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Differential fates of *Emiliania huxleyi*-derived fatty acids and alkenones in coastal marine sediments: Effects of the benthic crustacean *Palaemonetes pugio*

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ABSTRACT

In order to examine how benthic crustaceans affect the fates of phytoplankton-derived lipid biomarkers (fatty acids and alkenones) in coastal marine sediments, we incubated *Emiliania huxleyi* cells in microcosms (pre-sieved sediment cores with and without the grass shrimp *Palaemonetes pugio*) over six weeks. Crustacean, transport of surface sediments, and distributions of algal lipids were followed during incubations. Crustacean activities enhanced degradation of algal fatty acids (2–4× faster) but had a small impact on algal alkenone degradation (<1.4×) compared to the controls. During the first few days of incubations, alkenone concentrations were enriched while algal fatty acid concentrations were depleted in suspended particles in the overlying water of cores, indicating that *P. pugio* selectively grazed algal material from sediments and preferentially assimilated fatty acids over alkenones through digestion. Unlike algal fatty acids, alkenones were degraded primarily by microbial processes rather than by crustacean grazing. A substantial fraction (20–30%) of algal lipids was moved downward to the subsurface of sediments by *P. pugio* but algal fatty acids were more rapidly (3–6×) degraded than alkenones. In the presence of *P. pugio,* fatty acids bound in cell membrane and intracellular storage components degraded similarly, indicating that the crustacean activities minimized the effects of structural associations on fatty acid decomposition. Furthermore, there was no preferential degradation of 37:3 and 37:2 alkenones in both crustacean and control cores, suggesting that the $U^{k'}_{37}$ index (a paleotemperature indicator) was not significantly altered by *P. pugio*'s grazing or microbial decomposition.

1. Introduction

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 studies (Aller, 1982; Rice, 1986; Kristensen *et al.,* 1992; Green *et al.,* 2002) have demonstrated that benthic macrofauna greatly affect degradation of organic matter in sediments in numerous ways. For example, construction of burrows and irrigation by macrofauna enhance solute transport and substrate metabolism (Webb and Eyre, 2004). Macrofauna rework surface sediments and continuously mix particles across the oxic/anoxic boundary, creating an oscillating redox regime for organic matter degradation (Aller, 1994). Macro-

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fauna also degrade organic matter in sediments by direct grazing and release of extracellular enzymes (Lopez and Levinton, 1987). Additionally, macrofauna stimulate continuous rapid growth of microbial populations and accelerate microbial decomposition of organic matter by particle fragmentation (Plante *et al.,* 1990). Although the effects of macrofaunal activities on organic matter degradation have been examined extensively (Aller, 1982; Bianchi *et al.,* 1988; Andersen and Kristensen, 1992), limited studies have addressed the influence of specific macrofaunal activities on the fates of specific lipid biomarkers in marine sediments (Sun *et al.,* 1999).

Phytoplankton production in surface seawater is a major source of organic matter to the seafloor with lipids accounting for 5 to 20% of total organic carbon in phytoplankton (Parsons *et al.,* 1961). Due to the specificity in their structures and relatively higher stability than carbohydrates and proteins, lipids have been widely used as biomarkers to study carbon cycling and paleoceanography (Brassell *et al.,* 1986). For example, some studies have determined organic carbon fluxes from surface water to underlying sediments by following the distributions of specific lipid compounds (Wakeham *et al.,* 1997; Bac *et al.,* 2003). Other studies have used specific lipid compounds to trace origins of dissolved and particulate organic matter (Harvey, 1994; Jaffe *et al.,* 1995) and to characterize microbial community (Green and Scow, 2000; Boschker and Middelburg, 2002; Wakeham *et al.,* 2003). Furthermore, changes in relative concentrations of some specific biomarkers (e.g., long chain unsaturated alkenones) with sediment depth have been linked to the historic records of sea surface temperature (Brassell *et al.,* 1986; Prahl and Wakeham, 1987). However, applications of lipid biomarkers have been limited by an incomplete understanding of diagenetic processes which alter structures of original biomarkers (Hedges and Prahl, 1993). Consequently, there continues to be a debate about the effect of redox conditions and the role of benthic macrofauna on the fate of different algal lipids and the validity of biomarker applications (Prahl *et al.,* 1989; Hoefs *et al.,* 1998; Teece *et al.,* 1998; Gong and Hollander, 1999; Sun *et al.,* 2004).

Within algal cells, lipid compounds exist in different compartments associated with different molecular complexes which may affect their biochemical reactivities. For example, algal fatty acids are mostly bound in triacylglycerols (intracellular storage component) and in phospholipids (cell membrane). In addition, alkenones are biosynthesized as different components (metabolic storage vs. membrane) at different growth stages (Prahl *et al.,* 1988; Bell and Pond, 1996). Algal fatty acids bound in cell membrane and intracellular storage components degraded differently by microbial processes (Ding and Sun, 2005a). However, it remains unknown how benthic macrofaunal activities affect the degradation of various algal lipids bound in different cellular components.

This study was designed to specifically examine the effect of *P. pugio* (the grass shrimp, a common species of benthic crustacean) on the fate of two classes of lipids (fatty acids and alkenones) derived from *E. huxleyi* (a typical haptophyte alga) in coastal marine sediments. *P. pugio* is abundant in coastal environments along the Atlantic Ocean and the Gulf of Mexico (Williams, 1984) and feeds on a wide variety of organisms, including macro-

phytes, microalgae, meiofauna, and fungi (Reinsel *et al.,* 2001) and detritus. *P. pugio* resuspends surface sediments through its feeding, swimming, and crawling activities (Shenker and Dean, 1979). In our laboratory experiments, microcosms consisted of pre-sieved sediment cores with and without *P. pugio.* Cells of *E. huxleyi* were added into the microcosms to simulate the deposition of phytoplankton after a water column bloom. The concentrations of four major algal fatty acids [14:0, 16:0, 18:1(ω9) and 22:6(ω3)] and four alkenones (37:3, 37:2, 38:3 and 38:2) in the suspended particles of the overlying water and in the sediment were followed during six-week incubations to estimate the degradation rate constants of individual lipids. The effect of macrofaunal activities on degradation of fatty acids bound in different cellular components were examined by separating fatty acids bound in cell membrane and intracellular storage components. Variations in the $U^{k'}_{37}$ index [$C_{37:2}/(C_{37:2} + C_{37:3})$, where $C_{37:2}$ and $C_{37:3}$ are concentrations of 37:2 and 37:3 alkenones; Prahl and Wakeham, 1987], were monitored to test whether or not macrofaunal activities and microbial processes alter the paleotemperature signal.

