 1983; Volkman et al., 1987; Wakeham and Lee, 1993). Previous field studies have shown that lipids deposited in different environments with variable redox conditions degraded at different rates (McCaffrey et al., 1991; Sun and Wakeham, 1994; Canuel and Martens, 1996). Laboratory studies (Sun et al., 1997, 1998) further suggested that cell-associated fatty acids and free fatty acids (as tracer) degraded at similar rates under oxic conditions but at different rates under anoxic conditions (Table 1.2). Sun et al.'s (1993a) experiments observed that a small fraction of cell-associated chlorophyll-a (~20%) was degraded initially under anoxic conditions while a large fraction (~80%) remained stable over three months (Fig 1.1). In contrast, most of the chlorophyll-a degraded under oxic conditions in one month. These results implied that the molecular associations of lipids within algal cells play an important role on lipid degradation. However, there have been few studies focusing on these internal factors on lipid degradation in oxic and anoxic systems.

Diverse lipids have various structures and conduct different functions (Voet and Voet, 1995). Generally, the major natural lipids are fatty acids. Although more than 500 fatty acids have been found in plants and micro-organisms, only seven of them account for most of total

fatty acid in the tissue of organisms (Harwood and Russell, 1984; Derieux et al., 1998; Tolosa et al., 2004). Some fatty acids are useful indicators to track organic matter inputs from different sources. For instance, long chain saturated fatty acids are typical tracers of terrestrial plants (Meyers, 1997); polyunsaturated fatty acids (20:4, 20:5 and 22:6) are used as marine plankton biomarkers (Canuel and Martens, 1996; Colombo et al., 1996); and branched C15 and C17 fatty acids are primarily produced by bacteria (Kaneda, 1991). Some previous studies have demonstrated the effects of molecular structures on fatty acid degradation. For example, unsaturated fatty acids decay faster than saturated fatty acids, leading to a relatively higher preservation of saturated fatty acids in sediments (Farrington et al., 1977; Volkman et al., 1981; Haddad et al., 1992; Meyers and Eadie, 1993). It is well known that in plant cells, various fatty acids are complexed with glycerol, phosphate, alcohols, sugars or sterols (Hitchcock and Nichols, 1971). These fatty acid contained lipids account for the major lipids in higher plant and algae cells as storage and structural components (Harwood and Russell, 1984; Lehninger, et al., 1993). For example, the triacylglycerols are composed of three fatty acids esterified with a single glycerol, providing energy storage in plant cells (Lehninger, et al., 1993). Phospholipids contain two fatty acids esterified to a glycerol and there is a polar head group linked to the glycerol, froming a bilayer structure in cell membrane (Lehninger, et al., 1993). The basic structures of fatty acids, triacylglycerols and phospholipids are presented in Fig. 1.2.

The relationships among enzymes, bacteria and organic matter degradation. Generally, degradations of organic compounds, including triacylglycerol and phospholipids in pelagic and benthic ecosystems are enzymatic processes. Under both oxic and anoxic conditions, bacteria are involved in these enzymatic processes directly (Gajewski et al., 1993; Afi et al., 1996; Arnosti, et al., 2004). Heterotrophic bacteria in seawater and sediments are able

to decompose all kinds of organic compounds by producing various enzymes (Quemeneur and Marty, 1992). In fact, large macromolecules cannot be directly transported into microbes (Burns, 1980); hence they have to be hydrolyzed to small substrates before being utilized and remineralized by bacteria (Canuel and Martens, 1996; Henrichs, 1992; Arnosti, 1998; Kristensen et al., 1995). Hydrolysis of macromolecules is carried out by extracellualr enzymes released from organisms. This process is a rate-limiting step in the degradation of organic matter in both water column and sediments (Meyer-Riel, 1983, 1990; Kristensen et al., 1995; Podgórska and Mudryk, 2003). Degrading enzymes for proteins and carbohydrates in marine ecosystems have been well studied (Ladd and Bulter, 1972; Mayer, 1989; Billen, 1991; Mayer and Rice, 1992; Mayer et al., 1995; Arnosti, 1998), but less attention has been focused on the relationship between lipid-degrading enzyme activity and lipid degradation.

Since enzymatic reaction is generally structure dependent, molecular associations of various lipids in cells influence their degradation behavior dramatically. Therefore, the structural difference between tricylglycerol and phospholipids demands different enzymes to decompose them. Among these enzymes, lipases, which are capable of breaking down the fatty acid ester bonds, are the most important to hydrolyze tri-, di, and mono-acylglycerol and phospholipids with its broad substrate specificity (Mustranta et al., 1995). In contrast to other hydrolytic enzymes, lipase activity is controlled by both substrate concentration and surface area of substrate emulsion (Gajewski et al., 1993; Voet and Voet, 1995). For phospholipids, several different phospholipases are required to remove the phosphodiester bonds in the polar head group (Lehninger, et al., 1993). After hydrolysis of thiacylglycerol and phospholipids, the free fatty acids are oxidized to smaller metabolites or CO_2. A frame of triacylglycerol and phospholipids enzymatic hydrolysis is presented in Fig. 1.3. Although the mechanisms of

triacylglycerol and phospholipids hydrolysis and fatty acid decomposition are understood thoroughly, fewer studies have focused on the relationships and interactions among bacterial abundance, enzyme activity, and degradation of fatty acids bound in different structural complexes under natural redox conditions. Kristensen et al. (1995) found that when structural components (e.g. lipids) dominate the particulate detritus, their anaerobic degradation rates were hampered by inefficient and slow bacterial hydrolysis of structural complex macromolecules. Sun et al's studies (2000) suggested that lipase activity in estuarine sediments was related to specific fatty acids (e.g., branched-chain fatty acids), indicating that bacteria were probably a major lipase producer.

Previous studies have developed numerous methods for lipase activities assay. Overall, these methods can be classified as immunological methods and physico-chemical methods (Bensson, et al., 2000). Generally, immunological methods are used to assay lipase in biological media, while physico-chemical methods are applied for solid or liquid media by monitoring the disappearance of the substrate or the release of the product under lipase hydrolysis (Bensson et al., 2000). Disappearance of substrate can be detected by all kinds of instruments such as turbidimetry, atomic force microcopy or infrared spectroscopy directly (Walde and Luisi, 1989; Nielsen et al., 1999; Bensson, et al., 2000). However, a big issue for in these methods is that the substrate may not be specifically hydrolyzed by exclusive enzymes (von Tigerstrom and Stelmaschuk, 1989). On the other hand, detecting of products released from lipase hydrolytic reaction is another group of methods to analyze enzyme activity (Bensson, et al., 2000). These methods are more readily by measuring the variation of colors, fluorescence or proton concentration (Brockman, 1981; Rawyler and Siegenthaler, 1989; Wilton, 1991). However, colorimetric assay was sometimes interfered by background, and proton assay may be affected

by other acidic metabolites (Benssen, 2000). Although there are so many different ways to measure enzyme activity, fewer methods were used to analyze lipid-degrading enzymes (lipases) for sediment samples. Gajewski (1993) described a method to analyze lipase activity based on the transform of the colorless substrate *p*-nitrophenyl palmitate (PNPPal) to yellow end product, *p*-nitrophenol. This spectrophotometric method has been widely used to analyze lipase activities in water samples (Boon, 1989; Gajewski et al., 1993; Gajewski and Chróst, 1995). Recently, Sun et al. (2000) modified this method for analysis of sediment samples.

Moreover, to establish relationship between bacteria and lipase, the determination of bacteria abundance or biomass is critical. The most common method used to estimate bacteria abundance is direct cell counting by using epi-fluorescence microscopy (Porter and Feig, 1980; Suzuki et al., 1993; Gough and Stahl, 2003). In this method, the bacteria need to be collected onto filters and stained with fluorescent dye (acridine organe (AO) or 4',6 diamidino-2-phenylindole (DAPI)) (Hobbie et al., 1977; Porter and Feig, 1980). Although this method is relatively reliable to determine bacteria number in water samples, the abundance in sediment samples are sometimes underestimated due to masking and losses caused by bacteria extraction processes (Kepner and Pratt, 1993; Gough and Stahl, 2003). On the other hand, bacteria biomass can be evaluated from bacteria specific fatty acids. It is well known that bacteria can specifically biosynthesize branched odd numer (C15-C17) fatty acids and also 18:1(ω7) fatty acids (Kaneda, 1991). These specific fatty acids have been used to track bacterial source in carbon cycling studies (Cranwell et al., 1987; Wakeham and Beier, 1991). Two approaches are commonly used to measure bacteria biomass. One is phospholipids fatty acid (PLFA) analysis and the other one is fatty acid methyl ester (FAME) analysis (Green and Scow, 2000). As primary components of cell membrane, PLFA degrade rapidly with the death of cells (Harvey et al., 1986). Therefore,

PLFA assay is only useful to study viable organisms in large size sample (Green and Scow, 2000). In contrast, FAMEs are derived from both living and dead cells, so FAME analysis is suited for low biomass samples (Green and Scow, 2000). Although there are still some arguments on the reliabilities of lipase assay and bacterial biomass analysis, these methods have stimulated the studies of the interactions among enzymes, bacteria and lipids.

Specific application of alkenones in paleoceanography. Besides fatty acids, long chain methyl and ethyl ketones (alkenones) have been widely used as important indicators for paleoceanography (Brassell et al., 1986; Prahl and Wakeham, 1987; Villanueva et al., 2002). Alkenones are biosynthesized by a few species of haptophyte algae (e.g. *Emilinia huxleyi*) (Volkman et al, 1980). They were also observed in sediments from the Walvis Ridge by Boon et al. (1978). After more than two decades of their discovery, the relative concentration ratios of alkenones have been proved to be related to paleo sea surface temperature at which the algae grew (Conte, et al., 1992). Alkenone unsaturation index is defined as $U_{37}^{k} = (C_{37:2} + C_{37:4})/(C_{37:2}+C_{37:3}+C_{37:4})$ ($C_{37:2}$, $C_{37:3}$ and $C_{37:4}$ are concentrations of alkenone 37:2, 37:3 and 37:4) (Brassell et al., 1986) and the index became one of the most widely used molecular proxies in paleoceanography (Prahl and Wakeham, 1987; Conte et al., 2001). Since the $C_{37:4}$ is often very low or undetectable in some sediment samples (Prahl and Wakeham, 1987), a new index ($U_{37}^{k'} = C_{37:2} /(C_{37:2}+C_{37:3})$) has been applied more frequently as a robust proxy for reconstruction of paleo sea surface temperature (Prahl and Wakeham, 1987; Eglinton et al., 1992; Villanueva et al., 1998; Zhao et al., 2000). Despite this robustness, there are still some discrepancies on the relationship between $U_{37}^{k'}$ and temperature (Herbert, 2001). The *E. huxleyi* culturing experiments showed that non-thermal factors, such as nutrient concentration and cell growth rate can change $U_{37}^{k'}$ significantly (Epstein et al., 1998). $U_{37}^{k'}$ derived from various

clones responded differently to the same environmental factors (Popp et al., 1998). Besides environmental factors, the biological causes for $U_{37}^{k'}$ variations are still enigmatic. Some studies pointed out that alkenones were membrane-bound lipids to function as regulators of membrane fluidity and rigidity (Prahl et al., 1988; Brassell, 1993; Sawada and Shiraiwa, 2004). Other studies suggested that alkenons served as metabolic storage lipids (Bell and Pond, 1996; Epstein et al., 2001). If alkenones are membrane lipids, the $U_{37}^{k'}$ should depend on temperature because increasing unsaturation at lower temperature is helpful to keep membrane fluid (Epstein et al., 2001). If alkenones served as storage lipids, increasing number of double-bonds might decrease alkenone melting point. Alkenones with lower melting point are more easily catabolized (Epstein et al., 2001). The alternation in unsaturation extent may reflect the adaptation of *E. huxleyi* to ambient temperature variation. No matter alkenones are in membrane or intracellular structures, the synthesis processes may be affected by the environmental temperature.

On the other hand, degradation of alkenones during dingenetic processes may influence the alkenone unsaturation. The basic premise for $U_{37}^{k'}$ application is that alkenones with different mumber of double-bonds have no differential degradation in natural environments (Gong and Hollander, 1999). This premise has been argued for years, based on field and laboratory studies (Prahl et al., 1989; Teece et al., 1994; Madureira et al., 1995). For example, Prahl et al. (1989) suggested that $U_{37}^{k'}$ were constant under both oxic and anoxic conditions with remarkable degradation of alkenones. Laboratory of incubations of *E. hyxleyi* in oxic and anoxic systems (Teece et al., 1994; Sun et al., 2004) showed that aerobic and anaerobic microbial processes did not change $U_{37}^{k'}$ values significantly when a large fraction of alkenones degraded. Evidence from feeding experiments (Grice et al., 1998) showed that zooplankton grazing did not change the $U_{37}^{k'}$ in seawater. In contrast, Freeman and Wakeham's (1992) work observed that

$U_{37}^{k'}$ increased in water column when alknones degraded preferentially. Gong and Hollander (1999) indicated that differential degradation of alkenones 37:3 and 37:2 in oxic sediments with strong bioturbation caused a temperature shift up to 4°C compare to that in anoxic sediments. These contrasting observations implied that the impact of diagenesis on $U_{37}^{k'}$ is not fully understood (Sun, et al., 2004), especially in regimes with conbination of macrofaunal and microbial activities.

Advanced teniques in studying lipid degradation. To study early diagenesis of algae-derived lipids in water column and sediments, various techniques have been developed to extract, separate and analyze the lipid compounds bound in different structural matixes. Chromatographic technique is one of powerful tools to separate cell-associated lipids. For example, the two important cellular components, triglycerides in intracellular tissue and phospholipids in membrane can be separated based on various chromatographic procedures (Lehninger et al., 1993; Marty et al., 1996; Green and Scow, 2000). Although many procedures have been developed to extract and separate lipids well, fewer studies have been practically applied to examine the degradation of various lipids bound in different structural associations within natural organic matter matrixes.

Lipids in various structural cell associations. In general, both neutral lipids and fatty acids are served as energy or structural compounds in organism. Unlike these lipids, chloropigments in plant act as photoreceptors in photosynthesis (Hitchcock and Nichols, 1971; Voet and Voet, 1995). Previous studies have widely used chloropigment biomarkers to estimate algal biomass and assess carbon cycling in water column (Welschmeyer and Lorenzen, 1985; Carpenter et al., 1986) and in surface sediments (Furlong and Carpenter, 1988; Sun et al., 1991, 1993a, 1993b; Stephens et al., 1997). Among the chloropigments, chlorophyll-a (Chl-a) is the

most abundant pigment in almost all phytoplankton and higher plant (Hitchcock and Nichols, 1971). Chl-a is structurally a cyclic tetrapyrrole (Voet and Voet, 1995). During biochemical processes, Chl-a can be converted to several derivatives by two different pathways. One pathway involves cleavage of the macrocyclic ring system and decomposing Chl-a to small carbon or nitrogen fragments (Hendry et al., 1987). The other pathway converts Chl-a into chlorophyllide-a, phaeophytin-a (Ppt-a) and phaeophorbide-a (Ppb-a) (Fig. 1.4) by loss of magnesium and loss of phytol without breaking the ring system (Hendry et al., 1987; Matile et al., 1999). However, a number of studies showed that the redox conditions in natural environments strongly affect cell-derived chlorophyll degradation (Sun et al., 1993a, 1993b; Holmer, 1999; Bianchi et al., 2000). Bianchi et al.'s (2000) incubation experiment showed that cell-associated Chl-a degraded almost completely under oxic conditions in two months. They also found that bioturbation further enhanced degradation of cell-associated pigments in added phytoplankton but has less effect on pigments contained in bulk sediments (Bianchi, et al, 2000). Sun et al. (1993a) found that the cell-associated Chl-a degraded faster and more completely under oxic conditions than under anoxic conditions but variable redox conditions had insignificant effects on degradation of free Chl-a tracers. These studies imply that cell-associated Chl-a degraded differently from free pigment, but the mechanisms have remained unclear. It was suggested that structural Chl-a associations within algal cells might affect Chl-a degradation critically (Schoch and Brown, 1987; Louda et al., 1998), but few studies have focused on the effects of these structural associations on Chl-a degradation in natural environments.

In plant cells, it has been recognized that Chl-a exists in chloroplast to absorb light energy for photosynthesis (Voet and Voet, 1995). The chloroplast has two photosystems (PS-I

and PS-II) (Fig. 1.5) (Steer, 2003), which are embedded in the thylakoid membrane and carry out dark and light reactions respectively in photosynthesis (Voet and Voet, 1995). Both PS-I and PS-II are supercomplexes and compose of two functionally and structurally different domains: core protein complexes (CP) and the peripheral antenna — light-harvesting polypeptide complexes (LHC) (Green and Durnford, 1996; Scheller et al., 2001; Bumba and Vácha, 2003; Jensen et al., 2003).

In algal cells, the PS-I core complexes consist of thirteen individual proteins (Fromme et al., 2001; Scheller et al., 2001). About 100 Chl-a are non-covalently bound to the reaction center of PS-I as Chl-a complex I (CP-I), which is the largest Chl-a protein complex in the photosystem (Scheller et al., 2001). The CP-I is a chlorophyll dimmer, which has a larger molecular size (~160 kDa) and contains most pigments in PS-I (Scheller et al., 2001). In general, this chlorophyll dimmer is a heterodimer and consists of two large membrane intrinsic subunits — PSI-A and PSI-B (Scheller et al., 2001). Both PSI-A and PSI-B have thirteen α-helices, in which a fraction of Chl-a is located (Green and Durnford, 1996; Fromme et al., 2001). Within the PS-I system, there are four different polypeptide subunits, which are LHC-I complexes (Golbeck, 1992; Scheller et al., 2001). The molecular weights of these polypeptides range from about 25 to 30 kDa (Scheller et al., 2001). Each of these subunits has three α-helices, which bind ten Chl-a molecules to absorb and transfer light energy to reaction center (Green and Durnfold, 1996; Scheller et al., 2001; Croce et al., 2002; Germano et al., 2002). In general, the LHC-I subunits are located around one side of PS-I core complex (Scheller et al., 2001).

The core complexes in PS-II are different from those in PS-I. The core protein complexes in PS-II system include three chlorophyll-protein complexes: the reaction center (RC), CP43, and CP47 (Green and Durnford, 1996). The major Chl-a binding proteins in PS-II

core complex are CP43 and CP47 (Green and Durnford, 1996). They have similar structures and each possesses 6 α-helices (Barber et al., 2000, Bumba and Vácha, 2003). Both proteins bind about 20 Chl-a to form Chl-a protein complexes with molecular weight about 60 kDa (Green and Durnford, 1996). Unlike PS-I, most of Chl-a in PS-II are bound to its light harvesting complex — LHC-II, which exists as trimer in vivo and links to the PS-II core (Horton et al., 1996; Green and Durnford, 1996). In PS-II, LHC-II consists of polypeptides of 25-30 kDa that bind 13-15 Chl-a and Chl-b molecules (Peter and Thornber, 1991; Liu et al., 2004). Similar as LHC-I, the polypeptides in LHC-II also have 3 α-helices (Green and Durnford, 1996). In PS-II, LHC-II trimers are located on the edge of PS-II supercomplexes (Barber, 1998). Although the size, structures and functions of CP-I, CP43, CP47 and LHCs are generally known (Barber et al., 2000; Ben-Shem et al., 2003; Liu et al., 2004), few studies have been conducted to examine the effects of these protein and polypeptide complexes on their binding Chl-a degradation in natural oxic and anoxic environments.

To probe the compositions of various complexes in chloroplast, several methods have been developed to extract and separate various Chl-a complexes (Picuad et al., 1982; Schoch and Brown, 1987; Kashino, 2003). Principally, these complexes can be extracted by buffer solutions containing detergent and separated by gel electrophoresis. The high-performance liquid chromatography (HPLC) (Mantoura and Llewellyn, 1983) is often used to qualitatively and quantitatively analyze pigments extracted from these separated complexes (Zolla et al., 1997; Almela et al., 2000). Although the methods, which are used to extract, separate, purify, identify and quantify the Chl-a complexes in plant and algal cells, have been well developed, there is an uncertainty on the recovery of Chl-a from various complexes (Picuad et al., 1982; Caron and Brown, 1987). For most methods, only about 50%-60% of Chl-a were extracted from various

complexes, which were dissolved in detergent buffer (Picuad et al., 1982; Schoch and Brown, 1987). It is unclear how other unextractable Chl-a molecules are bound within the cells, besides Chl-a protein and Chl-a polypeptide complexes.

Effects of macrofuanal activities on organic matter degradation. Also, lipids have been widely used as tracers to study the bioturbation (Sun et al., 1999; Green et al., 2002). Generally, bioturbation refers to the overall mixing and displacement of sediment particles by activities of benthic organisms (Aller et al., 1982; 1994; Shull, 2001). Bioturbation causes transport of solutes and solids and affects the fluxes of chemicals through the sediment-seawater interface (Berg, et al., 2001). The bioturbation processes, including burrowing, irrigation, reworking and feeding by benthic fauna, create unique physical and biogeochemical conditions, which can dramatically influence the diagenetic processes (Aller et al., 1982, 1994, 1998).

Burrow dwelling macrofuana inhabit in most marine sediment (Hansen and Kristensen, 1997). A number of studies examined the effects of burrowing by different benthic animals on organic matter degradation. Burrowing can translocate fresh reactive organic matter and introduce oxygen from sediment surface to deep sediment strata (Forster and Graf, 1992; Hansen and Kristensen, 1998). The fluctuation between oxygen inducing and exhaust creates a unique microenvironment in and around the borrow wall (Dobbs and Guckert, 1988; Tomaszek, 1995) and provides a mosaic for both aerobic and anaerobic bacteria (Bird et al., 2002). It has been proved that the burrow walls are able to contain more organic carbon than the ambient sediments (Vaugelas and Buscail, 1990). The presence of labile organic matter and oxygen input in burrows enhance the microbial activities and then increase organic matter degradation (Aller and Aller, 1986; Kristensen, 1988; Reichardt, 1991; Steward et al., 1996).

Irrigation, which is the exchange of sediment pore water and bottom water by pump activities of macrofauna, enhances solute transport between sediment and overlying water (Berg et al., 2001). By solute transport, dissolved oxygen is introduced into anoxic sediments to promote organic matter decomposition (Bird et al., 2000). In a broad sense, burrowing is a kind of reworking activities by macrofauna (Caradec et al., 2004). The sediment reworking by benthic animal redistributes fluid and sediment particles, alters microbial community and adjusts the oxic/anoxic boundaries (Aller, 2001). As a result, the organic matter degradation pathway in bioturbated sediments may be different from that in sediments lacking macrofaunal reworking (Caradec et al., 2004).

Besides burrowing, irrigation and reworking, benthic animals also directly control organic matter decomposition by their feeding behavior. Previous studies have demonstrated the important role of animal feeding on organic matter degradation. For example, Heip et al. (2001) showed that in shelf and upper slope sediments, more than half of the organic matter flux was respired by macrofauna, with a lower contribution from metazoan meiofauna. However, some of organic matter in the sediment may be refractory and cannot be assimilated by benthic animals, especially deposit-feeder (Rice, 1982; Levinton and Stewart, 1988). Therefore, benthic animal (deposit-feeder) can selectively graze and ingest high quality food source to increase their feeding efficiency (Sun et al., 1999; Hansen and Josefson, 2004). The selective grazing and ingestion may affect the fates of various organic compounds in sediments.

In summary, bioturbation enhances degradation of organic matter by increasing oxygen availability, altering aerobic/anaerobic boundaries, fragmenting particles, regulating material transport, stimulating bacteria growth, and digesting organic matter (Aller, 1982, 1994; Lopez and Levinton, 1987; Bianchi et al., 1988; Sun, et al., 1999, 2002). Sun et al. (2000) indicated

that the presence of benthic macrofauna (*Yoldia limiatula*) would significantly enhance the degradation rates of microalgal lipids in sediment. Sun et al. (1998) also found the initial rate and pathway of phytol degradation were strongly influenced by benthic faunal activity. Bioturbation also increase degradation of old and relatively refractory detritus (Andersen and Kristensen, 1988; Kristensen et al., 1992). On the other hand, without the presence of animals, Wakeham and Ertel (1988) found little alteration of organic mater in anoxic Cariaco Trench bottom sediment. However, some reports indicated that biotrubation slow down the degradation of organic matter in sediments (Canfield, 1994; Josefson et al., 2002). For instance, Josefson et al. (2002) found a very low degradation of Chl-a in sediment despite the presence of abundant macrofauna. The slow degradation of organic matter was attributed to the burial of organic matter to deep anoxic sediment by benthic animals (Josefson, 2002). It is obvious that the role of bioturbatin on organic matter degradation depends on the activities and behaviors of benthic animals. However, we know not much about how activities of specific animal species affect the fates of specific algal biomarkers in sediments.

On the other hand, macrofaunal activities affect microbial community and then control organic matter degradation indirectly. Previous studies indicated that deposit-feeders could either control or stimulate bacterial growth and bacteria activities (Moriarty et al., 1985; Plante et al., 1990; Grossmann and Reichardt, 1991). Macrofauna grazing also decreases bacterial abundance or activities (Goñi-Urriza et al., 1999; Lucas, et al., 2003). On the other hand, bioturbation creates a fluctuation of oxygen conditions and transports reactive organic materials to deep sediment to promote the growth and production of both aerobic and anaerobic bacteria (Dobbs and Guckert, 1988; Aller and Aller, 1986; Steward et al., 1996). In addition, macrofaunal fecal pellets provide the substrate for bacteria growth (Hylleberg, 1975; Goñi-

Urriza et al., 1999). Despite these studies, few studies focused on how sediment reworking by specific benthic animals affected the bacterial community.

Hypotheses

Based on previous studies, four hypotheses were proposed to understand the intrinsic structure causes for organic matter degradation and the interactions between these molecular associations and environmental conditions:

(1) Fatty acids associated in membrane (e.g., phospholipids) and intracellular fractions (e.g., triacylglycerols) within algal cells may degrade differently under oxic and anoxic conditions due to the difference in their reactivities, structures or accessibilities by different degrading enzymes;

(2) Chl-a bound in various chlorophyll-protein complexes (e.g., CPI, CP43, CP47, and LHCs) may have different responses to oxic and anoxic degradation agents due to the differences in their size, solubility, association and molecular arrangement;

(3) Specific bioturbation effects of crustacean *Palaemonetes pugio* may alter the fates of different lipid biomarkers in surface sediment due to their selective grazing and differential assimilation for cell-associated lipid compounds;

(4) Aerobic and anaerobic microbes may play different roles in degradation of algal lipids in seawater and sediments due to the difference in their effectiveness on breaking compound structures via releasing different extracellular enzymes.

Experimental design

To test these hypotheses, three types of incubation experiments were designed to examine degradation of algal lipids from a haptophyte (*Emiliania huxleyi*) in three simulated natural

environments (Fig. 1.6). The alga selected in these incubation experiments was *E. huxleyi* (Fig. 1.7). This alga is a common coccolithophorid and occurs in most oceans except the polar areas (Brown and Yoder, 1994). In both oceanic and coastal waters, *E. huxleyi* can form extensive blooms (Blach et al., 1991, 1992). The sink of this alga to the sea floor removes a large amount of CO_2 from atmosphere and perturbs the ocean carbon cycle and then contribute to global climate change (Buitenhuis et al., 1996). On the other side, *E. huxleyi* can exclusively biosynthesize a series of specific biomarkers (long chain alkenones), which have been widely used as a temperature indicator in paleoceanography studies (Brassell et al., 1986; Prahl et al., 1988). However, the effects of degradation of alkenones on the $U_{37}^{k'}$ index have not been well understood (Gong and Hollander, 1999). Therefore, understanding degradation of *E. huxleyi* cells in marine systems has important impact on carbon cycling and paleoceanographic studies.

Experiment type-I stimulated permanent stratified oxic/anoxic seawater such as in Cariaco Trench and Black Sea. The seawater used in this experiment was collected from a typically stratified water column in Cariaco Trench (Muller-Karger et al., 2004). Since exchange of water and other material between the trench and adjacent ocean is restricted, the decomposition of sinking organic matter in water caused anoxia below about 350 m (Fig. 1.8). In these experiments, *E. huxleyi* cells were incubated in oxic (collect from 30 m) and anoxic (collect from 930 m) seawater. By tracking the degradation of Chl-a bound in various complexes, the effects of complex structures on Chl-a decay was investigated.

Experiment type-II stimulated sediment-seawater interface experienced seasonal hypoxia. The hypoxic conditions (O_2 < 3 mg/l) can occur naturally in bottom water of some estuaries, such as Long Island Sound (LIS) and Chesapeake Bay (Parker and O'Reilly, 1991). During summer time, the vertical mixing between surface and bottom water is prevented by increased

thermal and haline stratification (Valle-Levinson et al., 1995). The degradation of organic matter by biological and chemical processes reduces oxygen concentration dramatically to hypoxia (Valle-Levison et al., 1995). The reduction of oxygen content in bottom water not only induces stress to organisms' life, but also affects the accumulation, preservation and degradation of organic matter in sediments (Valle-Levison et al., 1995; Sun et al., 1998). In experiment type-II, algal material was incubated in oxic/anoxic sediment-seawater interface. The microcosms comprised of two tanks with oxic and anoxic seawater. A kind of "thin-plugs" loaded with sediments were put on the bottom of tanks to stimulate sediment-seawater interface. Focus of this type of experiment is to examine degradation of algal fatty acids bound in different cellular structural associations. In addition, the responses of aerobic and anaerobic bacteria to lipid degradation and the relationship between bacteria abundance and released enzymes (lipases) are also investigated by following the variations of these parameters during two-month incubations.

Experiment type-III simulated natural permanently oxygenated water and oxic surface sediment. In this kind of environment, the benthic animals are active in the sediments. In these experiments, the algal cells were incubated in the microcosms with and without presence of benthic macrofauna. The selected macrofauna is *Palaemonetes pugio* (Fig. 1.9), a delicate, translucent crustacean. This species abundantly inhabits in estuarine ecosystems along Atlantic and Gulf coasts (Ruppert and Fox, 1988; Williams, 1984). It greatly resuspend the surface sediments by various activities (Shenker et al., 1979). It can mechanically break refractory organic materials and assimilate microflora, microfuana and fungi (Adams et al., 1970). As an epibenthic predator and sediment disturber, it also alters infaunal community structure (Bell et al., 1978). Although a number of studies have demonstrated the predatory role of this species (Gregg and Fleeger, 1998; Kneib, 1985; Reinsel et al., 2002), few researches have focused on its

role in degradation of lipid biomarkers. As a non-burrowing deposit-feeder, *P. pugio* reworks surface sediment, resulting in resuspension and downward transport of sediment particles. The experiments examined how the specific activities by this crustacean alter lipid degradation and bacteria community. Since survival of the crustacean in the microcosms was critical, a preliminary feeding experiment was conducted to test its mortality under different food supplies (Fig. 1.10). The experimental results showed that with food supply (once every three days), all crustaceans could survive more than six weeks in sediment-seawater system. Even without any food supply, more than half of the crustacean could survive for up to six weeks. Thus, the incubation experiments lasted six weeks. Focus of these experiments is to examine the role the crustacean on the fates of various algal lipids, on the degradation of fatty acid structural associations, and on the alternation of the microbial community. In addition, these experiments also investigated the effect of benthic processes on $U_{37}^{k'}$ variations.

Organization of this dissertation

This dissertation consists of five chapters: one introduction chapter, three major chapters and a summary. Each chapter is independent but inherently linked to each other. Chapter 2 was recently submited to Geochimia Cosmochimia Acta. Chapter 3 is a manuscript accepted by Marine Chemistry in 2004. Chapter 4 will be sent to Limnology and Oceanography after further edit. The summary synthesizes findings in the three major chapters to describe the relationships between various control factors and organic matter degradation.

Chapter 2 focused on the effects of intracellular structural associations on degradation of Chl-a (Experiment I). By incubating *E.huxleyi* cells in natural oxic and anoxic seawater for 90 days, the time-dependent variations of Chl-a in different chloropigment-protein complexes were

followed. An extraction/separation procedure (modified based on Picuad's method, 1982) was used to separate various pigment-protein complexes in incubated cell detritus. The average degradation rate constants of Chl-a in different pigment-protein complexes were calculated by mulit-G model and the effect of the structural complexes on Chl-a degradation was examined. By following the variations of two Chl-a derivatives, Ppt-a and Ppb-a, a conceptual model was proposed to describe the degradation pathways of Chl-a from various pigment-protein complexes in oxic/anoxic seawater.

Charter 3 described a set of incubation experiments of *E. huxleyi* cells in simulated oxic/anoxic sediment-seawater interface microcosms for 56 days (Experiment II). In these experiments, a modified protocol was used to separate membrane and intracellular fatty acids in sediment samples. The multi-G model (Berner, 1964; Westrich and Berner, 1984) was used to quantify the degradation rates of algal fatty acids under various redox conditions. A new parameter, average degradation rate constant, was recalculated to compare the overall degradation of different fatty acids no matter how the pool sizes vary in different conditions. Based on the average degradation rate constants, the structural effects on the degradation of algal fatty acids bound in membrane and intracellular components under oxic and anoxic conditions were evaluated. By following time-dependent variations of lipase activity, bacteria abundance, and bacteria-specific fatty acids, the relationships between enzymes, microbial community and fatty acid degradation were discussed.

The major goal of Chapter 4 was to study how crustacean (*P. pugio*) affect the degradation of algal lipids. *E. huxleyi* cells were added into sediment-seawater microcosms with or without presence of crustacean for six-week incubations (Experiment III). The variations of algal lipids in suspended particles and surface sediments and the distribution of algal lipids along

sediment depth were tracked. By integrating the algal lipids in the whole incubation cores, the differential degradation of alkenones and algal fatty acids caused by crustacean's activities was observed. The specific roles of selective grazing and preferential assimilation in degradation of alkenones and algal fatty acids were examined. Moreover, the influence of crustacean's activities on $U_{37}^{k'}$ were investigated by following the time-dependent variations of these parameters. Finally, a conceptual model was established to assess the fates of various algal lipids in sediments with and without presence of crustacean.

In summary, through detailed study of degradation of cell associated lipid components and pigment-protein complexes, this work sheds light on the intrinsic control of various molecular associations on the organic matter degradation. This study also provides new information on the interactions between microorganisms and organic matter by comprehensive measurements of bacteria abundance, lipase activities and bacteria-specific biomarkers. With study of specific activities of macrofauna (resuspension, selective grazing and preferential assimilation), this work refines current understanding of effects of macrofaunal activities on degradation of lipid biomarkers in surface sediments. Finally, this study is likely to add new knowledge of different fates of organic biomarkers in marine ecosystems, which helps to understand global carbon cycling and paleoceanography researches.

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Table 1.1. Degradation rate constants of organic matter and its major fractions (Harvey et al., 1995).

T. weissflogii Biochemical fraction	$k_{ox}(year^{-1})$	$k_{an}(year^{-1})$	k_{ox}/k_{an}
POC	12.8	2.9	4.4
Protein	21.1	15.3	1.4
Carbohydrate	33.7	7.2	4.7
Total lipid	8.3	2.7	3.1

Synnechoccus sp. Biochemical fraction	$k_{ox}(year^{-1})$	$k_{an}(year^{-1})$	k_{ox}/k_{an}
POC	15.3	2.5	6.2
Protein	22	6.2	3.5
Carbohydrate	34.2	4.1	8.3
Total lipid	8.2	2.3	3.6

Tabel 1.2. Degradation rate constants of free cell-associated fatty acids under oxic and anoxic conditions (Sun et al., 1997).

Fatty acids		Degradation rate constants	
		oxic	anoxic
16:0	Free	0.23	0.06
	cell associated	0.107	0.005
18:1(ω9)	Free	0.21	0.1
	cell associated	0.14	0.009

Fig. 1.1. Degradation of cell-associated Chl-a in oxic/anoxic sediments (Sun et al., 1993a).

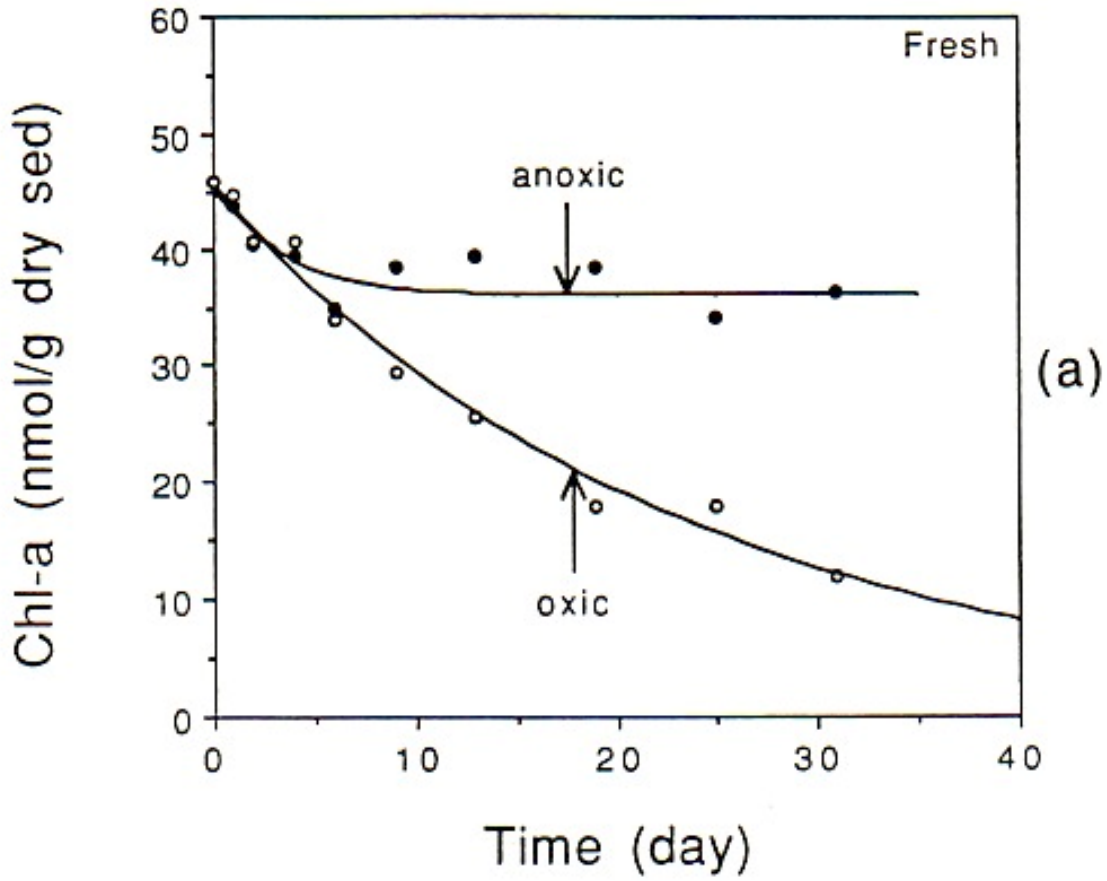

Fresh
anoxic
oxic
(a)
Chl-a (nmol/g dry sed)
Time (day)
0
10
20
30
40
50
60

Fig. 1.1.