2. Experimental

a. Sampling and materials

Seawater and sediment samples used in our experiments were collected in March 2002 from a site (31°23.52′N, 81°17.64′W) near Sapelo Island, Georgia, USA. Seawater (salinity ~28‰) was collected by pumping surface water through a set of Barnstead filter cartridges (pore size 25 μm) and sediment was collected using a box core sampler (surface area ~0.04 m^2). The top 0–2 cm and lower depth (2–15 cm) sediments were separately scraped from the box core. Both layers of sediments were separately passed through a 0.5 mm sieve (no water added) prior to incubation experiments. The benthic crustaceans, *P. pugio* (1.5–2 cm in length), were collected from an estuarine site near Skidaway Island, Georgia, USA.

A culture of *E. huxleyi* (clone CCMP1949) was obtained from the Provasoli-Guillard National Center for 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 in continuous growth mode. The culture was started in 50 ml medium and transferred to 250 ml of new medium in ten days (close to the end of exponential phase in its growth curve). The culture was transferred to 4 × 1000 ml medium and was grown for another 10 days. Algal cells were harvested by centrifugation. All harvested algal cells were stored frozen at −20°C for later incubation experiments.

b. Microcosm setup

Incubations were conducted in microcosms made up of 20 cylindrical PVC core liners (i.d. 7 cm and length 30 cm) with the bottom sealed. The liners were filled with the pre-sieved sediments: the bottom 15 cm with the lower depth sediments and upper 3 cm with the top layer sediments collected from the field. Macrofauna, shells, and large debris

were removed by sieving but the porosity was not significantly changed. About 320 ml of filtered seawater was gently added to each core to create an overlying water column. Thawed *E. huxleyi* cells (400 mg wet materials with ~90% water) were spiked equally into the overlying water of each core. Based on our previous pigment analysis for the alga (Ding and Sun, 2005b), addition of the algal material resulted in a ~6.6 μg Ch-*a*/cm^2 (corresponding to ~0.4 mg OC/cm^2) deposition in each core. The total organic carbon content in the top 1 cm sediment is 10–20 mg OC/g dry sediment and the porosity is 0.7–0.8 (Sun *et al.,* 2002). Therefore, addition of the algal material increased the OC content in the surface sediments (0–1 cm) by approximate 3–6%. After settling of algal cells (~30 min), two cores were immediately sampled as a starting point ($t = 0$ day). The crustaceans were added to 12 cores (2 animals in each) while the six other cores were used as controls (no animals). During incubations, the overlying seawater in all cores was continuously purged with air to keep the water oxygenated. In addition, the overlying seawater in each core was replaced weekly with fresh seawater to avoid excessive accumulation of harmful metabolites such as NH_4^+. Suspended particles in the overlying water were removed from the old seawater by centrifugation and added back into the new water to prevent physical loss of lipids from the system. All cores were incubated in the dark at 20°C (same as the *in situ* temperature in the field where the crustaceans were collected). At each sampling time (0, 4, 7, 14, 21, 28, and 42 days), one control and two crustacean cores (as duplicates) were removed from the incubator. The overlying water in each core was withdrawn gently by pipette. For crustacean cores, the suspended particles in overlying water were collected by centrifugation (4000 rpm). All crustaceans were removed from the sediments during sampling. Sediments were 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 and only the intervals in upper 3 cm were analyzed for lipids. About 0.5 g of suspended particles and sediments from each interval were dried at 60°C for 24 h for estimating water content and porosity. The remaining sediments were stored frozen at −40°C for lipid analysis.

c. Extraction and separation of lipids

Lipid extraction and separation were based on published procedures (Ding and Sun, 2005a). Briefly, ~0.5 g thawed suspended particles and ~2 g sediment samples were extracted first with 10 ml methanol, followed by 3 × 10 ml extraction with methylene chloride:methanol (2:1 v/v). The combined extracts were equally split into two aliquots. One aliquot was dried with a rotary evaporator and then saponified using KOH (0.5 mole) in MeOH/H_2O (95:5) at 100°C. Total neutral lipids and fatty acids were separately extracted from the solution with hexane under different pH conditions (pH > 13 for neutral and pH < 2 for acidic lipids). Another aliquot of the original extract was dried with a rotary evaporator and redissolved in 1 ml hexane. Lipid extracts in the latter aliquot were passed through a pre-cleaned Si-gel (EM Science, 63–200 mesh) column (6 mm i.d. and 14 cm in length) and separated into seven fractions by eluting with (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. We ran two standards (tripalmitin and *L*-α-phosphatidylcholine-β-oleoyl-γ-myristoryl) as representatives of natural intracellular storage and cell membrane fatty acids in the same column and confirmed that 100% tripalmitin (a triacylglycerol similar to other storage lipids) was eluted in fraction 3 and phosphatidylcholine-β-oleoyl-γ-myristoryl (a phosepholipid similar to membrane lipids) in fractions 6 (~70%) and 7 (~30%). Thus, for sample extracts, fractions (2)–(5) were combined as intracellular storage fatty acids while fractions (6)–(7) as cell membrane fatty acids. These two combined fractions were separately saponified with KOH in MeOH/H_2O to obtain neutral and acidic lipids through extractions under different pH conditions. Fatty acids in all fractions were methylated with BF_3-methanol to form fatty acid methyl esters (FAMEs).

d. Lipid analysis

Neutral lipids (alkenones) 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. Separation of lipid compounds were achieved by a 30 m × 0.25 mm i.d. column coated with 5%-diphenyl-95%-dimethyl polysiloxane (HP-5, Hewlett-Packard). The GC temperature program was: 50–170°C at 20°C/min, followed by 170–310°C at 4°C/min and held isothermally at the final temperatures for 5 min (for FAMEs) and for 35 min (for neutral lipids). Internal standards [5α(H)-cholestane for neutral lipids and nonadecanoic acid methyl ester for FAMEs] were separately added to samples prior to GC analysis to aid in quantification. The relative standard deviation of GC analyses for lipids was generally ±5% based on duplicate measurements. Selected samples were analyzed by a SHIMADZU QP-5000 gas chromatography-mass spectrometry (GC-MS) system to identify the compounds of interest. The GC-MS system was equipped with a split injector and a 30 m × 0.25 mm i.d. column coated with 5%-diphenyl-95%-dimethyl polysiloxane (XTI-5, Restek) and helium 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 50–150°C at 20°C/min followed by 150–310°C at 4°C/min and a 35 min hold for neutral lipids and a 5 min hold for FAMEs at 310°C.

To compare the differences between the control and crustacean cores, we used the student's *t*-test approach (Zar, 1999) based on two null hypotheses: H_0: $\mu_1 = \mu_2$ (two treatments are equal) and H_A: $\mu_1 \neq \mu_2$ (two treatments are different). The data sets include inventories of total lipid compounds, intracellular storage and membrane fatty acids in control and crustacean cores at each sampling time. The probabilities (P) in all tests were set at 0.05, and the t value at $P = 0.05$ was 12.706 when the total sample number of control and crustacean cores ($n_1 + n_2$) was 3 at each sampling time. If calculated t value for each sampling point was less than 12.706 ($P > 0.05$), the H_0 would be accepted and otherwise H_A would be accepted.