Fig. 1.2. Basic structures of fatty acids, triacrylglycerols and phospholipids.

Fatty acids

saturated

$CH_3(CH_2)_nCOOH$

alkyl chain (n ? 10)

mono-unsaturated

$CH_3(CH_2)_mCH=CH(CH_2)_nCOOH$

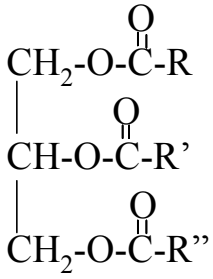
alkyl chain (n = 5 or 7, m+n ? 8)

poly-unsaturated

$CH_3(CH_2)_m(CH=CHCH_2)_x(CH_2)_nCOOH$

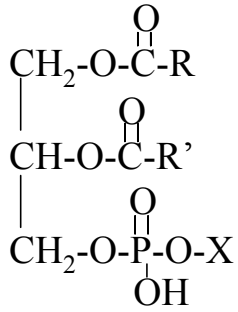
alkyl chain (x ? 2, m+n ? 4)

Triacylglycerols (intracellular)



R, R', R" representing different alkyl chains

Phospholipids (membrane)



R, R' representing different alkyl chains;
X is head-group substituent

Fig. 1.2.

Fig. 1.3. Degradation pathways of triacylglycerol and phospholipids under various enzyme attacks.

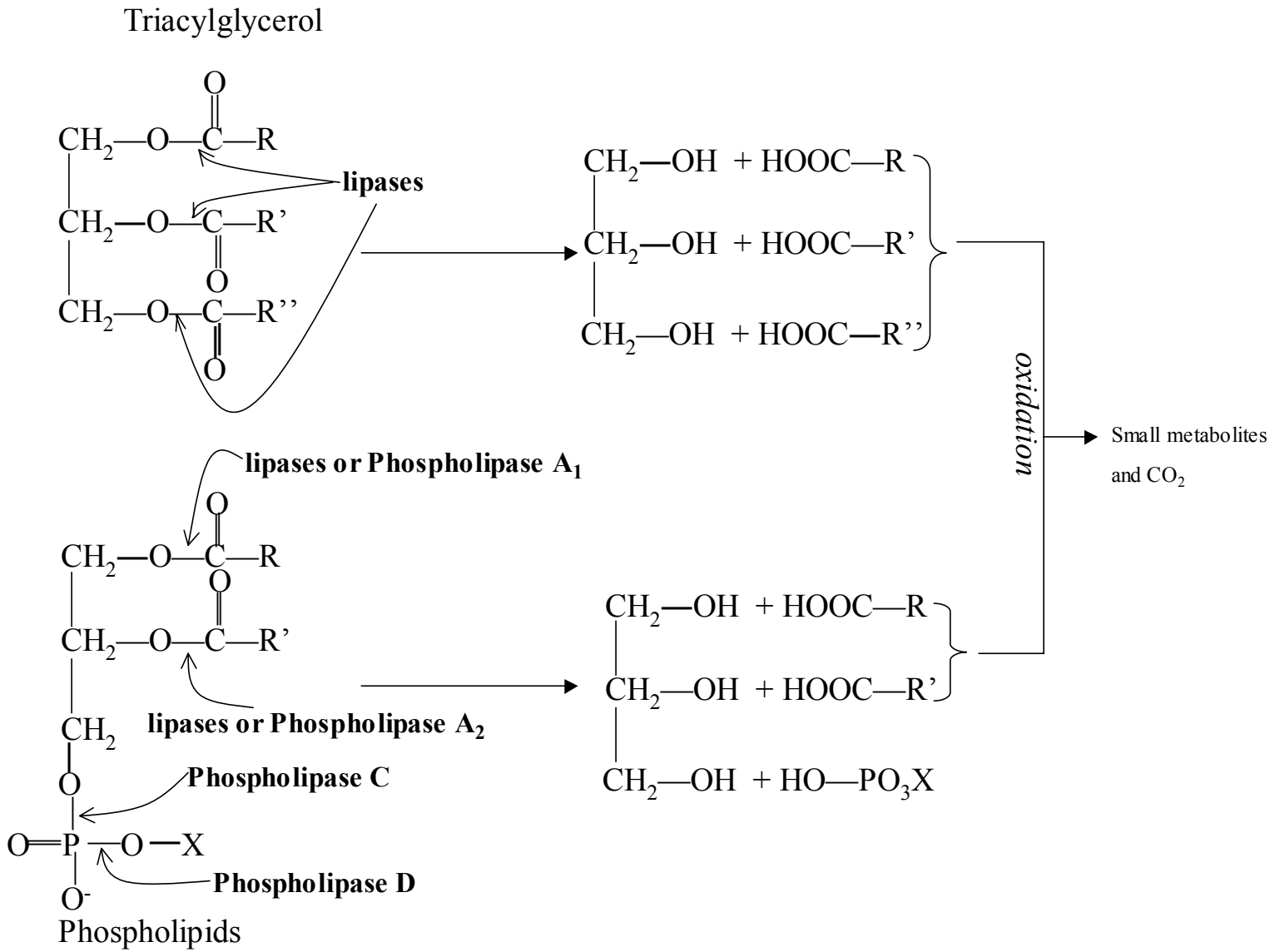
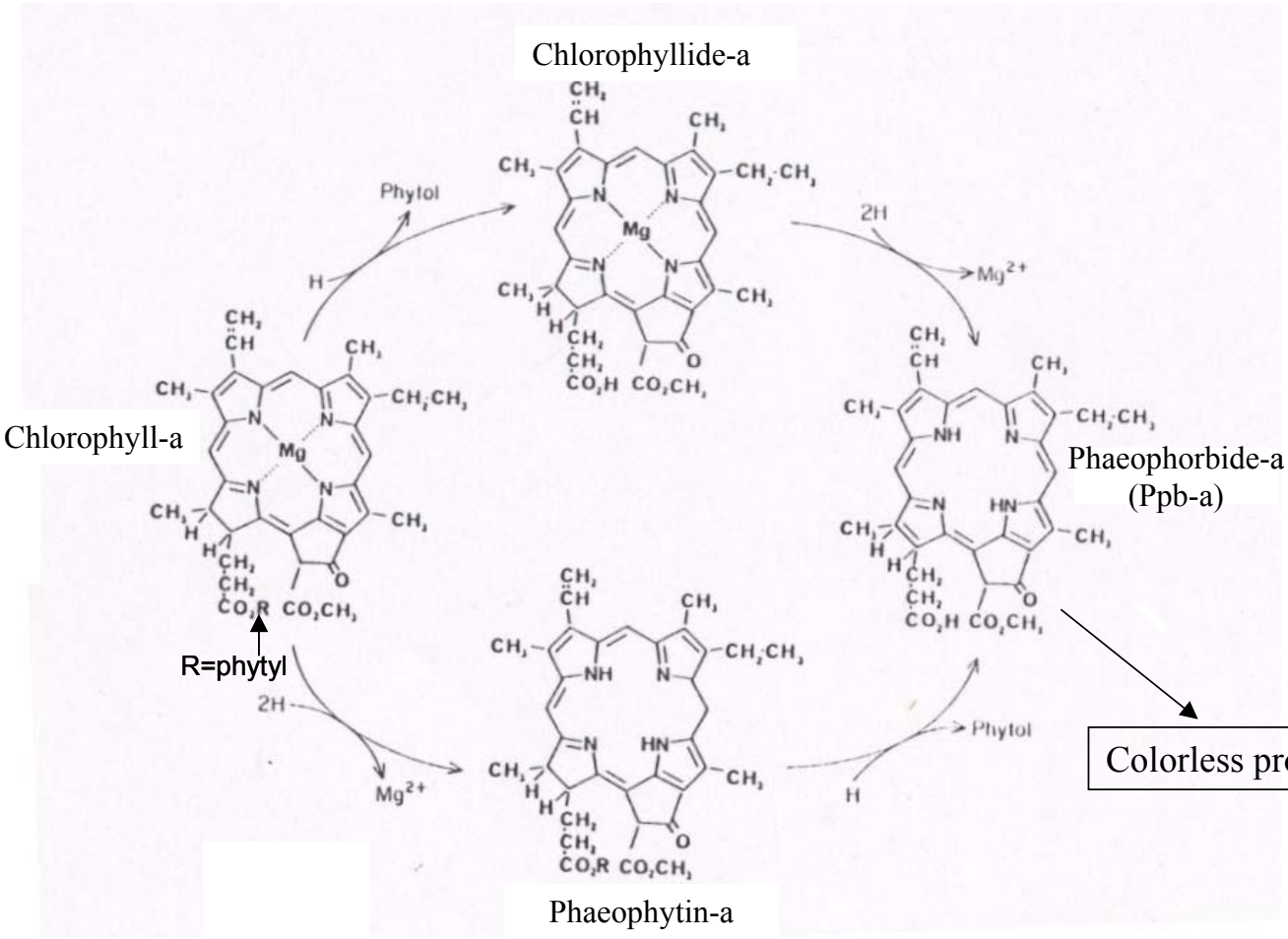

Triacylglycerol
lipases
lipases or Phospholipase A1
lipases or Phospholipase A2
Phospholipase C
Phospholipase D
Phospholipids
oxidation
Small metabolites and CO2

Fig. 1.3.

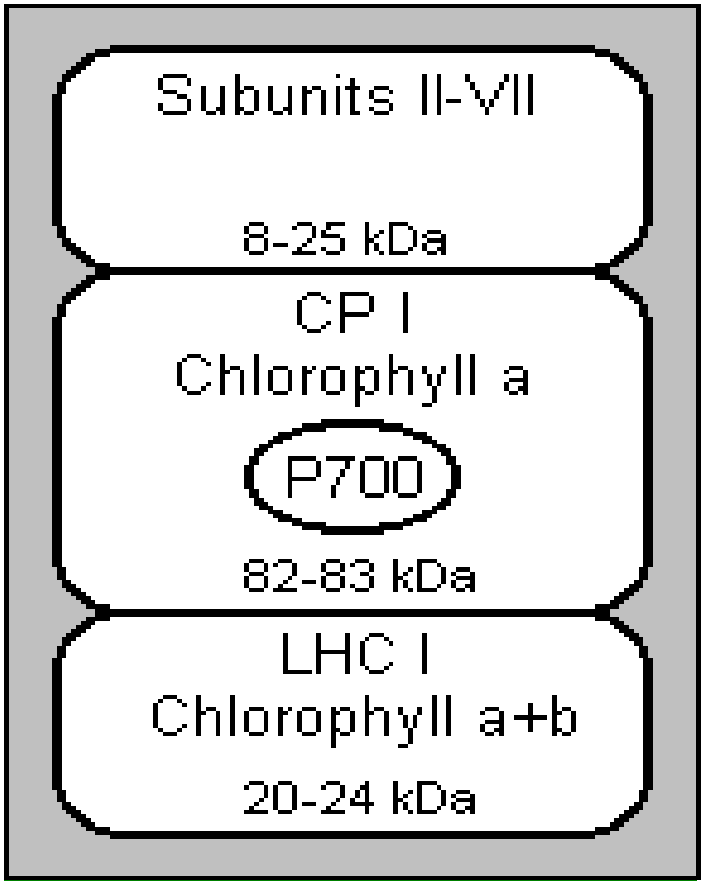
Fig. 1.4. Formation of Chl-a derivatives (Ppt-a and Ppb-a) by demetalation and dephytolation (Hendry et al., 1987).



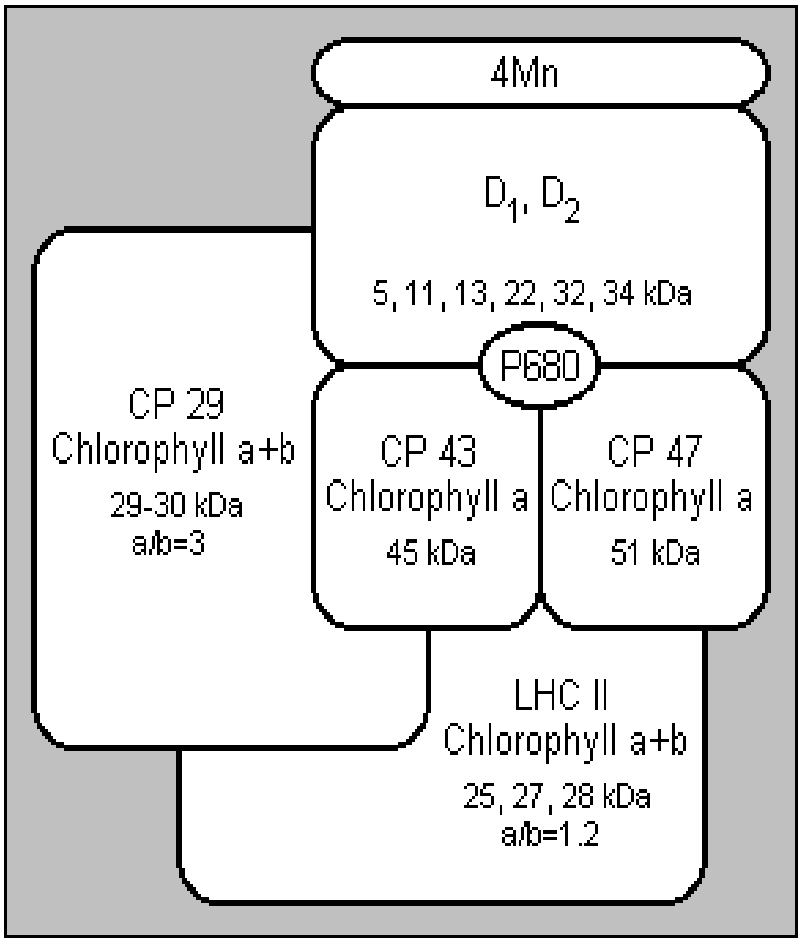
(Hendry et al., 1987)

Fig. 1.4.

Fig. 1.5. The pigment-protein complexes and other components in PS-I and PS-II (James Steer, 2003).



Photosystem I (PS-I)



Photosystem II (PS-II)

(James Steer, 2003)

Fig. 1.5.

Fig. 1.6. Picture of three marine ecosystems simulated in this study.

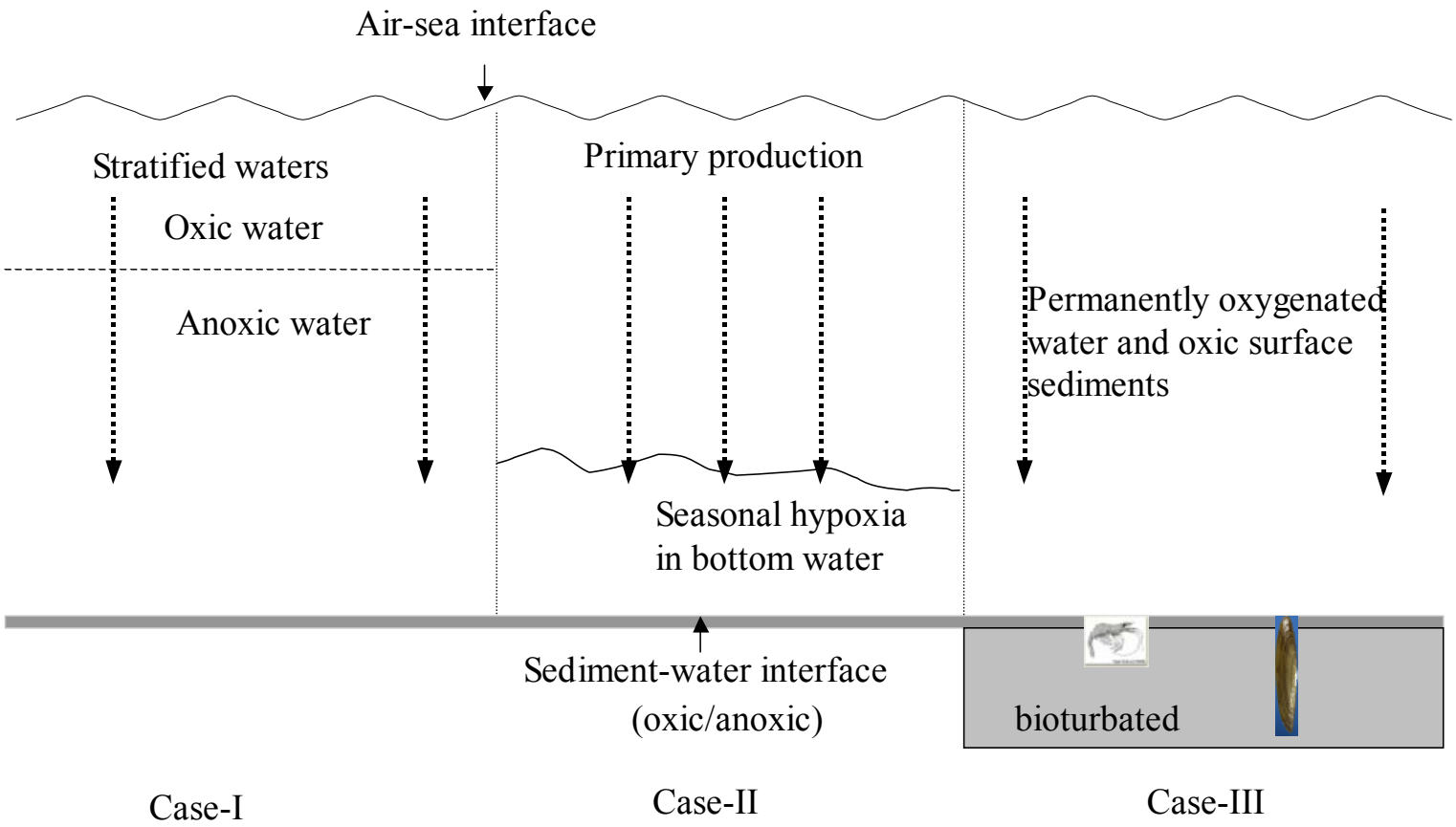
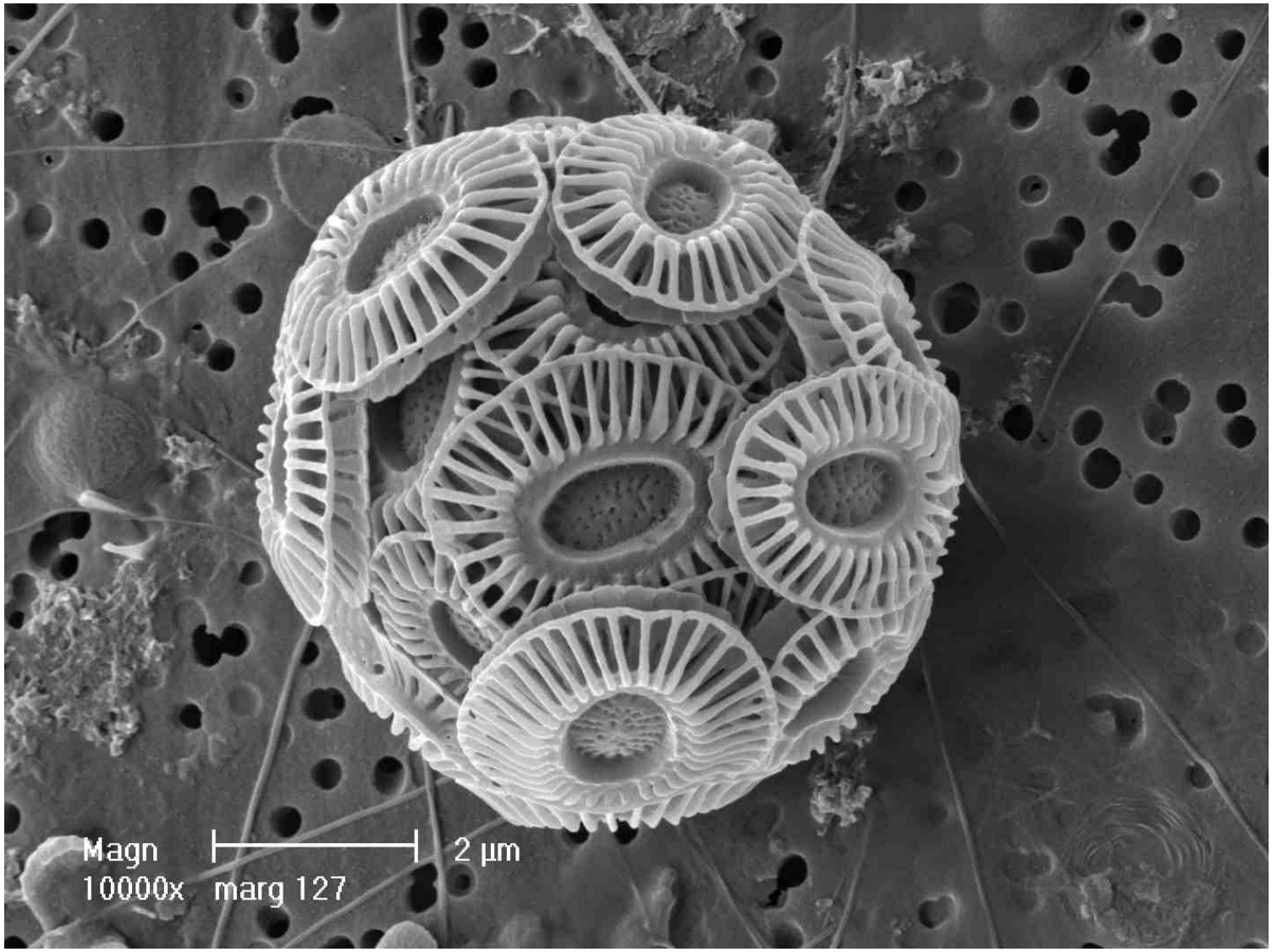

Air-sea interface
Stratified waters
Oxic water
Anoxic water
Primary production
Permanently oxygenated water and oxic surface sediments
Seasonal hypoxia in bottom water
Sediment-water interface (oxic/anoxic)
bioturbated
Case-I
Case-II
Case-III

Fig. 1.6.

Fig. 1.7. An SEM (Scan electronic microscopy) image of *E. huxleyi*.



(Picture from Jeremy Young)

Fig. 1.7.

Fig. 1.8. Depth profile of temperature and dissolved oxygen at a Cariaco Trench sampling site.

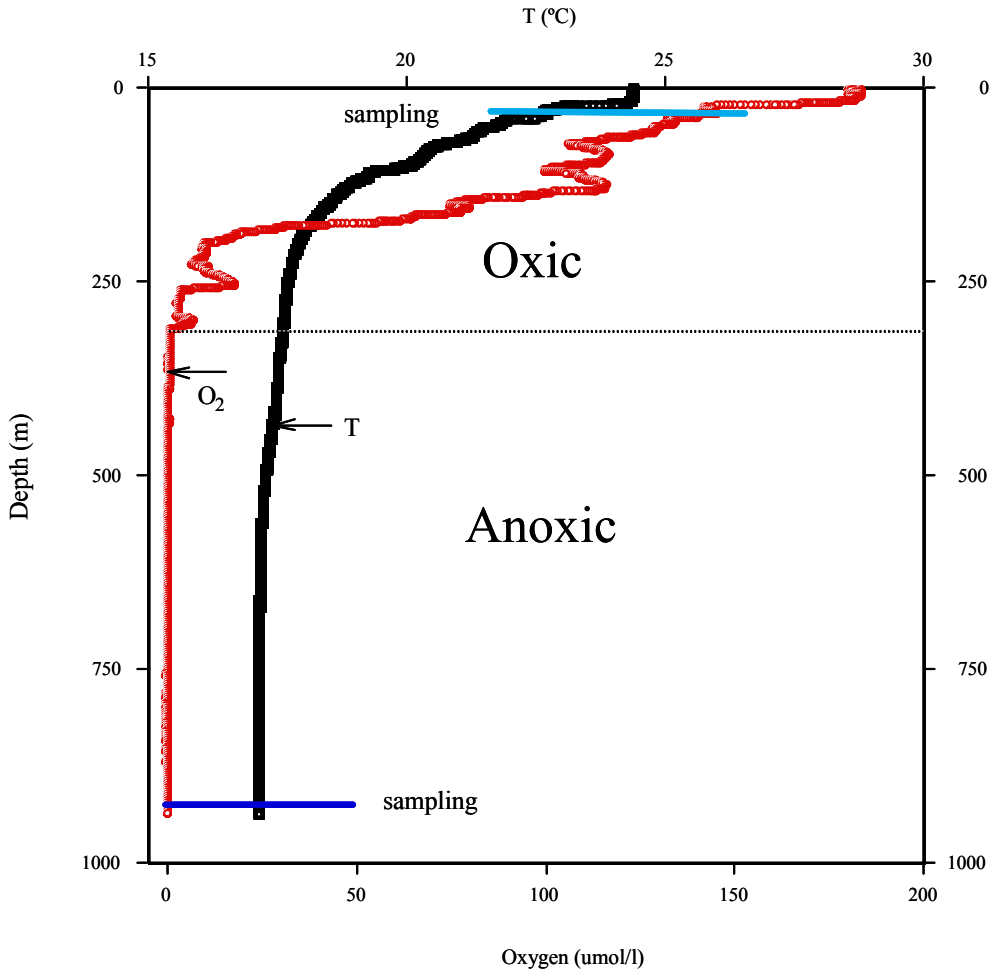
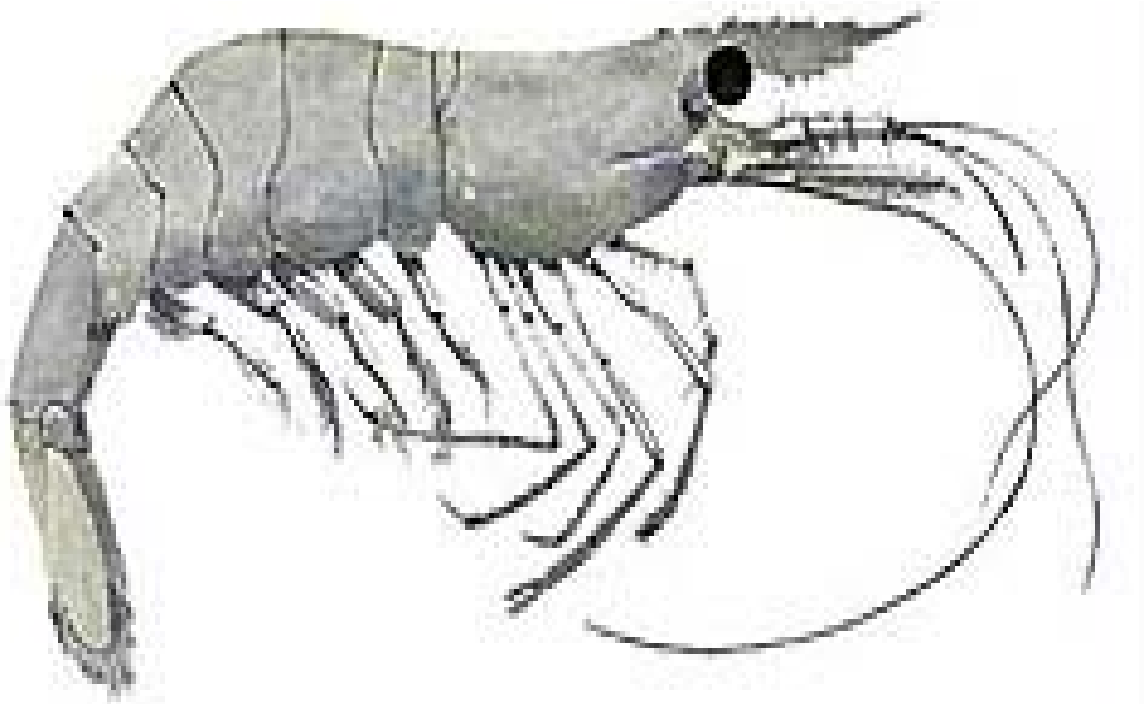

T (ºC)
15
20
25
30
0
sampling
Oxic
250
O_2
T
500
Anoxic
750
sampling
1000
Depth (m)
0
50
100
150
200
Oxygen (umol/l)

Fig. 1.8.

Fig. 1.9. Picture of crustacean *Palaemonetes pugio* (from Texas parks and wildlife website).



Palaemonetes pugio.

Picture from Texas parks and wildlife

Fig. 1.9.

Fig. 1.10. Time-dependent variations of *P. pugio* number in seawater and sediment-seawater systems with and without food supplies.

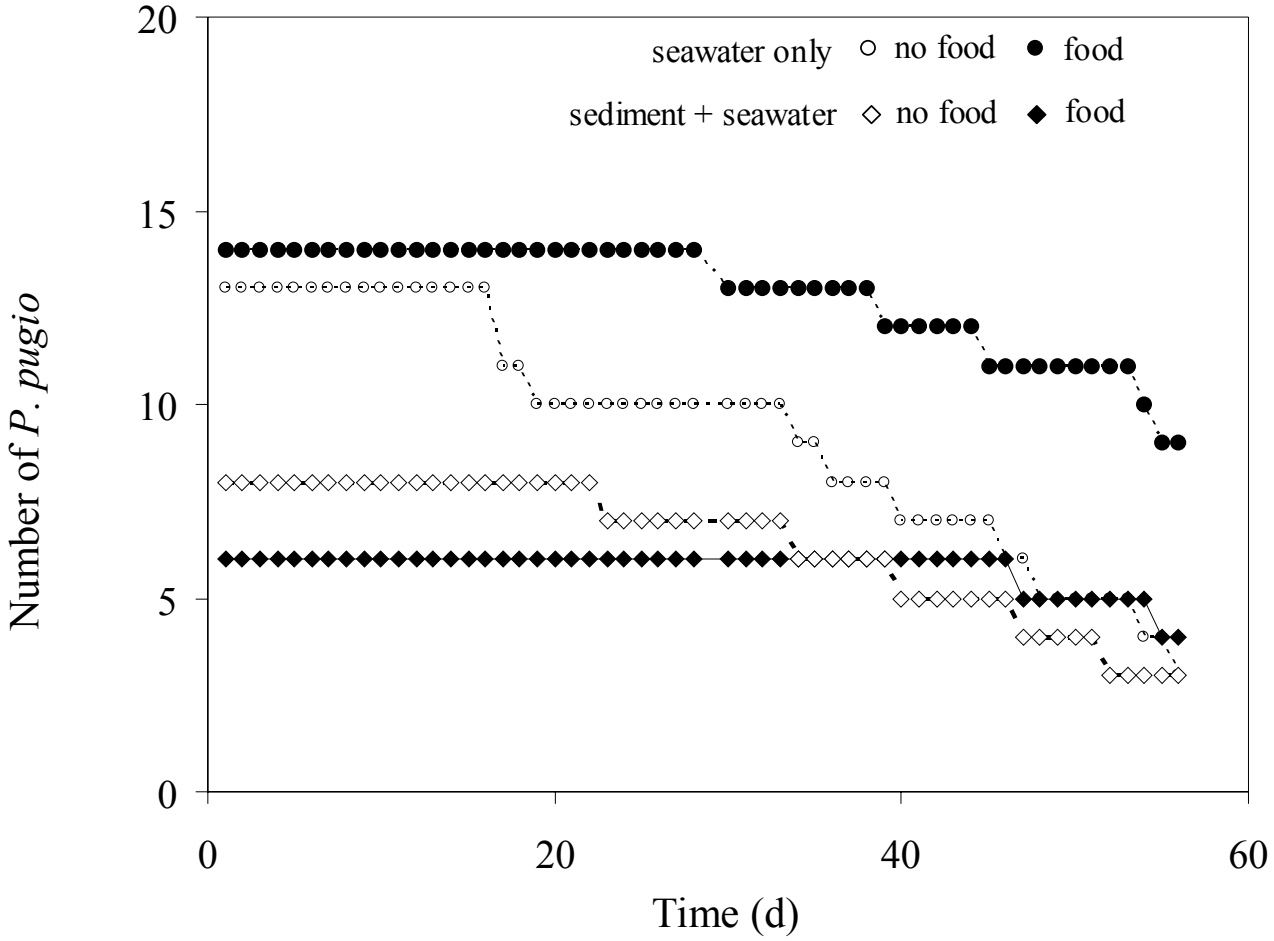

20
15
10
5
0
0
20
40
60
Number of P. pugio
Time (d)
seawater only
no food
food
sediment + seawater
no food
food

Fig. 1.10.

CHAPTER 2

EFFECTS OF INTRACELLULAR STRUCTRUAL ASSOCIATIONS ON DEGRADATION OF ALGAL CHLOROPIGMENTS IN NATURAL OXIC AND ANOXIC SEAWATERS[1]

[1] Haibing Ding and Ming-Yi Sun. Submit to *Geochimica et Cosmochimica Acta*, 09/02/2004

Abstract

To understand the effects of intracellular structural associations on degradation of algal chloropigments, we conducted a series of microcosm experiments by incubating *Emiliania huxleyi* cells (a marine haptophyte) in natural oxic and anoxic seawaters collected from a stratified water column in the Cariaco Basin. The incubated cell detritus were sequentially treated with two buffer solutions to separate pigment components into soluble and insoluble fractions. By using non-denaturing gel electrophoresis, several chlorophyll-complexes, free chlorophyll, and another unknown chlorophyll-containing component were further separated from the soluble fraction. The chlorophyll-complexes included those bound with high molecular weight core-proteins (CP-I and CP43+CP47) and low molecular weight polypeptides (LHC-I and LHC-II) in the cellular photosystems PS-I and PS-II. Pigment recovery from these fractions and gel bands was well equivalent to the total amount from direct acetone extraction of the cells. We followed the time-dependent concentration changes of chlorophyll-a (Chl-a), phaeophytin-a (Ppt-a) and phaeophorbide-a (Ppb-a) in all fractions and complexes to estimate the degradation rate constants of chloropigments in natural oxic and anoxic seawaters. Our experimental results demonstrated that the intracellular structural associations had important influences on degradation of chloropigments under different redox conditions. In general, total Chl-a (direct acetone extracted) degraded faster (~4x) in oxic seawater than in anoxic seawater. However, the rate differences between oxic and anoxic conditions varied among the fractions and complexes. Degradation rate constants of Chl-a in soluble fraction were much higher (>10x) than those in insoluble fraction under both oxic and anoxic conditions. Chl-a bound with the complexes in PS-II appeared to be more reactive (~2x) than that in PS-I under oxic conditions but the difference in degradation rate constants between two photosystems became indistinguishable

under anoxic conditions. Variations of Ppb-a in different fractions and complexes during incubation showed different patterns, implying that cellular Chl-a could degrade through two different pathways: (1) internal degradation into Ppb-a within insoluble pool and polypeptide complexes; and (2) release first from protein complexes and followed by external degradation.

Keywords: *Emiliania huxleyi*, chlorophyll-a, phaeophorbide-a, redox conditions, bacterial abundance, biochemical degradation, chlorophyll complexes

Introduction

Carbon cycling is one of the most important factors affecting global climate change (Berger and Keir, 1984; Ikeda and Tajika, 2003). Phytoplankton production and its degradation in marine systems consist of a biological pump, driving carbon cycling in water column and sediments (Keely and Brereton, 1985; Pedersen and Calvert, 1990; Harvey et al., 1995). Studies of carbon cycling have been greatly advanced by refined knowledge on the variations of individual organic compounds (biomarkers) in marine systems (Wakeham and Beier, 1991; Sun et al., 1991; Squier et al., 2002). For example, Chl-a, as a major pigment in almost all phytoplankton species, is commonly used as biomass index while its derivatives (phaeopigments), which are produced during various biochemical processes, have been often used as indicators to examine organic matter degradation (Welschmeyer and Lorenzen, 1985; Carpenter et al., 1986; Furlong and Carpenter, 1988). However, the degradation mechanisms of Chl-a, especially in variable environments, have not been completely understood (Hendry et al., 1987; Keely and Maxwell, 1991; Chen et al., 2003).

Previous studies have shown that natural cell-associated Chl-a degraded differently under variable redox conditions (Sun et al., 1993a, b; Bianchi et al., 2000). The Chl-a degraded faster and more completely under oxic conditions than under anoxic conditions. There was always a significant proportion of Chl-a remained stable under anoxic conditions. However, when free Chl-a tracers were incubated in oxic and anoxic sediments, the difference between oxic and anoxic degradation processes became insignificant (Sun et al., 1993a). It is unclear why the cell-associated Chl-a has different behavior from free Chl-a during degradation. However, it was suspected that the structural associations of Chl-a with other compounds within the cells might exert critical influences on Chl-a degradation (Schoch and Brown, 1987; Louda et al., 1998).

Within the algal cells, Chl-a and other pigments are non-covalently bound to various proteins or polypeptides to form several complexes and the assembly of these complexes builds up two photosystems (PS-I and PS-II), which are embedded in the thylakoid membrane and drive oxygenic photosynthesis (Golbeck, 1992; Barber, 1998, 2002; Fromme et al., 2001; Tetenkin, 2003). Both PS-I and PS-II photosystems are composed of core protein complexes (CP) and light-harvesting polypeptide complexes (LHC) (Green and Durnford, 1996; Scheller et al., 2001; Bumba and Vácha, 2003; Jensen et al., 2003). Although the sizes, structures and functions of various chlorophyll-protein and chlorophyll-polypeptide complexes are generally known (Barber et al., 2000; Ben-Shem et al., 2003; Liu et al., 2004), few studies have been conducted to understand how these complexes affect Chl-a degradation in natural oxic and anoxic environments.

Several methods have been developed to extract and separate chlorophyll-protein complexes (Picuad et al., 1982; Schoch and Brown, 1987; Kashino, 2003). Principally, four major steps are required to extract and separate chlorophyll-protein complexes from algal and plant cells: (1) break of cell wall by homogenization; (2) solubilization of the complexes from cell membrane with buffer solutions containing detergents; (3) separation and identification of complexes by gel electrophoresis; and (4) qualitative and quantitative analyses of pigments from the separated complexes after further extraction (Zolla et al., 1997; Almela et al., 2000). Although these methods have been successfully used to extract, separate, purify, identify and quantify the chlorophyll-protein complex compositions in various algal and plant cells, there is an uncertainty on the recovery of Chl-a from various complexes. It is also unclear if there are other potential structural components, which bind Chl-a within the cells, besides chlorophyll-protein and chlorophyll-polypeptide complexes.