3. Results

In our microcosms, two *P. pugio* were added to each core, equivalent to 520 fauna/m^2. The abundance and species of benthic crustaceans in Georgia coastal sediments vary seasonally over a large range (100–9000/m^2 of total number and 10–24 of species, Leiper, 1973). Following the addition of *P. pugio* to the microcosms, the overlying water quickly became turbid while in the control cores the overlying water was always clear. Throughout the six-week incubations, most *P. pugio* in the microcosms alive (only 3 of 24 died) and the overlying water remained turbid. In addition, we conducted a set of experiments in seawater and seawater/sediment systems to assess the survival rate of the crustacean. With a food (algae) supply (once every three days), all *P. pugio* lived while at least ~50% survived in the treatments without food supply after six weeks.

a. Lipid compositions in bulk and spiked sediments.

At the initial time ($t = 0$ d), the added algal material sank from the overlying water to the sediment-water interface. Thus, the top 0–0.5 cm sediments were considered as the spiked sediments while sediments from 0.5 to 3 cm were treated as the homogenized bulk sediments. In the bulk sediments, 17 fatty acids were found but there were no alkenones (Fig. 1). In the spiked sediments, four major alkenones (37:3, 37:2, 38:3, and 38:2) were isolated, indicating they were exclusive biomarkers of the added algal material. Compared to the bulk sediments, 22:6 fatty acid was a compound exclusively derived from the algal material while the concentrations of 14:0, 16:0 and 18:1(ω9 fatty acids in the spiked sediment were 2–5 fold greater than bulk sediment. These four fatty acids were also major components (each ~20% of the total fatty acids) in *E. huxleyi* cells (Ding and Sun, 2005a). Thus, these four fatty acids were chosen as typical algal fatty acids, with varying structural features (saturated, monounsaturated, and polyunsaturated).

b. Variations of algal fatty acids and alkenones in suspended particles.

In crustacean cores, a visible amount of particles was suspended in the overlying water from the beginning of incubation. The concentrations of algal lipids in the suspended particles generally decreased with time (Fig. 2). However, at $t = 4$ days, the concentrations of the alkenones in suspended particles were higher than the initial ($t = 0$) concentrations in the top 0–0.5 cm sediments (dotted lines in Fig. 2). In contrast, the concentrations of the four fatty acids in the suspended particles were lower than their initial concentrations (dotted lines in Fig. 2) in the top sediments on day 4. Meanwhile, the C_{ss}/C_0 ratios (concentrations in surface spiked sediment on day 4 to those on the initial day) of alkenones were less than 1 although the C_{sp}/C_0 ratios (concentrations in suspended particles on day 4 to the initial concentrations in spiked sediment) were greater than 1 (Table 1). On the other hand, fatty acid concentration ratios (C_{sp}/C_0 and C_{ss}/C_0) were all lower than 1 (Table 1). After the first week, the concentrations of all alkenones in the suspended particles were lower than the initial concentrations in the surface sediments. In the control cores, no suspended particles were observed in the overlying water.

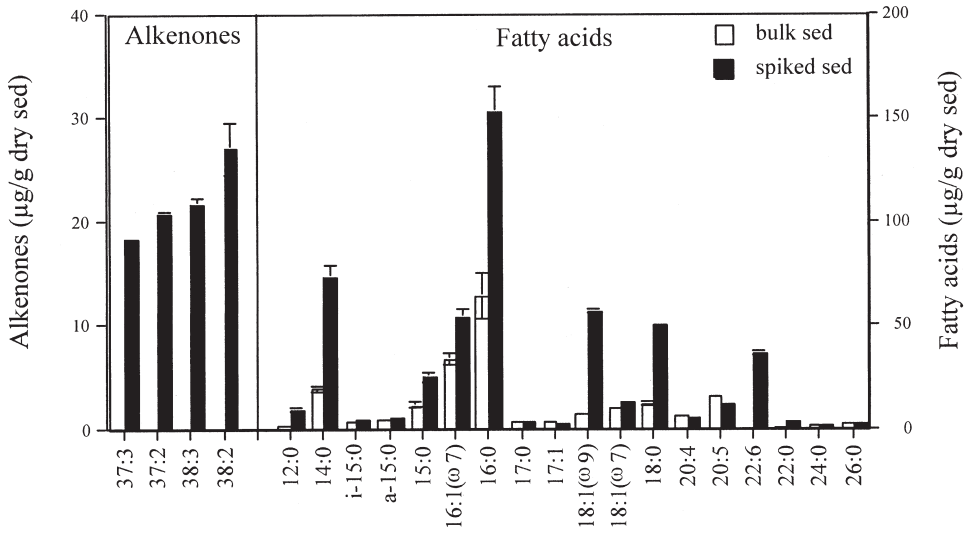

Figure 1. Comparison of initial concentrations of various lipids in the bulk and spiked (with addition of algae) sediments. Note: there were no 22:6 fatty acid and alkenones in the bulk sediments. The error ranges were determined based on measurements of duplicate samples.

c. Overall variations of algal lipids in microcosms

Distributions of the added algal lipids differed in the control and crustacean cores (Figs. 3 and 4). The algal fatty acids and alkenones in the control cores always remained in surface sediments and degraded gradually with time. In contrast, *P. pugio* resuspended the algal lipids from the surface sediments to particles in the overlying water and also downward to the subsurface (0.5–3 cm). To track the ultimate fates of algal lipids in the microcosms with crustacean, total amounts of algal lipids in suspended particles, surface 0–0.5 cm, and subsurface 0.5–3 cm were integrated and are reported as core inventory (μg/core). The amount in suspended particles was estimated by multiplying the lipid concentration (μg/g dry particle) by the total amount of suspended particles collected from the overlying water. The amount in the surface sediments was determined based on the lipid concentration (μg/g dry sediment) and total dry sediment in this layer. The amount in the subsurface sediments was determined by integrating the lipid amount in each depth interval (similar to the surface case). In the control cores, total amounts of alkenones were those in the surface (0–0.5 cm) sediments while total amounts of fatty acids (except 22:6) included a portion (~15–30%) from the background. Total amounts of all fatty acids decreased throughout the incubation while most alkenone concentrations declined after 4 days in both crustacean and control cores (Figs. 3 and 4). Moreover, the variations of alkenones and fatty acids in crustacean cores showed different patterns. For example, relatively larger portions of alkenones than algal fatty acids were found in suspended particles in the first week. Substantial amounts (20–30%) of alkenones in the crustacean

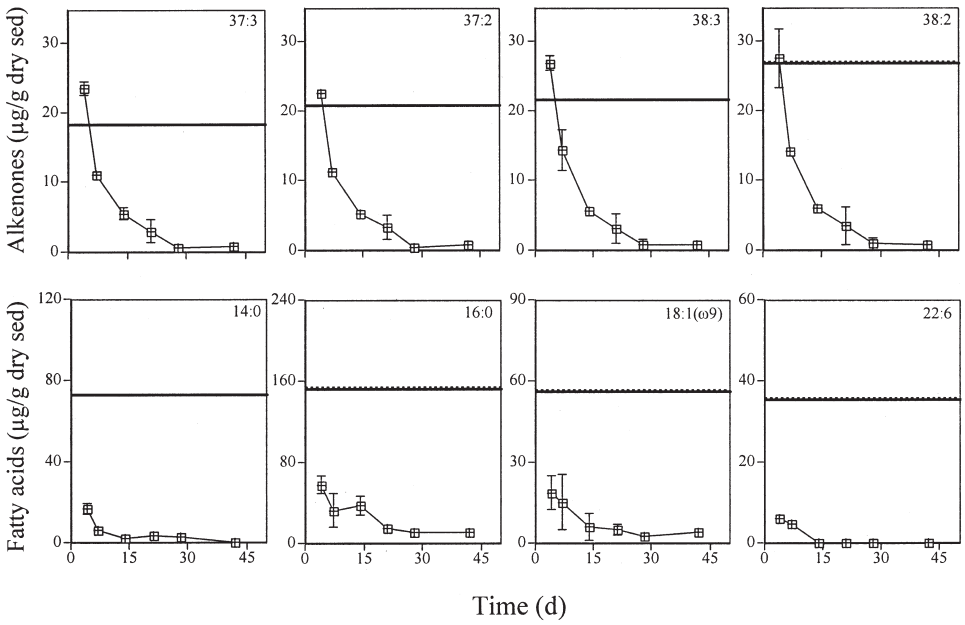

Figure 2. Variations of concentrations of alkenones and algal fatty acids in suspended particles in crustacean cores during incubations. Note: dotted lines are the initial ($t = 0$ day) concentrations of various lipids in surface sediments.

cores were moved to the subsurface and 22:6 fatty acid (exclusive algal component) was also observed in the subsurface in the first week. However, other algal fatty acids (other than 22:6) in the subsurface of crustacean cores were equal to or less than those in the control cores. At each sampling time, total amounts of fatty acids in the crustacean cores were significantly less than those in the control cores (t-test, $P < 0.05$) while the amounts of alkenones in the two microcosms were similar (t-test, $P > 0.05$).

d. Variations of intracellular storage and membrane fatty acids

In *E. huxleyi* cells, more fatty acids exist in membrane (~80% of the total) than in intracellular storage component (~20% of the total) (Ding and Sun, 2005a). During incubations, concentrations of fatty acids in membrane and intracellular storage component decreased continuously (Fig. 5). In the crustacean cores, fatty acids in both intracellular storage and membrane components degraded faster and more completely than those in the control cores. The only exception is the case of 22:6 fatty acid in the membrane fraction, where 22:6 in both control and crustacean cores degraded at similar rates and disappeared completely within three weeks. The variations of most fatty acids at each sampling time differed between the crustacean and control cores (t-test, $P < 0.05$).

Table 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 the day 4 of incubations in crustacean cores. The ratios of C_{sp} and C_{ss} to C_0 are listed. The error range is calculated based on duplicate samples ($n = 2$).

	Concentration (μg/g dry sed)			
Alkenones	37:3	37:2	38:3	38:2
C_0	18.3 ± 0.2	20.8 ± 0.2	21.6 ± 0.7	27.0 ± 2.4
C_{sp}	23.5 ± 1.0	22.5 ± 0.2	26.8 ± 1.1	27.5 ± 5.4
C_{ss}	11.2 ± 0.8	13.8 ± 0.4	11.0 ± 2.6	16.2 ± 2.8
C_{sp}/C_0	1.3	1.1	1.2	1.0
C_{ss}/C_0	0.6	0.7	0.5	0.6
Fatty acids	14:0	16:0	18:1(ω9)	22:6
C_0	73.3 ± 5.0	152.9 ± 11.6	56.5 ± 1.1	35.7 ± 1.0
C_{sp}	16.5 ± 2.7	57.5 ± 9.2	18.5 ± 1.1	6.0 ± 0.7
C_{ss}	17.6 ± 4.0	49.6 ± 8.8	10.3 ± 1.1	3.8 ± 0.3
C_{sp}/C_0	0.2	0.4	0.3	0.2
C_{ss}/C_0	0.2	0.3	0.2	0.1

e. Variations of $U^{k'}_{37}$ index

The $U^{k'}_{37}$ index in suspended particles and surface sediments in the control and crustacean cores was scattered in small ranges: 0.483–0.549 for surface sediments in control cores, and 0.485–0.552 for surface sediments and 0.477–0.536 for suspended particles in crustacean cores respectively (Fig. 6). Based on the correlation ($U^{k'}_{37} = 0.039 + 0.034T$, Prahl and Wakeham, 1987), these $U^{k'}_{37}$ values represented a temperature range of 13–15°C, closely corresponding to the mean culturing temperature (14°C).

4. Discussion

a. Processes controlling the distribution of algal lipids in microcosms

In the control cores (without *P. pugio*), all added algal lipids were present in the surface (0–0.5 cm) sediments throughout incubations because no mixing occurred in the sediments. By contrast, in the crustacean cores, algal lipids were observed in suspended particles, surface (0–0.5 cm) and subsurface (0.5–3 cm) sediments. Clearly, *P. pugio*'s activities were responsible for the different distributions of algal lipids between two microcosms.

P. pugio is typically a deposit-feeder (Reinsel *et al.,* 2001), playing an important role in recycling of organic matter from pulsed input (Welsh, 1975). Using its chelate pereiopods, *P. pugio* grasps and carries food particles to its mouth (Kneib, 1985). Although food sources for *P. pugio* are diverse, the grazing efficiencies vary greatly with the quality of food materials (Fleeger *et al.,* 1999). The crustacean also enhances its feeding efficiency by