This study was designed to examine the effects of intracellular structural associations of chloropigments with other molecular matrixes on Chl-a degradation in natural oxic and anoxic seawaters. Our specific goals are: (1) to differentiate structural pools containing Chl-a, including various complexes; (2) to test the recovery of Chl-a from various pools during extraction and separation processes; (3) to determine degradation rates of Chl-a in various pools under oxic and anoxic conditions; and (4) to examine the possible degradation pathways of Chl-a in different pools. We conducted a series of experiments by incubating *E. huxleyi* cells in natural oxic and anoxic seawaters collected from a stratified water column in the Cariaco Basin. We monitored the variations of bacterial abundances and three bacteria-specific fatty acids in two systems for understanding the bacteria community. We followed the time-dependent variations of Chl-a, Ppb-a and Ppt-a in total Chl-a pool (direct acetone extracted) and in different structural pools. Degradation rate constants of Chl-a in different pools were estimated by fitting data with a multi-G model (Berner, 1980) while the variations of Ppb-a (a major degradation product of Chl-a) in different pools were quantitatively described with a release-degradation model (Sun et al., 1993b). In addition, the degradation pathways of Chl-a in various pools were examined.

Experimental

Materials and Microcosm Setup. The natural oxic and anoxic seawaters used in our experiments were collected in May 2001 from a stratified water column in the western Cariaco Basin (10º30'N, 64º40'W) during cruise 66 of the CARIACO time series on the R/V Hermaño Gines. At this time, the oxic/anoxic boundary in the water column was at approximately 350 m depth. Oxic seawater was collected from 30 m and anoxic seawater from 930 m using a set of 12-L Teflon-coated Niskin bottles. A nitrogen line was fitted to the upper vent of the Niskin

bottles for collecting anoxic seawater. Seawater samples were unfiltered and directly transferred to pre-rinsed 2-L plastic screw-topped bottles. For anoxic seawater samples, the bottles were placed in sealed plastic bags filled with nitrogen. The laboratory incubation experiments were set up using these seawater samples after 10 days of sampling.

The marine haptophyte *E. huxleyi* (clone CCMP 1949) was obtained from the Provasoli-Guillard National Center for culture of marine phytoplankton, Booth Bay Harbor, Maine, USA. This alga was first cultured in 50 ml f/50 medium at 14°C and then transferred to 250 ml medium in 10 days (before the end of exponential growth phase). After another 10 days, the culture was separated into four flasks with 1000 ml medium in each. The algal cells in the final medium were harvested by centrifugation after 10 days and stored at -20°C for later incubation experiments. The culturing was carried out in a 12:12 light/dark regime.

The incubation microcosm consisted of a series of 500 ml flasks filled with 200 ml oxic or anoxic seawater. About 450 mg thawed (~90% of water) algal cells were added into each flask. Setup of anoxic incubations, including transfer of seawater and addition of cell materials, was conducted in a N_2-filled plastic bag. Oxic incubations were set up in open air. All flasks were sealed with stoppers (a switch being inserted for gas purging) and the flasks with anoxic seawater were flushed with N_2 for one minute before sealing. Oxic incubations were conducted in an open air chamber while anoxic incubations in a N_2-filled chamber. Two chambers were kept in an incubator at 15°C and in the dark. During incubation, water-saturated air and N_2 were separately purged into the oxic and anoxic seawater samples frequently (4-5 times per day) to maintain the original redox conditions. At 5, 10, 17, 24, 35, 50, 70 and 90 days, one oxic and one anoxic flask were taken out from each chamber. A subsample (100 ml) from each flask was used for extraction, separation, and analysis of pigment complexes while another subsample (50

ml) for lipid analysis. In addition, a small volume (10 ml) of sample was used for total pigment analysis by direct acetone extraction while another 1 ml for counting bacterial abundance. All detritus in the incubated samples was collected by centrifugation and stored frozen (-40°C) for later extraction and analysis.

Analysis of Bacteria-Specific Fatty acids and Bacteria Counting. Details for extraction and analysis of lipids in these incubated samples were reported elsewhere (Sun et al., 2004). Briefly, the detritus centrifuged from 50 ml incubated subsample was first extracted with 10 ml methanol, followed by 3×10 ml methylene chloride-methanol (2:1 v/v) extraction. Combined extracts were saponified with 0.5 M KOH in MeOH/H_2O (95:5) to separate neutral and acidic lipids. Neutral lipids were first extracted from the basic solution (pH>13) with hexane while fatty acids were then extracted with hexane after addition of HCl into the solution (pH<2). Fatty acids in the acidic extracts were methylated with 5% BF_3-methanol to form fatty acid methyl esters (FAMEs). FAMEs were analyzed by capillary gas chromatography (GC) using a Hewlett-Packard 6890 GC system with an on-column injector and a flame ionization detector. Compound separation was achieved by a 30 m×0.25 mm i.d. column coated with 5%-diphenyl-95%-dimethylsiloxane copolymer (HP-5, Hewlett-Packard). Selected samples were analyzed by gas chromatography-mass spectrometry (GC-MS) to identify bacteria-specific fatty acids. GC-MS analysis was performed on a SHIMADZU QP5000 GC-MS system using a 30 m×0.25 mm i.d. column coated with 5% phenyl methyl silicone (XTI-5, Restek).

Bacterial counting was performed based on published methods (Porter et al., 1980; Suzuki et al., 1993). First, 1 ml incubated seawater sample was mixed with 1 ml 4',6-diamidino-2-phenylindole solution (DAPI, 33 μg/ml) and then passed through 0.2 μm black filter. After filtration, the black filter was mounted on a slide and the bacterial numbers on the filter was

counted with an oil-immersion lens under a microscope. Bacterial abundance was estimated by averaging 10 countings.

Extraction and Separation of Pigments in Different Structural Pools. Total pigment concentrations (T) were determined by direct extraction of detritus, which was centrifuged from 10 ml incubated seawater, with 2×5 ml acetone. The extraction and separation of pigments in different structural components were carried out generally based on a modified Picaud et al's (1982) method. The detritus (centrifuged from 100 ml incubated seawater) were first treated in a 5 ml buffer-1 solution (2 mM Tris-maleate, 0.1 M sorbitol, 0.5 mM amino caproic acid and 1 mM phenylmethylsulfonyl fluoride) with a homogenizer for 2 min. After centrifugation at 4500 rpm at 4°C for 10 min, the supernatant was removed and acetone was added to the solution with a final volume of 20 ml as fraction 1 (F1). The detritus were then treated in a 2 ml buffer-2 solution (2 mM Tris-maleate and 20 mg/ml digitonin) with an ultrasonicator for 20 sec and followed by 1 h vortex. Solubilized pigment components were separated from the detritus by centrifugation at 5500 rpm (4°C) for 20 min. The extracted detritus were treated again in the same way. The combined supernatant, which contained solubilized pigment components, was defined as a soluble fraction (F2). Usually, 1 ml of F2 extract was diluted with acetone to 10 ml for direct pigment analysis while the rest F2 extract was used for further separation of soluble pigment components by electrophoresis. The final residues were further extracted with 20 ml acetone and centrifuged at 5500 rpm for 10 min. The acetone extract from the final residue was defined as insoluble fraction (F3). All operations were conducted under subdued light to avoid potential photodegradation.

Separation and Identification of Soluble Pigment Components. The soluble pigment components in F2 fraction were separated by polyacrylamide gel electrophoresis (PAGE)

(Picaud et al., 1982; Itagaki et al., 1986). The gel plate consisted of a stacking part and a separation part. The stacking gel was composed of 4% (w/v) acrylamide and 0.1% (w/v) N,N'-methylene-bis-acrylamide, buffered by 0.375 M tris-HCl (pH = 6.8). The separating gel contained 4.5% (w/v) acrylamide, 0.23% (w/v) N,N'-methylene-bis-acrylamide, 0.5% (w/v) deoxycholic acid sodium salt, buffered by 0.125 M tris-HCl (pH = 8.8). Ammonium persulfate (0.05%) and 0.005% N,N,N',N'-Tetramethylethylenediamine (TEMED) were used to polymerize both stacking and separating gels. The size ratio of stacking to separation gel was about 1:2.5.

To identify soluble pigment components, PAGE was first run on a small plate (10×10 cm, and 0.75 mm of thickness). Prior to PAGE separation, the fraction F2 was mixed with buffer-3 (60% glycerol in 0.5 M tris-HCl, pH = 6.8) (2:1, v/v). The mixture (40 µl) was loaded onto the top of gel plate and a BenchMark standard (Invigrogen) was parallelly loaded on the same gel plate as molecular weight markers. For comparison purpose, the extract from spinach leaves using the same procedure was also loaded on the same gel plate. Electrophoresis was carried out at 4°C and in dark with a 2 mA current for 40 min and then with a 4 mA current for 2 hours. The gel running buffer was made of 0.3% tris-base, 1.44% glycine and 0.05% sodium deoxycholate. To make the separated components visible, a Commassie Brilliant Blue dye (R-250) was used to stain the proteins and peptides in the gel.

To determine the pigment concentrations in various structural pools from the incubated samples, 1 ml mixture of F2 with buffer-3 (2:1, v/v) was loaded onto a large gel plate (15×25 cm, and 0.75 mm of thickness). Electrophoresis was conducted at a 2 mA current for 3 hrs and then a 4 mA current for 7 hrs respectively. After the separation was achieved, all green and green-yellow bands were separately cut off from the gel and ground in 1 ml water by

homogenizer. Then, acetone was added to extract pigments twice (2×5 ml). Each extract was centrifuged to separate pigments from the ground gel and then combined for HPLC analysis. The extraction and separation of pigment components, including total pigment (direct acetone-extracted) and various structural pools were schematically summarized in Fig. 2.1.

Pigment Analysis. Pigment concentrations in the extracts from all fractions and separated bands were determined by ion-pairing reverse-phase high-performance liquid chromatography (Mantoura and Llewellyn, 1983). The HPLC system consisted of a Hewlett Packard 1100 series with a quaternary pump and a variable wavelength detector. Column used was a 5 μm C-18 (ODS) Alltech column (250×4.6 mm i.d.) and detection was accomplished by measuring absorbance at a wavelength of 440 nm. After injection of 100 μl extract sample, a gradient program ramped from 100% eluant A (80% methanol and 20% aqueous solution of 0.5 mM tetrabutyl ammonium acetate and 10 mM ammonium acetate) to 100% eluant B (20% acetone in methanol) in 10 min with a hold for 30 min. Authentic Chl-a standard was obtained from Sigma Chemical Company. The Ppt-a standard was prepared by acidification of Chl-a standard. The Ppb-a standard was made from *Skeletonema costatum* cells according to the procedure used by Barrett and Jeffrey (1971). All standards were quantified spectrophotometrically (Shimadzu UV-2501 PC spectrophotometer) using published extinction coefficients (Mantoura and Liewellyn, 1983). To identify chloropigments in the incubation samples, three standards were co-injected with sample extracts in HPLC.

Results

Variations of Bacteria-Specific Fatty Acids and Bacterial Abundance. Three typical bacteria-specific fatty acids (iso-15:0, antesio-15:0 and 18:1(ω7)) were detected but they varied differently in oxic and anoxic systems during the incubation (Fig. 2.2). In the oxic system, little iso-15:0 and anteiso-15:0 fatty acids were detected throughout the incubation period while initial concentration of 18:1(ω7) fatty acid was relatively high but decreased continuously over the incubation. In the anoxic system, the concentrations of iso-15:0 and anteios-15:0 fatty acids declined continuously from the beginning of the incubation but there was a small amount of iso-15:0 survived after three months. By contrast, the concentration of 18:1(ω7) fatty acid increased slightly in the first a few days and then decreased. However, a significant proportion of 18:1(ω7) (40-50% of initial concentration) was remained stable after one-month under both oxic and anoxic conditions. Unlike the variations in bacteria-specific fatty acids, bacterial abundances in oxic and anoxic seawaters increased dramatically in the first week of incubation. After one week, the bacterial abundance decreased gradually to the initial levels (less than 109/L). It appeared that the bacterial abundance in anoxic seawater increased to a relatively higher level than in oxic seawater.

Characterization of Soluble Pigment Components by PAGE. To identify soluble pigment components, we run the protein standard (BenchMark) and the sample extracts (soluble fraction) from *E. huxleyi* cells and spinach leaves on the same PAGE plate. After separation and staining, six colored bands occurred in two sample channels and a series of proteins and polypeptides in the standard channel (Fig. 2.3). One narrow green band from the samples remained at the initial loading position (in the stacking gel) and thus defined as non-moving

band. Other five green and green-yellow bands with variable width were well separated in the separation gel. By comparing with BenchMark standard, the top narrow green band in the separation gel had a molecular weight close to 160 kDa. The next wide green band had a molecular weight between 55 kDa and 60 kDa. Other two wide bands had green-yellow color and their molecular weights were relatively small (~30 kDa and ~25 kDa respectively). One narrow green band in the bottom of the gel had a molecular weight far below 10 kDa. Based on the literature (Picaud et al., 1982; Green and Durnford, 1996; Croce et al., 2002; Jackowski et al., 2003), the bands in the separation gel were characterized as CP-I (~160 kDa), CP43+CP47 (~55-60 kDa), LHC-II (~30 kDa) and LHC-I (~25 kDa), which are known as core chlorophyll-binding complexes and antenna complexes in PS-I and PS-II photosystems. The bottom band is considered as free pigments due to their low molecular weights while the structural characterization of the band in the stacking gel (non-moving) is unknown.

Recovery of Chl-a from Various Fractions and Complexes. To test the recovery of Chl-a from various fractions and complexes during extraction and separation processes, we treated two identical cell samples as a duplicate measurement. Total Chl-a concentration was determined from direct acetone extraction of the cells. The Chl-a concentrations in three fractions (F1, supernatant 1; F2, supernatant 2; and F3, residue) were determined after extracting Chl-a from these fractions with acetone. The results showed that the recovery of Chl-a from three fractions was close to 100% (Table 2.1). The majority of Chl-a was present in F2 (60%) and F3 (37%) fractions while only <3% of Chl-a was in F1 fraction. Thus, the slight loss of Chl-a in F1 fraction was not considered in our estimation for degradation rate constants.

When the soluble pigment components in F2 fraction were further separated by the PAGE, the recovery of Chl-a from all bands reached to ~98% (Table 1) of the Chl-a in the F2.

However, the distribution of Chl-a in these bands was not uniform: a maximum percentage (36%) of soluble Chl-a bound to B3 band (CP43+CP47) while a minimum percentage (~7%) in B2 band (CP-I). Two LCH complexes (B4 and B5) had 17-19% of soluble Chl-a while approximately 9-11% were in non-moving band (B1) and free pigment band (B6) respectively.

Variations of Total Chloropigments. Concentrations of acetone extracted chloropigments varied differently during three-month incubation (Fig. 2.4). Chl-a degraded continuously in oxic and anoxic seawaters, but the initial degradation was fast in the first 10-20 days and followed by a slow degradation. There were differences in initial rate and remaining proportion between oxic and anoxic degradations of Chl-a: an apparently faster initial rate occurred in oxic seawater but a relatively larger proportion of Chl-a remained in anoxic seawater after three months. Variations of phaeopigments showed different patterns from Chl-a. In the first 10-20 days, the concentrations of Ppt-a increased slightly in both oxic and anoxic seawaters while the concentrations of Ppb-a increased apparently only in anoxic seawater. After that, the concentrations of these phaeopigments all decreased with time but relatively larger proportions remained in anoxic seawater than in oxic seawater.

Variations of Chloropigments in Soluble and Insoluble Fractions. Chl-a in soluble and insoluble fractions (F2 and F3) degraded continuously with time (Fig. 2.5). However, Chl-a in soluble fraction appeared to degrade much faster than that in insoluble fraction. There were also apparent differences between oxic and anoxic degradations in both soluble and insoluble fractions. After three-month incubation, soluble Chl-a was almost completely degraded in oxic seawater while approximately 20% of initial soluble Chl-a remained in anoxic seawater. By contrast, a large proportion (>50% relative to the initial concentrations) of insoluble Chl-a remained in both oxic and anoxic seawaters after three months. Two phaeopigments (Ppb-a and

Ppt-a) in the soluble and insoluble fractions followed the similar patterns to those observed in the total acetone extracted pool, but an exception (a small initial increase) was found for insoluble Ppb-a in oxic seawater (Fig. 2.5).

Variations of Chloropigments in Soluble Complex Pools. The concentrations of Chl-a in each pool declined continuously during incubation (Fig. 2.6). The Chl-a degradation in these structural components basically followed the same patterns as those observed in total acetone extracted pool and soluble fraction, that is, initial faster decrease followed by slower decrease. However, the differences between oxic and anoxic degradations varied with different pools. For example, a large difference between oxic and anoxic degradation was seen in the non-moving band while a small difference observed in CP-I band. In the most cases, Chl-a in the complexes degraded more completely in oxic seawater while there was always a proportion of Chl-a remained in anoxic seawater after three months. On the other hand, variations of Ppb-a in these soluble structural pools showed different patterns (Fig. 2.7). For example, in the LHC-I and LHC-II pools (polypeptide complexes), concentrations of Ppb-a initially increased in the first week and then gradually decreased. By contrast, in CP-I and CP43+CP47 pools (protein complexes), Ppb-a concentrations decreased from the beginning of the incubation. Continuous decrease trend of Ppb-a concentration was also observed in the non-moving band (both oxic and anoxic) and the free pigment pool although a slight increase of Ppb-a was observed in the free pool under anoxic conditions.

Discussion

Variations of Microbial Communities in the Incubation Systems. Different bacteria communities inhabit in the stratified oxic and anoxic seawaters in the Cariaco Basin (Tuttle et

al., 1977; Taylor et al., 2001). There have been intensive studies (Madrid et al., 2001; Neretin et al., 2003; Vetriani et al., 2003) demonstrating that specific microbial activities of different bacteria along the redox gradient in natural water column (e.g., the Cariaco Basin and the Black Sea) are responsible for different biochemical degradation of organic matter. The bacterial communities in oxic/anoxic water column have been characterized by several approaches, including lipid biomarkers (Saliot et al., 2002; Wakeham et al., 1995, 2004). It has been confirmed that aerobic and anaerobic bacteria have distinct fatty acid compositions (Parkes and Taylor, 1983; Keith-Roach et al., 2002). In this study, our experimental systems consisted of natural oxic and anoxic seawaters from the Cariaco water column and pure culture of *E. huxleyi* (bacteria free). The natural seawaters were unfiltered and used for the experiment in 10 days after the sampling. Thus, the bacterial communities in our experimental systems are generally assumed to be similar to those in natural environments. Our lipid analysis indeed showed the differences in bacteria-specific fatty acids between the oxic and anoxic systems (Fig. 2.3): little branched iso- and anteiso-15:0 fatty acids were found in the oxic system, contrasting to the anoxic system. This observation for the different fatty acid compositions between the experimental oxic and anoxic systems was consistent with the measurement for field samples (Wakeham and Ertel, 1987).

On the other hand, bacterial abundances in two systems varied in a similar way: initial increase in the first week and followed by a continuous decrease but greater variation was found in the anoxic system (Fig. 2.3). The increase of bacterial abundance in both systems was attributed to the addition of algal materials. It was demonstrated that introduction of fresh organic matter could stimulate bacterial growth (Danovaro et al., 1994; Zweifel et al., 1996), but the bacterial abundance declined when the most bioavailable materials were consumed (Harvey

et al., 1997; Puddu et al., 1998). Although the bacteria-specific fatty acids and bacterial abundance have been often used to assess bacterial biomass (Cranwell et al., 1987; Wakeham and Beier, 1991), their variations in our experimental systems were not correlated (Fig. 2.3). The inconsistence between bacteria-specific fatty acids and bacterial abundance (or biomass) was observed in other seawater and sediment systems (Harvey and Macko, 1997; Ding and Sun, 2004). However, the variations of the bacteria-specific fatty acids and bacterial abundance in the oxic and anoxic systems implied that different microbial processes might be involved in the degradation of cellular chloropigments.

Oxic vs. Anoxic Degradation of Chl-a in Various Fractions and Pools. During three-month incubation, the concentrations of total Chl-a (direct acetone extracted), soluble and insoluble fractions, and different soluble complex pools decreased rapidly in the first 10-20 days and then followed by a continuous slow decrease. This degradation pattern has been quantitatively described by a well-known multi-G model (Berner, 1980). In this model, different fractions of organic matter are assumed to have different reactivities and degrade at independent rates. The sum of each individual degradation rate equals to the overall organic matter degradation rate, as shown by the following equations:

$$-\frac{dG_t}{dt} = \sum k_i G_i \qquad (1)$$

$$G_t = \sum G_i = (G_1)_0 \exp(-k_1 t) + (G_2)_0 \exp(-k_2 t) + \ldots \qquad (2)$$

where, k_i is the first-order degradation rate constant of fraction i; G_i is the concentration of fraction i and $-dG_t/dt$ is the degradation rate of all fractions; $(G_1)_0$ and $(G_2)_0$ are initial concentrations of fraction 1 and fraction 2.

The validity of multi-G model was confirmed by testing natural organic matter degradation in natural seawater and sediment systems (Westrich and Berner, 1984). This model has been extended to studies of specific organic compounds, which are bound in different fractions of organic matter (Henrichs and Doyle, 1986; Sun and Wakeham, 1994; Teece et al., 1998). In the present study, intracellular Chl-a was bound in different structural components, as shown by our separation schemes (Fig. 2.1). Thus, Chl-a in these associations may have variable reactivities, resulting in differernt kinetic features during the degradation processes. Based on the G-model, we calculated the oxic and anoxic degradation rate constants of Chl-a in total acetone pool, soluble and insoluble fractions, and all complex pools. However, the relative pool sizes varied largely with fractions and redox conditions. In order to compare the Chl-a degradation rate constants between oxic and anoxic conditions no matter how pool sizes varied, we used a new parameter, $k_{av,}$ defined as the average degradation rate constant of two different pools:

$$k_{av} = k_1 \times f_1 + k_2 \times f_2 \qquad (3)$$

where, f_1 and f_2 are relative proportions of pool 1 and pool 2 ($f_1 = (G_1)_0/(G_t)_0$ and $f_2 = (G_2)_0/(G_t)_0$).

Comparison of the Chl-a degradation rate constants (Table 2.2) showed several interesting implications. First, the k_{ox}/k_{an} ratio of total Chl-a was approximately 4.0 but the ratios in various fractions and complexes ranged from ~2 to 9 (lower or higher than that of total Chl-a). This result implies that oxygen effects on Chl-a degradation are different among the various structural components. Second, Chl-a in soluble fraction degraded much faster (> 10x) than that in insoluble fraction under both oxic and anoxic conditions. This suggests that physical and chemical properties of the major structural fractions control the reactivity of Chl-a bound in them. Third, the oxic degradation rate constants of Chl-a complexes in PS-II photosystem

(LHC-II and CP43+CP47) were apparently (~2x) higher than those in PS-I photosystem (LHC-I and CP-I) while anoxic degradation rate constants of Chl-a complexes in two photosystems were almost the same. This indicates that two photosystems have different responses to oxic degradation processes but there are no different responses to anoxic degradation processes between them. Fourth, in the same photosystem, LHCs seemed to degrade somewhat faster than CP complexes under oxic conditions. This reflects that the binding features of polypeptides or proteins in these complexes affect Chl-a degradation in the complexes. And fifth, the oxic and anoxic degradation rate constants of Chl-a in free pigment pool were the smallest in almost all pools (except the case in anoxic non-moving pool). These rate constants may be underestimated because the decrease of free Chl-a concentration in the free pool is probably a net balance between addition of free Chl-a from other pools and its degradation within the pool.

Previous studies have demonstrated that Chl-a is a labile organic compound with a turnover time ranging from 10 to 100 days in different environments (Leavitt and Carpenter, 1990; Bianchi and Findlay, 1991; Sun et al., 1993a, b; Gerino et al., 1998; Bianchi et al., 2000). From this study, total cellular Chl-a turned over in approximately 6 days in oxic seawater and 24 days in anoxic seawater. This time scale is consistent with the estimation (44 turnovers per year) of global Chl-a degradation in the oceans (Whittaker, 1975). When intracellular Chl-a molecules were separated into different fractions, we could see that structural associations of Chl-a within the cells affected Chl-a reactivities and the responses to different redox conditions.

Relative Contributions of Various Pools to Total Chl-a Degradation. Degradation rate of Chl-a generally depends on the reactivity (*k*, degradation rate constant) and the pool size (G, concentration) when the first-order kinetics is followed (Sun et al., 1993a, b; Ahmed et al., 2002; Josefson et al., 2002). As shown by the estimates from the multi-G model, the degradation rate

constants of intracellular Chl-a varied with fractions and redox conditions (Table 2.2). In order to assess the relative contributions of each pool to the total degradation rate of Chl-a, we calculated the relative degradation rates of Chl-a in various fractions and complex pools by multiplying the degradation rate constants (k) with the relative pool sizes (concentration percentages relative to the total) (Table 2.3). In general, sum of relative degradation rates from two major fractions (soluble and insoluble) were close to the total degradation rates estimated from the total acetone extracted Chl-a. Although the relative size of insoluble fraction accounted for ~40% of the total Chl-a, the contributions to oxic and anoxic degradation rates of total Chl-a were very small (below 7%). The major cause for this low contribution is due to the high resistance of insoluble Chl-a to degradation processes. However, it is unclear what are structural configurations of insoluble Chl-a components within the cells and why they are refractory for oxic and anoxic degradations.

On the other hand, relative contributions of various Chl-a complexes to the oxic and anoxic degradation rates of soluble Chl-a fraction varied from 2% to 39% (Table 2.3). The largest contributions (36-39%) were from the CP43+CP47 complex pool in both oxic and anoxic systems. The reason for the largest contributions from the CP43+CP47 pool is because of higher pool size and higher reactivity. The contributions from LHC-I and LHC-II pools were moderate (from 18% to 26%). Under oxic conditions, the relative contribution from LHC-II was larger than that from LHC-I since the reactivity of Chl-a in LHC-II was ~1.5X larger than that in LHC-I although the relative pool size of LHC-II was somewhat smaller than that of LHC-I. In contrast, under anoxic conditions, the reactivities of Chl-a in both LHC-I and LHC-II were similar. Thus, the relative contributions from LHC-I and LHC-II depended more on the relative pool size, that is, higher contribution was from the LHC-I with a larger pool size. Other three

pools, CP-I, non-moving, and free pigment each contributed less than 7% of total degradation in the oxic system but their contributions ranged from 2% to 11% in the anoxic system. The lower contributions of three pools were presumably caused by lower pool sizes. Sums of relative rate contributions from six soluble sub-pools were well balanced with the rates from the soluble fraction in both oxic and anoxic systems. From these calculations, it is clear that the degradation of intracellular Chl-a was controlled by soluble components while the dominant contributions were from protein and polypeptide complexes (CP43+CP47, LHC-I and LHC-II).

Effects of Intracellular Structural Associations on Chl-a Degradation. Our experimental results showed that Chl-a complexes in PS-II had higher reactivities (larger k) than those in PS-I under oxic conditions. The difference in the reactivity of Chl-a complexes may be attributed to different structural configurations, functions and involved reactions between two photosystems. For example, PS-II performs a series of photochemical reactions to oxidize water during photosynthesis (Barber, 1998; Kraub, 2003) and a variety of toxic oxygen species such as singlet oxygen are generated simultaneously (Jung, 2004). To keep its stability and good performance, the PS-II system produces some antioxidants (e.g., carotenoids) and antioxidant enzymes (e.g., catalase and superoxide) or replaces damaged proteins with new synthesized compounds (Barber, 1998; Brown, 1987; Tracewell et al., 2001; Jung, 2004). However, when the cells die, the PS-II stops to produce antioxidants, antioxidant enzymes and new proteins. As a consequence, the PS-II is unable to protect itself anymore and the Chl-a complexes (LCH-II and CP43+CP47) in this photosystem may be easily attacked by oxygen species. Unlike PS-II, PS-I has a series of non-chlorophyll-bound proteins such as BtpA, PS-I-F, I, J and K, which surround and stabilize the Chl-a complexes even after the death of the cells (Zak and Pakrasi, 2000; Scheller et al., 2001; Chitnis, 2001; Jensen et al., 2003). Therefore, protection of Chl-a

complexes by other proteins in the PS-I may slow down the degradation of Chl-a bound in this system. However, all Chl-a complexes in both PS-I and PS-II had similar reactivities under anoxic conditions although they were less reactive than those under oxic conditions. At the present, it remains unknown how anoxic processes affect degradation of Chl-a complexes despite we observed a greater variation of bacterial abundance in the anoxic system.

Meanwhile, the differences in molecular size, structural binding mode, and location of various Chl-a complexes within the photsystems would be other reasons causing different degradation behaviors. The protein complex CP-I has a larger molecular size (160 kDa) than CP43+CP47 (~60 kDa) while the LHC-I and LHC-II are small polypeptide complexes with molecular weights of 25-30 kDa (Barber et al., 1998, 2000; Fromme et al., 2001; Germano et al., 2002; Jackowski et al., 2002). The CP complexes, as core proteins, usually locate in the center of the photosytems and are surrounded by many other proteins and LHC complexes (Barber et al., 1998, 2002; Ben-Shem et al., 2003; Bibby et al., 2003; Jensen et al., 2003). On the other hand, the CP proteins and LHC polypeptides have different number of α helices, which play a strong role in protecting Chl-a in these complex structures (Barber et al., 2000; Fromme et al., 2001; Tetenkin, 2003; Ben-Shem et al., 2003). The largest Chl-a protein complex (CP-I) in the PS-I generally has 13 helices (Green and Durnford, 1996; Fromme et al., 2001). In the PS-II, two CP complexes (CP43 and CP47) have similar structures and each possesses 6 helices (Barber et al., 2000, Bumba and Vácha, 2003). The LHC-I and LHC-II polypeptide complexes in the PS-I and PS-II photosystems have 3 helices for each (Green and Durnfold, 1996; Croce et al., 2002; Germano et al., 2002). Combination of the large molecular size, central location and surrounding by other molecules, and more helices in CP-I complex may provide an advantage for protecting the complex structure. By contrast, the Chl-a in LHC-I and LHC-II polypeptide

complexes may be more easily degraded due to their small sizes, less helices, and edge locations in the photosytems.

Possible Degradation Pathways of Chl-a in Different Intracellular Structural Pools. Algal Chl-a can be degraded by various biological and biochemical processes in nature (SooHoo and Kiefer, 1982; Welschmeyer and Lorenzen, 1985; Carpenter et al., 1986; Furlong and Carpenter, 1988; Louda et al., 1998). The primary degradation pathway of Chl-a involves production of several phaeopigments (Hendry et al., 1987). For example, Chl-a is altered to Ppt-a through loss of magnesium, to chlorophyllide-a through loss of phytol, and to Ppb-a through loss of both magnesium and phytol. In this study, we detected Ppt-a and Ppb-a as major phaeopigments in different intracellular fractions and complex pools, but the variations of Ppb-a were much greater and more closely related to Chl-a degradation than Ppt-a. Thus, we modeled the variations of Ppb-a in different pools to examine the possible degradation pathways of various Chl-a complexes.

During oxic and anoxic incubations, Ppb-a concentrations in different fractions and pools varied in two different patterns: (1) continuous decrease from the beginning of the incubations; and (2) initial increase in the first 10 days and followed by a continuous decrease over subsequent period. The first pattern was clearly observed in the CP complex pools and non-moving pool while the second pattern occurred in LHC complex pools and in insoluble fraction under both oxic and anoxic conditions. However, greater initial increases of Ppb-a concentration were always found in anoxic seawater, resulting in a Ppb-a concentration peak in the total pigment pool (direct acetone-extracted) only in anoxic seawater (Fig. 2.4c). The initial build-up of Ppb-a concentration is likely caused by prevailing production of Ppb-a over degradation

within some pools while continuous decrease of Ppb-a in other pools may be due to a lack of Ppb-a production.

Based on these results, we proposed a conceptual model to describe different degradation pathways of Chl-a in various structural pools (Fig. 2.8). The reverse correlation between initial Ppb-a increase and Chl-a decrease within the LHC pools and insoluble fraction indicates an internal degradation pathway, that is, Chl-a bound in these components might degrade inside the structural complexes remaining a link between Ppb-a and other molecular matrixes. In contrast, Chl-a molecules bound in CP complexes and in non-moving pool have to be released from the structural complexes into free pigment pool before they degrade into Ppb-a. In addition, the Ppb-a bound in LHC complexes need to be released into free pigment pool before further degradation into colorless or other small metabolites. Due to high labilities of free Chl-a and Ppb-a, both compounds will not largely accumulate in the free pool.

Sun et al. (1993a) applied a kinetic model to quantify release and degradation rates of bound and free Chl-a in sediments. In this study, we adopted this model to quantify the release and degradation rates of Ppb-a in various structural pools. Total Ppb-a concentration (C_t) is first separated into the released Ppb-a (C_a), which is from internal degradation of Chl-a, and the free Ppb-a (C_f). Within the free pool, there are two Ppb-a fractions: fast degradable C_{f1} and slow or non-degradable C_{f2}. The time-dependent variation of C_t (degradation rate) can be expressed as:

$$\frac{dC_t}{dt} = \frac{dC_a}{dt} + \frac{dC_f}{dt} = k_r C_a - k_{d1} C_{f1} - k_{d2} C_{f2} \qquad (4)$$

where, k_r is the first-order release rate constant of Ppb-a from internal Chl-a degradation; k_{d1} and k_{d2} are the first-order degradation rate constants of C_{f1} and C_{f2}. Since the k_{d2} is very small (close to 0) in the most cases, the solution of equation (4) becomes:

$$C_t = \frac{k_r (C_a)_0}{k_{d1} - k_r}[\exp(-k_r t) - \exp(-k_{d1} t)] + (C_{f1})\exp(-k_{d1} t) + (C_{f2})_0 \quad (5)$$

We assume that at $t = 0$, $(C_t) = (C_f)_0 = (C_{f1})_0 + (C_{f2})_0$; as $t \to \infty$, $(C_t) = (C_{f2})_t$; and $(C_a)_0$ is the potential concentration of Ppb-a if Chl-a in each pool is degraded to Ppb-a. Fitting the Ppb-a data from LHC pools to Equ. (5), the rate constants k_r and k_d (that is k_{d1} when $k_{d2} = 0$) can be estimated (Table 2.4). For the cases of continuous decrease in Ppb-a concentration during incubation, the degradation rate constants (Table 4) were estimated based on the Equ. (2).

Comparison of Ppb-a release and degradation rate constants between two polypeptide complexes (LHC-I and LHC-II) showed that a large difference (~3x) occurred only in oxic seawater and the rate constants were similar in anoxic seawater, which was consistent with the cases of Chl-a degradation. For protein complexes (CP-I and CP43+CP47), larger degradation rate constants (oxic and anoxic) occurred in PS-I than in PS-II. These results imply that the superstructures of photosystems may affect the production and degradation of Ppb-a, as they do for Chl-a. Moreover, chloropigments in PS-II may have a more sensitive response to oxic conditions than those in PS-I although they have similar behaviors under anoxic conditions.

Conclusion

Detailed examination of chloropigment degradation in natural oxic and anoxic seawaters reveals that the intracellular structural associations of pigments with various molecular matrixes have important influences on Chl-a degradation. Soluble protein and polypeptide complexes of Chl-a are major pools of reactive pigments, which contribute approximately 90% of overall degradation. Chl-a in the PS-II degraded generally faster than that in the PS-I, especially under oxic conditions, implying that the sizes, constitutions, and functions of these superstructural photosystems may control the fate of individual pigment compounds during biochemical

degradation processes. The structural features of various complexes also control the degradation pathways of Chl-a, leading to internal degradation of Chl-a to Ppb-a within the complex pools and external degradation after release from the complexes. In summary, the intracellular structural associations among organic compounds may be an important factor affecting degradation/preservation of organic matter in natural environments.

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Table 2.1. Initial concentrations (μg/g) of intracellular Chl-a bound in primary fractions* and following PAGE bands*. Recoveries and error ranges are estimated.

	Total Chl-a	Chl-a concentration in fractions			Sum of fractions	Recovery
	(T)	(F1)	(F2)	(F3)	(F1+F2+F3)	(F1+F2+F3)/T
Sample-I	645.84	18	391.07	227.91	636.98	98.6%
Sample-II	617.08	15.6	378.83	236.45	630.88	102.2%
Average	631.46	16.8	384.95	232.18	633.93	100.4%
(Error)	(±14.38)	(±1.2)	(±6.12)	(±4.27)	(±3.05)	(±1.80%)

	Chl-a concentration in PAGE bands						Sum of B1 to B6	Recovery
	(B1)	(B2)	(B3)	(B4)	(B5)	(B6)	(S)	(S)/(F2)
Sample-I	36.46	26.42	138.97	64.62	70.13	45.4	382	97.7%
Sample-II	33.58	27.06	129.91	62.44	72.75	43.42	369.16	97.5%
Average	35.02	26.74	134.44	63.53	71.44	44.41	375.58	97.6%
(Error)	(±1.44)	(±0.32)	(±4.53)	(±1.09)	(±1.31)	(±0.99)	(±6.42)	(±0.1%)

* Definitions of fractions and bands were shown in Fig. 2.1.

Table 2.2. Degradation rate constants (day-1) of Chl-a in total acetone extract pool, different fractions and bands, and the ratios of rate constants between oxic and anoxic conditions.

	Total Chl-a	Soluble fraction (F2)	Insoluble fraction (F3)	Non-moving (B1)	CP-I (B2)	CP43+CP47 (B3)	LHC-II (B4)	LHC-I (B5)	Free (B6)
k_{ox1}	0.27	0.328	0.055	0.261	0.248	0.415	0.445	0.302	0.173
k_{ox2}	0.006	0.022	0	0.024	0.037	0.047	0.069	0.017	0.012
f_{ox1} (%)	62.19	81.5	44.86	74.43	52.17	84.22	87.39	84.51	72.16
f_{ox2} (%)	37.81	18.5	55.14	25.57	47.83	15.78	12.61	15.85	27.84
$(k_{ox})_{av}$	0.17	0.271	0.025	0.200	0.147	0.357	0.398	0.258	0.128
k_{an1}	0.08	0.115	0.012	0.041	0.124	0.137	0.109	0.145	0.081
k_{an2}	0.004	0.009	0	0	0.015	0.012	0.004	0.004	0.005
f_{an1} (%)	49.05	73.84	42.62	54.62	70.67	82.24	93.33	74.62	71.95
f_{an2} (%)	50.95	26.16	57.38	45.36	29.33	17.76	6.67	25.38	28.05
$(k_{an})_{av}$	0.042	0.087	0.005	0.022	0.092	0.115	0.102	0.109	0.06
k_{ox}/k_{an}	4.05	3.11	5	9.09	1.6	3.1	3.9	2.37	2.13

Table 2.3. Relative degradation rate R' (concentration %/day) and relative contribution (%) of the Chl-a degradation rate in each pool to the total Chl-a degradation rate.