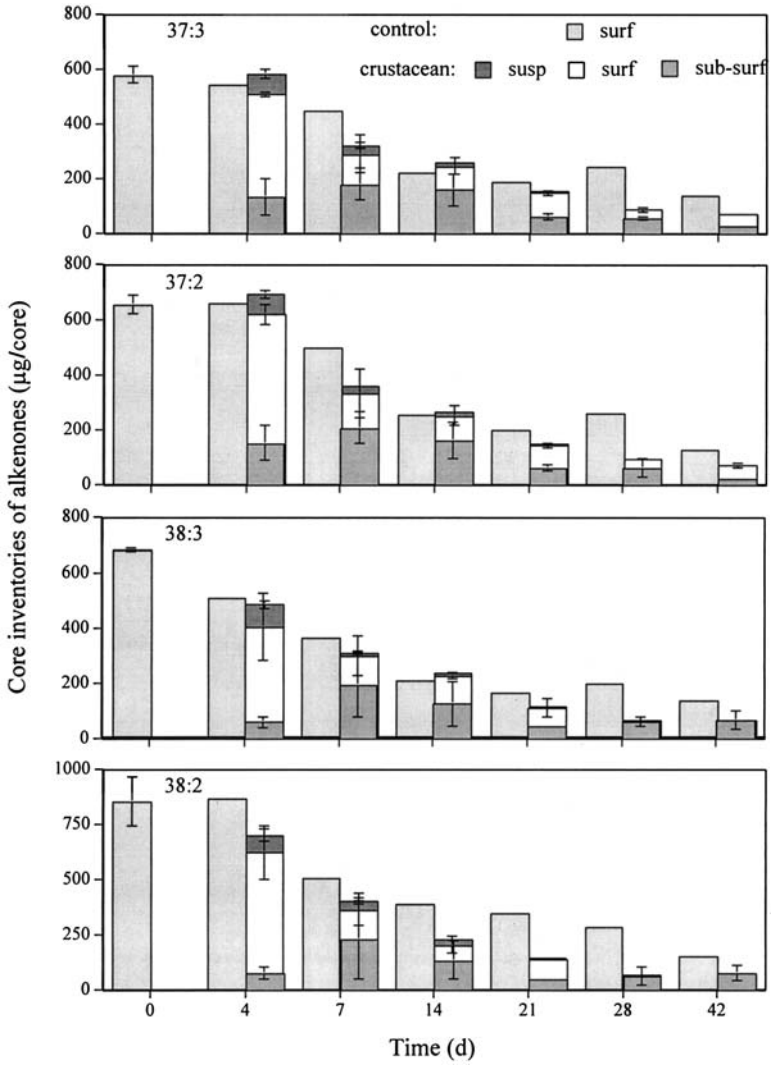

Figure 3. Variations of core inventories of alkenones (directly extracted) in control and crustacean cores during incubations. Alkenones in crustacean cores include those from suspended particles, surface (0–0.5 cm) and subsurface (0.5–3 cm) sediments. The error ranges in each fraction were determined based on measurements of duplicate samples.

selectively grazing organic detritus from sediments (Odum and Heald, 1972). When the crustacean ingests microalgae-rich particles, fecal materials are excreted into suspended particulate phase (Morgan, 1980). The crustacean also crawls on the sediment surface and directly lifts small particles into overlying water, resulting in measurable resuspension of sediment (Graf and Rosenberg, 1997). It is difficult to assess the relative portions of

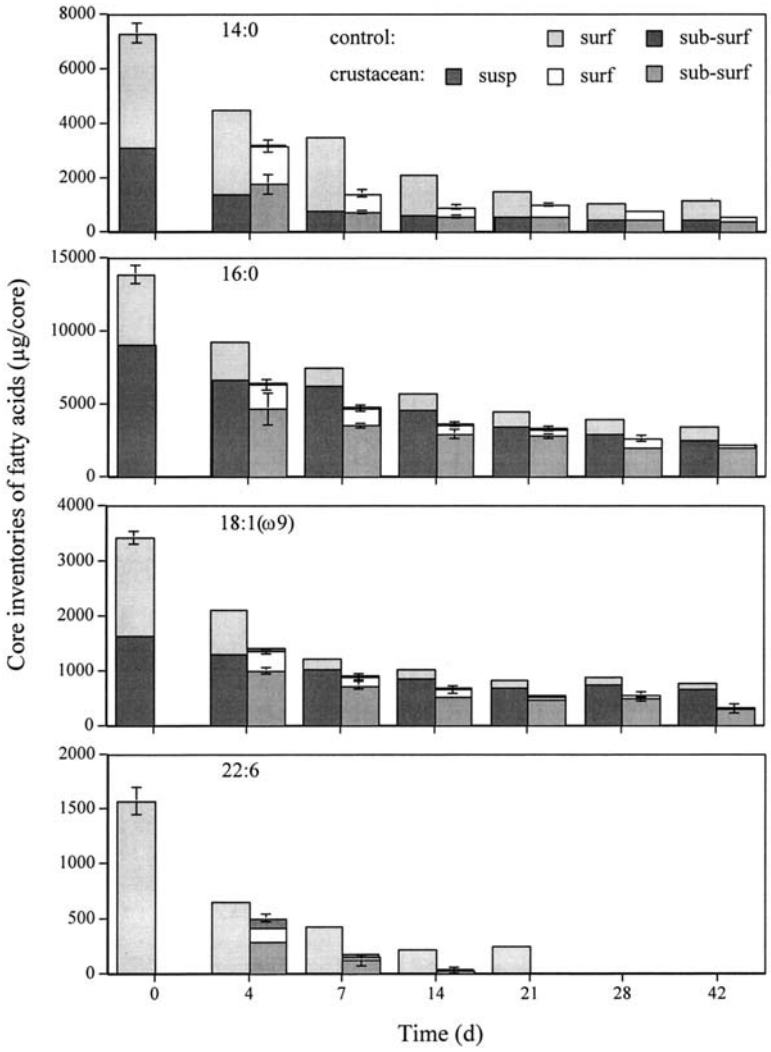

Figure 4. Variations of core inventories of fatty acids (directly extracted) in control and crustacean cores during incubations. The fatty acids in control cores include those from surface (0–0.5 cm) and subsurface (0.5–3 cm) sediments. The fatty acids in crustacean cores include those from suspended particles, surface (0–0.5 cm) and subsurface (0.5–3 cm) sediments.

suspended particles derived from physical movement and from excretion. However, in the first few days, the suspended particles were enriched in alkenones and depleted in fatty acids due to differential consumption by the crustacean, implying that excretion of feces might contribute more suspended particles than physical disturbance because physical processes could not fractionate lipid compounds between sediments and suspended

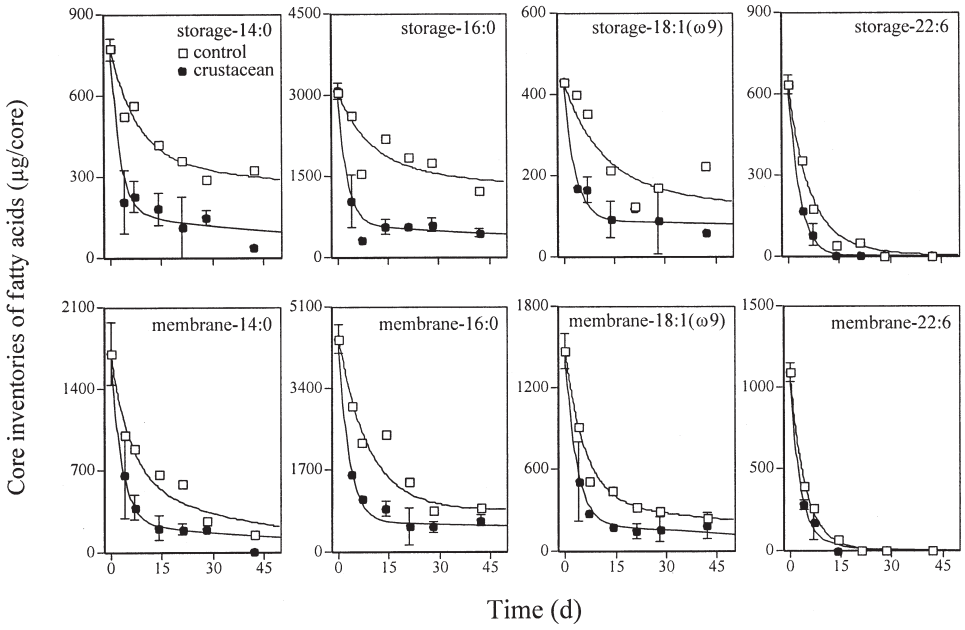

Figure 5. Variations of core inventories of storage and membrane fatty acids (separated by Si-gel) in control and crustacean cores during incubations. The curves are best fits of data using a two-component multi-*G* model.

particles. It also appears that the crustaceans do not assimilate alkenones but excrete them in their feces (Grice *et al.,* 1998).

Although the crustacean does not build burrows, it sometimes buries itself in sediments (Shenker and Dean, 1979). Frequent movements by the crustacean rework sediments, mixing surface sediments from the interface to subsurface. This downward transport was seen in the crustacean cores due to the occurrence of alkenones and 22:6 fatty acid in subsurface sediments. However, other fatty acids (14:0, 16:0 and 18:1(ω9), which are from both algal cells and bulk sediments) were depleted in the subsurface sediments of crustacean cores compared to those in control cores, implying that various lipid compounds might be differently utilized by crustacean during grazing and transport processes (Volkman *et al.,* 1980; Harvey *et al.,* 1987; Grice *et al.,* 1998).

Besides transport processes, the losses of algal lipids in the surface sediments were largely caused by degradation, including structure breakdown by decomposition and assimilation by organisms. In the control cores, most algal lipids were degraded in the surface sediments, likely mediated by microbial processes. In the crustacean cores, degradation of algal lipids was probably driven by both macrofaunal activities and microbial processes. It was observed that alkenones and algal fatty acids degraded at similar rates in experimental seawater systems, where only microbial processes were responsible for the degradation (Sun *et al.,* 2004). However, crustacean grazing may result

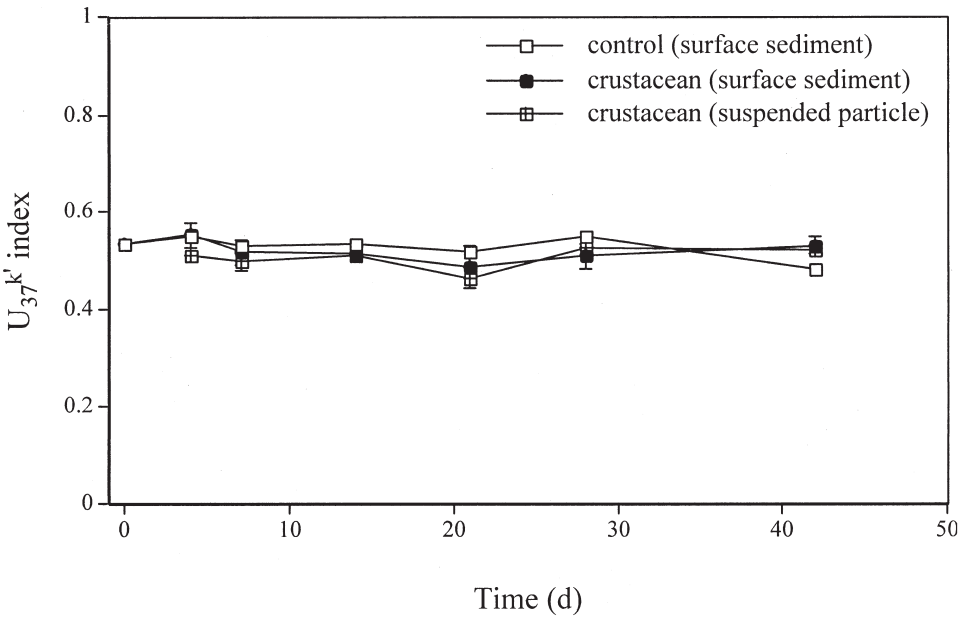

Figure 6. Variations of $U_{37}^{k'}$ index in surface sediments of control and crustacean cores and in suspended particles of crustacean cores during incubations.

in the differential degradation of algal lipids. For example, algal fatty acids, especially the polyunsaturated, were largely assimilated by crustacean during grazing (Harvey *et al.,* 1987) while most alkenones in diet material were not digested by crustacean and, instead, egested in fecal material (Volkman *et al.,* 1980; Grice *et al.,* 1998). Therefore, the contents of algal lipids in the subsurface sediments of crustacean cores were dependent on the relative rates of downward transport vs. degradation of individual compounds.

The microcosms in this study were constructed using pre-sieved sediments, which might change the physical and biochemical characteristics of natural sediments and disturb the original microbial community. Kristensen and Hansen (1995) observed that when new organic substrates were added into disturbed (pre-sieved) sediments, transient build-up of intermediate metabolic products in the DOC pool could occur before complete remineralization to CO_2, which was likely caused by adaptations of microbial community in such systems. However, the adaptations of microbial community were found to be dependent on sediment type (sand vs. mud) or quality of substrate materials (fresh vs. aged) (Hansen and Kristensen, 1998). As shown by the small loss of alkenones within the first a few days of incubations, the microbial community in our microcosms might need some time to adjust. However, rapid changes in fatty acid concentrations at the early period indicate that microbial processes can begin immediately after the systems are set up. A previous study (Sun *et al.,* 2002) also showed that ^{13}C was immediately incorporated into bacteria-specific fatty acids (iso-15:0) in similarly disturbed sediments when the ^{13}C-labeled algal material

was added. Thus, microbe species in the disturbed sediments may vary in their responses to different compounds although it is unclear how the adaptations proceed.

b. Effect of P. pugio's grazing on degradation of algal fatty acids and alkenones