	Total Chl-a (T)	Soluble (F2)	Insoluble (F3)	Sum (F2+F3)	Non-moving (B1)	CP-I (B2)	CP43+CP47 (B3)	LHC-II (B4)	LHC-I (B5)	Free (B6)	Sum (B1-B6)
Chl-a %	100	52.97	40.44	93.41	4.68	6.23	16.18	9.75	11.41	5.07	53.32
R'_{ox}	0.17	0.144	0.01	0.154	0.009	0.008	0.058	0.039	0.029	0.006	0.15
(Rel. Cont. %)		(93.5)	(6.49)		(6.00)	(6.00)	(38.67)	(26.00)	(19.33)	(4.00)	
R'_{an}	0.042	0.046	0.002	0.048	0.001	0.006	0.019	0.01	0.014	0.003	0.053
(Rel. Cont. %)		(95.83)	(4.17)		(1.89)	(11.32)	(35.85)	(18.87)	(26.41)	(5.66)	

$R' = k \times$(Chl-a %).

Table 2.4. Release and degradation rate constants (day-1) of Ppb-a in various soluble structural component pools.

	Release			Degradation		
	$(k_r)_{ox}$	$(k_r)_{an}$	$(k_r)_{ox}/(k_r)_{an}$	$(k_d)_{ox}$	$(k_d)_{an}$	$(k_d)_{ox}/(k_d)_{an}$
LHC-I	0.122	0.169	0.72	0.101	0.079	1.28
LHC-II	0.325	0.18	1.81	0.31	0.081	3.83
CP-I	na	na	na	0.067	0.035	1.92
CP43+CP47	na	na	na	0.245	0.099	2.48
Free	na	na	na	0.094	0.047	2.00
Non-moving	na	na	na	0.168	0.036	4.67

na: not available.

Fig. 2.1. An overview of extraction and separation schemes for intracellular pigment complexes from *Emiliania huxleyi* cells.

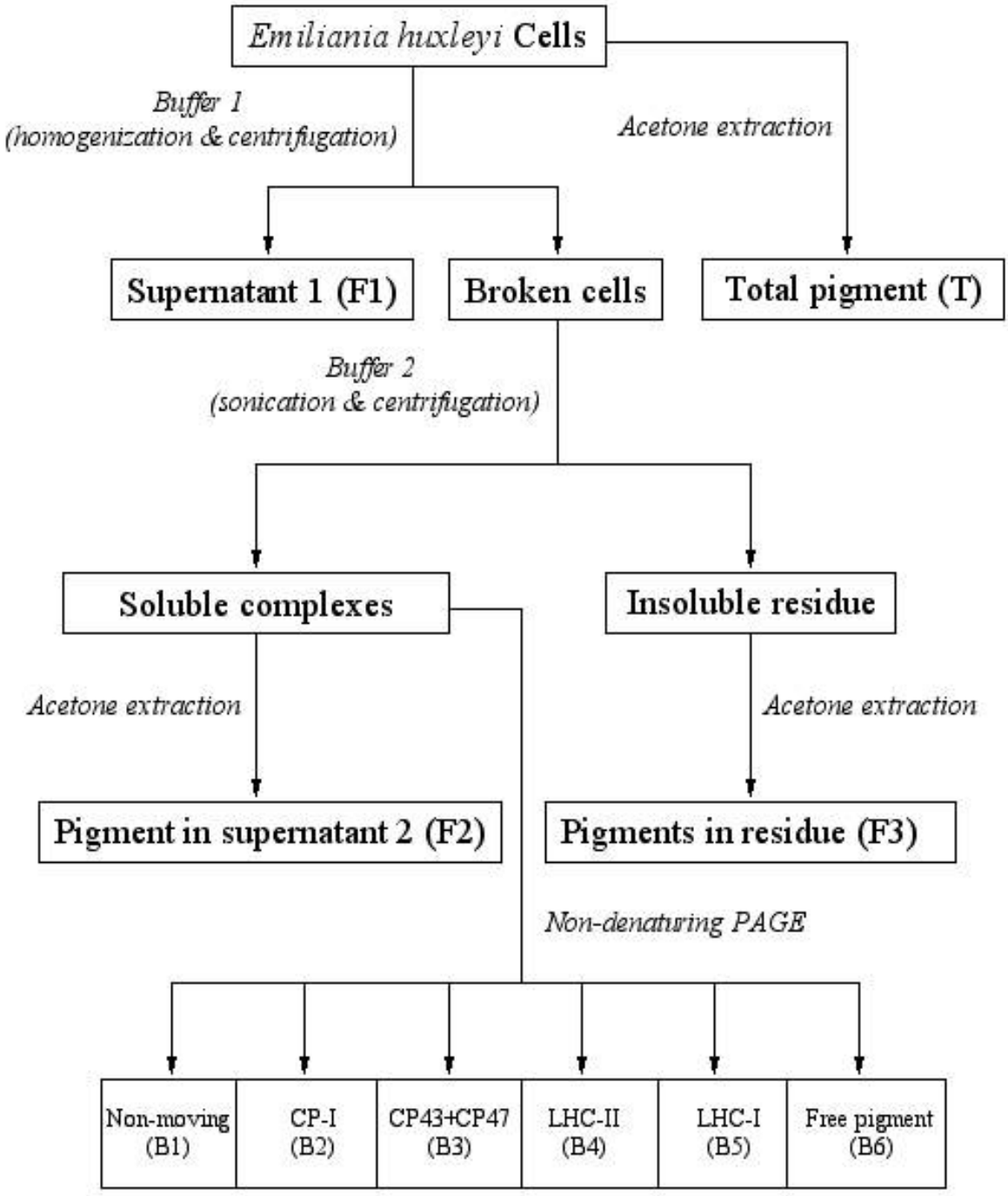
Emiliania huxleyi Cells
Buffer 1
(homogenization & centrifugation)
Acetone extraction
Supernatant 1 (F1)
Broken cells
Total pigment (T)
Buffer 2
(sonication & centrifugation)
Soluble complexes
Insoluble residue
Acetone extraction
Acetone extraction
Pigment in supernatant 2 (F2)
Pigments in residue (F3)
Non-denaturing PAGE
Non-moving (B1)
CP-I (B2)
CP43+CP47 (B3)
LHC-II (B4)
LHC-I (B5)
Free pigment (B6)

Fig. 2.1

Fig. 2.2. Variations of bacteria-specific fatty acids (iso-15:0+anteiso-15:0, and 18:1(ω7)) and bacterial abundance during incubations in oxic and anoxic seawaters.

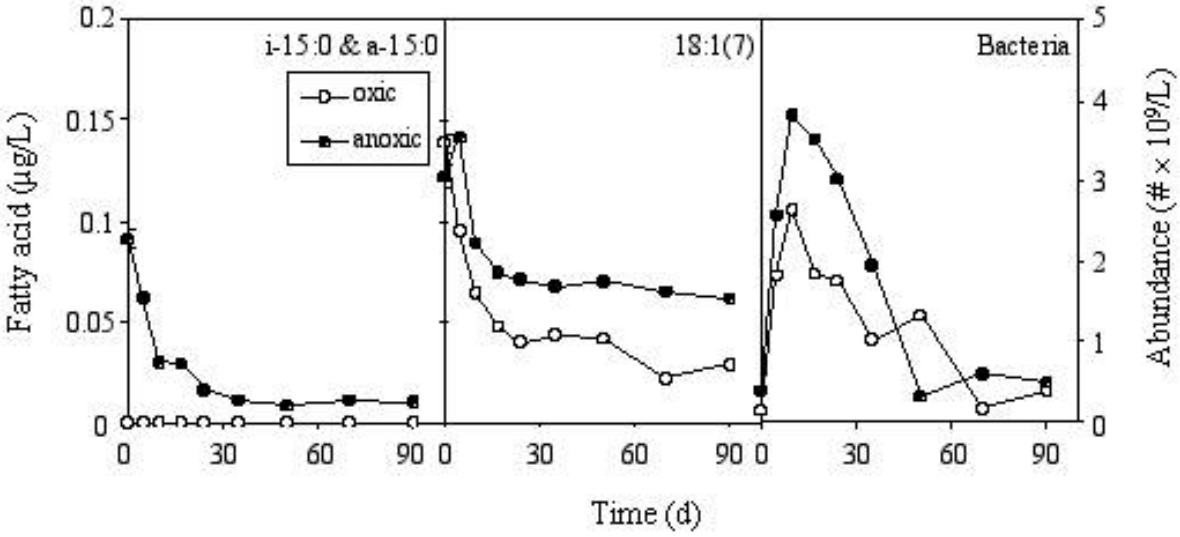
i-15:0 & a-15:0
18:1(7)
Bacteria
oxic
anoxic
Fatty acid (µg/L)
Abundance (# × 10⁹/L)
Time (d)
0.2
0.15
0.1
0.05
0
5
4
3
2
1
0
0
30
60
90

Fig. 2.2

Fig. 2.3. Characterization of soluble pigment complexes extracted from *E. huxleyi* cells and Spinach leaves in non-denaturing PAGE, comparing with the protein and polypeptide standard mixture.

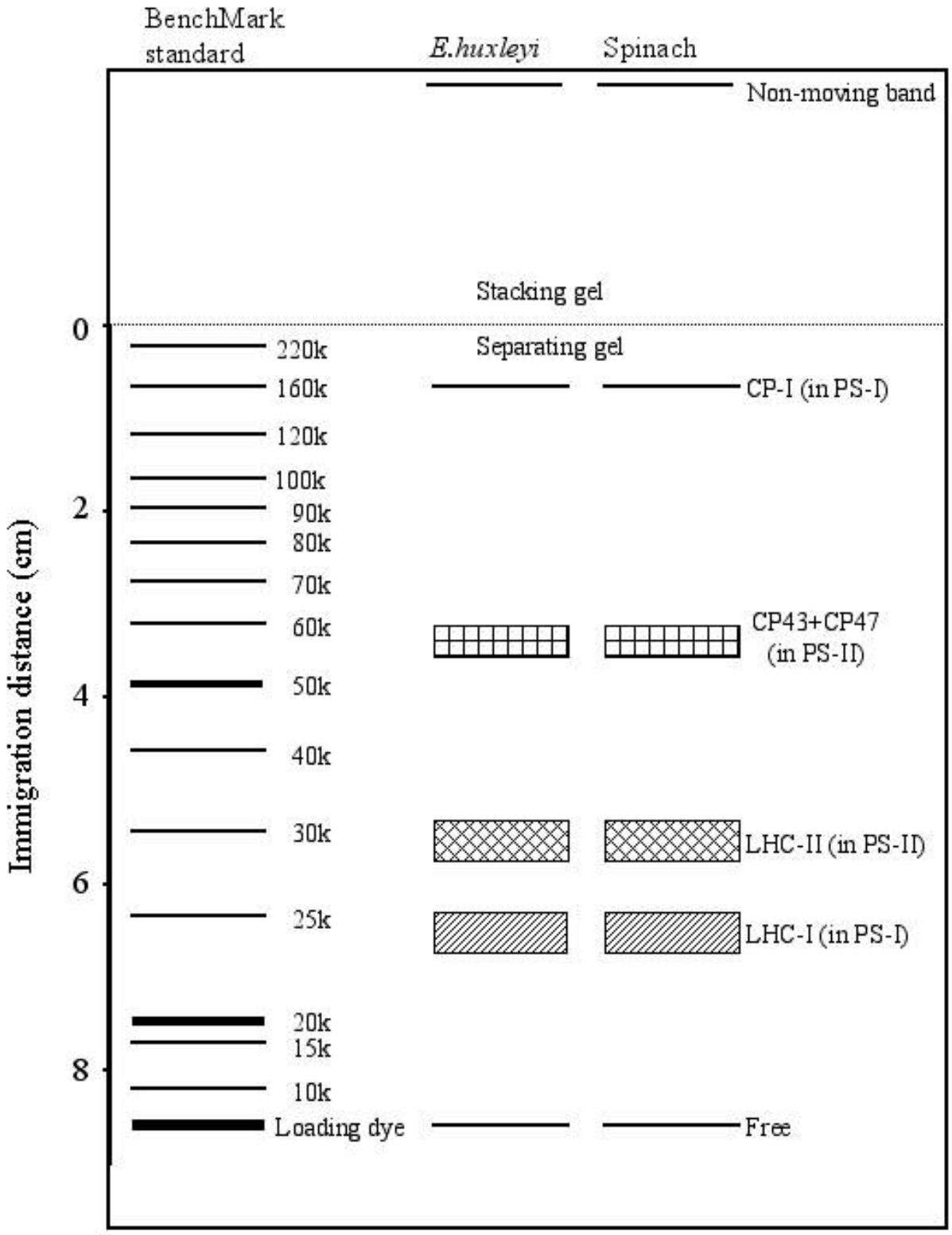
BenchMark standard
E.huxleyi
Spinach
Non-moving band
Stacking gel
Separating gel
Immigration distance (cm)
0
2
4
6
8
220k
160k
120k
100k
90k
80k
70k
60k
50k
40k
30k
25k
20k
15k
10k
Loading dye
CP-I (in PS-I)
CP43+CP47 (in PS-II)
LHC-II (in PS-II)
LHC-I (in PS-I)
Free

Fig. 2.3

Fig. 2.4. Variations of total acetone-extracted Chl-a, Ppt-a and Ppb-a concentrations during incubations in oxic and anoxic seawaters.

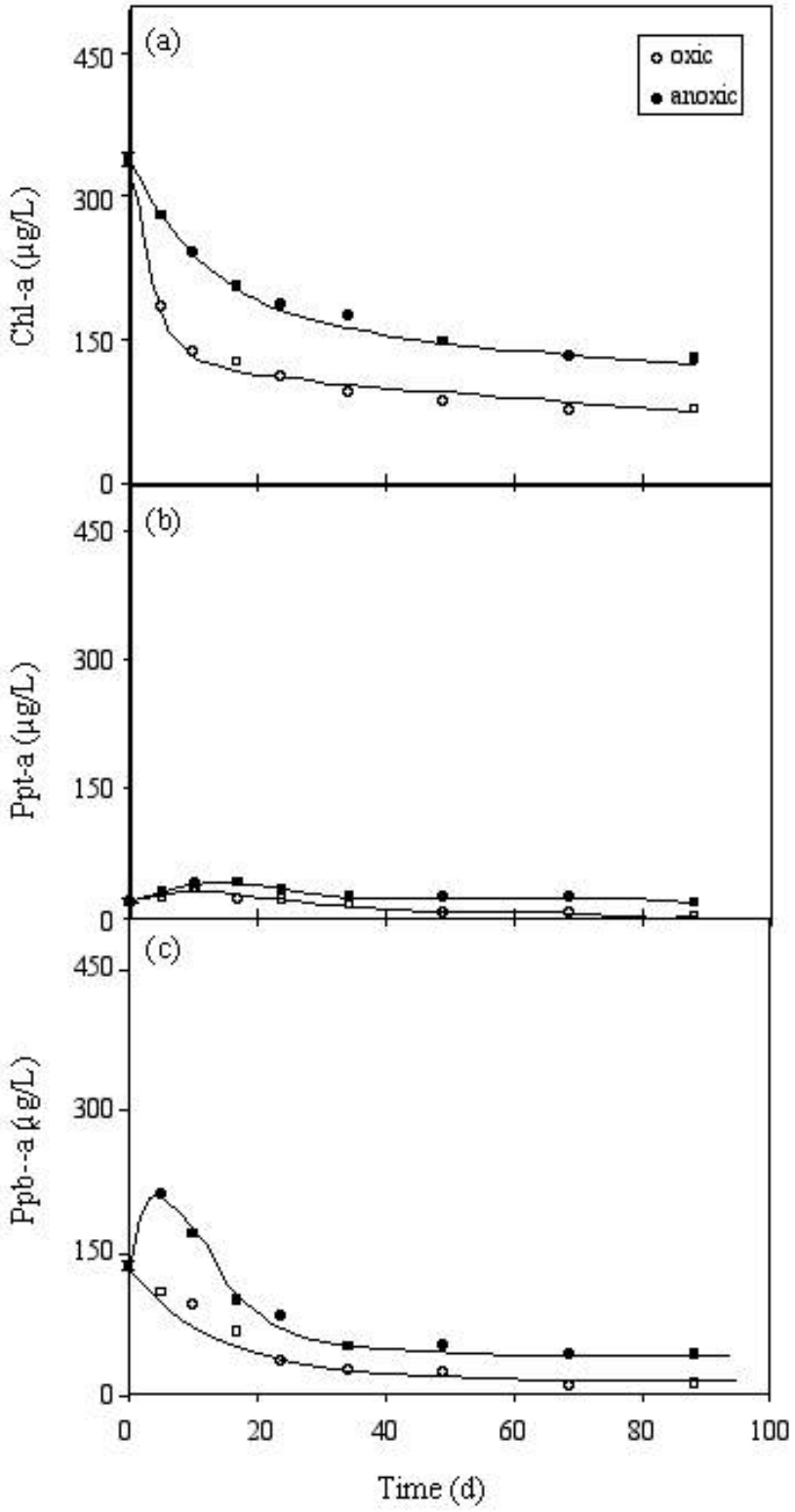
(a)
oxic
anoxic
Chl-a (µg/L)
450
300
150
0
(b)
Ppt-a (µg/L)
450
300
150
0
(c)
Ppb--a (µg/L)
450
300
150
0
0
20
40
60
80
100
Time (d)

Fig. 2.4.

Fig. 2.5. Variations of Chl-a, Ppt-a and Ppb-a concentrations in soluble and insoluble fractions during incubations in oxic and anoxic seawaters.

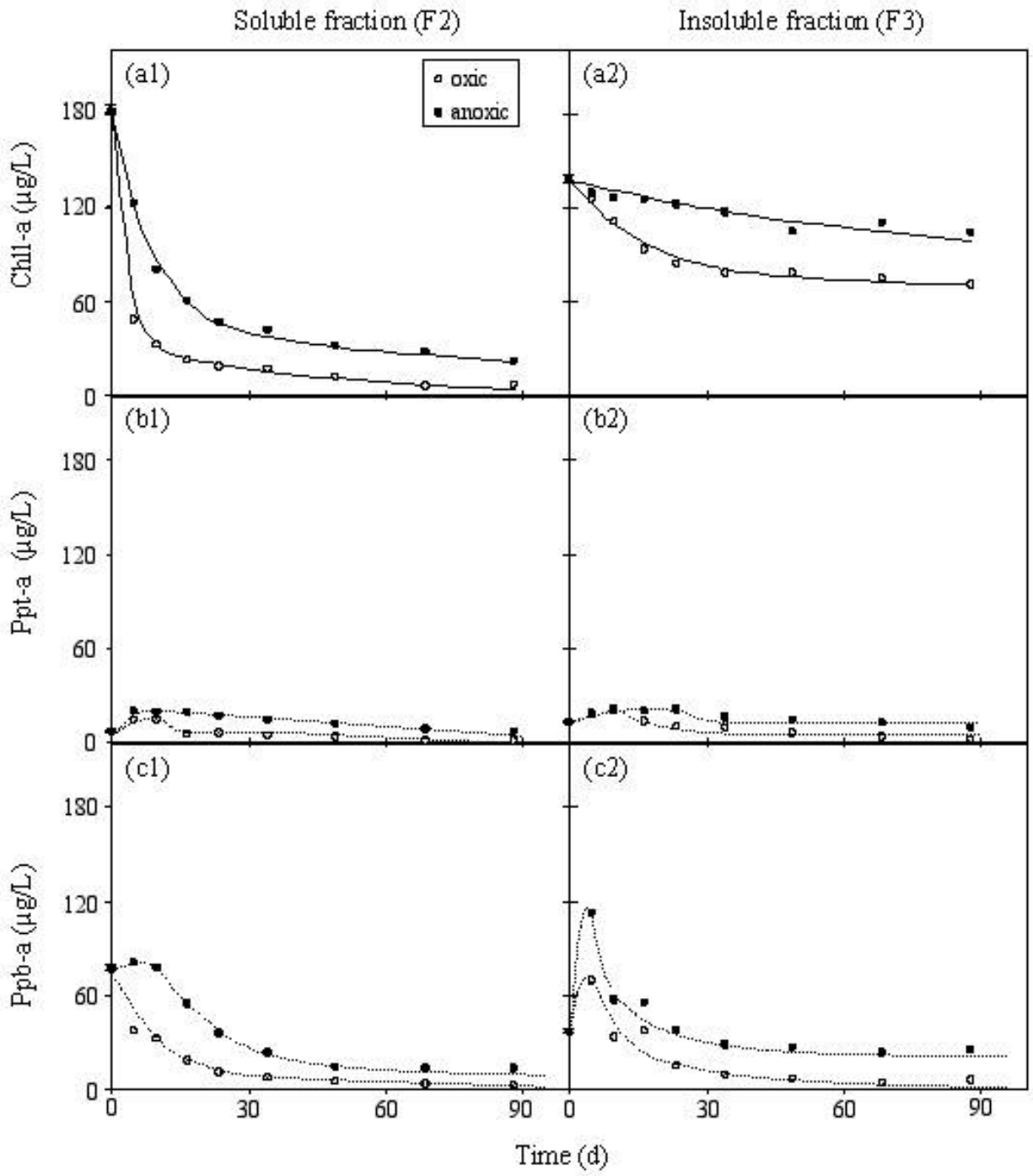
Soluble fraction (F2)
Insoluble fraction (F3)
(a1)
(a2)
(b1)
(b2)
(c1)
(c2)
oxic
anoxic
Chl1-a (µg/L)
Ppt-a (µg/L)
Ppb-a (µg/L)
0
60
120
180
0
30
60
90
Time (d)

Fig. 2.5.

Fig. 2.6. Variations of Chl-a concentrations in various soluble structural component pools during incubations in oxic and anoxic seawaters.

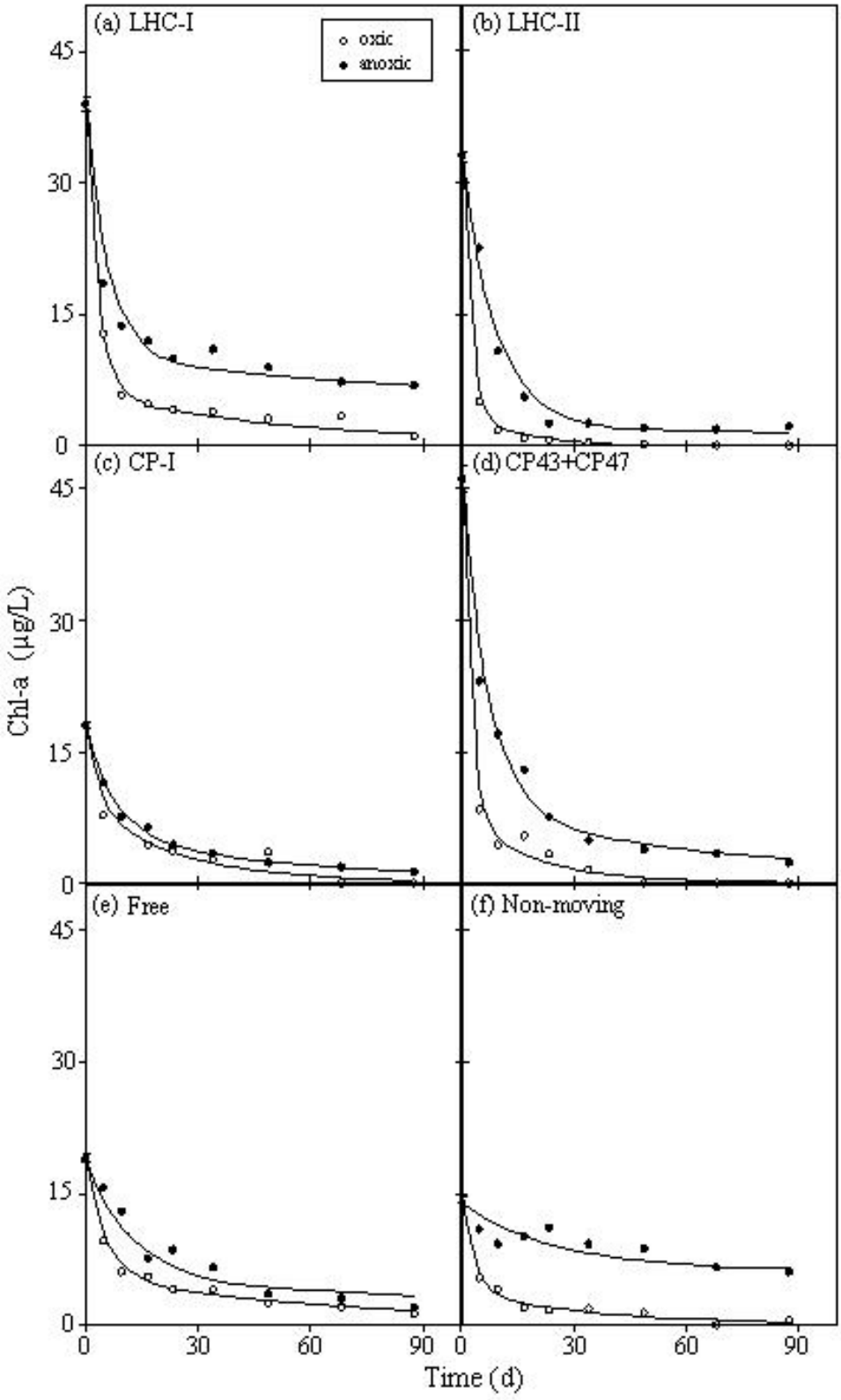
(a) LHC-I
oxic
anoxic
(b) LHC-II
(c) CP-I
(d) CP43+CP47
(e) Free
(f) Non-moving
Chl-a (μg/L)
Time (d)
0
15
30
45
0
30
60
90

Fig. 2.6.

Fig. 2.7. Variations of Ppb-a concentrations in various soluble structural component pools during incubations in oxic and anoxic seawaters.

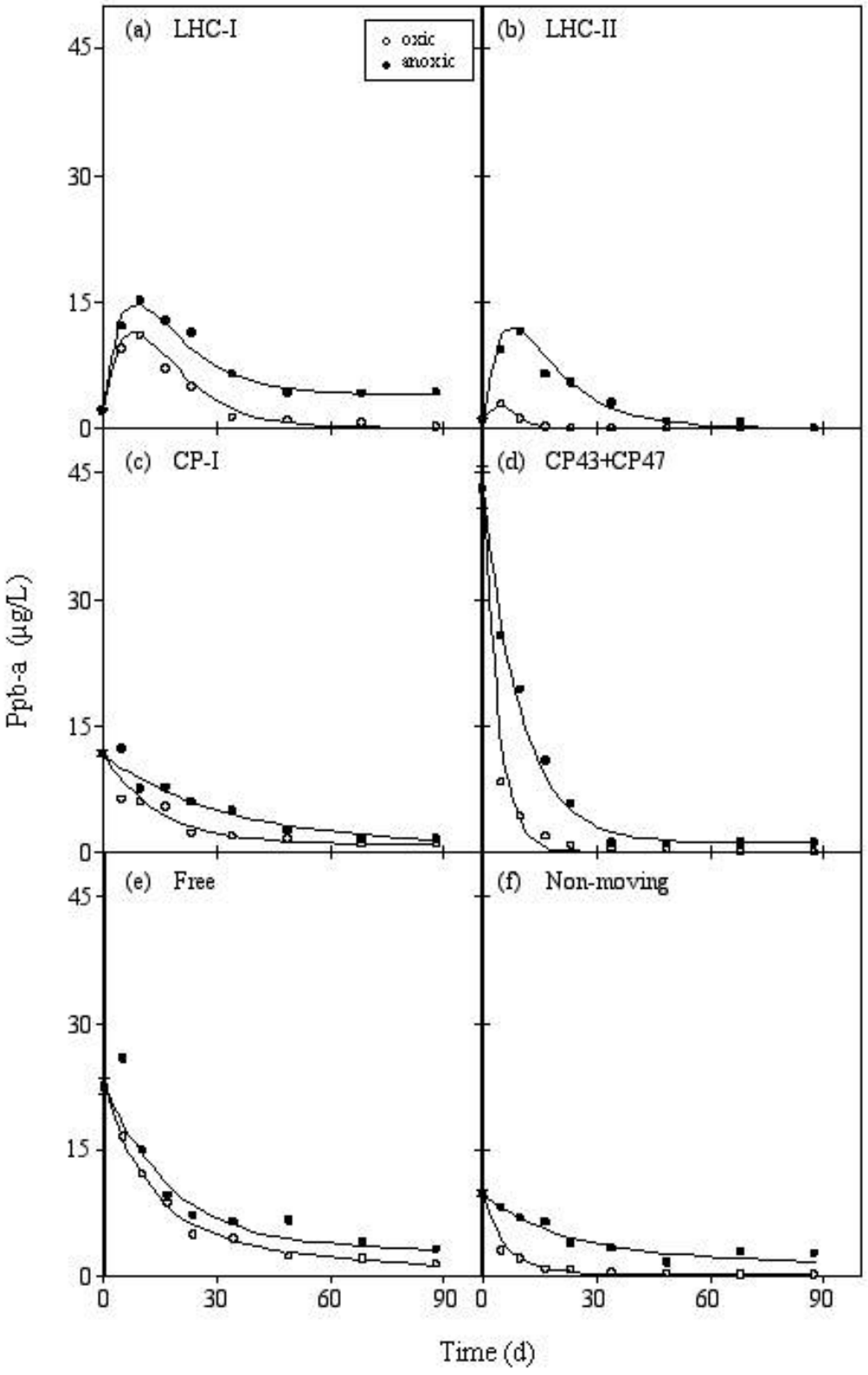
(a) LHC-I
oxic
anoxic
(b) LHC-II
(c) CP-I
(d) CP43+CP47
(e) Free
(f) Non-moving
Ppb-a (µg/L)
Time (d)
0
15
30
45
0
30
60
90

Fig. 2.7.

Fig. 2.8. Possible degradation pathways of chloropigments bound in various structural components of *E. hyxleyi* cells.

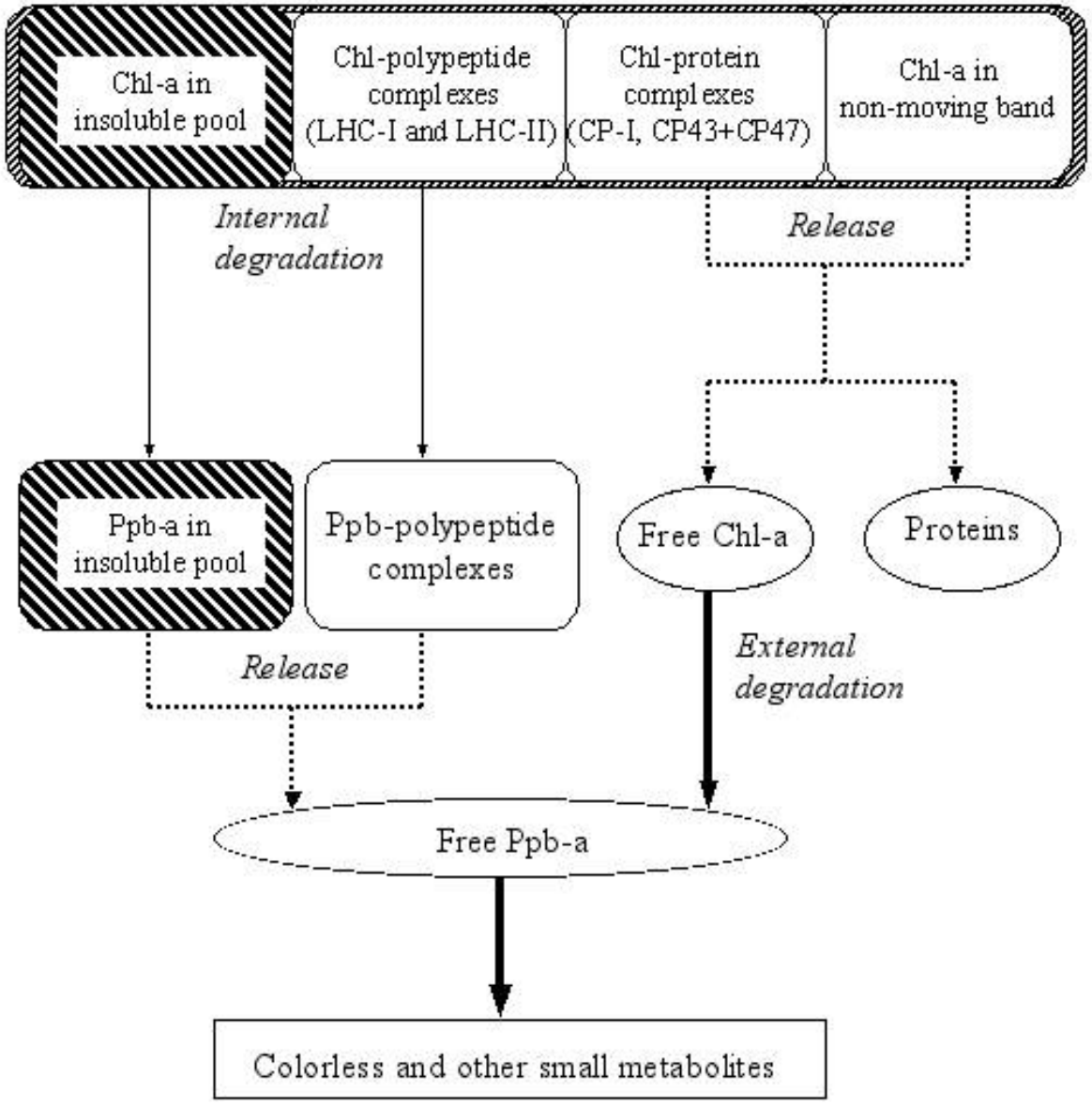
Chl-a in insoluble pool
Chl-polypeptide complexes (LHC-I and LHC-II)
Chl-protein complexes (CP-I, CP43+CP47)
Chl-a in non-moving band
Internal degradation
Release
Ppb-a in insoluble pool
Ppb-polypeptide complexes
Free Chl-a
Proteins
Release
External degradation
Free Ppb-a
Colorless and other small metabolites

Fig. 2.8.

CHAPTER 3

BIOCHEMICAL DEGRADATION OF ALGAL FATTY ACIDS IN OXIC AND ANOXIC SEDIMENT-SEAWATER INTERFACE SYSTEMS: EFFECTS OF STRUCTURAL ASSOCIATION AND RELATIVE ROLES OF AEROBIC AND ANAEROBIC BACTERIA[1]

[1] Haibing Ding and Ming-Yi Sun. Accepted by *Marine Chemistry*.
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Abstract

To examine microbially mediated degradation of algal fatty acids in marine environments, we conducted a series of microcosm experiments by incubating *Emiliania huxleyi* cells in simulated oxic/anoxic sediment-water interface systems. Variations in concentration of fatty acids, lipid-degrading enzyme (lipase) activity, and bacterial abundance over two month incubations were followed to determine degradation rate constants of major algal fatty acids and responses of bacteria. In the cell-spiked experiments, fatty acids bound in the membrane and intracellular components were separated to examine effects of structural association of fatty acids on their degradation. Experimental results showed that algal fatty acids generally degraded faster (2-4x) under oxic than under anoxic conditions. Most membrane fatty acids seemed to more readily degrade than intracellular ones under anoxic conditions but they degraded at similar rates under oxic conditions. Ratios of oxic to anoxic degradation rate constants were generally higher for intracellular fatty acids than for membrane fatty acids, implying that oxygen might play a more critical role on intracellular fatty acid degradation. Most algal fatty acids degraded almost completely under oxic conditions while there was always a significant fraction (10-40%) of initial added algal fatty acids remained not to degrade under anoxic conditions after two months. By contrast, variations in bacterial abundance during incubations were apparently greater under anoxic conditions compared to oxic conditions, suggesting that the function and relative effectiveness of aerobic vs. anaerobic bacteria rather than total bacterial abundance control biochemical degradation of algal fatty acids. Variations in potential lipase activity followed the similar pattern of bacterial abundance in oxic and anoxic systems, indicating that bacteria might be a major source for lipase in these experimental systems. Bacterial specific fatty acids varied differently during incubations and they were not directly linked to bacterial abundance.

Keywords: *Emiliania huxleyi*, membrane and intracellular fatty acids, lipase, bacterial abundance, redox conditions, biochemical degradation

Introduction

Degradation of organic matter in marine sediments plays an important role in global carbon and nutrient cycles. The degradation processes occur through either oxic or anoxic pathways, depending on *in situ* redox conditions. There have been numerous studies addressing the role of oxygen in organic matter degradation but controversial arguments still exist (Emerson, 1985; Lee, 1992; Canfield, 1994; Harvey et al., 1995; Sun et al., 2002). One major argument concerns whether anoxic decomposition of organic matter is intrinsically slower than oxic decomposition. Earlier experimental studies showed little difference between the aerobic and anaerobic decomposition of algal materials (Foree and McCarty, 1970; Jewell and McCarty, 1971; Otsuki and Hanya, 1972a; 1972b). More field evidence and laboratory results (Pedersen and Calvert, 1990; Henrichs and Reeburgh, 1987; Hansen and Blackburn, 1991) also suggested that anaerobic decomposition of organic matter could occur at rates comparable to aerobic decomposition. However, other studies indicated that oxic degradation of organic matter prevailed anoxic degradation and the exposure time of organic matter in oxic sediments might control the organic matter preservation (Emerson and Hedges, 1988; Harvey et al., 1995; Sun and Wakeham, 1997; Hartnett et al., 1998).

Plant organic matter consists of several classes of compounds, which have distinct degradation behaviors under different redox conditions (Westrich and Berner, 1984). For example, carbohydrate degraded more rapidly than proteins under oxic conditions but the situation was reversed under anoxic conditions (Harvey et al., 1995). Degradation rates of plant materials may depend on their ages and structural compositions. Kristensen et al. (1995) observed that the degradation rates were similar between soluble organics at initial leaking and early hydrolysis stages under both aerobic and anaerobic conditions, but anaerobic degradation

was hampered when the composition was dominated by structural components (e.g. lipids) in aged plant detritus. However, it is not clear why these structurally complex macromolecules degrade slowly and ineffectively under anaerobic conditions and how the structural associations between compounds affect their degradation behaviors.

Lipids, as one of major organic classes in phytoplankton (5-20%, Parsons et al., 1961), are less reactive than proteins and carbohydrates and have greater potential to be preserved in marine sediments. Therefore, lipids have been widely used as biomarkers in studies of carbon cycling and historic record (Gagosian et al., 1983; Volkman et al., 1987; Wakeham and Lee, 1993, Derieux et al., 1998). Lipids deposited in different environments with distinct redox conditions degraded at variable rates (McCaffrey et al., 1991; Sun and Wakeham, 1994; Canuel and Martens, 1996). Previous laboratory studies (Afi et al., 1996; Harvey and Macko 1997a; Sun et al., 2002) demonstrated that cell-associated lipids degraded faster under oxic than under anoxic conditions. However, other results (Sun et al., 1997; Sun and Wakeham, 1998) indicated that free lipids (added as tracers) degraded at less different rates under oxic and anoxic conditions. It seems that molecular association within the cells may exert a critical influence on lipid degradation under different redox conditions. However, there have been few studies focusing on the structural effects on organic matter degradation at molecular level.

In general, organic compounds in macromolecular complexes need to be released by extracellular enzymes (exoenzymes) before they are degraded to small metabolites, which can be further utilized by microbial community (Meyer-Reil, 1991; Arnosti, 1998). Exoenzymes are primarily produced by living bacteria such as lipolytic bacteria, which exist abundantly in marine systems (Podgorska and Mudryk, 2003). Other sources of exoenzymes are from lyses of dead and decaying cells. Some exoenzymes may retain activity by forming humic-enzymes complexes

bound to clay particles (Burns, 1980). The exoenzymes play a key role in modifying and transforming organic compounds in nature (Martinez et al., 1996; Gajewski et al., 1997). Protein and carbohydrate degrading enzymes in marine environments have been well investigated (Ladd and Bulter, 1972; King, 1986; Mayer, 1989; Billen, 1991; Mayer and Rice, 1992; Mayer et al., 1995; Arnosti, 1998) but fewer studies have focused on the relationship between lipid-degrading enzyme activity and lipid degradation. Lipases, which have broad substrate specificity, are capable of hydrolyzing fatty acid ester bonds in various macromolecules (Mustranta et al., 1995). However, little is known how lipase activities are related to aerobic and anaerobic processes and how they affect degradation of fatty acids bound in various structural complexes.

This study was designed to examine the influence of structural association within algal cells on fatty acid degradation under oxic and anoxic conditions. The alga used in our experiments is *Emiliania huxleyi*, which is one of the most abundant phytoplankton species in subpolar and low latitude oceanic regions (Brown and Yoder, 1994). This species biosynthesizes a set of specific lipids, which have been widely used as biomarkers in biogeochemical studies (Volkman et al., 1980; Marlowe et al., 1984; Riebesell et al., 2000). We cultured this alga in laboratory and incubated the dead cells in simulated oxic/anoxic sediment-water interface systems. The fatty acids associated with cell membrane (e.g. phospholipids) and intracellular component (e.g. triacylglycerols) were separated and the concentration variations during incubations were followed to determine the respective degradation rates. In addition, we monitored the changes in bacterial abundance and lipase activity during various incubations. The relationship among bacterial specific fatty acids, bacterial abundance and lipase activity was explored.

Experimental

Materials. Sediment and water samples used in the sediment-water interface simulation experiments were collected from a site in Doboy Sound near Sapelo Island, Georgia, USA. Sediment was collected using a box corer (surface area ~ 0.04 m^2). The top 2 cm sediments were scraped from the box core for use in the incubations. The sediments were passed through a 0.5 mm sieve to remove macrobenthos, large shells and detritus. Seawater (salinity ~28‰) used in the incubations was collected from the same site by pumping the water through a set of filters (25 μm).

Marine haptophyte *Emiliania huxleyi* (clone CCMP1949) was obtained from Provasoli-Guillard National Center for Culture of Marine Phytoplankton, Booth Bay Harbor, Maine, USA. It was cultured in f/50 medium using a 12:12 light/dark cycle at a mean temperature of 14°C. The culture was started in 50 ml medium and transferred to 250 ml new medium in ten days. The culture was then transferred to final 1000 ml medium after another 10 days. The dilution rates during entire culture period were ~0.5/d. The algal cells in final medium were harvested in ten days (before the end of exponential growth phase of the culture). The cells were collected by centrifugation and stored frozen (-20°C) for later incubation experiments.

Microcosm set up and control. The sediment-seawater interface system was simulated by setting a thin layer (~1.5 mm) of sediment in large water reservoirs with different redox conditions. The thawed algal cells (~5 g wet material with ~90% of water) were mixed with ~200 g of pre-sieved sediment (0.5 mm in mesh size) by hand stirring for 30 min. The well-mixed sediments (spiked sediment) were then inserted into 12 small plugs (1.5 mm thick, 5.7 cm i.d.) to form thin sediment layers. Another set of plugs was filled with the pre-sieved sediment without addition of algal cells (bulk sediment). These two groups of plugs (spiked and bulk

sediments) were placed in the bottom of two seawater reservoirs (~10 L), where oxic and anoxic conditions had already set up by purging with air (for oxic) and with N_2/CO_2 mixture (for anoxic) for 3 days. All samples were incubated at 28°C (*in situ* temperature at sampling site) in the dark. The advantage of this open system is to allow solute exchange between the sediment and the overlying water. The pH of the seawater in the anoxic tank was maintained same as original pH (8.1±0.5) by adjusting CO_2/N_2 ratio. One plug in each reservoir was taken out after 3, 7, 14, 28, 42 and 56 days. The sediments in the plug were transferred to a 50 ml plastic tube and frozen at -40°C for future analyses. About 1 g sediment was used for enzyme activity analysis, ~0.5 g for bacteria counting, 1-2 g for lipid analysis and ~0.5 g for water content measurement.

Extraction, separation and analysis of fatty acids. Lipid extraction and separation are based on published procedure (Sun et al., 1997). Briefly, the thawed sediment samples were first extracted with 10 ml methanol, followed by 3×10 ml methylene chloride-methanol (2:1 v/v) extraction. During each extraction, samples were sonicated for 10 min. Combined extracts were partitioned into a methylene chloride phase formed by the addition of 5% NaCl solution and then dried with a rotary evaporator. After redissolved in ~1 ml hexane, lipid extracts from the spiked sediment samples were passed through Si-gel column (6 mm i.d. and 14 cm in length) to separate membrane and intracellular fatty acids. Si-gel (EM Science, 63-200 mesh) was pre-cleaned by solvent extraction and high temperature combustion (at 450°C) and deactivated by 5% water. The extracts were separated into seven fractions by eluting with the following solvents: (1) 20 ml hexane; (2) 10 ml 5% ethyl acetate/hexane; (3) 10 ml 25% ethyl acetate/hexane; (4) 10 ml 50% ethyl acetate/hexane; (5) 10 ml ethyl acetate; (6) 20 ml methanol; and (7) 20 ml methylene chloride. Fractions (2)-(5) were combined as intracellular lipids and (6)-(7) fractions for

membrane lipids respectively. We confirmed the separation of two standards as representatives of intracellular and membrane components (tripalmitin eluting 100% in fraction 3 and L-α-phosphatidylcholine-β-oleoyl-γ-myristoyl ~70% in fraction 6 and ~30% in fraction 7). Two combined fractions were further saponified with KOH/MeOH to separate neutral and acidic lipids. Neutral lipids were extracted from the basic solution and fatty acids were extracted after addition of HCl (pH < 2). Fatty acids in the extracts were methylated with BF_3-methanol to form fatty acid methyl esters (FAMEs).

FAMEs were analyzed by capillary gas chromatography using a Hewlett-Packard 6890 GC system with an on-column injector and a flame ionization detector. Compound separation was achieved by a 30 m × 0.25 mm i.d. column coated with 5%-diphenyl-95%-dimethylsiloxane copolymer (HP-5, Hewlett-Packard). An internal standard (nonadecanoic acid methyl ester) was added to the samples immediately prior to GC analysis to aid in quantification. The operation temperature program was: 50-170ºC at 20ºC/min, followed by 170-310ºC at 4ºC/min and held at 310ºC for 5 min. Selected samples were analyzed by gas chromatography-mass spectrometry (GC-MS) to identify fatty acid structures. GC-MS analysis was performed on a SHIMADZU QP5000 GC-MS system using a 30 m × 0.25 mm i.d. column coated with 5% phenyl methyl silicone (XTI-5, Restek) and helium was used as carrier gas. Operating conditions of the GC-MS were: mass range 50-610 amu with a 0.4 s scan interval; 70 eV ionizing energy; GC temperature program 50-150ºC at 20ºC/min followed by 150-310ºC at 4ºC/min and a 5 min hold at 310ºC.

Lipase activity assays. Lipase activities in the incubated sediment samples were measured by spectrometric approach (Sun et al., 2000). In brief, lipase was extracted from ~1 g of wet sediment with 3×1.7 ml solution of 2% triton-X100 containing 2 g/L of polyvinylpyrrolidone in 0.2 molar tris-HCl buffer (pH = 8.0) (Reichardt, 1986). Each extraction

was conducted in ice-cold water bath for at least 10 minutes with hand shaking. Combined extracts were split into eight 4 ml vials, and then mixed with a series of substrate solutions with different concentrations. The stock substrate solution of p-nitrophenyl palmitate (PNPPal) was 100 mM in 95% ethanol. For lipase activity measurement, PNPPal substrate solutions were added to the eight vials and yield final substrate concentrations of 25, 50, 100, 200, 400, 600, 800 and 1000 μM. After adding the substrate, the absorbance of the samples was measured immediately and the initial readings served as blanks. Samples were incubated at 28°C (the same as the incubation temperature) in the dark for 5 h. The absorbance of the yellow product was measured at 410 nm using a SHIMADZU UV-2501 PC spectrophotometer. The enzyme reaction rate (ν) was calculated by the following equation:

$$\nu\ (\text{nmol/cm3/h}) = (\Delta A/\Delta t)\bullet(V_{cell}\bullet V_f)/(\varepsilon\bullet V_t\bullet V_{sed})\bullet 106 \qquad (1)$$

where, ΔA = absorbance change; Δt = incubation time (hours); V_{cell} = spectrophotometer cell volume (4 ml); ε = molar extinction coefficient (15772 l /mol/cm; John, 1993); V_t = total volume of sediment extract (ml); V_f = volume of sediment extract added to the cell (0.5 ml in this case); V_{sed} = total volume of sediment (cm^3). Enzymatic reaction rates measured in this study generally followed Michaelis-Menten kinetics:

$$\nu = (\nu_m\ [S])/(K_m + [S]) \qquad (2)$$

where, ν_m is the maximum rate of the enzyme reaction; [S] is the concentration of substrate; and K_m is the Michaelis constant. The values of ν_m were estimated using a Pharm-K v.2.1 software by plotting (1/ν) vs. (1/[S]).

Bacteria counting. The extraction and counting of bacteria were performed based on published methods (Porter and Feig, 1980; Suzuki et al., 1993). About 0.5 g thawed sediment was mixed with 1 ml 2% formaldehyde and 10 ml solution containing 0.1% deoxycholic acid (sodium salt) and 2.5% polyethylene glycol. The solvent and solution were pre-filtered through 0.2 μm sterile Tuffryn membrane non-pyrogenic filter to get rid of bacteria. The mixture was stirred by vortex for one minute. The sample was sonicated for 10 min and centrifuged at 5000 rpm for 15 min. The supernatant (containing bacteria) was separated and the sample was extracted for the second time. The combined supernatants were filtered through a 0.2 μm black filter. Prior to filtration, 1 ml 4',6-diamidino-2-phenylindole (DAPI, 33 μg/ml) was added into the extracts as DNA fluorescent groove-binding probe. After filtration, the black filter was put on a slide and the bacterial number on the filter was counted with an oil-immersion lens under a microscope. Bacterial abundance was calculated by averaging numbers from 10 countings.

Results

Fatty acid compositions in Emiliania huxleyi cell, bulk sediment and spiked sediment. There were seven detectable fatty acids identified in our cultured *Emiliania huxleyi* cells (Fig. 3.1a). Among them, 14:0, 16:0, 18:1(ω9) and 22:6 were major components and their relative percentages to total fatty acids accounted for approximately 20% by each. The relative percentages of other three fatty acids (15:0, 16:1(ω7), and 18:0) were below 10% of the total. There was neither branched iso- and anteiso-15:0 nor 18:1(ω7) fatty acids found in the cultured cells, indicating that our axenic culture had little bacterial input. In bulk sediments, eleven significant fatty acids were identified (Fig. 3.1b), including all except 22:6 found in *Emiliania huxleyi* cells plus three bacteria specific iso-15:0, anteiso-15:0, and 18:1(ω7) fatty acids, and two

polyunsaturated 20:4 and 20:5 fatty acids. The most abundant fatty acids in bulk sediments were 16:0 and 16:1(ω7) (>20% by each) and the relative percentages of rest fatty acids were below 10%. When the bulk sediments were spiked with *Emiliania huxleyi* cells, the relative percentages and concentrations of some fatty acids changed noticeably (Fig. 3.1b and 3.1c). For example, the relative percentages of 16:1(ω7), 20:5 and 20:4 in the spiked sediments decreased more than half relative to bulk sediment although their concentrations did not change. The similar changes also occurred for three bacteria specific fatty acids iso-15:0, anteiso-15:0 and 18:1(ω7). On the other hand, the relative percentage of 18:1(ω9) increased to 3-fold and 14:0 was almost doubled and their concentrations increased 7-fold and 3-fold respectively. The 16:0 fatty acid was the most abundant fatty acid in both bulk and spiked sediments. Although the relative percentage of 16:0 did not increase remarkably, its concentration increased more than 130%. The 22:6 fatty acid occurred in the spiked sediments as a unique compound exclusively from the algae. Based on their higher relative percentages and concentrations in the algal cells and spiked sediments, 14:0, 16:0, 18:1(ω9) and 22:6 were chosen as representative algal fatty acids for our degradation study.

Fatty acids associated with membrane and intracellular lipids. The membrane and intracellular fatty acids in *Emiliania huxleyi* cells and in the spiked sediments were separated by silica gel column chromatography. The membrane fatty acids in the cells accounted for approximately 80% of total fatty acids (Fig. 3.2a). The fatty acid ratios of membrane to intracellular components ranged from 3 to 9. The ratios of two major saturated fatty acids (14:0 and 16:0) were relatively higher (6-9) than those (3-4) of two major unsaturated fatty acids (18:1(ω9) and 22:6). In membrane, 16:0 was the most abundant fatty acid but 22:6 was the most abundant one in intracellular component. In the spiked sediments, membrane fatty acids

accounted for 68% of total fatty acids and the concentration ratios of individual fatty acid between membrane and intracellular component varied differently (Fig. 3.2b). For example, the ratios of 14:0 and 16:0 fatty acids decreased from 6-9 (in the cells) to 2-3 (in the sediments) but the ratios of 18:1(ω9) and 22:6 had little changes. Based on the relative percentages of membrane and intracellular fatty acids in the cells and in the spiked sediments, the relative percentages of membrane and intracellular fatty acids in the bulk sediments were calculated (Fig. 3.2c). In the bulk sediments, total intracellular fatty acids were close to the amount of membrane fatty acids (45% vs. 55%). In addition, the proportions of most saturated fatty acids (15:0, 16:0 and 18:0) in intracellular component were higher than those in membrane although the proportion of 14:0 was the same in the two components.

Variations of algal fatty acids during incubations. Concentrations of four major algal fatty acids declined continuously under both oxic and anoxic conditions during incubations (Fig. 3.3). In general, the concentrations of all algal fatty acids dropped dramatically in the first 2-3 weeks and then decreased slowly or even do not change. Although the degradation patterns of these algal fatty acids were similar, there were obvious differences between oxic and anoxic degradations of the algal fatty acids in all incubations. First, these fatty acids degraded faster under oxic conditions than under anoxic conditions. Second, most fatty acids degraded almost completely under oxic conditions but there was always a fraction (relative to initial value) not degraded under anoxic conditions. Exceptions were 16:0 fatty acids in all incubations, 18:1(ω9) in bulk sediment incubation and membrane 18:1(ω9) in the spiked sediment incubation, where there was a fraction (10-40% of initial concentrations) remained under both oxic and anoxic conditions (Table 3.1). Generally, higher percentages of initial fatty acids remained non-degradable in the bulk sediments than in the spiked sediment systems.

Variations of bacterial specific fatty acids during incubations. Unlike four algal fatty acids, three bacterial specific fatty acids (iso-15:0, anteiso-15:0 and 18:1(ω7)) showed different variation patterns during incubations (Fig. 3.4). In the bulk sediment incubation (Fig. 3.4), the three bacterial specific fatty acids varied similarly to the algal fatty acids: almost complete degradation under oxic conditions and incomplete under anoxic conditions. In the spiked sediment incubation, these bacterial fatty acids had different variation patterns in intracellular component and membrane (Fig. 3.4). The concentrations of the bacterial specific fatty acids in intracellular component increased apparently in the first week, with more increase under anoxic than under oxic conditions. After the first week, the concentrations decreased continuously until the end of incubation. The bacterial specific fatty acids in membrane increased under anoxic conditions in the first week and then decreased similarly as intracellular ones. Under oxic conditions, however, anteiso-15:0 and 18:1(ω7) declined from the beginning of incubation and only iso-15:0 increased a little bit in the first three days (Fig. 3.4).

Variations of bacterial abundance and lipase activity during incubations. Bacterial abundance varied dramatically during all incubations (Fig. 3.5). Initial bacterial abundances in the bulk and spiked sediments were low but the abundances increased quickly in the first 2-3 weeks: higher under anoxic conditions than under oxic conditions. After 2-3 weeks, bacterial abundances declined gradually to the initial level in the bulk sediments but remained relatively higher abundances in the spiked sediments (especially under anoxic conditions) at the end of incubations. Apparently higher bacterial abundances occurred in the spiked sediments than in the bulk sediments.

The maximum lipase activity (ν_m) varied almost coincidentally with the bacterial abundance in the bulk and spiked sediment incubations (Fig. 3.5). The peaks in ν_m occurred in all incubations but higher ν_m peaks were observed in anoxic sediments than in oxic sediments.

Discussion

Degradation rate constant of algal fatty acids. Variations of four major algal fatty acids in all incubations showed a general pattern: initial faster decrease in the first 2-3 weeks and followed by slower or little changes. This pattern has been previously observed for organic matter degradation and can be quantitatively described by the well-known multi-G model proposed by Berner (1964). In this model, fractions of organic matter are assumed to degrade independently at distinct rates, depending on their reactivities. Overall degradation rate of organic matter is simply a sum of individual degradation rates of each fraction, as shown by the following equations:

$$-(dG_t/dt) = \Sigma\, k_i G_i \qquad (3)$$

$$G_t = \Sigma\, G_i = (G_1)_0 \exp(-k_1 t) + (G_2)_0 \exp(-k_2 t) + \ldots\ldots \qquad (4)$$

where, k_i is the first-order degradation rate constant of fraction i; G_i is the concentration of fraction i; $-(dG_t/dt)$ is the degradation rate of all fractions; $(G_1)_0$ and $(G_2)_0$ are initial concentrations (at $t = 0$) of fraction 1 and fraction 2.

Many field observations and laboratory studies have confirmed the validity of the multi-G model (Westrich and Berner, 1984; Henrichs and Doyle, 1986). In this study, individual fatty

acids showed variable kinetic characteristics during their degradation course, implying that same molecules can degrade at different rates if they are associated with different structural components. However, pool size of various structural components may vary with redox conditions and for different compounds. Under oxic conditions, some fatty acids (e.g. 14:0 and 22:6) seemed to degrade completely as one pool. By contrast, all algal fatty acids apparently degraded as two pools under anoxic conditions. In many cases, the k_2 is so small (close to zero) that the second pool can be assumed to be non-degradable (G_{NR}). Thus, the equation (4) can be simplified as:

$$G_t = (G_1)_0 \exp(-k_1 t) + G_{NR} \quad (5)$$

This simplified model has been used to treat field profile data and laboratory experimental results (Sun and Wakeham, 1994; Teece et al., 1998). The modeling processes involved two steps to determine different parameters. The first step was to plot $\ln(G_t)$ versus incubation time (t). There was a sudden change in the curve (breaking point), which could be used to estimate relative pool sizes ($(G_1)_0$ and $(G_2)_0$). The second step was to fit the original (G_t) data to the two-pool model by adjusting kinetics parameters (k_1 and k_2). In order to directly compare overall degradation rate constants no matter how many pools were involved, we introduced a new parameter (k_{av}), which is defined as the average degradation rate constant from two different pools:

$$k_{av} = k_1 \times f_1 + k_2 \times f_2 \quad (6)$$

where, f_1 and f_2 are relative proportions of pool 1 and pool2 ($f_1 = (G_1)_0/(G_t)_0$ and $f_2 = (G_2)_0/(G_t)_0$.

Comparison of degradation rate constants (Table 3.2) showed several interesting implications for degradation of algal fatty acids in the bulk and spiked sediments. First, oxic degradation rate constants were generally 2-4x higher than those of anoxic degradation in both systems, indicating that there is a general trend in nature with preference of oxic over anoxic degradation for algal fatty acids. Second, the ratios of oxic to anoxic degradation rate constants (k_{ox}/k_{an}) were higher for the intracellular fatty acids than those of membrane fatty acids, implying that redox conditions might be more critical for degradation of intracellular fatty acids compared to membrane fatty acids. Third, the fatty acids in both membrane and intracellular components seemed to degrade at similar rates under oxic conditions (exception was 14:0) while membrane fatty acids degraded apparently faster (~2x) than intracellular fatty acids under anoxic conditions (exception was 22:6). Fourth, unsaturated fatty acids (18:1(ω9) and 22:6) in all cases had higher degradation rate constants than saturated fatty acids (14:0 and 16:0), indicating an effect of structure (double bond) on lability of fatty acids.

In this study, the degradation rate constants (k_{av}) of algal fatty acids ranged from 0.026 d^{-1} to 0.256 d^{-1}. These values were comparable with those reported by other studies although the experimental systems, substrate types, environmental conditions were remarkably different. For example, Harvey and Macko (1997a) conducted a series of experiments by incubating two phytoplankton species (*Thalassiosira weissflogii* and *Synechococcus sp*.) in oxic and anoxic seawater. The degradation rate constants of saturated, monounsaturated, and polyunsaturated fatty acids of two algae were estimated to be 0.01 d^{-1} - 0.1 d^{-1}, with oxic/anoxic ratios from 3 to 7. Degradation rate constants of algal fatty acids estimated from sediment incubations were in a wider range from 0.007 d^{-1} to 0.381 d^{-1} (Sun et al., 1997, 2002; Grossi et al., 2001, 2003), with

higher values in oxic sediments and with presence of macrofauna. In permanent anoxic basin sediments (e.g. Black Sea), fatty acids degraded at very slow rates (with rate constants of <0.001 d-1), estimated by a diagenetic model (Sun and Wakeham, 1994). Canuel and Martens (1996) found that reactivity of fatty acids was dramatically reduced (rate constants from ~0.05 d^{-1} to ~0.005 d^{-1}) with burial depth in coastal marine sediments.

Structural effect on fatty acid degradation. The structural effects of molecular matrixes on fatty acid degradation vary with environmental redox conditions. As shown by our estimated rate constants (Table 2.2), under oxic conditions, degradation rate constant ratios of membrane to intracellular fatty acids (k_m/k_{in}) were similar but these ratios were apparently higher under anoxic conditions. Moreover, k_{ox}/k_{an} ratios of intracellular fatty acids were generally larger than those of membrane fatty acids. These facts suggest that presence of oxygen strongly affect the responses of structural matrixes to degradation processes. Biotic and abiotic processes in oxic environments can produce large quantity of oxygen reactive radicals, which can readily depolymerize relatively refractory materials (Canfield, 1994). Thus, degradation of intracellular fatty acid might be accelerated by these radicals, resulting in similar rates between membrane and intracellular fatty acids under oxic conditions. By contrast, lack of these reactive radicals under anoxic conditions hinders the breakup of more refractory matrixes such as intracellular components. Furthermore, the location of structural matrixes in natural environments may affect the interactions between the fatty acids and bacteria. For example, the phospholipids in algal membrane have more chance Our results demonstrated that algal fatty acids in membrane and intracellular components had different degradation behaviors under variable redox conditions. These differences may be partly caused by their structural associations within the cells. Fatty acids are a major fraction of lipids biosynthesized by living algal cells and they are generally

bound in different structural complexes such as triacylglycerols, phospholipids, and wax esters. These fatty acid complexes exist in different compartments within the cells and play different functions during cell growth and metabolism. When cells die, these complexes will experience biochemical degradation in different environments. Fatty acids bound in different complexes may have different responses to degrading agents due to the differences in their molecular size, structural bond, polarity, and chemical lability. For example, cell membrane backbone is built up by phospholipids, in which two fatty acids are esterified with two hydroxyl groups of glycerol while a phosphate group is linked to the third hydroxyl group. Thus, phospholipids have both polar and non-polar ends, which make phospholipids packing into membrane with bilayer configuration in aquatic conditions. Major intracellular fatty acids bound in cell cytoplasm include triacylglycerols and wax esters although the relative proportions may vary from species to species (Volkman et al., 1989). These complexes are hydrophobic and are used as energy storage for cell metabolisms (Voet and Voet, 1995). The different fatty acids with variable length and unsaturation extent are bound in the membrane and intracellular structures. As shown by analysis of fatty acid composition in the cells (Fig. 2.2a), polyunsaturated 22:6 is the dominant fatty acid in intracellular component while saturated 16:0 is the most abundant one in the membrane.

In general, cell-associated fatty acids degrade via two steps: (1) esterified fatty acids are first released from the structural matrixes by enzymatic hydrolysis; and (2) the free fatty acids further decompose to CO_2 or other smaller organic molecules. Structural effects of the matrixes may affect the first step (hydrolysis) and have little influence on free fatty acid decomposition. Therefore, the first step, which is a limiting step and very complicated, controls the overall degradation of fatty acids. In this step, different enzymes may be involved for releasing fatty

acids from various matrixes. Lipases can break the ester link between fatty acid and glycerol in both triacylglycerols and phospholipids (Mustranta et al., 1995). Several different phospholipases can either attack the ester link between fatty acids and glycerol (e.g. phospholipase A_1 and A_2) or tear the phosphate group away from glycerol (e.g. phospholipase C) (Lehninger et al., 1993). As digestive enzymes, both lipases and phospholipases are water-soluble, so lipid digestion occurs at lipid-water interface (Voet and Voet, 1995). Thus, amphipathic phospholipids may react more readily than hydrophobic triacylglycerols in natural environments while the hydrophobic feature of triacylglycerols obstructs the release of fatty acids from the molecular matrixes. In addition, triacylglycerols are coated by phospholipids and associated with other hydrophilic proteins to form lipoprotein for keeping them stable in cytoplasm (Voet and Voet, 1995). These coated phospholipids and associated proteins may provide an additional protection for fatty acids from degradation (Voet and Voet, 1995). To break lipoprotein, some special enzymes such as lipoprotein lipases are needed (Hans et al., 1998). It appears that the faster degradation of membrane fatty acids than intracellular fatty acids observed in our experiments is likely caused by the differences in their physiochemical properties, which are dependent on different structural associations, and their susceptibility to degrading enzymes.exposing to surrounding bacteria than triacylglycerols, which are bound in cytoplasm and protected by cell membrane (Voet and Voet, 1995).

Although triacylglycerols are a major source of intracellular fatty acids, other compounds may contribute significant amounts of fatty acids into this pool. For example, diacylglycerols and monoacylglycerols, which have similar polarity as triacylglycerols, are intermediate products from initial hydrolysis of triacylglycerols and phospholipids (Goutx et al., 2003). Thus, part of these intermediate products in the intracellular pool might be derived from the membrane pool.

By our separation scheme, the intermediate products from membrane pool could not be separated from those derived from intracellular pool. These intermediate products would further degrade in intracellular pool. Only when hydrolysis rates of triacylglycerols and phospholipids are much greater than degradation rates of these intermediate products, fatty acids in the intracellular pool can be accumulated. Fortunately, no accumulation of fatty acids was observed in the intracellular pool in our incubations, implying that the intermediate products from membrane pool might degrade faster enough before they accumulate in the intracellular pool.

Relative roles of aerobic and anaerobic bacteria in fatty acid degradation. Generally, bacteria have dual roles in organic matter cycling: either as consumers of both dissolved and particulate organic compounds or as contributors for newly produced organic matter (Azam et al., 1983; Deming and Baross, 1993). Many field and laboratory experiments have demonstrated the activities of bacterial community during early diagenesis in oxic and anoxic depositional environments (Henrichs and Reeburgh, 1987; Lee, 1992; Harvey and Macko, 1997b), yet the understanding of relative efficiency of aerobic vs. anaerobic degradation remains to be controversial. Two agreements have been achieved based on a number of studies (Kristensen and Blackburn, 1987; Kristensen et al., 1995; Hulthe et al., 1998; Sun and Wakeham, 1999): (1) degradation rates under different redox conditions depend on the chemical composition and lability of organic matter, and (2) presence of animals (grazer of bacteria) in oxic environments, rather than the presence of oxygen per se, may accelerate degradation processes.

In this study, we observed a rapid decrease in concentration of algal fatty acids during the first 2-3 weeks of incubation in both bulk and spiked sediments (Fig. 3.3). On the other hand, a general increase in bacterial abundance was also found in the same period (Fig. 3.5). Reverse variations in organic compound concentration and bacterial abundance or biomass were observed

by other studies (Harvey and Macko, 1997b; Teece et al., 1998). Degradation of algal fatty acids represents the loss of organic substrate (algal materials) via biochemical processes while increase of bacterial abundance indicates the new production of organic matter by bacterial biosynthesis. However, the greater loss of algal fatty acids in oxic systems does not accompany higher bacterial abundance. This fact implies that degradation effectiveness of aerobic and anaerobic bacteria on algal fatty acids may not be simply related to their abundance. Growth of aerobic and anaerobic bacteria during degradation of algal organic matter may depend on different labile compounds. There was evidence showing that metabolisms of dissolved amino acids and monosaccharides corresponded with changes in bacterial abundance during incubation (Harvey et al., 1995). Thus, the faster degradation of algal fatty acids under oxic conditions was not caused only by microbial processes since lower bacterial abundance occurred in the oxic systems. It is likely that aerobic bacteria, combined with abiotic processes, more effectively degrade fatty acids associated in algal cells.

Relationship between bacterial abundance and bacterial specific fatty acids. Bacteria can specifically biosynthesize a series of odd number C15-C17 (branched) and also 18:1(ω7) fatty acids (Kaneda, 1991), which have been widely used as bacterial source indicators in carbon cycling studies (Cranwell et al., 1987; Wakeham and Beier, 1991). However, previous attempts to quantitatively link bacterial specific fatty acids with bacterial biomass have been equivocal (Harvey and Macko, 1997b). When we plotted the sum of three bacterial specific fatty acids (iso-15:0, anteiso-15:0, and 18:1(ω7)) vs. bacterial abundance (Fig. 3.6), a general variation pattern between these two variables was found in all incubations under both oxic and anoxic conditions. The variation pattern was characterized by a peak in bacterial abundance with varying

concentrations of the fatty acids, although the magnitudes and positions of the peaks in different systems might change.

Similar pattern does not necessarily suggest that the relationships between bacterial specific fatty acids and bacterial abundance in different incubation systems are the same since the concentrations of the bacterial specific fatty acids varied differently in these systems (Fig. 3.4). Two possible causes may be attributed to these variations in bacterial specific fatty acids. One is the difference in bacterial community from different systems, which can make different bacterial fatty acids. Parkes and Taylor (1983) demonstrated that fatty acid compositions are closely linked with bacterial respiratory types (aerobic, facultative anaerobic, and strictly anaerobic bacteria). In natural stratified oxic/anoxic seawaters (e.g. the Black Sea and the Cariaco Trench), branched fatty acids were found to be insignificant components in oxygenated water column but their concentrations dramatically increased below the oxic/anoxic interface (Wakeham and Beier, 1991). Another cause is a stimulation of fresh organic matter on growth of sedimentary bacteria (Danovaro et al., 1994). With addition of algal materials into the sediments, both bacterial abundance and bacterial specific fatty acids rapidly increased in the first few weeks (Figs. 3.4 and 3.5), implying that the fresh organic matter was efficiently used by bacteria. However, without addition of fresh organic materials, the bacterial abundance in the bulk sediments had a slow and less increase while the bacterial fatty acids did not increase at all.

The formation of peak in bacterial abundance with varying concentration of bacterial fatty acids may be controlled by several opposite processes: production vs. turnover of bacterial biomass, and biosynthesis vs. degradation of bacterial fatty acids. Only when new bacteria biosynthesize the specific fatty acids and the production is over the turnover process, the concentration of bacterial fatty acids can vary positively with the increase of bacterial

abundance. If the turnover of bacteria exceeds the production but the specific fatty acids do not degrade completely within a given time period, the bacterial abundance will vary inversely with the concentration of bacteria-produced fatty acids. Therefore, there is no simple relationship between bacterial biomass and bacterial specific fatty acids. More complicated variations of bacterial biomass with total bacterial fatty acids were observed in other study (Harvey and Macko, 1997b). There are several uncertainties in our measurements, which may influence the assessment of the relationship. For example, the DAPI counting method for bacterial number may include active, inactive and even dead species (Zweifel and Hagström, 1995) while the concentrations of bacterial fatty acids in them may differ. On the other hand, the activities of bacteria grazers (e.g. protozoans and meiofauna) were not quantified in this study. Bacteria grazers may incorporate bacterial fatty acids into their biomass when bacteria are consumed (Ederington et al., 1995). Thus, bacterial fatty acids may remain as membrane components of grazers even after digestion of the bacterial cells, resulting in extra "bacterial" fatty acids with the absence of bacteria.

Relationship between bacterial abundance and lipase activity. Bacteria play a primary role in organic matter degradation since they produce variety of enzymes, which can alter structures of a wide range of particulate and dissolved organic compounds (Deming and Baross, 1993). However, contrary evidence showed that the relationship between bacterial abundance and enzyme activity was complicated. For example, it was observed that seasonal variations in extracellular protease activity were not related to changes in bacterial abundance but appeared to relate to the seasonal temperature changes (Mayer, 1989). Reichardt (1986) showed that there were different relationships between redox conditions (different bacterial community) and protease (positive correlation) and cellulase (inverse correlation). Bourguet et al. (2003)

observed that hydrolysis rates of lipids by lipase varied consistently with bacterial production in a Lagoon area. Sun et al. (2000) found that variations of lipase activity in estuarine sediments were associated with changes in bacterial specific fatty acids, suggesting a close coupling between them. In this study, the maximum enzymatic rate is a potential rate due to the presence of naturally occurring substrates which can compete with added substrates for enzyme sites. The maximum rates of enzymatic reaction (by lipase) varied at similar pattern as bacterial abundance during sediment incubations (Fig. 3.5), suggesting that bacteria might be a major source of lipid-degrading enzymes.

Conclusions

Determination of degradation rate constants in the sediment-water interface systems demonstrates that oxic degradations of algal fatty acids are generally faster than anoxic degradations. Most algal fatty acids degrade completely as one pool under oxic conditions but there are at least two pools involved in anoxic degradations. Examination of structural effects of fatty acids within algal cells on their degradation shows that membrane and intracellular fatty acids have similar degradation behaviors under oxic conditions but they degrade at apparently different rates under anoxic conditions. Although bacterial abundance and lipase activity derived from bacteria are higher under anoxic conditions than under oxic conditions, fatty acids associated in both membrane and intracellular components degrade slower under anoxic rather than under oxic conditions. These results indicate that degradation of cell-associated fatty acids is more likely controlled by relative effectiveness of aerobic vs. anaerobic bacteria rather than their abundance. During incubations, bacterial specific fatty acids in different systems varied in different ways despite of the similar variations in bacterial abundance, implying that bacterial

fatty acids are not simply related to the biomass. Relatively higher variations in enzymatic reaction rate and bacterial abundance were found in the spiked sediments than in the bulk sediments, indicating that addition of fresh organic matter may stimulate bacterial growth.

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Table 3.1. Initial concentrations of various algal fatty acids in bulk and spiked sediments and the remained fractions (%) at the end of incubations.

Fatty acids	14:0		16:0		18:1(ω9)		22:6	
Bulk sediments								
Initial conc. (μg/g)	16.9		46.6		8.3			
Remained % after 56 days								
Oxic	0.0		22.8		18.5			
Anoxic	25.1		39.5		43.3			
Spiked sediments	intra*	mem#	intra*	mem#	intra*	mem#	intra*	mem#
Initial conc. (μg/g)	11.2	34.3	39.9	62.7	6.9	35.8	12.5	25.8
Remained % after 56 days								
Oxic	0.0	0.0	11.3	12.3	0.0	8.5	0.0	0.0
Anoxic	23.2	9.5	20.1	23.0	21.0	29.9	8.1	8.0

intra*: intracellular fatty acids;

mem#: membrane fatty acids

Table 3.2. Averaged degradation rate constants (day^{-1}) of various algal fatty acids in bulk and spiked sediments and the ratios of rate constants between oxic and anoxic conditions and between membrane and intracellular fatty acids.

Fatty acids	14:0			16:0			18:1(ω9)			22:6		
Bulk sediments												
$(k_{ox})_{av}$	0.178			0.135			0.187					
$(k_{an})_{av}$	0.119			0.052			0.074					
k_{ox}/k_{an}	(1.50)			(2.60)			(2.53)					
Spiked sediments	intra*	mem#	k_m/k_{in}	intra*	mem#	k_m/k_{in}	intra*	mem#	k_m/k_{in}	intra*	mem#	k_m/k_{in}
$(k_{ox})_{av}$	0.108	0.199	(1.84)	0.160	0.186	(1.16)	0.227	0.245	(1.06)	0.227	0.210	(0.92)
$(k_{an})_{av}$	0.026	0.067	(2.58)	0.050	0.101	(2.02)	0.063	0.165	(2.62)	0.119	0.151	(1.27)
k_{ox}/k_{an}	(4.15)	(2.97)		(3.20)	(1.84)		(3.60)	(1.48)		(1.91)	(1.39)	

Note that the correlation coefficients of *k* from individual modeling are: r2 = 0.8-0.99 for 72% cases, r2 = 0.6-0.8 for 18% cases, and r2 < 0.6 for 10% cases.

intra*: intracellular fatty acids;

mem#: membrane fatty acids.

Fig. 3.1. Fatty acid compositions in *Emiliania huxleyi* cells (a) and in bulk and spiked sediments (b), and fatty acid concentrations in spiked sediments (c).