To examine the differential effects of crustacean activities on overall degradation of alkenones and algal fatty acids, the variations of individual lipid compounds in suspended particles, surface and subsurface sediments were followed to estimate degradation rate constants. Considering kinetic features of lipid degradation for most compounds (a faster degradation in the first two weeks and followed by a slow degradation), a two-component multi-*G* model (Westrich and Berner, 1984) was used to fit the original data:

$$G_t = (G_1)_0\exp(-k_1t) + (G_2)_0\exp(-k_2t) \quad (1)$$

where, G_t, $(G_1)_0$, and $(G_2)_0$, are total amount of lipid compounds, initial amount of pool 1 and initial amount of 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), in which there was a breaking point to distinguish two pools. The k_1 and k_2 were estimated based on the Eq. (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:

$$k = f_1k_1 + f_2k_2 \quad (2)$$

where, f_1 is the proportion of pool 1 to total (G_1/G_t) and f_2 the proportion of pool 2 to total (G_2/G_t). For rate constant estimates of some fatty acids (14:0, 16:0 and 18:1(ω9), which were from added algal cells and bulk sediments), the background concentrations (averaged from three depths in subsurface sediments) were subtracted, which minimized the content of fatty acids from the bulk sediments.

A comparison of degradation rate constants of alkenones and algal fatty acids between control and crustacean cores (Table 2) suggested that crustacean activities had 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 generally smaller (1.2–1.4) than those for algal fatty acids (1.9–4.9). The difference in the rate constant ratios between two classes of lipids is likely 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, a large portion of algal fatty acids was readily assimilated by the crustacean, as indicated by initial depletion of fatty acids in suspended particles (Fig. 2). Conversely, the crustacean rejected alkenones into fecal materials, leading to an initial enrichment of alkenones in suspended particles. The initial enrichment of alkenones also indicated that crustacean selectively grazed the fresh algal materials from the surface sediments. Therefore, the degradation of alkenones in both control and crustacean cores was largely controlled by microbial processes.

Table 2. Degradation rate constants of alkenones and algal fatty acids in control (k_c) and crustacean (k_p) cores. The ratios of k_p to k_c, and the correlation coefficient (r^2) are listed.

	Alkenones			
	37:3	37:2	38:3	38:2
k_p (d^{-1})	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 (d^{-1})	0.039	0.043	0.071	0.059
(r^2)	(0.92)	(0.94)	(0.90)	(0.88)
k_p/k_c	1.38	1.44	1.24	1.27
	Fatty acids			
	14:0	16:0	18:1(ω9)	22:6
k_p (d^{-1})	0.315 ± 0.060	0.238 ± 0.046	0.304 ± 0.036	0.363 ± 0.043
(r^2)	(0.95)	(0.92)	(0.94)	(0.99)
k_c (d^{-1})	0.065	0.070	0.123	0.194
(r^2)	(0.99)	(0.91)	(0.91)	(0.99)
k_p/k_c	4.85	3.40	2.47	1.87

It is interesting to note that degradation rate constants of unsaturated fatty acids are higher than those of saturated ones in control cores but they are similar in crustacean cores. Previous field observations (Haddad *et al.,* 1992; Meyers and Eadie, 1993; Canuel and Martens, 1996) and laboratory experiments (Harvey and Macko, 1997; Sun *et al.,* 1997) have demonstrated that unsaturated (especially polyunsaturated) fatty acids generally degrade faster than saturated fatty acids. However, with the presence of crustacean, the effect of structural unsaturation on fatty acid degradation was minimized or eliminated. Although many animals preferentially assimilate dietary polyunsaturated fatty acids over saturated fatty acids (Tanoue *et al.,* 1982; Harvey *et al.,* 1987), some animals (e.g., annelids and bivalves) assimilate all fatty acids to a high degree (Bradshaw *et al.,* 1990; Sun *et al.,* 1999). Bradshaw and Eglinton (1993) suggested that the extent of assimilation of unsaturated and saturated fatty acids was dependent on the total quantity of algal material ingested. 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.

Benthic fauna produce surface-active agents (or surfactants) in their enclosed digestive system, which provides an advantage relative to bacteria in degrading hydrophobic organic compounds (Mayer *et al.,* 2001). Many studies (Mayer *et al.,* 1996; Voparil and Mayer, 2000; Ahrens *et al.,* 2001) have shown that surfactants enhance bioavailability of polycyclic aromatic hydrocarbons and halogenated hydrocarbons to marine benthos. Similarly, we can expect that the crustacean might use the surfactants in its digestive system to more rapidly degrade hydrophobic lipid compounds than bacteria. However, in our experiments, the effect of crustacean (k_p/k_c ratio) on lipid degradation seemed to decrease with increasing molecular weight of lipid compounds (Table 2). One possible

reason for this trend is related to the structures and concentrations of surfactants produced by the crustacean. Generally, the surfactants are amphiphilic molecules with hydrophobic and hydrophilic ends, which have chain length of C_8–C_{12} (Vonk, 1969). When surfactants accumulate to a threshold level, they assemble into micelles (molecular aggregates) in the digestive system, which can dissolve some insoluble organic compounds with similar chain length (Smoot *et al.,* 2003). However, when the chain length of hydrophobic compounds increases (e.g., $>C_{12}$), the ability of surfactants to solubilize hydrophobic compounds in digestive fluids would decrease. This trend may, at least partially, explain why the crustacean has poor ability to digest the long chain (C_{37} and C_{38}) alkenones. Unlike most natural lipids, the geometry of double bonds in long chain alkenones were determined to be *trans* rather than *cis* (Rechka and Maxwell, 1988; Volkman *et al.,* 1988), which may also affect their susceptibility to degrading agents (fauna and bacteria). However, there has been no direct evidence for this structural factor (Rontani *et al.,* 2005).

c. Influence of P. pugio's activities on structural associations of fatty acids

Fatty acids are mostly bound in different molecular complexes such as triacylglycerols and phospholipids within algal cells. These complexes are present in the cells as intracellular storage components and membrane constituents, which differ in molecular size, structural linkage, polarity, and chemical lability. Microbial degradation of fatty acids bound in cell membrane components was faster than those bound in intracellular storage components (Ding and Sun, 2005a). In the present study, fatty acids were degraded primarily by microbial processes in control cores while their degradation in crustacean cores was driven by both crustacean activities and microbial processes. To examine the influence of *P. pugio*'s grazing on the degradation of fatty acids bound in different structural associations, the degradation rate constants of membrane and storage fatty acids were estimated using the same two-component multi-*G* model (Eqs. 1 and 2).