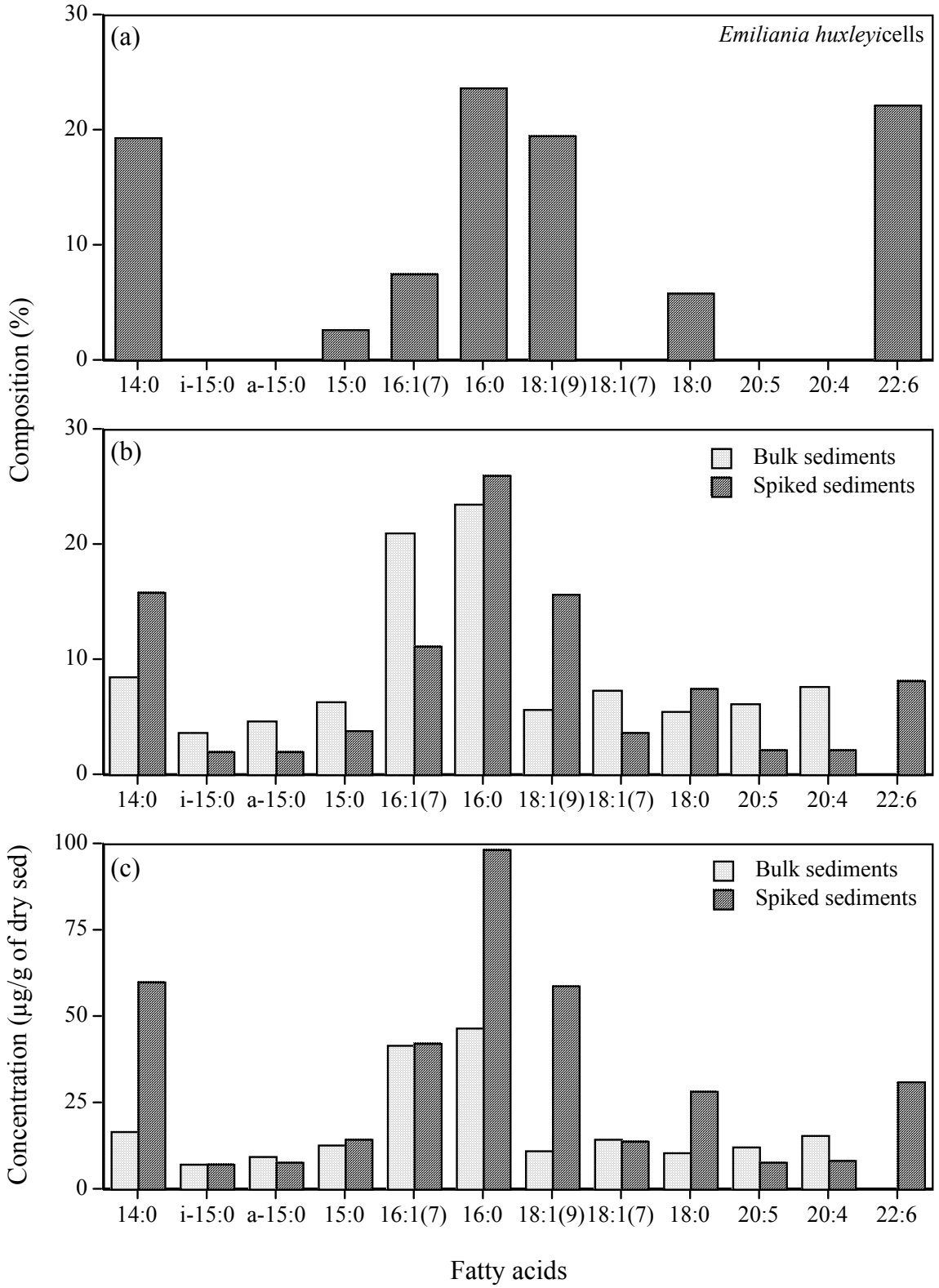
(a)
Emiliania huxleyi cells
(b)
Bulk sediments
Spiked sediments
(c)
Bulk sediments
Spiked sediments
Composition (%)
Concentration (µg/g of dry sed)
14:0
i-15:0
a-15:0
15:0
16:1(7)
16:0
18:1(9)
18:1(7)
18:0
20:5
20:4
22:6
Fatty acids

Fig. 3.1.

Fig. 3.2. Relative percentages of intracellular and membrane associated fatty acids in *Emiliania huxleyi* cells (a), in spiked sediments (b), and in bulk sediments (c). The result of (c) was calculated based on (a) and (b).

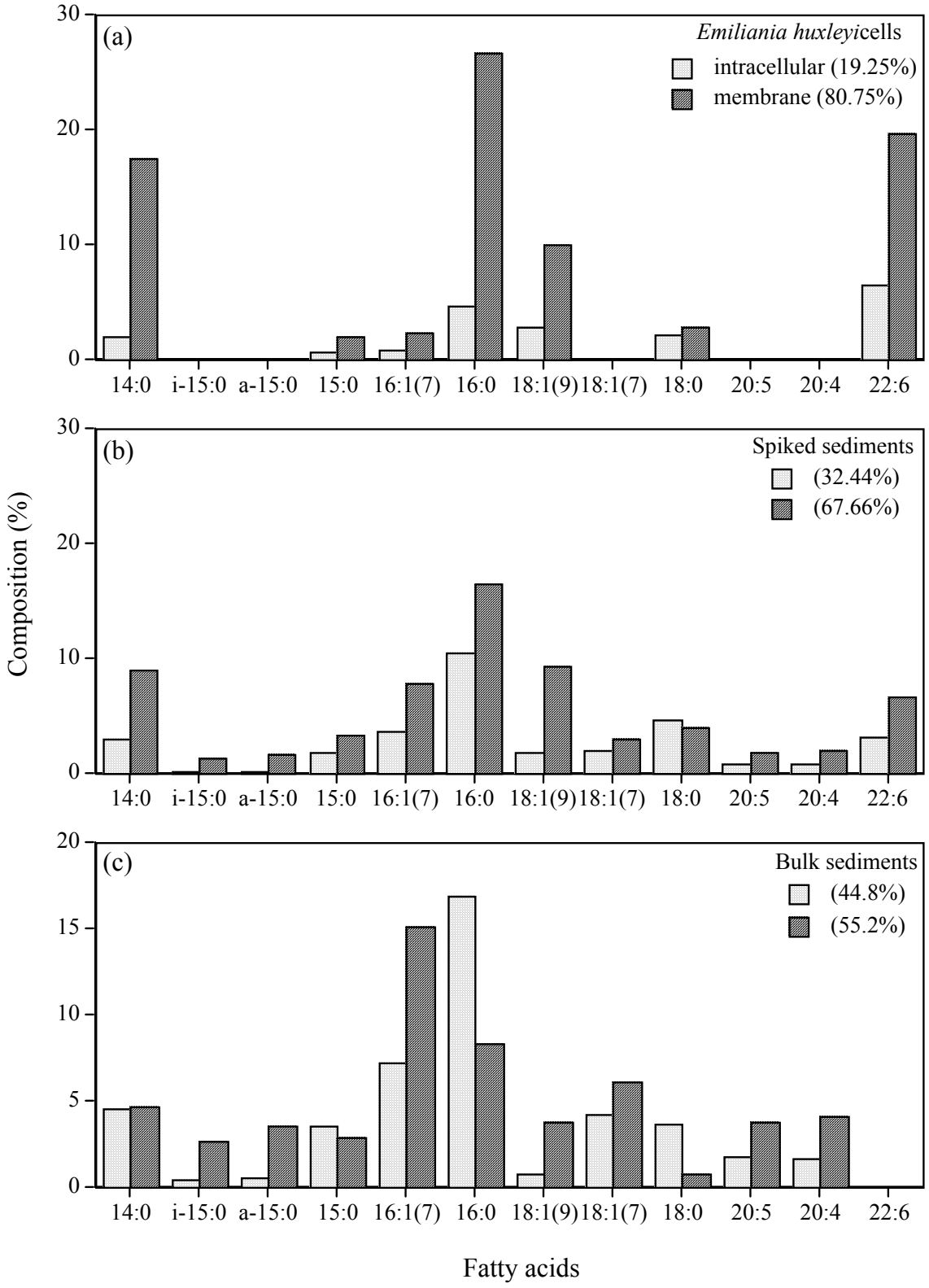
(a)
Emiliania huxleyicells
intracellular (19.25%)
membrane (80.75%)
(b)
Spiked sediments
(32.44%)
(67.66%)
(c)
Bulk sediments
(44.8%)
(55.2%)
Composition (%)
Fatty acids
14:0
i-15:0
a-15:0
15:0
16:1(7)
16:0
18:1(9)
18:1(7)
18:0
20:5
20:4
22:6

Fig. 3.2.

Fig. 3.3. Variations of algal fatty acid concentrations during incubations in bulk and spiked sediments (intracellular and membrane components). The curves in the figures are modeling results.

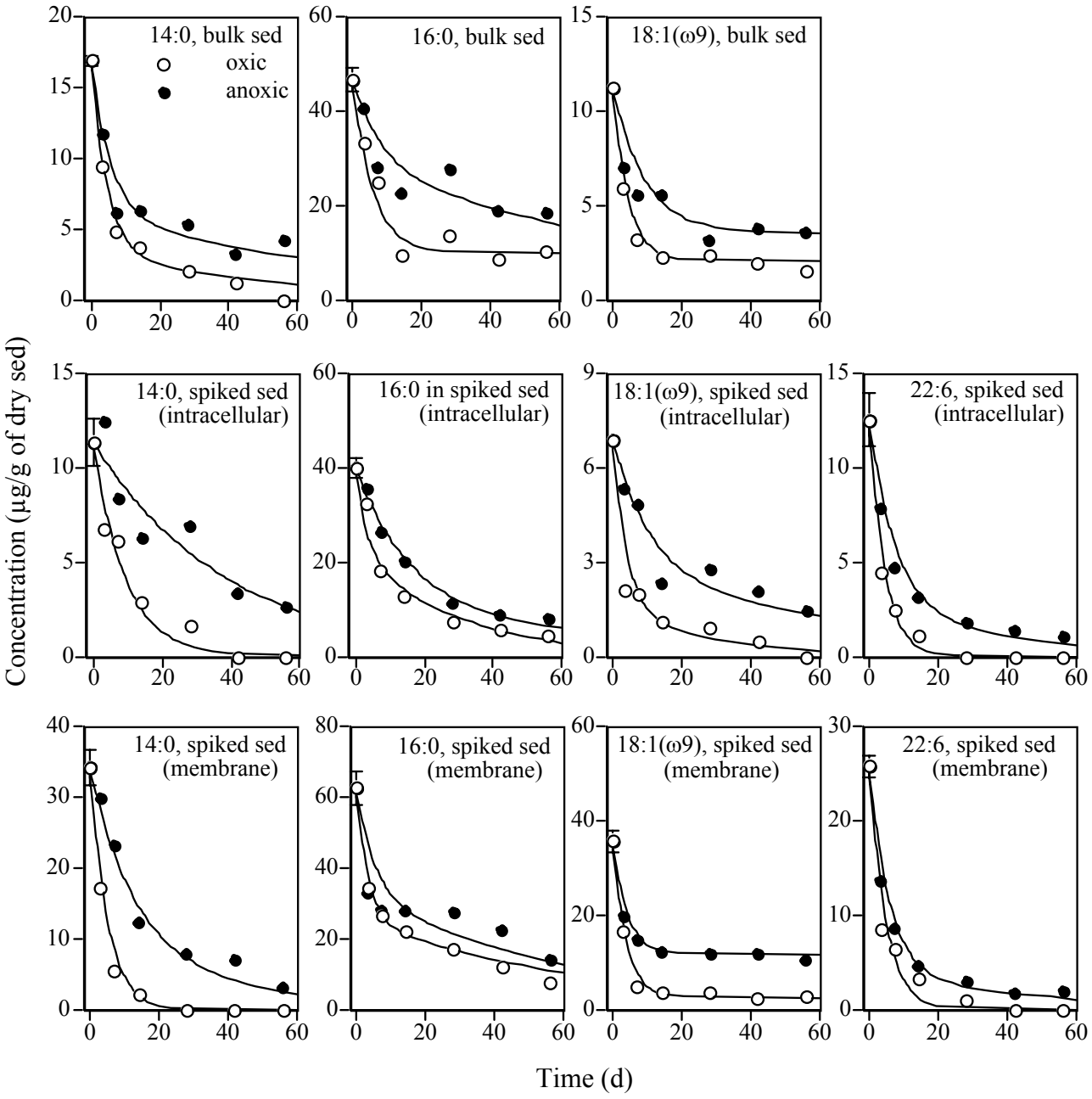
14:0, bulk sed
oxic
anoxic
16:0, bulk sed
18:1(ω9), bulk sed
14:0, spiked sed (intracellular)
16:0 in spiked sed (intracellular)
18:1(ω9), spiked sed (intracellular)
22:6, spiked sed (intracellular)
14:0, spiked sed (membrane)
16:0, spiked sed (membrane)
18:1(ω9), spiked sed (membrane)
22:6, spiked sed (membrane)
Concentration (µg/g of dry sed)
Time (d)

Fig. 3.3

Fig. 3.4. Variations of bacterial specific fatty acid concentrations during incubations in bulk and spiked sediments (intracellular and membrane components).

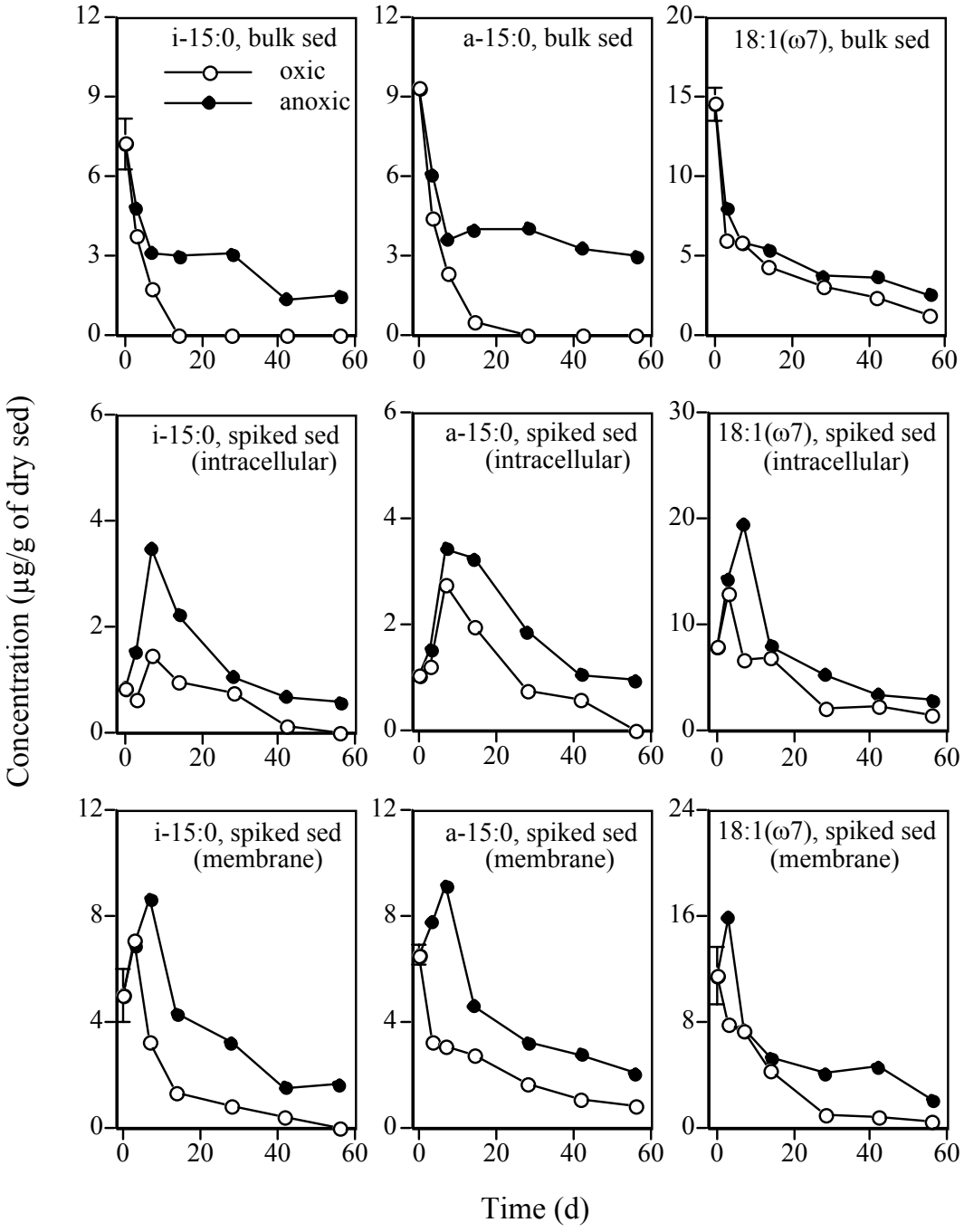
i-15:0, bulk sed
oxic
anoxic
a-15:0, bulk sed
18:1(ω7), bulk sed
i-15:0, spiked sed (intracellular)
a-15:0, spiked sed (intracellular)
18:1(ω7), spiked sed (intracellular)
i-15:0, spiked sed (membrane)
a-15:0, spiked sed (membrane)
18:1(ω7), spiked sed (membrane)
Concentration (µg/g of dry sed)
Time (d)

Fig. 3.4

Fig. 3.5. Variations of bacterial abundance and maximum enzymatic reaction rate (ν_m) during incubations in bulk and spiked sediments.

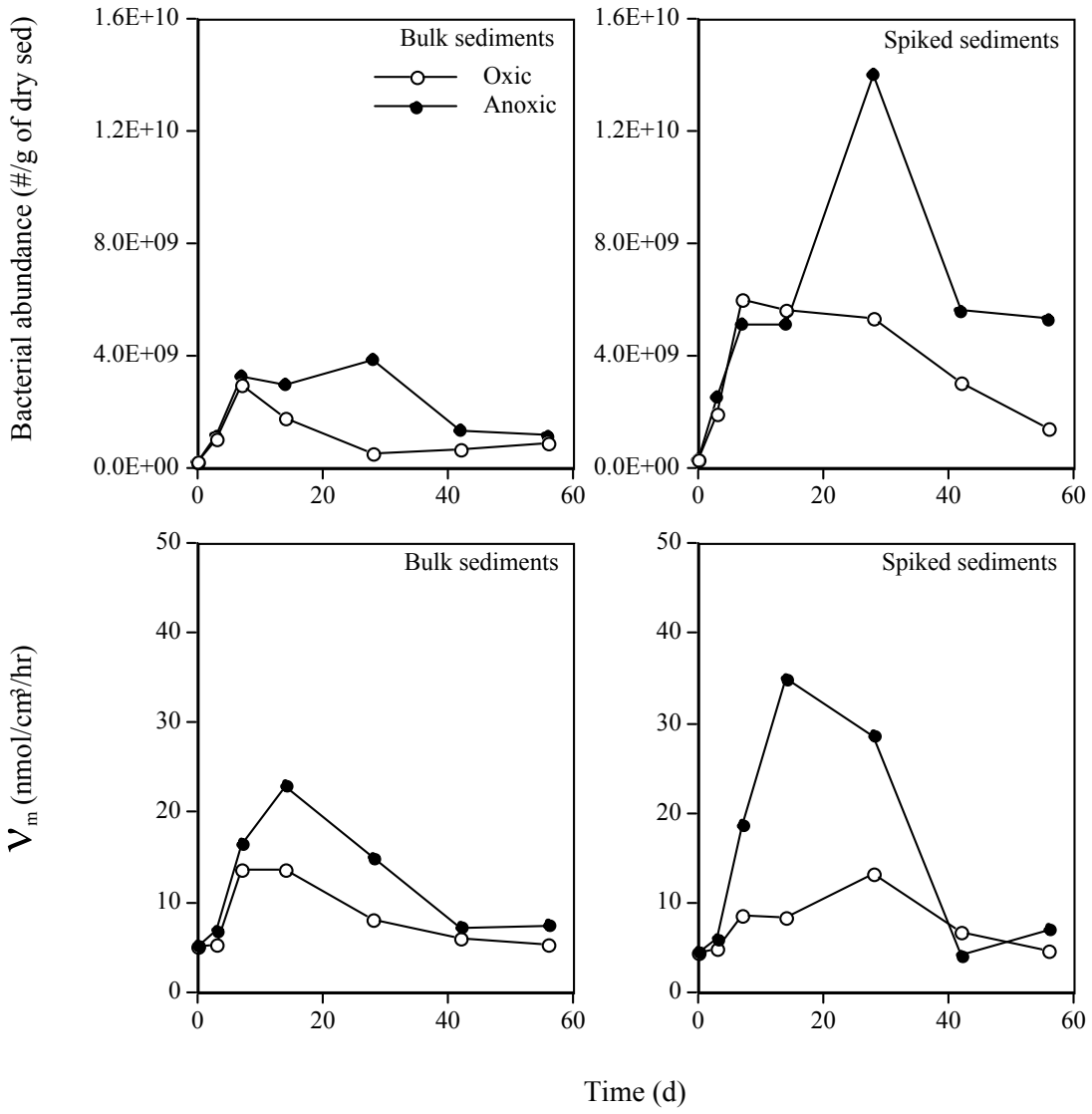
Bacterial abundance (#/g of dry sed)
1.6E+10
1.2E+10
8.0E+09
4.0E+09
0.0E+00
Bulk sediments
Oxic
Anoxic
Spiked sediments
0
20
40
60
V_m (nmol/cm³/hr)
50
40
30
20
10
0
Time (d)

Fig. 3.5

Fig. 3.6. Variations of total bacterial specific fatty acid concentrations (sum of iso-15:0; anteiso-15:0 and 18:1(ω7)) vs. bacterial abundance in bulk and spiked sediments (intracellular and membrane components). Note that the initial (t = 0) data were not connected to others.

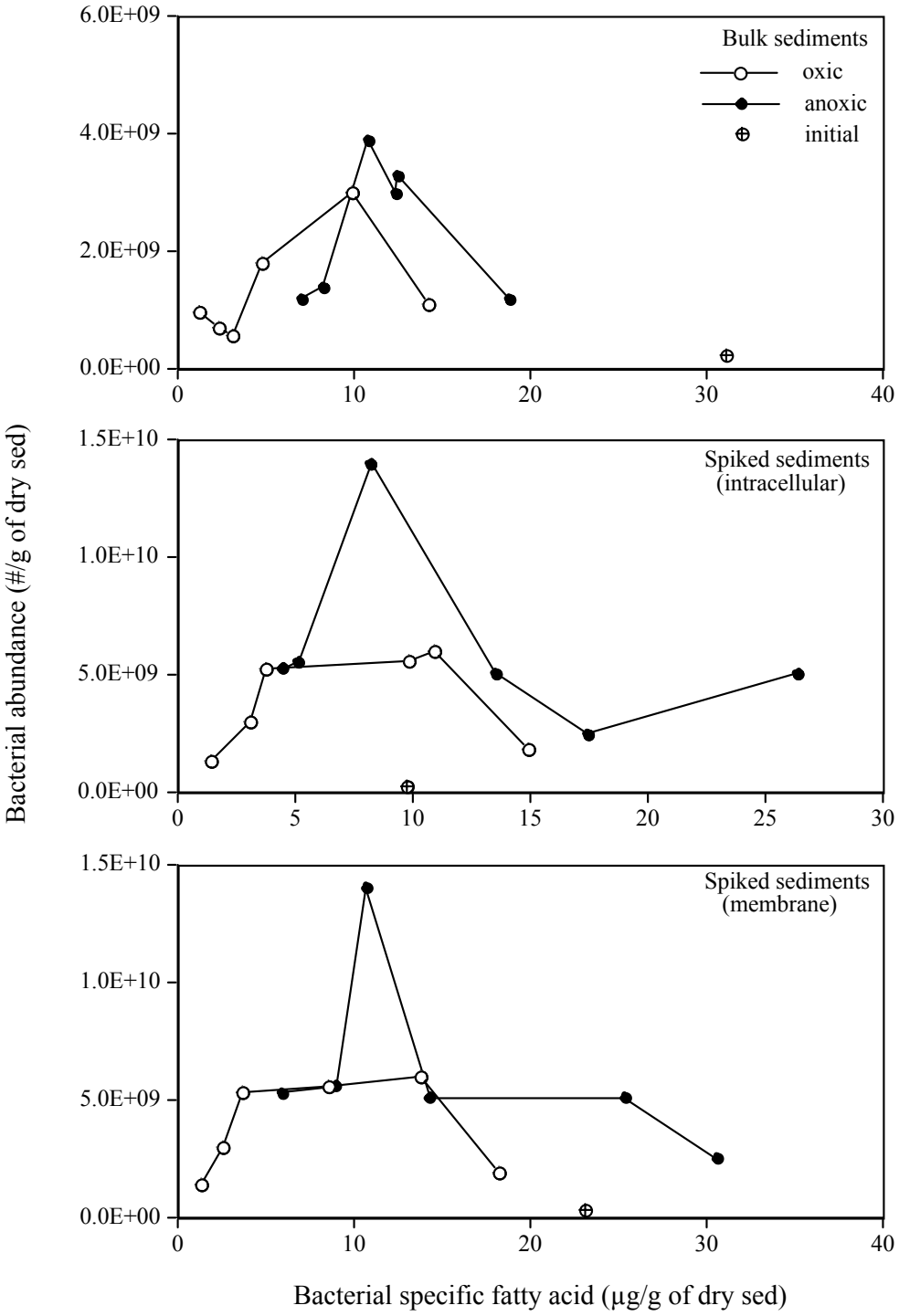
Bulk sediments
oxic
anoxic
initial
Spiked sediments
(intracellular)
Spiked sediments
(membrane)
Bacterial abundance (#/g of dry sed)
Bacterial specific fatty acid (µg/g of dry sed)
6.0E+09
4.0E+09
2.0E+09
0.0E+00
1.5E+10
1.0E+10
5.0E+09
0
5
10
15
20
25
30
40

Fig. 3.6.

CHAPTER 4

DIFFERENTIAL EFFECTS OF BENTHIC MACROFAUNAL ACTIVITIES ON FATES OF ALGAL FATTY ACIDS AND ALKENONES IN COASTAL MARINE SEDIMENTS[1]

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Abstract

Incubation experiments were conducted to specifically examine the effects of one species of benthic crustacean, *Palaemonetes pugio*, on degradation of various algal lipids in coastal marine sediments. The laboratory microcosm systems consisted of pre-sieved sediment cores with overlying seawater. *Emiliania huxleyi* (marine haptophyte) cells were added to the systems as a simulation of phytoplankton deposition after a water column bloom. The crustaceans were present in two sets of cores (duplicate) while another set of cores without animals was used as control. Activities of macrofauna, transport of surface sediments, and degradation of algal lipids were followed during six-week incubations. The experimental results showed that algal lipids were rapidly lost from the surface (0-0.5 cm) sediments, where the added algal material was initially present, at a faster rate in animal cores than in control cores. This fast loss was largely attributed to grazing and downward transport by crustaceans and biochemical degradation by microbial processes. Integration of algal lipids along the surface and subsurface of sediment core and in suspended particles showed that crustacean's activities caused differential degradations for alkenones and fatty acids derived from the same algal material. The crustacean enhanced degradation of algal fatty acids (2-4x faster) but had less impact on alkenone degradation (<1.5x) compared to the controls. Enrichment of alkenones and depletion of algal fatty acids observed in suspended particles during the first week indicated that the crustacean could selectively graze the algal material from sediments and preferentially assimilate fatty acids over alkenones during digestion. Unlike algal fatty acids, degradation of alkenones was largely controlled by microbial processes rather than by crustacean's grazing. With the presence of crustacean, the degradation rate constants of intracellular and membrane fatty acids were similar, implying that the crustacean's activities minimized the effects of structural association on fatty

acid decomposition, which was noticeable in control cores (microbial processes dominated). On the other hand, the 37:3 and 37:2 alkenones degraded at similar rates in both crustacean and control cores, implying that the $U_{37}^{k'}$ index (an important paleotemperature indicator) might not be altered by microbial processes or crustacean's activities.

Keywords: *Palaemonetes pugio*, *Emiliania huxleyi*, lipid degradation, alkenones, algal fatty acids, membrane and intracellular fatty acids, bacteria-specific fatty acids, $U_{37}^{k'}$ index

Introduction

Global carbon cycling is one of major processes influencing climate change (Berger and Keir, 1984; Ikeda and Tajika, 2002). Degradation of organic matter in marine sediments plays an important role in controlling preservation of organic carbon (Hedges and Keil, 1995; Ogrinc et al., 2003). Many previous studies have demonstrated that benthic macrofauna significantly affect the fates of organic matter in marine sediments by a number of ways: burrowing, irrigation, reworking, direct grazing and controlling microbial community (Aller, 1982, 1988, 1994; Rice, 1986; Kristensen, 1988, 1992; Green et al., 1992, 2002; Goñi-Urriza et al., 1999).

Construction of burrows in sediments influences redox condition, solute transports, and substrate metabolism (Kristensen et al., 1985; Webb and Eyre, 2004). Irrigation accelerates the exchange between sediment pore water and overlying seawater, leading to an increase of the flux of dissolved oxygen into the sediments (Stamhuis and Videler, 1998; Ferro et al., 2003; Webb and Eyre, 2004). Reworking causes upward or downward transport of particles through oxic/anoxic boundary, creating an oscillating redox regime in sediments (Aller, 1994; Canfield, 1994; Sun et al., 1999, 2002a; Caradec et al., 2004). The alternative changes of redox conditions significantly influences degradation and preservation of organic matter in marine sediment (Sun et al., 1993; 2002b; Aller, 1994). Benthic macrofauna also greatly enhance organic matter degradation in sediment by direct digestion and by release of extracellular enzymes (Bock and Miller, 1997; Cohen and Pechenik, 1999; Hansen and Josefson, 2004). To feed efficiently, macrofauna can selectively ingest organic detritus (Aller, 1982; Lopez and Levinton, 1987; Graf, 1989; Clough et al., 1997; Hansen and Kristensen, 1998; Gribsholt and Kristensen, 2002). On the other hand, macrofauna influence organic matter degradation by controlling activities of microbial community. For example, presence of benthic animals increases substrate availability

for bacteria by fragmentation, which breaks large particles into small particles and provides more surface area for bacterial colonization (Daumas and Bianchi, 1984; Goñi-Urrize et al., 1999). Therefore, the consequence of fragmentation is to increase bacterial density and activities (Daumas and Bianchi, 1984; Reichardt et al., 1991; Goñi-Urrize et al., 1999). In addition, macrofauna are capable of stimulating bacterial growth by exerting a grazing pressure (Plante et al., 1990). Although the effects of macrofaunal activities on organic matter degradation and preservation have been extensively examined (Stamhuis and Videler, 1998; Ferro et al., 2003; Webb and Eyre, 2004), few studies focus on the differential influences of specific macrofuanal activities on the fates of different organic biomarkers in marine sediments.

Lipids account for 5 to 20% of organic carbon in phytoplankton (Parsons et al., 1961) and have been widely used as biomarkers to study carbon cycling and paleoceanography (Brassell et al., 1986; Wakeham et al., 1997; Grossi et al., 2001, 2003). For example, many studies have determined carbon flow between estuarine, coastal and oceanic systems by following the distribution, transport and degradation of specific lipid compounds (Canuel et al., 1996; Derieux et al., 1998; Mannino and Harvey, 1999; Bac et al, 2003). Some studies have used specific lipid compounds to track origins of dissolved and particulate organic matter (DOM and POM) (Volkman, 1986; Harvey, 1994; Jaffe et al., 1995; Mannino and Harvey, 1999) and to characterize microbial community (Harvey et al., 1986; Rajendran et al., 1997; Green and Scow, 2000; Wakeham et al., 2003). In paleoceanography studies, long chain unsaturated alkenones produced by haptophyte have been widely used to reconstruct ancient sea surface temperature (Volkman et al., 1980; Brassell et al., 1986; Prahl and Wakeham, 1987; Villanueva et al., 2002). However, there has been a controversial discussion about the effect of diagenetic processes, especially the role of benthic macrofauna, on the stability of the $U_{37}^{k'}$ index [$U_{37}^{k'} = C_{37:2}/(C_{37:2} +$

$C_{37:3}$)] ($C_{37:2}$ and $C_{37:3}$ are concentrations of 37:2 and 37:3 alkenones in sediment or water samples). Gong and Hollander (1999) found that the differential degradation of two alkenones in oxic sediments with strong bioturbation could cause a significant shift of the $U_{37}^{k'}$. However, evidence from grazing experiments showed that zooplankton herbivory in water column did not alter the $U_{37}^{k'}$ index (Grice et al., 1998). More laboratory experiments (Teece et al., 1998; Sun et al., 2004) indicated that aerobic and anaerobic degradation of alkenones in seawater and sediment systems by microbial processes did not significantly change the $U_{37}^{k'}$ index. However, it is unclear how the presence of specific benthic macrofauna in surface sediments affects the degradation of different lipid compounds derived from algal material.

Lipid compounds exist in different compartments within algal cells and associate with different molecular matrixes. For example, algal fatty acids are mostly bound in triacylglycerols (intracellular tissue) and in phospholipids (algal membrane). It was also observed that alkenones are biosynthesized as different components (metabolic storage vs. membrane) at different growth stages (Prahl et al., 1988; Brassell, 1993; Bell and Pond, 1996; Pond and Harris, 1996). Our recent study (Ding and Sun, 2004) indicated that algal fatty acids bound in membrane and intracellular components degraded differently by microbial processes, which might be caused by different structural associations within algal cells. However, it remains unknown what benthic macrofauna can do to break different structural associations of various lipids within organic matter matrixes.

The present study was designed to investigate the effects of a specific benthic crustacean species, *Palaemonetes pugio* (*P. Pugio*), on the fates of two classes of lipids (fatty acids and alkenones) derived form a typical haptophyte alga (*E. huxleyi*) in coastal marine sediments. *P. pugio* abundantly inhabits in estuaries along the Atlantic and Gulf coasts (Williams, 1984;

Reinsel et al., 2001) and feeds a wide variety of aquatic foods, including macrophytes, microalgae, meiofauna and fungi (Odum and Heald, 1972; Gore et al., 1981; Chambers, 1981; Reinsel et al., 2001). As a sediment bioturbator, *P. pugio* intensively resuspends surface sediments through its feeding, swimming and crawling activities (Shenker and Dean., 1979). In our laboratory experiments, microcosms consisted of sediment cores with and without the presence of *P. pugio*. *E. huxleyi* cells were added into the microcosms as a simulation of deposited phytoplankton after a water column bloom. The concentration variations of four major algal fatty acids [14:0, 16:0, 18:1(ω9) and 22:6] and four alkenones (37:3, 37:2, 38:3 and 38:2) were followed in suspended particles in the overlying water and along sediment depth to estimate the degradation rate constants of individual lipids. The effects of macrofaunal activities on degradation of fatty acids bound in different structural associations were examined by separating fatty acids into intracellular and membrane components. Variations of bacteria-specific fatty acids [iso-15:0, anteiso-15:0 and 18:1(ω7)] in all microcosm cores were determined to examine the influence of macrofaunal activities on microbial community. The $U_{37}^{k'}$ index was calculated based on the changes in 37:3 and 37:2 alkenones in suspended particles and in surface sediments to test if the benthic processes can alter the paleoceanographic signals.

Experimental

Materials. Seawater, sediment, and marcofauna (*P. pugio*) used in this study were collected in March 2002 from Georgia coast, USA. A box corer (surface area ~ 0.04 m^2) was used to collect sediment from a site in Doboy Sound near Sapelo Island. The top 0-2 cm and lower (< 2 cm) depth sediments were separately scraped from the box core as surficial and subsurficial sediments. Both surficial and subsurficial sediments were passed through a 0.5 mm

sieve respectively to remove macrobenthos, large shells and detritus for incubation experiments. The solid particle density was measured by drying 5 ml sieved wet sediment at 100°C for 24 h. Seawater (salinity ~28‰) used in the experiments was obtained from the same site by pumping the water through a set of filters (25 μm). *P. pugio* (1.5-2 cm in length) used in the experiments was collected from an estuarine site near Skidaway Island.

Marine haptophyte *E. huxleyi* (clone CCMP1949) was obtained from Provasoli-Guillard National Center, Booth Bay Harbor, Maine, USA. It was cultured in f/50 medium using a 12:12 light/dark cycle at a mean temperature of 14°C with continuous growth mode. The culture was started in 50 ml medium and transferred to 250 ml new medium in ten days (close to the end of exponential phase in its growth curve). The culture was then transferred to final 1000 ml medium after another 10 days. The dilution rates during entire culture period were ~0.5/d. The algal cells in final 1000 ml medium were harvested in ten days by centrifugation and the culturing was repeated several times to obtain enough algal cells for the experiments. All harvested algal cells were stored frozen at -20°C for later incubation experiments.

Microcosm setup and sampling. Incubations were conducted in microcosms made up by 20 cylindrical PVC core liners (i.d. 7 cm, length 30cm) with the bottom sealed. The pre-sieved sediments were filled into the liners: bottom 15 cm with the subsurficial sediments and the upper 3 cm with the surficial sediments collected from the field. About 320 ml filtered seawater was gently added into each core to create overlying water column. *E. huxleyi* cells (400 mg wet materials) were equally spiked into the overlying water of each core. After settling of algal cells (~30 min), two cores were immediately sacrificed as an initial starting point (t = 0 day). The crustaceans were added into 12 cores (2 animals in each) while other six cores were used as control (without crustaceans). During incubations, the overlying seawater in all cores was

continuously purged with air to keep the overlying water oxygenated. Overlying seawater in each core was changed weekly with fresh seawater to avoid excessive accumulation of harmful metabolites such as NH_4^+. Preliminary experiments indicated that the crustaceans could be alive more than 7 weeks in the microcosm system. All set-up cores were incubated in a temperature-controlled incubator in the dark at 20°C (same as *in situ* temperature in field where the crustacean were collected). At each sampling time (4, 7, 14, 21, 28 and 42 days), one control and two crustacean cores (as duplicate) were taken out from the incubator. The overlying water in each core was gently sucked out. For crustacean cores, the suspended particles in the overlying water were collected by centrifugation (4000 rpm). The total mass of suspended particles in each core was determined by weighing. All living crustaceans were removed from the sediments and stored frozen. Sediments in each core was extruded and sliced into 0-0.5, 0.5-1, 1-2, 2-3, 3-4, 4-5, 5-7 and >7 cm depth intervals. About 0.5 g of suspended particles and sediments from each sampling interval were dried at 100°C for 24 h to estimate water content and porosity. The rest sediments will be frozen at -40°C for lipid analysis.

Extraction and separation of lipids. Lipid extraction and separation were based on published procedures (Sun et al., 1997, 2000; Ding and Sun, 2004). About 1-2g thawed suspended particles and sediment samples were first extracted with 10 ml methanol, followed by 3×10 ml methylene chloride:methanol (2:1 v/v) extraction. During each extraction, the samples were sonicated for 10 min in a water bath. The combined extracts were partitioned into a methylene chloride phase by addition of 5% NaCl solution. After separation, organic solvent was removed by a rotary evaporator. The lipids were redissolved in 2 ml hexane and equally split into two parts. One part was dried again and saponifed using 0.5 mol/L KOH in $MeOH/H_2O$ at 100°C. Total neutral lipids and fatty acids were separately extracted from the

solution under different pH conditions (pH>13 for neutral and pH<2 for acid). Another part of the original extract was further separated into 3 fractions (hydrocarbons, intracellular and membrane components) via Si-gel column chromatography (Ding and Sun, 2004). Si-gel (EM Science, 63-200 mesh) was cleaned by washing with organic solvents (hexane and methylene chloride) and combustion at high temperature (450°C). The cleaned Si-gel was deactivated by addition of water (5%) before loading into the column (~ 1 g/column). The lipid extracts were eluted by following solvents: (1) 20 ml hexane; (2) 10 ml 5% ethyl acetate/hexane; (3) 10 ml 25% ethyl acetate/hexane; (4) 10 ml 50% ethyl acetate/hexane; (5) 10 ml ethyl acetate; (6) 20 ml methanol; and (7) 20 ml methylene chloride. Elution (1) was collected as hydrocarbons. Elutions (2)-(5) were combined as intracellular (non-membrane) lipids and (6)-(7) elutions as membrane lipids, based on the elution of two standards (tripalmitin and L-α-phosphatidylcholine-β-oleoyl-γ-myristoyl). Intracellular and membrane fractions were then saponified with KOH in MeOH/H_2O to separate neutral and acidic lipids. Neutral lipids were extracted from the basic solution (pH>13) and fatty acids were extracted from acidic solution after addition of HCl (pH<2). Neutral lipids were treated with N,-bis(trimethylsilyl)trifluoroacetamide (BSTFA) in actonitrile at 100°C, and the alcohols and sterols in the extract were converted to TMS(trimethylsily)-ethers. Fatty acids in another extract were methylated with BF_3-methanol to form FAMEs.

Lipid analysis. Neutral lipids (alkenones and TMS-ethers) and FAMEs were analyzed by capillary gas chromatography using a Hewlett-Packard 6890 GC system with an on-column injector and a flame ionization detector. Lipid compounds were separated by a 30 m×0.25 mm i.d. column coated with 5%-diphenyl-95%-dimethylsiloxane copolymer (HP-5, Hewlett-Packard). The operation temperature program was: 50-170ºC at 20ºC/min, followed by 170-

310ºC at 4ºC/min and held isothermally at the final temperatures for 5 min (for FAMEs) and for 35 min (for neutral lipids). Internal standards [5 α(H)-cholestan for neutral lipids and nonadecanoic acid methyl ester for FAMEs] were respectively added to samples prior to GC analysis to aid in quantification. The initial samples (t = 0) were selected for lipid identification by a SHIMADZU QP5000 GC-MS system using a 30 m×0.25 mm i.d. column coated with 5% phenyl methyl silicone (XTI-5, Restek). Helium was used as carrier gas. Operating conditions of the GC-MS were: mass range 50-610 amu with a 0.4 s scan interval; 70 eV ionizing energy; GC temperature program 50-150ºC at 20ºC/min followed by 150-310ºC at 4ºC/min and a 35 min hold for neutral lipids and a 5 min hold for FAMES at 310ºC.

Results

Lipid compositions in bulk and spiked sediments. At initial time (t = 0), the top 0-0.5 cm sediment received sinking *E. hyxleyi* cells from the overlying water. Thus, the top sediment was considered as spiked sediments, while the sediments between 0.5 and 3 cm was treated as bulk sediments. In the bulk sediments, 17 fatty acids were found but alknones were absent (Fig. 4.1). In the spiked sediments, four major alkenones (37:3, 37:2, 38:3, and 38:2) occurred as neutral lipids, so they were exclusive biomarkers from algal organic matter. Compared to the bulk sediments, the concentrations of 14:0, 16:0 and 18:1(ω9) fatty acids in the spiked sediments increased more than 3-fold, 2-fold and 5-fold respectively, and 22:6 fatty acid occurred as a unique component exclusively from the algal material. It was observed that these fatty acids were major fatty acid components in the *E. huxleyi* cells (Ding and Sun, 2004). Thus, these four fatty acids were chosen as typical algal components with distinct structural features (saturated, monounsaturated, and polyunsaturated). At the initial time, the concentrations of three typical

bacteria-specific fatty acids [iso-15:0, anteiso-15:0 and 18:1(ω7)] in both bulk and spiked sediments were the same, implying that our axenic culture had little bacterial input. However, the bacteria-specific fatty acids varied during incubations, which were useful for assessment of microbial processes in different microcosms.

Variations of alkenone and algal fatty acids in surface (0-0.5 cm) sediment during incubations. Since the added algal cells were settled on the sediment-water interface from the overlying water, the concentrations of algae-derived lipids (alkenones and algal fatty acids) were much higher in the top 0-0.5 cm of the cores than the bulk concentrations below 0.5 cm at the beginning of the experiments. During incubations, the concentrations of all algal lipids in the surface sediments decreased continuously in both control and crustacean cores (Fig. 4.2). The general pattern is a faster decrease in the first week and followed by a slow decrease. There are clear differences in concentrations of algal lipids between control and crustacean cores. With the presence of crustaceans, algal lipids disappeared from the surface sediments (0-0.5 cm) faster and more completely. However, larger differences for alkenones between two treatments were observed compared to those for algal fatty acids. For unsaturated fatty acid [18:1(ω9) and 22:6], the differences between control and crustacean cores were markedly minimized.

Variations of alkenones and algal fatty acids in suspended particles during incubations. In crustacean cores, a lot of particles were resuspended in the overlying water since the beginning of incubations. The concentrations of algal lipids in the suspended particles varied during incubations (Fig. 4.3). At the first sampling time (4 days), the concentrations of all lipids in the suspended particles were highest in the course of incubations. When the concentrations of algal lipids in the suspended particles were compared with the initial concentrations (dotted line in Fig. 3) in top 0-0.5 cm sediments, all alkenones were enriched while all fatty acids were

apparently depleted in the suspended particles. Meanwhile, the concentration ratios of alkenones in surface sediments between initial and 4 days were apparently higher than those of algal fatty acids (Table 4.1). After the first week, the concentrations of all lipids in the suspended particles decreased with time. In the control cores, no suspended particles were observed in the overlying water.

Vertical distributions of alkenones and algal fatty acids in sediments during incubations. Vertical distributions of algal lipids in the experimental cores varied during incubations (Fig. 4.4). Different patterns were observed for alkenones (37:3 and 37:2) and fatty acids [16:0 and 18:1(ω9)]. At the beginning of incubations, alkenones were present only at the top surface (0-0.5 cm) sediments. In the control cores, the concentrations of alkenones in the surface sediments decreased with time but no alkenones were observed in the subsurface sediment below 0.5 cm during incubations. In contrast, in the crustacean cores, some alkenones were moved down to 2 cm in the first week. After that, the concentrations of alkenones in all depths between 0 and 3 cm decreased with time. On the other hand, a small and uniform background concentration was observed for fatty acids at the beginning of the experiments. During incubations, the fatty acid concentrations in the top 0-0.5 cm sediments decreased rapidly. However, in the subsurface (0.5-3 cm), the fatty acid concentrations in the crustacean cores were lower than those in the control cores. The background concentrations of fatty acids in the control cores gradually decreased with time.

Overall variations of alkenones and algal fatty acids in microcosm systems. To track the ultimate fates of algal lipids in the microcosms with presence of crustacean, overall amounts of various lipids in suspended particles, in surface 0-0.5 cm, and in subsurface 0.5-3 cm were integrated. The amount in suspended particles was estimated by multiplying the lipid

concentration (μg/g dry sed) with total dry particles collected from the overlying water at each sampling time. The amount in top surface was determined based on the lipid concentration and total sediment in this layer. The amount in subsurface 0.5-3 cm was integrated the lipids in each depth intervals. In the control cores, total amounts of alkenones were those in the surface (0-0.5 cm) sediments while total amounts of fatty acids included a fraction from the background. Variations of total amounts of individual lipid compounds in crustacean cores were similar to the control (Fig. 4.5 and Fig. 4.6). However, the variations in each fraction of crustacean cores showed different patterns for alkenones and fatty acids. For example, a relatively larger proportion of alkenones than algal fatty acids were found in suspended particles. In the subsurface (0.5-3 cm) of crustacean cores, there were significant amounts of alkenones while no alkenones were observed in subsurface of control cores. However, the background fatty acids in subsurface sediments of control cores were more than those in crustacean cores. At the end of experiments, the total amounts of all lipid compounds were lower in the crustacean cores than those in the control cores, although the distributions in three fractions might vary differently with individual compounds.

Variations of intracellular and membrane fatty acids during incubations. More fatty acids exist in *E. huxleyi* cells as membrane than as intracellular component, and the initial ratios of various fatty acids in membrane to intracellular components ranged from 1.4 to 3.4 (Fig. 4.7). During incubations, concentrations of fatty acids in membrane and intracellular components decreased continuously (Fig. 4.7). In the crustacean cores, however, fatty acids in each structural component degraded apparently faster and more completely than in the control cores. Only exception is the case of polyunsaturated 22:6 fatty acid in membrane component, where 22:6 in

both control and crustacean cores degraded at similar rates and disappeared completely within three weeks.

Overall variations of bacteria-specific fatty acids in microcosm systems. Overall amounts of three bacteria-specific fatty acids [iso-15:0, anteiso-15:0 and 18:1(ω7)] in suspended particles, surface and subsurface sediment gradually decreased during incubations (Fig. 4.8). However, variations of bacteria-specific fatty acids in different fractions were somewhat different between control and crustacean cores. For example, the bacteria-specific fatty acids in the surface sediments (0-0.5 cm) of control cores varied slightly during incubations but these fatty acids in suspended and surface fractions of crustacean cores increased in the first week and then decreased until the end of incubations. On the other hand, the bacteria-specific fatty acids integrated in subsurface (0.5-3 cm) of crustacean cores were generally less than those integrated in the subsurface of control cores.

Variations of $U_{37}^{k'}$ index during incubations. The values of $U_{37}^{k'}$ measured from suspended particles and surface sediments in control and crustacean cores varied slightly during incubations (Fig. 4.9). The fluctuation ranges were 0.483-0.549 for surface sediments of control cores, 0.485-0.552 for surface sediments of crustacean cores, and 0.477-0.536 for suspended particles of crustacean cores respectively. Based on the calibration ($U_{37}^{k'} = 0.039 + 0.034T$, Prahl and Wakeham, 1987; Prahl et al., 1988), these $U_{37}^{k'}$ values gave a temperature range of 13 to 15ºC, which were consistent with the temperature of the culturing (14ºC).

Discussion

Processes controlling the distributions of algal lipids in microcosm systems. There were apparent differences in distributions of algal lipids between control and crustacean cores

during incubations. In control cores (without macrofauna), most algal lipids were present in the surface (0-0.5 cm) sediments because the added algal cells deposited on the sediment-water interface from the overlying water and no mixing occurred in the sediments. In contrast, in crustacean cores, algal lipids were observed in suspended particles, surface (0-0.5 cm) and subsurface (0.5-3 cm) sediments. Obviously, activities of crustacean were responsible for the different distributions of algal lipids in two microcosm systems.

The crustacean, *P. pugio*, is typically a deposit-feeder (Shenker and Dean, 1979; Reinsel et al., 2001). Using its chelate pereiopods, *P. pugio* grasps and carries food particles to its mouth (Kneib, 1995). Although the food sources for the crustacean are diverse, including detritus, algae, macrophyte, and meiofauna, the grazing efficiencies vary greatly with the quality of food materials (Morgan, 1980; Fleeger et al., 1999). The capability of selective grazing helps crustacean to feed food materials more efficiently (Odum and Heald, 1972). When the crustacean ingests microalgae-rich particles, some particles were ejected into overlying water as fecal materials, which is one way to resuspend the surface sediments (Morgan, 1980). The crustacean also crawls on the sediment surface and directly uplifts small particles into overlying water, which elicits sizable resuspension (Graf and Rosenberg, 1997). Thus, these processes transport fresh and digested algal materials from the surface sediments to the overlying water with other sediment particles.

On the other hand, *P. pugio* is a strong sediment bioturbator (Williams, 1984). Although the crustacean does not build burrows, sometimes it buries itself in sediments (Shenker and Dean, 1979). The frequent movement of this crustacean reworks sediments and transports surface particles including algal material from the sediment-water interface to subsurface sediments. This downward transport was clearly seen in the crustacean cores by occurrence of

alkenones in subsurface sediments during incubations since the alkenones were exclusively from the added algal material, which was initially absent in the subsurface sediments. However, it seems to be contradictory for the downward transport that less algal fatty acids were present in the subsurface sediments of crustacean cores than those in control cores during incubations. The reason for this may be due to a difference in degradation behaviors between different lipid compounds in surface sediments.

Besides transport processes, the distributions of algal lipids in the microcosms were also affected by biochemical degradation. In control cores, most algal lipids degraded in the surface sediments, which was likely mediated by microbial processes. In the crustacean cores, degradation of algal lipids might be driven by both macrofaunal activities and microbial processes. It was observed that alkenones and fatty acids derived from *E. huxleyi* cells degraded at similar rates in oxic and anoxic seawaters, where only microbial processes were responsible for the degradation (Sun et al., 2004). However, crustacean's grazing may cause differential degradations for different algal lipids. For example, algal fatty acids, especially polyunsaturated ones, were largely assimilated by crustaceans (Harvey et al., 1987) while most alkenones in diet material were egested in fecal material (Grice et al., 1998). Therefore, the occurrence of algal lipids in the subsurface sediments of crustacean cores may depend on the relative rates of transport vs. degradation of individual compounds.

Effects of crustacean's grazing on degradation of alkenones and algal fatty acids. In the first week of incubations, alkenones in the suspended particles of crustacean cores were enriched but algal fatty acids were largely depleted relative to the initial concentrations in the surface sediments (Fig. 4.3 and Table 4. 1). The enrichment and depletion of different lipids in the suspended particles provides a direct evidence of differential effects of crustacean's grazing.

On the other hand, a higher proportion of alkenones than algal fatty acids was transported down to subsurface sediments (Fig. 4.4), which might be partly affected by differential degradation in the surface sediments. To examine the differential effects of crustacean's activities on overall degradations of alkenones and algal fatty acids in the experimental microcosms, the variations of individual lipid compounds in three fractions (suspended particles, surface and subsurface sediments) were followed to estimate degradation rate constants.

Considering kinetic features of lipid degradation (a faster degradation in the first week and followed by a slow degradation), a two-pool model (Westrich and Berner, 1984) was used to fit the original data:

$$G_t = (G_1)_0 \exp(-k_1 t) + (G_2)_0 \exp(-k_2 t) \quad (1)$$

where, G_t, $(G_1)_0$, and $(G_2)_0$, are total amount of lipid compounds during incubations, amount in initial pool 1 and amount in initial pool 2 respectively, and k_1 and k_2 are the first-order degradation rate constants for pool 1 and pool 2 respectively. The initial pool sizes were determined by plotting ln (G_t) vs. time (t), and k_1 and k_2 were estimated based on the equation (1). In order to directly compare overall degradation rates of lipids between control and crustacean microcosms with variable pool size, k_1 and k_2 were combined into a mean constant based on the following equation:

$$k = f_1 k_1 + f_2 k_2 \quad (2)$$

where, f_1 is the proportion of pool 1 to total (G_1/G_t) and f_2 is the proportion of pool 2 to total (G_2/G_t). For algal fatty acids, a subtraction of background concentration was made, which minimized the influence from fatty acids in bulk sediments.

A comparison of degradation rate constants of alkenones and algal fatty acids between control and crustacean cores (Table 4.2) showed that crustacean's activities played a more

important influence on fatty acid degradation than on alkenone degradation. The ratios of k_p (rate constant in *P. pugio* core) to k_c (rate constant in control core) for alkenones are 1.2-1.4 while those ratios for algal fatty acids are in a larger range of 1.9-4.9. The difference in the rate constant ratios between two classes of lipids is presumably caused by differential effects of grazing activities of crustacean because the rate constants of alkenones and algal fatty acids (especially saturated ones) in control cores are similar. During grazing processes, more algal fatty acids are assimilated, implied by depletion of fatty acids in the suspended particles. Enrichment of alkenones in suspended particles indicates that crustacean can selectively graze the fresh algal materials from the surface sediments but cannot assimilate alkenones during digestion. Therefore, the degradation of alkenones might be largely controlled by microbial processes, which dominated in the control cores. Similar differential effects of grazing by a bivalve deposit feeder (*Yoldia limatula*) on algal fatty acids and phytol were observed in other studies (Sun et al., 1999).

It is interesting to note that degradation rate constants of unsaturated fatty acids are apparently higher than those of saturated ones in control cores but they are similar in crustacean cores. Previous field observations (Farrington et al., 1977; Haddad et al., 1992; Meyers and Eadie, 1993) and laboratory experiments (Harvey and Macko, 1997; Sun et al., 1997; Grossi et al., 2003) have demonstrated that unsaturated (especially polyunsaturated) fatty acids generally degrade faster than saturated fatty acids. However, with presence of crustacean, the effect of unsaturation on fatty acid degradation was minimized or eliminated. It is again resulted from crustacean's grazing and assimilation processes. Although many animals preferentially assimilate dietary polyunsaturated fatty acids over other saturated fatty acids, some animals (e.g., annelids and bivalves) assimilate all fatty acids to a high degree (Tanoue et al., 1982; Harvey et

al., 1987; Bradshaw et al., 1990; 1991; Sun et al., 1999). It was suggested that the extent of assimilation of unsaturated and saturated fatty acids was dependent on the total quantity of algal material ingested (Bradshaw and Eglinton, 1993). In our experiments, *P. pugio* appeared to efficiently assimilate all fatty acids to a high degree, resulting in a large depletion of all fatty acids in suspended particles.

Influences of crustacean's activities on structural associations of fatty acids. Fatty acids are mostly associated in different molecular complexes such as triacylglycerols and phospholipids within algal cells (Voet and Voet, 1995). These complexes are present as membrane and intracellular components, which have different molecular size, structural linkage, polarity, and chemical lability. Our recent studies (Ding and Sun, 2004) found that microbial degradation of fatty acids bound in cell membrane was faster than that bound in intracellular components, especially in anoxic sediment-water interface system. In present study, fatty acids were degraded by microbial processes in the control cores while their degradation in crustacean cores was dominated by crustacean's activities. To examine the influences of crustacean's grazing on the degradation of different structural associations, the degradation rate constants of membrane and intracellular fatty acids were estimated using the same two-pool model (Table 4. 3).

A comparison of rate constants of membrane and intracellular fatty acids between control and crustacean cores showed that the rate constants from crustacean cores were markedly (2-6x) higher than those from control cores, implying that crustacean's activities accelerated the degradation of both membrane and intracellular fatty acids. On the other hand, the ratios of rate constants of membrane to intracellular fatty acids in control cores ranged from 1.4 to 2.3 for different compounds. However, the rate constant ratios of membrane to intracellular fatty acids

in crustacean cores were close to 1 in all cases. These results suggested that crustacean's activities minimized the effects of structural associations on fatty acid degradation. As discussed above, grazing and assimilation by crustaceans are major processes degrading algal fatty acids. To digest algal fatty acids bound in different structural complexes during passage of particles through the gut, crustacean might produce various enzymes such as thiokinase, dehydrogenase, lipase, and phospholipase (Voet and Voet, 1995). These enzymes release fatty acids from various ester complexes, which is the first and limiting step for fatty acid degradation, no matter how fatty acids are bound within algal cells. In contrast, bacteria in the sediments might produce limited enzymes, which would be influenced by a variety of environmental factors (Jaeger et al., 1994; Arnosti, 2004). In addition, bacteria-produced enzymes are generally very specific, which might hydrolyze some esters but not all ester complexes in algal cells (Finnerty, 1989; Jaeger et al., 1999).

Influences of crustacean's activities on microbial processes. Bacteria play different roles in organic matter cycling either as decomposer to remineralize organic carbon to CO_2 or as producer to synthesize new organic biomass (Pomeroy, 1974; Azam et al., 1983; Kemp, 1990). Presence of benthic macrofauna has dual influences on microbial activities. A positive influence is to stimulate rapid growth of microbial populations (Plante et a., 1990) while a negative influence is to reduce bacterial abundance by direct ingestion (Newell, 1965; Fenchel, 1970; Wetzel, 1976; Lopez et al., 1977; Cammen, 1980). On the other hand, bacterial abundance is also dependent on availability of labile substrate. Many studies have demonstrated that an input of fresh organic matter always results in an increase of bacterial abundance (Danovaro et al., 1994; Zweifel et al., 1996). During growth, bacteria synthesize a series of specific lipids such as branched and monounsaturated fatty acids [e.g., iso-15:0, anteiso-15:0, and 18:1(ω7), Parkers

and Taylor, 1983; Kaneda, 1991]. Thus, the variations of these bacteria-specific fatty acids in our microcosms might provide insight into the influences of crustacean's activities on microbial processes.

A general variation pattern of bacteria-specific fatty acids observed during incubations is a continuous decrease with time (Fig. 4.8). However, relatively higher amounts of bacteria-specific fatty acids were present in control cores than in crustacean cores (integrated from suspended particles, surface and subsurface sediments), implying that crustacean's grazing exerted a high pressure on microbial community. In the first two weeks of incubations, a noticeable amount of bacteria-specific fatty acids was observed in suspended particles, which might be caused by rapid colonization of bacteria on freshly egested feces (Pomeroy and Deibel, 1980; Jacobsen and Azam, 1984) or from enteric microbial production of ingested algal material (Sochard et al., 1979; Nagasawa and Nemoto, 1988; Bradshaw et al., 1990, 1991).

On the other hand, the amounts of bacteria-specific fatty acids in surface (0-0.5 cm) sediments of crustacean cores were equal to or slightly greater than those in the surface of control cores during the first two weeks but they were apparently lower after three weeks of incubations, which might be resulted from a net balance between production and consumption. In the first two weeks, rapid degradation of fresh algal material probably stimulated bacterial growth (same in the control and crustacean cores). During this time, the food source for crustacean might not depend on bacteria; instead, they might enhance bacterial activities through fragmentation and bioturbation (Aller, 1982; Plante et al., 1990). When most fresh organic matter was consumed after two weeks, the crustacean probably ingest bacteria as a supplement food source, leading to a substantial decrease in bacterial biomass. In subsurface (0.5-3 cm) sediments, amounts of bacteria-specific fatty acids always greater in control than in crustacean

cores, presumably caused by crustacean's grazing activities in this depth. However, the influences of crustacean's activities may be more complicated than discussion based on variations of bacteria-specific fatty acids because there are no simple relationships between bacteria-specific fatty acids and bacterial biomass (Harvey and Macko, 1997).

Influences of crustacean's activities on $U_{37}^{k'}$. There has been a strong argument about the stability of $U_{37}^{k'}$ during diagenetic processes, supporting by contrasting evidence. For example, Hoefs et al. (1998) found that the oxidative processes in Pliocene and Micicene Madeira Abyssal Plain sediments selectively degraded 37:2 and 37:3 alkenones, resulting in a significant change in the $U_{37}^{k'}$. Based on profiles of alkenones in oxic and anoxic sediment cores from the Santa Bonica Basin, Gong and Hollander (1999) suggested that intensive activities of benthic macrofauna in oxic sediments caused differential degradation of 37:3 vs. 37:2 alkenones, resulting in a shift of 2-4°C in the estimated temperature. However, other field measurements (Prahl et al., 1989; Madureira et al., 1995) showed that in spite of extensive bioturbation and remarkable degradation of alkenones, the $U_{37}^{k'}$ is largely unaltered.

Teece et al. (1998) conducted a series of laboratory experiments to test the stability of the $U_{37}^{k'}$ during microbial degradation. Their experimental systems consisted of sediment slurries (without macrofauna) under defined conditions (oxic, sulphate-reducing, and methanogenic) with addition of *E. huxleyi* cells. The experimental results demonstrated that decomposition of >80% of initial alkenones would not yield significant bias of the $U_{37}^{k'}$. Recently, we incubated *E. huxleyi* cells in natural oxic and anoxic seawaters collected from stratified water column in the Cariaco Basin (Sun et al., 2004). When 80-90% of initial alkenones degraded in the water systems (without macrofauna), the $U_{37}^{k'}$ remained almost constant. In the present study, *E. huxleyi* cells were incubated in sediments with and without presence of benthic macrofauna.

During six-week incubations, although large proportions of alkenones degraded, the $U_{37}^{k'}$ values varied slightly in both control and crustacean cores, and also in suspended particles of crustacean cores (Fig. 4.9). These results suggested that microbial and macrofaunal processes did not significantly change the $U_{37}^{k'}$. In fact, the degradation rate constants of various alkenones in control and crustacean cores were similar while those of algal fatty acids between these cores differ markedly (Table 4.2), suggesting that the crustacean's activities had little impact on alkenone degradation. This conclusion is consistent with the results from previous feeding experiments (Volkman et al., 1980; Rowland and Volkman, 1982; Grice et al., 1998).

Fates of algal lipids in sediments with and without presence of crustacean. Our experimental results clearly revealed the important role of one species of benthic macrofauna (*P. pugio*) in controlling the fates of different lipids from algal source. Major processes in control and crustacean cores are compared based on a conceptual model (Fig. 4.10). Without mixing due to absence of macrofauna, degradation of algal lipids in control cores is dominated by microbial processes in the surface sediments. Alkenones and fatty acids derived from algal cells degrade at similar rates in this regime. In contrast, presence of crustacean affects algal lipid degradation in sediments by several ways: (1) selective grazing for algal material in surface sediments; (2) preferential assimilation of fatty acids over alkenones during digestion; and (3) transport of detritus containing alkenones from surface to subsurface sediments. In this regime, both macrofaunal activities and microbial processes are responsible for algal lipid degradation.

However, differential effects of crustacean on alkenones and algal fatty acids may control the ultimate fates of these important biomarkers in sediments. For example, preferential assimilation or rapid degradation of algal fatty acids in surface sediments with presence of macrofauna may result in little transport of these compounds from the surface to subsurface

sediments, where degradation is inhibited. On the other hand, alkenones are not efficiently degraded by macrofaunal grazing, so they can be partially transported down to subsurface sediments when bioturbation is faster enough than microbial degradation. Therefore, the preservation of biomarkers in sediments may in part depend on relative rates of degradation in the surface sediments vs. downward transport.

Conclusions

Detailed examination of distributions and variations of algae-derived lipids in microcosm sediments showed that *P. pugio* (benthic crustacean) greatly affected the fates of different biomarker compounds by grazing and bioturbation activities. All major algal fatty acids (polyunsaturated, monounsaturated, and saturated) were preferentially assimilated by the crustacean during digestion, leading to an enhanced degradation of algal fatty acids in the surface sediments. In contrast, the alkenones were not assimilated by the crustacean while only microbial processes were responsible for alkenone degradation. A significant proportion of alkenones and little algal fatty acids were transported down to subsurface when bioturbation rate was fast enough before all alknones were destroyed by microbial processes in the surface sediments. The activities of crustacean also minimized the structural effects of algal fatty acids, including unsaturation extent and ester complexes. In addition, both macrofaunal activities and microbial processes in sediments did not significantly alter the $U_{37}^{k'}$, ensuring the applications of this indicator in paleoceanography.

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Table 4.1. Concentrations of alkenones and algal fatty acids in surface sediments at initial time (C_0), in suspended particles (C_{sp}) and in surface sediment (C_{ss}) on 4 days of incubation in crustacean cores. The ratios of C_{sp} and C_{ss} to C_0 are listed.

Concentration ($\mu g\ g^{-1}$)				
Alkenone	37:3	37:2	38:3	38:2
C_0	18.34±0.18	28.03±0.2	21.61±0.72	26.99±2.44
C_{sp}	23.49±0.96	22.47±0.17	26.82±1.09	27.49±5.39
C_{ss}	11.22±0.82	13.81±0.43	10.98±2.63	16.15±2.79
C_{sp}/C_0	1.28	1.08	1.24	1.02
C_{ss}/C_0	0.61	0.66	0.51	0.6
Fatty acid	14:0	16:0	18:1(ω9)	22:6
C_0	73.29±4.97	152.92±11.61	56.5±1.14	35.74±0.95
C_{sp}	16.54±2.68	57.46±9.18	10.27±1.08	6±0.65
C_{ss}	17.55±4.03	49.57±8.79	10.27±1.08	3.82±0.25
C_{sp}/C_0	0.23	0.38	0.33	0.17
C_{ss}/C_0	0.24	0.32	0.18	0.11

Table 4.2. Degradation rate constants (day^{-1}) of alkenones and algal fatty acids in the control (k_c) and crustacean (k_p) cores. The ratios of k_p to k_c, and r^2 are listed.

	Alkenone			
	37:3	37:2	38:3	38:2
k_p	0.054±0.019	0.062±0.013	0.088±0.009	0.075±0.01
r^2	(0.96)	(0.95)	(0.91)	(0.93)
k_c	0.039	0.043	0.071	0.059
r^2	(0.92)	(0.94)	(0.9)	(0.88)
k_p/k_c	1.38	1.44	1.24	1.27
	Fatty acid			
	14:0	16:0	18:1(ω9)	22:6
k_p	0.316±0.06	0.242±0.046	0.304±0.036	0.363±0.043
r^2	(0.95)	(0.92)	(0.94)	(0.99)
k_c	0.065	0.07	0.123	0.194
r^2	(0.99)	(0.91)	(0.91)	(0.99)
k_p/k_c	4.85	3.46	2.47	1.87

Table 4.3. Degradation rate constants (day^{-1}) of intracellular and membrane fatty acids in the control (k_c) and crustacean (k_p) cores. The ratios of k_p to k_c, and r^2 are listed.

Fatty acid	14:0			16:0			18:1(ω9)			22:6		
	intra*	mem**	mem/intra	intra	mem	mem/intra	intra	mem	mem/intra	intra	mem	mem/intra
k_p	0.279	0.275	0.99	0.291	0.273	0.94	0.233	0.269	1.15	0.315	0.356	1.13
	±0.038	±0.077		±0.103	±0.005		±0.012	±0.087		±0.035	0.047	
r^2	(0.96)	(0.94)		(0.96)	(0.91)		(0.96)	(0.96)		(0.99)	(0.99)	
k_c	0.07	0.117	1.67	0.049	0.092	1.88	0.056	0.131	2.34	0.16	0.229	1.43
r^2	(0.85)	(0.88)		(0.7)	(0.82)		(0.83)	(0.93)		(0.99)	(0.99)	
k_p/k_c	3.99	2.35		5.94	2.97		4.16	2.05		1.97	1.55	

*: intracellular fatty acid

**: membrane fatty acid

Fig. 4.1. Comparison of initial concentrations of various lipids between the bulk and spiked (with addition of algae) sediments.

Note: there are no 22:6 fatty acid and alkenones in the bulk sediments.

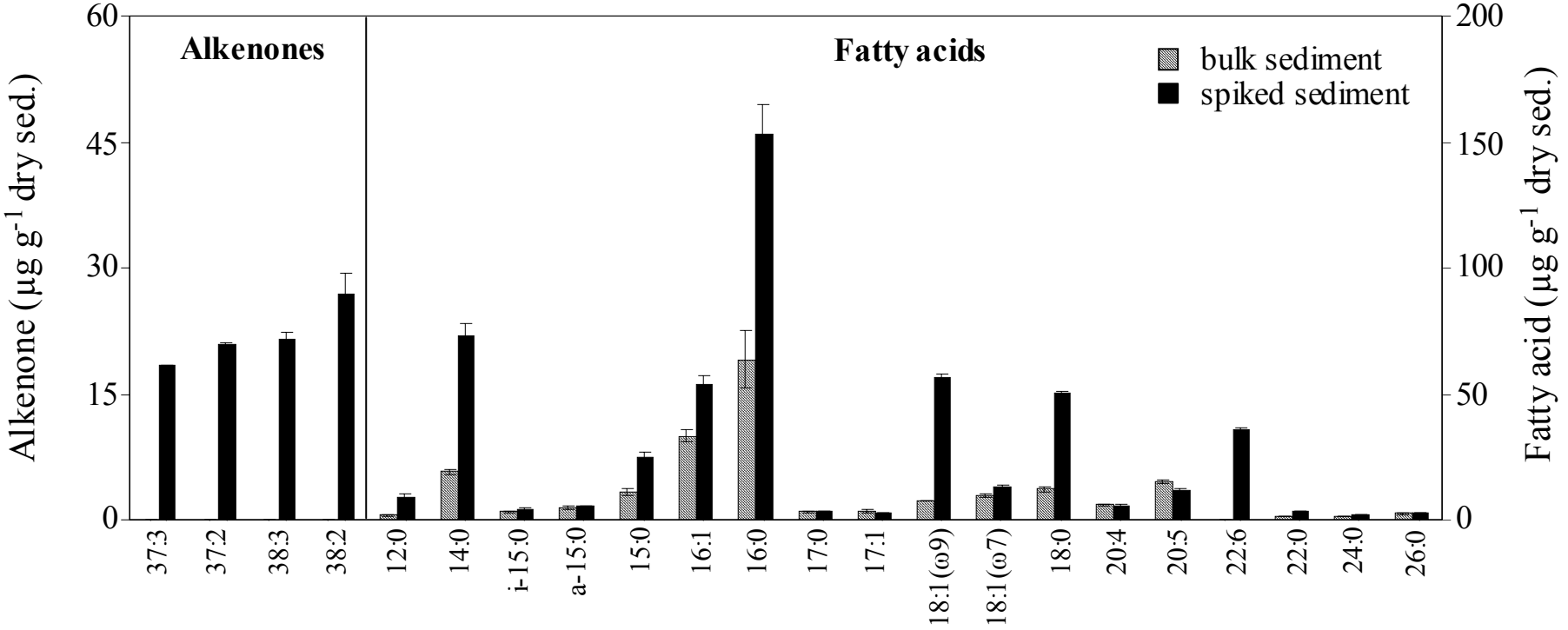
Alkenones
Fatty acids
bulk sediment
spiked sediment
Alkenone (µg g-1 dry sed.)
Fatty acid (µg g-1 dry sed.)
60
45
30
15
0
200
150
100
50
0
37:3
37:2
38:3
38:2
12:0
14:0
i-15:0
a-15:0
15:0
16:1
16:0
17:0
17:1
18:1(ω9)
18:1(ω7)
18:0
20:4
20:5
22:6
22:0
24:0
26:0

Fig. 4.1.

Fig. 4.2. Variations of alkenones and algal fatty acids in surface (0-0.5 cm) sediments during incubations.

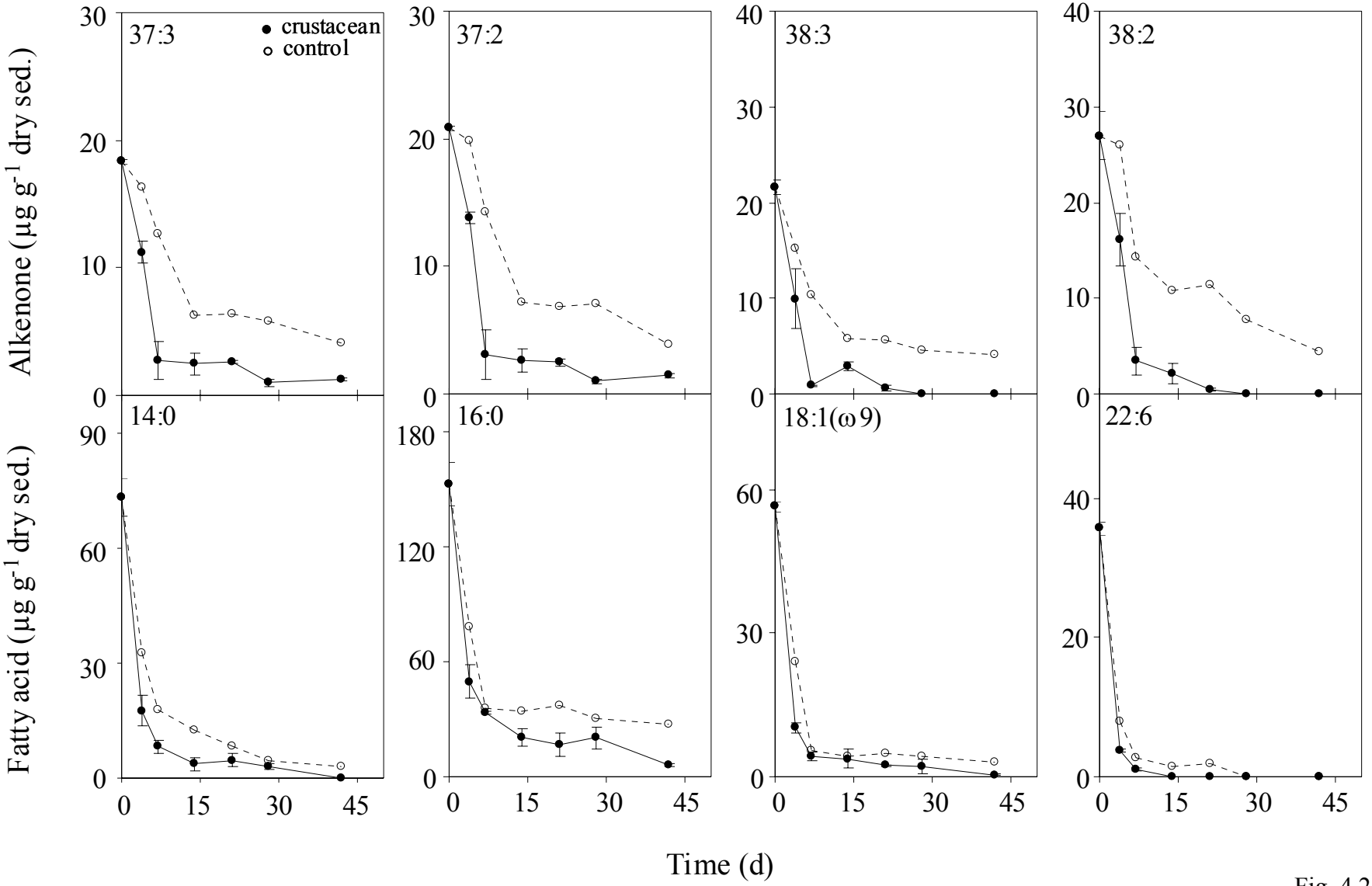
37:3
crustacean
control
37:2
38:3
38:2
14:0
16:0
18:1(ω9)
22:6
Alkenone (μg g-1 dry sed.)
Fatty acid (μg g-1 dry sed.)
Time (d)

Fig. 4.2.

Fig. 4.3. Variations of alkenones and algal fatty acids in suspended particles in crustacean cores during incubations.

Note: dotted line is the initial concentrations of various lipids in surface sediments.