A comparison of rate constants of membrane and storage fatty acids between control and crustacean cores (Table 3) showed that the rate constants from crustacean cores were two to six times higher than those from control cores, indicating that crustacean activities accelerated the degradation of both membrane and storage fatty acids. On the other hand, the rate constant ratios of membrane to storage fatty acids in control cores ranged from 1.4 to 2.3 for various compounds whereas these ratios in crustacean cores were close to 1 for all fatty acids. These results suggest that *P. pugio* minimized the effects of structural associations on fatty acid degradation. As discussed above, grazing and assimilation by crustaceans are the primary processes degrading algal fatty acids. To digest algal fatty acids bound in different structural complexes during passage through the gut, crustacean produce various enzymes such as lipases, phospholipases, and other esterases in their digestive system (Dall and Moriarty, 1983). These enzymes release fatty acids from various ester complexes bound in the different cellular components, which is the first and limiting step for fatty acid degradation (Billen, 1982). In contrast, bacteria in sediments produce limited enzymes and the production of these enzymes is influenced by a variety of

Table 3. Degradation rate constants of intracellular storage and membrane fatty acids in control (k_c) and crustacean (k_p) cores. The ratios of k_p to k_c, and the correlation coefficient (r^2) are listed.

	14:0			16:0			18:1(ω9)			22:6		
Fatty acids	stor*	mem**	mem/stor	stor*	mem**	mem/stor	stor*	mem**	mem/stor	stor*	mem**	mem/stor
k_p (d^{-1})	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	± 0.005		± 0.035	± 0.047	
(r^2)	(0.96)	(0.94)		(0.96)	(0.91)		(0.96)	(0.96)		(0.99)	(0.99)	
k_c (d^{-1})	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)		(0.70)	(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	

*: storage fatty acids
**: membrane fatty acids

environmental factors (Jaeger *et al.,* 1994; Arnosti, 2004). In addition, bacteria-released enzymes are very specific, whereby they might efficiently hydrolyze some esters but may not be general to all ester complexes in algal cells (Finnerty, 1989; Jaeger *et al.,* 1999).

Based on our separation scheme, the storage fatty acid pool might include some intermediate products (e.g., diacylglycerols, monoacylglycerols, and free fatty acids) from initial hydrolysis of triacylglycerols and phospholipids during incubations. On the other hand, bacterial growth during incubations may produce some new phospholipids (e.g., di- and mono-phosphoglycerides and phosphatidylethanolamines), which are bound in the membrane fatty acid pool (Goutz *et al.,* 2003). In addition, some studies (Parrish, 1987; Parrish *et al.,* 1988) found acetone-mobile polar lipids (e.g., glyceryl-1-monohexadecanate) in dissolved and particulate marine lipid pools, which are potential intermediate products from various ester complexes. However, no accumulations of these fatty acids were observed in either pool throughout the incubations (Fig. 5), and our previous study (Sun *et al.,* 1997) demonstrated that free fatty acids generally degraded $>2\times$ faster than the cell-associated analogues in marine sediments.

d. Influence of P. pugio's activities on $U^{k'}_{37}$ index

There has been a long-standing argument about the stability of $U^{k'}_{37}$ during diagenetic processes, supported by contrasting evidence. For example, Hoefs *et al.* (1998) found that the oxidative processes in Pliocene and Miocene Madeira Abyssal Plain sediments selectively degraded 37:3 over 37:2 alkenones, resulting in a significant change in the $U^{k'}_{37}$. Based on profiles of alkenones in oxic and anoxic sediments in the Santa Monica Basin, Gong and Hollander (1999) indicated that intensive activities of benthic macrofauna in oxic sediments caused differential degradation of 37:3 vs. 37:2 alkenones, leading to a shift of 2–4°C in the predicted 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 $U^{k'}_{37}$ was largely unaltered in marine sediments.

Teece *et al.* (1998) conducted a series of experiments to incubate *E. huxleyi* cells in sediment slurries (without macrofauna) under oxic, sulphate-reducing, and methanogenic conditions. Their results demonstrated that decomposition of $>80\%$ initial alkenones would not yield significant bias of the $U^{k'}_{37}$. Our previous experiments also showed that when 80–90% of initial alkenones from *E. huxleyi* cells degraded in natural oxic and anoxic seawaters (without animals), the $U^{k'}_{37}$ remained almost constant (Sun *et al.,* 2004). However, a recent study (Rontani *et al.,* 2005) found that environmental conditions (oxic, denitrifying, and anoxic non-denitrifying conditions) affected alkenone degradation differently, resulting in a potential alteration in the $U^{k'}_{37}$. In our study, although a large portion of alkenones degraded, the $U^{k'}_{37}$ index showed no consistent change over the incubation (Fig. 6), with variations corresponding to a calculated growth temperature change of ± 1°C. These results indicate that microbial processes and crustacean activities did not significantly change the $U^{k'}_{37}$ index.

e. Fates of algal lipids in sediments

Our experimental results reveal the important role of one species of benthic macrofauna (*P. pugio*) in controlling the fates of different lipid biomarkers derived from algal source. In the absence of the crustacean, degradation of algal lipids in control cores was mediated mostly by microbial processes in the surface sediments. Alkenones and fatty acids derived from algal cells degraded at similar rates in this regime. In contrast, the presence of crustacean affected algal lipid degradation in sediments by several ways: (1) selectively grazing algal material from surface sediments; (2) preferentially assimilating fatty acids over alkenones during digestion; and (3) transporting lipid containing detritus from the surface sediments upward to the overlying water and downward to the subsurface sediments. In this regime, both macrofaunal activities and microbial processes were responsible for fates of algal lipids.

Differential effects of crustacean on alkenones and algal fatty acids may affect the ultimate fates of these important biomarkers in sediments. For example, preferential assimilation of algal fatty acids by *P. pugio* in surface sediments may lead to a less amount of these compounds transporting to the subsurface sediments. On the other hand, alkenones were not efficiently degraded by the crustacean grazing in the surface sediments, so relatively larger amount of alkenones can be moved to the less active subsurface zone. Therefore, the preservation of algal lipid biomarkers in sediments may in part depend on the relative rates of degradation and downward transport by macrofauna.

5. Conclusions

Detailed examination of distributions and variations of algae-derived lipids in sediment microcosms showed that *P. pugio* (benthic crustacean) affected the fates of algal fatty acids and alkenones by grazing and bioturbation activities. All major algal fatty acids (polyunsaturated, monounsaturated, and saturated) were preferentially assimilated by the crustacean during digestion, causing an enhanced degradation of algal fatty acids in sediments. In contrast, the alkenones were not assimilated by the crustacean while microbial processes were largely responsible for alkenone degradation. A significant portion of alkenones was transported down to the subsurface sediments before all alkenones were degraded by microbial processes in the surface sediments while the algal fatty acids were more rapidly and completely utilized by the crustacean during grazing and transport processes. The crustacean activities also minimized the structural effects of algal fatty acids, including unsaturation extent and labilities of various ester complexes. In addition, both crustacean activities and microbial processes in sediments did not significantly alter the $U^{k'}_{37}$, supporting the application of this index in paleoceanographic studies.

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