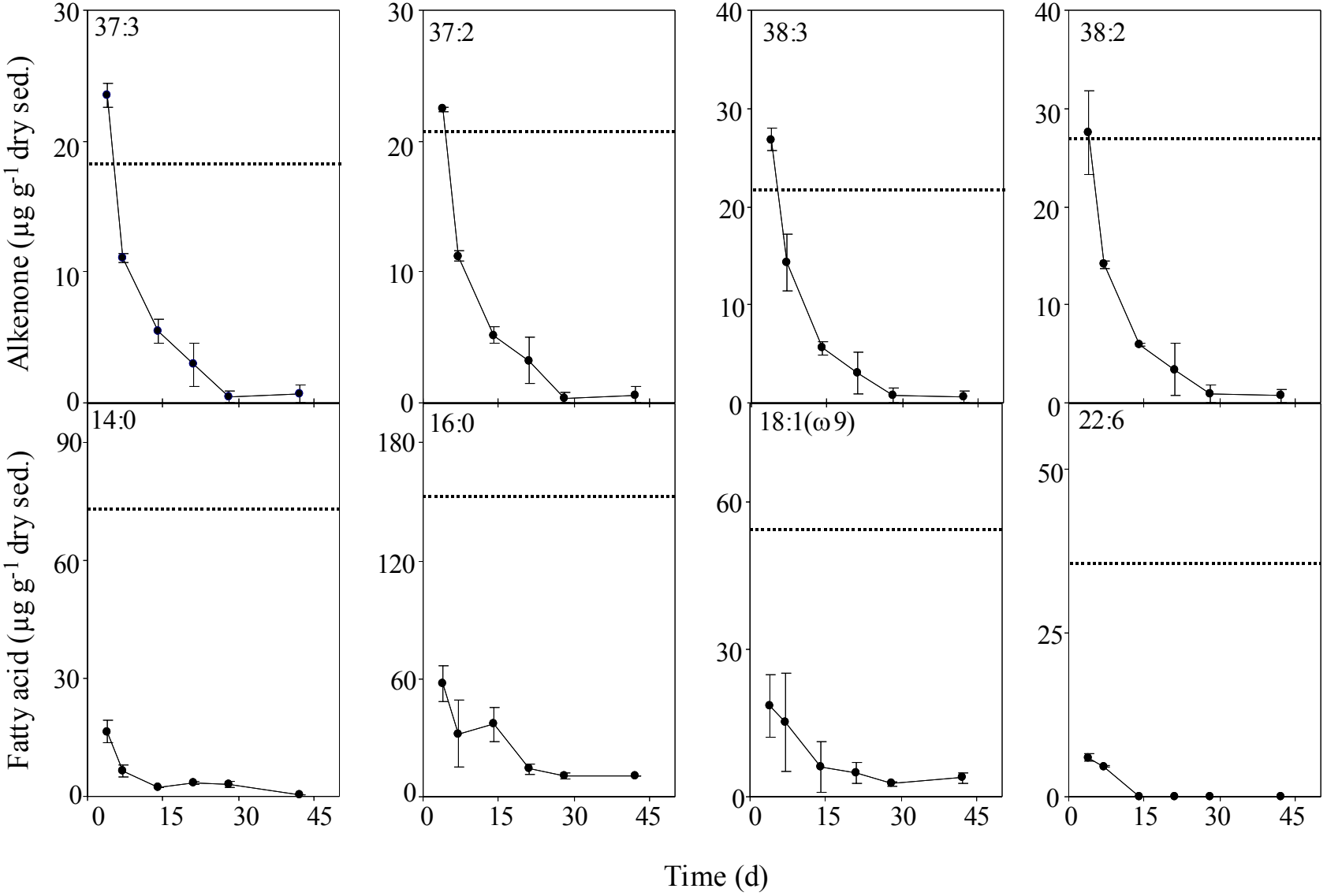
37:3
37:2
38:3
38:2
14:0
16:0
18:1(ω9)
22:6
Alkenone (µg g-1 dry sed.)
Fatty acid (µg g-1 dry sed.)
Time (d)

Fig. 4.3.

Fig. 4.4. Vertical distributions of two alkenones and two algal fatty acids in the control and crustacean cores at different sampling time.

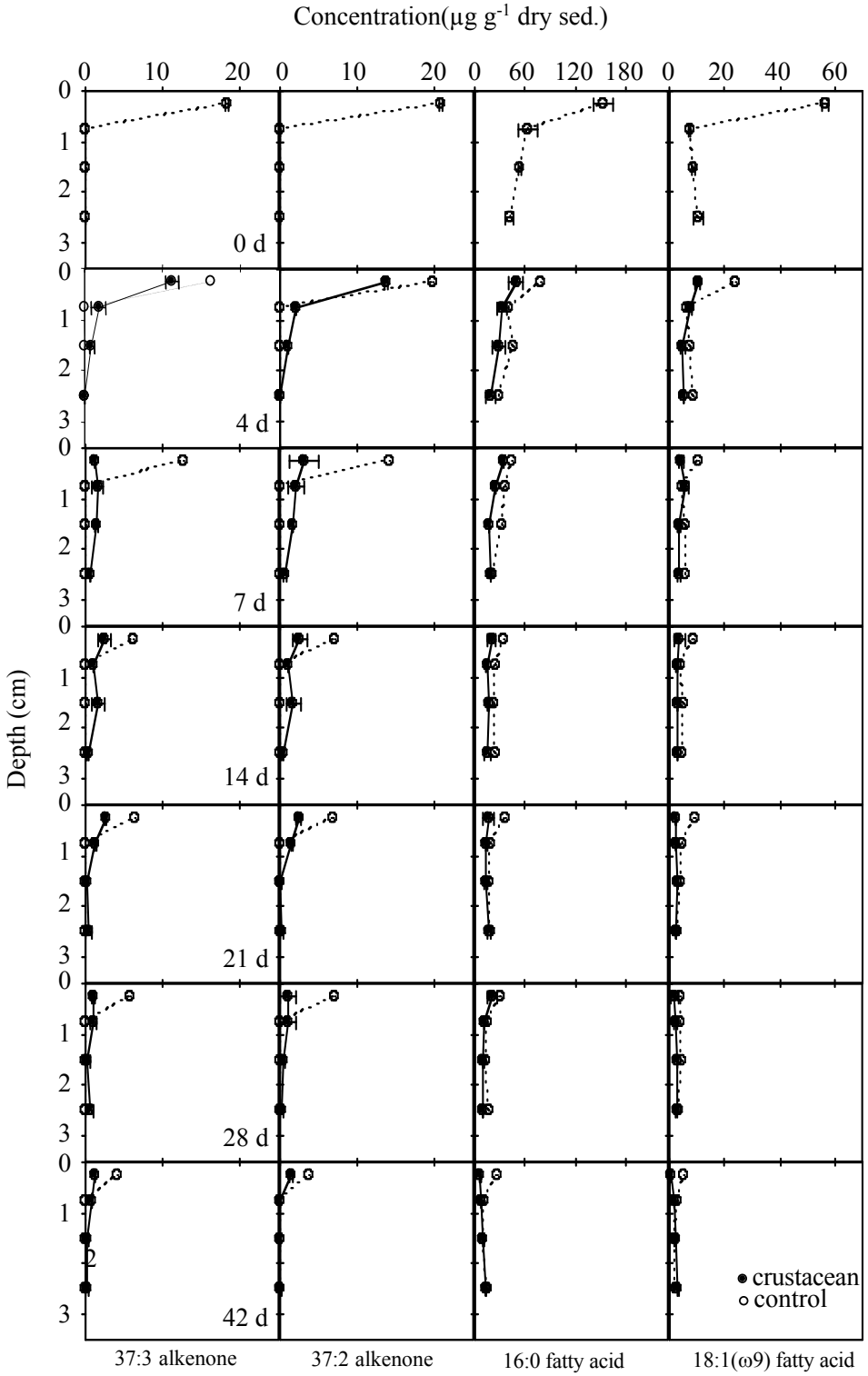
Concentration(μg g-1 dry sed.)
Depth (cm)
0 d
4 d
7 d
14 d
21 d
28 d
42 d
crustacean
control
37:3 alkenone
37:2 alkenone
16:0 fatty acid
18:1(ω9) fatty acid

Fig. 4.4.

Fig. 4.5. Overall variations of alkenones in the control and crustacean cores during incubations. Alkenones in crustacean cores include those from suspended particles, surface (0-0.5 cm) and subsurface (0.5-3 cm) sediments.

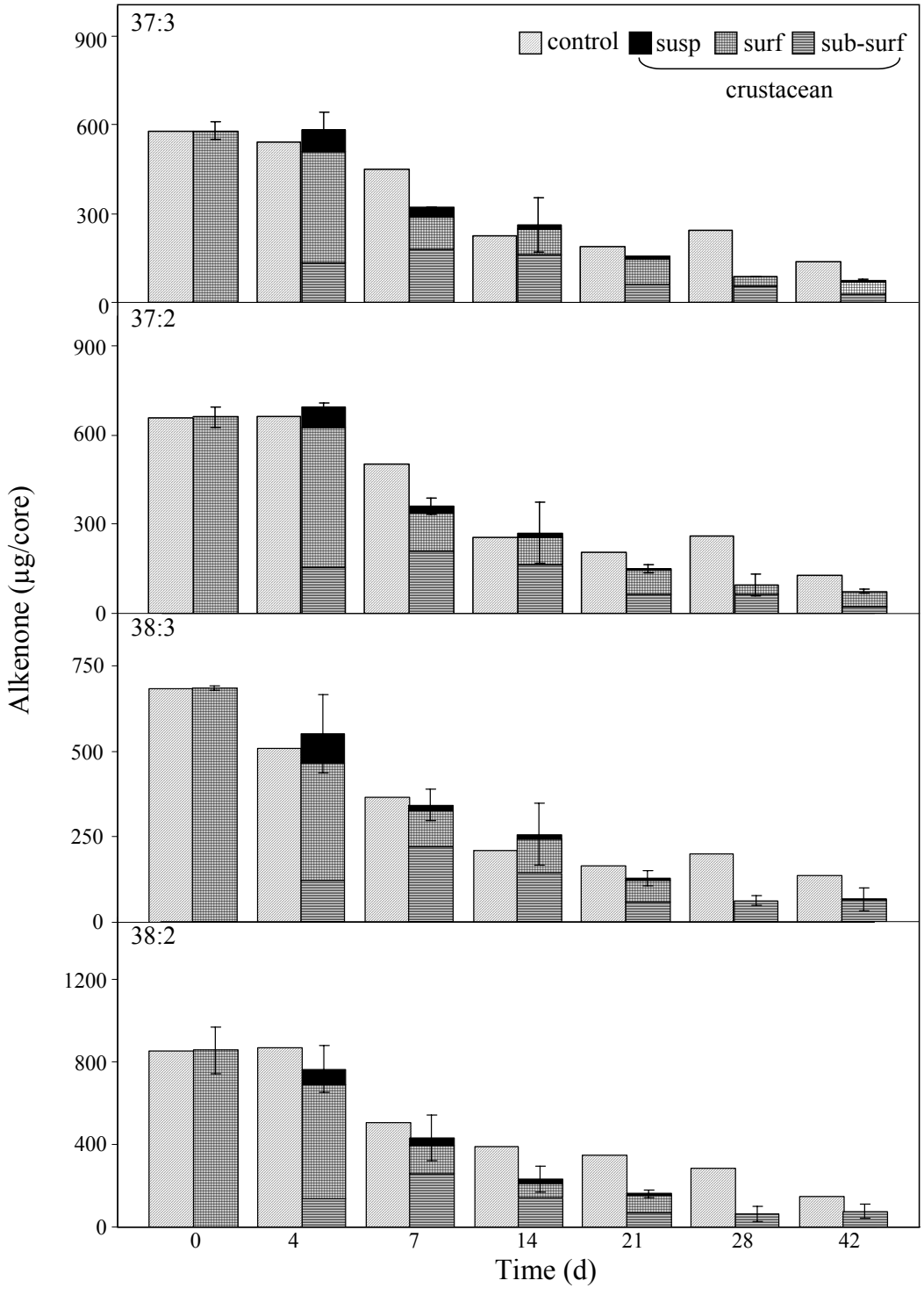
37:3
control
susp
surf
sub-surf
crustacean
900
600
300
0
37:2
900
600
300
0
38:3
750
500
250
0
38:2
1200
800
400
0
Alkenone (µg/core)
0
4
7
14
21
28
42
Time (d)

Fig. 4.5.

Fig. 4.6. Overall variations of fatty acids in the control and crustacean cores during incubations. Fatty acids in the control cores include those from surface (0-0.5 cm) and subsurface (0.5-3 cm) sediments. Fatty acids in crustacean cores include those from suspended particles, surface (0-0.5 cm) and subsurface (0.5-3 cm) sediments.

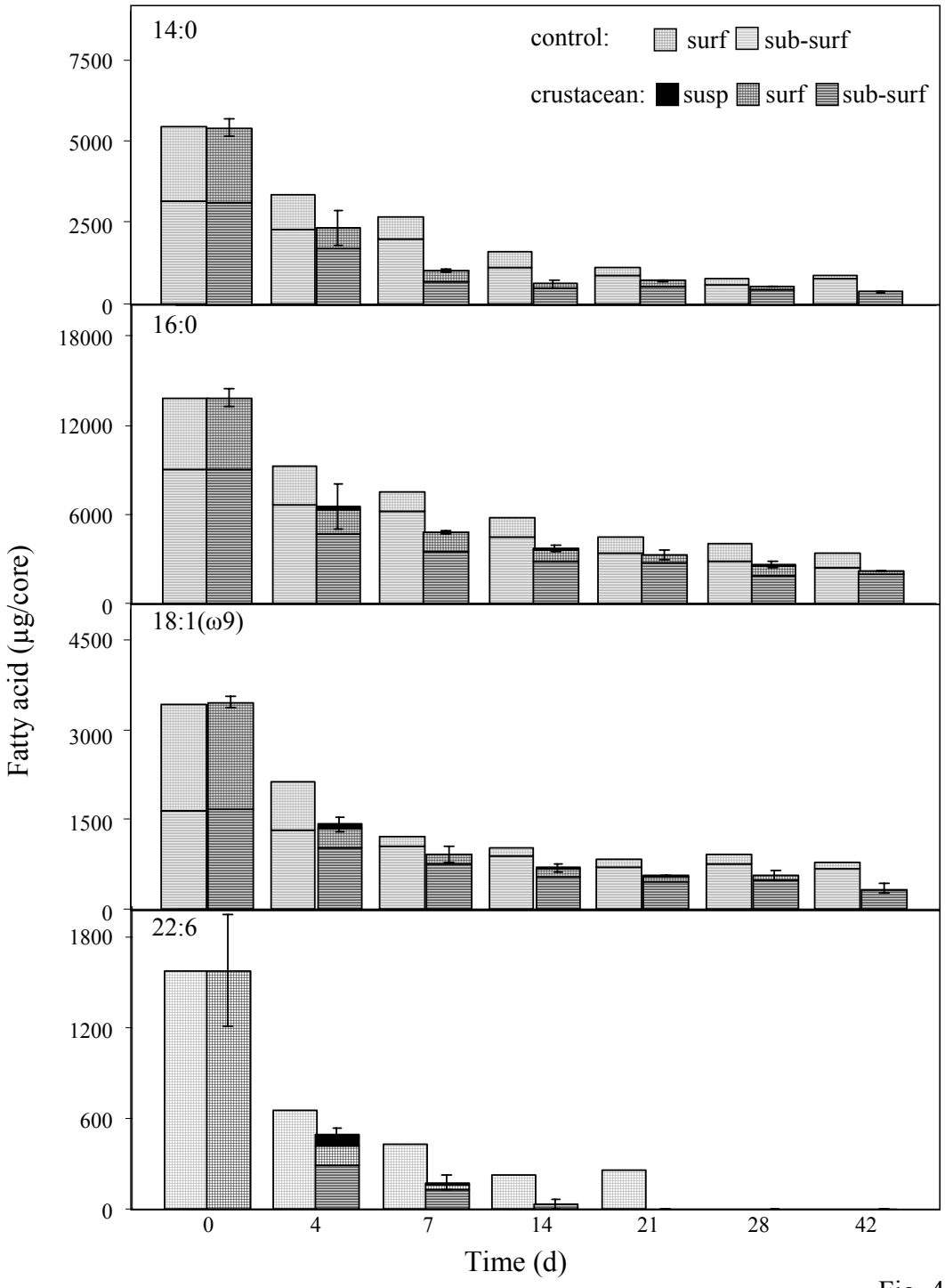
14:0
control: surf sub-surf
crustacean: susp surf sub-surf
7500
5000
2500
0
16:0
18000
12000
6000
0
18:1(ω9)
4500
3000
1500
0
22:6
1800
1200
600
0
Fatty acid (µg/core)
0
4
7
14
21
28
42
Time (d)

Fig. 4.6.

Fig. 4.7. Variations of intracellular and membrane fatty acids in the control and crustacean cores during incubations. The curves are best fits of a two-pool G model to the data.

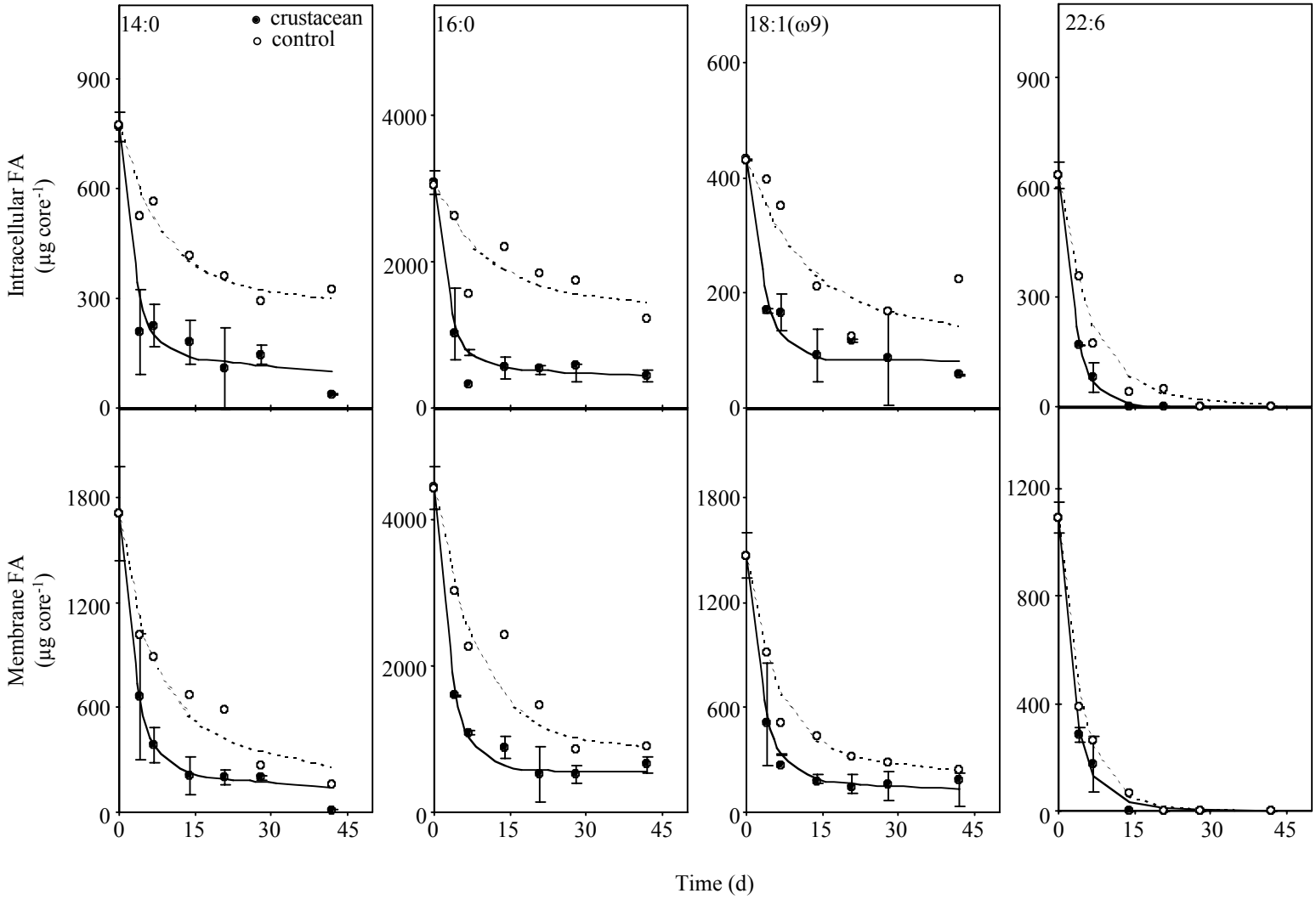
14:0
crustacean
control
16:0
18:1(ω9)
22:6
Intracellular FA (µg core-1)
Membrane FA (µg core-1)
Time (d)

Fig. 4.7.

Fig. 4.8. Variations of bacteria-specific fatty acids in the control and crustacean cores during incubations.

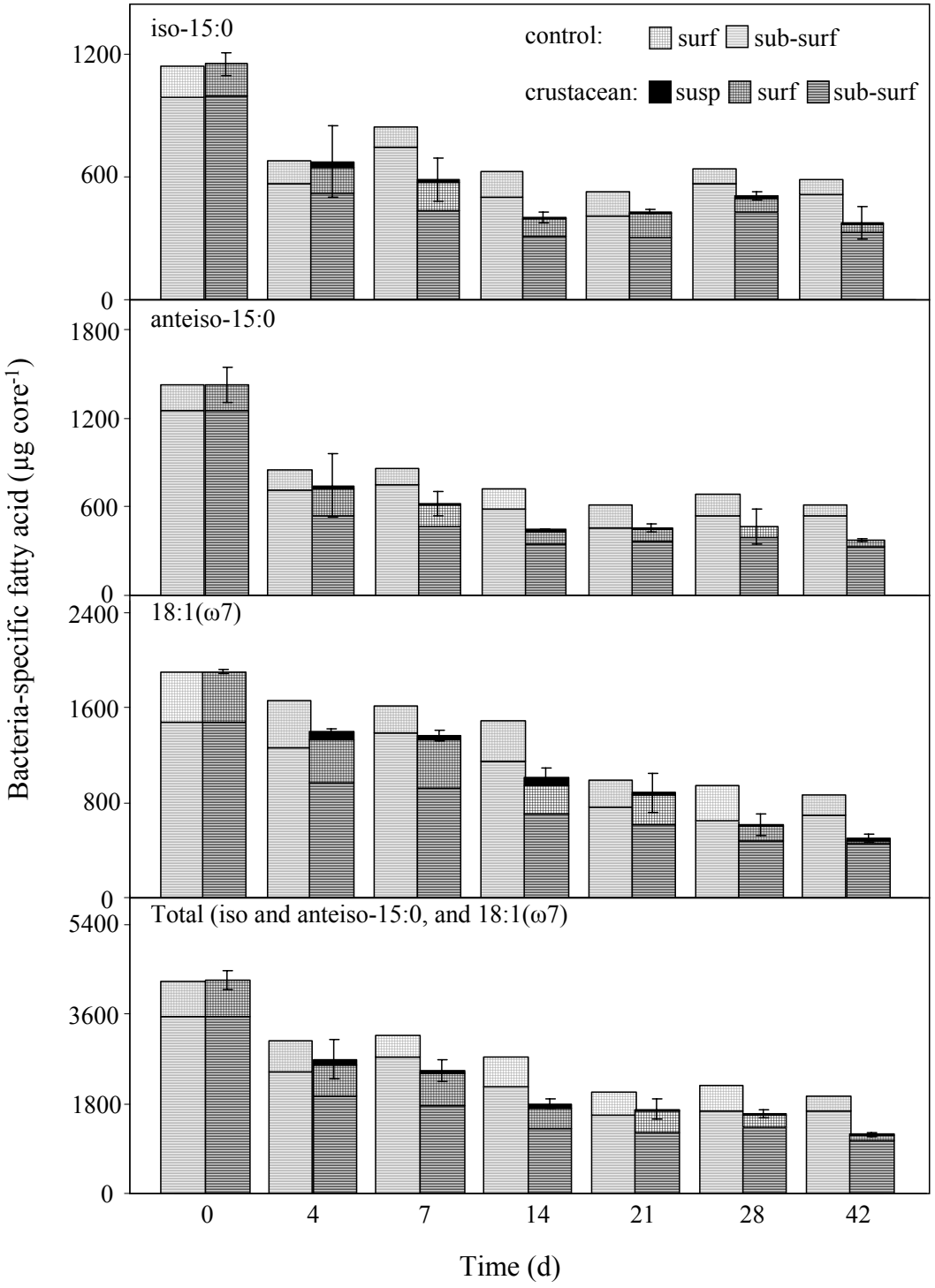
iso-15:0
control: surf sub-surf
crustacean: susp surf sub-surf
1200
600
0
anteiso-15:0
1800
1200
600
0
18:1(ω7)
2400
1600
800
0
Total (iso and anteiso-15:0, and 18:1(ω7)
5400
3600
1800
0
0
4
7
14
21
28
42
Time (d)
Bacteria-specific fatty acid (μg core-1)

Fig. 4.8.

Fig. 4.9. Variations of $U_{37}^{k'}$ index in surface sediments of control and crustacean cores and in suspended particles of crustacean cores during incubations.

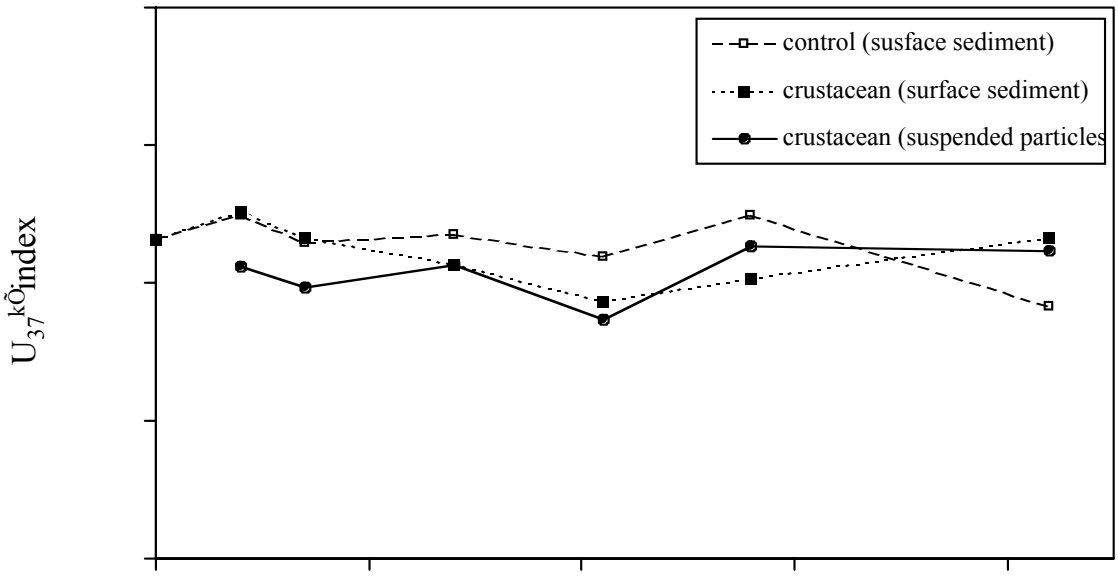
control (susface sediment)
crustacean (surface sediment)
crustacean (suspended particles)
$U_{37}^{k\tilde{O}}$index
Time (d)

Fig. 4.9.

Fig. 4.10. Fates of algal alkenones and fatty acids in the experimental microcosms with and without presence of *P. pugio*.

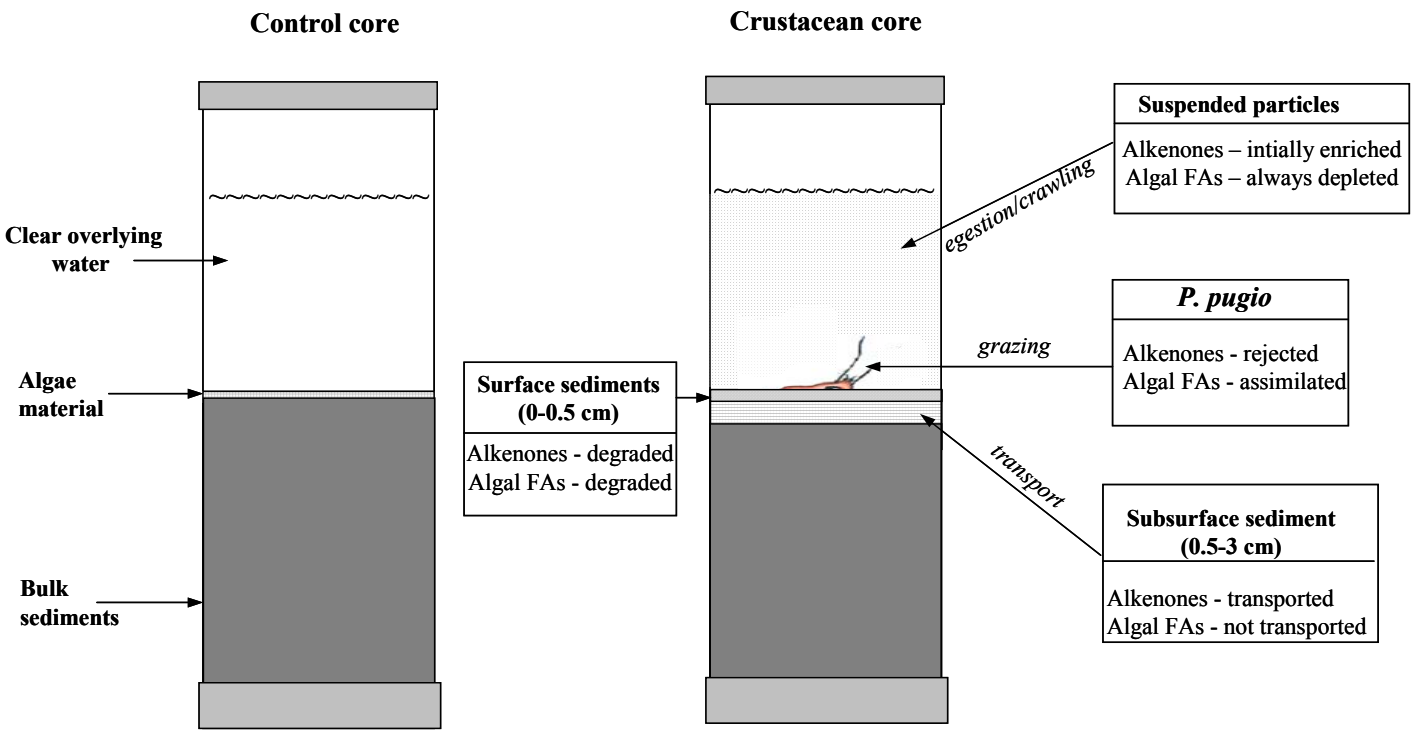
Control core
Crustacean core
Clear overlying water
Algae material
Bulk sediments
Surface sediments (0-0.5 cm)
Alkenones - degraded
Algal FAs - degraded
Suspended particles
Alkenones – intially enriched
Algal FAs – always depleted
egestion/crawling
P. pugio
grazing
Alkenones - rejected
Algal FAs - assimilated
transport
Subsurface sediment (0.5-3 cm)
Alkenones - transported
Algal FAs - not transported
Microbial processes — responsible for algal lipid degradation
Macrofaunal activities and microbial processes — responsible for algal lipid degradation

Fig. 4.10

CHAPTER 5

GENERAL SUMMARY

Comprehensive examination of degradation processes of three classes of important biomarkers (alkenones, fatty acids, and chloropigments) derived from a common alga (*Emiliania huxleyi*) in three simulated microcosms (oxic/anoxic seawater systems, oxic/anoxic sediment-water interface systems, and sediment cores with and without presence of benthic macrofauna) clearly demonstrated that both internal and external factors significantly control the fates of different algal biomarkers in marine systems. The internal factors arise from structural associations of different molecules within cellular components while the external factors result from variable environmental conditions. These factors are interlinked to rework individual compounds, and then affect the distributions and biogeochemical cycling of organic matter in natural environments.

Effects of intercellular structural associations on degradation of fatty acids and Chl-a. Fatty acids from different structural associations of cellular components have various degradation behaviors under different redox conditions. Membrane and intracellular fatty acids degraded at similar rates under oxic conditions; but membrane fatty acids degraded faster than intracellular fatty acids under anoxic conditions. Major components of intracellular fatty acids are non-polar, hydropobic triacylglycerols. Intracellular fatty acids are coated with cell membrane. The backbone of cell membrane is a bilayer of amphipathic phospholipids, which are more readily response to water soluble enzymes such as lipases and phospholipases. Therefore, overall, membrane fatty acids tend to degrade faster than intracellular fatty acids.

However, reactive oxygen species (such as oxygen radicals) can accelerate depolymerization of relatively refractory materials, as a result, degradation of more refractory intracellular fatty acids was enhanced and similar degradation rates between membrane and intracellular fatty acids were detected under oxic condition in this study. On the other side, mono or polyunsaturated fatty acids degraded faster than saturated fatty acids under both oxic and anoxic conditions in seawater and sediments. This faster degradation was attributed to the presence of double bonds in the fatty acids.

Compared to fatty acid degradation, Chl-a in different structural associations degraded similar under anoxic conditions but totally different under oxic conditions. Generally, Chl-a bound in PS-II complexes degraded faster than in PS-I complexes. In the same photosystem, Chl-a in LHC complexes had higher degradation rate constants than that in CP complexes. The degradation differences were determined by both internal factors such as the size, structural binding mode, and location of various Chl-a complexes, and external factors — redox conditions. Unlike cell-associated fatty acid, cell-associated Chl-a obtained protection from proteins or polypeptides in Chl-a complexes and some other proteins in the photosystems. Therefore, Chl-a bound to large size proteins with more α helices and located in central photosystem had lower degradation rate constants. The structural differences also caused the degradations of the Chl-a in LHC complexes and CP complexes through different pathways. However, compared to living cells, dead cells could not provide enough protection to toxic oxygen species for the complexes in PS-II. Thus, Chl-a in PS-II could not resist these oxygen species well and had faster degradation rates. On the other hand, the extremely slow degradation of Chl-a in insoluble complexes implied the existence of other potential structural components in chloroplast.

Effects of oxygen on biomarker degradation. Overall, oxygen presence increases degradation of algal biomarker compounds in seawater and sediments. This degradation enhancement was caused by various reactive oxygen species produced from chemical or biochemical processes. For algal fatty acids and Chl-a, oxic degradation rate constants were generally 2-4x higher than those of anoxic degradation in natural seawater and sediments. Compared to anoxic condition, much less algal biomarkers were preserved under oxic condition. For various algal compounds, the effects of oxygen are different. For example, degradation rate constants of fatty acid is less than 2x for 22:6 fatty acid but more than 9 for Chl-a in a kind of structural association, indicating the role of oxygen is dependent in part on the structure of biomarkers and other biochemical factors in different environments.

Without bioturbation, bacteria dominate degradation of algal biomarkers. The bacterial processes controlled the degradation of organic matter in seawater, seawater-sediment interface and subsurface sediments by producing different hydrolysis enzymes. Moreover, almost all anoxic degradations of algal fatty acids and Chl-a were carried out by heterotrophic bacteria. In this study, to track the variations of bacteria, several different ways were applied. By direct DAPI counting, the increase of bacterial abundance followed rapid decrease of fatty acids was detected, implying the contribution of bacteria on the loss of algal compounds. On the other side, there is no general patterns to describe the variations of bacteria specific fatty acids (iso-15:0, anteiso-15:0 and 18:1(7)) in all three experiments, implying bacteria biomass and activities was altered by multiple factors, for instance, redox condition, substrate availability, variations of bacterial community and bioturbation. Although degradation of organic matter was controlled by microbial processes, this study indicated that the quantitative link among algal biomarkers, bacterial enzymes and bacterial abundance and biomass are equivocal.

Effect of hydrolysis enzymes on algal biomarker degradation. Degradation of algal biomarkers involved series enzymes such as lipase, phospholipase and dehydrogenase. Animals and heterotrophic microbes are the major producer of these enzymes. Bacteria cannot utilize macromolecules directly and have to secret extracellular enzymes to hydrolysis various organic compounds. Enzyme reaction is highly specific, so different bacterial enzymes are necessary to hydrolyze algal compounds. This study demonstrated that microbes could produce all necessary enzymes to decompose fatty acids, alkenones and Chl-a. In general, aerobic microbes are capable of respiring a substrate completely to CO_2 (Gottschalk, 1986), and then most of algal biomarkers disappeared under oxic condition. However, this study did not find any simple relationship between enzymes (lipases) and lipid degradation, indicating the complicated reactions between extracellular enzymes and their substrates. In contrast, animal conducted enzymatic macromolecular hydrolysis in its body, but animal could not produce some necessary enzymes to decompose all algal biomarkers such as alkenones, which cause obvious differential degradation of algal biomarkers.

Alternation of degradation of algal biomarkers by macrofauna. This study demonstrated the important roles of crustacean *Palaemonentes pugio* on the degradation and transport of algal compounds. Crustacean's resuspension and downward transport sent a part of algal material to overlying water and subsurface sediment. Their bioturbation enriched alkenones in suspended particles. Presence of crustacean also enhanced algal fatty acid degradation but had little influence on alkenone degradation in the microcosms. The differential degradation of algal fatty acids and alkenones was attributed to crustaceans' selective grazing and preferential assimilation of algal material. Generally, crustacean utilized and assimilated fatty acids as their carbon source and then increased fatty acid degradation. However, since

lacking of corresponding enzymes, it is reasonable that crustacean could not digest alkenones as their carbon source. The activities of crustacean also minimized the structural effects on decomposition of membrane and intracellular fatty acids and caused similar degradation rates of saturated and unsaturated fatty acids. On the other hand, crustacean altered microbial community by three different ways: directly grazing, stimulating bacteria growth and competing for substrate availability. In this study, the lower amounts of bacterial specific fatty acids with presence of crustacean implied that crustacean may exerted a high pressure on bacteria growth. It is possible that crustacean grazed on bacteria as their food source after algal material was consumed and decrease labile material obtained by bacteria. Moreover, in paleoceanography, this study indicated that little effects of microbial processes on $U_{37}^{k'}$ values with or without presence of crustacean though most of alkenones were degraded during the incubation.

Degradation rate constants of various biomarkers in different incubation systems. The multi-G model was commonly used to quantitatively describe degradation of fatty acids, alkenones and Chl-a in all three microcosms. The calculation of average degradation rate constants (k_{av}) provides a valuable way to compare the overall degradation rate constants of all algal biomakers (fatty acids, alkenones and Chl-a) in different environments no matter how many degradation pools involved. In this study, the degradation rate constants of all algal lipids ranged from as low as 0.005 (anoxic degradation of Chl-a in insoluble fraction) to as high as 0.363 (degradation of 22:6 fatty acid with bioturbation). Generally, for all algal biomarkers, their degradation rate constants are higher under oxic condition than under anoxic conditions. Overall, oxygen presence and bioturbation were most important external factors to increase algal biomarker degradation rate constants. However, this enhancement was markedly influenced by

their structural cell associations (e.g. membrane and intracellular fatty acid, Chl-a in various pigment complexes) and biochemical functions (e.g. fatty acids and alkenones).

Fates of various biomarkers in seawater and sediment. Generally, algal biomarkers were consumed by microbial and animal processes in seawater or in sediments. Microbial processes could decompose all algal biomarkers under both oxic and anoxic conditions but anoxic condition was an advantage to preserve more organic matter. Bioturbation had differential effects on degradation of various algal biomarkers and could transport algal material upward to overlying water or downward to subsurface sediments.

Overall, this study provides a basic picture of degradation of algal material from seawater to subsurface sediments. By examining the role of different factors on lipid degradation, the fates and degradation pathways of various algal biomarkers in seawater and marine sediments were revealed. The new knowledge and information obtained from this work is helpful in understanding global carbon cycling and in paleoceanographic research.